# ISOLATION AND CHARACTERISATION OF SECONDARY METABOLITES OF TWO ASTERACEAE SPECIES, *ARTEMISIA AFRA* AND *ELYTROPAPPUS RHINOCEROTIS*

Submitted in fulfilment of the requirements

for the degree of

**MASTER OF SCIENCE**

By

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May 2009

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**Pietermaritzburg**

# DECLARATION

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

Signed……

Emmanuel Gakuba

I hereby certify that this statement is correct

Signed…….....

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Pietermaritzburg

May 2009

## DEDICATION

This research is dedicated to my mother Mrs. Bernadette Mukaruyonza and my father Mr. Nicodème Rushishi, for unconditional and everlasting love they offered me.

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# ABSTRACT

In this study two medicinal plant species, namely *Artemisia afra* Jacq. ex. Willd and *Elytropappus rhinocerotis* Less. (L.f) (= *Dicerotamnus rhinocerotis* Koekemoer), both belonging to the family Asteraceae, have been investigated and different compounds isolated and characterised. Both species are important plants used in traditional medicine in general in Africa and particularly in South Africa.

*A. afra*, commonly called "African wormwood" is one of 400 species belonging to the genus *Artemisia* and it is the only one indigenous to Africa. *E. rhinocerotis* is one of eight *Elytropappus* species which are all restricted to the Cape floristic region.

The aim of this study was to investigate the phytochemistry of these species. In total fifteen compounds were isolated and characterised. From the *E. rhinocerotis* extract, four known compounds, labdanolic acid, methyl labdanolate, 6, 7- dimethoxycoumarin and a sesquiterpene viridiflorol were isolated. These compounds were not previously reported from *E. rhinocerotis*. Two different chemotypes of *A. afra* were studied and eleven compounds were isolated. These compounds include sesquiterpenes such as taurin, artesin, maritimin, artemin, norsantolinifolide, santolinifolide A, and reynosin, a flavonoid, 5-hydroxy-7,4′-dimethoxyflavone, a coumarin called scopoletin or 7-hydroxy-6-methoxycoumarin and other aromatic compounds such as *p*-hydroxyacetophenone, and 2,4-dihydroxy-6-methoxyacetophenone**.** Except for taurin, scopoletin and 5-hydroxy-7, 4′-dimethoxyflavone , none of these other compounds has been reported previously from *A. afra*. This study has shown that *A. afra* contains a large number of sesquiterpenoids, mostly from the eudesmane-type.

Structural elucidation of different compounds was performed using mainly NMR spectroscopy. Other methods used for identification include LC-MS and infrared spectroscopy. The major compound, labdanolic acid, is known to selectively inhibit cyclooxygenase-2, an enzyme associated with inflammation. The presence of labdanolic acid in the plant may account for its traditional use as an anti-inflammatory.

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# LIST OF SCHEMES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| br. s | broadened singlet |
| C | concentration in g/100 mL |
| COSY | correlated spectroscopy |
| COX | cyclooxygenase |
| CINC | cytokine-induced neutrophil chemoattractant-1 |
| d | doublet |
| dd | doublet of doublets |
| DEPT | distortionless enhancement polarization transfer |
| dq | doublet of quartets |
| dt | doublet of triplet |
| EI-MS | electron impact-mass spectrometry |
| ESI-MS | electrospray ionisation-mass spectrometry |
| EtOAc | ethyl acetate |
| GC | gas chromatography |
| HMBC | heteronuclear multiple bond correlation |
| HPLC | high-performance liquid chromatography |
| HR-MS | high-resolution mass spectrometrometry |
| HSQC | heteronuclear multiple quantum coherence |
| IR | infrared |
| *J* | spin-spin coupling constant in Hz |
| LPS | lipopolysaccharides |
| lit. | literature |
| m | multiplet |
| *m/z* | mass-to-charge ratio |
| $M^+$ | molecular ion |
| MeOH | methanol |
| Mp | melting point |
| MS | mass spectrometry |
| NMR | nuclear magnetic resonance |
| ppm | parts per million |
| q | quartet |

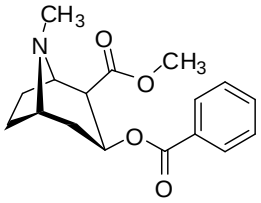
| | |
|---|---|
| $R_f$ | retarding factor |
| s | singlet |
| sp. | species |
| t | triplet |
| TLC | thin-layer chromatography |
| TMS | tetramethylsilane |
| TNF-alpha | tumor necrosis factor-alpha |
| UV | ultraviolet |
| [α] | specific rotation |

# CHAPTER ONE

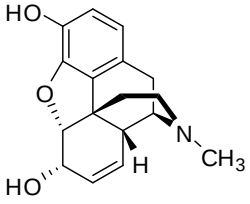
# INTRODUCTION

## 1.1 BIOACTIVE NATURAL PRODUCTS FROM PLANTS

The isolation of bioactive pure compounds from medicinal plants began in the 19th century. Before that time, medicine was based on herbs and potions.[1] The isolation of natural bioactive compounds was the first step towards the birth of the pharmaceutical industry. Natural products isolated in the early 19th century include cocaine **(1.1)** from leaves of *Erythroxylum coca* Lam. (Erythroxylaceae), morphine **(1.2)** from *Papaver somniferum* Linnaeus (Papaveraceae), called the opium poppy, and quinine **(1.3)** from the bark of *Cinchona pubescens* Vahl (Rubiaceae).




**1.1**

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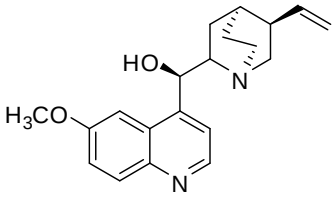
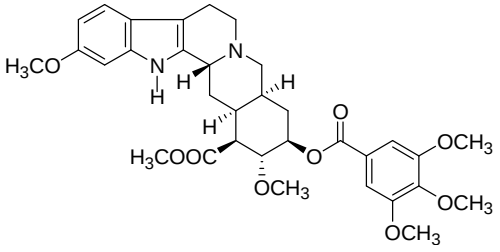



**1.3**

Since then, plants have become a biofactory of different natural substances used for different purposes, especially in natural medicine. Chinese people are among the pioneers who explored the flora for medicinal purposes. Many other societies in the world also valued plant natural products for their life-giving properties. A survey conducted by Lev and Amar[2] showed that 85% of traditional drugs sold in Israel are derived from plants. In their article, Scott *et al*. mentioned that 70% of all South Africans use plant-derived traditional medicine.[3] The World Health Organization estimated that approximately 80% of Africa's inhabitants use traditional medicine for primary health care.[4] In 1999, Grabley and Thiericke reported that about 30% of drug sales in the world were based on natural products.[5] Nowadays, pharmaceutical organisations are able to produce plant-based proteins that play an important role in human body metabolism.[6]

Many drugs available today were discovered through the isolation and analysis of the active principles from natural sources. For example, reserpine **(1.4)**, used in the control of hypertension, was isolated from the roots of a shrub called *Rauvolfia littolaris* ex. Pitard [7] and *Rauvolfia serpentina* (L.) Benth. ex. Kurz (Apocynaceae);[8,9] salicin **(1.5)**, a suitable agent for fever control, was isolated from the bark of *Salix alba* Linnaeus (Salicaceae); codeine **(1.6)**, an important analgesic and cough suppressant, was obtained from *P. somniferum* [10, 11] digitoxin **(1.7)**, used to regulate cardiac function, was isolated from *Digitalis purpurea* Linnaeus (Scrophulariaceae), commonly called foxglove; taxol **(1.8)**, an anticancer agent, was extracted from the bark of *Taxus brevifolia* Nutt. (Taxaceae), known as the pacific yew tree.[12] Emetine **(1.9)**, which induces apoptosis in human tumour cell lines, was obtained from *Psychotria ipecacuanha* (Brot.) Standl (Rubiaceae).[13,14]

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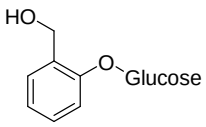


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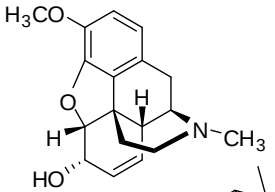
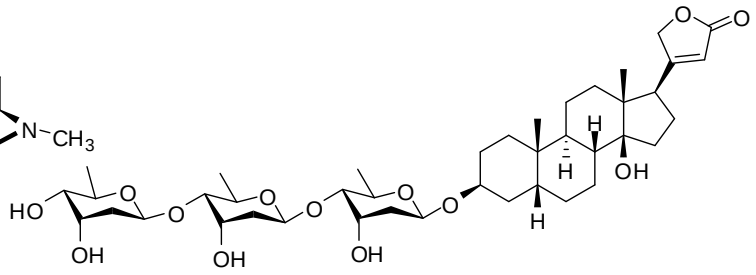
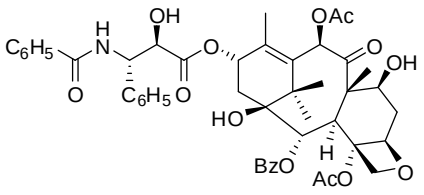
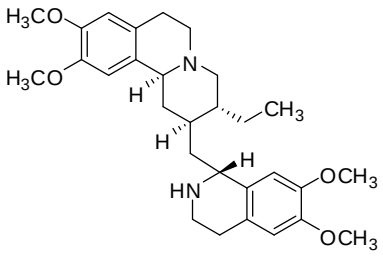


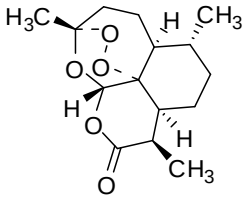

**1.6** **1.7**




**1.8** **1.9**

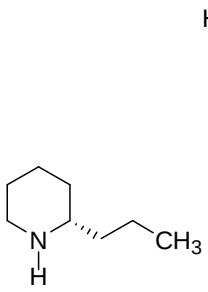
Although many pharmaceuticals are synthesized in the laboratory currently, the isolation of natural products remains crucial because many of them have radically new and sophisticated structures, which no chemist would have dreamt of designing. Such an example is one of the recently isolated compounds, artemisinin **(1.10)**, a powerful antimalarial drug isolated from *Artemisia annua* Linneaus. (Asteraceae), bearing an unusual trioxane ring.[1,15]



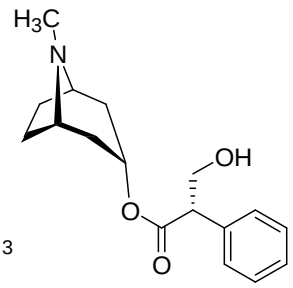
**1.10**

## 1.2 THE PURPOSE OF ISOLATION AND CHARACTERISATION OF ACTIVE NATURAL PRODUCTS

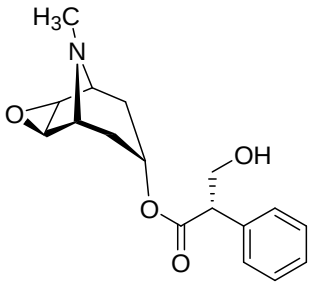
Both traditional and modern societies have used natural products for survival.[16] The first drugs used by humans were derived from plants.[17] However, plants have also been used to poison humans; for example, the alkaloid coniine **(1.11)** produced by a poisonous plant known as *Conium maculatum* Linneaus (Apiaceae) was ingested by Socrates, in form of hemlock in 399 BC, and was the cause of his death. Other natural compounds that can cause death include atropine **(1.12)** from *Atropa belladonna* Linneaus (Solanaceae), scopolamine **(1.13)** from *Datura stramonium* Linneaus (Solanaceae).[17] In developing countries, such as those in Africa, with low living standards, pharmaceutical industries are not well developed. Yet, it is common to see people chewing leaves, flowers and bark and swallowing crude plant extracts to cure diseases.



**1.11**



**1.12**



**1.13**

The lack of information about active ingredients contained in different parts of medicinal plants, is a big problem for users of natural products in their natural state. Also, in most cases, the necessary dose to be taken by adults and given to children is not known. Apart from the lead compound, herbal medicines contain numerous other compounds which may be biologically active and can cause side effects and toxicity.[1] This problem may have serious and/or fatal consequences. Therefore, the study of natural products from medicinal plants is of great importance in both developing and developed countries. Many people accept using drugs from traditional healers rather than modern drugs because they are cheaper and available at all times in their immediate environment.

It is on the basis of these numerous reasons that the project ***"Isolation and characterisation of secondary metabolites of two Asteraceae species, Artemisia afra and Elytropappus rhinocerotis"*** has been undertaken. The preparations from these two medicinal plants have been used for many years by Africans for the treatment of many diseases.

## 1.3 AIMS OF THE PROJECT

*Artemisia afra* Jacq. ex. Willd (African wormwood) and *Elytropappus rhinocerotis* (L.f) Less. (*Dicerothamnus rhinocerotis* Koekemoer[*,18,19]) (rhinoceros bush) are both medicinal plants in South Africa. *A. afra* is the only *Artemisia* species indigenous to sub-Saharan Africa. Many sesquiterpenes have been reported from different *Artemisia* species. However, limited information is available on the sesquiterpenes of *A. afra*. Furthermore, based on the essential oil composition, different chemotypes of *A. afra* have been identified. The variation of sesquiterpenes in the different chemotypes has not been investigated. Preparations from *E. rhinocerotis* were used to treat illnesses such as dyspepsia, stomach cancer, lack of appetite diarrhoea in children, influenza and fevers[20,21] but little information is available on the phytochemistry of this species.

[*] A name change to *Dicerothamnus rhinocerotis* Koekemoer has been proposed in a thesis,[18] but upon writing this thesis, the reclassification of the species has not been published in a botanical journal.

The aims of this project were the:

- Isolation of sesquiterpenes and other compounds from two chemotypes of *A. afra*, named the ketone chemotype and the thujone chemotype
- Structure elucidation of the different compounds isolated from *A. afra*
- Isolation of different secondary metabolites from *E. rhinocerotis*
- Structure elucidation of isolated compounds from *E. rhinocerotis*

In the second Chapter, the phytochemical investigation of *A. afra* will be discussed, while Chapter 3 will deal with the phytochemistry of *E. rhinocerotis*. Finally, in Chapter 4, concluding remarks will be made.

## 1.4 REFERENCES


1. Patrick, G.L. 2005. An Introduction to Medicinal Chemistry. Third Ed. Oxford University Press Inc., New York, pp. 161-184.
2. Lev, E., Amar, Z. 2000. Ethnopharmacological survey of traditional drugs sold in Israel at the end of the 20th century. *Journal of Ethnopharmacology*, **72**, 191-205.
3. Scott, G., Springfield, E.P., Coldrey, N. 2004. Pharmaceutical study of 26 South African plants. *Journal of Pharmaceutical Biology*, **42**, 186-213.
4. World Health Organization website: http:/www.who.int/mediacentre/factsheets/fs134/en Accessed on 22nd December 2008.
5. Grabley, S., Thiericke, R. 1999. Bioactive agents from natural sources: trends in discovery and application. In: Advances in Biochemical Engineering/ Biotechnology. Springer-Verlag, Berlin.
6. http://www.cpmp2003.org/pages/en/home.html Accessed on 22nd December 2008.
7. Nguyen, K.C. 1990. Alkaloids of *Rauvolfia littoralis*. *Khimiya Priodnykh Soedinenii*, **2**, 282-283 (Abstract obtained from Scifinder Scholar).
8. Sehrawat, A.R., Sanjogta, U., Chowdhury, J.B. 2002. Establishment of plantlet and evaluation of differentiated roots for alkaloids in *Rauvolfia serpentina*. *Journal of Plant Biochemistry and Biotechnology*, **11**, 105-108.
9. Nguyen K.C., Nikolaeva, L.A. 1991. Alkaloids of *Rauwolfia* species growing in Vietnam. *Khimiya Priodnykh Soedinenii*, **6**, 813-816 (Abstract obtained from Scifinder Scholar).

10. Costin, J.W., Lewis, S.W., Purcell, S.D., Waddell, L.R., Francis, P.S., Barnett, N.W. 2007. Rapid determination of *Papaver somniferum* alkaloids in process streams using monolithic column, high-performance liquid chromatography with chemiluminescence detection. *Analitica Chimica Acta*, **597**, 19-23.
11. Schulz, H., Baranska, M., Quilitzsch, R., Schutz, W. 2004. Determination of alkaloids in capsules, milk and ethanolic extract of poppy (*Papaver somniferum L.*) by ATR-FTR-FR and FT-Raman spectroscopy. *Analyst*, **129**, 917-920.
12. Bruice, P.Y. 2004. Organic Chemistry, Fourth Ed., Pearson Education, Inc. pp. 1208-1209.
13. Maren, M., Michael, W. 2007. Characteristics of apoptosis induction by the alkaloid emetine in human tumour cell lines. *Planta Medica*, **73**, 1389-1396.
14. Maren, M., Kerstin, H., Till, W., Michael, W. 2007. The alkaloid emetine as a promising agent for the induction and enhancement of drug-induced apoptosis in leukemia cells. *Oncology Reports*, **18**, 737-744.
15. Covello, P.S. 2008. Molecules of interest: Making artemisinin. *Phytochemistry*, **69**, 2881- 2885.
16. Hendrickson, J.B. 1965. The Molecules of Nature. A Survey of the Biosynthesis and Chemistry of Natural Products. W.A. Benjamin, Inc. New York- Amsterdam. p.1.
17. Liska, K. 1981. Drugs and the Human Body: with Implication for Society. Macmillan Publishing Co., Inc. New York. pp. 23-24.
18. Koekemoer, M. 2002. Systematics of the *Metalasia* group in the Relhaniinae (Asteraceae-Gnaphaliae). Ph. D. Thesis, Rand Afrikaans University, Johannesburg.
19. Germishuizen, G., Meyer, N.L. (Ed.). 2003. Plants of Southern Africa: an Annotated Checklist. National Botanical Institute, Pretoria, p. 186.
20. Dekker, T.G., Fourie, T.G., Matthee, E., Snyckers, F.O., van der Schyf, C.J. 1988. Studies of South African medicinal plants. Part 7. Rhinocerotinoic acid: a labdane diterpene with anti-inflammatory properties from *Elytropappus rhinocerotis*. *South African Journal of Chemistry*, **41**, 33-35.
21. http://www.plantzafrica.com/medmonographs/elytraprhino.pdf Date of access: 22 December 2008.

# CHAPTER TWO

# A PHYTOCHEMICAL INVESTIGATION OF *ARTEMISIA AFRA*

## 2.1 INTRODUCTION AND LITERATURE REVIEW

### 2.1.1 The family Asteraceae

In the 19th century, the family Asteraceae or Compositae was investigated by the Frenchman Henri Cassini, who is considered to be the founder of this family due to his effort in classifying its members. Asteraceae forms the largest family of the plant kingdom; it comprises 1535 genera and 2300 known species, without considering the unknown and microspecies.[1] They are grouped in three subfamilies, namely Asteroideae, Cichorioideae, and Barnadesiodeae; and 17 tribes: Barnadesieae, Mutisieae, Cardueae, Lactuceae, Vernonieae, Liabeae, Arctoteae, Inuleae, Plucheeae, Gnaphalieae, Calenduleae, Astereae, Anthemideae, Senecioneae, Helenieae, Heliantheae and Eupatorieae.[1,2]

Many of Asteraceae species have served as sources of rubber, pesticides, medicines, edible oil, food and vegetables; while others are used as ornamental plants.[3] Numerous species of this family are known as the producers of sesquiterpene lactones.[4] The characteristics common to all species are the following: [1,5]

- All species comprise either perennial or annual herbs or shrubs
- Regeneration and propagation of most of the species are by seed
- Flowers are clustered on a common base surrounded by bracts
- Bracts are presented in different colours, mainly yellow, pink and orange but may also appear as white
- Pollination is effected mainly by insects and wind.

The genus *Artemisia* is one of the genera grouped under the tribe known as Anthemideae. This tribe is mainly found in the Mediteranean region and central Asia. Most of the genera are distributed in the northern hemisphere; in the southern hemisphere, it has representatives in South Africa, Australia and South America[1]. Members of this tribe are characterised by their aromatic scent which is due to the high concentration of

monoterpenes and sesquiterpenes. The tribe has been reported to have anti-infectious activities; and its largest genus is *Artemisia* with 400 species.[6,7]

### 2.1.2 The genus *Artemisia*

#### 2.1.2.1 Description

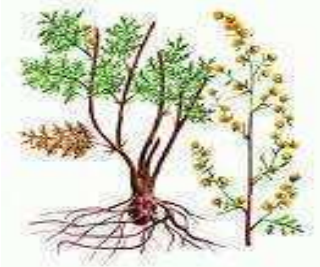
*Artemisia* members are annual or perennial herbs or shrubs. Most of them bear lobed or dissected leaves with the inflorescence in the form of a panicle.[8] The genus is mostly found in the northern hemisphere; in Eurasia, and Western North America; but some others occur in South America, south of the Sahara in Africa and also on the Island of Hawaii.[8]

The genus has been of great importance in botany, pharmacy and in the food industry.[7] Many species of *Artemisia* have been investigated by researchers and it has been reported that they are chemically characterized by numerous sesquiterpenes lactones, acetylenic compounds, phenylpropanes, coumarin derivatives and flavonoids.[9,10]

#### 2.1.2.2 Phytochemistry of some *Artemisia* species

Most of the compounds isolated from *Artemisia afra* Jacq. ex. Willd in this study have been isolated either from other *Artemisia* species or have the same general structure as those occurring in other members of the genus. A few examples of other related *Artemisia* species are given hereunder.

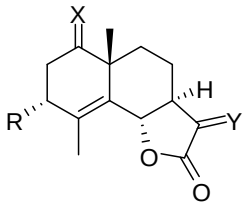
- *Artemisia santolinifolia* Trucz ex. Besser

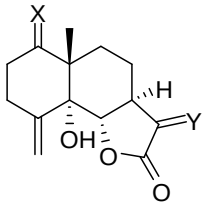


http://images.google.com/images?hl=en&q=artemisia+santolinifolia+photo&um=1&ie=UTF-8&sa=X&oi=image_result_group&resnum=1&ct=title

**Figure 2.1.** *A. santolinifolia.*[11]

This species has been found in Mongolia and has been investigated chemically by Jakupovic *et al.*[12], who isolated three types of compounds. These are the eudesmanolides **(2.1** to **2.4)**, coumarins (**2.5** to **2.8)** and glucopyranosides (**2.9** to **2.15)**. The author also isolated other types of sesquiterpene lactones with an unusual carbon skeleton and he named them "*nor*-santolinidilactones" **(2.16** to **2.17).**



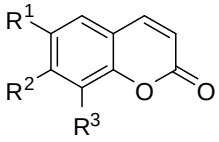




**2.1** R=H, X=O, Y=H, β-Me

**2.2** R=OH, X= H, β-OH, Y= H, α-Me

**2.3** X= H, β-OH, Y=H, β-Me

**2.4** X=O, Y= H, α-Me

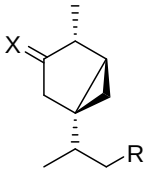



**2.5** $R^1$= H, $R^2$= OGlc, $R^3$= H

**2.6** $R^1$= OMe, $R^2$= OH, $R^3$= OMe

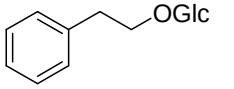
**2.7** $R^1$= OGlc, $R^2$= OMe, $R^3$= H

**2.8** $R^1$= H, $R^2$=OH, $R^3$=H

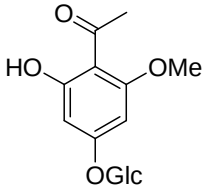



**2.9** R= OGlc, X=O

**2.10** R=H, X=H, β-OGlc




**2.11**

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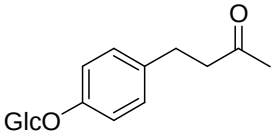


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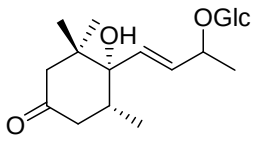


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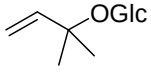


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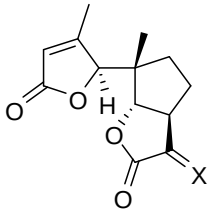



**2.16** X=H, α-Me

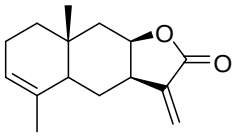
**2.17** X=H, β-Me

- *Artemisia iwayomogi* Kitamura

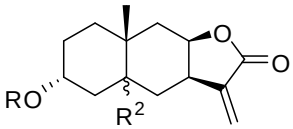
http://www.google.co.za/search?hl=enq=artemisia+iwayomogi=picture&btnG=google=search&meta

**Figure 2.2.** *A. iwayomogi*.[13]

*A. iwayomogi* occurs in Japan and it has been reported by Greger *et al*.[13] that it contains interesting eudesmanolides. Some of them are mentioned hereunder, for instance, eudesma-4,11-dien-12,8β-olide **(2.18)**, 3α-peroxyisoalantolactone **(2.19)**, 3α,5α-dihydroxyisoalantolactone **(2.20)**, 3α-hydroperoxy-5α-hydroxyisoalantolactone **(2.21)**, 3α-hydroperoxy-5β-hydroxyisoalantolactone (**2.22**), 3α-peroxyeudesma-4,11-dien-12,8β-olide **(2.23)**, 3α-hydroxyeudesma-4,11-dien-12,8β-olide **(2.24)**, 3-oxoeudesma-4,11-dien-12,8β-olide (**2.25**), 4α-peroxyeudesma-2,11-dien-12,8β-olide (**2.26**) 3α-hydroxy-4α,5α-epoxyeudesm-11-en-12,8β-olide (**2.27**), and 3β-hydroxy-4α,5α-epoxyeudesm-11-en-12,8β-olide (**2.28**)



**2.18**

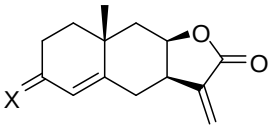


**2.19** R=OH, $R^2$= α-H

**2.20** R=H, $R^2$=α-OH

**2.21** R=OH, $R^2$=α-OH

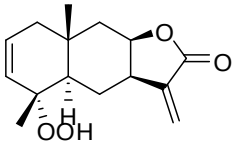
**2.22** R=OH, $R^2$=β-OH



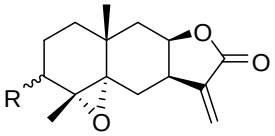
**2.23** X=α-OOH, H

**2.24** X= α-OH, H

**2.25** X= O



**2.26**



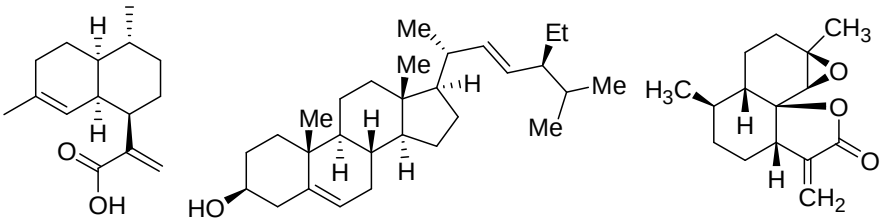
**2.27** R= α-OH

**2.28** R= β-OH

- *Artemisia annua* Linnaeus

**Figure 2.3.** *A. annua*.[14]

*A. annua* is a sweet-scented annual herb native of Asia and of Eastern Europe.[8] It is well known, due to the presence, in its tissues, of artemisinin, an important drug against malaria.[15] It is 97% effective against *Plasmodium falciparum*, a protozoan parasite that causes malaria and it is now distributed worldwide, especially in Africa, where one child dies from malaria every 30 seconds.[16] The structure of artemisinin **(1.10)** has been given in Chapter 1. Kohler *et al.*[17] isolated both artemisinin and artemisinic acid **(2.29)** by supercritical fluid extraction. In their investigation, Yougen *et al.*[18] isolated stigmasterol **(2.30)** and arteannuin **B (2.31)** from the leaf by solvent extraction.



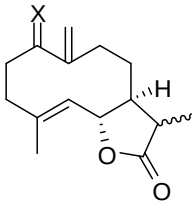

**2.29** **2.30** **2.31**

- *Artemisia herba-alba* Asso

http://upload.wikimedia.org/wikipedia/commons/9/98/Artemisia-alba.jpg

**Figure 2.4.** *A. herba-alba*.[19]

*A. herba-alba*, also called white wormwood, is one of the species belonging to the genus *Artemisia* growing in Spain and the semi-arid regions of Tunisia. As it has been underlined by Haouari and Ferchichi,[20] it is a herbaceous, aromatic, and therapeutic plant. The research carried out by Marco yielded some germacranolides **(2.32** to **2.40)** and eudesmanolide **(2.41).**[21]

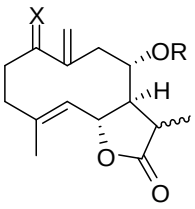



**2.32** X=O (11β-H)

**2.33** X=O (11α-H)

**2.34** X=H, β-OH (11β-H)

**2.35** X= H, β-OH (11α-H)



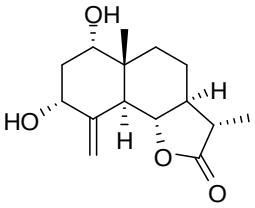

**2.36** X=O, R=H (11β-H)

**2.37** X=H, β-OH, R=H (11β-H)

**2.38** X=H, β-OH, R=H (11α-H)

**2.39** X=H, β-OOH, R=H (11β-H)

**2.40** X=O, R=Ac (11β-H)



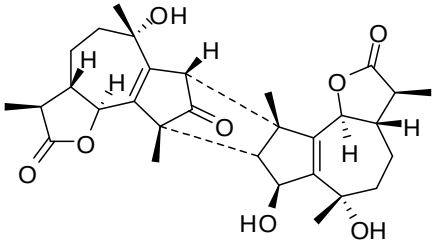

**2.41**

- *Artemisia absinthium* Linnaeus

http://en.wikipedia.org/wiki/Artemisia_(plant)

**Figure 2.5.** *A. absinthium*.[22]

It is an aromatic plant; native to warm Mediterranean countries; its extracts and essential oil are used in the treatment of different diseases and as an insect repellent.[23] The chemical investigation carried out by Beauhaire *et al*.[24] showed that the *A. absinthium* contains interesting compounds, such as the guaianolide dimer absintholide **(2.42)** and its derivatives.




**2.42**

*A. absinthium* was the main herb used in the manufacturing of an alcoholic drink called absinthe. This drink was created in French-speaking Switzerland in the late 18$^{th}$ century.[25] The mixture of the leaves and flowers of *A. absinthium*, anise, fennel and alcohol (85% vol) was macerated and water was added. A distillation was done to yield a colourless distillate. To the distillate, other herbs such as *A. pontica* were added to give it a characteristic green colour and a mild bitter taste. Finally, the distillate was diluted with water to achieve a drinkable concentration (absinthe 74% vol).[25] Absinthe was the most popular drink in Europe until the late 19$^{th}$ century.[26]

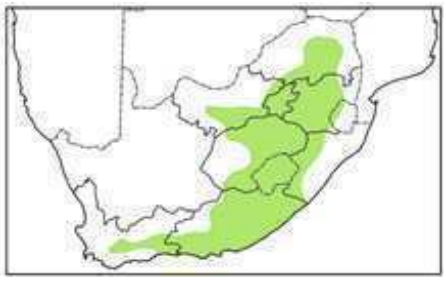
The analysis by GC/MS-SIM, GC/MS and HPTLC of the spirit absinthe showed that it contained the monoterpene thujone, one of the main constituents of *A. absinthium*, in a high concentration, especially its β-isomer.[26,27] In the 20th century it was experimentally discovered that thujone was the cause of a mental disorder known as absinthism and absinthe was classified as a narcotic poison.[28] The liquor was then prohibited in many parts of Europe and America and considered as an illegal drink.[29,30]

### 2.1.3 Review of *Artemisia afra* Jacq. ex. Willd

#### 2.1.3.1 Historical background

Being a common medicinal plant in southern Africa,[31] and easy to grow, *A. afra* is a herb that is an attractive garden plant due to its silver and grey foliage. The genus name "*Artemisia*" comes in honour of Artemis, the Greek goddess of hunting, according to Jackson[32]. This name is also linked to Artemisia, the wife of the Greek King Mausolus, who took power after his death in 353 BC. The species name "*Afra*" means "from Africa".[32]

*A. afra* has been given different common names by different societies, such as wild wormwood, African wormwood (English), wilde-als (Afrikaans), umhlonyane (Xhosa), muhlonyane (Zulu), lengana (Tswana) and zengana (Southern Sotho).[33] It is a common species in Africa and its distribution extends from the Cederberg Mountains in the Cape, to tropical East Africa and continues north to Ethiopia. Its favourite altitude is 2000 to 2400 m.[33] Among the 400 species of the genus *Artemisia*, only *A. afra* is indigenous to Africa.[34] It has been described by Huxley *et al.*[35] as a condiment and it is also believed to have some magical properties!



http://www.plantzafrica.com/medmonographs/artemisiaafra.pdf

**Figure 2.6.** Distribution of *A. afra* in South Africa.[36]

### 2.1.3.2 Description

*A. afra*, commonly known as African wormwood, grows as a thick, bushy and untidy mass. Its height varies from 0.6 m to 2 m. The basal stems of the plant are thick, firm and woody compared with the upper parts of the plant. Leaves are finely divided. The general appearance of *A. afra* is grey; this is due to the fact that its leaves bear two different colours. The upper surface is dark green, whilst small white hairs cover the underside. Small flowers appear in late summer. It is an aromatic plant and when it is touched or cut, it sprays a sweet smell in its surroundings.

http:/www.plantzafrica.com/plantab/artemisafra.htm

**Figure 2.7.** Grey foliage of *A. afra*.[37]

### 2.1.3.3 Uses

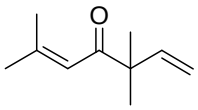
From early times, *A. afra* was exploited by some societies for different purposes. Egyptians, Greeks, and Romans used it not only for medicinal purposes, but also in their religious rites. It was also considered to be a love charm.[33]

Nowadays in Africa, in general, and in South Africa in particular, *A. afra* is among the most known medicinal plants and several biological activities, such as antimicrobial,[38,39] antifungal and antioxidant activities,[40,41,42] have been observed experimentally for extracts of *A. afra*. It has been used by people from all cultures to treat different diseases such as fever, intestinal worms, colic, headache, malaria, coughs, etc. Different parts of the plants have different uses. For example roots, stems and leaves are used as poultices, infusions, body washes and lotions. Roberts[33] mentioned that the Zulus prepare an infusion of ground leaves in hot water. This is used as an enema for children with worms and for constipation,

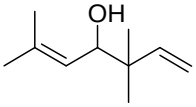
while the Vendas and Twanas use it to cure skin ailments. The author also listed other usages such as its use in natural insecticide sprays and as a moth repellent, as well as its painkilling and relaxation properties.

### 2.1.3.4 Previous chemical investigation

Previous investigations of the chemical composition of *A. afra* yielded interesting compounds. Garnero[43] detected artemisia ketone **(2.43)**, that is 3,3,6-trimethylhepta-1,5-dien-4-one, and artemisia alcohol **(2.44)** in an *A. afra* crude extract by gas chromatography. He confirmed that the latter represents 15% of the *A. afra* essential oil, while the first represents 20% and it is also the main component of *Santolina chamaecyparissus* Linnaeus essential oil.

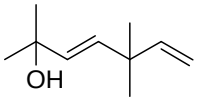



**2.43**




**2.44**

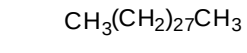
It has been reported by the same author that these two compounds are present in the essential oil of *A. annua* and in the extracts of *Achillea ageratum* Linnaeus (Asteraceae) leaves and flowers. After isolation of the above-mentioned compounds, the researcher believed that "yomogi" **(2.45),** an alcohol which is formed by allylic rearrangement of artemisia alcohol and known as an important constituent of essential oil of *Artemisia feddei* H. Lev. & Vanihot, may also be present in the essential oil of *A. afra.*




**2.45**

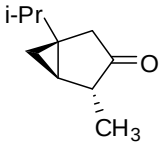
In 1990, Silbernagel's investigation yielded two important compounds, respectively known as nonacosane **(2.46)** and ceryl cerotinate **(2.47).**[34] Researchers found that the species contains monoterpenes such as α-thujone **(2.48)** and β-thujone **(2.49)**, and sesquiterpenes with glaucolide **(2.50)** and guaianolide **(2.51)** skeletons.[37,44] An eudesmanolide derivative, eudesmaafraglaucolide **(2.52)**, were isolated by Jakupovich *et al.*[44] Using GC-MS, the

above-cited compounds were also detected by Graven *et al.*[40] in *A. afra.* Other compounds which have been detected from *A. afra* include 1,8-cineol **(2.53)**, camphor **(2.54)** and borneol **(2.55)**.[7,45]

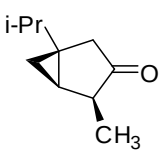



**2.46**

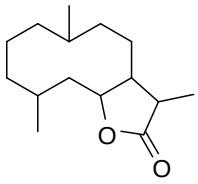

**2.47**



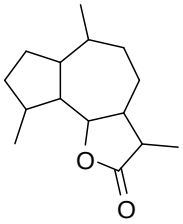

**2.48**



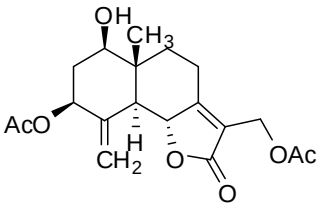

**2.49**



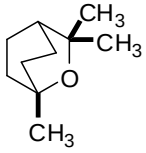

**2.50**



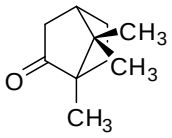

**2.51**



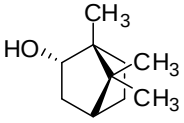

**2.52**




**2.53**




**2.54**




**2.55**

Moody *et al.*[46], in their analysis by GC and GC-MS of the essential oil of *A. afra*, found that the oil is composed of 21 components. The main constituents were found to be *cis*-2,7-dimethyl-4-octene-2,7-diol (19.03%), 1,8-cineol **(2.53)** (17.55%), tricosane (13.92%) and artemisia ketone **(2.43)** (11.67%). It was confirmed that the next important series of compounds which fall in relative intermediate constituent categories were: camphor **(2.54)** (6.21%), linalyl propionate (4.96%) and styrene (4.03%).

Another investigation has been carried out recently by Viljoen *et al.*[47] on the geographical variation of some major oil constituents. A total of 17 plants from 4 different geographic locations were investigated. A large variation in the essential oil composition was observed, with one of cineole (**2.53**), artemisia ketone **(2.43**), α- and β-thujone **(2.48**, **2.49)** or camphor **(2.54)** present as the major component in the majority of the oils. In a previous investigation, it was concluded that the major components of *A. afra* essential oil were

artemisyl acetate (24.4-32.1%) from Ethiopian oil, 1,8-cineol **(2.53)** (63.4%) in Kenyan oils, α- and β-thujone **(2.48**, **2.49)** (52%) in Zimbabwean oil and α-thujone (52.5-54.2%) **(2.48)** in the South African oil.[48]

From the results discussed above, it is clear that a large variation in the chemical constitution of *A. afra* essential oil has been observed. This may be due to many factors, such as the age of the plant, the season in which the plant material has been collected, the organ of the plant investigated and mainly the geographical location of the wild plant.

## 2.2 RESULTS AND DISCUSSION

### 2.2.1 Introduction

*A. afra* essential oil has been investigated and most of its main constituents are known. Although most researchers were more interested in the essential oil composition, the species also produces important non-essential oil components. The aim of our study was to identify the non-volatile compounds in an *A. afra* extract. Other researchers have also detected non-essential oil compounds, for example, Silbernagel isolated nonacosane **(2.46)**,[34] and Jakupovic *et al.*[44] detected some derivatives of guaianolide **(2.50),** glaucolide (**2.51)** and a eudesmanolide **2.52**.

Dr. Maria de Figueiredo, Agriculture Research Centre, Cedara have identified two chemotypes of *A. afra*, based on essential oil composition. The thujone chemotype has cineole **(2.53)**, camphor **(2.54)** and thujone **(2.48**, **2.49)** as the major components of the essential oil, whereas cineole **(2.53)**, camphor **(2.54)** and artemisia ketone **(2.43)** are the major constituents of the essential oil of the artemisia ketone chemotype of *A. afra*. Under the guidance of Dr de Figueiredo, the two chemotypes have been cultivated under identical conditions. The aim of this project was to investigate the less polar components and specifically the sesquiterpenoids of *A. afra* and to ascertain whether the variation in essential oil composition is reflected in the sesquiterpene composition of *A. afra*.

Different crude extracts obtained from *A. afra* plant material were subjected to silica gel column chromatography, where various eluents were prepared using different organic solvents namely hexane, dichloromethane, ethyl acetate, acetone and methanol. Repeated

column chromatography, centrifugal thin-layer chromatography (chromatotron) and TLC, led to the isolation of taurin **(2.56)**, artesin **(2.57)**, maritimin **(2.58)**, artemin **(2.59)**, norsantolinifolide (**2.61)**, santolinifolide **A (2.62)**, reynosin **(2.65)**, scopoletin **(2.66)**, *p*-hydroxyacetophenone **(2.67)**, 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**, and 5-hydroxy-7,4′-dimethoxyflavone **(2.69)**.

The first extraction of *A. afra* plant material of the ketone chemotype was effected by suspending it in 100% hexane for 24 hours. Given that hexane is a non-polar solvent with polarity index of zero (0),[49,50] the resulting extract was expected to contain less polar components of the extract, and **2.56** and **2.57** were isolated.

### 2.2.2 Structural elucidation of taurin (2.56)

The first compound was isolated from the hexane extract of the ketone chemotype. The NMR data of **2.56** are summarized in Table 2.1. The $^{13}$C NMR spectrum (Plate 2) of the compound displayed fifteen signals, which indicated that this compound might be a sesquiterpene. Two quaternary carbon signals were observed at $\delta_C$ 129.6 and 127.0, indicating the presence of a tetrasubstituted double bond. The peak at $\delta_C$ 178.7 was characteristic of a lactone carbonyl carbon, and the one at $\delta_C$ 213.6 indicated that a ketone group is present in the structure. Three methyl signals at $\delta_C$ 14.4, 20.0 and 23.5 were also observed.

The $^1$H NMR spectrum (Plate 1) exhibited signals of a secondary methyl ($\delta_H$ 1.21, 3H, d, *J* 7.0 Hz) and two tertiary methyl groups ($\delta_H$ 1.94 and 1.30), both appearing as singlets. The same spectrum had a doublet at $\delta_H$ 4.57 (1H, d, *J* 11.0 Hz), which is characteristic of a lactone proton[51] (H-6). In the HMBC spectrum (Plate 7), $^1$H,$^{13}$C correlations were observed between the methyl protons resonating at $\delta_H$ 1.30 and the carbonyl carbon ($\delta_C$ 213.6), two quaternary carbons ($\delta_C$ 48.9 and $\delta_C$ 129.6) and a methylene carbon ($\delta_C$ 34.8). The methyl proton resonating at $\delta_H$ 1.94 correlated with the two olefinic carbons at $\delta_C$ 127.0 and $\delta_C$ 129.6 and a methylene carbon resonating at $\delta_C$ 36.0. The protons coupled directly to the last-mentioned carbon, resonating at $\delta_H$ 2.36 and $\delta_H$ 2.30, also showed a correlation to the ketone carbonyl resonating at $\delta_C$ 213.6. This information enabled us to construct the molecular fragment indicated in Fig. 2.8.

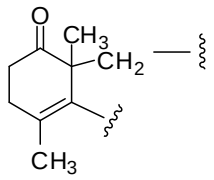
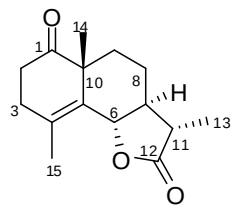



**Figure 2.8.** A fragment of compound **2.56**

The evidence for the presence of an α-substituted methyl lactone in **2.56** was also derived from the NMR data. In the HMBC spectrum (Plate 7), the secondary methyl protons ($\delta_H$ 1.21) showed $^{1}H,^{13}C$ correlations with the lactone carbonyl carbon ($\delta_C$ 178.7) and two methine carbons resonating at $\delta_C$ 41.0 and $\delta_C$ 52.9, respectively. In the COSY spectrum (Plate 5), the coupling between the proton attached to the last-mentioned carbon ($\delta_H$ 1.71), and the proton on an acyl-bearing carbon ($\delta_H$ 4.57) was observed. In the HMBC spectrum, the proton resonating at $\delta_H$ 4.57 correlates with the olefinic carbon resonating at $\delta_C$ 127.0, thereby connecting the lactone ring to the molecular fragment given in Fig. 2.8. The structure of the compound **2.56** was assigned as (6β,7α,11β)-1-oxoselin-4(5)-en-6,12-olide or taurin, a eudesmanolide which have been isolated from several *Artemisia* species. The structure was confirmed by the agreement between the NMR data of **2.56** and values published by Gonzalez *et al.*[51], who isolated taurin from *A. maritima.*

The relative stereochemistry of **2.56** was confirmed by a NOESY experiment (Plate 5a), NOE correlations were observed between H-6, $H_\beta$-8 and H-11, respectively, thereby proving that all these protons are on the same side of the molecule. The large coupling constant between H-6 and H-7 (*J* 11.0) confirmed that these two protons were *trans* to each other. Therefore, **2.56** had the same relative configuration as taurin. Although the specific rotation obtained for **2.56** was lower than that reported for taurin in the literature, both rotations were negative and it was assumed that **2.56** had the same absolute configuration as that reported for taurin.




**2.56**

**Table 2.1. $^{1}H$ (400 MHz) and $^{13}C$ (100 MHz) NMR data of taurin (2.56) in $CDCl_3$.**

| Experimental values | | | | | | | Literature values[51] | |
|---|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **NOESY** | **HMBC*** | **$\delta_C$ (20 MHz)** | **$\delta_H$ (90 MHz)** |
| 1 | 213.6 | C | | | 2 | 2, 3, 9, 14 | 212.5 | |
| 2 | 33.0 | $CH_2$ | 2.45 (m)<br>2.61 (m) | 2 | 1, 2 | 3 | 35.0† | |
| 3 | 36.0 | $CH_2$ | 2.36 (m)<br>2.30 (m) | | 6, 15 | 2, 15 | 35.9 | |
| 4 | 127.0 | C | | | | 2, 15 | 130.2† | |
| 5 | 129.6 | C | | | | 3, 9, 14, 15 | 126.6† | |
| 6 | 81.8 | CH | 4.57 (1H, d, *J* 11.0 Hz) | 7 | 7, 8, 11, 14 | 7, 8 | 81.5 | 4.6 (1H, d, *J* 9.0 Hz, |
| 7 | 52.9 | CH | 1.70 (qd, *J* 11.8, 3.3 Hz) | 6 | 6 | $9_{ax}$, $9_{eq}$, 11, 13 | 55.0 | |
| 8 | 23.9 | $CH_2$ | *ax* 1.58 (qd, *J* 12.8, 3.5 Hz)<br>*eq* 1.97 (m) | 9 | 6 | 9 | 23.8 | |
| 9 | 34.8 | $CH_2$ | *ax* 1.50 (td, *J* 13.3, 4.2 Hz)<br>*eq* 1.83 (dt, *J* 13.3, 2.6 Hz) | 8 | 9 | 7, 8, 14 | 32.9† | |
| 10 | 48.9 | C | | | | 14 | 48.8 | |
| 11 | 41.0 | CH | 2.27 (dq, *J* 12.2, 7.0 Hz) | | 6, 13 | 7, 8, 13 | 40.8 | |
| 12 | 178.7 | C | | | | 11, 13 | 177.8 | |
| 13 | 12.4 | $CH_3$ | 1.21 (3H, d, *J* 7.0 Hz) | | 11 | | 12.3 | 1.23 (3H, d, *J* 7.0 Hz |
| 14 | 23.5 | $CH_3$ | 1.30 (3H, s) | 6 | 6 | | 23.3 | 1.33 (3H, s) |
| 15 | 20.0 | $CH_3$ | 1.94 (3H, s) | | 3 | | 19.7 | 1.98 (3H, s) |

* HMBC Correlations are from the protons indicated in this column to the carbons indicated in column 1.
† Assignments wrong in the literature.

The high-resolution mass spectrum showed a peak at *m/z* 271.1300, corresponding to the sodium adduct of the molecular ion, $[M+Na]^+$, which was in agreement with the calculated value of 271.1310 for $C_{15}H_{20}O_3Na$.

The isolation of taurin **(2.56)** from *A. afra* was previously reported by Nkunya *et al.*[52] Jakupovic *et al.*[44] reported the isolation of one other eudesmanolide (**2.50**), but apart from these two compounds, none of the other sesquiterpenoids reported from *A. afra* are eudesmanolide derivatives.

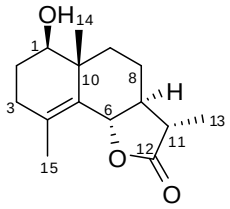
Different biological activities have been reported for taurin (**2.56**). An article by Batuev *et al.* [53] mentioned that it decreased the incidence and severity of audiogenically induced convulsions in rat. According to Doksina *et al*.,[54] it showed inhibition of proteolytic ferments in radiation sickness. In conjugation with ursodeoxycholic acid, taurin exercises inhibitory effect on the normal growth of human keratinocytes.[55] Taurin **(2.56)** was also used in dentifrice manufacture (Kasuno *et al*.).[56]

### 2.2.3 Structural elucidation of artesin (2.57)

Artesin or (6β,7α,11β)-1β-hydroxyselin-4(5)-en-6,12-olide **(2.57)** was obtained from the hexane extract of the ketone chemotype after repeated column chromatography using hexane - ethyl acetate solvent mixtures of increasing polarities. The NMR data of **2.57** are collated in Table 2.2.

The isolated compound displayed a total of fifteen carbon atoms in the $^{13}C$ NMR spectrum (Plate 9), which indicated that the compound was a sesquiterpene. The $^1H$ NMR spectrum (Plate 8) exhibited a doublet at $\delta_H$ 4.57 (1H, d, *J* 11.0 Hz) due to the presence of a lactone proton H-6. Methyl proton signals were observed at $\delta_H$ 1.12 (3H, s), $\delta_H$ 1.24 (3H, d. *J* 7.0 Hz, 13-$CH_3$) and at $\delta_H$ 1.85 (3H, s). These are the three methyl groups as was evident from both the HSQC (Plate 13) and DEPT135 (Plate 10) spectra. In the HSQC spectrum (Plate 13), direct correlations were observed between C-13 at $\delta_C$ 12.3 and H-13 at $\delta_H$ 1.24, C-14 at $\delta_C$ 18.4 and H-14 at $\delta_H$ 1.12 and between C-15 at $\delta_C$ 19.7 and H-15 at $\delta_H$ 1.85.

A comparison of the $^{13}C$ NMR data of **2.56** and **2.57** showed that these two compounds were closely related. The major difference between the two spectra was that the ketone peak at $\delta_C$ 213.6 (C-1) in the spectrum of **2.56** was replaced by a peak at $\delta_C$ 77.7, which is characteristic of an aliphatic carbon bonded to oxygen. Therefore, it was concluded that the ketone group in taurin **(2.56)** was reduced to an alcohol. The coupling constants of H-1 (*J* 11.4 and 4.4 Hz) indicated that this proton had an axial-axial and an axial-equatorial relationship with the two C-2 protons and is therefore in the axial position and *trans* to 14-$CH_3$. The structure of **2.57** was assigned as artesin. The NMR data of **2.57** was in agreement with those reported for artesin.[21]




**2.57**

In the high-resolution mass spectrum, the sodium adduct of the molecular ion was observed at m/z 273.1472, which is in close agreement with the calculated value of 273.1467 for $C_{15}H_{22}O_3Na$. Artesin **(2.57)** has been isolated from a number of other *Artemisia* species, for example *A. herba-alba* Asso, *A. santolinifolia* Trucz ex. Besser [21,51] but the presence of artesin **(2.57)** in *A. afra* has not been reported previously.

### 2.2.4 Structural elucidation of maritimin (2.58)

The residue obtained after extraction of plant material with hexane, was re-extracted with a mixture of 50% $CH_2Cl_2$ in MeOH. These two solvents, being more polar than hexane with polarity indexes of 3.1 and 5.1,[49,50] respectively, for $CH_2Cl_2$ and MeOH, could extract more polar components from the plant material. The compounds isolated from this extract were **2.58** - **2.59**, **2.61**-**2.62** and **2.65**-**2.69**.

The component with $R_f$ 0.28 (hexane–ethyl acetate, 7:3) was obtained after repeated column chromatography of the $CH_2Cl_2$-MeOH crude extract from the *A. afra* ketone chemotype. After recrystallization from methanol, the compound was obtained as small white crystals. The NMR data of **2.58** are summarized in Table 2.3.

**Table 2.2. $^{1}H$ and $^{13}C$ NMR data of artesin (2.57) in $CDCl_3$ (400 MHz).**

| Isolated compound | | | | | | Artesin ( lit.[21]) | |
|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC** | **$\delta_C$** | **$\delta_H$** |
| 1 | 77.7 | CH | 3.54 (dd, *J* 11.4, 4.4 Hz) | 2 | 14 | 77.7 | 3.52 (dd) |
| 2 | 27.1 | $CH_2$ | 1.70 (m) | 1, 3 | | 27.1 | |
| 3 | 33.2 | $CH_2$ | 2.35 (m)<br>2.04 (m) | 2 | 15 | 33.3 | |
| 4 | 126.0 | C | | | 15 | 126.0 | |
| 5 | 129.0 | C | | | 14, 15 | 128.9 | |
| 6 | 83.2 | CH | 4.57 (d, *J* 11.0 Hz) | | | 83.0 | 4.58 (1H, ddq) |
| 7 | 52.8 | CH | 1.73 (m) | | 13 | 52.8 | |
| 8 | 24.5 | $CH_2$ | 1.96 (m)<br>1.26 (m) | | | 24.4 | |
| 9 | 38.3 | $CH_2$ | 2.05 (m)<br>1.26 (m) | | 14 | 38.3 | |
| 10 | 41.9 | C | | | 14 | 41.9 | |
| 11 | 41.1 | CH | 2.25 (m) | | 13 | 41.1 | |
| 12 | 178.9 | C | | | 13 | 179.0 | |
| 13 | 12.3 | $CH_3$ | 1.24 (3H, d, *J* 7.0 Hz) | | | 12.4 | 1.24 (1H,d) |
| 14 | 18.4 | $CH_3$ | 1.12 (3H, s) | | | 18.5 | 1.1 (3H, s) |
| 15 | 19.7 | $CH_3$ | 1.85 (3H, s) | | | 19.8 | 1.84 (3H, s) |

The $^{1}H$ NMR spectrum (Plate 15) showed a doublet at $\delta_H$ 4.32 (1H, d, *J* 11 Hz), which is characteristic of the lactone proton H-6. The presence of two tertiary methyl groups ($\delta_H$1.69 and 1.24) and a secondary methyl ($\delta_H$ 1.27, d, *J* 7.0 Hz) was observed. In the $^{13}C$ NMR spectrum (Plate 16), the absorptions of 15 carbon atoms were detected, including three methyl groups resonating at $\delta_C$ 12.3, $\delta_C$ 19.4 and $\delta_C$ 20.7; four methylene carbons appearing at $\delta_C$ 23.0, $\delta_C$ 28.0, $\delta_C$ 31.0, and $\delta_C$ 33.5; two methine carbons at $\delta_C$ 40.6 and $\delta_C$ 48.4; one quaternary carbon at $\delta_C$ 49.2, three carbons joined to oxygen at $\delta_C$ 63.7 (C) $\delta_C$ 66.9 (C), and $\delta_C$ 77.2 (CH); a ketone carbon resonating at $\delta_C$ 210.8 and a lactone carbonyl at $\delta_C$ 177.9.

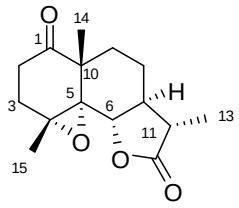
In the HMBC experiment, $^{1}H,^{13}C$-correlations were observed between the methyl protons resonating at $\delta_H$ 1.24 and the ketone carbon ($\delta_C$ 210.8), an aliphatic quaternary carbon ($\delta_C$ 49.2), a methylene carbon ($\delta_C$ 28.0), and one of the oxygen-containing quaternary carbons ($\delta_C$ 63.7). The protons of the other quaternary methyl ($\delta_H$ 1.69) correlate to a methylene carbon ($\delta_C$ 33.5) and the two quaternary carbon atoms bonded to oxygen ($\delta_C$ 63.7, $\delta_C$ 66.9). From the NMR data, it is clear that the structure of **2.58** is closely related to that of taurin **(2.56)**, the only difference being that the 4,5-double bond of taurin **(2.56)** is oxidized to either a diol or an epoxide. Differentiation between these two structures was possible by IR spectroscopy and MS spectrometry.

Analysis of the IR spectrum of the isolated compound gave useful information. Firstly, it exhibited two absorption bands, one at 1781 $cm^{-1}$ and another at 1708 $cm^{-1}$; these carbonyl bands are characteristic of a γ-lactone and a cyclohexanone. Secondly, it did not show any absorption band for a hydroxy group; this would suggest the presence of an 4,5-epoxy group. An α-epoxide and a β-epoxide will have the same NMR characteristics and, therefore, we were unable to confirm the configuration of the epoxide by this method. The mass spectrum showed the sodium adduct of the molecular ion at *m/z* 287.1259 $[M + Na]^{+}$, which was the same as the mass calculated for $C_{15}H_{20}O_4Na$ (287.1259).

**Table 2.3. $^{1}H$ and $^{13}C$ NMR data of maritimin (2.58) in $CDCl_3$ (400 MHz).**

| Experimental results | | | | | | Literature results[51] | |
|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC correlation** | **$\delta_C$** | **$\delta_H$** |
| 1 | 210.8 | C | | | 14 | 210.7 | |
| 2 | 31.0 | $CH_2$ | 1.99 (m)<br>1.74 (m) | | | 31.0 | |
| 3 | 33.5 | $CH_2$ | 2.38 (m) | | 15 | 33.4 | |
| 4 | 66.9 | C | | | 6, 15 | 66.0 | |
| 5 | 63.7 | C | | | 14, 15 | 63.6 | |
| 6 | 77.2 | CH | 4.32 (1H, d, *J* 11.0 Hz) | 7 | | 76.6 | 4.34 (1H,d, *J* 9.0 Hz) |
| 7 | 48.4 | CH | 2.00 (m) | 6 | 6, 13 | 48.5 | |
| 8 | 23.0 | $CH_2$ | 1.50 (m) | | 6 | 22.9 | |
| 9 | 28.0 | $CH_2$ | 2.18 (m) | | 14 | 27.9 | |
| 10 | 49.2 | C | | | 14 | 49.2 | |
| 11 | 40.6 | CH | 2.25 (m) | 13 | 6, 13 | 40.4 | |
| 12 | 177.9 | C | | | | 178.0 | |
| 13 | 12.3 | $CH_3$ | 1.27 (3H, d, *J* 7.0 Hz) | 11 | | 12.3 | 1.25 (3H, d, *J* 7.0 Hz) |
| 14 | 20.7 | $CH_3$ | 1.24 (3H, s) | | | 20.7 | 1.27 (3H, s) |
| 15 | 19.4 | $CH_3$ | 1.69 (3H, s) | | | 19.4 | 1.68 (3H, s,) |

However, Gonzalez *et al*. derived the stereochemistry of the epoxide by chemical degradation reactions.[51] Therefore, the structure of **2.58** was assigned as that of maritimin, previously isolated from *A. maritima gallica* by Gonzalez *et al*.[51] This thesis is the first report of the isolation of maritimin **(2.58)** from *A. afra.*




**2.58**

### 2.2.5 Structural elucidation of artemin (2.59)

Seven grams of crude fraction were subjected to column chromatography and centrifugal thin-layer chromatography eluting with hexane-EtOAc (6:4) and (7:3), respectively, and finally the solvent was removed to yield (292 mg) of crystals. Different NMR experiments were run for the compound and the spectra were analysed to obtain the data summarised in Table 2.4.

