SYNTHESIS AND ANALYSIS OF *ERIOSEMA* ISOFLAVONOIDS AND DERIVATIVES THEREOF

Submitted in fulfillment of the academic requirements for the degree of

Doctor of Philosophy

in the

College of Agriculture, Engineering and Science

School of Chemistry and Physics

at the

UNIVERSITY OF KWAZULU-NATAL

INYUVESI YAKWAZULU-NATALI

(Pietermaritzburg)

By

MAMOALOSI ALIX-MARIA SELEPE

Supervisor: Prof. Fanie R. van Heerden

Co-Supervisor: Prof. Siegfried E. Drewes

December 2011

ABSTRACT

Kraussianone 1 and kraussianone 2 were previously isolated as active compounds from the roots of *Eriosema kraussianum* Meisn., a plant used for the treatment of male impotence and urinary complaints in KwaZulu-Natal. The objectives of this study were firstly, to develop a method for the analysis of metabolites from *E. kraussianum* and other *Eriosema* plants that are used for erectile dysfunction and secondly, to develop synthetic methods for kraussianone 1 and structurally related compounds.

A reversed-phase HPLC-PDA method was developed for the analysis of the extracts of plants from different sources, two of which were authentic *E. kraussianum* collected from the Drakensberg and Pietermaritzburg. The roots of other *Eriosema* species called *ubangalala* and *uqonsi* in Isizulu were also analysed. These plants were bought from the local herbal traders. The extracts of the two *E. kraussianum* plants and one *uqonsi* sample showed a similar chemical profile, even though there were variations in the relative concentrations of the metabolites within each plant. In these three plants, kraussianone 1, the most active metabolite of *E. kraussianum*, occurred in relatively low quantities, whereas kraussianone 2 was one of the major constituents. The other commercial plants that were analysed contained different compounds from those found in *E. kraussianum*. The HPLC method developed herein facilitates rapid identification and relative quantification of metabolites from *E. kraussianum*.

Strategies based on semi-synthesis and total synthesis were employed for the preparation of kraussianone 1. The semi-synthetic route was based on the transformation of the prenyl side chain of kraussianone 2 into a linear dimethylpyran scaffold fused to the A-ring. Two routes were investigated for the semi-synthesis of kraussianone 1 from kraussianone 2. In the first route, the dimethylchromene ring was to be prepared by the acid-catalysed cyclisation of the prenyl group of kraussianone 2, followed by dehydrogenation of the resulting dimethylchroman chromophore. This route was abandoned due to poor regioselectivity of the cyclisation reaction and the difficulty of oxidising the dimethylchroman scaffold on the phloroglucinol moiety into a dimethylchromene. The second strategy involved selective protection of the OH-2', followed by DDQ-mediated

oxidative cyclisation of the prenyl group to OH-7. This was the most viable route and kraussianone 1 was prepared in an overall yield of 54% from kraussianone 2.

The total synthesis of kraussianone 1, on the other hand, employed the Suzuki-Miyaura reaction for the construction of the isoflavone nucleus and the regioselective introduction of the dimethylpyran scaffolds to the A- and B-rings. The key precursors in this synthesis were 3-iodo-5,7-dimethoxymethoxychromone and a boronic acid coupling partner, 7-benzyloxy-2,2-dimethylchromene-6-boronic acid, already bearing the prerequisite chromene scaffold attached to the B-ring. The isoflavones genistein, 2-hydroxygenistein, eriosemaone D and a geranyl analogue of kraussianone 1 were prepared via the route developed for the total synthesis of kraussianone 1 by structural modifications of rings A and B. Furthermore, this synthetic approach was expanded to the synthesis of the coumarochromones lupinalbin A and lupinalbin H.

The development of the feasible semi-synthetic and total synthetic routes described herein for kraussianone 1 is of importance for the production of material for an in depth study of the pharmacological activities and the structure-activity relationship studies of kraussianone 1 and related compounds.

DECLARATION

I hereby certify that this research is a result of my own investigation, which has not already been accepted in substance for any other degree and is not being in candidature for any other degree.

Signed:_____(Mamoalosi A. Selepe) Date:_____

I hereby certify that this statement is correct

Signed:_____ Professor F.R. van Heerden Date:_____
(Supervisor)

Signed:_____ Professor S. E. Drewes Date:_____
(Co-supervisor)

School of Chemistry and Physics
University of KwaZulu-Natal
Pietermaritzburg
December 2011

PLAGIARISM

I, *Mamoalosi A. Selepe* declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original work.

2. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other sources.

3. The thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted:
    - Their words have been rewritten but the general information attributed to them has been referenced.
    - Where their exact words have been used, their writing has been placed inside quotation marks and referenced.

4. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source therefore being detailed in the thesis.

Signed:_____(Mamoalosi A. Selepe) Date:_____

ACKNOWLEDGEMENTS

I express my sincere gratitude to my supervisor, Professor Fanie van Heerden, for her supervision, guidance, and encouragement throughout this work.

I am also grateful to my co-supervisor, Professor Siegfried E. Drewes, for the motivation, the valuable discussions and great involvement in every single step of this project. I was indeed privileged to have you as my co-supervisor.

I am thankful to Dr. Molahlehi Sonopo, for being a good friend and a mentor to me. Thank you for sharing your knowledge of chemistry with me and for teaching me the tactics I needed most for my laboratory work. Your support and brilliant ideas are highly appreciated.

My thanks are also due to the following people:

The Frank Warren laboratory colleagues, for creating a pleasant working environment for me.

Mr. C. Grimmer for assistance with NMR experiments.

Mrs. C. Janse van Rensburg and Mr. L. Mayne for helping with mass spectrometry.

Mr. R. Somaru and Mr. F. Shaik for their technical assistance.

Dr. C. Southway and Ms. P. Lebunyane for assistance with HPLC.

Mr. B. Dlamini for helping with the IR spectrophotometer.

My greatest thanks go to my family, my mother 'Ma-Agatha Selepe, my sisters and my brother (Agatha, Mapaulina and Julias) for their prayers, their support and the great love that helps me endure through hardships.

I thank the government of Lesotho, the Univesity of KwaZulu-Natal and the National Reseach Foundation (South Africa) for the financial Support.

Above All, I thank the Almighty God, for the gift of life, for the blessings, protection and guidance.

Glory be to the Father, to the Son and to the Holy Spirit.

TABLE OF CONTENTS

APPENDIX I. COPIES OF NMR SPECTRA

APPENDIX II. COPIES OF PUBLICATIONS

ABBREVIATIONS

Ac_2O	Acetic anhydride
AcOH	Acetic acid
BnBr	Benzyl bromide
BuLi	Butyllithium
^oC	Degree Celsius
CAN	Ceric ammonium nitrate
cGMP	Cyclic guanosine monophosphate
DBU	1,8-Diazabycyclo[5.4.0]undec-7-ene
DDQ	2,3-Dichloro-5,6-dicyanobenzoquinone
DIA	Diisopropylamine
DIEA	Diisopropylethylamine
DMSO-d_6	Deuterated dimethylsulfoxide
DMAPP	Dimethylallyl pyrophosphate
DME	Dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMF-DMA	*N*,*N*-dimethylformamide dimethyl acetal
Et	Ethyl
Hex	Hexanes (bp 68 – 70 ^oC)
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry
Hz	Hertz
IR	Infrared
LRMS	Low-resolution mass spectrometry
MOMCl	Methoxymethyl chloride
m/z	Mass/Charge ratio
NADPH	Nicotinamide adenine dinucleotide phosphate
OAc	Acetate
NIDMM	Non-insulin-dependent diabetes mellitus
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser effect spectroscopy
PCC	Pyridinium chlorochromate

PDA	Photo-diode array
PDE-5	Phosphodiesterase-5
PT	Prenyl transferase
p-TsOH	*para*-Toluenesulfonic acid
Rha	Rhamnosyl
rt	Room temperature
SET	Single-electron transfer
SPhos	2-(2,6-Dimethoxybiphenyl)dicyclohexylphosphane
t-BuOH	*tert*-Butanol
t-BuOOH	*tert*-Butyl hydroperoxide
TBDMS	*tert*-Butyldimethylsilyl
TBDPS	*tert*-Butyldiphenylsilyl
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMSBr	Trimethylsilyl bromide
TTN	Thallium(III) nitrate
UV	Ultraviolet

LIST OF FIGURES

LIST OF TABLES

LIST OF SCHEMES

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

A number of important medicinal drugs are derived from natural sources. These include the antitumour drug taxol from the yew tree (*Taxus brevifolia*), huperzine A from Qian Ceng Ta (*Huperzia serrata*) used for treatment of Alzheimer's disease and an antimalarial drug artemisinin from qinghao (*Artemisia annua*).[1] However, the supply of compounds from their natural sources is often limited and not enough material for intensive tests and clinic trials is available. Total synthesis, semi-synthesis and structural modification of the biologically-active compounds from plants play a vital role in the studies of biological activities of the compounds. It makes structure-activity relationship studies possible, which in turn lead to the development of compounds with higher activity, improved physical properties, bioavailability, specificity and lower toxicity.[1,2]

Our interest is centred on the synthesis of biologically-active isoflavonoids. Isoflavonoids are secondary metabolites, which occur mostly in leguminous plants.[3-6] They consist of two phenolic rings (A-ring and B-ring), which are linked by a three carbon heterocyclic ring (C-ring).[3-5] They are distinguished from other classes of flavonoids by the position of attachment of the phenolic B-ring at C-3.[3-5] The isoflavonoid framework is established by enzyme-mediated migration of the aryl B-ring of flavanones.[3-5] Further biochemical transformations of the isoflavone skeleton lead to the formation of advanced subclasses of isoflavonoids such as pterocarpanoids, coumaronochromones, rotenoids as well as coumestans as illustrated in Figure 1.1.[3,7-9] More complex natural isoflavonoid structures are attained by the incorporation of additional substituents by *C*- or *O*- alkylation and/or alkenylation (e.g. methylation, prenylation and geranylation), cyclisation of alkyl and alkenyl side chains to adjacent hydroxy groups and oxidation of additional substituents as well as phenolic rings A and B.[3,7-9]

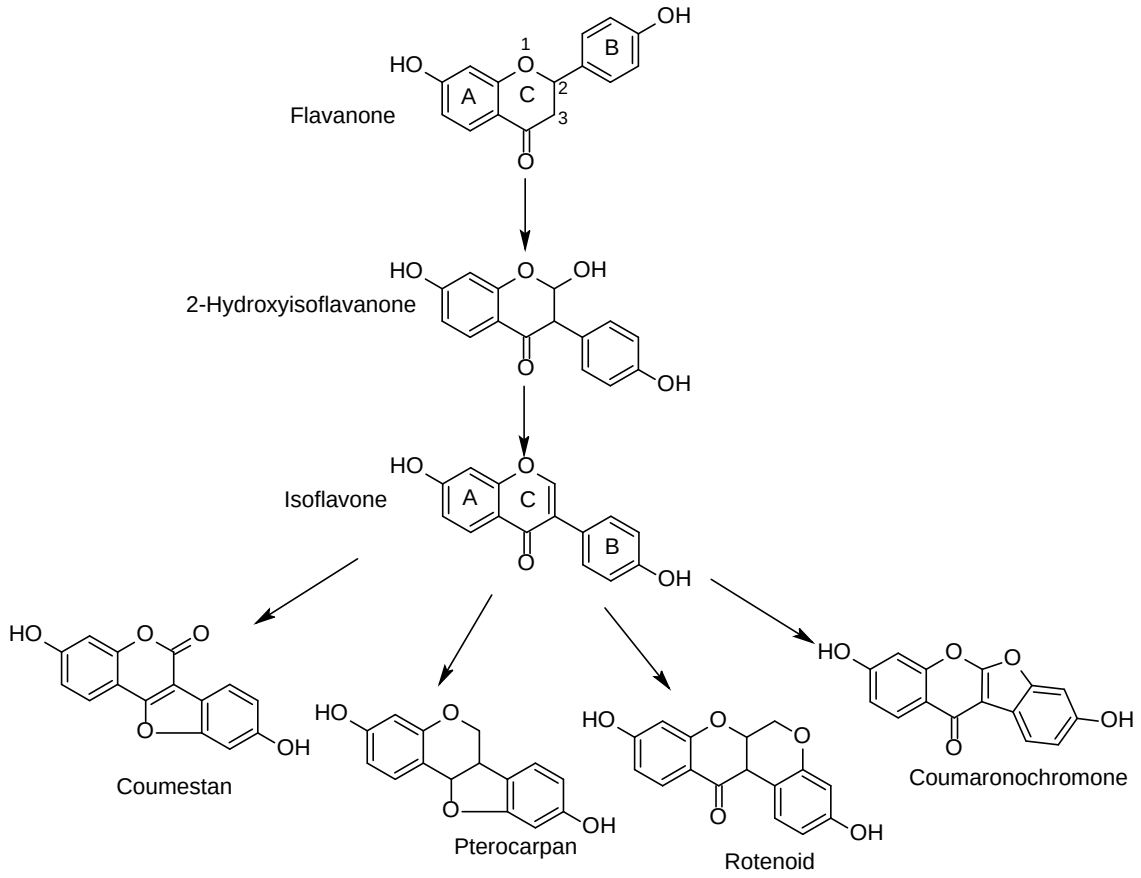


Figure 1.1. Major isoflavonoid classes and their biosynthetic precursors[3,4,7]

The isoflavonoids are frequently referred to as phytoestrogens,[10,11] because they are non-steroidal plant-derived compounds which have the ability to bind to estrogen receptors and modulate estrogenic responses.[10-14] The estrogenic properties of isoflavonoids, particularly isoflavones, have been extensively studied with regards to health benefits.[6,11,12,15] The consumption of phytoestrogen-rich food has been linked to protection against hormone-dependent breast cancer and prostate cancer, alleviation of postmenopausal disorders, osteoporosis as well as cardiovascular protection.[11-16] Besides hormonal action, the health benefits of isoflavonoids have also been attributed to their antioxidant properties, which involve scavenging of reactive oxygen species and metal chelating capabilities.[13,16] Thus, isoflavonoids have been implicated in protection against neurodegenerative diseases.[11-13]

Contrary to the health benefits which have been reported in favour of isoflavonoids, some reports have indicated their potential to trigger adverse health effects, especially when consumed in large quantities.[12-14]

1.2 AIMS AND OBJECTIVES

The aims of this study were:

- To investigate the chemical profile of *Eriosema kraussianum* Meisn. and other plants from different sources, which are used for the treatment of male impotence.
- The second objective was to synthesise the pyranoisoflavone kraussianone 1 (**1**) and structurally related compounds, for investigation of their pharmacological properties.

Kraussianone 1 (**1**) and other related pyranoisoflavonoids were first isolated by Drewes and co-workers from *E. kraussianum*, a plant used in a traditional remedy for male impotence and urinary complaints in KwaZulu-Natal.[17] Preliminary studies on the biological activities of the isolated compounds revealed that **1** and kraussianone 2 (**2**) respectively, possess 85% and 65% erectile dysfunction activity compared to sildenafil (Viagra®) (**3**) (Figure 1.2).[17] Moreover, the compounds were determined to exhibit significant hypoglycaemic and secondary vasorelaxant effects.[18] In addition, they demonstrated improved foetal weights and decreased foetal absorption in a study carried out on pregnant rats.[19] However, the mode of action of the compounds for these activities is still unknown.[17,18] Further biochemical investigations of **1** were limited by the small amount of material obtained from the plant and the difficulty in isolating the compound in its pure form.

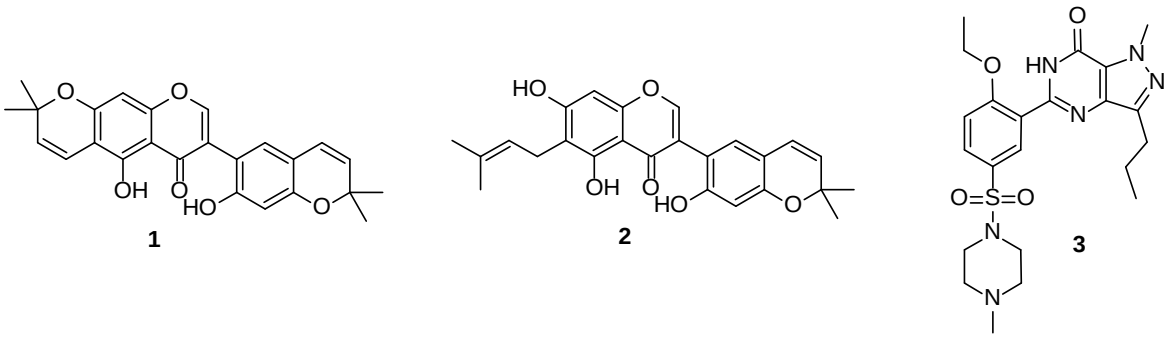


Figure 1.2. Compounds with erectile dysfunction activity

The successful synthesis of kraussianone 1 (**1**) and related isoflavonoids would enable us to perform studies leading to the determination of the molecular basis of action of **1** for erectile dysfunction activity, test the synthesised compounds for other important biological

activities as well as unwanted cytotoxicity, and establish structure-activity relationships of the synthesised compounds.

1.3 ORGANISATION OF THESIS

Following Chapter 1, this thesis is organised into five more chapters. Chapter 2 examines the HPLC profile of some plants used as aphrodisiacs in KwaZulu-Natal. Chapter 3 presents the synthetic routes employed for the semi-synthesis of kraussianone 1 (**1**). This is followed by Chapters 4 and 5, which deal with the development of a strategy for the total synthesis of pyranoisoflavonoids and its application to the synthesis of natural and unnatural isoflavonoids. These include the total synthesis of **1** and structural analogues, discussed in Chapter 4 and the total synthesis of a pyranocoumaronochromone lupinalbin H and related isoflavonoids in Chapter 5. The overall conclusions drawn from the different chapters are given in Chapter 6.

1.4 REFERENCES

(1) Liang, X.-T.; Fang, W.-S. *Medicinal Chemistry of Bioactive Natural Products*; John-Wiley and Sons: New Jersey, 2006.

(2) Walsh, G. *Biopharmaceuticals*; Second ed.; Wiley and Sons: Southern Gate, England, 2003.

(3) Tahara, S.; Ibrahim, R. K. *Phytochemistry* **1995**, *38*, 1073-1094.

(4) Dewick, P. M. In *The Flavonoids: Advances in Research Since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994, p 117 - 238.

(5) Donnelly, D. M. X.; Boland, G. M. *Natural Product Reports* **1995**, 321 - 338.

(6) Botta, B.; Menendez, P.; Zappia, G.; Lima, R. A. d.; Torge, R.; Monache, G. D. *Current Medicinal Chemistry* **2009**, *16*, 3414-3468.

(7) Lapcík, O. *Phytochemistry* **2007**, *68*, 2909-2916.

(8) Veitch, N. C. *Natural Product Reports* **2007**, *24*, 417-464.

(9) Veitch, N. C. *Natural Product Reports* **2009**, *26*, 776-802.

(10) Cornwell, T.; Cohick, W.; Raskin, I. *Phytochemistry* **2004**, *65*, 995-1016.

(11) Miadokova, E. *Toxicology* **2009**, *2*, 211 - 218.

(12) Andersen, Ø. M.; Markham, K. R. *Flavonoids - Chemistry, Biochemistry and Applications*; Taylor and Francis: New York, 2006.

(13) Rice-Evans, C. A.; Packer, L. *Flavonoids in Health and Disease*; Second ed.; Marcel Dekker: Basel, New York, 2003.

(14) Cos, P.; Apers, S.; Vanden Berghe, D.; De Bruyne, T.; Pieters, L.; Vlietinck, A. J. *Planta Medica* **2003**, *69*, 589 - 599.

(15) Sarkar, F. H.; Li, Y. *Cancer Treatment Reviews* **2009**, *35*, 597-607.

(16) Yao, H.; Xu, W.; Shi, X.; Zhang, Z. *Journal of Environmental Science and Health Part C-Environmental Carcinogenesis & Ecotoxicology Reviews* **2011**, *29*, 1-31.

(17) Drewes, S. E.; Horn, M. H.; Munro, O. R.; Dhlamini, J. T. B.; Meyer, J. J. M.; Rakuambo, N. C. *Phytochemistry* **2002**, *59*, 739-747.

(18) Ojewole, J. A.; Drewes, S. E.; Khan, F. *Phytochemistry* **2006**, *67*, 610-617.

(19) Ramesar, S. V.; Gathiram, P.; Drewes, S. E. *Phytotherapy Research* **2012**, *in press*.

CHAPTER 2

HPLC PROFILE OF PLANTS USED FOR TREATMENT OF ERECTILE DYSFUNCTION

2.1 INTRODUCTION

Chemical studies on plants used traditionally for treatment of male impotence have revealed several compounds that offer potential leads for the development of drugs for erectile dysfunction and associated ailments.[1-7] As a result of growing scientific evidence on potency of plants as aphrodisiacs,[2-4,8] there is great interest on commercialisation of these plants, and formulations of some of the extracts are already on the market as "over-the-counter" products. However, it has been reported that the relative composition of the active ingredients vary greatly among plant sources.[9,10] These variations are influenced amongst others by the regions where the plants are grown, the period of harvesting and the age of the plant.[10] Moreover, the metabolites within a plant can manifest synergistic or antagonist effects.[11-15] Therefore, knowledge of the nature, levels and distribution of compounds within a plant is of outmost importance. Several analytical techniques have been employed for qualitative and quantitative analysis of plant metabolites to facilitate the safe and best use of the plant material for medicinal purposes and to guide selection of the optimal material for commercialisation.[9,10,16-18] Furthermore, knowledge of the identity and content of the plant metabolites helps to ensure consistency of the phytotherapeuticals and aids in identification of adulterants.[10,17]

Previous phytochemical studies on the roots of *E. kraussianum* led to the isolation of seven prenylated isoflavonoids (**1**, **2**, **4**-**8**) (Figure 2.1).[11,19] The stereochemistry of compounds **4**-**8** has not been defined. These compounds were tested for erectile dysfunction activity. The assay was based on the ability of the compounds to relax the *corpus cavernosum* of rabbits relative to Viagra®.[11,19] Two compounds, kraussianone 1 (**1**) and kraussianone 2 (**2**), were found to be the most active compounds, whereas kraussianone 3 (**4**) and kraussianone 5 (**6**) manifested the opposite effect, by contracting muscles.[11,19] Our aim was to develop an analytical method that would facilitate rapid identification and relative quantification of

metabolites from *E. kraussianum.* The developed method would then be employed for the analysis of extracts of *E. kraussianum* plants from different origins. This would aid in identification of a source that is rich in the most active ingredients.

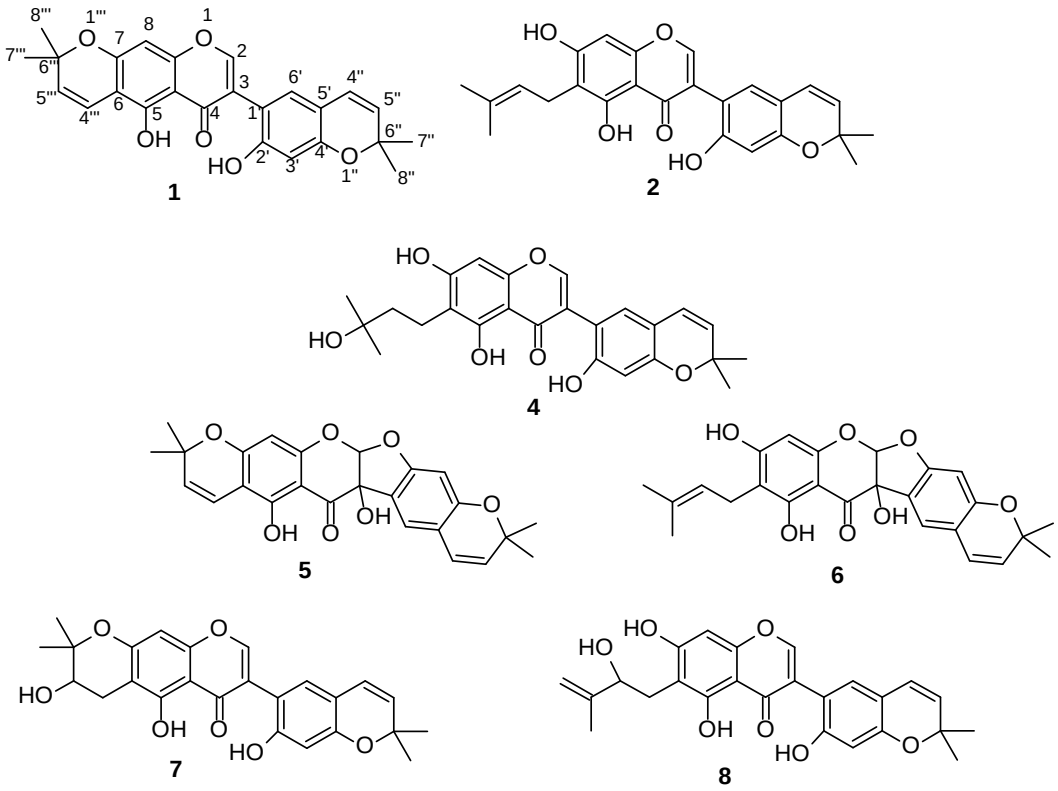


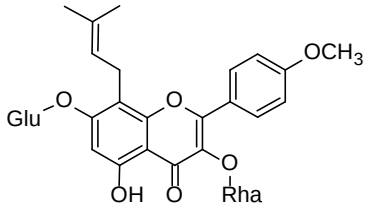
Figure 2.1. Compounds isolated from *E. kraussianum*

The second objective was to analyse other plants which are also used traditionally for treatment of male impotence and urinary complaints in KwaZulu-Natal. There are several plants that are used for male impotence in KwaZulu-Natal. The most common ones are called *iqonsi* or *uqonsi*, *uqontsi* and *ubangalala* in Isizulu.[20-22] However, there is considerable overlap in their scientific names. For instance, the name *ubangalala* is given to three species of the genus *Eriosema*, which are *E. cordatum* E. Mey,[21] *E. salignum* E. Mey[22] and *E. distictum* N.E.Br. (also called *ubangalala omkhulu*, meaning large *ubangalala*).[22] *Iqonsi*/*uqonsi*, on the other hand, refers to *E. salignum*[20-22] and *uqontsi* refers to *E. cordatum* (also called *uqontsi olukhulu*, meaning large *uqontsi*)[20-22] or *E. salignum* (*uqontsi olucane*, meaning small *uqontsi*).[20,22] The roots of these plants are sold commercially and *ubangalala* is sold in the form of tablets called Erex™ u'Bangalala.[23-25] *E. kraussianum* is also marketed as a tincture and capsules called Pro-Erex.[26]

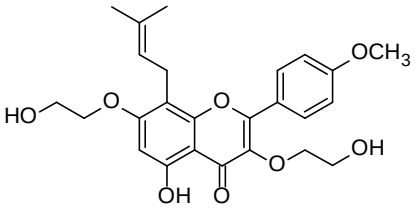
2.2 IMPOTENCE

Impotence, or erectile dysfunction (ED), refers to the failure to achieve or maintain erection for satisfying sexual activity. Chronic ED is often associated with health disorders such as diabetes mellitus, hormonal imbalances, cardiovascular diseases, hypertension, neurological disorders, disseminated sclerosis, Alzheimer's disease and dyslipidemia.[27,28] Selective phosphodiesterase-5 (PDE-5) inhibitors, sildenafil (Viagra®) (**3**), vardenafil (Levitra®), and tadalafil (Cialis®) are currently used as oral anti-impotence drugs.[27,29] They manifest this activity via the nitric oxide/cyclic guanosine monophosphate (NO/cGMP) pathway, which commences with the release of NO from the parasynthetic nerve endings. The released NO activates the production of cGMP, causing relaxation of the arteries of the *Corpus cavernosum* while simultaneously preventing the venous backward flow of the blood. These result in the increased influx of blood into the penile tissue and increased pressure that effect stiffening and enlargement of the male sexual organ. PDE enzymes impede this process by hydrolysing molecules of cGMP. PDE-5 inhibitors therefore promote accumulation of cGMP by preventing its metabolism, leading to a prolonged erection.[27,30,31] However, they cannot stimulate production of nitric oxide, and they are reported to be incompatible with nitrate medications which are often prescribed for patients with diabetes, hypertension, hyperlipedemia or ischemic heart disease, conditions associated with ED.[32,33] Furthermore, they are reported to cause some side effects due to interference with other types of phosphodiesterases.[32]

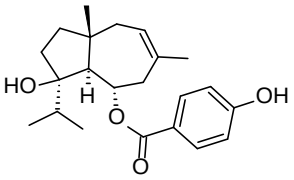
The continuing search for anti-impotence drugs with maximal efficacy has revealed several potential drug leads of natural origin which can overcome the shortcomings of PDE-5 inhibitors via the NO/cGMP pathway or via hormonal action. A good example is the development of an icariin analogue **10** with enhanced PDE-5 inhibitory potency over the parent compound icariin (**9**), and higher selectivity for PDE-5 enzymes than Viagra® (**3**).[1] Icariin (**9**) is an active component isolated from *Epemedium brevicornum* Maxim.[1] Also, ferutinin (**11**), a phytoestrogen[34] from *Ferula hermonis* Boiss., has been reported to boost the levels of gonadotropin in the blood stream of male rats.[35] However, Zanoli and colleagues report both positive and negative sexual effects of ferutinin (**11**) with respect to duration of administration on potent rats, but most importantly, it is reported to increase levels of testosterone in impotent rats.[36] Similarly, *Tribulus terrestris* L., through its active component protodioscin (**12**), promotes the production of testosterone.[37]



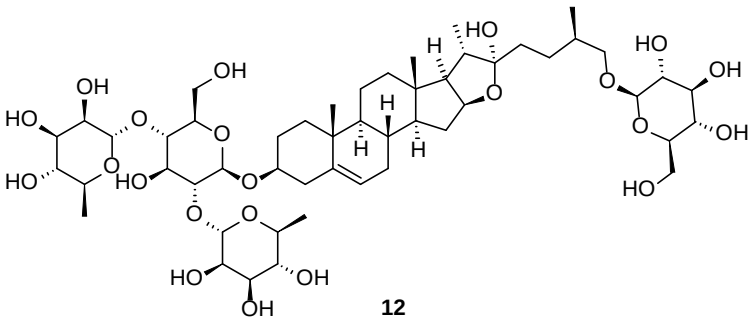
9



10



11



12

In certain instances, the anti-impotence activity of plant extracts is attributed to the protection and restoration of the penile vascular endothelial cells via antioxidative and radical scavenging effects. For example, experiments on non-insulin-dependent diabetes mellitus (NIDDM) rats suggest that Korean red ginseng restores sexual potency in NIDDM rats via antioxidative activity.[38]

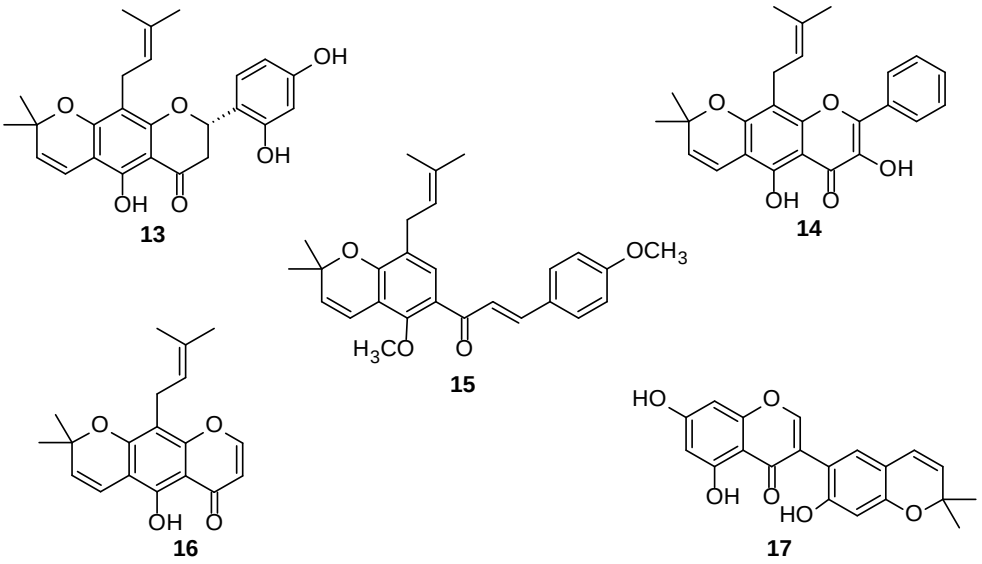
More recently, the human *Corpus cavernosum* smooth muscle tissue has been found to produce a novel neurotransmitter H_2S, which facilitates erectile function.[39,40] A statement was made that ‘‘scientists believe that this discovery could lead to the development of a new class of drugs to combat erectile dysfunction by affecting a different biochemical pathway to the one targeted by Viagra®’’ (Cape Times 3/5/2009).[41] As a result of this finding, H_2S-donating PDE-5 inhibitors have been developed.[42] The biochemical study of these compounds proves them to be as potent as PDE-5 inhibitors in promoting erection. However, unlike classical PDE-5 inhibitors, these compounds have the additional effect of preventing oxidative stress.[42]

2.3 THE GENUS *ERIOSEMA*

The genus *Eriosema* DC.Desv. (Leguminosae) consists of erect or climbing subshrubs or herbs, which occur in tropical and subtropical areas. There are 130 species belonging to this genus and 46 of them originate from Southern Africa.[43,44] The plants in this genus possess leaves with 1 or 3 foliolate and leaflets that often have small glands underneath. The flowers are reflexed, have 5-lobed calyx and mainly yellow corolla.[43,45] *Eriosema* is distinguished from other closely related genera such as *Rhynchosia* Gear. and *Flemingia* Roxb. by the morphology of its seed.[44,45]

The plants of the genus *Eriosema* have several ethnomedical and ethnobotanical uses besides treatment of infertility and related disorders. These include treatment of diarrhoea, orchitis, hydrophobia, detoxification, conjuctivities, cough, emetic, and scabies.[46-48] Other *Eriosema* species have been found to be mildly poisonous and have been used for fish poisoning. These include *E. glomerata* Guill., Perr., *E. griseum* Baker and *E. psoraleoides* (Lam.) G.Don.[49]

The genus *Eriosema* is rich in prenylated polyphenols, which range from flavonoids, flavonoid glycosides, chromones and chalcones.[11,47,50-53] The striking feature in most compounds isolated from different species of *Eriosema* is the prevalence of a dimethylpyran ring, which results from the cyclisation of the prenyl group with the adjacent hydroxy group. Despite the observed structural similarities, the compounds exhibit a variety of biological activities. For example, an extract from the leaves of *E. montanum* Baker F. exhibits high activity against the Semliki forest virus.[48] Flavonoids **13** and **14** from *E. chinense* Vogel are potent antimycobacterial agents against *Mycobacterium tuberculosis* H37Ra and cytotoxic against small cell lung (NCI-H187) and oral epidermal carcinoma (KB) human cell lines.[50] Awouafack *et al.* isolated prenylated chalcones from *E. glomerata* Guill. and Perr., which manifest antimicrobial activity against *Bacillus megaterium*, *Escherichia coli*, *Chlorella fusca*, and *Microbotryum violaceum*.[51] *E. tuberosum* A. Rich., which is traditionally used for treatment of diarrhoea, orchitis, hydrophobia, and for detoxification in China, is the most studied *Eriosema* species.[46,47] Most compounds isolated from *E. tuberosum* are fungitoxic, e.g. **15 – 17**.[47,52,54] It is noteworthy that compound **1** (*E. kraussianum*) differs from **17** (*E. tuberosum*) only in the absence of the dimethylpyran moiety attached to the A-ring.



As already mentioned, metabolites from *E. kraussianum* manifest contradictory activities. The kraussianones, **1** and **2**, induce relaxation of *Corpus cavernosum* tissue; by contrast, **4** and **6** considerably contract the penile tissue regardless of the structural resemblances.[11] The overall effect of *E. kraussianum* as a traditional remedy for male impotence and urinary complaints in KwaZulu-Natal is therefore attributed to the kraussianones **1** and **2**.[11] These observations are a strong motivation to study the chemical profile of *E. kraussianum* and other species of *Eriosema*, which are used to enhance virility.

2.4 RESULTS AND DISCUSSION

Reversed-phase HPLC coupled with a PDA detector was employed for the analysis of the plant extracts. This technique has been used successfully for qualitative and quantitative analysis of plant metabolites in several instances.[9,10,55,56] Although HPLC-PDA does not give detailed structural information, identification of analytes from the matrix can be facilitated by comparison of the UV spectra as well as retention times of the eluted peaks with those of the pure analytes ran under the same conditions.[9,57,58] Furthermore, one can have an idea of the classes of compounds present in a sample by comparing the UV spectra of eluates with those from the literature profiles.[9,59] Different classes of compounds have been reported to exhibit UV absorptions at different wavelengths depending on the degree of conjugation.[9,59]

2.4.1 Analysis of *E. kraussianum* from the Drakensberg and Pietermaritzburg

The first step in our analysis was to develop a method that would separate the different constituents of *E. kraussianum*. Kraussianone 1 (**1**), kraussianone 2 (**2**), kraussianone 3 (**4**), kraussianone 4 (**5**) and kraussianone 5 (**6**) were used as reference samples. These compounds were isolated from the CH_2Cl_2 extract of the roots of *E. kraussianum* (Figure 2.2) by the procedure established by Drewes and co-workers.[19]

Figure 2.2. The roots of *E. kraussianum* from the Drakensberg

The five analytes were separated by reversed-phase HPLC by a step-wise gradient/isocratic programme using CH_3OH/ 0.02% AcOH in H_2O as mobile phase. As expected for the reversed-phase HPLC, the more polar components, kraussianone 3 (**4**), kraussianone 2 (**2**) and kraussianone 5 (**6**) were eluted first and the less polar fractions kraussianone 1 (**1**) and kraussianone 4 (**5**) were retained for a longer time as shown in Figure 2.3. To establish the reproducibility of the retention times, the reference samples were analysed several times, and the results were consistent.

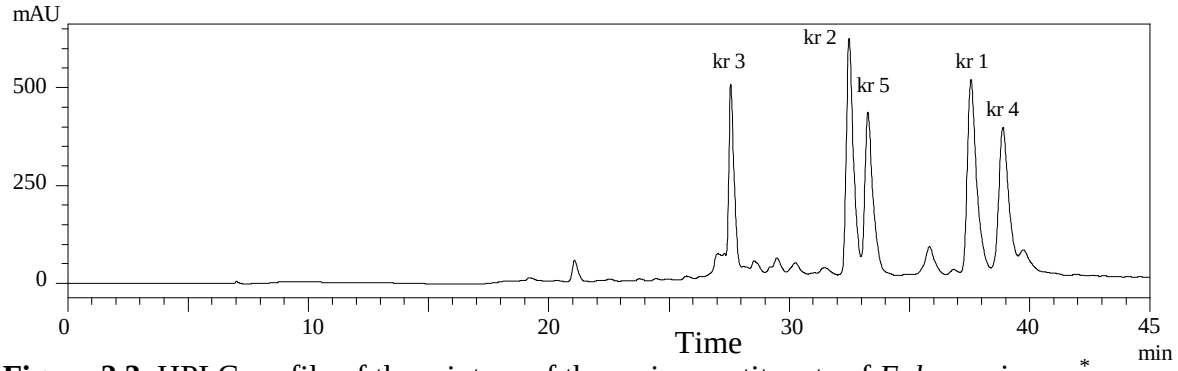


Figure 2.3. HPLC profile of the mixture of the main constituents of *E. kraussianum*[*]

[*] kr refers to kraussianone

The UV spectra of the compounds displayed maximum absorbance peaks at shorter wavelengths, characteristic for isoflavonoids. Isoflavonoids are reported to exhibit maximum UV absorptions at shorter wavelengths than other classes of flavonoids due to substitution of the phenyl B-ring at C-3.[9,59] This is said to prevent flow of electrons between the B-ring and the chromone (A-ring and C-ring) chromophore.[9,59] The UV spectra of kraussianone 1 (**1**), kraussianone 2 (**2**), kraussianone 3 (**4**) and kraussianone 4 (**5**) displayed two main absorbance bands at 215 - 235 nm and 260 - 280 (max) nm and a shoulder at around 300 nm. The UV spectrum of kraussianone 5 (**6**), on the other hand, showed two major absorption peaks, whereby the maximum absorbance was observed at shorter wavelength (220 nm) and the second absorbance at 300 nm (Figure 2.4).

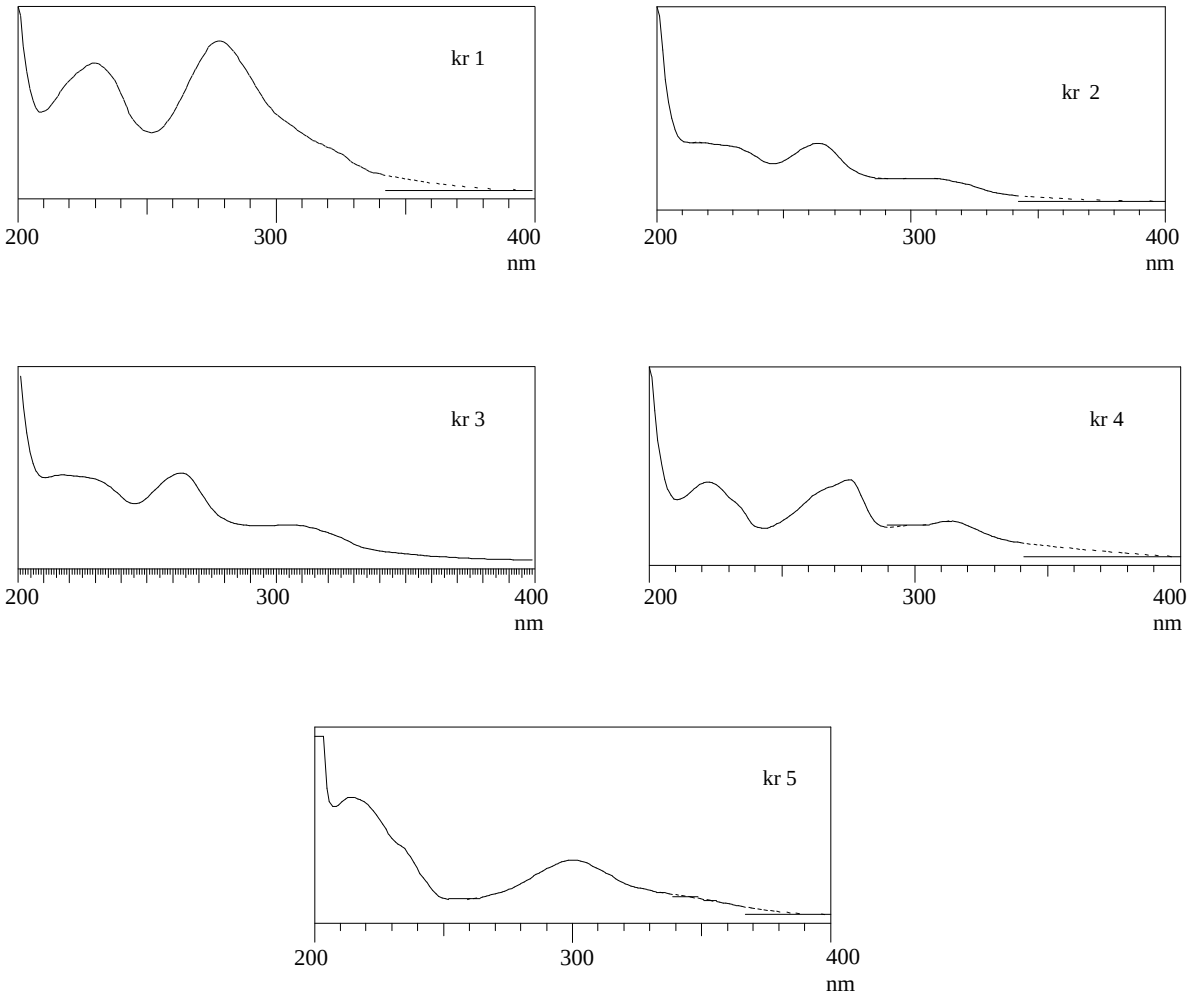


Figure 2.4. UV absorbance spectra of the constituents of *E. kraussianum*

Having developed a method for the separation and identification of the five analytes, the next step was to analyse the plant extracts from different sources. Two samples of *E. kraussianum*, which were identified by the curator of the Bews Herbarium (UKZN), Dr Trevor Edwards, were investigated. These plants were collected from the Drakensberg (Monk's Cowl Gate) and from Pietermaritzburg (northern boundary of the National Botanical Gardens). The peaks for all the five analytes were observed in the chromatograms of the root extracts from Drakensberg and Pietermarizburg as shown in Figure 2.5. The main difference observed from the chromatograms of the two samples was in the relative amounts of the analytes. There were two main constituents in the plant from Pietermaritzburg, kraussianone 2 (**2**) and kraussianone 3 (**4**), and the other kraussianones occurred in comparably low amounts. Conversely, the sample from the Drakensberg contained mainly kraussianone 2 (**2**).

.

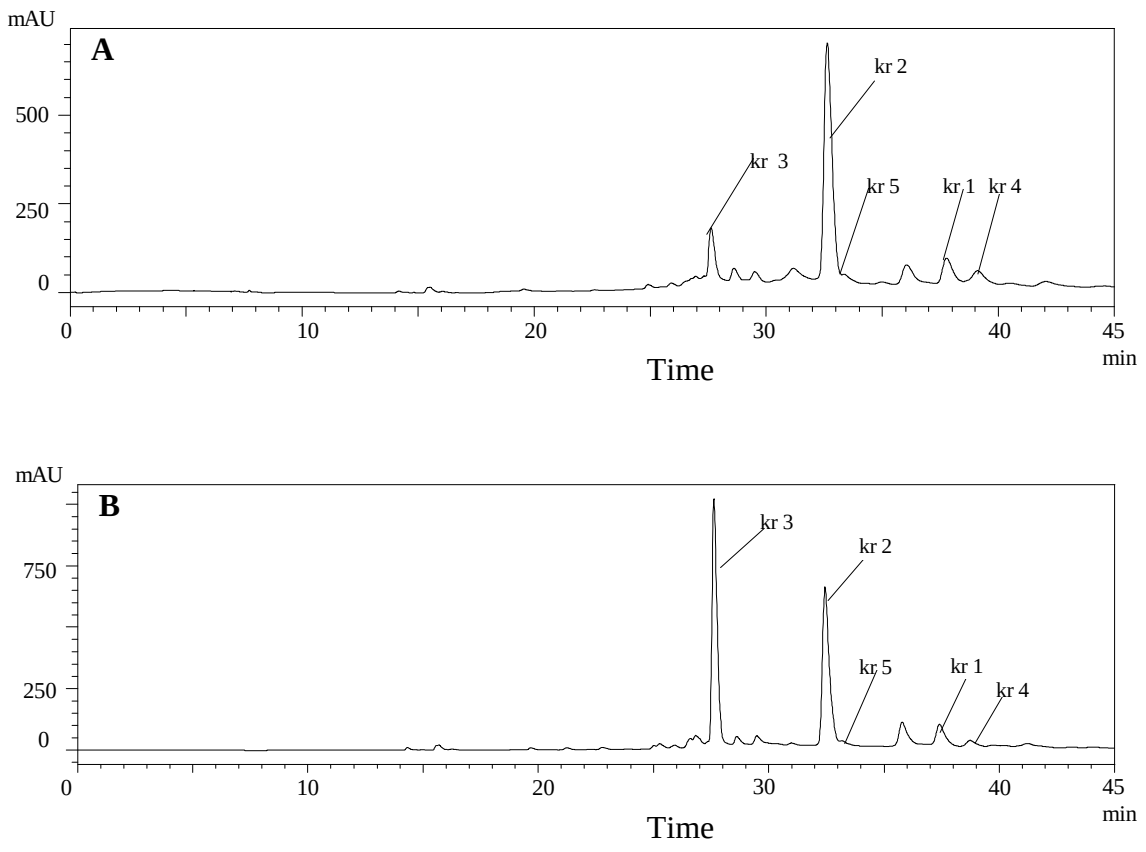


Figure 2.5. HPLC chromatograms of *E. kraussianum* from Drakensberg (**A**) and Pietermaritzburg (**B**)

The variations observed in the authentic *E. kraussianum* plants from different regions are not unexpected. It has been reported that secondary metabolites play a vital role in the plant physiology, and some of them are produced to help the plant adapt to the environment.[60,61] Thus, the chemical profile of the plant is influenced by its habitat.[10,56,61] Other factors would include the age and the genotype of the plant.[10,56] From these results, it can be deduced that the plant from the Drakensberg provides good material for the treatment of male impotence, as an active metabolite, kr 2 (**2**) was the major constituent. Further investigations are recommended to determine the safety in using the plant from Pietermaritzburg as kraussianone 3 (**4**), the metabolite that led to contraction of the *corpus cavernosum*, was prevalent in relatively high quantities.

2.4.2 Analysis of Commercial Samples *Uqonsi* and *Ubangalala*

Following the successful analysis of the CH_2Cl_2 extracts of *E. kraussianum*, the chemical compositions of other plants which are used for the treatment of urinary complaints and male impotence in KwaZulu-Natal were examined. The plants that were investigated are called *uqonsi* and *ubangalala* in Isizulu. The roots of the plants were purchased from street vendors and from an herbal shop in Retief Street in Pietermaritzburg.

The CH_2Cl_2 extracts of root samples of *uqonsi*, from the street vendor (already chopped) and from the herbal shop (Figure 2.6), were analysed with reversed-phase HPLC under the conditions developed for *E. kraussianum*. As seen from Figure 2.7, the HPLC profiles of the two samples were completely different. The retention times and the UV spectra of the two samples did not match. There was no outstanding major component in the extract from the street vendor and almost all the components were in relatively similar concentrations.

A **B**

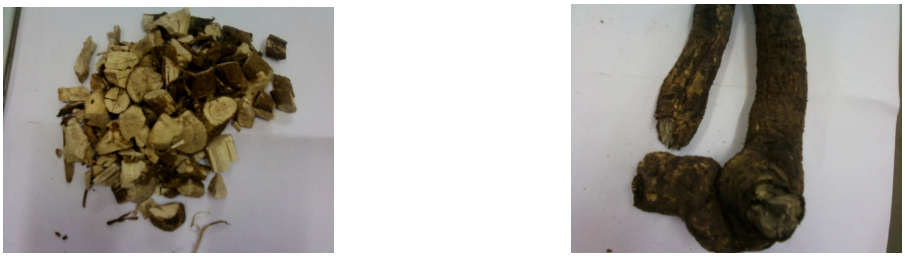


Figure 2.6. Roots of *uqonsi* from a street vendor (**A**) and an herbal shop (**B**)

On the other hand, the chromatogram of the extract from the herbal shop displayed peaks with retention times that corresponded to those of the five analytes, kraussianone 1 (**1**), kraussianone 2 (**2**), kraussianone 3 (**4**), kraussianone 4 (**5**) and kraussianone 5 (**6**) (Figure 2.7). The identity of the compounds was confirmed by comparison of the UV spectra of the eluted peaks with those of the pure analytes. Furthermore, **2** was found to be the major compound in this extract and all other kraussianone peaks were minute, including kraussianone 3 (**4**).

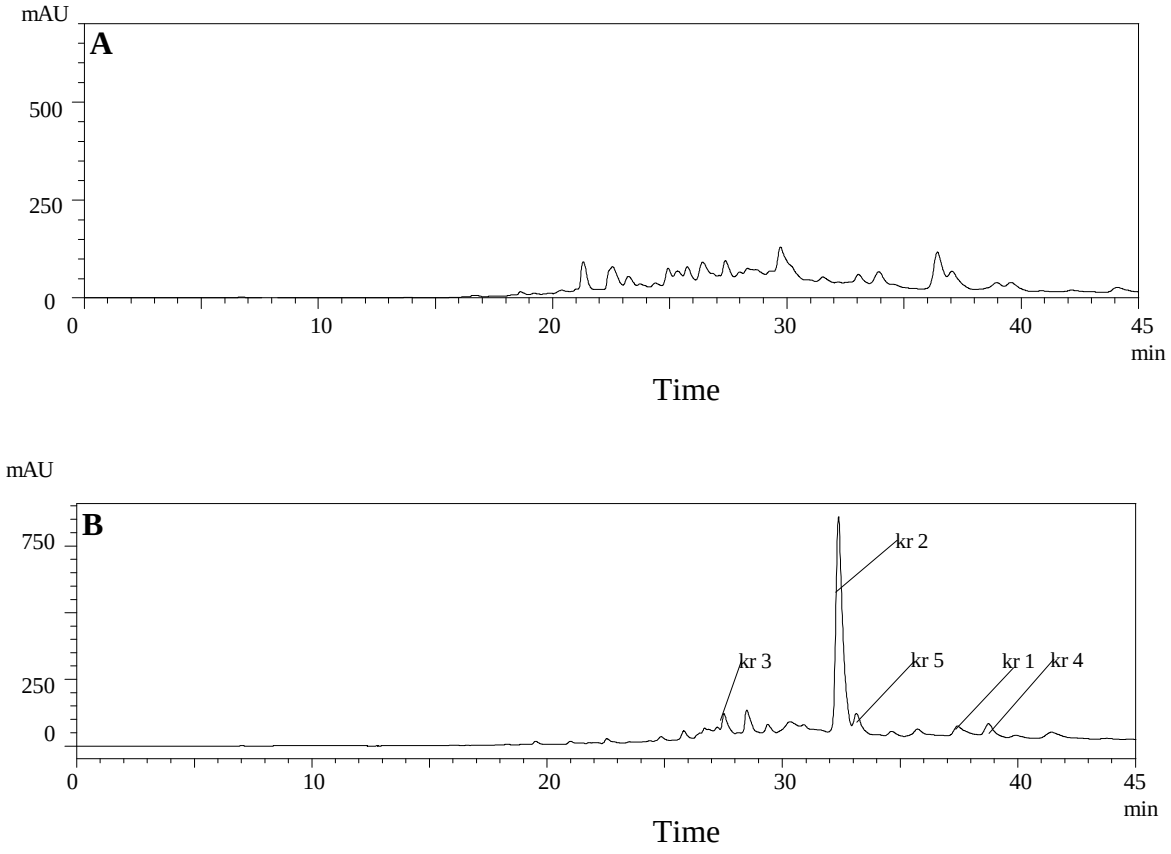


Figure 2.7. Chromatograms of *uqonsi* from the street vendor (**A**) and the herbal shop (**B**)

The similarities observed in the chemical composition of the *uqonsi* from the herbal shop and *E. kraussianum* suggest that the two plants may be the same. *Uqonsi* normally refers to *E. salignum*. There is a possibility that some commercial suppliers may fail to make a clear distinction between the two species, and market both *E. kraussianum* and *E. salignum* as *uqonsi*. Nevertheless, the species of *Eriosema* investigated so far contain mostly prenylated flavonoids,[46,47,50-54] thus, it is also probable that *E. kraussianum* and *E. salignum* contain

similar metabolites. However, no compounds have been isolated from *E. salignum* up to date, thus it would be essential to conduct phytochemical studies of an identified authentic *E. salignum* plant in order to clear this controversy. Although *uqonsi* from the herbal shop cannot be unambiguously identified as *E. kraussianum* or *E. salignum* at this stage, it was interesting to note that its extract had the same metabolites as *E. kraussianum*, and that kraussianone 2 (**2**), one of the two compounds that are believed to be responsible for erectile dysfunction activity in *E. kraussianum*, was the major constituent.

The second commercial plant that was studied was *ubangalala*. The roots of *ubangalala* were also bought from the street vendor and the herbal shop. The physical appearance of the roots was similar, except that those from the herbal shop were smaller than those from the street vendor, and both gave an intensely orange extract (Figure 2.8). Judging from the physical appearance, one would expect the two samples to have similar chemical profiles. However, the similar constituents in the CH_2Cl_2 extracts of the two *ubangalala* samples could only be observed with a close scrutiny of the chromatograms and the UV spectra of the samples as shown in Figures 2.9 and 2.10.

Figure 2.8. Roots of *ubangalala* from the herbal shop (**A**) and the street vendor (**B**), and the CH_2Cl_2 extract (**C**).

The chromatogram of the sample from the herbal shop displayed two major peaks with retention times 27.1 and 28.8 min. The compound that eluted at 27.1 min exhibited a maximum UV absorbance at 308 nm, indicative of a more conjugated system and the one at 28.8 min exhibited a maximum UV absorbance at 255 nm and a shoulder at 310 nm (Figure 2.9). These peaks were also observed, but in trace amounts, in the chromatogram

of the *ubangalala* sample from the street vendor. The main constituent of the sample from the vendor was eluted at a shorter retention time (15.3 min). The UV spectrum of the peak at 15.3 min displayed four absorption bands at 240, 257 (max), 317 and 365 nm as shown in Figure 2.10.

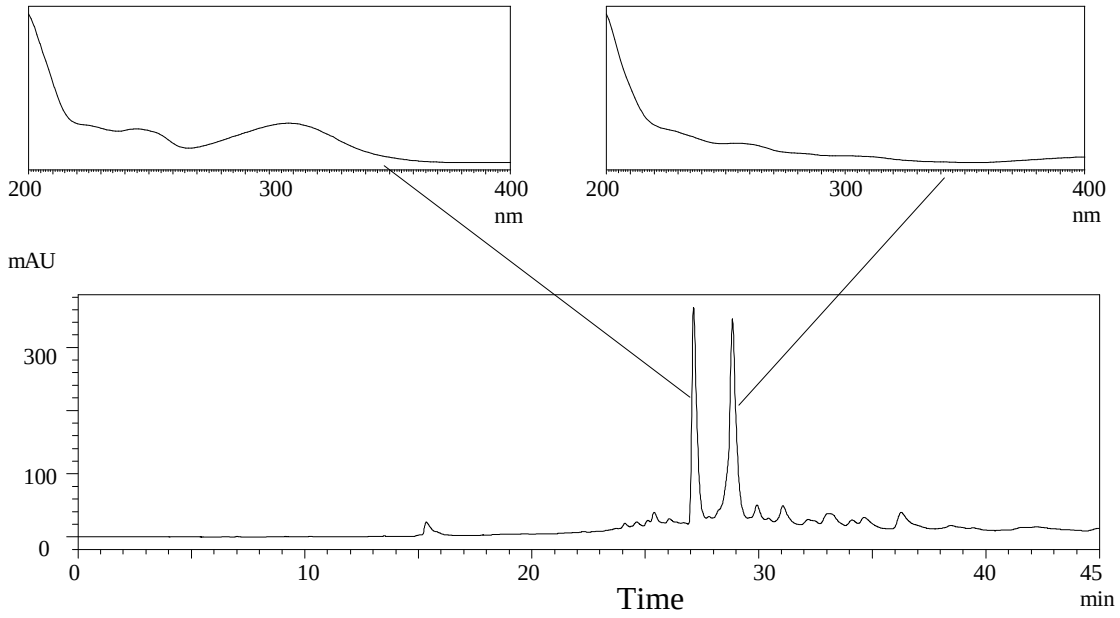


Figure 2.9. The chromatogram and UV absorbance spectra of *ubangalala* from the herbal shop

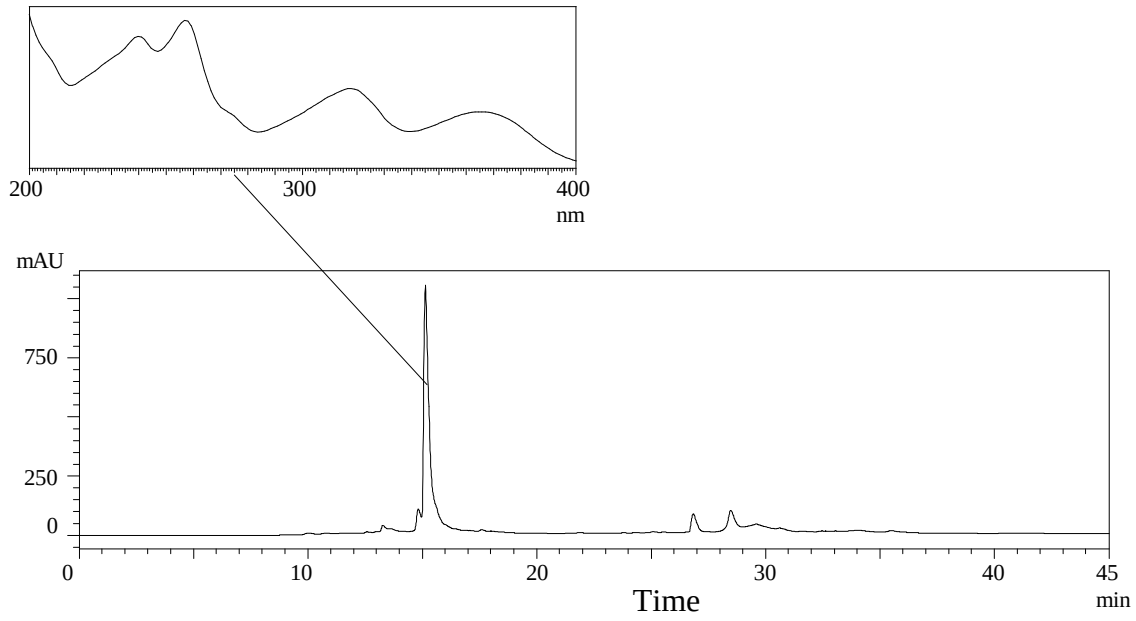


Figure 2.10. The chromatogram and UV absorbance spectra of *ubangalala* from the street vendor

The differences observed in the two samples of *ubangalala* may be attributed to several factors. Firstly, the name *ubangalala* covers a wide range of plant species under the genus *Eriosema*. Thus, it may be possible that the two *ubangalala* plants were two different species of *Eriosema* or not *Eriosema* species at all.[21,22] It should be noted that it was not possible to identify the plant species as the commercial samples consisted of root material only and there were no leaves or seeds accompanying them. Secondly, it is well known that the levels and distribution of plant metabolites vary with the age, the region from which the plant was collected and the season of collection.[9,10,56,60,61]

2.5 CONCLUSION

Six plants from different sources, which were categorised as *E. kraussianum*, *uqonsi* and *ubangalala* were analysed by reversed-phase HPLC-PDA. Of the six plants, three displayed a similar chemical profile, even though there were variations in the relative abundance of the metabolites within each plant. These were the two plants identified as *E. kraussianum* and the commercial *uqonsi* from the herbal shop. In all three plants, kraussianone 2 (**2**) was found to be the major constituent. The three other plants, *uqonsi* from the street vendor and the two commercial *ubangalala* plants, did not contain any of the compounds that are found in *E. kraussianum*. Since all six plants are claimed to be used for the treatment of male impotence, the differences observed in their constituents may suggest that the active metabolites in the *ubangalala* and *uqonsi* from the street vendor are different from those identified in *E. kraussianum*. Several classes of compounds, alkaloids, terpenoids, xanthones and prenylated flavonoids have been reported as active ingredients in plants used for treatment of male impotence.[1-7] Therefore, biochemical studies are required for identification of the compounds that are responsible for enhancing virility in the authentic plants that are used for this purpose. This will help to monitor the quality of the commercial plants, identify adulteration and hence protect consumers.

2.6 EXPERIMENTAL

2.6.1 Materials

Kraussianone 1 (**1**), kraussianone 2 (**2**), kraussianone 3 (**4**), kraussianone 4 (**5**) and kraussianone 5 (**6**) used as reference samples were isolated from the CH_2Cl_2 extract of the roots of *E. kraussiaum* following the procedure established by Drewes *et al*.[19] The plant was previously collected from the Drakensberg. *E. kraussianum* plants used for the analysis were collected in October 2011 from the Drakensberg (Monk's Cowl Gate) and from the northern boundary of the National Botanical Gardens in Pietermaritzburg. The commercial samples of *uqonsi* and *ubangala* were purchased from a street vendor and from an herbal shop in Retief Street in Pietermaritburg. HPLC-grade CH_3OH and AcOH were bought from Aldrich. Ultra-pure water was obtained from a Labotec Pure LAB ultra water purification system.

2.6.2 Sample Preparation

Reference solutions of 0.84 mg/mL kraussianone 1 (**1**), 0.8 mg/mL kraussianone 2 (**2**), 1.2 mg/mL kraussianone 3 (**4**), 0.82 mg/mL kraussianone 4 (**5**) and 0.82 mg/mL kraussianone 5 (**6**) were prepared in HPLC-grade CH_3OH. Four µL volumes of these solutions were analysed in multiple injections.

Approximately 2 g of ground dry root material was extracted with CH_2Cl_2 for 24 h. The extract was filtered, evaporated under reduced pressure, and redissolved in 5 mL HPLC-grade CH_3OH. Four µL volumes of these samples were analysed in triplicate.

2.6.3 Chromatographic Conditions

Samples were analysed on a Shimadzu Prominence HPLC system equipped with an LC-20AB pump, SIL-20A autosampler and an SPD-M20A PDA detector. The chromatographic separations were achieved with 5 µm particle size, 250 mm × 4.60 mm i.d, C_{18} reversed-phase Phenomenex Luna column. The mobile phase consisted of solvents

A and B, where A was 0.02% AcOH (v/v) in H_2O and B was CH_3OH. The following elution programme was employed: 1-5 min linear gradient from 25 to 40% B, 5-10 min linear gradient from 40 to 70% B, 10-20 min linear gradient from 70 to 90% B, 20-40 min isocratic on 90%, 40-45 min linear gradient from 90 to 25%. The flow rate was maintained at 0.4 mL/min and the column oven temperature was adjusted to 40 ^{o}C. The PDA detector was set to measure at the range 200 nm – 400 nm.

2.7 REFERENCES

(1) Dell'Agli, M.; Galli, G. V.; Dal Cero, E.; Belluti, F.; Matera, R.; Zironi, E.; Pagliuca, G.; Bosisio, E. *Journal of Natural Products* **2008**, *71*, 1513-1517.

(2) Malviya, N.; Jain, S.; Gupta, V. B.; Vyas, S. *Acta Poloniae Pharmaceutica* **2011**, *68*, 3-8.

(3) Iacono, F.; Prezioso, D.; Ruffo, A.; Di Lauro, G.; Romis, L.; Illiano, E. *Journal of Men's Health* **2011**, *8*, 282-287.

(4) McKay, D. *Alternative Medicine Review : a Journal of Clinical Therapeutics* **2004**, *9*, 4-16.

(5) Campos, A. R.; Lima, R. C. P.; Uchoa, D. E. A.; Silveira, E. R.; Santos, F. A.; Rao, V. S. N. *Journal of Ethnopharmacology* **2006**, *104*, 240-244.

(6) Meyer, J. J. M.; Rakuambo, N. C.; Hussein, A. A. *Journal of Ethnopharmacology* **2008**, *119*, 599 - 603.

(7) Drewes, S. E.; George, J.; Khan, F. *Phytochemistry* **2003**, *62*, 1019-1025.

(8) Rakuambo, N. C.; Meyer, J. J. M.; Hussein, A.; Huyser, C.; Mdlalose, S. P.; Raidani, T. G. *Journal of Ethnopharmacology* **2006**, *105*, 84-88.

(9) Andersen, Ø. M.; Markham, K. R. *Flavonoids - Chemistry, Biochemistry and Applications*; Taylor and Francis: New York, 2006.

(10) Ma, X. Q.; Shi, Q.; Duan, J. A.; Dong, T. T. X.; Tsim, K. W. K. *Journal of Agricultural and Food Chemistry* **2002**, *50*, 4861-4866.

(11) Drewes, S. E.; Horn, M. M.; Khan, F.; Munro, O. Q.; Dhlamini, J. T. B.; Rakuambo, C.; Meyer, J. J. M. *Phytochemistry* **2004**, *65*, 1955-1961.

(12) Nguefack, J.; Tamgue, O.; Lekagne Dongmo, J. B.; Dakole, C. D.; Leth, V.; Vismer, H. F.; Amvam Zollo, P. H.; Nkengfack, A. E. *Food Control* **2012**, *23*, 377-383.

(13) Wagner, H.; Ulrich-Merzenich, G. *Phytomedicine* **2009**, *16*, 97-110.

(14) Junio, H. A.; Sy-Cordero, A. A.; Ettefagh, K. A.; Burns, J. T.; Micko, K. T.; Graf, T. N.; Richter, S. J.; Cannon, R. E.; Oberlies, N. H.; Cech, N. B. *Journal of Natural Products* **2011**, *74*, 1621-1629.

(15) Joray, M. B.; González, M. L.; Palacios, S. M.; Carpinella, M. C. *Journal of Agricultural and Food Chemistry* **2011**, *59*, 11534-11542.

(16) Simons, R.; Vincken, J. P.; Bohin, M. C.; Kuijpers, T. F. M.; Verbruggen, M. A.; Gruppen, H. *Rapid Communications in Mass Spectrometry* **2011**, *25*, 55-65.

(17) Wu, Q. L.; Wang, M. F.; Simon, J. E. *Journal of Chromatography A* **2003**, *1016*, 195-209.

(18) De Souza, L. M.; Cipriani, T. R.; Iacomini, M.; Gorin, P. A. J.; Sassaki, G. L. *Journal of Pharmaceutical and Biomedical Analysis* **2008**, *47*, 59-67.

(19) Drewes, S. E.; Horn, M. M.; Munro, O. Q.; Dhlamini, J. T. B.; Meyer, J. J. M.; Rakuambo, N. C. *Phytochemistry* **2002**, *59*, 739-747.

(20) Bryant, A. T. *Zulu Medicine and Medicine Men*; Centaur Press: Cape Town, 1983.

(21) Cunningham, A. B. *An Investigation of the Herbal Medicine Trade in Natal*; Institute of Natal Resources, University of KwaZulu-Natal Pietermaritzburg, 1988.

(22) Hutchings, A.; Scott, A. H.; Lewis, G.; Cunningham, A. B. *Zulu Medicinal Plants - An Inventory*; University of Natal Press: Pietermaritzburg, 1996.

(23) Williams, V.; Balkwill, K.; Witkowski, E. *Economic Botany* **2000**, *54*, 310-327.

(24) www.mayaethnobotanicals.com Accessed on 14 December 2011.

(25) www.moretolifehealth.com Accessed on 12 December 2011.

(26) www.proerex.co.nz Accessed on 12 December 2011.

(27) Turkoski, B. B. *Orthopaedic Nursing* **2008**, *27*, 201-206.

(28) Seftel, A. D.; Sun, P.; Swindle, R. *Journal of Urology* **2004**, *171*, 2341 - 2345.

(29) Soler, J. M.; Previnaire, G. J.; Denys, P.; Chartier-Kastler, E. *Spinal Cord* **2007**, *45*, 169 -173.

(30) Chuang, A. T.; Strauss, J. D.; Murphy, R. A.; Steers, W. D. *Journal of Urology* **1998**, *160*, 257-261.

(31) Drewes, S. E.; George, J.; Khan, F. *Phytochemistry* **2003**, *62*, 1019 -1025.

(32) Krenzelok, E. *Journal of Toxicology - Clinical Toxicology* **2000**, *38*, 645-651.

(33) Webb, D. J.; Muirhead, G. J.; Wulff, M.; Sutton, J. A.; Levi, R.; Dinsmore, W. W. *Journal of American College of Cardiology* **2000**, *36*, 25 -31.

(34) Appendino, G.; Spagliardi, P.; Cravotto, G.; Pocock, V.; Milligan, S. *Journal of Natural Products* **2002**, *65*, 1612-1615.

(35) Ignatkov, V.; Akhmedkhodzheava, K. S.; Babichev, V. N. *Farmakol Toksikol* **1990**, *53*, 37-38.

(36) Zanoli, P.; Rivasi, M.; Zavatti, M.; Brusiani, F.; Vezzalini, F.; Baraldi, M. *International Journal of Impotence Research* **2005**, *17*, 513-518.

(37) Adimoelja, A. *Journal of Andrology* **2000**, *23*, 82 - 84.

(38) Ryu, J. K.; Lee, T.; Kim, D. J.; Park, I. S.; Yoon, S. M.; Lee, H. S.; Song, S. U.; Suh, J. K. *Urology* **2005**, *65*, 611-615.

(39) Srilatha, B.; Adaikan, P. G.; Moore, P. K. *European Journal of Pharmacology* **2006**, *535*, 280-282.

(40) Srilatha, B.; Adaikan, P. G.; Ling, L.; Moore, P. K. *Journal of Sexual Medicine* **2007**, *4*, 1304-1311.

(41) Connor, S. In *Cape Times*; 2nd ed.; Cape Times & Independent Online (Pty) Ltd.: Cape 2009.

(42) Nilima, S.; Giuseppe, R.; Matthew, H.; Anna, S.; Piero Del, S.; Valerio, T.; Raj, P.; Gianni, D. A.; Jamie, Y. J. *BJU International (Formerly: British Journal of Urology)* **2009**, *103*, 1522-1529.

(43) www.biodiversityexplorer.com Accessed on 12 December 2011.

(44) Grear, J. W.; Dengler, N. G. *Brittonia* **1976**, *28*, 281-288.

(45) Edmund, G.; Baker, F. L. S. *Journal of Botany* **1895**, *33*, 97-100.

(46) Ma, W. G.; Fukushi, Y.; Hostettmann, K.; Tahara, S. *Phytochemistry* **1998**, *49*, 251-254.

(47) Ma, W. G.; Fuzzati, N.; Sheng, Q.; Yang, C. R.; Stoeckli-Evans, H.; Hostettmann, K. *Phytochemistry* **1995**, *39*, 1049-1061.

(48) Cos, P.; Hermans, N.; De Bruyne, T.; Apers, S.; Sindambiwe, J. B.; Vanden Berghe, D.; Pieters, L.; Vlietinck, A. J. *Journal of Ethnopharmacology* **2002**, *79*, 155-163.

(49) Neuwinger, H. D. *Toxicon* **2004**, *44*, 417-430.

(50) Sutthivaiyakit, S.; Thongnak, O.; Lhinhatrakool, T.; Yodchun, O.; Srimark, R.; Dowtaisong, P.; Chuankamnerdkarn, M. *Journal of Natural Products* **2009**, *72*, 1092-1096.

(51) Awouafack, M. D.; Kouam, S. F.; Hussain, H.; Ngamga, D.; Tane, P.; Schulz, B.; Green, I. R.; Krohn, K. *Planta Medica* **2008**, *74*, 50-54.

(52) Hostettmann, K.; Marston, A. *Pure and Applied Chemistry* **1994**, *66*, 2231-2234.

(53) Ma, W. G.; Fuzzati, N.; Lu, S. L.; Gu, D. S.; Hostettmann, K. *Phytochemistry* **1996**, *43*, 1339 - 1343.

(54) Ma, W. G.; Fukushi, Y.; Ducrey, B.; Hostettmann, K.; Tahara, S. *Phytochemistry* **1999**, *51*, 1087-1093.

(55) Ribas-Agusti, A.; Gratacos-Cubarsi, M.; Sarraga, C.; Garcia-Regueiro, J.-A.; Castellari, M. *Phytochemical Analysis*, *22*, 555-563.

(56) Cherdshewasart, W.; Sriwatcharakul, S. *Bioscience Biotechnology and Biochemistry* **2007**, *71*, 2527-2533.

(57) Meyer, V. R. *Practical High-Perfomance Liquid Chromatography*; John Wiley & Sons: Chichester, 1988.

(58) Beesley, T. E.; Scott, R. P. W. *Chiral Chromatography*; John Wiley & Sons: Chischester, 1998.

(59) Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The Systematic Identification of Flavonoids;* Springler-Verlag: New York, 1970.

(60) Orians, C. M.; Lower, S.; Fritz, R. S.; Roche, B. M. *Biochemical Systematics and Ecology* **2003**, *31*, 233-247.

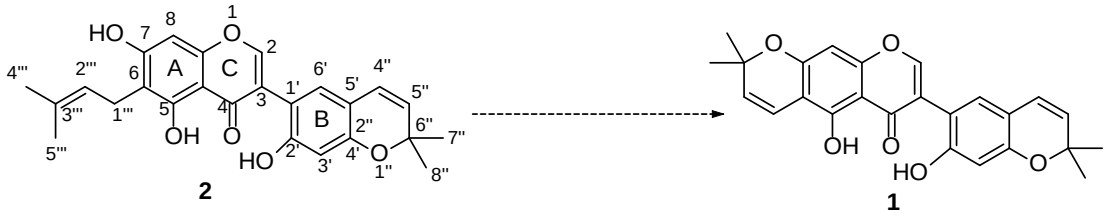
(61) Maver, M.; Queiroz, E. F.; Wolfender, J. L.; Hostettmann, K. *Journal of Natural Products* **2005**, *68*, 1094-1098.

CHAPTER 3

SEMI-SYNTHESIS OF KRAUSSIANONE 1

3.1 INTRODUCTION

Kraussianone 2 (**2**) was determined to be the major constituent of the CH_2Cl_2 extract of the roots of *E. kraussianum* by HPLC study and also by quantitative isolation, thus appreciable quantities of **2** can be obtained from the plant. However, kraussianone 1 (**1**), the most active metabolite, occurs in relatively low concentration in the plant (See Chapter 2), therefore isolation of large amounts of kraussianone 1 (**1**) from the plant would not be sustainable. Since **2** is the major constituent in the roots of *E. kraussianum* and can be readily isolated by the established procedure,[1,2] it presents an ideal starting material for the synthesis of **1**. The main operation in this endeavour would involve regioselective formation of the dimethylchromene ring by cyclisation of the prenyl side chain on the A-ring of kraussianone 2 (**2**) as shown in Scheme 3.1.



Scheme 3.1. Conversion of kraussianone 2 (**2**) into kraussianone 1 (**1**)

Studies on the chemical nature of naturally-derived and synthetic compounds have revealed that a substantial number of bioactive compounds possess the dimethylpyran scaffold. This qualifies the dimethylpyran moiety as a ‘‘privileged structure’’ from which compounds with favourable bioactivities and bioavailability can be developed.[3] Thus, it is not surprising that kraussianone 1 (**1**) was the most active metabolite. K.C. Nicolaou has been particularly successful in basing the synthesis of compounds with interesting pharmacological properties on the dimethylchromene scaffold.[4,5] The formation of this

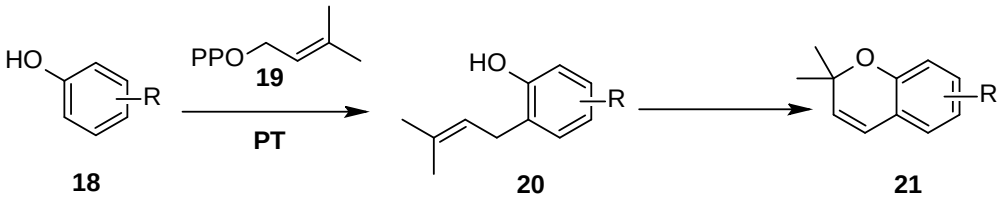
scaffold thus constitutes subsections in total and partial syntheses of a number of bioactive compounds. As a result of the wide distribution of the scaffold in natural and synthetic compounds, several synthetic procedures for synthesis of the dimethylpyran ring have been developed.

In this chapter the methods for the formation of the dimethylchromene ring will be reviewed, followed by a discussion of the results on the semi-synthesis of kraussianone 1 (**1**) and, finally, the conclusion and experimental procedures.

3.2 AN OVERVIEW OF METHODS FOR SYNTHESIS OF THE DIMETHYLPYRAN MOIETY

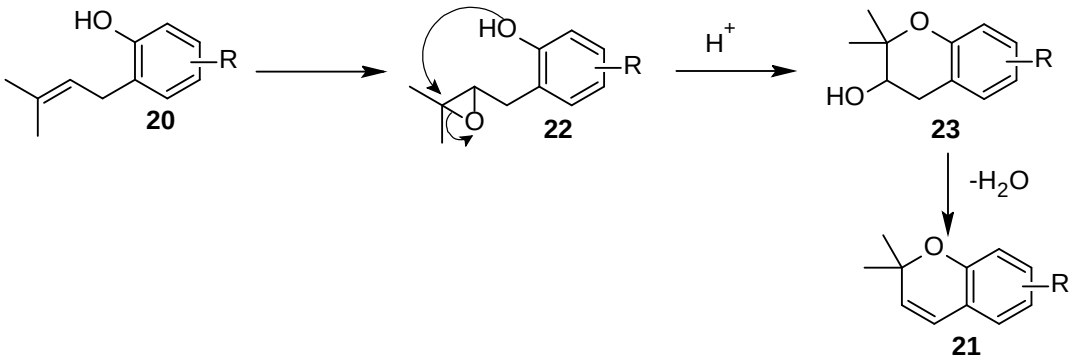
3.2.1. Biosynthetic Pathway

The dimethylpyran ring is believed to originate biosynthetically from the enzyme-catalysed cyclisation of a prenyl group with an adjacent hydroxy group of a phenol (Scheme 3.2).[6,7] The isoprene unit is introduced onto the aromatic ring in the form of dimethylallyl pyrophosphate (DMAPP) (**19**) with the aid of the enzyme prenyl transferase (PT).[6]



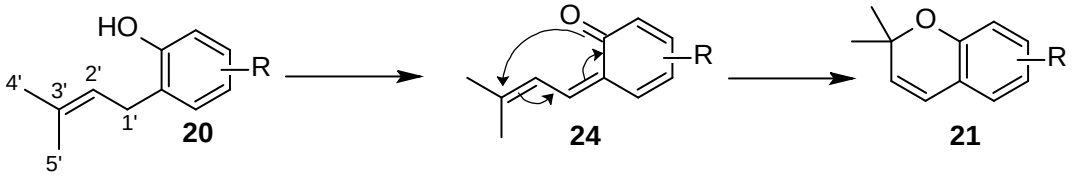
Scheme 3.2. Formation of the dimethylpyran ring

The mechanism by which the prenyl cyclisation occurs remains under debate. The mechanism proposed initially is via an epoxide intermediate **22**, followed by nucleophilic attack of the *ortho*-hydroxy group,[6,7] thereby forming the hydroxy-substituted dihydrodimethylpyran **23**. Dehydration of **23** under the prevailing acidic conditions results in the dimethylchromene ring **21**[6,7] (Scheme 3.3).