The $^{1}$H NMR (Plate 22) showed a signal at $\delta_H$ 4.26 (1H, d, *J* 10.5 Hz) characteristic of a γ-lactone. The doublet appearing at $\delta_H$ 1.23 (3H, d, *J* 6.5 Hz) indicated a secondary methyl group. The singlet at $\delta_H$ 0.89 was assigned to the angular methyl group. The signals at $\delta_H$ 5.03 and 4.98 corresponded to two methylene alkene protons. The $^{13}$C NMR spectrum (Plate 23) showed fifteen carbon atoms, implying that the compound was a sesquiterpene. The signal at $\delta_C$ 179.4 was characteristic of a carbonyl group (C=O). Three signals were detected at $\delta_C$ 71.8, $\delta_C$ 77.1 and $\delta_C$ 81.9, indicating the presence of three carbon atoms bearing oxygen atoms. The peak at $\delta_C$ 112.4 was assigned to the olefinic methylene carbon.

The HMBC spectrum (Plate 28) indicated that the protons resonating at $\delta_H$ 4.26, 4.17 and $\delta_H$ 0.89 were all correlating with a carbon resonating at $\delta_C$ 44.5. Correlations were also observed between protons resonating at $\delta_H$ 5.03, 4.98, 4.26 and a carbon having an OH group, resonating at $\delta_C$ 77.1. Also, protons at $\delta_H$ 2.15 and $\delta_H$ 2.65 correlated

with the olefinic carbon at $\delta_C$ 112.4. The above-mentioned information enabled us to construct the following fragment.

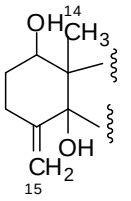
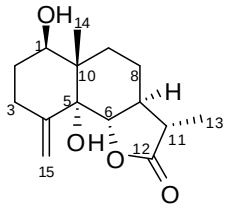


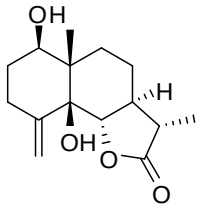

**Figure 2.9.** A fragment of compound **2.59**

In the HMBC, a $^1H,^{13}C$ correlation between the methyl resonating at $\delta_H$ 1.23 and the lactone carbonyl at $\delta_C$ 179.4 was observed. The COSY spectrum (Plate 26), revealed $^1H,^1H$ coupling between the proton at $\delta_H$ 4.26 and $\delta_H$ 2.34. In the HMBC spectrum, a correlation was observed between the carbon resonating at $\delta_C$ 12.4 and the proton at $\delta_H$ 4.26 (γ-lactone), attaching the lactone ring to the fragment above (Fig. 2.9). Hence, the isolated compound was characterised as one of the two epimers, artemin **(2.59)** or epi-artemin **(2.60)**. The latter differs from the first only by the configuration of the OH group attached to C-5. To avoid the ambiguity, the $^1H$ NMR data of the isolated compound was compared to that of **2.59** and **2.60** (Table 2.9).[57] The comparison revealed that the isolated compound was artemin **(2.59)** rather than *epi*-artemin **(2.60)**.

The present work is the first report of the isolation of artemin **(2.59)** from *A. afra.* In the literature, artemin has been reported from other *Artemisia* species, such as *A. pontica* from Bulgaria and *A. hugueti* from Marocco.[57, 58]




**2.59**




**2.60**

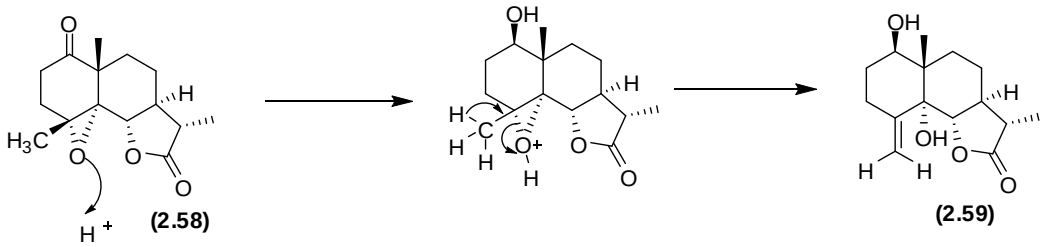
The HRMS revealed the molecular mass of the compound at *m/z* 289.1423 $[M + Na]^+$, which agreed with the calculated mass of 289.1416 for $C_{15}H_{22}O_4Na$.

**Table 2.4. $^{1}H$ and $^{13}C$ NMR data of artemin (2.59) in $CDCl_3$ (400 MHz).**

| Experimental data | | | | | | Literature data[57,*] | |
|---|---|---|---|---|---|---|---|
| Isolated compound | | | | | | Artemin (2.59) | 5-*Epi*-artemin (2.60) |
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC correlation** | **$\delta_H$** | **$\delta_H$** |
| 1 | 71.8 | CH | 4.17 (dd, *J* 11.5, 5.3 Hz ) | 2, 9 | | 4.15 (dd) | 3.45 (br s) |
| 2 | 29.9 | $CH_2$ | 1.73 (m) | 1 | | 1.75 (m)<br>1.60 (m) | 1.90 (m)<br>1.80 (m) |
| 3 | 29.6 | $CH_2$ | 2.65 (m)<br>2.15 (m) | 3 | | 2.65 (m)<br>2.17 (ddd) | 2.07 (ddd)<br>2.90 (m) |
| 4 | 145.1 | C | | | | | |
| 5 | 77.1 | C | | | 6, 15 | | |
| 6 | 81.9 | CH | 4.26 (d, *J* 10.2 Hz) | 7 | | 4.25 (d) | 4.35 (d) |
| 7 | 41.2 | CH | 2.34 (m) | 6, 13 | 6, 13 | 2.35 (m) | 2.32 (dt) |
| 8 | 30.3 | $CH_2$ | 1.50 (m) | | | 1.85(m)<br>1.55 (m) | 1.85 (m)<br>1.60 (m) |
| 9 | 22.8 | $CH_2$ | 1.84 (m)<br>1.49 (m) | 1 | | 1.85 (m)<br>1.60 (m) | 1.60 (m)<br>1.30 (m) |
| 10 | 44.5 | C | | | 1, 6, 14 | | |
| 11 | 45.5 | CH | 2.35 (m) | 13 | 7, 13 | 2.40 (dq) | 2.32 (dq) |
| 12 | 179.4 | C | | | 13 | | |
| 13 | 12.4 | $CH_3$ | 1.23 (d, *J* 3.8 Hz) | 7, 11 | | 1.24 (d) | 1.23 (d) |
| 14 | 13.3 | $CH_3$ | 0.89 (s) | | | 0.90 (s) | 1.34 (s) |
| 15 | 112.4 | $CH_2$ | 5.03 (d, 19.4 Hz)<br>4.98 (s) | | 3 | 5.03 (d)<br>4.98 (br s) | 5.14 (d)<br>5.08 (br s) |

*$^{13}C$ NMR data are not available in the literature.

Artemin **(2.59)** can be formed by reduction of the C-1 carbonyl and the acid-catalysed rearrangement of maritimin **(2.58)** (Scheme 2.1). Although this reaction is chemically possible, artemin **(2.59)**, is mostly likely formed by an enzyme-catalysed reaction and is not an artefact.




**Scheme 2.1.** Proposed formation of artemin **(2.59)** from maritimin **(2.58)**.

Indirectly, the α-configuration of the 5-hydroxy group in artemin **(2.59)** confirmed the α-configuration the 4,5-epoxide assigned to maritimin **(2.58)**.

### 2.2.6 Structural elucidation of norsantolinifolide (2.61)

Norsantolinifolide **(2.61)** was isolated from the aerial parts of the ketone chemotype of *A. afra.* After filtration of the crude extract by a plug, various columns were run and 19 mg of white crystals were obtained and dried prior to spectroscopic analysis. The $^{1}$H NMR spectrum (Plate 29, Table 2.5) was much simpler than those of the sesquiterpenoids discussed earlier. The COSY spectrum (Plate 33) confirmed that the characteristic resonances of the lactone protons were still present, *i.e.* a secondary methyl resonating at $\delta_H$ 1.29 (3H, d, *J* 6.6 Hz, 13-H), multiplets at $\delta_H$ 2.36 and $\delta_H$ 2.39, assigned to H-11 and H-7, respectively, and a doublet observed at $\delta_H$ 3.60 (1H, d, *J* 11.5 Hz). The $^{13}$C NMR spectrum (Plate 30) showed a signal at $\delta_C$ 181.0 characteristic of a lactone carbonyl carbon. In the HMBC spectrum (Plate 35), $^{1}$H,$^{13}$C correlations were observed between the lactone proton ($\delta_H$ 3.60), methine proton ($\delta_H$ 2.39), secondary methyl protons ($\delta_H$ 1.29) and a methine carbon resonating at $\delta_C$ 51.0. The above-obtained information allowed us to construct the fragment in Fig. 2.10.

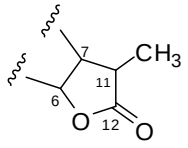



**Figure 2.10.** First fragment of compound **2.61**.

The $^{13}C$ NMR spectrum of **2.61**, unlike those of the sesquiterpenes discussed earlier, displayed a total number of only ten carbon signals. Among them, a tertiary methyl and a quaternary carbon signal were observed at $\delta_C$ 24.0 ($CH_3$) and $\delta_C$ 46.6 (C), respectively. Two methylene carbons resonated at $\delta_C$ 21.0 and 38.0 and a carbon resonating at $\delta_C$ 179.0 was assigned to a carboxy group (COOH). This information suggested that the compound might be a sesquiterpene that has been subjected to degradation. The HMBC spectrum (Plate 35) showed $^{1}H,^{13}C$ correlations between the tertiary methyl proton resonating at $\delta_H$ 1.53 and a quaternary carbon ($\delta_C$ 46.6), methylene group ($\delta_C$ 38.0), a lactone carbon ($\delta_C$ 90.8) and a carboxy group carbon ($\delta_C$ 179.0). The COSY spectrum (Plate 33) showed $^{1}H$-$^{1}H$ coupling between the protons of the two above-mentioned methylene groups ($\delta_H$ 1.92 and $\delta_H$ 2.80). The lactone proton ($\delta_H$ 3.60) was coupled to a methine group proton at $\delta_H$ 2.39. The above-mentioned data enabled us to construct the fragment in Fig. 2.11.

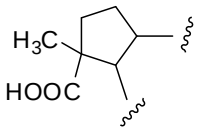
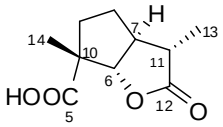



**Figure 2.11.** Second fragment of compound **(2.61)**

The fragments presented in Fig. 2.10 and 2.11, joined together, gave the structure of norsantolinifolide **(2.61)**.




**2.61**

The molecular mass obtained by the high-resolution mass spectrometry, *m/z* 221.0784 $[M+Na]^+$, corresponded to the molecular formulae of $C_{10}H_{14}O_4Na$. The mass calculated for this formula was 221.0790. The isolation of norsantolinifolide **(2.61)** was only reported once before, by Jakupovic *et al.*[12], who isolated it from *A. santolinifolia*.

**Table 2.5. $^{1}H$ and $^{13}C$ NMR data of norsantolinifolide (2.61) in $CDCl_3$ (400 MHz).**

| Experimental | | | | | | Literature[12] | |
|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC correlation** | **$\delta_H$** | **Multiplicity** |
| 5 | 179.0 | C | | | 9, 14 | | |
| 6 | 90.8 | CH | 3.60 (1H, d, *J* 11.5 Hz) | 7 | 9, 14 | 3.58 | d |
| 7 | 43.4 | CH | 2.39 (m) | 6 | 6, 13 | 2.20 | dddd |
| 8 | 21.0 | $CH_2$ | 1.92 (m)<br>1.45 (m) | 9, 8 | 9 | 1.92<br>1.42 | dddd<br>ddd |
| 9 | 38.0 | $CH_2$ | 1.80 (1H, ddd, *J* 14.0, 10.0, 2.0 Hz)<br>2.80 (1H, dt, *J* 14.0, 8.6 Hz) | 8, 9 | 14 | 1.76<br>2.82 | ddd<br>ddd |
| 10 | 46.6 | C | | | 9, 14 | | |
| 11 | 51.0 | CH | 2.36 (m) | 13 | 6, 7, 13 | 2.36 | dq |
| 12 | 181.0 | C | | | 6, 7, 13 | | |
| 13 | 13.4 | $CH_3$ | 1.29 (3H, d, *J* 6.6 Hz) | 11, 14 | 8 | 1.28 | d |
| 14 | 24.0 | $CH_3$ | 1.53 (3H, s) | 13 | 6 | 1.51 | s |

The assignment of the above-mentioned structure was corroborated by the close agreement of the spectroscopic data of the isolated compound with the published values for the compound.[12]

### 2.2.7 Structural elucidation of santolinifolide A (2.62)

Santolinifolide **A (2.62)** was not obtained pure but was isolated as a mixture of the **2.62** and **2.59**, as explained in experimental section. The mixture was obtained after subjecting the crude extract of *A. afra* (ketone chemotype) to multiple column chromatographies and finally, to separation on a chromatotron. Although the material was still a mixture, the NMR experiments and comparison with published results allowed the identification of the compound.

The $^{13}C$ NMR (Plate 37) exhibited 29 signals, but further examination revealed the presence of one more signal underneath the solvent ($CDCl_3$) peak, resulting from a non-protonated carbon. This would suggest either a dimer or a mixture of two sesquiterpenes. After scrutinising the spectrum, it was discovered that fifteen signals were those of santolinifolide A **(2.62**) and fifteen other were due to the presence of artemin **(2.59)**, which was isolated from the same fraction. The NMR data of **2.62** are assembled in Table 2.6. The $^{1}H$ NMR spectrum (Plate 36) of the mixture, similarly to other sesquiterpenoids discussed, exhibited characteristic resonances of the lactone ring protons. A doublet at $\delta_H$ 3.72 (1H, d, *J* 11.5 Hz) indicated that a lactone proton was present. A secondary methyl resonated at $\delta_H$ 1.25 and a tertiary methyl signal was observed at $\delta_H$ 1.58 (3H, s). Signals characteristic of exocyclic methylene protons were observed at $\delta_H$ 6.01(1H, br. s) and 5.86 (1H, br. s). Aliphatic methylene protons were found at $\delta_H$ 2.58 (m) and 2.61(m), indicating the presence of an aliphatic chain.

A comparison of the $^{13}C$ NMR spectra of **2.61** and **2.62** showed that similar carbon signals to those of **2.61** were found in the spectrum for **2.62**. In addition to the **2.61** carbon signals, **2.62** had 5 other carbon signals. A carbon resonating at $\delta_C$ 204.0 was assigned to a ketone carbonyl group, a carbon signal at $\delta_C$ 126.0 was attributed to an exocyclic methylene group, a signal at $\delta_C$ 144.5 indicated an unsaturated carbon (a double bond), and peaks observed at $\delta_C$ 28.5 and 32.9 were assigned to aliphatic methylene groups. The HMBC spectrum (Plate 42) showed $^{1}H$-$^{13}C$ correlations

between the exocyclic methylene protons ($\delta_H$ 6.01, $\delta_H$ 5.86) and the ketone carbonyl group at $\delta_C$ 204.0. The methylene protons resonating at $\delta_H$ 2.55 correlated with the quaternary carbon resonating at $\delta_C$ 144.5. All these data enabled us to constitute the aliphatic fragment presented in Fig. 2.12

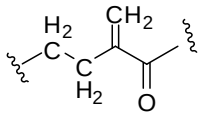
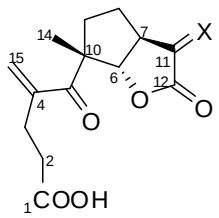


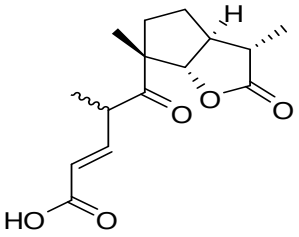

**Figure 2.12.** A fragment of compound (**2.62)**.

Finally, the comparison of **2.61** and **2.62**, revealed that the carbonyl goup (C=O) of aliphatic chain of **2.62** was oxidised to a carboxy group (COOH) to form **2.61**. Joining **2.61** and the fragment presented in Fig. 2.12, the structure of **2.62** (santolinifolide A) was obtained. The spectroscopic data of the isolated compound were also compared to those of other isomers of santolinifolide A, santolinifolides B **(2.63)** and C **(2.64)**.




**2.62** X= H, α-Me

**2.63** X= H, β-Me



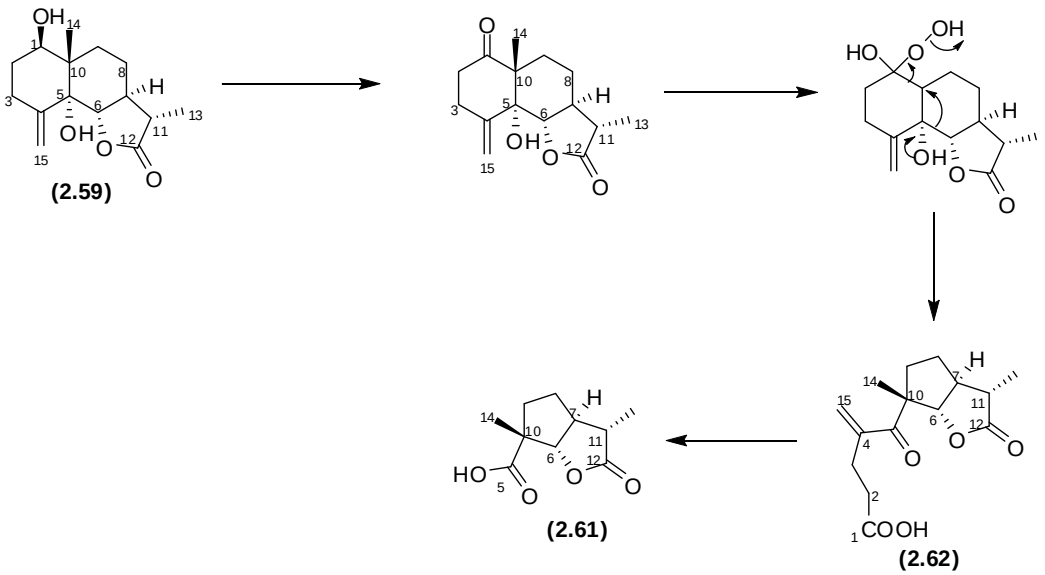

**2.64**

From the Table 2.6, it is clear that the chemical shifts of the isolated compound were in close agreement to those of santolinifolide A **(2.62)** and not the other of those isomers. Jakupovic *et al*.[12] reported the isolation of santolinifolide A from *A. santolinifolia* collected in Mongolia in June 1988 and subsequently Marco *et al*. have isolated it from *A. hugueti* collected in Marocco.[58] Prior to the present research, it was not reported from *A. afra*. Jakupovic *et al.*[12] proposed a transformation of artemin **(2.59)** to santolinifolide A **(2.62)** and norsantolinifolide **(2.61)** as illustrated in Scheme 2.2.

**Table 2.6. $^{1}H$ and $^{13}C$ NMR data of santolinifolide A (2.62) in $CDCl_3$ (400 MHz).**

| Experimental data | | | | | | Literature data[12] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolated compound | | | | | | Santolinifolide A | | Santolinifolide B | | Santolinifolide C | |
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC correlation** | **$\delta_C$** | **$\delta_H$** | **$\delta_C$** | **$\delta_H$** | **$\delta_C$** | **$\delta_H$** |
| 1 | 176.8 | C | | | 3 | 173.1* | | 173.1 | | 166.5 | |
| 2 | 32.9 | $CH_2$ | 2.58 m | | | 32.9 | 2.40 t | 33.0 | 2.42 t | 122.0 | 5.87 dd |
| 3 | 28.5 | $CH_2$ | 2.61 m<br>2.55 m | 15 | | 28.6 | 2.61 br dt<br>2.51 br dt | 28.7 | 2.63 br ddt<br>2.53 br ddt | 144.8 | 6.86 dd |
| 4 | 144.5 | C | | | 3 | 144.7 | | 144.8 | | 46.7 | 3.91 ddq |
| 5 | 204.0 | C | | | 15, 14 | 204.1 | | 204.0 | | 210.6 | |
| 6 | 90.5 | CH | 3.72 (d *J* 11.5 Hz) | 8 | 14 | 96.0* | 3.71 d | 88.9 | 3.95d | 89.9 | 3.65 d |
| 7 | 43.2 | CH | 2.32 m | 13 | 13 | 42.9 | 1.88 m | 38.6 | 2.32 dddd | 42.9 | 1.66 dddd |
| 8 | 20.5 | $CH_2$ | 1.88 m<br>1.39 m | 6 | | 20.1 | 1.88 m<br>1.39 m | 17.0 | 1.69 dddd<br>1.43 dddd | 20.5 | 1.86 dddd<br>1.38 dddd |
| 9 | 38.7 | $CH_2$ | 1.72 m<br>3.03 (ddd, *J* 2.3, 6.8,13.7 Hz) | | | 38.5 | 1.59 ddd<br>3.01 ddd | 38.1 | 1.57 ddd<br>3.02 ddd | 34.4 | 1.51 ddd<br>3.00 ddd |
| 10 | 52.8 | C | | | 9 | 52.5 | | 52.6 | | 54.3 | |
| 11 | 50.8 | CH | 2.33 m | | 13 | 50.6 | 2.32 dq | 56.4 | 2.71 dq | 51.7 | 2.36 dq |
| 12 | 181.0 | C | | | 13 | 180.8 | | 181.4 | | 180.7 | |
| 13 | 13.3 | $CH_3$ | 1.23 m | 7 | | 13.2 | 1.21 d | 8.6 | 1.15 d | 13.3 | 1.24 d |
| 14 | 26.0 | $CH_3$ | 1.58 s | | | 26.0 | 1.55 s | 25.9 | 1.57 s | 23.0 | 1.51 s |
| 15 | 126.0 | $CH_2$ | 6.01 br. s | 3 | | 125.8 | 5.99 br s | 126.0 | 5.01 br s | 17.6 | 1.15 d |

* Assignment wrongly reported in the literature



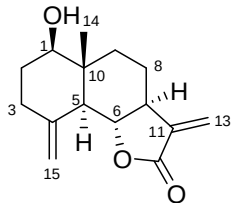

**Scheme 2.2.** Proposed transformation of artemin **(2.59)** to santolinifolide **A (2.62)** and norsantolinifolide **(2.61)**.[12]

### 2.2.8 Structural elucidation of reynosin (2.65)

Reynosin **(2.65)** was isolated from the $CH_2Cl_2$-MeOH extract of the thujone chemotype of *A. afra*. The solvent systems used as eluents were prepared from hexane and ethyl acetate. The crude extract was filtered before being subjected to repeated column chromatography to yield 22 mg of yellowish material. The data obtained from NMR experiments are summarised in Table 2.7. The $^1$H NMR spectrum (Plate 43) showed some impurities, but fifteen carbon signals could clearly be identified in the $^{13}$C NMR spectrum (Plate 44, Table 2.7). The $^{13}$C peaks could be assigned to a lactone carbonyl ($\delta_C$ 170.7), two exocyclic olefinic methylene ($\delta_C$ 110.6 and $\delta_C$ 142.4), two oxygen-containing methines ($\delta_C$ 78.3 and 79.6), one quaternary carbon ($\delta_C$ 43.0), two methines ($\delta_C$ 53.1 and 49.6), four methylene carbons ($\delta_C$ 35.8, 33.6, 31.3 and 21.5) and one methyl group ($\delta_C$ 11.7). It was clear that this compound was related to artemin **(2.59)**, but that the 5-hydroxy group was absent and that a double bond was present between C-11 and C-13. Structure **2.65** was proposed for this compound. A literature search revealed that **2.65** is the structure of reynosin, a compound first isolated in 1970 by Yoshioka *et al*. from *Ambrosia concertiflora* collected in Mexico.[59] This compound was also isolated from several other *Artemisia* species including *A. hispanica*,[60] *A. anomala*.[61] and *A. genipi* Weber, a highly-valued commercially plant due to its uses in liqueur-producing industries in Italy.[62]

**Table 2.7. $^{1}H$ and $^{13}C$ NMR of reynosin (2.65) in $CDCl_3$ (400 MHz).**

| Experimental results | | | | | | Literature results[62,63] | |
|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC** | **$\delta_C$** | **$\delta_H$** |
| 1 | 78.3 | CH | 3.52 (1H,dd, *J*a 4.6 Hz; *J*b 11.5 Hz) | | 14 | 78.3 | 3.56 (1H, dd, *J* 4.0, 10.2 Hz) |
| 2 | 31.3 | $CH_2$ | 1.84 (1H, m)<br>1.56 (1H, m) | | | 31.3 | |
| 3 | 33.6 | $CH_2$ | 2.32 (1H, m)<br>2.11 (1H, m) | | | 33.6 | |
| 4 | 142.4 | C | | | | 142.4 | |
| 5 | 53.1 | CH | 2.17 (1H, m) | 6 | 14, 15 | 53.0 | |
| 6 | 79.6 | CH | 4.02 (, t, *J* 10.8) | | | 79.7 | 4.08 (1H, t, *J* 10.8 Hz) |
| 7 | 49.6 | CH | 2.54 (1H, m) | | 13 | 49.6 | |
| 8 | 21.5 | $CH_2$ | 1.58 (1H, t)<br>0.87 (1H, t) | 5 | | 21.5 | |
| 9 | 35.8 | $CH_2$ | 2.09 (1H, m)<br>1.32(1 H, m) | | 14 | 35.7 | |
| 10 | 43.0 | C | | | 14 | 43.0 | |
| 11 | 139.3 | C | | | | 139.3 | |
| 12 | 170.7 | C | | | 13 | 170.7 | |
| 13 | 117.0 | $CH_2$ | 5.42 (1H, d, *J* 3.1 Hz)<br>6.08 (1H, d, *J* 3.3 Hz) | 7 | | 117.2 | 5.46 (1H, d, J 3.0Hz)<br>6.12 (1H, d, *J* 3.5 Hz) |
| 14 | 11.7 | $CH_3$ | 0.81 (3H, s) | | | 11.7 | 0.85 (3H, s) |
| 15 | 110.6 | $CH_2$ | 4.99 (1H, br s)<br>4.86 (1H, br s) | | | 110.7 | 5.03 (1H, br s, H-$15_a$)<br>4.90 (1H, br s, H-$15_b$) |




**2.65**

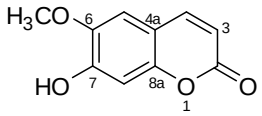
The $^{1}$H NMR and HMBC data indicated in Table 2.7 are in agreement with the structure and with the published data. The spectrum obtained from the HRMS showed the molecular ion (+ Na) peak of the isolated compound at m/z 271. 1309 $[M + Na]^{+}$. The calculated mass for $[C_{15}H_{20}O_3Na]$ was 271.1310. Studies proved that reynosin **(2.65)** has important biological activities such as inhibition of CINC-1 induction in LPS-stimulated rat kidney epithelioid NRK-52E cells.[64] It also showed inhibitory effect on the production of TNF-alpha in a dose-dependent manner.[65]

### 2.2.9 Structural elucidation of scopoletin (2.66)

Scopoletin (7-hydroxy-6-methoxycoumarin) (**2.66**) was isolated from the $CH_2Cl_2$-MeOH crude extract of the aerial parts of the *A. afra*, ketone chemotype. The extract was subjected to repeated silica gel column chromatography to yield white needles. When stained with anisaldehyde spray, the compound appeared blue in colour with $R_f$ 0.2 (hexane-EtOAc, 3:2). The NMR data of **2.66** are collated in Table 2.8.

Analysis of the $^{1}$H NMR spectrum of **2.66** (Plate 50) revealed that the compound has six different types of protons. A singlet was observed at $\delta_H$ 3.96 (3H, s) due to the presence of a methoxy group (OC**H$_3$**). Two doublets were observed at $\delta_H$ 7.60 (1H, d, *J* 9.5 Hz) and at $\delta_H$ 6.27 (1H, d, *J* 9.5 Hz). Two aromatic singlets resonated at $\delta_H$ 6.92 (1H, s) and at $\delta_H$ 6.85 (1H, s). A broadened signal resonating at $\delta_H$ 6.03 (1H, s) was indicative of a hydroxy group. The COSY spectrum (Plate 54, Table 2.8) showed $^{1}$H-$^{1}$H correlation between protons resonating at $\delta_H$ 6.27 and 7.60, respectively. These data are characteristics of a coumarin bearing two substituents, a methoxy group ($OCH_3$) and a hydroxy group (OH), on its aromatic ring. This was confirmed by the HMBC spectrum (plate 56) which showed $^{1}$H,$^{13}$C correlations between the methoxy group protons at $\delta_H$ 3.96 and a carbon resonating at $\delta_C$ 144.1. This indicates that the

methoxy group is attached to the last-mentioned carbon. The aromatic proton resonating at $\delta_H$ 6.85 correlated with carbons resonating at $\delta_C$ 111.4, 144.1, 149.8 and $\delta_C$ 150.3. The $^{13}C$ NMR spectrum (Plate 51) supported the above information and displayed 10 carbons at different chemical shifts such as the aromatic carbons at $\delta_C$ 103.3, $\delta_C$ 107.6, $\delta_C$ 111.4, $\delta_C$ 144.1, $\delta_C$ 149.8 and at $\delta_C$ 150.3. The carbonyl carbon of the lactone ring of the coumarin appeared at $\delta_C$ 161.6, while the methoxy group carbon resonated at $\delta_C$ 56.4 (O**C**$H_3$). The two other carbons of the lactone ring were located at $\delta_C$ 113.4 and at $\delta_C$ 143.3, respectively. The compound was assigned 7-hydroxy-6-methoxycoumarin **(2.66)**.




**2.66**

The mass spectrum of the isolated compound gave the accurate molecular mass for scopoletin **(2.66)** as 215.0317 $[M + Na]^+$, which is in agreement with the calculated value of 215.0320 for $C_{10}H_8O_4Na$.

Scopoletin **(2.66)** was also isolated by other researchers from *A. afra*, the first being Goodson[66] in 1922. Marco *et al*.[21] reported the isolation of this compound from *A. herba-alba* in 1989 and recently, scopoletin **(2.66)** has been isolated from *A. campestris subsp. maritima* by Vasconclos *et al*.[67] Scopoletin **(2.66)** also occurs in many other Asteraceae species.

The different biological activities and other uses of scopoletin **(2.66)** have been reported. Khan *et al*. and Chin-Ying *et al*. isolated the compound from *Cotoneaster racemiflora* and *Sinomonium actum*, respectively, and showed that it has antioxidant activity. [68, 69] The compound also manifested significant anti-inflammatory and antimicrobial activities when isolated by Yu *et al*. from *Molinda citrifolia* fruits and Kashihara from *Euphorbia humifuza*,[70,71] respectively. Panda and Kar reported its antithyroid and antihyperglycemic activity in hyperthyroid rats, after isolating **2.66** from *Aegle marmelos.*[72] Other properties include immunomodulatory effects and hepatoprotective action, as demonstrated by Manuele *et al*. and Kang *et al*.,[73,74] respectively.

**Table 2.8. $^{1}H$ and $^{13}C$ NMR data of scopoletin (2.66) in $CDCl_3$ (400 MHz).**

| Experimental | | | | | | Literature[67] | |
|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC correlation** | **$\delta_C$** | **$\delta_H$** |
| 2 | 161.6 | C | | | 3, 4 | 161.5 | |
| 3 | 113.4 | CH | 6.27 (d, *J* 9.5 Hz) | 4 | | 113.4 | 6.28 (d, *J* 9.5) |
| 4 | 143.3 | CH | 7.60 (d, *J* 9.5 Hz) | 3 | 5 | 143.3 | 7.60 (d, *J* 9.5 Hz) |
| 4a | 111.4 | C | | | 8, 3 | 111.5 | |
| 5 | 107.6 | CH | 6.92 (1H, s) | | 4 | 107.4 | 6.85 (s) |
| 6 | 144.1 | C | | | 8, 6-$OCH_3$ | 144.0 | |
| 7 | 149.8 | C | | | 8, 5 | | |
| 8 | 103.3 | CH | 6. 85 (1H, s), | | | 103.2 | 6. 92 (s), |
| 8a | 150.3 | C | | | 8 | 150.2 | |
| 6-$OCH_3$ | 56.4 | $CH_3$ | 3.96 (3H, s) | | | 56.4 | 3.95 (s) |
| 7-OH | | | 6.03 (1H, s) | | | | 6.17 (s) |

### 2.2.10 Structural elucidation of *p*-hydroxyacetophenone (2.67)

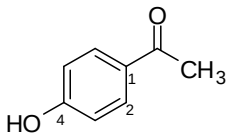
*p*-Hydroxyacetophenone was isolated from the ketone chemotype crude extract. After filtration, the crude extract was repeatedly chromatographed on silica gel using columns of different sizes, eluting with solvent system of increasing polarities from hexane-EtOAc (8:2) to (3:2). Both isocratic and stepwise elution were used and finally 65 mg of white needles were obtained and dried before spectroscopic analysis. The NMR data are given in Table 2.9.

The $^{1}$H NMR spectrum (Plate 57) was simple and informative. In the aromatic region two distinct doublets were observed at $\delta_H$ 7.90 (1H, d, *J* 8.8 Hz, and at $\delta_H$ 6.90 (1H, d, *J* 8.8 Hz). The presence of only two doublets with a coupling constant characteristic of *ortho* coupling indicated that the aromatic ring is substituted at the *para* position and hence the ring has a plane of symmetry. This was confirmed by the COSY spectrum (Plate 61) of **2.67**, whereby a proton resonating at $\delta_H$ 7.90 was coupled to proton resonating at $\delta_H$ 6.90 and *vice versa*. A singlet observed at $\delta_H$ 2.56 (3H, s) was assigned to an acetyl methyl group attached to the aromatic ring as one of the two substituents. A broad singlet was observed at $\delta_H$ 13.94 (1H, s) and was attributed to an OH group. Hence the second substituent was identified and the structure of **2.67** was assigned.

In the $^{13}$C NMR spectrum (Plate 58), six signals were observed; but according to the above-obtained information, the compound has eight carbon atoms. Due to the symmetry, as has been mentioned above, only six of them were observed on the spectrum. The DEPT135 spectrum (plate 59) showed three signals, two aromatic signals attributed to four protonated aromatic carbon atoms, two resonating at $\delta_C$ 131.1 and the other two at $\delta_C$ 115.5. The third signal was at high field of the spectrum ($\delta_C$ 26.5) and was obviously due to the acetyl methyl group. The signal at $\delta_C$ 197.2 was attributed to a carbonyl carbon of the acetyl group. These data agreed with the structure of *p*-hydroxyacetophenone **(2.67)**.

The mass spectrum exhibits a signal at *m/z* 159.0420 $[M + Na]^+$, confirming the molecular formula of $C_8H_8O_2Na$ (calculated value 159.0446). This is the first report

on the isolation of this compound from *A. afra*, but it has been isolated from several other *Artemisia* species.




**2.67**

**Table 2.9. $^{1}$H and $^{13}$C NMR data of *p*-hydroxyacetophenone (2.67) in $CDCl_3$ (400 MHz).**

| Experimental results | | | | | |
|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC Correlation** |
| 1 | 130.3 | C | | | 2, 3, OC-**$CH_3$** |
| 2, 6 | 131.1 | CH | 7.90 (2H, d, *J* 8.8 Hz) | 3 | 3, OC-**$CH_3$** |
| 3, 5 | 115.5 | CH | 6.90 ( 2H, d, *J* 8.8Hz) | 2 | 2 |
| 4 | 160.6 | C | | | 2, 3 |
| O=C | 197.2 | C | | | 2, OC-**$CH_3$** |
| OC-**$CH_3$** | 26.5 | $CH_3$ | 2.56 (3H, s) | | |
| 4-OH | | | 13.94 (1H, s) | | |

### 2.2.11 Structural elucidation of 2,4-dihydroxy-6-methoxyacetophenone (2.68)

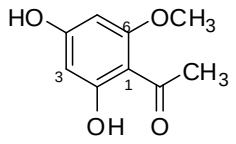
2,4-Dihydroxy-6-methoxyacetophenone was isolated from the $CH_2Cl_2$-MeOH extract of the dried aerial parts of the ketone chemotype of *A. afra*. Using column chromatography and eluting with different solvents of increasing polarities, 27 mg of whitish crystals were obtained, giving a single spot on a TLC plate with a $R_f$ 0.32 in hexane-EtOAc (7:3). The compound appeared orange when stained with the anisaldehyde stain reagent.

The NMR data of **2.68** are collated in Table 2.10. An analysis of $^{1}$H NMR spectrum (Plate 64) showed four main signals. There were two aromatic doublets, one at $\delta_H$ 5.98 (1H, d, *J* 2.4 Hz) and another at $\delta_H$ 5.91 (1H, d, *J* 2.4 Hz). The weak coupling (2.4 Hz) indicated that the two protons are in a *meta* position to each other on the

aromatic ring. A singlet was observed at $\delta_H$ 3.86 (3H, s), which demonstrated the presence of a methoxy group attached to the aromatic ring. Finally, a singlet appeared at $\delta_H$ 2.60 (3H, s), due to the presence of the acetyl methyl protons. A broad singlet at $\delta_H$ 13.94 was assigned to the OH group.

The $^{13}C$ NMR spectrum (Plate 65) exhibited nine peaks of which four are quaternary carbons; one signal at $\delta_C$ 203.4 was assigned to the carbonyl carbon of the acyl group. while the chemical shifts of aromatic carbon were at $\delta_C$ 106.5, 167.2, 96.6, 162.7, 90.8, and 163.7. The methoxy carbon resonated at $\delta_C$ 55.6, while the acyl methyl carbon was observed at $\delta_C$ 32.9. The fact that all six aromatic carbons were observed separately, indicated that the aromatic ring was not substituted symmetrically.

Three different oxygenation patterns for compound **2.68** were considered: 2,4,6, 2,3,5 and 3,4,5. A chemical shift of $\delta_H$ 13.94 for a hydroxyl proton indicated hydrogen bonding with the carbonyl oxygen, therefore excluding a 3,4,5 oxygenation pattern. Furthermore, if a hydrogen was present on C-6, the anisotropic effect of the carbonyl would have caused deshielding of this proton. Since no substantial chemical shift difference was observed for the two *meta*-coupled aromatic protons, the 2,3,5-oxygenation pattern was also excluded and the structure of the compound was assigned as 2,6-dihydroxy-6-methoxyacetophenone **(2.68)**. The molecular mass was obtained from the GC-MS spectrum in which a peak was observed at $[M]^+$ 182.02, which is the molecular mass for $C_9H_{10}O_4$. Although **2.68** was isolated from other *Artemisia* species, the presence of the compound in *A. afra* has not been reported previously.




**2.68**

**Table 2.10. $^{1}H$ and $^{13}C$ NMR data of 2,4-dihydroxy-6-methoxyacetophenone (2.68) in $CDCl_3$ (400 MHz).**

| Experimental results | | | | | |
|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC correlation** |
| 1 | 106.5 | C | | | |
| 2 | 167.2 | C | | | |
| 3 | 96.6 | CH | 5.98 (1H, d, *J* 2.4 Hz) | 5 | |
| 4 | 162.7 | C | | | |
| 5 | 90.8 | CH | 5.91 (1H, d, *J* 2.4 Hz) | 3 | |
| 6 | 163.7 | C | | | |
| O=**C**-$CH_3$ | 203.4 | C | | | OC-**$CH_3$** |
| OC-**C**$H_3$ | 32.9 | $CH_3$ | 2.60 (3H, s) | | |
| 6-**$OCH_3$** | 55.6 | $OCH_3$ | 3.86 (3H, s) | | |
| O**H** | | | (13.94, s) | | |

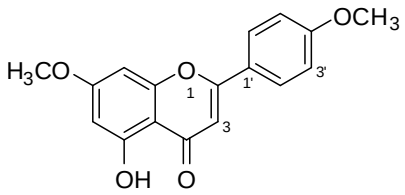
### 2.2.12 Structural elucidation of 5-hydroxy-7,4′-dimethoxyflavone (2.69)

A $CH_2Cl_2$-MeOH (1:1) extract of the aerial parts of thujone chemotype of *A. afra* was chromatographed on silica gel, using successively hexane-EtOAc (8:2) and (7:3) by isocratic and stepwise elution. This process afforded reddish crystals of **2.69**, which was one of the major compounds isolated from the extract. The NMR data of **2.69** are given in Table 2.11.

The $^{1}H$ NMR spectrum (Plate 71) showed four aromatic doublets, two with *ortho* coupling, at $\delta_H$ 7.85 (1H, d, *J* 8.0 Hz) and at $\delta_H$ 7.02 (1H, d, *J* 8.0 Hz) and two with *meta* coupling at $\delta_H$ 6.37 (1H, d, *J* 2.2 Hz) and at $\delta_H$ 6.49 (1H, d, *J* 2.2 Hz). Two methoxy singlets resonated at $\delta_H$ 3.89 and $\delta_H$ 3.88, respectively. Another singlet was observed at 12.83 (1H, br. s) indicating the presence of a hydroxy group (OH). The HMBC spectrum (Plate 77) exhibited $^{1}H$-$^{13}C$ correlations between aromatic protons resonating at $\delta_H$ 7.85, 7.02, 7.02, and the aromatic carbon resonating at $\delta_C$ 162.6. Protons at $\delta_H$ 7.85, 7.02, 6.58 were also correlated to a carbon resonating at $\delta_C$ 124.0. The methoxy group protons at $\delta_H$ 3.88 correlated with the quaternary carbon at $\delta_C$ 165.7. The above-obtained experimental data were characteristic of a flavonoid bearing one methoxy group on each of its two aromatic ring. The *meta* coupling observed between protons resonating at $\delta_H$ 6.37 and $\delta_H$ 6.50 indicated that the position in between them was occupied by a methoxy group on one of the aromatic ring of the flavonoid. The presence of two doublets, with *ortho* coupling, instead of four,

indicated that there was a plane of symmetry created by a methoxy group between protons of the second aromatic ring of the flavonoid.

The $^{13}C$ NMR spectrum (Plate 72) of **2.69** contained 15 carbons atoms instead of 17. This supported the idea of the existence of the symmetry in the second aromatic ring of the isolated compound due to the methoxy group resonating at $\delta_C$ 55.5 and attached to C-4′ ($\delta_C$ 162.6). This would mean that C-2′ and C-6′ both resonated the same chemical shift ($\delta_C$ 128.1). This assumption might also be true for C-3′ and C-5′ both resonating at $\delta_C$ 114.7. The peak observed at $\delta_C$ 182.7 might be due to a carbonyl group of the flavonoid. Therefore, the isolated compound was assigned as 5-hydroxy-7,4′-dimethoxyflavone **(2.69)**.




**2.69**

The HR-MS spectrum exhibited a molecular ion peak (+Na) at 321.0735$[M + Na]^+$. The calculated mass for $C_{17}H_{14}O_5Na$ was 321.0739. The isolation of 5-hydroxy-7,4′-dimethoxyflavone or the 7,4′-dimethyl ether of apigenin called 4′-*O*-methylgenkwanin **(2.69)** was previously reported by Nkunya *et al.* from *A. afra*.[52] This compound was also isolated from *Artemisia pontica* collected in Kazakhstan by Talzhanov *et al.*[75] The published NMR data are similar to the experimental data obtained in the current investigation.

### 2.2.13 Comparison of the thujone and ketone chemotypes of *A. afra*

Based on the present investigation, the phytochemistry of the two chemotypes does not differ significantly. Most of compounds isolated from one chemotype could be obtained from the other. However, the TLC analysis of $CH_2Cl_2$-MeOH extract (Fig. 2.15) revealed some minor differences. These include unequal concentration of chemical components and a slight difference in the number of constituents. For instance, a component at $R_f$ 0.29 is more concentrated in the ketone type than in the thujone type.

**Table 2.11. $^{1}H$ and $^{13}C$ NMR data of 5-hydroxy-7,4′-dimethoxyflavone (2.69) in $CDCl_3$ (400 MHz).**

| Experimental: *A. afra* | | | | | | Litterature[75]: *A. pontica* | |
|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC correlation** | **$\delta_C$** | **$\delta_H$** |
| 2 | 164.0 | C | | | 3 | 163.5 | |
| 3 | 104.5 | CH | 6.58 (1H, s,) | | 6 | 103.6 | 6.9 (1H, s) |
| 4 | 182.7 | C | | | 3 | 181.8 | |
| 4a | 105.6 | C | | | OH, 3, 6 | 104.5 | |
| 5 | 162.3 | C | | | OH, 8 | 157.2* | |
| 6 | 98.2 | CH | 6.37 (1H, d, *J* 2.2 Hz) | | OH, 8 | 97.9 | 6.38 (1H, d, *J* 2.2 Hz) |
| 7 | 165.5 | C | | | $OCH_3$ | 165.1 | |
| 8 | 92.8 | CH | 6.49 (1H, d, *J* 2.2 Hz) | | 6 | 92.6 | 6.78 (1H, d, *J* 2.2 Hz) |
| 8a | 157.8 | C | | | 6 | 161.1* | |
| 1′ | 124.0 | C | | | 3, 3′, 5′ | 122.6 | |
| 2′, 6′ | 128.1 | CH | 7.85 (1H,d, *J* 8.0 Hz) | 3′, 5′ | 6′ | 128.2 | 8.06 (2H, d, *J* 8.8 Hz) |
| 3′,5′ | 114.7 | CH | 7.02 (1H, d, *J* 8.0 Hz) | 2′, 6′ | 5′ | 114.5 | 7.13 (2H, d, *J* 8.8 Hz) |
| 4′ | 162.6 | C | | | $OCH_3$, 2′, 3′ 5′ | 162.3 | |
| 7-$OCH_3$ | 55.8 | $CH_3$-C7 | 3.88 (3H, s) | | | 55.9 | 3.87 (3H, s) |
| 4′-$OCH_3$ | 55.5 | $CH_3$-C8 | 3.89 (3H, s) | | | 55.4 | 3.88 (3H, s) |
| 5-OH | | | 12.83 (1H, br. s) | | | 5-OH | 12.89 (1H, br. s, 5-OH) |

* Assignments wrong in the literature

The one at $R_f$ 0.38 is present in the thujone type and almost absent in the ketone type. The number of observable main components on stained and heated TLC is 10 for the thujone and 8 for the ketone type.

Careful examination of the TLC of $CH_2Cl_2$-MeOH crude extracts spotted together with isolated compounds (Fig. 2.13), although not a very accurate method, lead to the following observations:

- ☞ Isolated compounds such as artesin, artemin, *p*-hydroxyacetophenone, 2,4-dihydroxy-6-methoxyacetophenone, are present in higher concentration in the ketone type than in the thujone type
- ☞ The scopoletin (7-hydroxy-6-methoxycoumarin) is present in higher concentration in the thujone than in the ketone chemptype.

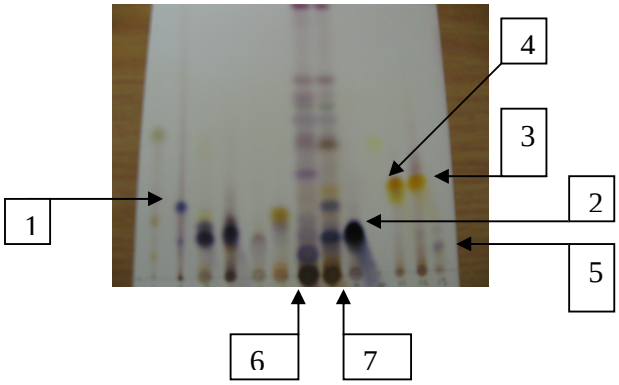



**Figure 2.13.** TLC (hexane-EtOAc, 7:3): the $CH_2Cl_2$-MeOH thujone (6) and ketone (7) crude extracts spotted together with isolated compounds.

Where: 1= artesin 2 = artemin, 3 = 2,4-dihydroxy-6-methoxyacetophenone
4 = *p*-hydroxyacetophenone 5 = scopoletin

Based on the appearance of spots as observed on TLC (Fig. 2.13), and the yield of isolated compounds, Table 2.12 could be constructed.

**Table 2.12**. Presence of isolated compounds in the ketone and thujone chemotypes of *A. afra*.

| N$^{o}$ of isolated compounds | **2.56** | **2.57** | **2.58** | **2.59** | **2.61** | **2.62** | **2.65** | **2.66** | **2.67** | **2.68** | **2.69** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Presence in ketone type | + | ++ | + | + | +- | +- | +- | + | ++ | ++ | + |
| Presence in thujone type | + | + | + | ++ | +- | +- | +- | ++ | + | + | + |

Where: + = Compound present in reasonable concentration

++ = Compound present at high concentration

+- = Compound present, but the extent at which it occurs cannot be predicted on TLC plate

## 2.3. CONCLUSION

Eleven compounds have been identified in the *A. afra* extracts. These include seven eudesmane-type sesquiterpenoids, taurin **(2.56)**, artesin **(2.57)**, maritimin **(2.58)**, artemin **(2.59)**, norsantolinifolide **(2.61)**, santolinifolide **(2.62)** and reynosin **(2.65)**, the coumarin scopoletin **(2,66)**, two acetophenones, *p*-hydroxyacetophenone **(2.67)** and 2.4-dihydroxy-6-methoxyacetophenone **(2.68)**, and a flavonoid, 5-hydroxy-7,4′-dimethoxyflavone **(2.69)**. Only compounds **2.56**, **2.60** and **2.69** have been reported previously from *A. afra*.

Preliminary TLC investigations indicated that the isolated compounds are present in both the ketone and thujone chemotypes, although at different concentrations. The aim of this investigation, i.e. to identify the non-volatile secondary metabolites in two chemotypes of *A. afra*, has been accomplished.

Time did not permit the development of a HPLC-MS method in order to determine the accurate concentrations of the metabolites in the two extracts. Future studies on this project will include:

- Development of a HPLC-MS method for the quantification of sesquiterpenes and phenolic compounds in *A. afra*.
- Analyse samples of *A. afra* collected monthly for one year and determine the annual fluctuation in concentration of the sesquiterpenoids in the two chemotypes.

## 2.4 EXPERIMENTAL PROCEDURES

### 2.4.1 General methods

All separations were monitored by thin-layer chromatography (TLC), using silica gel plates (Kieselgel 60 $F_{254}$ Merck alminium sheets) cut into strips of various sizes. After development, TLC Plates were visualised with the aid of UV radiation (254 nm or 366 nm) and were subsequently stained with reagents such as iodine or anisaldehyde (0.5 mL *p*-anisaldehyde in 85 mL of methanol containing 4 mL of sulfuric acid and 10 mL of glacial acetic acid). Sprayed plates were heated by means of a heat gun to allow for colour development.

Normal and flash column chromatography were carried out using silica gel 60 (Merck 0.040-0.063 mm). Columns of various sizes, ranging from 2 cm to 5 cm in diameter were used.[76] Crude extracts were filtered through a plug to remove streaking and solid material, prior to proper column chromatography. The filtered extract and combined fractions were dissolved in a small amount of solvent and carefully loaded onto the column using Pasteur pipettes. Both dry and slurry-packed columns were used and the eluents were composed of a mixture of two or more of the following solvents: hexane, dichloromethane, ethyl acetate, acetone and methanol; in different ratios. Isocratic and stepwise types of elution were employed. Mixed fractions were subsequently separated with centrifugal thin-layer chromatography on a Chromatotron 7924T-01. The chromatotron plates were prepared using silica gel Merck 7749 with gypsum binding agent.

Nuclear magnetic resonance spectroscopy (NMR) experiments of the isolated compounds were performed on a Bruker Avance III 400 MHz spectrometer. Samples were dried on a high-vacuum pump before being subjected to NMR. All NMR spectra were recorded in deuterated chloroform ($CDCl_3$) at 25 $^{o}$C. Tetramethylsilane (TMS)

was used as internal reference and all coupling constants (*J)* are reported in Hz. Structures were determined by analysis of 1D ($^{1}$H and $^{13}$C NMR spectra) and 2D (HSQC, HMBC and COSY) NMR spectra. A comparison of experimental values with reported data was also established.

Infrared spectra of isolated compounds were recorded on a Perkin-Elmer spectrometer as a nujol mix. Mass spectra were obtained on an ion trap Thermofinnigan Polaris Q mass spectrometer with electron-impact ionisation (low resolution) or on a time-of-flight Waters LCT spectrometer with electrospray ionisation (positive or negative mode, high resolution). Optical rotations were measured, using suitable spectroscopic solvents, on a Perkin-Elmer 241 polarimeter. Rotations were recorded using the Na lamp at 589 nm. Melting points (Mp) were recorded using a Kofler hot stage melting point apparatus.

### 2.4.2 Plant material

Two chemotypes of *Artemisia afra*, the ketone type and the thujone type were cultivated on a farm in Tongaat, KwaZulu-Natal under the supervision of Dr. Maria de Figueiredo, Agriculture Research Centre, Cedara. The cultivated plants originated from two plants which were collected from the wild in KwaZulu-Natal. The aerial parts of *A. afra* were collected October 2007, air dried at room temperature and ground to yield 1.7 kg and 1.6 kg powdered material of the ketone type and thujone, respectively.

### 2.4.3 Extraction

The milled plant material was extracted using organic solvents for 24 hours at room temperature, filtered, and the solvent evaporated by rotavapor to yield the crude extract.

For each chemotype, a small scale extraction has been carried out using different solvents such as hexane, ethyl acetate, dichloromethane, methanol and a mixture of dichloromethane and methanol (1:1) to establish the optimum solvent(s) for extraction. Every time, 20 g of the plant material were soaked in solvent, left overnight and the yield of crude extract determined.

After the small-scale extraction, a large-scale extraction was performed. The powder obtained for the two chemotypes was extracted consecutively with hexane and a 1:1 mixture of $CH_2Cl_2$ and MeOH. After being extracted with one solvent, the residue of the plant material was air dried in the laboratory and extracted again with another solvent more polar than the previous one (Fig. 2.14). The average extraction period for each solvent was 24 hours. From this extraction, the yield of hexane crude extract was 45 g and 30 g, respectively, for the ketone and the thujone chemotypes, whereas the $CH_2Cl_2$-MeOH extracts were 128 and 100 g. The following figure illustrates the process.

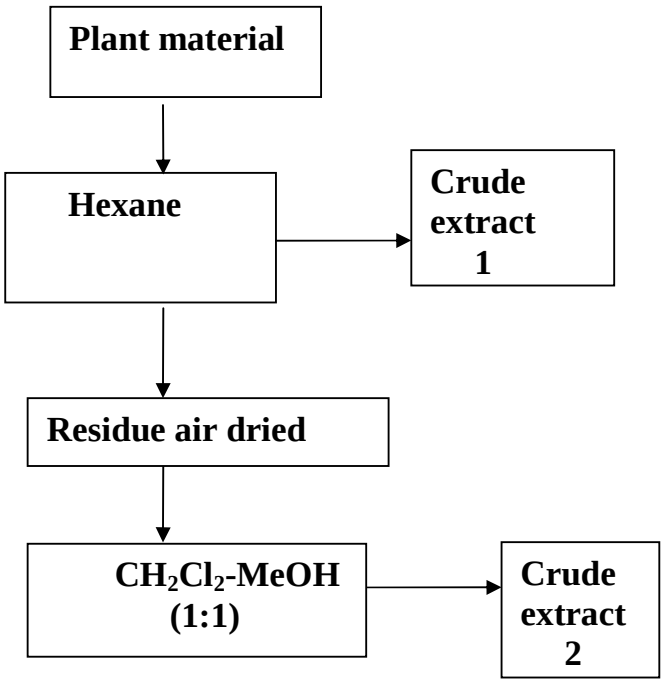



**Figure 2.14.** Differential solvent extraction.

A portion of the hexane extract (10 g) was then dissolved in small volume of hexane and filtered through a short silica gel column eluting with hexane. This separation removed all solid and streaking impurities. The resulting fraction was spotted on a TLC plate using hexane-EtOAc (7:3) as the solvent system. The plate was visualized under UV (366 nm and 254 nm) and stained with anisaldehyde stain reagent. The thujone hexane extract showed about 10 spots with different colours, while for the

ketone extract, about 7 coloured components were observed. Among them 7 for thujone chemotype and 5, for ketone chemotype, were the major components.

For $CH_2Cl_2$-MeOH extract, a small amount (10 g) was dissolved in small volume of ethyl acetate and also filtered through a plug, eluting with methanol. The plug was pressurized with a compressor to overcome the problem of high content in streaking material, preventing the mobile phase from moving down through the column. The obtained fraction was spotted on a TLC plate and when visualized under UV, and heated after being soaked in anisaldehyde dip reagent (Fig 2.15), it showed 10 components for thujone and 8 for ketone chemotypes. 7 constituents at $R_f$ 0.11, $R_f$ 0.33, $R_f$ 0.38, $R_f$ 0.55, $R_f$ 0.63, $R_f$ 0.78 and $R_f$ 0.92 (hexane-EtOAc, 7:3), were major for thujone, while for ketone all 8 constituents at $R_f$ 0.15, $R_f$ 0.29, $R_f$ 0.33, $R_f$ 0.48, $R_f$ 0.55, $R_f$ 0.63, $R_f$ 0.78 and $R_f$ 0.92 (hexane-EtOAc,7:3), appeared to be major.


**Figure 2.15**. TLC (hexane-EtOAc, 7:3) of *A. afra* $CH_2Cl_2$-MeOH crude extracts. At left: three different concentrations of the thujone type extract. At right: three different concentrations of the ketone type extract.

The main constituents observed in both the hexane and the $CH_2Cl_2$-MeOH extracts could be isolated using suitable methods with selected solvent systems.

### 2.4.4 Isolation of compounds

#### 2.4.4.1 Isolation of taurin (2.56)

Taurin or 1-oxo-6β,7α,11β-H-selin-4(5)-en-6,12-olide **(2.56)** was isolated from the hexane extract of the ketone type. After filtration of 10 g of crude extract as described above, the solvent was evaporated from the fraction obtained. The material was again dissolved in small quantity of hexane and carefully loaded onto a slurry packed silica gel column, eluting with hexane-ethyl acetate (3:2). 90 Fractions were collected, 10 mL each. After inspection by TLC, fractions 25-48 showed one spot under UV light and were combined and the solvent removed under vacuum. The resulting material was again inspected with TLC to make sure of a single spot. The TLC plate was stained with anisaldehyde spray reagent and heated with a heat gun. A single yellow spot with $R_f$ 0.6 (hex-EtOAc, 3:2) appeared. The material was left in a round-bottomed flask for a while and it crystallized. It was dried on high vacuum pump and the yield was determined to be 205 mg of white needle-like crystals. The identification of the compound was based on spectroscopic data.

| | |
|---|---|
| Appearance: | white needle-like crystals |
| Empirical formula: | $C_{15}H_{20}O_3$ |
| Melting point: | 110-112 $^{o}$C, hexane (lit.[51] 116-118 $^{o}$C) |
| Optical rotation: | $[\alpha]_D^{30}$ -56 (c = 0. 615, MeOH) (lit.[51] , -115) |
| $^{1}$H NMR ($CDCl_3$) $\delta_H$: | |
| | 4.57 (1H, d, *J* 11.0 Hz, H-6), 1.70 (qd, *J* 11.8, 3.3 Hz) 2.61 (1H, m), 2.45 (1H, m), 2.36 (1H, m), 2.30 (1H, m), 1.97 (1H, m, 8-H *eq*), 1.83 (1H, dt, *J* 13.3, 2.6 Hz, H-9 *eq*), 1.58 (1H, qd *J* 12.8, 3.5 Hz, H-8 *ax*) 1 1.50 (1H, td, *J* 13.3, 4.2 H-9 *ax*), 2.27(1H, dq, *J* 12.2, 7.0 Hz), 1.21 (3H, d, *J* 7.0 Hz, 13-$CH_3$), 1.30 (3H, s, 15-$CH_3$), 1.94 (3H, s, 14-$CH_3$). |
| $^{13}$C NMR ($CDCl_3$) $\delta_C$: | |
| | 213.6 (s, C=O), 33.0 (t, C-2, $CH_2$), 36.0 (t, C-3, $CH_2$), 127.0 (s, C-4), 129.6 (s, C-5), 81.8 (d, C-O), 52.9 (d, C-7), 23.9 (t, C-8), 34.8 (t, C-9), 48.9 (s, C-10), 41.0 (d, C- |

11), 178.7 (s, O**C=O**), 12.4 (q, C-13), 23.5 (q, C-14), 20.0 (C-15)

ESI-MS (positive mode) *m/z*: 271.1300 $[M + Na]^+$ (Calc. for $C_{15}H_{20}O_3Na$: 271.1310).

### 2.4.4.2 Isolation of artesin (2.57)

A filtered fraction (10 g) was separated by column chromatography. It was dissolved in small volume of hexane and transferred into a slurry packed silica gel column. A hexane–EtOAc (6:4) solvent mixture was used as the eluent and 10 mL fractions were collected in vials. Fractions 65-81 showed a single spot on a TLC plate and were combined in a round-bottomed flask to remove solvent on a rotavapor. 20 mg of pure material with $R_f$ 0.4 hexane-EtOAc (3:2), were obtained and dried. The pure compound isolated **(2.57)** had the following physical characteristics:

| | |
|---|---|
| Appearance: | white feather-like crystals |
| Empirical formula: | $C_{15}H_{21}O_3$ |
| Melting point: | 150-152 $^oC$, hexane (Lit.[21] 177 $^oC$) |
| Optical rotation: | $[\alpha]_D^{30}$ +18 (c = 0. 0.260, MeOH) |
| $^1H$ NMR ($CDCl_3$) $\delta_H$: | |
| | 4.57 (1H, d, *J* 11.0 Hz, H-6), 3.54 (1H, dd, *J* 14.4, 4.4 Hz, H-1), 2.35 (1H, m), 2.25 (m) 2.05 (m) 2.04 (m), 1.96 (m), 1.85 (3H, s, 15-$CH_3$), 1.73 (m), 1.26 (m) 1.24 (1H, d, *J* 7.0 Hz, 13-$CH_3$), 1.12 (3H, s, 14-$CH_3$). |
| $^{13}C$ NMR ($CDCl_3$) $\delta_C$: | |
| | 77.7 (d, COH), 27.1 (t, C-2), 33.2 (t, C-3), 126.0 (s, C-4), 129.9 (s, C-5), 83.2 (d, CH-O), 52.8 (d, C-7), 24.5 (t, C- 8), 38.3 (t, C-9), 41.9 (s, C-10), 41.1 (d, C-11), 178.9 (s, C-12), 12.3 (q, C13), 18.4 (q, C-14), 19.7 (q, C-15). |

ESI-MS (positive mode) *m/z*: 273.1472 $[M + Na]^+$ (Calc. for $C_{15}H_{21}O_3Na$: 273.1467)

IR $\nu_{max}$ /$cm^{-1}$: 3350 (OH), 1625 (C=O), 1115, 1076, 966

### 2.4.4.3 Isolation of maritimin (2.58)

Maritimin **(2.58)** was obtained after repeated column chromatography of 10 g of crude extract of the ketone chemotype. After filtration, the extract was dissolved in a minimum volume of ethyl acetate and transferred with a Pasteur pipette into slurry packed silica gel column, eluting with hexane–EtOAc (7:3) 52 fractions, 15 mL each, were collected. After inspection of all fractions on TLC plate, fractions 31 to 52 were combined and solvent removed to give impure material. The above-mentioned procedure was repeated using hexane-EtOAc (8:2) as the solvent system to obtain 239 mg of impure crystals. This material was subsequently purified on a small silica gel column (2 cm in diameter) using hexane-EtOAc (7:3) as eluent. 50 vials, 10 mL each were collected. Inspection of different fractions by TLC revealed the identity of fractions 37-50. The latter were combined and the solvent evaporated to yield 15 mg of maritimin **(2.58)**. The $R_f$ was measured to be 0.2 in hexane-EtOAc (7:3).

| | |
|---|---|
| Appearance: | tiny powder- like white crystals |
| Empirical formula: | $C_{15}H_{20}O_4$ |
| Melting point: | 169-171 $^oC$, MeOH (lit.[51] 176-178 $^oC$) |
| Optical rotation: | $[\alpha]_D^{30}$ -12 (c = 0. 480 , MeOH) (lit.[51], -42) |
| $^1H$ NMR ($CDCl_3$) $\delta_{H:}$ | |
| | 4.32 (1H, d, *J* 11.0 Hz, H-6), 2.38 (2H, m), 2.25 (m), 2.18 (2H, m), 2.00 (1H, m), 1.99 (m), 1.74 (m), 1.69 (3H, s, 15-$CH_3$), 1.27 (3H, d, *J* 7.0 Hz, 13-$CH_3$), 1.24 (3H, s, 14-$CH_3$). |
| $^{13}C$ NMR ($CDCl_3$) $\delta_C$: | |
| | 210.8 (s, C=O), 31.0 (t, C-2), 33.5 (t, C-3), 66.9(s, C-O), 63.7 (s, C-O), 77.2 (d, CH-O), 48.4 (d, C-7), 23.0 (t, C-8), 28.0 (t, C-9), 49.2 (s, C-10), 40.6 (d, C-11), 177.9 (C=12), 12.3 (q, C-13), 20.7 (q, C-14), 19.4 (q, C-15). |

ESI-MS (positive mode) *m/z*: 287.1259 $[M + Na]^+$ (Calc. for $C_{15}H_{20}O_4Na$: 287.1259

IR $\nu_{max}$ /$cm^{-1}$: 3368, 3171, 1781 and 1708 (γ-lactones) 3368, 3171, 1781, 1708, 1624 (ketone), 1035, 1156, 1624, 1035, 1156.

#### 2.4.4.4 Isolation of scopoletin (2.66)

Scopoletin or 7-hydroxy-6-methoxycoumarin **(2.66)** was isolated from the ketone chemotype. The $CH_2Cl_2$-$CH_3OH$ (1:1) extract was filtered through a plug and a portion (10 g) was repeatedly separated by column chromatography using varying polarity eluents prepared from hexane-EtOAc respectively (7:3, 8:2, 7:3) and 6:4). For the last column chromatography, 201 vials, 5 mL each, were collected. Fractions in vials 81-100, after being spotted on TLC plate, were visualised with UV radiations and a single blue spot was clearly observed. They were combined and the solvent removed to afford 10 mg of needles. The obtained material was re-spotted together with the crude extracts of both thujone and ketone chemotype, to make sure of the presence of single component (Fig. 2.16).

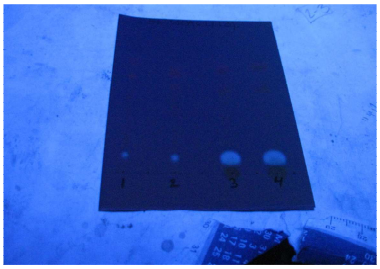


**Figure 2.16.** Scopoletin TLC visualised under UV radiation (254 nm): at the left, crude extracts respectively of thujone and ketone chemotype. At the right, pure scopoletin.

TLC revealed the presence of scopoletin **(2.66)** in both the chemotypes of *A. afra*. Its physical characteristics are summarized hereunder.

Appearance: white needle-like crystals
Empirical formula: $C_{10}H_8O_4$
Melting point: 201-203 $^oC$, MeOH (lit.[67] 205-207 $^oC$)

$^{1}$H NMR ($CDCl_3$) $\delta_{H:}$

7.60 (1H, d, *J* 9.5 Hz, H-4), 6.92 (1H, s, H-5), 6.85 (1H, s, H-8), 6.27 (1H, d, *J* 9.5 Hz, H-3), 6.03 (1H, s, 7-OH), 3.96 (3H, s, OC**H$_3$**).

$^{13}$C NMR ($CDCl_3$) $\delta_C$:

161.6 (s, C=O), 113.4 (d, C-3), 143.3 (d, C-4), 111.4 (s, C-4a), 107.6 (d, C-5), 144.1 (s, C-6), 149.8 (s, C-7), 103.3 (d, C-8), 150.3 (s, C-8a), 56.4 (q, $OCH_3$)

ESI-MS (positive mode) *m/z*: 215.0317 $[M + Na]^+$ (Calc. for $C_{10}H_8O_4Na$ : 215.0320)

### 2.4.4.5 Isolation of *p*-hydroxyacetophenone (2.67)

Ten grams of $CH_2Cl_2$-MeOH extract was subjected to repeated liquid chromatography eluting with hexane-EtOAc solvent systems of different polarities. 380 mg of impure material was obtained and again chromatographed using hexane-EtOAc (7:3) and 65 mg of needles were obtained with $R_f$ 0.32 hexane-EtOAc (7:3). The spectroscopic analysis showed the compound to be *p*-hydroxyacetophenone **(2.67)**.

Appearance: white needle-like crystals

Empirical formula: $C_8H_8O_2$

Melting point: 111-113 $^{o}$C, MeOH (lit.[77] 109-111 $^{o}$C)

$^{1}$H NMR ($CDCl_3$) $\delta_{H:}$

7.90 (1H, d, *J* 8.8 Hz), 6.90 (1H, d, *J* 8.8 Hz), 2.56 (3H, s, OC-C**H$_3$**)

$^{13}$C NMR ($CDCl_3$) $\delta_C$:

160.6 (s, C-1), 115.5 (d, C-2, C-6), 131.1 (d, C-3, C-5), 130.3 (s, C-4), 197.2 (s, C=O), 26.5 (q, OC-**C**$H_3$)

ESI-MS (positive mode) *m/z*: 159.0420 $[M + Na]^+$ (Calc. for $C_8H_8O_2$ Na: 159.0446)

IR $\nu_{max}$ /cm$^{-1}$: 3306 (O-H), 3172, 1769, 1710, 1663 (C=O), 1603, 1281, 1167.

### 2.4.4.6 Isolation of norsantolinifolide (2.61)

The ketone chemotype crude extract (10g), was repeatedly fractionated with hexane-EtOAc solvent systems (8:3) to (6:4), using column chromatography to yield 3 g of impure material. It was again subjected to column chromatography eluting with hexane-EtOAc-acetone (7:2:1). This process afforded 19 mg of colourless needles. The $R_f$ was calculated to be 0.5 (acetone-MeOH, 97:3).

| | |
|---|---|
| Appearance: | white needle-like crystals |
| Empirical formula: | $C_{10}H_{14}O_4$ |
| Melting point: | 157-159 $^oC$ |
| Optical rotation: | $[\alpha]_D^{30}$ +27 (c = 0. 140, MeOH) |
| $^1H$ NMR ($CDCl_3$) $\delta_{H:}$ | 3.60 (1H, d, *J* 11.5 Hz), 2.80 (1H, dt, *J* 14.0, 8.6 Hz) 2.39 (m), 2.36 (m), 1.92 (m), 1.80 (1H, ddd, *J* 14.0, 10.0, 2.0 Hz), 1.53 (3H, s, 14-$CH_3$), 1.45 (m), 1.29 (3H, d, *J* 6.6 Hz, 13-$CH_3$), |
| $^{13}C$ NMR ($CDCl_3$) $\delta_C$: | 179.0 (s, C-5), 90.8 (d, C-6), 43.4 (d, C-7), 21.0 (t, C-8), 38.0 (t, C-9), 46.6 (s, C-10), 51.0 (d, C-11), 181.0 (s, C=O), 13.4 (q, C-13), 24.0(q, C-14). |

ESI-MS (positive mode) *m/z*: 221.0784 $[M + Na]^+$ (Calc. for $C_{10}H_{14}O_4Na$: 221.0790).

### 2.4.4.7 Isolation of 2,4-dihydroxy-6-methoxyacetophenone (2.68)

The crude fraction of 380 mg containing the compound **(2.61)** was chromatographed with hexane-EtOAc (7:3) and 27 mg of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)** were obtained. Its $R_f$ was 0.33 hexane-EtOAc (7:3).

| | |
|---|---|
| Description: | whitish needles |
| Empirical formula: | $C_9H_{10}O_4$ |
| Melting point: | 166-168 $^oC$ |

$^1H$ NMR ($CDCl_3$) $\delta_{H:}$

5.98 (1H, d, *J* 2.4), 5.91(1H, d, *J* 2.4), 2.60 (3H, s, OC-C**H$_3$)**, 3.86 (3H, s, 6-OC**H$_3$**)

$^{13}$C NMR ($CDCl_3$) $\delta_C$:

106.5 (s, C-1), 167.2 (s, C-2), 96.6 (d, C-3), 162.7 (s, C-4), 90.8 (d, C-5), 163.7 (s, C-6), 203.4 (C=O), 32.9 (OC-**C**$H_3$), 55.6 (6-$OCH_3$)

EI-MS *m*/*z*: $[M]^+$ 182.02

IR $\nu_{max}$ /$cm^{-1}$: 3369 (O-H), 1718 (C=O), 1718, 1627, 1032, 1167.

### 2.4.4.8 Isolation of santolinifolide (2.62)

Seven grams of the $CH_2Cl_2$-MeOH crude fraction were subjected to column chromatography eluting with hexane-EtOAc (6:4). 201 vials, 5 mL each, were collected. Vials 81-120 were combined and solvent evaporated to obtain a mixture of crystals of two compounds with $R_f$ of 0.19 and 0.20 respectively, hexane-EtOAc (7:3). The mixture was subsequently separated on a chromatotron, using hexane-EtOAc (7:3). Two overlapping bands were observable but difficult to separate. 5mL fractions were collected and fractions 1 to 20 were combined and solvent was removed on a rotavap to give 8 mg of impure crystals of **2.62** with $R_f$ 0.20. Although its signals were clearly shown in all NMR spectra, further inspection with TLC revealed that it was still mixed with artemin, which was in higher concentration in the fraction subjected to chromatotron, as it was evidenced by its yield (292 mg) when isolated later. Also, the signals of artemin were observable on the NMR spectra of **2.62**.

Empirical formula: $C_{15}H_{20}O_5$

Melting point: 135.5 $^oC$

$^1$H NMR ($CDCl_3$) $\delta_{H:}$

6.01(1H, br s, 15-H) 5.86 (1H, br s, 15-H′), 3.72 (1H, d, *J* 11.5 Hz), 3.03 (1H, ddd) 2.61 (1H, m), 2.55 (1H, m), 2.58 (2H, m), 2.33, (dq) 2.32 (2H, m), 1.88 (1H, m), 1.59 (1H, ddd), 1.58 (3H, s) 1.39 (1H, m), 1.23 (t ,*J* 3.3 Hz)

$^{13}$C NMR ($CDCl_3$) $\delta_C$:

176.8 (s, C-1), 32.9 (t, C-2), (t, 28.5, C-3), 144.5 (s, C-4), 204.0 (s, C=O), 90.5 (d, C-6), 43.2 (d, C-7), 20.5 (t, C-8), 38.7 (t, C-9), 52.8 (s, C-10), 50.8 (d, C-11), 181.0 (s, C=O),13.3 (q, C-13), 26.0 (q, C-14), 126.0 (t, C-15)

#### 2.4.4.9 Isolation of artemin (2.59)

During the above-mentioned process in Section 2.4.4.8, for isolation of **2.62,** fractions 22 to 46, were also combined and the solvent evaporated to afford 292 mg of crystals of **2.59**.

| | |
|---|---|
| Description: | white feather-like crystals |
| Empirical formula: | $C_{15}H_{22}O_4$ |
| Melting point: | 227-229 $^{o}$C, EtOAc (lit.[57] 238-240 $^{o}$C) |
| Optical rotation: | $[\alpha]_D^{30}$ +62 (c = 0. 520 , MeOH) (lit.[57] +157) |
| $^{1}$H NMR ($CDCl_3$) $\delta_{H:}$ | 5.03 (2H, d, 15-H), 4.98(s, H-15), 4.26 (1H, d, *J* 11.5 Hz, 6-H), 4.17 (1H, dd, 1-H), 2.65 (1H, m), 2.34 (1H, m), 2.15 (1H, m), 1.84 (1H, m), 1.73 (2H, m), 1.50 (1H, m), 1.49 (m), 1.23 (3H, d, *J* 6.47 Hz, 13-$CH_3$), 0.89 (3H, s, 14-$CH_3$) |
| $^{13}$C NMR ($CDCl_3$) $\delta_C$: | 71.8 (d, C-1), 29.9 (t, C-2), 29.6 (t, C-3), 145.1 (s, C-4), 77.1(s, C-5), 81.9 (d, C-6), 41.2 (d, C-7), 30.3 (t, C-8), 22.8 (t, C-9), 44.5 (s, C-10), 45.5 (d, C-11), 174.4 (s, C=O),12.4 (q, C-13), 13.3 (q, C-14), 112.4 (t, C-15). |
| ESI-MS (positive mode) *m/z*: | 289.1416 $[M + Na]^+$ (Calc. for $C_{15}H_{22}O_4$ Na: 289.1416). |

#### 2.4.4.10 Isolation of reynosin (2.65)

A portion of $CH_2Cl_2$-MeOH crude extract (10 g) from the thujone type was filtered and chromatographed on silica gel slurry packed column, using isocratic elution with hexane-EtOAc (8:2). 51 fractions, 20 mL each, were collected. The last fraction was obtained with methanol to remove the more polar compounds. The same procedure

was repeatedly applied to the last fraction and 34 mg of **2.65** were obtained with $R_f$ 0.73 in acetone-MeOH (97:3).

| | |
|---|---|
| Description: | yellowish needles |
| Empirical formula: | $C_{15}H_{20}O_3$ |
| Melting point: | 132-134 $^oC$, MeOH (lit.[62] 144-145 $^oC$) |
| Optical rotation: | $[\alpha]_D^{30}$ +54 (c = 0.140, MeOH) (lit. [62] +122) |
| $^1H$ NMR ($CDCl_3$) $\delta_{H:}$ | 6.08 (1H, d, *J* 3.3 Hz, 13-H), 5.42 (1H, d, *J* 3.1 Hz, 13-H′), 4.99 (1H, br s, 15-H), 4.86 (1H, br s, 15-H′), 4.03 (1H, m), 4.02 (t, *J* 10.8), 3.52 (1 H, dd, $J_a$ 4.6 Hz; $J_b$ 11.5 Hz, H-1), 2.54 (1H, m), 2.32 (1H, m), 2.17 (1H, m), 2.11 (1H, m), 2.09 (1H, m), 1.84 (1H, m), 1.58 (1H, m), 1.32 (1H, m) 0.87 (1H, m), 0.81(3H, s, 14-$CH_3$) |
| $^{13}C$ NMR ($CDCl_3$) $\delta_C$: | 79.6 (d, C-1), 33.6 (t, C-2), 35.8 (t, C-3), 142.4 (s, C-4), 53.1 (d, C-5), 21.5 (t, C-6), 78.3 (d, C-7), 49.6 (d, C-8), 31.34 (t, C-9), 43.0 (s, C-10), 139.3 (s, C-11), 170.7 (s, C=O), 117.0 (t, C- 13), 11.7 (q, C-14), 110.6 (t, C-15). |

ESI-MS (positive mode) *m/z*: 271.1309 $[M + Na]^+$ (Calc. for $C_{15}H_{22}O_4$ Na: 271.1310).

**2.4.4.11 Isolation of 5-hydroxy-7,4′-dimethoxyflavone (2.69)**

Ten grams of filtered crude extract were subjected to flash column chromatography using both isocratic and stepwise elution with hexane-EtOAc 8:2. Twenty mL fractions were collected. The last fraction was rechromatographed using hexane - EtOAc (7:3) and 120 fractions, 10 mL each were collected. Fractions 101-110 were combined and solvent removed to afford 139 mg of pure reddish crystals of the flavone **(2 .69).** The $R_f$ was 0.6 hexane-EtOAc (3:2). The compound was isolated four different times and the total yield was measured to be 504 mg.

| | |
|---|---|
| Description: | reddish needles |
| Empirical formula: | $C_{17}H_{14}O_5$ |
| Melting point: | 165-167 $^oC$, MeOH (lit.[75] 170-174 $^oC$) |

$^{1}$H NMR ($CDCl_3$) $\delta_{H:}$ 12.3 (1H, br s, 5-OH), 7.85 (1H, d, *J* 8.0 Hz, H-2′, H-6′), 7.02 (1H, d, *J* 8.0 Hz, H-3′, H-5′), 6.58 (1H, s, H-3), 6.49 (1H, d, *J* 2.2 Hz, H-8), 6.37 (1H, d, *J* 2.24 Hz, H-6), 3.89 (3H, s, 4′-$OCH_3$), 3.88 (3H, s, 7-$OCH_3$)

$^{13}$C NMR ($CDCl_3$) $\delta_C$: 164.2 (s, C-2), 104.5 (d, C-3), 182.7 (s, C=O), 105.6 (s, C-4a), 162.3 (s, C-5), 98.2 (d, C-6), 165.5 (s, C-7), 92.8 (d, C-8), 157.8 (s, C-8a), 124.0 (s, C-1′), 128.1 (d, C-2′, C-6′), 114.7 (d, C-3′, C-5′), 162.6 (s, C-4′), 55.8 (q, 7-$OCH_3$), 55.5 (q, 4′-$OCH_3$)

ESI-MS (positive mode) *m/z*: 321.0735 $[M + Na]^+$ (Calc. for $C_{17}H_{14}O_5Na$)

IR $\nu_{max}$ /$cm^{-1}$: 3368, 1671, 1606, 1509, 1271, 1196, 1165, 833, 722.

## 2.5 REFERENCES


1 . Bremer, K. 1994. Asteraceae, Cladistic and Classification, Timber Press, Inc, Portland, p. 435-458.

2 . Wu, Q.X., Shi, Y.P., Jia, Z.J. 2006. Eudesmane sesquiterpenoids from the Asteraceae family. *Natural Product Reports*, **23**, 699-734.

3. Wagner, H. 1977. Pharmaceutical and economic uses of the Compositae. *Biological Chemistry of Compos* [sympos.], Meeting Date 1975, **1,** 411-433.

4 . Wagner, S., Arce, R., Murillo, R., Terfloth, L., Gasteiger, J., Merfort, I. 2007. Neural networks as valuable tools to differentiate between sesquiterpene lactones inhibitory activity on serotonin release and on NF-κB. *Journal of Medicinal Chemistry*, **51**, 1324-1332.

5. www.botany.hawaii.edu/Faculty/Carr/aster.htm (date of access: 15th July 2008).

6 . Abad, M.G., Lazaro, R.M., Bedoya Del Olmo, L.M., Bermejo, Benito, P. 2008. Anti-infectious activity in the Anthemideae tribe. *Studies in Natural Products Chemistry*. **35**, 445-516.

7. Vagionas, K., Graikou, K., Chinou, I.B., Runyoro, D., Ngassapa, O. 2007. Chemical analysis and antimicrobial activities of essential oils from the

aromatic plants *Artemisia afra* Jacq. and *Leonotis osmifolia* (Burm.F.) *Iwassron* var. *raineriana* (vision1) Iwarsson growing in Tanzania. *Journal of Essential Oil Research*, **19**, 396-400.

8. Hall, H.M., Clements, F.E. 1923. The Phylogenetic Method in Taxonomy. Carnegie Institution of Washington, Washington, p. 31-45.
9. Bohlmann, F., Ang, W., Trinks, C., Jakupovic, J., Huneck, S. 1985. Dimeric guaianolide from *Artemisia sieversiana*. *Phytochemistry*, **24**, 1009-1015.
10. Marco, J.A, Sanz, J.F, Yuste, A., Carda, M., Jakupovic, J. 1991. Sesquiterpene lactones from *Artemisia barrelieri. Phytochemistry*, **20**, 3661-3668.
11. http://images.google.com/images?hl=en&q=artemisia+santolinifolia+photo&um=1&ie=UTF-8&sa=X&oi=image_result_group&resnum=1&ct=title (Date of access: 15th July 2008).
12. Jakupovic, J., Tan, R.X., Bohlmann, F., Jia, Z.J., Huneck, S. 1991. Seco- and *nor*-sesquiterpene lactones with a new carbon skeleton from *Artemisia santolinifolia*. *Phytochemistry*, **30**, 1941-1946.
13. http://www.google.co.za/search?hl=enq=artemisia+iwayomogi=picture&btnG=google =search&meta = (Date of access: 15th July 2008).
14. Greger, H., Zdero, C., Bohlmann, F. 1986. Eudesman-12,8β-olides and other terpenes from *Artemisia* species. *Phytochemistry*, **25**, 891-897.
15. Dana, A., Dalrymple, G. 2006. *Artemisia*, Agriculture and Malaria in Africa: The Interplay of Tradition, Science and Public Policy. p.19. http://www.icuciwmi.org/files/Publications/Artemisia%20Agriculture%20and%20Malaria%20in%20Africa%20Consolidated-WHO%20.pdf (Date of access, 15th July 2008).
16. Teoh, H.K., Polichuk, D.R., Reed, D.W., Nowak, G., Covello, P.S. 2006. *Artemisia annua* L. (Asteraceae) trichome-specific cDNA reveal CYP71AV1, a cytochrome P450 with a key role in the biosynthesis of the antimalarial sesquiterpene lactone artemisinin. FEBS Letters, **580**, 1411-1416.
17. Kohler, M., Haerdi, W., Christen, P., Veuthey, J.L. 1997. Extraction of artemisinin and artemisinic acid from *Artemisia annua* L. using supercritical carbon dioxide. *Journal of Chromatography* A, **785**, 353-360.

18. Chen, Y., Yu, B., Dong, L., Zhou, L. 2001. Isolation and identification of arteannuin and its precursor. *Zhongcaoyao*, **32**, 302-303 (Abstact obtained from SciFinder Scholar).
19. http://upload.wikimedia.org/wikipedia/commons/9/98/Artemisia-alba.jpg (Date of access: 15th July 2008).
20. Haouari, M., Ferchichi, A. 2007. Study of genetic polymorphism of *Artemisia herba-alba* from Tunisia using ISSR markers. *African Journal of Biotechnology*, **7**, 44-50.
21. Marco, J.A. 1989. Sesquiterpene lactones from *Artemisia herba-alba* subsp. *herba-alba*. *Phytochemistry*, **28**, 3121- 3126.
22. http://en.wikipedia.org/wiki/Artemisia_(plant) (Date of access: 15th July 2008).
23. Rezaeinodehi, A., Khangholi, S. 2008. Chemical composition of *Artemisia absinthium* growing wild in Iran. *Pakistan Journal of Biological Sciences*, **11**, 946-949.
24. Beauhaire, J., Fourrey, J.L., Guittet, E. 1984. Structure of absintholide, a new guaianolide dimer of *Artemisia absinthium* L. *Tetrahedron Letters*, **25**, 2751-2754.
25. Lachenmeier, D.W., Walch, S.G., Padosch, S.A., Kroner, L.U. 2006. Absinthe - A review. *Critical Review in Food Science and Nutrition*, **46**, 365-377.
26. Lachenmeier, D.W., Emmert, J., Kuballa, T., Sartor, J. 2006. Thujone - Cause of absinthism? *Forensic Science International*, **158**, 1-8.
27. Lachenmeier, D.W. 2005. Authentication of alcoholic drinks by GC/MS and HPTLC. Absinthe - cult drink or bad spirit? *CLB Chemie in Labor und Biotechnik*, 56, 18-20 (Abstract extracted from SciFinder Scholar).
28. Dettling, A., Grass, H., Schuff, A., Skopp, G., Strohbeck-Kuehner, P., Haffner, H.Th. 2004. Absinth: attention performance and mood under the influence of thujone. *Journal of Studies on Alcohol*, **65**, 573-581.
29. Gimpel, M., Hoenersch, Y., Altmann, H.J., Wittkowski, R., Fauhl-Hassek, C. 2006. Absinthe: thujone content of absinthe spirits using historical recipes. *Deutsche Lebensmittel-Rundschau*, **102**, 457-463 (Abstract obtained from SciFinder Scholar).

30. The Swiss Law Prohibiting the Manifacture and Sale of Absinth. 1911. Schweizerische Wochenschrift Fuer Chemie und Pharmacie. 48-677 (abstract available on SciFinder Scholar).
31. Mukinda, J.T., Sice, J.A. 2007. Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. *Journal of Ethnopharmacology*, **112**, 138-144.
32. Jackson, W.P.U. 1987. Origins and Meanings of Names of South African plant genera. University of Cape Town.
33. Roberts, M. 1990. Indigenous Healing Plants. Southern Book Publishers, Halfway House, pp 226-228.
34. Silbernagel, E. Spreitzer, H. Buchbauer, G. 1990. Nichtflüchtige Inhaltsstoffe von *Artemisia afra*. *Monatshefte für Chemie*, **121**, 433-436.
35. Huxley. A., Griffiths, M., Levy, M. (Editors) 1992. The New Royal Horticultural Society Dictionary of Gardening. Macmillan Press, London.
36. http://www.plantzafrica.com/medmonographs/artemisiaafra.pdf (Date of access: 10 December 2008).
37. http:/www.plantzafrica.com/plantab/artemisafra.htm (Date of access: 10 December 2008).
38. Huffman, A., Klepser. M.E., Ernst, E.J., Keele, D., Roling, E., Viljoen, A.M. 2002. Susceptibility of opportunistic bacteria and yeasts to the volatile oils of *Artemisia afra, Lippia javanica, Lippia scaberrima, Myrothamnus flabellifolius* and *Osmitopsis arteriscoides*. *Journal of Infectious Disease Pharmacotherapy*, **5**, 43-50.
39. Burits, M., Asres, K., Bucar, F. 2001. The antioxidant activity of essential oil of *Artemisia afra, Artemisia abyssinica* and *Juniperus procera*. *Phytotherapy Research*, **15**, 103-108.
40. Graven, O.H., Deans, S.G., Svoboda, K.P., Mavi, S., Gundidza, M.G. 1992. Antimicrobiol and antioxidative properties of volatile essential oil of *Artemisia afra*. *Flavour and Fragrance Journal*, **7**, 121-123.
41. Rabe, T., van Staden, J. 1997. Antibacterial activity of southern African plants used for medicinal purposes. *Journal of Ethnopharmacology*, **56**, 81-87.
42. Gundidza, M. 1993. Antifungal activity of the essential oil from *Artemisia afra* Jacq. *Central Africa Journal of Medicine*, **39**, 140-142.

43. Garnero, J., 1977. Huile essentielle de lanyana. *Parfums, Cosmetiques, Aromes*, **16**, 45-47.

44. Jakupovic, J., Klemeyer, H., Bohlmann, F., Graven, E.H. 1988. Glaucolides and guaianolides from *Artemisia afra*. *Phytochemistry*, **27**, 1129-1133.

45. Libbey, L.M., Sturtz, G. 1989. Unusual essential oils grown in Oregon. I. *Artemisia afra* Jacq. *Journal of Essential Oil Research*, **1**, 29-31.

46. Moody, J.O., Adeleye, S.A., Gundidza, M.G., Wyllie, G., 1994. Analysis of essential oil of *Artemisia afra*. *Pharmazie*, **49**, 935-938.

47. Viljoen, A.M., van Vuuren, S.F., Gwebu, T., Demirci, B., Husnu Baser, K.C. 2006. The geographical variation and antimicrobial activity of African wormwood (*Artemisia afra* Jacq.) essential oil. *Journal of Essential Oil Research*, **18**, 19-25.

48 Changonda, L.S., Makanda, C., Chalchat, J.C. 1999. The essential oil of cultivated *A. afra* (Jacq) from Zimbabwe. *Flavour and Fragrance Journal*, **8**, 140-142.

49. Meyer, V.R. 1994. Practical High-Performance Liquid Chromatography, 2nd Ed., John Wiley & Sons, Chichester.

50. Gailliot, F.P. 1998. Natural Products Isolation, Humana Press, Towawa, p. 63.

51. Gonzalez, A.J., Galindo, A., Mansilla, H., Guitierrez, A. 1981. Structure of maritimin, a sesquiterpene lactone from *Artemisia maritima gallica*. *Phytochemistry*, **20**, 2367-2369.

52. Nkunya, M. H. H., Weenen, H., Kinabo, L.S. 1992. Constituents of *Artemisia afra*. Fitoterapia, **63**, 279-280.

53. Batuev, A.S., Ryabinskaya, E.A., Gudimova, N.V. 1979. Effects of taurin on audiogenic convulsive fits in rats. *Doklady Akademii Nauk* SSSR, **248**, 1496-1499 (Abstract obtained from SciFinder Scholar).

54. Doksina, G.A., Climova, A.D., Jarcev, E.I., Jakovlev, V.G. 1975. Inhibition of proteolytic ferments by taurin in radiation sickness. *Radiobiologia Radiotherapia*, **16**, 339-345 (Abstract obtained from Scifinder Scholar).

55. Yamaguchi, Y., Itami, S., Nishida, K., Ando, Y. 1998. Taurin – conjugated ursodeoxycholic acid has a reversible inhibitory effect on human keratinocytes growth. *Journal of Dermatological Science*, **18**, 35- 42.

56. Kasuno, K., Nagahata, T. 1994. Dentifrice containing taurin and aromatic essences. *Japan Kokai Tokkyo Koho*. 6 pp (Abstract obtained from SciFinder Scholar).
57. Trendafilova, A.B., Todorova, M.N., Gussev, C.V. 1996. Eudesmanolides from Artemisia pontica. Phytochemistry, **42**, 496-471.
58. Marco J.A., Sanz-Cervera, J.F., Pareja, J.M., Sancenon, F., Valles-Xirau, J. 1994. Sesquiterpene lactones from North African *Atemisia* species, *Phytochemistry*, **37**, 477-485.
59. Yoshioka, H., Renold, W., Fischer, N.H., Higo, A., Mabry, T.J. 1970. Sesquiterpene lactone from *Ambrosia confertiflora* (compositae). Phytochemistry, **9**, 823-832.
60. Sanz, J.F., Barbera, O., Marco, J.A. 1989. Sesquiterpene lactone from *Artemisia hispanica*. *Phytochemistry*, **28**, 2163-2167.
61. Jakupovic, J., Chen, Z.L., Bohlmann, F. 1987. Artanomaloide, a dimeric guaianolide and phenylalanine derivatives from *Artemisia anomala*. *Phytochemistry*, **26**, 2777-2779.
62. Appendino, G., Belliardo, F., Nano, G.M., Stefenelli, S. 1982. Sesquiterpene lactones from *Artemisia genipi* Weber: isolation and determination in plant material and in liqueurs. *Journal of Agricultural Food Chemistry*, **30**, 518-521.
63. Barrero, A.F., Enrique Oltra, J., Cuerva, J.M., Rosales, A. 2002. Effects of solvents and water in Ti (III)-mediated radical cyclisations of epoxygermacrolides. Straightforward synthesis and absolute stereochemistry of (+)-3α-hydroxyreonosin and related eudesmanolides. Journal of Organic Chemistry, **67**, 2566-2571.
64. Jung, J.H., Ha, J.Y., Min, K.R., Shibata, F. Nakagawa, H., Kang, S.S., Chang, I-M., Kim, Y. 1998. Reynosin from *Sassurea lappa* as inhibitor on CINC-1 induction in LPS-stimulated NRK-52E cells. *Planta Medica*, **64**, 454-455.
65. Cho, J.Y., Park, J., Yoo, E.S., Baik, K.U, Jung, J.H., Lee, J., Park, M.H. 1998. Inhibitory effect of sesquiterpene lactones from *Saussurea lappa* on tumor necrosis factor-alpha production in murine macrophage-like cells. *Planta Medica*, **64**, 594-597.
66. Goodson, J.A. 1922. The constituents of the flowering tops of *Artemisia afra*, Jacq. *Biochemical Journal*, **16**, 489-493.

67. Vasconselos, J.M.J., Silva, A.M.S and Cavaleiro, J.A.S. 1998. Chromones and flavanones from *Artemisia campestris* subsp. *maritima*. *Phytochemistry*, **49**, 1421-1424.

68. Khan. S., Riaz, N., Afza. N., Malik, A., Ikbal, N., Lateef, M. 2009. Antioxidant constituents from *Cotoneaster racemiflora*. *Journal of Asian Natural Products Research*, **11**, 44-48.

69. Chin-Ying, S., Chen- Hui, C., Chih-Chieh, H., Chien-Chih, C., Ying-Chieh, T. 2003. Antioxidant properties of scopoletin isolated from *Sinomonium acutum*, *Phytotherapy Research*, **17**, 823-825.

70. Yu, H., Li, S., Huang, M.T., Ho, C.T. 2008. Anti-inflammatory constituents in noni (*Morinda citrifolia*) fruits. *ACS Symposium Series*, **987** (Dietary Supplements), 179- 190.

71. Kashihara, M., Ishiguro, K., Amok, M., Takagi, S. 1986. Antimicrobiol constituents of *Euphorbia humifusa* willd. Shoyakugaku Zasshi, **40**, 427-428 (Abstract obtained from SciFinder Scholar).

72. Panda, S., Kar, A. 2006. Evaluation of antithyroid, antioxydative and antihyperglycemic activity of scopoletin from *Aegle mameros* leaves in hyperthyroid rats. *Phytochemistry Research*, **12**, 1103-1105.

73. Manuele, M.G., Ferraro, G., Barreiro Arcos, M.L., Lopez, P., Cremaschi, G., Anesini, C. 2006. Comparative immunomodulatory effect of scopoletin on tumoral and normal lymphocytes. *Journal of Life Sciences*, **79**, 2043-2048.

74. Kang, S.Y., Sung, S.H., Park, J.H., Kim, Y.C. 1998. Hepatoprotective activity of scopoletin, a constituent of *Solanum lyratum*. *Archives of Pharmacology Research*, **21**, 718-722.

75. Talzhanov, N.A., Sadyrbekov, D.T., Smagulov, F.M., Mukanov, R.M., Radioing, V.A., Shakirov, M.M., Tkachev, A.V., Atzhanova, G.A., Tuleuov, B.I., Adekenov, S.M. 2005. Components of *Artemisia pontica*. *Chemistry of Natural Compounds*, **41**, 178-181.

76. Still, W.C., Khan, M., Mitra, A. 1978. Rapid chromatographic technique for preparative separation with moderate resolution. *Journal of Organic Chemistry* **43**, 2923-2925.

77. Aldrich Chemical Company. 2005-2006. Aldrich: Advancing science, p. 1369.

# CHAPTER THREE

# A PHYTOCHEMICAL INVESTIGATION OF *ELYTROPAPPUS RHINOCEROTIS*

## 3.1 INTRODUCTION

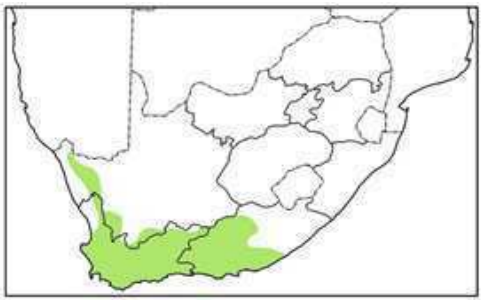
The genus *Elytropappus* Cass. belongs to the family Asteraceae (described in Section 2.1.1), tribe Gnaphalieae. This tribe is one of the largest in the family, with 180 genera and 2000 species. Geographically, the Gnaphalieae are distributed worldwide, but with a high occurrence in South Africa and Australia.[1] The name *Elytropappus* is derived from the Greek word "elytron" meaning a "sheath" and "pappos" meaning "down" or "fluff", referring to the small cup-like rim around the basis of feathery pappus.[2]

The genus consists of eight species, *E. rhinocerotis*, *E. glandulosus*, *E. hispidus*, *E. longifolius*, *E. gnaphaloides*, *E. scaber*, *E. adpressus*, and *E. ruschianus*.[3,4] A recent thesis proposed that *Elytropappus* must be split into three genera, *Elytropappus* and two new genera *Myrovernix* and *Dicerothamnus*.[5] However, this reclassification of the genus has not been published formally, and we will be using the older classification in this thesis.

*Elytropappus rhinocerotis* (L.f.) Less., commonly called rhinoceros bush or renoster bush, is a single-stemmed small plant growing to about 1 m high (Fig. 3.1). A new name, *Dicerothamnus rhinocerotis* Koekemoer, has been suggested for this species by Koekemoer,[5] but as mentioned, this classification has not been published formally. The species name "*rhinocerotis*" refers to the association of this species with the rhinoceros. The very old branches of *E. rhinocerotus* are gnarled and the bark is smooth and greyish in colour.[1] Old branches normally do not bear leaves but have many thin, whip-like twigs which are held erect and enveloped by small triangular leaves pressed tightly to the stem. In between the leaves, there is a layer of fine white, hair-like stuff, which gives a woolly appearance. Hence the overall plant appears as greyish. Numerous flowerheads (capitulae) are borne towards the end of the twigs; these are very small and inconspicuous, which makes it very difficult to recognize on a flowering bush. In Africa, *E. rhinocerotis* occurs in South Africa and Namibia (Fig. 3.2).[1]

http://www.plantzafrica.com/medmonographs/elytraprhino.pdf

**Figure 3.1.** *Elytropappus rhinocerotis.*



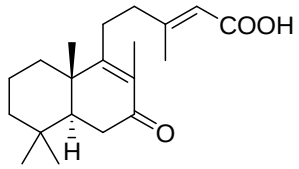
http://www.plantzafrica.com/medmonographs/elytraprhino.pdf

**Figure 3.2.** Geographical distribution of *E. rhinocerotis* in South Africa.[2]

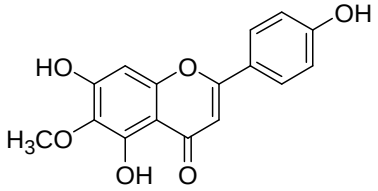
*E. rhinocerotis* is widely used as a medicinal plant in South Africa. Studies indicate that it is used in the treatment of many ailments such as digestive upsets, hyperthemia, acidity in children, stomach cancer and fevers.[6] In the case of lack of appetite, it is taken as a tonic and its preparations are believed to stimulate sweating. It served as a remedy of krimpsiekte in sheep. In Western Cape, people use it for the treatment of diarrhoea and convulsions in children and typhoid fever in both child and adult.[7,8] Reports confirm it to have been a famous remedy during the 1918 influenza epidemic.[7,8,9]

Limited information is available on the phytochemistry of *E. rhinocerotis*. Dekker *et al.*[6] isolated a compound known as rhinocerotinoic acid **(3.1)**. Proksch *et al.*[10] reported that *E. rhinocerotis* secretes a resin from its leaves from glandular trichomes located on the leaf surface. He mentioned that 80% of this ether-soluble resin consisted of acidic and phenolic

material. The phenolic fraction contained four flavonoids, hispidulin (6-methoxyapigenin) **(3.2)**, cirsimaritin (7-*O*-methylhispidulin) **(3.3)**, eupafolin **(3.4)** and quercetin **(3.5)**.[*]




**3.1**

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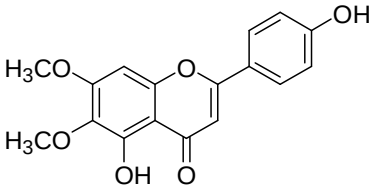


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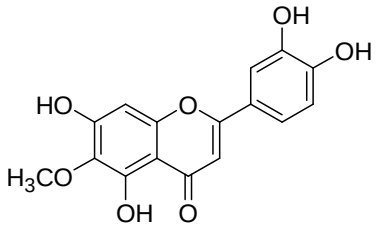
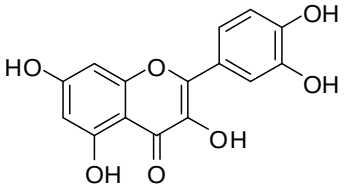
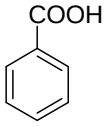



**3.4**

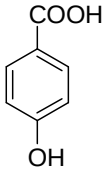



**3.5**

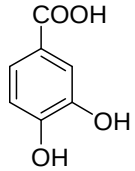
Apart from the four major components of the leaf resin, it has been confirmed that benzoic acid **(3.6)** and its derivatives, *p*-hydroxybenzoic acid **(3.7),** protocatechuic acid **(3.8)** and veratric acid **(3.9)**, are present in minor amounts, as well as cinnamic acid derivatives, namely *p*-coumaric acid **(3.10)**, ferulic acid **(3.11)** and sinapic acid **(3.12).**



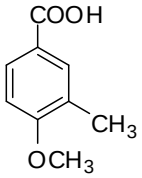

**3.6**




**3.7**

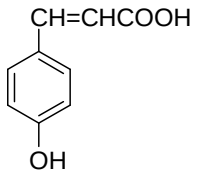



**3.8**

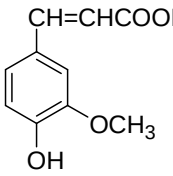



**3.9**

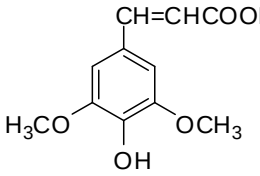
[*] The author of this thesis finds it difficult to believe that a polar compound such as quercetin will be soluble in diethyl ether.




**3.10**




**3.11**




**3.12**

## 3.2 RESULTS AND DISCUSSION

### 3.2.1 Preparation of plant material and isolation of compounds

The aerial parts of *E. rhinocerotis* were obtained from the farm Weltevrede in the Murraysburg district, Western Cape. The plant material was ground to yield 1.9 kg of material. A mixture of $CH_2Cl_2$ and MeOH was used for extraction to yield 189 g of crude extract. This extract was examined by thin-layer chromatography using various solvent systems in order to have an idea of the number and polarity of the components in a mixture before isolation.

The obtained crude extract was subjected to column chromatography and subsequently to centrifugal thin-layer chromatography on a chromatotron, using solvent systems prepared from different organic solvents, namely hexane, dichloromethane, ethyl acetate, acetone and methanol. As a result, the compounds labdanolic acid **(3.13)**, methyl labdanolate **(3.14)**, scoparone **(3.15)** and viridiflorol **(3.16)**, have been isolated and their structures determined.

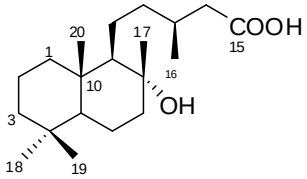
### 3.2.2 Structural elucidation of labdanolic acid (3.13)

The first compound isolated from the *E. rhinocerotis* extracts exhibited twenty signals in its $^{13}C$ NMR spectrum (Plate 79, Table 3.1). This indicated that the compound was a diterpene. The DEPT spectrum (Plate 80) revealed the presence of five methyl groupes at $\delta_C$ 22.1, 21.5, 33.4, 19.9 and 15.4, resepctively. The signal at $\delta_C$ 74.6 was attributed to a carbon bearing a hydroxy group, while the one resonating at $\delta_C$ 178.0 clearly resulted from a carboxyl group (COOH). This means that the compound was a carboxylic acid. No signal was observed between $\delta_C$ 100 and $\delta_C$ 150. Therefore, it was concluded that the diterpene was saturated.

The $^{1}H$ NMR spectrum (Plate 78) showed five methyl proton signals, four singlets at $\delta_H$ 0.79 (6H), 0.87 (3H) and 1.16 (3H), and a doublet at $\delta_H$ 0.99 (3H, d, *J* 6.6 Hz). In the HMBC spectrum (Plate 84), two of the methyl group protons ($\delta_H$ 0.87 and one of the methyls resonating at $\delta_H$ 0.79) correlate to the carbons of each other and both show correlations with three carbons resonating at $\delta_C$ 42.1 ($CH_2$), at $\delta_C$ 33.3 (C) and at $\delta_C$ 56.1 (CH), respectively. These two methyls are characteristic of a *gem*-4,4-dimethyl substituted decahydronaphthalene system.

The methyl protons at $\delta_H$ 1.16 correlate with the oxygen-bearing quaternary carbon ($\delta_C$ 74.6), a methylene carbon resonating at $\delta_C$ 44.6 and a methine carbon resonating at $\delta_C$ 61.9. These correlations are in agreement with a diterpene containing the above-mentioned decahydronaphthalene system and with 8-hydroxy and 8-methyl substituents.

The $^{1}H$ chemical shift and size of the *geminal* $^{1}H,^{1}H$-coupling of the methylene group with protons resonating at $\delta_H$ 2.17 (1H, dd, *J* 14.8 and 7.2 Hz) and $\delta_H$ 2.41 (1H, dd, *J* 14.8 and 6.4 Hz), indicated that this group is located adjacent to the carboxylic acid. Furthermore, an HMBC correlation was observed between this methylene carbon ($\delta_C$ 40.9) and the secondary methyl group resonating at $\delta_H$ 0.99. The secondary methyl group protons also show a correlation with the methane carbon at $\delta_C$ 30.8 and the methylene carbon at $\delta_C$ 39.9. These results, together with the COSY (Plate 82) and HSQC (Plate 83) spectra, confirmed the structure of the isolated compound to be labdanolic acid **(3.13)**. The experimental data were in agreement with the reported data for this compound (Table 3.1).[10,11]




**3.13**

The IR spectrum showed some informative signals. The peak at $\nu_{max}$ 1693 $cm^{-1}$ was assigned to the acidic carbonyl (COOH) group and the signal at $\nu_{max}$ 1075 $cm^{-1}$ was attributed to the C-O stretching vibration of the OH group bonded to C-8.[12] The HR-ESI-MS spectrum gave a peak at 347.2570 $[M + Na]^+$, corresponding to the molecular ion ($+Na^+$) which is not significantly different from the calculated mass of 347.2562 for $C_{20}H_{36}O_3Na$.

**Table 3.1. $^{1}H$ and $^{13}C$ NMR of labdanolic acid (3.13) in $CDCl_3$ (400 MHz).**

| Experimental values | | | | | | Literaturevalues[11] | |
|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC correlation** | **$\delta_C$** | **$\delta_H$** |
| 1 | 39.7 | $CH_2$ | 1.63<br>0.99 | | 20 | 39.6 | |
| 2 | 18.5 | $CH_2$ | 1.61 | | | 18.4 | |
| 3 | 42.1 | $CH_2$ | 1.39<br>1.15 | | 18, 19 | 41.9 | |
| 4 | 33.3 | C | | | 18, 19 | 33.2 | |
| 5 | 56.1 | CH | 0.90 | | 18, 19 | 56.0 | |
| 6 | 20.5 | $CH_2$ | 1.28 | | | 20.4 | |
| 7 | 44.6 | $CH_2$ | 1.86 (1H, dt, *J* 12.3, 3.3 Hz)<br>1.39 | | 17 | 43.7 | 1.85 (1H, dt, *J* 12.3, 3.3 Hz, 7-βH) |
| 8 | 74.6 | C | | | 17 | 74.9 | |
| 9 | 61.9 | CH | 1.03 | | 17, 20 | 62.0 | |
| 10 | 39.2 | C | | | 20 | 39.0 | |
| 11 | 22.1 | $CH_2$ | 1.26 | | 20 | 23 | |
| 12 | 39.9 | $CH_2$ | 1.42 | | 16 | 40.5 | |
| 13 | 30.8 | CH | 2.01 | 14, 16 | 16 | 31.2 | |
| 14 | 40.9 | $CH_2$ | 2.17 (1H, dd, *J* 14.8, 7.2 Hz)<br>2.41 (1H, dd, *J* 14.8, 6.4 Hz | 13, 14 | 16 | 41.5 | 2.19 (1H, dd, *J* 14.8, 7.2 Hz, 14-H), 2.30 (1H, dd, *J* 14.8, 6.4Hz, 14-H′) |
| 15 | 178.0 | C | | | | 177.9 | |
| 16 | 19.9 | $CH_3$ | 0.99 (3H, d, *J* 6.6 Hz) | 13 | | 19.9 | 0.98 (3H, d, *J* 6.6 Hz) |
| 17 | 24.0 | $CH_3$ | 1.16 (3H, s) | | | 23.8 | 1.15 (3H, s) |
| 18 | 33.4 | $CH_3$ | 0.87 (3H, s) | | 19 | 33.3 | 0.86 (3H, s) |
| 19 | 21.5 | $CH_3$ | 0.79 (6H, s) | | | 21.4 | 0.78 (6H,s) |
| 20 | 15.4 | $CH_3$ | 0.79 (6H, s ) | | | 15.5 | 0.78 (6H,s) |

Labdanolic acid **(3.13)** was first isolated from gum labdanum (*Cistus ladaniferus*), well known in the perfume industry for its balsamic odour and its fixative properties.[13,14] It was recently reported that at a concentration of 100 μg/mL, **3.13** selectively inhibited the COX-2 enzyme.[13] At this concentration, the COX-2 inhibition was 43%, whereas no inhibition of COX-1 was observed. In the same experiment, acetylsalicylic acid (aspirin), at a concentration of 180 μg/mL, showed inhibition of 61% and 24% for COX-1 and COX-2, respectively. COX-2 is an important enzyme in inflammation. The excellent inhibition activity of labdanolic acid **(3.13)** of COX-2 may explain the traditional uses of *E. rhinocerotis* as an anti-inflammatory.

### 3.2.3 Structural elucidation of methyl labdanolate (3.14)

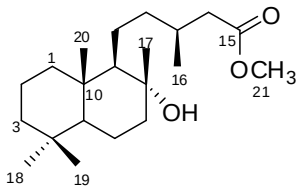
The determination of the structure of methyl labdanolate **(3.14)** was easy, since it is similar to that of labdanolic acid. The $^{1}$H NMR spectrum (Plate 85) gave some informative signals. It showed five methyl groups respectively at $\delta_H$ 0.95 (3H, d, *J* 6.7 Hz, 16-$CH_3$), $\delta_H$ 1.15 (3H, s, 17-$CH_3$), $\delta_H$ 0.87 (3H, s, 18-$CH_3$), $\delta_H$ 0.79 (6H, s, 19, 20-$CH_3$). The singlet observed at $\delta_H$ 3.66 (3H, s) indicated the presence of a methoxy group, which was assigned as a methyl ester ($COOCH_3$). The doublet of a triplets at 1.86 (1H, dt, *J* 12.0 and 3.1 Hz) was assigned to the equatorial proton of the methylene group at C-7. Two doublet of doublets appearing at $\delta_H$ 2.14 (1H, dd, *J* 14.6 and 8.0 Hz) and $\delta_H$ 2.36 (1H, dd, *J* 14.6 and 5.8 Hz), resulted from the non-equivalent C-14 protons.

The $^{13}$C NMR spectrum (Plate 86) displayed twenty-one signals. A comparison with the $^{13}$C NMR spectrum of labdanolic acid (**3.13**), revealed an extra carbon resonating at $\delta_C$ 51.4, which could be assigned to the methyl of an ester group. This was confirmed by HSQC spectrum (Plate 90) where the direct coupling between the proton resonating at $\delta_H$ 3.66 and the carbon resonating at $\delta_C$ 51.4, was observed. The five methyl groups were also observed in the DEPT spectrum (Plate 87) at $\delta_C$ 20.0, 24.0, 33.4, 21.5 and 15.5. The signal at $\delta_C$ 74.3 resulted from C-8, a quaternary carbon bearing an OH group. In addition to this information, the COSY (Plate 89) and HMBC (Plate 91) spectra supported the structure of methyl labdanolate **(3.14)**. The spectroscopic data of the isolated compound were in good agreement with the reported data for methyl labdanoate **(3.14)**. (Table 3.2).[10]

**Table 3.2. $^{1}H$ and $^{13}C$ NMR of methyl labdanolate (3.14) in $CDCl_3$ (400 MHz).**

| Experimental values | | | | | | Literature values[11] | |
|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC correlation** | **$\delta_C$** | **$\delta_H$** |
| 1 | 37.2 | $CH_2$ | 1.61<br>0.93 | | 20 | 39.6 | |
| 2 | 18.5 | $CH_2$ | 1.59<br>1.40 | | 18 | 18.4 | |
| 3 | 42.0 | $CH_2$ | 1.37<br>1.13 | **7,** 6 | 18 | 41.9 | |
| 4 | 33.3 | C | | | 18,19 | 33.2 | |
| 5 | 56.2 | CH | 0.91, 1.27 | 6, 5, 11 | 18 | 56.0 | |
| 6 | 20.6 | $CH_2$ | 1.64<br>1.27 | 3, 18 | 18 | 20.4 | |
| 7 | 44.6 | $CH_2$ | 1.86 (1H, dt, *J* 12.0, 3.1 Hz)<br>1.38 | 3, 17 | 17 | 44.2 | 1.86 (1H, dt, *J* 12.0, 3.1 Hz) |
| 8 | 74.3 | C | | | 17 | 74.1 | |
| 9 | 62.1 | CH | 1.01 | | 17 | 62.2 | |
| 10 | 39.2 | C | | | 20 | 39.0 | |
| 11 | 22.4 | $CH_2$ | 1.40<br>1.27 | 5, 19 | | 22.7 | |
| 12 | 40.3 | $CH_2$ | 1.40 | | | 40.6 | |
| 13 | 31.1 | CH | 2.11 | 14 | 16 | 31.2 | |
| 14 | 41.3 | $CH_2$ | 2.14 (1H, dd, *J* 14.6, 8.0 Hz, 14-H)<br>2.36 (1H, dd, *J* 14 .6, 5.8 Hz, 14-H′) | 13, 14 | 16 | 41.4 | 2.16 (1H, dd, *J* 14.6, 8.0 Hz, 14-H), 2.34 (1H, dd, *J* 14.6, 5.8 Hz) |
| 15 | 174.0 | C | | | 21 | 173.8 | |
| 16 | 20.0 | $CH_3$ | 0.95 (3H, d, *J* 6.7 Hz) | | | 19.8 | 0.95 (3H, d, *J* 6.7 Hz) |
| 17 | 24.0 | $CH_3$ | 1.15 (3H, s) | 7 | | 23.9 | 1.14 (3H, s) |
| 18 | 33.4 | $CH_3$ | 0.87 (3H, s) | 6 | 19 | 33.3 | 0.86 (3H, s) |
| 19 | 21.5 | $CH_3$ | 0.79 (6H, s) | 11 | 18 | 21.4 | 0.78 (6H, s) |
| 20 | 15.5 | $CH_3$ | 0.79 (6H, s) | | | 15.4 | 0.78 (6H, s) |
| 21 | 51.4 | $CH_3$ | 3.66 (3H, s) | | | 51.4 | 3.66 (3H, s, $OCH_3$ |

In the HR-ESI-MS spectrum, the peak of the molecular ion (+$Na^+$) was observed at 361.2716 $[M + Na]^+$, which agreed with the calculated mass of 361.2719 for $C_{21}H_{38}O_3Na$.

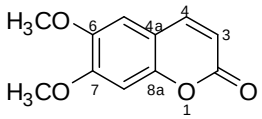



**3.14**

### 3.2.4 Structural elucidation of scoparone (6,7-dimethoxycoumarin) (3.15)

6,7-Dimethoxycoumarin or scoparone, was isolated and its structure was elucidated by analysis of NMR spectra. The $^1H$ NMR spectrum (Plate 92) showed a tall signal at $\delta_H$ 3.92 (6H, s, 6-$OCH_3$, 7-$OCH_3$), which is typical of two methoxy groups, both resonating at the same chemical shift. The proton on C-3 appeared as a doublet at $\delta_H$ 6.29 (1H, d, *J* 9.5 Hz, H-3), while the proton on C-4 resonates as a doublet at $\delta_H$ 7.62 (1H, d, *J* 9.5 Hz, H-4). Two singlets with closely related chemical shifts occurred at $\delta_H$ 6.847 (1H, s) and 6.854 and were assigned to the two aromatic protons located at C-5 and C-8. The COSY spectrum (Plate 96) confirmed these arrangements by showing a correlation between the protons at $\delta_H$ 7.62 and $\delta_H$ 6.85.