Scheme 3.3. Suggested mechanism for the formation of the dimethylpyran scaffold

On the other hand, Crombie and colleagues argued that the dimethylpyran ring is formed in a single 6π-electrocyclisation step, which involves dienone **24** as an intermediate as shown in Scheme 3.4.[8-10] They proposed that the formation of the dienone **24** is intitiated by a radical abstraction of one of the hydrogens at C-1', followed by delocalisation and finally decomposition to a state approaching **24**.[8-10]

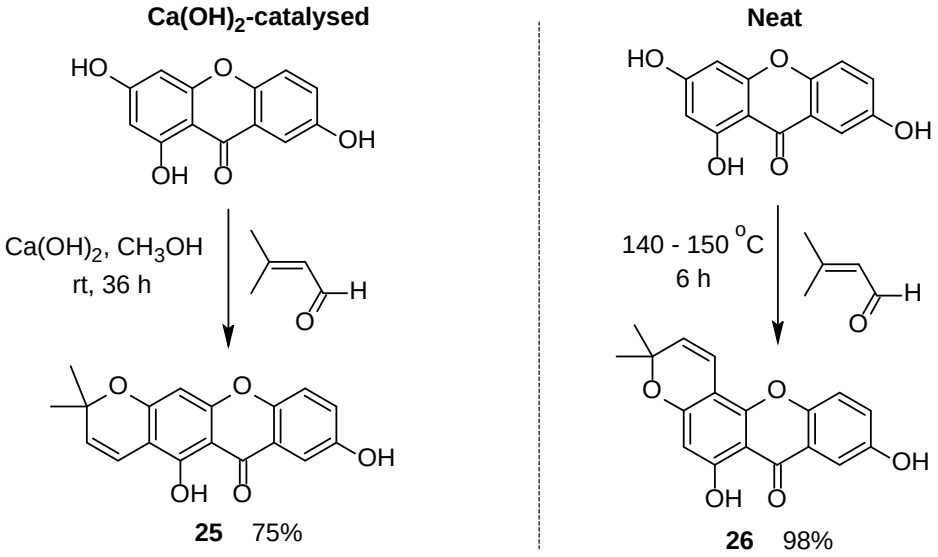


Scheme 3.4. Alternative mechanism for the formation of the dimethylpyran scaffold

3.2.2 Synthetic Methods

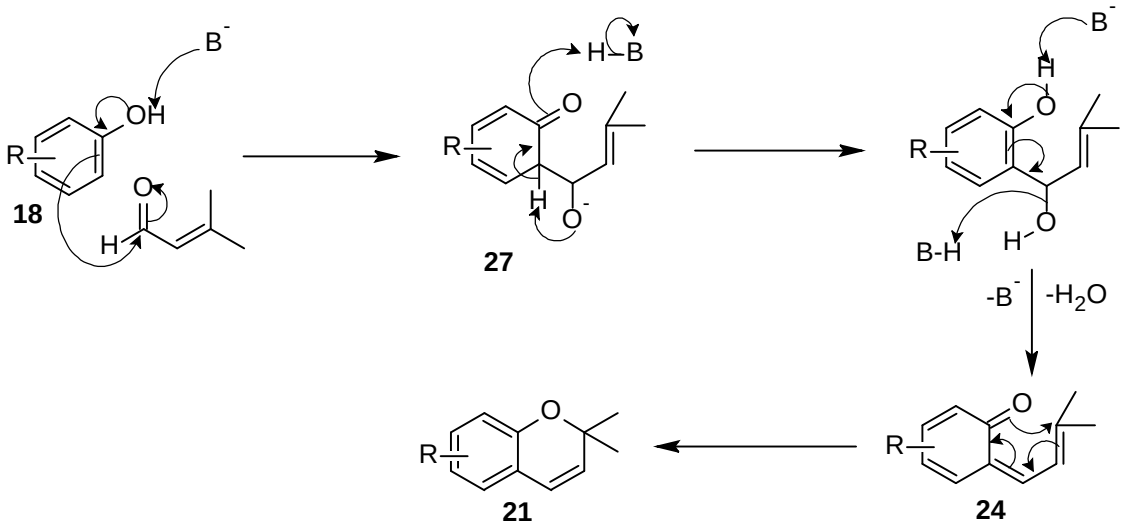
3.2.2.1 Aldol-type condensation with 3-methylbut-2-enal

The most commonly employed procedure for the synthesis of the 2,2-dimethylchromene ring is an aldol-type condensation of a phenol with 3-methylbut-2-enal (prenal) or prenal acetal.[11-17] Different reaction conditions have been used to facilitate this reaction, and it has been found that the regioselectivity of the reaction on substrates bearing a phloroglucinol core greatly depends on the experimental conditions.[14,15] Mondal *et al*. demonstrated the exclusive formation of the linear and the angular pyranoxanthenones, osajaxanthone (**25**) and nigrolineaxanthone (**26**) respectively, under the conditions shown in Scheme 3.5.[15]



Scheme 3.5. Formation of 2,2-dimethylchromenes from prenal

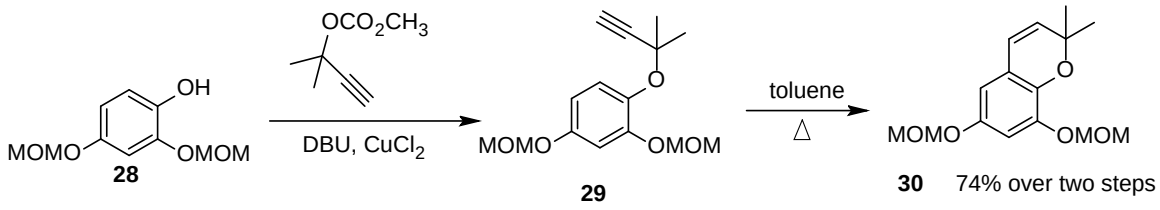
The reaction mechanism is envisaged to proceed via a 6π-electrocyclisation as shown in Scheme 3.6. The first step involves the aldol-type condensation of the phenol with prenal. Tautomerisation and dehydration of **27** give an intermediate **24**, which undergoes a 6π-electrocyclic reaction to furnish the 2,2-dimethylchromene **21**. This method has an advantage over others in that all steps involved occur in one pot.[17] However, prenal is reported to be prone to polymerisation, which makes isolation of the product difficult.[18]



Scheme 3.6. Proposed mechanism for the condensation of prenal with phenols

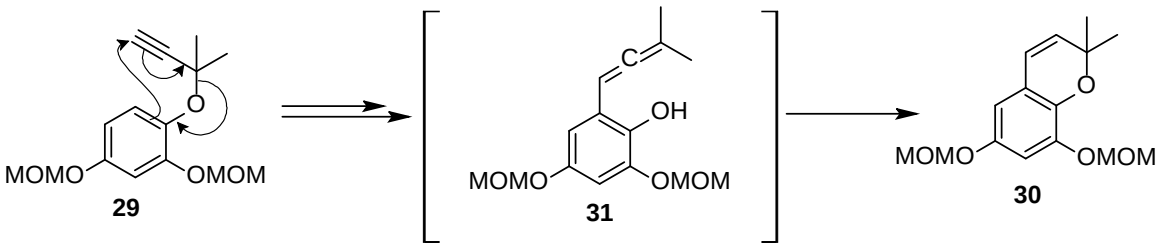
3.2.2.2 Harfenist-Tom rearrangement

The formation of the dimethylpyran ring by the Harfenist-Tom rearrangement proceeds in two steps, which involve first the *O*-propargylation of phenol **28** to give 1,1-dimethylpropargyl ether **29**, followed by a Harfenist-Tom rearrangement to give the 2,2-dimethylchromene **30** (Scheme 3.7).[17,19] Other than the conditions shown in Scheme 3.7, propargyl halides can also be used with potassium carbonate as a base in refluxing acetone to furnish propargyl ethers.[12] The drawback of this method is the poor regioselectivity when the condensation is performed on polyphenolic compounds with alternating hydroxy groups.[16,20]



Scheme 3.7. Formation of dimethylchromenes by the Harfenist-Tom rearrangement

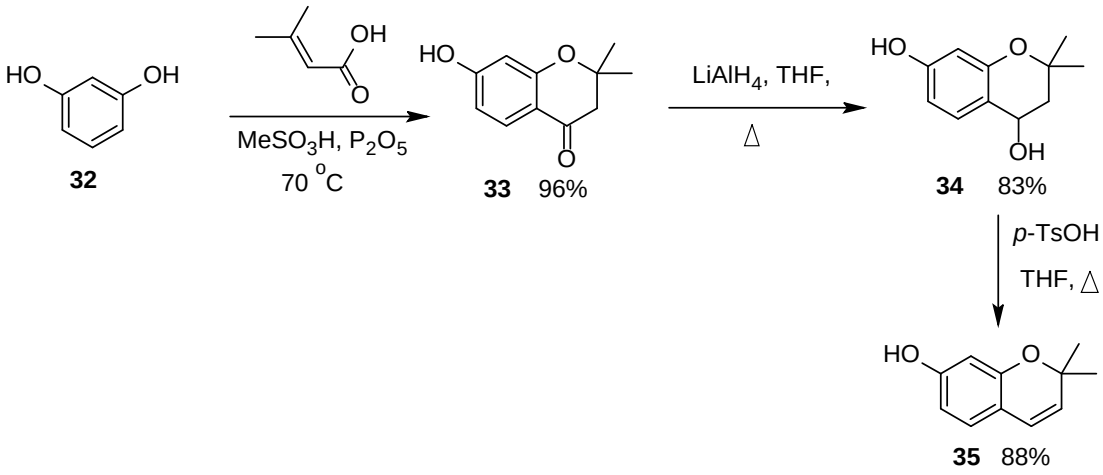
The conversion of the propagyl ether **29** into the dimethylchromene **30** is proposed to follow a Claisen-type rearrangement to give an intermediate diene **31**, followed by conjugate addition,[19] as shown in Scheme 3.8.



Scheme 3.8. Proposed mechanism for the cyclisation of the propagyl ether **29** into dimethylpyran **30**

3.2.2.3 2,2-Dimethylchromenes from 3-methylbut-2-enoic acid.

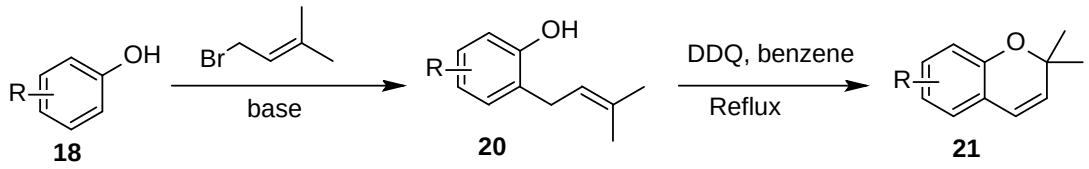
To circumvent the regioselectivity problem inherent to the procedures described above, 6- and 7-alkoxy or hydroxy-2,2-dimethylchromenes have been synthesised elegantly from the corresponding 2,2-dimethylchromanones.[21-23] This is illustrated in the synthesis of 7-hydroxy-2,2-dimethylchromene (**35**) from resorcinol (**32**) (Scheme 3.9).[23] The formation of 2,2-dimethylchromanone **33** by condensation of a resorcinol **32** with but-2-enoic acid proceeds via a Friedel-Crafts acylation, followed by a 1,4-addition reaction. Reduction of the chromanone **33** to the alcohol **34** and subsequent dehydration give the 2,2-dimethylchromene **35**. Regardless of the many synthetic steps involved in this route, the 2,2-dimethylpyran scaffold has been obtained in good yields from phenols in several instances.[22,23]



Scheme 3.9. Regioselective synthesis of 7-hydroxy-2,2-dimethylchromene (**35**)

3.2.2.4 2,2-Dimethylchromene by *O*-cyclisation of prenyl group

Alternatively, the 2,2-dimethylchromene ring can be synthesised based on the sequence of its biogenesis, which involves C-prenylation of phenol followed by ring closure of the prenyl group to the *ortho*-hydroxy group (Scheme 3.10).[24] This ring closure is often accomplished by cyclodehydrogenation with DDQ or under palladium-catalysed conditions.[24-27]



18 **20** **21**

Scheme 3.10. Synthesis of 2,2-dimethylchromenes by oxidative cyclisation of a prenylated phenol

The major shortcomings of this procedure are incurred when base-catalysed electrophilic aromatic substitution with prenyl halides is employed for C-prenylation. This often results in diprenylation, *O*-prenylation or prenylation at an unwanted position.[24,28] The regioselective methodologies developed for C-prenylation proceed via multiple steps such as Mitsunobu coupling followed by Claisen-Cope rearrangement, and regioselective iodination followed by Pd-catalysed coupling reactions.[29-32]

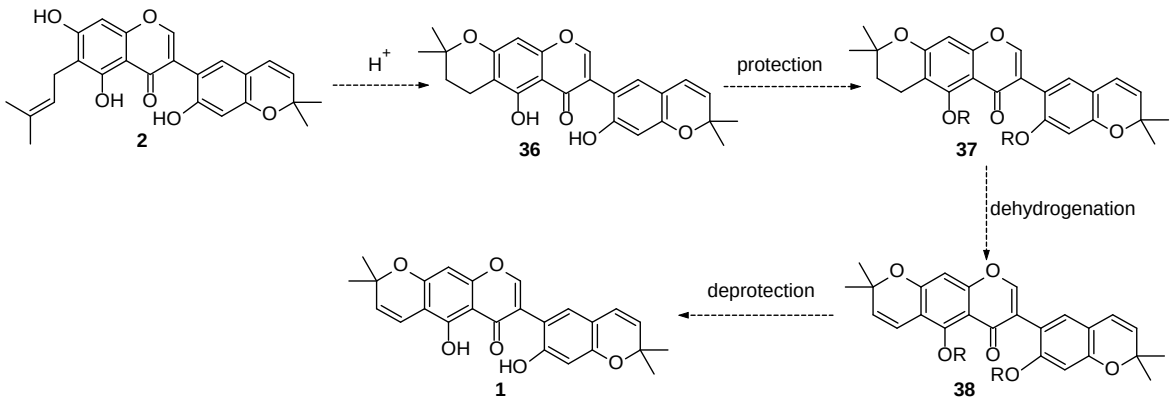
3.3 RESULTS AND DISCUSSION

3.3.1 Introduction

As seen from Scheme 3.1, the main step in the synthesis of kraussianone 1 (**1**) from kraussianone 2 (**2**) is oxidative cyclisation of the prenyl chain at C-6 to OH-7 to give the dimethylpyran scaffold on the A-ring. This step can be facilitated by quinone-based reagents such as DDQ and chloranil or by an Ullmann-type coupling reaction.[25-27] This method has advantages over other procedures in that the dimethylchromene ring is formed in one step. However, kraussianone 2 (**2**) possesses a hydroxy group at C-2', which is prone to cyclisation with the C-ring under oxidative conditions to give coumaronochromones (see Chapter 5).[33] Therefore, OH-2' would have to be selectively protected if this route were to be followed. Nevertheless, selective protection of OH-2' would not be an easy endeavour in the presence of the hydroxy group at C-7 and would require multiple protection and deprotection steps. It was essential to devise a synthetic route that would effect transformation at the targeted positions, and leave the rest of the molecule intact. Two synthetic strategies for the conversion of **2** into **1** were investigated.

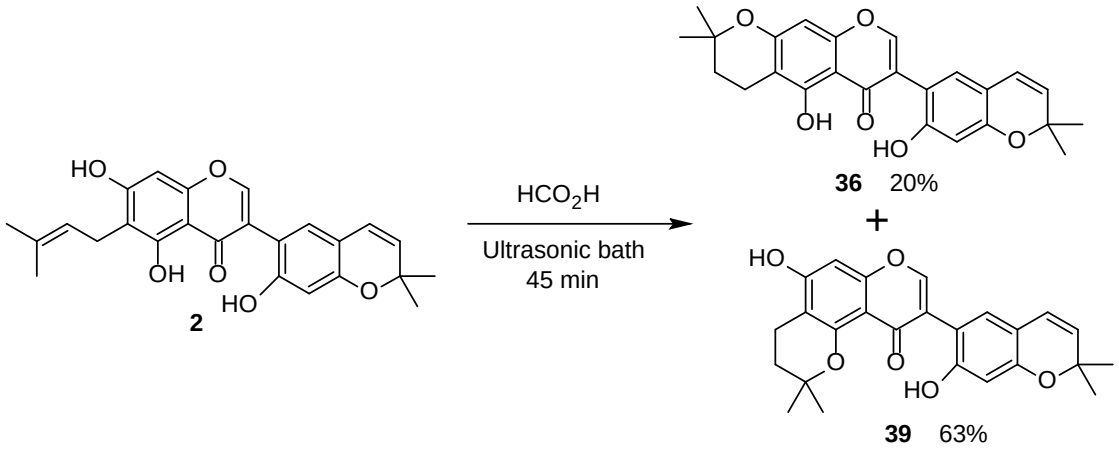
3.3.2 The First Synthetic Approach

In order to avoid several protection and deprotection steps, we initially envisaged to transform **2** into **1** as shown in Scheme 3.11. We planned to convert the prenyl group on **2** into a dimethylchroman ring to give **36**. This would allow protection of OH-2' and OH-5 without interference of OH-7. Dehydrogenation of the dimethylchroman ring into the dimethylchromene scaffold and subsequent deprotection of **38** would render **1**. Although there are two hydroxy groups adjacent to the prenyl chain in **2**, it was anticipated that cyclisation would occur favourably at OH-7 to give **36**. It is well established that the carbonyl oxygen forms a hydrogen bond with *ortho*-hydroxy groups, thus we expected OH-5 to be less reactive.



Scheme 3.1. Proposed synthetic route for kraussianone 1 (**1**)

Cyclisation of the prenyl group to an adjacent hydroxy group to give a chroman ring has been reported to occur under acidic conditions.[34-37] The mechanism involves a standard acid-catalysed addition of an alcohol to an alkene. Therefore, **2** was dissolved in HCO_2H (98-100%) and stirred in an ultrasonic bath for 45 min (Scheme 3.12). The reaction gave two compounds, which were identified as the linear isomer **36** and the angular isomer **39** in 20 and 63% yields, respectively.



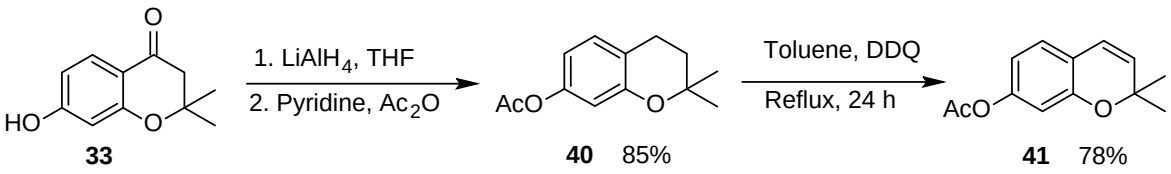
Scheme 3.12. Cyclisation of prenyl group under acidic conditions

The structures of the two compounds were derived from NMR data. The ^{1}H NMR spectrum of the linear isomer **36** displayed a one-proton singlet at δ_H 12.59 (Plate 1a), indicative of the presence of a hydrogen-bonded hydroxy group (OH-5). The signal of OH-2' appeared as a singlet at δ_H 8.39. The aromatic protons of rings A and B were displayed as singlets, at δ_H 6.41 (1H, H-8), 6.53 (1H, H-3), and 6.76 (1H, H-6). The signals for the dimethylchromene scaffold attached to the B-ring were displayed as a six-proton singlet at δ_H 1.44 (H-7" and H-8") and two *cis*-olefinic doublets at δ_H 5.52 (J = 9.8 Hz, H-5") and 6.28 (J = 9.8 Hz, H-4") and those for the dimethylchroman ring attached to ring A appeared at δ_H 1.38 (6H, s, H-7'" and H-8'"), 1.86 (2H, t, J = 6.8 Hz, H-5'") and 2.75 (2H, t, J = 6.8 Hz, H-4'"). The ^{1}H NMR spectrum of compound **39** showed similar signals to those of **36** (Plate 2a), except for the absence of the signal for OH-5, confirming cyclisation at that position. The structure of **39** was further confirmed by HRMS, which gave an m/z of 443.1460 $[M + Na]^+$ in agreement with the calculated formula weight of 443.1471 for $C_{25}H_{24}NaO_6$.

These results are not in accordance with our initial hypothesis, as the unexpected isomer was the major constituent of the reaction mixture. Since the reaction was carried out under acidic conditions, it is postulated that the carbonyl oxygen was protonated by the acid, thereby preventing the formation of the hydrogen bond with the OH-5. The two hydroxy groups on the A-ring were therefore equally available for cyclisation. The reaction was repeated with 1:1 THF/HCO_2H. This left **2** untouched after stirring in an ultrasonic bath for

12 h at rt. Refluxing the mixture gave multiple products. Other acids, *p*-TSOH and HCl, were tested under different conditions, but in vain.[36,37]

Because the targeted isomer **36** was obtained in low yields, we decided to test the feasibility of the main subsequent step, which was dehydrogenation. A model reaction was performed with chroman **40**. As shown in Scheme 3.13, chroman **40** was prepared by reduction of the available chromanone **33** with $LiAlH_4$ followed by acetylation.[38] The chromanone **33** was prepared in the total synthesis of kraussianone 1 (**1**), discussed in Chapter 4.



33 **40** 85% **41** 78%

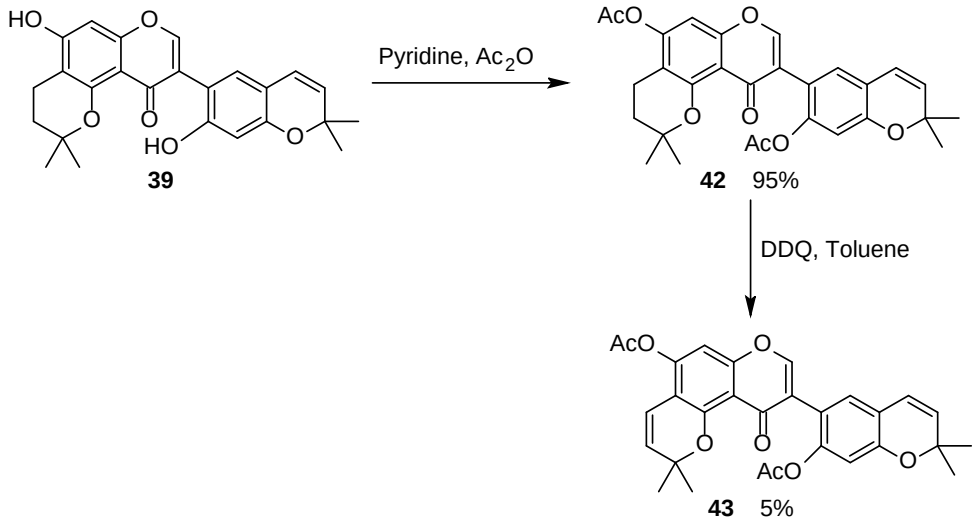
Scheme 3.13. Model reaction for dehydrogenation of chromans into chromenes.

The ^{1}H NMR spectrum of **40** showed an ABX spin system for the aromatic protons at δ_H 7.03 (1H, d, J = 8.3 Hz, H-5), 6.56 (1H, dd, J = 2.5 and 8.3 Hz, H-6), 6.53 (1H, d, J = 2.5 Hz, H-8), and the signals for the dimethylchroman ring were displayed as a pair of triplets integrating for two protons each at δ_H 2.75 (H-4) and 1.79 (H-3), and a six-proton singlet at δ_H 1.33 (2 × CH_3). The resonance for the acetate group appeared as a singlet at δ_H 2.26.

Two oxidising agents, chloranil and DDQ were employed for the dehydrogenation of chroman **40** into chromene **41**. The best results were obtained from the DDQ reaction, whereby **41** was prepared in 78% yield, Scheme 3.13.[39] Chloranil afforded 10% of chromene **41**, and most of the starting material was recovered. The formation of the dimethylchromene moiety was confirmed by the appearance of the two doublets with coupling constant of 10.0 Hz, indicative of the *cis*-double bond in the ^{1}H NMR spectrum of **41**, and the absence of the two triplets observed in the ^{1}H NMR of **40**.

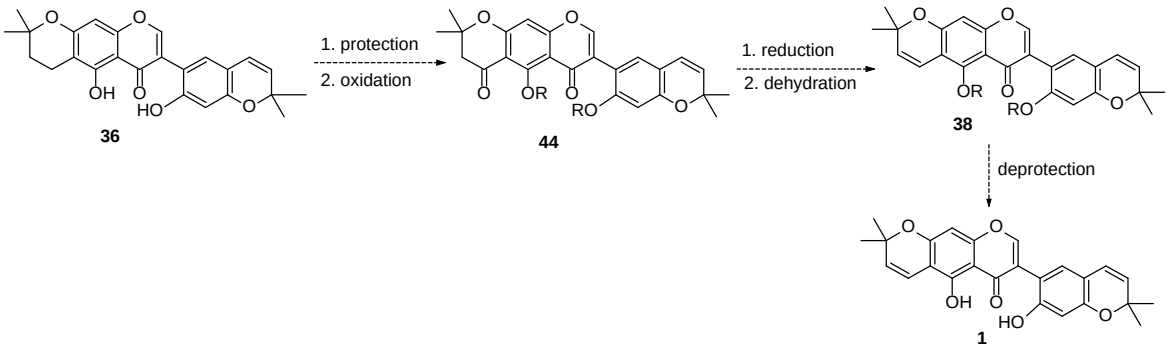
The optimum conditions were applied to the isoflavone **42**, prepared by acetylation of **39**, Scheme 3.14. DDQ was added to a solution of **39** in toluene, and the mixture was refluxed for 24 h.[39] Disappointingly, the starting material was left intact after 24 h reflux. Increasing

the reaction time to 5 days facilitated 5% conversion of the starting material into the targeted product **43**, as observed from the ^{1}H NMR spectrum of the reaction mixture.



Scheme 3.14. Dehydrogenation of the isoflavone **42**

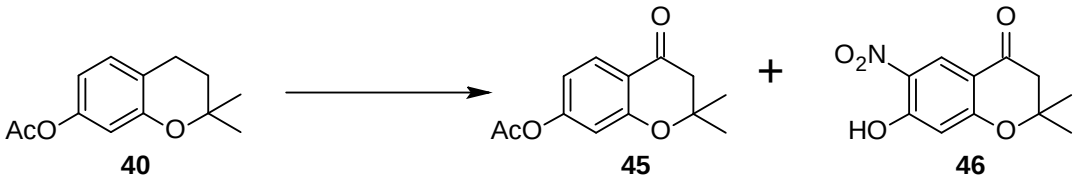
The failure to obtain good yields from the dehydrogenation of **42**, prompted us to investigate alternative methods for preparing chromenes from chromans. It has been reported that chromans can be oxidised at the benzylic position to give chromanones using reagents such as CAN, DDQ, PCC and IBX.[40-43] Chromanones can be readily converted into chromenes by reduction and dehydration as seen from Section 3.2.2.3. Therefore, we planned to alter the initial route slightly, and prepare kraussianone 1 (**1**) from **36** as shown in Scheme 3.15.



Scheme 3.15. Modified route towards the synthesis of kraussianone 1 (**1**)

Since compound **36** was obtained in a low yield, it was important to establish ideal conditions for the oxidation of the chroman to the chromanone. Model reactions were performed with chroman **40**. A series of CAN oxidation reactions were set under different conditions as shown in Table 3.1. The best yield (82%) of **45** was obtained upon treatment of a CH_3CN/H_2O solution of **40** with 2 *eq.* CAN. Addition of CH_3CO_2H to the reaction led to formation of the nitrated site product **46**, which was obtained in low yields when the reaction was conducted for a shorter time (30 min) and increased with long reaction time (12 h).

Table 3.1. Oxidation of chroman **40** to chromanone **45**

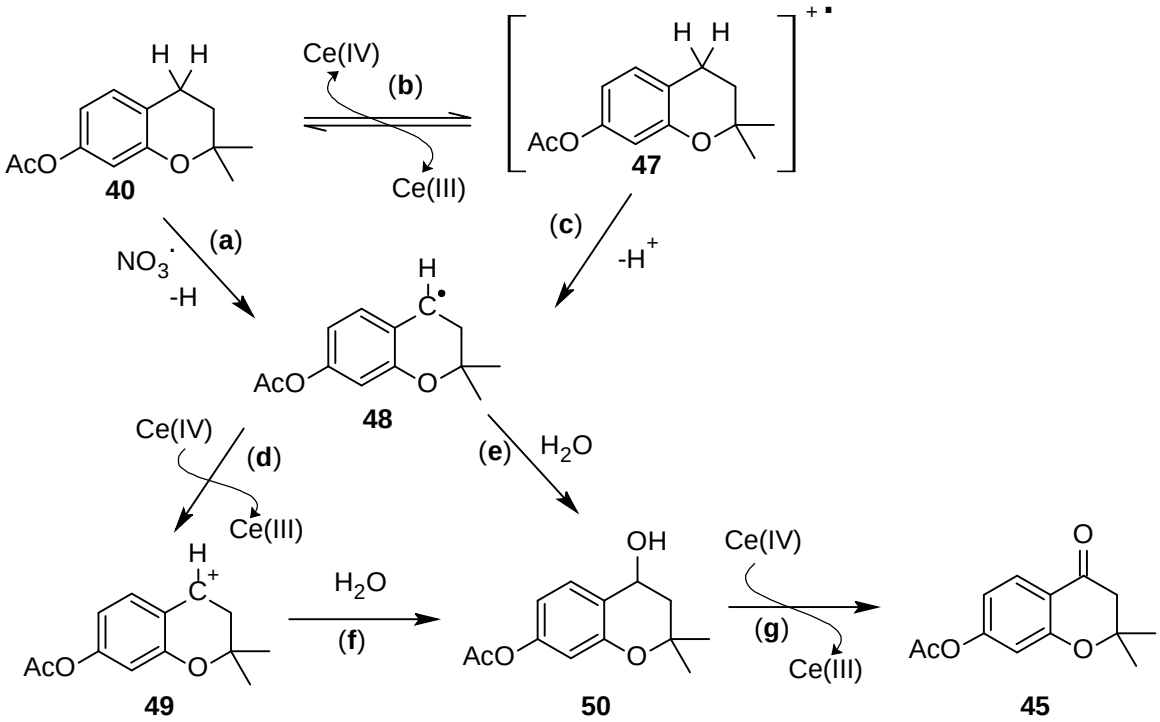


Reagent	**Mole Equiv**	**Solvent**	**Time h**	**Isolated Product**	**% yield**
CAN	6	Ether/H_2O/ CH_3CO_2H	12	**46**	56%
CAN	6	Ether/H_2O/ CH_3CO_2H	0.5	**45** **46**	45% 10%
CAN	6	CH_3CN/ H_2O	2	**45**	78%
CAN	**2**	**CH_3CN/ H_2O**	**1**	**45**	**82%**
CAN	1.5	CH_3CN/ H_2O	1	**45**	74%
CAN	1.0	CH_3CN/ H_2O	1	**45**	70%

The success of the oxidation of chroman **40** to chromanone **46** was confirmed by the absence of a pair of triplets and the presence of a singlet integrating for two protons at δ_H 2.67 (H-3) in the ^1H NMR spectrum of compound **45**. The ^{13}C NMR spectrum indicated the presence of a carbonyl functional group by displaying a signal at δ_C 191.2. The ^1H NMR spectrum of compound **46** displayed similar signals to those of compound **45**. However, the aromatic protons exhibited an AX spin system at δ_H 8.74 (1H, s, H-5) and

6.60 (1H, s, H-8), instead of the ABX system observed for **45**. In addition the signal for the acetate group was absent in the ^{1}H NMR spectrum of **46**.

The oxidation of chromans into chromanones by CAN is proposed to proceed via a SET mechanism. There are several plausible pathways by which chromans can be converted into chomanones by a SET mechanism as illustrated in Scheme 3.16.[44-46] All the probable pathways involve formation of a radical **48**.[44-46] The radical **48** can be generated via two routes. Route a involves removal of a hydrogen atom by a nitrate radical. The second route follows two steps, which involve formation of a cationic radical **47** and subsequent abstraction of a proton (route b-c).[46] The mechanistic studies conducted by Dinctürk *et al*. in CH_3CN suggested the latter route to be the most probable one, whereby the loss of the proton from the radical cation was determined to be rate-determining to a certain extend.[46] The benzylic radical **48** can then be attacked by oxygen or nucleophiles,[45] in the present case, by H_2O to give an alcohol **50**, which is further oxidised by Ce(IV) to give the ketone (route e-g). Alternatively, the radical **48** can be further oxidised to the cation **49**,[44,46] which undergoes nucleophilic addition with H_2O and subsequent oxidation to give the ketone **45** (route d-f-g).

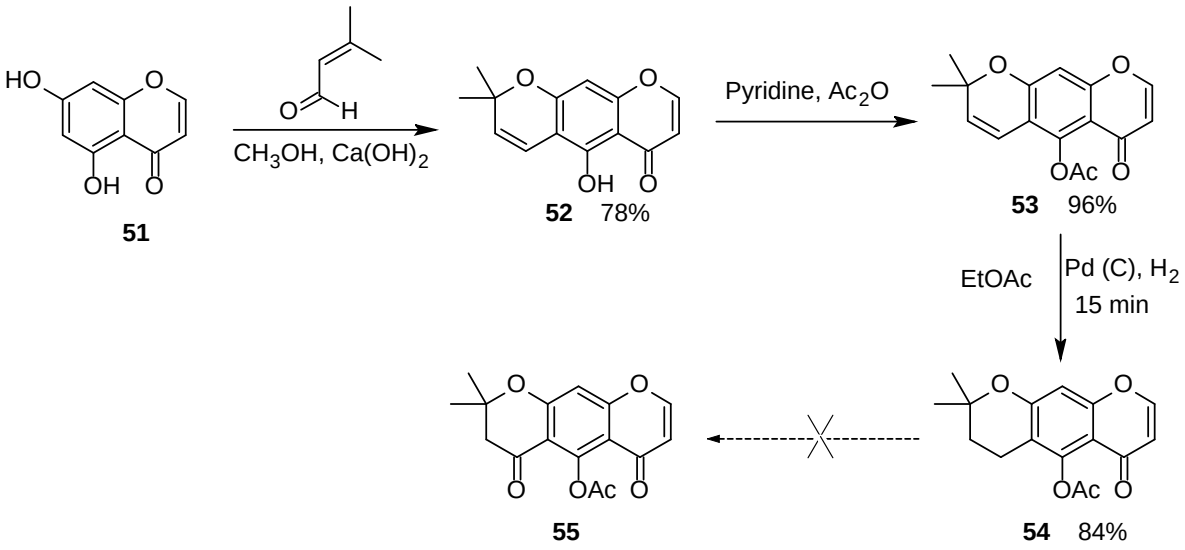


Scheme 3.1. Proposed pathways for oxidation of chroman **40** to chromanone **45**

Nitration of aromatic rings with CAN is not a new phenomenon. Different mechanisms have been suggested for the nitration of aromatic compounds with CAN.[47] Dinctürk and colleagues proposed a mechanism that involves generation of a nitronium ion (NO_2^+) from the Ce(IV) complex, whereby the aromatic substrate participates as a 'spectator' as shown in Equation 3.1.[47] Alternatively, Mellor *et al.* suggested that simple aromatic compounds such as toluene and halobenzenes are nitrated a via simple electrophilic aromatic substitution reaction with nitronium cation (NO_2^+), whereas nitration of naphthalene involves attack on nitronium radical (NO_2•).[48] Dinctürk and Mellor conducted their mechanistic studies under different conditions, thus a conclusive remark cannot be drawn from their arguments. In the present case, the nitrated by-product **46** was observed when the reaction was performed in a mixture of ether/AcOH/H_2O, but not in a solution of CH_3CN/H_2O. Therefore, further studies are required to unravel the role of different solvents in the nitration of aromatic compounds with CAN.

$$ArH.\ NO_2^+.\ CeO(NO_3)_5^{3-} \longrightarrow ArNO_2 + Ce(OH)(NO_3)_5^{2-} \quad \text{(Equation 3.1)}$$

Since the chroman scaffold of **36** is attached to the phloroacetophenone moiety (A-ring), it was important to test the optimised reaction conditions for the conversion of the chroman into the chromanone on a model compound that bears the phloroglucinol moeity. The optimised reaction conditions were applied to compound **54** that closely resembles the western half of **36**. The model compound **54** was prepared by selective reduction of the chromene **53** by hydrogenation as shown in Scheme 3.17. Compound **53** was in turn prepared by condensation of the available chromone **51** (the synthesis of which is discussed in Chapter 4) with prenal following Mondal's protocol and subsequent acetylation of OH-5. Treatment of a solution of **54** in CH_3CN/H_2O with CAN gave a mixture of unidentified compounds.

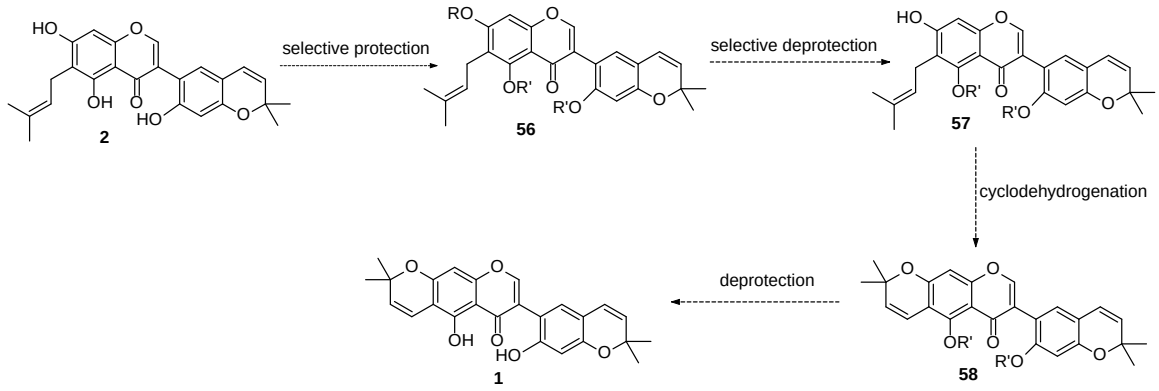


Scheme 3.17. Attempted oxidation of chroman **54** into a diketone **55**

Due to poor yields of the isomer **36**, obtained by the acid-catalysed cyclisaton of prenyl moiety of kraussianone 2 (**2**) and the difficulty experienced in the oxidation of the chroman with a phloroglucinol moiety into a chromene, this route was abandoned for the preparation of kraussianone 1 (**1**) from kraussianone 2 (**2**). Therefore, a second synthetic approach was devised.

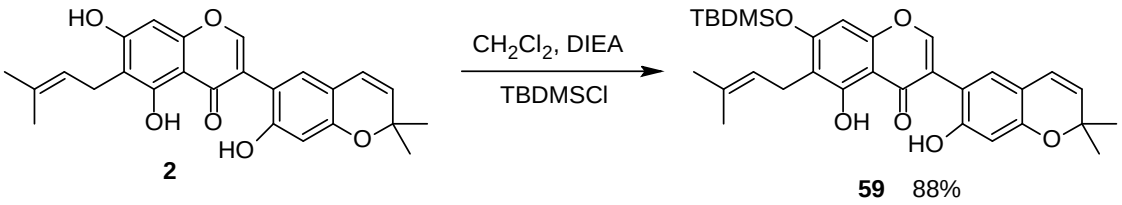
3.3.3 The Second Synthetic Approach

An alternative route was based on selective protection and deprotection of the hydroxy groups and cyclodehydrogenation of the dimethylallyl chain as shown in Scheme 3.18. As already mentioned, the main challenge in this route was to protect OH-5 and OH-2' selectively and leave OH-7 free for cyclisation. Since most protection reactions are conducted under basic conditions, it was envisaged that OH-7 and OH-2' would be more susceptible to protection than OH-5. Regioselectivity between OH-7 and OH-2' could be achieved by using bulkier bases and protecting groups, which would make OH-2' less accessible.



Scheme 3.18. The second synthetic route

The first step involved protecting OH-7 with bulky TBDMSCl using DIEA as a base. The reaction exclusively gave **59** in 88% yields (Scheme 3.19). The structure of the compound was confirmed by the ^{1}H, ^{13}C and 2D NMR spectroscopy. The ^{1}H and the ^{13}C NMR spectra of **59** showed signals similar to those of kraussianone 2 (**2**). In addition, signals for the TBDMS protons resonated at δ_H 1.04 [9H, s, $SiC(CH_3)_3$] and 0.33 [6H, s, $Si(CH_3)_2$] in the ^{1}H NMR spectrum (Plate 4a) and at δ_C 25.6 [3 × CH_3, $SiC(\underline{C}H_3)_3$], 18.4 [C, $Si\underline{C}(CH_3)_3$], -4.1 [2× CH_3, $Si(CH_3)_2$] in the ^{13}C NMR spectrum of compound **59** (Plate 4b). The attachment of the TBDMS group at OH-7 was confirmed by the presence of signals for the OH-5 and OH-2' at δ_H 12.43 and 8.32, respectively.



Scheme 3.19. Selective protection of kraussianone 2 (**2**) with TBDMS

The regiochemistry of compound **59** was further substantiated by HMBC correlations shown in Figure 3.1. The resonances of the oxygen-linked carbons on the A- and B-rings could be distinguished from the HMBC correlations. For instance, the proton signal at δ_H 6.75 (H-6') showed connections to carbon resonances at δ_C 157.2 (C-2') and 155.9 (C-4'), and the hydroxy group at δ_H 8.32 (OH-2') correlated with C-2' at δ_C 157.2. From these connections, the two oxygen-linked carbon resonances on the B-ring could be assigned,

and it could be deduced that the silyl protecting group was attached to the A-ring and not the B-ring. As already mentioned, the ^{1}H NMR spectrum of **59** exhibited a one-proton singlet characteristic of a hydrogen-bonded hydroxy group at δ_H 12.43 (OH-5), thus it was clear that the TBDMS group was attached to OH-7. Furthermore, the OH-5 signal at δ_H 12.43 and the methylene signal at δ_H 3.38 (H-1'") showed connections to the carbon signal at δ_C 159.5 (C-5), and the one-proton singlet at δ_H 7.95 (H-2) showed a correlation to the signal of C-8a at δ_C 155.5. The remaining oxygen-linked carbon resonance at δ_C 161.0 was therefore assigned to C-7. The C-7 resonance correlated to the proton signals at δ_H 3.38 (H-1'") and 6.43 (H-8).

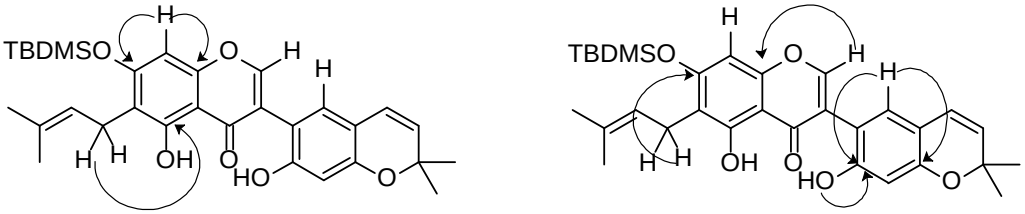
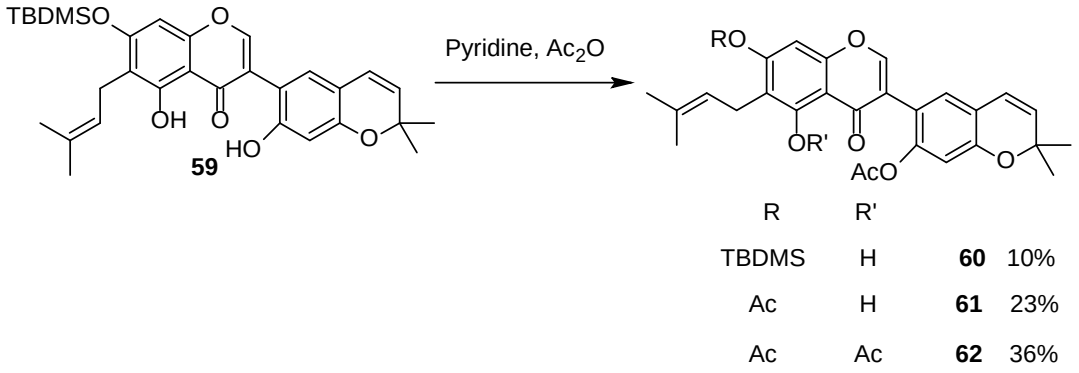


Figure 3.1. Key HMBC correlations of **59**

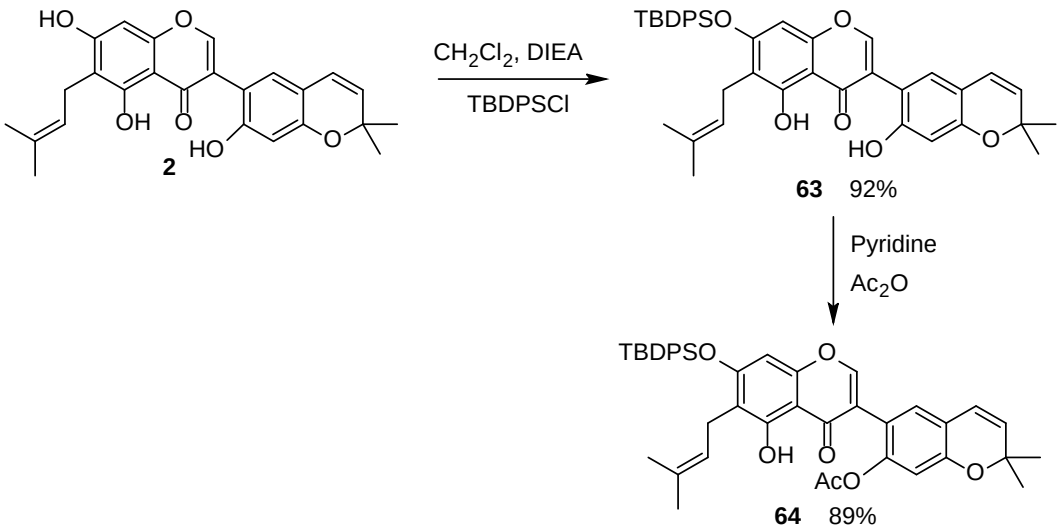
The subsequent step involved protection of the OH-5 and OH-2'. The choice of the second protecting group was motivated by the ease of introduction of the group, its stability to conditions that were to be employed for the deprotection of TBDMS and the ease of removal of the group itself. Thus we opted for acetyl protection. Compound **59** was reacted with acetic anhydride in the presence of pyridine as shown in Scheme 3.20. The reaction gave a mixture of compounds identified as **60**, **61** and **62** by ^{1}H NMR experiments. The ^{1}H NMR spectrum of compound **60** resembled that of **59**, however, the peak for the OH-2' was no observed, instead there was a three-proton singlet at δ_H 2.34 for the acetate group. On the other hand, the resonances of the TBDMS group were absent in the ^{1}H NMR spectra of **61** and **62**, instead additional signals were observed for the acetate groups at δ_H 2.35 (3H, s), 2.14 (3H, s) for **61**, and δ_H 2.40 (3H, s), 2.35 (3H, s), 2.13 (3H, s) for **62**.



Scheme 3.20. *O*-acetyl protection of **59**

Owing to the instability of the TBDMS ether under the basic acetylation conditions, we opted for the TBDPS protecting group. This silyl protecting group was chosen due to its stability to acidic conditions, especially AcOH, which is generated during the reaction.[49] Therefore, kraussianone 2 (**2**) was reacted with TBDPSCl in the presence of DIEA as shown in Scheme 3.21. As anticipated, the reaction gave **63** in excellent yield. An attempt to acetylate the two remaining hydroxy groups resulted in the formation of **64** as a sole product, Scheme 3.21. The C-5 hydroxy group was found to be resistant to esterification.

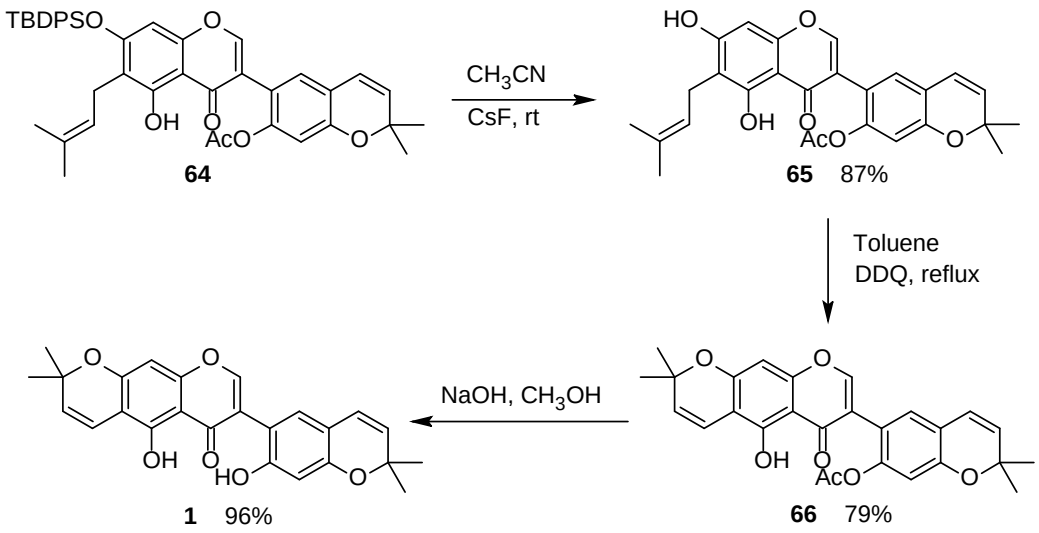
The ^{1}H NMR spectrum of **64** (Plate 6a) exhibited the characteristic isoflavone one-proton singlet at δ_H 7.54 for H-2, a signal for OH-5 resonated further downfield at δ_H 12.85 and the resonances for the aromatic hydrogens were displayed at δ_H 5.95 (1H, s, H-8), 6.83 (1H, s, H-6') and 6.59 (1H, s, H-3'). The signals for the dimethylpyran ring attached to ring B were observed at δ_H 6.25 (1H, d, *J* = 9.8 Hz, H-4''), 5.58 (1H, d, *J* = 9.8 Hz, H-5'') and 1.42 (6H, s, H-7'' and H-8''), and those of the prenyl chain on ring A were observed at δ_H 5.34 (1H, t, *J* = 6.5 Hz, H-2'''), 3.58 (2H, d, *J* = 6.5 Hz, H-1'''), 1.83 (3H, s, H-4'''/H-5''') and 1.72 (3H, s, H-4'''/H-5'''). The presence of the TBDPS and the acetate protecting groups was confirmed by resonances at δ_H 7.74 (4H, dd, *J* = 8.0 and 1.5 Hz, $SiPh_2$), 7.50-7.40 (6H, m, $SiPh_2$), 1.12 [9H, s, $SiC(CH_3)_3$] and 2.09 (3H, s, $COCH_3$). The HRMS spectrum gave an *m/z* of 723.2740 in agreement with the calculated pseudo molecular weight of 723.2754 for $C_{43}H_{44}NaO_7Si$.



Scheme 3.21. Preparation of **64**

Although OH-5 was not protected, we decided to proceed with the subsequent steps, with the hope that DDQ-induced *O*-cyclisation of prenyl group would occur favourably at OH-7. This was motivated by the literature reports that indicated that cyclodehydrogenation of the prenyl chain between two hydroxy groups in phloroacetophenone-like moieties occurs preferably at the *para*-hydroxy group than the *ortho*-hydroxy group relative to the carbonyl moiety.[25,27,35] Therefore, the TBDPS group was selectively removed with CsF in CH_3CN at rt to give **65** as shown in Scheme 3.21. The penultimate steps involved oxidation of **65** with DDQ to give **66**.

The formation of compound **66** was confirmed by the absence of the signals for the TBDPS and the prenyl groups in the ^{1}H NMR spectrum of **66** (Plate 8a). Instead, the resonances for the second dimethylchromene ring, comprising two olefinic doublets at δ_H 6.71 (1H, d, J = 10.0 Hz, H-4'''), 5.62 (1H, d, J = 10.0 Hz, H-5''') and a six-proton singlet for the enantiotopic methyl groups at δ_H 1.47 (H-7''' and H-8''') were observed. The presence of a singlet integrating for one proton at δ_H 13.00 in the ^{1}H NMR spectrum of **66** showed that OH-5 was left intact during the reaction, which confirmed that cyclisation occurred at OH-7 as anticipated. Finally, removal of the acetyl group from **66** under basic conditions rendered the target molecule, kraussianone 1 (**1**). The structure of the compound was confirmed by the ^{1}H NMR spectrum, which was identical to that of the isolated natural compound (Plate 9a).



Scheme 3.22. Synthesis of kraussianone 1 (**1**)

3.4. CONCLUSION

Two synthetic routes have been investigated for the synthesis of kraussianone 1 (**1**) from kraussianone 2 (**2**). The main transformation in the two strategies involved cyclisation of the prenyl side chain on kraussianone 2 (**2**) into a dimethylchromene ring. In the first synthetic strategy, the dimethylchomene ring was to be prepared in two major steps, which were acid-catalysed cyclisation of the prenyl group into a dimethylchroman moiety and subsequent dehydrogenation. This route was abandoned due to poor regioselectivity of the prenyl cycloadditon reaction under acidic conditions, and the difficulty of oxidation of the dimethylchroman into dimethylchromene on substrates with a phloroglucinol moiety. The second pathway was based on selective protection and deprotection of the hydroxy groups, followed by oxidative cyclisation of the prenyl goup to OH-7 to give the dimethylchromene scaffold. This was determined to be the most viable route for conversion of kraussianone 2 (**2**) into kraussianone 1 (**1**). Following this route, kraussianone 1 (**1**) was prepared in an excellent overall yield of 54% in five steps from kraussianone 2 (**2**).

3.5 EXPERIMENTAL

3.5.1 General Experiental Procedures

All reactions requiring anhydrous conditions were performed under nitrogen in oven-dried glassware. Toluene was distilled under N_2 from sodium wire and benzophenone. CH_3OH was distilled under N_2 from CaO. Other anhydrous solvents were distilled under N_2 from Technologies (Newburyport, MA) Pure-Solv 800 Solvent Purification System. Hex used for chromatographic purifications was distilled at 70 ^oC prior to use. Reagent grade reagents used for syntheses were purchased from Fluka, Sigma-Aldrich or Merck and were used without further purification unless otherwise stated. Kraussianone 2 (**2**) used for the synthesis was isolated from *E. kraussianum* following the procedure reported by Drewes *et al*.[1,2]

TLC was performed on Merck silica gel plates (60 F_{254}) and visualised under UV light (254 nm) and/or upon treatment with iodine vapour. Alternatively, detection of the TLC spots was achieved by heating with a heat gun after treatment with a solution of anisaldehyde in concentrated sulfuric acid and ethanol prepared in volume ratios of 1:1:18, respectively. Column chromatography was performed on columns with 25 mm or 40 mm column diameter using Merck Kieselgel 60 (230-400 mesh). Centrifugal chromatography was performed on a Harrison Research Chromatotron Model 7924T on glass plates coated with Merck silica gel with particle size 0.040-0.063 mm, 2-4 mm thick.

Synthesised compounds were characterised by mass spectrometry, infrared spectroscopy and NMR spectroscopy. The mass spectra were recorded on an ion trap Thermo Finnigan GC-MS using electron-impact ionization or on a time-of-flight Waters LCT Premier MS using electrospray ionisation in the positive or negative mode, and the IR spectra on an FT-IR Perkin Elmer spectrophotometer (Spectrum 100 and Spectrum one).

The NMR spectra were recorded on a Bruker AVANCE DPX spectrometer. The chemical shifts from ^1H NMR and ^{13}C NMR spectra are reported in parts per million relative to the residual protonated or deuterated solvents peaks. The chemical shifts for the solvents used are as follows:

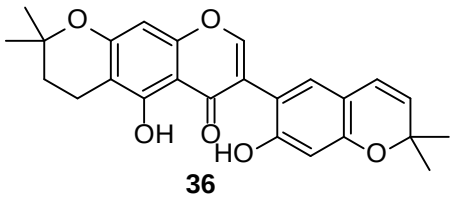
Solvents	^{1}H Chemical Shift	^{13}C Chemical Shift
$CDCl_3$	7.26	77.0
DMSO-d_6	2.50	39.5
CD_3OD	4.79	49.0
Acetone-d_6	2.05	205.1

The coupling constants were measured in Hz. Abbreviations used for the multiplicity of signals are as follows:

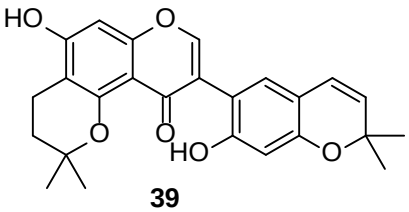
s	singlet	dd	doublet of doublets
d	doublet	br	broadened
t	triplet	m	multiplet
q	quartet		

The melting points of crystalline compounds were measured on a Reichert electrothermal melting point apparatus, and were uncorrected.

3.5.2 Acid-Catalysed Cyclisation of the Prenyl Group of Kraussianone 2 (2) into a Chroman Moiety



36



39

A solution of kraussianone 2 (**2**) (50 mg, 0.119 mmol) in HCO_2H (15 mL, 98%-100%) was immersed in an ultrasonic bath fitted with a magnetic stirrer. The solution was stirred for 45 min, diluted with H_2O and extracted with EtOAc. The combined organic extracts were washed with H_2O, brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude mixture was purified by column chromatography using Hex:EtOAc (3:2) to give **36** (10 mg, 20%) and **39** (31.5 mg, 63%). Compound **39** was recrystallised from CH_3OH to give cream white crystals.

2',5-Dihydroxy-[6",6"-dimethylpyrano(2",3":4',5')][4"',5"'-dihydro-6"',6"' dimethylpyrano(2"',3"':7,6)]isoflavone (**36**)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.59 (1H, s, OH-5), 8.39 (1H, s, OH-2'), 7.93 (1H, s, H-2), 6.76 (1H, s, H-6'), 6.53 (1H, s, H-3'), 6.41 (1H, s, H-8), 6.28 (1H, d, *J* = 9.8 Hz, H-4"), 5.52 (1H, d, *J* = 9.8 Hz, H-5"), 2.73 (1H, t, *J* = 6.8 Hz, H-4"'), 1.86 (1H, t, *J* = 6.8 Hz, H-5"'), 1.44 (6H, s, H-7"' and H-8"'), 1.38 (6H, s, H-7" and H-8"); (Plate 1a)

^{13}C NMR (DMSO-d_6, 100 MHz) δ 182.0 C, C-4), 161.4 (C, C-7), 159.3 (C, C-5), 157.1 (C, C-2'), 155.7 (C-8a), 155.5 (C, C-4'), 154.9 (CH, C-2), 128.7 (CH, C-5"), 127.1 (CH, C-6'), 122.6 (C, C-3), 121.4 (CH, C-4"), 115.2 (C, C-5'), 112.4 (C, C-1'), 107.3 (CH, C-3'), 106.1 (C, C-6), 104.4 (C, C-4a), 95.0 (CH, C-8), 76.6 (C, C-6"), 76.7 (C, C-6"'), 31.7 (CH_2, C-5"'), 28.1 (2 × CH_3, C-7" and C-8"), 26.7 (2 × CH_3, C-7"' and C-8"'), 16.1 (CH_2, C-4"'). (Plate 1b)

2',7-Dihydroxy-[6",6"-dimethylpyrano(2",3":4',5')][4"',5"'-dihydro-6"',6"'-dimethylpyrano(2"',3"':5,6)]isoflavone (**39**)

mp > 270 ^{o}C (decomposes)

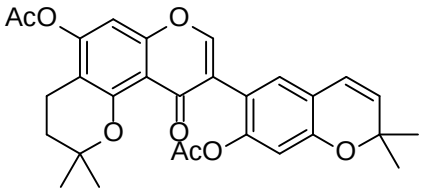
IR (neat) ν_{max} 3336, 2958, 2926, 1725, 1620, 1547, 1462, 1298, 1261, 1149, 1068 cm^{-1};

^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.67 (1H, brs, OH-2'), 7.84 (1H, s, H-2), 6.78 (1H, s, H-6'), 6.38 (1H, s, H-8), 6.28 (1H, d, *J* = 9.8 Hz, H-4"), 6.24 (1H, s, H-3'), 5.52 (1H, d, *J* = 9.8 Hz, H-5"), 2.52 (1H, t, *J* = 6.9 Hz, H-4"'), 1.70 (1H, t, *J* = 6.8 Hz, H-5"'), 1.32 (6H, s, H-7" and H-8"), 1.25 (6H, s, H-7"' and H-8"'); (Plate 2a)

^{13}C NMR (DMSO-d_6, 100 MHz) δ 174.7 (C, C-4), 160.6 (C, C-7), 157.9 (C-8a), 156.8 (C, C-2'), 155.6 (C, C-5), 153.9 (C, C-4'), 152.4 (CH, C-2), 129.9 (CH, C-6'), 128.3 (CH, C-5"), 123.0 (C, C-3), 120.0 (CH, C-4"), 113.5 (C, C-5'), 113.0 (C, C-1'), 108.1 (C, C-4a), 106.4 (C, C-6), 103.9 (CH, C-3'), 93.9 (CH, C-8), 76.8 (C, C-6"), 75.8 (C, C-6"'), 31.3 (CH_2, C-5"'), 28.3 (2 × CH_3, C-7" and C-8"), 26.8 (2 × CH_3, C-7"' and C-8"'), 17.3 (CH_2, C-4"'); (Plate 2b)

HR-ESIMS *m/z* $[M + Na]^+$ 443.1460 (Calcd. for $C_{25}H_{24}NaO_6$ 443.1471).

3.5.3 2',7-Diacetoxy-[6'',6''-dimethylpyrano(2'',3'':4',5')][4''',5'''-dihydro-6''',6''' dimethylpyrano(2''',3''':5,6)]isoflavone (42)



A solution of **39** (20 mg, 0.0476 mmol), pyridine (0.20 mL, 2.53 mmol) and acetic anhydride (0.16 mL, 1.65 mmol) was stirred at rt for 6 h. The reaction was quenched with ice-water and stirred for an additional 20 min. The resulting mixture was extracted with EtOAc, washed with H_2O and brine. The organic layer was dried over anhydrous $MgSO_4$. The solvent was evaporated with a rotary evaporator and the crude product was purified on a chromatotron using Hex:EtOAc (3:2) to give compound **42** (22.8 mg, 95%) as a colourless oil.

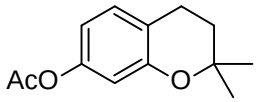
IR (neat) ν_{max} 2975, 2930, 1761, 1652, 1607, 1426, 1368, 1182, 1127 cm^{-1};

^1H NMR ($CDCl_3$, 400 MHz) 7.65 (1H, s, H-2), 6.97 (1H, s, H-6'), 6.71 (1H, s, H-8), 6.58 (1H, s, H-3'), 6.28 (1H, d, *J* = 9.8 Hz, H-4''), 5.57 (1H, d, *J* = 9.8 Hz, H-5''), 2.62 (1H, t, *J* = 6.8 Hz, H-4'''), 2.36 (3H, s, $OCOCH_3$), 2.13 (3H, s, $OCOCH_3$), 1.82 (1H, t, *J* = 6.9 Hz, H-5'''), 1.43 (6H, s, H-7'' and H-8''), 1.41 (6H, s, H-7''' and H-8'''); (Plate 3a)

^{13}C NMR ($CDCl_3$,100 MHz) δ 175.4 (C, C-4), 169.4 (C, O<u>C</u>OCH_3), 168.3 (C, O<u>C</u>OCH_3), 156.8 (C-8a), 156.1 (C, C-5), 153.8 (C, C-4'), 152.5 (C, C-7), 151.5 (CH, C-2), 149.2 (C, C-2'), 130.3 (CH, C-5''), 129.3 (CH, C-6'), 122.3 (C, C-3), 121.5 (CH, C-4''), 119.1 (C, C-5'), 117.0 (C, C-1'), 113.2 (C, C-4a), 110.8 (CH, C-3'), 110.6 (C, C-6), 102.0 (CH, C-8), 76.7 (C, C-6''), 75.9 (C, C-6'''), 31.0 (CH_2, C-5'''), 28.3 (2 × CH_3, C-7'' and C-8''), 26.6 (2 × CH_3, C-7''' and C-8'''), 21.0 (CH_3, OCO<u>C</u>H_3), 20.8 (CH_3, OCO<u>C</u>H_3), 17.3 (CH_2, C-4'''); (Plate 3b)

HR-ESIMS *m/z* $[M + Na]^+$ 527.1672 (Calcd. for $C_{29}H_{28}NaO_8$ 527.1682).

3.5.4 7-Acetoxy-2,2-dimethylchroman (40)



$LiAlH_4$ (1.58 g, 41.6 mmol) was added portion-wise to a solution of chromanone **33** (2.01 g, 10.4 mmol) in THF (50 mL) at 0 ^oC. The mixture was stirred at this temperature for 10 min and then refluxed for 12 h. The reaction was quenched with H_2O and filtered. The filtrate was extracted with EtOAc, washed with brine and dried over ahydrous $MgSO_4$. The solvent was evaporated to give a yellow oil. Pyridine (2.03 mL, 25.3 mmol) and acetic anhydride (1.86 mL, 19.8 mmol) were added to the oil and the mixture was stirred at rt for 6 h. Ice was added and the mixture was stirred for an additional 20 min, after which a white solid precipitated. The solid was filtered and washed with H_2O to render **40** (1.95 g, 83%). The compound was recrystallised from Hex to give colourless needles.

mp 56.6 – 57.2 ^oC

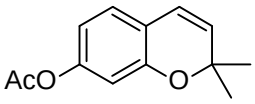
IR (neat) ν_{max} 2972, 1755, 1498, 1201, 1142, 771 cm^{-1};

^1H NMR ($CDCl_3$, 400 MHz) δ 7.03 (1H, d, *J* = 8.3 Hz, H-5), 6.56 (1H, dd, *J* = 2.5 and 8.3 Hz, H-6), 6.53 (1H, d, *J* = 2.5 Hz, H-8), 2.75 (2H, t, *J* = 6.8, H-4), 2.26 (3H, s, $OCOCH_3$), 1.79 (2H, t, *J* = 6.8, H-3), 1.33 (6H, s, 2 × CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.3 (C, O<u>C</u>OCH_3), 154.5 (C, C-8a), 149.6 (C, C-7), 129.6 (CH, C-5), 118.4 (C, C-4a), 112.7 (CH, C-6), 110.2 (CH, C-8), 74.3 (C, C-2), 32.5 (CH_2, C-3), 26.8 (2 × CH_3), 22.0 (CH_2, C-4), 20.9 (CH_3, OCO<u>C</u>H_3);

EIMS *m/z* 220 (20%), 178 (55), 163 (20), 123 (100).

3.5.5 7-Acetoxy-2,2-dimethylchromene (41)



To a solution of **40** (52.0 mg, 0.236 mmol) in toluene (5.00 mL), was added DDQ (0.161 g, 0.708 mmol) under N_2. The mixture was refluxed for 24 h. The solid was filtered and the filtrate was extracted with EtOAc, washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude product was purified by column chromatography using Hex:EtOAc (8:2) to give chromene **41** as a yellow oil (40 mg, 78%).

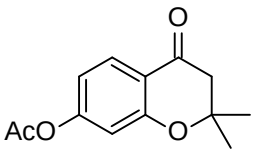
IR (neat) ν_{max} 2976, 1759, 1610, 1494, 1368, 1195, 1115, 990 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.94 (1H, d, *J* = 8.2 Hz, H-5), 6.57 (1H, dd, *J* = 2.3 and 8.2 Hz, H-6), 6.52 (1H, d, *J* = 2.3 Hz, H-8), 6.29 (1H, d, *J* = 10.0 Hz, H-4), 5.57 (1H, d, *J* = 10.0 Hz, H-3), 2.26 (3H, s, $OCOCH_3$), 1.43 (6H, s, 2 × CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.2 (C, O<u>C</u>OCH_3), 153.8 (C, C-8a), 151.1 (C, C-7), 130.1 (CH, C-3), 126.6 (CH, C-5), 121.6 (CH, C-4), 119.0 (C, C-4a), 113.6 (CH, C-6), 109.9 (CH, C-8), 76.5 (C, C-2), 28.1 (2 × CH_3), 21.1 (CH_3, OCO<u>C</u>H_3);

EIMS *m/z* 218 (10%), 203 (20), 161 (100).

3.5.6 7-Acetoxy-2,2-dimethylchroman-4-one (46)



CAN (0.248 g, 0.454 mmol) was added to a solution of chroman **40** (50.0 mg, 0.227 mmol) in CH_3CN/H_2O (3:1, 8 mL). The mixture was stirred for 3 h at rt, H_2O and EtOAc were added and the two phases were partitioned. The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude product was

purified by column chromatography using Hex:EtOAc (7:3) to give chromanone **46** as a white solid (43.6 mg, 82%). Recrystallisation of the solid from EtOH gave colourless crystals.

mp 78.3 – 79.8 ^{o}C;

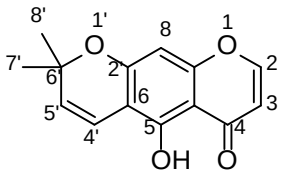
IR (neat) ν_{max} 2983, 1685, 1659, 1606, 1219, 1150, 772 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84 (1H, d, *J* = 8.5 Hz, H-5), 6.67-6.69 (2H, m, H-6 and H-8), 2.67 (2H, s, H-3), 2.26 (3H, s, $OCOCH_3$), 1.42 (6H, s, 2 × CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.3 (C, C-4), 168.5 (C, O<u>C</u>OCH_3), 161.0 (C, C-8a), 156.7 (C, C-7), 127.9 (CH, C-5), 118.0 (C, C-4a), 114.6 (CH, C-6), 111.2 (CH, C-8), 79.9 (C, C-2), 48.6 (CH_2, C-3), 26.6 (2 × CH_3), 21.1 (CH_3, OCO<u>C</u>H_3);

EIMS *m/z* 192 (42%), 177 (100), 137 (30), 108 (18).

3.5.7 5-Hydroxy-6',6'-dimethylpyrano[2',3':7,6]chromone (52)

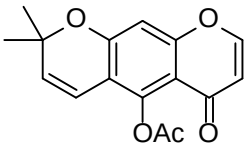


To a solution of **51** (50.2 mg, 0.282 mmol) in CH_3OH (15 mL) was added $Ca(OH)_2$ (42.0 mg, 0.564 mmol) followed by prenal (0.12 mL, 1.41 mmol). The mixture was stirred under an N_2 atmosphere for 3 days at rt, the CH_3OH evaporated and the reaction mixture diluted with EtOAc and 1 M HCl. The two phases were partitioned and the aqueous phase was extracted with EtOAc. The combined organic extracts were washed with H_2O and brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude product purified by column chromatography using Hex:EtOAc (3:2) to give **52** as a cream white solid (53.7 mg, 78%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.9 (1H, s, OH-5), 7.69 (1H, d, *J* = 6.0 Hz, H-2), 6.70 (1H, d, *J* = 10.0 Hz, H-4'), 6.30 (1H, s, H-8), 6.18 (1H, d, *J* = 6.0 Hz, H-3), 5.61 (1H, d, *J* = 10.0 Hz, H-5'), 1.47 (6H, s, 2 × CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.9 (C, C-4), 159.6 (C, C-7), 157.4 (C, C-8a), 156.5 (C, C-5), 155.4 (CH, C-2), 128.3 (CH, C-5'), 115.4 (CH, C-4'), 111.2 (CH, C-3), 106.6 (C, C-4a), 105.7 (C, C-6), 95.1 (CH, C-8), 78.1 (C, C-6'), 28.4 (2 × CH_3).

3.5.8 5-Acetoxy-6',6'-dimethylpyrano[2',3':7,6]chromone (53)



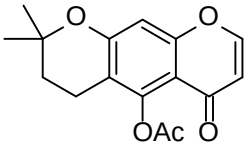
Compound **53** was prepared from **52** (50 mg, 0.205 mmol) according to the procedure given in **3.5.3**. The crude product was purified on a chromatotron using Hex:EtOAc (3:1) to give compound **53** (56.3 mg, 96%) as a colourless oil, which solidified on cooling.

IR (neat) ν_{max} 3064, 2989, 1765, 1640, 1620, 1609, 1293, 1190, 1114, 780 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (1H, d, *J* = 6.0 Hz, H-2), 6.68 (1H, s, H-8), 6.48 (1H, d, *J* = 10.0 Hz, H-4'), 6.10 (1H, d, *J* = 6.0 Hz, H-3), 5.75 (1H, d, *J* = 10.0 Hz, H-5'), 2.45 (3H, s, $OCOCH_3$), 1.47 (6H, s, 2 × CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.8 (C, C-4), 169.3 (C, O<u>C</u>OCH_3), 158.2 (C, C-8a), 157.8 (C, C-7), 153.5 (CH, C-2), 144.6 (C, C-5), 132.0 (CH, C-5'), 115.1 (CH, C-4'), 113.7 (CH, C-3), 113.0 (C, C-6), 112.1 (C, C-4a), 102.3 (CH, C-8), 78.1 (C, C-6'), 28.4 (2 × CH_3), 21.0 (CH_3, OCO<u>C</u>H_3).

3.5.9 5-Acetoxy-4',5'-dihydro-6',6'-dimethylpyrano[2',3':7,6]chromone (54)



To a solution of **53** (20.0 mg, 0.070 mmol) in EtOAc (15 mL) was added 10% Pd(C) (11.2 mg, 15 mol%). The mixture was purged with H_2 at 60 kPa with stirring for 15 min. The Pd(C) was filtered off and washed with EtOAc and the filtrate was evaporated to give a yellow oil. The oil was purified on a chromatotron using Hex:EtOAc (7:3) to give **54** as a white solid (17 mg 84%).

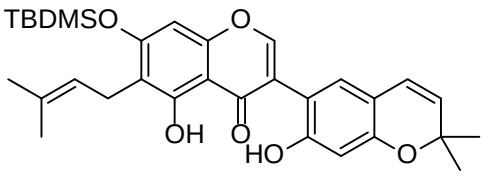
IR (neat) ν_{max} 2985, 2936, 1761, 1626, 1456, 1280, 1198, 1154, 1087 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (1H, d, *J* = 6.0 Hz, H-2), 6.71 (1H, s, H-8), 6.09 (1H, d, *J* = 6.0 Hz, H-3), 2.68 (2H, brs, H-4'), 2.44 (3H, s, $OCOCH_3$), 1.84 (2H, t, *J* = 6.9 Hz, H-5'), 1.36 (6H, s, 2 × CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.0 (C, C-4), 169.2 (C, O<u>C</u>OCH_3), 159.0 (C, C-7), 156.9 (C, C-8a), 153.6 (CH, C-2), 147.7 (C, C-5), 113.7 (C, C-6), 113.4 (CH, C-3), 111.3 (C, C-4a), 102.6 (CH, C-8), 76.2 (C, C-6'), 31.6 (CH_2, C-5'), 26.7 (2 × CH_3), 21.0 (CH_3, OCO<u>C</u>H_3), 16.9 (CH_2, C-4');

HR-ESIMS *m/z* $[M + Na]^+$ 311.0892 (Calcd. for $C_{16}H_{16}NaO_5$ 311.0895).

3.5.10 7-*tert*-Butyldimethylsilyloxy-2',5-dihydroxy-6-prenyl-[6'',6''-dimethylpyrano(2'',3'':4',5')]isoflavone (59).

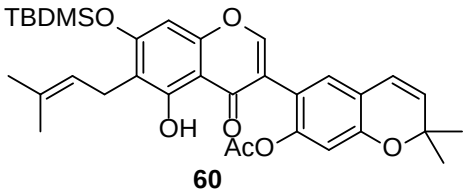


To a solution of kraussianone 2 (**2**) (50 mg, 0.119 mmol) in CH_2Cl_2 (15 mL) was added DIEA (0.02 mL, 0.119 mmol) and TBDMSCl (21.4 mg, 0.142 mmol) under N_2. The solution was stirred at rt for 3 h, H_2O added and the two phases partitioned. The aqueous phase was extracted with CH_2Cl_2 and the combined organic extracts were washed with H_2O, brine and dried over anhydrous $MgSO_4$. The crude product was purified on a chromatotron using Hex:EtOAc (4:1) to afford **59** (56.0 mg, 88%) as a yellow oil.

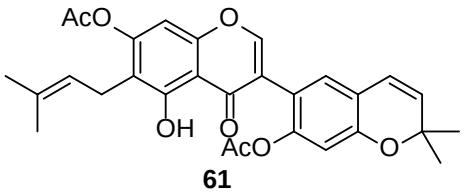
^{1}H NMR ($CDCl_3$, 400 MHz) 12.42 (1H, s, OH-5), 8.32 (1H, s, OH-2'), 7.95 (1H, s, H-2), 6.74 (1H, s, H-6'), 6.53 (1H, s, H-3'), 6.43 (1H, s, H-8), 6.28 (1H, d, *J* = 9.9 Hz, H-4"), 5.52 (1H, d, *J* = 9.9 Hz, H-5"), 5.17 (1H, t, *J* = 6.7 Hz, H-2'"), 3.38 (2H, d, *J* = 6.7 Hz, H-1'"), 1.78 (3H, s, H-4'" or H-5'"), 1.68 (3H, s, H-4'" or H-5'"), 1.44 (6H, s, H-7" and H-8"), 1.04 [9H, s, $SiC(CH_3)_3$], 0.33 [6H, s, $Si(CH_3)_2$]; (Plate 4a)

^{13}C NMR ($CDCl_3$,100 MHz) δ 180.1 (C, C-4), 161.0 (C, C-7), 159.5 (C-5), 157.2 (C, C-2'), 155.6 (C, C-4'), 155.5 (C, C-8a), 154.7 (CH, C-2), 132.2 (C, C-3'"), 128.7 (CH, C-5"), 127.1 (CH, C-6'), 123.1 (C, C-3), 121.9 (CH, C-2'"), 121.4 (CH, C-4"), 117.0 (C, C-6), 115.2 (C, C-5'), 112.3 (C, C-1'), 107.3 (CH, C-3'), 105.8 (C, C-4a), 96.7 (CH, C-8), 76.6 (C, C-6"), 28.1 (2 × CH_3, C-7" and C-8"), 25.7 (CH_3, C-4'" or C-5'"), 25.6 [3 × CH_3, $SiC(\underline{CH_3})_3$], 22.0 (CH_2, C-1'"), 18.4 [C, $Si\underline{C}(CH_3)_3$], 18.0 (CH_3, C-4'" or C-5'"), -4.1 [2 × CH_3, $Si(CH_3)_2$]. (Plate 4b)

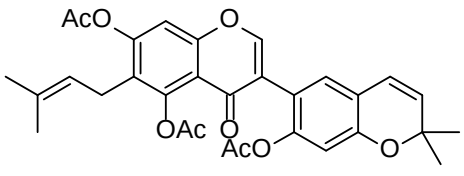
3.5.11 Acetylation of compound 59



60



61



62

Compound **59** (50 mg, 0.0935 mmol) was acetylated according to the procedure given in **3.5.3** to give compounds **60** (5.4 mg, 10%), **61** (10.8 mg 23%) and **62** (18.4 mg, 36%) after column chromatographic purification.

2'-Acetoxy-7-*tert*-butyldimethylsilyloxy-5-hydroxy-6-prenyl-[6",6"-dimethylpyrano(2",3":4',5')]isoflavone (**60**)

^{1}H NMR ($CDCl_3$, 400 MHz) 13.16 (1H, s, OH-5), 7.90 (1H, s, H-2), 6.95 (1H, s, H-6'), 6.72 (1H, s, H-3'), 6.38 (1H, s, H-8), 6.27 (1H, d, *J* = 9.8 Hz, H-4"), 5.50 (1H, d, *J* = 9.8 Hz, H-5"), 5.16 (1H, t, *J* = 6.8 Hz, H-2'"), 3.33 (2H, d, *J* = 6.8 Hz, H-1'"), 2.34 (3H, s, $COCH_3$), 1.78 (3H, s, H-4'" or H-5'"), 1.68 (3H, s, H-4'" or H-5'"), 1.44 (6H, s, H-7" and H-8"), 0.83 [9H, s, $SiC(CH_3)_3$], 0.13 [6H, s, $Si(CH_3)_2$].

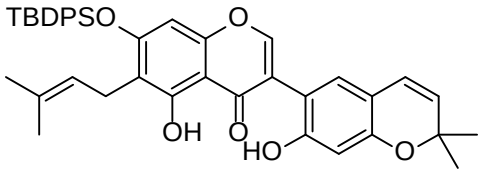
2',7-Diacetoxy-5-hydroxy-6-prenyl-[6",6"-dimethylpyrano(2",3":4',5')] isoflavone (**61**)

^{1}H NMR ($CDCl_3$, 400 MHz) 12.98 (1H, s, OH-5), 7.82 (1H, s, H-2), 6.91 (1H, s, H-6'), 6.71 (1H, s, H-3'), 6.64 (1H, s, H-8), 6.30 (1H, d, *J* = 9.8 Hz, H-4"), 5.61 (1H, d, *J* = 9.8 Hz, H-5"), 5.14 (1H, t, *J* = 7.0 Hz, H-2'"), 3.32 (2H, d, *J* = 7.0 Hz, H-1'"), 2.35 (3H, s, $COCH_3$), 2.14 (3H, s, $COCH_3$), 1.78 (3H, s, H-4'" or H-5'"), 1.68 (3H, s, H-4'" or H-5'"), 1.45 (6H, s, H-7" and H-8").

2',5,7-Triacetoxy-6-prenyl-[6",6"-dimethylpyrano(2",3":4',5')]isoflavone (**62**)

^{1}H NMR ($CDCl_3$, 400 MHz) 7.75 (1H, s, H-2), 7.19 (1H, s, H-6'), 6.85 (1H, s, H-3'), 6.63 (1H, s, H-8), 6.27 (1H, d, *J* = 9.8 Hz, H-4"), 5.58 (1H, d, *J* = 9.8 Hz, H-5"), 5.02 (1H, t, *J* = 6.7 Hz, H-2'"), 3.29 (2H, d, *J* = 6.7 Hz, H-1'"), 2.40 (3H, s, $COCH_3$), 2.35 (3H, s, $COCH_3$), 2.13 (3H, s, $COCH_3$), 1.74 (3H, s, H-4'" or H-5'"), 1.67 (3H, s, H-4'" or H-5'"), 1.44 (6H, s, H-7" and H-8").

3.5.12 7-*tert*-Butyldiphenylsilyloxy-2',5-dihydroxy-6-prenyl[6'',6''-dimethylpyrano(2'',3'':4',5')]isoflavone (63)



To a solution of **2** (50 mg, 0.119 mmol) in CH_2Cl_2 (15 mL) was added DIEA (0.024 mL, 0.142 mmol) and TBDPSCl (0.033 mL, 0.142 mmol) under N_2. The solution was stirred at rt for 3 h. H_2O was added and the two phases were partitioned. The aqueous phase was extracted with CH_2Cl_2 and the combined organic extracts were washed with H_2O, brine and dried over anhydrous $MgSO_4$. The crude product was purified on a chromatotron using Hex:ether (4:1) to afford **63** (72 mg, 92%) as a yellow solid.

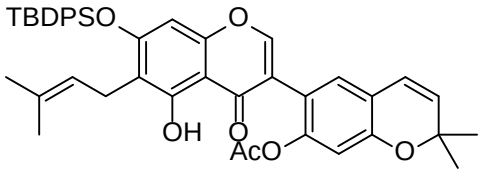
IR (neat) ν_{max} 2931, 2859, 1649, 1639, 1574, 1472, 1108, 820 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) 12.46 (1H, s, OH-5), 8.26 (1H, s, OH-2'), 7.75-7.74 (5H, m, H-2, Si$\underline{Ph_2}$), 7.50-7.40 (6H, m, Si$\underline{Ph_2}$), 6.64 (1H, s, H-6'), 6.49 (1H, s, H-3'), 6.22 (1H, d, *J* = 9.8 Hz, H-4''), 6.02 (1H, s, H-8), 5.49 (1H, d, *J* = 9.8 Hz, H-5''), 5.33 (1H, t, *J* = 6.6 Hz, H-2'''), 3.60 (2H, d, *J* = 6.7 Hz, H-1'''), 1.85 (3H, s, H-4''' or H-5'''), 1.73 (3H, s, H-4''' or H-5'''), 1.42 (6H, s, H-7'' and H-8''), 1.13 [9H, s, $SiC(CH_3)_3$]; (Plate 5a)

^{13}C NMR ($CDCl_3$,100 MHz) δ 182.0 (C, C-4), 160.6 (C, C-7), 159.3 (C-5), 157.2 (C, C-2'), 155.6 (C, C-4'), 155.1 (C, C-8a), 154.7 (CH, C-2), 135.3 (4 × CH, Si$\underline{Ph_2}$), 132.2 (C, C-3'''), 131.5 (2 × C, Si$\underline{Ph_2}$), 130.4 (2 × CH, Si$\underline{Ph_2}$), 128.7 (CH, C-5''), 128.1 (4 × CH, Si$\underline{Ph_2}$), 127.1 (CH, C-6'), 123.0 (C, C-3), 122.0 (CH, C-2'''), 121.3 (CH, C-4''), 116.6 (C, C-6), 115.2 (C, C-5'), 112.3 (C, C-1'), 107.3 (CH, C-3'), 105.8 (C, C-4a), 97.6 (CH, C-8), 76.6 (C, C-6''), 28.1 (2 × CH_3, C-7'' and C-8''), 26.3 [3 × CH_3, SiC($\underline{CH_3}$)$_3$], 25.8 (CH_3, C-4''' or C-5'''), 22.0 (CH_2, C-1'''), 19.5 [C, Si$\underline{C}(CH_3)_3$], 18.0 (CH_3, C-4''' or C-5'''); (Plate 5b)

HR-ESIMS *m/z* $[M+H]^+$ 659.2830 (Calcd. for $C_{41}H_{43}O_6Si$ 659.2829).

3.5.13 2'-Acetoxy-7-*tert*-butyldiphenylsilyloxy-5-hydroxy-6-prenyl[6'',6''-dimethylpyrano(2'',3'':4',5')]isoflavone (64)



Compound **63** (65.0 mg, 0.0987 mmol) was acetylated according to the procedure given in **3.5.3** to afford **64** (61.5 mg, 89%) as a white solid after purification on a chromatotron using Hex:ether (4:1).

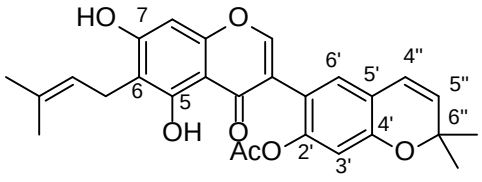
IR (neat) ν_{max} 2932, 2854, 1757, 1651, 1469, 1281, 1887, 1107, 824, 701 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) 12.85 (1H, s, OH-5), 7.74 (4H, dd, *J* = 8.0 and 1.5 Hz, Si$\underline{Ph_2}$), 7.54 (1H, s, H-2), 7.50-7.40 (6H, m, Si$\underline{Ph_2}$), 6.83 (1H, s, H-6'), 6.59 (1H, s, H-3'), 6.25 (1H, d, *J* = 9.8 Hz, H-4''), 5.95 (1H, s, H-8), 5.58 (1H, d, *J* = 9.8 Hz, H-5''), 5.34 (1H, t, *J* = 6.5 Hz, H-2'''), 3.58 (2H, d, *J* = 6.5 Hz, H-1'''), 2.09 (3H, s, $COCH_3$), 1.83 (3H, s, H-4''' or H-5'''), 1.72 (3H, s, H-4''' or H-5'''), 1.42 (6H, s, H-7'' and H-8''), 1.12 [9H, s, $SiC(CH_3)_3$]; (Plate 6a)

^{13}C NMR ($CDCl_3$,100 MHz) δ 180.2 (C, C-4), 169.0 (C, $\underline{C}OCH_3$), 159.7 (C, C-7), 159.5 (C-5), 155.1 (C, C-8a), 154.1 (C, C-4'), 153.8 (CH, C-2), 149.3 (C, C-2'), 135.3 (4 × CH, Si$\underline{Ph_2}$), 132.4 (C, C-3'''), 131.7 (2 × C, Si$\underline{Ph_2}$), 130.5 (CH, C-5''), 130.3 (2 × CH, Si$\underline{Ph_2}$), 128.7 (CH, C-6'), 128.1 (4 × CH, Si$\underline{Ph_2}$), 122.4 (CH, C-2'''), 121.2 (CH, C-4''), 120.3 (C, C-3), 119.2 (C, C-5'), 115.89 (2 × C, C-6, C-1'), 111.1 (CH, C-3'), 106.2 (C, C-4a), 97.6 (CH, C-8), 76.9 (C, C-6''), 28.3 (2 × CH_3, C-7'' and C-8''), 26.3 [3 × CH_3, SiC($\underline{CH_3}$)$_3$], 25.8 (CH_3, C-4''' or C-5'''), 22.0 (CH_2, C-1'''), 21.0 (CH_3, CO$\underline{CH_3}$), 19.5 [C, Si$\underline{C}(CH_3)_3$], 18.0 (CH_3, C-4''' or C-5'''); (Plate 6b)

HR-ESIMS *m/z* $[M+Na]^+$ 723.2740 (Calcd. for $C_{43}H_{44}O_7NaSi$ 723.2754).

3.5.14 2'-Acetoxy-5,7-dihydroxy-6-prenyl[6'',6''-dimethylpyrano (2'',3'':4',5')]isoflavone (65)



To a stirred solution of **64** (50 mg, 0.0713 mmol) in CH_3CN (5 mL) was added CsF (21.7 mg, 0.143 mmol). The mixture was stirred at rt for 12 h and then diluted with H_2O and EtOAc. The two phases were partitioned and the aqueous phase was extracted with EtOAc. The organic layers were combined, washed with H_2O, brine and dried over anhydrous $MgSO_4$. The crude product was purified on a chromatotron using Hex:EtOAc (3:2) for afford **65** as a cream white solid (28.7 mg, 87%).

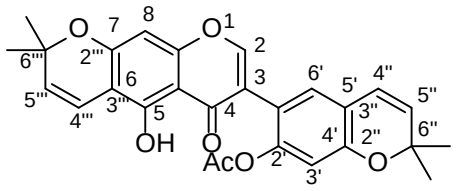
IR (neat) ν_{max} 3208, 2971, 2925, 2950,1762, 1641, 1281, 1196, 1130 cm^{-1};

^1H NMR ($CDCl_3$, 400 MHz) 13.08 (1H, s, OH-5), 7.82 (1H, s, H-2), 6.92 (1H, s, H-6'), 6.64 (1H, s, H-3'), 6.38 (1H, s, H-8), 6.29 (1H, d, J = 9.9 Hz, H-4''), 5.60 (1H, d, J = 9.9 Hz, H-5''), 5.28 (1H, t, J = 7.0 Hz, H-2'''), 3.46 (2H, d, J = 7.0 Hz, H-1'''), 2.12 (3H, s, $COCH_3$), 1.84 (3H, s, H-4''' or H-5'''), 1.78 (3H, s, H-4''' or H-5'''), 1.45 (6H, s, H-7'' and H-8''); (plate 7a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.3 (C, C-4), 169.1 (C, $\underline{C}OCH_3$), 161.5 (C, C-7), 159.6 (C-5), 156.2 (C, C-8a), 154.2 (C, C-4'), 154.0 (CH, C-2), 149.4 (C, C-2'), 136.2 (C, C-3'''), 130.6 (CH, C-5''), 128.7 (CH, C-6'), 121.2 (CH, C-4''), 121.0 (CH, C-2'''), 120.4 (C, C-3), 119.3 (C, C-5'), 115.8 (C, C-1'), 111.2 (CH, C-3'), 110.1 (C, C-6), 105.6 (C, C-4a), 94.1 (CH, C-8), 77.2 (C, C-6''), 28.3 (2 × CH_3, C-7'' and C-8''), 25.8 (CH_3, C-4''' or C-5'''), 21.5 (CH_2, C-1'''), 21.0 (CH_3, CO$\underline{C}H_3$), 17.9 (CH_3, C-4''' or C-5'''); (Plate 7b)

HR-ESIMS m/z $[M+Na]^+$ 485.1570 (Calcd. for $C_{27}H_{26}O_7NaSi$ 485.1576).

3.5.15 2'-Acetoxy-5-hydroxy-[6'',6''-dimethylpyrano(2'',3'':4',5')][6''',6'''-dimethylpyrano(2''',3''':7,6)]isoflavone (66)



DDQ (17.7 mg, 0.0778 mmol) was added to a solution of **65** (30.0 mg, 0.0649 mmol) in toluene (20 mL). The mixture was refluxed under an N_2 atmosphere for 4 h. A solution of 10% $NaHCO_3$ was added and the mixture was extracted with EtOAc. The organic layer was washed with H_2O, brine and dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography using Hex:EtOAc (7:3) to afford **66** as a cream white solid (23.6 mg, 79%)

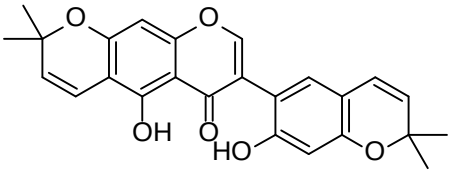
IR (neat) ν_{max} 2975, 2919, 1764, 1650, 1617, 1184, 1130 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.00 (1H, s, OH-5), 7.73 (1H, s, H-2), 6.92 (1H, s, H-6'), 6.71 (1H, d, *J* = 10.0 Hz, H-4'''), 6.64(1H, s, H-3'), 6.33 (1H, s, H-8), 6.29 (1H, d, *J* = 9.8 Hz, H-4''), 5.62 (1H, d, *J* = 10.0 Hz, H-5'''), 5.60 (1H, d, *J* = 9.8 Hz, H-5''), 2.13 (3H, s, $COCH_3$), 1.47 (6H, s, H-7''' and H-8'''), 1.45 (6H, s, H-7'' and H-8''); (Plate 8a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.3 (C, C-4), 169.0 (C, $\underline{C}OCH_3$), 159.6 (C, C-7), 157.3 (C-8a), 156.8 (C, C-5), 154.2 (C, C-4'), 153.9 (CH, C-2), 149.3 (C, C-2'), 130.6 (CH, C-5''), 128.6 (CH, C-6'), 128.2 (CH, C-5'''), 121.2 (CH, C-4''), 120.6 (C, C-3), 119.3 (C, C-5'), 115.7 (C, C-1'), 115.4 (CH , C-4'''), 111.2 (CH, C-3'), 105.8 (C, C-4a), 105.7 (C, C-6), 94.9 (CH, C-8), 78.1 (C, C-6'''), 77.2 (C, C-6''), 28.3 (4 × CH_3, C-7''', C-8''', C-7'' and C-8''), 21.0 (CH_3, $CO\underline{CH}_3$); (Plate 8a)

HR-ESIMS *m/z* $[M+Na]^+$ 483.1404 (Calcd. for $C_{27}H_{24}O_7NaSi$ 483.1420).

3.5.16 Kraussianone 1 (1)



To a solution of **66** (15 mg, 0.0326 mmol) in CH_3OH (10 mL) was added NaOH (2 mL, 2 M). The solution was stirred at rt for 3 h. CH_3OH was evaporated and crude mixture was diluted with DCM and HCl (10 mL, 1M). The two phases were partitioned and the aqueous phase was back-extracted with DCM. The combined organic extracts were washed with H_2O, brine and dried over anhydrous $MgSO_4$. The product was purified on a chromatotron using Hex:EtOAc (4:1) to give **1** as a yellow solid (13.1 mg, 96%).

IR (KBr) ν_{max} 3336, 2975, 2872, 1649, 1615, 1460, 1132, 773 cm^{-1};

^1H NMR ($CDCl_3$, 400 MHz) δ 12.55 (1H, s, OH-5), 8.27 (1H, s, OH-2'), 7.73 (1H, s, H-2), 6.75 (1H, s, H-6'), 6.74 (1H, d, *J* = 10.0 Hz, H-4'''), 6.53 (1H, s, H-3'), 6.40 (1H, s, H-8), 6.28 (1H, d, *J* = 9.8 Hz, H-4''), 5.65 (1H, d, *J* = 10.0 Hz, H-5'''), 5.52 (1H, d, *J* = 9.8 Hz, H-5''), 1.49 (6H, s, H-7''' and H-8'''), 1.44 (6H, s, H-7'' and H-8''); (Plate 9a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.0 (C, C-4), 160.4 (C, C-7), 157.2 (C, C-2'), 157.1 (C-8a), 156.4 (C, C-5), 155.7 (C, C-4'), 154.7 (CH, C-2), 128.8 (CH, C-5''), 128.6 (CH, C-5'''), 127.1 (CH, C-6'), 123.1 (C, C-3), 121.3 (CH, C-4''), 115.28 (CH and C, C-4''' and C-5'), 112.1 (C, C-1'), 107.3 (CH, C-3'), 106.1 (C, C-6), 105.5 (C, C-4a), 95.0 (CH, C-8), 78.5 (C, C-6'''), 77.2 (C, C-6''), 28.4 (2 × CH_3, C-7''' and C-8'''), 28.2 (2 × CH_3, C-7'' and C-8''); (Plate 9b)

HR-ESIMS *m*/*z* $[M + Na]^+$ 441.1317 (Calcd. for $C_{25}H_{22}NaO_6$ 441.1314).

3.5. REFERENCES

(1) Drewes, S. E.; Horn, M. M.; Munro, O. Q.; Dhlamini, J. T. B.; Meyer, J. J. M.; Rakuambo, N. C. *Phytochemistry* **2002**, *59*, 739-747.

(2) Drewes, S. E.; Horn, M. M.; Khan, F.; Munro, O. Q.; Dhlamini, J. T. B.; Rakuambo, C.; Meyer, J. J. M. *Phytochemistry* **2004**, *65*, 1955-1961.

(3) Newman, D. J. *Journal of Medicinal Chemistry* **2008**, *51*, 2589-2599.

(4) Nicolaou, K. C.; Pfefferkorn, J. A.; Barluenga, S.; Mitchell, H. J.; Roecker, A. J.; Cao, G. Q. *Journal of the American Chemical Society* **2000**, *122*, 9968-9976.

(5) Nicolaou, K. C.; Pfefferkorn, J. A.; Mitchell, H. J.; Roecker, A. J.; Barluenga, S.; Cao, G. Q.; Affleck, R. L.; Lillig, J. E. *Journal of the American Chemical Society* **2000**, *122*, 9954-9967.

(6) Dewick, P. M. *Medicinal Natural Products-A Biosynthetic Approach*; Second ed.; John Wiley and Sons: NewYork, 2001.

(7) Tahara, S.; Ibrahim, R. K. *Phytochemistry* **1995**, *38*, 1073-1094.

(8) Crombie, L. *Natural Product Reports* **1984**, *1*, 3-19.

(9) Bhandari, P.; Vanbruggen, N.; Crombie, L.; Whiting, D. A. *Journal of the Chemical Society, Chemical Communications* **1989**, 982-984.

(10) Bhandari, P.; Crombie, L.; Harper, M. F.; Rossiter, J. T.; Sanders, M.; Whiting, D. A. *Journal of the Chemical Society, Perkin Transactions 1* **1992**, 1685-1697.

(11) Narkhede, D.; Iyer, R. R.; Iyer, C. S. R. *Tetrahedron* **1990**, *46*, 2031 - 2034.

(12) North, J. T.; Kronenthal, D. R.; Pullockaran, A. J.; Real, D. S.; Chen, H. Y. *The Journal of Organic Chemistry* **1995**, *60*, 3397 - 3400.

(13) Subburaj, K.; Murugesh, G. M.; Trivedi, G. K. *Journal of the Chemical Society, Perkin Transactions I* **1997**, 1875 - 1878.

(14) Lee, R. Y.; Wang, X.; Xia, L. *Molecules* **2007**, *12*, 1420 - 1429.

(15) Mondal, M.; Puranik, G. V.; Argade, N. P. *The Journal of Organic Chemistry* **2006**, *71*, 4992-4995.

(16) Khupse, R. S.; Erhardt, P. W. *Organic Letters* **2008**, *10*, 5007-5010.

(17) Jeso, V.; Nicolaou, K. C. *Tetrahedron Letters* **2009**, *50*, 1161-1163.

(18) Bandaranayake, W. M.; Crombie, L.; Whiting, D. A. *Journal of the Chemical Society (C)* **1971**, 811-816.

(19) Harfenist, M.; Thom, E. *The Journal of Organic Chemistry* **1972**, *37*, 841-848.

(20) Sittisombut, C.; Boutenouchet, S.; Van-Dufat, H. T.; Tian, W.; Michel, S.; Koch, M.; Tillequin, F.; Pfeiffer, B.; Pierre, A. *Chemical Pharmaceutical Bulletin* **2006**, *54*, 1113-1118

(21) Camps, F.; Coll, J.; Messeguer, A.; Pericas, M. A.; Ricart, S. *Synthesis* **1980**, 725-728.

(22) Lichtenfels, A. R.; Coelho, A. L.; Costa, R. R. *Journal of Chemical Society, Perkin Transactions 1* **1995**, 949 - 951.

(23) Lim, J.; Kim, H.-I.; Kim, H. H.; Ahn, K.-S.; Han, H. *Tetrahedron Letters* **2001**, *42*, 4001-4003.

(24) Yang, J. H.; Zhao, Y. M.; Ji, C. B. *Chinese Chemical Letters* **2008**, *19*, 658-660.

(25) Tan, W. F.; Li, W. D. Z.; Li, Y. L. *Synthetic Communications* **2002**, *32*, 1077-1083.

(26) Larock, C. R.; Wei, L.; Hightower, R. T. *Synlett* **1998**, 522-524.

(27) Li, N. G.; You, Q. D.; Huang, X. F.; Wang, J. X.; Guo, Q. L.; Chen, X. G.; Li, Y.; Li, H. Y. *Chinese Chemical Letters* **2007**, *18*, 659-662.

(28) Jain, A. C.; Kumar, A.; Gupta, C. R. *Journal of Chemical Society, Perkin Transactions 1* **1979**, 279-282.

(29) Gester, S.; Metz, P.; Zierau, O.; Vollmer, G. *Tetrahedron* **2001**, *57*, 1015-1018.

(30) Vogel, S.; Ohmayer, S.; Brunner, G.; Heilmann, J. *Bioorganic and Medicinal Chemistry* **2008**, *16*, 4286-4293.

(31) Tsukayama, M.; Li, H.; Tsurumoto, K.; Nishiuchi, M.; Kawamura, Y. *Bulletin of the Chemical Society of Japan* **1998**, *71*, 2673-2680.

(32) Gerbino, D. C.; Mandolesi, S. D.; Schmalz, H.-G.; Podestá, J. C. *European Journal of Organic Chemistry* **1999**, 3964-3972.

(33) Dewick, P. M. In *The Flavonoids: Advances in Research Since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994, p 117-238.

(34) Narender, T.; Reddy, K. P.; Kumar, B. *Tetrahedron Letters* **2008**, *49*, 4409-4415.

(35) Vogel, S.; Heilmann, J. *Journal of Natural Products* **2008**, *71*, 1237-1241.

(36) Macone, A.; Lendaro, E.; Comandini, A.; Rovardi, I.; Matarese, R. M.; Carraturo, A.; Bonamore, A. *Bioorganic and Medicinal Chemistry* **2009**, *17*, 6003-6007.

(37) Rao, G. V.; Swamy, B. N.; Chandregowda, V.; Reddy, G. C. *European Journal of Medicinal Chemistry* **2009**, *44*, 2239-2245.

(38) Miranda, M. A.; Primo, J.; Tormos, R. *Tetrahedron* **1989**, *45*, 7593-7600.

(39) Patel, A.; Netscher, T.; Gille, L.; Mereiter, K.; Rosenau, T. *Tetrahedron* **2007**, *63*, 5312-5318.

(40) Kanvinde, M. N.; Kulkarni, S. A.; Paradkar, M. V. *Synthetic Communications* **1990**, *20*, 3259-3264.

(41) Wang, Q. L.; She, X. G.; Ren, X. F.; Ma, J. Y.; Pan, X. F. *Tetrahedron-Asymmetry* **2004**, *15*, 29-34.

(42) Chen, L. Y.; Li, S. R.; Chen, P. Y.; Chang, H. C.; Wang, T. P.; Tsai, I. L.; Wang, E. C. *Arkivoc* **2010**, 64-76.

(43) Nicolaou, K. C.; Baran, P. S.; Zhong, Y. L. *Journal of the American Chemical Society* **2001**, *123*, 3183-3185.

(44) Doyle, M. P.; Zuidema, L. J.; Bade, T. R. *The Journal of Organic Chemistry* **1975**, *40*, 1454-1456.

(45) Baciocchi, E.; Rol, C.; Mandolini, L. *The Journal of Organic Chemistry* **1977**, *42*, 3682-3686.

(46) Dinctürk, S.; Ridd, J. H. *Journal of the Chemical Society, Perkin Transactions 2* **1982**, 961-964.

(47) Dinctürk, S.; Ridd, J. H. *Journal of the Chemical Society, Perkin Transactions 2* **1982**, 965-969.

(48) Mellor, J. M.; Mittoo, S.; Parkes, R.; Millar, R. W. *Tetrahedron* **2000**, *56*, 8019-8024.

(49) Wuts, P. G. M.; Greene, T. W. *Protective Groups in Organic Synthesis* 4th ed.; John Wiley & Sons: New Jersey, 2007.

CHAPTER 4

TOTAL SYNTHESIS OF THE PYRANOISOFLAVONE KRAUSSIANONE 1 AND RELATED ISOFLAVONES

4.1 INTRODUCTION

Pyranoisoflavones have been isolated from different plants, mostly belonging to the Leguminosae.[1-4] This class of compounds has been reported to possess biological activities such as cytotoxicity against cancer cell lines, hypertensive, hypoglyceamic, antifungal, antiplasmodial, anti-inflammatory, estrogenic and antiestrogenic activities.[2-4] The pyranoisoflavones are believed to originate biosynthetically from the enzyme-catalysed cyclisation of a prenyl group with the adjacent hydroxy group of the isoflavone.[5] Prenylation can occur after the isoflavone skeleton has been assembled or during the early stages in the biosynthetic pathway.[5,6] The early formation of the dimethylpyran ring is in part supported by the prevalence of prenylated chalcones and flavanones, which are biosynthetic precursors of the isoflavones.[6]

Most synthetic routes for the pyranoisoflavones followed either the deoxybenzoin or the chalcone route for the construction of the isoflavone core and a number of methods have been followed for the synthesis of the dimethylpyran moeity.[7-12] More recently, Zheng and Shen synthesised a pyranoisoflavone as precursor to hirtellanine A by the Suzuki-Miyaura reaction.[13] Poor regioselectivity in the introduction of the dimethylpyran ring, which often resulted in the formation of both the linear and angular isomers of the pyranoisoflavones, has been a problem in most synthetic routes.[7-12]

In this Chapter, the methods for the synthesis of the isoflavone skeleton are reviewed, followed by the discussion of the results on the synthesis of the pyranoisoflavone kraussianone 1 (**1**) and related isoflavones. The conclusions drawn from the discussions and the experimental procedures are given in the last sections of the chapter.

4.2 AN OVERVIEW OF SYNTHETIC METHODS FOR THE SYNTHESIS OF ISOFLAVONES

As a result of the wide range of important biological activities exhibited by the isoflavones, many synthetic routes towards these compounds have been investigated since the 1950's.[14] New methods are still being developed in a quest for efficient methodologies that will tolerate substitution patterns found in most naturally-occurring isoflavones. There are two long-established procedures for the preparation of isoflavones which are still widely used. These are the deoxybenzoin and chalcone routes.[1,14-17] Other methods include the Suzuki-Miyaura coupling of halochromones with arylboronic acids, reductive cleavage of isoxazoles, intramolecular ketene cycloaddition followed by decarboxylation, rearrangement and cyclisation of chalcone epoxides, rearrangement of flavanones and many others.[1] The methods that have been developed more recently, are the Wacker-Cook tandem conversion of α-methylene deoxybenzoins into isoflavones and the Cu(I)-mediated cyclisation of 3-(2-bromophenyl)-3-oxopropanal.[18,19] Regardless of the many new synthetic approaches presented, the general applicability of most of them has not been demonstrated. This is particularly the case in the synthesis of polyhydroxyisoflavones and isoflavones bearing other naturally-occurring substitution patterns.

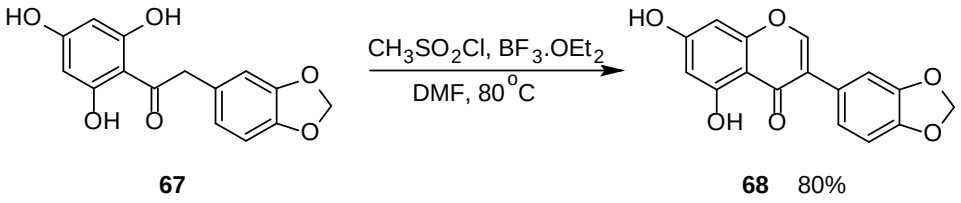
The subsections that follow will review the methods that are widely employed for the synthesis of isoflavones, i.e. the deoxybenzoin and chalcone routes. The synthesis of isoflavones by the Suzuki-Miyaura coupling, which is now gaining acceptance, and the newly developed methods, the Wacker-Cook and the Cu(I) catalysed intramolecular cyclisation of oxophenylpropanal, will also be reviewed.

4.2.1 The Deoxybenzoin Route

The deoxybenzoin method involves formylation and subsequent cyclisation of 2-hydroxyphenyl benzyl ketone (deoxybenzoin) with a reagent containing an activated C-1 unit. Initial procedures employed reagents such as ethyl formate and sodium, ethyl orthoformate with pyridine or piperidine, and ethoxalyl chloride with pyridine. The two former conditions were reported to give poor yields of isoflavones bearing a phloroglucinol nucleus, thus the ethoxalyl chloride procedure was developed to overcome this

shortcoming. However, ethoxalyl chloride gives 2-carboethoxyisoflavone, which requires subsequent decarboxylation.[14,15]

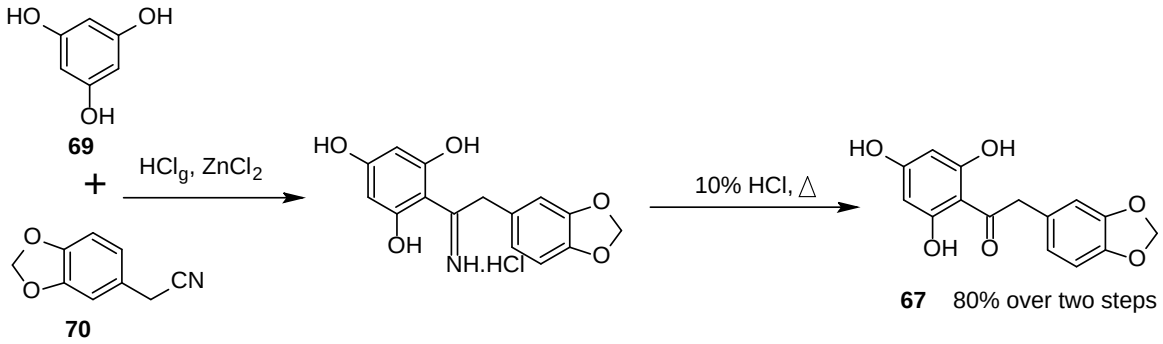
Pelter and Foot later developed a formylation procedure for large-scale synthesis, employing DMF-DMA in benzene or DMF at high temperatures.[20] Again, with this method, very low yields were reported with benzyl ketones containing a phloroglucinol moiety.[20,21] Bredereck's reagent, *bis*(dimethylamino)-*t*-butoxymethane [$HC[N(CH_3)_2]_2O$-^tBu] was employed for the synthesis of calopogonium isoflavone-B and jamaicin.[9,1] DMF has also been used as a source of an activated C-1 unit in conjunction with the Lewis acids $BF_3.OEt_2$ and CH_3SO_2Cl.[22] A number of polyhydroxyisoflavones have been successfully synthesised under these conditions at higher temperatures or under microwave irradiation.[23-25] The application of this route is demonstrated in Scheme 4.1 in the synthesis of the derrubone precursor, 5,7-dihydroxy-3',4'-methylenedioxyisoflavone (**68**).[23]



67 **68** 80%

Scheme 4.1. Preparation of an isoflavone by the deoxybenzoin route

The benzyl ketone **67** was synthesised from phloroglucinol (**69**) and phenylacetonitrile **70** by the Houben-Hoesch reaction using $ZnCl_2$ and gaseous HCl. Hydrolysis of the resulting iminium chloride gave compound **67** in good yields, Scheme 4.2.[23,24,26]



Scheme 4.2. Preparation of a deoxybenzoin by the Houben-Hoesch reaction

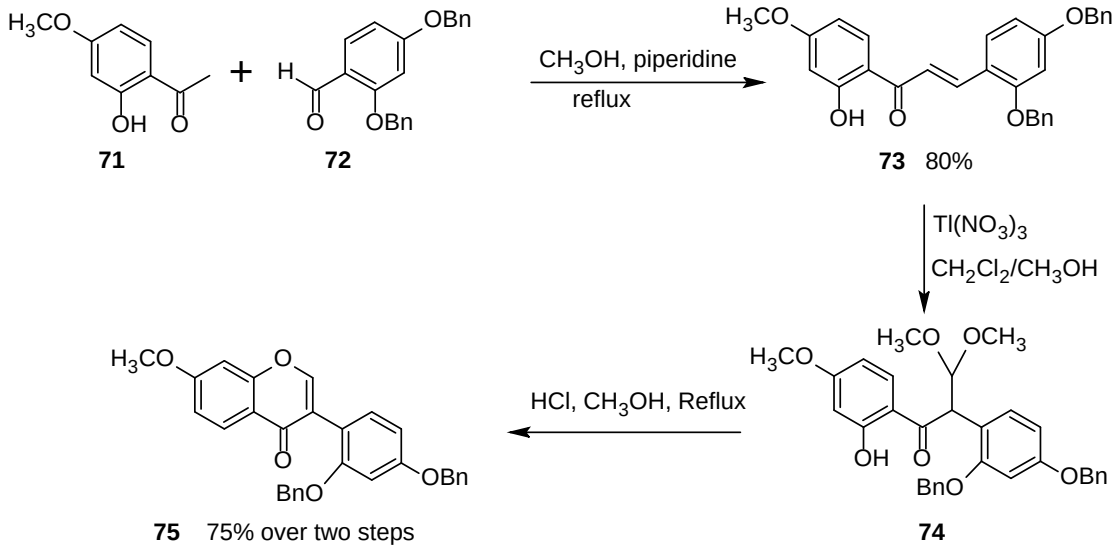
Alternatively, the deoxybenzoin can be synthesised by Friedel-Crafts acylation of an appropriately substituted phenol with a phenylacetic acid or acyl chloride. This condensation is facilitated by Lewis acids such as $AlCl_3$, $TiCl_4$ or $BF_3.Et_2O$.[1,21,22,24,27,28]

A proper choice of the reagents and conditions for the preparation of isoflavones via the deoxybenzoin route allows for the preparation of polyhydroxyisoflavones without prior protection of the free hydroxy groups.[21-23] This eliminates the protection and deprotection steps often required when other synthetic methods are employed. However, sensitive substituents such as dimethylchromenes and prenyls do not survive the harsh conditions employed for the preparation of the deoxybenzoin via Friedel-Crafts acylation and Houben-Hoesch reaction.[29] As a result such functional groups are often incorporated upon complete construction of the isoflavone skeleton, making it difficult to control the regiochemistry of the final compound.[7,23]

4.2.2 The Chalcone Route

Prior to 1974, oxidative rearrangement of chalcones with TTN in CH_3OH was the most widely used method for the synthesis of isoflavones.[16] However, the use of TTN made the method unattractive due to its high cost, toxicity and adverse environmental effects.[30,31] Some environmentally friendly and less toxic hypervalent iodine reagents have been developed to facilitate the important aryl shift. They include phenyliodine(III) bis(trifluoroacetate), (diacetoxyiodo)benzene/*p*-toluenesulfonic acid, [hydroxy(tosyloxy) iodo]benzene and [bis(trifluoroacetoxy)iodo]benzene.[17,30,31] Unlike deoxybenzoins, chalcones can be readily prepared by a Claisen-Schmidt condensation of a benzylaldehyde and an acetophenone. Good yields of the chalcones have been reported when the reaction is performed in an alcoholic KOH solution or in piperidine and anhydrous CH_3OH.[32]

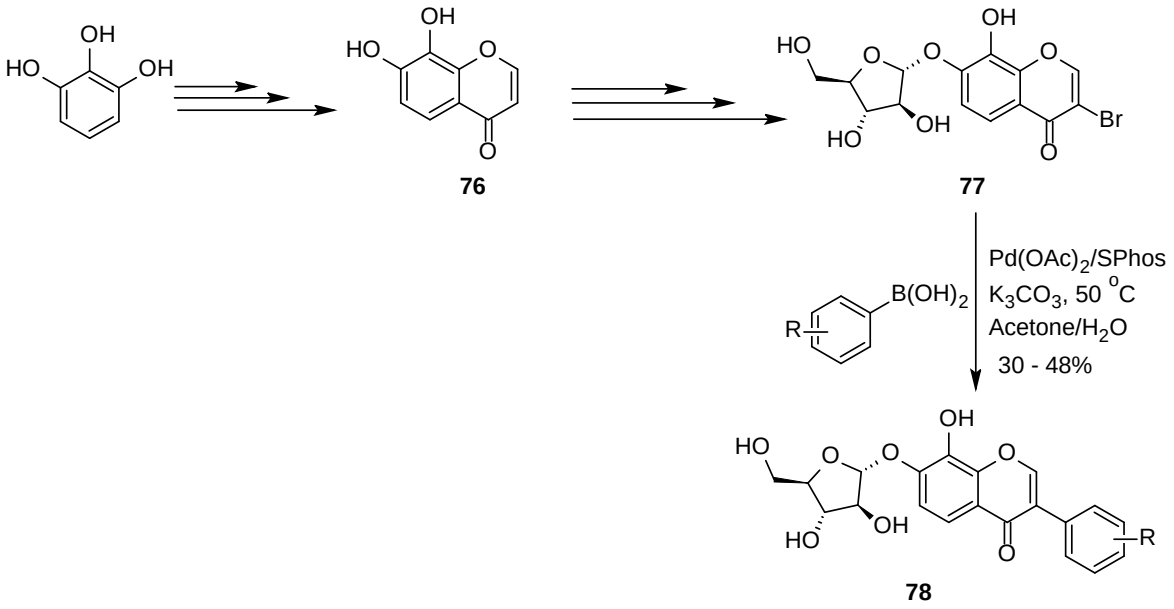
Scheme 4.3 shows the synthesis of 2',4'-dibenzyloxy-7-methoxyisoflavone (**75**) by the oxidative rearrangement of chalcone **73**. The chalcone **73** was obtained by condensation of acetophenone **71** and benzaldehyde **72** in dry CH_3OH using piperidine as the base. Treatment of the chalcone **73** with TTN gave the intermediate acetal **74**, which was cyclised to the isoflavone **75**.[32]



Scheme 4.3. Synthesis of isoflavone **75** by the oxidative rearrangement of chalcone **73**

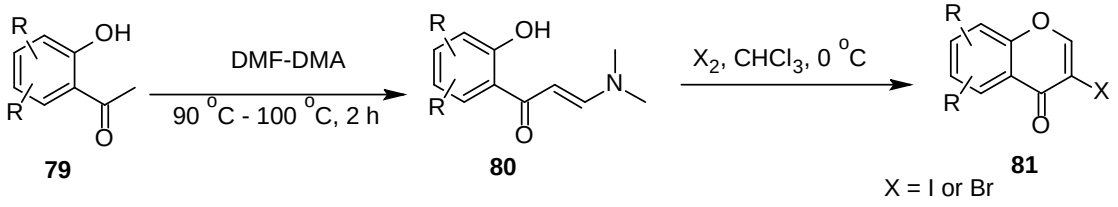
4.2.3 The Suzuki-Miyaura Cross Coupling

In 1988, Suzuki *et al*. demonstrasted the versatility of the palladium-catalysed cross-coupling reaction for the synthesis of isoflavones from 3-bromochromones and arylboronic acids.[33] Following this, the Suzuki-Miyaura cross-coupling reaction has been applied in several instances for the synthesis of isoflavones in the presence of palladium(0) or palladium(II) catalysts.[34-38] Examples of catalysts which have successfully facilitated this C-C bond formation are $Pd(PPh_3)_4$, Pd(C), trans-[$PdCl_2$(2-ethyl-2-oxazoline-κ^1N)$_2$], benzothiozole-oxime-based Pd(II) catalyst, and $Pd(OAc)_2$ in the presence of 2-(2,6-dimethoxybiphenyl)dicyclohexylphosphane (SPhos) as a ligand.[34-38] The mild reaction conditions employed by the Suzuki-Miyaura coupling have the advantage of the reaction occurring in the presence of sensitive substituents found in most isoflavones. Moreover, a large number of isoflavones can be synthesised from a 3-halochromone precursor by varying the substituents on the boronic acid partner. This has been well demonstrated by Wei and Yu in the synthesis of a number of isoflavone glycoside derivatives (Scheme 4.4).[39]



Scheme 4.4. Synthesis of isoflavone glycosides by the Suzuki-Miyaura reaction

In this sequence, a 3-bromochromone **77** was coupled to different aryl boronic acids in the presence of $Pd(OAc)_2$ and SPhos to give the corresponding isoflavones **78**. The 3-bromochromone was prepared in a sequence of steps employing the condensation of triethyl orthoformate with an acetophenone to give chromone **76**. Subsequent bromination of the chromone at C-3 by treatment with PhI(OAc), TMSBr and pyridine gave 3-bromochromone **77**.[39]

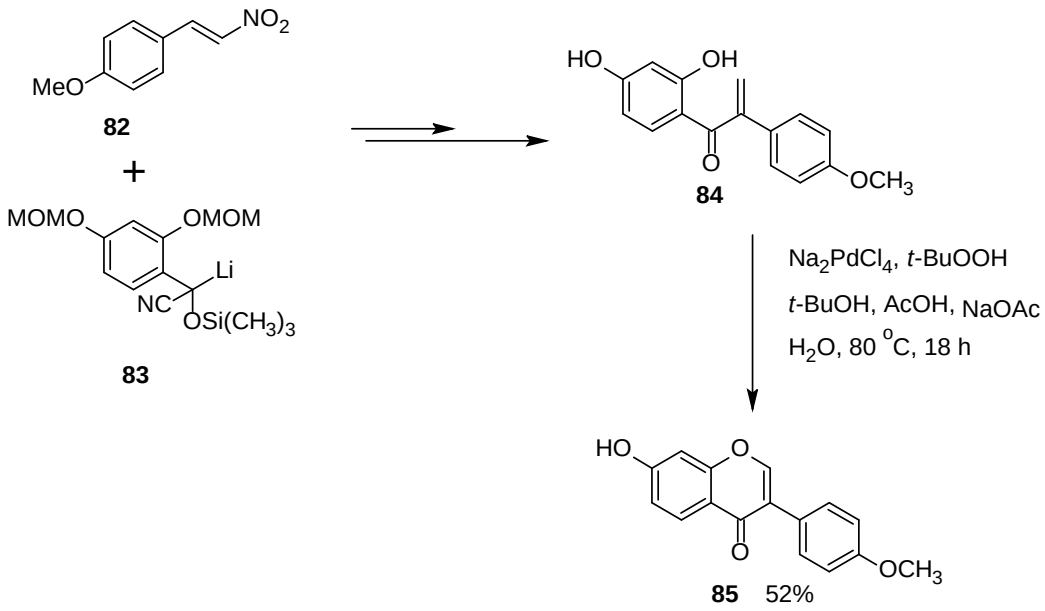


Scheme 4.5. Preparation of 3-halochromone

More conveniently, the 3-halochromones can also be obtained by Gammill's protocol,[40] which involves condensation of an appropriately substituted 2'-hydroxyacetophenone **79** with DMF-DMA to form an enaminoketone **80**. Halogen-mediated ring closure of the enamino ketone then gives the corresponding 3-halochromone **81**, Scheme 4.5.[40] Good yields of the 3-halochromones have been reported for mono- and dihydroxyacetophenones. However, the yields drop considerably when 2',4',6'-trihydroxyacetophenone is used as the starting material.[41-43]

4.2.4 The Wacker-Cook Synthesis

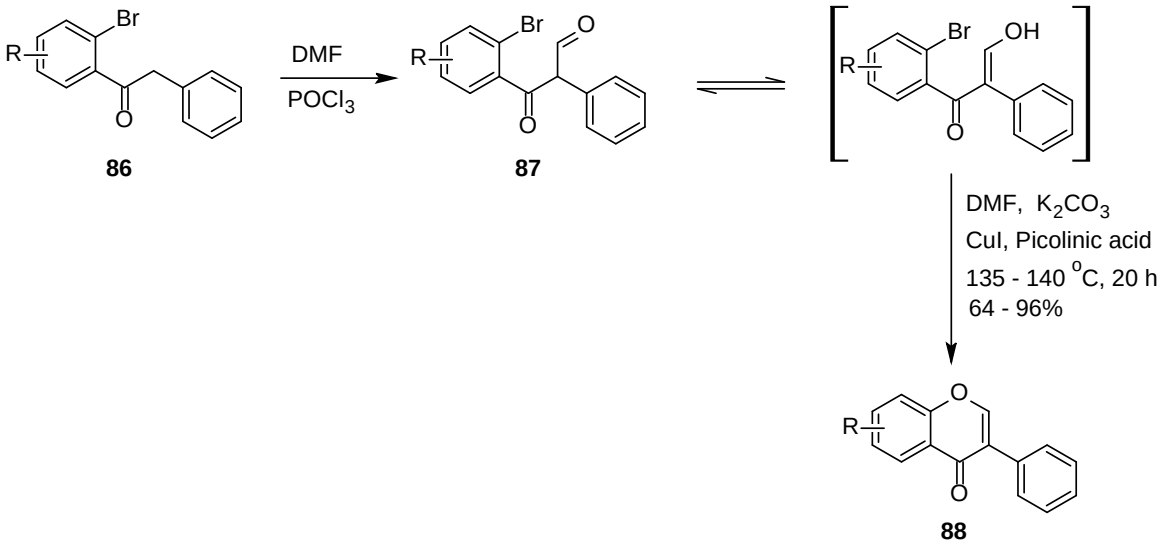
In 2009 Granados-Covarrubias and Maldonado adapted the Wacker-Cook method for the synthesis of formononetin (**85**). The reaction occurred in one pot, via oxidative cyclisation of α-methylenedeoxybenzoin **84** in the presence of a Pd(II) catalyst. The methylenedeoxybenzoin was obtained by conjugate addition of the cyanohydrin **83** to the nitrostyrene **82** as shown in Scheme 4.6.[18]



Scheme 4.6. A Wacker-Cook synthesis of formononetin

4.2.5 Cu(I)-Catalysed Intramolecular Cyclisation

Most recently (2011), Qiu-Lian Li and co-workers developed a new method for the synthesis of isoflavones based on Cu(I)-mediated intramolecular cyclisation of 3-(2-bromophenyl)-3-oxo-2-phenylpropanal (**87**), Scheme 4.7. The bromophenylpropanal **87** was prepared by formylation of bromodeoxybenzoin **86**. The application of this method was demonstrated for isoflavones **88** with various substitution patterns, however, no natural isoflavones have yet been prepared by this method.[19]



Scheme 4.7. Preparation of isoflavones **88** by Cu(I)-catalysed intramolecular cyclisation

Despite the many new synthetic methods that are being developed for the isoflavones, the deoxybenzoin route is used most often, mainly because it involves a smaller number of synthetic steps.[22-25,27-28] As already mentioned, the isoflavones can be prepared by this method without prior protection of the hydroxy groups.[21-23] Moreover, most starting materials (i.e. polyphenols and phenylacetic acids/phenylacetonitriles) are commercially available. However, the reaction employs strong acidic conditions, which are incompatible with some naturally-occuring substituents such as prenyl groups.[7,21-24,27-29] Thus, introduction of such groups is restricted to the late stages of the synthesis, when the isoflavone nucleus has been assembled. Therefore, it becomes difficult to control the regioselectivity in the synthesis of isoflavones bearing acid-sensitive substituents.[7,23]

The two newly developed methods, the Wacker-Cook synthesis and Cu(I)-catalysed cyclisation of bromopropanals are variations of the deoxybenzoin route.[18,19] The main disadvantage of these methods is that they introduce extra synthetic steps to the original deoxybenzoin route. Moreover, the Cu(I)-catalysed method still employs strong Lewis acids such as $POCl_3$ for formylation of the bromodeoxybenzoin. On the other hand, the synthesis of isoflavones by oxidative rearrangement of chalcones has been discouraged due to the high cost and toxicity of TTN.[30,31] Even though the less toxic hypervalent iodine reagents have been developed to facilitate aryl migration of chalcones, the rearrangement proceeds only when the 2'-hydroxy group is protected with robust protective groups, as most acid-sensitive protective groups do not survive.[17,30,31] Furthermore, olefinic substituents cannot withstand the conditions employed for the aryl-shift, thus such

substituents can only be incorporated after the isoflavone skeleton has been completely constructed.

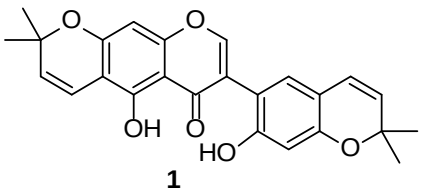
4.3 RESULTS AND DISCUSSION

4.3.1 Introduction

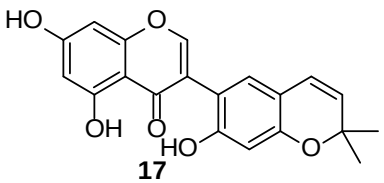
Several synthetic pathways for the pyranoisoflavonoids can be designed based on the isoflavone and the dimethylchromene synthetic methods discussed in Sections 4.2 and 3.2.2 (Chapter 3), respectively. However, a thoughtful combination of the methods for the preparation of the isoflavone skeleton and the dimethylpyran ring is required for the development of an ideal synthetic procedure. The choice of the methods may be guided by a number of factors which include:

- the structural variations of the target pyranoisoflavonoids,
- the regiochemistry of the target compounds,
- the sensitivity of the functional groups on the target molecules towards the reagents used in the method of choice, and
- the toxicity of the reagents involved

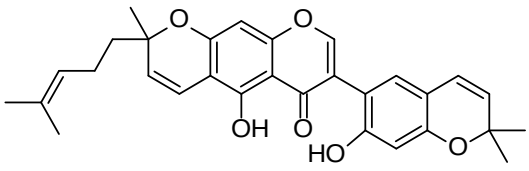
Our aims were to develop a synthetic route for the total synthesis of kraussianone 1 (**1**) and to prepare other isoflavonoids, which are structurally related to kraussianone 1 (**1**). These were the pyranoisoflavone eriosemaone D (**17**),[44,45] and the geranyl derivative *rac*-**89**.



1



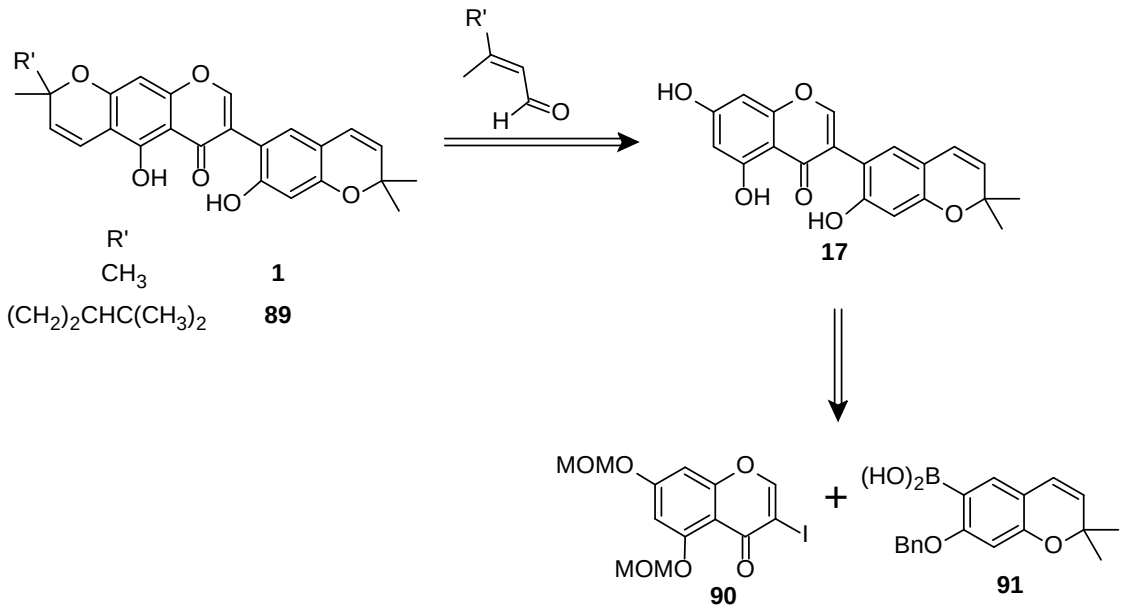
17



***rac*-89**

For the syntheses of these compounds, we took advantage of the Suzuki-Miyaura protocol.[33] As already seen from the literature review, this method employs less toxic reagents and offers access to a number of different isoflavones in the final stages of the synthetic pathway, by using different boronic acid partners or by incorporating additional substituents to the A-ring of the isoflavone.[38,39] We also hoped that the mild conditions often employed in the Suzuki-Miyaura protocol would permit the syntheses of the pyranoisoflavones from precursors bearing the dimethylchromene scaffolds. This would allow the regioselective construction of the dimethylpyran rings, which has been a problem in several synthetic routes for the pyranoisoflavones.[7,8,10]

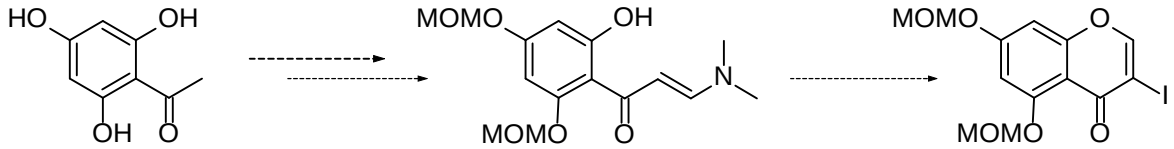
We envisaged the syntheses of the target compounds to proceed as outlined in the retrosynthetic route (Scheme 4.8). As seen from Scheme 4.8, the precursors for all the target compounds were 3-iodochromone **90** and arylboronic acid **91**. Eriosemaone D (**17**) was planned to be synthesised by the Suzuki-Miyaura cross-coupling reaction of **90** and **91**, followed by deprotection. Kraussianone 1 (**1**) and **89** were envisaged to arise from aldol-type condensation and subsequent 6π-electrocyclisation of eriosemaone D (**17**) with prenal and citral, respectively.



Scheme 4.8. Retrosynthetic analysis of eriosemaone D (**17**), kraussianone 1 (**1**) and the geranyl derivative **89**

4.3.2 Preparation of 3-Iodochromone 90

We planned to construct 3-iodochromone **90** by an aldol-type condensation of the commercially available 2',4',6'-trihydroxyacetophenone (**92**) with the dimethylacetal of dimethylformamide (DMF-DMA), followed by iodine-mediated cyclisation of the resulting enaminoketone **93** (Scheme 4.9).[40]



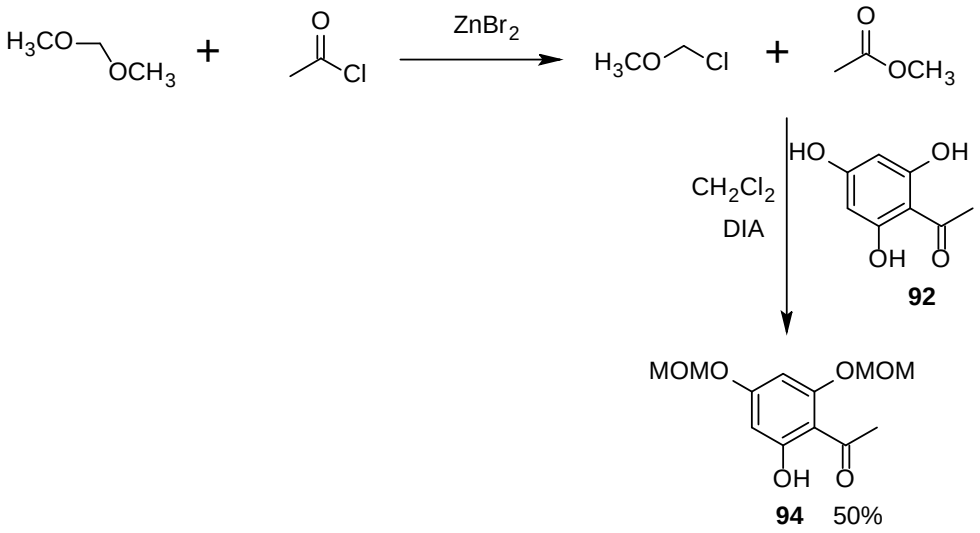
92 **93** **90**

Scheme 4.9. The proposed synthetic pathway for the precursor **90**

Although the synthesis of the 3-iodochromones via an enaminoketone intermediate is a well-established procedure,[40] there are only a few reports on its application using phloroacetophenone as the starting material. Vasselin and colleagues attempted the synthesis of 3-iodochromones from the corresponding 4',6'-dibenzyloxy-2'-hydroxyacetophenone and 2'-hydroxy-4',6'-dimethoxyacetophenone.[41] However, they only managed to recover an unidentified mixture of products from the dibenzylated acetophenone, and 29% of the 3-iodo-5,7-dimethoxychromone from the latter.[41] Similarly, low yields (34.5%) of the 3-iodo-5,7-dimethoxychromone have been obtained by Watanabe *et al*.[43] In both instances, the enaminoketones were obtained in good yields and the main problem was encountered in the iodine-mediated cyclisation step.[41,43]

Therefore, it was required that other protecting groups be investigated for the preparation of the 3-iodochromone. The protection of the 4'- and 6'-hydroxy groups of the phloroacetophenone is essential for the successful condensation of the 2',4',6'-trihydroxyacetophenone (**92**) with DMF-DMA, because it decreases the nucleophilicity of the aromatic ring and prevents side reactions that may occur between the free hydroxy groups and DMF-DMA.

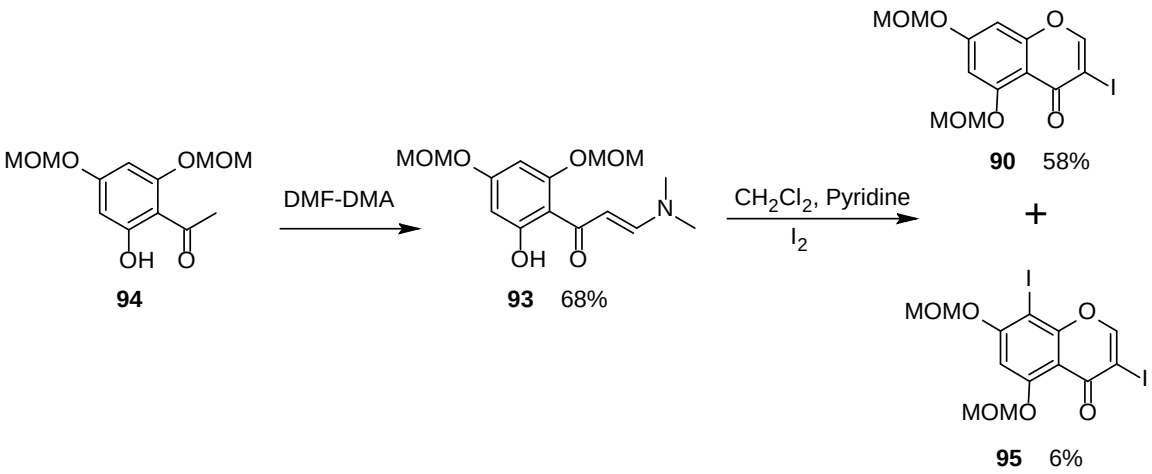
2',4',6'-Trihydroxyacetophenone (**92**) was regioselectively protected with MOMCl generated from the reaction of dimethoxymethane, acetyl chloride and catalytic $ZnBr_2$ to give **94** (Scheme 4.10).[46] The ^{1}H NMR spectrum of compound **94** showed two *meta*-coupled doublets at δ_H 6.24 and 6.26 arising from the aromatic protons H-3' and H-5', respectively, a three-proton singlet at δ_H 2.65 due to the C-2 methyl group, and a characteristic hydrogen-bonded hydroxy signal at δ_H 13.68. The methoxymethyl ether pair of signals appeared as singlets at δ_H 3.47 (OC<u>H</u>$_3$), 3.52 (OC<u>H</u>$_3$), 5.16 (OC<u>H</u>$_2$O) and 5.25 (OC<u>H</u>$_2$O).[46]



Scheme 4.10. MOM protection of trihydroxyacetophenone **92**

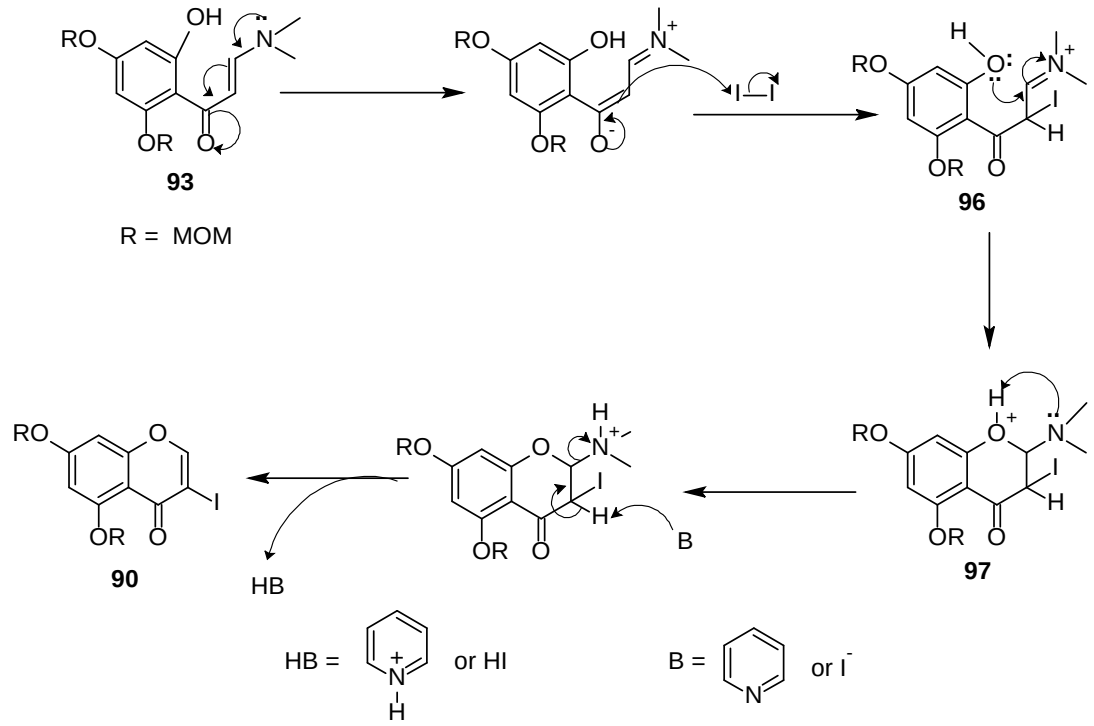
Condensation of **94** with DMF-DMA gave enaminoketone **93**, albeit in modest yield (68%) (Scheme 4.11).[40] The structure of compound **93** was confirmed by the appearance of broad singlets at δ_H 2.94 and 3.13, integrating for three protons each for the dimethylamino protons, and two olefinic doublets, δ_H 6.29 and 7.92 (J = 12.0 Hz, H-2 and H-3, respectively) in the ^{1}H NMR spectrum of the compound (Plate 10a). The relative small coupling constant of 12.0 Hz for the *E*-configuration in this case is due to the electronegative nitrogen substituent, which weakens the through-bond interaction between the two olefinic protons. The ^{13}C NMR spectrum of the compound showed an upfield shift for the carbonyl signal to δ_C 190.6 due to the resulting conjugation (Plate 10b), relative to the chemical shift of δ_C 203.1 observed for the carbonyl signal of acetophenone **94**.

Treatment of **93** with pyridine and iodine yielded an inseparable mixture of the targeted iodochromone **90** as the major component and the unwanted diiodinated chromone **95** as the minor product (Scheme 4.11). The ^{1}H NMR spectrum of the iodochromone **90** exhibited, apart from the aromatic protons and the MOM ether signals, a characteristic H-2 signal resonating as a one-proton singlet at δ_H 8.09 (Plate 11a). The ^{1}H NMR spectrum of the diiodinated chromone **95** displayed a singlet at δ_H 6.96 for the one aromatic proton instead of the two *meta*-coupled doublets observed for **90**.



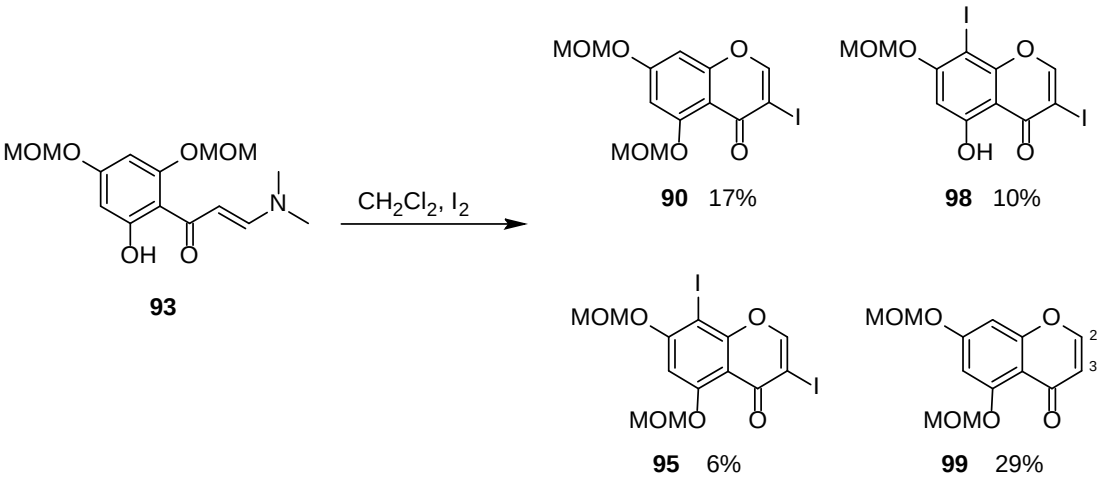
Scheme 4.11. Preparation of 3-iodochromone **90** from enaminoketone **93**

The cyclisation of the intermediate **93** into 3-iodochromone **90** in the presence of iodine and pyridine, is believed to follow the mechanism depicted in Scheme 4.12. The reaction is initiated by addition of iodine to the α-position, followed by nucleophilic attack of the *ortho*-hydroxy group to the iminium moiety **96** to afford **97**. Abstraction of the α-proton by pyridine or iodide ion and subsequent elimination of the labile quarternary amine renders 3-iodochromone **90**.



Scheme 4.12. Proposed mechanism for the formation of 3-iodochromones

Addition of pyridine was found to be essential for the conversion of **93** to **90** in moderate yields in the present case. It was observed that without pyridine, the reaction yielded multiple products whereby non-iodinated chromone **99** was obtained as the major product (Scheme 4.13). The ^{1}H NMR spectrum of **99** showed a pair of coupled doublets at δ_H 6.23 and 7.76 (J = 6.0 Hz) for the chromone protons (H-2 and H-3).



Scheme 4.13. Preparation of iodochromone **90** in the absence of pyridine

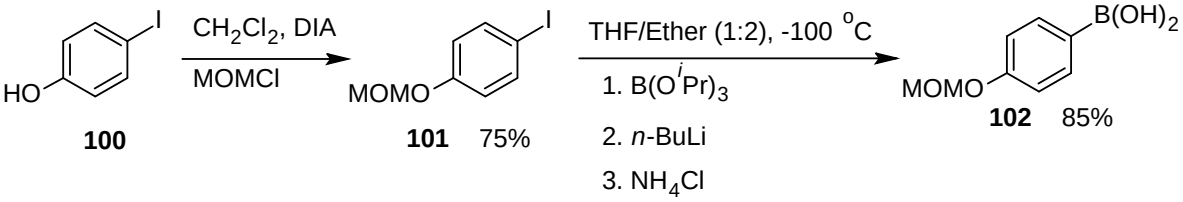
The formation of **99** in the absence of pyridine may be attributed to the generation of HI in the reaction mixture. It is known that *ortho*-hydroxy enaminoketones readily cyclise to form chromones under acidic conditions.[20,47] It is therefore proposed that pyridine promotes the formation of the target 3-iodochromone by neutralising the generated HI. A similar phenomenon has been observed by Hong *et al*., who established that nitrogen-containing bases such as piperidine and pyridine promote the conversion of enaminoketones into 3-iodochromones in good yields.[48]

4.3.3 Preparation of Genistein (103)

Having accomplished the synthesis of the 3-iodochromone precursor **90**, we decided to perform a model reaction for optimisation of the reaction conditions for the Suzuki-Miyaura coupling using phenylboronic acid **102**. Not only did this reaction help us to establish the ideal conditions for the synthesis of a boronic acid from an aryl iodide and for the Suzuki-Miyaura coupling, but it also gave access to an important phytoestrogen, genistein (**103**).

Although most phenylboronic acids are commercially available, we opted to synthesise the boronic acid **102** in order to pave the way for the synthesis of the boronic acid **91**, which was required for eriosemaone D (**17**), kraussianone 1 (**1**) and the geranyl analogue **89**. Arylboronic acids are traditionally prepared by the reaction of arylmagnesium halides or aryllithium with trialkyl borates. Alternatively, they can be synthesised by the Pd-catalysed cross-coupling reaction of aryl halides with alkoxydiboron or alkoxy hydroboranes.[49-52] However, the boron reagents used in the latter procedure are expensive and unattractive for large scale synthesis.[53] On the other hand, the direct metal-halogen exchange reactions involved in the former procedures, are reported to have low functional group tolerance.[49] The magnesium-halogen exchange procedures reported to be applicable in the presence of sensitive substituents, require alkylmagnesium halide reagents, such as isopropylmagnesium chloride, to facilitate the insertion of magnesium to the aryl halide.[54] We opted to synthesise the arylboronic acids from an aryllithium using the readily-available *n*-BuLi to facilitate lithium-halogen exchange.

The synthesis of **102** was initially attempted by a normal sequential procedure whereby a solution of aryl iodide **101** in THF was successively treated with *n*-BuLi, then triisopropyl borate and the resulting boronate ester hydrolysed with NH_4Cl. Following this procedure, a small amount of boronic acid **102** was obtained and an appreciable quantity of the de-iodinated starting material was recovered. It was evident from these results that the lithium-iodine exchange occurred rapidly as expected, and that the generated aryllithium was very unstable. It was therefore necessary to change the reaction conditions to accommodate the substrate in use.

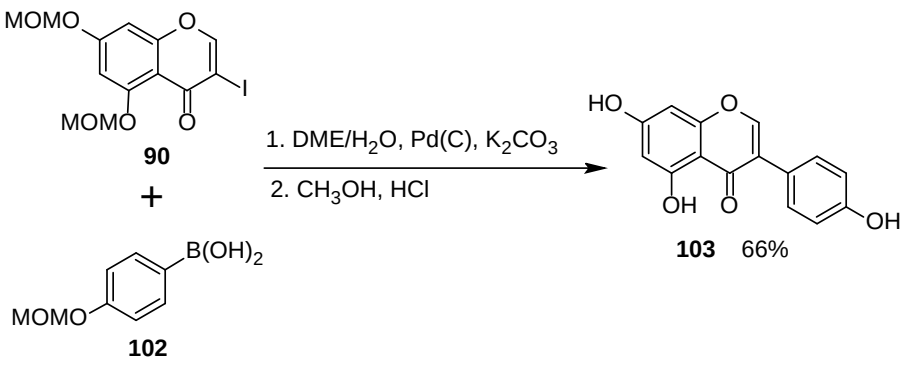


Scheme 4.14. Preparation of boronic acid **102**

Optimum results were obtained by changing the solvent system to THF:diethyl ether (1:2), and the sequence in which the reagents were added, by employing the ‘‘*in situ* quench’’ procedure developed by Li and co-workers.[53] Therefore, the boronic acid **102** was prepared in a one-pot sequence, which involved addition of *n*-BuLi to a solution of aryl iodide **101** and triisopropyl borate in THF:diethyl ether (1:2), followed by the hydrolysis of the boronate ester with an ammonium chloride solution (Scheme 4.14). The improvement observed with this procedure may be attributed to the immediate *in situ* quench of the generated phenyllithium with triisopropyl borate thus preventing side reactions of the phenyllithium.[53] The role played by the THF:ether solvent system is not fully understood. However, it has been reported that different coordinating solvents affect the stability, the aggregation and the reactivity of the lithiated species.[55,56]

The ^{1}H NMR spectrum of compound **102** displayed two *ortho*-coupled doublets, each integrating for two protons, δ_H 7.15 and 8.16 (J = 8.2 Hz), and the protons of the methoxymethyl protecting group resonating at δ_H 5.07 (2H, s, OCH_2O) and 3.52 (3H, s OCH_3). The presence of boron in the molecule was confirmed by the disappearance of a signal at around δ_C 84.3 in the ^{13}C NMR spectrum, indicating the displacement of iodine in the starting material. The ^{11}B spectrum of **102** further confirmed the presence of a boronic

acid by a resonance at δ_B 28.52. The IR spectrum of compound **102** displayed an OH stretch band at 3436 cm^{-1}.

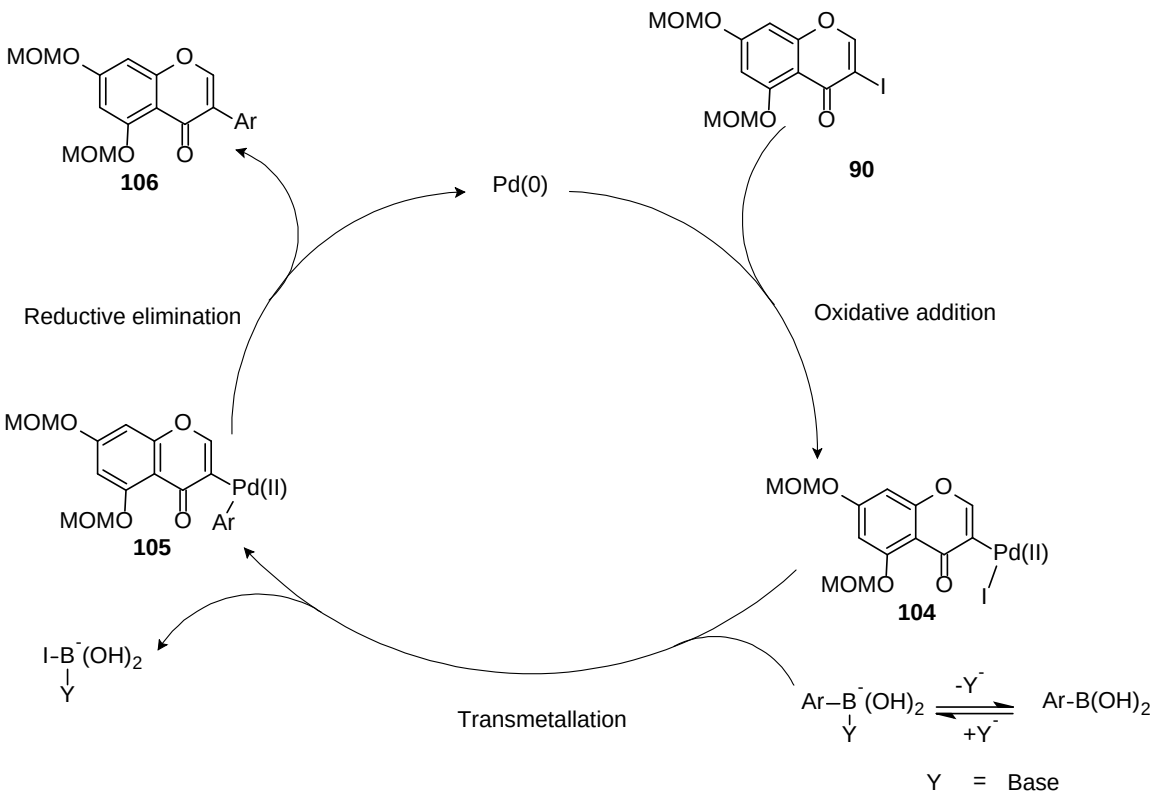


Scheme 4.15. Synthesis of genistein (**103**)

The Suzuki-Miyaura cross-coupling of boronic acid **102** with 3-iodochromone **90** using a heterogeneous Pd(C) catalyst and subsequent removal of the MOM protecting groups furnished genistein (**103**) in 66% yield (Scheme 4.15).[35] The use of the ligandless Pd(C) for the C-C bond formation is said to have advantages over other methods in that the reaction is conducted successfully under hydrous conditions. Moreover, the Pd(C) can be readily removed from the reaction mixture by filtration.[35]

The ^{1}H NMR spectrum of compound **103** displayed a characteristic isoflavone one-proton singlet at δ_H 8.29 for the hydrogen at C-2, two *meta*-coupled doublets for the aromatic protons of the A-ring at δ_H 6.22 (1H, d, *J* = 2.1 Hz, H-8) and δ_H 6.38 (1H, d, *J* = 2.1 Hz, H-6), and the protons of the B-ring exhibited an AA'BB' spin system at δ_H 6.81 (2H, d, *J* = 8.8 Hz, H-3' and H-5') and 7.36 (2H, d, *J* = 8.8 Hz, H-2' and H-6') (Plate 12). The HRMS spectrum of the compound showed an *m/z* peak of 269.0452 $[M-H]^-$, consistent with the calculated molecular mass of 269.0450 for $C_{15}H_9O_5$.

Using 3-iodochromone **90**, the proposed mechanism for the Suzuki coupling reaction is shown in Scheme 4.16. It involves oxidative addition of iodochromone **90** to Pd(0) to give Pd(II) intermediate **104**. The intermediate **104** undergoes transmetallation with the arylboronic acid to afford the organopalladium species **105**, which upon reductive elimination gives the coupled product **106** and regenerates Pd(0).[49]

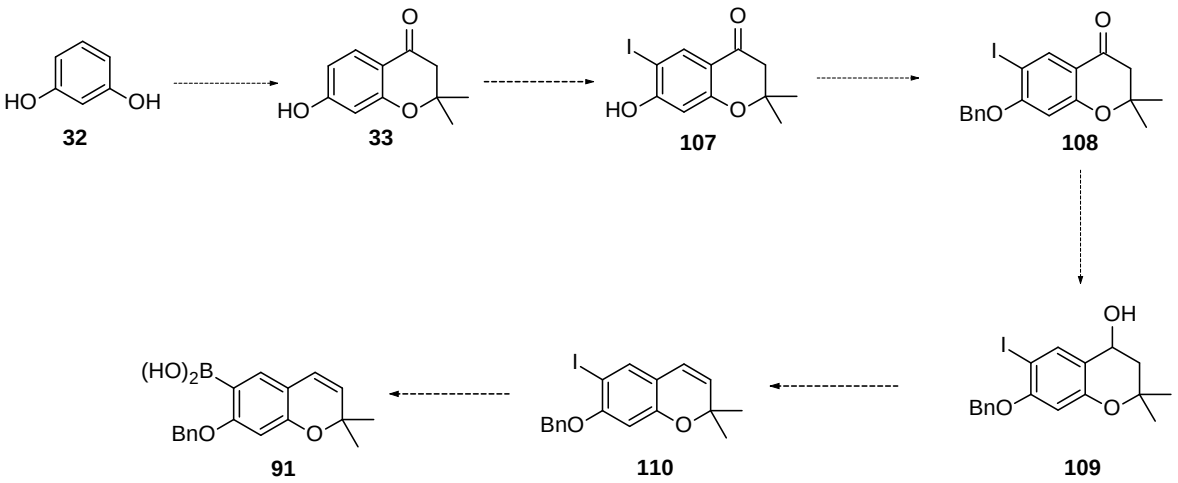


Scheme 4.16. Mechanism for the Pd-catalysed C-C bond formation

Although genistein **(103)** was synthesised in a model reaction, this synthesis is of utmost importance since **103** has been associated with a number of biological activities that include inhibition of tyrosine kinase, phytoestrogenic, antioxidative, anticancer activities and protection against cardiovascular diseases and ostereoporosis.[57,58] Furthermore, it may serve as an important precursor for a large number of biologically-active isoflavonoids by modifications of rings A, B and C.

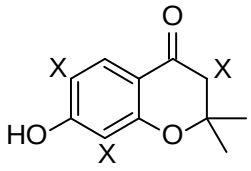
4.3.4 Preparation of the Boronic Acid Derivative 91

As seen from the proposed synthetic route, Scheme 4.8, the target compounds **17**, **1** and **89** were planned to be prepared from 3-iodochromone **90** and the boronic acid **91**. The boronic acid **91** on the other hand, was to be prepared from resorcinol **32** as outlined in Scheme 4.17.



Scheme 4.17. Proposed synthetic pathway for the boronic acid **91**

In this synthetic route, it was envisaged that a major challenge would be encountered in the regiospecific C-6 iodination of 7-hydroxy-2,2-dimethylchromanone **33** to form 7-hydroxy-6-iodo-2,2-dimethylchromanone **107**. Iodination of chromanone **33** has not been reported previously. Except for the nucleophilic aromatic substitution of a diazonium salt with iodide ion,[59] most iodination protocols for electron-rich aromatic rings proceed via electrophilic aromatic substitution. Nevertheless, there are three sites susceptible to electrophilic substitution in compound **33**, as shown in Figure 4.1, which may give multiple products depending on the reaction conditions.

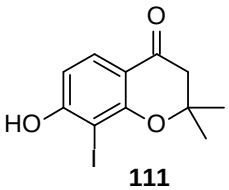


X = nucleophilic site

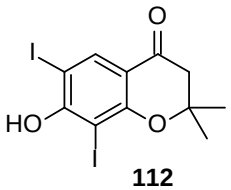
Figure 4.1. Active sites for electrophilic substitution

The dimethylchromanone **33** was prepared in good yields by condensation of resorcinol (**32**) with 3-methylbut-2-enoic acid.[60] The iodinating reagents, KIO_3/KI, HIO_4/I_2 and ICl were initially screened for C-6 iodination of **33** under different conditions,[31,61-63] but 7-hydroxy-8-iodo-2,2-dimethylchroman-4-one (**111**) and 7-hydroxy-6,8-diiodo-2,2-dimethylchroman-4-one (**112**) predominated. Benzyl protection of the 7-hydroxy group of chromanone **33**, with the aim of preventing di-iodination through decreased nucleophilicity

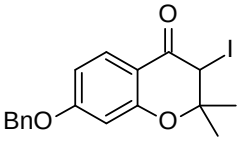
of the aromatic ring, and subsequent iodination of the resulting benzyloxychromanone with HIO_3/I_2,[64] resulted in the formation of 7-benzyloxy-3-iodochromanone **113** (Figure. 4.2).



111



112

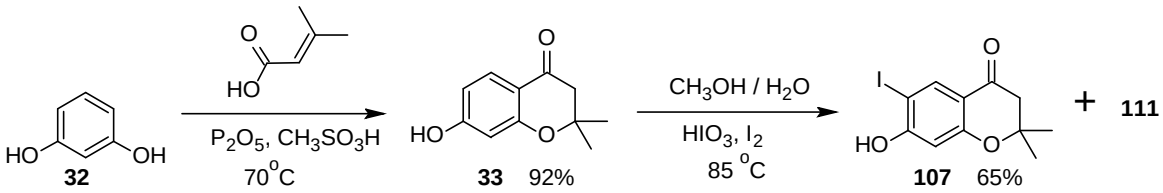


***rac*-113**

Figure 4.2. Unwanted iodinated chromanones

The structures of the iodinated chromanones **111**, **112** and **113** were elucidated by ^{1}H NMR spectroscopy. The spectrum of **111** displayed two *ortho*-coupled doublets for the aromatic protons at δ_H 6.71 and 7.83 (J = 8.4, H-6 and H-5, respectively) and two singlets for the aliphatic protons at δ_H 1.55 (2 x CH_3) and 2.73 (2H, H-3), while that of **112** exhibited a one-proton singlet for the aromatic proton H-5 at δ_H 8.26. Besides the signals of the benzyl protecting group, the ^{1}H NMR spectrum of **113** on the other hand, displayed an ABX spin system in the aromatic region [δ_H 6.54 (1H, d, J = 1.8, H-8), 6.71 (1H, dd, J = 1.8 and 8.4, H-6) and 7.89 (1H, J = 8.4, H-5)]. This indicated that the aromatic ring of the starting material was not iodinated. However, a deshielded one-proton singlet was observed at δ_H 4.65 instead of the two-proton singlet at around δ_H 2.73, observed in the ^{1}H NMR spectrum of the starting material and other iodinated chromanones **111** and **112**. The resonances of the diastereotopic methyl groups in **113** gave two distinct singlets, intergrating for three protons each at δ_H 1.59 and 1.67. Therefore, it was deduced that iodination of 7-benzyloxy-2,2-dimethylchromanone occurred at C-3, giving a racemic mixture of **113**.

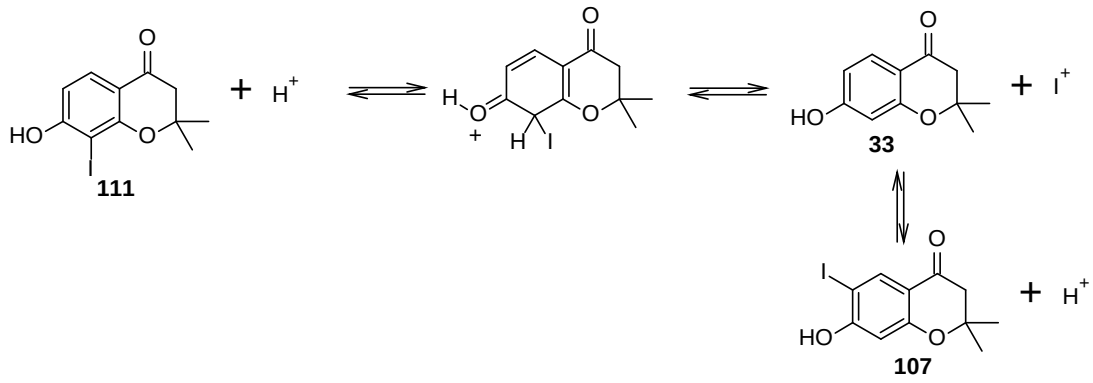
Promising results were obtained upon treatment of a methanolic solution of **33** with iodine and iodic acid at 85 ^{o}C. This gave a mixture of the intended C-6 iodinated chromanone **107** as the major component and the unwanted iodochromanone **111** as a minor component, Scheme 4.18. The two compounds were difficult to separate with column chromatography, but could be purified based on their solubility properties. It was established that the two isomers had different solubility properties, whereby **111** dissolved in CH_2Cl_2 and $CHCl_3$, and **107** was insoluble in these solvents.



Scheme 4.18. Preparation of 7-hydroxy-6-iodo-2,2-dimethylchroman-4-one (**107**)

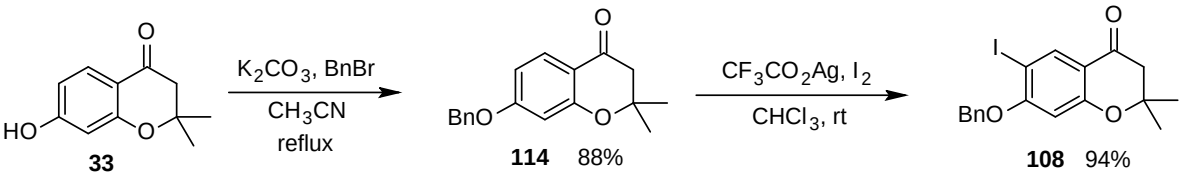
The ^{1}H NMR spectrum of **107** displayed two aromatic singlets at δ_H 6.40 (1H, s, H-8) and 7.96 (1H, s, H-5), and the aliphatic protons resonated at δ_H 1.36 (6H, s, 2 x CH_3) and 2.67 (2H, s, H-3). The ^{13}C NMR spectrum displayed a signal at δ_C 189.2 assigned to the carbonyl carbon, in addition to the signals for the six aromatic carbons (δ_C 76.4, 102.8 – 163.1) and the four aliphatic carbons (δ_C 26.1, 47.4 and 79.8) with the methyl carbons overlapping. A sharp C=O band appeared at 1641 cm^{-1} in the IR spectrum of the compound.

It was necessary to synthesise **107** in larger quantities in order to continue with the steps leading to boronic acid **91**. However, scaling up to 1.5 g scale gave rise to some reproducibility problems, whereby we obtained a mixture of **107**, **111**, **112** and the starting material **33**, repeatedly, the major compound being the unwanted 8-iodochromanone **111**. From these results, we suspected that the formation of 6-iodochromanone **107** by treatment of **33** with iodine and iodic acid did not proceed via a simple electrophilic aromatic substitution as expected. We deduced that compound **107** may be formed via migration of I^+ from C-8 of **111** to C-6 as shown in Scheme 4.19. This kind of rearrangement, also known as the ‘‘iodine dance’’, has been reported by Thomsen and Torsell, in the transformation of 2-iodoresorcinol to 4-iodoresorcinol in acidic media.[65]



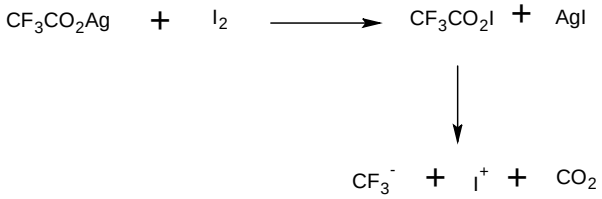
Scheme 4.19. Acid-catalysed rearrangement of **111** to **107**

The targeted 6-iodochromanone was obtained upon treatment of a $CHCl_3$ solution of 7-benzyloxy-2,2-dimethylchromanone (**114**) with silver trifluoroacetate and iodine at room temperature. This gave 7-benzyloxy-6-iodo-2,2-dimethylchromanone (**108**) as the sole product in 94% yield (Scheme 4.20).[66] This procedure allowed for the large scale synthesis of **108** in good yields. The ^{1}H NMR spectrum of compound **108** displayed signals similar to those of compound **107** and the additional signals for the benzyl protecting group.