The $^{13}C$ NMR spectrum displays 10 signals corresponding to 11 carbon atoms (the two $OCH_3$ appearing at the same chemical shift). The DEPT spectrum (Plate 94) showed that of the 11 carbon atoms, 6 are protonated. These are found at $\delta_C$ 56.5 (2 $OCH_3$), two methoxy groups bonded to C-6 and C-7; at $\delta_C$ 100.3 (CH) and 108.2 (CH), which are aromatic carbons, and $\delta_C$ 113.7 (CH) and $\delta_C$ 143.4 (CH), the lactone ring carbons C-3 and C-4 of the coumarin. Obviously, the 5 carbon atoms remaining are not protonated; they are quaternary carbons of the molecule and are found at $\delta_C$ 111.6, 146.6, 150.2, 153.3 and 161.5, respectively. The above-mentioned spectra together with HSQC (Plate 97) and HMBC (Plate 98) agreed with the structure of scoparone **(3.15).**




**3.15**

**Table 3.3. $^{1}H$ and $^{13}C$ NMR of 6,7-dimethoxycoumarin (3.15) in $CDCl_3$ (400 MHz).**

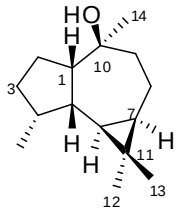
| Experimental values | | | | | | Literature values[15] | |
|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC correlation** | **$\delta_C$** | **$\delta_H$** |
| 2 | 161.5 | C | | | 4 | 161.4 | |
| 3 | 113.7 | CH | 6.29 (1H, d, *J* 9.5 Hz) | 4 | | 113.4 | 6.28 |
| 4 | 143.4 | CH | 7.62 (1H, d, *J* 9.5 Hz) | 3 | 5 | 143.3 | 7.62 |
| 4a | 111.6 | C | | | 3, 5, 8 | 111.5 | |
| 5 | 108.2 | CH | 6.85 (2H, s) | 6-$OCH_3$ 7-$OCH_3$ | 4 | 108.1 | 6.86 |
| 6 | 146.6 | C | | | 5, 8, 6-$OCH_3$ | 146.4 | |
| 7 | 153.3 | C | | | 7-$OCH_3$ | 152.9 | |
| 8 | 100.3 | CH | 6.85 (2H, s) | 6-$OCH_3$ 7-$OCH_3$ | 7-$OCH_3$ | 100.0 | 6.84 |
| 8a | 150.2 | C | | | 5, 8, 4 | 150.1 | |
| 6-$OCH_3$, 7-$OCH_3$ | 56.5 | $OCH_3$ | 3.92 (6H, s) | 8 | | 56.4 | 3.95 |

The HR-ESI-MS showed a molecular ion at 229.0473 $[M + Na]^+$ that is in agreement with the calculated mass for $C_{11}H_{10}O_4Na$ 229.0477. The experimental data were in agreement with the literature values.[15]

Different biological activities have been observed for scoparone (6,7-dimethoxycoumarin) **(3.15)**. For example, its biosynthesis in some species such as *Citrus paradis*, was associated with resistance to *Phytophtora* infection.[16] Its activities also include vasodilatation,[17] inhibitory effect on cardiohemodynamic functions,[18] immunosuppressive effect,[19] radioprotective effect,[20,21] and antifungal activity.[22] The coumarin derivatives are used as anticoagulants which depress the synthesis of four vitamin K-dependant clotting factors in the liver.[23]

### 3.2.5 Structural elucidation of viridiflorol (3.16)

A sesquiterpene alcohol was obtained from the $CH_2Cl_2$-MeOH extract after column chromatography, using hexane-$CH_2Cl_2$ (3:1). The analysis of the spectroscopic data of the isolated compound led to the assignment of the structure of the compound as viridiflorol or (1*S*,4*R*,5*S*,6*R*,7*R*)-4,10,11,11-tetramethyltricycloundecan-10α-ol (**3.16)**.




**3.16**

The $^{13}C$ NMR spectrum (Plate 100) displayed fifteen signals, indicating that the compound was a sesquiterpene. The signal at $\delta_C$ 74.7 was assigned to the tertiary carbon (C-10) bearing the hydroxy group. The DEPT spectrum (Plate 101) revealed that the isolated compound have four methyl groups; two of them at $\delta_C$ 28.6 and $\delta_C$ 16.1, both bonded to the quaternary carbon C-11; one at $\delta_C$ 16.3, connected to the methine carbon in C-4; the fourth methyl group was observed at $\delta_C$ 32.1 and it was linked to the tertiary carbon having the hydroxy group (C-10). Another feature was that the spectrum did not show any signals at lower field, indicating that there was neither a carbonyl group nor a double bond in the

structure of the isolated compound. The singlet appearing at an unusually high field ($\delta_C$ 18.4), was assigned to the quaternary carbon C-11.

The $^1$H NMR spectrum (Plate 99) supported the information given by the $^{13}$C NMR spectrum by exhibiting three 3H singlets at $\delta_H$ 1.00 (3H, s, H-13), $\delta_H$ 1.02 (3H, s, H-12) and $\delta_H$ 1.15 (3H, s, H-14), respectively, confirming the presence of methyl groups at $\delta_C$ 16.1 (C-13), $\delta_C$ 28.6 (C-12) and $\delta_C$ 32.1 (C-14). The doublet observed at $\delta_H$ 0.93 (3H, d, *J* 6.8 Hz, H-15), was attributed to the methyl group at $\delta_C$ 16.3 (C-15). The experimental data were in agreement with the reported data in by Bombarda *et al.*[24] for viridiflorol **(3.16)** isolated from *Melaleuca quinquenervia*.

The molecular mass of viridiflorol **(3.16)** was obtained by a GC-EI-MS experiment. The spectrum showed a signal at *m/z* 204, while the expected mass was 222. This indicated that on the GC inlet, the compound might have eliminated $H_2O$.

It was found that when viridiflorol **(3.16)** is applied to dog food bait, it reduces efficiently fire ant consumption and visitation. At concentration of 18 μg/mg of dog food, fire ants would not consume the bait within the period of six hours after application of viridiflorol **(3.16)**.[25,26] This suggests that this compound can act as an antifeedant for some insects. Viridiflorol **(3.16)** was also reported to occur in other different species, including *Kunzea ambigua*, a therapeutic and insect repellent species,[27] *Mentha piperita* L.,[28] and in different chemotypes of *Melaleuca quiquenervia,*[29] a species considered as aggressive to other species,[30,31] but having antiseptics with bioactivity against human pathogens.[32]

## 3.3 CONCLUSION

In the current phytochemical study, four compounds have been isolated from *E. rhinocerotis*. None of these compounds have been reported previously from this species. The isolation of large amount of labdanolic acid **(3.13)** is interesting, since it has been shown that this compound is a selective of COX-2 inhibitor. This activity may account for the traditional uses of the plant as an anti-inflamatory.[8]

**Table 3.4. $^{1}H$ and $^{13}C$ NMR of viridiflorol (3.16) in $CDCl_3$ (400 MHz).**

| Experimental | | | | | | Literature[24] | |
|---|---|---|---|---|---|---|---|
| **Position** | **$\delta_C$** | **DEPT** | **$\delta_H$** | **COSY** | **HMBC correlation** | **$\delta_C$** | **$\delta_H$** |
| 1 | 58.3 | CH | 1.77 (m) | | 14 | 58.4 | 1.80 |
| 2 | 25.8 | $CH_2$ | 1.64<br>1.59 | | 14 | 25.9 | 1.63<br>1.57 |
| 3 | 29.1 | $CH_2$ | 1.79<br>1.24 | 3 | 15 | 29.3 | 1.78<br>1.27 |
| 4 | 38.6 | CH | 1.94 (m) | 15 | 15 | 38.5 | 1.98 |
| 5 | 39.8 | CH | 1.80 (m) | 8 | | 39.9 | 1.84 |
| 6 | 22.4 | CH | 0.13 (t, *J* 9.2 Hz) | | 12, 13 | 22.5 | 0.11 |
| 7 | 28.7 | CH | 0.61 (m) | | 12, 13 | 28.7 | 0.61 |
| 8 | 18.9 | $CH_2$ | 1.60<br>1.44 | 5 | 12, 13, 14 | 18.9 | 1.60<br>1.45 |
| 9 | 37.8 | $CH_2$ | 1.70<br>1.51 | | 14 | 37.9 | 1.66<br>1.57 |
| 10 | 74.7 | C | | | 14 | 74.5 | |
| 11 | 18.4 | C | | | | 18.4 | |
| 12 | 28.6 | $CH_3$ | 1.02 (s) | 15 | 13 | 28.7 | 1.03 |
| 13 | 16.1 | $CH_3$ | 1.00 (s) | 14 | 12 | 16.1 | 1.00 |
| 14 | 32.1 | $CH_3$ | 1.15 (s) | 13 | | 32.1 | 1.13 |
| 15 | 16.3 | $CH_3$ | 0.93 (d, *J* 6.8 Hz) | 4, 12 | | 16.3 | 0.93 |

## 3.4 EXPERIMENTAL PROCEDURES

### 3.4.1 General experimental procedure

The general experimental procedures have been described in Section 2.4.1.

### 3.4.2 Plant material and extraction

Aerial parts from healthy and mature *E. rhinocerotis* were collected on the farm Weltevrede in the Murraysburg district, Western Cape, and were air dried and then ground to a powder (1.9 kg). The powder was extracted with a 1:1 mixture of $CH_3OH$-$CH_2Cl_2$ at room temperature for 24 h. The plant material was removed by filtration and the solvent was evaporated on a rotavapor. The yield of crude extract was 189 g.

A small portion of crude extract (15 g) was redissolved in a small volume of ethyl acetate and filtered through a short silica gel column eluting with methanol. The resulting fraction was analysed by TLC. 7 main components at $R_f$ 0.12, $R_f$ 0.17, $R_f$ 0.25, $R_f$ 0.71, $R_f$ 0.82, $R_f$ 0.91 and $R_f$ 0.99 (hexane-EtOAc, 7:3) (Fig. 3.3) were observed using suitable solvent systems. The extremely polar compounds were not investigated at this stage.

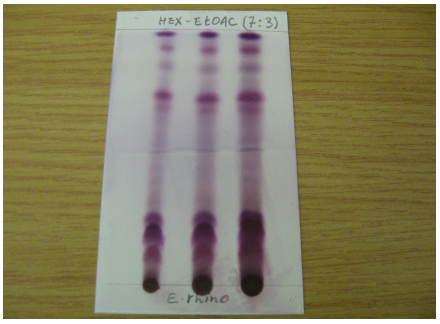



**Figure 3.3.** TLC (hexane-EtOAc, 7:3) of the $CH_2Cl_2$-MeOH crude extract of *E. rhinocerotis*.

### 3.4.3 Isolation of labdanolic acid (3.13)

The filtered fraction mentioned above was then fractionated by column chromatography. It was concentrated by removing some solvent and transferred carefully into a silica gel

column packed as slurry. Mixtures of hexane and ethyl acetate were used as eluents. Initially 100% hexane (200 mL) was used as a single solvent. Thereafter, the polarity of the solvent was gradually increased stepwise, from 90% hexane-10% EtOAc to 10% hexane-90% EtOAc. Finally, 100% methanol was used to wash the column to obtain the very polar components. This process is summarized in the Fig. 3.4.

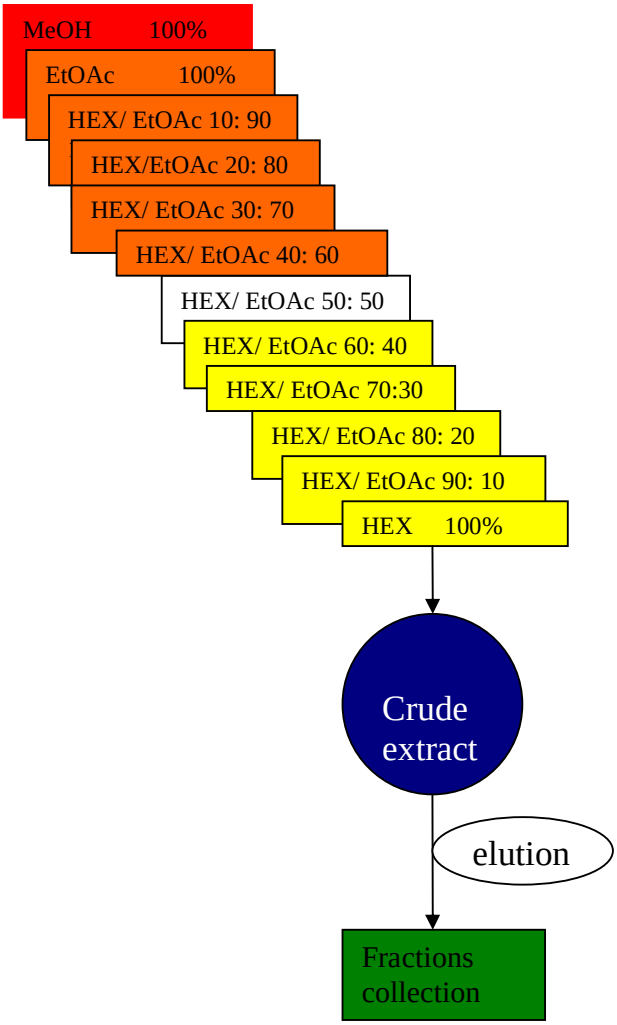



**Figure 3.4.** Stepwise elution of *E. rhinocerotis* crude extract column chromatography

Fifteen fractions, 120 mL each, were collected. Fractions 5, 6 and 7 were combined and the solvent removed. The resulting material was again chromatographed, using hexane-EtOAc (3:2) as eluent. Sixty-eight vials, 10 mL each, were collected. Vials 52 to 68 were combined and the solvent evaporated. This process yielded 273 mg of white needle-like crystals, with $R_f$ 0.24 (hexane-EtOAc-acetone, 8:1:1). The spectroscopic data analysis revealed the compound to be labdanolic acid (8-hydroxylabdan-15-oic acid) **(3.13)**, with the following physical characteristics:

| | |
|---|---|
| Description: | white needles |
| Empirical formula: | $C_{20}H_{36}O_3$ |
| Melting point: | 66-69 $^oC$ (lit.[11] 68 $^oC$) |
| Optical rotation: | $[\alpha]_D^{30}$ +4 (c 1.600, MeOH) (lit.[11], +5) |
| $^1H$ NMR ($CDCl_3$) $\delta_H$: | |
| | 2.17 (1H, dd, *J* 14.8, 7.2 Hz, 14-H), 2.41(1H, dd, *J* 14.8, 6.4 Hz, 14-H′), 1.86 (1H, dt, *J* 12.3, 3.3 Hz, 7 β-H), 1.16 (3H, s, 17-$CH_3$), 0.99 (3H, d, *J* 6.6 Hz, 16-$CH_3$), 0.87 (3H, s, 18-$CH_3$), 0.79 (6H, s, 19-$CH_3$, 20-$CH_3$) |
| $^{13}C$ NMR ($CDCl_3$) $\delta_C$: | |
| | 39.7 (t, C-1), 18.5 (t, C-2), 42.1 (t, C-3), 33.3 (s, C-4), 56.1 (d, C-5), 20.5 (t, C-6), 44.6 (t, C-7), 74.6 (s, C-8), 61.9 (d, C-9), 39.2 (s, C-10), 22.1 (t, C-11), 39.9 (t, C-12), 30.8 (d, C-13), 40.9 (t, C-14), 178.0 (s, C-15), 19.9 (q, C-16), 24.0 (q, C-17), 33.4 (q, C-18), 21.5 (q, C-19), 15.4 (q, C-20) |
| ESI-MS (positive mode) *m/z*: 347.2570 $[M + Na]^+$ (Calc. for $C_{20}H_{36}O_3Na$: 347.2562) | |
| IR $\nu_{max}$ ($cm^{-1}$): | 1693 (COOH) |

### 3.4.4 Isolation of scoparone (6,7-dimethoxycoumarin) (3.15)

From the 15 fractions of 120 mL each, collected previously from the above-mentioned stepwise elution (Fig. 3.4), fraction 10 was subjected to column chromatography, using $CH_2Cl_2$-EtOAc-acetone (8:1:1) as a suitable solvent system. In total, 57 fractions were collected, 10 mL each. Fractions 7, 8 and 9 were combined and solvent removed to give impure crystals (28 mg).

The above-mentioned quantity of material was redissolved in a small volume of ethyl acetate and purified by means of a chromatotron, using hexane-EtOAc (1:1) as eluent. Twelve fractions, 15 mL each, were collected, concentrated in a fume hood, spotted on TLC plates and visualized with UV radiations. Fractions 6, 7 and 8 showed a clear big blue spot under UV light (254 nm) (Fig. 3.5). These fractions were combined and the solvent evaporated with a rotavapor to yield 10 mg of white needle-like crystals, with $R_f$ = 0.45 (hexane-EtOAc, 6:4). Analysis of the NMR and MS data led to the structure of scoparone or 6,7-dimethoxycoumarin **(3.15).**

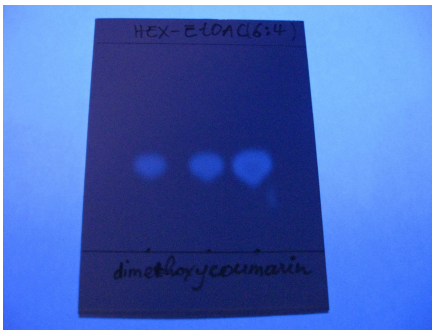



**Figure 3.5.** TLC of scoparone (6,7-dimethoxycoumarin).

| | |
|---|---|
| Description: | white needles |
| Empirical formula: | $C_{11}H_{10}O_4$ |
| Melting point: | 139-141$^{o}$C (lit.[33] 143-145 $^{o}$C) |
| $^{1}$H NMR ($CDCl_3$) $\delta_H$: | |
| | 6.29 (1H, d, *J* 9.5 Hz, H-3), 7.62 (1H, d, *J* 9.5 Hz, H-4), 6.847 (1H, s, H-5) 6.854 (1H, s, H-8), 3.92 (6H, s, 6-$OCH_3$), 7-$OCH_3$) |
| $^{13}$C NMR ($CDCl_3$) $\delta_C$: | |
| | 161.5 (s, C-1), 113.7 (d, C-3), 143.4 (d, C-4), 111.6 (s, C-4a), 108.2 (d, C-5), 146.6 (s, C-6), 153.3 (s, C-7), 100.3 (d, C-8), 150.2 (s, C-8a), 56.5 (q, 6-$OCH_3$, 7-$OCH_3$) |

ESI-MS (positive mode) *m/z*: 229.0473 $[M + Na]^+$ (Calc. for $C_{11}H_{10}O_4Na$: 229. 0477)

### 3.4.5 Isolation of methyl labdanolate (3.14)

The isolation of this compound necessitated the separation of the acidic and non-acidic components of the crude extract. This was done because it was observed that labdanolic acid was present in almost all the fractions, either pure or mixed, with other constituents which prevented us from obtaining other components in their pure form. The procedure used to separate both acidic and none acidic components is described below.

10 g of filtered crude extract were dissolved in 200 mL of ethyl acetate and transferred to a separating funnel. A saturated solution of sodium bicarbonate (10mL) was added and the two layers were mixed gently. The aqueous layer was removed, acidified with aqueous HCl and poured back onto the separating funnel and extracted with ethyl acetate. The equations below illustrate what happened.

$$\text{R-COOH (in crude extract)} + NaHCO_3\,(aq) \longrightarrow \text{RCOO-Na}\,(aq) + H_2CO_3 + \text{Non acidic components}$$

$$\text{RCOO-Na}\,(aq) + HCl\,(aq) \longrightarrow \text{RCOOH} + Na^+Cl^-(aq)$$

The non-acidic component was subjected to column chromatography, eluting with hexane-EtOAc (8:2). 136 fractions, 10 mL each were collected. Fractions 84-110 were combined and the solvent removed to yield 242 mg of impure gum-like material. The gum was purified on a chromatotron to yield 44 mg of greenish crystals of methyl labdanolate **(3.14)** with $R_f$ 0.7 (hexane-EtOAc, 7:3).

Description: greenish crystals

Empirical formula: $C_{21}H_{38}O_3$

Melting point: 62-64 $^oC$ (lit.[10] 68.3-71.3)

Optical rotation: $[\alpha]_D^{30}$ +12 (c 0. 200, MeOH) (lit.[34], +10)

$^1H$ NMR ($CDCl_3$) $\delta_H$: 1.86 (1H, dt, *J* 12.0, 3.1 Hz, 7-βH), 2.14 (1H, dd, *J* 14.6, 8.0 Hz, 14-H), 2.36 (1H, dd, *J* 14.6, 5.8 Hz, 14-H′), 0.95 (3H, d, *J* 6.7 Hz, 16-$CH_3$), 1.15 (3H, s, 17-$CH_3$), 0.87 (3H, s, 18-α$CH_3$), 0.79 (6H, s, 19-β$CH_3$, 20-$CH_3$), 3.66 (3H, s, $OCH_3$)

$^{13}C$ NMR ($CDCl_3$) $\delta_C$:

37.2 (t, C-1), 18.5 (t, C-2), 42.0 (t, C-3), 33.3(s, C-4),
56.2 (d, C-5), 20.6 (t, C-6), 44.6 (t, C-7), 74.3 (s, C-8),
62.1(d, C-9), 39.2 (s, C-10), 22.4 (t, C-11), 40.3 (t, C12)
31.1(d, C-13), 41.3 (t, C-14), 174.0 (s, C-15), 20.0 (q, C-16),
24.0 (q, C-17), 33.4 (q, C-18), 21.5 (q, C-19), 15.5 (q, C-20),
51.4 (q, $OCH_3$)

ESI-MS (positive mode) *m/z*: 361.2716 $[M + Na]^+$ (Calc. for $C_{21}H_{38}O_3Na$: 361.2719)

### 3.4.6 Isolation of viridiflorol (3.16)

The above-mentioned process in Section 3.4.5 was repeated and the non-acidic material of the crude extract was chromatographed using isocratic elution with hexane-$CH_2Cl_2$ (15:5). 138 fractions, 10 mL each, were collected and concentrated in a fume hood. Each fraction was inspected by TLC. Fractions 85-102 showed only one component under UV light and when the TLC plates were stained. They were then combined and the solvent evaporated.

This process afforded 34 mg of colourless crystals of viridiflorol or (1*S*,4*R*,5*S*,6*R*,7*R*)-4,10,11,11-tetramethyltricycloundecan-10α-ol (**3.16)**.

| | |
|---|---|
| Description: | colourless crystals |
| Empirical formula: | $C_{15}H_{26}O$ |
| Melting point: | 63-65 $^{o}$C (lit.[35] 74 $^{o}$C) |
| Optical rotation: | $[\alpha]_D^{30}$ +10 (c 0. 100, MeOH) (lit.[36], +5.4) |
| $^{1}$H NMR ($CDCl_3$) $\delta_{H:}$ | 0.13 (1H, t, *J* 9.2 Hz, H-6), 0.61(1H, m, H-7), 1.02 (3H, s, H-12), 1.00 (3H, s, H-13), 1.15 (3H, s, H-14), 0.93(3H, d, *J* 6.8 Hz, H-15), 1.77 (m), 1.94 (m), 1.80 (m). |
| $^{13}$C NMR ($CDCl_3$) $\delta_C$: | 58.3 (d, C-1), 25.8 (t, C-2), 29.1 (t, C-3), 38.6 (d, C-4), 39.8 (d, C-5), 22.4 (d, C-6), 28.7 (d, C-7), 18.9 (t, C-8), 37.8 (t, C-9), 74.7 (s, C-10), 18.4 (s, C-11), 28.6 (q, 12), 16.1(q, C-13), 32.1(q, C-14), 16.3 (q, C-15) |
| EI-MS *m*/*z*: | 204.12 $[M - H_2O]^+$ |

## 3.5 REFERENCES


1. Bremer, K. 1994. Asteraceae: Cladistics & Classification. Timber Press, Inc. Portland, p 304.
2. http://www.plantzafrica.com/medmonographs/elytraprhino.pdf Date of access: 20 February 2009.
3. Germishuizen, G. and Meyer, N.L. (Ed.). 2003. Plants of Southern Africa. An Annotated Checklist. National Botanical Institute, Pretoria, p. 186.
4. Plants of Southern Africa, online checklist. http://www.sanbi.org/frames/posafram.htm Accessed on 20 February 2009.
5. Koekemoer, M. 2002. Systematics of the *Metalasia* group in the Relhaniinae (Asteraceae – Gnaphaliae). Ph. D. Thesis, Rand Afrikaans University, Johannesburg.
6. Dekker, T.G., Fourie, T.G., Matthee, E., Snyckers, F.O., van der Schyf, C.J., Boeyens, J.C.A., Denner, L. 1988. Studies of South African medicinal plants. Part

7. Rhinocerotinoic acid: a labdane diterpene with anti-inflammatory properties from *Elytropappus rhinocerotis*. *South African Journal of Chemistry*, **41**, 33-35.

7. Watt, J.M., Breyer-Brandwijk, M.G. 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd edition. Livingston, London. p. 226.

8. van Wyk, B.-E., van Oudtshoorn, B., Gericke, N. 1997. Medicinal Plants of South Africa, 1st Ed. Briza Publications, Pretoria. p. 118-119.

9. Smith, C.A. 1966. Common Names of South African Plants. Botanical Survey Memoir **35** p. 387.

10. Proksch, P., Proksch, M., Rundel, P.W., Rodriguez, E. 1982. Ecological significance of the chemistry of the leaf resin of *Elytropappus rhinocerotis*. *Biochemical Systematics and Ecology*, **10**, 49-53.

11. Ramirez, M., Tellez, N., Torrenegra, R. 1998. (+)-8-Hydroxylabdan-15-oic acid. *Molecules*, **3**, M48.

12. Castro, J.M., Salido, S., Altarejos, J., Nogueras, M., Sanchez, A. 2002. Synthesis of ambrox from labdanolic acid. *Tetrahedron*, **58**, 5941-5949.

13. Jayaprakasam, B., Alexander-Lindo, R.L., DeWitt, D.L., Nair, M.G. 2007. terpenoids from stinking toe (Hymneae courbaril) fruits with cyclooxygenase and lipid peroxydation inhibitory activities. *Food Chemistry*, **105,** 485-490.

14. Cocker, J.D., Harsall, T.G. 1956. The chemistry of gum labdanum. II. The structure of labdanolic acid. *Journal of the Chemical Society*, 4262-4271.

15. Integrated spectral data base for organic compounds. http:/www.riodb01.ibase.aist.go.jp/sdbs/cgi-bin,direct-frame-top.cgi Date of access: 5thJuly 2008.

16. Ortuno, A., Botia, J.M., Fuster, M.P., Porras, I., Garcia-Lidon, A., Del Rio, J.A. 1997. Effect of scoparone (6,7-dimethoxycoumarin) biosynthesis on the resistance of Tangelo nova, *Citrus paradis*, and *Citrus aurantium* fruits against *Phytophtora parasitica*. *Journal of Agricultural and Food Chemistry*, **45**, 2740-2743.

17. Huang, H.-C., Lee, C.-R., Weng, Y.-I., Lee, M.-C., Lee, Y.-T. 1992. Vasodilator effect of scoparone (6,7-dimethoxycoumarin) from a Chinese herb. *European Journal of Pharmacology*, **218**, 123-128.

18. Huang, J., Wan, X., Sun, A. 1993. Effect of 6,7-dimethoxycoumarin on left ventricular function and hemodynamics in anesthetized cats. *Zhongguo Yaoli Xuebao*, **14**, 253-256. (obtained from Scifinder Scholars)
19. Huang, H.-C., Huang, Y.L., Chang, J.H., Chen C.C., Lee, Y.T. 1992. Possible mechanism of immunosuppressive effect of scoparone (6,7- dimethoxycoumarin). *European Journal of Pharmacology*, **217**, 143-148.
20. Wan, Y., Liu, J., Xu, J. 1989. Preventive effect of 6,7-dimethoxy-coumarin for rats subjected to whole- body single γ-irradiation. *Hejishu*, **12**, 121-126. (abstract available on Scifinder Scholar)
21. Wan, Y., He, G., Zang, Q., Liu, H.1988. Radioprotective action of 6,7-dimethoxycoumarin. *Zhonghua Fangshe Yixue Yu Fanghu Zazhi* **8**, 94-99. (abstract obtained from Scifinder Scholar)
22. Dubery, I.A., Schabort, J.C. 1987. 6,7-Dimethoxycoumarin: a stress metabolite with antifungal activity in γ-irradiated citrus peel. *South African Journal of Science*, **83**, 440-441.
23. Malseed, R.T. 1982. Pharmacology: Therapy and Nusing Considerations, Lippincott, Philadelphia.
24. Bombarda, I., Raharivelomanana, P., Ramanoelina, P.A.R., Faure, R., Bianchini, J.P., Gaydou, E.M. 2001. Spectrometric identifications of sesquiterpene alcohols from niaouli (*Melaleuca quinqenervia*) essential oil. *Analytica Chimica Acta*, **447**, 113-123.
25. Wheeler, G.S., Massey, L.M., Southwell, I.A. 2002. Antipredator defense of biological control agent Oxyops vitiosa is mediated by plant volatiles sequestered from the host plant *Melaleuca quinquenervia*. *Journal of Chemical Ecology*, **28**, 297-315.
26. Wheeler, G.S., Massey, L.M., Southwell, I.A. 2003. Dietary influences on terpenoids sequestered by biological control agent oxyops vitiosa: effect of plant volatiles from different *Melaleuca quinquenervia* chemotypes and laboratory host species. *Journal of Chemical Ecology*, **29**, 189-208.
27. Hood, J.J.D. 1998. *Kunzea ambigua* oil for therapeutic and insect repellent uses, PCT Int. Appl., Australia.

28. Rojahn, W., Hammmerschmidt, F.G., Ziegler, E., Hefendehl, F.W. 1977. Presence of viridiflorol in peppermint oil (*Mentha piperita* L.). Dragoco Report (German Edition), **24**, 230-232 (abstract obtained from Scifinder Scholar).
29. Gbenou, J.D., Moudachirou, M., Chalchat, J-C., Figueredo, G. 2007. Chemotypes in *Malaleuca quinquenervia* (Cav.) S.T. Blake (Niaouli) from Benin using multivariate statistical analysis of their essential oils. *Journal of Essential oil Research*, **19**, 101-104.
30. Porazinska, D.L., Pratt, P.D., Glblin-Davis, R.M. 2007. Consequences of *Melaleuca quinquinervia* invasion on soil nematodes in the Florida Everglades. *Journal of Nematology*, **37**, 305-312.
31. Lopez-Zamora, I., Comerford, N.B., Muchovj, R.M. 2004. Root development and competitive ability of the invasive species *Melaleuca quinquenervia* (Cav.) S.T. Blake in the South Florida flatwoods. *Plant and Soil*, **263**, 239-247.
32. Trilles, B., Bouraima-Madjebi, S., Valet, G. 1999. *Malaleuca quinquenervia* (Cavanilles) S.T. Blake, Niaouli. *Medicinal and aromatic Plants-Industrial profiles*, **9**, 237-245.
33. Aldrich Chemical Company. 2005-2006. Aldrich: Advancing science, p. 972
34. Dictionary of Organic Compounds, 5th Ed., Chapman and Hall, New York, p. 3532.
35. Feliciano, A.S., Medarde, M., Gordaliza, M., Del Olmo, E., Miguel Del Corral, J.M. 1989. Sesquiterpenoids and phenolics of *Puricaria parudosa*. *Phytochemistry*, **28**, 2717-2721.
36. Dictionary of Organic Compounds, 5th Ed., Chapman and Hall, New York, p. 5719.

# CHAPTER FOUR

# CONCLUSIONS

## 4.1 GENERAL CONCLUSIONS

Naturally occurring organic substances are of vital importance in everyday life. These substances are derived from numerous sources, such as plants, animals, marine- and micro-organisms. Natural products are used in different domains of life, for instance in medicine as drugs, in agriculture as insecticides, pesticides, in construction as paints, in food industry as food, in the beauty industry as lotions and perfumes, etc. All of these uses of natural products are possible only if the active / relevant compounds are isolated and identified. Knowledge of their structures allows their synthesis, transformation and bioassays.

It is in this framework that the current study, the isolation and characterisation of naturally occurring compounds from *Artemisia afra* and *Elytropappus rhinocerotis*, two Asteraceae species, was undertaken. Previous investigations have shown that numerous *Artemisia* species contain important biologically active compounds, for example, artemisinin from *Artemisia annua* is being used worldwide as important antimalarial drug, especially for cerebral malaria.[1,2,3,4] Extracts of *A. afra* showed antimicrobial, antifungal and antioxidant activities.[5,6,7] In this project, a chemical investigation has been carried out and compounds belonging to different classes were isolated and characterised. These were for example, maritimin, a sesquiterpene, scopoletin, a coumarin and 7,4′-dimethoxyflavone, a flavonoid. After obtaining these compounds, the first and second objectives of this project, namely the isolation of compounds from two chemotypes of *A. afra* and their characterisation, respectively, were achieved. Inspection by TLC of the hexane and $CH_2Cl_2$-MeOH extracts for both chemotypes showed no significant difference between the two chemotypes.

Little information regarding the chemical components of *Elytropappus rhinocerotis*, which grows in the Eastern Cape and Western Cape provinces in South Africa, is known. The species showed a wide range of activities, including enzyme inhibition, photoprotection against UV radiation and anti-inflammatory activities. [8, 9]

Motivated by the biological activities of *E. rhinocerotis*, its chemical investigation was carried out during this project. Compounds isolated and characterised include an acid, labdanolic acid; an ester, methyl labdanolate; a coumarin, scoparone or 6,7-dimethoxycoumarin; and a sesquiterpene alcohol, viridiflorol or (1*S*,4*R*,5*S*,6*R*,7*R*)-4,10,11,11-tetramethyltricyclo-undecan-10α-ol. The isolation and characterisation of these compounds was mentioned in the objectives three and four of the project, and hence these were achieved. The known inhibitory activity of labdanolic acid on the enzyme COX-2 may explain the ethnobotanical use of the plant as an anti-inflammatory.

All compounds isolated from both *A. afra* and *E. rhinocerotis*, are known compounds but have not previously been reported from these species, except the sesquiterpene taurin, the flavone, 5-hydroxy-7,4′-dimethoxyflavone and the coumarin, 7-hydroxy-6-methoxycoumarin.

## 4.2 REFERENCES


1. Paramanik, R.C., Chikkaswamy, B.K., Debojyoti, G.R., Paramanik, A., Venkatesh, K. 2008. Effects of biochemicals of *Artemisia annua* in plants. *Journal of Phytological Research*, **21**, 11-18.
2. Lapanna, S., Bengozi, M.C., Vincieri, F.F., Bilia, A. R. 2008. Comparative analysis of antimalarial principles in *Artemisia annua* L. herbal drug from East Africa. *Natural Products Communications*, **3**, 2003-2006.
3. Namdeo, A.G., Mahadik, K.R., Kadam, S.S. 2006. Antimalarial drug - *Artemisia* annua. *Pharmacognosy Magazine*, **2**, 106-111.
4. Iwu, M.M. 1993. Handbook of African Medicinal plants, CRC Press, Inc, London. pp 121-122.
5. Mangena, T., Muyima, N.Y.O. 1999. Comparative evaluation of antimicrobial activities of essential oils of *Artemisia afra*, *Pteronia incana* and *Rosmarinus officinalis* on selected bacteria and yeast strains. *Letters in Applied Microbiology*, **28**, 291-296.
6. Muyima, N.Y.O., Nkata, L. 2005. Inhibition of growth of dermatophyte fungi and yeast associated with dandruff and related scalp inflammatory conditions by the

essential oils of *Artemisia afra*, *Pteronia incana*, *Lavandula officinalis* and *Rosmarinus officinalis*. *Journal of Essential Oils- Bearing Plants*, **8**, 224-232.

7. Watt, J.M., Breyer-Brandwijk, M.G. 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd edition. Livingston, London.

8 . Proksch, P., Proksch, M., Rundel, P.W., Rodriguez, E. 1982. Ecological significance of the chemistry of the leaf resin of *Elytropappus rhinocerotis*, *Biochemical Systematics and Ecology*, **10**, 49-53.

9. Dekker, T.G., Fourie, T.G., Matthee, E., Snyckers, F.O., van der Schyf, C. J., Boeyens, J.C.A., Denner, L. 1988. Studies of South African medicinal plants. Part 7. Rhinocerotinoic acid: a labdane diterpene with anti-inflammatory properties from *Elytropappus rhinocerotis*. *South African Journal of Chemistry*, **41**, 33-35.