Scheme 4.20. Preparation of iodochromanone **108**

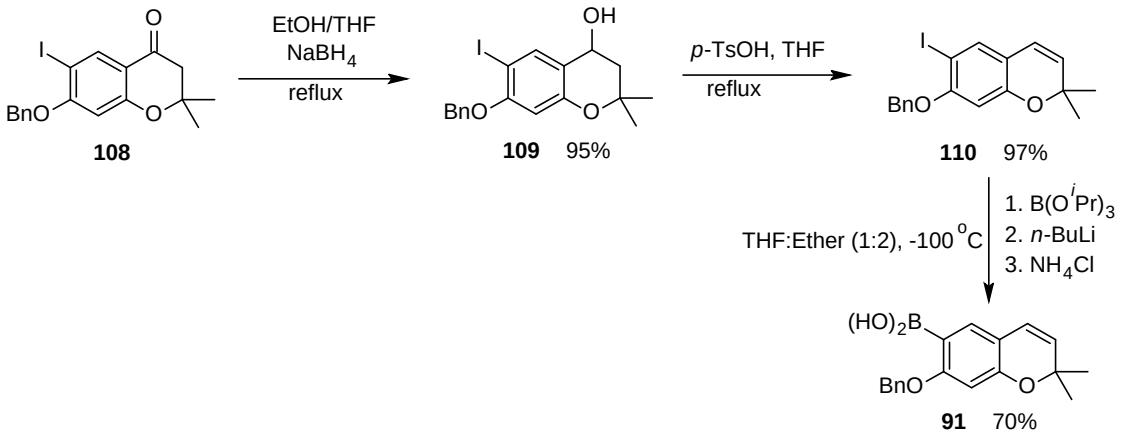
These results support the Haszeldine and Sharpe findings, who reported that iodination of substituted aromatic rings with silver trifluroacetate and iodine gives predominantly *para*-iodinated compounds.[66] Iodination with these reagents is said to be facilitated by the generation of the electrophilic iodine trifluoroacetate, which decomposes into carbon dioxide and trifluoroiodomethane (Scheme 4.21).[66]



Scheme 4.21. The generation of electropositive iodine from silver trifluoroacetate and iodine

Reduction of **108** with sodium borohydride and the subsequent dehydration proceeded cleanly to give 7-benzyloxy-6-iodo-2,2-dimethylchromene (**110**) (Scheme 4.22).[67,68] Reduction of the carbonyl group was confirmed by the presence of a signal at δ_H 4.75 (1H, ddd, J = 8.5, 7.3 and 6.0 Hz) for H-4, indicating a coupling to the two C-3 protons and an adjacent OH in the ^{1}H NMR spectrum of racemic **109**. The two C-3 protons were displayed as a pair of doublet of doublets at δ_H 1.80 (J_{gem} = 13.6 and $J_{3,4}$ = 8.5 Hz) and 2.10 (J_{gem} = 13.6 and $J_{3,4}$ = 6.0 Hz), and the OH as a broad doublet at δ_H 2.17 (J = 7.3 Hz). The

reduction was further confirmed by the disappearance of the carbonyl absorption peak at 1641 cm^{-1} and the appearance of the broad OH band at 3467 cm^{-1} in the IR spectrum of compound **109**. The ^{1}H NMR spectrum of chromene **110**, on the other hand, exhibited olefinic doublet signals at δ_H 5.48 and 6.22 (J = 9.9 Hz, H-3 and H-4, respectively). The IR spectrum showed sharp absorption bands at 1602 – 1713 and 3032 cm^{-1} for the C=C and =C-H absorptions, respectively.

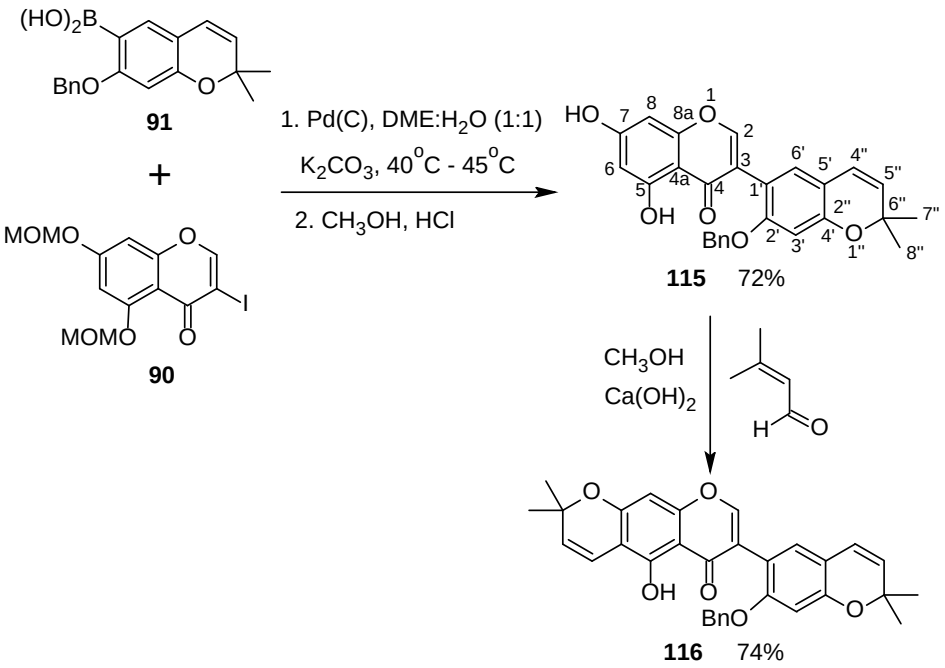


Scheme 4.22. Preparation of the boronic acid derivative **91**

Boronic acid **91** was prepared from iodochromene **110** under the same reaction conditions to those employed in the synthesis of boronic acid **102** (Scheme 4.22).[53] The ^{1}H NMR spectrum of **91** (Plate 13a) exhibited an additional two-proton singlet at δ_H 5.54 (-$B(OH)_2$) and a slight downfield shift of the aromatic proton signals, δ_H 6.46 and 7.47 (H-8 and H-5, respectively) instead of δ_H 6.41 and 7.37 observed in the ^{1}H NMR spectrum of the iodochromene **110** due to the electron-withdrawing effect of boron in the molecule. The ^{13}C NMR spectrum confirmed the substitution of iodine by the absence of the iodine-bearing C-6 signal at δ_C 74.8 (Plate 13b). The presence of the boronic acid group was further confirmed by the IR spectrum which displayed a broad band at 3367 cm^{-1} due to the OH stretches and by the appearance of a characteristic arylboronic acid signal at δ_B 28.74 in the ^{11}B NMR spectrum of compound **91**.

4.3.5 Preparation of Eriosemaone D (17) and Kraussianone 1 (1)

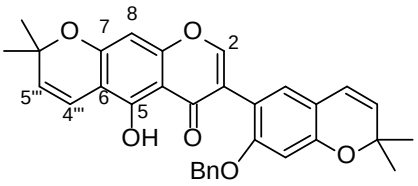
Following the synthesis of the boronic acid **91**, the next step was to couple it to the 3-iodochromone precursor **90**. Thus, Pd(C)-catalysed cross coupling of boronic acid **91** with 3-iodochromone **90** and subsequent cleavage of the MOM protecting groups with HCl gave 2'-*O*-benzyleriosemaone D (**115**) (Scheme 4.23).[35] The ^{1}H NMR spectrum of **115** (Plate 14a) displayed the characteristic isoflavone one-proton singlet at δ_H 7.77 (H-2), instead of a downfield shift δ_H 8.09 for H-2 observed in the spectrum of the 3-iodochromone **90**. The aromatic protons of the A-ring appeared as two *meta*-coupled doublets at δ_H 6.19 and 6.21 (J = 2.2, H-6 and H-8, respectively) and those of the B-ring displayed an AM spin system, δ_H 6.50 and 6.92 (s, H-3' and H-6', respectively). The dimethylchromene ring signals were observed at δ_H 1.40 (2 x CH_3), 5.44 and 6.23 (d, J = 9.8 Hz, for the *cis* olefinic protons H-5" and H-4", respectively) and those of the benzyl group at δ_H 5.03 (2H, s, PhC$\underline{H}_2$) and 7.22-7.34 (5H, m, $\underline{Ph}CH_2$). The HRMS spectrum gave an *m/z* ion at 465.1312 $[M+Na]^+$ in agreement with the calculated pseudo molecular mass of 465.1314 for $C_{27}H_{22}NaO_6$.



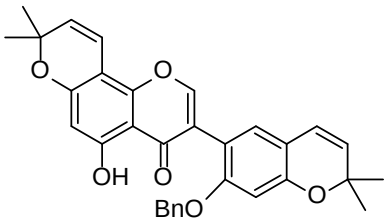
Scheme 4.23. Preparation of benzyl derivatives of eriosemaone D and kraussianone 1

Base-catalysed condensation of the pyranoisoflavone **115** with prenal gave the benzyl derivative of kraussianone 1 **116** (Scheme 4.23).[69] The structure of **116** was confirmed by the presence of additional signals in the ^{1}H NMR spectrum for the dimethylchromene fused to the A-ring at δ_H 1.47 (2 x CH_3), 5.61 and 6.73 (d, J = 10.0 Hz, H-5''' and H-4''', respectively) (Plate 15a). The HRMS spectrum gave an *m/z* of 531.1782 $[M+Na]^+$ in agreement with the calculated pseudo molecular mass of 531.1784 for $C_{32}H_{28}NaO_6$.

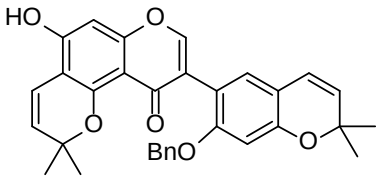
Three regioisomers are possible for this reaction. These are the linear **116** and the angular isomers **117** and **118**, respectively. From the ^{1}H NMR results, it could be readily deduced that the isomer **118** was not formed, due to the presence of the characteristic hydrogen-bonded OH-5 resonance at δ_H 13.22 in the ^{1}H NMR spectrum. Thus, the product could either be **116** or **117**.



116

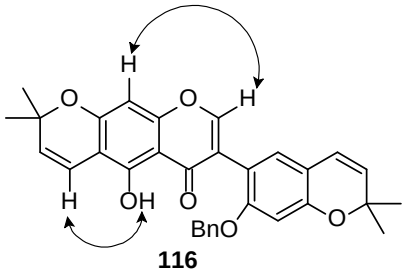


117



118

The fusion of prenal to C-6 and OH-7 of **115** to give the linear isomer **116** was confirmed by the presence of the slight downfield H-8 signal at δ_H 6.31 in the ^{1}H NMR spectrum of compound **116**, consistent with the literature results.[70-72] If the angular isomer **117** was formed, we expected a more shielded H-6 signal at around δ_H 6.27, which was not observed in the present case. The structure of **116** was further corroborated by the ^{13}C NMR spectrum, which displayed the characteristic non-alkylated C-8 signal at δ_C 94.8, instead of the C-6 signal, which would appear at around 100.3.[70,71] The regiochemistry of compound **116** was also confirmed by the NOESY experiment, which showed weak through-space correlations between OH-5 and H-4''', and H-8 and H-2, in agreement with the structure of the linear isomer **116** (Figure 4.3).



116

Figure 4.3. Observed NOESY correlations for the linear isomer **116**

The formation of the second dimethylpyran ring by the condensation of the pyranoisoflavone **115** with prenal follows an aldol-type condensation and subsequent 6π-electrocyclisation as discussed in Chapter 3, Section 3.2.2.1. It has been reported that the regioselectivity in the synthesis of linear and angular isomers by condensation of prenal to the phloroglucinol moiety of chromone-based systems depends on the reaction conditions.[69,73] Jeso and Nicolaou introduced the linear dimethylpyran scaffold on the phloroacetophenone moiety of tovophyllin B by a CaO-induced aldol-type condensation with prenal.[73] Mondal and co-workers also reported the regioselective formation of the linear and angular pyranoxanthones. From their investigation, the angular isomer was formed in good yields when the reaction was performed neat at high temperatures (140-150 ^{o}C) whereas the linear isomer was formed when the reaction was performed at rt in CH_3OH, using $Ca(OH)_2$ as a base.[69] Therefore, we opted for the latter conditions for the synthesis of **116** from **115**. Under these conditions, it is postulated that Ca^{2+} chelates with the carbonyl group and the adjacent OH-5 (Figure 4.4), thereby increasing the nucleophilicity of C-6 relative to C-8.[69]

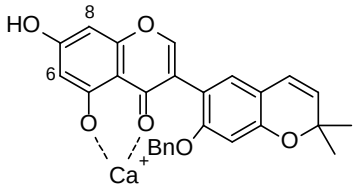
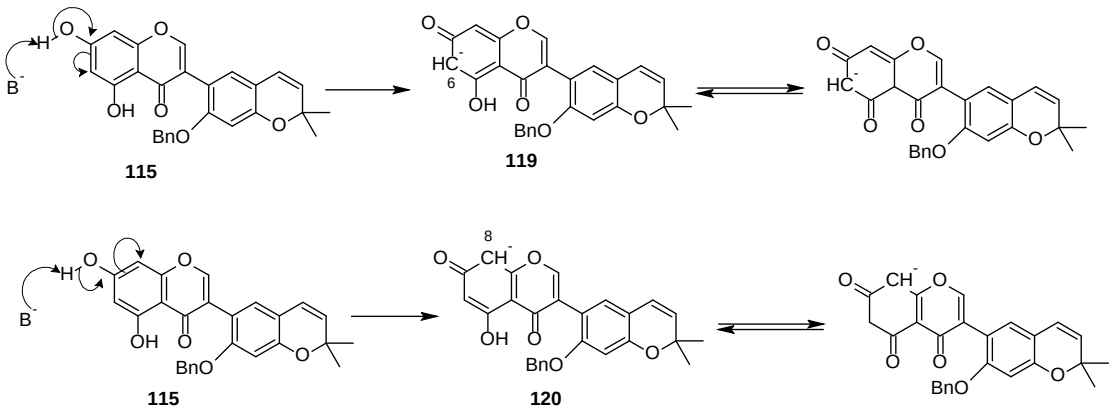


Figure 4.4. Chelation of 2'-*O*-benzyleriosemaone D (**115**) with Ca^{2+}

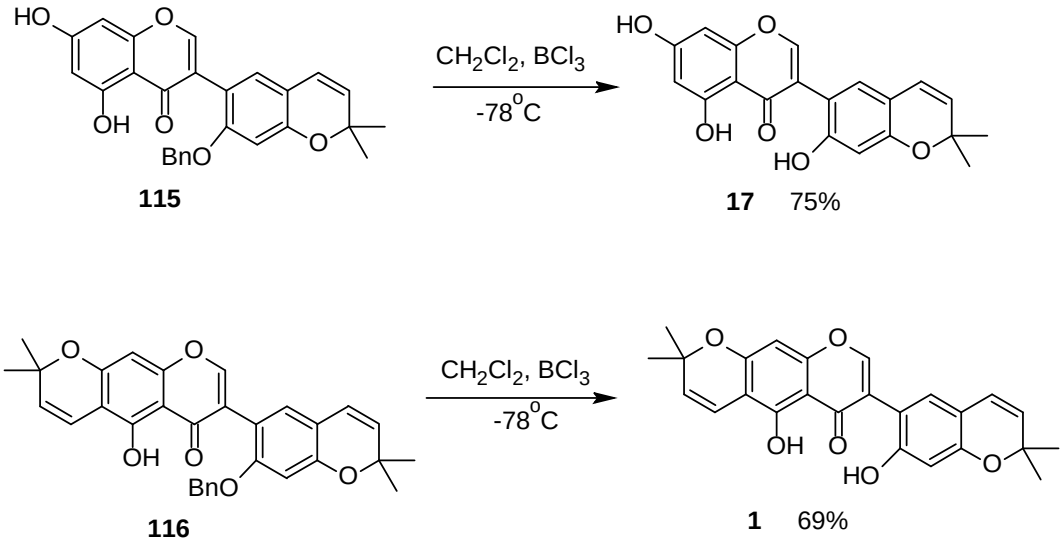
Furthermore, two hypothetical carbanions **119** and **120** may result from deprotonation of the OH-7 under basic conditions as shown in Scheme 4.24. Upon keto-enol tautomerisation, the carbanion **119** is flanked between two carbonyl systems, therefore it is less stable and more reactive than the carbanion **120**, which is stabilised by conjugation with the α,β unsaturated carbonyl.[69] The regioselective formation of the linear isomer **116**

may therefore be attributed to high reactivity of C-6 relative to C-8 under the current conditions.



Scheme 4.24. Formation of the linear and the angular isomers via carbanions **119** and **120**, respectively

Having successfully synthesised the benzyl derivatives of eriosemaone D and kraussianone 1, we were now in a position to cleave the benzyl protecting group. A careful selection of the debenzylating conditions was crucial for the successful conversion of **115** and **116** into **17** and **1**, respectively, since the compounds possess dimethylchromene scaffolds. Thus palladium-catalysed hydrogenation, which is often employed for cleavage of the benzyl protecting group, could not be employed in the present case as the double bonds would be reduced. The choice of the reagents for the debenzylation was restricted to Lewis acids such as $TiCl_4$ and $AlCl_3$, $BF_3.OEt_2$ and BCl_3.[11,74,75,76,77] The successful debenzylation of benzylpyranoisoflavones has been reported by Tsukayama and co-workers, who employed BCl_3 in CH_2Cl_2. Therefore, similar conditions were employed in the synthesis of eriosemaone D (**17**) and kraussianone 1 (**1**) from **115** and **116**, respectively (Scheme 4.25).[11] The structures of the targeted compounds were confirmed by the absence of the signals of the benzyl group in their ^1H and ^{13}C NMR spectra and by comparison with reported data.[44,45,72]

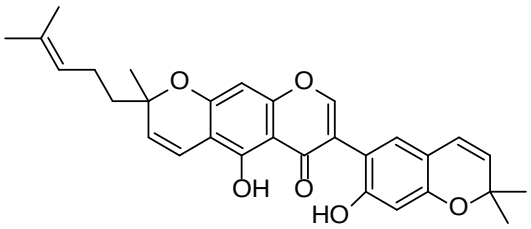


Scheme 4.25. Synthesis of eriosemaone D (**17**) and kraussianone 1 (**1**)

4.3.6 Preparation of a Geranyl Analogue of Kraussianone 1

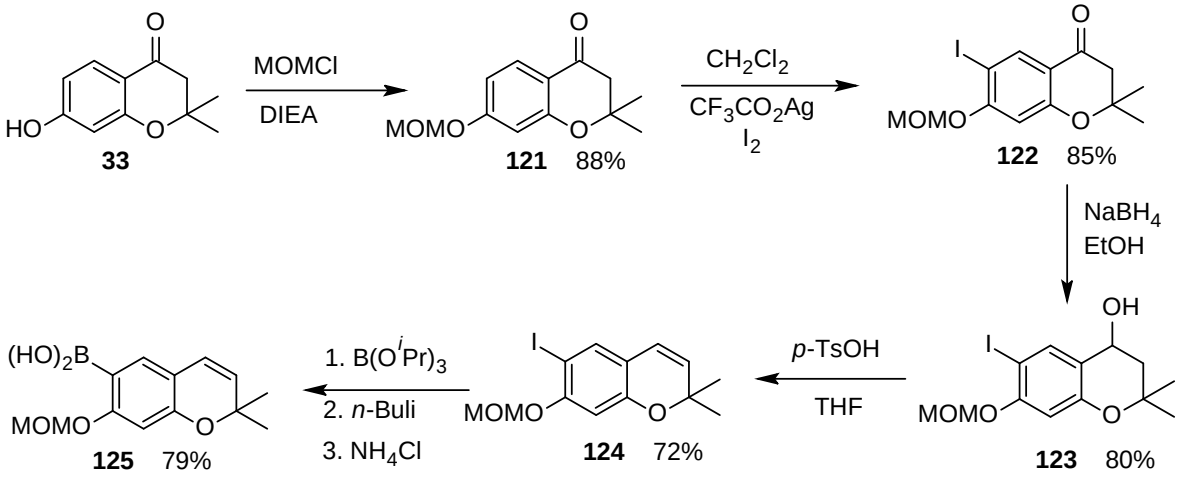
The geranyl moiety occurs sporadically amongst the different classes of flavonoids and is mostly prevalent in flavanones, chalcones and other classes of polyphenolic compounds such as xanthones,.[78-82] Even though the geranylated isoflavonoids are uncommon, the few reported ones exhibit important biological activities such as anti-inflammatory, estrogenic, cytotoxicity against human cancer cell lines, antiviral, antimicrobial, immunosuppressive as well as anti-oxidative activities.[2-4,83,84]

Following the successful synthesis of kraussianone 1 (**1**), eriosemaone D (**17**) and genistein (**103**), the focus was shifted to the synthesis of **89**, a derivative of kraussianone 1 with an *O*-cyclised geranyl moiety attached to the A-ring. More often, prenylation of biologically-active compounds has been determined to enhance the activity of the compounds,[85,86] and in certain instances, geranylated compounds have been found to be more active than the prenylated ones.[78,80,85-87] Therefore, we planned to prepare **89**, the geranyl derivate of kraussianone 1 (**1**) in order to evaluate its influence on the activities manifested by **1**.



***rac*-89**

As shown in Scheme 4.26, the synthesis commenced with preparation of the dimethylchromene boronic acid **125**, which would render the B-ring and the dimethylpyran scaffold fused to it. This time, the MOM protecting group was used instead of a benzyl group, because of the ease of cleavage of the MOM group and in order to eliminate the debenzylation step. Chromanone **33** was thus converted into methoxymethyl ether **121**,[46] which was iodinated by I_2 and CF_3CO_2Ag in CH_2Cl_2 to give **122**. Iodochromanone **122** was reduced and dehydrated to give iodochromene **124**.[66] Sequential treatment of the solution of **124** in ether:THF with triisopropylborate, *n*-BuLi and NH_4Cl gave boronic acid **125** in 79% yield.[53]

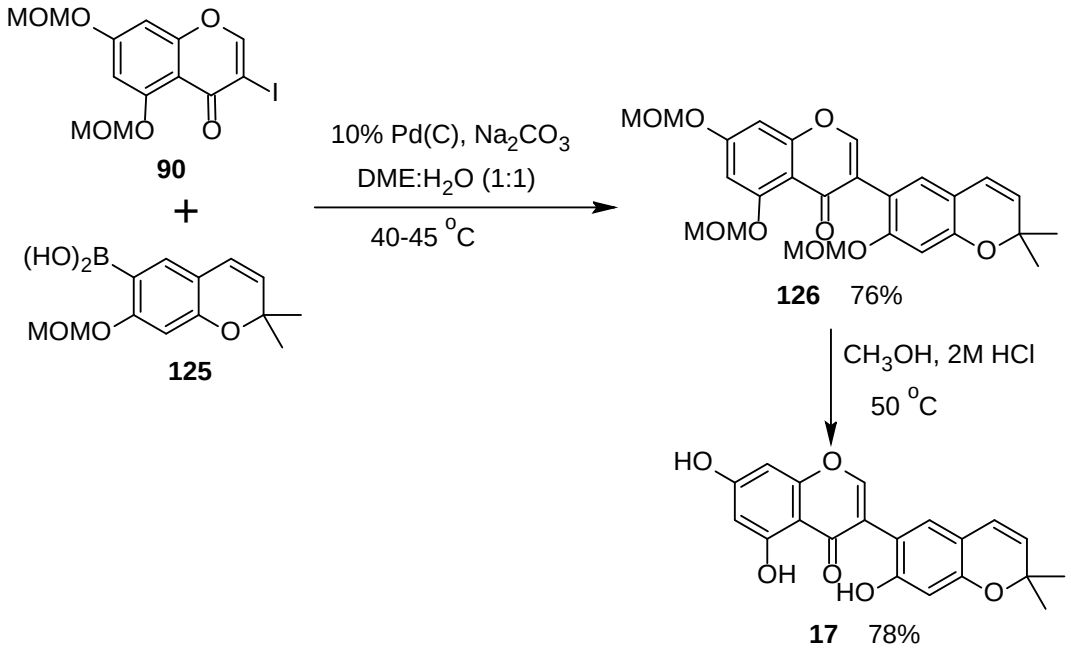


Scheme 4.26. Preparation of boronic acid **125**

The ^{1}H NMR spectrum of the boronic acid **125** displayed an AM spin system at δ_H 7.45 (1H, s, H-5) and 6.58 (1H, s, H-8) for the aromatic protons, two olefinic doublets at δ_H 5.50 and 6.31 (J = 9.9 Hz, H-3 and H-4, respectively) and a six-proton singlet at δ_H 1.43 (6H, s, 2 × CH_3) for the dimethylpyran scaffold. In addition, a broad singlet intergrating for two protons was observed at δ_H 5.63 ($B(OH)_2$) and the signals for the methoxymethyl protecting group resonated at δ_H 5.24 (2H, s, OCH_2O) and 3.50 (3H, s, OCH_3) in the ^{1}H

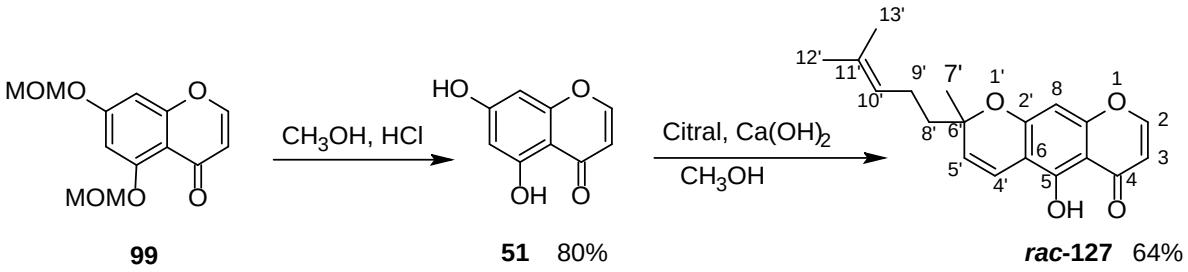
NMR spectrum of **125** (Plate 16a). The presence of the boronic acid group was further confirmed by the IR spectrum which displayed a broad band at 3380 cm^{-1} due to the OH stretches and by the appearance of a characteristic arylboronic acid signal at δ_B 29.06 in the ^{11}B NMR spectrum of compound **125**.

The Suzuki-Miyaura coupling of boronic acid **125** with 3-iodochromone **90** in the presence of Pd(C) and Na_2CO_3 in DME:H_2O gave the isoflavone **126**.[35] Cleavage of the MOM protecting groups with HCl rendered eriosemaone D (**17**)[44,45] in a 78% yield (Scheme 4.27).



Scheme 4. 27. Preparation of eriosemaone D (**17**)

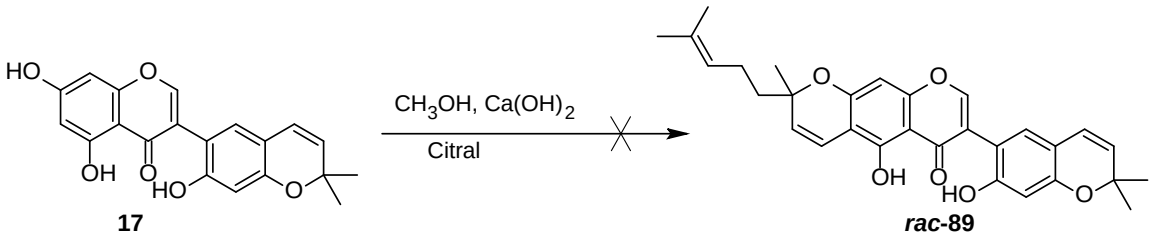
Having prepared **17**, the next step was to condense it with citral to give the geranyl derivative **89**. The feasibility of this reaction was first tested on chromone **51**. Condensation of **51** with citral gave racemic **127** in 64% yield as shown in Scheme 4.28. The presence of the *O*-cyclised geranyl moiety was evidenced by the presence of signals at δ_H 5.57 (1H, d, *J* = 10.0 Hz, H-5'), 6.75 (1H, d, *J* = 10.0 Hz, H-4'), 5.10 (1H, t, *J* = 7.2 Hz, H-10'), 2.09 (2H, dd, J = 16.1 and 8.0, H-9'), 1.75-1.81 (1H, m, H-8'b), 1.62-1.69 (4H, m, H-8'a and H-12' or 13'), 1.57 (3H, s, H-12' or 13'), 1.43 (3H, s, H-7'), indicative of the pyran chromophore with a methyl and 4-methylpent-3-enyl substituents in the ^1H NMR spectrum of **127** (Plate 19a).



99 **51** 80% ***rac*-127** 64%

Scheme 4.28. Model reaction for condensation of chromone **51** with citral

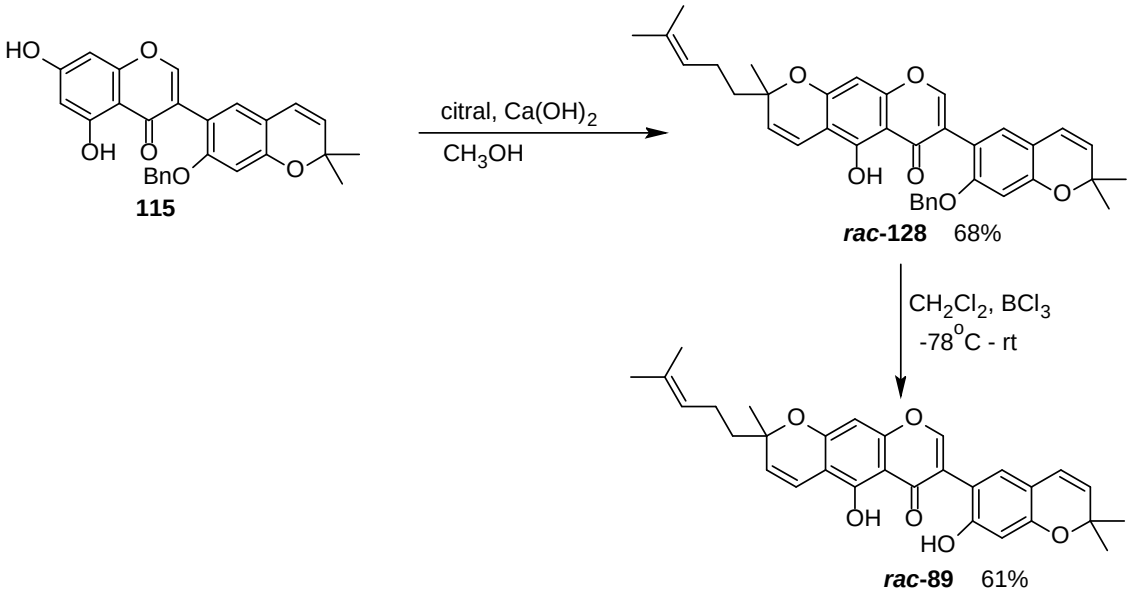
Motivated by the results from the model reaction, the condensation was performed on eriosemaone D (**17**). $Ca(OH)_2$-induced condensation of **17** with citral gave an inseparable mixture of products in which polymerised citral dominated (Scheme 4.29).



17 ***rac*-89**

Scheme 4.29. Attempted synthesis of **89**

In order to overcome the aforementioned problem, we opted to condense citral with 2'-*O*-benzyleriosemaone D (**115**) as the polarity of the resulting product would be different from that of **89**. Thus, 2'-*O*-benzyleriosemaone D **115** was reacted with citral under similar conditions to give racemic compound **128** in 68% (Scheme 4.30). Cleavage of the benzyl protecting group with BCl_3 in CH_2Cl_2 gave the targeted racemic compound **89** in a 61% yield. The ^{1}H NMR spectrum of **89** (Plate 21) showed signals similar to those of kraussianone 1 (**1**) except for the appearance of additional signals of the 4-methylpent-3-enyl and the methyl substituents [δ_H 5.09 (1H, t, *J* = 7.0 Hz, H-10'''), 2.11 (2H, dd, J = 15.8 and 7.9, H-9'''), 1.84 (1H, m, H-8'''b), 1.66-1.70 (4H, m, H-8'''a, H-12''' or 13'''), 1.57 (3H, s, H-12''' or 13'''), 1.45 (3H, s, H-7''')] instead of two equivalent methyl groups on the chromene ring attached to the A-ring. The structure was confirmed by HRMS, which gave an *m/z* of 509.1934 $[M+Na]^+$ in agreement with the calculated pseudo molecular mass of 509.1940 for $C_{30}H_{30}NaO_6$.



Scheme 4.30. Successful synthesis of **89**

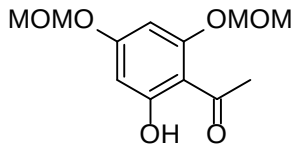
4.4 CONCLUSION

In conclusion, we have successfully synthesised the two biologically-active pyranoisoflavones, the anti-impotence pyranoisoflavone kraussianone 1 (**1**) and the anti-fungal pyranoisoflavone eriosemaone D (**17**), the simple isoflavone genistein (**103**) and the geranyl derivative of kraussianone 1 **89** by application of the Suzuki-Miyaura protocol as the key step. This route readily gave access to structurally-related isoflavones by using different boronic acid partners or by incorporation of additional substituents to the A-ring of the isoflavones. Secondly, it employed mild reaction conditions, which tolerated highly sensitive functional groups, such as the dimethylpyran ring. For instance the dimethylpyran ring constructed in the early stages of the synthetic pathway survived all the subsequent reaction conditions leading to targeted isoflavones. Furthermore, the present synthetic strategy allowed the regioselective introduction of the dimethylpyran scaffolds to the resorcinol (B-ring) and the phloroglucinol (A-ring) moieties of kraussianone 1 (**1**) and its derivatives. Therefore, this route offers an ideal synthetic strategy for the synthesis of other related bioactive isoflavonoids.

4.5 EXPERIMENTAL

The general experimental procedures are given in Chapter 3, Section 3.5.1.

4.5.1 2'-Hydroxy-4',6'-dimethoxymethoxyacetophenone (94)



$ZnBr_2$ (13.4 mg, 0.0594 mmol) was dissolved in dimethoxymethane (2.62 mL, 29.7 mmol) under an N_2 atmosphere, and then AcCl (2.12 mL, 29.7 mmol) was added dropwise to the stirred solution. The solution was stirred for an additional 2 h at room temperature and transferred *via* a cannula to an ice-cold solution of the pre-dried phloroacetophenone (**92**) (2.00 g, 11.9 mmol) and DIA (3.6 mL, 23.59 mmol) in CH_2Cl_2 (100 mL) under an N_2 atmosphere. The mixture was stirred for 3 h, diluted with saturated NH_4Cl solution and stirred for an additional 15 min. The two phases were partitioned and the aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated to give a yellow oil which was purified by column chromatography using Hex:EtOAc (7:3) as eluent to afford **94** as a colourless oil (1.53 g, 50%).

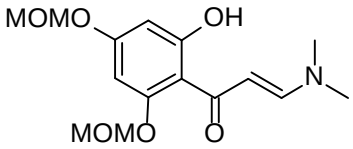
IR (neat) ν_{max} 3100, 2925, 1618 cm^{-1};

^1H NMR ($CDCl_3$, 400 MHz) δ 13.68 (1H, s, OH), 6.26 (1H, d, J = 2.4 Hz, H-3'), 6.24 (1H, d, J = 2.4 Hz, H-5'), 5.25 (2H, s, OCH_2O), 5.16 (2H, s, OCH_2O), 3.52 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 2.65 (3H, s, $COCH_3$);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.1 (C, C-1), 167.3 (C, C-2'), 163.3 (C, C-4'), 160.5 (C, C-6'), 107.3 (C, C-1'), 97.3 (CH, C-3'), 94.5 (CH, C-5'), 94.0 (CH_2, 2 × OCH_2O), 56.7 (CH_3, OCH_3), 56.4 (CH_3, OCH_3), 32.9 (CH_3, -$COCH_3$);

EIMS *m/z* 225 (10%), 209 (29), 207 (100), 191 (22), 44 (40), 40 (62).

4.5.2 3-(*N*,*N*-Dimethylamino)-1-(2'-hydroxy-4',6'-dimethoxymethoxyphenyl)propenone (93)



DMF-DMA (0.29 mL, 2.19 mmol) was added to **94** (0.28 g, 1.09 mmol) at 95 ^{o}C. The mixture was stirred at the same temperature for 1.5 h. The volatiles in the orange oil were evaporated on a rotary evaporator and the crude solid was subjected to flash chromatography using Hex:EtOAc (1:1) as eluent. Evaporation of the solvent gave **93** as a yellow oil, which solidified upon cooling (0.23 g, 68%). The solid was recrystallized to give yellow needle-like crystals (1:1 Hex:Et_2O).

mp 85-86 ^{o}C;

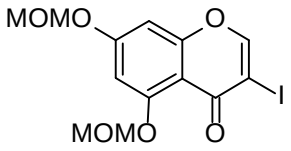
IR (KBr) ν_{max} 3445, 3163, 2913, 1606, 1233 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 15.08 (1H, s, OH-2'), 7.92 (1H, d, *J* = 12.0 Hz, H-3), 6.29 (1H, d, *J* = 12.0 Hz, H-2), 6.26 (1H, d, *J* = 2.4 Hz, H-3'), 6.17 (1H, d, *J* = 2.4 Hz, H-5'), 5.20 (2H, s, OCH_2O), 5.14 (2H, s, OCH_2O), 3.51 (3H, s, OCH_3), 3.46 (3H, s, OCH_3), 3.13 [3H, brs, $N(CH_3)_2$], 2.94 [3H, brs, $N(CH_3)_2$]; (Plate 10a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 190.6 (C, C-1), 166.9 (C, C-2'), 161.3 (C, C-4'), 158.9 (C, C-6'), 154.4 (CH, C-3), 107.1 (C, C-1'), 97.9 (CH, C-3'), 97.0 (CH, C-2), 95.2 (CH_2, OCH_2O), 94.5(CH, C-5'), 94.1 (CH_2, OCH_2O), 56.7 (CH_3, OCH_3), 56.3 (CH_3, OCH_3), 45.5 [CH_3, $N(CH_3)_2$], 37.2 [CH_3, $N(CH_3)_2$]; (Plate 10b)

HR-ESIMS *m/z* $[M + Na]^+$ 334.1267 (Calcd. for $C_{15}H_{21}NNaO_6$ 334.1267).

4.5.3 3-Iodo-5,7-dimethoxymethoxychromone (90)



To a solution of propenone **93** (0.12 g, 0.38 mmol) in CH_2Cl_2 (20 mL) was added pyridine (0.06 mL, 0.74 mmol) followed by I_2 (0.11 g, 0.44 mmol). The resulting solution was stirred at room temperature for 12 h. The reaction was quenched with saturated $Na_2S_2O_3$ solution and the two phases partitioned. The aqueous phase was extracted with CH_2Cl_2. The organic layers were combined, washed with H_2O, brine and dried over anhydrous $MgSO_4$. The solvent was evaporated to give a yellow solid. The crude product was purified on a chromatotron using Hex:EtOAc (7:3) as eluent. The solvent was evaporated to afford a cream-white solid (94 mg), which was identified (^{1}H NMR) as a mixture of **90** and 3,8-diiodo-5,7-dimethoxymethoxychromone (**95**) in the ratio of 50:7, respectively. Therefore, the percentage yield of compound **90** was 58%.

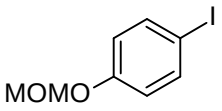
IR (KBr) ν_{max} 3179, 3099, 3058, 2958, 2904, 1622, 1445, 1275, 1140, 1036, 921, 844 cm^1;

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.09 (1H, s, H-2), 6.75 (1H, d, *J* = 2.3 Hz, H-8), 6.71 (1H, d, *J* = 2.3 Hz, H-6), 5.29 (2H, s, OCH_2O), 5.22 (2H, s, OCH_2O), 3.54 (3H, s, OCH_3), 3.49 (3H, s, OCH_3); (Plate 11a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2 (C, C-4), 161.5 (C, C-7), 159.2 (C, C-5), 158.3 (C, C-8a), 155.6 (CH, C-2), 108.8 (C, C-4a), 102.1 (CH, C-6), 96.8 (CH, C-8), 95.5 (CH_2, OCH_2O), 94.4 (CH_2, O<u>C</u>H_2O), 89.4 (C, C-3), 56.7 (CH_3, OCH_3), 56.5 (CH_3, OCH_3); (Plate 11b)

HR-ESIMS *m*/*z* $[M + Na]^+$ 392.9834 (Calcd. for $C_{13}H_{14}INaO_6$ 392.9835).

4.5.4 1-Iodo-4-methoxymethoxybenzene (101)



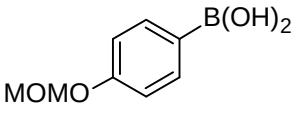
AcCl (1.94 mL, 27.3 mmol) was added dropwise to the stirred solution of dimethoxymethane (2.40 mL, 27.3 mmol) and $ZnBr_2$ (12.3 mg, 0.0546 mmol) under N_2. The solution was stirred for an additional 2 h at room temperature and transferred via a cannula to the ice-cold solution of 4-iodophenol (**100**) (2.00 g, 9.09 mmol) and DIA (4.16 mL, 27.27 mmol) in CH_2Cl_2 (100 mL) under an N_2 atmosphere. The mixture was stirred for 4 h, diluted with saturated NH_4Cl solution and stirred for an additional 15 min. The two phases were partitioned and the aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the product was purified with column chromatography using Hex:EtOAc (8:2) to afford **101** as a colorless oil (1.8 g, 75%).

IR (neat) ν_{max} 2954, 2901, 2825, 1585, 1483, 1231, 1147, 987, 818 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.56 (2H, d, *J* = 9.0 Hz**,** H- 2 and H-6), 6.82 (2H, d, *J* = 9.0 Hz, H-3 and H-5), 5.14 (2H, s, OCH_2O), 3.46 (3H, OCH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.0 (C, C-4), 138.2 (CH, C-2 and 6), 118.5 (CH, C-3 and C-5), 94.3 (CH_2, OCH_2O), 84.3 (C, C-1), 55.9 (CH_3, OCH_3).

4.5.5 4-Methoxymethoxyphenylboronic acid (102)



Triisopropyl borate (2.21 mL, 9.47 mmol) was added in one portion to the stirred solution of iodobenzene **101** (1.0 g, 3.79 mmol) in THF:Et_2O (60 mL, 1:2) under N_2. The solution was cooled to -100 ^oC using liquid N_2 and a CH_3OH bath, and then *n*-BuLi (3.79 mL of a

1.5 M solution in hexanes) was added with stirring over 5 min. After 1 h of stirring at a temperature below -78 ^{o}C, saturated NH_4Cl solution was added. The mixture was stirred for an additional 1 h and the two phases were partitioned. The aqueous phase was extracted with Et_2O. The organic phases were combined, washed with H_2O and brine, and dried over anhydrous $MgSO_4$. The solvent was evaporated to give a white solid which was subjected to column chromatography using Hex:EtOAc (4:1) as the mobile phase. The solvent was evaporated to give boronic acid **102** as a white solid (0.59 g, 85%).

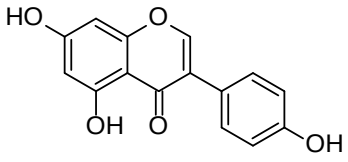
IR (KBr) ν_{max} 3436, 3359, 1651, 1606, 1235, 1016, 773 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.16 (2H, d, *J* = 8.6 Hz, H- 2 and H-6), 7.15 (2H, d, *J* = 8.6 Hz, H-3 and H-5), 5.27 (2H, s, OCH_2O), 3.52 (3H, OCH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.9 (C, C-4), 137.5 (CH, C-2 and 6), 115.6 (CH, C-3 and C-5), 94.1 (CH_2, OCH_2O), 56.1 (CH_3, OCH_3), (C-1 signal not observed);

^{11}B NMR (128 MHz, $CDCl_3$) δ 28.52.

4.5.6 Genistein (103)



4-Methoxymethoxyphenylboronic acid (**102**) (42 mg, 0.23 mmol), K_2CO_3 (51 mg, 0.37 mmol), and catalytic 10% Pd/C were added to a solution of 3-iodochromone **90** (50 mg, 0.13 mmol) in DME (2 mL) and H_2O (2 mL). The resulting mixture was stirred at 40-45 ^{o}C for 12 h. The catalyst was filtered and washed with H_2O and Et_2O. The organic solvents were evaporated and the crude product was diluted in CH_3OH (10 mL) and HCl (3 M, 5 mL). The resulting solution was refluxed for 30 min. CH_3OH was evaporated and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with H_2O, brine, and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude

product was purified by column chromatography using Hex:EtOAc (3:2) and recrystallized from EtOH to give the isoflavone **103** as a yellow crystals (23 mg, 66%).
mp 300-303 ^{o}C (Lit.[88] 301-302 ^{o}C);

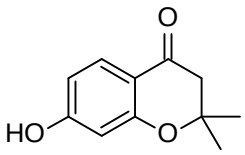
IR (KBr) ν_{max} 3434, 3181, 3069, 2922, 1652, 1621, 1176, 809, 784 cm^{-1};

^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.93 (1H, s, OH-5), 9.65 (1H, brs, OH-4'), 8.29 (1H, s, H-2), 7.36 (2H, d, J = 8.8 Hz, H-2' and 6'), 6.81 (2H, d, J = 8.8 Hz, H-3' and H-5'), 6.38 (1H, d, J = 2.1 Hz, H-6), 6.22 (1H, d, J = 2.1 Hz, H-8); (Plate 12)

^{13}C NMR (DMSO-d_6, 100 MHz,) δ 182.3 (C, C-4), 166.0 (C, C-7), 163.9 (C, C-5), 159.8 (C, C-4'), 158.9 (C, C-8a), 154.8 (CH, C-2), 131.2 (CH, C-2' and 6'), 124.8 (C, C-1'), 123.3 (C, C-3), 116.8 (CH, C-3' and 5'), 106.3 (C, C-4a), 100.1 (CH, C-6), 94.8 (CH, C-8);

HR-ESIMS (m/z) [M-H]$^{-}$ 269.0452 (Calcd. for $C_{15}H_9O_5$ 269.0450).

4.5.7 7-Hydroxy-2,2-dimethylchroman-4-one (33)



Resorcinol (**32**) (1.00 g, 9.10 mmol) and 3-methyl-2-butenoic acid (0.910 g, 9.10 mmol) were added simultaneously under N_2 to a stirred mixture of CH_3SO_3H (14.5 mL, 0.218 mol) and P_2O_5 (0.70 g, 5.10 mmol) at 70 ^{o}C. The reaction mixture was stirred at the same temperature for 30 min, cooled to room temperature and poured into ice-water. The aqueous phase was extracted with Et_2O, and the combined organic extracts were washed with H_2O, brine, and dried over anhydrous $MgSO_4$. The solvent was evaporated to afford a yellow solid. The solid was purified by flash chromatography using Hex:EtOAc (3:2) as mobile phase to give chromanone **33** as a light yellow solid (1.61 g, 92%). Recrystallization of the solid afforded white crystals (9:1 CH_2Cl_2:Et_2O).

mp 170.0-171.5 ^{o}C (Lit.[60] 172-174 ^{o}C);

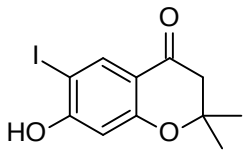
IR (KBr) v_{max} 3129, 2967, 2837, 1578, 1252, 1169, 1126, 854 cm^{-1};

^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.58 (1H, d, *J* = 8.4 Hz, H-5), 6.43 (1H, dd, *J* = 1.9 and 8.4 Hz, H-6), 6.24 (1H, d, *J* = 1.9 Hz, H-8), 2.65 (2H, s, H-3), 1.36 (6H, s, 2 × CH_3);

^{13}C NMR (DMSO-d_6, 100 MHz) δ 190.1 (C, C-4), 164.7 (C, C-7), 161.4 (C, C-8a), 127.9 (CH, C-5), 112.8 (C, C-4a), 109.8 (CH, C-6), 102.8 (CH, C-8), 79.3 (C, C-2), 47.8 (CH_2, C-3), 26.2 (2 × CH_3);

HR-ESIMS *m*/*z* $[M + Na]^+$ 215.0687 (Calcd. for $C_{11}H_{12}NaO_3$ 215.0684).

4.5.8 7-Hydroxy-6-iodo-2,2-dimethylchroman-4-one (107)



A solution of chromanone **33** (0.15 g, 0.78 mmol), HIO_3 (0.03 g, 0.17 mmol), and I_2 (0.08 g, 0.32 mmol) in CH_3OH:H_2O (3:1, 10 mL) was heated to 85 ^{o}C for 12 h. The CH_3OH was evaporated to give a yellow residue, which was diluted with EtOAc and H_2O. The two phases were partitioned, and the aqueous phase was extracted with EtOAc. The combined organic extracts were washed with saturated $Na_2S_2O_3$ solution, H_2O and brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave a yellow solid, which was washed with hot CH_2Cl_2, cooled and filtered. The residue was recrystallized from CH_2Cl_2:EtOAc (8:2) to give pure **107** (0.16 g, 65%). The mother liquor, which contained mostly 7-hydroxy-8-iodo-2,2-dimethyl-4-chromanone (**111**), was evaporated to give a yellow solid (49 mg).

mp 195-197 ^{o}C;

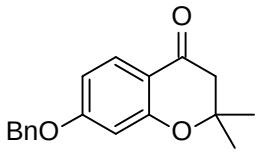
IR (KBr) v_{max} 2978, 2922, 2702, 1641, 1563, 1327, 1259, 849 cm^{1};

^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.96 (1H, s, H-5), 6.40 (1H, s, H-8), 2.67 (2H, s, H-3), 1.36 (6H, s, 2 × CH_3);

^{13}C NMR (DMSO-d_6, 100 MHz) δ 189.2 (C, C-4), 163.1 (C, C-7), 161.1 (C, C-8a), 136.3 (CH, C-5), 114.9 (C, C-4a), 102.8 (CH, C-8), 79.8 (C, C-2), 76.4 (C, C-6), 47.4 (CH_2, C-3), 26.1 (2 × CH_3);

HR-ESIMS *m/z* $[M + Na]^+$ 340.9656 (Calcd. for $C_{11}H_{11}INa\ O_3$ 340.9651).

4.5.9 7-Benzyloxy-2,2-dimethylchroman-4-one (114)



K_2CO_3 (2.16 g, 15.6 mmol) and BnBr (1.20 mL, 10.4 mmol) were added under N_2 to a stirred solution of chromanone **33** (1.00 g, 5.21 mmol) in dry CH_3CN (30 mL). The reaction mixture was refluxed for 8 h under N_2. The mixture was cooled to room temperature, acidified with 2 M HCl (40 mL) and extracted with EtOAc. The organic extracts were combined and washed with H_2O, brine, then dried over anhydrous $MgSO_4$. The solvent was removed with a rotary evaporator and the crude product purified on a chromatotron using Hex:EtOAc (7:3) solvent mixture. The product **114** was obtained as white crystals after evaporation of the solvent and recrystallization from Hex (1.30 g, 88%).

mp 55-56 ^{o}C;

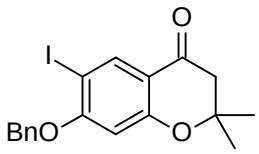
IR (KBr) ν_{max} 3324, 2982, 2964, 2890, 1672, 1604, 1441, 1319, 1258, 1164, 1000, 988, 840 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81 (1H, d, *J* = 8.4 Hz, H-5), 7.32-7.43 (5H, m, $\underline{Ph}CH_2$), 6.62 (1H, dd, *J* = 1.9 and 8.4 Hz, H-6), 6.46 (1H, d, *J* = 1.9 Hz, H-8), 5.08 (2H, s, $PhC\underline{H_2}$), 2.66 (2H, s, H-3), 1.45 (6H, s, 2 × CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.2 (C, C-4), 165.7 (C, C-7), 161.8 (C, C-8a), 136.2 ($\underline{Ph}CH_2$), 128.7 ($\underline{Ph}CH_2$), 128.3 (CH, C-5), 128.2 ($\underline{Ph}CH_2$), 127.5 ($\underline{Ph}CH_2$), 113.1 (C, C-4a), 109.8 (CH, C-6), 102.2 (CH, C-8), 79.6 (C, C-2), 70.2 (CH_2, $PhCH_2$), 48.6 (CH_2, C-3), 26.7 (2 × CH_3);

HR-ESIMS *m*/*z* $[M + Na]^+$ 305.1149 (Calcd. for $C_{18}H_{18}NaO_3$ 305.1154).

4.5.10 7-Benzyloxy-6-iodo-2,2-dimethylchroman-4-one (108)



CF_3CO_2Ag (94.0 mg, 0.426 mmol) was added to a solution of **114** (100 mg, 0.355 mmol) in $CHCl_3$ (10 mL). The mixture was stirred for 5 min, and then a solution of I_2 (90.0 mg, 0.355 mmol) in $CHCl_3$ (20 mL) was added dropwise to the stirred suspension. The resulting mixture was stirred at room temperature for 12 h. The solution was filtered to remove the AgI, and the filtrate was washed with 10% $Na_2S_2O_3$ solution, $NaHCO_3$ solution, H_2O, and brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent was evaporated to give a cream-white solid. The solid was purified by flash chromatography using Hex:EtOAc (6:4) as eluent to afford iodochromanone **108** as a white solid (136 mg, 94%). Recrystallization of the solid from CH_3OH gave colorless needles.

mp 128-130 ^{o}C;

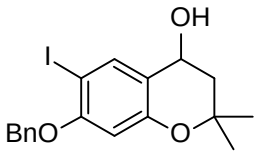
IR (KBr) ν_{max} 2983, 1661, 1583, 1262 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.28 (1H, s, H-5), 7.48 (2H, d, *J* = 7.5 Hz, PhCH_2), 7.41 (2H, t, *J* = 7.5 Hz, PhCH_2), 7.34 (1H, t, *J* = 7.3 Hz, PhCH_2), 6.40 (1H, s, H-8), 5.14 (2H, s, PhCH_2), 2.65 (2H, s, H-3), 1.44 (6H, s, 2 × CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.2 (C, C-4), 162.8 (C, C-7), 162.1 (C, C-8a), 137.3 (CH, C-5), 135.6 (PhCH_2), 128.7 (PhCH_2), 128.2 (PhCH_2), 127.0 (PhCH_2), 116.0 (C, C-4a), 101.3 (CH, C-8), 80.1 (C, C-2), 77.2 (C, C-6), 71.1 (CH_2, PhCH_2), 48.2 (CH_2, C-3), 26.6 (2 × CH_3);

HR-ESIMS *m/z* $[M + Na]^+$ 431.0118 (Calcd. for $C_{18}H_{17}INaO_3$ 431.0120).

4.5.11 7-Benzyloxy-6-iodo-2,2-dimethylchroman-4-ol (109)



To a solution of **108** (0.295 g, 1.05 mmol) in THF (5 mL) was added $NaBH_4$ (0.200 g, 5.25 mmol) in EtOH (20 mL). The resulting solution was refluxed for 3 h. The reaction mixture was cooled to room temperature, quenched with saturated NH_4Cl solution and extracted with EtOAc. The organic phases were combined, washed with saturated $NaHCO_3$ solution, brine, H_2O, and dried over anhydrous $MgSO_4$. The organic layer was concentrated and the product was purified by flash chromatography using Hex:EtOAc (1:1) solvent system to afford chromanol **109** as a colorless oil (0.282 g, 95%), which eventually solidified. The solid was recrystallized from Hex:Et_2O (3:2) to give white crystals.

mp 90-92 ^{o}C;

IR (KBr) ν_{max} 3782, 3467, 2982, 2929, 1579, 1460, 1278, 1163, 1129, 1035 cm^{-1};

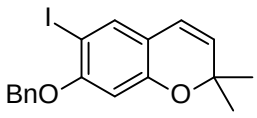
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83 (1H, s, H-5), 7.49 (2H, d, *J* = 7.5 Hz, PhCH_2), 7.39 (2H, t, *J* = 7.6 Hz, PhCH_2), 7.32 (1H, t, *J* = 7.3 Hz, PhCH_2), 6.36 (1H, s, H-8), 5.07 (2H, s, PhCH_2), 4.75 (1H, ddd, *J* = 8.5, 7.3 and 6.0 Hz, H-4), 2.17 (1H, br d, *J* = 7.3 Hz, OH-4),

2.10 (1H, dd, J = 6.0 and 13.6 Hz, H-3b), 1.80 (1H, dd, J = 8.5 and 13.6 Hz, H-3a), 1.43 (3H, s, CH_3), 1.30 (3H, s, CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.8 (C, C-7), 154.9 (C, C-8a), 138.0 (CH, C-5), 136.6 (PhCH_2), 128.5 (PhCH_2), 127.9 (PhCH_2), 127.0 (PhCH_2), 119.5 (C, C-4a), 101.8 (CH, C-8), 76.2 (C, C-2), 75.2 (C, C-6), 70.8 (CH_2, PhCH_2), 62.9 (C, C-4), 42.6 (CH, C-3), 28.8 (CH_3), 25.9 (CH_3);

HR-ESIMS m/z $[M + Na]^+$ 433.0280 (Calcd. for $C_{18}H_{19}INaO_3$ 433.0277).

4.5.12 7-Benzyloxy-6-iodo-2,2-dimethylchromene (110)



p-TSOH (0.012 g, 0.058 mmol) was added to a solution of chromanol **109** (0.120 g, 0.290 mmol) in THF (15 mL) under N_2. The resulting solution was refluxed under N_2 for 2 h. A 10% NaOH solution was added to the cooled reaction mixture, and the mixture was extracted with Et_2O. The combined organic extracts were washed with H_2O, brine solution, and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded a colourless oil, which solidified upon cooling. The solid was successively recrystallized from Hex and CH_3OH to give iodochromene **110** as white fluffy crystals (0.112 g, 97%).

mp 63-65 ^oC;

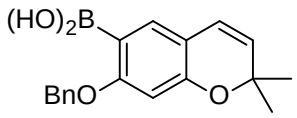
IR (KBr) ν_{max} 3032, 2972, 2924, 1713, 1602, 1480, 1454, 1358, 1158, 736 cm^{-1};

^1H NMR ($CDCl_3$, 400 MHz) δ 7.49 (2H, d, J = 7.3 Hz, PhCH_2), 7.40 (2H, t, J = 7.3 Hz, PhCH_2), 7.37 (1H, s, H-5), 7.32 (1H, t, J = 7.2 Hz, PhCH_2), 6.41 (1H, s, H-8), 6.22 (1H, d, J = 9.9 Hz, H-4), 5.48 (1H, d, J = 9.9 Hz, H-3), 5.10 (2H, s, PhCH_2), 1.42 (6H, s, 2 × CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.9 (C, C-7), 154.6 (C, C-8a), 136.5 (<u>Ph</u>CH_2), 136.0 (CH, C-5), 128.7 (CH, C-3), 128.5 (<u>Ph</u>CH_2), 127.9 (<u>Ph</u>CH_2), 127.0 (<u>Ph</u>CH_2), 120.9 (CH, C-4), 117.0 (C, C-4a), 102.0 (CH, C-8), 76.8 (C, C-2), 74.8 (C, C-6), 70.9 (CH_2, Ph<u>C</u>H_2), 28.0 (2 × CH_3);

HR-ESIMS *m/z* $[M + Na]^+$ 415.0176 (Calcd. for $C_{18}H_{17}INaO_2$ 415.0171).

4.5.13 7-Benzyloxy-2,2-dimethylchromene-6-boronic acid (91)



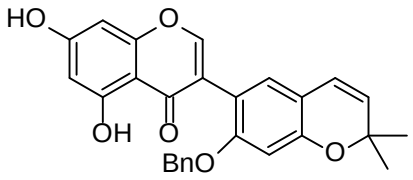
Boronic acid **91** was prepared from aryl iodide **110** (1.00 g, 2.55 mmol) according to the procedure given in **4.5.5**. The title compound was obtained as a fluffy white solid (0.55 g, 70%) after purification with column chromatography using Hex:EtOAc (4:1) as the mobile phase.

IR (KBr) ν_{max} 3367, 3027, 2969, 2928, 1643, 1607, 1567, 1449 cm^{-1};
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.47 (1H, s, H-5), 7.36-7.42 (5H, m, <u>Ph</u>CH_2), 6.46 (1H, s, H-8), 6.32 (1H, d, *J* = 10.1 Hz, H-4), 5.54 (2H, s, $B(OH)_2$), 5.49 (1H, d, *J* = 10.1 Hz, H-3), 5.08 (2H, s, PhC<u>H</u>$_2$), 1.44 (6H, s, 2 × CH_3); (Plate 13a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.6 (C, C-7), 157.4 (C, C-8a), 135.8 (<u>Ph</u>CH_2), 134.8 (CH, C-5), 128.9 (<u>Ph</u>CH_2), 128.6 (<u>Ph</u>CH_2), 127.9 (C, C-3), 127.8 (<u>Ph</u>CH_2), 121.8 (CH, C-4), 114.9 (C, C-4a), 100.1 (CH, C-8), 77.1 (C, C-2), 70.9 (CH_2, Ph<u>C</u>H_2), 28.3 (2 × CH_3), (C-6 signal not observed); (Plate 13b)

^{11}B NMR ($CDCl_3$, 128 MHz) δ_B 28.74.

4.5.14 2'-Benzyloxy-5,7-dihydroxy-6'',6''-dimethylpyrano[2'',3'':4',5']isoflavone (115)



Compound **115** was prepared from 3-iodochromone **90** (0.500 g, 1.28 mmol) and boronic acid **91** (0.550 g, 1.77 mmol) according to the procedure in **4.5.6.** The reaction mixture was subjected to column chromatography using Hex:EtOAc (3:2) as eluent to give **115** as a yellowish oil which solidified upon cooling (0.41 g, 72%). Recrystallization of the solid from Hex:Et_2O (1:1) gave white crystals.

mp 93-95 ^{o}C;

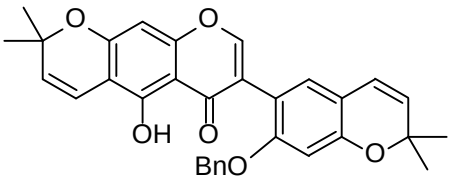
IR (KBr) ν_{max} 3255, 2974, 2929, 1650, 1615, 1497, 1277, 1151, 1038, 832, 699 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.79 (1H, s, OH-5), 7.77 (1H, s, H-2), 7.22-7.34 (5H, m, PhCH_2), 6.92 (1H, s, H-6'), 6.50 (1H, s, H-3'), 6.23 (1H, d, *J* = 9.8 Hz, H-4''), 6.21 (1H, d, *J* = 2.2 Hz, H-8), 6.19 (1H, d, *J* = 2.2 Hz, H-6), 5.44 (1H, d, *J* = 9.8 Hz, H-5''), 5.03 (2H, s, PhCH_2), 1.40 (6H, s, H-7'' and H-8''); (Plate 14a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.0 (C, C-4), 163.0 (C, C-7), 162.5 (C, C-5), 158.0 (C, C-8a), 157.5 (C, C-2'), 154.8 (C, C-4'), 154.6 (CH, C-2), 136.6 (PhCH_2), 129.2 (CH, C-6'), 128.5 (PhCH_2), 128.2 (CH, C-5''), 127.9 (PhCH_2), 127.3 (PhCH_2), 121.5 (CH, C-4''), 120.8 (C, C-3), 114.6 (C, C-5'), 112.0 (C, C-1'), 105.8 (C, C-4a), 101.8 (CH, C-3'), 99.6 (CH, C-6), 94.1 (CH, C-8), 76.8 (C, C-6''), 70.9 (CH_2, PhCH_2), 28.2 (2 × CH_3, C-7'' and C-8''); (Plate 14b)

HR-ESIMS *m*/*z* [M + Na]$^{+}$ 465.1312 (Calcd. for $C_{27}H_{22}NaO_6$ 465.1314).

4.5.15 2'-*O*-Benzylkraussianone 1 (116)



3-Methyl-2-butenal (0.18 mL, 1.85 mmol) was added under an N_2 atmosphere to a stirred mixture of $Ca(OH)_2$ (55.0 mg, 0.740 mmol) and **115** (82.0 mg, 0.185 mmol) in CH_3OH (10 mL). The mixture was stirred for 3 days at room temperature. CH_3OH was evaporated and the reaction mixture was diluted with EtOAc and H_2O. The two phases were partitioned and the aqueous phase was extracted with EtOAc**.** The organic layers were combined, washed with 1M HCl, H_2O, brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent followed by column chromatography gave **116** as a yellowish oil (70 mg, 74%).

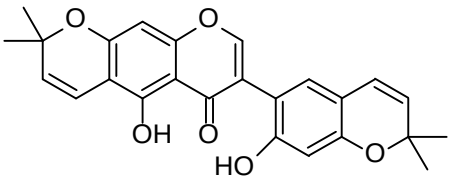
IR (KBr) ν_{max} 3422, 3034, 2974, 2927, 1651,1615, 1496, 1463, 1287, 1146, 1058, 828, 696 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.22 (1H, s, OH-5), 7.79 (1H, s, H-2), 7.27-7.34 (5H, m, PhCH_2), 6.96 (1H, s, H-6'), 6.73 (1H, d, *J* = 10.0 Hz, H-4'''), 6.52 (1H, s, H-3'), 6.31 (1H, s, H-8), 6.28 (1H, d, *J* = 9.8 Hz, H-4''), 5.61 (1H, d, *J* = 10.0 Hz, H-5'''), 5.49 (1H, d, *J* = 9.8 Hz, H-5''), 5.04 (2H, s, PhCH_2), 1.47 (6H, s, H-7''' and H-8'''), 1.44 (6H, s, H-7'' and H-8''); (Plate 15a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.9 (C, C-4), 159.3 (C, C-7), 157.5 (C, C-2'), 157.3 (C, C-8a), 156.9 (C, C-5), 154.7 (C, C-4'), 154.4 (CH, C-2), 136.8 (PhCH_2), 129.1 (CH, C-6'), 128.5 (PhCH_2), 128.1 (CH, C-5''), 128.0 (CH, C-5'''), 127.8 (PhCH_2), 127.2 (PhCH_2), 121.6 (CH, C-4''), 120.6 (C, C-3), 115.6 (CH, C-4'''), 114.5 (C, C-5'), 112.2 (C, C-1'), 106.2 (C, C-4a), 105.5 (C, C-6), 101.8 (CH, C-3'), 94.8 (CH, C-8), 77.9 (C, C-6'''), 76.8 (C, C-6''), 70.8 (CH_2, PhCH_2), 28.3 (2 × CH_3, C-7''' and C-8'''), 28.2 (2 × CH_3, C-7'' and C-8''); (Plate 15b)

HR-ESIMS *m*/*z* $[M + Na]^+$ 531.1782 (Calcd. for $C_{32}H_{28}NaO_6$ 531.1784).

4.5.16 Kraussianone 1 (1)



BCl_3 (0.5 mL, 1M in heptane) was added to a solution of **116** (50.0 mg, 0.0983 mmol) in CH_2Cl_2 (5 mL) cooled to -80 ^oC. The mixture was stirred under N_2 at the temperature below -68 ^oC for 30 min. The reaction was quenched with H_2O and extracted with CH_2Cl_2. The combined organic extracts were washed with H_2O, brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave a yellow oil which was purified by chromatotron using CH_2Cl_2 to give **1.1** (24 mg, 69%) as a yellow solid. The solid was recrystallized from Hex:CH_3OH (4:1).

mp 186-188 ^oC (Lit. 185-187 ^oC);

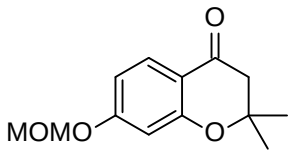
IR (KBr) ν_{max} 3336, 2975, 2872, 1649, 1615, 1460, 1132, 773 cm^{-1};

^1H NMR ($CDCl_3$, 400 MHz) δ 12.55 (1H, s, OH-5), 8.27 (1H, s, OH-2'), 7.73 (1H, s, H-2), 6.75 (1H, s, H-6'), 6.74 (1H, d, *J* = 10.0 Hz, H-4'''), 6.53 (1H, s, H-3'), 6.40 (1H, s, H-8), 6.28 (1H, d, *J* = 9.8 Hz, H-4''), 5.65 (1H, d, *J* = 10.0 Hz, H-5'''), 5.52 (1H, d, *J* = 9.8 Hz, H-5''), 1.49 (6H, s, H-7''' and H-8'''), 1.44 (6H, s, H-7'' and H-8''); (Plate 9a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.0 (C, C-4), 160.4 (C, C-7), 157.2 (C, C-2'), 157.1 (C-8a), 156.4 (C, C-5), 155.7 (C, C-4'), 154.7 (CH, C-2), 128.8 (CH, C-5''), 128.6 (CH, C-5'''), 127.1 (CH, C-6'), 123.1 (C, C-3), 121.3 (CH, C-4''), 115.28 (CH and C, C-4''' and C-5'), 112.1 (C, C-1'), 107.3 (CH, C-3'), 106.1 (C, C-6), 105.5 (C, C-4a), 95.0 (CH, C-8), 78.5 (C, C-6'''), 77.2 (C, C-6''), 28.4 (2 × CH_3, C-7''' and C-8'''), 28.2 (2 × CH_3, C-7'' and C-8''); (Plate 9b)

HR-ESIMS *m/z* $[M + Na]^+$ 441.1317 (Calcd. for $C_{25}H_{22}NaO_6$ 441.1314).

4.5.17 7-Methoxymethoxy-2,2-dimethylchroman-4-one (121)



Compound **121** was prepared from **33** (6.00 g, 31.3 mmol) according to the procedure given in **4.5.4**. The title compound was obtained as a white solid (6.5 g, 88%) after purication by silica gel column chromatography using Hex:EtOAc (7:3) solvent system.

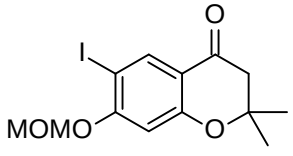
IR (neat) ν_{max} 3097, 2970, 2929, 1672, 1605, 1256, 1149, 998, 967 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.73 (1H, d, *J* = 8.8 Hz, H-5), 6.56 (1H, dd, *J* = 2.3 and 8.8 Hz, H-6), 6.24 (1H, d, *J* = 2.3 Hz, H-8), 5.12 (2H, s, OCH_2O), 3.40 (3H, s, OCH_3), 2.59 (2H, s, H-3), 1.38 (6H, s, 2 × CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 190.8 (C, C-4), 163.5 (C, C-7), 161.5 (C, C-8a), 128.0 (CH, C-5), 114.8 (C, C-4a), 110.0 (CH, C-6), 103.7 (CH, C-8), 93.9 (CH_2, OCH_2O), 79.3 (C, C-2), 56.1 (CH_3, OCH_3), 48.4 (CH_2, C-3), 26.5 (2 × CH_3);

HR-ESIMS *m*/*z* $[M + Na]^+$ 237.1128 (Calcd. for $C_{11}H_{12}NaO_3$ 237.1127).

4.5.18 6-Iodo-7-methoxymethoxy-2,2-dimethylchroman-4-one (122)



Compound **121** (5.00 g, 21.2 mmol) was iodinated according to the procedure given in **4.5.10** to give 6-iodochromanone **122** (6.5 g, 85%) as a white solid after flash chromatography with Hex:EtOAc (7:3).

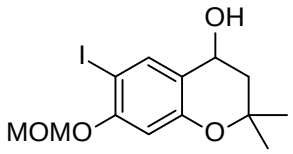
IR (neat) ν_{max} 2948, 1678, 1585, 1152 cm^{1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.25 (1H, s, H-5), 6.60 (1H, s, H-8), 5.25 (2H, s, OCH_2O), 3.50 (3H, s, OCH_3), 2.67 (2H, s, H-3), 1.44 (6H, s, 2 × CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 190.0 (C, C-4), 161.7 (C, C-7), 161.5 (C, C-8a), 137.3 (CH, C-5), 116.6 (C, C-4a), 103.2 (CH, C-8), 94.9 (CH_2, OCH_2), 80.0 (C, C-2), 77.2 (C, C-6), 56.7 (CH_3, OCH_3), 48.3 (CH_2, C-3), 26.6 (2 × CH_3);

HR-ESIMS *m*/*z* $[M + H]^+$ 363.0092 (Calcd. for $C_{13}H_{16}IO_4$ 363.0093).

4.5.19 6-Iodo-7-methoxymethoxy-2,2-dimethylchroman-4-ol (123)



Iodochromanone **122** (5.00 g, 13.8 mmol) was reduced following the procedure given in **4.5.11** to afford chromanol **123** as a colourless oil (4.0 g, 80%) after flash chromatography with Hex:EtOAc (1:1).

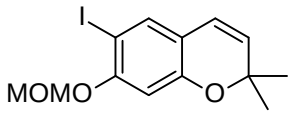
IR (neat) ν_{max} 3377, 2974, 1704, 1602, 1568, 1478, 1151, 1013 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (1H, s, H-5), 6.55 (1H, s, H-8), 5.18 (2H, s, OCH_2O), 4.78 (1H, dd, *J* = 6.2 and 8.5 Hz, H-4), 3.49 (3H, s, OCH_3), 2.12 (1H, dd, *J* = 6.2 and 13.6 Hz, H-3b), 1.81 (1H, dd, *J* = 8.5 and 13.6 Hz, H-3a), 1.79 (1H, brs, OH-4), 1.43 (3H, s, CH_3), 1.30 (3H, s, CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.8 (C, C-7), 154.9 (C, C-8a), 138.0 (CH, C-5), 119.5 (C, C-4a), 101.8 (CH, C-8), 94.9 (CH_2, OCH_2), 76.2 (C, C-2), 75.2 (C, C-6), 62.9 (C, C-4), 56.7 (CH_3, OCH_3), 42.6 (CH, C-3), 28.8 (CH_3), 25.9 (CH_3);

HR-ESIMS *m*/*z* $[M + Na]^+$ 387.0071 (Calcd. for $C_{13}H_{17}INaO_4$ 387.0069).

4.5.20 6-Iodo-7-methoxymethoxy-2,2-dimethylchromene (124)



Dimethylchromene **124** was synthesised by dehydration of chromanol **123** (3.80 g, 10.4 mmol) according to the procedure in **4.5.12**. The title compound was obtained as a colourless oil (2.6 g, 72%) after purification with column chromatography using Hex:EtOAc (8:2) as eluent.

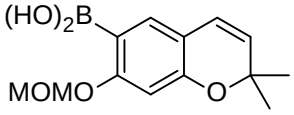
IR (neat) ν_{max} 2973, 2931, 1713, 1600, 1481, 1353, 1147, 1009 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33 (1H, s, H-5), 6.58 (1H, s, H-8), 6.20 (1H, d, J = 9.9 Hz, H-4), 5.49 (1H, d, J = 9.9 Hz, H-3), 5.17 (2H, s, OCH_2O), 3.49 (3H, s, OCH_3), 1.41 (6H, s, 2 × CH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.3 (C, C-7), 154.4 (C, C-8a), 135.6 (CH, C-5), 129.1 (CH, C-3), 120.7 (CH, C-4), 117.7 (C, C-4a), 103.9 (CH, C-8), 94.9 (CH_2, OCH_2O), 76.7 (C, C-2), 75.1 (C, C-6), 56.3 (CH_3, OCH_3), 28.0 (2 × CH_3);

HR-ESIMS m/z $[M + Na]^+$ 368.991 (Calcd. for $C_{13}H_{15}INaO_3$ 368.9964).

4.5.21 7-Methoxymethoxy-2,2-dimethylchromene-6-boronic acid (125)



Compound **125** was prepared from iodochromene **124** (2.30 g, 6.64 mmol) according to the procedure given in **4.5.5**. Pure **125** was obtained in 79% (1.32 g, white solid) after purification with column chromatography using Hex:EtOAc (7:3).

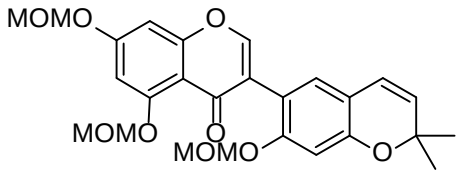
IR (neat) ν_{max} 3380, 2945, 1644, 1609, 1569, 1448, 1014, 979 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.45 (1H, s, H-5), 6.58 (1H, s, H-8), 6.31 (1H, d, *J* = 9.9 Hz, H-4), 5.63 (2H, s, $B(OH)_2$), 5.50 (1H, d, *J* = 9.9 Hz, H-3), 5.24 (2H, s, OCH_2O), 3.50 (3H, s, OCH_3), 1.43 (6H, s, 2 × CH_3); (Plate 16a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.6 (C, C-7), 157.2 (C, C-8a), 134.5 (C, C-6), 128.3 (C, C-3), 121.7(CH, C-4), 115.6 (C, C-4a), 102.0 (CH, C-8), 94.8 (CH_2, OCH_2O), 77.1 (C, C-2), 56.5 (CH_3, OCH_3), 28.3 (2 × CH_3), (C-6 signal not observed); (Plate 16b)

^{11}B NMR ($CDCl_3$, 128 MHz) δ_B 29.06.

4.5.22 2',5,7-Trimethoxymethoxy-6'',6''-dimethylpyrano[2'',3'':4',5']isoflavone (126)



Boronic acid **125** (1.30 g, 4.92 mmol), K_2CO_3 (1.32 g, 9.33 mmol), and catalytic 10% Pd/C were added to a solution of 3-iodochromone **90** (1.29 g, 3.28 mmol) in DME (10 mL) and H_2O (10 mL). The resulting mixture was stirred at 40-45 ^{o}C for 12 h. The catalyst was filtered and washed with H_2O and Et_2O. The mixture was extracted with Et_2O, washed with water and brine. The solvent was evaporated and the crude product was purified by column chromatography using Hex:EtOAc (7:3) to give compound **126** (1.2 g, 76%) as a yellow oil.

IR (neat) ν_{max} 3450, 2974, 2932, 1645, 1611, 1271, 1138, 1029 cm^{-1};

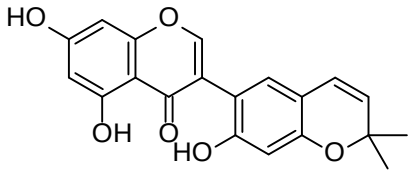
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (1H, s, H-2), 6.93 (1H, s, H-6'), 6.73 (1H, d, *J* = 2.2 Hz, H-8), 6.72 (1H, d, *J* = 2.2 Hz, H-6), 6.63 (1H, s, H-3'), 6.24 (1H, d, *J* = 9.8 Hz, H-4''), 5.47 (1H, d, *J* = 9.8 Hz, H-5''), 5.28 (2H, s, OCH_2O), 5.21 (2H, s, OCH_2O), 5.06 (2H, s,

OCH_2O), 3.51 (3H, s, OCH_3), 3.48 (3H, s, OCH_3), 3.38 (3H, s, OCH_3), 1.41 (6H, s, H-7" and H-8"); (Plate 17a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.2 (C, C-4), 161.0 (C, C-7), 159.3 (C, C-5), 158.4 (C, C-8a), 155.9 (C, C-2'), 154.1 (C, C-4'), 152.1 (CH, C-2), 129.2 (CH, C-6'), 128.3 (CH, C-5"), 122.9 (CH, C-4"), 121.6 (C, C-3), 115.1 (C, C-5'), 113.6 (C, C-1'), 111.4 (C, C-4a), 103.5 (CH, C-3'), 101.9 (CH, C-6), 97.1 (CH, C-8), 95.4 (CH_2, OCH_2O), 95.0 (CH_2, OCH_2O), 94.2 (CH_2, OCH_2O), 76.5 (C, C-6"), 56.4 (CH_3, OCH_3), 56.3 (CH_3, OCH_3), 56.0 (CH_3, OCH_3), 28.2 (2 × CH_3, C-7" and C-8"); (Plate 17b)

HR-ESIMS *m/z* $[M + Na]^+$ 507.1632 (Calcd. for $C_{26}H_{28}NaO_9$ 507.1631).

4.5.23 2',5,7-Trihydroxy-6'',6''-dimethylpyrano[2'',3'':4',5']isoflavone (Eriosemaone D) (17)



Method A

Compound **115** (10 mg, 0.023 mmol) was debenzylated under similar conditions to those in procedure **4.5.16** and the product was purified on a chromatotron to give **17** as a yellow solid (6 mg, 75%).

Method B

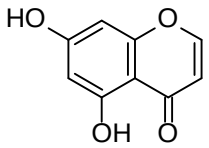
To a solution of **126** (1.0 g, 2.06 mmol) in CH_3OH (25 ml) was added HCl (3 M, 10 mL). The resulting solution was refluxed for 20 min, then CH_3OH was evaporated and the reaction mixture was diluted with EtOAc and water. The two phases were partitioned and the organic layer was washed with water, brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude product was purified by column chromatography using Hex:EtOAc (7:3) to afford eriosemaone D (**17**) as a yellow solid (0.56 g, 78%).

^{1}H NMR (CD_3OD, 400 MHz) δ 8.04 (1H, s, H-2), 6.92 (1H, s, H-6'), 6.39 (1H, d, *J* = 2.3 Hz, H-8), 6.36 (1H, s, H-3'), 6.33 (1H, d, *J* = 10.0 Hz, H-4''), 6.27 (1H, d, *J* = 2.3 Hz, H-6), 5.55 (1H, d, *J* = 9.8 Hz, H-5''), 1.43 (6H, s, H-7'' and H-8''); (Plate 18a)

^{13}C NMR (CD_3OD, 100 MHz) δ 182.8 (C, C-4), 166.2 (C, C-7), 163.9 (C, C-5), 160.0 (C, C-2'), 158.0 (C, C-8a), 157.1 (C, C-4'), 156.2 (CH, C-2), 130.5 (CH, C-6'), 129.1 (CH, C-5''), 123.1 (CH, C-4''), 122.4 (C, C-3), 115.6 (C, C-5'), 112.2 (C, C-1'), 106.6 (C, C-4a), 105.3 (CH, C-3'), 100.5 (CH, C-6), 95.2 (CH, C-8), 77.8 (C, C-6''), 28.6 (2 × CH_3, C-7'' and C-8''); (Plate 18b)

HR-ESIMS *m*/*z* $[M + Na]^+$ 375.0845 (Calcd. for $C_{20}H_{16}NaO_6$ 375.0845).

4.5.24 5,7-Dihydroxychromone (51)

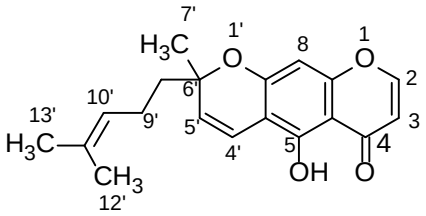


Chromone **99** (50 mg, 0.188 mmol) was deprotected according to the procedure in **4.5.23** to afford **51** (27 mg, 80%) as a cream white solid after column chromatographic purification with Hex:EtOAc (4:6).

^{1}H NMR (CD_3OD, 400 MHz) δ 7.97 (1H, d, *J* = 6.0 Hz, H-2), 6.34 (1H, d, *J* = 2.2 Hz, H-8), 6.22 (1H, d, *J* = 2.2 Hz, H-6), 6.20 (1H, d, *J* = 6.0 Hz, H-3);

^{13}C NMR (CD_3OD, 100 MHz) δ 182.0 (C, C-4), 164.7 (C, C-7), 162.0 (C, C-5), 158.5 (C, C-8a), 154.6 (CH, C-2), 110.2 (CH, C-3), 105.2 (C, C-4a), 98.9 (CH, C-6), 93.7 (CH, C-8).

4.5.25 *rac*-5-Hydroxy-[6'-methyl-6'-(4-methylpent-3-enyl)pyrano]-2',3':7,6-chromone (127)

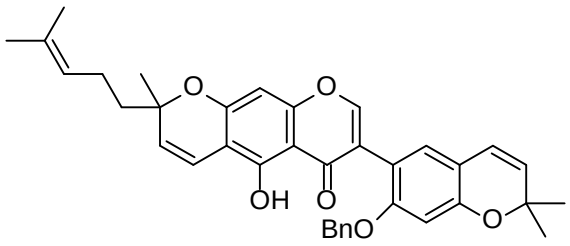


Citral (0.1 mL, 0.58 mmol) was added under an N_2 atmosphere to a stirred mixture of $Ca(OH)_2$ (16.6 mg, 0.0.225 mmol) and chromone **51** (20 mg, 0.112 mmol) in CH_3OH (5 mL). The mixture was stirred for 3 days at room temperature. CH_3OH was evaporated and the reaction mixture was diluted with EtOAc and H_2O. The two phases were partitioned and the aqueous phase was extracted with EtOAc**.** The organic layers were combined, washed with 1M HCl, H_2O, brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent followed by column chromatography gave racemic **127** as a yellow oil (22 mg, 64%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.86 (1H, s, OH-5), 7.70 (1H, d, *J* = 6.0 Hz, H-2), 6.75 (1H, d, *J* = 10.0 Hz, H-4'), 6.31 (1H, s, H-8), 6.19 (1H, d, *J* = 6.0 Hz, H-3), 5.57 (1H, d, *J* = 10.0 Hz, H-5'), 5.10 (1H, t, *J* = 7.2 Hz, H-10'), 2.09 (2H, dd, J = 16.1 and 8.0, H-9'), 1.75-1.81 (1H, m, H-8'b), 1.62-1.69 (4H, m, H-8'a and H-12' or 13'), 1.57 (3H, s, H-12' or 13'), 1.43 (3H, s, H-7'); (Plate 19a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.8 (C, C-4), 159.9 (C, C-7), 157.5 (C, C-8a), 156.4 (C, C-5), 155.3 (CH, C-2), 132.0 (C, C-11'), 127.1 (CH, C-5'), 123.7 (CH, C-10'), 115.9 (CH, C-4'), 111.3 (CH, C-3), 106.5 (C, C-6), 105.5 (C, C-4a), 94.8 (CH, C-8), 80.6 (C, C-6'), 41.7 (CH_2, C-8'), 27.1 (CH_3, C-12' or 13'), 25.6 (CH_3, C-7'), 22.6 (CH_2, C-9'), 17.6 (CH_3, C-12' or 13'). (Plate 19b)

4.5.26 *rac*-2'-Benzyloxy-5-hydroxy-[(6'',6''-dimethylpyrano(2'',3'':4',5')][(6'''-methyl-6'''-(4-methylpent-3-enyl)pyrano(2''',3''':7,6)]isoflavone (128)



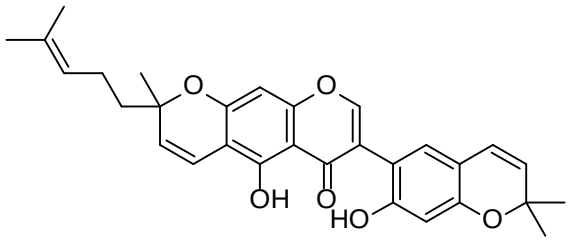
The title compound was prepared in 68% yield (40 mg, yellow oil) from **115** (45.0 mg, 0.102 mmol) under similar reaction conditions to those in procedure **4.5.25.**

^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.22 (1H, s, OH-5), 7.79 (1H, s, H-2), 7.27-7.33 (5H, m, PhCH_2), 6.96 (1H, s, H-6'), 6.77 (1H, d, *J* = 10.0 Hz, H-4'''), 6.52 (1H, s, H-3'), 6.31 (1H, s, H-8), 6.28 (1H, d, *J* = 9.8 Hz, H-4''), 5.59 (1H, d, *J* = 10.0 Hz, H-5'''), 5.49 (1H, d, *J* = 9.8 Hz, H-5''), 5.09 (1H, t, *J* = 7.2 Hz, H-10'''), 5.02 (2H, s, PhCH_2), 2.10 (2H, dd, J = 16.0 and 8.3, H-9'''), 1.76-1.81 (1H, m, H-8'''b), 1.66-1.70 (4H, m, H-8'''a, H-12''' or 13'''), 1.59 (3H, s, H-12''' or 13'''), 1.44 (6H, s, H-7'' and H-8''), 1.43 (3H, s, H-7'''); (Plate 20a)

^{13}C NMR ($CDCl_3$,100 MHz) δ 180.9 (C, C-4), 159.7 (C, C-7), 157.5 (CH, C-2'), 157.4 (C, C-8a), 156.9 (C, C-5), 154.7 (C, C-4'), 154.3 (CH, C-2), 136.8 (PhCH_2), 129.2 (CH, C-6'), 128.5 (PhCH_2), 128.1 (CH, C-5''), 127.8 (PhCH_2), 127.2 (PhCH_2), 126.8 (CH, C-5'''), 123.8 (CH, C-10'''), 121.6 (CH, C-4''), 120.6 (C, C-3), 115.6 (CH, C-4'''), 114.5 (C, C-5'), 112.2 (C, C-1'), 106.2 (C, C-4a), 105.5 (C, C-6), 101.8 (CH, C-3'), 94.8 (CH, C-8), 80.4 (C, C-6'''), 76.8 (C, C-6''), 70.8 (CH_2, PhCH_2), 41.7 (CH_2, C-8'''), 28.3 (2 × CH_3, C-7'' and C-8''); 27.1 (CH_3, C-12''' or 13'''), 25.6 (CH_3, C-7'), 22.6 (CH_2, C-9'''), 17.7 (CH_3, C-12''' or 13'''). (Plate 20b)

HR-ESIMS *m/z* $[M + Na]^+$ 599.2411 (Calcd. for $C_{37}H_{36}NaO_6$ 599.2410).

4.5.27 *rac*-2',5-Dihydroxy-[(6'',6''-dimethylpyrano(2'',3'':4',5')][(6'''-methyl-6'''-(4-methylpent-3-enyl)pyrano(2''',3''':7,6)]isoflavone (89)



Compound **89** as a racemic mixture was prepared from **128** (25 mg, 0.0433 mmol) according to the procedure in **4.5.16**. The title compound was obtained as a yellow oil (13 mg, 61%) after purification on a chromatotron.

12.54 (1H, s, OH-5), 8.29 (1H, brs, OH-2'), 7.93 (1H, s, H-2), 6.77 (1H, d, *J* = 10.0 Hz, H-4'''), 6.75 (1H, s, H-6'), 6.53 (1H, s, H-3'), 6.38 (1H, s, H-8), 6.28 (1H, d, *J* = 9.8 Hz, H-4''), 5.60 (1H, d, *J* = 10.0 Hz, H-5'''), 5.52 (1H, d, *J* = 9.8 Hz, H-5''), 5.09 (1H, t, *J* = 7.0 Hz, H-10'''), 2.11 (2H, dd, J = 15.8 and 7.9, H-9'''), 1.84 (1H, m, H-8'''b), 1.66-1.70 (4H, m, H-8'''a, H-12''' or 13'''), 1.57 (3H, s, H-12''' or 13'''), 1.45 (3H, s, H-7'''), 1.44 (6H, s, H-7'' and H-8''); (Plate 21)

HR-ESIMS *m/z* $[M + Na]^+$ 509.1934 (Calcd. for $C_{30}H_{30}NaO_6$ 509.1940.

4.6 REFERENCES

(1) Dewick, P. M. In *The Flavonoids: Advances in Research Since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994, p 117-238.

(2) Botta, B.; Menendez, P.; Zappia, G.; de Lima, R. A.; Torge, R.; Delle Monache, G. *Current Medicinal Chemistry* **2009**, *16*, 3414-3468.

(3) Veitch, N. C. *Natural Product Reports* **2007**, *24*, 417-464.

(4) Veitch, N. C. *Natural Product Reports* **2009**, *26*, 776-802.

(5) Tahara, S.; Ibrahim, R. K. *Phytochemistry* **1995**, *38*, 1073-1094.

(6) Lapcík, O. *Phytochemistry* **2007**, *68*, 2909-2916.

(7) Jain, A. C.; Jain, S. M. *Tetrahedron* **1972**, *28*, 5063-5067.

(8) Rao, K. S. R. M.; Iyer, C. S. R.; Iyer, P. R. *Tetrahedron* **1987**, *43*, 3015-3019.

(9) Schuda, P. F.; Price, A. W. *The Journal of Organic Chemistry* **1987**, *52*, 1972-1979.

(10) Tsukayama, M.; Horie, T.; Iguchi, Y.; Nakayama, M. *Chemical Pharmaceutical Bulletin* **1988**, *36*, 592-600.

(11) Tsukayama, M.; Kawamura, Y.; Tamaki, H.; Horie, T. *Chemical Pharmaceutical Bulletin* **1991**, *39*, 1704-1706.

(12) Tsukayama, M.; Kawamura, Y.; Tamaki, H.; Kubo, T.; Horie, T. *Bulletin of the Chemical Society of Japan* **1989**, *62*, 826-832.

(13) Zheng, S.-Y.; Shen, Z.-W. *Tetrahedron Letters* **2010**, *51*, 2883-2887.

(14) Ollis, W. D. In *The Chemistry of Flavanoids*; Geissman, T. A., Ed.; Pergamon Press Inc.: London, 1962, p 353 - 440.

(15) Baker, W.; Chadderton, J.; Harborne, B. J.; Ollis, D. W. *Journal of the Chemical Society, Part I* **1953**, 1852-1854.

(16) Farkas, L.; Gottsegen, A.; Nogradi, M. *Journal of Chemical Society, Perkin Transactions I* **1974**, 305 - 312.

(17) Miki, Y.; Fujita, R.; Matsushita, K. *Journal of the Chemical Society, Perkin Transactions I* **1998**, 2533 - 2536.

(18) Granados - Covarrubias, E. H.; Maldonado, L. A. *Tetrahedron Letters* **2009**, *50*, 1542-1545.

(19) Li, Q.-L.; Liu, Q.-L.; Ge, Z.-Y.; Zhu, Y.-M. *Helvetica Chimica Acta* **2011**, *94*, 1304-1309.

(20) Pelter, A.; Foot, S. *Synthesis* **1976**, 326-329.

(21) Oldfield, F. M.; Chen, L.; Botting, N. P. *Tetrahedron* **2004**, *60*, 1887-1893.

(22) Wahalala, K.; Hase, A. T. *Journal of the Chemical Society, Perkin Transactions 1* **1991**, 3005-3008.

(23) Hastings, J. M.; Hadden, M. K.; Blagg, B. S. J. *The Journal of Organic Chemistry* **2007**, *73*, 369-373.

(24) Xiao, Z.-P.; Shi, D.-H.; Li, H.-Q.; Zhang, L.-N. *Bioorganic and Medicinal Chemistry* **2007**, *15*, 3703-3710.

(25) Whalley, J. L.; Oldfield, F. M.; Botting, N. P. *Tetrahedron* **2000**, *56*, 455 460.

(26) Pelter, A.; Ward, R. S.; Whalley, J. L. *Synthesis* **1998**, 1793-1802.

(27) Miller, C. P.; Collinin, D. M.; Harris, A. H. *Bioorganic and Medicinal Chemistry Letters* **2003**, *13*, 2399 - 2403.

(28) Hakala, U.; Wahalala, K. *Tetrahedron Letters* **2006**, *47*, 8375-8378.

(29) Mohamed, S. E. N.; Thomas, P.; Whiting, D. A. *Journal of Chemical Society, Perkin Transactions I* **1987**, 431-437.

(30) Kawamura, Y.; Maruyama, M.; Tokuoka, T.; Tsukayama, M. *Synthesis* **2002**, 2490-2496.

(31) Hossain, M. M.; Tokuoka, T.; Yamashita, K.; Kawamura, Y.; Tsukayama, M. *Synthetic Communications* **2006**, *36*, 1201-1211.

(32) Khupse, R. S.; Erhardt, P. W. *Journal of Natural Products* **2008**, *71*, 275-277.

(33) Hoshino, Y.; Miyaura, N.; Suzuki, A. *Bulletin of the Chemical Society of Japan* **1988**, *61*, 3008-3010.

(34) Ito, F.; Iwasaki, M.; Watanabe, T.; Ishikawa, T.; Higuchi, Y. *Organic and Biomolecular Chemistry* **2005**, *3*, 674 - 681.

(35) Felpin, F.-X. *The Journal of Organic Chemistry* **2005**, *70*, 8575-8578.

(36) Eisnor, C. R.; Gossage, R. A.; Yadav, P. N. *Tetrahedron* **2006**, *62*, 3395-3401.

(37) Dawood, K. M. *Tetrahedron* **2007**, *63*, 9642 - 9651.

(38) Felpin, F.-X.; Lory, C.; Sow, H.; Acherar, S. *Tetrahedron* **2007**, *63*, 3010-3016.

(39) Wei, G.; Yu, B. *European Journal of Organic Chemistry* **2008**, *2008*, 3156-3163.

(40) Gammill, B. R. *Synthesis* **1979**, 901-903.

(41) Vasselin, A. D.; Wetwell, A. D.; Matthews, C. S.; Bradshaw, D. T.; Stevens, F. G. M. *Journal of Medicinal Chemistry* **2006**, *49*, 3973-3981.

(42) Igarashi, Y.; Kumazawa, H.; Ohshima, T.; Satomi, H.; Terabayashi, S.; Takeda, S.; Aburada, M.; Miyamoto, I.-K. *ChemicalPharmaceutical Bulletin* **2005**, *53*, 1088-1091.

(43) Watanabe, K.; Saito, T.; Niimura, K.; Kureha Chemical Industry Co., Ltd., Tokyo, Japan: United States, 1997; Vol. 6,698,575, p 1-20.

(44) Hostettmann, K.; Marston, A. *Pure and Applied Chemistry* **1994**, *66*, 2231-2234.

(45) Eicher, T.; Hauptmann, S. *The Chemistry of Heterocycles*; Georg Thieme Verlag: Stuttgart, 1995.

(46) Berliner, M.; Belecki, K. *Organic Syntheses* **2007**, *84*, 102.

(47) Föhlisch, B. *Chemische Berichte* **1971**, *104*, 348-349.

(48) Hong, R.; Feng, J.; Hoen, R.; Lin, G.-Q. *Tetrahedron* **2001**, *57*, 8685-8689.

(49) Miyaura, N.; Suzuki, A. *Chemical Reviews* **1995**, *95*, 2457-2483.

(50) Murata, M.; Watanabe, S.; Masuda, Y. *The Journal of Organic Chemistry* **1997**, *62*, 6458-6459.

(51) Murata, M.; Oyama, T.; Watanabe, S.; Masuda, Y. *The Journal of Organic Chemistry* **1999**, *65*, 164-168.

(52) Baudoin, O.; Guenard, D.; Gueritte, F. *The Journal of Organic Chemistry* **2000**, *65*, 9268-9271.

(53) Li, W.; Nelson, D. P.; Jensen, M. S.; Hoerrner, R. S.; Cai, D.; Larsen, R. D.; Reider, P. J. *The Journal of Organic Chemistry* **2002**, *67*, 5394-5397.

(54) Knochel, P.; Dohle, W.; Gommermann, N.; Kneisel, F. K.; Korn, T.; Sapountzis, I.; Vu, V. A. *Angewandte Chemie, International Edition* **2003**, *42*, 4302-4320.

(55) Remenar, J. F.; Lucht, B. L.; Collum, D. B. *Journal of the American Chemical Society* **1997**, *119*, 5567-5572.

(56) Reich, H. J.; Green, P. D.; Medina, M. A.; Goldenburg, W. S.; Gudmundsson, B. O.; Dykstra, R. R.; Phillips, N. H. *Journal of American Chemical Society* **1998**, *120*, 7201-7210.

(57) Andersen, Ø. M.; Markham, K. R. *Flavonoids*; Taylor and Francis: New York, 2006.

(58) Rice-Evans, C. A.; Packer, L. *Flavonoids in Health and Disease*; Second ed.; Marcel Dekker: Basel, New York, 2003.

(59) Elsohly, M. A.; Slatkin, D. J.; Knapp, J. E.; Doorenbos, N. J.; Quimby, M. W.; Schiff, P. L.; Gopalakrishna, E. M.; Watson, W. H. *Tetrahedron* **1977**, *33*, 1711-1715.

(60) Camps, F.; Coll, J.; Messeguer, A.; Pericas, A. M.; Ricart, S. *Synthesis* **1980**, 725-728.

(61) Weitl, F. L. *The Journal of Organic Chemistry* **1976**, *41*, 2044-2045.

(62) Adimurthy, S.; Ramachandraiah, G.; Ghosh, P. K.; Bedekar, A. V. *Tetrahedron Letters* **2003**, *44*, 5099-5101.

(63) Aslam, S. N.; Stevenson, P. C.; Phythian, S. J.; Veitch, N. C.; Hall, D. R. *Tetrahedron* **2006**, *62*, 4214-4226.

(64) Mujahidin, D.; Doye, S. *European Journal of Organic Chemistry* **2005**, 2689-2693.

(65) Thomsen, I.; Torssel, K. B. G. *Acta Chemica Scandinavica* **1991**, *45*, 539-542.

(66) Haszeldine, R. N.; Sharpe, A. G. *Journal of the Chemical Society* **1952**, 993-1001.

(67) Lichtenfels, A. R.; Coelho, A. L.; Costa, R. R. *Journal of Chemical Society, Perkin Transactions 1* **1995**, 949-951.

(68) Lim, J.; Kim, H.-I.; Kim, H. H.; Ahn, K.-S.; Han, H. *Tetrahedron Letters* **2001**, *42*, 4001-4003.

(69) Mondal, M.; Puranik, G. V.; Argade, N. P. *The Journal of Organic Chemistry* **2006**, *71*, 4992-4995.

(70) Russell, G. B.; Sirat, H. M.; Sutherland, O. R. W. *Phytochemistry* **1990**, *29*, 1287-1291.

(71) Máximo, P.; Lorenço, A.; Feio, S. S.; Roseiro, J. C. *Zeitschrift Für Naturforschung C - A Journal of Biosciences* **2000**, *55*, 506-510.

(72) Drewes, S. E.; Horn, M. M.; Munro, O. Q.; Dhlamini, J. T. B.; Meyer, J. J. M.; Rakuambo, N. C. *Phytochemistry* **2002**, *59*, 739-747.

(73) Jeso V.; Nicolaou K. C. *Tetrahedron Letters* **2009**, *50*, 1161-1163.

(74) Srihari, P.; Bhasker, E. V.; Yadav, A. B. R. *Tetrahedron Letters* **2009**, *50*, 2420-2424.

(75) Akiyama, T.; Hirofuji, H.; Ozaki, S. *The Chemical Society of Japan* **1992**, *65*, 1932-1938.

(76) Brar, A.; Vankar, Y. D. *Tetrahedron Letters* **2006**, *47*, 5207-5210.

(77) Fuji, K.; Kawabata, T.; Fujita, E. *Chemical Pharmaceutical Bulletin* **1980**, *28*, 3662-3664.

(78) Zima, A.; Hosek, J.; Treml, J.; Muselik, J.; Suchy, P.; Prazanova, G.; Lopes, A.; Zemlicka, M. *Molecules* **2010**, *15*, 6035-6049.

(79) Dat, N. T.; Binh, P. T. X.; Quynh, L. T. P.; Van Minh, C.; Huong, H. T.; Lee, J. J. *Fitoterapia* **2010**, *81*, 1224-1227.

(80) Morikawa, T.; Funakoshi, K.; Ninomiya, K.; Yasuda, D.; Miyagawa, K.; Matsuda, H.; Yoshikawa, M. *Chemical and Pharmaceutical Bulletin* **2008**, *56*, 956-962.

(81) Ha, L. D.; Hansen, P. E.; Vang, O.; Duus, F.; Pham, H. D.; Nguyen, L.-H. D. *Chemical and Pharmaceutical Bulletin* **2009**, *57*, 830-834.

(82) Lannang, A. M.; Komguem, J.; Ngninzeko, F. N.; Tangmouo, J. G.; Lontsi, D.; Ajaz, A.; Choudhary, M. I.; Sondengam, B. L.; Atta ur, R. *Bulletin of the Chemical Society of Ethiopia* **2006**, *20*, 247-252.

(83) Shou, Q.-Y.; Fu, R.-Z.; Tan, Q.; Shen, Z.-W. *Journal of Agricultural and Food Chemistry* **2009**, *57*, 6712-6719.

(84) Lapcik, O. *Phytochemistry* **2007**, *68*, 2909-2916.

(85) Sugamoto, K.; Matsusita, Y.-I.; Matsui, K.; Kurogi, C.; Matsui, T. *Tetrahedron* **2011**, *67*, 5346-5359.

(86) BruyÃ¨re, C. l.; Genovese, S.; Lallemand, B.; Ionescu-Motatu, A.; Curini, M.; Kiss, R.; Epifano, F. *Bioorganic and Medicinal Chemistry Letters* **2011**, *21*, 4174-4179.

(87) Tsuji, F.; Kobayashi, K.; Okada, M.; Yamaguchi, H.; Ojika, M.; Sakagami, Y. *Bioorganic and Medicinal Chemistry Letters* **2011**, *21*, 4041-4044.

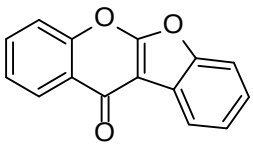
(88) Bradbury, R. B. *Journal of the Chemical Society* **1951**, 3447-3449.

CHAPTER 5

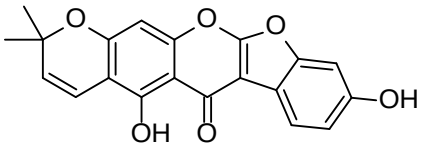
TOTAL SYNTHESIS OF THE PYRANOCOUMARONOCHROMONE LUPINALBIN H

5.1 INTRODUCTION

Coumaronochromones are a subclass of isoflavonoids with general stucture **129**.[1] They have been isolated from different plant genera, mostly of the *Leguminosae*,[1-5] although a few coumaronochromones have been reported from non-leguminous plants.[6-8] The striking feature of most naturally-occurring coumaronochromones is the presence of prenyl side chains, which in most instances are cyclised to adjacent hydroxy groups to give pyrano or furano rings.[1-3,5,7,9,10] Most pyranocoumaronochromones exhibit important biological activities such as anthelminthic, oestrogenic, neuroprotective, antiplatelet aggregation, anti-HIV, immunosuppressive activities, as well as cytotoxicity against certain cancer cell lines.[2,3,5,9-13] Despite their biological importance, the synthesis of these compounds has received little attention.



129



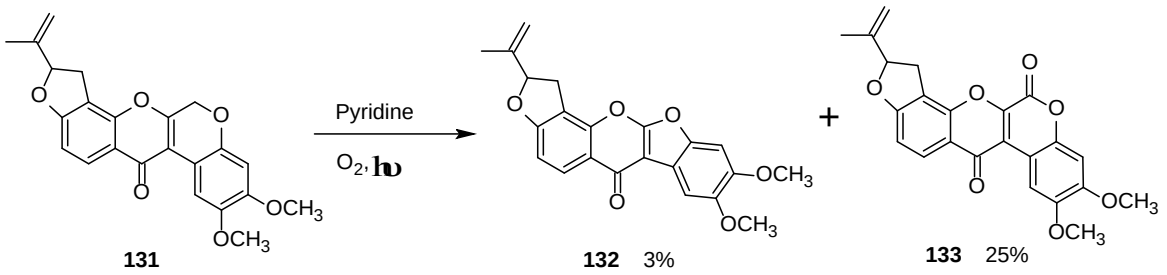
130

In continuation of our studies on the regioselective synthesis of pyranoisoflavonoids,[14] this Chapter reports the first total synthesis of lupinalbin H (**130**). With this synthesis, we further demonstrate the usefulness of the Suzuki-Miyaura reaction in the synthesis of isoflavonoids. A review on the methods for the synthesis of coumaronochromones and the total syntheses of individual pyranocoumaronochromones is given in the beginning sections of this Chapter, followed by discussions on the total synthesis of lupinalbin H (**130**), and finally the conclusions and experimental procedures.

5.2 METHODS FOR THE SYNTHESIS OF COUMARONOCHROMONES - AN OVERVIEW

Coumaronochromones are commonly known to co-exist with structurally-similar 2'-hydroxyisoflavones,[1,15] thus 2'-hydroxyisoflavones, although not yet proven, are proposed to be biosynthetic precursors of coumaronochromones.[1,15,16] However, in 2008, Lawson and co-workers isolated two new coumaronochromones along with the closely related rotenoids dehydrodeguelin and dehydrorotenone.[17] Therefore, they proposed the rotenoids to be probable biosynthetic precursors of the coumaronochromones.[17] These prospective precursors have been chemically transformed into the corresponding coumaronochromones under oxidative conditions.[1,16,18]

Chubachi and Hamada reported the synthesis of a coumaronochromone **132** by photo-oxidative ring contraction of rotenoid **131** (Scheme 5.1).[18] In their experiment, they irradiated a solution of **131** in pyridine with a high pressure mercury lamp in the presence of oxygen. This gave a mixture of products, whereby 3% of the coumaronochromone **132** was isolated, and the major product was rotenonone **133** obtained in 25% yield.[18] Although this method was developed four decades ago, it has received scant acceptance due to poor selectivity and low yields.

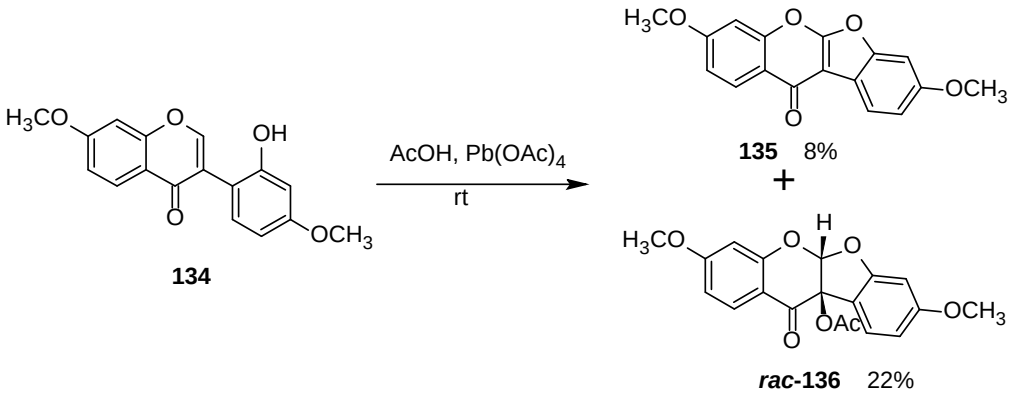


131 **132** 3% **133** 25%

Scheme 5.1. Synthesis of coumaronochromones from rotenoids

The most commonly employed procedure for the synthesis of coumaronochromones is oxidative cyclisation of 2'-hydroxyisoflavones. Metal salts such as K_3FeCN_6, Ag_2CO_3, $Pb(OAc)_4$ and $Mn(OAc)_3.2H_2O$ were initially employed to facilitate this cyclodehydrogenation albeit in low yields.[1,16,19] The major drawback with $Pb(OAc)_4$ and $Mn(OAc)_3.2H_2O$ was the formation of the acetylated by-product **136** along with the

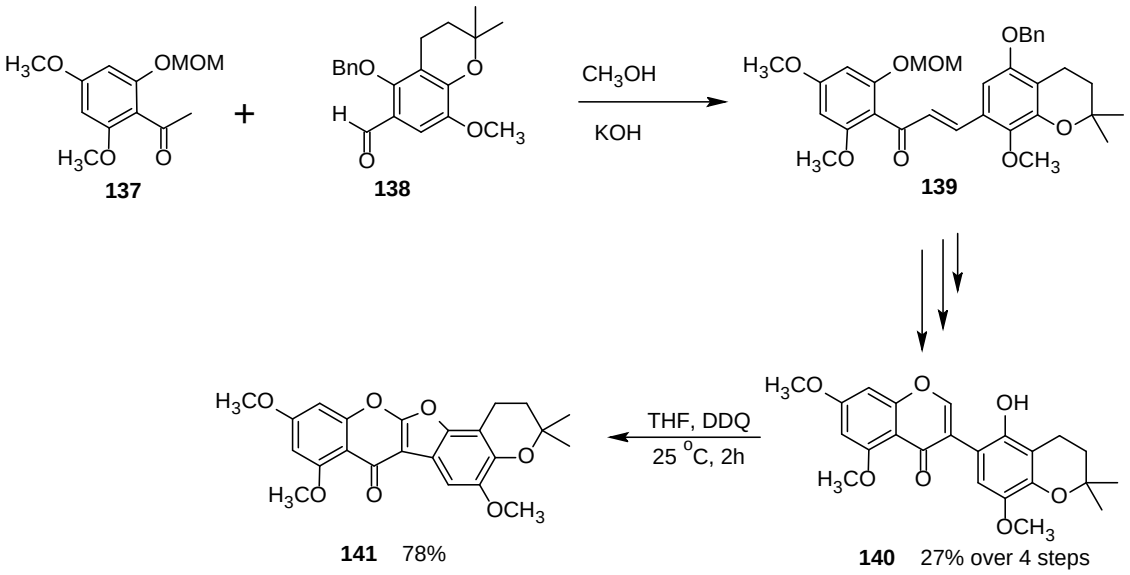
targeted coumaronochromone **135** when the reaction was conducted at low temperatures (Scheme 5.2).[19] In 1983, the method was greatly improved by using SeO_2 as an oxidising agent and later (2001) by using quinone-based oxidants such as DDQ and chloranil.[20,21] Under these conditions, coumaronochromones were obtained in yields that range from 70-82%.[20,21]



Scheme 5.2. Preparation of coumaronochromones from 2'-hydroxyisoflavones

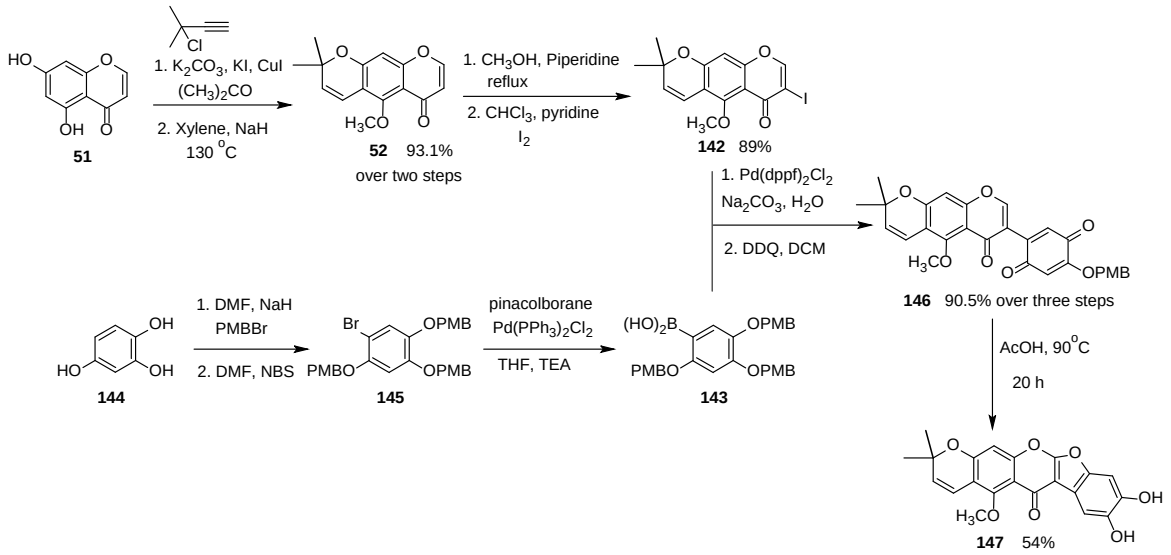
5.3 TOTAL SYNTHESES OF PYRANOCOUMARONOCHROMONES – AN OVERVIEW

Although coumaronochromones with dimethylpyran scaffolds continue to be reported as new compounds with interesting biological properties, there are only a few reports on their syntheses. Currently, there are two reports on the total synthesis of pyranocoumarochromones.[21,22] The first report was on the unnatural compound **141** prepared by Tsukayama and co-workers (Scheme 5.3).[21] In their synthetic strategy, the coumaronochromone nucleus was assembled by cyclodehydrogenation of 2'-hydroxyisoflavone **140** with DDQ. The isoflavone **140** was prepared by TTN oxidative rearrangement of chalcone **139**, which was in turn prepared by the Claisen-Schmidt condensation of acetophenone **137** and aldehyde **138**, bearing the dimethyldihydropyran scaffold. The dimethyldihydropyran moiety was introduced in the early stages of the synthesis by condensation of benzaldehyde with 2-methyl-3-buten-2-ol in the presence of $BF_3.OEt_2$ to give chroman **138**.[21]



Scheme 5.3. Synthesis of the pyranocoumaronochromone **141**

The second synthesis targeted the immunossuppresive agent, hirtellanine A (**147**) shown in Scheme 5.4.[22] The synthesis was based on the Suzuki-Miyaura reaction for the construction of the isoflavone nucleus.[22] The main precursors were 3-iodochromone bearing a dimethylchromene scaffold **142** and boronic acid **143**. The 3-iodochromone **142** was prepared in a sequence of steps, which involved regioselective formation the chromene scaffold by a Tom-Harfenist rearrangement of the propagyl ether, prepared by *O*-alkylation of the 7-hydroxy group of chromone **51** with 3-chloro-3-methylbut-1-yne. Iodination of the resulting pyranochromone **52** at C-3 following Gammill's protocol rendered **142**. The boronic acid **143** was prepared in three steps from trihydroxybenzene **144**. The Suzuki coupling of **143** with **142** and subsequent oxidative cleavage of the *p*-methoxybenzyl protecting groups gave a quinone **146**, which upon treatment with acetic acid gave hirtellanine A (**147**).[22]



Scheme 5.4. Synthesis of hirtellanine A (**147**)

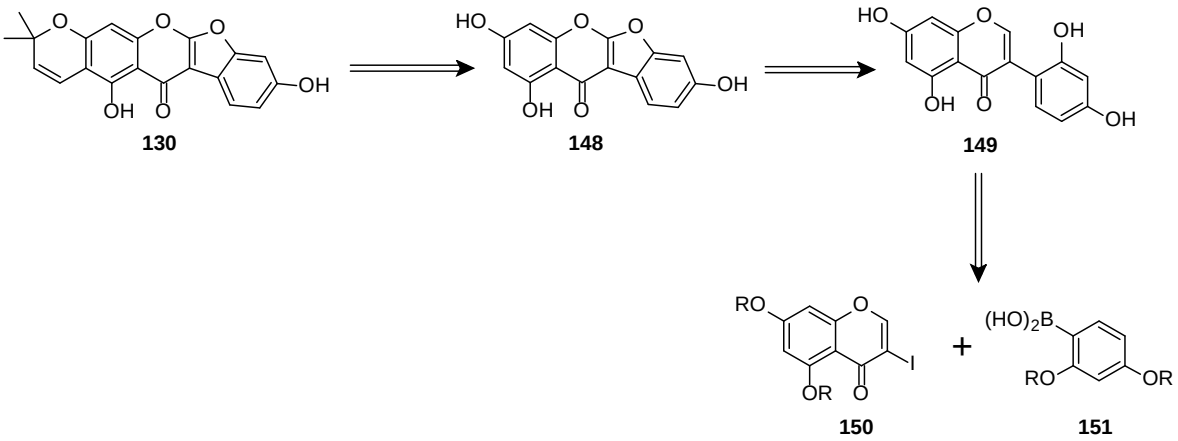
5.4 RESULTS AND DISCUSSION

5.4.1 Introduction

Lupinalbin H (**130**) was isolated together with other flavonoids from the methanolic extract of the roots of yellow lupin (*Lupinus luteus* cv Topaz) by Tahara *et al*.[23] The assignment of the structure of **130** was based mainly on comparison of its ^{1}H NMR signals with those of related compounds. The ^{13}C NMR data was not reported. Furthermore, the accompanying flavonoids were reported to exhibit antifungal activity; however, **130** was not assayed for its activity. The lack of detailed structural characterisation and bioactivity studies of lupinalbin H (**130**) can be attributed to the low quantity obtained from the plant source.[23] Therefore, our aim was to develop a synthetic route that can readily give access to **130** in a good yield, to confirm its structure unambiguously, and provide material for future biological studies.

Scheme 5.5 shows the retrosynthetic analysis for lupinalbin H (**130**). Lupinalbin H (**130**) was planned to be synthesised by the regiospecific condensation of lupinalbin A (**148**)[15,24] with prenal. Compound **148** could be prepared by oxidative cyclisation of 2'-hydroxygenistein (**149**),[25,26] which could readily be accessed by the Suzuki-Miyaura reaction of 3-iodochromone **150** and boronic acid **151**. The synthesis of the penultimate

precursor **148** from 2'-hydroxyisoflavones has been reported previously.[21,27] However, the former syntheses were based on the deoxybenzoin and the chalcone routes for the construction of the isoflavone core.[21,27] Unlike the previous synthetic routes on pyranocoumaronochromones,[21,22] the current synthetic pathway was designed to give naturally-occurring isoflavonoids, lupinalbin A (**148**)[15] and 2'-hydroxygenistein (**149**)[25] as intermediates.



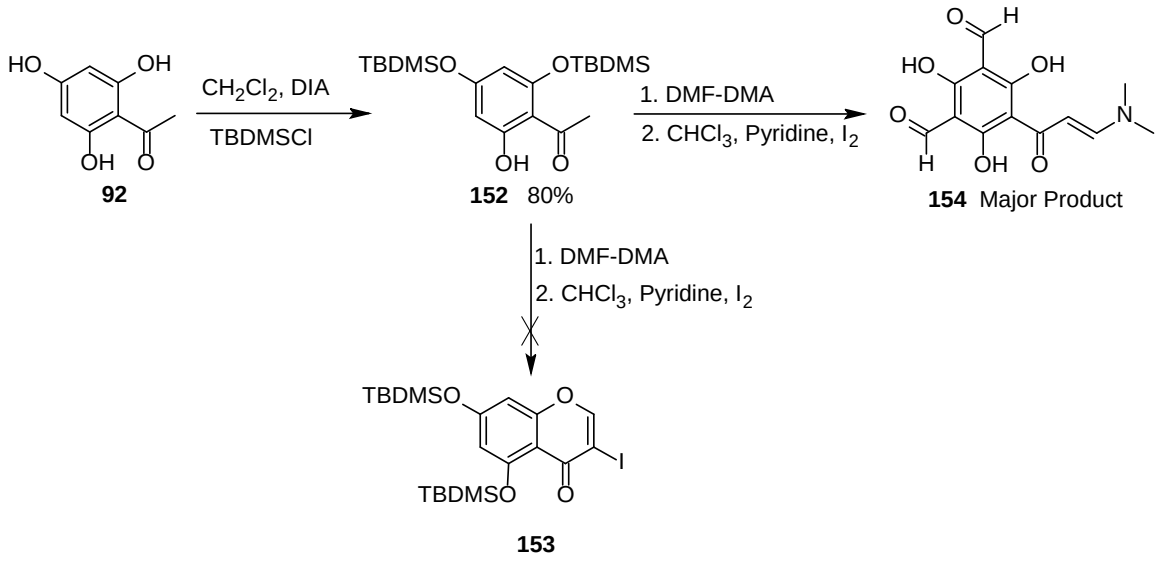
Scheme 5.5. Retrosynthetic route for lupinalbin H (**130**)

5.4.2 Optimisation of Conditions for the Synthesis of 3-Iodochromone Precursor

As seen from the retrosynthetic route (Scheme 5.5), the main precursors were 3-iodochromone **150** and boronic acid **151**. In Chapter 4, we reported the synthesis of the dimethoxymethyl protected 3-iodochromone **90**. However, the product was obtained in poor overall yield, resulting from two low-yielding steps, which were the protection of the phloroacetophenone **92** with MOMCl, and the iodine-mediated cyclisation of an enaminoketone **93** into 3-iodochromone **90**. Therefore, we decided to investigate other protecting groups for the synthesis of 3-iodochromone **150**.

Esters (acetyl and benzoyl) were initially employed for this protection. However, they were immediately abandoned due to poor regioselectivity, which led to the formation of four products identified as the tri-, di- and two monoprotected phloroacetophenones. Secondly, condensation of either the *O*-diacetyl or *O*-dibenzoyl protected phloroacetophenone with DMF-DMA failed to give the target enaminoketone.

From the literature, good regioselectivity and high yields of the diprotected phloroacetophenone have been reported when TBDMS was employed for protection of the 2'- and 4'-hydroxy groups of **92**.[28] Motivated by these reports, phloroacetophenone (**92**) was then protected with TBDMS to give **152** in 80% yield (Scheme 5.6). Encouraged by the good yield, **152** was subsequently condensed with DMF-DMA.[29] The reaction gave an orange oil, which on TLC indicated complete consumption of the starting material. However, the product could not be isolated as it trailed on TLC. DMF-DMA was then evaporated under reduced pressure and the crude product reacted with iodine in the presence of pyridine.[30] Upon completion of the reaction, the targeted product **153** could not be obtained from the reaction mixture; instead the diformylated enaminoketone **154** was isolated as the major component (Scheme 5.6).

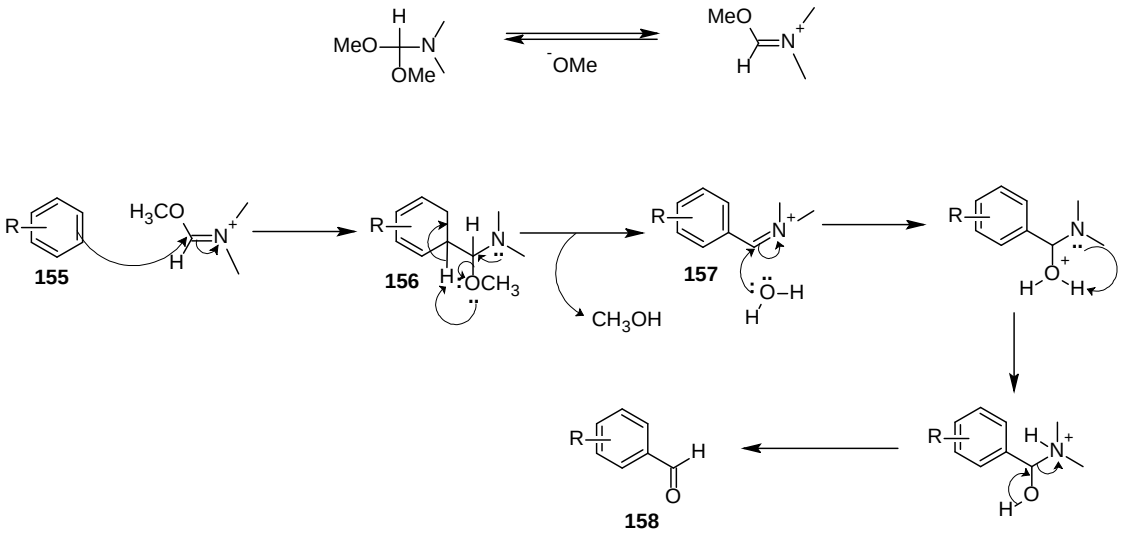


Scheme 5.6. Attempted synthesis of TBDMS protected 3-iodochromone **153**

The structure of **154** was deduced from the ^{1}H NMR spectrum (Plate 22a), which showed characteristic signals of the dimethylenaminoketone, in the form of two three-proton singlets at δ_H 3.04 and 3.29, and two olefinic doublets at δ_H 6.45 and 8.10 (J = 12.0 Hz, H-2 and H-3, respectively). The signals for the aromatic protons and the TBDMS groups were not observed. Instead, two one-proton singlets appeared at δ_H 10.02 and 10.11, indicative of two formyl groups. Furthermore, two hydrogen-bonded hydroxy protons at δ_H 14.19 and 15.26 were also observed in the spectrum of **154**. The presence of formyl

functional groups was further confirmed by the ^{13}C NMR spectrum, which displayed two signals at δ_C 191.2 and 193.3 in addition to the 11 other carbon resonances (Plate 22b).

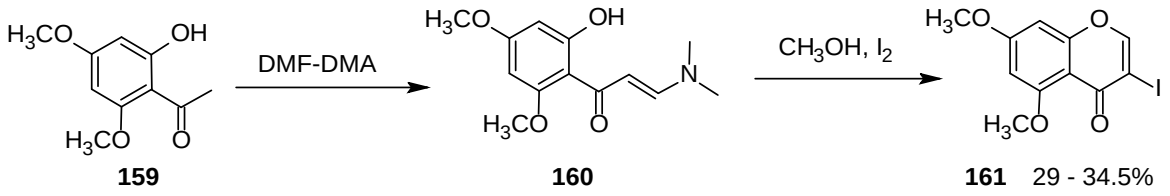
DMF-DMA is known to exist in equilibrium with its iminium counterpart, thereby releasing methoxide ions as shown in Scheme 5.7.[31] It is therefore deduced that the generated methoxide ions led to cleavage of the TBDMS protecting groups, and hence activated the phloroglucinol moiety for formylation. However, there are currently no reports on formylation of electron-rich aromatic rings with DMF-DMA. The reaction is proposed to proceed via electrophilic aromatic substitution of **155** to give **156**, followed by elimination of CH_3OH to give an iminium intermediate **157**, which upon hydrolysis with water, gives the aldehyde **158** as shown in Scheme 5.7.



Scheme 5.7. Proposed mechanism for formylation with DMF-DMA

Failure to obtain the target intermediate from the TBDMS-protected phloroacetophenone, prompted the investigation of other protecting groups. The choice of the protecting group was motivated by its stability under basic conditions, especially resistance to the methoxide ions which are generated from DMF-DMA.[31] Therefore, we planned to prepare 3-iodochromone from 2'-hydoxy-4',6'-dimethoxyacetophenone (**159**). The reaction was previously reported by Watanabe *et al.*[32] and Vassseline *et al.*[33] who independently prepared 3-iodo-5,7-dimethoxychromone (**161**) from the corresponding enaminoketone **160** in 34.5 and 29% yields, respectively (Scheme 5.8).[32,33] However, their reaction was

performed in CH_3OH, which partially dissolves propenone **160**. Therefore, the solution had to be warmed to about 60 ^oC for complete dissolution and cooled to rt before addition of iodine.[32]



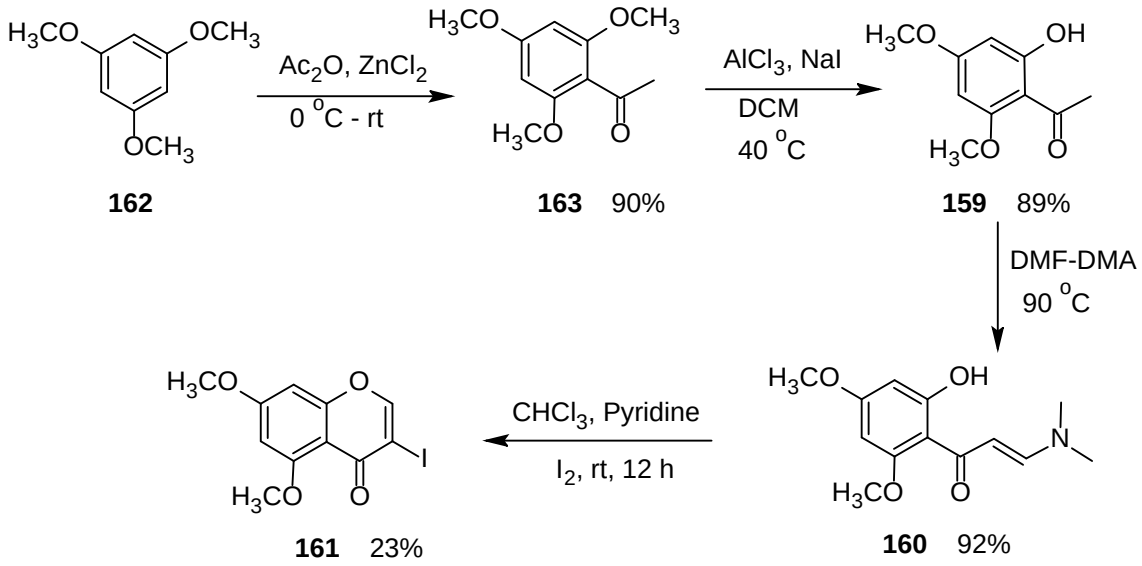
159 **160** **161** 29 - 34.5%

Scheme 5.8. Preparation of 3-iodochromone **161** by Watanabe *et al*. and Vasseline *et al*.[32,33]

We envisaged that using $CHCl_3$, which readily dissolves the enaminoketone **160**, and addition of pyridine to the reaction mixture, would improve the results. Pyridine and other nitrogen-containing bases have been reported to prevent conversion of an enaminoketone into the non-iodinated chromone, which is often formed as a major side product.[30]

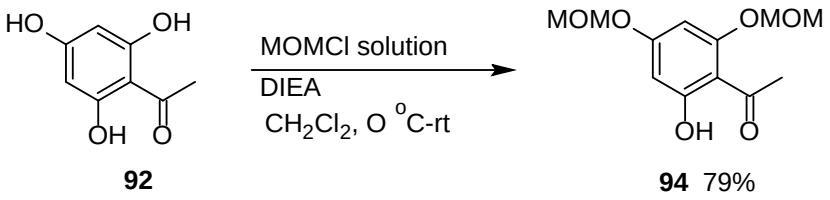
As illustrated in Scheme 5.9, we prepared 3-iodo-5,7-dimethoxychromone (**161**) from 1,3,5-trimethoxybenzene (**162**). Friedel-Crafts acylation of **162** with Ac_2O and $ZnCl_2$ gave acetophenone **163** in a 90% yield. Selective cleavage of the *ortho*-methoxy group of **163** was initially attempted by treatment of a CH_3CN solution of **163** with $AlCl_3$ and NaI.[34] This gave **159** in unsatisfactory yields and a diacylated by-product. Changing the solvent to CH_2Cl_2 cleanly rendered **159** in 89%. It was therefore deduced that CH_3CN promoted the double acylation via a Houben-Hoesch-type reaction. Condensation of **159** with DMF-DMA gave the enaminoketone **160** (92%),[29] which was subsequently converted into 3-iodochromone **161** (23%) by treatment with pyridine and I_2 in $CHCl_3$.[30]

The ^1H NMR spectrum of **161** displayed two singlets intergrating for three protons at δ_H 3.91 and 3.87 for the methoxy groups, two *meta*-coupled doublets at δ_H 6.41 and 6.37, for the aromatic protons and a one-proton singlet at δ_H 8.06 (H-2) (Plate 23a). The HRMS gave an *m/z* peak of 354.9442 $[M + Na]^+$, in agreement with the calculated pseudo molecular weight of 354.9443 for $C_{11}H_9INaO_4$.



Scheme 5.9. Preparation of 3-iodochromone

Although the steps that preceded the formation 3-iodochromone **161** gave good yields, the low yield obtained for **161** from iodine-mediated cyclisation of enaminoketone **160** prompted us to reconsider MOM protecting groups for the synthesis of the 3-iodochromone. Previously, we obtained the MOM-protected iodochromone **90** in a moderate yield of 58% (Chapter 4, Section 4.4.2), which is much better than the 23% obtained for the dimethoxy-protected 3-iodochromone **161**. However, the regioselective protection of the phloroacetophenone **92** gave 50% of 2'-hydroxy-4',6'-dimethoxymethoxyacetophenone (**94**). Denis *et al*. recently reported high yields of **94** when DIEA was used as a base.[35] Motivated by these reports, we also used DIEA instead of DIA for the regioselective protection of the 4'- and 6'-hydroxy groups of **92**. This gave the expected product **94** in 79% yield (Scheme 5.10). Having improved the yields of **94**, the next step was to optimise the reaction conditions for the iodine-mediated cyclisation of enaminoketone into 3-iodochromone **90**. The reaction was performed under different conditions; nevertheless there was no significant improvement in all the attempts.

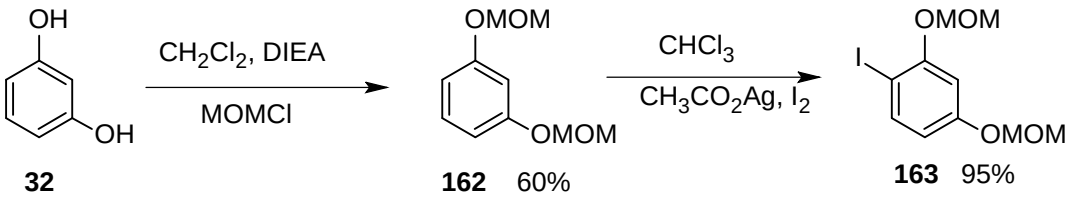


Scheme 5.10. Protection of phloroacetophenone with MOMCl

5.4.3 Preparation of Boronic Acid Precursor

As seen from the retrosynthetic Scheme 5.5, the second precursor for the synthesis of lupinalbin H (**130**) was the boronic acid **151**. The synthesis of the boronic acid coupling partner commenced with protection of the hydroxy groups of resorcinol (**32**) with MOMCl.[36] This rendered the methoxymethyl ether **162** in 60% yield. The MOM protecting groups were used in the present case as they would be removed simultaneously after the Suzuki-Miyaura coupling reaction with 3-iodochromome **90**. Regioselective iodination of **162** at C-4 by I_2 in the presence of CH_3CO_2Ag gave an aryl iodide **163** in 95% yield.[37] In our earlier investigation, we demonstrated the successful regioselective iodination of a chromanone containing a resorcinol moiety using CF_3CO_2Ag and I_2 (Chapter 4, section 4.4.4).[14] CH_3CO_2Ag gave comparable results in the present case. Other readily available silver salts ($AgNO_3$ and Ag_2SO_4) were tested for C-4 iodination of **162**, but afforded **163** in low yields when $CHCl_3$ was used as the solvent and in moderate yields in EtOH (Table 1).[38,39]

Table 5.1. Preparation of aryl iodide 163 by iodination of **162** with I_2 and silver salts[a]



32 **162** 60% **163** 95%

Silver Salt	Solvent	Time (h)	Yields[b] (%)
$AgNO_3$	$CHCl_3$	5	40
Ag_2SO_4[c]	$CHCl_3$	5	35
$AgNO_3$	EtOH	1	62
Ag_2SO_4[c]	EtOH	1	58
CH_3CO_2Ag	**$CHCl_3$**	**5**	**95**

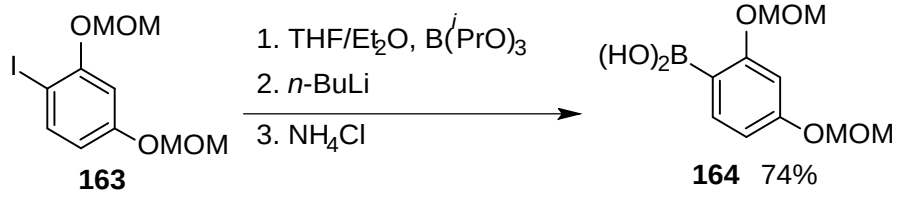
[a] 1.2 equiv silver salt, 1.1 equiv I_2

[b] Isolated yields

[c] 0.6 equiv Ag_2SO_4, 1.1 equiv I_2

The ^{1}H NMR spectrum of **163** displayed an ABX spin system at δ_H 7.62 (1H, d, J = 8.5 Hz, H-6), 6.80 (1H, d, J = 2.5 Hz, H-3), 6.53 (1H, dd, J = 2.5 and 8.5 Hz, H-5), confirming substitution at C-4 of **162**, and the signals for the methoxymethyl ether were displayed at δ_H 5.21 (2H, s, OCH_2O), 5.13 (2H, s, OCH_2O), 3.50 (3H, s, OCH_3), 3.46 (3H, s, OCH_3). The presence of iodine was further confirmed by an up-field resonance at δ_C 77.4 for the carbon *ipso* to iodine. The HRMS gave an *mz* of 354.9442 $[M + Na]^+$, which corresponded with the calculated pseudo molecular mass of 354.9443 for $C_{11}H_9INaO_4$.

The aryl iodide **163** was converted into boronic acid **164** by lithium-iodine exchange, followed by the immediate *in situ* nucleophilic attack of the generated aryl lithium species on triisopropyl borate and hydrolysis of the resulting boronate ester with NH_4Cl in a one pot reaction (Scheme 5.11).[40,41] The substitution of iodine by a boronic acid was proven by a down-field shift of the aromatic proton signals in the ^{1}H NMR spectrum of **164** and the disappearance of iodine-bearing carbon signal at δ_C 77.4 in the ^{13}C NMR spectrum of **164**. The ^{11}B NMR spectrum further confirmed borylation by the presence of a signal at δ_B 28.55.

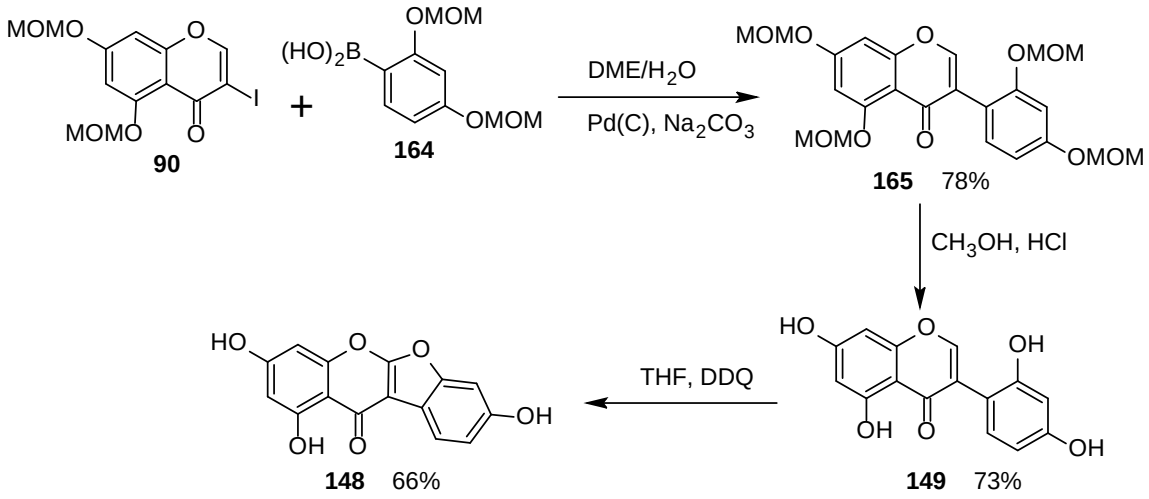


163 **164** 74%

Scheme 5.11. Synthesis of boronic acid **164**

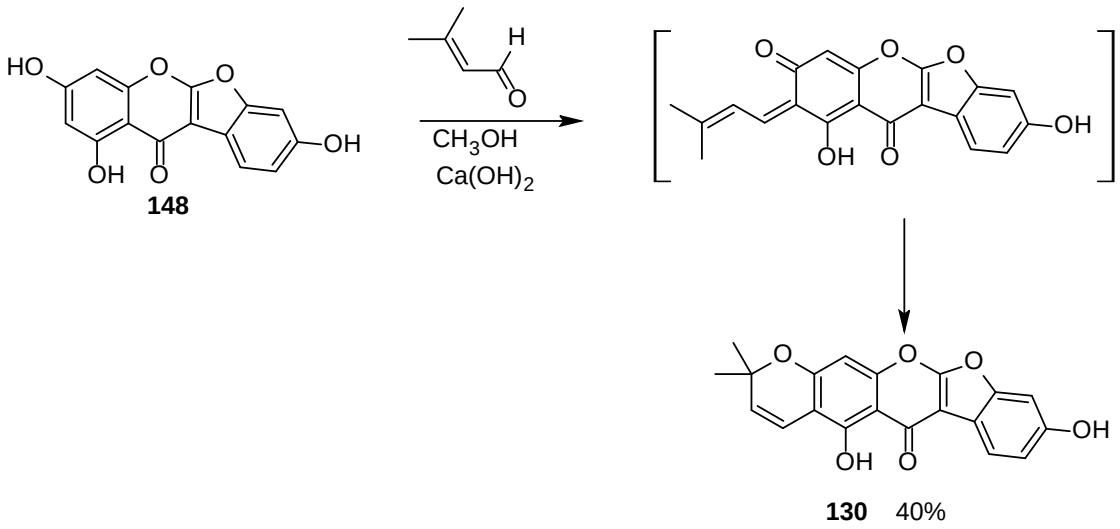
5.4.4 Synthesis of 2'-Hydroxygenistein, Lupinalbin A and Lupinalbin H

Having successfully synthesised the boronic acid **164**, the next step involved coupling of **164** with 3-iodochromone **90** in the presence of 10% Pd(C) to give the isoflavone **165** (Scheme 5.12).[42] Cleavage of the MOM protecting groups of **165** under acidic conditions and subsequent oxidative cyclisation of 2'-hydroxygenistein (**149**) with DDQ afforded the phytoestrogen lupinalbin A (**148**)[16] in a 66% yield.[21]



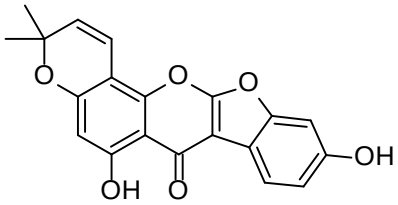
Scheme 5.12. Preparation of 2'-hydroxygenistein (**149**) and lupinalbin A (**148**)

The last step was the regioselective introduction of the dimethylpyran scaffold to the phloroglucinol moiety of lupinalbin A (**148**) to give lupinalbin H (**130**) as shown in Scheme 5.13. This was planned to be achieved by base-catalysed coupling of **148** with prenal, which proceeds via an aldol-type reaction and a 6π-electrocylisation.[43] Thus, treatment of the methanolic solution of **148** with $Ca(OH)_2$ and prenal (2.5 equiv) gave lupinalbin H (**130**) in 40% yield and 35% of **148** was recovered.[44] Addition of a large excess of prenal (5 equiv) effected complete consumption of **148** as observed on TLC but required tedious chromatographic isolation of the targeted product from the reaction mixture due to side products resulting from the polymerisation of prenal.

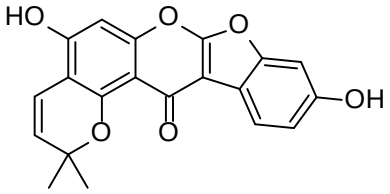


Scheme 5.13. Synthesis of lupinalbin H (**130**)

Lupinalbin A (**148**) has four nucleophilic sites at positions 6, 8, 3' and 5', which can condense with prenal to give multiple products. As anticipated, the reaction favoured the more nucleophilic phloroglucinol moiety (A-ring) rather than the resorcinol moiety (B-ring). Nevertheless, three possible regioisomers can result from condensation of prenal with the A-ring of **148**, i.e. the targeted linear isomer **130** and two angular isomers **166** and **167**.



166



167

From the ^{1}H NMR results, it could be readily deduced that the isomer **167** was not formed due to the presence of a signal characteristic for a hydrogen-bonded OH at δ_H 13.38 (1H, OH-5). Furthermore, signals for four aromatic protons, comprising an ABX spin system for the B-ring protons at δ_H 7.82 (1H, d, J = 8.3 Hz, H-6'), 7.14 (1H, d, J = 2.0 Hz, H-3'), 7.02 (1H, dd, J = 2.0 and 8.3 Hz, H-5'), and a one-proton singlet for the A-ring proton at δ_H 6.54 (H-8) were present in the ^{1}H NMR spectrum (Plate 26a). The dimethylpyran protons occurred as a singlet integrating for six protons at δ_H 1.48 (2 × CH_3) and two one-proton doublets for the olefinic protons at δ_H 6.70 (J = 10.0 Hz, H-4") and 5.79 (1H, d, J = 10.0 Hz, H-5"). These ^{1}H NMR results were in agreement with those reported for **130**.[23] The ^{13}C NMR spectrum displayed 19 carbon resonances (Plate 26b), which were identified as two methyl carbons overlapping at δ_C 27.3, six methine carbons at δ_c 128.6, 121.3, 114.6, 113.6, 98.5 and 95.3 and twelve quaternary carbons at δ_C 178.6, 164.7, 158.5, 157.0, 156.4, 154.0, 150.4, 114.0, 105.9, 104.0, 97.5 and 80.0 by DEPT and HSQC experiments.

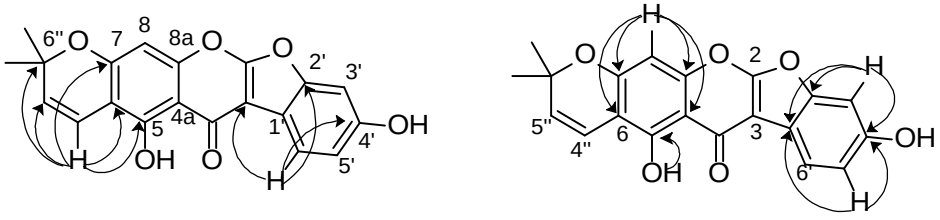


Figure 5.1. Key HMBC Correlations of (**130**)

To distinguish between the linear and angular isomers, it was crucial to assign the oxygen-bonded aromatic carbons attached to the A-ring unambiguously using the HMBC experiment. As shown in Figure 5.1, a two-bond HMBC correlation was established between the hydroxy proton at δ_H 13.38 (OH-5) and the aromatic carbon at δ_C 157.0 (C, C-5). The olefinic proton at δ_H 6.70 (H-4") showed correlations to carbon signals at δ_C 80.0 (C, C-6"), 105.9 (C, C-6), 157.0 (C, C-5) and 158.5 (C, C-7) and the aromatic proton at δ_H 6.54 (H-8) showed connections to carbon resonances at δ_C 104.0 (C, C-4a), 105.9 (C, C-6), 154.0 (C, C-8a) and 158.5 (C, C-7) (Figure 5.1). From these correlations, it was confirmed that the dimethylpyran ring was attached to C-6 and OH-7, giving the targeted product **130** and not the angular isomer **166**. The assignments of the quaternary carbons on the B-ring were also based on HMBC correlations. The proton at δ_H 7.82 (H-6') displayed correlations to carbon signals at 97.5 (C, C-3), 150.4 (C, C-2') and 156.4 (C, C-4'), whereas H-5' (δ_H 7.02) showed correlations to carbons at δ_C 114.0 (C, C-1') and 156.4 (C, C-4'), and the proton at δ_H 7.14 (H-3') showed correlations to signals at δ_C 114.0 (C, C-1'), 150.4 (C, C-2') and 156.4 (C, C-4') (Figure 5.1). The structure of **130** was confirmed by HRMS, which gave an *m/z* peak of 349.0710 $[M-H]^-$, in agreement with the calculated pseudo molecular weight of 349.0712 for $C_{20}H_{13}O$.

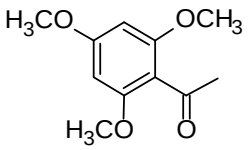
5.5 CONCLUSION

In conclusion, lupinalbin H (**130**) has been successfully synthesised by a highly convergent route. The synthesis featured the Suzuki-Miyaura coupling reaction for the construction of the isoflavone nucleus in good yields, and a highly regioselective introduction of the dimethylpyran scaffold to the coumaronochromone core. Furthermore, it gave access to other naturally-occurring phytoestrogens, 2'-hydroxygenistein (**149**) and lupinalbin A (**148**). Owing to the potential pharmacological properties of the pyranocoumaronochromones, the development of the practical synthetic route described herein for lupinalbin H (**130**) represents a significant advance towards the synthesis of other structurally related compounds and exploration of their biological properties.

5.6 EXPERIMENTAL PROCEDURES

The general procedures are given in Chapter 3, Section 3.5.1.

5.6.1 2',4',6'-Trimethoxyacetophenone (163)



To a round bottom flask cooled in an ice bath, was added 1,3,5-trimethoxybenzene (**162**) (5.00 g, 29.7 mmol), $ZnCl_2$ (4.05 g, 29.7 mmol) and Ac_2O (20 mL). The resulting mixture was stirred for 1 h, quenched with H_2O and stirred for an additional 30 min. A white solid precipitated. It was filtered by suction and washed with H_2O to give 2',4',6'-trimethoxyacetophenone (**163**) as a white solid (5.9 g, 95%). The solid was recrystallised from EtOH to give **163** as white needles.

mp 103.4 – 104.5 ^oC (Lit.[45] mp 93.5 – 104 ^oC);

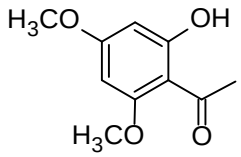
IR (neat) ν_{max} 2954, 1688, 1583, 1417, 1158, 1122, 822 cm^{-1};

^1H NMR ($CDCl_3$, 400 MHz) δ 6.08 (2H, s, H-3' and H-5'), 3.79 (3H, s, OCH_3), 3.76 (6H, s, 2 × OCH_3), 2.43 (3H, s, $COCH_3$);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.6 (C, C-1), 162.3 (C, C-4'), 158.3 (2 × C, C-2' and C, C-6'), 113.6 (C, C-1'), 90.5 (2 × CH, C-3' and C-5'), 55.7 (2 × CH_3, 2 × OCH_3), 55.3 (CH_3, OCH_3), 32.4 (CH_3, -$COCH_3$);

EIMS *m/z* 210 (9 %, M^+), 195 (100), 207 (100), 137 (21).

5.6.2 2'-Hydroxy-4',6'-dimethoxyacetophenone (159)



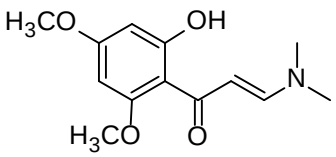
To an ice cold mixture of **163** (5.00 g, 23.7 mmol) and NaI (1.80 g, 11.8 mmol) in CH_2Cl_2 50 mL), was added $AlCl_3$ (15.8 g, 0.237 mol) in small portions with stirring. The mixture was stirred in an ice bath for additional 10 min and then heated to 40 ^oC for 24 h. The reaction mixture was cooled in an ice bath and HCl (1 M, 40 mL) was added slowly with stirring. The organic and the aqueous phases were partitioned. The aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were washed with H_2O, brine and dried over anhydrous $MgSO_4$. The solvent was evaporated to give a yellow solid, which was purified by silica gel column chromatography using Hex:EtOAc (7:3) to give **159** as a white solid (4.1 g, 89%). Recrystallisation of the compound from EtOH gave colourless crystals.

mp 83.6 – 85.4 ^oC (Lit.[46] mp 84 - 85 ^oC);

^1H NMR ($CDCl_3$, 400 MHz) δ 13.96 (1H, s, OH), 5.99 (1H, d, *J* = 2.4 Hz, H-3'), 5.86 (1H, d, *J* = 2.4 Hz, H-5'), 3.80 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 2.55 (3H, s, $COCH_3$);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.9 (C, C-1), 167.4 (C, C-2'), 166.0 (C, C-4'), 162.8 (C, C-6'), 105.8 (C, C-1'), 97.4 (CH, C- 3'), 90.4 (CH, C-5'), 55.3 (CH_3, OCH_3), 55.2 (CH_3, OCH_3), 32.6 (CH_3, -$COCH_3$).

5.6.3 3-(*N*,*N*-Dimethylamino)-1-(2'-hydroxy-4',6'-dimethoxyphenyl)propenone (160)



DMF-DMA (13.1 mL, 0.1 mol) was added to **159** (4.0 g, 20.0 mmol) at 95 ^{o}C. The mixture was stirred at the same temperature for 12 h to give an orange solid. The volatiles were evaporated on a rotary evaporator and the solid was recrystallised from CH_3OH to give **160** as yellow needles (4.6 g, 92%).

mp 152.6 – 154.5 ^{o}C

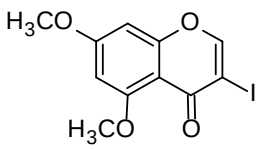
IR (neat) ν_{max} 3171, 2920, 1582, 1539, 1412, 1359, 1207, 1115, 889 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 15.59 (1H, s, OH-2'), 7.88 (1H, d, *J* = 12.4 Hz, H-3), 6.23 (1H, d, *J* = 12.4 Hz, H-2), 6.04 (1H, d, *J* = 2.4 Hz, H-3'), 5.89 (1H, d, *J* = 2.4 Hz, H-5'), 3.81 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.09 (3H, br s, $N(CH_3)_2$), 2.92 (3H, br s, $N(CH_3)_2$);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 189.9 (C, C-1), 167.7 (C, C-2'), 163.9 (C, C-4'), 161.6 (C, C-6'), 154.2 (CH, C-3), 105.3 (C, C-1'), 96.7 (CH, C-2), 93.9 (CH, C-3'), 90.5 (CH, C-5'), 55.6 (CH_3, OCH_3), 55.2 (CH_3, OCH_3), 45.0 (CH_3, $N(CH_3)_2$), 37.2 (CH_3, $N(CH_3)_2$);

HR-ESIMS *m/z* $[M + Na]^+$ 274.1054 (Calcd. for $C_{13}H_{17}NNaO_4$ 274.1055).

5.6.4 3-Iodo-5,7-dimethoxychromone (161)



To a solution of propenone **160** (4.50 g, 17.9 mmol) in $CHCl_3$ (100 mL) was added pyridine (2.90 mL, 35.8 mmol) followed by I_2 (9.09 g, 35.8 mmol). The resulting solution was stirred at rt for 12 h. The reaction was quenched with saturated $Na_2S_2O_3$ solution and the two phases partitioned. The aqueous phase was extracted with CH_2Cl_2. The organic layers were combined, washed with H_2O, brine and dried over anhydrous $MgSO_4$. The solvent was evaporated to give a yellow solid. The crude product was purified by silica gel column chromatography using Hex:EtOAc (1:1) as eluent. The solvent was evaporated to

afford **161** (1.4 g, 23%) as a white solid, which was recrystallised from EtOH to give white fluffy crystals.

mp 157.6 – 159.8 ^{o}C (Lit.[47] mp 157 - 159 ^{o}C);

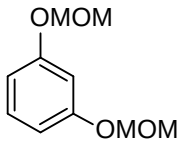
IR (neat) ν_{max} 2938, 1644, 1622, 1597, 1548, 1217, 1154, 1068, 831 cm^{1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.06 (1H, s, H-2), 6.41 (1H, d, J = 2.3 Hz, H-8), 6.37 (1H, d, J = 2.3 Hz, H-6), 3.91 (3H, s, OCH_3), 3.87 (3H, s, OCH_3); (Plate 23a)

^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1 (C, C-4), 164.1 (C, C-7), 160.8 (C, C-5), 159.6 (C, C-8a), 155.3 (CH, C-2), 107.3 (C, C-4a), 96.5 (CH, C-6), 92.3 (CH, C-8), 89.6 (C, C-3), 56.3 (CH_3, OCH_3), 55.7 (CH_3, OCH_3); (Plate 23b)

HR-ESIMS m/z $[M + Na]^+$ 354.9442 (Calcd. for $C_{11}H_9INaO_4$ 354.9443).

5.6.5 1,3-Dimethoxymethoxybenzene (162)



$ZnBr_2$ (10.25 mg, 0.045 mmol) was dissolved in dimethoxymethane (16.0 mL, 0.182 mol) under an N_2 atmosphere, and then AcCl (12.9 mL, 0.182 mol) was added dropwise to the stirred solution. The solution was stirred for an additional 2 h at rt and then transferred via a cannula to an ice-cold solution of resorcinol (**32**) (5.00 g, 45.4 mmol) and DIEA (23.3 mL, 0.136 mol) in CH_2Cl_2 (100 mL) under an N_2 atmosphere. The mixture was stirred for 3 h, diluted with saturated NH_4Cl solution and stirred for an additional 15 min. The two phases were partitioned and the aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated to give a yellow oil, which was purified by column chromatography using Hex:EtOAc (9:1) to give **162** as a colorless oil (5.4 g, 60%).

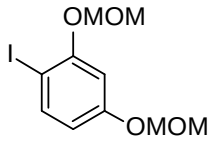
IR (neat) ν_{max} 2955, 2902, 2827, 1768, 1591, 1487, 1219, 1138, 1004, 772 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.19 (1H, t, *J* = 8.3 Hz, H-5), 6.75 (1H, t, *J* = 2.3 Hz, H-2), 6.71 (2H, dd, *J* = 2.3 and 8.3 Hz, H-4 and H-6), 5.16 (4H, s, 2 × OCH_2O), 3.48 (6H, s, 2 × OCH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.3 (2 × C, C-1 and C-3), 129.9 (CH, C-5), 109.6 (2 × CH, C-4 and C-6), 105.0 (CH, C-2), 94.5 (2 × OCH_2O), 55.9 (2 × OCH_3);

LR-ESIMS *m*/*z* 198.1 $[M]^+$.

5.6.6 1-Iodo-2,4-dimethoxymethoxybenzene (163)



CH_3CO_2Ag (5.10 g, 30.3 mmol) was added to a solution of **162** (5.00 g, 25.2 mmol) in $CHCl_3$ (50 mL). The mixture was stirred for 5 min, and then a solution of I_2 (7.04 g, 27.7 mmol) in $CHCl_3$ (150 mL) was added dropwise to the stirred suspension. The resulting mixture was stirred at rt for 5 h and filtered to remove the AgI. The filtrate was washed with 10% $Na_2S_2O_3$ solution, 5% $NaHCO_3$ solution, H_2O and brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent evaporated to give a colourless oil. The oil was purified by flash chromatography using Hex:EtOAc (9:1) to afford the iodinated compound **163** as a colorless oil (7.76 g, 95%).

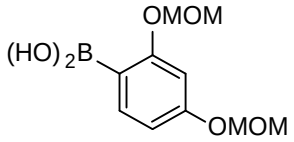
IR (neat) ν_{max} 2956, 2902, 2826, 1716, 1577, 1567, 1474, 1219, 1149, 982, 772 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 (1H, d, *J* = 8.5 Hz, H-6), 6.80 (1H, d, *J* = 2.5 Hz, H-3), 6.53 (1H, dd, *J* = 2.5 and 8.5 Hz, H-5), 5.21 (2H, s, OCH_2O), 5.13 (2H, s, OCH_2O), 3.50 (3H, s, OCH_3), 3.46 (3H, s, OCH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.7 (C, C-4), 156.7 (C, C-2), 139.2 (CH, C-6), 111.4 (CH, C-5), 104.4 (CH, C-3), 95.0 (CH_2, OCH_2O), 94.5 (CH_2, OCH_2O), 77.4 (C, C-1), 56.4 (CH_3, OCH_3), 56.0 (CH_3, OCH_3);

HR-ESIMS *m*/*z* $[M + Na]^+$ 346.9754 (Calcd. for $C_{10}H_{13}INaO_4$ 346.9756).

5.6.7 2,4-Dimethoxymethoxyphenylboronic acid (164)



Triisopropyl borate (10.7 mL, 46.3 mmol) was added to the stirred solution of aryl iodide **163** (5.00 g, 15.4 mmol) in THF:Et_2O (1:2, 100 mL) under N_2. The solution was cooled to -100 ^{o}C using liquid N_2 and a CH_3OH bath, and then *n*-BuLi (14.5 mL of a 1.6 M solution in hexanes) was added slowly with stirring. After 1 h of stirring at a temperature below -78 ^{o}C, a saturated NH_4Cl solution was added. The solution was stirred for an additional 1 h at rt and the two phases partitioned. The aqueous phase was extracted with Et_2O. The combined organic phases were washed with H_2O and brine, and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to give an orange oil. Purification of the oil by column chromatography (8:2 Hex:EtOAc) and evaporation of the solvent afforded the boronic acid **164** as an orange solid (2.76 g, 74%).

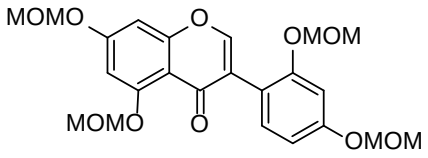
IR (neat) ν_{max} 3385, 2957, 1726, 1603, 1142, 993 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (1H, d, *J* = 8.4 Hz, H-6), 6.80 (1H, d, *J* = 2.0 Hz, H-3), 6.76 (1H, dd, *J* = 2.0 and 8.4 Hz, H-5), 6.68 (2H, br s, $B(OH)_2$), 5.27 (2H, s, OCH_2O), 5.19 (2H, s, OCH_2O), 3.50 (3H, s, OCH_3), 3.47 (3H, s, OCH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.5 (C, C-2), 160.9 (C, C-4), 137.8 (CH, C-6), 109.3 (CH, C-5), 102.1 (CH, C-3), 94.6 (OCH_2O), 94.0 (OCH_2O), 56.4 (OCH_3), 56.0 (OCH_3), (C-1 signal not observed);

^{11}B NMR ($CDCl_3$, 128 MHz) δ_B 28.55.

5.6.8 2',4',5,7-Tetramethoxymethoxyisoflavone (165)



To a solution of 3-iodochromone **90** (1.20 g, 3.06 mmol) in 1:1 DME:H_2O (50 mL) were added 10% Pd/C (0.160 g, 5 mol%), Na_2CO_3 (0.970 g, 9.18 mmol), and phenylboronic acid **164** (1.11 g, 4.59 mmol). The resulting mixture was stirred at 40-45 ^oC overnight. The catalyst was filtered and washed with H_2O and EtOAc. The aqueous phase was extracted with EtOAc, and the combined organic layers were washed with H_2O, brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude product was purified by silica gel column chromatography using Hex:EtOAc (7:3) to afford the isoflavone **168** (1.1 g, 78%) as a yellow oil:

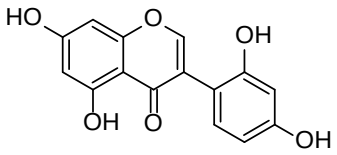
IR (neat) ν_{max} 2905, 2828, 1647, 1609, 1570, 1256, 1150, 999, 918 cm^{-1};

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (1H, s, H-2), 7.22 (1H, d, *J* = 8.5 Hz, H-6'), 6.88 (1H, d, *J* = 2.3 Hz, H-3'), 6.76-6.72 (3H, m, H-6, H-8, H-5'), 5.27 (2H, s, OCH_2O), 5.23 (2H, s, OCH_2O), 5.17 (2H, s, OCH_2O), 5.10 (2H, s, OCH_2O), 3.52 (3H, s, OCH_3), 3.50 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 3.41 (3H, s, OCH_3);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.9 (C, C-4), 160.9 (C, C-7), 159.4 (C, C-8a), 158.5 (C, C-5), 158.4 (C, C-4'), 156.2 (C, C-2'), 151.8 (CH, C-2), 132.4 (CH, C-6'), 123.1 (C, C-3), 115.5 (C, C-1'), 111.5 (C, C-4a), 108.9 (CH, C-5'), 104.1 (CH, C-3'), 102.4 (CH, C-6), 97.3 (CH, C-8), 95.8 (CH_2, OCH_2O), 95.1 (CH_2, OCH_2O), 94.5 (CH_2, OCH_2O), 94.3 (CH_2, OCH_2O), 56.5 (CH_3, OCH_3), 56.4 (CH_3, OCH_3), 56.1 (CH_3, OCH_3), 56.0 (CH_3, OCH_3);

HR-ESIMS *m/z* $[M + Na]^+$ 485.1415 **(**Calcd for $C_{23}H_{26}NaO_{10}$ 485.1424).

5.6.9 2',4',5,7-Tetrahydroxyisoflavone (149)



HCl (3M, 15 mL) was added to a solution of **165** (1.00 g, 2.16 mmol) in CH_3OH (30 mL) and stirred at 50 ^oC for 12 h. CH_3OH was evaporated and the reaction mixture was extracted with EtOAc. The combined organic phases were washed with H_2O and brine. The organic layer was dried over anhydrous $MgSO_4$ and the solvent evaporated under reduced pressure to give a yellow solid. Purification of the solid by column chromatography using Hex:EtOAc (1:1) afforded **149** as a pale yellow solid (0.45 g, 73%). Recrystallisation of **149** from Hex:EtOAc (1:4) gave pale yellow needles.

mp 268.4 - 270.1 ^oC (Lit.[26] mp 272.0 ^oC);

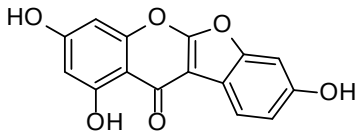
IR (neat) ν_{max} 3319, 1652, 1613, 1502, 1254, 1171 cm^{-1};

^1H NMR (CD_3OD, 400 MHz) δ 8.00 (1H, s, H-2), 7.04 (1H, d, J = 8.2 Hz, H-6'), 6.40 (1H, d, J = 2.3 Hz, H-3'), 6.37 (1H, dd, J = 2.3 and 8.2 Hz, H-5'), 6.36 (1H, d, J = 2.3 Hz, H-8), 6.23 (1H, d, J = 2.3 Hz, H-6); (Plate 24a)

^{13}C NMR (CD_3OD, 100 MHz) δ 182.7 (C, C-4), 166.2 (C, C-7), 163.7 (C, C-5), 160.2 (C, C-4'), 159.8 (C, C-8a), 157.8 (C, C-2'), 156.7 (CH, C-2), 133.2 (CH, C-6'), 122.6 (C, C-3), 110.9 (C, C-1'), 108.2 (CH, C-5'), 106.2 (C, C-4a), 104.4 (CH, C-3'), 100.3 (CH, C-6), 94.9 (CH, C-8); (Plate 24b)

HR-ESIMS m/z $[M - H]^-$ 285.0399 (Calcd. for $C_{15}H_9O_6$ 285.0399).