# APPENDIX

**NMR spectra of isolated compounds ($CDCl_3$, 400 MHz)**

**Plate 1:** $^{1}H$ NMR of taurin **(2.56)**

**Plate 2:** $^{13}C$ NMR spectrum of taurin **(2.56)**

**Plate 3:** DEPT 135 spectrum of taurin **(2.56)**

**Plate 4:** DEPT 90 spectrum of taurin **(2.56)**

**Plate 5:** COSY spectrum of taurin **(2.56)**

**Plate 5a:** NOESY spectrum of taurin **(2.56)**

**Plate 6:** HSQC spectrum of taurin **(2.56)**

**Plate 7:** HMBC spectrum of taurin **(2.56)**

**Plate 8:** $^{1}H$ NMR spectrum of artesin **(2.57)**

**Plate 9:** $^{13}C$ NMR spectrum of artesin **(2.57)**

**Plate 10:** DEPT 135 spectrum of artesin **(2.57)**

**Plate 11:** DEPT 90 spectrum of artesin **(2.57)**

**Plate 12:** COSY spectrum of artesin **(2.57)**

**Plate 13:** HSQC spectrum of artesin **(2.57)**

**Plate 14:** HMBC spectrum of artesin **(2.57)**

**Plate 15:** $^{1}H$ NMR spectrum of maritimin **(2.58)**

**Plate 16:** $^{13}C$ NMR spectrum of maritimin **(2.58)**

**Plate 17:** DEPT135 spectrum of maritimin **(2.58)**

**Plate 18:** DEPT 90 spectrum of maritimin **(2.58)**

**Plate 19:** COSY spectrum of maritimin **(2.58**

**Plate 20:** HSQC spectrum of maritimin **(2.58)**

**Plate 21:** HMBC spectrum of maritimin **(2.58)**

**Plate 22:** $^{1}H$ NMR spectrum of artemin **(2.59)**

**Plate 23:** $^{13}C$ NMR spectrum of artemin **(2.59)**

**Plate 24:** DEPT135 spectrum of artemin **(2.59)**

**Plate 25:** DEPT90 spectrum of artemin **(2.59)**

**Plate 26:** COSY spectrum of artemin **(2.59)**

**Plate 27:** HSQC spectrum of artemin **(2.59)**

**Plate 28:** HMBC spectrum of artemin **(2.59)**

**Plate 63:** HMBC spectrum of *p*-hydroxyacetophenone **(2.67)**

**Plate 64.** 1H NMR spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

**Plate 65.** $^{13}$C NMR spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

**Plate 66.** DEPT135 spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

**Plate 67.** DEPT90 spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

**Plate 68.** COSY spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

**Plate 69.** HSQC spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

**Plate 70.** HMBC spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

**Plate 71:** $^{1}$H NMR spectrum of 5-hydroxy-7,4'-dimethoxyflavone **(2.69)**

**Plate 72:** $^{13}$C NMR spectrum of 5-hydroxy-7,4'-dimethoxyflavone **(2.69)**

**Plate 73:** DEPT135 spectrum of 5-hydroxy-7,4'-dimethoxyflavone **(2.69)**

**Plate 74:** DEPT90 spectrum of 5-hydroxy-7,4'-dimethoxyflavone **(2.69)**

**Plate 75:** COSY spectrum of 5-hydroxy-7,4'-dimethoxyflavone **(2.69)**

**Plate 76:** HSQC spectrum of 5-hydroxy7,4'-dimethoxyflavone **(2.69)**

**Plate 77:** HMBC spectrum of 5-hydroxy-7,4'-dimethoxyflavone **(2.69)**

**Plate 78:** $^{1}$H NMR spectrum of labdanolic acid **(3.13)**

**Plate 79:** $^{13}$C NMR spectrum of labdanolic acid **(3.13)**

**Plate 80:** DEPT135 spectrum of labdanolic acid **(3.13)**

**Plate 81:** DEPT90 spectrum of labdanolic acid **(3.13)**

**Plate 82:** COSY spectrum of labdanolic acid **(3.13)**

**Plate 83:** HSQC spectrum of labdanolic acid **(3.13)**

**Plate 84:** HMBC spectrum of labdanolic acid **(3.13)**

**Plate 85:** $^{1}$H NMR spectrum of methyl labdanolate **(3.14)**

**Plate 86:** $^{13C}$ NMR spectrum of methyl labdanolate **(3.14)**

**Plate 87:** DEPT135 spectrum of methyl labdanolate **(3.14)**

**Plate 88:** DEPT90 spectrum of methyl labdanolate **(3.14)**

**Plate 89:** COSY spectrum of methyl labdanolate **(3.14)**

**Plate 90:** HSQC spectrum of methyl labdanolate **(3.14)**

**Plate 91:** HMBC spectrum of methyl labdanolate **(3.14)**

**Plate 92:** $^{1}$H NMR spectrum of scoparone (6,7-dimethoxycoumarin) **(3.15)**

**Plate 93:** $^{13}$C NMR spectrum of scoparone (6,7-dimethoxycoumarin) **(3.15)**

**Plate 94:** DEPT135 spectrum of scoparone (6,7-dimethoxycoumarin) **(3.15)**

**Plate 95:** DEPT90 spectrum of scoparone (6,7-dimethoxycoumarin) **(3.15)**

**Plate 96:** COSY spectrum of scoparone (6,7-dimethoxycoumarin) **(3.15)**

**Plate 1:** $^{1}$H NMR of taurin **(2.56)**

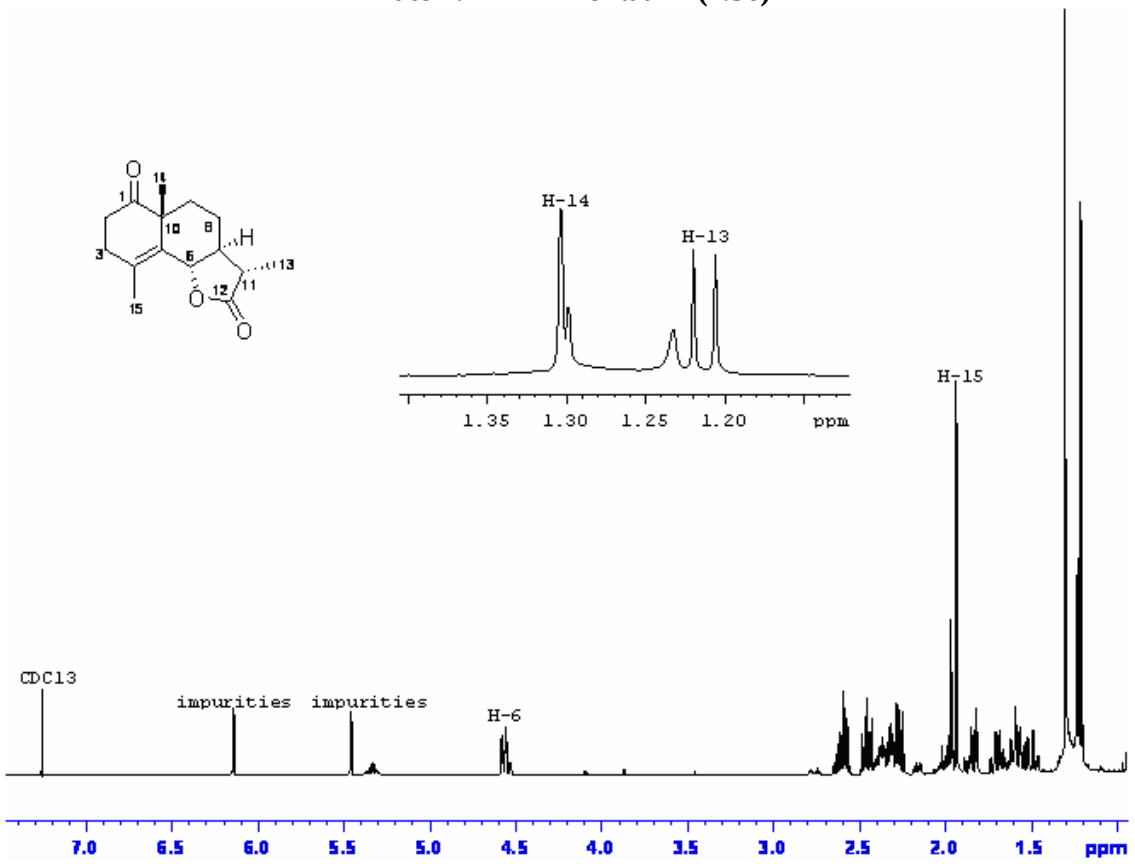


**Plate 2:** $^{13}$C NMR spectrum of taurin **(2.56)**

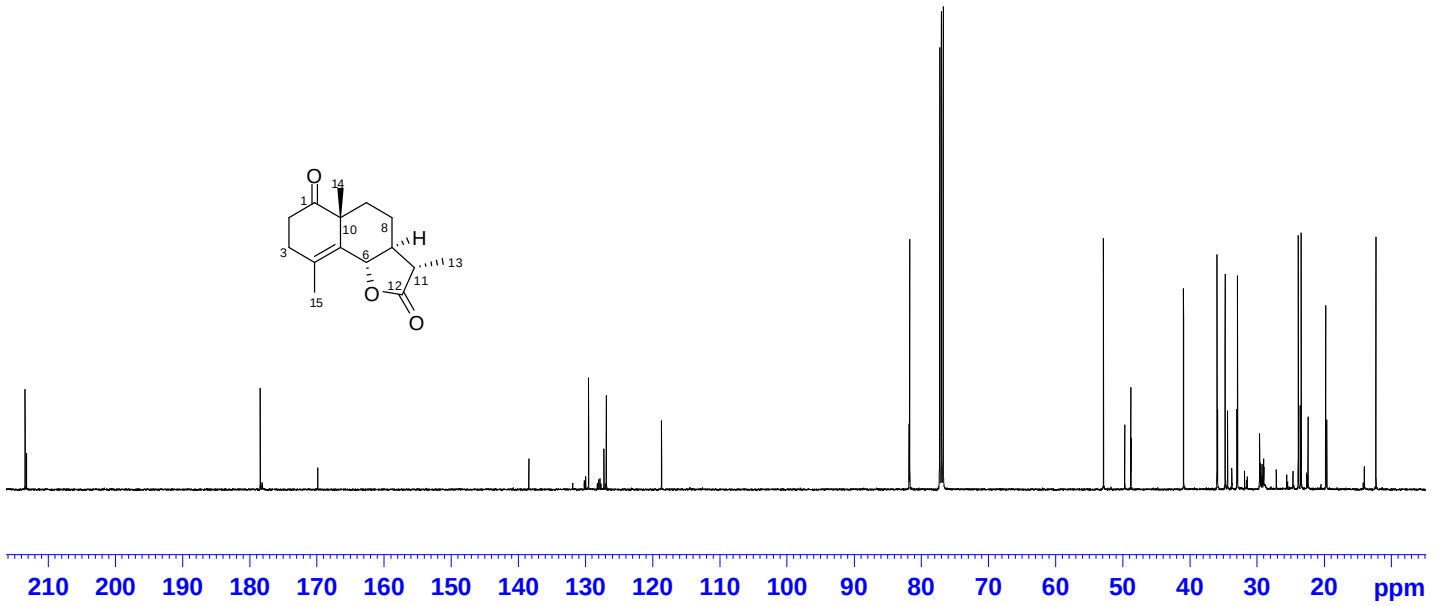


**Plate 3:** DEPT 135 spectrum of taurin **(2.56)**

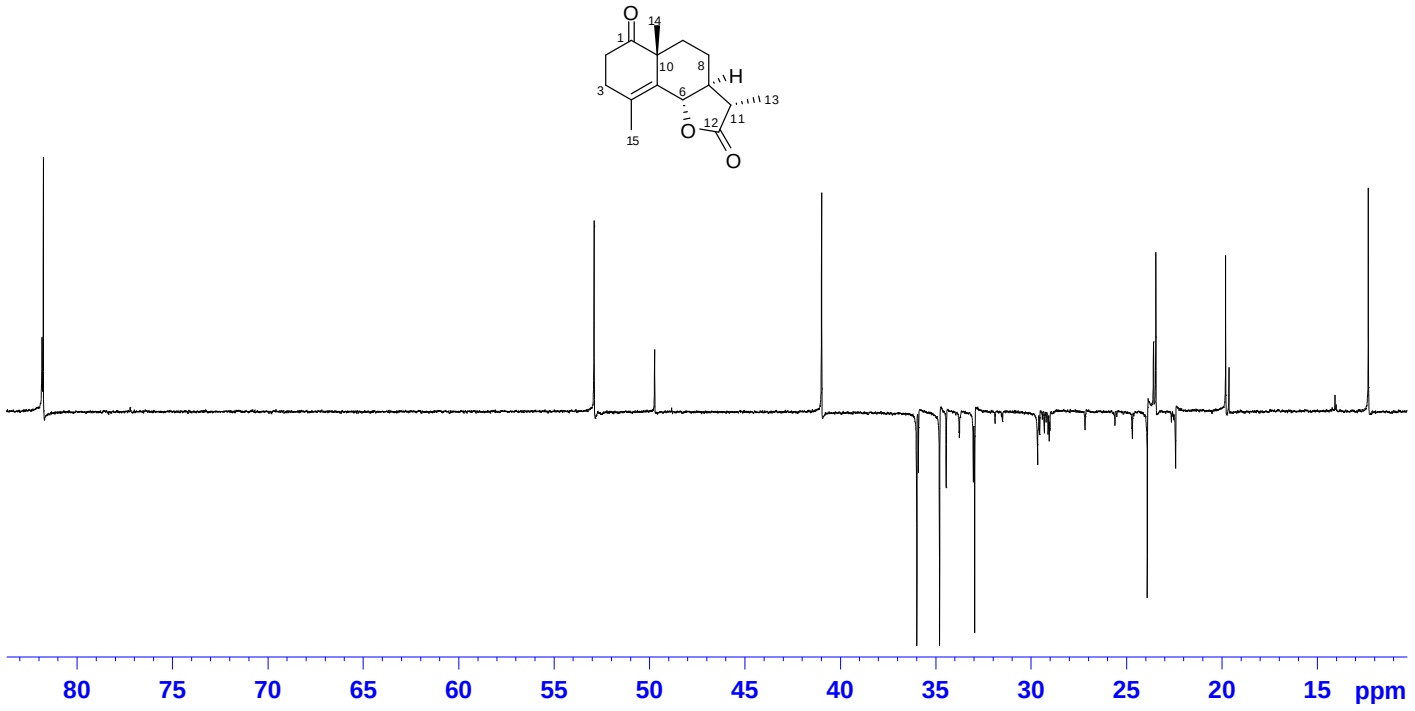


**Plate 4:** DEPT 90 spectrum of taurin **(2.56)**

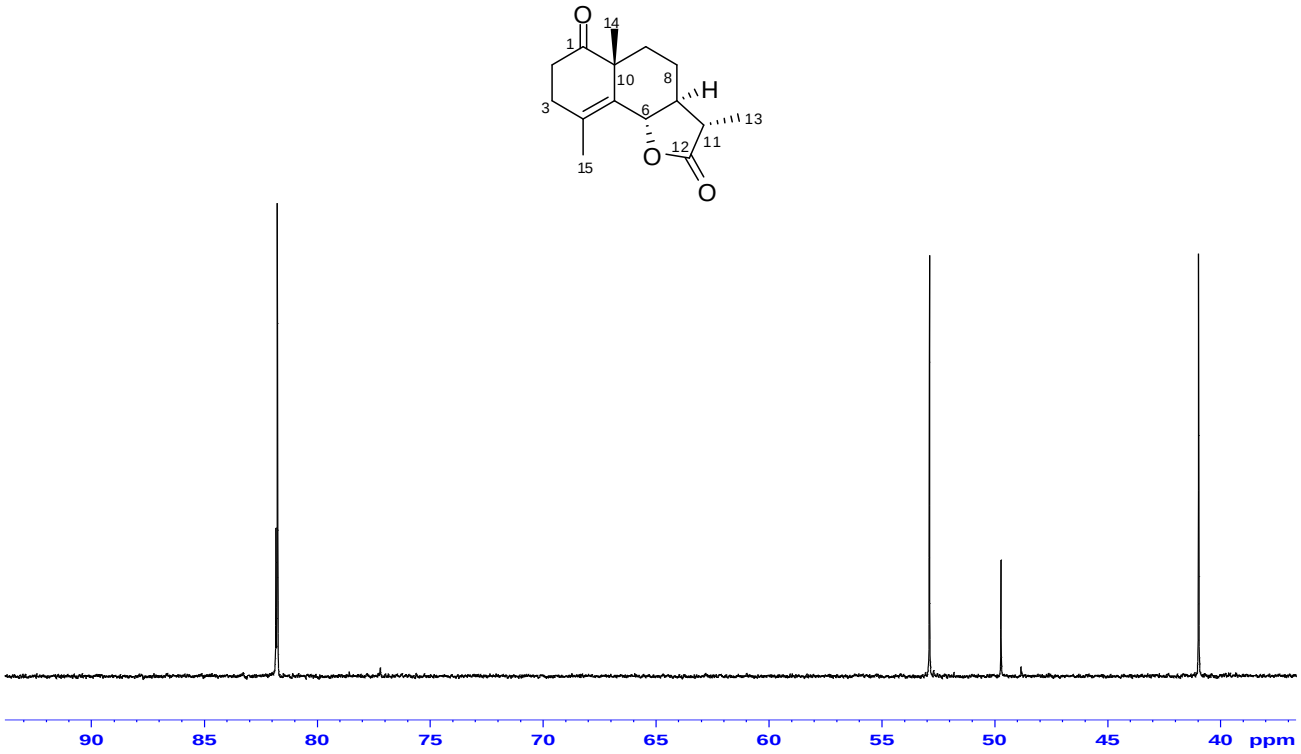


**Plate 5:** COSY spectrum of taurin **(2.56)**

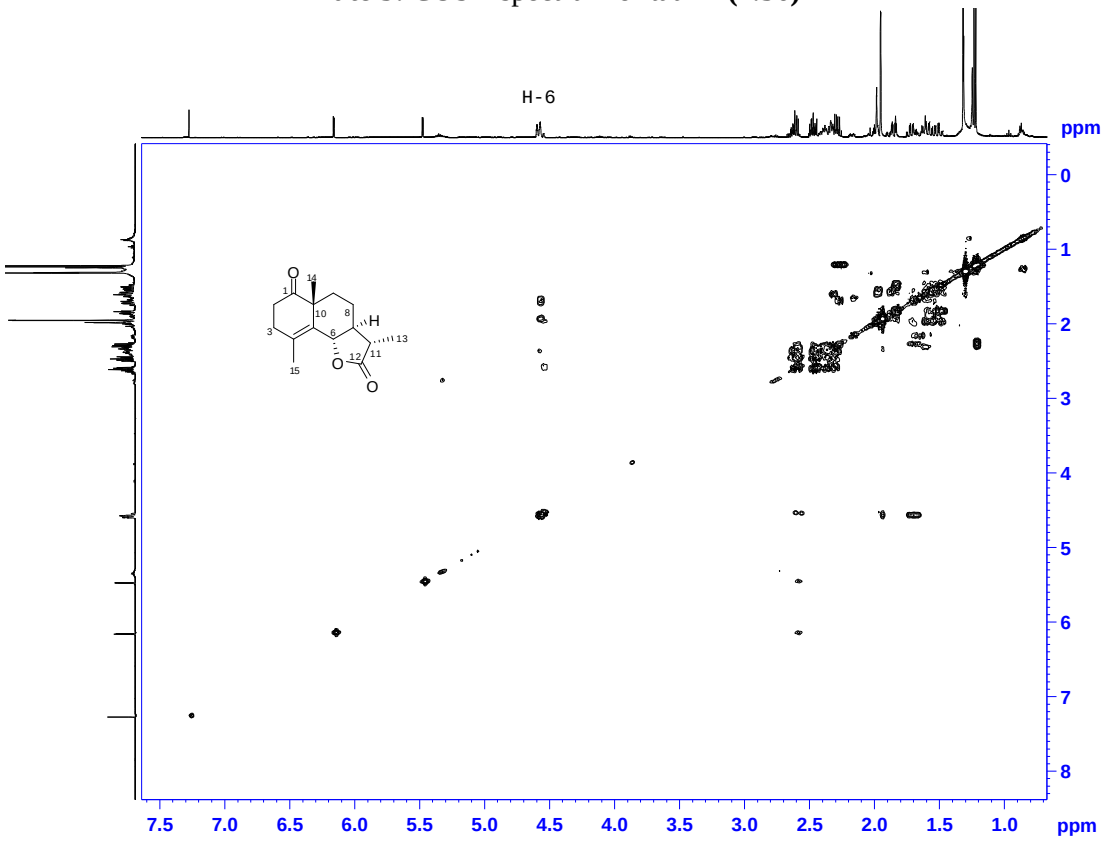


**Plate 5a:** NOESY spectrum of taurin **(2.56)**

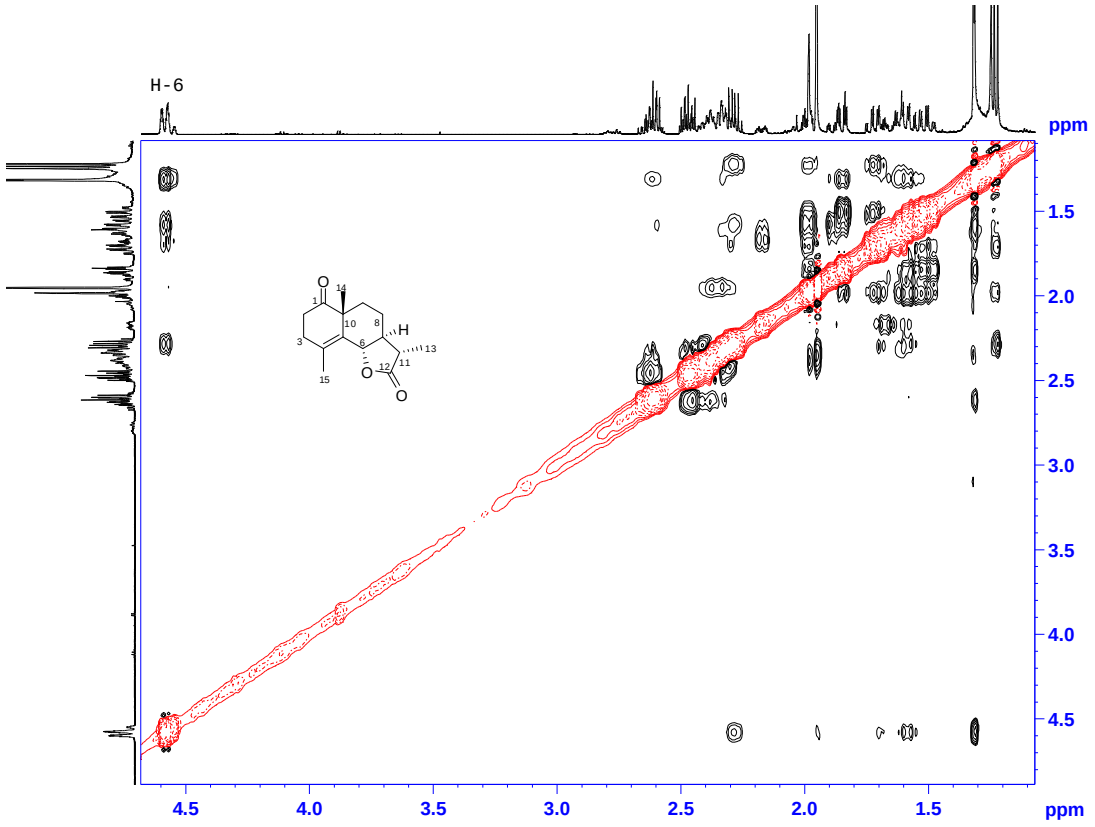


**Plate 6:** HSQC spectrum of taurin **(2.56)**

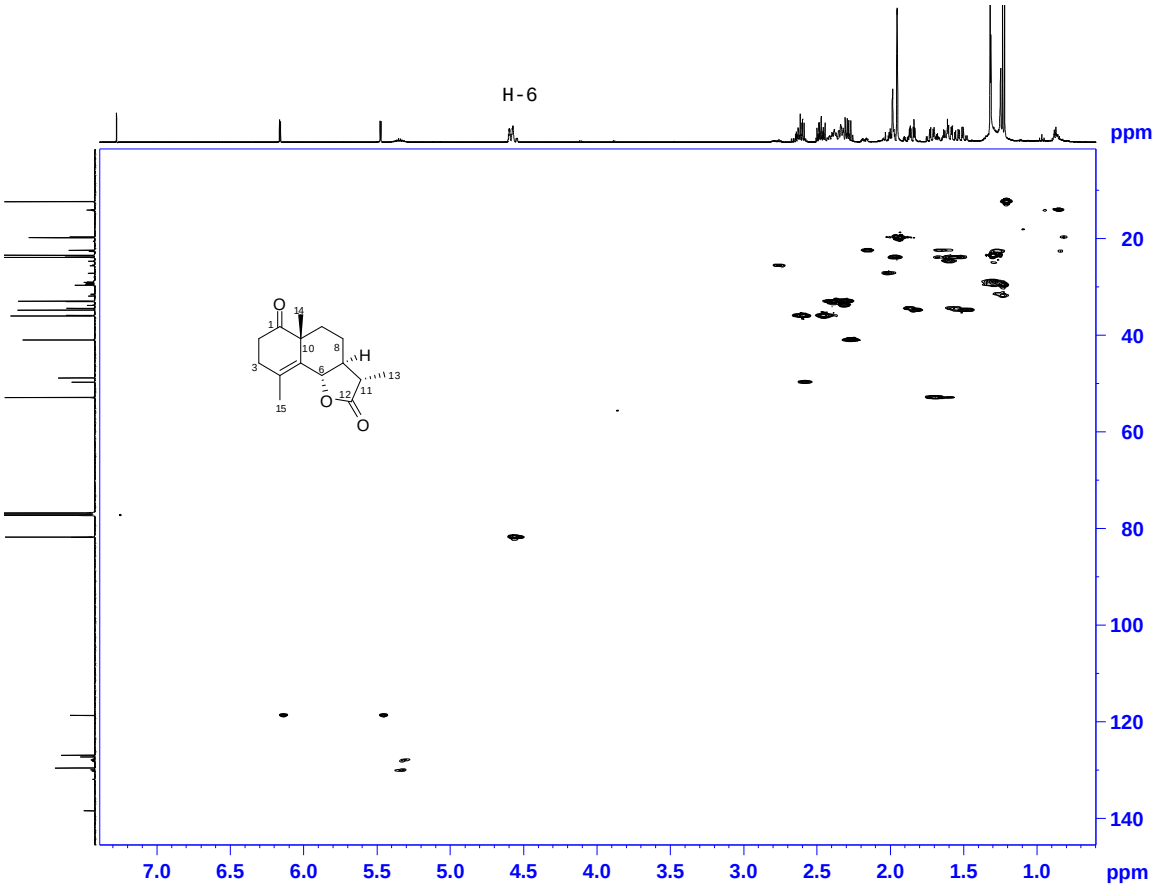


**Plate 7:** HMBC spectrum of taurin **(2.56)**

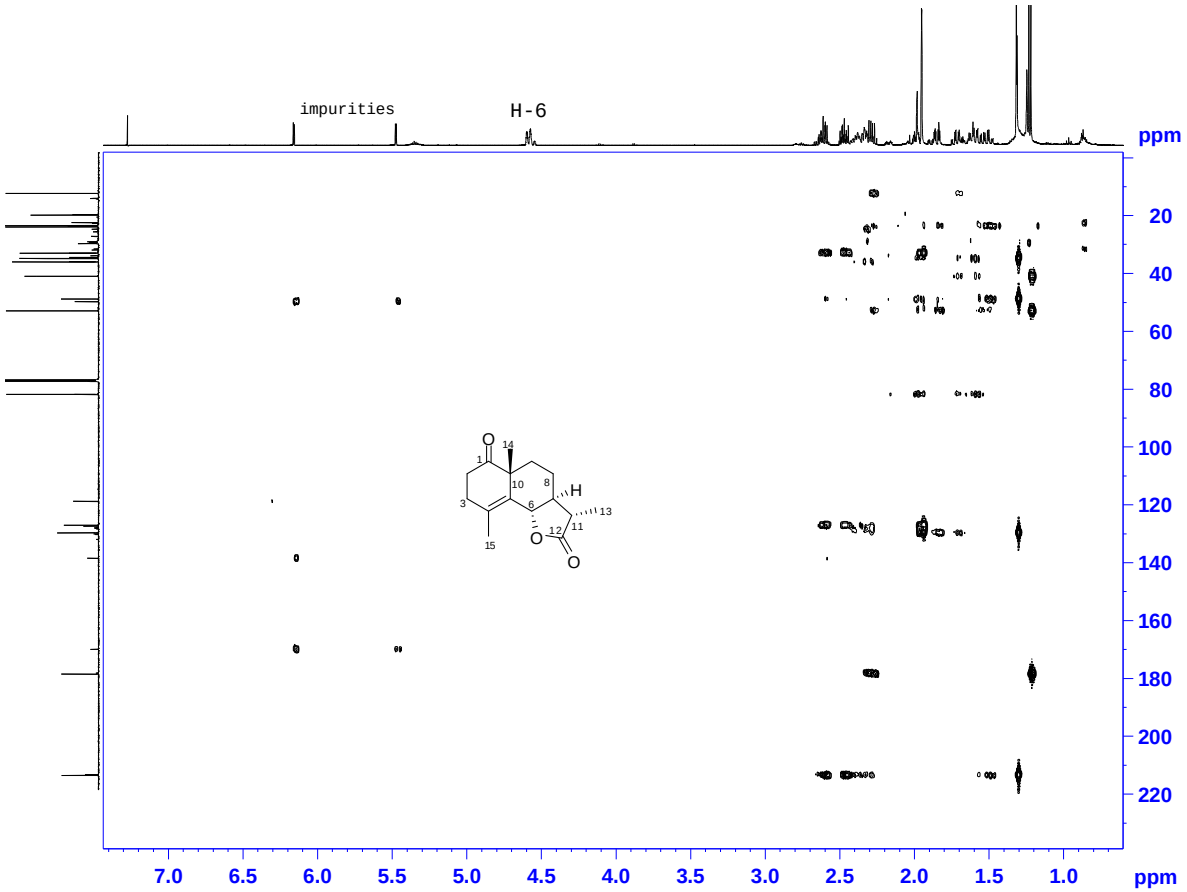


**Plate 8:** $^{1}$H NMR spectrum of artesin **(2.57)**

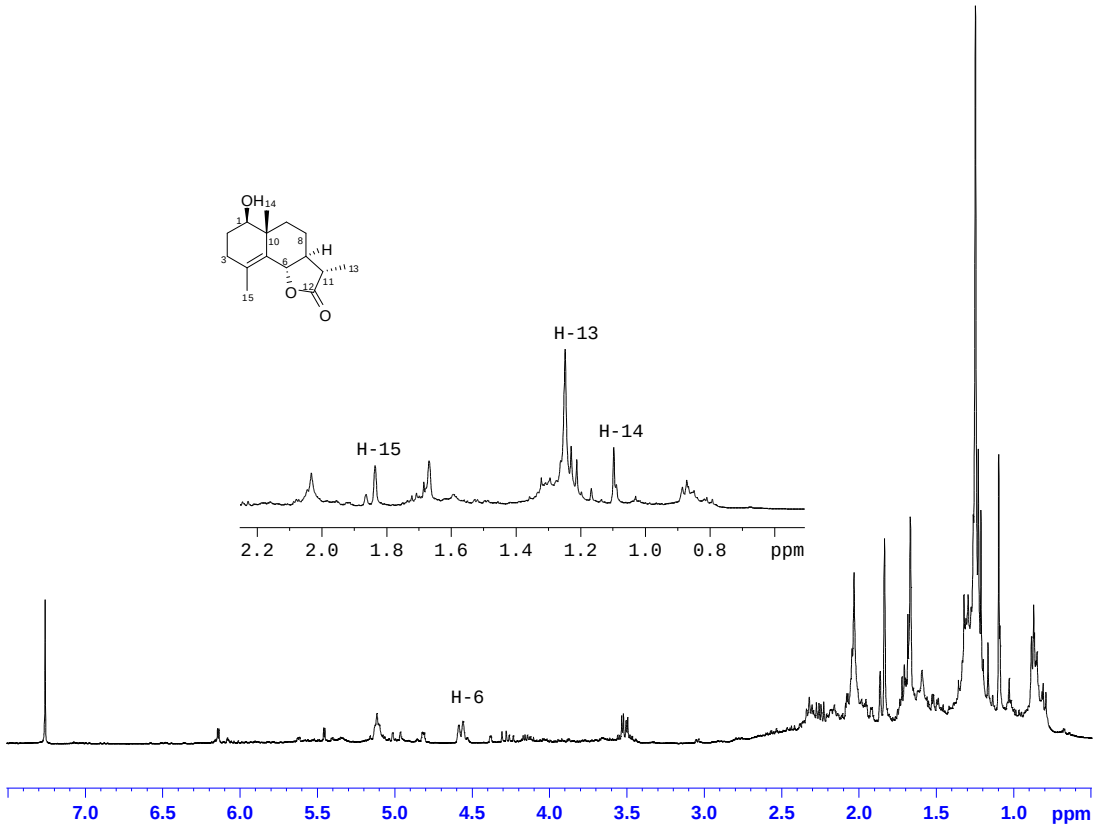


**Plate 9:** $^{13}$C NMR spectrum of artesin **(2.57)**

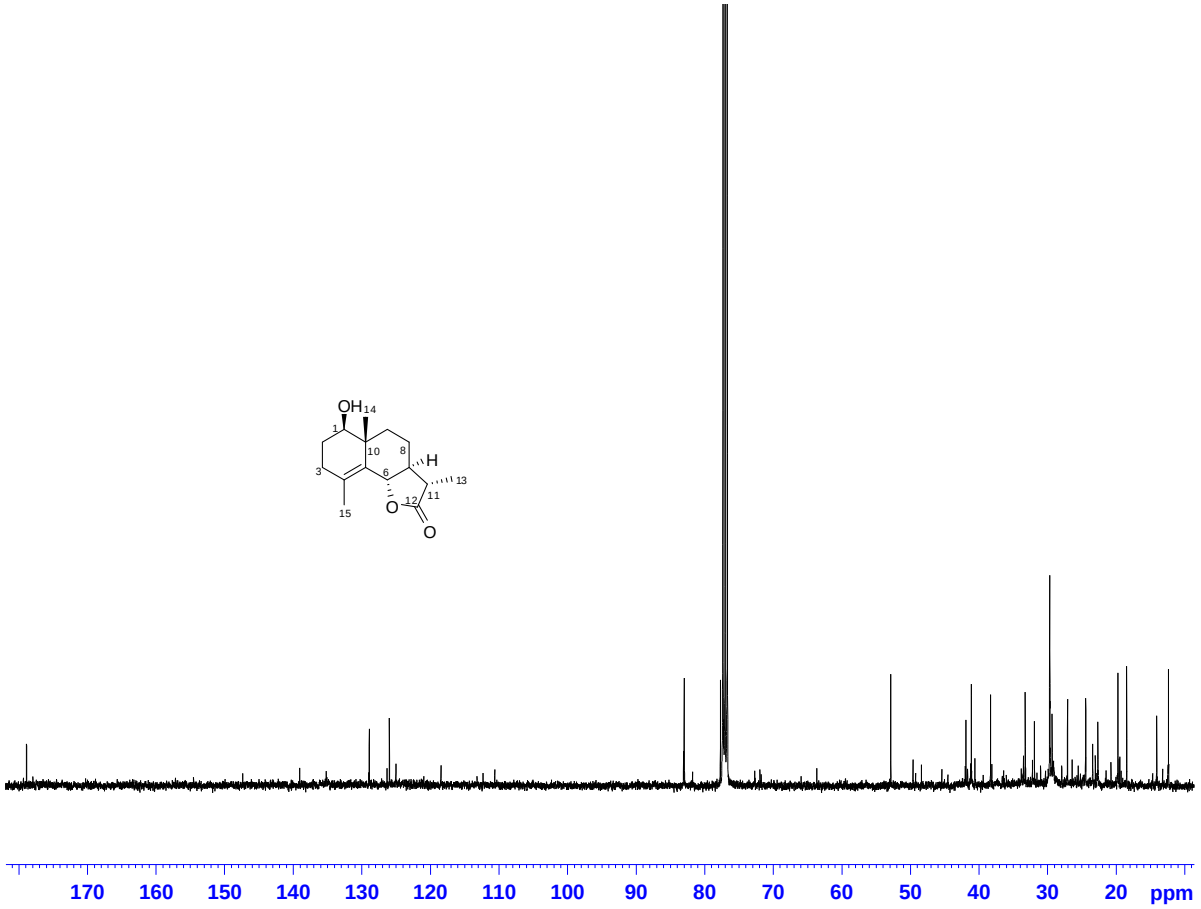


**Plate 10:** DEPT 135 spectrum of artesin **(2.57)**

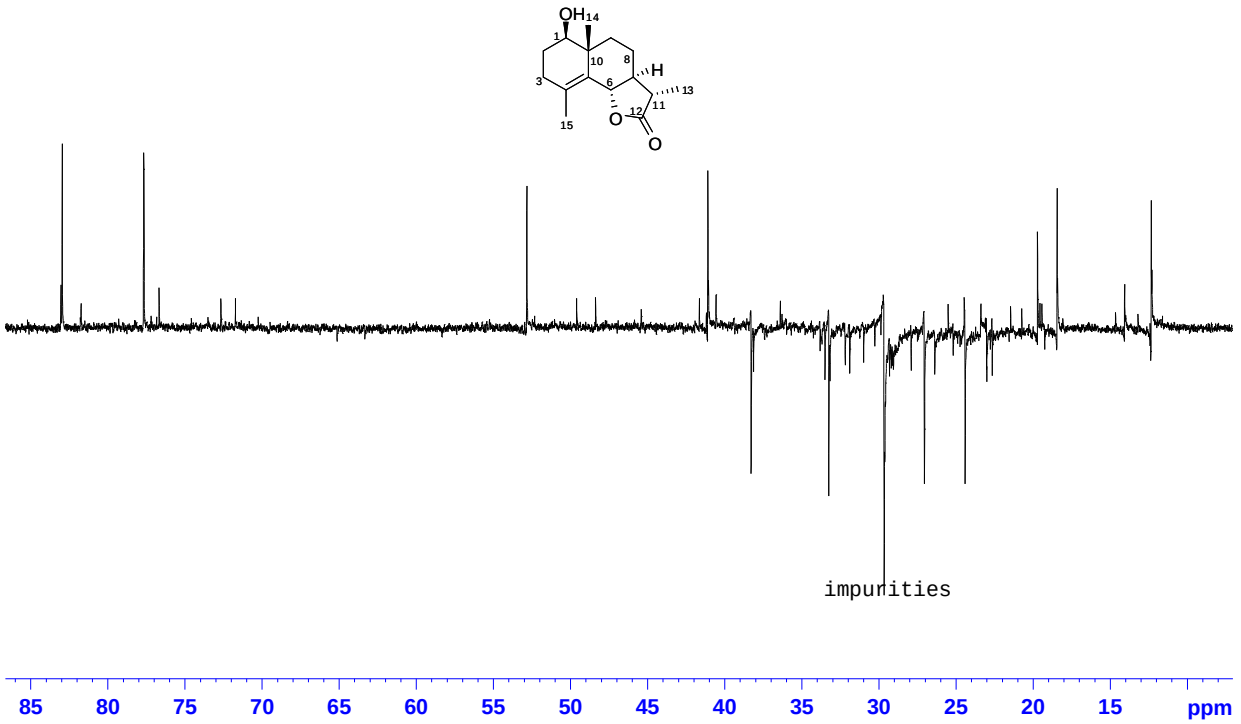


**Plate 11:** DEPT 90 spectrum of artesin **(2.57)**

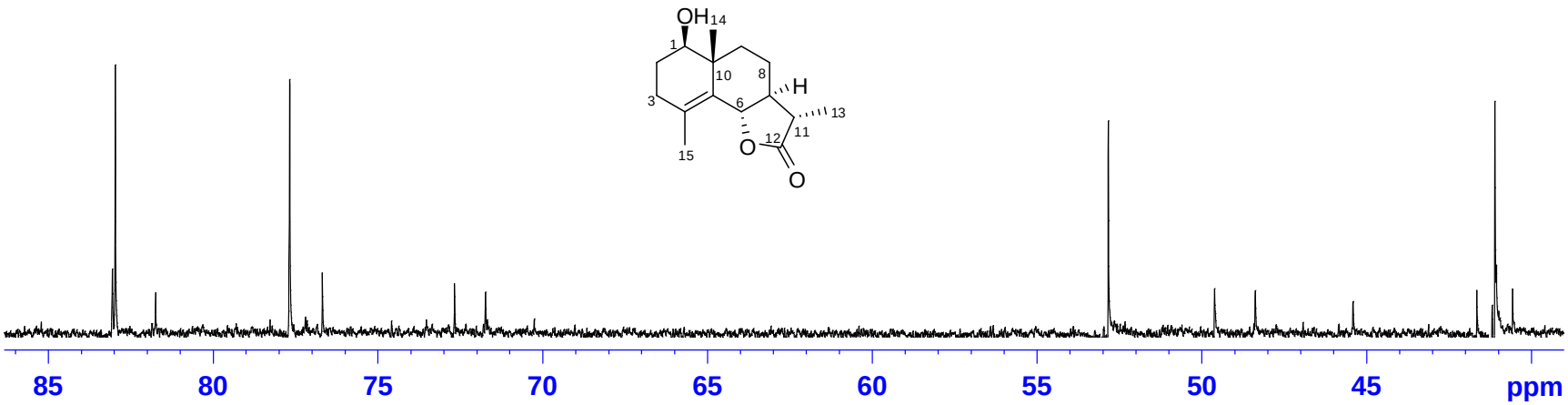


**Plate 12:** COSY spectrum of artesin **(2.57)**

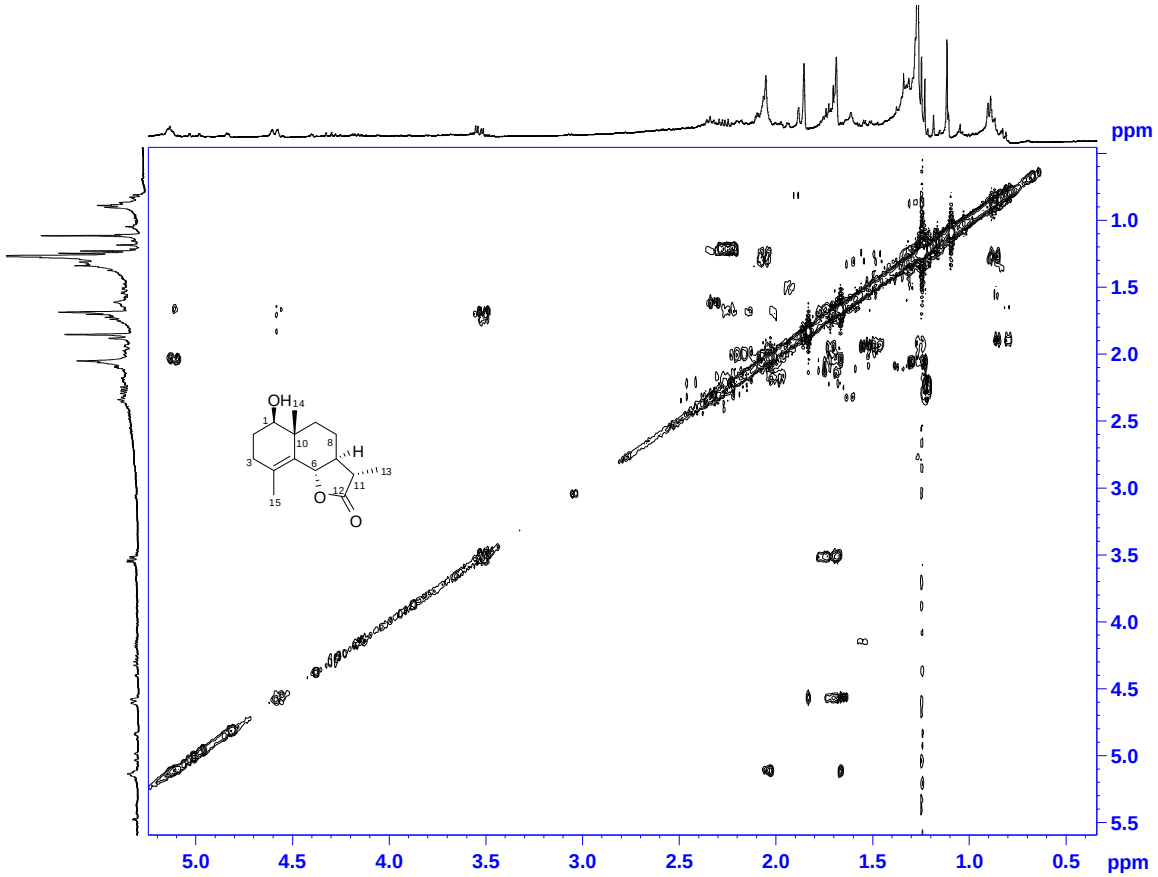


**Plate 13:** HSQC spectrum of artesin **(2.57)**

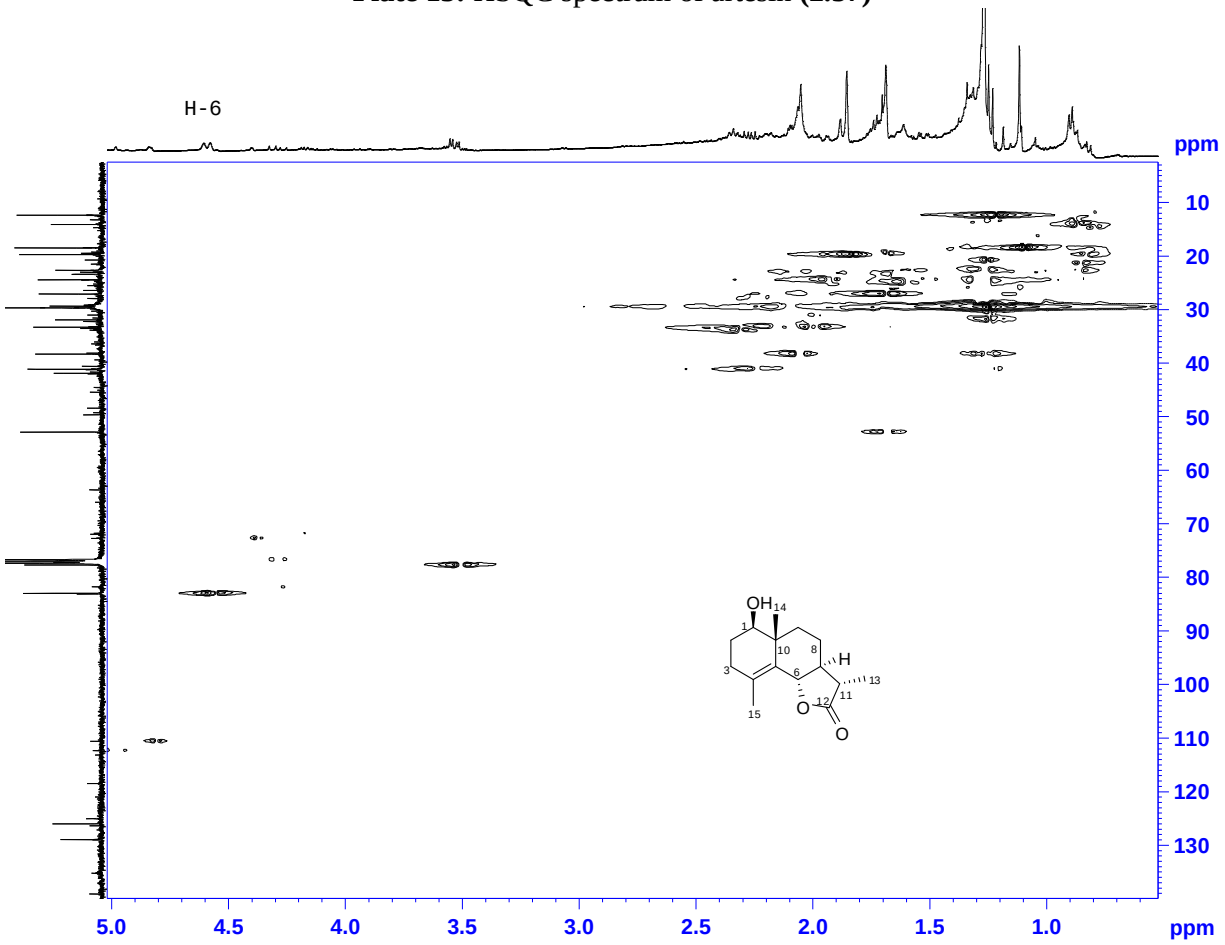


**Plate 14:** HMBC spectrum of artesin **(2.57)**

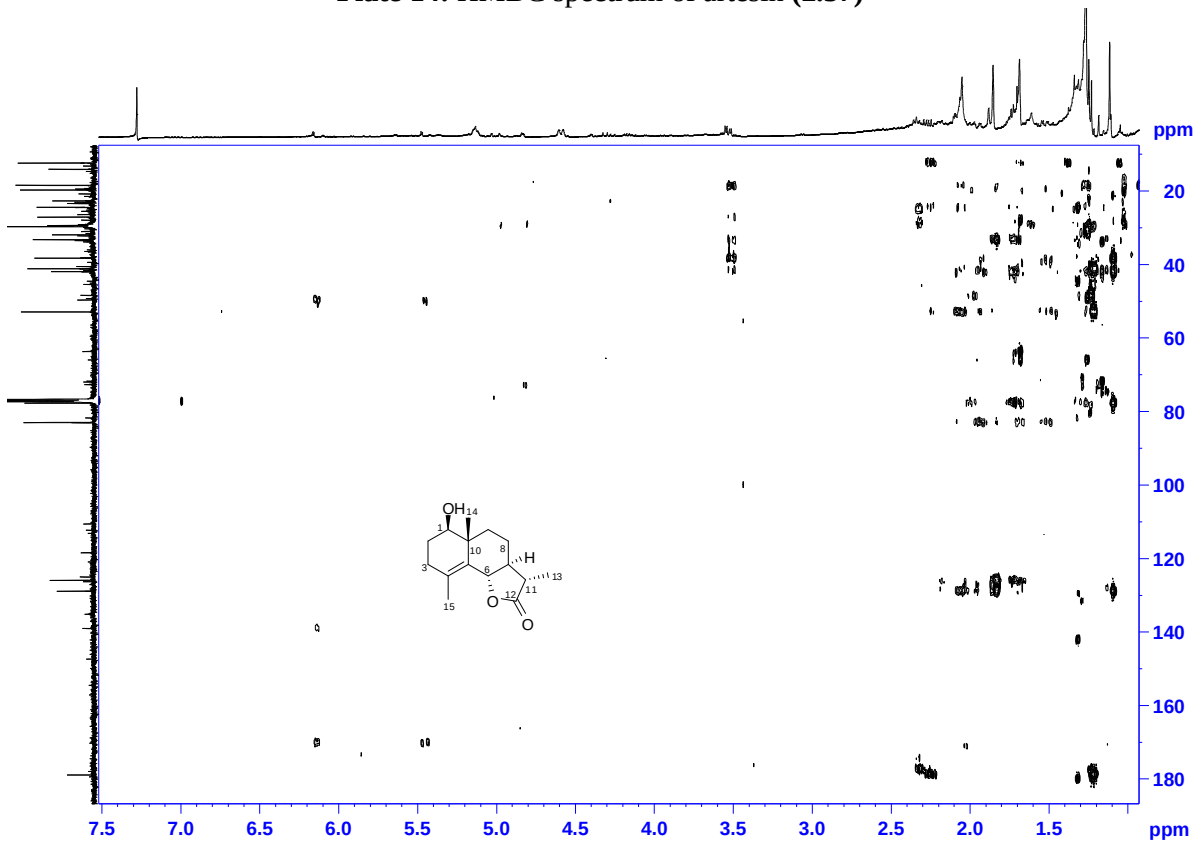


**Plate 15:** $^{1}$H NMR spectrum of maritimin **(2.58)**

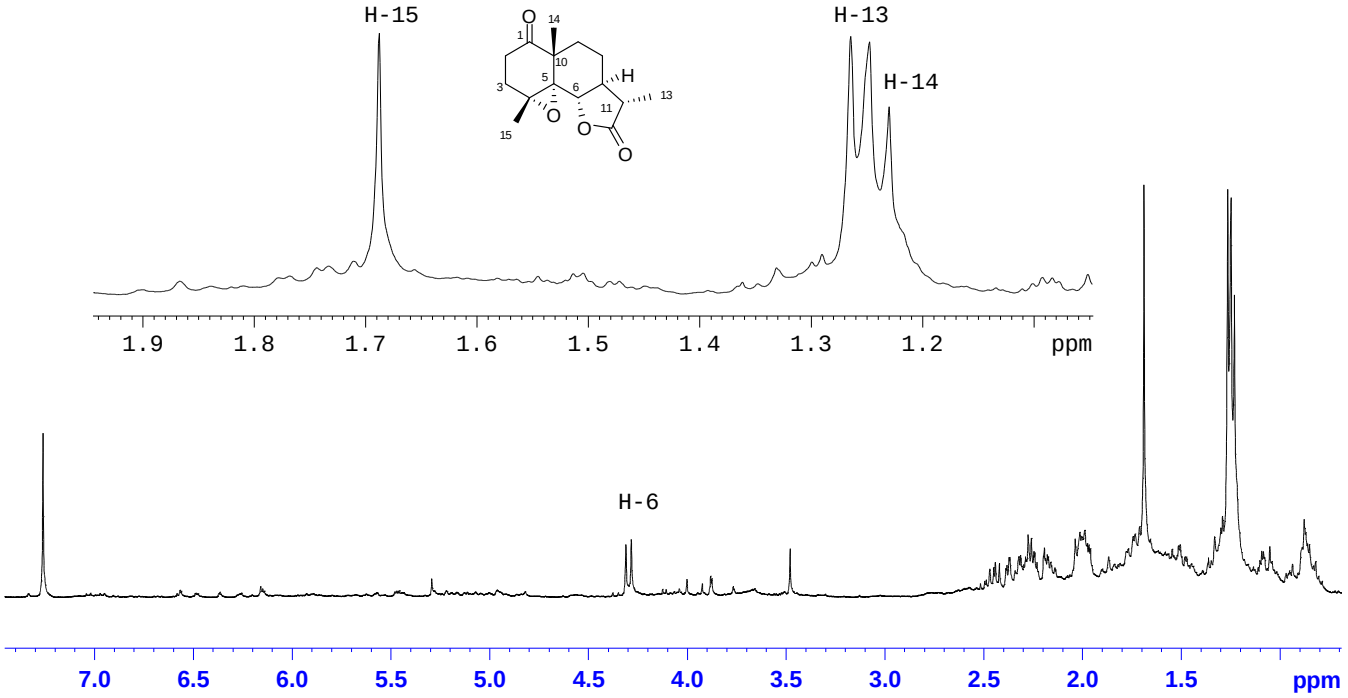


**Plate 16:** $^{13}$C NMR spectrum of maritimin **(2.58)**

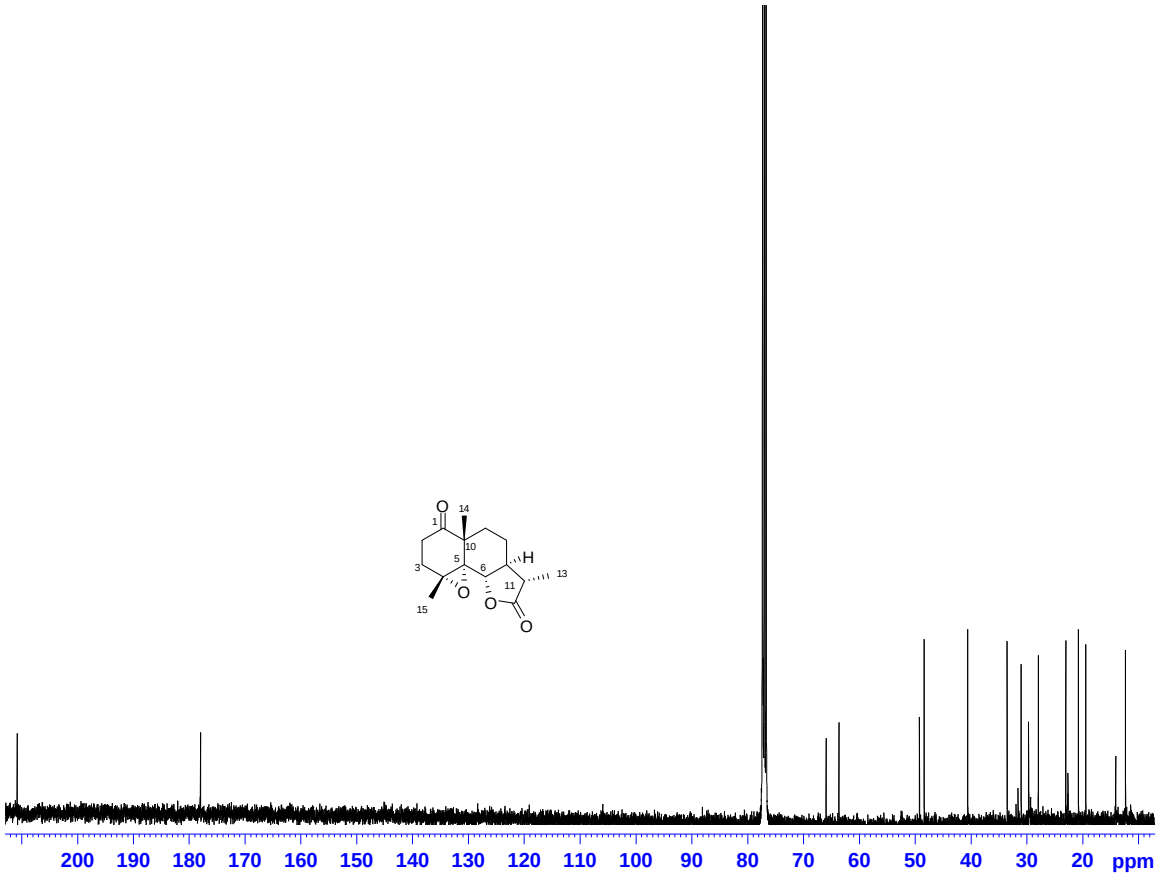


**Plate 17:** DEPT135 spectrum of maritimin **(2.58)**

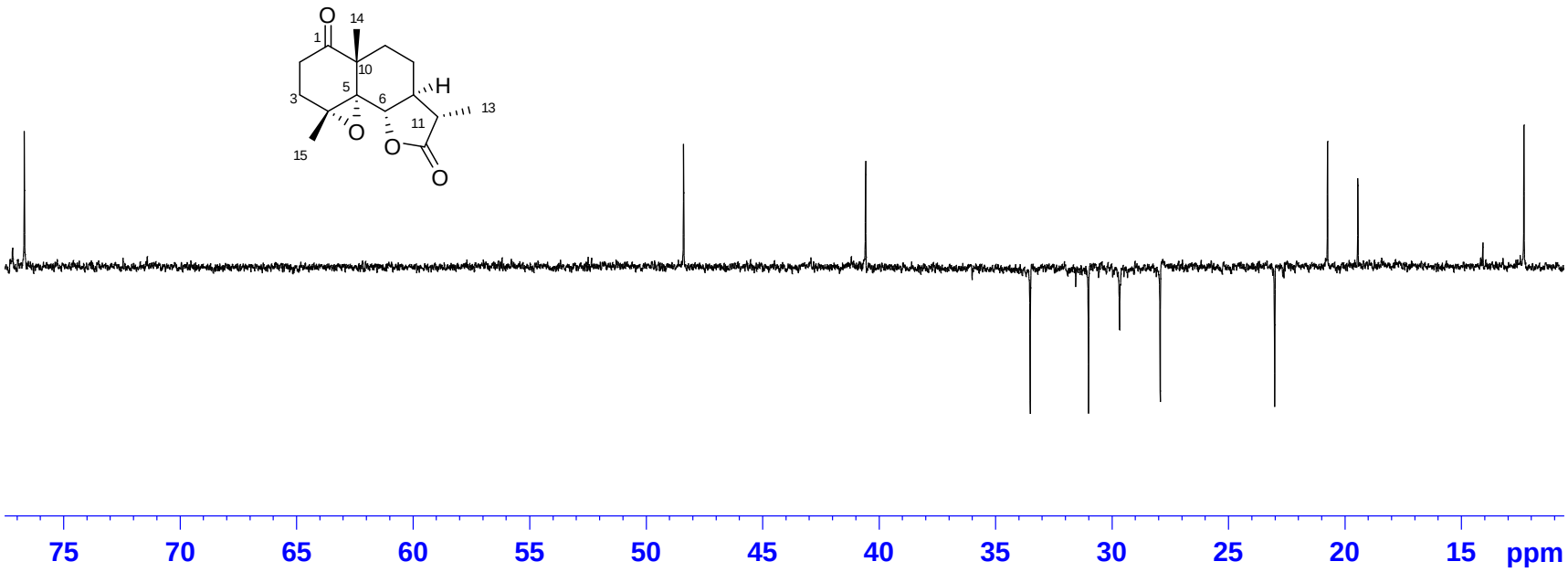


**Plate 18:** DEPT 90 spectrum of maritimin **(2.58)**

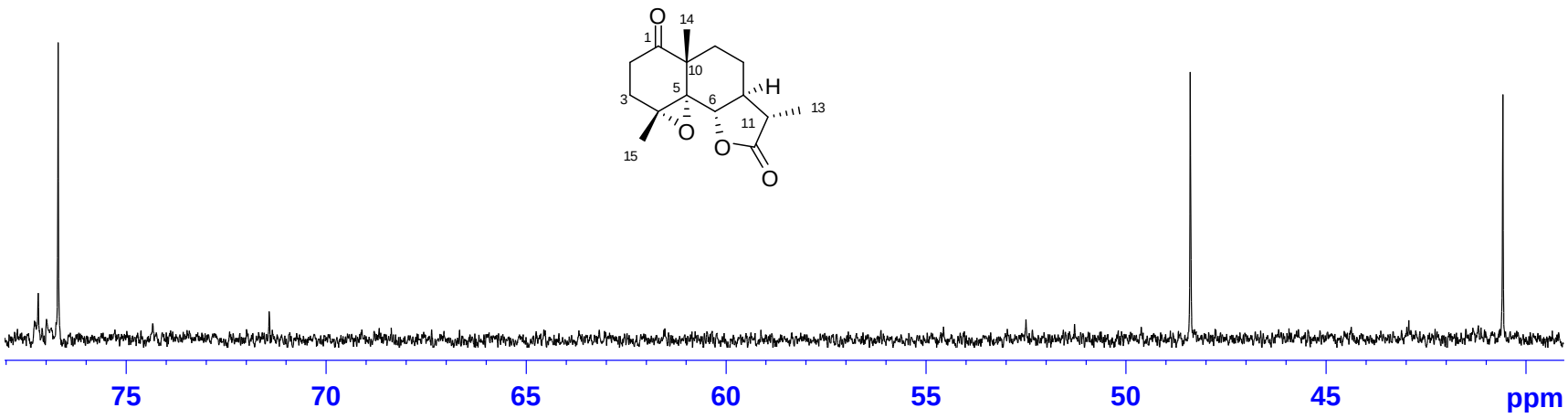


**Plate 19:** COSY spectrum of maritimin **(2.58)**

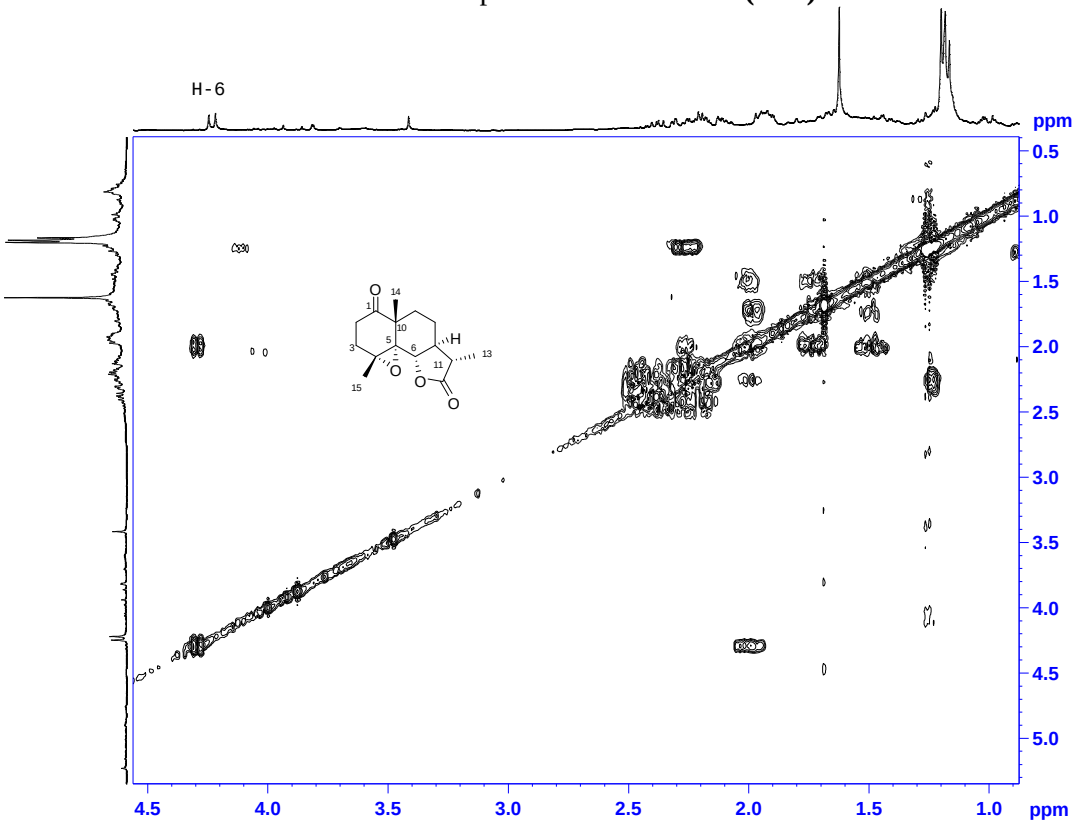


**Plate 20:** HSQC spectrum of maritimin **(2.58)**

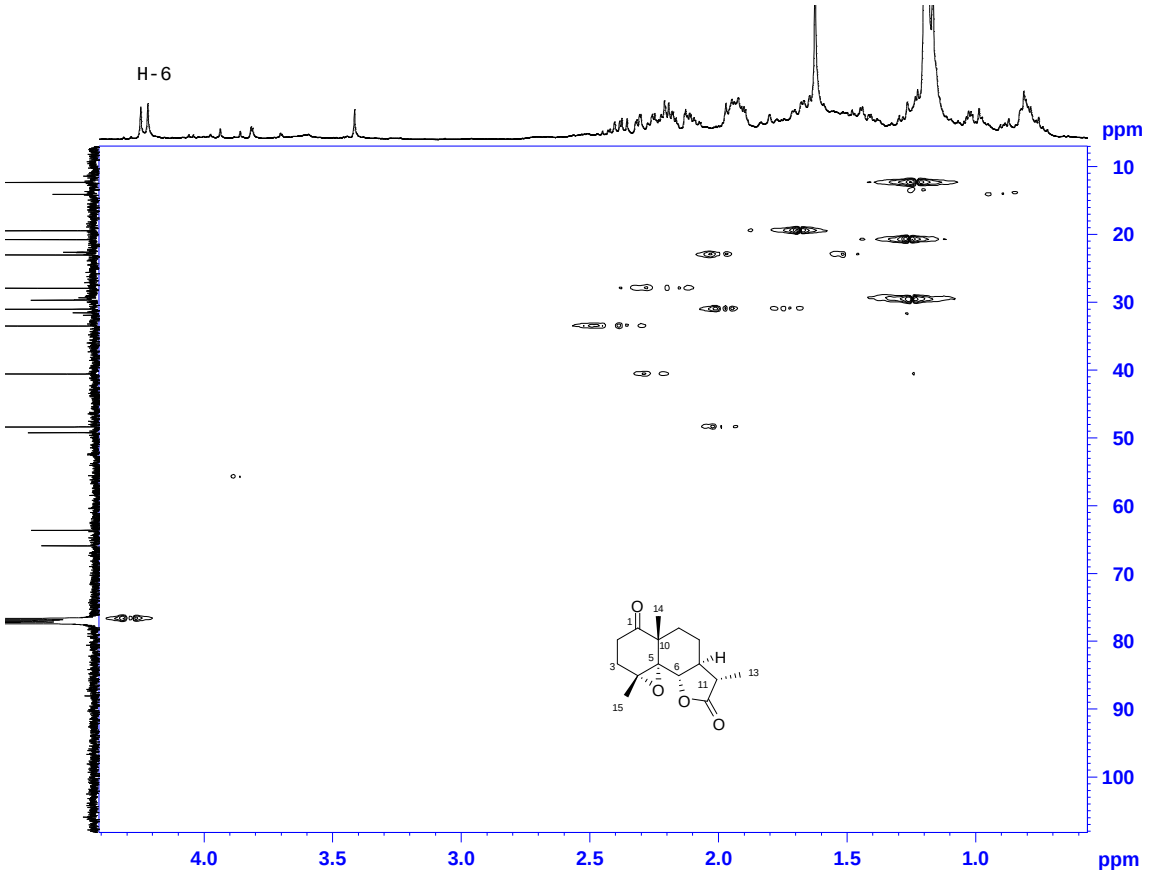


**Plate 21:** HMBC spectrum of maritimin **(2.58)**

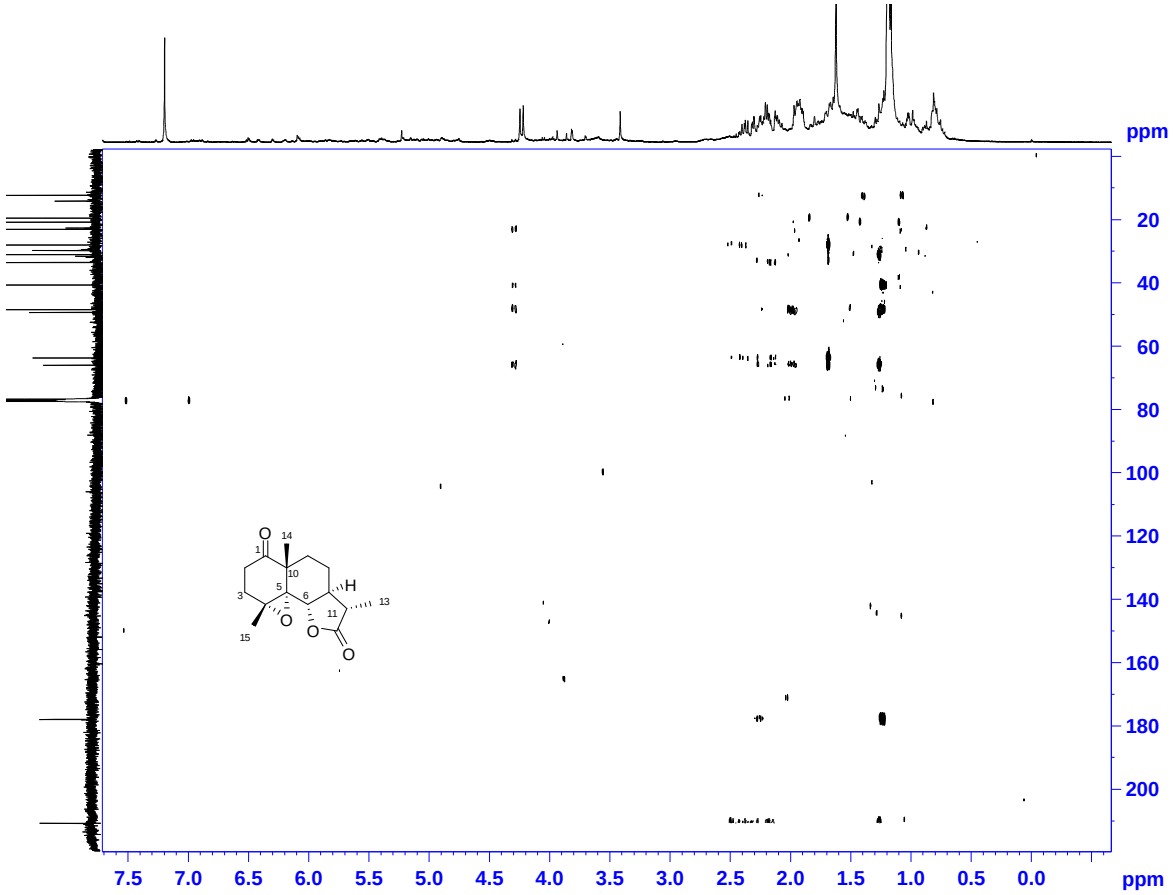


**Plate 22:** $^{1}$H NMR spectrum of artemin **(2.59)**

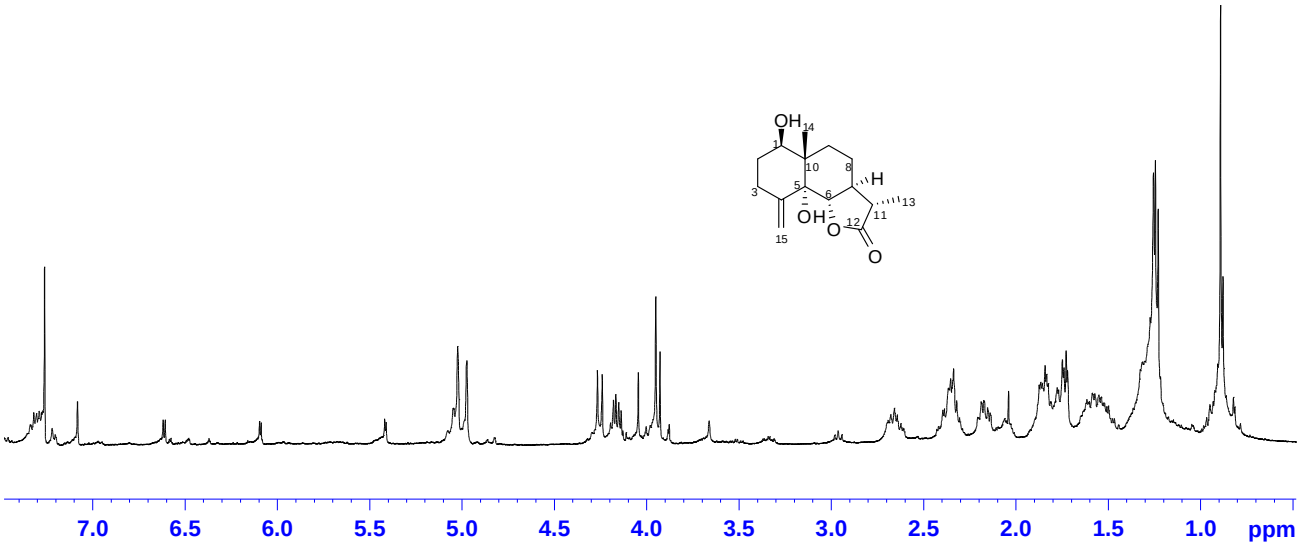


**Plate 23:** $^{13}$C NMR spectrum of artemin **(2.59)**

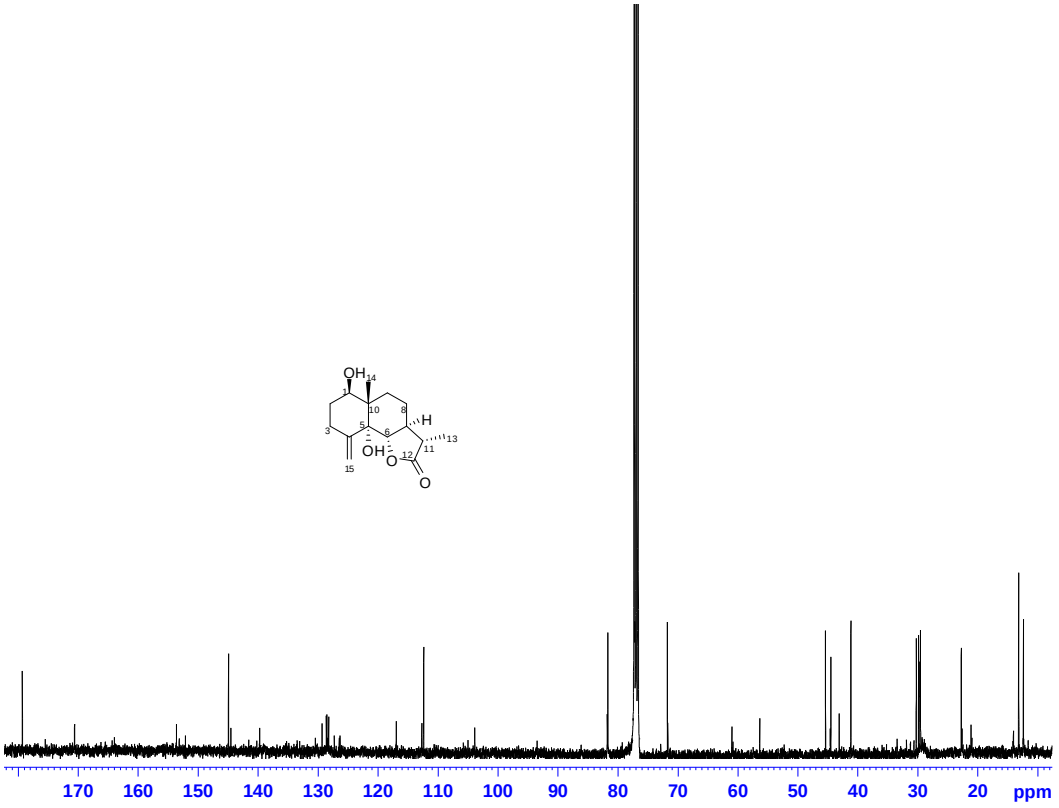
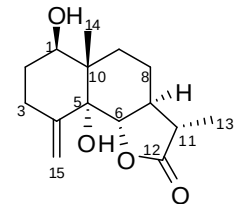


**Plate 24:** DEPT135 spectrum of artemin **(2.59)**



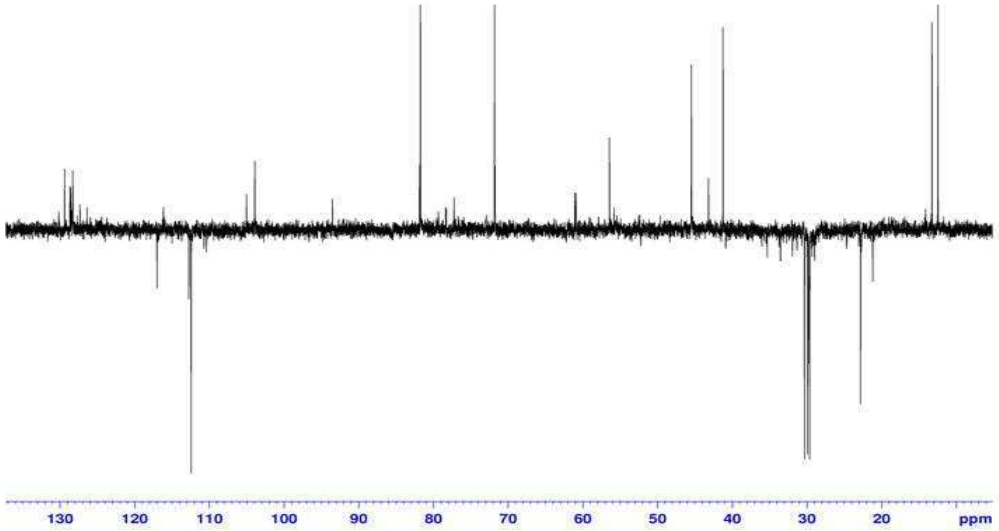



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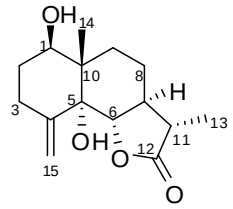
**Plate 25:** DEPT90 spectrum of artemin **(2.59)**