5.9.10 Lupinalbin A (148)



DDQ (79.0 mg, 0.349 mmol) was added under N_2 to a solution of **149 (**100 mg, 0.350 mmol**)** in THF (20 mL). The reaction mixture was heated to 60 ^{o}C with stirring for 15 min. Additional DDQ (79.0 mg, 0.349 mmol) was added and stirring was continued at the same temperature for 30 min. The solvent was evaporated and the crude mixture purified by column chromatography using Hex:EtOAc as eluent to give coumaronochromone **148** (66 mg, 66%) as a white solid. **148** was recrystallised from Hex:EtOAc (1:4) to afford white needle-like crystals, which decomposed at 278.4 - 280.0 ^{o}C (Lit.[21] mp > 300 ^{o}C);

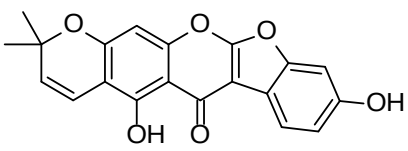
IR (neat) ν_{max} 3327, 3101, 2922, 1622, 1436, 1029, 822 cm^{-1};

^{1}H NMR (CD_3OD, 400 MHz) δ 7.75 (1H, d, *J* = 8.4 Hz, H-6'), 6.99 (1H, d, *J* = 1.9 Hz, H-3'), 6.89 (1H, dd, *J* = 1.9 and 8.4 Hz, H-5'), 6.46 (1H, d, *J* = 2.0 Hz, H-8), 6.27 (1H, d, *J* = 2.0 Hz, H-6); (Plate 25a)

^{13}C NMR (CD_3OD, 100 MHz) δ 180.0 (C, C-4), 166.2 (C, C-2), 165.2 (C, C-7), 164.0 (C, C-5), 157.8 (C, C-4'), 156.6 (C, C-8a), 151.9 (C, C-2'), 122.5 (CH, C-6'), 115.2 (CH, C-5'), 114.7 (C, C-1'), 104.5 (C, C-4a), 100.9 (CH, C-6), 99.5 (C, C-3), 98.7 (CH, C-3'), 95.8 (CH, C-8); (Plate 25b)

HR-ESIMS *m*/*z* [M - H]$^{-}$ 283.0239 (Calcd for $C_{15}H_7O_6$ 283.0243).

5.6.10 Lupinalbin H (130)



To a solution of **148** (40 mg, 0.141 mmol) in CH_3OH (15 mL) was added $Ca(OH)_2$ (21.0 mg, 0.282 mmol) followed by prenal (0.03 mL, 0.353 mmol). The mixture was stirred under an N_2 atmosphere for 3 days at rt. The CH_3OH was evaporated and the reaction mixture was diluted with EtOAc and 1 M HCl. The two phases were partitioned and the aqueous phase was extracted with EtOAc. The combined organic extracts were washed with H_2O and brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude product purified by column chromatography using Hex:EtOAc:acetone (6:1:1), followed by chromatotron using CH_2Cl_2 to give **130** as a cream white solid (20 mg, 40%) and lupinalbin A (**148**) (14 mg, 35%) was recovered. Recrystallisation of **130** from CH_3OH gave cream white crystals, which decomposed at 245.5-247.2 ^oC (Lit.[23] mp 248.0-250.0 ^oC);

IR (neat) ν_{max} 3501, 3230, 2923, 2853, 1729, 1640, 1594, 1456, 1393, 1116, 811 cm^{-1};

^1H NMR (acetone-d_6), 500 MHz) δ 13.38 (1H, s, OH-5), 8.98 (1H, brs, OH-4'), 7.82 (1H, d, *J* = 8.3 Hz, H-6'), 7.14 (1H, d, *J* = 2.0 Hz, H-3'), 7.02 (1H, dd, *J* = 2.0 and 8.3 Hz, H-5'), 6.70 (1H, d, *J* = 10.0 Hz, H-4"), 6.54 (1H, s, H-8), 5.79 (1H, d, *J* = 10.0 Hz, H-5"), 1.48 (6H, s, H-7" and H-8"); (Plate 26a)

^{13}C NMR (acetone-d_6), 125 MHz) δ 178.6 (C, C-4), 164.7 (C, C-2), 158.5 (C, C-7), 157.0 (C, C-5), 156.4 (C, C-4'), 154.0 (C, C-8a), 150.4 (C, C-2'), 128.6 (CH, C-5"), 121.3 (CH, C-6'), 114.6 (CH, C-4"), 114.0 (C, C-1'), 113.6 (CH, C-5'), 105.9 (C, C-6), 104.0 (C, C-4a), 98.5 (CH, C-3'), 97.5 (C, C-3), 95.3 (CH, C-8), 80.0 (C, C-6"), 27.3 (2 × CH_3, C-7" and C-8"); (Plate 26b)

HR-ESIMS *m/z* $[M - H]^-$ 349.0710 (Calcd for $C_{20}H_{13}O_6$ 349.0712).

5.7 REFERENCES

(1) Dewick, P. M. In *The Flavonoids: Advances in Research Since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994, p 117 - 238.

(2) Veitch, N. C. *Natural Product Reports* **2007**, *24*, 417-464.

(3) Veitch, N. C. *Natural Product Reports* **2009**, *26*, 776-802.

(4) Lawson, M. A.; Kaouadji, M.; Chulia, A. J. *Tetrahedron Letters* **2008**, *49*, 2407-2409.

(5) Shou, Q. Y.; Tan, Q.; Shen, Z. W. *Bioorganic and Medicinal Chemistry Letters* **2009**, *19*, 3389-3391.

(6) Reynaud, J.; Guilet, D.; Terreux, R.; Lussignol, M.; Walchshofer, N. *Natural Product Reports* **2005**, *22*, 504-515.

(7) Shu, P.; Qin, M. J.; Shen, W. J.; Wu, G. *Biochemical Systematics and Ecology* **2009**, *37*, 20-23.

(8) Mackova, Z.; Koblovska, R.; Lapcik, O. *Phytochemistry* **2006**, *67*, 849-855.

(9) Xiang, W.; Li, R. T.; Mao, Y. L.; Zhang, H. J.; Li, S. H.; Song, Q. S.; Sun, H. D. *Journal of Agricultural and Food Chemistry* **2005**, *53*, 267-271.

(10) Botta, B.; Menendez, P.; Zappia, G.; de Lima, R. A.; Torge, R.; Delle Monache, G. *Current Medicinal Chemistry* **2009**, *16*, 3414-3468.

(11) Lo, W. L.; Chang, F. R.; Liaw, C. C.; Wu, Y. C. *Planta Medica* **2002**, *68*, 146-151.

(12) Lo, W. L.; Wu, C. C.; Chang, F. R.; Wang, W. Y.; Khalil, A. T.; Lee, K. H.; Wu, Y. C. *Natural Product Research* **2003**, *17*, 91-97.

(13) Shiao, Y. J.; Wang, C. N.; Wang, W. Y.; Lin, Y. L. *Planta Medica* **2005**, *71*, 835-840.

(14) Selepe, M. A.; Drewes, S. E.; van Heerden, F. R. *Journal of Natural Products* **2010**, *73*, 1680-1685.

(15) Tahara, S.; Ingham, J. L.; Mizutani, J. *Agricultural and biological chemistry* **1985**, *49*, 1775-1783.

(16) Falshaw, C. P.; Harmer, R. A.; Ollis, W. D.; Wheeler, R. E.; Lalitha, V. R.; Rao, N. V. S. *Journal of the Chemical Society C* **1969**, 374-382.

(17) Lawson, M. A.; Kaouadji, M.; Chulia, A. J. *Tetrahedron Letters* **2008**, *49*, 2407-2409.

(18) Chubachi, M.; Hamada, M. *Tetrahedron Letters* **1971**, *12*, 3537-3540.

(19) Kurosawa, K.; Araki, F. *Bulletin of the Chemical Society of Japan* **1979**, *52*, 529-532.

(20) Chubachi, M.; Hamada, M.; Kawano, E. *Agricultural and Biological Chemistry* **1983**, *47*, 619-621.

(21) Tsukayama, M.; Oda, A.; Kawamura, Y.; Nishiuchi, M.; Yamashita, K. *Tetrahedron Letters* **2001**, *42*, 6163-6166.

(22) Zheng, S. Y.; Shen, Z. W. *Tetrahedron Letters* **2010**, *51*, 2883-2887.

(23) Tahara, S.; Katagiri, Y.; Ingham, J. L.; Mizutani, J. *Phytochemistry* **1994**, *36*, 1261-1271.

(24) Hanawa, F.; Tahara, S.; Mizutani, J. *Phytochemistry* **1991**, *30*, 157-163.

(25) Prasad, J. S.; Varma, R. S. *Phytochemistry* **1977**, *16*, 1120-1120.

(26) Whalley, W. B. *Journal of the Chemical Society* **1957**, *2*, 1833-1837.

(27) Miller, C. P.; Collini, M. D.; Harris, H. A. *Bioorganic and Medicinal Chemistry Letters* **2003**, *13*, 2399-2403.

(28) Minassi, A.; Giana, A.; Ech-Chahad, A.; Appendino, G. *Organic Letters* **2008**, *10*, 2267-2270.

(29) Gammill, R. B. *Synthesis* **1979**, 901-903.

(30) Hong, R.; Feng, J.; Hoen, R.; Lin, G.-Q. *Tetrahedron* **2001**, *57*, 8685-8689.

(31) Kim, T. Y.; Kim, H. S.; Lee, K. Y.; Kim, J. N. *Bulletin Korean Chemical Society* **1999**, *20*, 1255-1256.

(32) Watanabe, K.; Saito, T.; Niimura, K.; Kureha chemical industry Co., Ltd., Tokyo, Japan: United States, 1997; Vol. 6,698,575, p 1-20.

(33) Vasselin, A. D.; Wetwell, A. D.; Matthews, C. S.; Bradshaw, D. T.; Stevens, F. G. M. *Journal of Medicinal Chemistry* **2006**, *49*, 3973-3981.

(34) Node, M.; Kajimoto, T.; Nishide, K.; Fujita, E.; Fuji, K. *Bulletin of Institute for Chemical Research, Kyoto University* **1992**, *70*, 308-318.

(35) Denis, J. D. S.; Gordon, J. S.; Carroll, V. M.; Priefer, R. *Synthesis* **2010**, 1590-1592.

(36) Berliner, M.; Belecki, K. *Organic Syntheses* **2007**, *84*, 102-110.

(37) Zheng, X.; Meng, W.-D.; Qing, F.-L. *Tetrahedron Letters* **2004**, *45*, 8083-8085.

(38) Hoye, T. R.; Chen, M. *Tetrahedron Letters* **1996**, *37*, 3099-3100.

(39) Al-Zoubi, R. M.; Hall, D. G. *Organic Letters* **2010**, *12*, 2480-2483.

(40) Brown, H. C.; Cole, T. E. *Organometallics* **1983**, *2*, 1316-1319.

(41) Li, W.; Nelson, D. P.; Jensen, M. S.; Hoerrner, R. S.; Cai, D.; Larsen, R. D.; Reider, P. J. *The Journal of Organic Chemistry* **2002**, *67*, 5394-5397.

(42) Felpin, F.-X.; Lory, C.; Sow, H.; Acherar, S. *Tetrahedron* **2007**, *63*, 3010-3016.

(43) North, J. T.; Kronenthal, D. R.; Pullockaran, A. J.; Real, S. D.; Chen, H. Y. *The Journal of Organic Chemistry* **1995**, *60*, 3397-3400.

(44) Mondal, M.; Puranik, V. G.; Argade, N. P. *The Journal of Organic Chemistry* **2006**, *71*, 4992-4995.

(45) Horton, W. J.; Spence, J. T. *Journal of the American Chemical Society* **1955**, *77*, 2894-2896.

(46) Mathur, K. B. L.; Sharma, J. N.; Venkataramanan, K.; Krishnamurty, H. G. *Journal of the American Chemical Society* **1957**, *79*, 3582-3586.

(47) Vasselin, D. A.; Westwell, A. D.; Matthews, C. S.; Bradshaw, T. D.; Stevens, M. F. G. *Journal of Medicinal Chemistry* **2006**, *49*, 3973-3981.

CHAPTER 6

CONCLUSION

The main aims of this investigation were to develop an HPLC method for the analysis of *Eriosema* plants used for the treatment of male impotence and to develop methods for the synthesis of kraussianone 1 (**1**) and related isoflavonoids. Six plants from different sources, which were grouped as *E. kraussianum*, *uqonsi* and *ubangalala* were analysed by reversed-phase HPLC-PDA. Three plants, i.e., the two authentic *E. kraussianum* plants from the Drakensberg and Pietermaritzburg, and *uqonsi* from the herbal shop in Pietermaritzburg showed similar chemical profiles. However, there were variations in the relative amounts of the metabolites within each plant. In all the three plants, kraussianone 1 (**1**) occurred in relatively low concentrations, while kraussianone 2 (**2**) was one of the major constituents in the plant extracts. Other plant extracts contained different components from those found in *E. kraussianum*. This may suggest that the active ingredients in these plants are different compounds from those found in *E. kraussianum*. However, further chemical and biological investigations are required to reveal the active components in these plants.

The synthesis of kraussianone 1 (**1**) was accomplished both by a semi-synthetic route and a total synthetic strategy. Two routes were explored for the semi-synthesis of kraussianone 1 (**1**) from kraussianone 2 (**2**). The key step in the two semi-synthetic strategies was the conversion of the prenyl side chain of kraussianone 2 (**2**) into a dimethylchromene scaffold fused to OH-7. The formation of the dimethylchromene ring in the first route was based on acid-catalysed cyclisation of the prenyl group to give the dimethylchroman ring and subsequent dehydrogenation. This route was abandoned due to poor regioselectivity of the acid-catalysed cycloaddition reaction and the difficulty of oxidising the chroman fused to phloroglucinol moiety into a chromene. The best results were obtained from the second route, which was based on selective protection of the 2'-hydroxy group and oxidative cyclisation of the prenyl

side chain to OH-7 of kraussianone 2 (**2**) to give the dimethylpyran scaffold. Following this route, kraussianone 1 (**1**) was prepared in a 54% overall yield over five steps.

Although kraussianone 1 (**1**) could be prepared in good yields by the semi-synthetic route, there are several limitations inherent with semi-syntheses in general. Firstly, semi-synthetic routes depend on natural compounds as starting materials; this may increase the demand of the plant material and lead to illegal and unsustainable harvesting of the plant. Secondly, isolation of the pure material from the plant may be difficult and costly. Furthermore, the application of semi-synthetic strategies is narrow, especially in the preparation of diverse structural analogues.

The total synthesis of kraussianone 1 (**1**) and related isoflavonoids featured the Suzuki-Miyaura coupling reaction for the construction of the isoflavone nucleus and two methods for the regioselective introduction of the dimethylpyran scaffolds to the A- and B-rings. This route has advantages over the semi-synthetic one in that it allowed for the preparation of compounds structurally related to kraussianone 1 (**1**) by modifications of rings A, B and C. Following this protocol, six more isoflavonoids, eriosemaone D (**17**), genistein (**103**), geranylated isoflavonoid **89**, 2'-hydroxygenistein (**149**), lupinalbin A (**148**) and lupinalbin H (**130**), as well as two pyranochromones **52** and **127** were prepared in good to excellent yields by structural modifications of rings A, B and C (Figure 6.1).

The developed synthetic routes for kraussianone 1 (**1**) and related compounds provide us with material to conduct biological activity studies. Therefore, future work will involve investigations that will lead to the revelation of the mode of action of **1** for the erectile dysfunction activity. Furthermore, prenylated isoflavonoids are reported to exhibit many important biological activities, thus the synthesised compounds will be tested for other pharmacological activities.

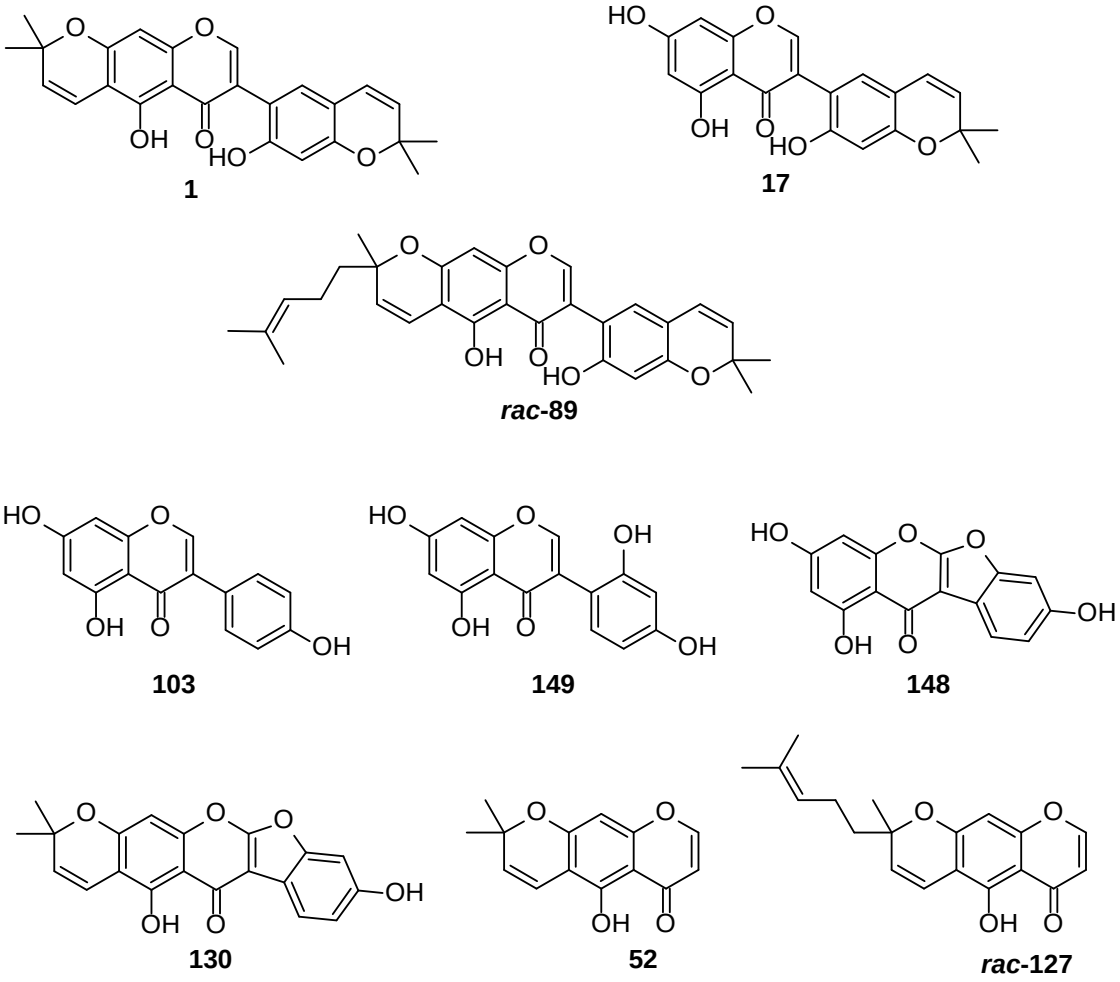


Figure 6.1. Synthesised isoflavonoids and chromones

APPENDIX I

COPIES OF NMR SPECTRA

Plate 1a. ^{1}H NMR spectrum of 2',5-dihydroxy-[6",6"-dimethylpyrano(2",3":4',5')][4"',5"'-dihydro-6"',6"' dimethylpyrano(2"',3"':7,6)]isoflavone (**36**) in $CDCl_3$

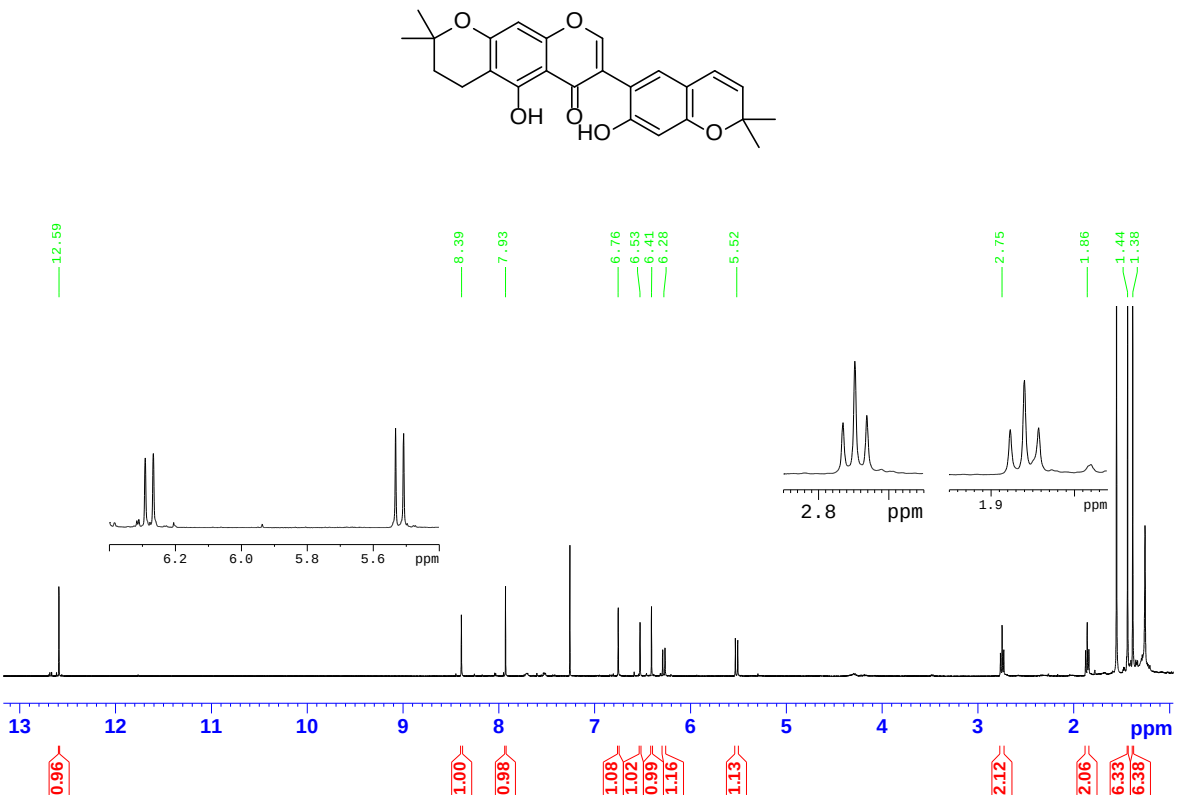


Plate 1b. ^{13}C NMR spectrum of 2',5-dihydroxy-[6",6"-dimethylpyrano(2",3":4',5')][4"',5"'-dihydro-6"',6"' dimethylpyrano(2"',3"':7,6)]isoflavone (**36**) in $CDCl_3$

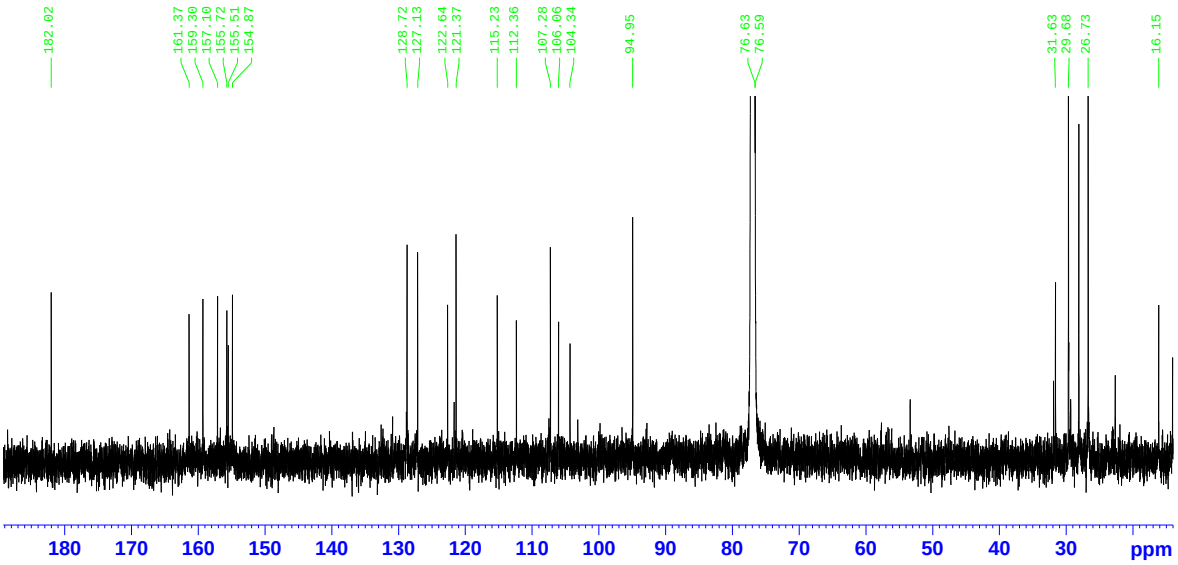


Plate 2a. ^{1}H NMR Spectra of 2',7-dihydroxy-[6",6"-dimethylpyrano(2",3":4',5')][4"',5"'-dihydro-6"',6"'-dimethylpyrano(2"',3"':5,6)]isoflavone (**39**) in DMSO-d_6

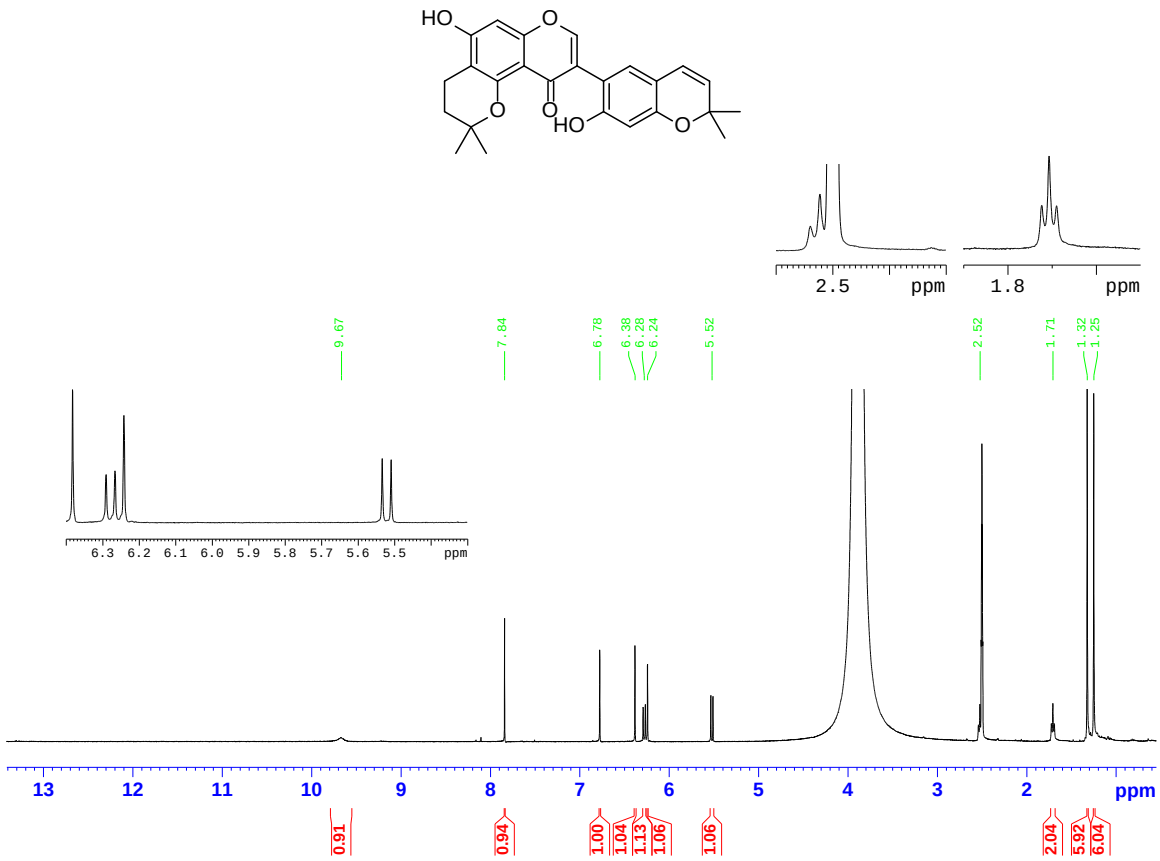


Plate 2b. ^{13}C NMR Spectra of 2',7-dihydroxy-[6",6"-dimethylpyrano(2",3":4',5')][4"',5"'-dihydro-6"',6"'-dimethylpyrano(2"',3"':5,6)]isoflavone (**39**) in DMSO-d_6

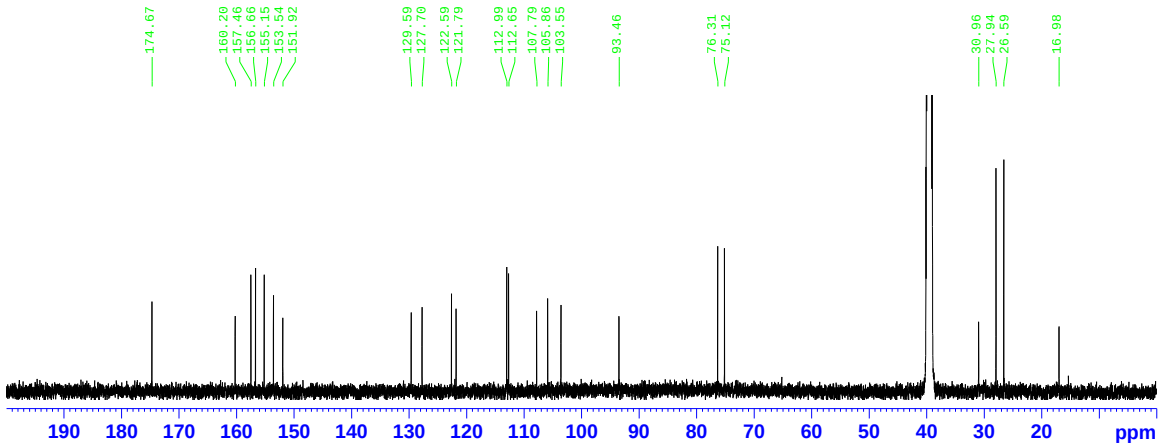


Plate 3a. ^{1}H NMR spectrum of 2',7-diacetoxy-[6",6"-dimethylpyrano(2",3":4',5')][4"',5"'-dihydro-6"',6"' dimethylpyrano(2"',3"':5,6)]isoflavone (**42**) in $CDCl_3$

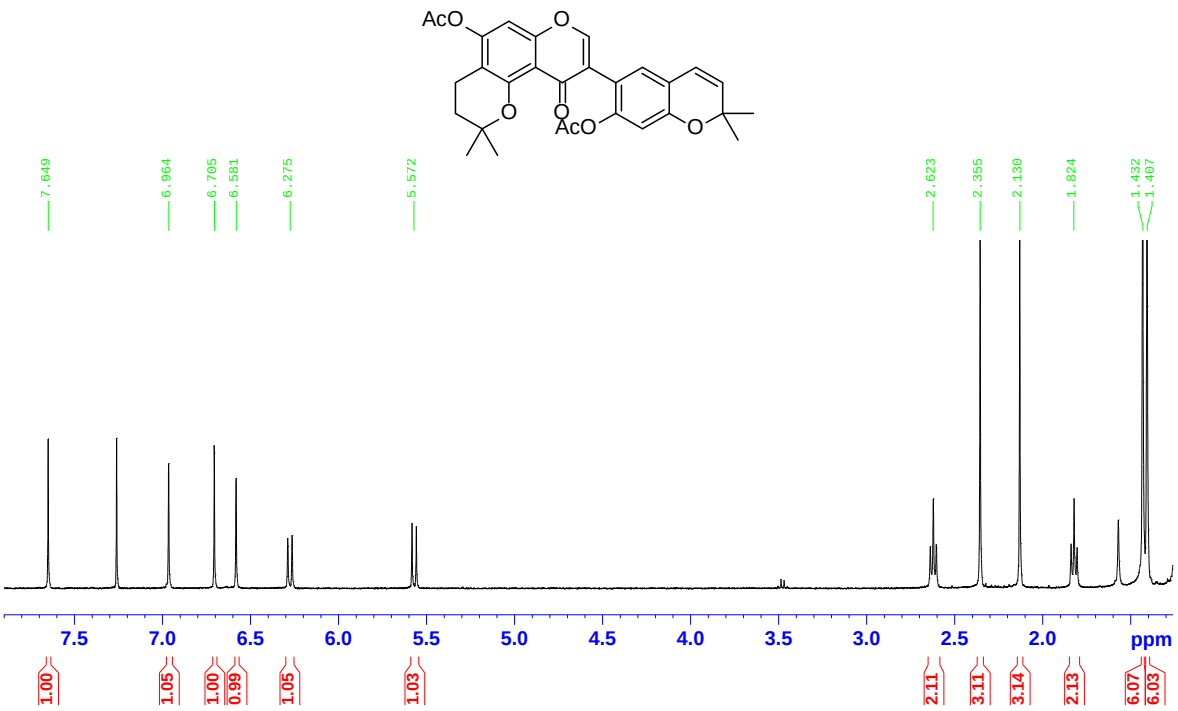


Plate 3b. ^{13}C NMR spectrum of 2',7-diacetoxy-[6",6"-dimethylpyrano(2",3":4',5')][4"',5"'-dihydro-6"',6"' dimethylpyrano(2"',3"':5,6)]isoflavone (**42**) in $CDCl_3$

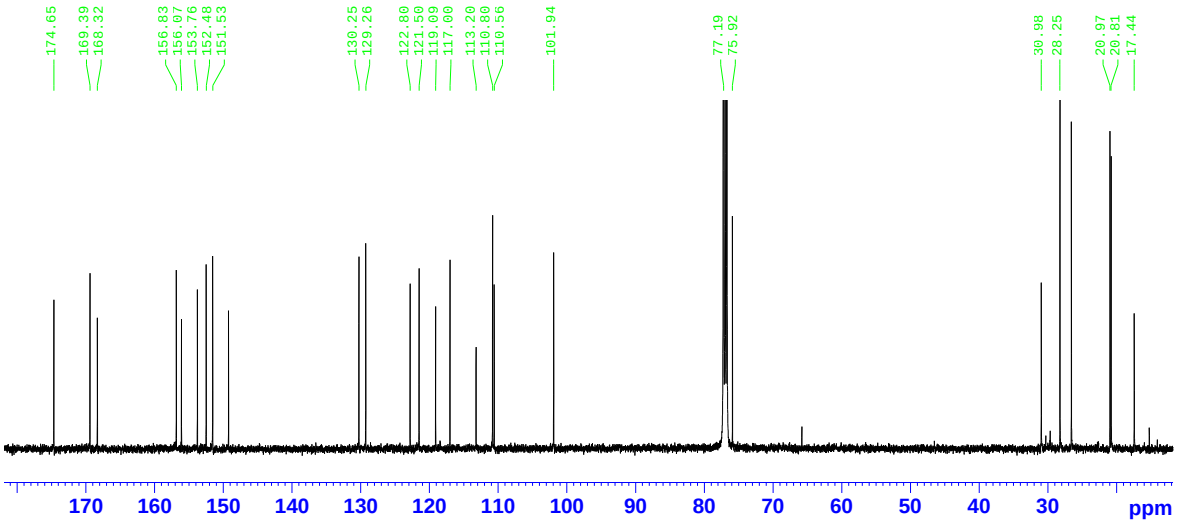


Plate 4a. ^{1}H NMR spectrum of 7-*tert*-butyldimethylsiloloxy-2',5-dihydroxy-6-prenyl-[6",6"-dimethylpyrano(2",3":4',5')]isoflavone (**59**) in $CDCl_3$

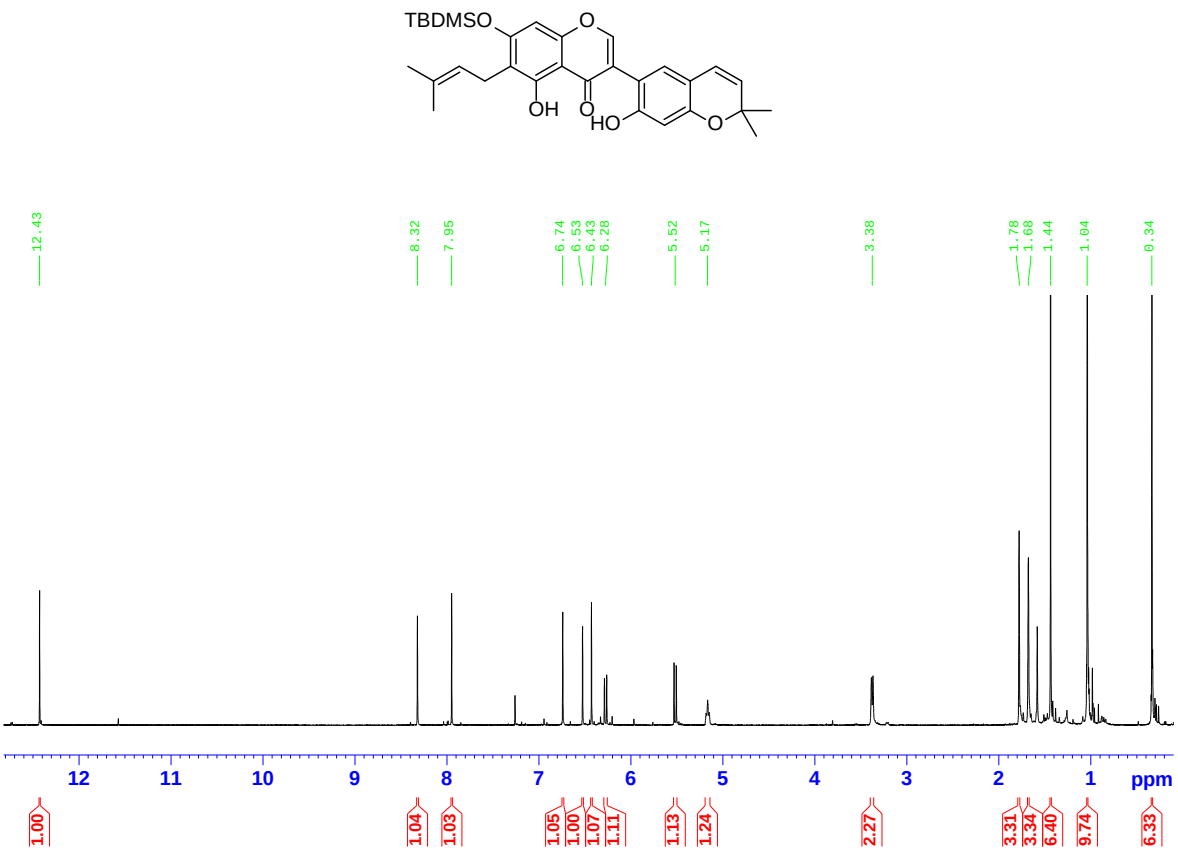


Plate 4b. ^{13}C NMR spectrum of 7-*tert*-butyldimethylsiloloxy-2',5-dihydroxy-6-prenyl-[6",6"-dimethylpyrano(2",3":4',5')]isoflavone (**59**) in $CDCl_3$

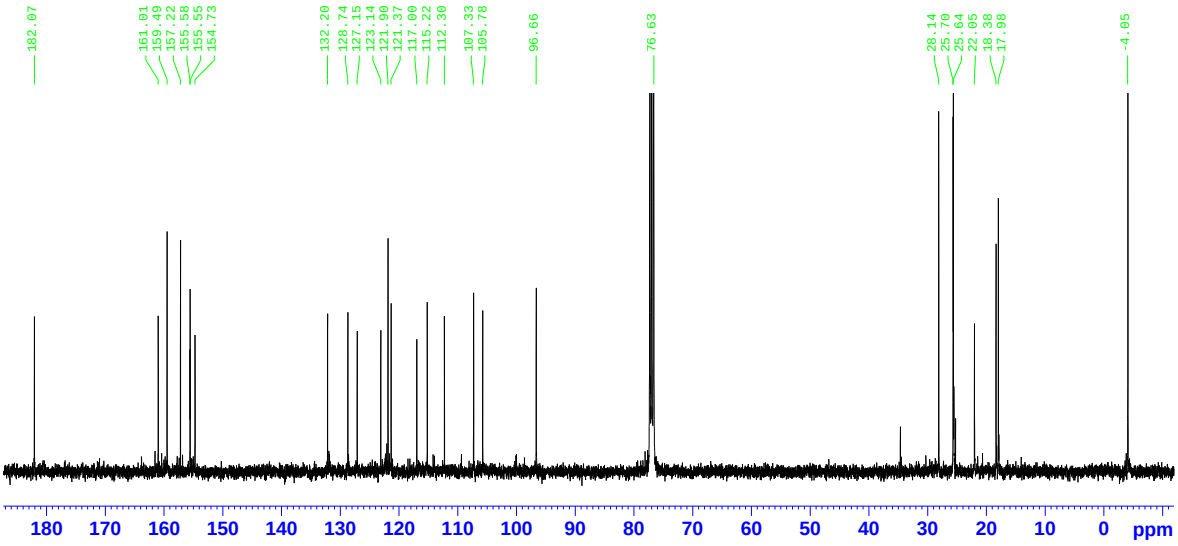


Plate 5a. ^{1}H NMR spectrum of 7-*tert*-butyldiphenylsilyloxy-2',5-dihydroxy-6-prenyl[6",6"-dimethylpyrano(2",3":4',5')]isoflavone (**63**) in $CDCl_3$

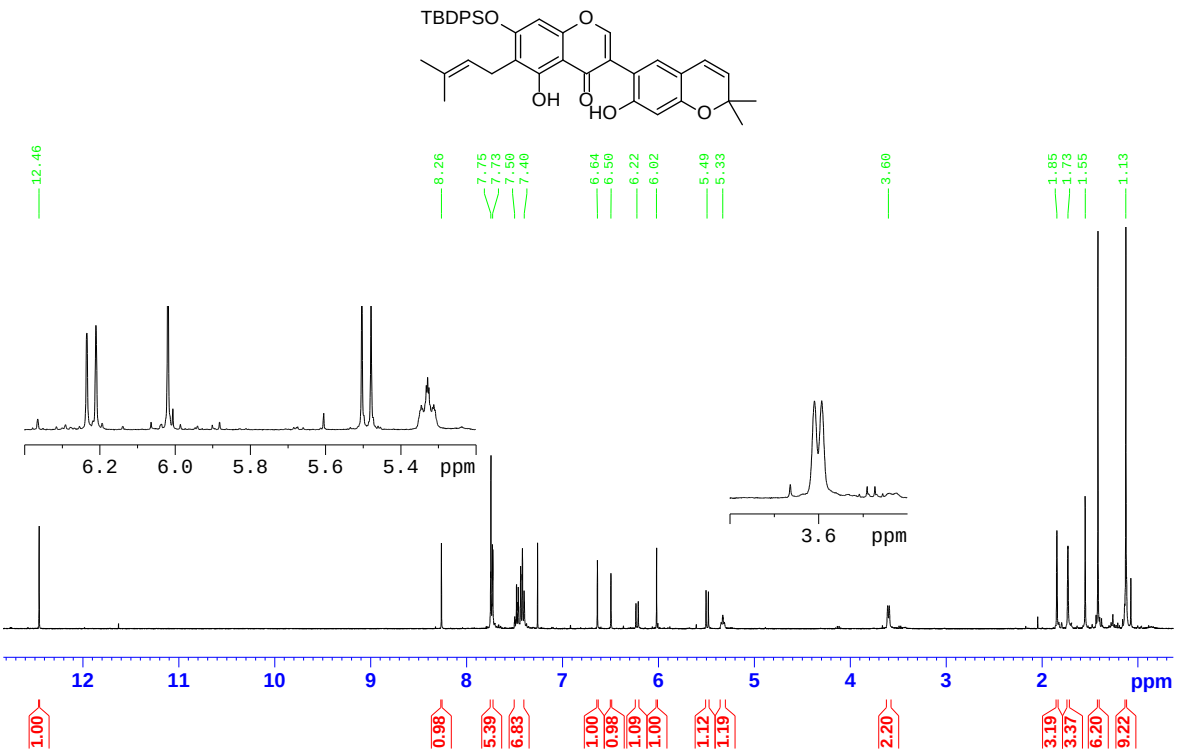


Plate 5b. ^{13}C NMR spectrum of 7-*tert*-butyldiphenylsilyloxy-2',5-dihydroxy-6-prenyl[6",6"-dimethylpyrano(2",3":4',5')]isoflavone (**63**) in $CDCl_3$

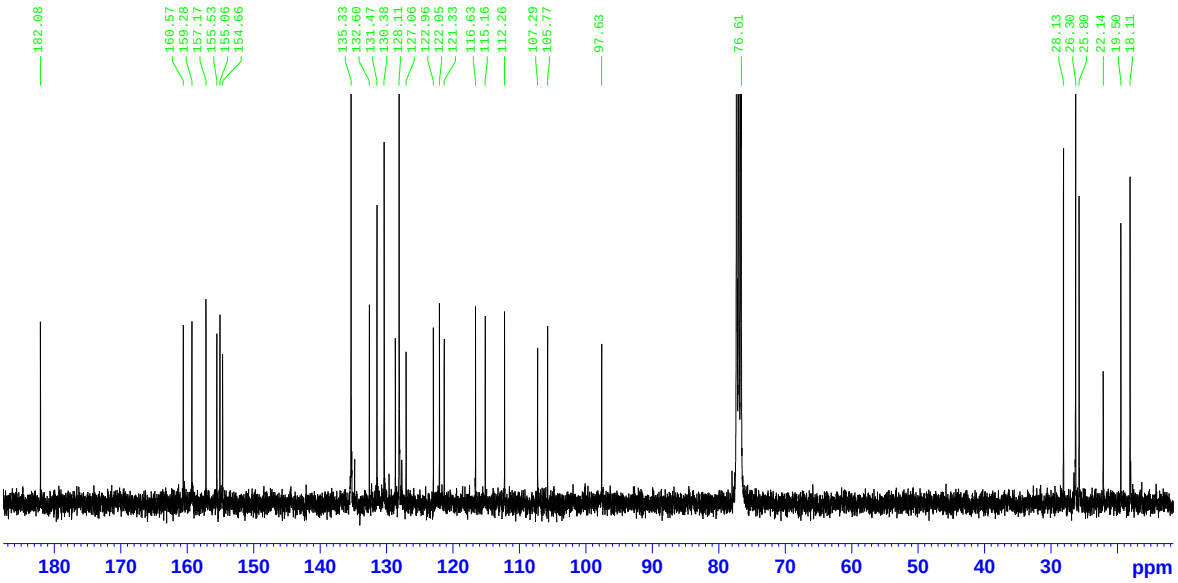


Plate 6a. ^{1}H NMR spectrum of 2'-acetoxy-7-*tert*-butyldiphenylsilyloxy-5-hydroxy-6-prenyl[6",6"-dimethylpyrano(2",3":4',5')]isoflavone (**64**) in $CDCl_3$

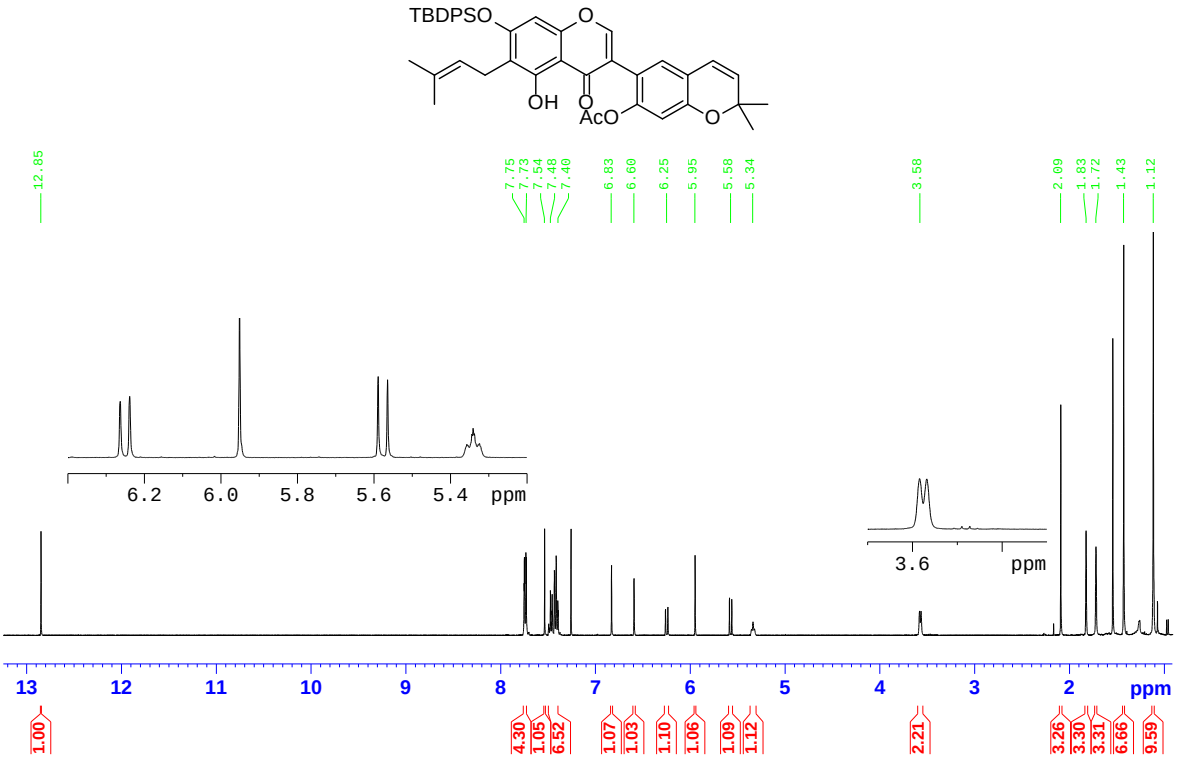


Plate 6b. ^{13}C NMR spectrum of 2'-acetoxy-7-*tert*-butyldiphenylsilyloxy-5-hydroxy-6-prenyl[6",6"-dimethylpyrano(2",3":4',5')]isoflavone (**64**) in $CDCl_3$

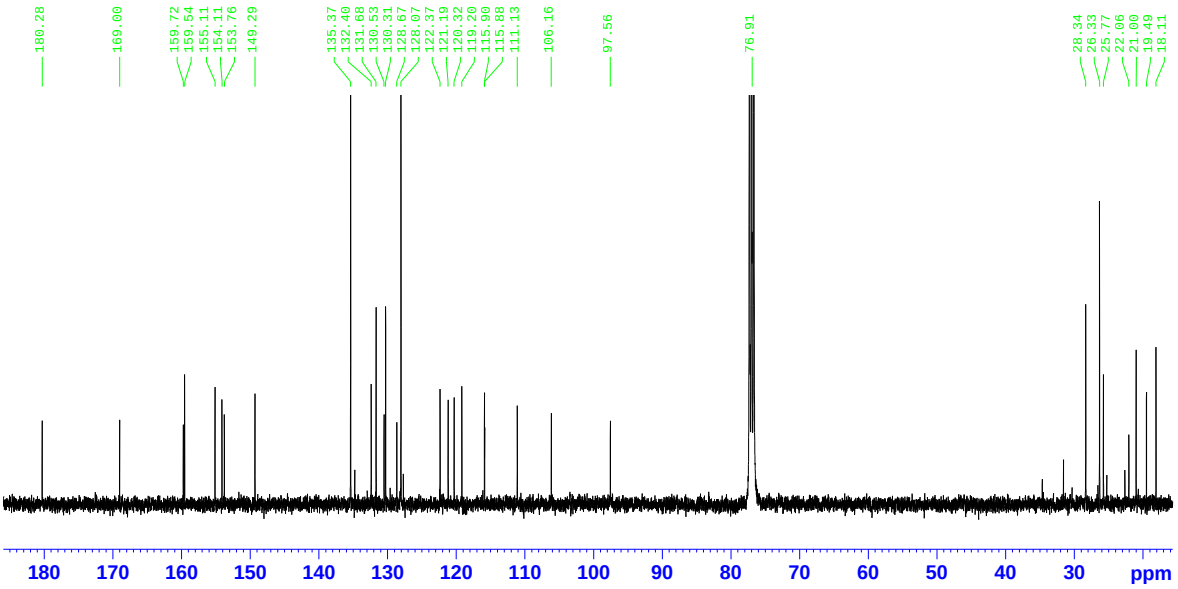
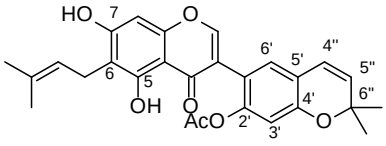


Plate 7a. ^{1}H NMR spectrum of 2'-acetoxy-5,7-dihydroxy-6-prenyl[6",6"-dimethylpyrano (2",3":4',5')]isoflavone (**65**) in $CDCl_3$



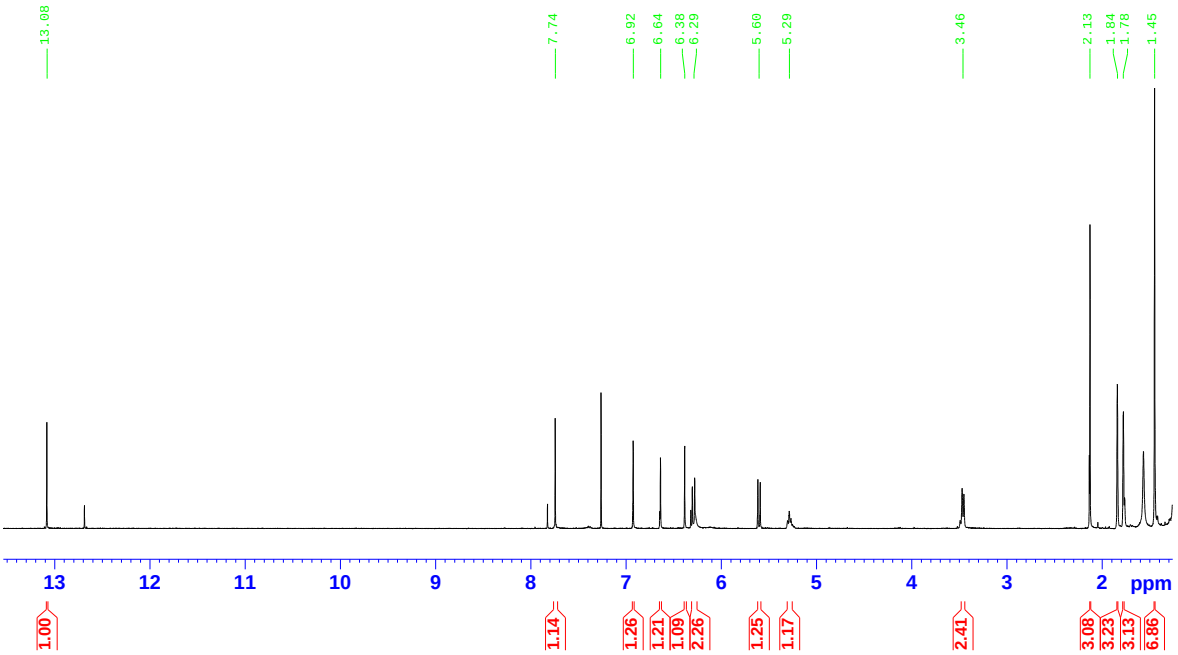


Plate 7b. ^{13}C NMR spectrum of 2'-acetoxy-5,7-dihydroxy-6-prenyl[6",6"-dimethylpyrano (2",3":4',5')]isoflavone (**65**) in $CDCl_3$

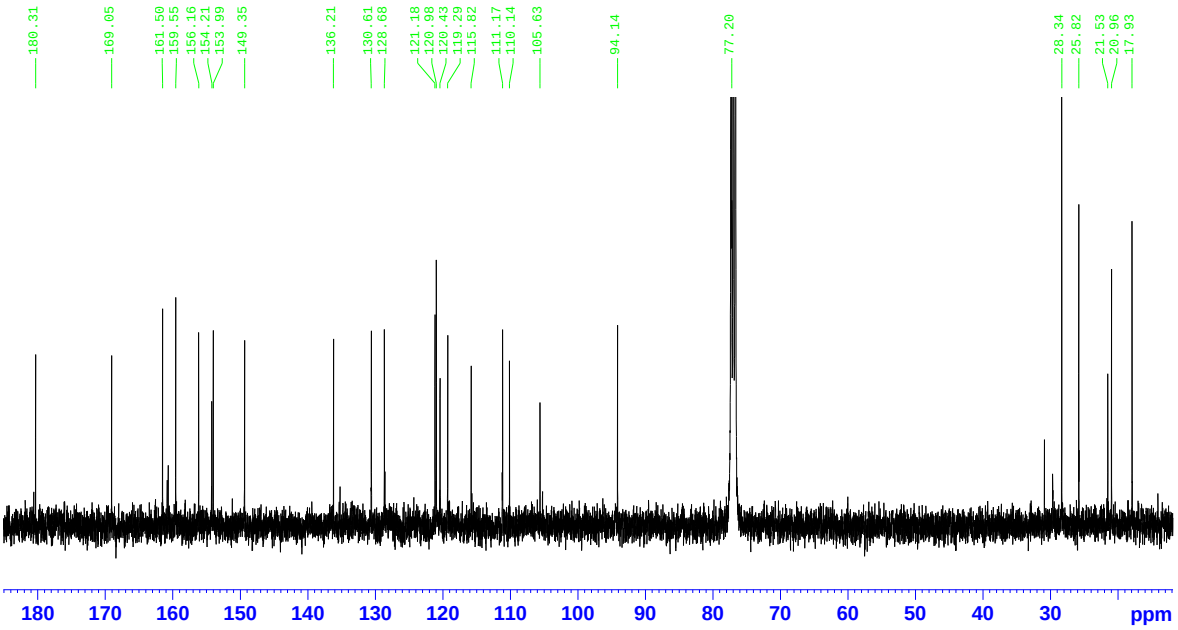


Plate 8a. ^{1}H NMR spectrum of 2'-acetoxy-5-hydroxy-[6",6"-dimethylpyrano(2",3":4',5')][6"',6"'-dimethylpyrano(2"',3"':7,6)]isoflavone (**66**) in $CDCl_3$

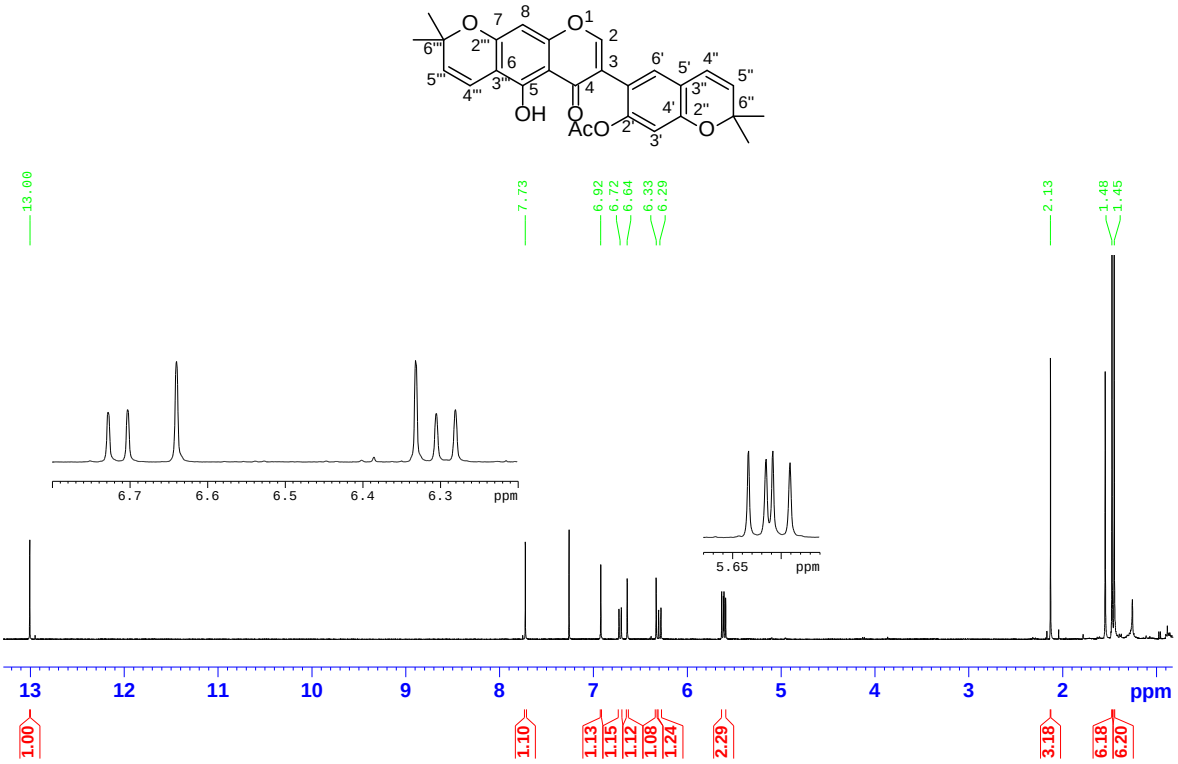


Plate 8b. ^{13}C NMR spectrum of 2'-acetoxy-5-hydroxy-[6",6"-dimethylpyrano(2",3":4',5')][6"',6"'-dimethylpyrano(2"',3"':7,6)]isoflavone (**66**) in $CDCl_3$

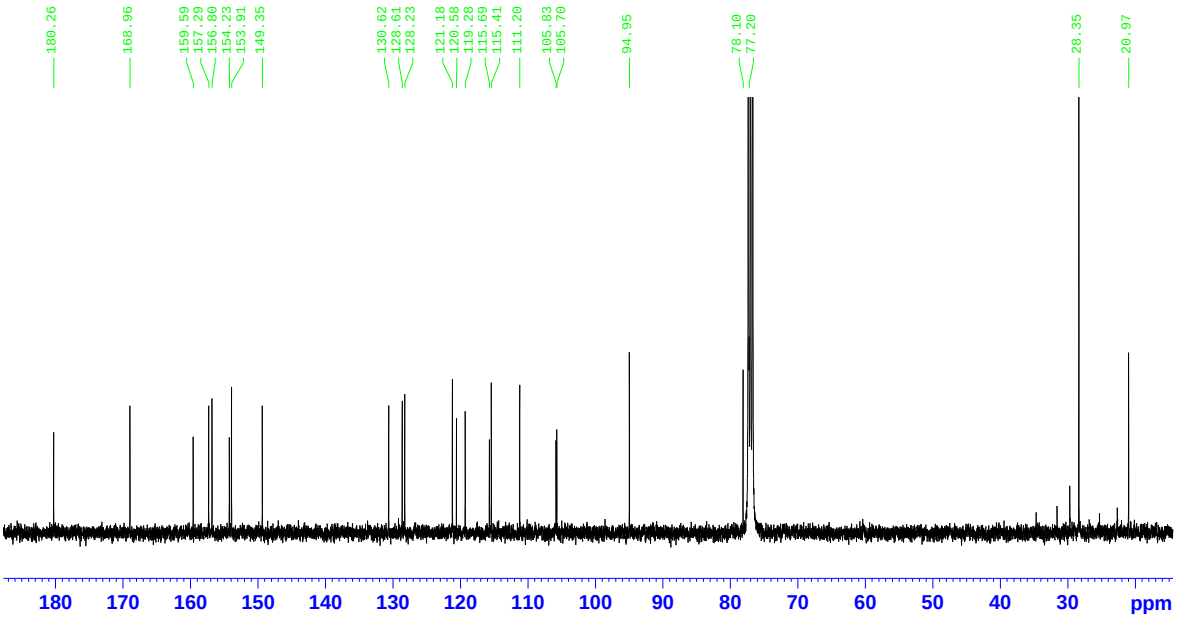


Plate 9a. ^{1}H NMR spectrum of kraussianone 1 (**1**) in $CDCl_3$

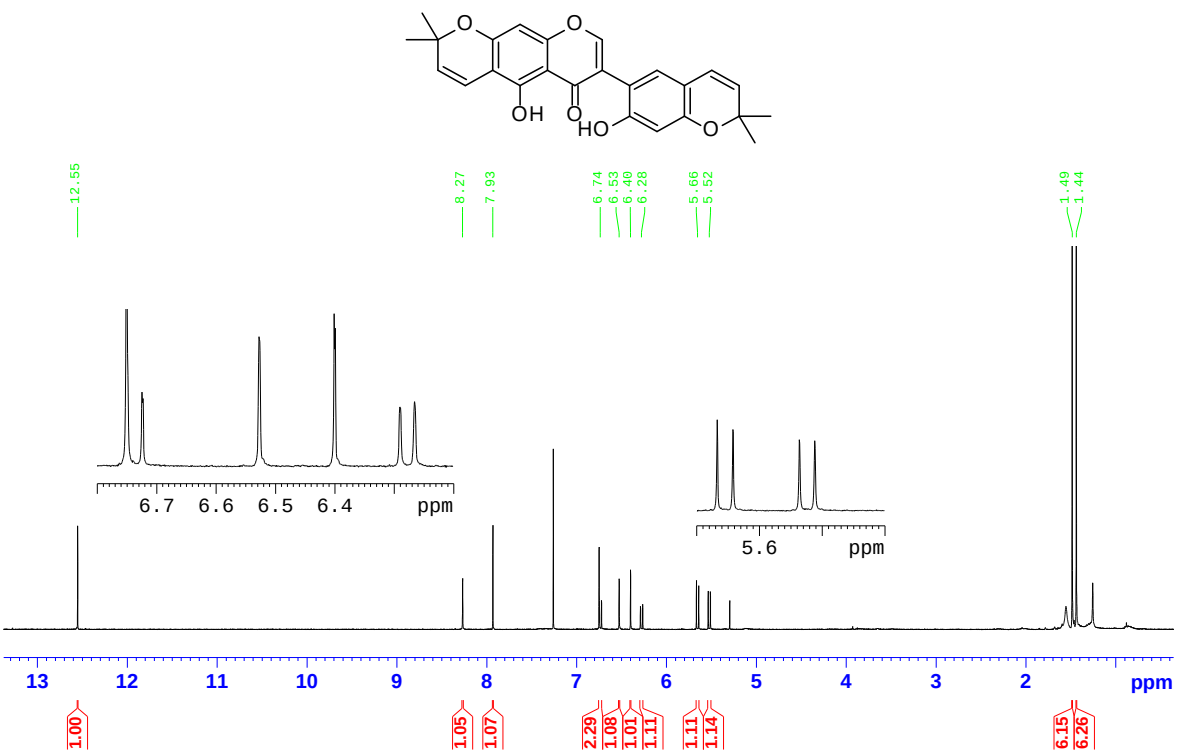


Plate 9b. ^{13}C NMR spectrum of kraussianone 1 (**1**) in $CDCl_3$

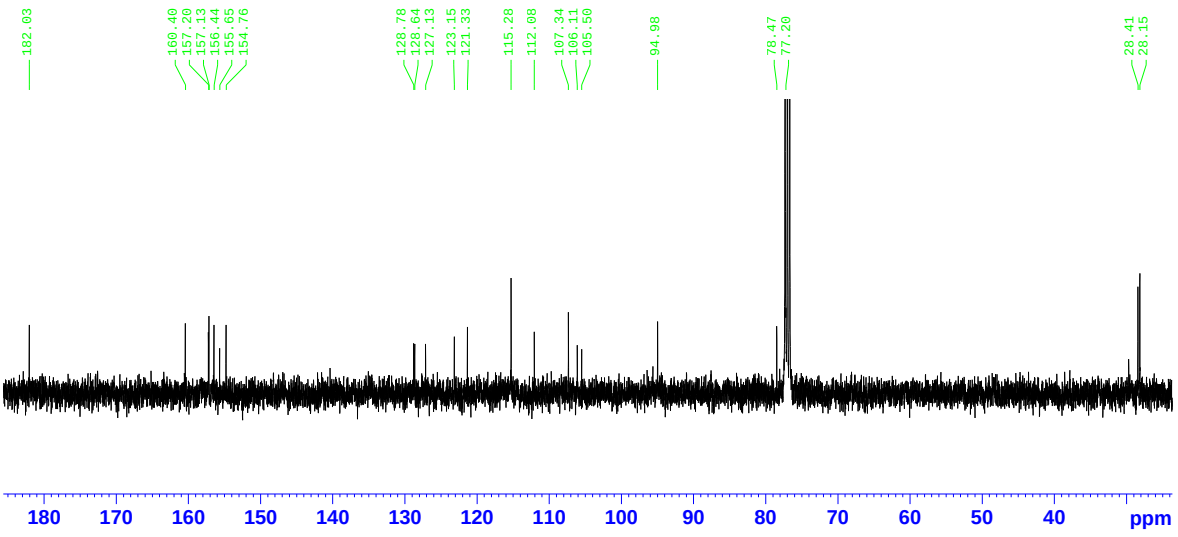


Plate 10a. ^{1}H NMR spectrum of 3-(*N*,*N*-dimethylamino)-1-(2'-hydroxy-4',6'-dimethoxymethoxyphenyl)propenone (**93**) in $CDCl_3$

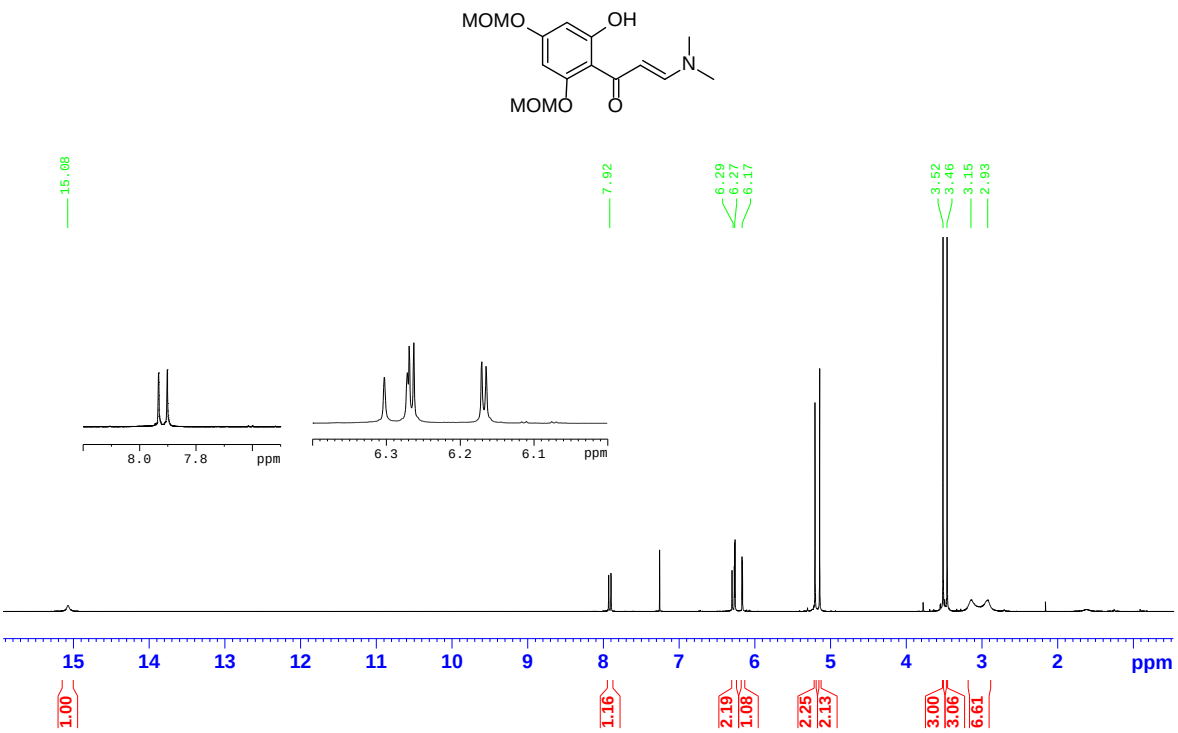


Plate 10b. ^{13}C NMR spectrum of 3-(*N*,*N*-dimethylamino)-1-(2'-hydroxy-4',6'-dimethoxymethoxyphenyl)propenone (**93**) in $CDCl_3$

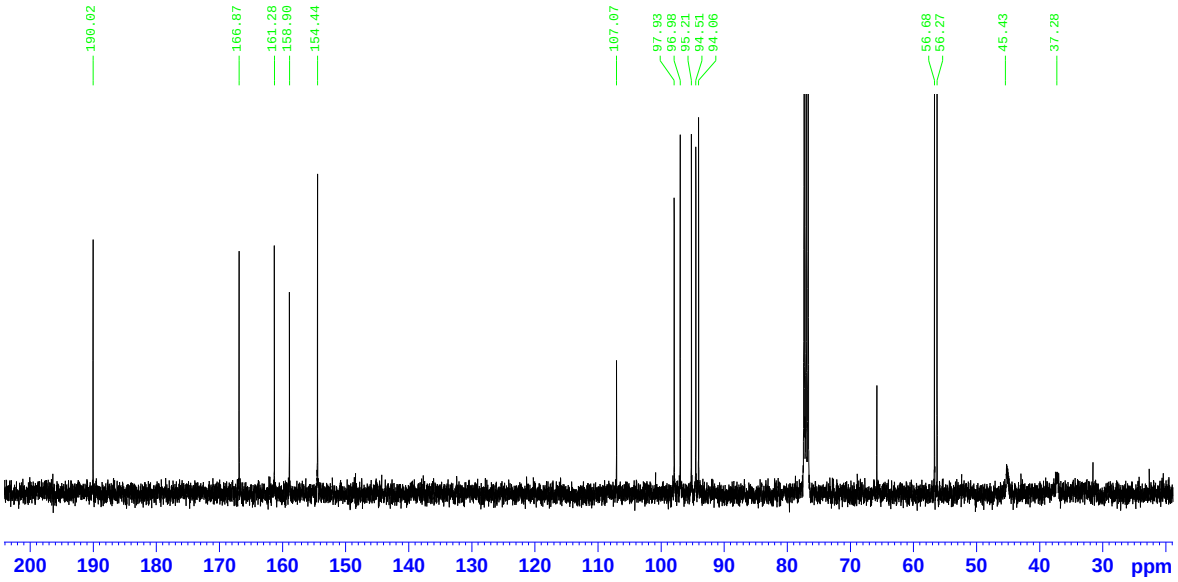
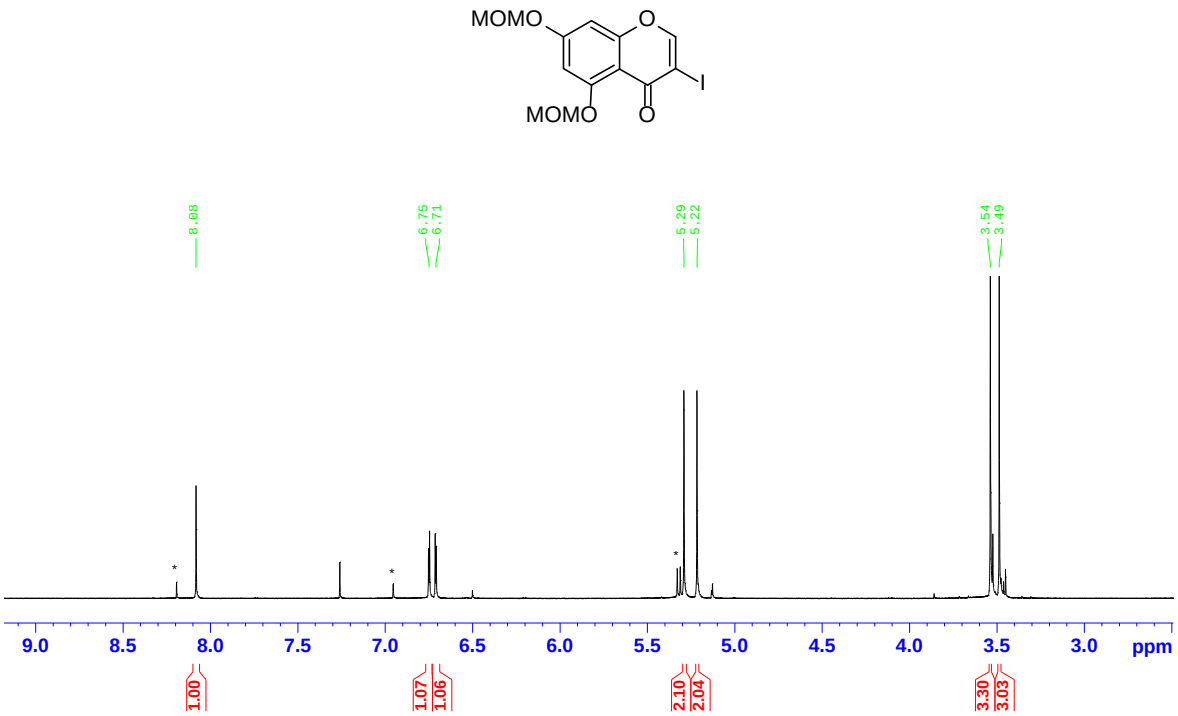


Plate 11a. ^{1}H NMR spectrum of 3-iodo-5,7-dimethoxymethoxychromone (**90**) in $CDCl_3$



* impurities of **95**

Plate 11b. ^{13}C NMR spectrum of 3-iodo-5,7-dimethoxymethoxychromone (**90**) in $CDCl_3$

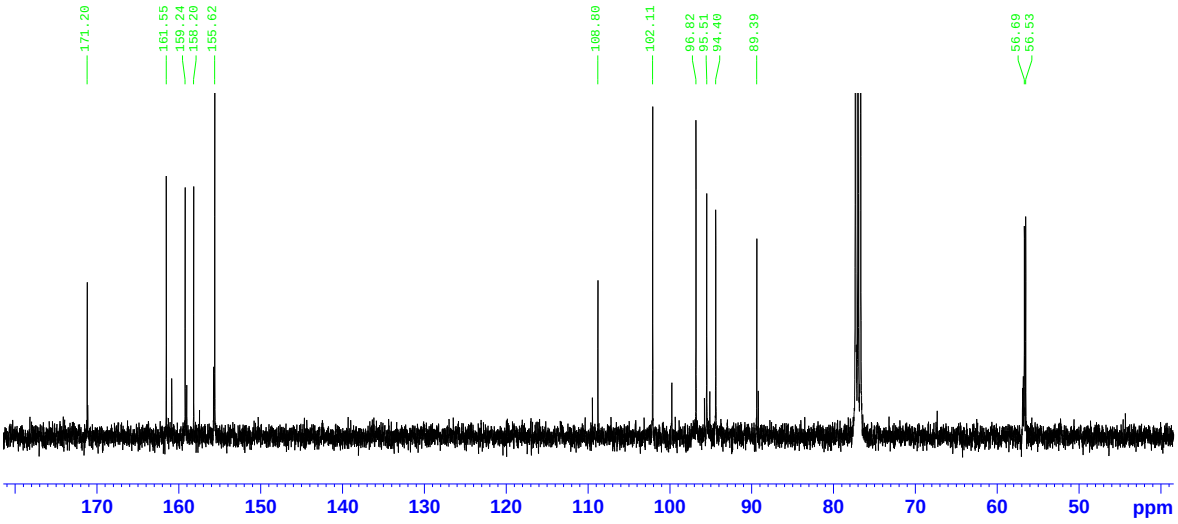


Plate 12. ^{1}H NMR spectrum of genistein (**103**) in DMSO-d_6

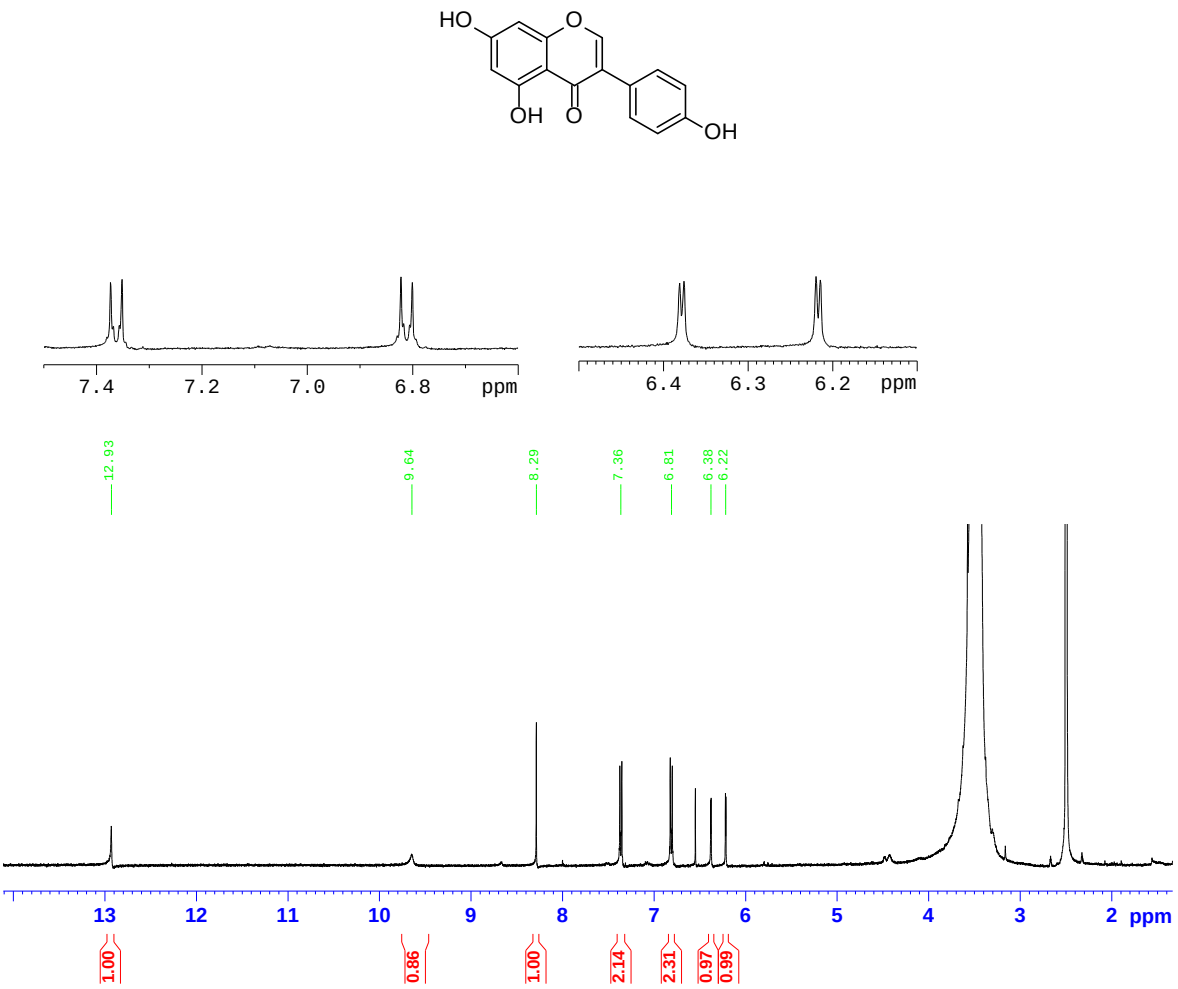


Plate 13a. ^{1}H NMR spectrum of 7-benzyloxy-2,2-dimethylchromene-6-boronic acid (**91**) in $CDCl_3$