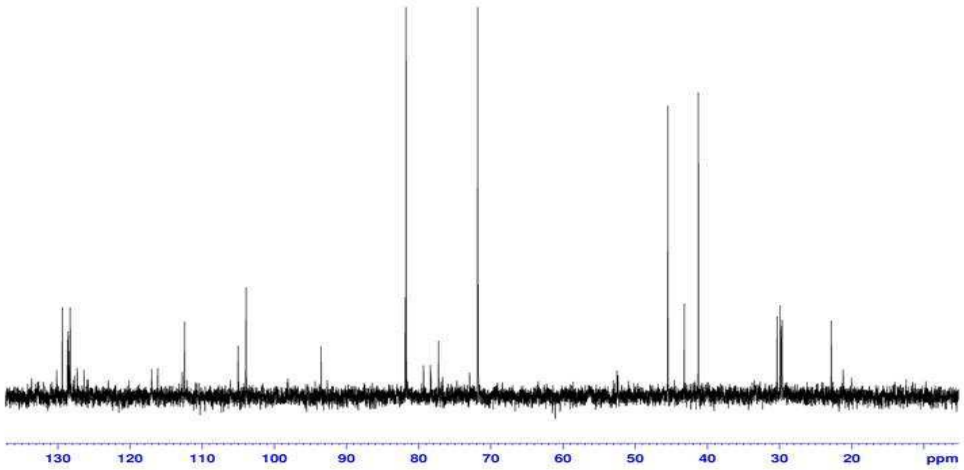



**Plate 26:** COSY spectrum of artemin **(2.59)**

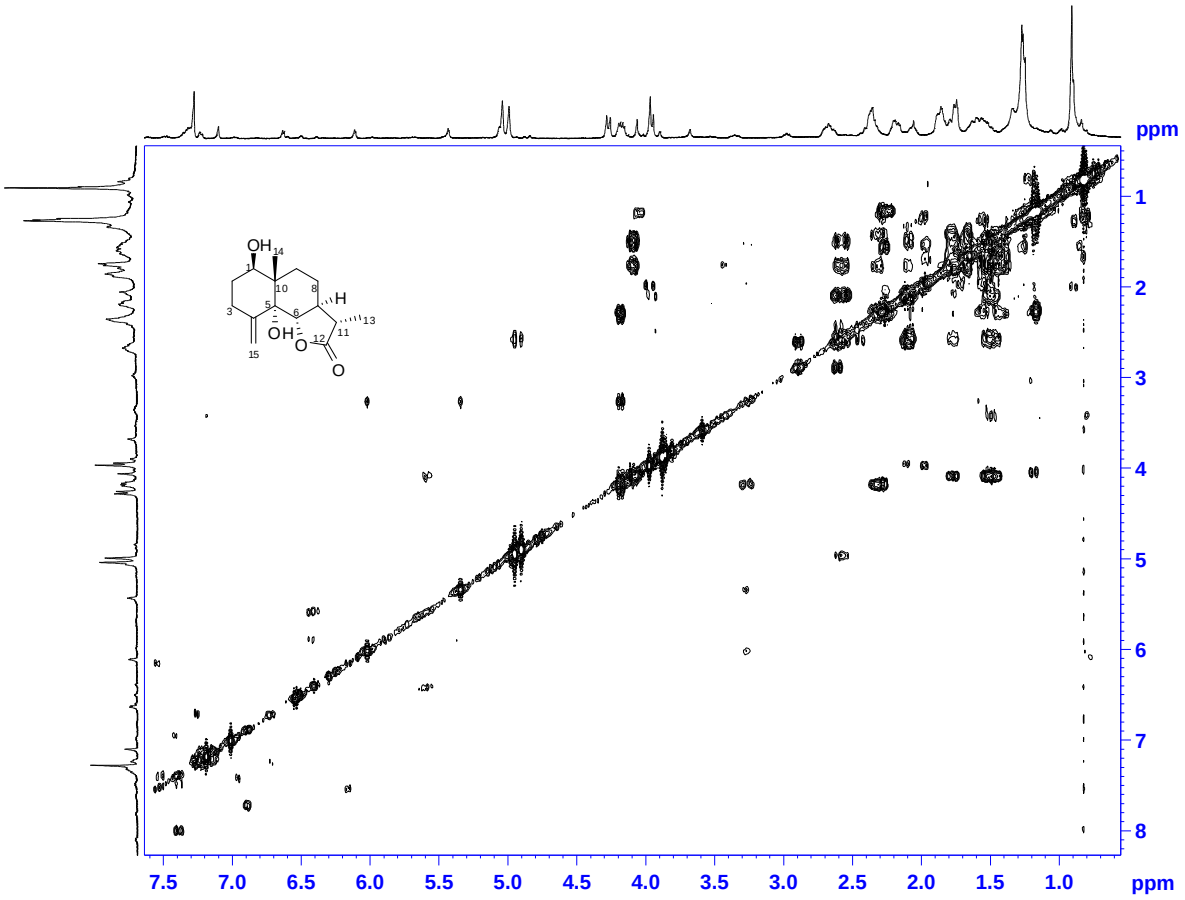


**Plate 27:** HSQC spectrum of artemin **(2.59)**

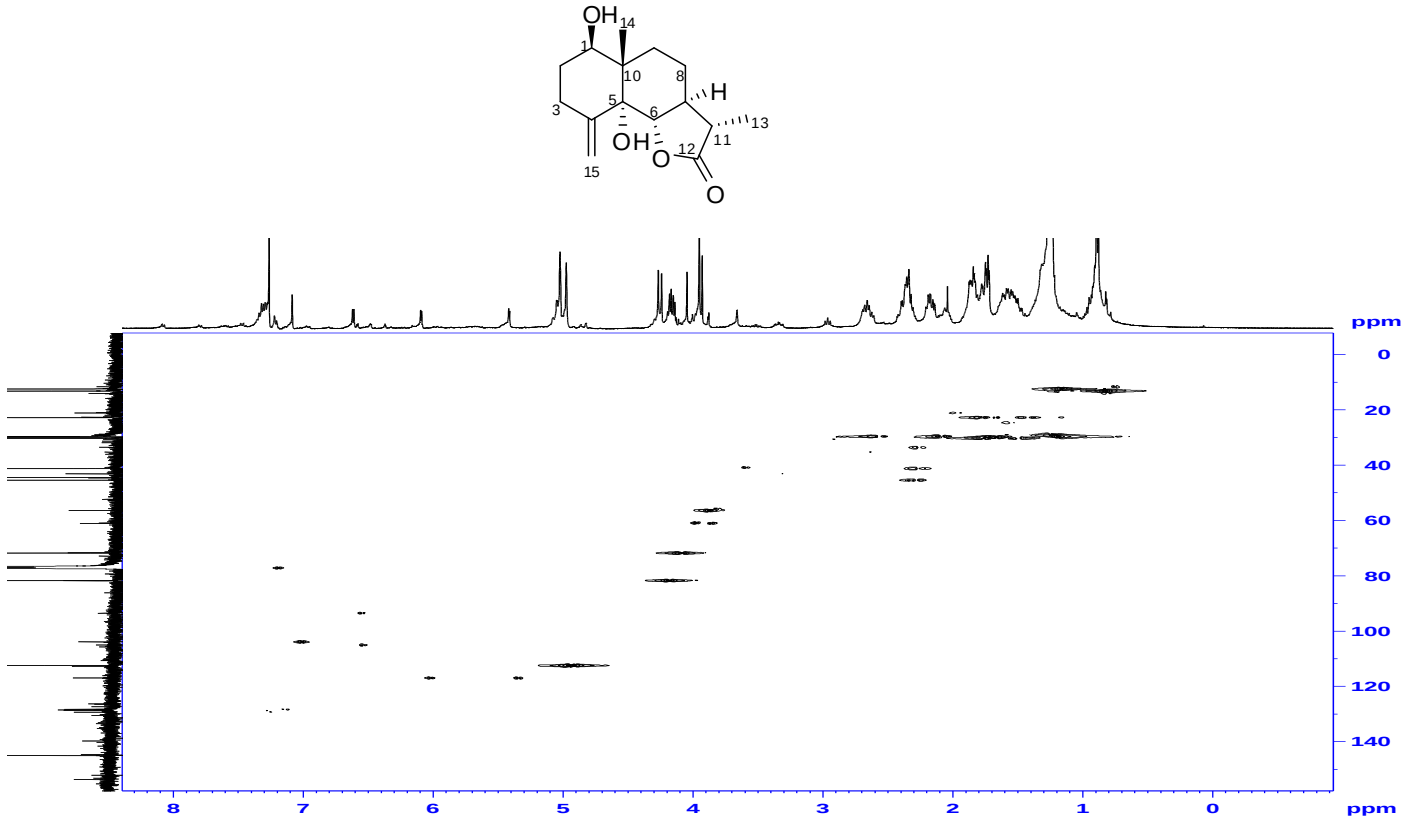


**Plate 28:** HMBC spectrum of artemin **(2.59)**

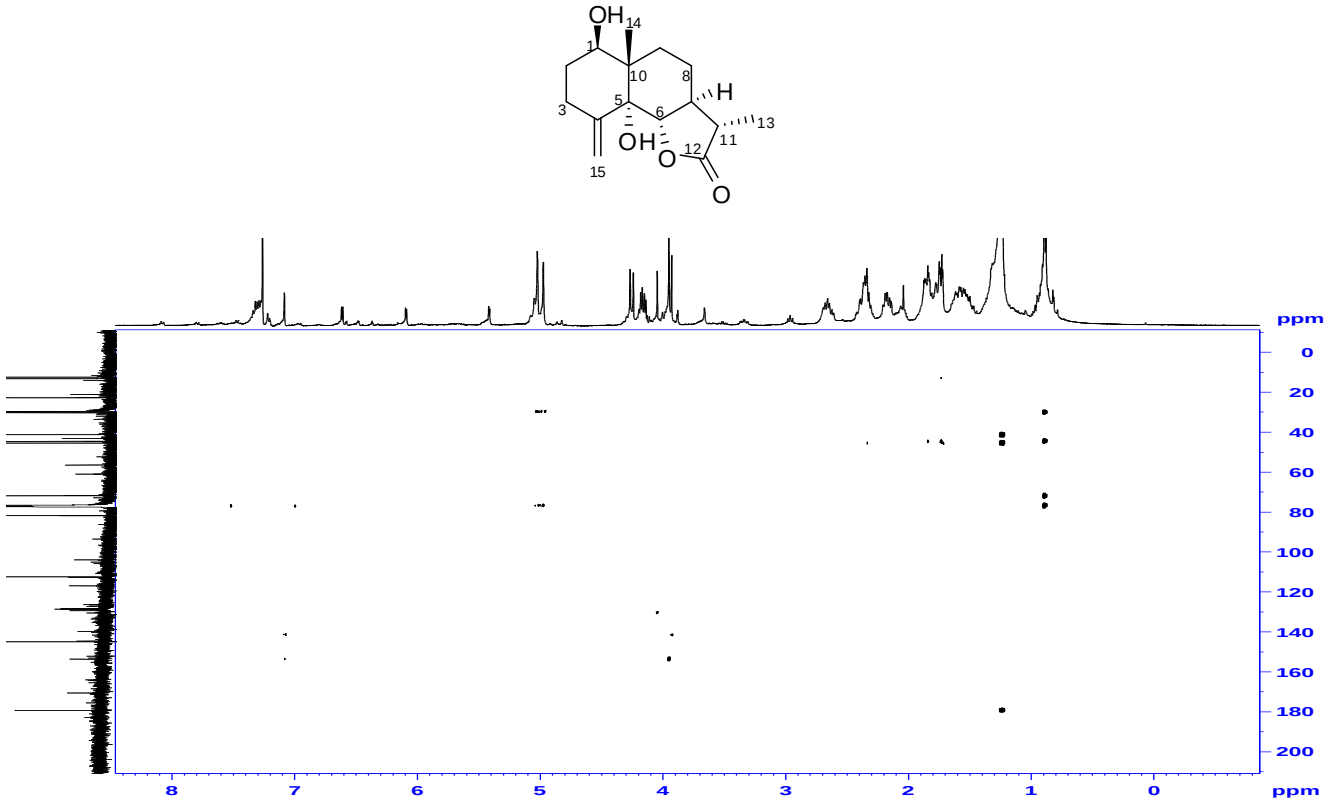


**Plate 29:** 1H NMR spectrum of norsantolinifolide **(2.61)**

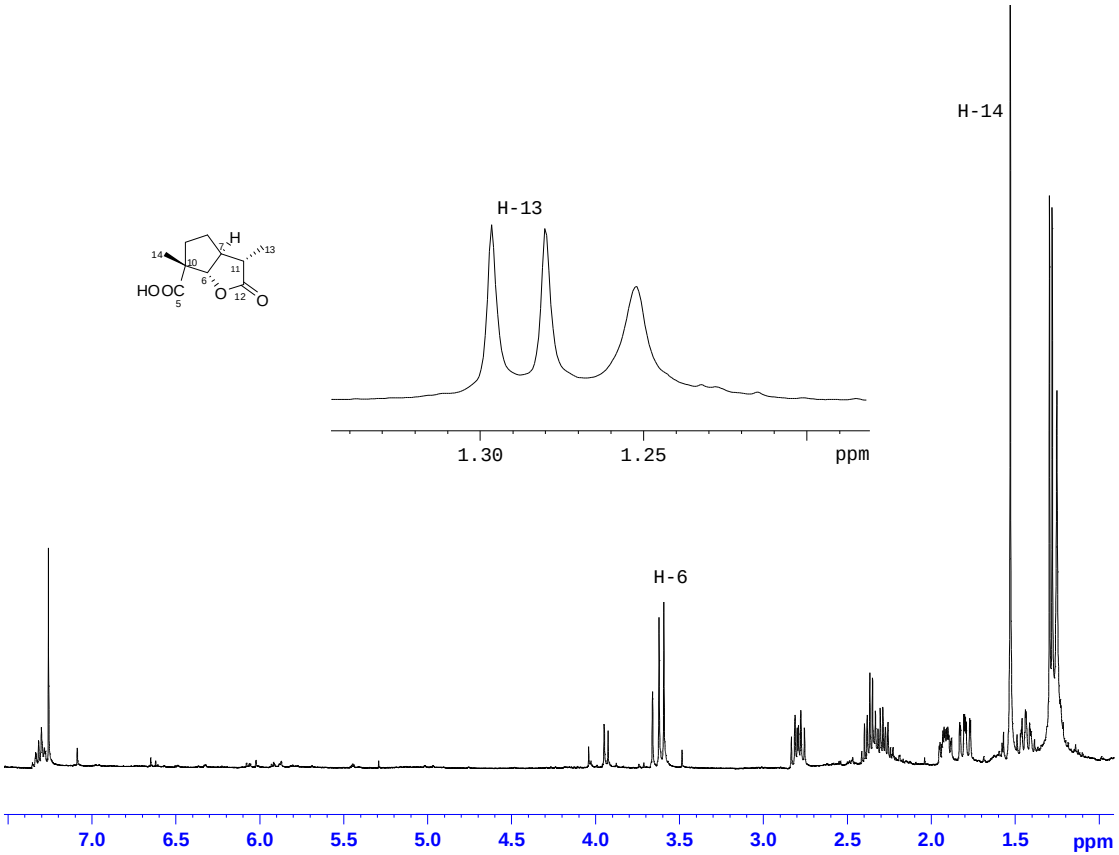


**Plate 30:** $^{13}$C NMR spectrum of norsantolinifolide **(2.61)**

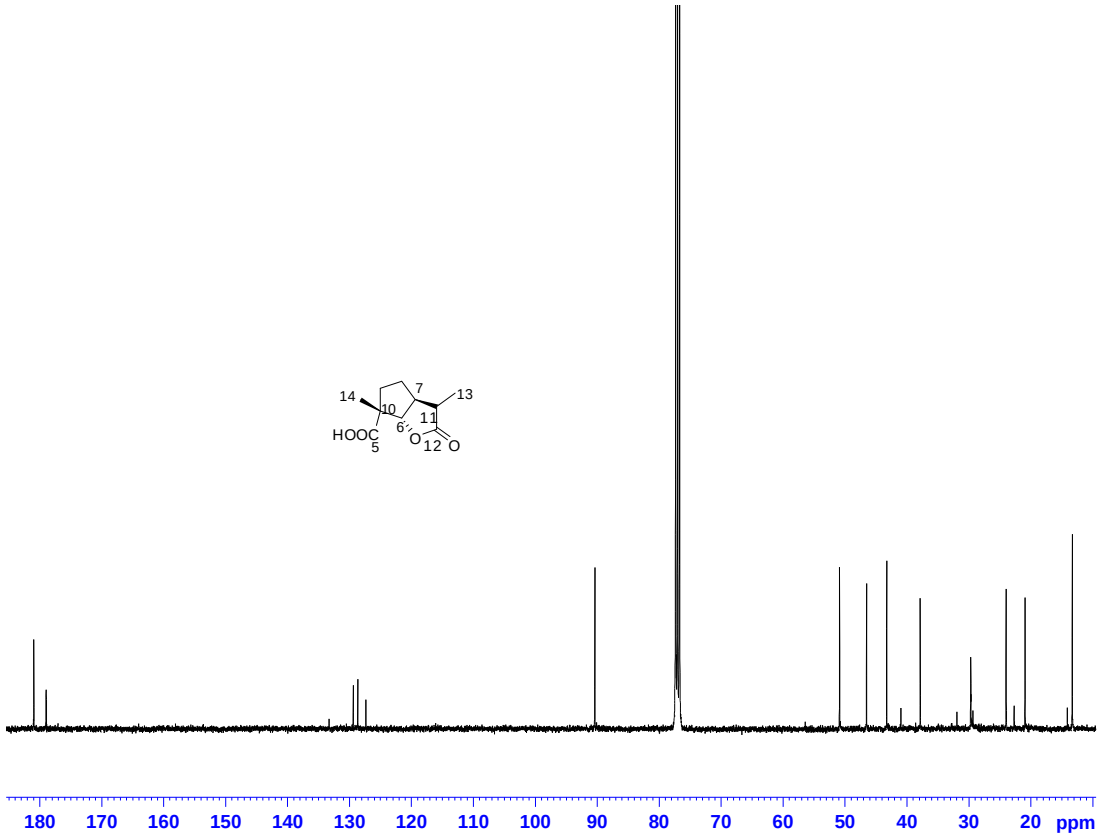


**Plate 31:** DEPT135 spectrum of norsantolinifolide **(2.61)**

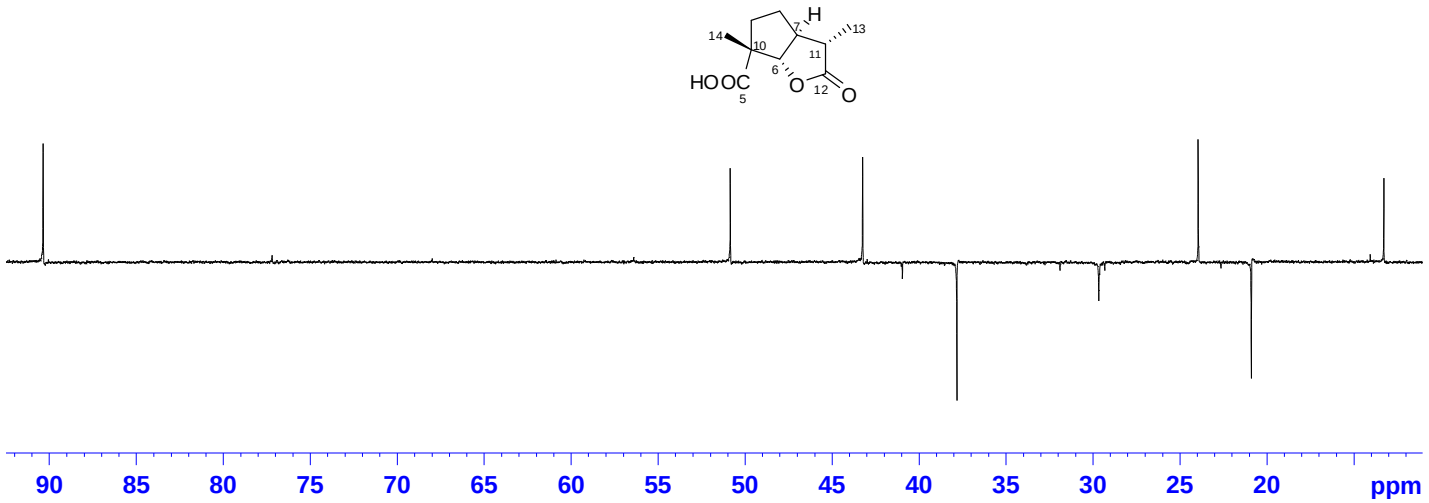


**Plate 32:** DEPT90 spectrum of norsantolinifolide **(2.61)**

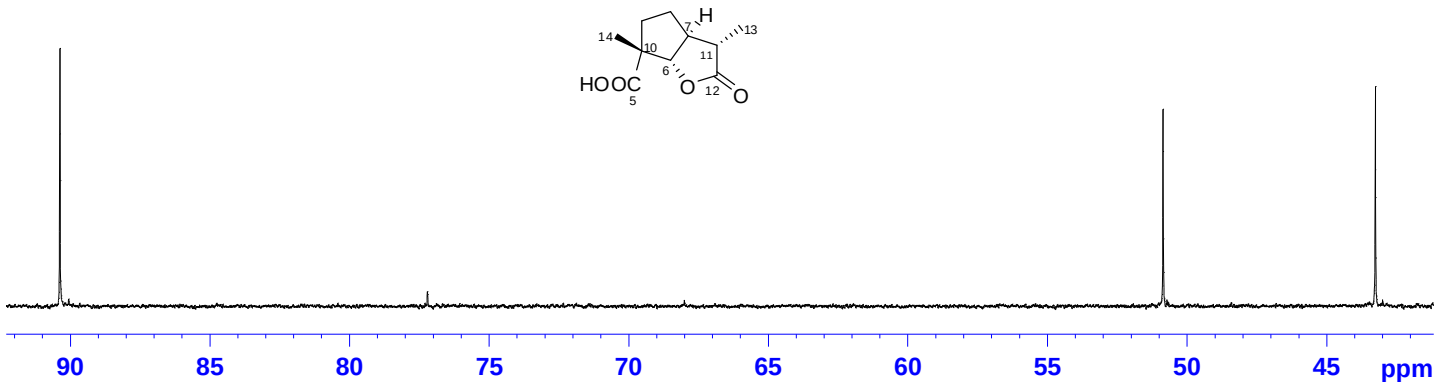


**Plate 33:** COSY spectrum of norsantolinifolide **(2.61)**

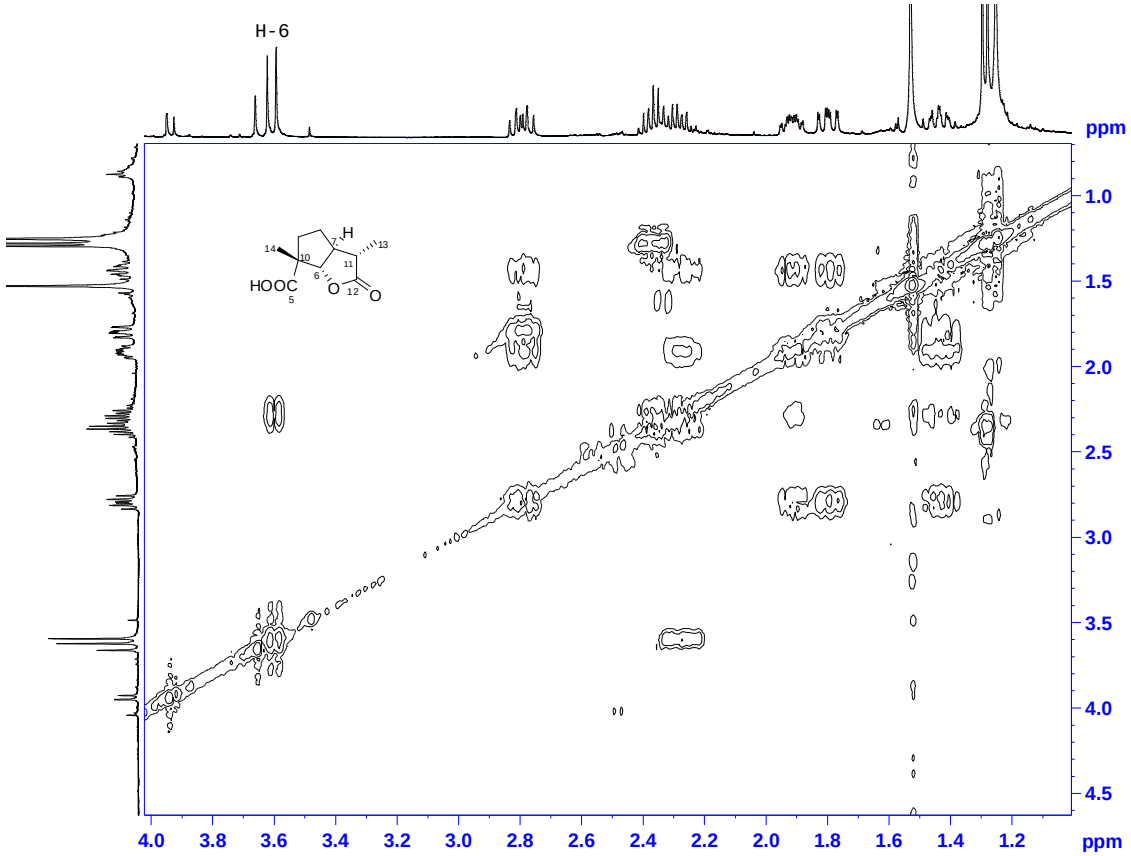


**Plate 34:** HSQC spectrum of norsantolinifolide **(2.61)**

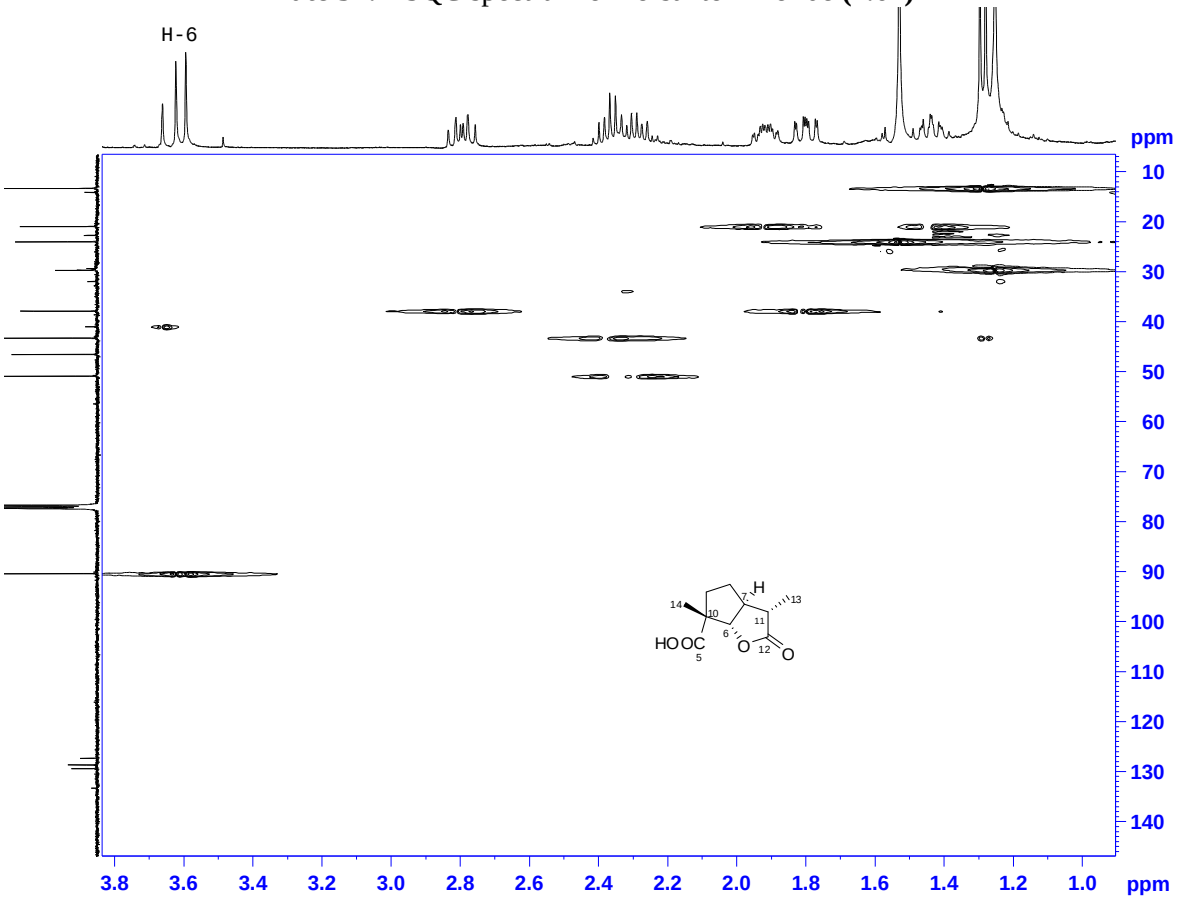


**Plate 35:** HMBC spectrum of norsantolinifolide **(2.61)**

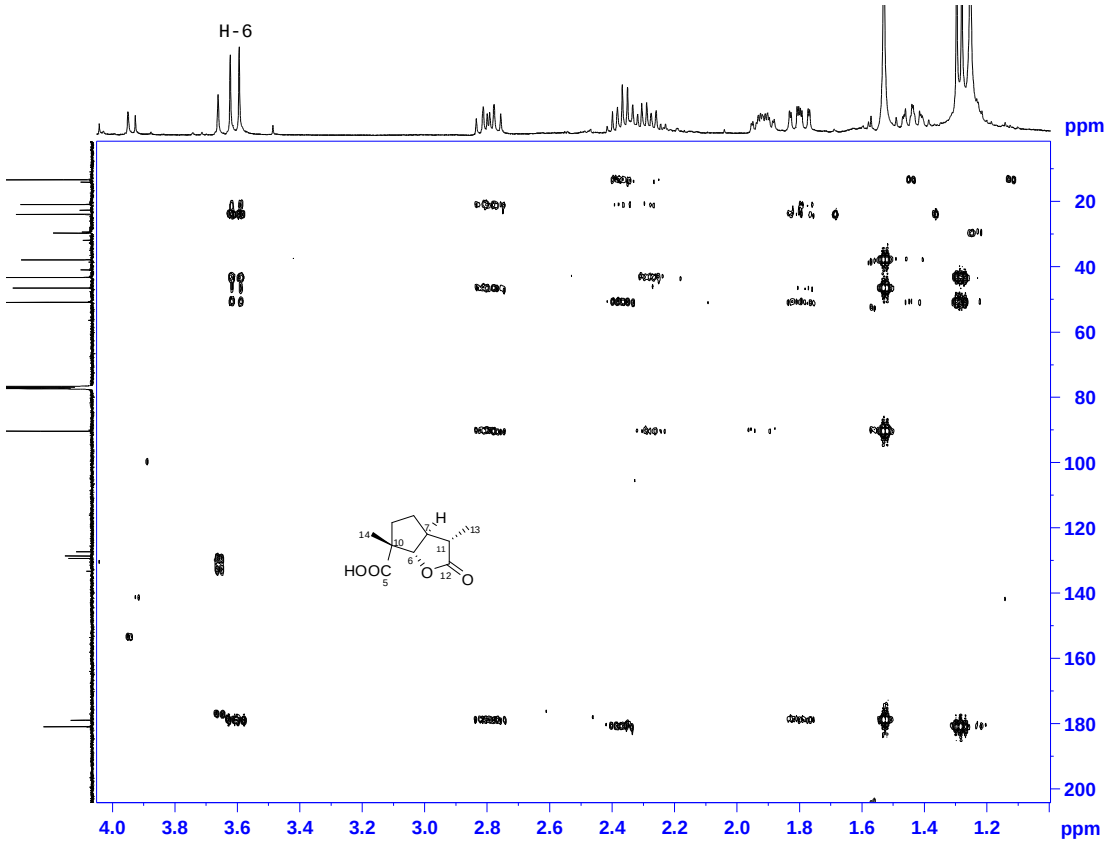


**Plate 36**: $^{1}$H NMR spectrum of santolinifolide A **(2.62)**

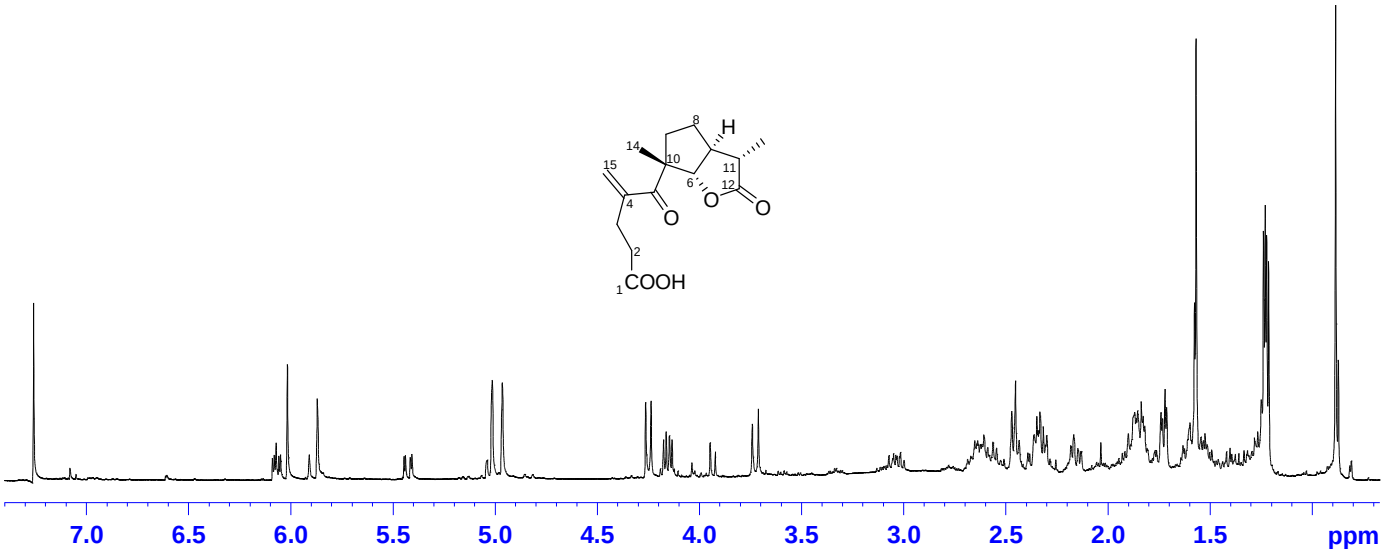


**Plate 37**: $^{13}$C NMR spectrum of santolinifolide A **(2.62)**

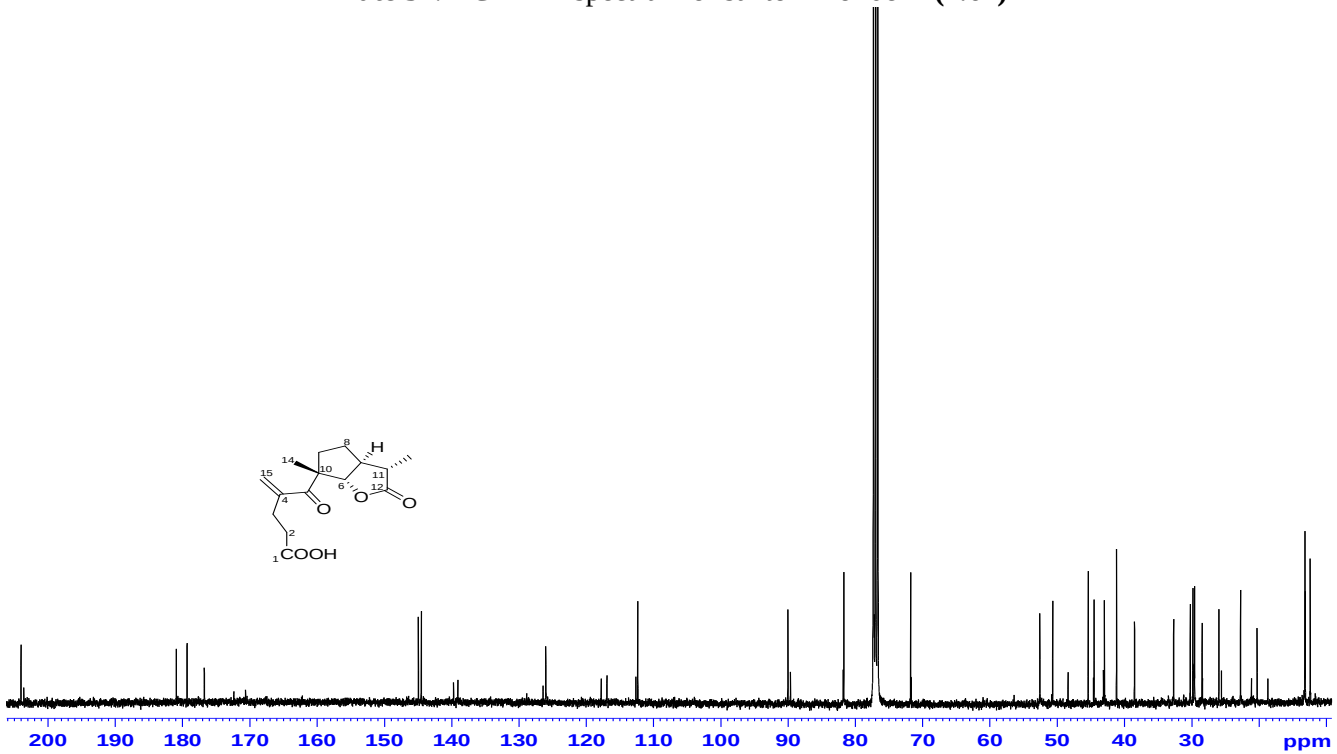


**Plate 38**: DEPT135 spectrum of santolinifolide A **(2.62)**

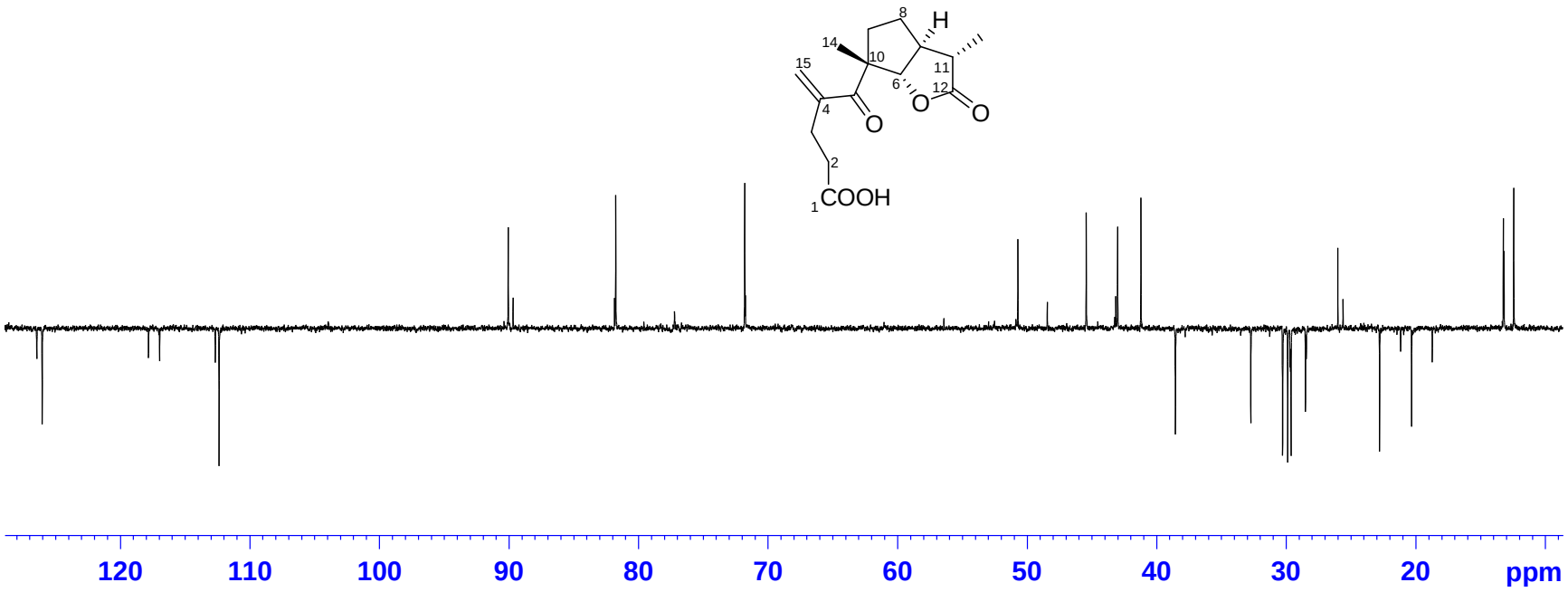


**Plate 39**: DEPT90 spectrum of santolinifolide A **(2.62)**

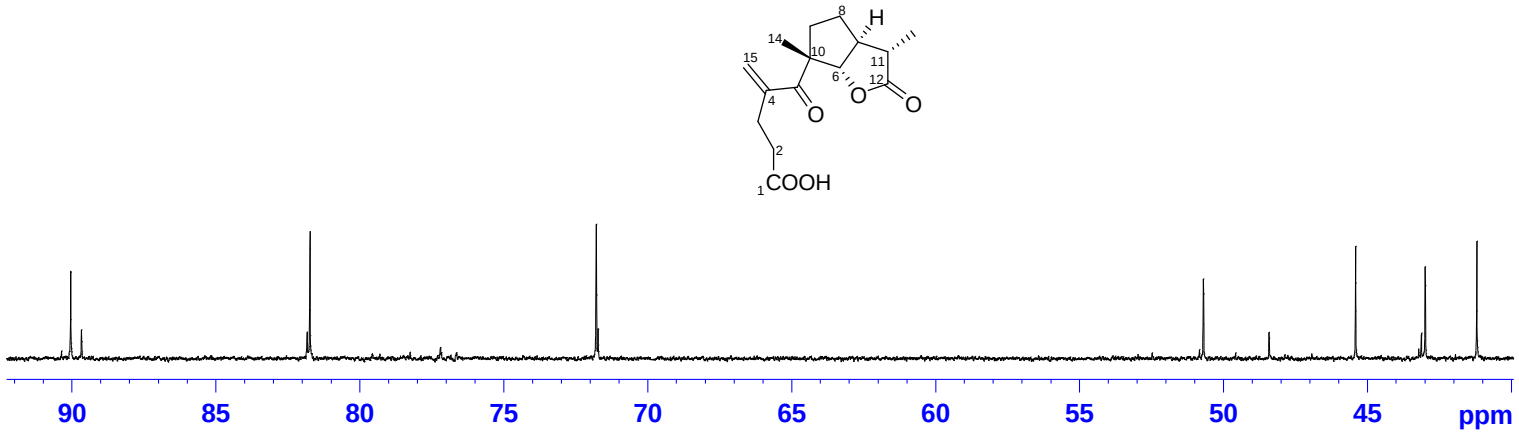


**Plate 40**: COSY spectrum of santolinifolide A **(2.62)**

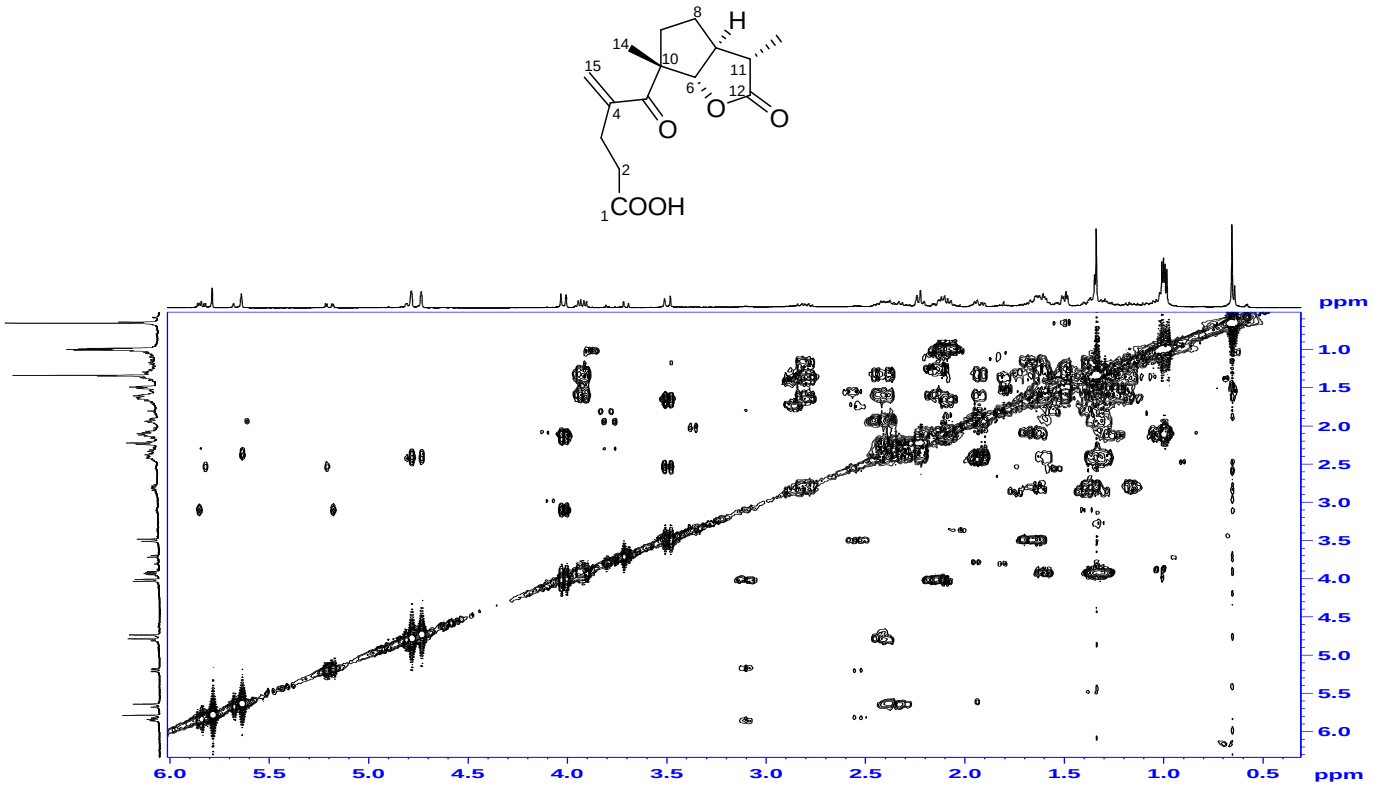


**Plate 41**: HSQC spectrum of santolinifolide A **(2.62)**

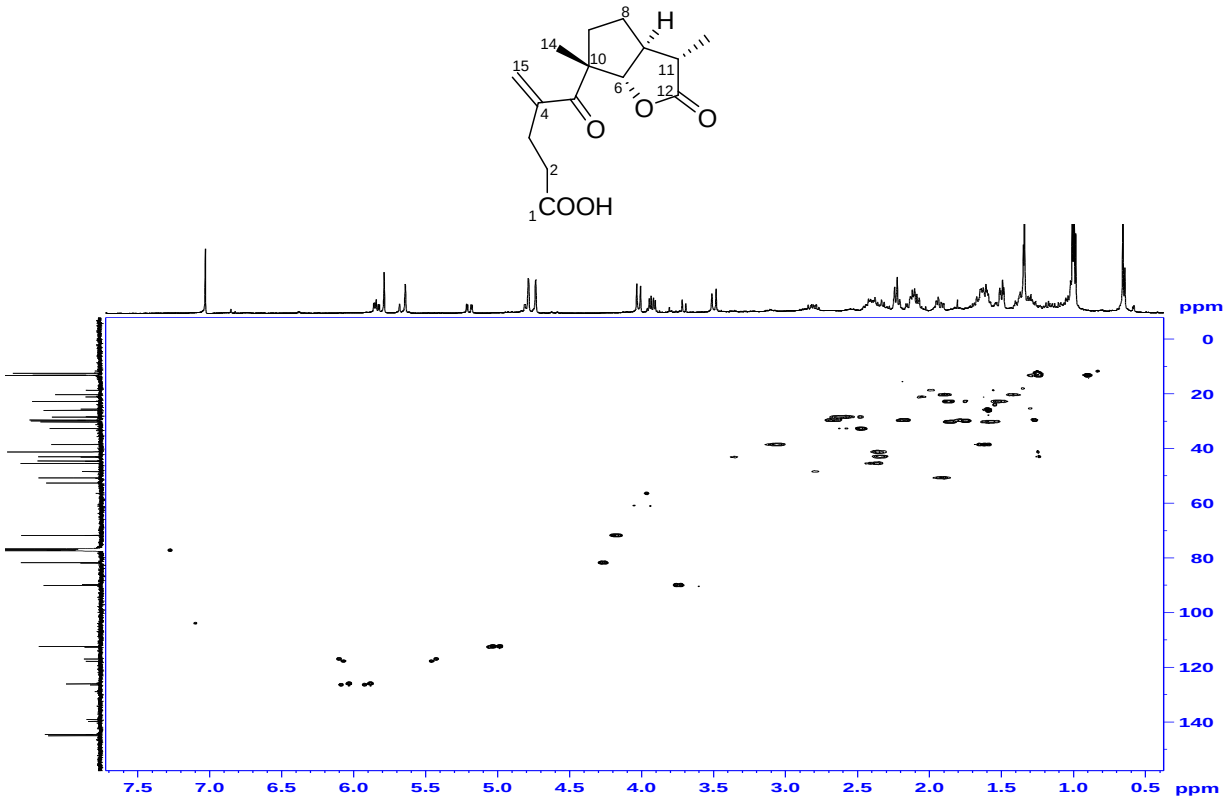


**Plate 42**: HMBC spectrum of santolinifolide A **(2.62)**

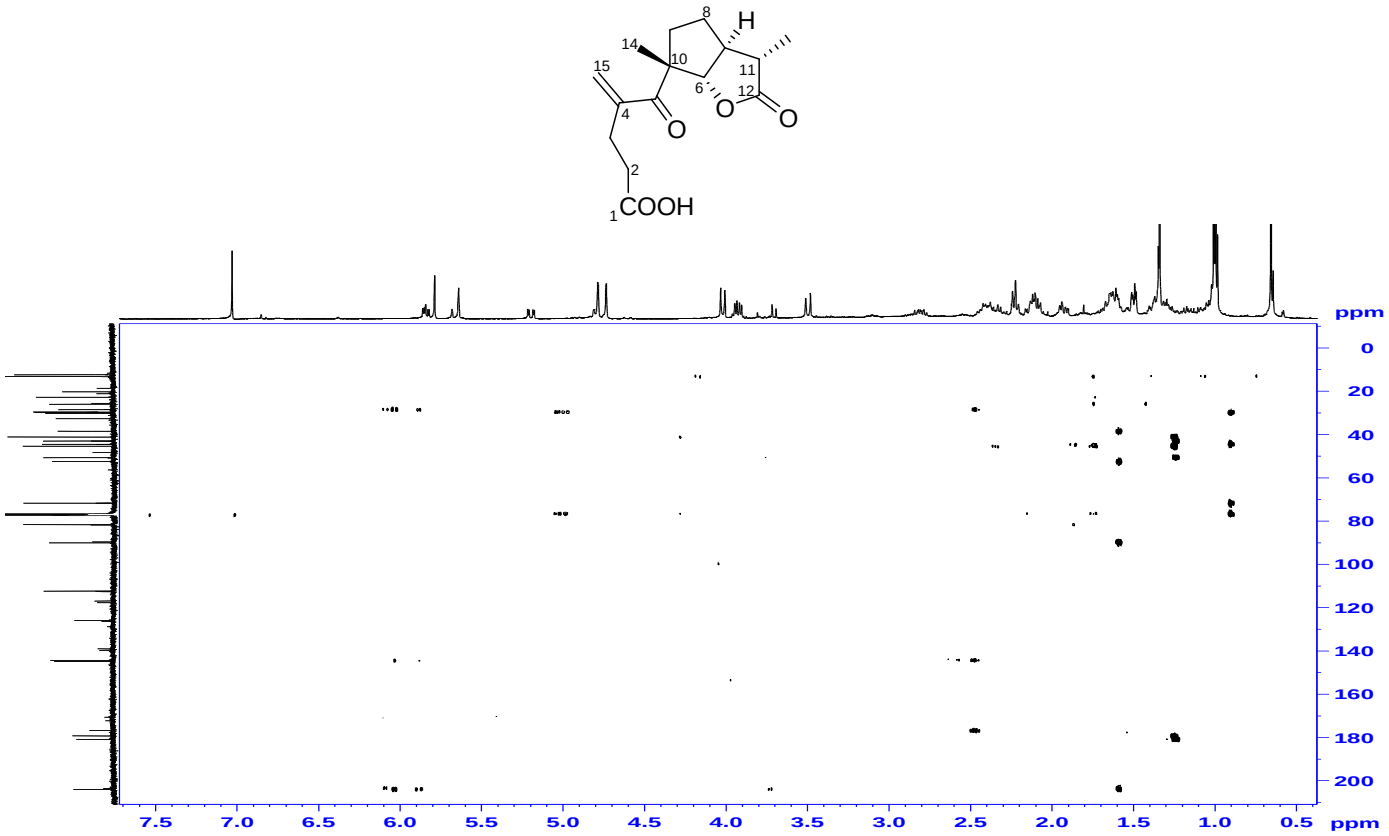


**Plate 43:** $^{1}$H NMR spectrum of reynosin **(2.65)**

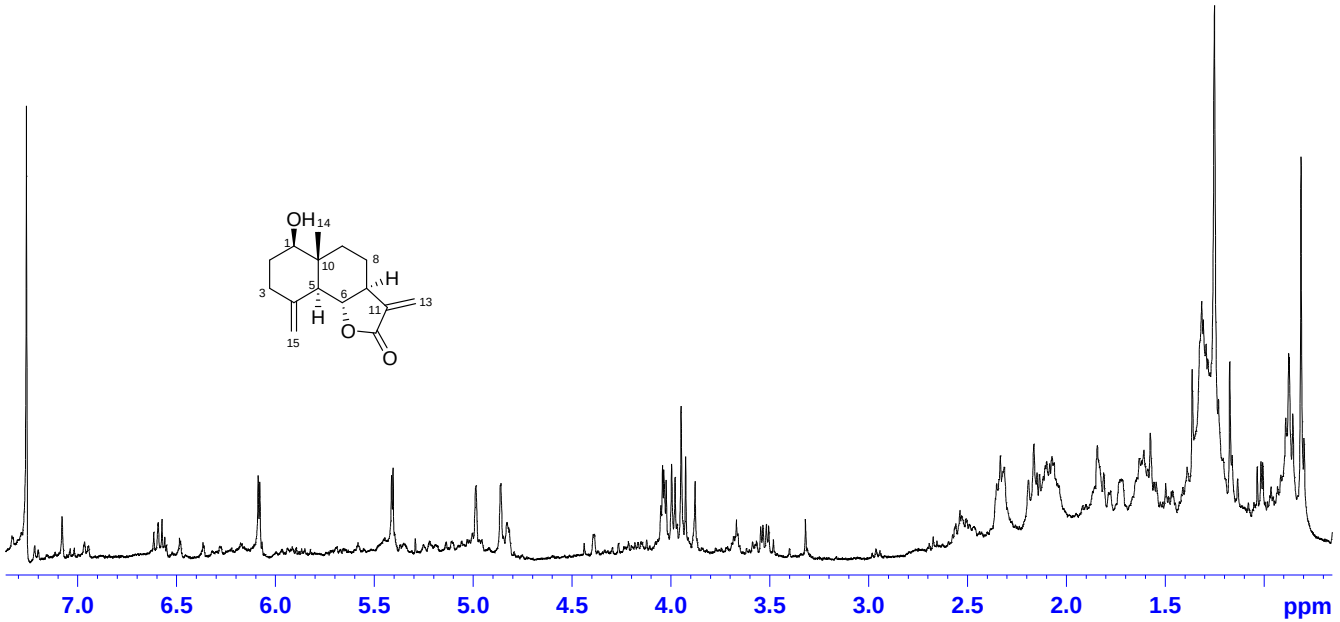


**Plate 44:** $^{13}$C NMR spectrum of reynosin **(2.65)**

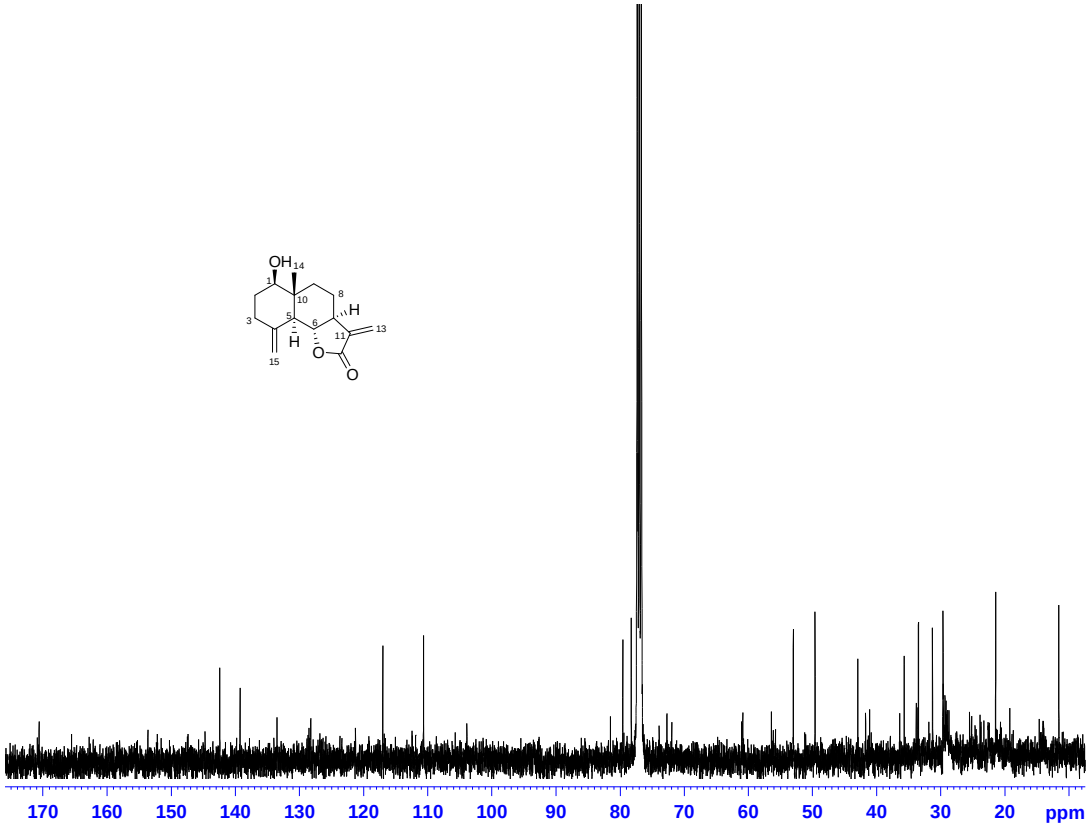


**Plate 45:** DEPT135 spectrum of reynosin **(2.65)**

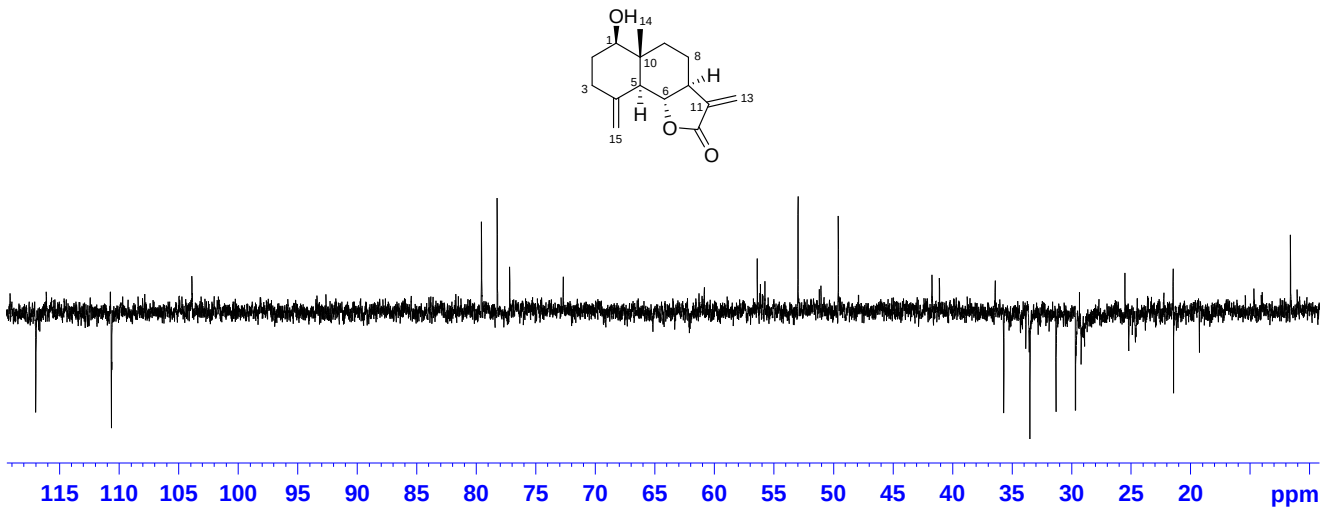


**Plate 46:** DEPT90 spectrum of reynosin **(2.65)**

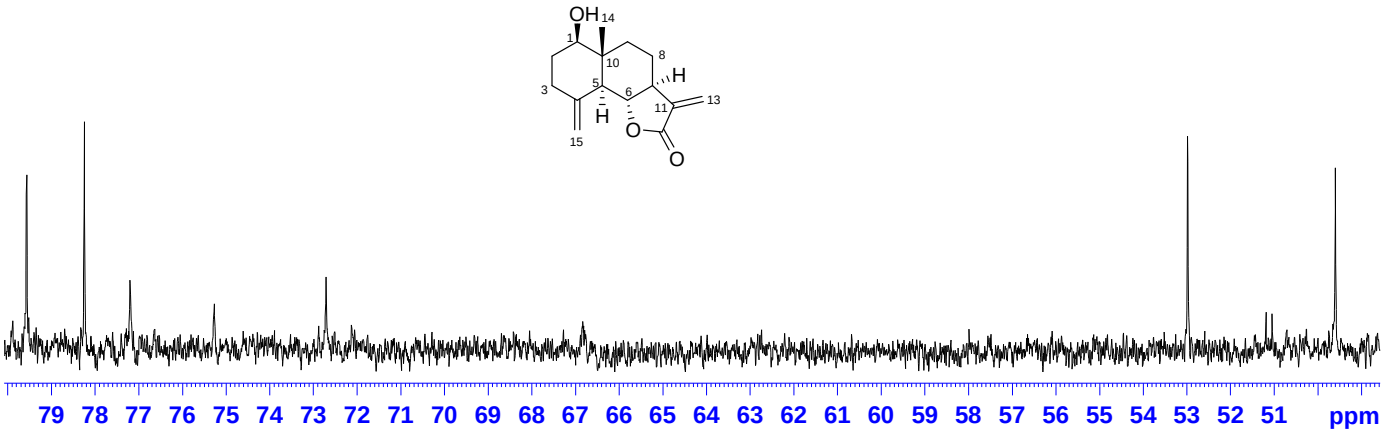


**Plate 47:** COSY spectrum of reynosin **(2.65)**

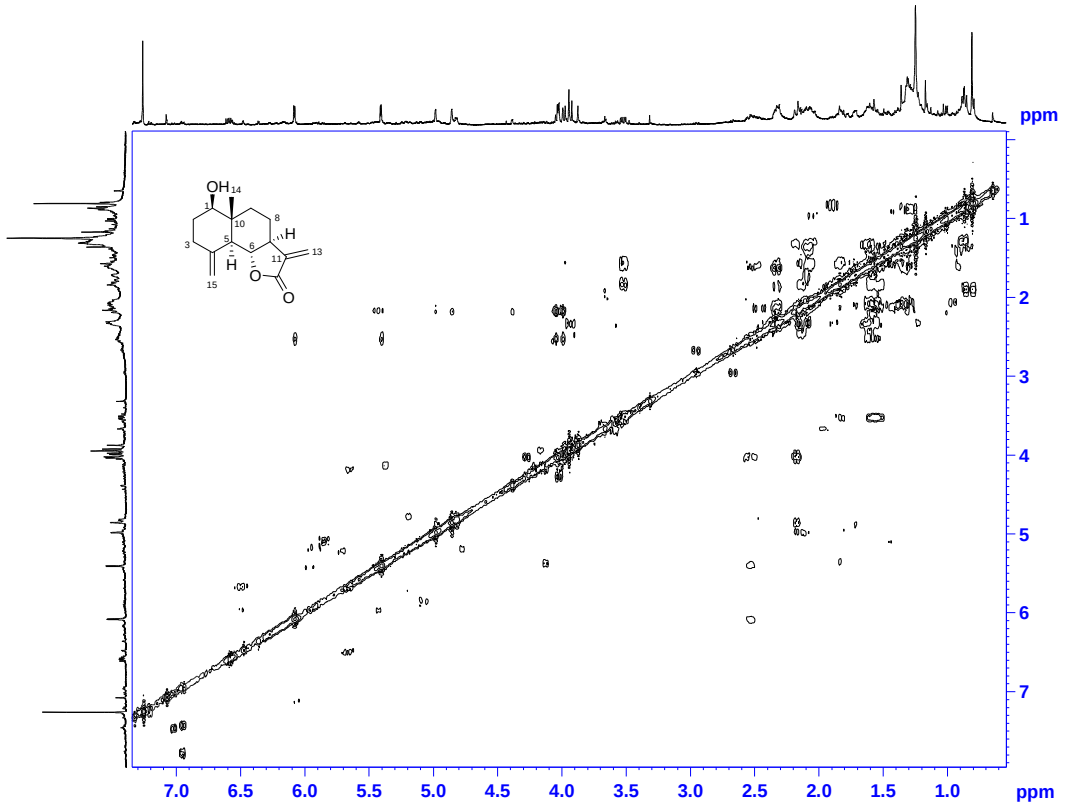
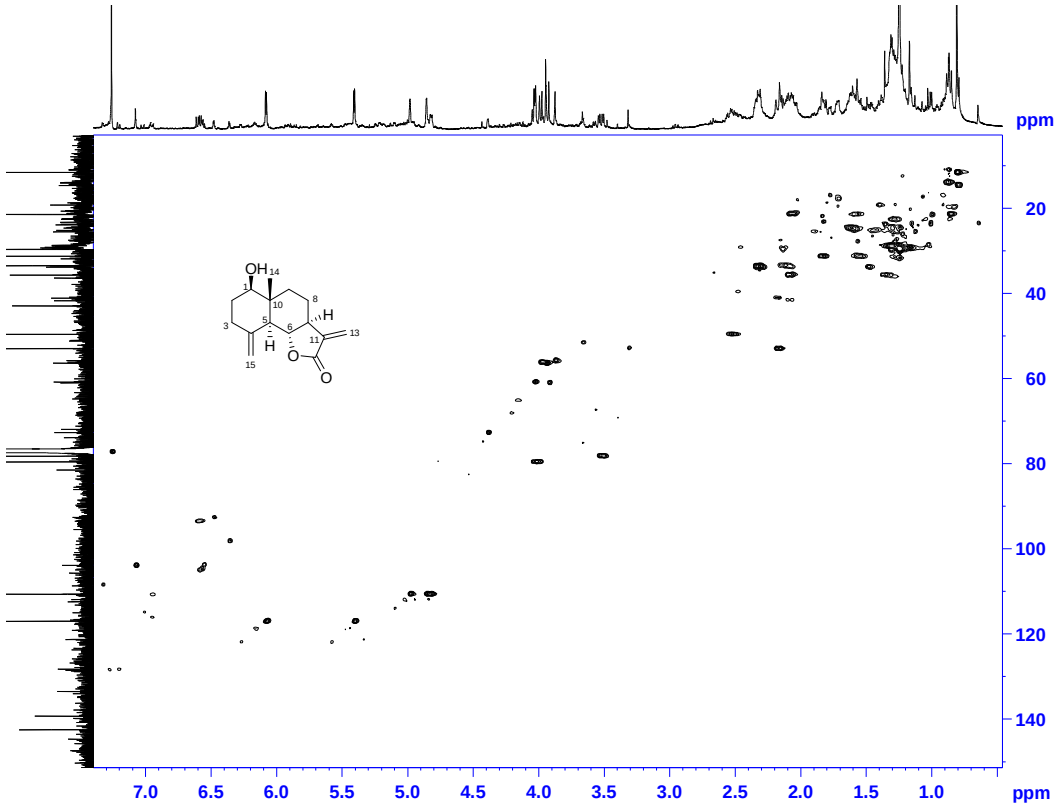