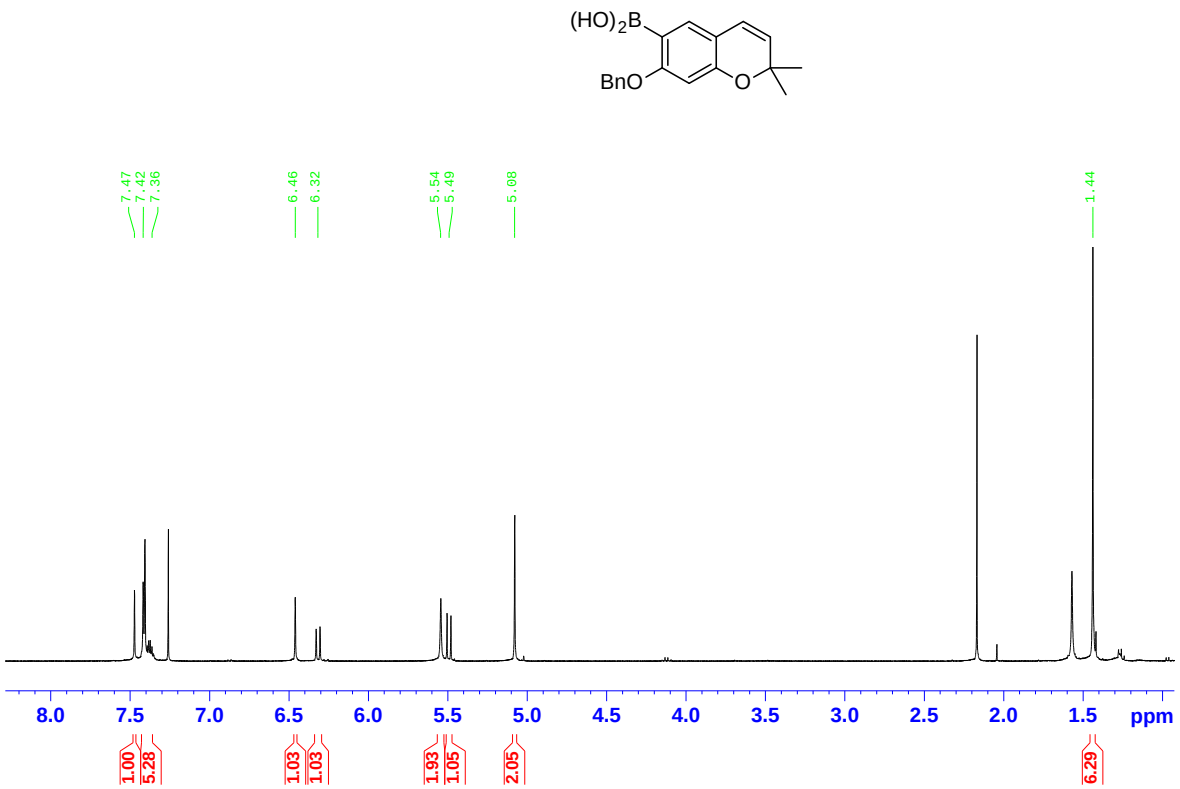


Plate 13b. ^{13}C NMR spectrum of 7-benzyloxy-2,2-dimethylchromene-6-boronic acid (**91**) in $CDCl_3$

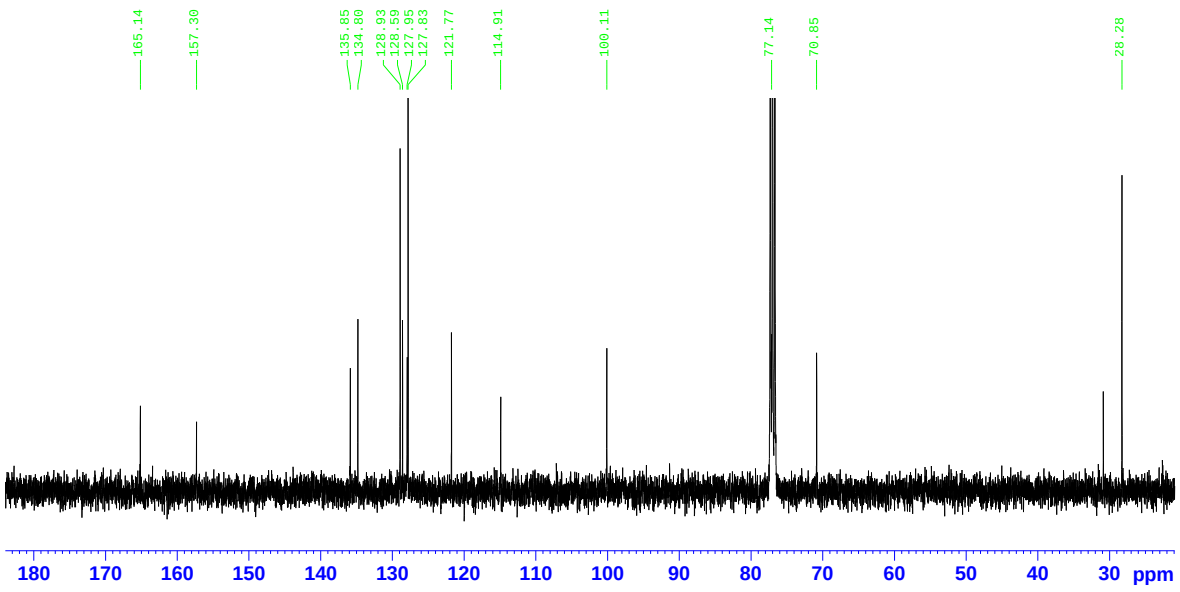


Plate 14a. ^{1}H NMR spectrum of 2'-benzyloxy-5,7-dihydroxy-6",6"-dimethylpyrano[2",3":4',5']isoflavone (**115**) in $CDCl_3$

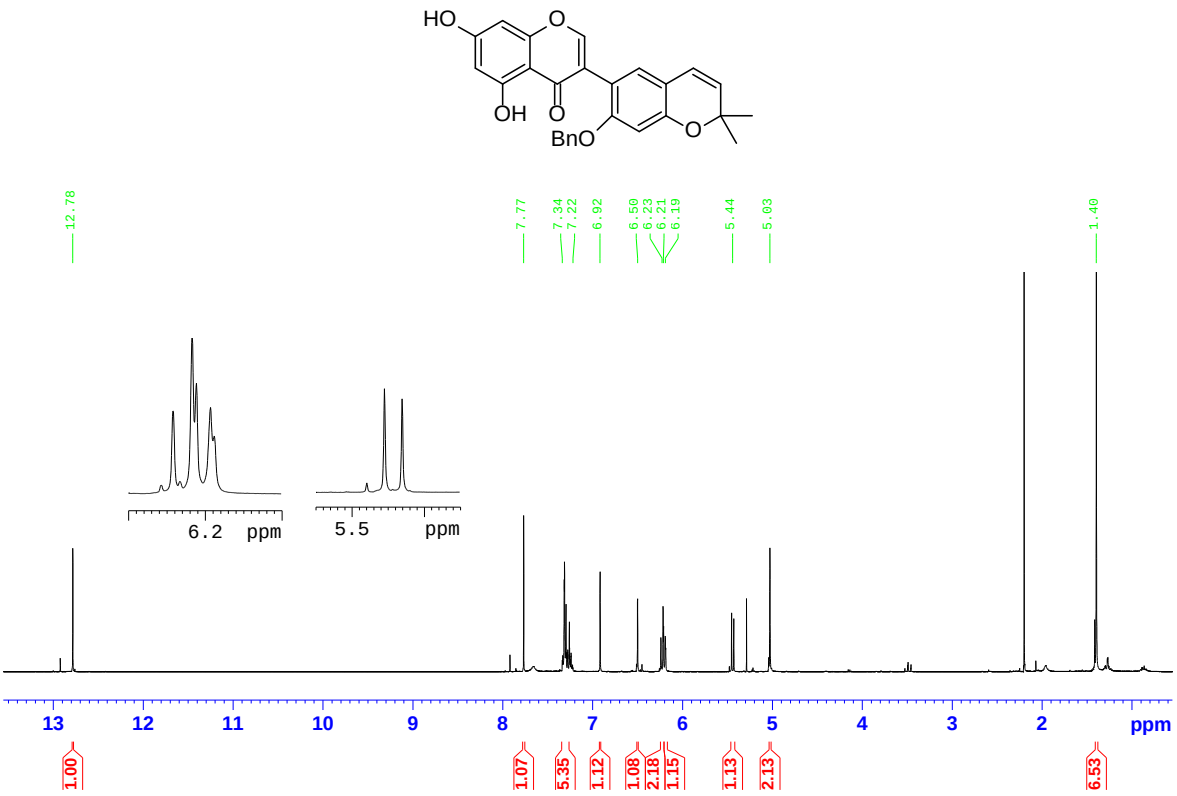


Plate 14b. ^{13}C NMR spectrum of 2'-benzyloxy-5,7-dihydroxy-6",6"-dimethylpyrano[2",3":4',5']isoflavone (**115**) in $CDCl_3$

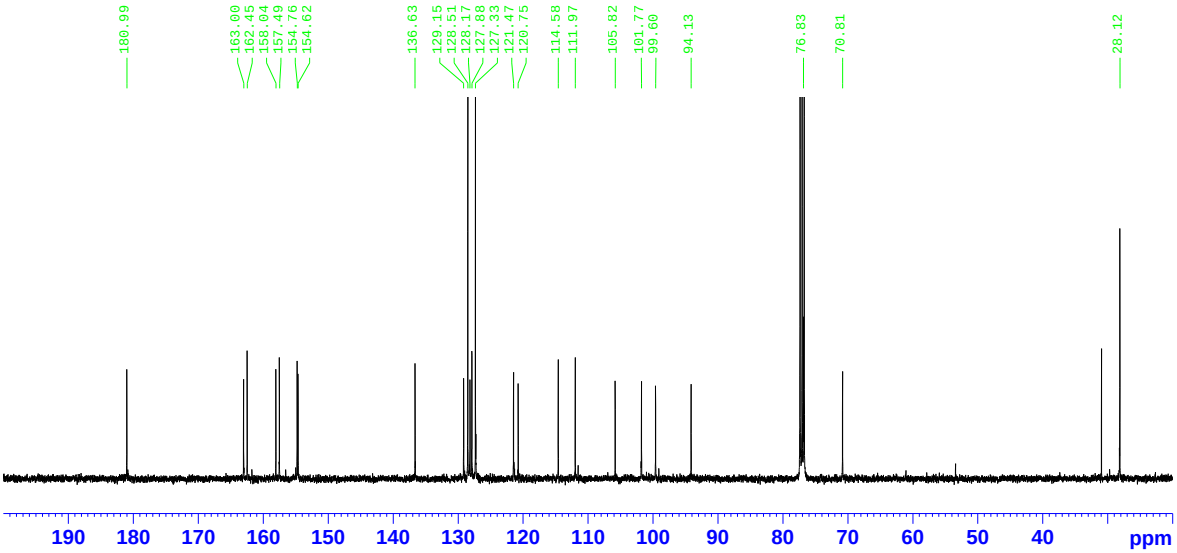


Plate 15a. ^{1}H NMR spectrum of 2'-*O*-benzylkraussianone 1 (**116**) in $CDCl_3$

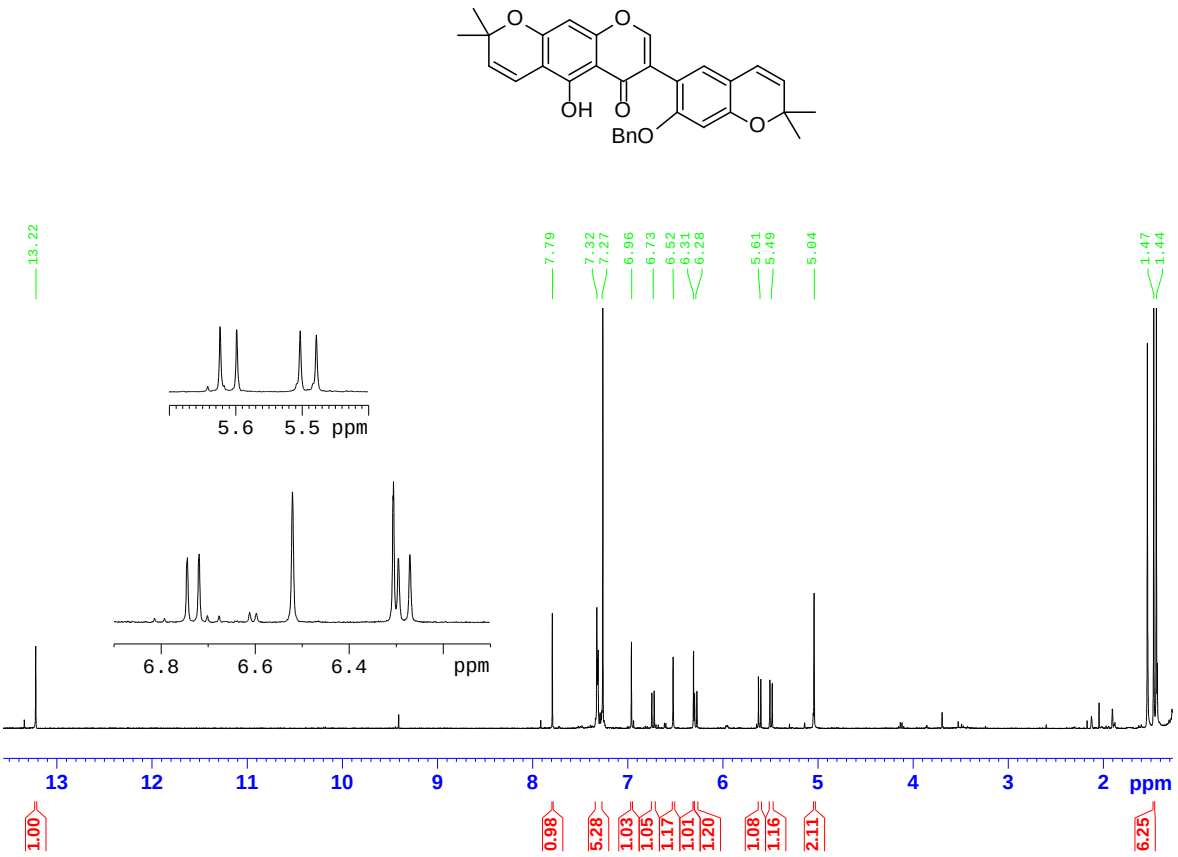


Plate 15b. ^{13}C NMR spectrum of 2'-*O*-benzylkraussianone 1 (**116**) in $CDCl_3$

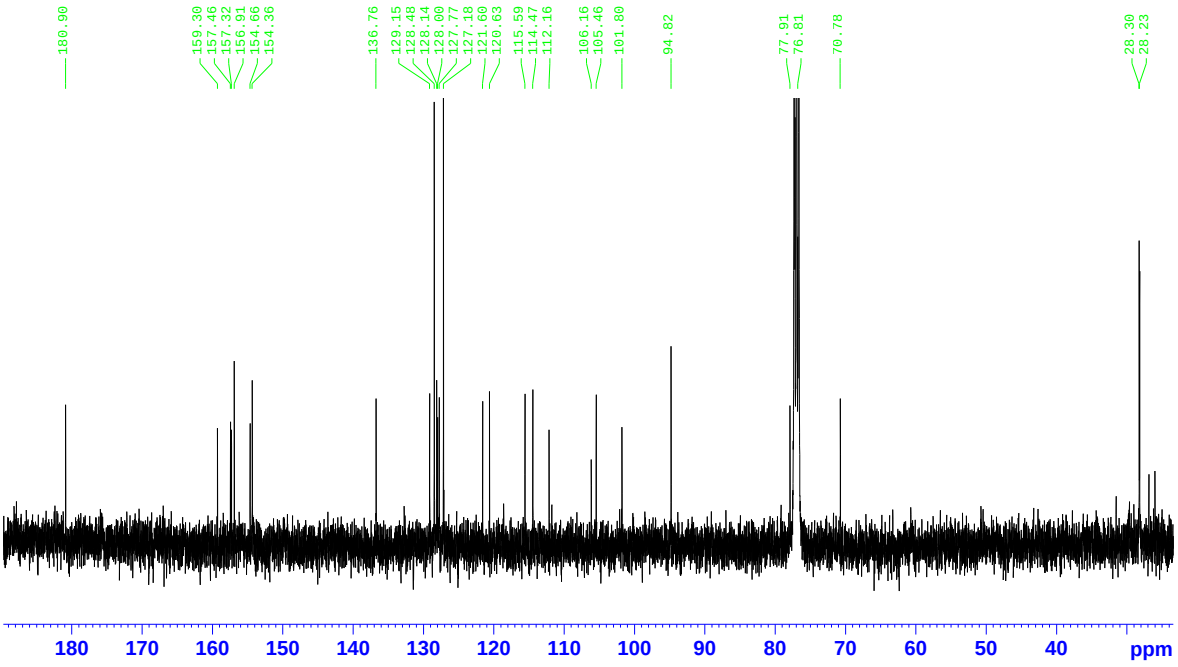


Plate 16a. ^{1}H NMR spectrum of 7-methoxymethoxy-2,2-dimethylchromene-6-boronic acid (**125**) in $CDCl_3$

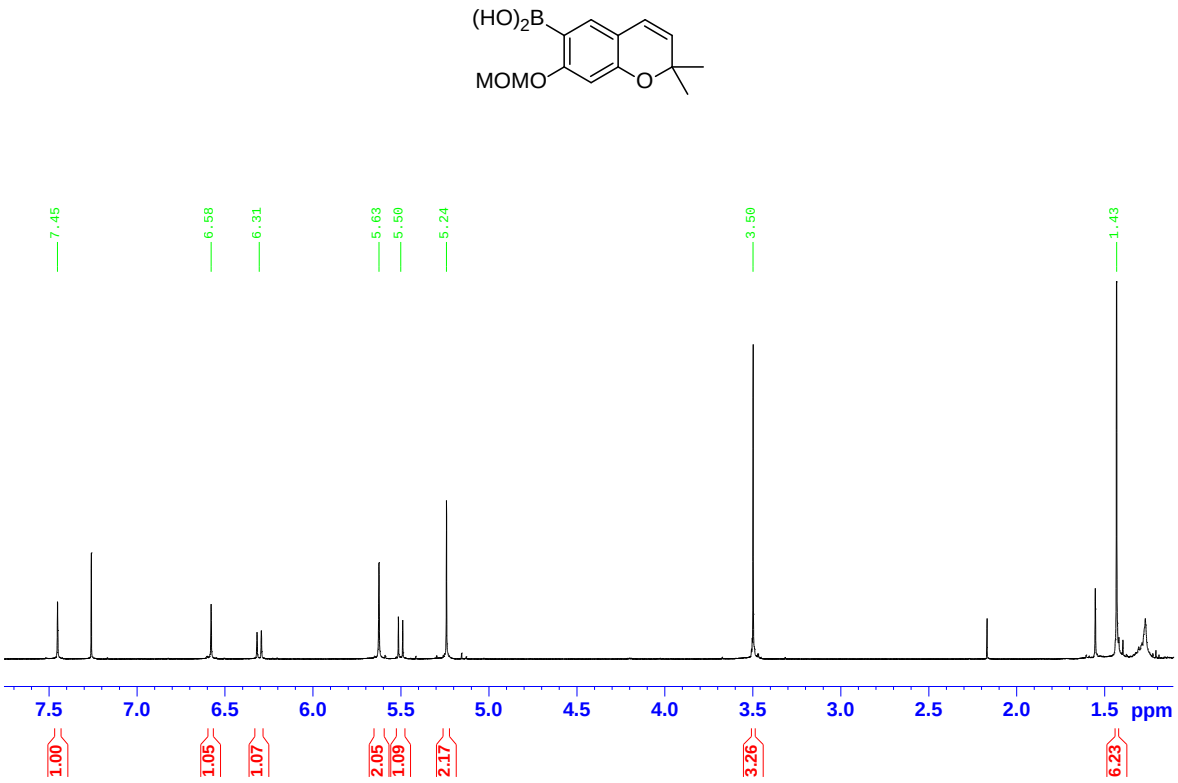


Plate 16b. ^{13}C NMR spectrum of 7-methoxymethoxy-2,2-dimethylchromene-6-boronic acid (**125**) in $CDCl_3$

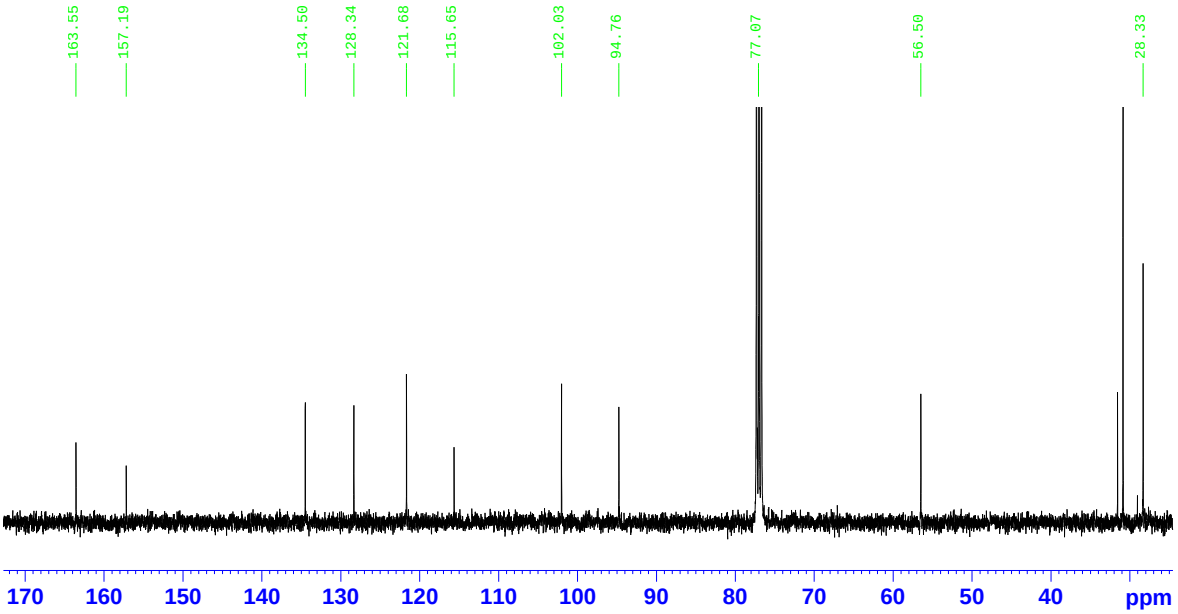


Plate 17a. ^{1}H NMR spectrum of 2',5,7-trimethoxymethoxy-6",6"-dimethylpyrano[2",3":4',5']isoflavone (**126**) in $CDCl_3$

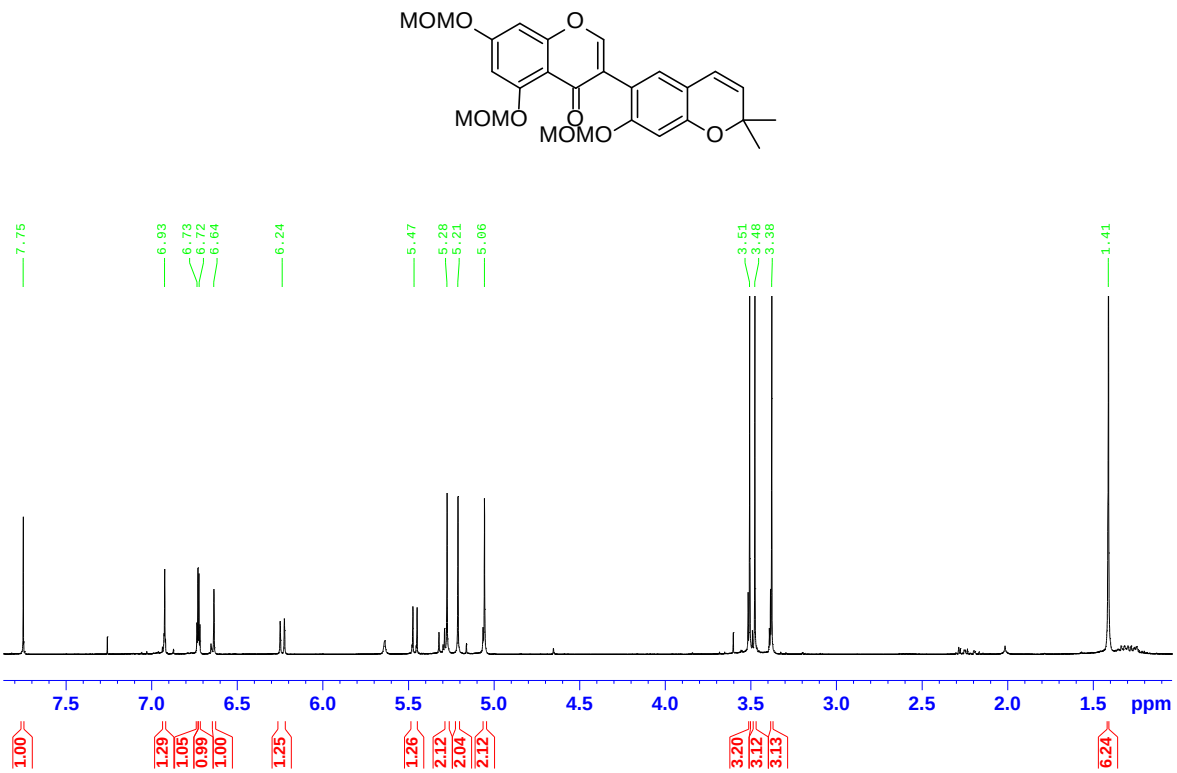


Plate 17b. ^{13}C NMR spectrum of 2',5,7-trimethoxymethoxy-6",6"-dimethylpyrano[2",3":4',5']isoflavone (**126**) in $CDCl_3$

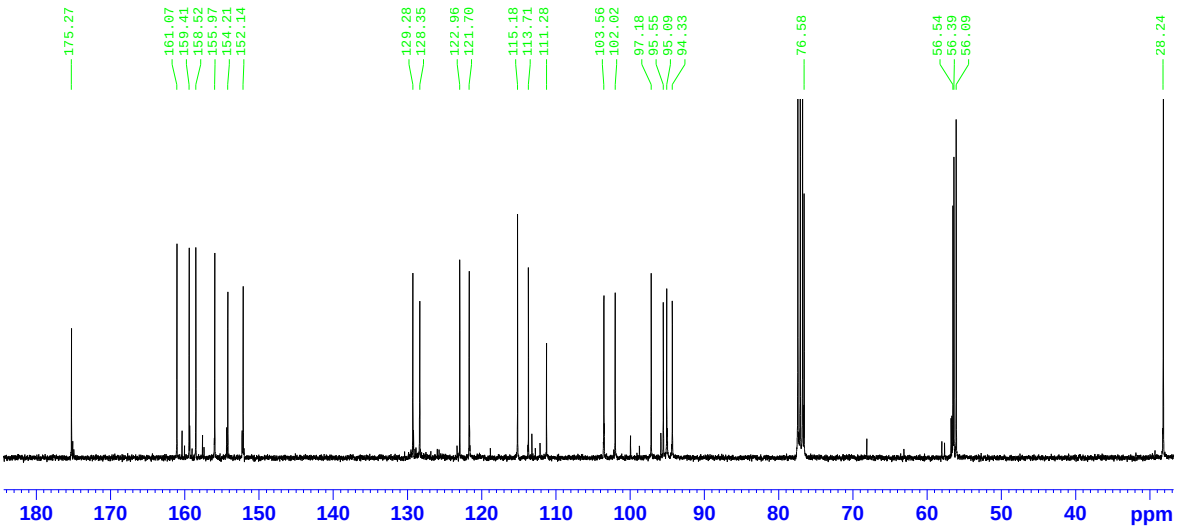


Plate 18a. ^{1}H NMR spectrum of eriosemaone D (**17**) in CD_3OD

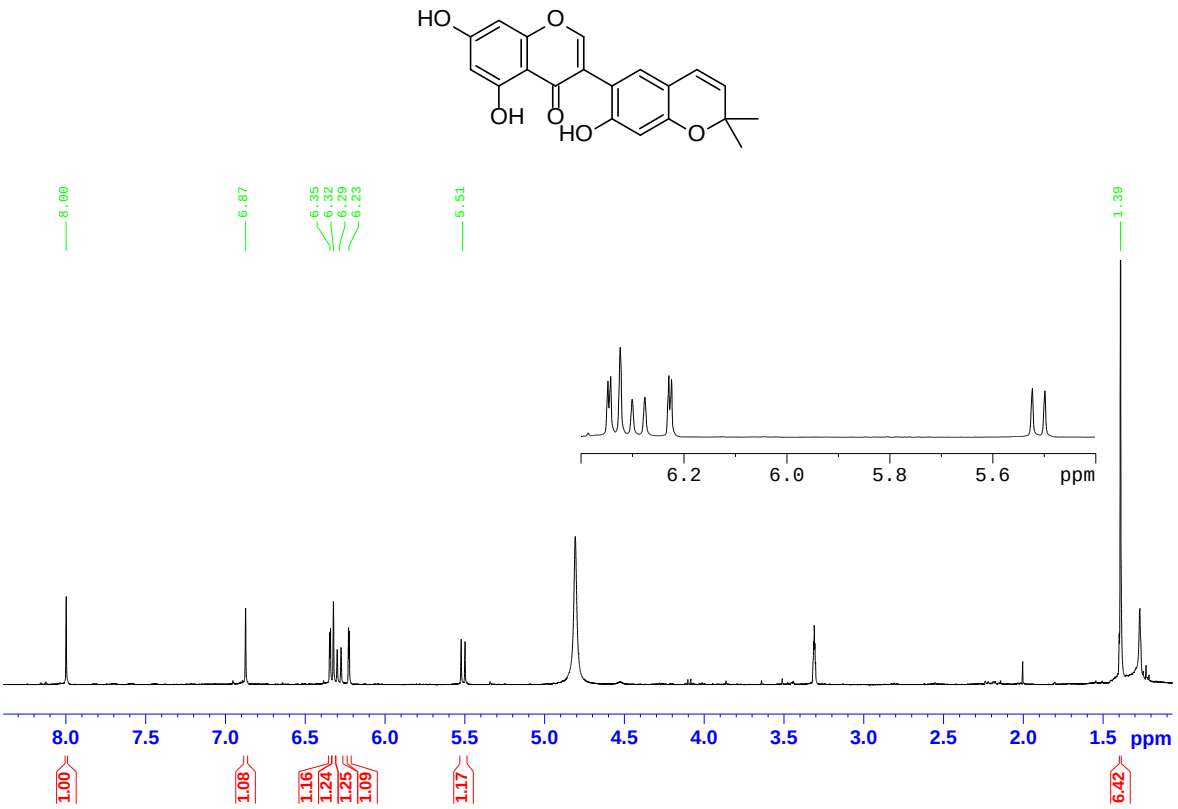


Plate 18b. ^{13}C NMR spectrum of eriosemaone D (17) in CD_3OD

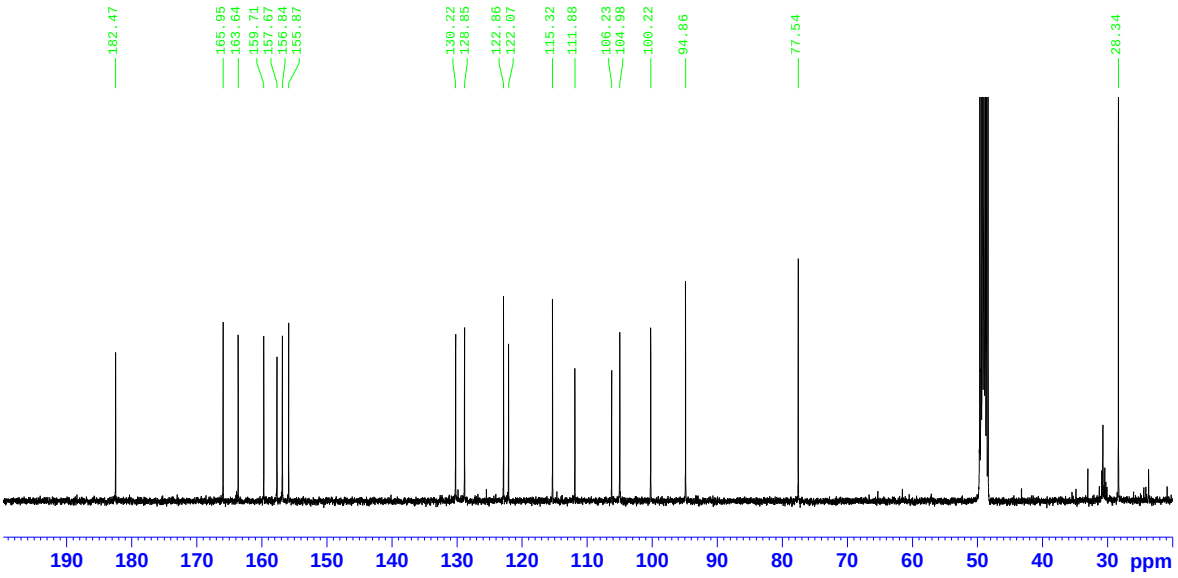


Plate 19a. ^{1}H NMR spectrum of *rac*-5-hydroxy-[6'-methyl-6'-(4-methylpent-3-enyl)pyrano]-2',3':7,6-chromone (**127**) in $CDCl_3$

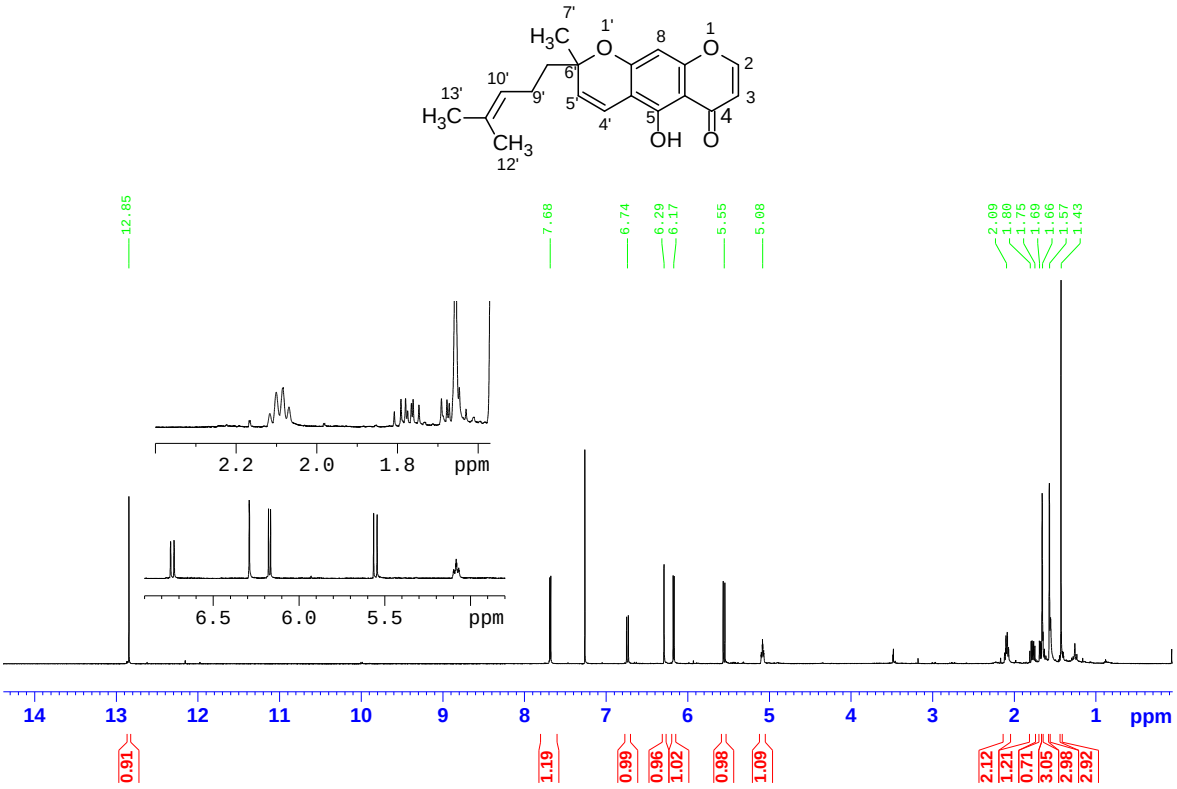


Plate 19b. ^{13}C NMR spectrum of *rac*-5-Hydroxy-[6'-methyl-6'-(4-methylpent-3-enyl)pyrano]-2',3':7,6-chromone (**127**) in $CDCl_3$

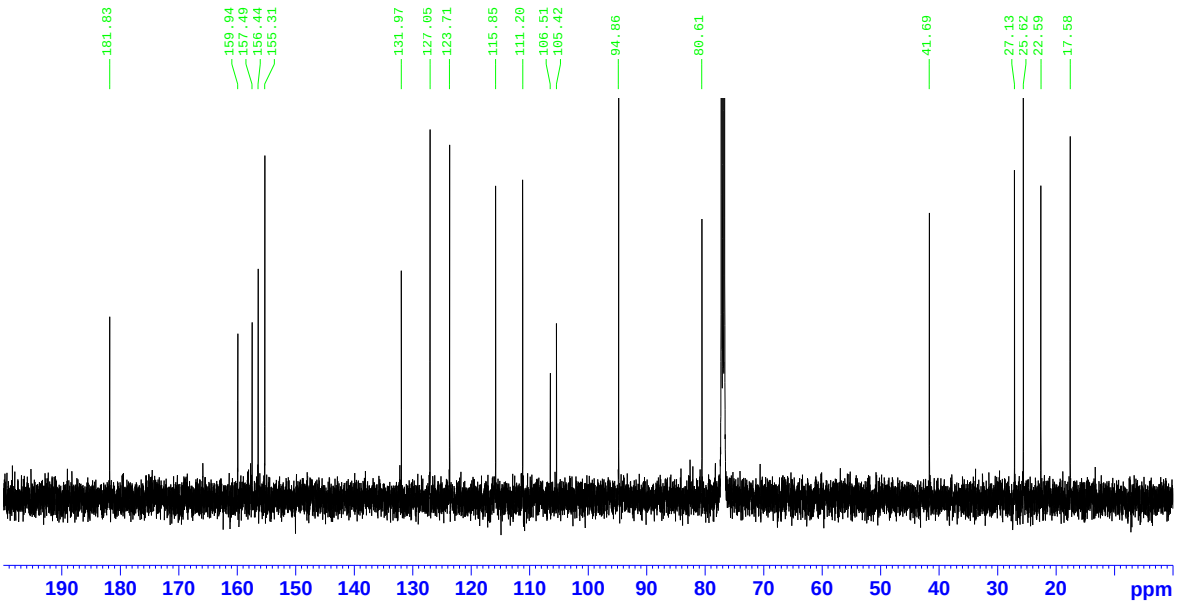


Plate 20a. ^{1}H NMR spectrum of *rac*-2'-benzyloxy-5-hydroxy-[(6",6"-dimethylpyrano (2",3":4',5')][(6"'-methyl-6"'-(4-methylpent-3-enyl)pyrano(2"',3"':7,6)]isoflavone (**128**) in $CDCl_3$

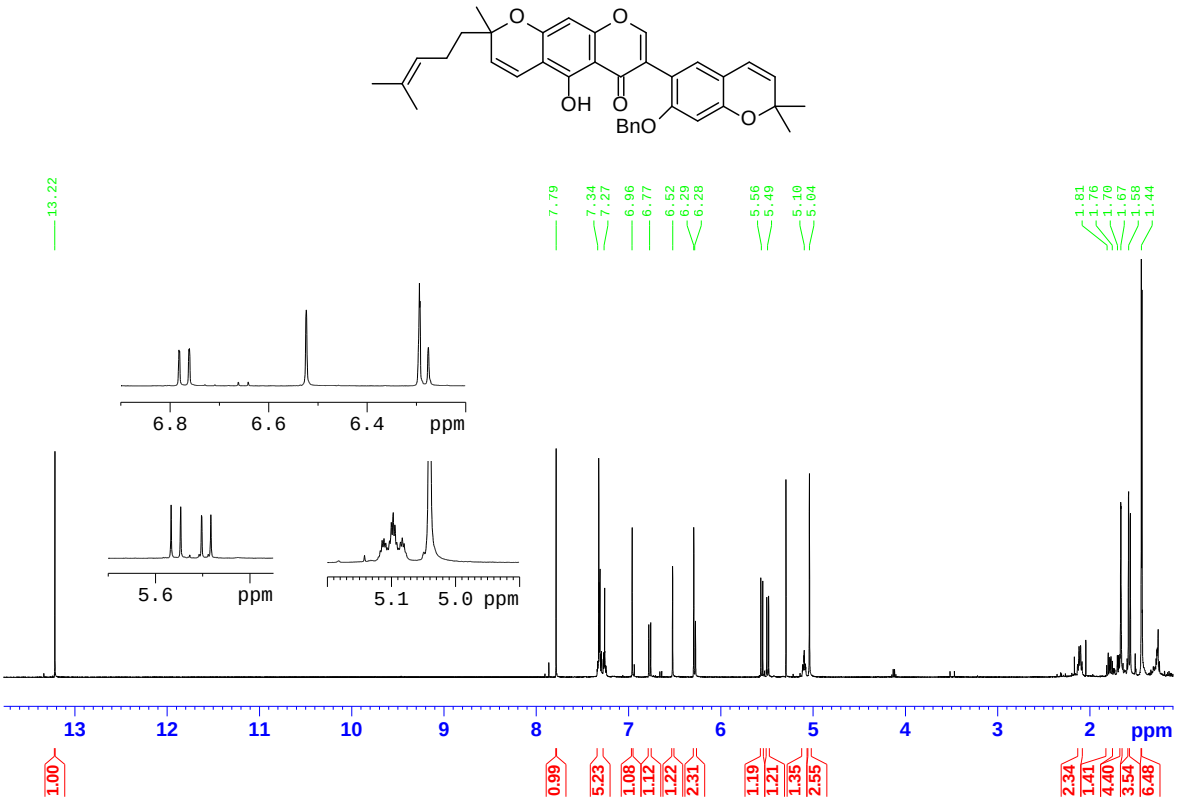


Plate 20b. ^{13}C NMR spectrum of *rac*-2'-benzyloxy-5-hydroxy-[(6",6"-dimethylpyrano (2",3":4',5')][(6"'-methyl-6"'-(4-methylpent-3-enyl)pyrano(2"',3"':7,6)]isoflavone (**128**) in $CDCl_3$

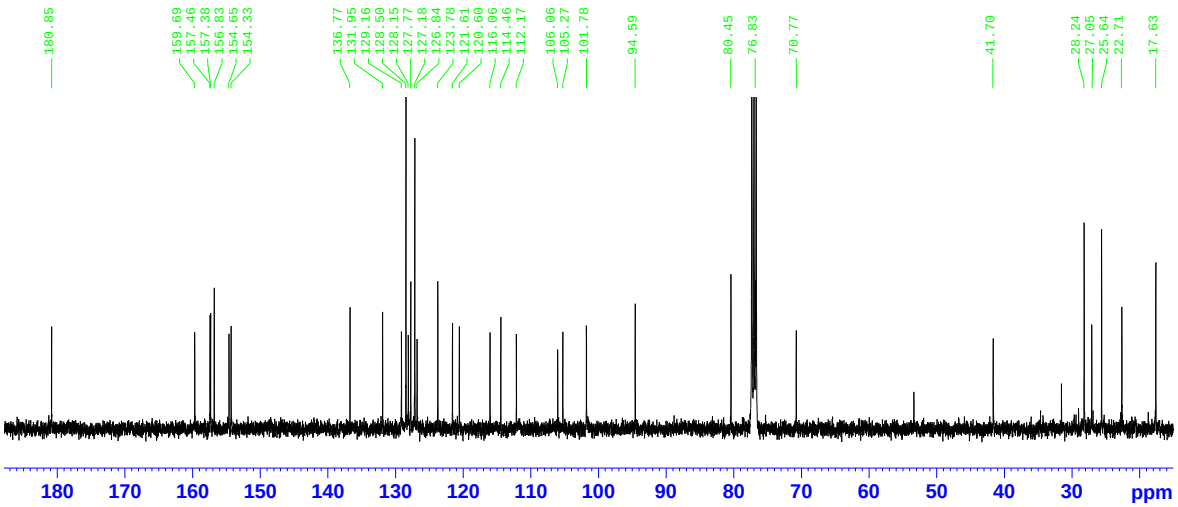


Plate 21. ^{1}H NMR spectrum of *rac*-2',5-dihydroxy-[(6",6"-dimethylpyrano(2",3":4',5')][(6"'-methyl-6"'-(4-methylpent-3-enyl)pyrano(2"',3"':7,6)]isoflavone (**89**) in $CDCl_3$

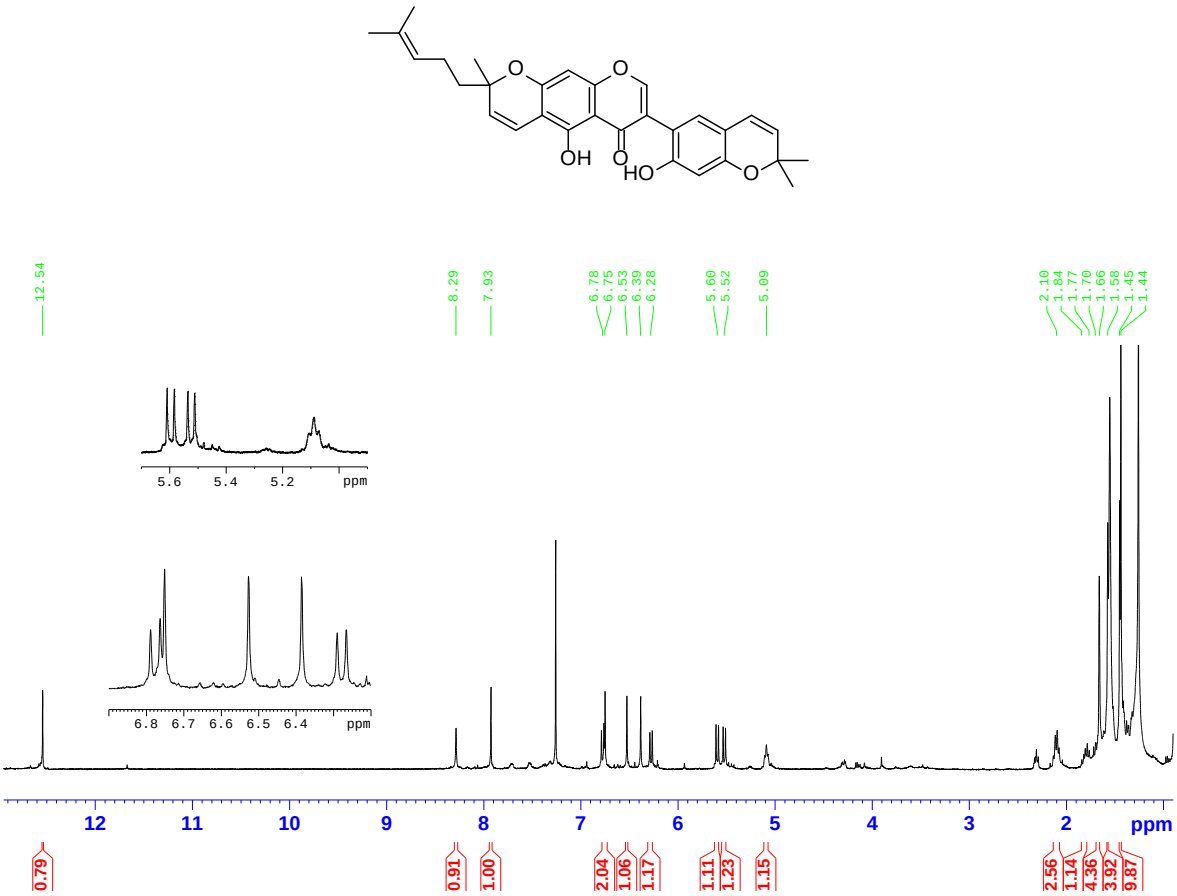


Plate 22a. ^{1}H NMR spectrum of diformylated aminoketone **154** in $CDCl_3$

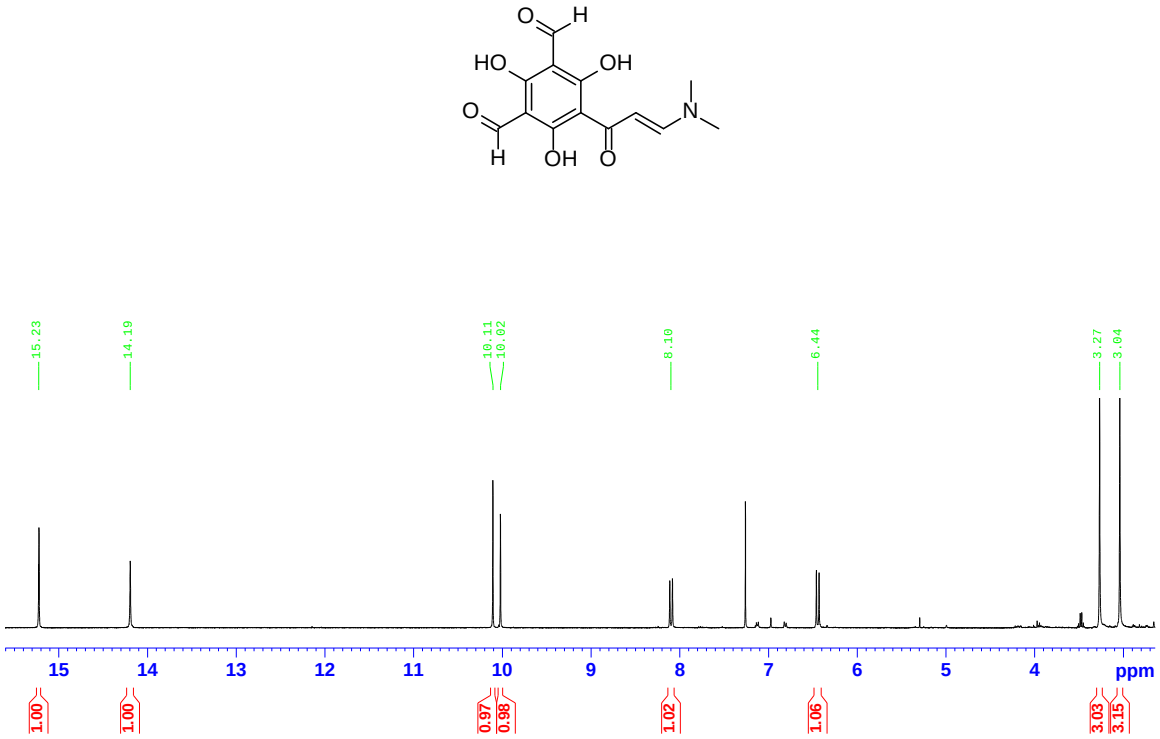


Plate 22b. ^{13}C NMR spectrum of diformylated aminoketone **154** in $CDCl_3$

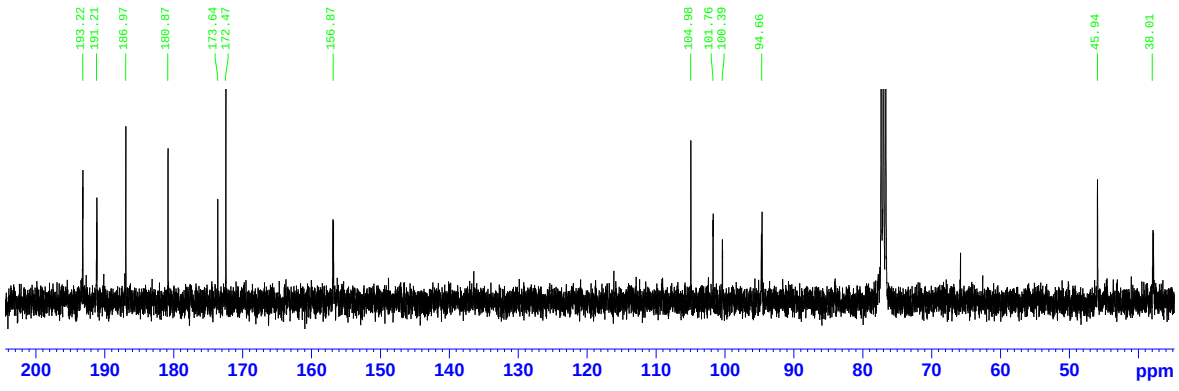


Plate 23a. ^{1}H NMR spectrum of 3-iodo-5,7-dimethoxychromone (**161**) in $CDCl_3$

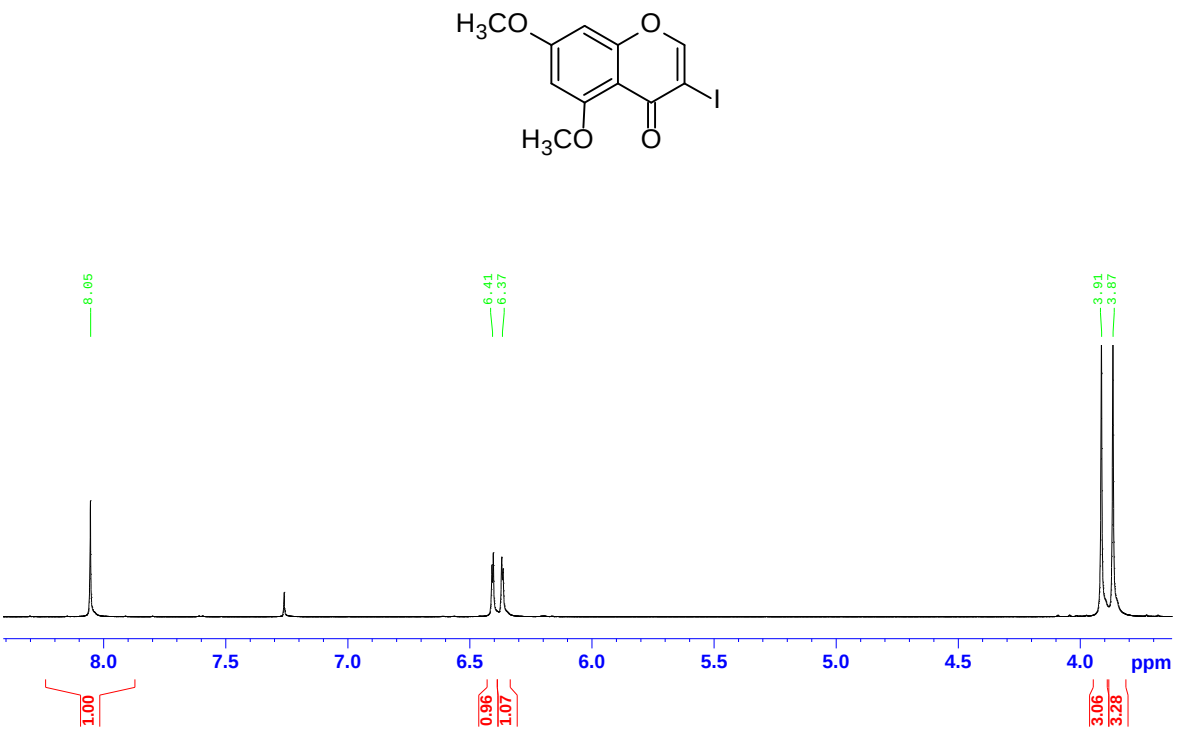


Plate 23b. ^{13}C NMR spectrum of 3-iodo-5,7-dimethoxychromone (161) in $CDCl_3$

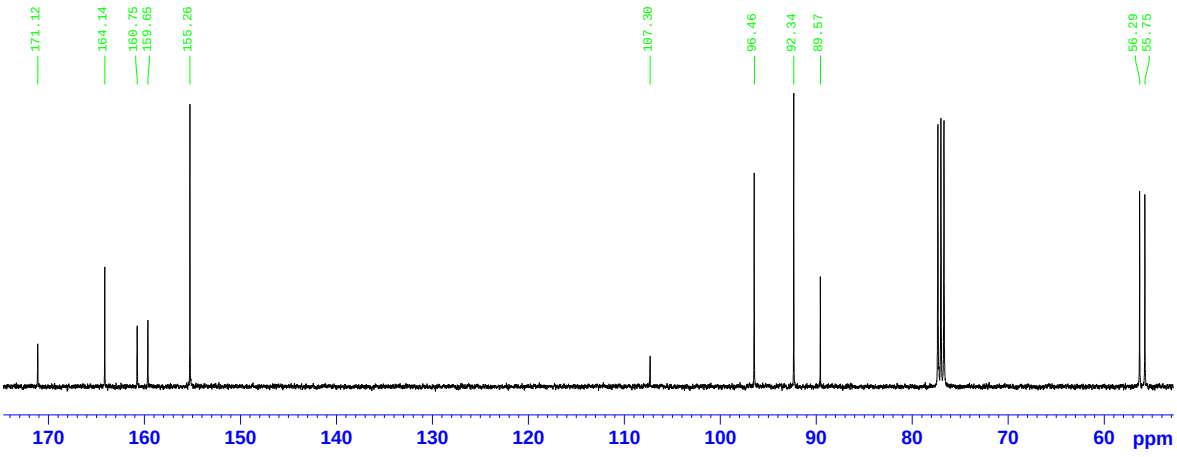


Plate 24a. ^{1}H NMR spectrum of 2',4',5,7-tetrahydroxyisoflavone (**149**) in CD_3OD

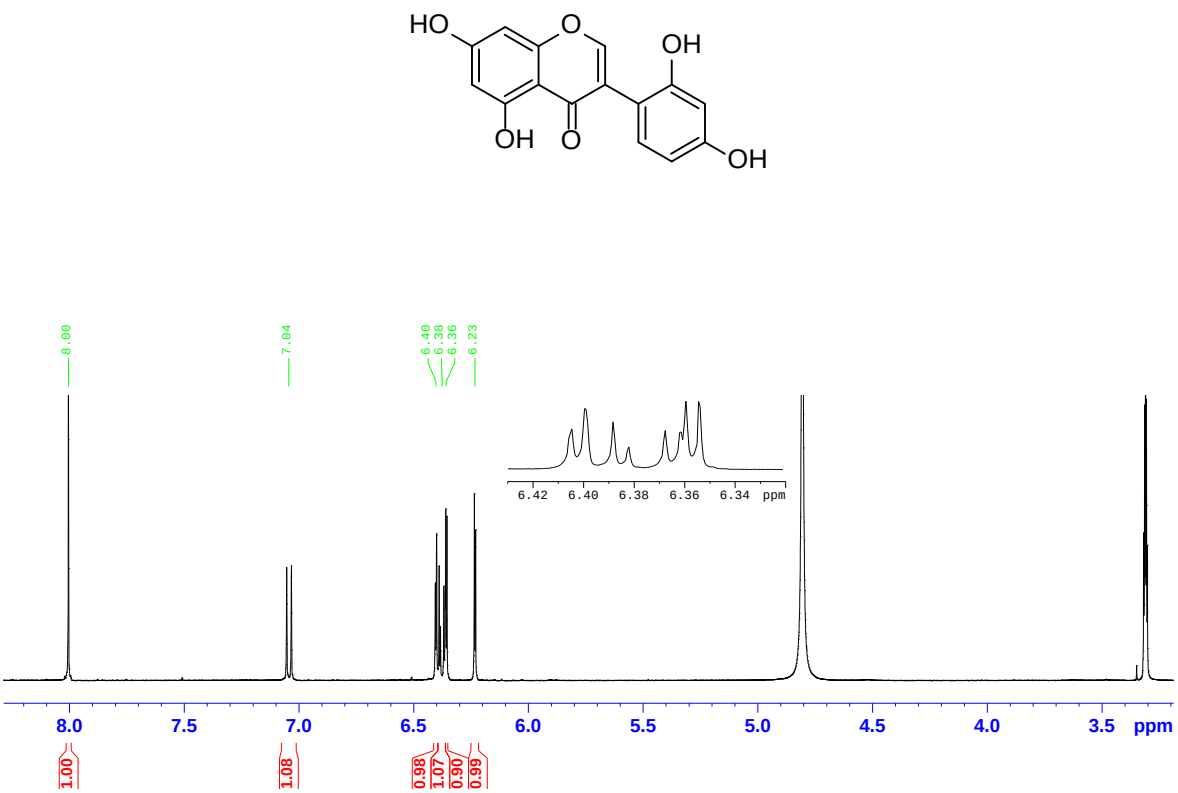


Plate 24b. ^{13}C NMR spectrum of 2',4',5,7-tetrahydroxyisoflavone (149) in CD_3OD

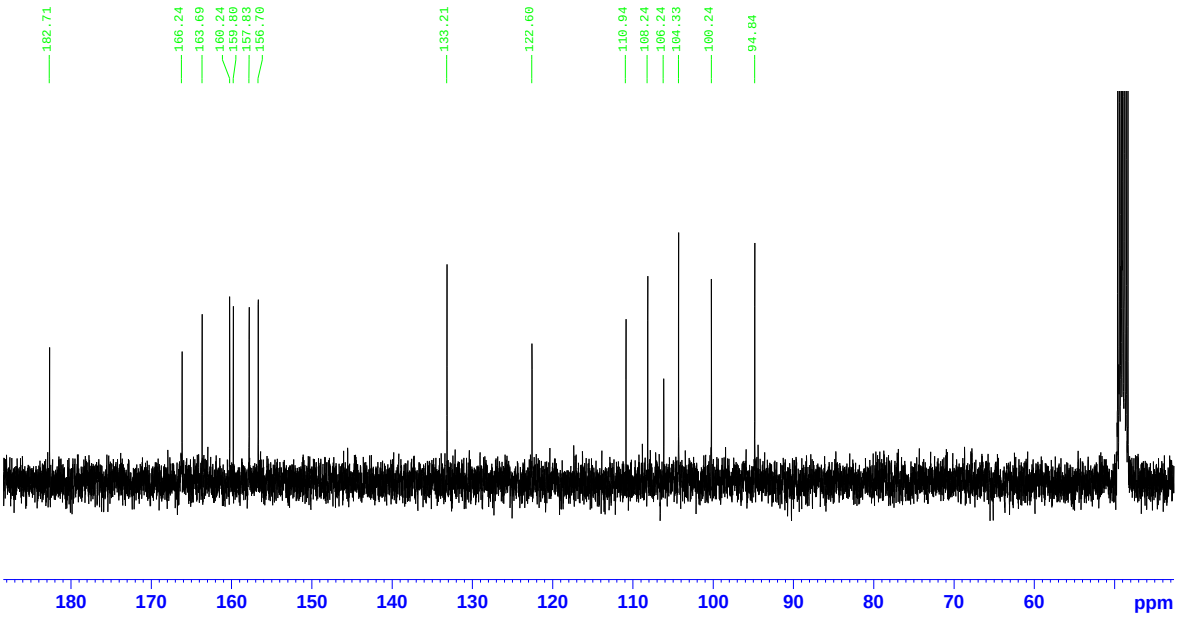


Plate 25a. ^{1}H NMR spectrum of lupinalbin A (**148**) in CD_3OD

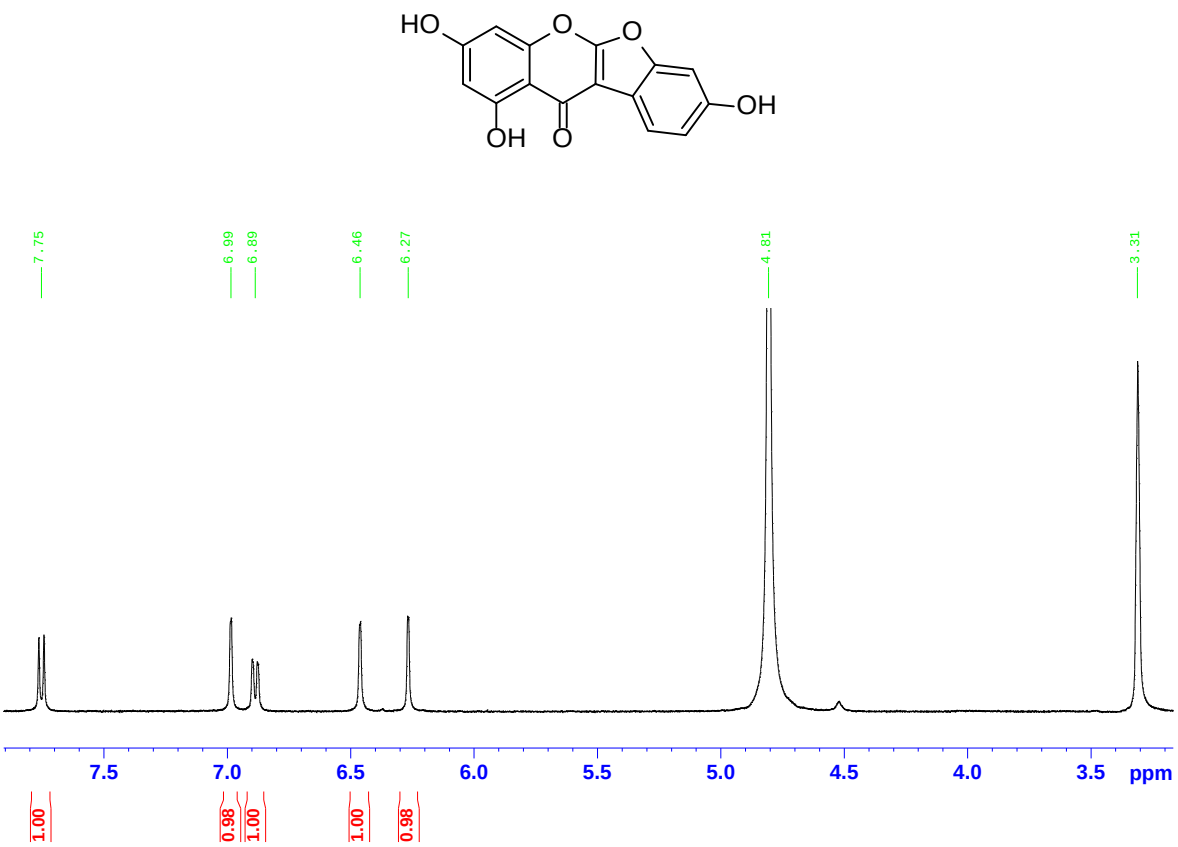


Plate 25b. ^{13}C NMR spectrum of lupinalbin A (**148**) in CD_3OD

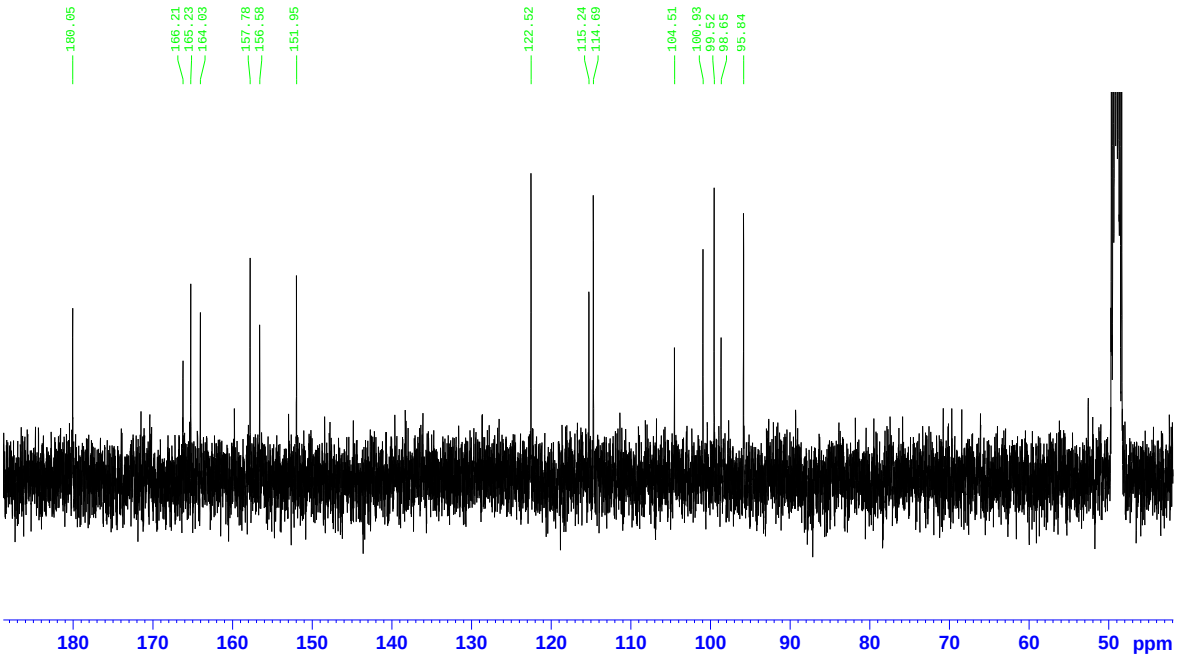


Plate 26a. ^{1}H NMR spectrum of lupinalbin H (**130**) in acetone-d_6

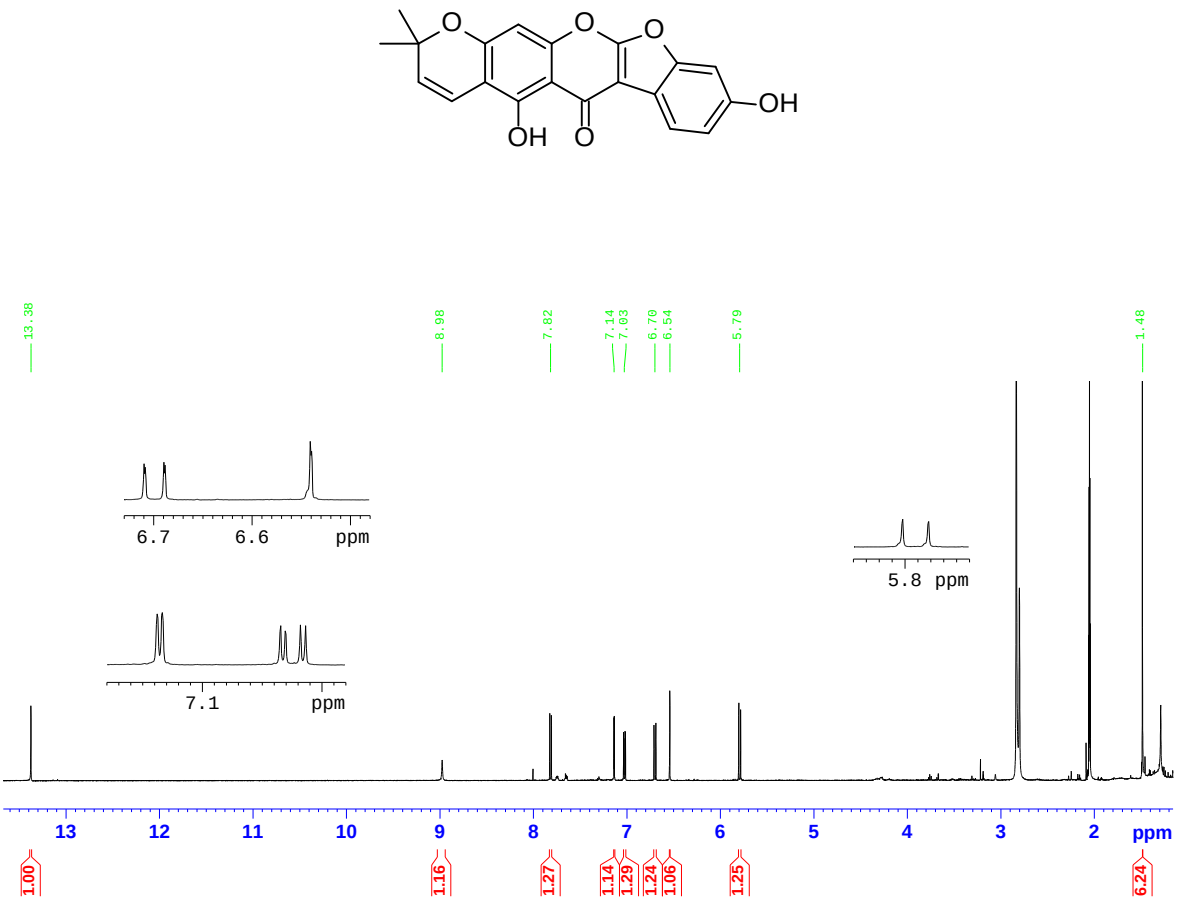
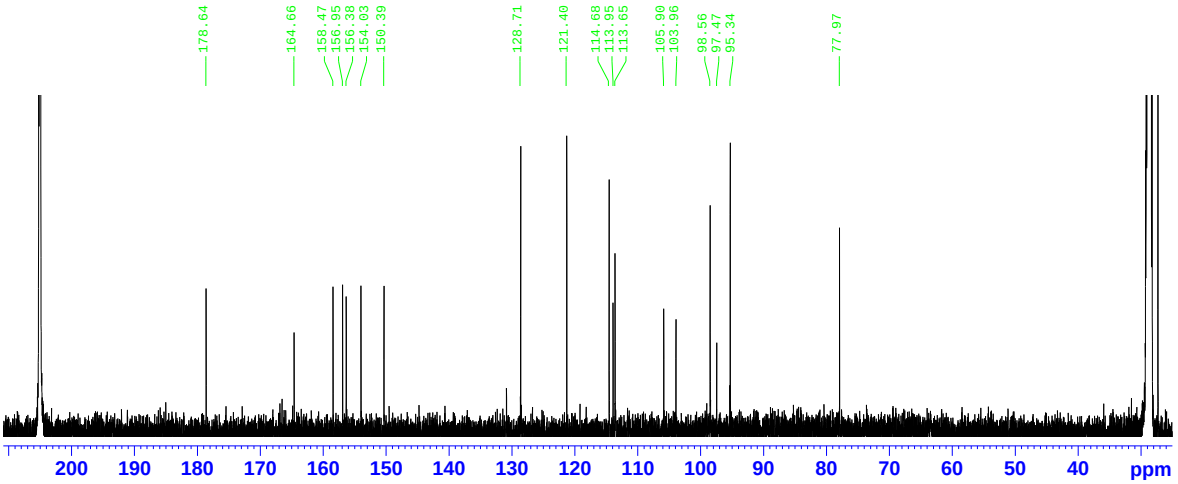


Plate 26b. ^{13}C NMR spectrum of lupinalbin H (**130**) in acetone-d_6



APPENDIX II

COPIES OF PUBLICATIONS

Total Synthesis of the Pyranoisoflavone Kraussianone 1 and Related Isoflavones

Mamoalosi A. Selepe, Siegfried E. Drewes, and Fanie R. van Heerden*

School of Chemistry, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, Pietermaritzburg, South Africa

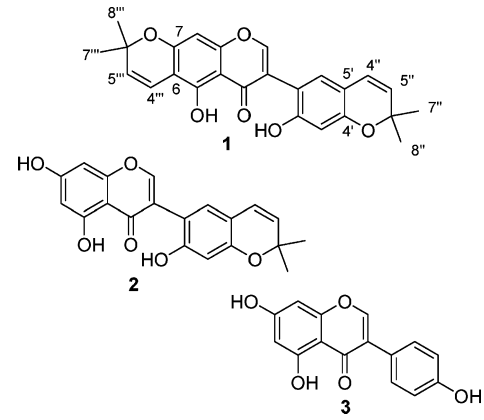
Received June 22, 2010

The first total synthesis of the pyranoisoflavone kraussianone 1 (**1**) is described. The key steps involved the Suzuki−Miyaura reaction for the construction of the isoflavone core and the regioselective formation of the dimethylpyran scaffolds to the phloroglucinol (ring A) and resorcinol (ring B) moieties of kraussianone 1 (**1**). This route also provided access to the related isoflavones eriosemaone D (**2**) and genistein (**3**) via simple structural modifications.

Kraussianone 1 (**1**) is a pyranoisoflavone that was isolated from the bark of *Eriosema kraussianum* Meisn. (Fabaceae), a plant used traditionally for the treatment of male impotence and urinary complaints in KwaZulu-Natal, South Africa.[1] Preliminary studies on the biological activity of the compounds isolated from *E. kraussianum* revealed **1** to be the most potent metabolite for the relaxation of carvenosal smooth muscle, an assay used to evaluate drugs for erectile dysfunction. In this assay, **1** showed an activity of 85% compared to sildenafil (Viagra) at 78 ng/mL.[1,2] Compound **1** further demonstrated significant hypoglycemic and secondary vasorelaxant effects.[3] Therefore, **1** serves as an ideal lead compound for the development of drugs to combat erectile dysfunction and related disorders. However, **1** awaits in-depth biochemical studies to reveal its molecular basis of action for these activities. This has been hampered by the limited supply of the compound from *E. kraussianum*, its sole natural source to date. In order to provide access to sufficient quantities of **1** for further pharmacological studies, we have developed a convenient synthetic route for the preparation of **1** and related compounds, based on the Suzuki−Miyaura reaction for the construction of the isoflavone core.[4] While the application of the Suzuki−Miyaura reaction in the synthesis of isoflavones was demonstrated more than two decades ago,[4] there are not many reports on the synthesis of isoflavones with naturally occurring substitution patterns by this method, especially those with ring A possessing a phloroglucinol moiety.[5,6] Except for the pyranoisoflavone recently synthesized as a precursor to hirtellanine A,[6] no natural pyranoisoflavones have been reported by this method. The previous synthetic procedures for pyranoisoflavones were based on the deoxybenzoin or the chalcone route, which often suffered from poor regioselectivity in the introduction of the dimethylpyran ring to the isoflavone skeleton.[7−13] We herein report the first regioselective total synthesis of kraussianone 1 (**1**), employing the Suzuki−Miyaura reaction as the key step for the construction of the isoflavone nucleus.[4] This route also gave access to a structurally related antifungal pyranoisoflavone, eriosemaone D (**2**),[14,15] whose synthesis has not been disclosed, and to an important phytoestrogen, genistein (**3**).

Results and Discussion

The retrosynthetic analysis of kraussianone 1 (**1**) is shown in Scheme 1. We envisaged that the regioselective aldol-type condensation of the pyranoisoflavone **4** with prenal and the subsequent 6π electrocyclization would give the dimethylpyran ring fused to ring A of kraussianone 1 (**1**). The isoflavone **4** would in turn be synthesized by the Suzuki−Miyaura cross-coupling of 3-iodochromone **5** and the boronic acid derivate **6**, with the requisite dimethylpyran scaffold. The latter would be prepared from readily available resorcinol (**8**) and the former from phloroacetophenone (**7**). Following this route, a number of related isoflavones can be synthesized from the same key building blocks. This has been demonstrated by the synthesis of eriosemaone D (**2**) from the kraussianone 1 precursor **4** and by the synthesis of genistein (**3**) from the 3-iodochromone **5** (*vide infra*).



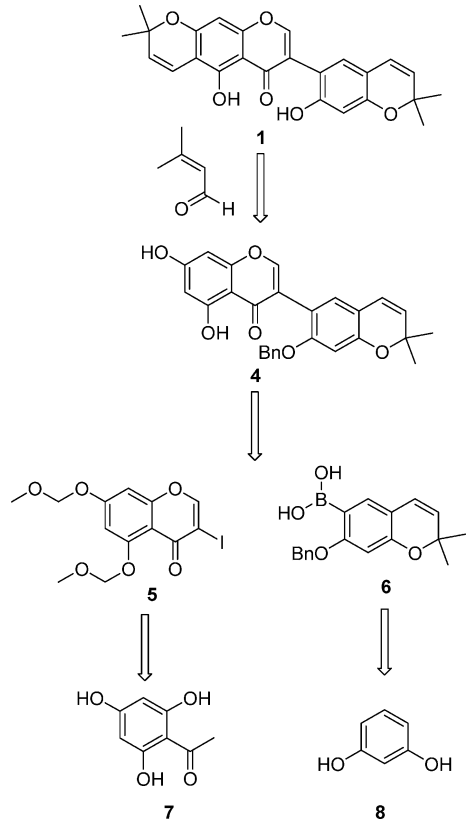
As illustrated in Scheme 2, the synthesis of the intermediate **5** commenced with regioselective protection of the hydroxy groups of phloroacetophenone (**7**) by MOMCl generated from the reaction of dimethoxymethane, AcCl, and catalytic $ZnBr_2$[16] to give **9** in 50% yield. Condensation of **9** with DMF-DMA gave the enaminoketone **10** (68%), which was cyclized in the presence of pyridine and I_2 to afford an inseparable mixture of **5** and **11** in the ratio of 50:7 (by ^{1}H NMR), giving 58% and 6% overall yields, respectively.[17−19] 3-Iodochromone **5** is thus appropriately functionalized for the ultimate conversion to an isoflavone via C−C bond formation by the Suzuki−Miyaura reaction.

Having synthesized the first key building block, **5**, the next step was to prepare its boronic acid partner, **6**. This was achieved in a sequence of steps starting from resorcinol (**8**) (Scheme 3). Thus, condensation of **8** with 3-methyl-2-butenoic acid gave chromanone **12** in 92% yield.[20] Iodination of **12** with I_2 and HIO_3 in CH_3OH/H_2O[21] gave a mixture of the targeted 6-iodochromanone **13** as the major component in 65% yield together with 8-iodochromanone **14** as a minor component. These compounds were inseparable by silica gel column chromatography. However, their purification could be effected by their different solubility properties. The 6-iodochromanone **13** was insoluble in CH_2Cl_2 and $CHCl_3$, whereas compound **14** readily dissolved in these solvents. Although compound **13** was initially obtained in moderate yields under these conditions, this method was not reproducible on a larger scale. Therefore, alternative iodinating reagents, KIO_3/KI, HIO_4/I_2, and ICl, were screened for the regioselective C-6 iodination of chromanone **12** under different conditions,[22−25] but these mostly gave the 8-iodo isomer **14** and 7-hydroxy-6,8-diiodo-2,2-dimethyl-4-chromanone (**15**). Benzyl protection of the 7-hydoxy group of

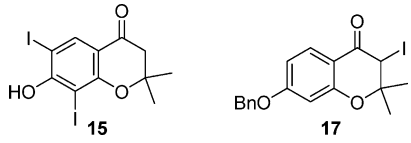
* To whom correspondence should be addressed. Tel: +27 33 2605886. Fax: +27 33 2605009. E-mail: vanheerdenf@ukzn.ac.za.

10.1021/np100407n
Published on Web 08/18/2010

Scheme 1. Retrosynthetic Analysis of Kraussianone 1 (**1**)



chromanone **12** and subsequent iodination of the resulting benzyloxychromanone **16** with HIO_3/I_2[21] resulted in the formation of 7-benzyloxy-3-iodo-2,2-dimethyl-4-chromanone (**17**).

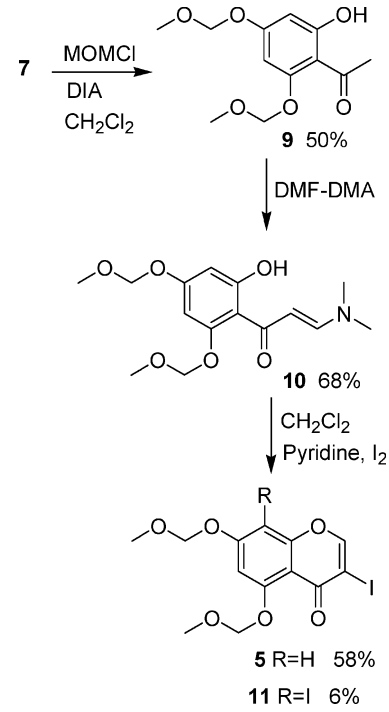


Eventually, the targeted C-6-iodinated chromanone was successfully synthesized by treatment of the $CHCl_3$ solution of 7-benzyloxy-2,2-dimethyl-4-chromanone (**16**) with CF_3CO_2Ag and I_2 at room temperature.[26] This reaction gave 7-benzyloxy-6-iodo-2,2-dimethyl-4-chromanone (**18**) as the sole product in 94% yield. Reduction of **18** with $NaBH_4$ and subsequent dehydration proceeded smoothly to afford the iodochromene **20** in 96% overall yield.[27,28]

We envisioned that the boronic acid **6** could be synthesized from **20** by lithium−iodine exchange with *n*-BuLi, followed by treatment of the phenyllithium with triisopropyl borate and hydrolysis of the resulting boronate ester.[29] The viability of this method was tested on *p*-methoxymethylphenyl iodide (**21**) (Scheme 4), synthesized by protection of the hydroxy group of *p*-iodophenol as the methoxymethyl ether.[16] A solution of phenyl iodide **21** in THF was therefore sequentially treated with *n*-BuLi, triisopropyl borate, and NH_4Cl in one pot. Following this procedure, a small amount of the targeted boronic acid **22** was obtained plus appreciable quantities of the deiodinated material. It was evident from these results that the lithium−iodine exchange occurred rapidly as expected and that the generated aryllithium was very unstable.