**Plate 48:** HSQC spectrum of reynosin **(2.65)**



**late 49:** HMBC spectrum of reynosin **(2.65)**

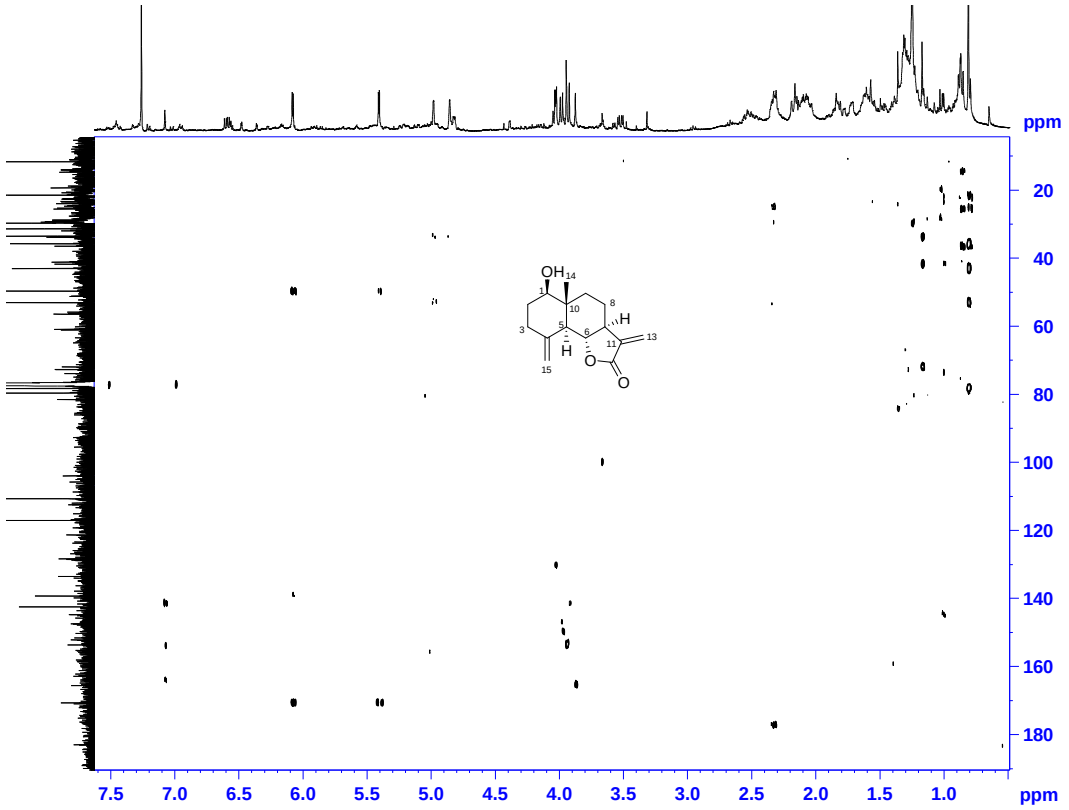


**Plate 50:** $^{1}H$ NMR spectrum of scopoletin **(2.66)**

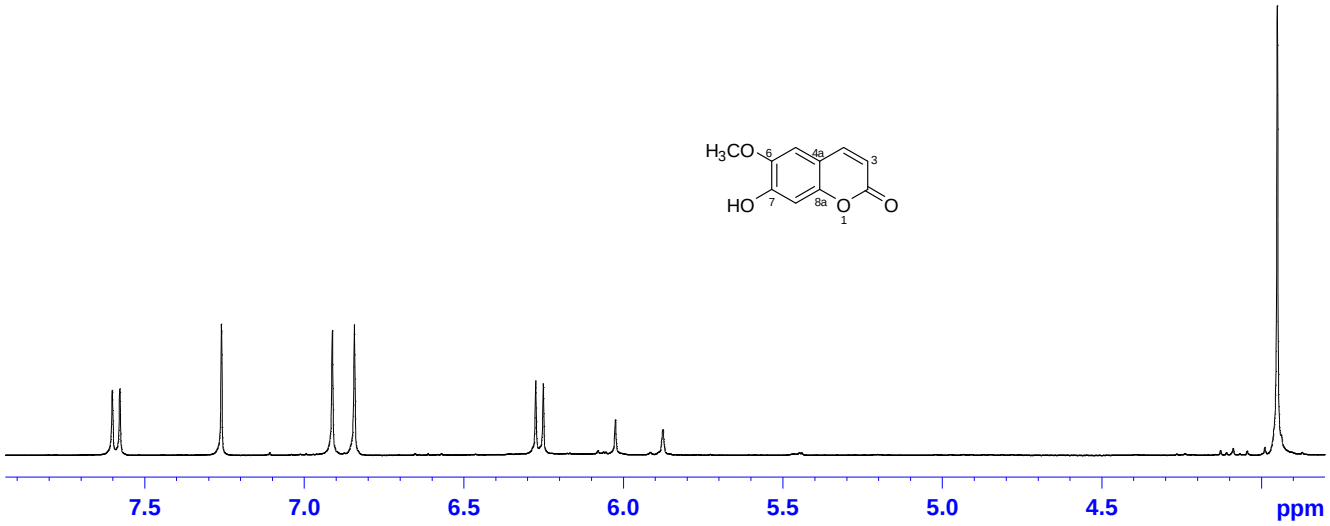


**Plate 51:** $^{13}C$ NMR spectrum of scopoletin **(2.66)**

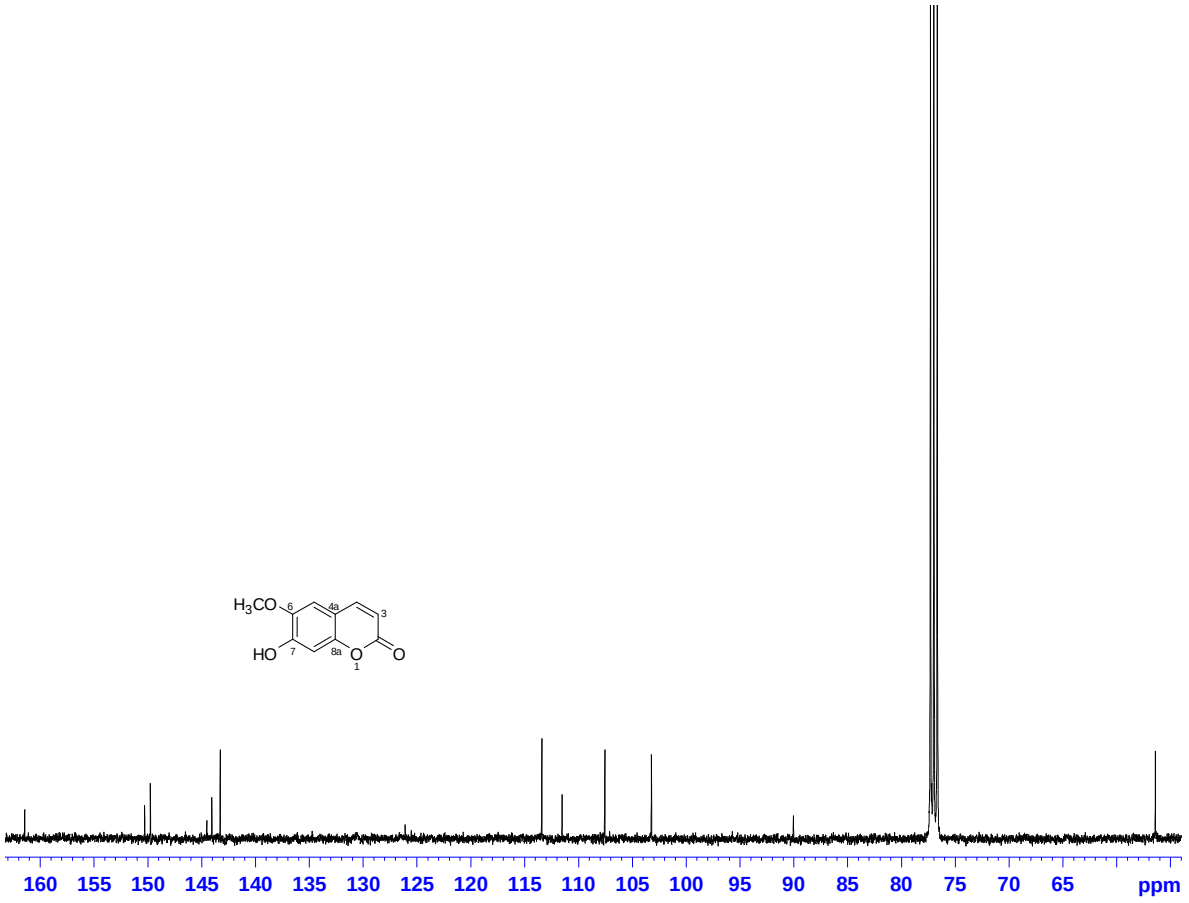
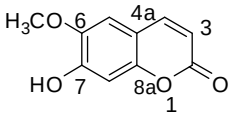


**Plate 52:** DEPT135 spectrum of scopoletin **(2.66)**



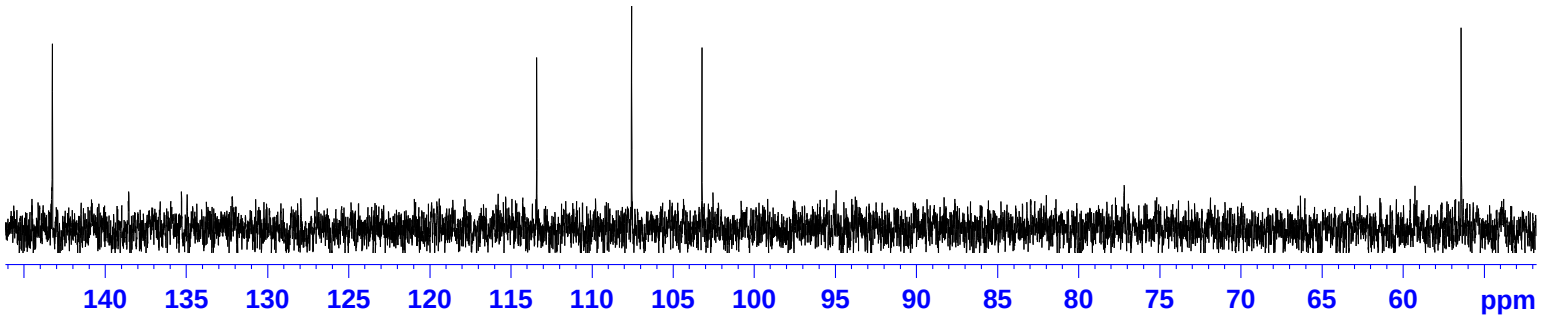



**Plate 53:** DEPT90 spectrum of scopoletin **(2.66)**

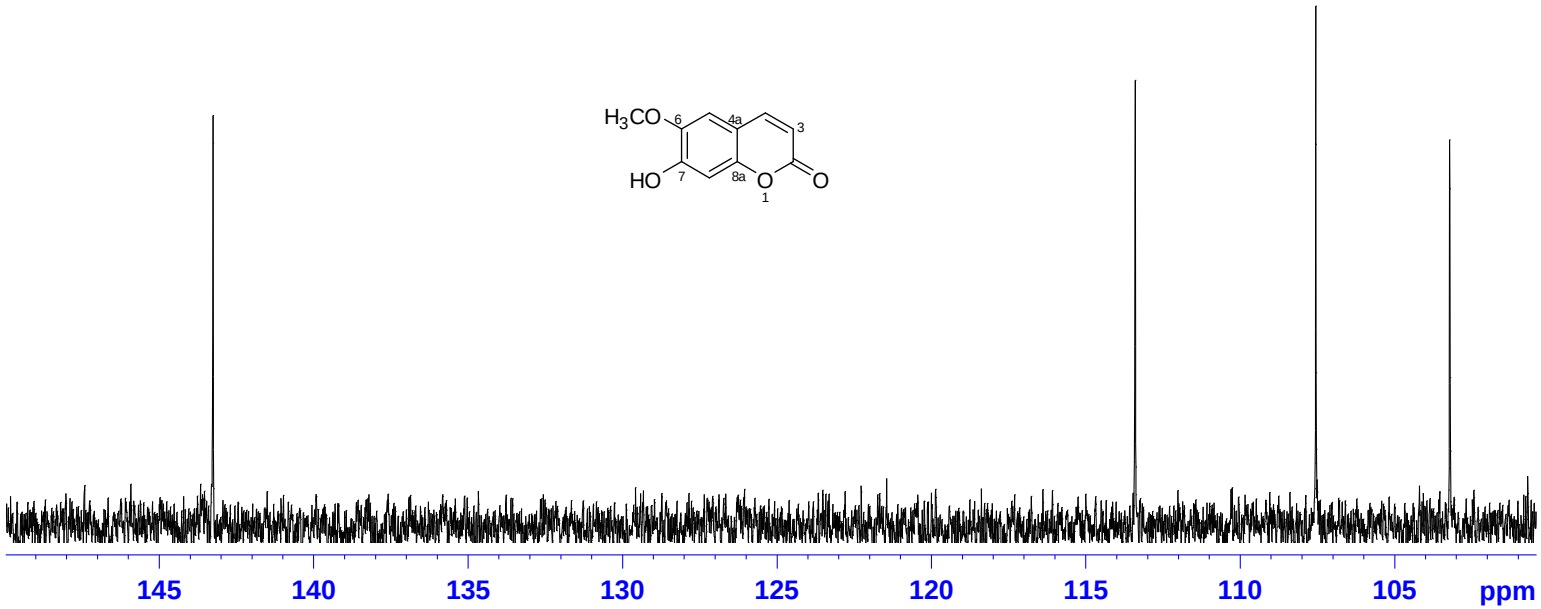


**Plate 54:** COSY spectrum of scopoletin **(2.66)**

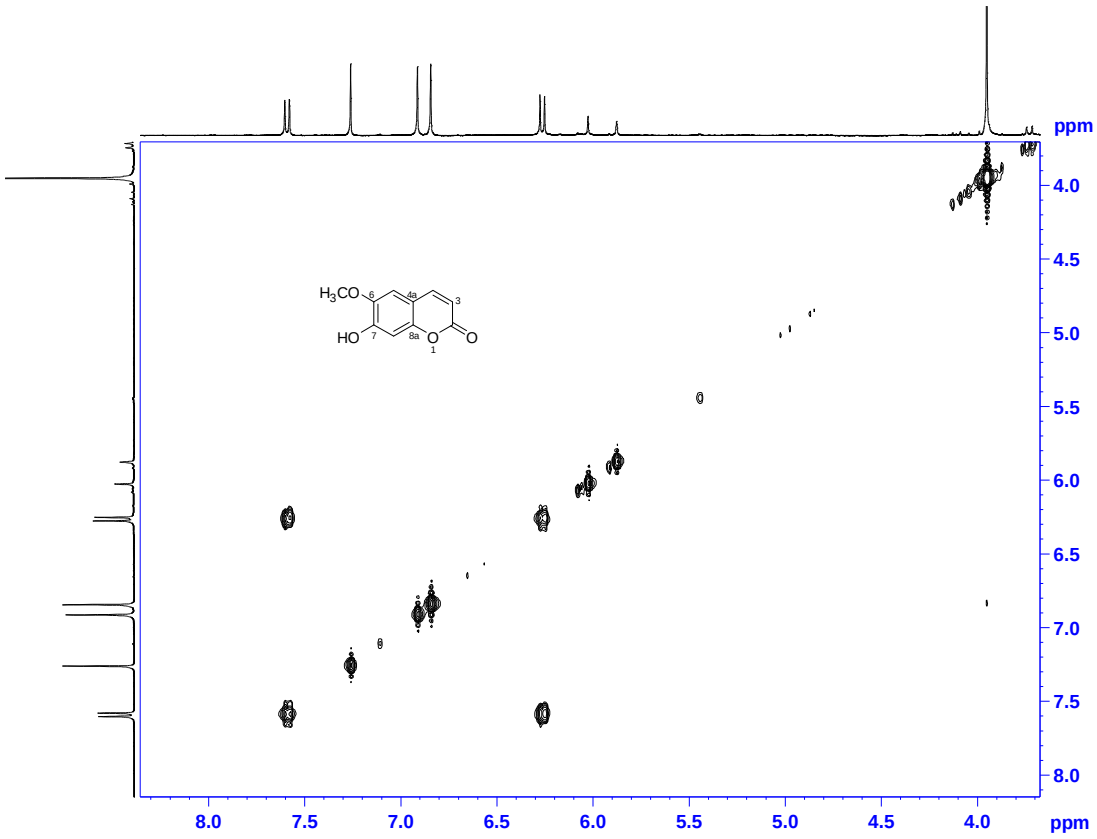


**Plate 55:** HSQC spectrum of scopoletin **(2.66)**

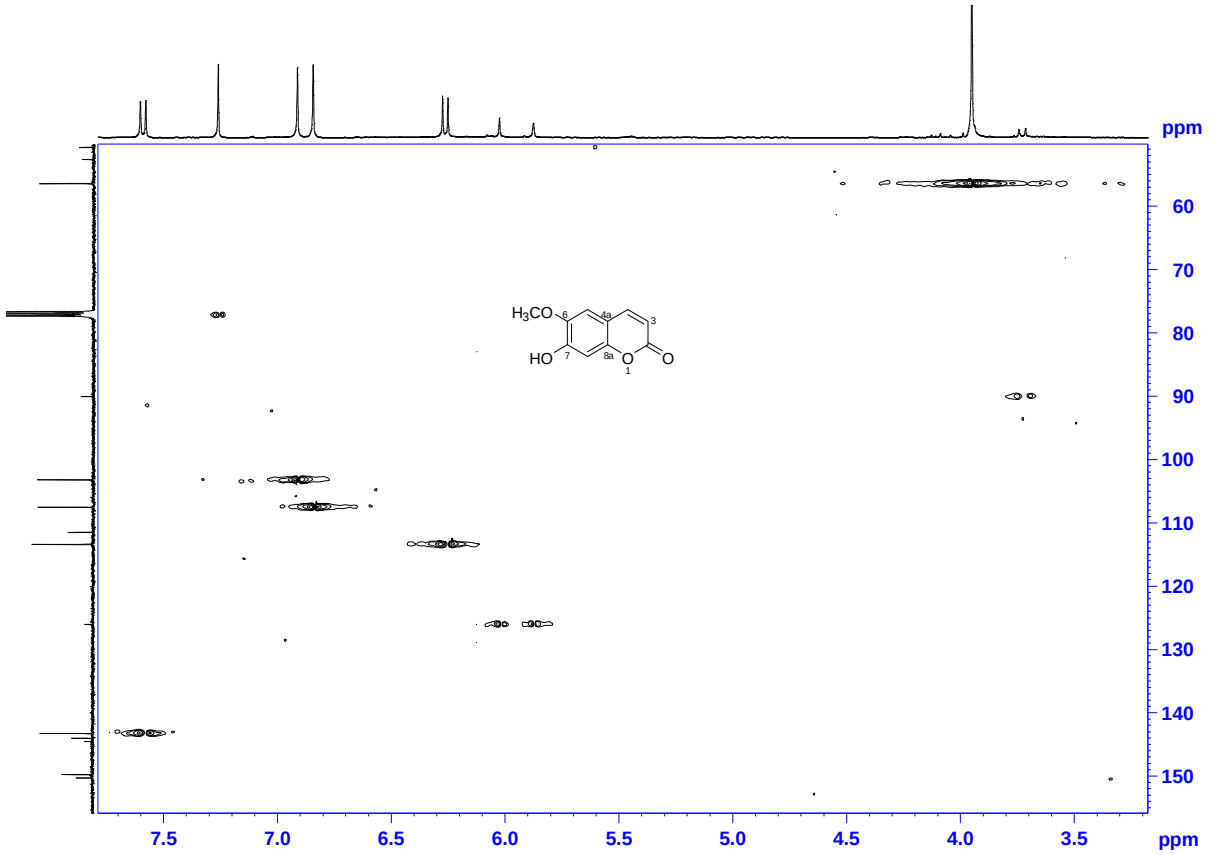


**Plate 56:** HMBC spectrum of scopoletin **(2.66)**

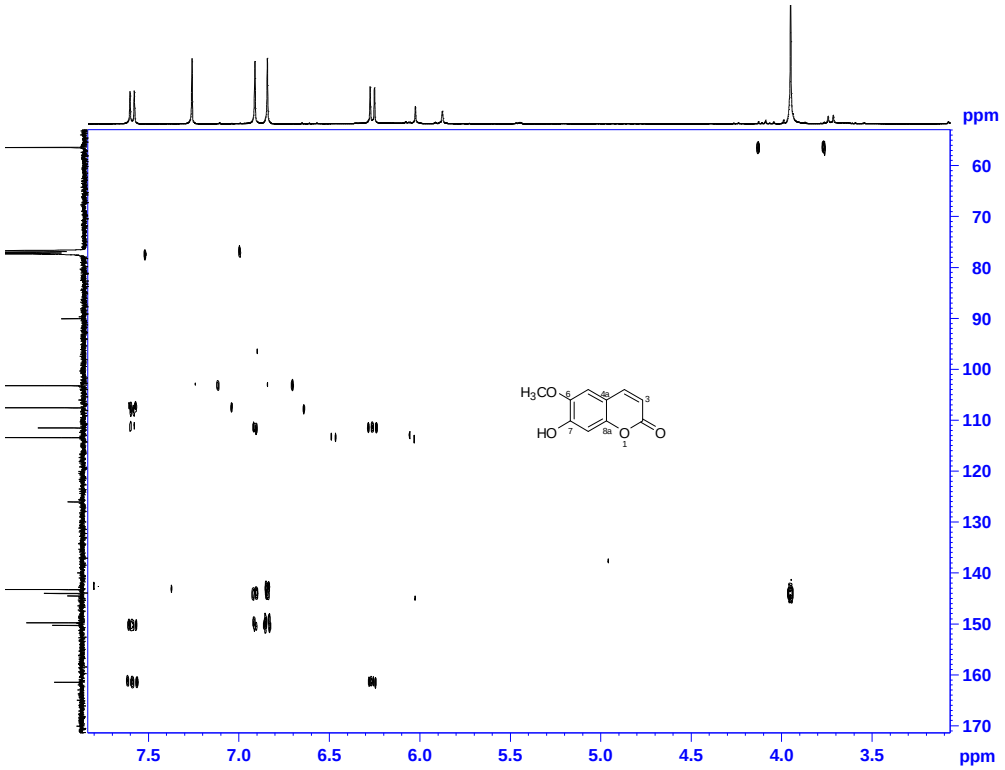


**Plate 57:** $^{1}$H NMR spectrum of *p*-hydroxyacetophenone **(2.67)**

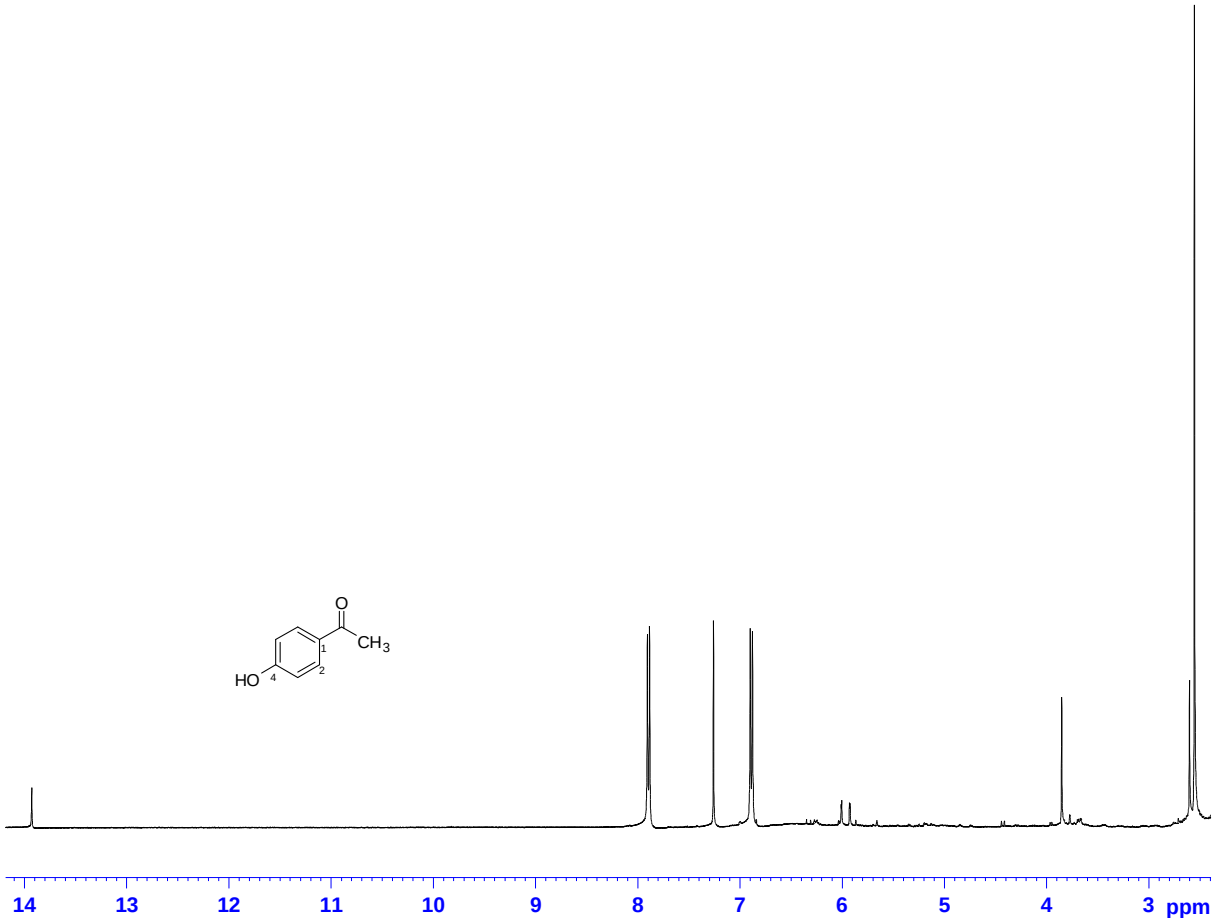


**Plate 58:** $^{13}$C NMR spectrum *of p*-hydroxyacetophenone **(2.67)**

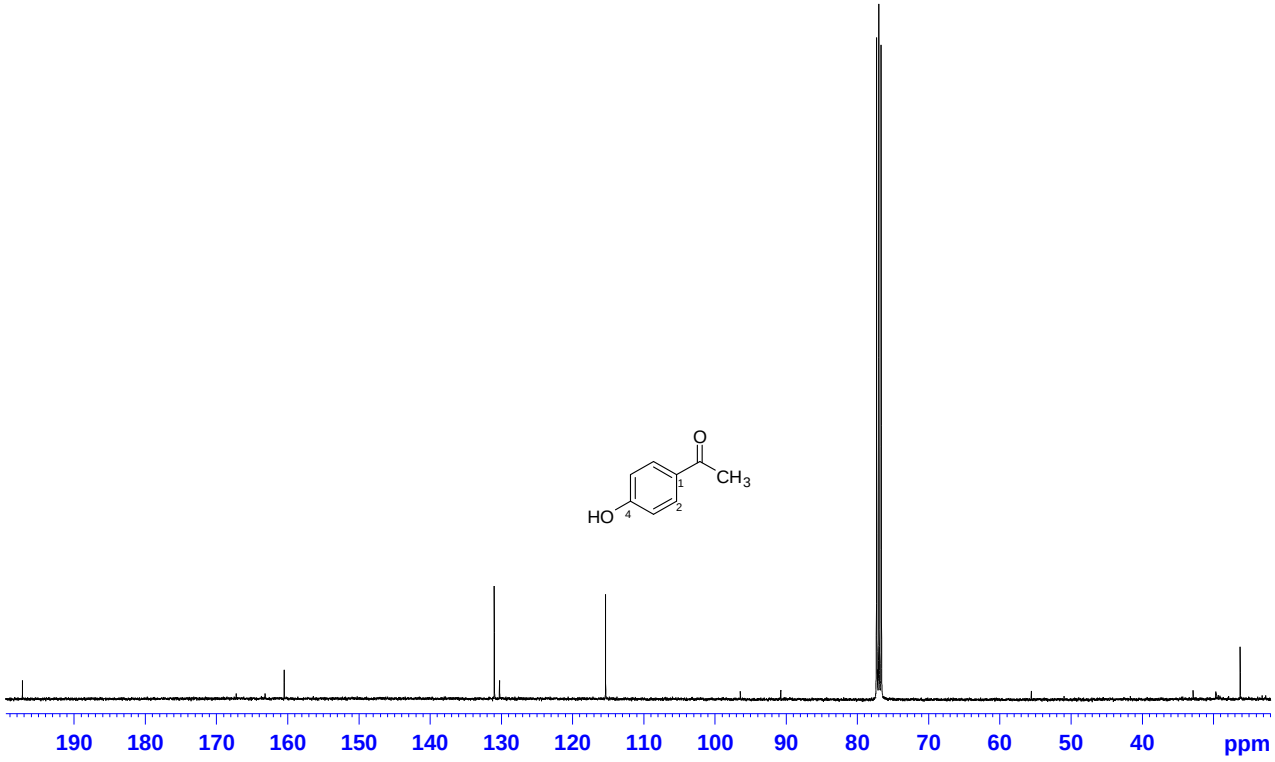


**Plate 59:** DEPT135 spectrum of *p*-hydroxyacetophenone **(2.67)**

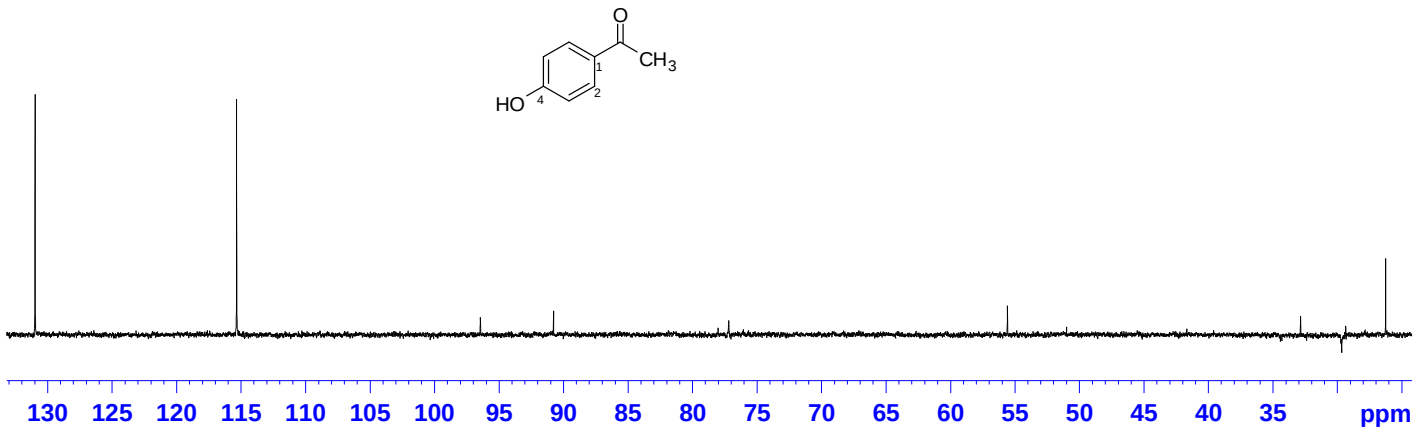


**Plate 60:** DEPT90 spectrum of *p*-hydroxyacetophenone **(2.67)**

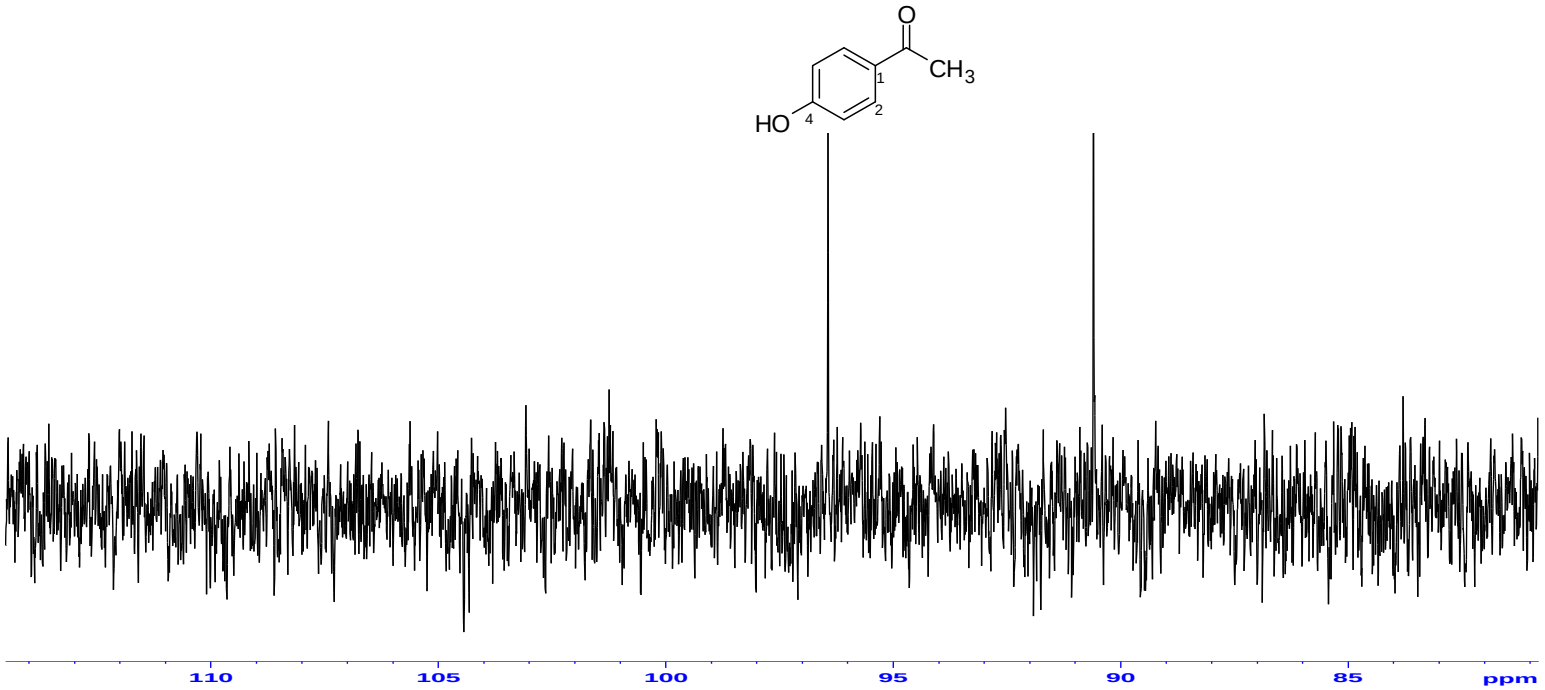


**Plate 61:** COSY spectrum of *p*-hydroxyacetophenone **(2.67)**

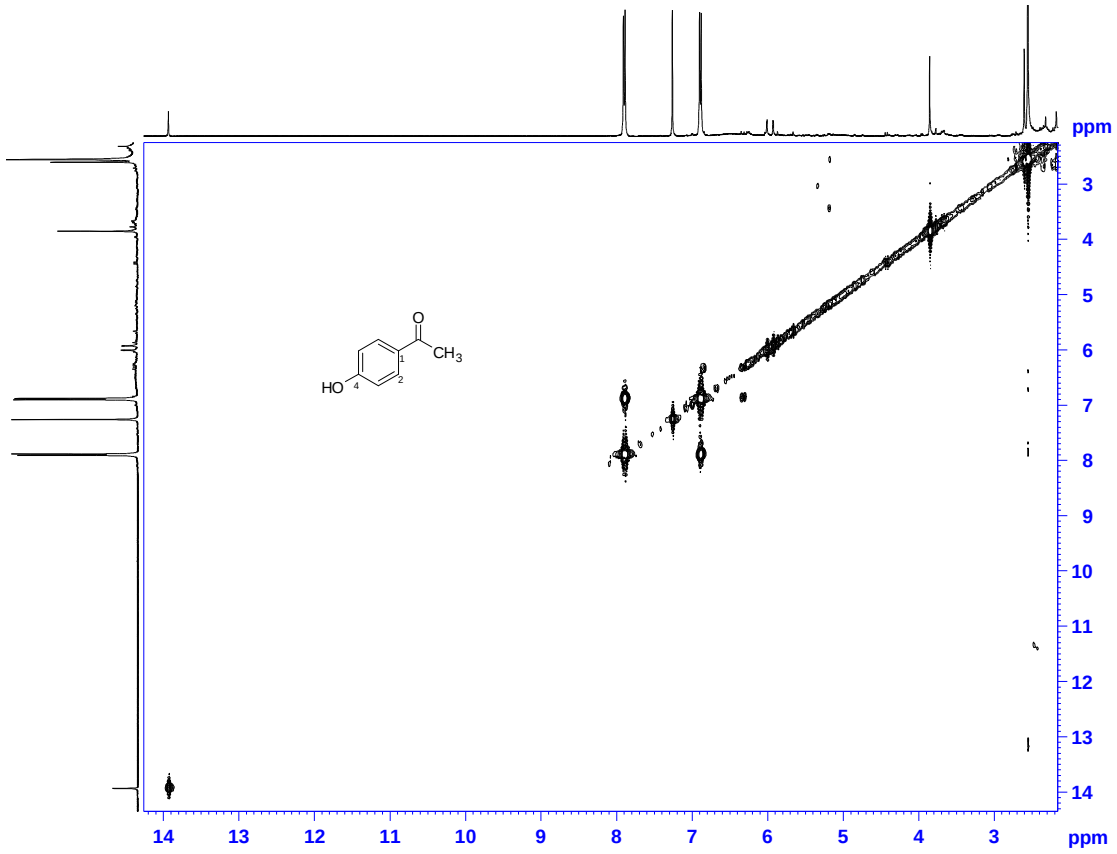
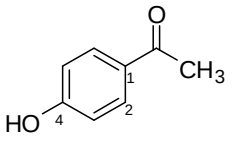


**Plate 62:** HSQC spectrum of *p*-hydroxyacetophenone **(2.67)**



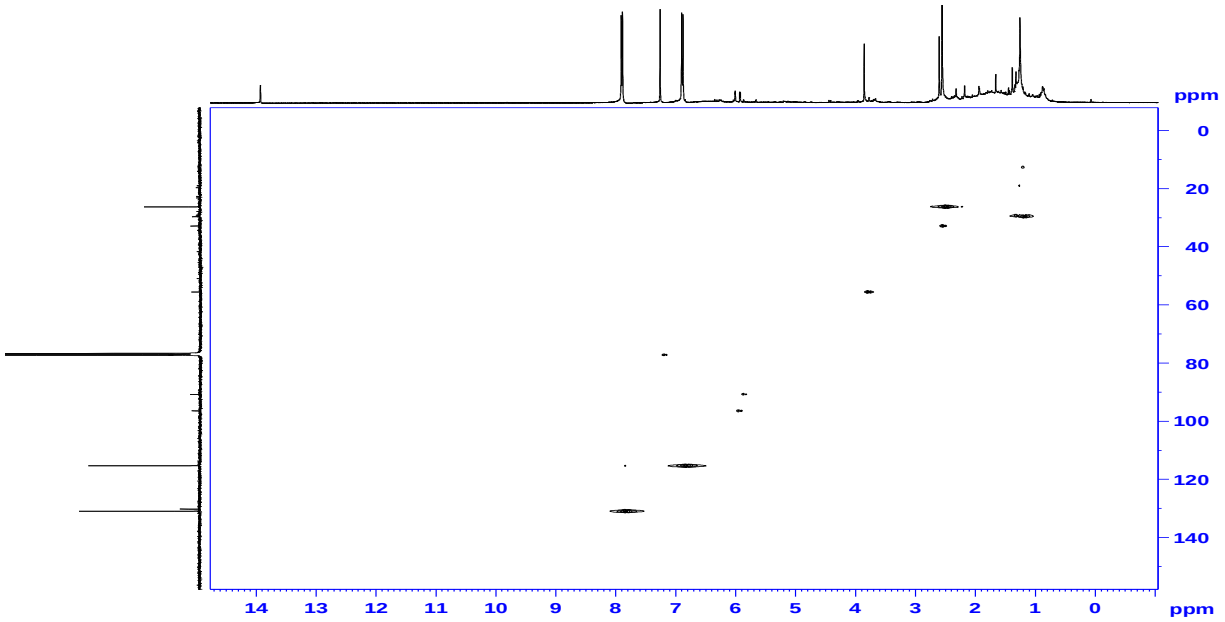
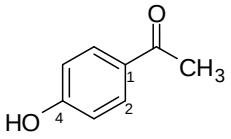