Optimum results were obtained by changing the solvent system to THF/Et_2O (1:2) and the sequence in which the reagents were added, by employing the "*in situ* quench" procedure developed by Li and co-workers.[30] Accordingly, the boronic acid **22** was prepared in a one-pot sequence that involved addition of *n*-BuLi to a solution of aryl iodide **21** and triisopropyl borate in THF/Et_2O (1:2), followed by the hydrolysis of the boronate ester with an NH_4Cl solution (Scheme 4). The improvement observed with this procedure may be attributed to the immediate *in situ* quench of the generated phenyllithium with triisopropyl borate, thus preventing side reactions of the phenyllithium.[30] These optimized reaction conditions were subsequently employed for the synthesis of the boronic acid **6** in 70% yield from iodochromene **20**, as shown in Scheme 3.

Scheme 2. Synthesis of 3-Iodochromone **5**

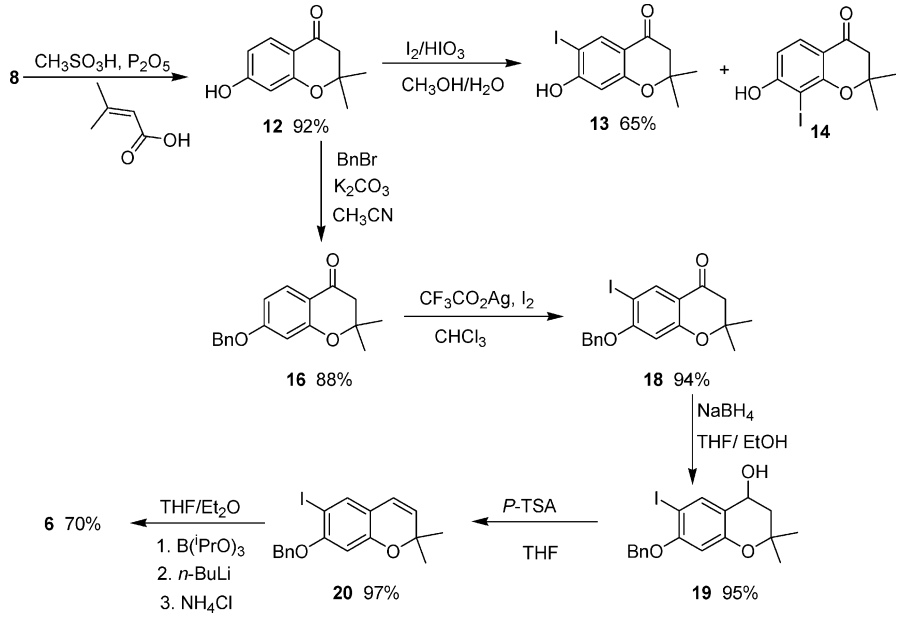


The Suzuki−Miyaura cross-coupling of 3-iodochromone **5** with boronic acid **6** using a heterogeneous Pd(C) catalyst[19,31] and subsequent cleavage of the MOM protecting groups with HCl furnished the pyranoisoflavone **4** in 72% yield. Base-catalyzed condensation of the pyranoisoflavone **4** with prenal[32] gave the benzyl derivative of kraussianone 1, **23** (74% yield), which was finally deprotected with BCl_3[13] to afford kraussianone 1 (**1**) in 69% yield (Scheme 5).

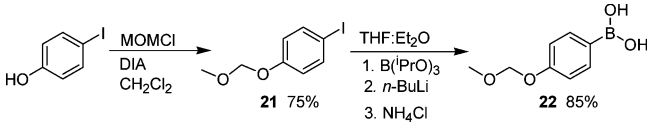
Having successfully synthesized kraussianone 1 (**1**), we took advantage of the available materials and the optimized reaction conditions to synthesize genistein (**3**) and eriosemaone D (**2**). Eriosemaone D (**2**) was isolated from *E. tuberosum* A.Rich,[14,15] but its total synthesis has not been disclosed. The synthesis of genistein (**2**) by the Suzuki−Miyaura reaction has also not been published. As shown in Scheme 5, genistein (**3**) was synthesized in 66% yield over two steps, by coupling the boronic acid **22** to 3-iodochromone **5**,[19,31] followed by removal of MOM protecting groups under similar conditions to those employed for **4**. Eriosemaone D (**2**) on the other hand was synthesized by benzyl deprotection of the pyranoisoflavone **4** with BCl_3.[13] The physical properties and the spectroscopic data of all the synthesized compounds were in agreement with those of the isolated natural compounds. This represents the first total synthesis of kraussianone 1 (**1**) and eriosemaone D (**2**) and the first synthesis of genistein using the Suzuki−Miyaura reaction.

In conclusion, kraussianone 1 (**1**) and the structurally related isoflavones eriosemaone D (**2**) and genistein (**3**) have been successfully synthesized by employing the Suzuki−Miyauara reaction as the key step. The successful synthesis of kraussianone 1 (**1**), via a route that readily gives entry to analogues, will allow for further investigations of its pharmacological properties and structure−activity relationship studies.

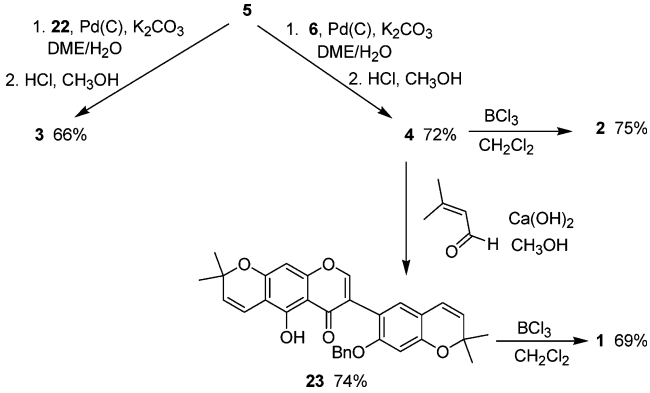
Scheme 3. Synthesis of Boronic Acid **6**



Scheme 4. Model Reaction for Synthesis of Boronic Acid



Scheme 5. Synthesis of Kraussianone 1 (**1**), Eriosemaone D (**2**), and Genistein (**3**)



Experimental Section

General Experimental Procedures. Abbreviations: DIA, diisopropylamine; DMF-DMA, *N*,*N*-dimethylformamide dimethylacetal; Hex, hexanes (bp 68−70 °C); *p*-TSA, *p*-toluenesulfonic acid. Hex used for chromatographic purifications was distilled prior to use. Anhydrous CH_2Cl_2, CH_3CN, THF, and Et_2O were obtained from Innovative Technologies (Newburyport, MA) Pure-Solv 800 solvent purification system. Reagents used for syntheses were purchased from Fluka, Sigma-Aldrich, or Merck and were used without further purification unless otherwise stated. 2′,4′,6′-Trihydroxyacetophenone monohydrate was dried in an oven at 90 °C for 24 h prior to use. Reactions were monitored by TLC. The TLC was performed on Merck silica gel plates (60 F_{254}) and visualized under UV light (254 nm). Alternatively, detection of spots on the TLC was achieved by heating with a heat gun after treatment with a solution of anisaldehyde in concentrated H_2SO_4 and EtOH prepared in the volume ratio 1:1:18, respectively. Column chromatography was performed using Merck Kieselgel 60 (230−400 mesh). Centrifugal chromatography was performed on a Harrison Research Chromatotron model 7924T on glass plates coated with Merck silica gel with particle size 0.040−0.063 mm, 2−4 mm thick.

The melting points were measured on a Reichert electrothermal melting point apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer spectrophotometer, and NMR spectra on a Bruker AVANCE DPX_{400} spectrometer. ^{11}B NMR spectra were referenced against an external standard of neat $BF_3 \cdot OEt_2$ containing a capillary tube of acetone-d_6 for deuterium lock. The chemical shifts from ^1H NMR and ^{13}C NMR spectra are reported in parts per million relative to the residual protonated or deuterated solvents peaks ($CDCl_3$: δ_H 7.26, δ_C 77.0 and DMSO-d_6: δ_H 2.50, δ_C 39.5). The spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), br (broad), or m (multiplet). The mass spectra were recorded on a Thermo Finnigan GC-MS ion trap using electron-impact ionization or on a time-of-flight Waters LCT Premier MS using electrospray ionization in the positive or negative mode.

2′-Hydroxy-4′,6′-dimethoxymethoxyacetophenone (9). Catalytic $ZnBr_2$ was dissolved in dimethoxymethane (2.62 mL, 29.74 mmol) under an N_2 atmosphere, and then AcCl (2.12 mL, 29.74 mmol) was added dropwise to the stirred solution. The solution was stirred for an additional 2 h at room temperature and transferred via a cannula to an ice-cold solution of the predried phloroacetophenone (**7**) (2.0 g, 11.89 mmol) and DIA (3.6 mL, 23.59 mmol) in CH_2Cl_2 (100 mL) under an N_2 atmosphere. The mixture was stirred for 3 h, diluted with saturated NH_4Cl solution (40 mL), and stirred for an additional 15 min. The two phases were partitioned, and the aqueous phase was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic extracts were washed with brine (3 × 100 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated to give a yellow oil, which was purified by column chromatography using Hex/EtOAc (7:3) as eluent to afford **9** as a colorless oil (1.53 g, 50%): IR (neat) ν_{max} 3100, 2925, 1618 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 13.68 (1H, s, OH), 6.26 (1H, d, J = 2.4 Hz, H-3′), 6.24 (1H, d, J = 2.4 Hz, H-5′), 5.25 (2H, s, OCH_2O), 5.16 (2H, s, OCH_2O), 3.52 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 2.65 (3H, s, $COCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.1 (C, C-1), 167.3 (C, C-2′), 163.3 (C, C-4′), 160.5 (C, C-6′), 107.3 (C, C-1′), 97.3 (CH, C-3′), 94.5 (CH, C-5′), 94.0 (CH_2, 2 × OCH_2O), 56.7 (CH_3, OCH_3), 56.4 (CH_3, OCH_3), 32.9 (CH_3, −$COCH_3$); EIMS *m*/*z* 225 (10%), 209 (29), 207 (100), 191 (22), 44 (40), 40 (62).

3-(*N*,*N*-Dimethylamino)-1-(2′-hydroxy-4′,6′-dimethoxymethoxyphenyl)propenone (10). DMF-DMA (0.29 mL, 2.19 mmol) was added to **9** (0.28 g, 1.09 mmol) at 95 °C. The mixture was stirred at the same temperature for 1.5 h. The volatiles in the orange oil were evaporated on a rotary evaporator, and the crude solid was subjected to flash chromatography using Hex/EtOAc (1:1) as eluent. Evaporation of the solvent gave **10** as a yellow oil, which solidified upon cooling (0.23 g, 68%). The solid was recrystallized to give yellow needle-like crystals (1:1 Hex/Et_2O): mp 85−86 °C; IR (KBr) ν_{max} 3445, 3163, 2913, 1606, 1233 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 15.08 (1H, s, OH-2′), 7.92 (1H, d, J = 12.0 Hz, H-3), 6.29 (1H, d, J = 12.0 Hz, H-2), 6.26 (1H,

d, J = 2.4 Hz, H-3′), 6.17 (1H, d, J = 2.4 Hz, H-5′), 5.20 (2H, s, OCH_2O), 5.14 (2H, s, OCH_2O), 3.51 (3H, s, OCH_3), 3.46 (3H, s, OCH_3), 3.13 (3H, brs, $N(CH_3)_2$), 2.94 (3H, brs, $N(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 190.6 (C, C-1), 166.9 (C, C-2′), 161.3 (C, C-4′), 158.9 (C, C-6′), 154.4 (CH, C-3), 107.1 (C, C-1′), 97.9 (CH, C-3′), 97.0 (CH, C-2), 95.2 (CH_2, OCH_2O), 94.5(CH, C-5′), 94.1 (CH_2, OCH_2O), 56.7 (CH_3, OCH_3), 56.3 (CH_3, OCH_3), 45.5 (CH_3, $N(CH_3)_2$), 37.2 (CH_3, $N(CH_3)_2$); HRMS-ES m/z $[M + Na]^+$ 334.1267 (calcd for $C_{15}H_{21}NO_6Na$ 334.1267).

3-Iodo-5,7-dimethoxymethoxychromone (5). To a solution of propenone **10** (0.12 g, 0.38 mmol) in CH_2Cl_2 (20 mL) was added pyridine (0.06 mL, 0.74 mmol) followed by I_2 (0.11 g, 0.44 mmol). The resulting solution was stirred at room temperature for 12 h. The reaction was quenched with a saturated $Na_2S_2O_3$ solution (10 mL), and the two phases were partitioned. The aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL). The organic layers were combined, washed with H_2O (15 mL) and brine (15 mL), and dried over anhydrous $MgSO_4$. The solvent was evaporated to give a yellow solid. The crude product was purified on a chromatotron using Hex/EtOAc (7:3) as eluent. The solvent was evaporated to afford a cream-white solid (94 mg), which was identified (^{1}H NMR) as a mixture of **5** and **11** in the ratio 50:7, respectively. Therefore, the percentage yield of compound **5** was 58%: IR (KBr) ν_{max} 3179, 3099, 3058, 2958, 2904, 1622, 1445, 1275, 1140, 1036, 921, 844 cm^1; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.09 (1H, s, H-2), 6.75 (1H, d, J = 2.3 Hz, H-8), 6.71 (1H, d, J = 2.3 Hz, H-6), 5.29 (2H, s, OCH_2O), 5.22 (2H, s, OCH_2O), 3.54 (3H, s, OCH_3), 3.49 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2 (C, C-4), 161.5 (C, C-7), 159.2 (C, C-5), 158.3 (C, C-8a), 155.6 (CH, C-2), 108.8 (C, C-4a), 102.1 (CH, C-6), 96.8 (CH, C-8), 95.5 (CH_2, OCH_2O), 94.4 (CH_2, OCH_2O), 89.4 (C, C-3), 56.7 (CH_3, OCH_3), 56.5 (CH_3, OCH_3); HRMS-ES m/z $[M + Na]^+$ 392.9834 (calcd for $C_{13}H_{14}O_6NaI$ 392.9835).

7-Hydroxy-2,2-dimethyl-4-chromanone (12). Resorcinol (**8**) (1.0 g, 9.1 mmol) and 3-methyl-2-butenoic acid (0.91 g, 9.1 mmol) were added simultaneously under N_2 to a stirred mixture of CH_3SO_3H (14.5 mL, 218.2 mmol) and P_2O_5 (0.7 g, 5.1 mmol) at 70 °C. The reaction mixture was stirred at the same temperature for 30 min, cooled to room temperature, and poured into ice−water (150 mL). The aqueous phase was extracted with Et_2O (3 × 25 mL), and the combined organic extracts were washed with H_2O (2 × 50 mL) and brine (25 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated to afford a yellow solid. The solid was purified by flash chromatography using Hex/EtOAc (3:2) as mobile phase to give chromanone **12** as a light yellow solid (1.61 g, 92%). Recrystallization of the solid afforded white crystals (9:1 CH_2Cl_2/Et_2O): mp 170.0−171.5 °C (lit.[20] 172−174 °C); IR (KBr) ν_{max} 3129, 2967, 2837, 1578, 1252, 1169, 1126, 854 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.58 (1H, d, J = 8.4 Hz, H-5), 6.43 (1H, dd, J = 1.9 and 8.4 Hz, H-6), 6.24 (1H, d, J = 1.9 Hz, H-8), 2.65 (2H, s, H-3), 1.36 (6H, s, 2 × CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 190.1 (C, C-4), 164.7 (C, C-7), 161.4 (C, C-8a), 127.9 (CH, C-5), 112.8 (C, C-4a), 109.8 (CH, C-6), 102.8 (CH, C-8), 79.3 (C, C-2), 47.8 (CH_2, C-3), 26.2 (2 × CH_3); HRMS-ES m/z $[M + Na]^+$ 215.0687 (calcd for $C_{11}H_{12}O_3Na$ 215.0684).

7-Hydroxy-6-iodo-2,2-dimethyl-4-chromanone (13). A solution of chromanone **12** (0.15 g, 0.78 mmol), HIO_3 (0.03 g, 0.17 mmol), and I_2 (0.08 g, 0.32 mmol) in CH_3OH/H_2O (3:1, 10 mL) was heated to 85 °C for 12 h. The CH_3OH was evaporated to give a yellow residue, which was diluted with EtOAc (20 mL) and H_2O (25 mL). The two phases were partitioned, and the aqueous phase was extracted with EtOAc (2 × 20 mL). The combined organic extracts were washed with saturated $Na_2S_2O_3$ solution (20 mL), H_2O (40 mL), and brine (30 mL) and dried over anhydrous $MgSO_4$. Evaporation of solvent gave a yellow solid, which was washed with hot CH_2Cl_2, cooled, and filtered. The residue was recrystallized from CH_2Cl_2/EtOAc (8:2) to give pure **13** (0.16 g, 65%). The mother liquor, which contained mostly 7-hydroxy-8-iodo-2,2-dimethyl-4-chromanone (**14**), was evaporated to give a yellow solid (49 mg). Compound **13** had mp 195−197 °C; IR (KBr) ν_{max} 2978, 2922, 2702, 1641, 1563, 1327, 1259, 849 cm^1; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.96 (1H, s, H-5), 6.40 (1H, s, H-8), 2.67 (2H, s, H-3), 1.36 (6H, s, 2 × CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 189.2 (C, C-4), 163.1 (C, C-7), 161.1 (C, C-8a), 136.3 (CH, C-5), 114.9 (C, C-4a), 102.8 (CH, C-8), 79.8 (C, C-2), 76.4 (C, C-6), 47.4 (CH_2, C-3), 26.1 (2 × CH_3); HRMS-ES m/z $[M + Na]^+$ 340.9656 (calcd for $C_{11}H_{11}O_3NaI$ 340.9651).

7-Benzyloxy-2,2-dimethyl-4-chromanone (16). K_2CO_3 (2.16 g, 15.63 mmol) and BnBr (1.2 mL, 10.42 mmol) were added under N_2 to a stirred solution of chromanone **12** (1.00 g, 5.21 mmol) in dry CH_3CN (30 mL). The reaction mixture was refluxed for 8 h under N_2. The mixture was cooled to room temperature, acidified with 2 M HCl (40 mL), and extracted with EtOAc (3 × 20 mL). The organic extracts were combined and washed with H_2O (25 mL) and brine (25 mL), then dried over anhydrous $MgSO_4$. The solvent was removed with a rotary evaporator and the crude product purified on a chromatotron using a Hex/EtOAc (7:3) solvent mixture. The product **16** was obtained as white crystals after evaporation of the solvent and recrystallization from Hex (1.30 g, 88%): mp 55−56 °C; IR (KBr) ν_{max} 3324, 2982, 2964, 2890, 1672, 1604, 1441, 1319, 1258, 1164, 1000, 988, 840 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81 (1H, d, J = 8.4 Hz, H-5), 7.32−7.43 (5H, m, PhCH_2), 6.62 (1H, dd, J = 1.9 and 8.4 Hz, H-6), 6.46 (1H, d, J = 1.9 Hz, H-8), 5.08 (2H, s, PhCH_2), 2.66 (2H, s, H-3), 1.45 (6H, s, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.2 (C, C-4), 165.7 (C, C-7), 161.8 (C, C-8a), 136.2 (PhCH$_2$), 128.7 (PhCH_2), 128.3 (CH, C-5), 128.2 (PhCH$_2$), 127.5 (PhCH_2), 113.1 (C, C-4a), 109.8 (CH, C-6), 102.2 (CH, C-8), 79.6 (C, C-2), 70.2 (CH_2, PhCH_2), 48.6 (CH_2, C-3), 26.7 (2 × CH_3); HRMS-ES m/z $[M + Na]^+$ 305.1149 (calcd for $C_{18}H_{18}O_3Na$ 305.1154).

7-Benzyloxy-6-iodo-2,2-dimethyl-4-chromanone (18). CF_3CO_2Ag (94 mg, 0.426 mmol) was added to a solution of **16** (100 mg, 0.355 mmol) in $CHCl_3$ (10 mL). The mixture was stirred for 5 min, and then a solution of I_2 (90 mg, 0.355 mmol) in $CHCl_3$ (20 mL) was added dropwise to the stirred suspension. The resulting mixture was stirred at room temperature for 12 h. The solution was filtered to remove the AgI, and the filtrate was washed with $Na_2S_2O_3$ solution (10%, 30 mL), $NaHCO_3$ solution (5%, 30 mL), H_2O (50 mL), and brine (30 mL). The organic phase was dried over anhydrous $MgSO_4$, and the solvent was evaporated to give a cream-white solid. The solid was purified by flash chromatography using Hex/EtOAc (6:4) as eluent to afford iodochromanone **18** as a white solid (136 mg, 94%). Recrystallization of the solid from CH_3OH gave colorless needles: mp 128−130 °C; IR (KBr) ν_{max} 2983, 1661, 1583, 1262 cm^1; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.28 (1H, s, H-5), 7.48 (2H, d, J = 7.5 Hz, PhCH_2), 7.41 (2H, t, J = 7.5 Hz, PhCH_2), 7.34 (1H, t, J = 7.3 Hz, PhCH_2), 6.40 (1H, s, H-8), 5.14 (2H, s, PhCH_2), 2.65 (2H, s, H-3), 1.44 (6H, s, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.2 (C, C-4), 162.8 (C, C-7), 162.1 (C, C-8a), 137.3 (CH, C-5), 135.6 (PhCH$_2$), 128.7 (PhCH_2), 128.2 (PhCH_2), 127.0 (PhCH_2), 116.0 (C, C-4a), 101.3 (CH, C-8), 80.1 (C, C-2), 77.2 (C, C-6), 71.1 (CH_2, PhCH_2), 48.2 (CH_2, C-3), 26.6 (2 × CH_3); HRMS-ES m/z $[M + Na]^+$ 431.0118 (calcd for $C_{18}H_{17}O_3NaI$ 431.0120).

7-Benzyloxy-6-iodo-2,2-dimethyl-4-chromanol (19). To a solution of **18** (0.295 g, 1.05 mmol) in THF (5 mL) was added $NaBH_4$ (0.20 g, 5.25 mmol) in EtOH (20 mL). The resulting solution was refluxed for 3 h. The reaction mixture was cooled to room temperature, quenched with saturated NH_4Cl solution (40 mL), and extracted with EtOAc (3 × 20 mL). The organic phases were combined, washed with saturated $NaHCO_3$ solution (20 mL), brine (20 mL), and H_2O (20 mL), and dried over anhydrous $MgSO_4$. The organic layer was concentrated, and the product was purified by flash chromatography using a Hex/EtOAc (1:1) solvent system to afford chromanol **19** as a colorless oil (0.282 g, 95%), which eventually solidified. The solid was recrystallized from Hex/Et_2O (3:2) to give white crystals: mp 90−92 °C; IR (KBr) ν_{max} 3782, 3467, 2982, 2929, 1579, 1460, 1278, 1163, 1129, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83 (1H, s, H-5), 7.49 (2H, d, J = 7.5 Hz, PhCH_2), 7.39 (2H, t, J = 7.6 Hz, PhCH_2), 7.32 (1H, t, J = 7.3 Hz, PhCH_2), 6.36 (1H, s, H-8), 5.07 (2H, s, PhCH_2), 4.75 (1H, brq, J = 7.5 Hz, H-4), 2.17 (1H, brd, J = 7.3 Hz, OH-4), 2.10 (1H, dd, J = 6.0 and 13.6 Hz, H-3b), 1.80 (1H, dd, J = 8.5 and 13.6 Hz, H-3a), 1.43 (3H, s, CH_3), 1.30 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.8 (C, C-7), 154.9 (C, C-8a), 138.0 (CH, C-5), 136.6 (PhCH$_2$), 128.5 (PhCH$_2$), 127.9 (PhCH$_2$), 127.0 (PhCH$_2$), 119.5 (C, C-4a), 101.8 (CH, C-8), 76.2 (C, C-2), 75.2 (C, C-6), 70.8 (CH_2, PhCH_2), 62.9 (C, C-4), 42.6 (CH, C-3), 28.8 (CH_3), 25.9 (CH_3); HRMS-ES m/z $[M + Na]^+$ 433.0280 (calcd for $C_{18}H_{19}O_3NaI$ 433.0277).

7-Benzyloxy-6-iodo-2,2-dimethylchromene (20). *p*-TSA (0.012 g, 0.058 mmol) was added to a solution of chromanol **19** (0.12 g, 0.29 mmol) in THF (15 mL) under N_2. The resulting solution was refluxed under N_2 for 2 h. A NaOH solution (10%, 15 mL) was added to the cooled reaction mixture, and the mixture was extracted with Et_2O (3 × 15 mL). The combined organic extracts were washed with H_2O (40 mL) and brine solution (30 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded a colorless oil, which solidified upon cooling. The solid was successively recrystallized from Hex and

CH_3OH to give iodochromene **20** as white fluffy crystals (0.112 g, 97%): mp 63−65 °C; IR (KBr) ν_{max} 3032, 2972, 2924, 1713, 1602, 1480, 1454, 1358, 1158, 736 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.49 (2H, d, J = 7.3 Hz, PhCH_2), 7.40 (2H, t, J = 7.3 Hz, PhCH_2), 7.37 (1H, s, H-5), 7.32 (1H, t, J = 7.2 Hz, PhCH_2), 6.41 (1H, s, H-8), 6.22 (1H, d, J = 9.9 Hz, H-4), 5.48 (1H, d, J = 9.9 Hz, H-3), 5.10 (2H, s, PhCH_2), 1.42 (6H, s, 2 × CH_3); ^{13}C NMR ($CDCl_3$,100 MHz) δ 157.9 (C, C-7), 154.6 (C, C-8a), 136.5 (PhCH_2), 136.0 (CH, C-5), 128.7 (CH, C-3), 128.5 (PhCH_2), 127.9 (PhCH_2), 127.0 (PhCH_2), 120.9 (CH, C-4), 117.0 (C, C-4a), 102.0 (CH, C-8), 76.8 (C, C-2), 74.8 (C, C-6), 70.9 (CH_2, PhCH_2), 28.0 (2 × CH_3); HRMS-ES m/z $[M + Na]^+$ 415.0176 (calcd for $C_{18}H_{17}O_2NaI$ 415.0171).

7-Benzyloxy-2,2-dimethylchromene-6-boronic acid (6). Triisopropyl borate (1.8 mL, 7.65 mmol) was added to a stirred solution of **20** (1.0 g, 2.55 mmol) in THF/Et_2O (1:2, 75 mL) under N_2. The solution was cooled to −100 °C using a liquid N_2 and CH_3OH bath, and then *n*-BuLi (2.4 mL of a 1.6 M solution in hexanes) was added slowly with stirring. After 1 h of stirring at a temperature below −78 °C, saturated NH_4Cl solution was added (50 mL). The solution was stirred for an additional 1 h at room temperature, and the aqueous phase was extracted with Et_2O (3 × 30 mL). The combined organic phases were washed with H_2O (2 × 60 mL) and brine (60 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated to give a white solid, which was purified by column chromatography using Hex/EtOAc (4:1) as the mobile phase. Evaporation of the solvent gave boronic acid **6** as a fluffy white solid (0.55 g, 70%): IR (KBr) ν_{max} 3367, 3027, 2969, 2928, 1643, 1607, 1567, 1449 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.47 (1H, s, H-5), 7.36−7.42 (5H, m, PhCH_2), 6.46 (1H, s, H-8), 6.32 (1H, d, J = 10.1 Hz, H-4), 5.54 (2H, s, $B(OH)_2$), 5.49 (1H, d, J = 10.1 Hz, H-3), 5.08 (2H, s, PhCH_2), 1.44 (6H, s, 2 × CH_3); ^{13}C NMR ($CDCl_3$,100 MHz) δ 165.6 (C, C-7), 157.4 (C, C-8a), 135.8 (PhCH_2), 134.8 (CH, C-5), 128.9 (PhCH_2), 128.6 (PhCH_2), 127.9 (C, C-3), 127.8 (PhCH_2), 121.8 (CH, C-4), 114.9 (C, C-4a), 100.1 (CH, C-8), 77.1 (C, C-2), 70.9 (CH_2, PhCH_2), 28.3 (2 × CH_3) (C-6 signal not observed); ^{11}B NMR ($CDCl_3$, 128 MHz) δ_B 28.74.

2′-Benzyloxy-5,7-dihydroxy-6″,6″-dimethylpyrano[2″,3″:4′,5′]isoflavone (4). To a solution of **5** (0.5 g, 1.28 mmol) in DME/H_2O (1:1, 60 mL) were added K_2CO_3 (0.52 g, 3.82 mmol), boronic acid **6** (0.55 g, 1.77 mmol), and 10% Pd(0)/C (68 mg, 5 mol %). The reaction mixture was stirred at 40−45 °C for 12 h. The catalyst was filtered and washed with Et_2O and H_2O. The organic solvents were evaporated, and the crude product was diluted with CH_3OH (25 mL) and HCl (3 M, 10 mL). The resulting solution was stirred at 50 °C for 18 h. CH_3OH was evaporated, and the reaction mixture was diluted with CH_2Cl_2 (15 mL) and H_2O (10 mL). The two phases were partitioned, and the aqueous phase was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic phases were washed with $NaHCO_3$ solution (10%, 50 mL), H_2O (50 mL), and brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$. Evaporation of the solvent with a rotary evaporator gave a yellow oil, which was subjected to column chromatography using Hex/EtOAc (3:2) as eluent. The solvent was evaporated to give **4** as a yellowish oil, which solidified upon cooling (0.41 g, 72%). Recrystallization of the solid from Hex/Et_2O (1:1) gave white crystals: mp 93−95 °C; IR (KBr) ν_{max} 3255, 2974, 2929, 1650, 1615, 1497, 1277, 1151, 1038, 832, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 12.79 (1H, s, OH-5), 7.77 (1H, s, H-2), 7.22−7.34 (5H, m, PhCH_2), 6.92 (1H, s, H-6′), 6.50 (1H, s, H-3′), 6.23 (1H, d, J = 9.8 Hz, H-4″), 6.21 (1H, d, J = 2.2 Hz, H-8), 6.19 (1H, d, J = 2.2 Hz, H-6), 5.44 (1H, d, J = 9.8 Hz, H-5″), 5.03 (2H, s, PhCH_2), 1.40 (6H, s, H-7″ and H-8″); ^{13}C NMR ($CDCl_3$,100 MHz) δ 181.0 (C, C-4), 163.0 (C, C-7), 162.5 (C, C-5), 158.0 (C, C-8a), 157.5 (C, C-2′), 154.8 (C, C-4′), 154.6 (CH, C-2), 136.6 (PhCH_2), 129.2 (CH, C-6′), 128.5 (PhCH_2), 128.2 (CH, C-5″), 127.9 (PhCH_2), 127.3 (PhCH_2), 121.5 (CH, C-4″), 120.8 (C, C-3), 114.6 (C, C-5′), 112.0 (C, C-1′), 105.8 (C, C-4a), 101.8 (CH, C-3′), 99.6 (CH, C-6), 94.1 (CH, C-8), 76.8 (C, C-6″), 70.9 (CH_2, PhCH_2), 28.2 (2 × CH_3, C-7″ and C-8″); HRMS-ES m/z $[M + Na]^+$ 465.1312 (calcd for $C_{27}H_{22}O_6Na$ 465.1314).

2′-*O*-Benzylkraussianone 1 (23). 3-Methyl-2-butenal (0.18 mL, 1.85 mmol) was added under an N_2 atmosphere to a stirred mixture of $Ca(OH)_2$ (55 mg, 0.74 mmol) and **4** (82 mg, 0.185 mmol) in CH_3OH (10 mL). The mixture was stirred for 3 days at room temperature. CH_3OH was evaporated, and the reaction mixture was diluted with EtOAc (15 mL) and H_2O (20 mL). The two phases were partitioned, and the aqueous phase was extracted with EtOAc (2 × 15 mL). The organic layers were combined, washed with 1 M HCl (40 mL), H_2O (40 mL), and brine (40 mL), and dried over anhydrous $MgSO_4$. Evaporation of the solvent followed by column chromatography gave **23** as a yellowish oil (70 mg, 74%): IR (KBr) ν_{max} 3422, 3034, 2974, 2927, 1651,1615, 1496, 1463, 1287, 1146, 1058, 828, 696 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 13.22 (1H, s, OH-5), 7.79 (1H, s, H-2), 7.27−7.34 (5H, m, PhCH_2), 6.96 (1H, s, H-6′), 6.73 (1H, d, J = 10.0 Hz, H-4‴), 6.52 (1H, s, H-3′), 6.31 (1H, s, H-8), 6.28 (1H, d, J = 9.8 Hz, H-4″), 5.61 (1H, d, J = 10.0 Hz, H-5‴), 5.49 (1H, d, J = 9.8 Hz, H-5″), 5.04 (2H, s, PhCH_2), 1.47 (6H, s, H-7‴ and H-8‴), 1.44 (6H, s, H-7″ and H-8″); ^{13}C NMR ($CDCl_3$,100 MHz) δ 180.9 (C, C-4), 159.3 (C, C-7), 157.5 (CH, C-2′), 157.3 (C, C-8a), 156.9 (C, C-5), 154.7 (C, C-4′), 154.4 (CH, C-2), 136.8 (PhCH_2), 129.1 (CH, C-6′), 128.5 (PhCH_2), 128.1 (CH, C-5″), 128.0 (CH, C-5‴), 127.8 (PhCH_2), 127.2 (PhCH_2), 121.6 (CH, C-4″), 120.6 (C, C-3), 115.6 (CH, C-4‴), 114.5 (C, C-5′), 112.2 (C, C-1′), 106.2 (C, C-4a), 105.5 (C, C-6), 101.8 (CH, C-3′), 94.8 (CH, C-8), 77.9 (C, C-6‴), 76.8 (C, C-6″), 70.8 (CH_2, PhCH_2), 28.3 (2 × CH_3, C-7‴ and C-8‴), 28.2 (2 × CH_3, C-7″ and C-8″); HRMS-ES m/z $[M + Na]^+$ 531.1782 (calcd for $C_{32}H_{28}O_6Na$ 531.1784).

Kraussianone 1 (1). BCl_3 (0.5 mL, 1 M in heptane) was added to a solution of **23** (50 mg, 0.1 mmol) in CH_2Cl_2 (5 mL) cooled to −80 °C. The mixture was stirred under N_2 at a temperature below −68 °C for 30 min. The reaction was quenched with H_2O and extracted with CH_2Cl_2 (2 × 15 mL). The combined organic extracts were washed with H_2O (20 mL) and brine (20 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave a yellow oil, which was purified on a chromatotron using CH_2Cl_2 to give **1** (24 mg, 69%) as a yellow solid. The solid was recrystallized from Hex/CH_3OH (4:1): mp 186−188 °C (lit.[1] 185−187 °C); IR (KBr) ν_{max} 3336, 2975, 2872, 1649, 1615, 1460, 1132, 773 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 12.55 (1H, s, OH-5), 8.27 (1H, s, OH-2′), 7.73 (1H, s, H-2), 6.75 (1H, s, H-6′), 6.74 (1H, d, J = 10.0 Hz, H-4‴), 6.53 (1H, s, H-3′), 6.40 (1H, s, H-8), 6.28 (1H, d, J = 9.8 Hz, H-4″), 5.65 (1H, d, J = 10.0 Hz, H-5‴), 5.52 (1H, d, J = 9.8 Hz, H-5″), 1.49 (6H, s, H-7‴ and H-8‴), 1.44 (6H, s, H-7″ and H-8″); ^{13}C NMR ($CDCl_3$,100 MHz) δ 182.0 (C, C-4), 160.4 (C, C-7), 157.2 (CH, C-2′), 157.1 (C-8a), 156.4 (C, C-5), 155.7 (C, C-4′), 154.7 (CH, C-2), 128.8 (CH, C-5″), 128.6 (CH, C-5‴), 127.1 (CH, C-6′), 123.1 (C, C-3), 121.3 (CH, C-4″), 115.28 (CH and C, C-4‴ and C-5′), 112.1 (C, C-1′), 107.3 (CH, C-3′), 106.1 (C, C-6), 105.5 (C, C-4a), 95.0 (CH, C-8), 78.5 (C, C-6‴), 77.2 (C, C-6″), 28.4 (2 × CH_3, C-7‴ and C-8‴), 28.2 (2 × CH_3, C-7″ and C-8″); HRMS-ES m/z $[M + Na]^+$ 441.1317 (calcd for $C_{25}H_{22}O_6Na$ 441.1314).

Eriosemaone D (2). Compound **4** (10 mg, 0.023 mmol) was debenzylated under similar conditions to those applied for **1**, and the product was purified on a chromatotron to give **2** as a yellow solid (6 mg, 75%): ^1H NMR ($CDCl_3$, 400 MHz) δ 12.32 (1H, s, OH-5), 8.21 (1H, brs, OH-7 or OH-2), 7.96 (1H, s, H-2), 6.76 (1H, s, H-6′), 6.53 (1H, s, H-3′), 6.44 (1H, d, J = 2.3 Hz, H-8), 6.37 (1H, d, J = 2.3 Hz, H-6), 6.27 (1H, d, J = 9.8 Hz, H-4″), 5.53 (1H, d, J = 9.8 Hz, H-5″), 1.44 (6H, s, H-7″ and H-8″); LRMS-ES m/z $[M - H]^+$ 351.0.

4-Methoxymethoxy-1-iodobenzene (21). AcCl (1.94 mL, 27.27 mmol) was added dropwise to the stirred solution of dimethoxymethane (2.40 mL, 27.27 mmol) and catalytic $ZnBr_2$ under N_2. The solution was stirred for an additional 2 h at room temperature and transferred via a cannula to an ice-cold solution of 4-iodophenol (2.0 g, 9.09 mmol) and DIA (4.16 mL, 27.27 mmol) in CH_2Cl_2 (100 mL) under an N_2 atmosphere. The mixture was stirred for 4 h, diluted with saturated NH_4Cl solution (40 mL), and stirred for an additional 15 min. The two phases were partitioned, and the aqueous phase was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic extracts were washed with brine (3 × 100 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the product was purified with column chromatography using Hex/EtOAc (8:2) to afford **21** as a colorless oil (1.8 g, 75%): IR (neat) ν_{max} 2954, 2901, 2825, 1585, 1483, 1231, 1147, 987, 818 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.56 (2H, d, J = 9.0 Hz, H-2 and H-6), 6.82 (2H, d, J = 9.0 Hz, H-3 and H-5), 5.14 (2H, s, OCH_2O), 3.46 (3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.0 (C, C-4), 138.2 (CH, C-2 and C-6), 118.5 (CH, C-3 and C-5), 94.3 (CH_2, OCH_2O), 84.3 (C, C-1), 55.9 (CH_3, OCH_3).

4-Methoxymethoxyphenylboronic acid (22). Triisopropyl borate (2.21 mL, 9.47 mmol) was added in one portion to the stirred solution of iodobenzene **21** (1.0 g, 3.79 mmol) in THF/Et_2O (60 mL, 1:2) under N_2. The solution was cooled to −100 °C using a liquid N_2 and CH_3OH bath, and then *n*-BuLi (3.79 mL of a 1.5 M solution in hexanes) was

added with stirring over 5 min. After 1 h of stirring at a temperature below −78 °C, a saturated NH_4Cl (30 mL) solution was added. The mixture was stirred for an additional 1 h, and the two phases were partitioned. The aqueous phase was extracted with Et_2O (2 × 20 mL). The organic phases were combined, washed with H_2O (40 mL) and brine (40 mL), and dried over anhydrous $MgSO_4$. The solvent was evaporated to give a white solid, which was subjected to column chromatography using Hex/EtOAc (4:1) as the mobile phase. The solvent was evaporated to give boronic acid **22** as a white solid (0.59 g, 85%): IR (KBr) ν_{max} 3436, 3359, 1651, 1606, 1235, 1016, 773 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.16 (2H, d, J = 8.6 Hz, H-2 and H-6), 7.15 (2H, d, J = 8.6 Hz, H-3 and H-5), 5.27 (2H, s, OCH_2O), 3.52 (3H, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.9 (C, C-4), 137.5 (CH, C-2 and C-6), 115.6 (CH, C-3 and C-5), 94.1 (CH_2, OCH_2O), 56.1 (CH_3, OCH_3) (C-1 signal not observed); ^{11}B NMR (128 MHz, $CDCl_3$) δ 28.52.

Genistein (3). 4-Methoxymethoxyphenylboronic acid (**22**) (42 mg, 0.23 mmol), K_2CO_3 (51 mg, 0.37 mmol), and catalytic 10% Pd/C were added to a solution of 3-iodochromone **5** (50 mg, 0.13 mmol) in DME (2 mL) and H_2O (2 mL). The resulting mixture was stirred at 40−45 °C for 12 h. The catalyst was filtered and washed with H_2O and Et_2O. The organic solvents were evaporated, and the crude product was diluted in CH_3OH (10 mL) and HCl (3 M, 5 mL). The resulting solution was refluxed for 30 min. CH_3OH was evaporated, and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (30 mL) and brine (30 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude product was purified by column chromatography using Hex/EtOAc (3:2) and recrystallized from EtOH to give the isoflavone **3** as a yellow solid (23 mg, 66%): mp 300−303 °C (lit.[33] 301−302 °C); IR (KBr) ν_{max} 3434, 3181, 3069, 2922, 1652, 1621, 1176, 809, 784 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.93 (1H, s, OH-5), 9.65 (1H, brs, OH-4′), 8.29 (1H, s, H-2), 7.36 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 6.81 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 6.38 (1H, d, J = 2.1 Hz, H-6), 6.22 (1H, d, J = 2.1 Hz, H-8); ^{13}C NMR (DMSO-d_6, 100 MHz,) δ 182.3 (C, C-4), 166.0 (C, C-7), 163.9 (C, C-5), 159.8 (C, C-4′), 158.9 (C, C-8a), 154.8 (CH, C-2), 131.2 (CH, C-2′ and C-6′), 124.8 (C, C-1′), 123.3 (C, C-3), 116.8 (CH, C-3′ and C-5′), 106.3 (C, C-4a), 100.1 (CH, C-6), 94.8 (CH, C-8); HRMS-ES (m/z) $[M - H]^-$ 269.0452 (calcd for $C_{15}H_9O_5$ 269.0450).

Acknowledgment. We are grateful to the National Research Foundation (South Africa) for financial assistance.

Supporting Information Available: Copies of NMR spectra of kraussianone 1 (**1**), eriosemaone D (**2**), genistein (**3**), and synthetic intermediates **4**, **5**, **6**, **10**, **13**, **18**, **19**, **20**, and **23**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Drewes, S. E.; Horn, M. M.; Munro, O. Q.; Dhlamini, J. T. B.; Meyer, J. J. M.; Rakuambo, N. C. *Phytochemistry* **2002**, *59*, 739–747.
(2) Drewes, S. E.; Horn, M. M.; Khan, F.; Munro, O. Q.; Dhlamini, J. T. B.; Rakuambo, C.; Marion Meyer, J. J. *Phytochemistry* **2004**, *65*, 1955–1961.
(3) Ojewole, J. A. O.; Drewes, S. E.; Khan, F. *Phytochemistry* **2006**, *67*, 610–617.
(4) Hoshino, Y.; Miyaura, N.; Suzuki, A. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3008–3010.
(5) Wei, G.; Yu, B. *Eur. J. Org. Chem.* **2008**, 3156–3163.
(6) Zheng, S.-Y.; Shen, Z.-W. *Tetrahedron Lett.* **2010**, *51*, 2883–2887.
(7) Jain, A. C.; Jain, S. M. *Tetrahedron* **1972**, *28*, 5063–5067.
(8) Jain, A. C.; Sharma, B. N. *J. Org. Chem.* **1974**, *39*, 2215–2217.
(9) Jain, A. C.; Kumar, A.; Gupta, R. C. *J. Chem. Soc., Perkin Trans. 1* **1979**, 279–282.
(10) Rao, K. S. R. M.; Iyer, C. S. R.; Iyer, P. R. *Tetrahedron* **1987**, *43*, 3015–3019.
(11) Tsukayama, M.; Horie, T.; Iguchi, Y.; Nakayama, M. *Chem. Pharm. Bull.* **1988**, *36*, 592–600.
(12) Tsukayama, M.; Kawamura, Y.; Tamaki, H.; Kubo, T.; Horie, T. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 826–832.
(13) Tsukayama, M.; Kawamura, Y.; Tamaki, H.; Horie, T. *Chem. Pharm. Bull.* **1991**, *39*, 1704–1706.
(14) Hostettmann, K.; Marston, A. *Pure Appl. Chem.* **1994**, *66*, 2231–2234.
(15) Ma, W. G.; Fuzzati, N.; Li, Q. S.; Yang, C. R.; Stoeckli-Evans, H.; Hostettmann, K. *Phytochemistry* **1995**, *39*, 1049–1061.
(16) Berliner, M.; Belecki, K. *Org. Synth.* **2007**, *84*, 102.
(17) Gammill, B. R. *Synthesis* **1979**, 901–903.
(18) Hong, R.; Feng, J.; Hoen, R.; Lin, G.-Q. *Tetrahedron* **2001**, *57*, 8685–8689.
(19) Felpin, F.-X.; Lory, C.; Sow, H.; Acherar, S. *Tetrahedron* **2007**, *63*, 3010–3016.
(20) Camps, F.; Coll, J.; Messeguer, A.; Pericas, M. A.; Ricart, S. *Synthesis* **1980**, 725–728.
(21) Mujahidin, D.; Doye, S. *Eur. J. Org. Chem.* **2005**, 2689–2693.
(22) Adimurthy, S.; Ramachandraiah, G.; Ghosh, P. K.; Bedekar, A. V. *Tetrahedron Lett.* **2003**, *44*, 5099–5101.
(23) Aslam, S. N.; Stevenson, P. C.; Phythian, S. J.; Veitch, N. C.; Hall, D. R. *Tetrahedron* **2006**, *62*, 4214–4226.
(24) Hossain, M. M.; Tokuoka, T.; Yamashita, K.; Kawamura, Y.; Tsukayama, M. *Synth. Commun.* **2006**, *36*, 1201–1211.
(25) Weitl, F. L. *J. Org. Chem.* **1976**, *41*, 2044–2045.
(26) Haszeldine, R. N.; Sharpe, A. G. *J. Chem. Soc.* **1952**, 993–1001.
(27) Lichtenfels, A. R.; Coelho, A. L.; Costa, R. R. *J. Chem. Soc., Perkin Trans. 1* **1995**, 949–951.
(28) Lim, J.; Kim, H.-I.; Kim, H. H.; Ahn, K.-S.; Han, H. *Tetrahedron Lett.* **2001**, *42*, 4001–4003.
(29) Brown, H. C.; Cole, T. E. *Organometallics* **1983**, *2*, 1316–1319.
(30) Li, W.; Nelson, D. P.; Jensen, M. S.; Hoerrner, R. S.; Cai, D.; Larsen, R. D.; Reider, P. J. *J. Org. Chem.* **2002**, *67*, 5394–5397.
(31) Felpin, F.-X. *J. Org. Chem.* **2005**, *70*, 8575–8578.
(32) Mondal, M.; Puranik, G. V.; Argade, N. P. *J. Org. Chem.* **2006**, *71*, 4992–4995.
(33) Bradbury, R. B. *J. Chem. Soc.* **1951**, 3447–3449.

NP100407N

Tetrahedron 67 (2011) 8654–8658

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Total synthesis of the pyranocoumaronochromone lupinalbin H

Mamoalosi A. Selepe, Siegfried E. Drewes, Fanie R. van Heerden *

School of Chemistry, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, Pietermaritzburg, South Africa

ARTICLE INFO

Article history:
Received 5 July 2011
Received in revised form 30 August 2011
Accepted 12 September 2011
Available online 17 September 2011

Keywords:
Lupinalbin H
Lupinalbin A
Pyranocoumaronochromone
Isoflavonoid
2′-Hydroxygenistein
Suzuki–Miyaura reaction

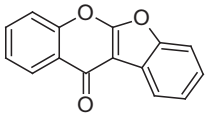
ABSTRACT

The pyranocoumaronochromone lupinalbin H was synthesized in three major steps, which involved preparation of 2′-hydroxygenistein by the Suzuki–Miyaura reaction, followed by oxidative cyclodehydrogenation into lupinalbin A. The final step was the regiospecific introduction of the dimethylpyran moiety to ring A of lupinalbin A via an aldol-type condensation with 3-methyl-2-butenal and 6π-electrocyclization.

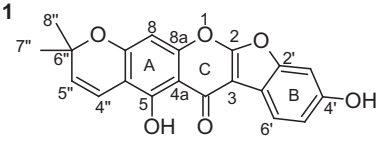
1. Introduction

Coumaronochromones are a subclass of isoflavonoids with general structure **1**.[1] They have been isolated from different plant genera, mostly of the Leguminosae,[1–5] although a few coumaronochromones have been reported from non-leguminous plants.[6–8] The striking feature of most naturally-occurring coumaronochromones is the presence of prenyl side chains, which in most instances are cyclized to adjacent hydroxy groups to give pyrano or furano rings.[1–3,5,7,9,10] Most pyranocoumaronochromones exhibit important biological activities, such as anthelminthic, oestrogenic, neuroprotective, antiplatelet aggregation, anti-HIV, immunosuppressive activities as well as cytotoxicity against certain cancer cell lines.[2,3,5,9–13] Despite their biological importance, the synthesis of these compounds has received little attention. Methods, which have been employed for the synthesis of the coumaronochromone nucleus are photochemical contraction of rotenoids and oxidative cyclization of 2′-hydroxyisoflavones, the latter being the strategy employed mostly.[14–16] The dimethylpyran scaffold, on the other hand, can be accessed via several synthetic approaches, which involve cycloaddition reactions of *C*-prenylated phenols, aldol-type condensation of phenols with prenal (3-methyl-2-butenal) or prenal acetal, dehydration of chromanols, Harfenist–Thom rearrangement of propargyl ethers and one-pot Wittig reactions of *o*-naphthoquinones with allyltriphenylphosphonium salts and subsequent electrocyclization.[17–20]

Despite the numerous synthetic procedures that have been developed for the dimethylpyran moiety, the regioselective introduction of the dimethylpyran system to the synthetic precursors or to the coumaronochromone core has been a major challenge in the synthesis of pyranocoumaronochromones.[14,15]



1



2

In continuation of our studies on the regioselective synthesis of pyranoisoflavonoids,[21] the present paper reports the first total synthesis of lupinalbin H (**2**) and confirmation of its structure on the basis of 1D and 2D NMR techniques. Lupinalbin H (**2**) was isolated together with other flavonoids from the methanolic extract of the roots of yellow lupin (*Lupinus luteus* cv Topaz) by Tahara et al.[22] The assignment of the structure of **2** was based mainly on comparison of its ^{1}H NMR signals with those of related compounds and the ^{13}C NMR data was not reported. Furthermore, the other flavonoids isolated along with it were reported to exhibit antifungal activity; however, **2** was not assayed for its activity. The lack of detailed structural characterization and bioactivity studies of lupinalbin H (**2**) can be attributed to the low quantity obtained from

* Corresponding author. Tel.: +27 33 2605886; fax: +27 33 2605009; e-mail address: vanheerdenf@ukzn.ac.za (F.R. van Heerden).

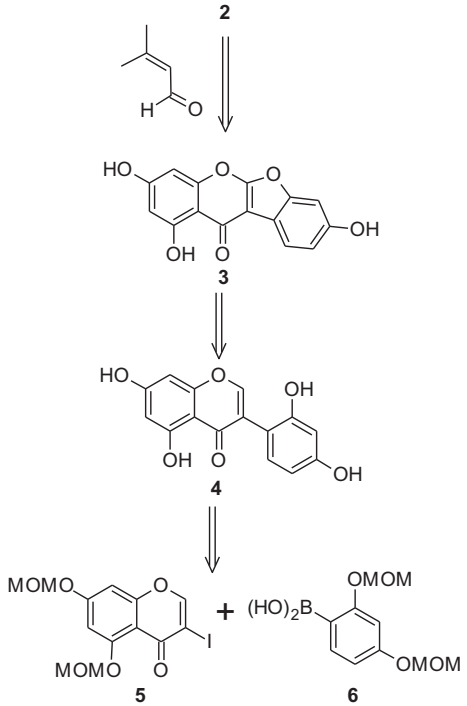
0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.09.042

the plant source.[22] Therefore, our aim was to develop a synthetic route that can readily give access to **2** in a good yield, to confirm its structure unambiguously and provide material for future biological studies.

With this synthesis we hope to demonstrate the usefulness of the Suzuki–Miyaura reaction in the synthesis of isoflavonoids. Previous methods for the synthesis of this class of compounds were based mainly on chalcones and deoxybenzoins as intermediates.[14,16] However, the chalcone route depends on the use of the highly toxic thallium trinitrate whereas the deoxybenzoin route employs harsh reaction conditions.

2. Results and discussion

Scheme 1 shows the retrosynthetic analysis of lupinalbin H (**2**). Lupinalbin H (**2**) was planned to be synthesized by the regiospecific condensation of lupinalbin A (**3**)[23,24] with prenal. Compound **3** could be prepared by oxidative cyclization of 2′-hydroxygenistein (**4**),[25,26] which could readily be accessed by the Suzuki–Miyaura reaction of 3-iodochromone **5**[21] and boronic acid **6**.[27] The synthesis of the penultimate precursor **3** from 2′-hydroxyisoflavones has been reported previously.[14,16] However, the former syntheses were based on the deoxybenzoin and the chalcone routes for the construction of the isoflavone core.[14,16]



Scheme 1. Retrosynthetic analysis of lupinalbin H (**2**).

The synthesis of intermediate **5** was described in a previous paper.[21] Vasselin et al.[28] reported that with the widely used benzyl protecting group, the 3-iodobenzopyran-4-one could not be prepared and therefore we opted for methoxymethyl as protecting group in the synthesis of **5**. Boronic acid **6**[27] was prepared from commercially available resorcinol (**7**), as illustrated in Scheme 2. Protection of **7** with MOMCl, prepared in situ from the readily available dimethoxymethane and acetyl chloride,[29] rendered methoxymethyl ether **8** (60%),[30] which was regioselectively iodinated at C-4 by I_2 in the presence of CH_3CO_2Ag to give an aryl iodide **9** in 95% yield.[31] In our previous investigation, we demonstrated the successful regioselective iodination of a chromanone containing resorcinol moiety using CF_3CO_2Ag and I_2.[21] CH_3CO_2Ag gave comparable results in the present case. Other readily available silver salts ($AgNO_3$ and Ag_2SO_4) were tested for C-4 iodination of **8**, but rendered **9** in low yields when $CHCl_3$ was used as the solvent and in moderate yields in EtOH (Table 1).[32,33] The aryl iodide **9** was converted into boronic acid **6** by lithium–iodine exchange, followed by immediate in situ nucleophilic attack of the generated aryl lithium species on triisopropyl borate and hydrolysis of the resulting boronate ester with NH_4Cl in a one-pot reaction.[34,35]

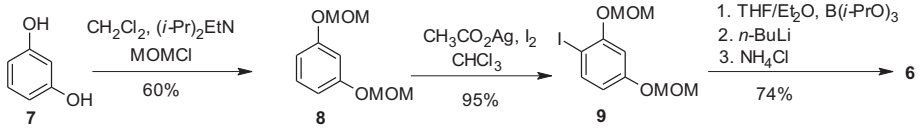
Table 1
Preparation of aryl iodide **9** by iodination of **8** with I_2 and silver salts[a]

Silver salt	Solvent	Time (h)	Yields[b] (%)
$AgNO_3$	$CHCl_3$	5	40
Ag_2SO_4[c]	$CHCl_3$	5	35
$AgNO_3$	EtOH	1	62
Ag_2SO_4[c]	EtOH	1	58
CH_3CO_2Ag	$CHCl_3$	5	95

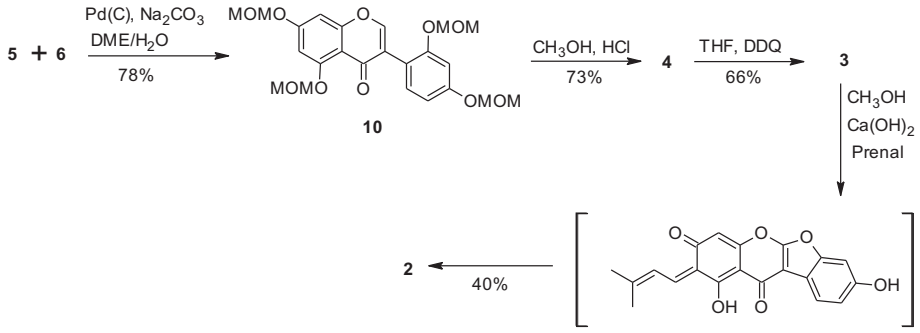
[a] Silver salt (1.2 equiv), 1.1 equiv I_2.
[b] Isolated yields.
[c] Ag_2SO_4 (0.6 equiv), 1.1 equiv I_2.

Having successfully synthesized the boronic acid **6**, the next step involved coupling of **6** with 3-iodochromone **5**[21] in the presence of 10% Pd(C) to give an isoflavone **10** (Scheme 3).[36] Cleavage of the MOM protecting groups of **10** under acidic conditions and subsequent oxidation of 2′-hydroxygenistein (**4**) with DDQ afforded the phytoestrogen lupinalbin A (**3**)[16] in a 66% yield.[14] The last step was regioselective introduction of the dimethylpyran scaffold to the phloroglucinol moiety of lupinalbin A (**3**) to give lupinalbin H (**2**). This was planned to be achieved by base-catalyzed coupling of **3** with prenal, which proceeds via aldol-type reaction and 6π-electrocylization.[18] Thus, treatment of the methanolic solution of **3** with $Ca(OH)_2$ and prenal (2.5 equiv) rendered lupinalbin H (**2**) in 40% yield and 35% of **3** was recovered.[37] Addition of a large excess of prenal (5 equiv) effected complete consumption of **3** as observed on TLC but required tedious chromatographic isolation of the targeted product from the reaction mixture due to side products resulting from the polymerization of prenal.

Lupinalbin A (**3**) has four nucleophilic sites at positions 6, 8, 3′ and 5′, which can condense with prenal to give multiple products. As anticipated, the reaction favoured the more nucleophilic phloroglucinol moiety (ring A) rather than the resorcinol moiety (ring B). Nevertheless, three possible regioisomers can result from condensation of prenal with ring A of **3**, i.e., the targeted linear isomer **2** and two angular isomers **11** and **12**. From the ^1H NMR results, it could be readily deduced that the isomer **12** was not



Scheme 2. Synthesis of boronic acid **6**.



Scheme 3. Total synthesis of lupinalbin H (**2**).

formed due to the appearance of a signal characteristic for a hydrogen-bonded OH at δ_H 13.38 (1H, OH-5). Furthermore, in the ^{1}H NMR spectrum was present four aromatic protons, which gave an ABX spin system for ring B protons at δ_H 7.82 (1H, d, J=8.3 Hz, H-6′), 7.14 (1H, d, J=2.0 Hz, H-3′), 7.02 (1H, dd, J=2.0 and 8.3 Hz, H-5′),and a one-proton singlet for the ring A proton at δ_H 6.54 (H-8). The dimethylpyran protons displayed a singlet integrating for six protons at δ_H 1.48 (2× CH_3) and two one-proton doublets for the olefinic protons at δ_H 6.70 (J=10.0 Hz, H-4″) and 5.79 (1H, d, J=10.0 Hz, H-5″). These ^{1}H NMR results were in agreement with those reported in the literature for **2**.[22] The ^{13}C NMR spectrum displayed 19 carbon resonances, which were identified as two methyl carbons overlapping at δ_C 27.3, six methine carbons at δ_c 128.6, 121.3, 114.6, 113.6, 98.5 and 95.3 and 12 quaternary carbons at δ_C 178.6, 164.7, 158.5, 157.0, 156.4, 154.0, 150.4, 114.0, 105.9, 104.0, 97.5 and 80.0 by DEPT and HSQC experiments. From the HMBC spectrum, the olefinic proton at δ_H 6.70 (H-4″) displayed correlations to carbon signals at δ_C 80.0 (C, C-6″), 105.9 (C, C-6), 157.0 (C, C-5) and 158.5 (C, C-7) and the aromatic proton at δ_H 6.54 (H-8) showed connections to carbon resonances at δ_C 104.0 (C, C-4a), 105.9 (C, C-6), 154.0 (C, C-8a) and 158.5 (C, C-7) (Fig. 1). From these correlations, it was confirmed that the dimethylpyran ring was attached to C-6 and OH-7, giving the targeted product **2** and not the angular isomer **11**. The assignments of the quaternary carbons on ring B were also based on HMBC correlations. The proton at δ_H 7.82 (H-6′) displayed correlations to carbon signals at δ_C 97.5 (C, C-3), 150.4 (C, C-2′) and 156.4 (C, C-4′), whereas H-5′ (δ_H 7.02) showed correlations to carbons at δ_C 114.0 (C, C-1′) and 156.4 (C, C-4′), and the proton at δ_H 7.14 (H-3′) showed correlations to signals at δ_C 114.0 (C, C-1′), 150.4 (C, C-2′) and 156.4 (C, C-4′) (Fig. 1). The structure of **2** was confirmed by HRMS-ESI, which gave an m/z peak of 349.0710 $[M-H]^-$, in agreement with the calculated molecular weight of 349.0712 for $C_{20}H_{13}O_6$.

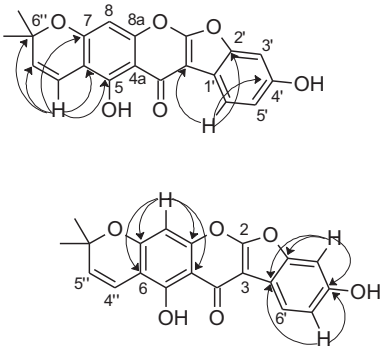
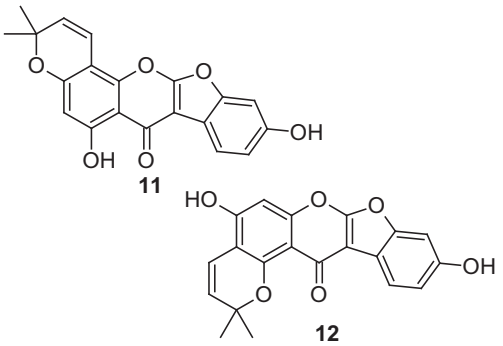


Fig. 1. Key HMBC correlations of (**2**).



3. Conclusion

In conclusion, lupinalbin H (**2**) has been successfully synthesized by a highly convergent route. The synthesis featured the Suzuki–Miyaura coupling reaction for the construction of the isoflavone nucleus in good yields, and a highly regioselective introduction of the dimethylpyran scaffold to the coumaronochromone core. Furthermore, it gave access to other naturally-occurring phytoestrogens, 2′-hydroxygenistein (**4**) and lupinabin A (**3**). Owing to the potential pharmacological properties of the pyranocoumaronochromones, the development of the practical synthetic route described herein for lupinalbin H (**2**) represents a significant advance towards the synthesis of other structurally related compounds and exploration of their biological properties.

4. Experimental procedures

4.1. General

All moisture-sensitive reactions were performed using dried anhydrous solvents in oven-dried glassware under an atmosphere of N_2. Hexanes (Hex) used for chromatographic purifications was distilled prior to use. Reactions were monitored by TLC, performed on silica gel plates (60 F_{254}) and visualized under UV light. Alternatively, detection of spots on the TLC was achieved by heating with a heat gun after treatment with a solution of anisaldehyde in concentrated H_2SO_4 and EtOH prepared in volume ratios 1:1:18, respectively. Column chromatographic purifications were effected using silica gel (60 Å, 230–400 mesh). Centrifugal chromatography (chromatotron) was performed on glass plates coated with silica gel with particle size 0.040–0.063 mm, 2–4 mm thick. ^{1}H, ^{11}B, ^{13}C NMR spectra were recorded on 400 or 500 MHz spectrometer. DEPT and 2D NMR (COSY, HSQC and HMBC) were used for assignments of individual protons and carbons resonances. ^{11}B NMR spectra were referenced against an external standard of neat BF_3.OEt_2 containing a capillary tube of acetone-d_6 for deuterium

lock. The chemical shifts from ^{1}H NMR and ^{13}C NMR spectra are reported in parts per million relative to the residual protonated or deuterated solvents peaks ($CDCl_3$: δ_H 7.26, δ_c 77.0; CD_3OD: δ_H 3.31, δ_c 49.0 and acetone-d_6: δ_H 2.05, δ_c 205.1). The mass spectra were recorded on a time-of-flight mass spectrometer using electrospray ionisation in the positive or negative mode. IR spectra were recorded with FT-IR spectrophotometer.

4.2. Synthesis

*4.2.1. 1,3-Dimethoxymethoxybenzene (**8**)*[30]. Catalytic $ZnBr_2$ was dissolved in dimethoxymethane (16.0 mL, 0.182 mol) under nitrogen atmosphere, and then acetyl chloride (12.9 mL, 0.182 mol) was added dropwise to the stirred solution. The solution was stirred for an additional 2 h at rt then transferred via a cannula to the ice-cold solution of resorcinol (**7**) (5.00 g, 45.4 mmol) and (i-Pr)$_2$EtN (23.3 mL, 0.136 mol) in CH_2Cl_2 (100 mL) under a nitrogen atmosphere. The mixture was stirred for 3 h, diluted with saturated NH_4Cl solution and stirred for an additional 15 min. The two phases were partitioned and the aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated to give a yellow oil, which was purified by column chromatography using Hex/EtOAc (9:1) to give **8** as a colorless oil (5.4 g, 60%): IR (neat) ν_{max} 2955, 2902, 2827, 1768, 1591, 1487, 1219, 1138, 1004, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.19 (1H, t, J=8.3 Hz, H-5), 6.75 (1H, t, J=2.3 Hz, H-2), 6.71 (2H, dd, J=2.3 and 8.3 Hz, H-4 and H-6), 5.16 (4H, s, 2× OCH_2O), 3.48 (6H, s, 2× OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.3 (2× C, C-1 and C-3), 129.9 (CH, C-5), 109.6 (2× CH, C-4 and C-6), 105.0 (CH, C-2), 94.5 (2× OCH_2O), 55.9 (2× OCH_3); LRMS-ESI m/z [M]$^+$ 198.1.

*4.2.2. 1-Iodo-2,4-dimethoxymethoxybenzene (**9**).* CH_3CO_2Ag (5.10 g, 30.3 mmol) was added to a solution of **8** (5.00 g, 25.2 mmol) in $CHCl_3$ (50 mL). The mixture was stirred for 5 min, and then a solution of I_2 (7.04 g, 27.7 mmol) in $CHCl_3$ (150 mL) was added dropwise to the stirred suspension. The resulting mixture was stirred at rt for 5 h and filtered to remove the AgI. The filtrate was washed with 10% $Na_2S_2O_3$ solution, 5% $NaHCO_3$ solution, H_2O and brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent evaporated to give a colourless oil. The oil was purified by flash chromatography using Hex/EtOAc (9:1) to afford the iodinated compound **9** as a colorless oil (7.76 g, 95%): IR (neat) ν_{max} 2956, 2902, 2826, 1716, 1577, 1567, 1474, 1219, 1149, 982, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 (1H, d, J=8.5 Hz, H-6), 6.80 (1H, d, J=2.5 Hz, H-3), 6.53 (1H, dd, J=2.5 and 8.5 Hz, H-5), 5.21 (2H, s, OCH_2O), 5.13 (2H, s, OCH_2O), 3.50 (3H, s, OCH_3), 3.46 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.7 (C, C-4), 156.7 (C, C-2), 139.2 (CH, C-6), 111.4 (CH, C-5), 104.4 (CH, C-3), 95.0 (CH_2, OCH_2O), 94.5 (CH_2, OCH_2O), 77.4 (C, C-1), 56.4 (CH_3, OCH_3), 56.0 (CH_3, OCH_3); HRMS-ESI m/z [M+Na]$^+$ calcd for $C_{10}H_{13}INaO_4$ 346.9756, found 346.9754.

*4.2.3. 2,4-Dimethoxymethoxyphenylboronic acid (**6**)*[27]. Triisopropyl borate (10.7 mL, 46.3 mmol) was added to the stirred solution of aryl iodide **9** (5.00 g, 15.4 mmol) in THF/Et_2O (1:2, 100 mL) under N_2. The solution was cooled to −100 °C using liquid N_2 and CH_3OH bath, and then n-BuLi (14.5 mL of a 1.6 M solution in hexanes, 23.2 mmol) was added slowly with stirring. After 1 h of stirring at the temperature below −78 °C, saturated NH_4Cl solution was added. The solution was stirred for an additional 1 h at rt and the two phases partitioned. The aqueous phase was extracted with Et_2O. The combined organic phases were washed with H_2O and brine, and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to give an orange oil. Purification of the oil by column chromatography (8:2 Hex/EtOAc) and evaporation of the solvent afforded the boronic acid **6** as an orange soild (2.76 g, 74%): IR (neat) ν_{max} 3385, 2957, 1726, 1603, 1142, 993 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (1H, d, J=8.4 Hz, H-6), 6.80 (1H, d, J=2.0 Hz, H-3), 6.76 (1H, dd, J=2.0 and 8.4 Hz, H-5), 6.68 (2H, br s, $B(OH)_2$), 5.27 (2H, s, OCH_2O), 5.19 (2H, s, OCH_2O), 3.50 (3H, s, OCH_3), 3.47 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.5 (C, C-2), 160.9 (C, C-4), 137.8 (CH, C-6), 109.3 (CH, C-5), 102.1 (CH, C-3), 94.6 (OCH_2O), 94.0 (OCH_2O), 56.4 (OCH_3), 56.0 (OCH_3), (C-1 signal not observed); ^{11}B NMR ($CDCl_3$, 128 MHz) δ_B 28.55.

*4.2.4. 2′,4′,5,7-Tetramethoxymethoxyisoflavone (**10**).* To a solution of 3-iodochromone **5** (1.20 g, 3.06 mmol) in 1:1 DME/H_2O (50 mL) were added 10% Pd/C (0.160 g, 5 mol %), Na_2CO_3 (0.970 g, 9.18 mmol) and phenylboronic acid **6** (1.11 g, 4.59 mmol). The resulting mixture was stirred at 40–45 °C overnight. The catalyst was filtered and washed with water and EtOAc. The aqueous phase was extracted with EtOAc, and the combined organic layers were washed with water, brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude product was purified by silica gel column chromatography using Hex/EtOAc (7:3) to afford an isoflavone **10** (1.1 g, 78%) as a yellow oil: IR (neat) ν_{max} 2905, 2828, 1647, 1609, 1570, 1256, 1150, 999, 918 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (1H, s, H-2), 7.22 (1H, d, J=8.5 Hz, H-6′), 6.88 (1H, d, J=2.3 Hz, H-3′), 6.76–6.72 (3H, m, H-6, H-8, H-5′), 5.27 (2H, s, OCH_2O), 5.23 (2H, s, OCH_2O), 5.17 (2H, s, OCH_2O), 5.10 (2H, s, OCH_2O), 3.52 (3H, s, OCH_3), 3.50 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 3.41 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.9 (C, C-4), 160.9 (C, C-7), 159.4 (C, C-8a), 158.5 (C, C-5), 158.4 (C, C-4′), 156.2 (C, C-2′), 151.8 (CH, C-2), 132.4 (CH, C-6′), 123.1 (C, C-3), 115.5 (C, C-1′), 111.5 (C, C-4a), 108.9 (CH, C-5′), 104.1 (CH, C-3′), 102.4 (CH, C-6), 97.3 (CH, C-8), 95.8 (CH_2, OCH_2O), 95.1 (CH_2, OCH_2O), 94.5 (CH_2, OCH_2O), 94.3 (CH_2, OCH_2O), 56.5 (CH_3, OCH_3), 56.4 (CH_3, OCH_3), 56.1 (CH_3, OCH_3), 56.0 (CH_3, OCH_3); HRMS-ESI m/z [M+Na]$^+$ calcd for $C_{23}H_{26}NaO_{10}$ 485.1424, found 485.1415.

*4.2.5. 2′,4′,5,7-Tetrahydroxyisoflavone (**4**)*[25,26]. HCl (3 M, 15 mL) was added to a solution of **10** (1.00 g, 2.16 mmol) in CH_3OH (30 mL) and stirred at 50 °C for 12 h. CH_3OH was evaporated and the reaction mixture was extracted with EtOAc. The combined organic phases were washed with H_2O and brine. The organic layer was dried over anhydrous $MgSO_4$ and the solvent evaporated under reduced pressure to give a yellow solid. Purification of the solid by column chromatography using Hex/EtOAc (1:1) afforded **4** as a pale yellow solid (0.45 g, 73%): recrystallization of **4** from Hex/EtOAc (1:4) gave pale yellow needles. Mp 268.4–270.1 °C (lit.[26] mp 272.0 °C); IR (neat) ν_{max} 3319, 1652, 1613, 1502, 1254, 1171 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.00 (1H, s, H-2), 7.04 (1H, d, J=8.2 Hz, H-6′), 6.40 (1H, d, J=2.3 Hz, H-3′), 6.37 (1H, dd, J=2.3 and 8.2 Hz, H-5′), 6.36 (1H, d, J=2.3 Hz, H-8), 6.23 (1H, d, J=2.3 Hz, H-6); ^{13}C NMR (CD_3OD, 100 MHz) δ 182.7 (C, C-4), 166.2 (C, C-7), 163.7 (C, C-5), 160.2 (C, C-4′), 159.8 (C, C-8a), 157.8 (C, C-2′), 156.7 (CH, C-2), 133.2 (CH, C-6′), 122.6 (C, C-3), 110.9 (C, C-1′), 108.2 (CH, C-5′), 106.2 (C, C-4a), 104.4 (CH, C-3′), 100.3 (CH, C-6), 94.9 (CH, C-8), HRMS-ESI m/z [M−H]$^-$ calcd for $C_{15}H_9O_6$ 285.0399, found 285.0399.

*4.2.6. Lupinalbin A (**3**)*[23]. DDQ (79.0 mg, 0.349 mmol) was added under N_2 to a solution of **4** (100 mg, 0.35 mmol) in THF (20 mL). The reaction mixture was heated to 60 °C with stirring for 15 min. Additional DDQ (79.0 mg, 0.349 mmol) was added to the mixture and stirring was continued at the same temperature for 30 min. The solvent was evaporated and the crude mixture purified by column chromatography using Hex/EtOAc as eluent to give coumaronochromone **3** (66 mg, 66%) as a white solid: **3** was recrystallized from Hex/EtOAc (1:4) to afford white needle-like crystals, which

decomposed at 278.4–280.0 °C (lit.[14] mp >300 °C); IR (neat) ν_{max} 3327, 3101, 2922, 1622, 1436, 1029, 822 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.75 (1H, d, J=8.4 Hz, H-6′), 6.99 (1H, d, J=1.9 Hz, H-3′), 6.89 (1H, dd, J=1.9 and 8.4 Hz, H-5′), 6.46 (1H, d, J=2.0 Hz, H-8), 6.27 (1H, d, J=2.0 Hz, H-6); ^{13}C NMR (CD_3OD, 100 MHz) δ 180.0 (C, C-4), 166.2 (C, C-2), 165.2 (C, C-7), 164.0 (C, C-5), 157.8 (C, C-4′), 156.6 (C, C-8a), 151.9 (C, C-2′), 122.5 (CH, C-6′), 115.2 (CH, C-5′), 114.7 (C, C-1′), 104.5 (C, C-4a), 100.9 (CH, C-6), 99.5 (C, C-3), 98.7 (CH, C-3′), 95.8 (CH, C-8); HRMS-ESI m/z $[M-H]^-$ calcd for $C_{15}H_7O_6$ 283.0243, found 283.0239.

*4.2.7. Lupinalbin H (**2**)*[22]. To a solution of **3** (40.0 mg, 0.141 mmol) in CH_3OH (15 mL) was added $Ca(OH)_2$ (21.0 mg, 0.282 mmol) followed by prenal (0.03 mL, 0.353 mmol). The mixture was stirred under an N_2 atmosphere for 3 days at rt. CH_3OH was evaporated and the reaction mixture was diluted with EtOAc and 1 M HCl. The two phases were partitioned and the aqueous phase was back-extracted with EtOAc. The combined organic extracts were washed with H_2O and brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude product purified by column chromatography using Hex/EtOAc/acetone (6:1:1), followed by centrifugal chromatography using CH_2Cl_2 to give **2** as a cream white solid (20 mg, 40%) and lupinalbin A (**3**) (14 mg, 35%) was recovered. Recrystalization of **2** from CH_3OH gave cream white crystals, which decomposed at 245.5–247.2 °C (lit.[22] mp 248–250 °C); IR (neat) ν_{max} 3501, 3230, 2923, 2853, 1729, 1640, 1594, 1456, 1393, 1116, 811 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 13.38 (1H, s, OH-5), 8.98 (1H, br s, OH-4′), 7.82 (1H, d, J=8.3 Hz, H-6′), 7.14 (1H, d, J=2.0 Hz, H-3′), 7.02 (1H, dd, J=2.0 and 8.3 Hz, H-5′), 6.70 (1H, d, J=10.0 Hz, H-4″), 6.54 (1H, s, H-8), 5.79 (1H, d, J=10.0 Hz, H-5″), 1.48 (6H, s, H-7″ and H-8″); ^{13}C NMR (acetone-d_6, 125 MHz) δ 178.6 (C, C-4), 164.7 (C, C-2), 158.5 (C, C-7), 157.0 (C, C-5), 156.4 (C, C-4′), 154.0 (C, C-8a), 150.4 (C, C-2′), 128.6 (CH, C-5″), 121.3 (CH, C-6′), 114.6 (CH, C-4″), 114.0 (C, C-1′), 113.6 (CH, C-5′), 105.9 (C, C-6), 104.0 (C, C-4a), 98.5 (CH, C-3′), 97.5 (C, C-3), 95.3 (CH, C-8), 80.0 (C, C-6″), 27.3 (2× CH_3, C-7″ and C-8″); HRMS-ESI m/z $[M-H]^-$ calcd for $C_{20}H_{13}O_6$ 349.0712, found 349.0710.

Acknowledgements

We are grateful to the National Research Foundation (South Africa) and the University of Kwazulu-Natal for financial assistance.

Supplementary data

Copies of ^1H and ^{13}C NMR spectra of **8**, **9**, **6**, **10**, **4**, **3** and lupinalbin H (**2**) as well as copies of HSQC and HMBC NMR spectra of **2**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.09.042.

References and notes

1. Dewick, P. M. In *The Flavonoids: Advances in Research since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994; pp 117–238.
2. Veitch, N. C. *Nat. Prod. Rep.* **2007**, *24*, 417–464.
3. Veitch, N. C. *Nat. Prod. Rep.* **2009**, *26*, 776–802.
4. Lawson, M. A.; Kaouadji, M.; Chulia, A. J. *Tetrahedron Lett.* **2008**, *49*, 2407–2409.
5. Shou, Q. Y.; Tan, Q.; Shen, Z. W. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3389–3391.
6. Reynaud, J.; Guilet, D.; Terreux, R.; Lussignol, M.; Walchshofer, N. *Nat. Prod. Rep.* **2005**, *22*, 504–515.
7. Shu, P.; Qin, M. J.; Shen, W. J.; Wu, G. *Biochem. Syst. Ecol.* **2009**, *37*, 20–23.
8. Mackova, Z.; Koblovska, R.; Lapcik, O. *Phytochemistry* **2006**, *67*, 849–855.
9. Xiang, W.; Li, R. T.; Mao, Y. L.; Zhang, H. J.; Li, S. H.; Song, Q. S.; Sun, H. D. *J. Agric. Food Chem.* **2005**, *53*, 267–271.
10. Botta, B.; Menendez, P.; Zappia, G.; de Lima, R. A.; Torge, R.; Delle Monache, G. *Curr. Med. Chem.* **2009**, *16*, 3414–3468.
11. Lo, W. L.; Chang, F. R.; Liaw, C. C.; Wu, Y. C. *Planta Med.* **2002**, *68*, 146–151.
12. Lo, W. L.; Wu, C. C.; Chang, F. R.; Wang, W. Y.; Khalil, A. T.; Lee, K. H.; Wu, Y. C. *Nat. Prod. Res.* **2003**, *17*, 91–97.
13. Shiao, Y. J.; Wang, C. N.; Wang, W. Y.; Lin, Y. L. *Planta Med.* **2005**, *71*, 835–840.
14. Tsukayama, M.; Oda, A.; Kawamura, Y.; Nishiuchi, M.; Yamashita, K. *Tetrahedron Lett.* **2001**, *42*, 6163–6166.
15. Zheng, S. Y.; Shen, Z. W. *Tetrahedron Lett.* **2010**, *51*, 2883–2887.
16. Miller, C. P.; Collini, M. D.; Harris, H. A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2399–2403.
17. Harfenist, M.; Thom, E. *J. Org. Chem.* **1972**, *37*, 841–848.
18. North, J. T.; Kronenthal, D. R.; Pullockaran, A. J.; Real, S. D.; Chen, H. Y. *J. Org. Chem.* **1995**, *60*, 3397–3400.
19. Eicher, T.; Hauptmann, S. *The Chemistry of Heterocycles*; Georg Thieme: Stuttgart, 1995.
20. da Silva, F. D. C.; Jorqueira, A.; Gouvea, R. M.; de Souza, M.; Howie, R. A.; Wardell, J. L.; Wardell, S.; Ferreira, V. F. *Synlett* **2007**, 3123–3126.
21. Selepe, M. A.; Drewes, S. E.; van Heerden, F. R. *J. Nat. Prod.* **2010**, *73*, 1680–1685.
22. Tahara, S.; Katagiri, Y.; Ingham, J. L.; Mizutani, J. *Phytochemistry* **1994**, *36*, 1261–1271.
23. Tahara, S.; Ingham, J. L.; Mizutani, J. *Agric. Biol. Chem.* **1985**, *49*, 1775–1783.
24. Hanawa, F.; Tahara, S.; Mizutani, J. *Phytochemistry* **1991**, *30*, 157–163.
25. Prasad, J. S.; Varma, R. S. *Phytochemistry* **1977**, *16*, 1120–1120.
26. Whalley, W. B. *J. Chem. Soc.* **1957**, *2*, 1833–1837.
27. Ikegashira, K.; Oka, T.; Hirashima, S.; Noji, S.; Yamanaka, H.; Hara, Y.; Adachi, T.; Tsuruha, J.-I.; Doi, S.; Hase, Y.; Noguchi, T.; Ando, I.; Ogura, N.; Ikeda, S.; Hashimoto, H. *J. Med. Chem.* **2006**, *49*, 6950–6953.
28. Vasselin, D. A.; Westwell, A. D.; Matthews, C. S.; Bradshaw, T. D.; Stevens, M. F. G. *J. Med. Chem.* **2006**, *49*, 3973–3981.
29. Berliner, M.; Belecki, K. *Org. Synth.* **2007**, *84*, 102–110.
30. Yagoub, A. K.; Iskander, G. M. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1043–1045.
31. Zheng, X.; Meng, W.-D.; Qing, F.-L. *Tetrahedron Lett.* **2004**, *45*, 8083–8085.
32. Hoye, T. R.; Chen, M. *Tetrahedron Lett.* **1996**, *37*, 3099–3100.
33. Al-Zoubi, R. M.; Hall, D. G. *Org. Lett.* **2010**, *12*, 2480–2483.
34. Brown, H. C.; Cole, T. E. *Organometallics* **1983**, *2*, 1316–1319.
35. Li, W.; Nelson, D. P.; Jensen, M. S.; Hoerrner, R. S.; Cai, D.; Larsen, R. D.; Reider, P. J. *J. Org. Chem.* **2002**, *67*, 5394–5397.
36. Felpin, F.-X.; Lory, C.; Sow, H.; Acherar, S. *Tetrahedron* **2007**, *63*, 3010–3016.
37. Mondal, M.; Puranik, V. G.; Argade, N. P. *J. Org. Chem.* **2006**, *71*, 4992–4995.