**Plate 63:** HMBC spectrum of *p*-hydroxyacetophenone **(2.67)**



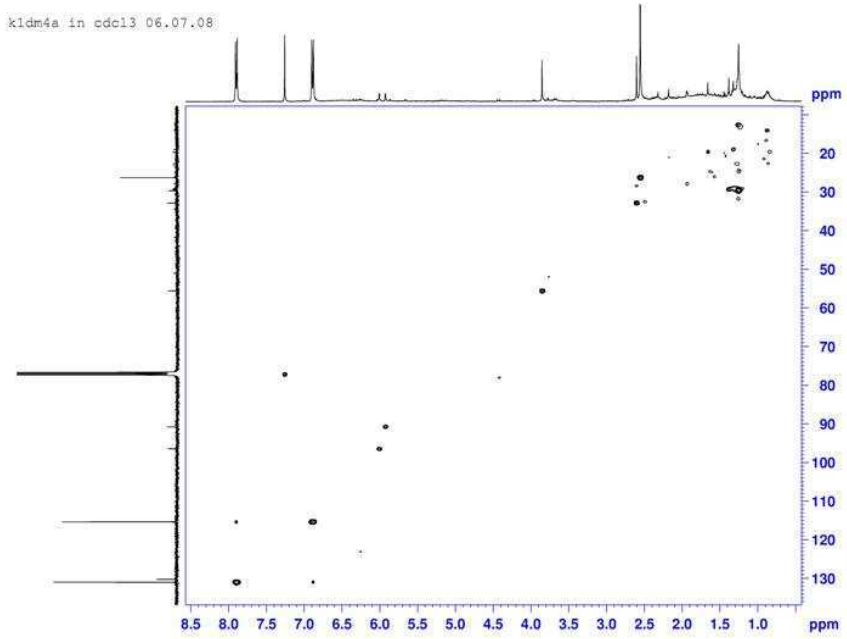



**Plate 64.**1H NMR spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

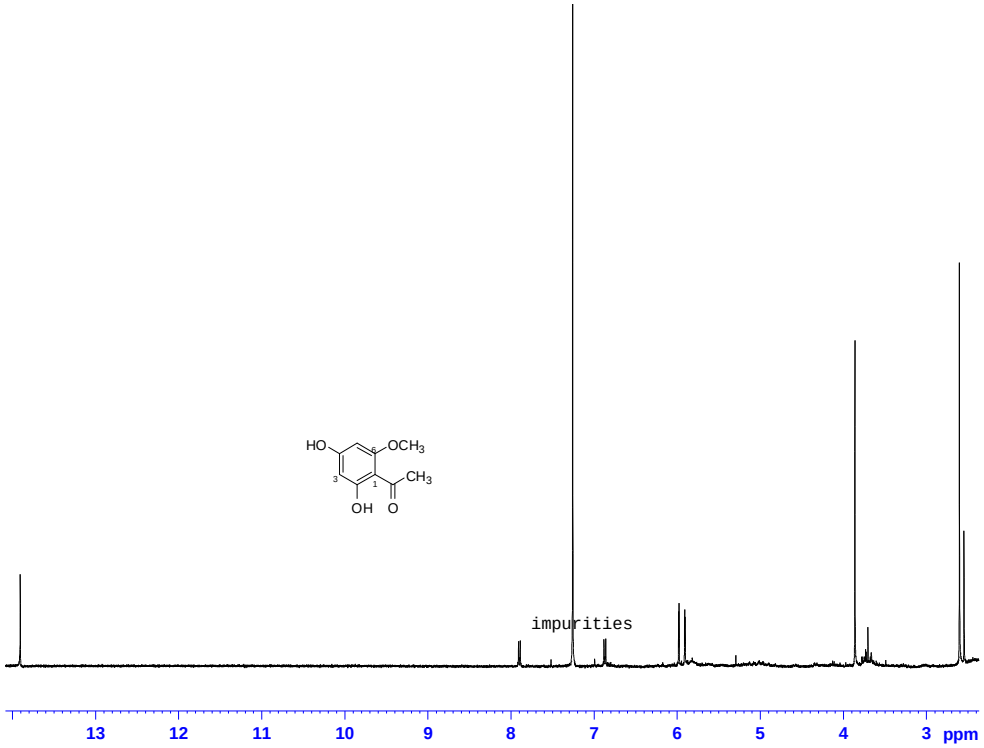


**Plate 65.** $^{13}$C NMR spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

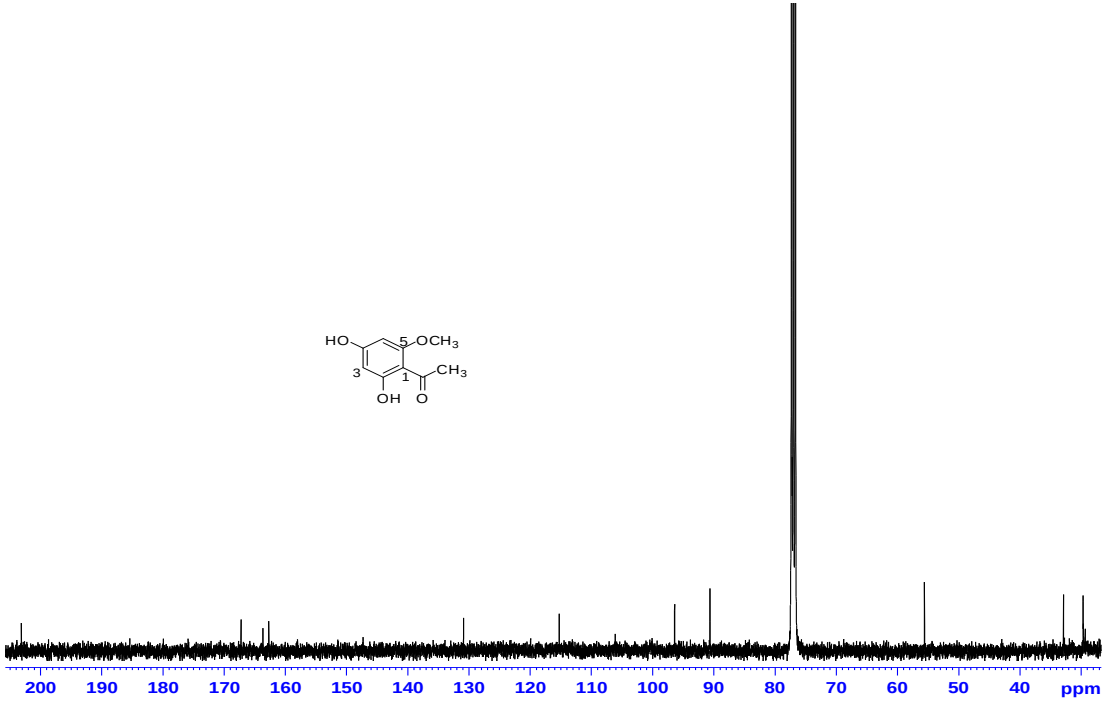


**Plate 66.** DEPT135 spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

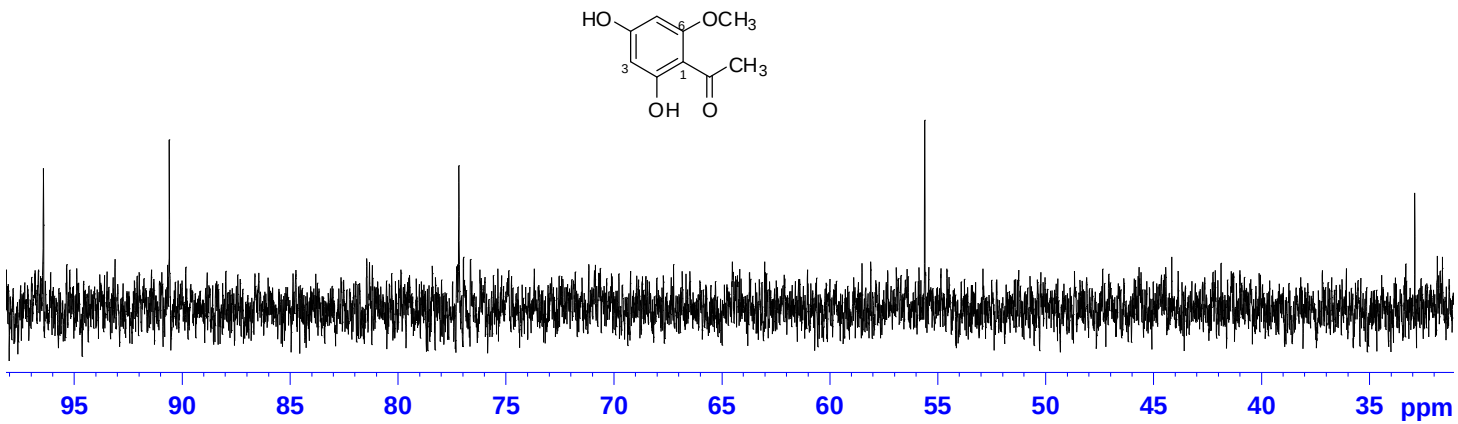


**Plate 67.** DEPT90 spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

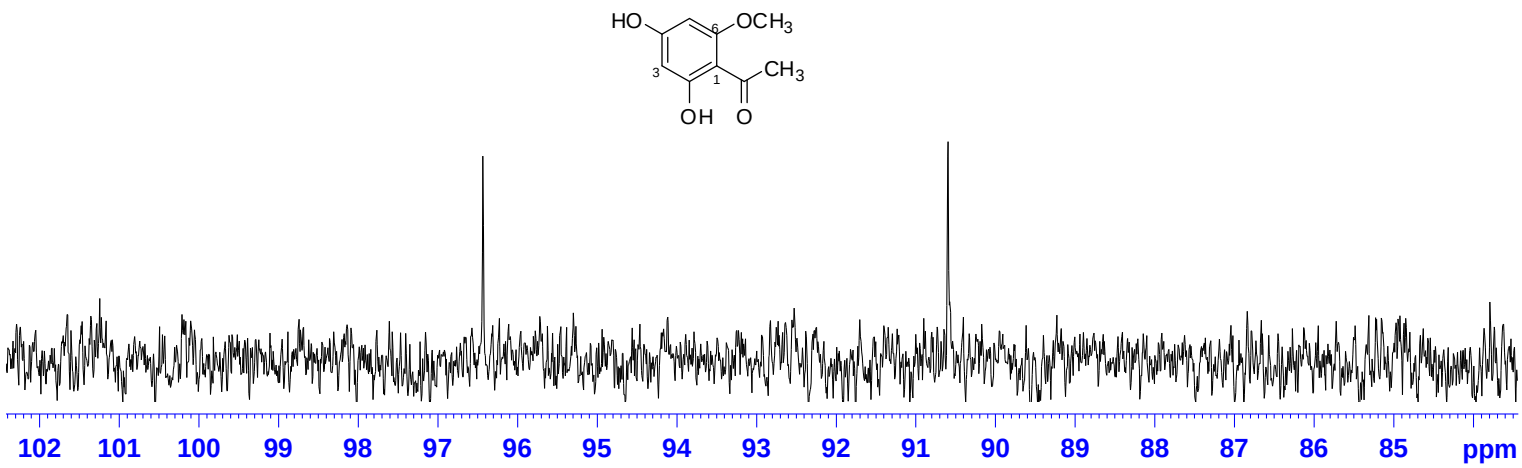


**Plate 68.** COSY spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

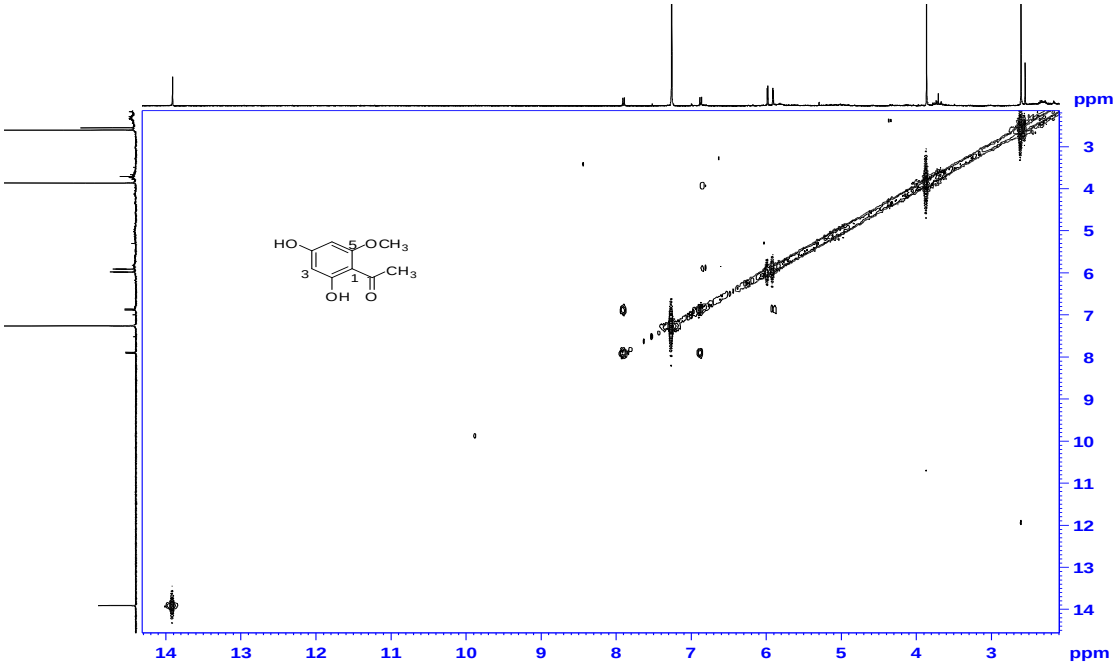


**Plate 69.** HSQC spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

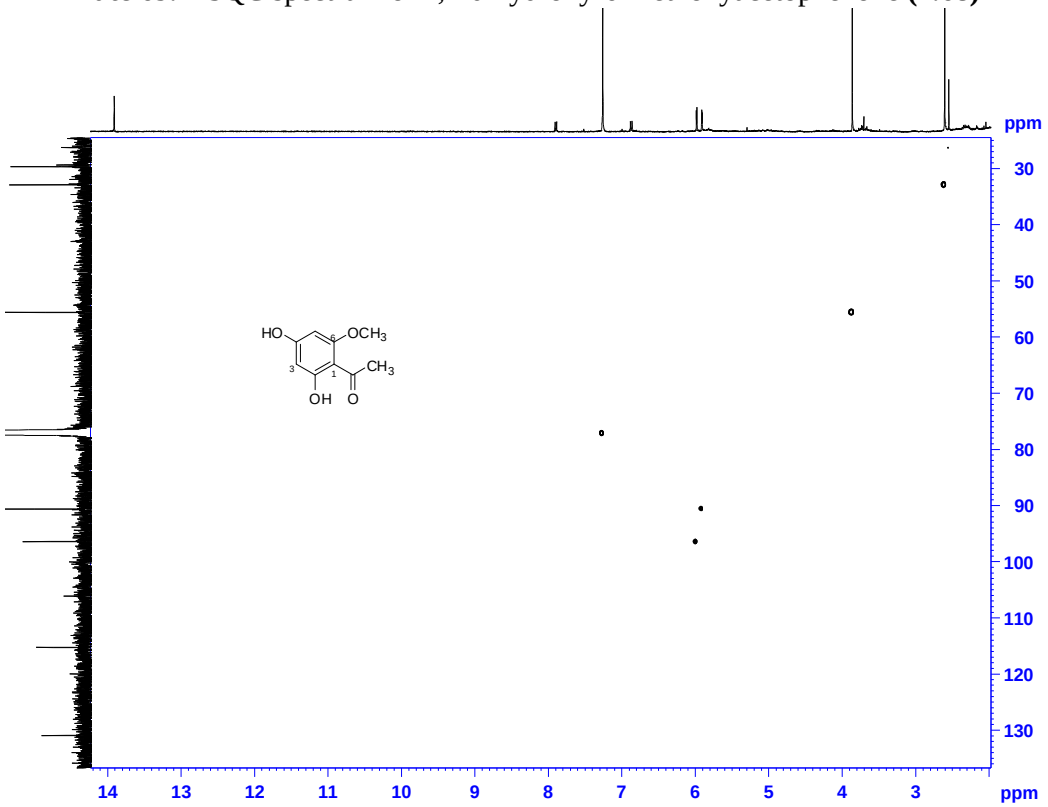


**Plate 70.** HMBC spectrum of 2,4-dihydroxy-6-methoxyacetophenone **(2.68)**

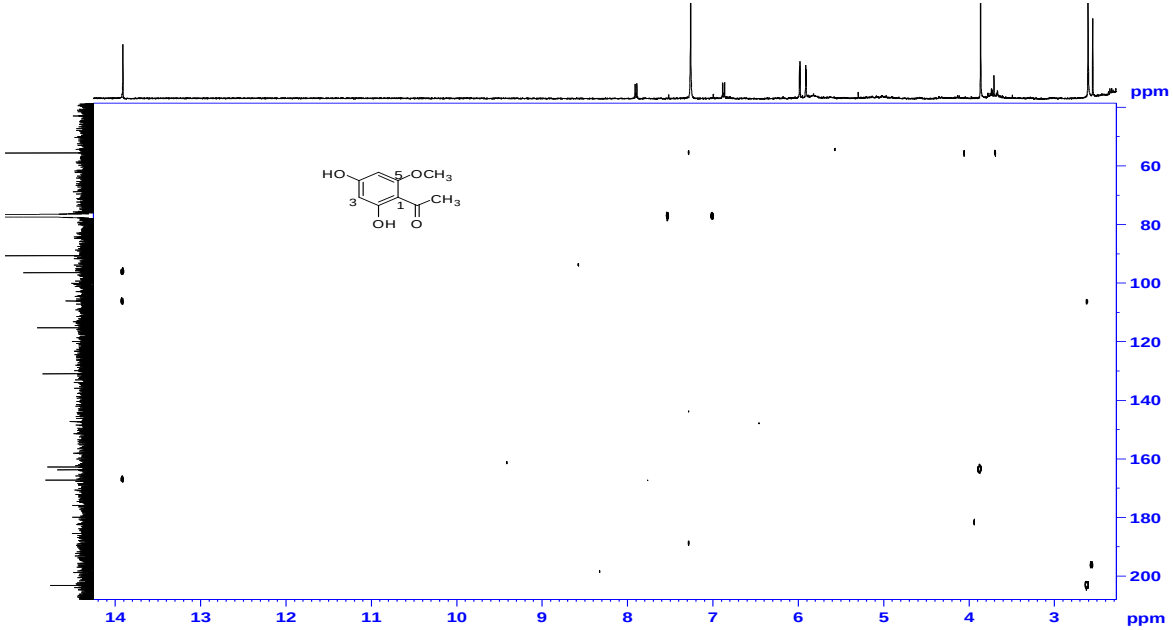


**Plate 71:** $^{1}$H NMR spectrum of 5-hydroxy-7,4'-dimethoxyflavone **(2.69)**

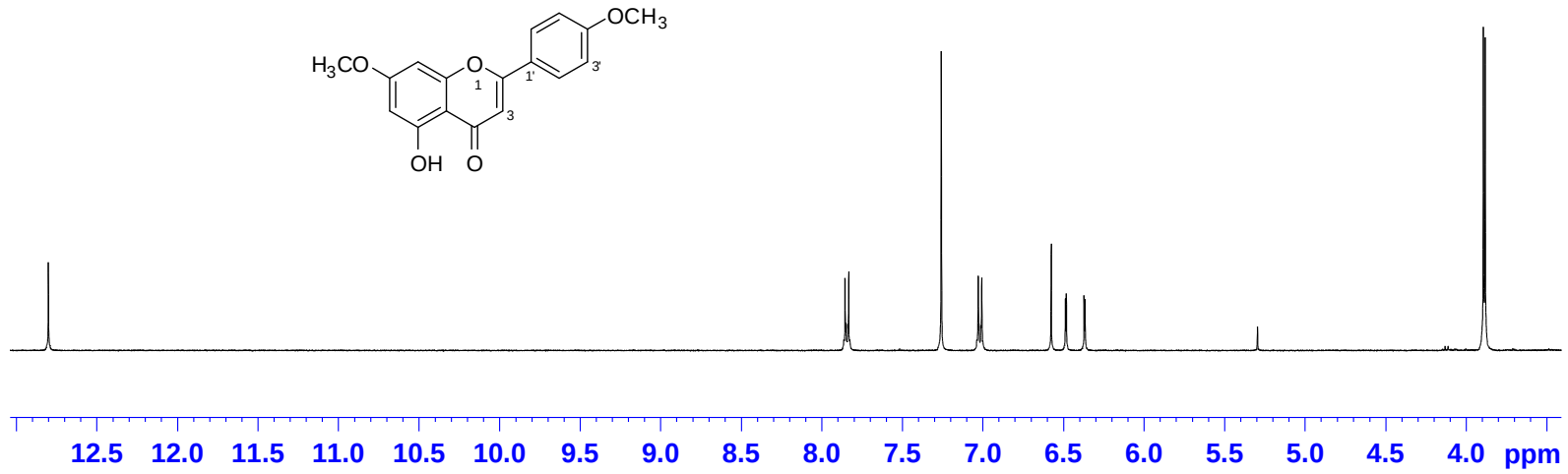


**Plate 72:** $^{13}$C NMR spectrum of 5-hydroxy-7,4'-dimethoxyflavone **(2.69)**

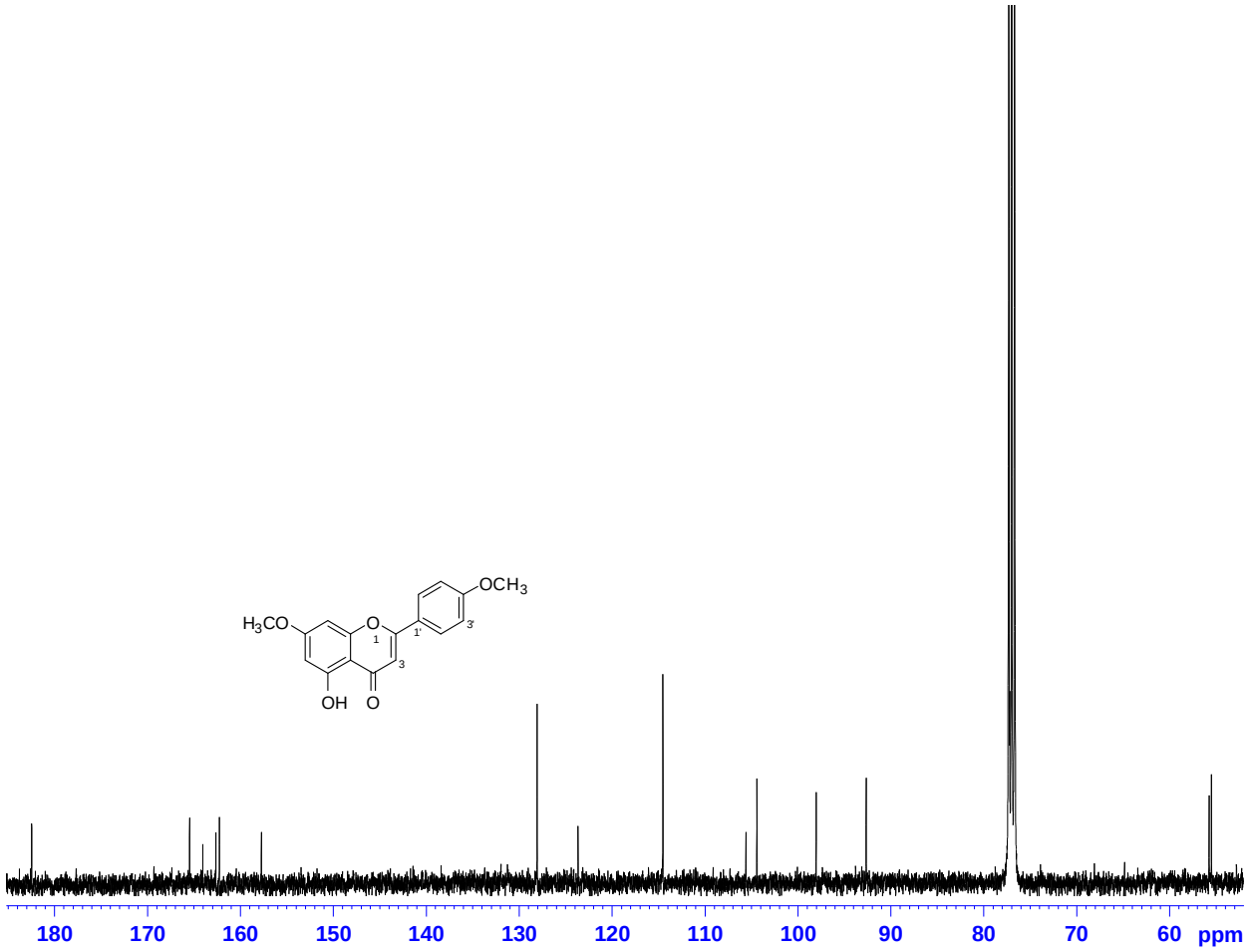


**Plate 73:** DEPT135 spectrum of 5-hydroxy-7,4'-dimethoxyflavone **(2.69)**

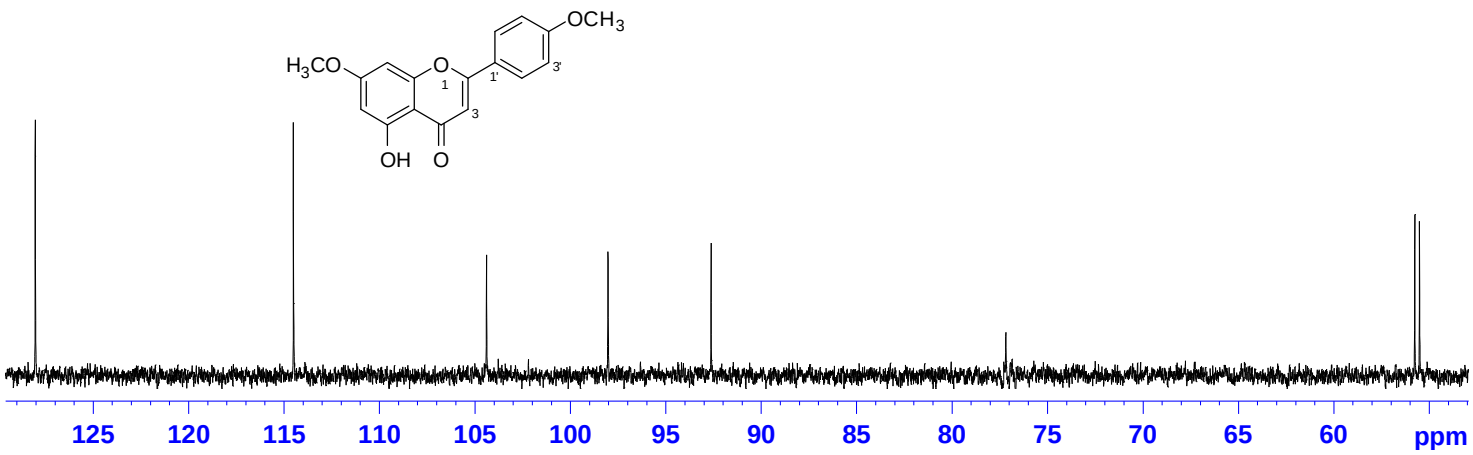


**Plate 74:** DEPT90 spectrum of 5-hydroxy-7,4'-dimethoxyflavone **(2.69)**

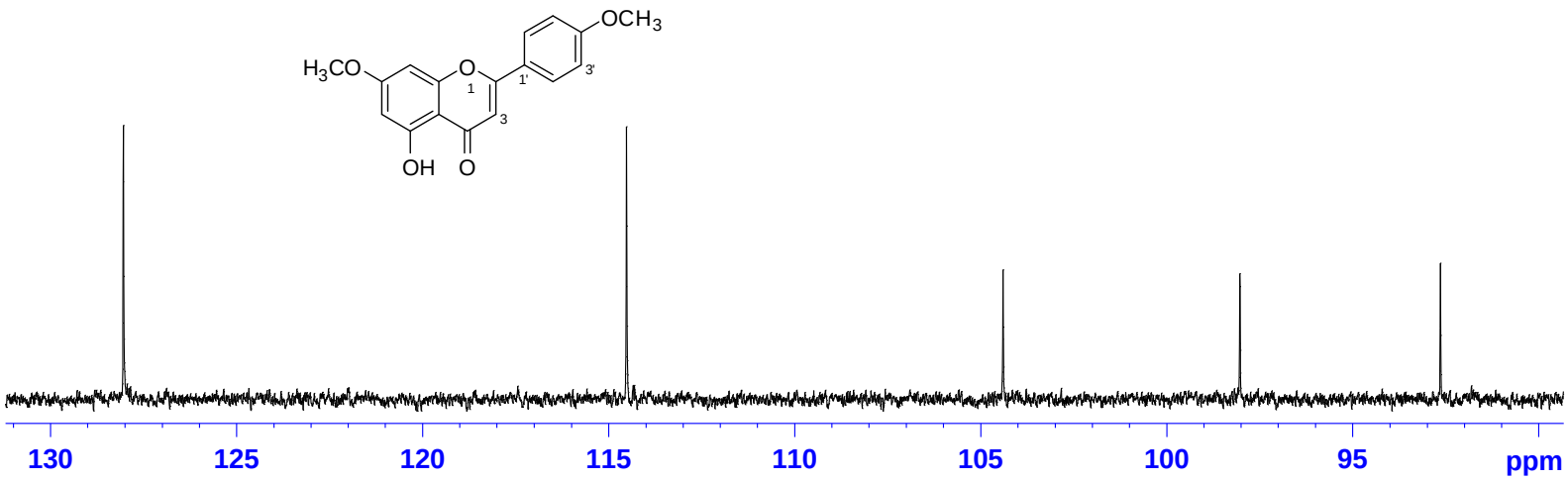


**Plate 75:** COSY spectrum of 5-hydroxy-7,4'-dimethoxyflavone **(2.69)**

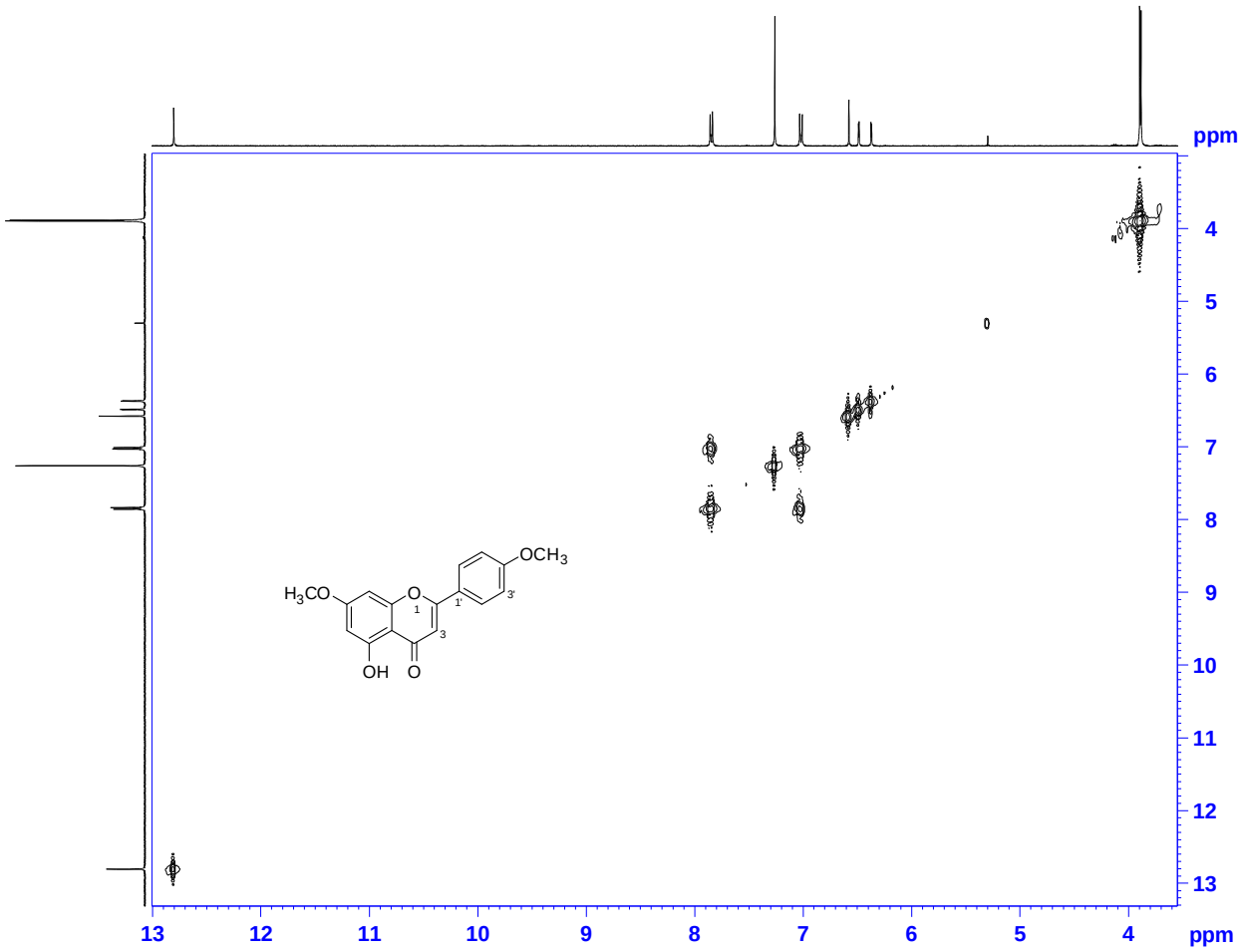


**Plate 76:** HSQC spectrum of 5-hydroxy-7,4'-dimethoxyflavone **(2.69)**

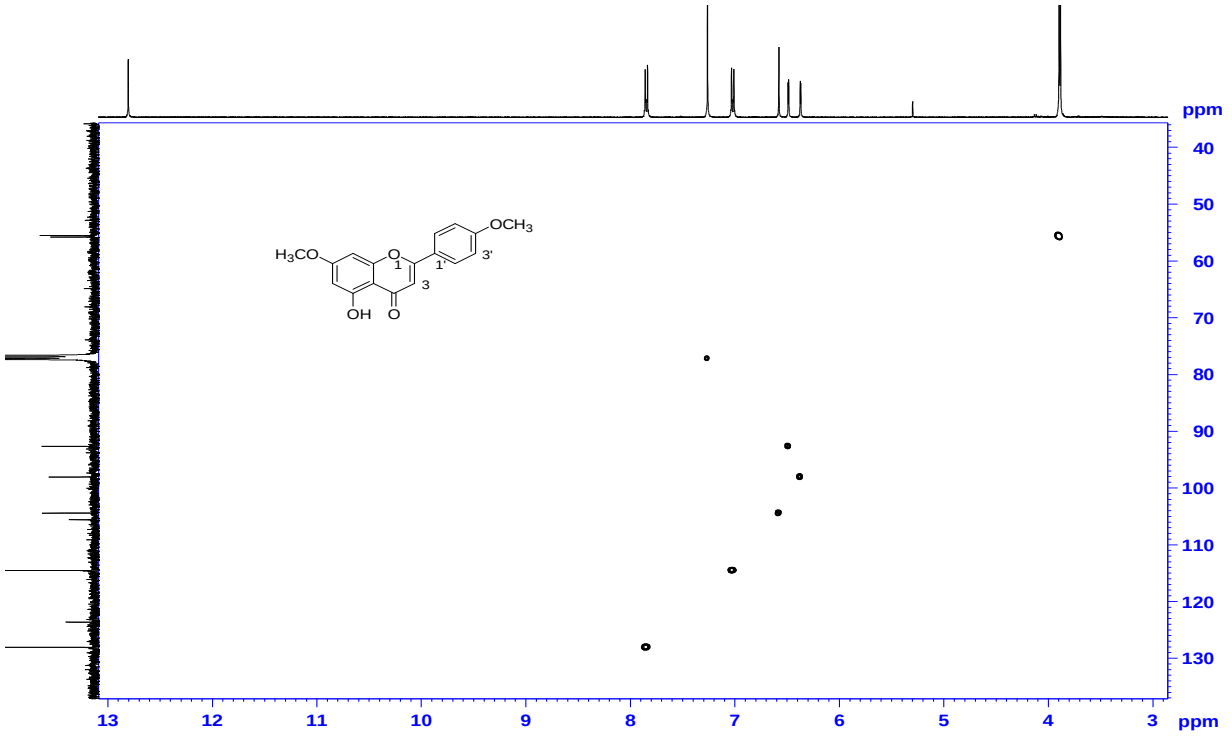


**Plate 77:** HMBC spectrum of 5-hydroxy-7, 4'-dimethoxyflavone **(2.69)**

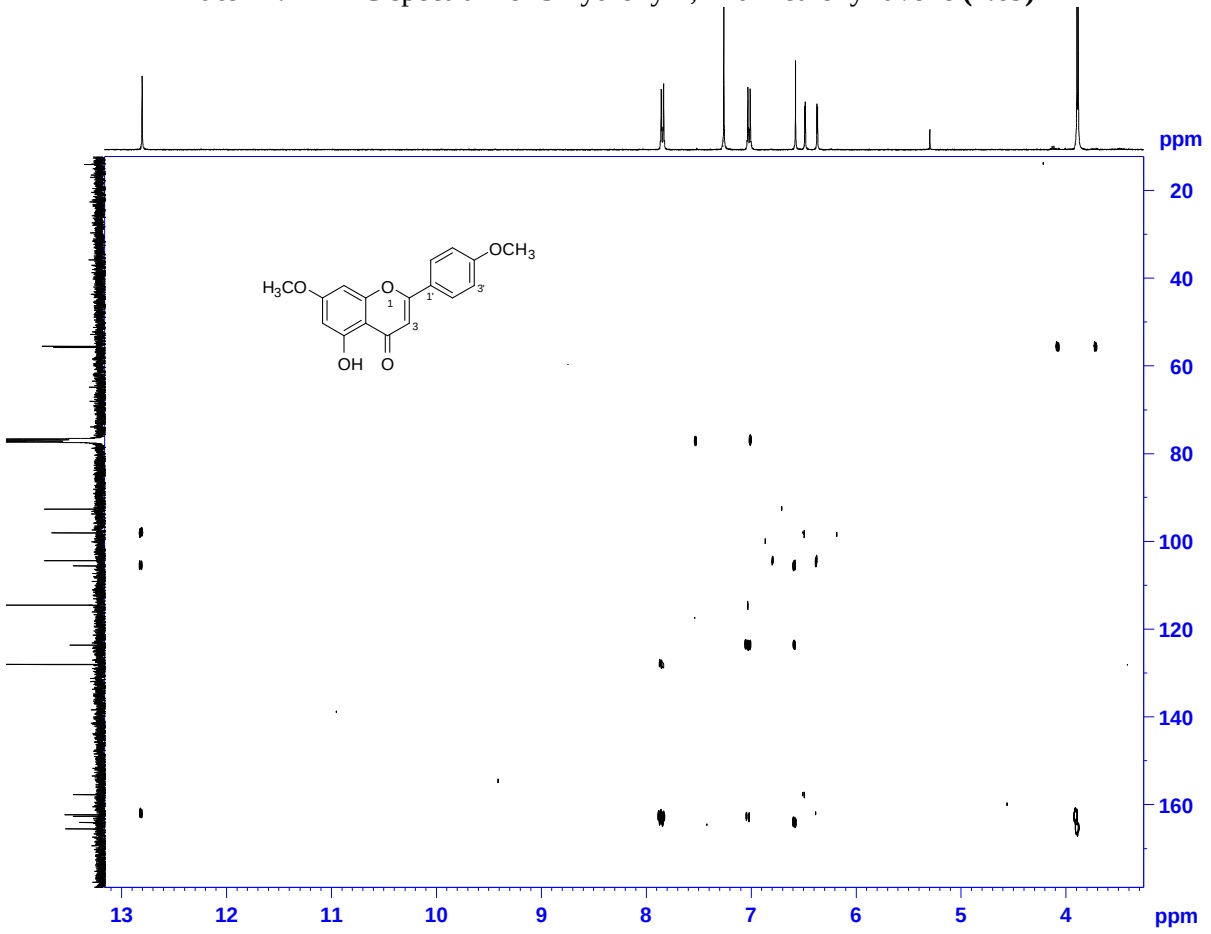


**Plate 78:** $^{1}$H NMR spectrum of labdanolic acid **(3.13)**

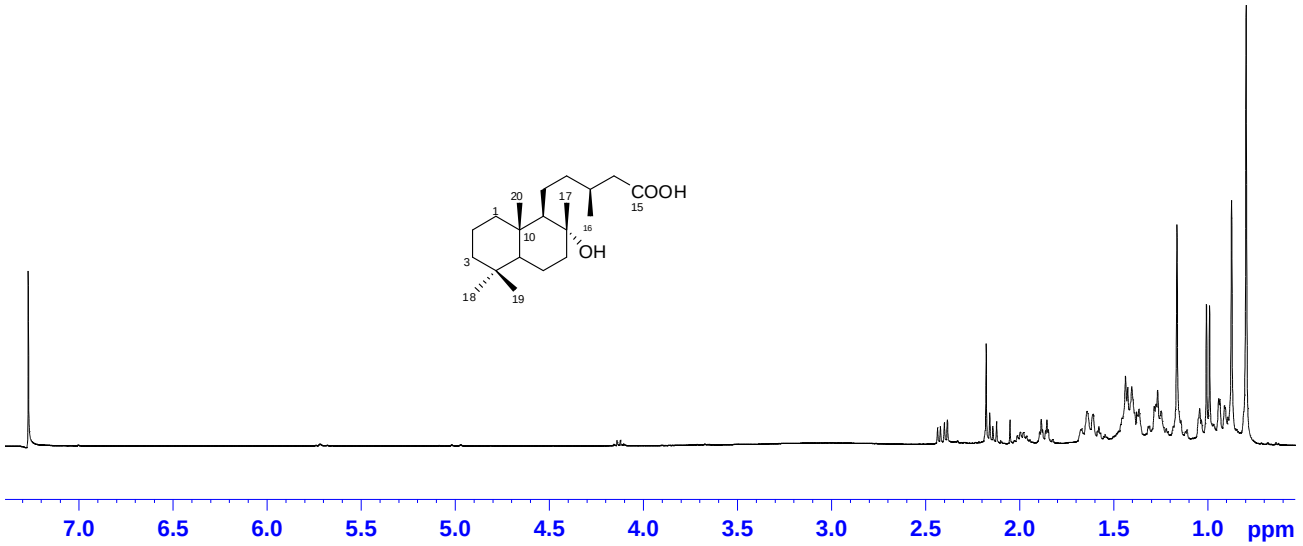


**Plate 79:** $^{13}$C NMR spectrum of labdanolic acid **(3.13)**

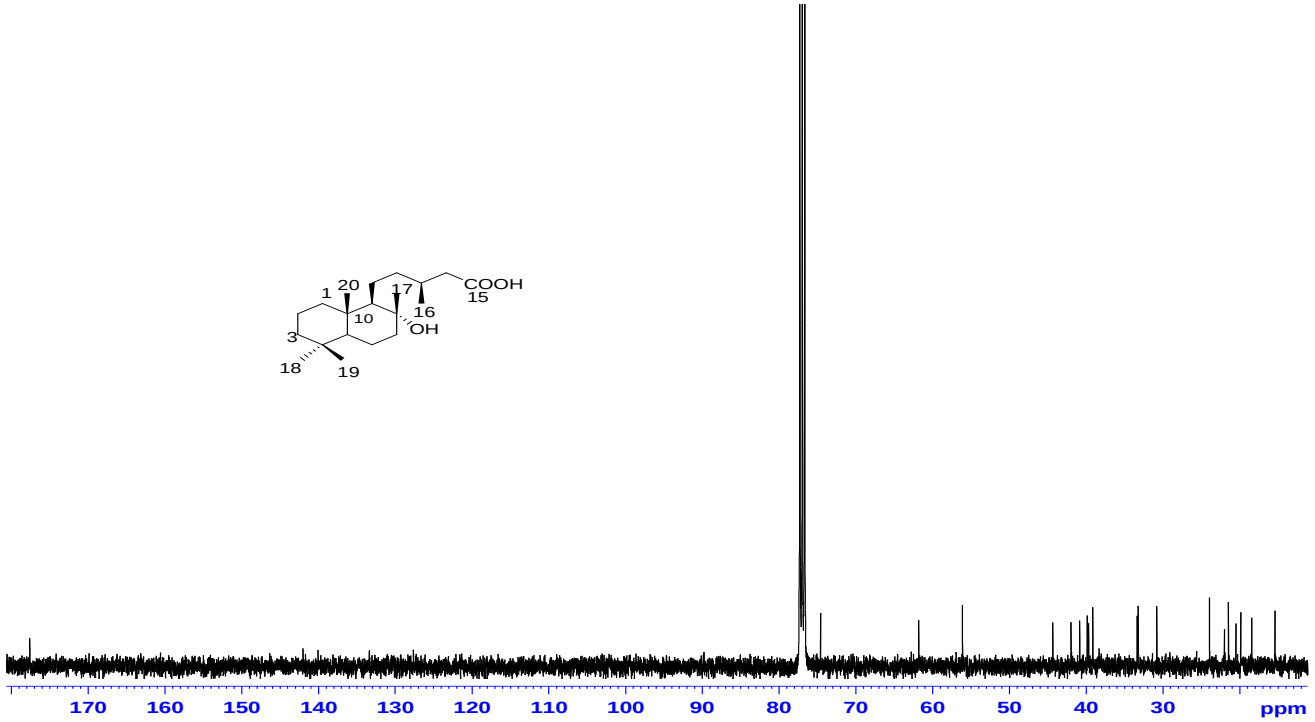


**Plate 80:** DEPT135 spectrum of labdanolic acid **(3.13)**

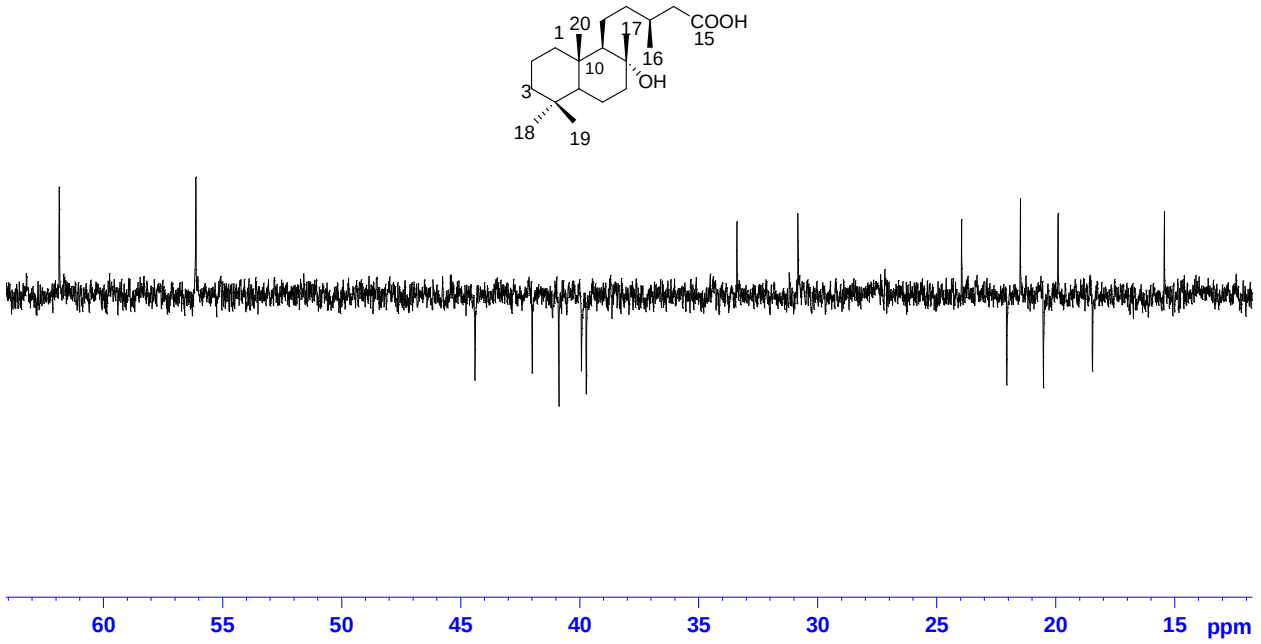


**Plate 81:** DEPT90 spectrum of labdanolic acid **(3.13)**

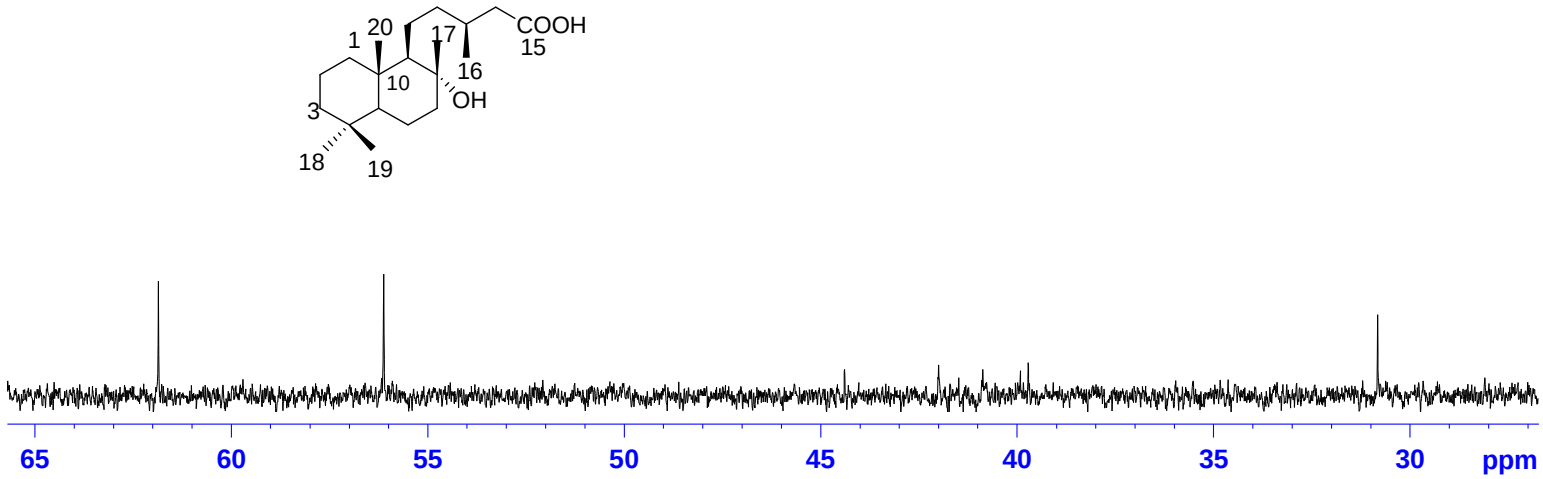


**Plate 82:** COSY spectrum of labdanolic acid **(3.13)**

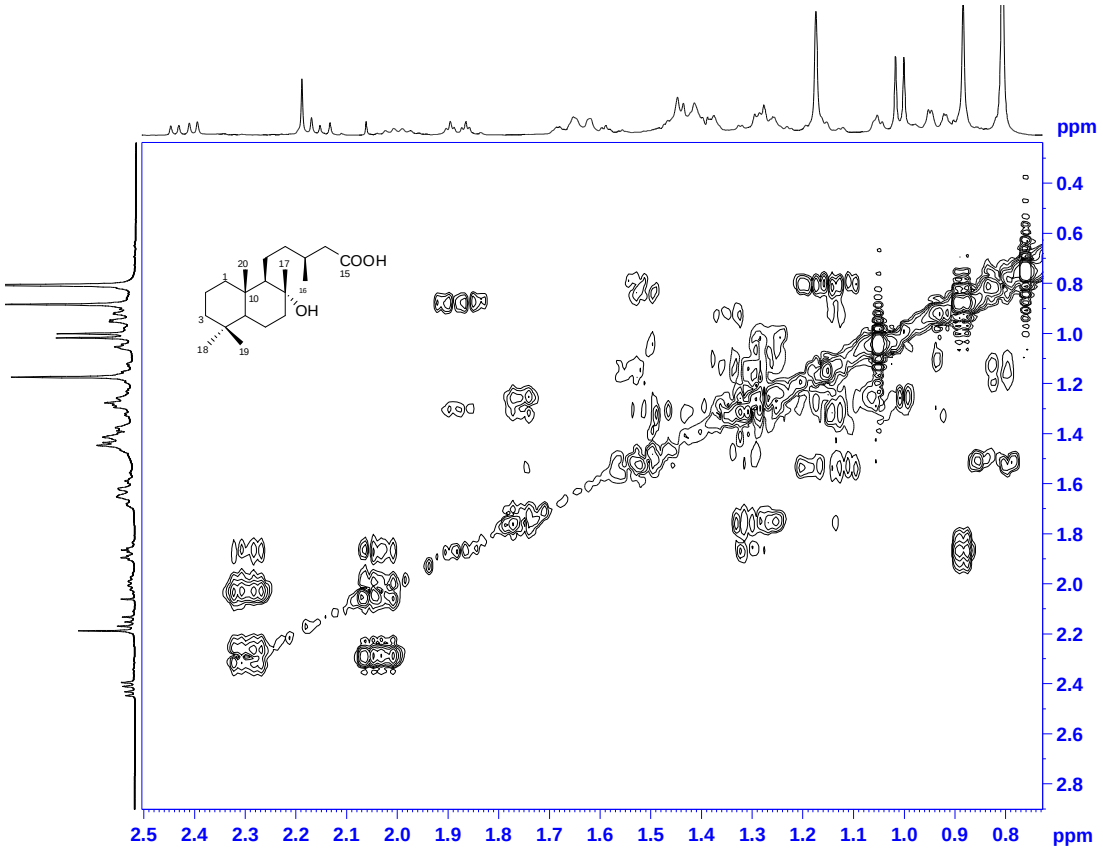


**Plate 83:** HSQC spectrum of labdanolic acid **(3.13)**

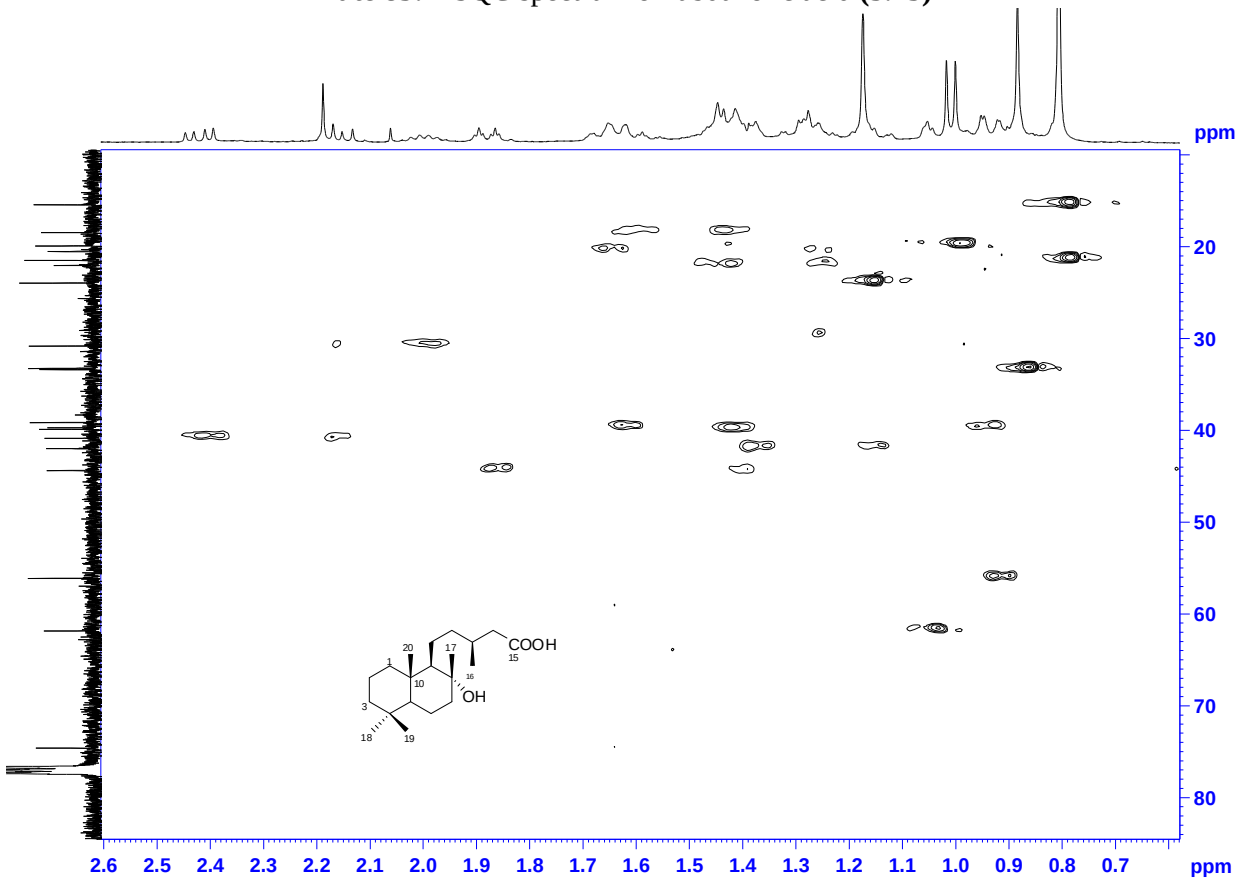


**Plate 84:** HMBC spectrum of labdanolic acid **(3.13)**

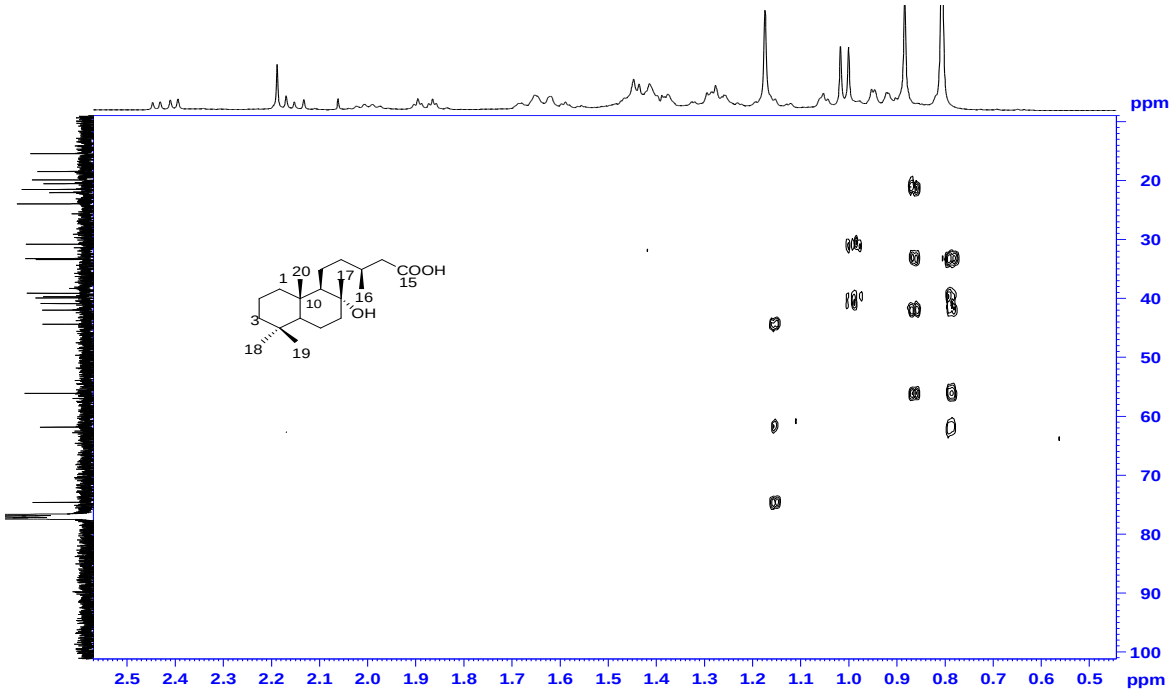


**Plate 85:** $^{1}$H NMR spectrum of methyl labdanolate **(3.14)**

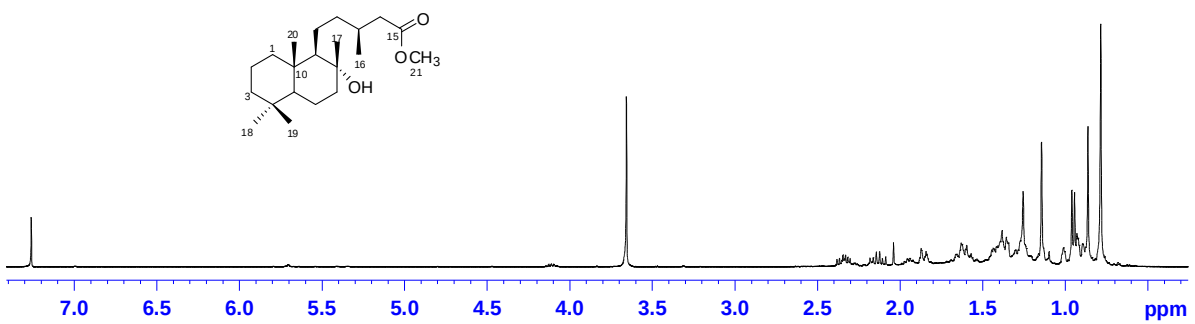


**Plate 86:** $^{13C}$ NMR spectrum of methyl labdanolate **(3.14)**

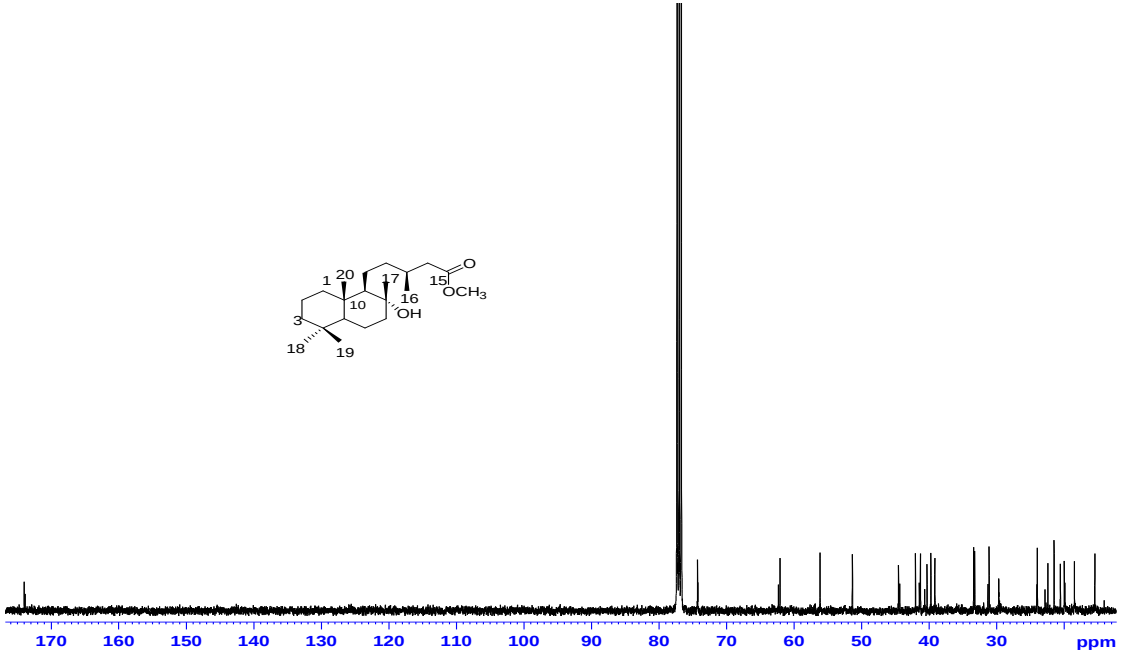


**Plate 87:** DEPT135 spectrum of methyl labdanolate **(3.14)**

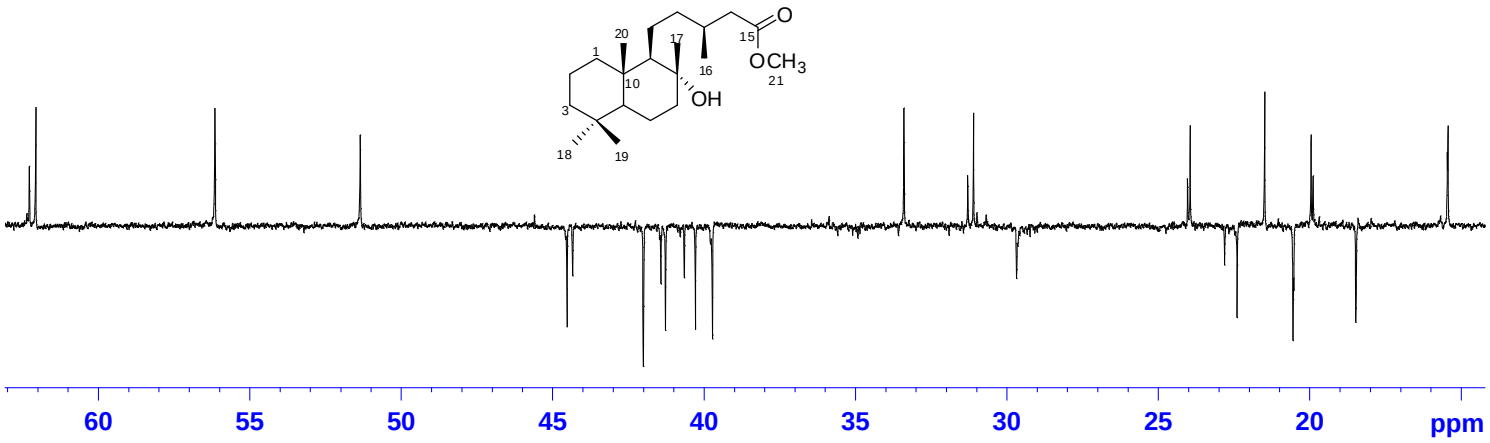


**Plate 88:** DEPT90 spectrum of methyl labdanolate **(3.14)**

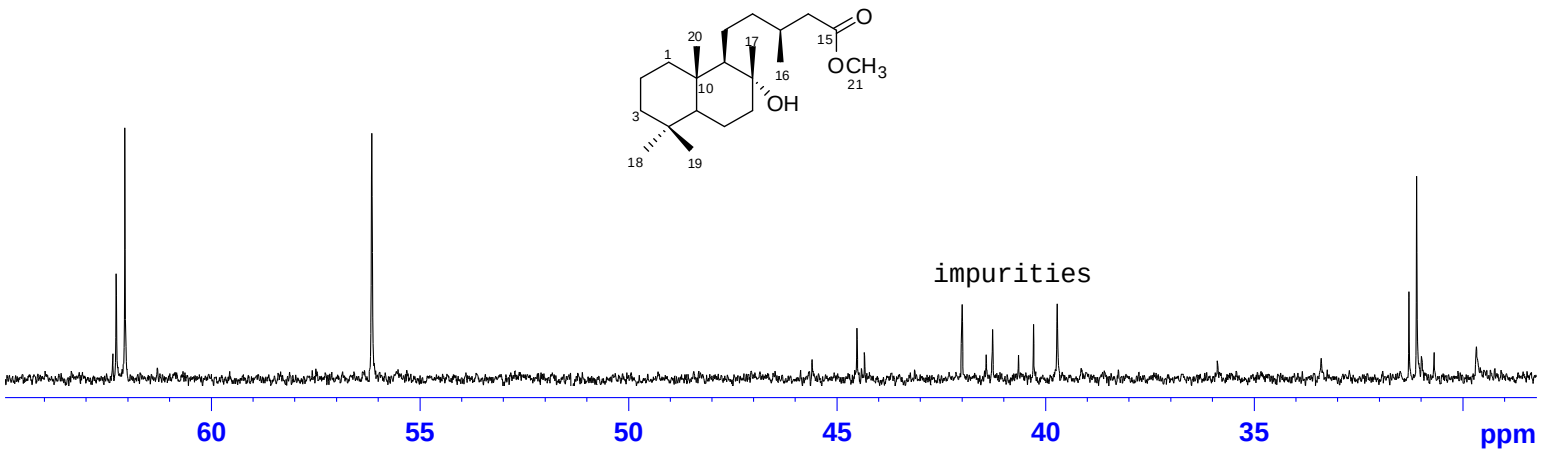


**Plate 89: COSY** spectrum of methyl labdanolate **(3.14)**

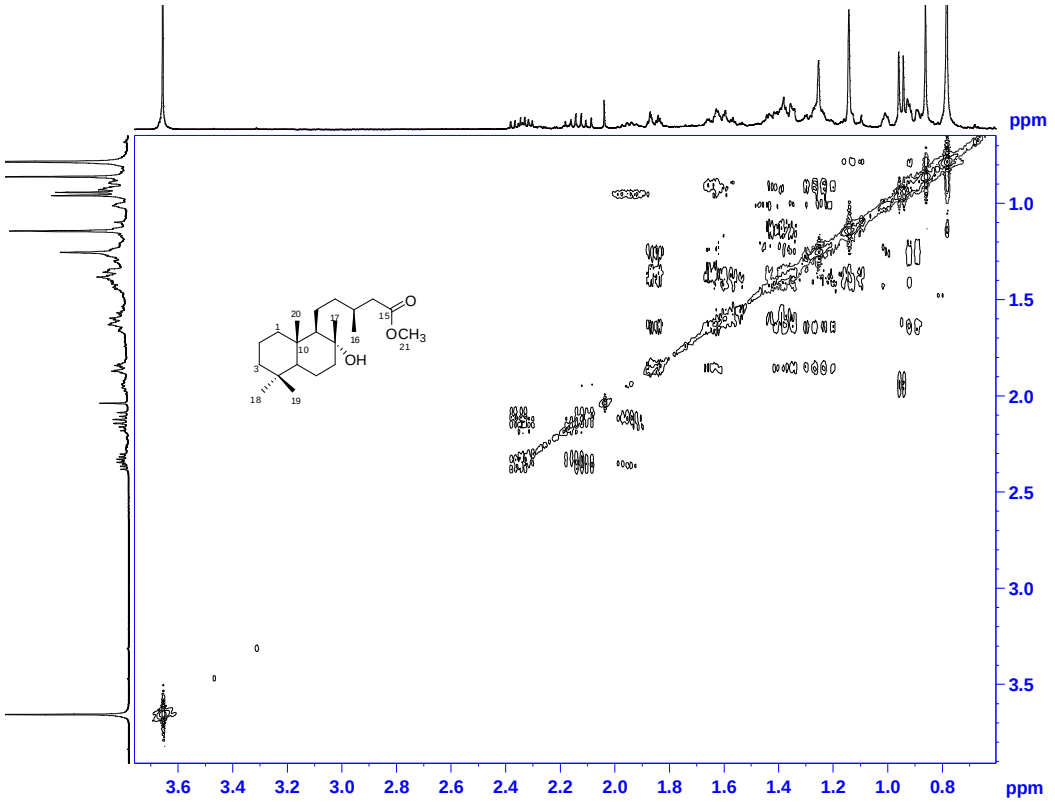


**Plate 90:** HSQC spectrum of methyl labdanolate **(3.14)**

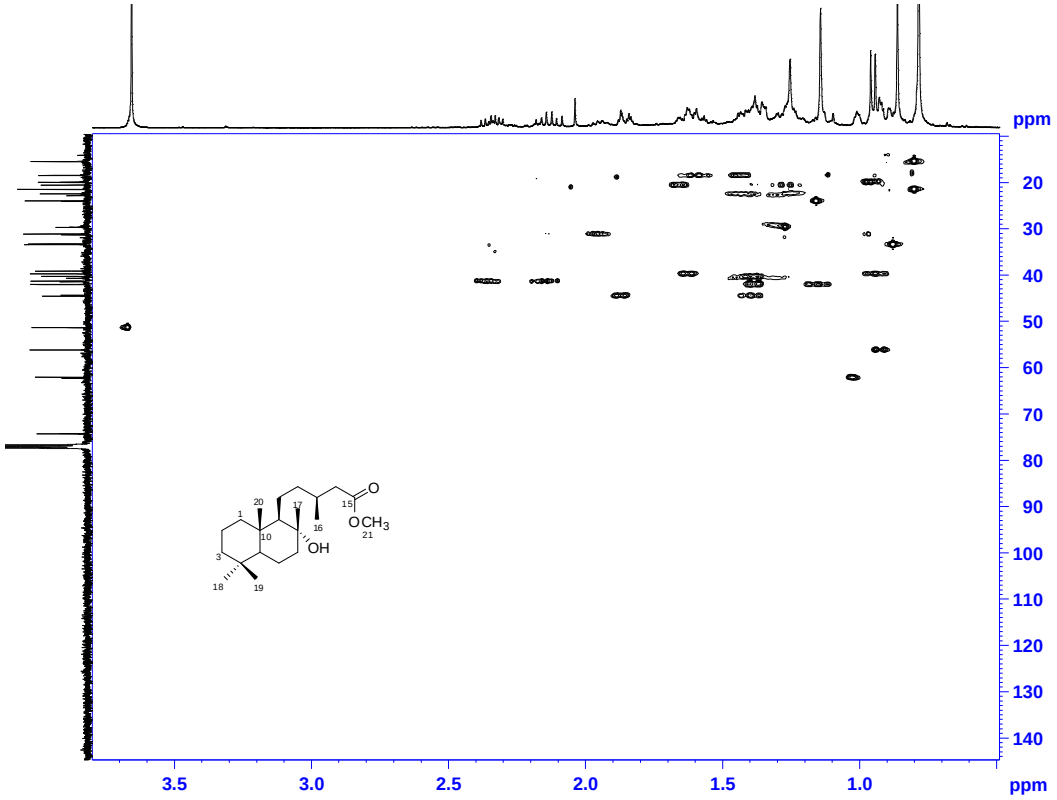


**Plate 91:** HMBC spectrum of methyl labdanolate **(3.14)**

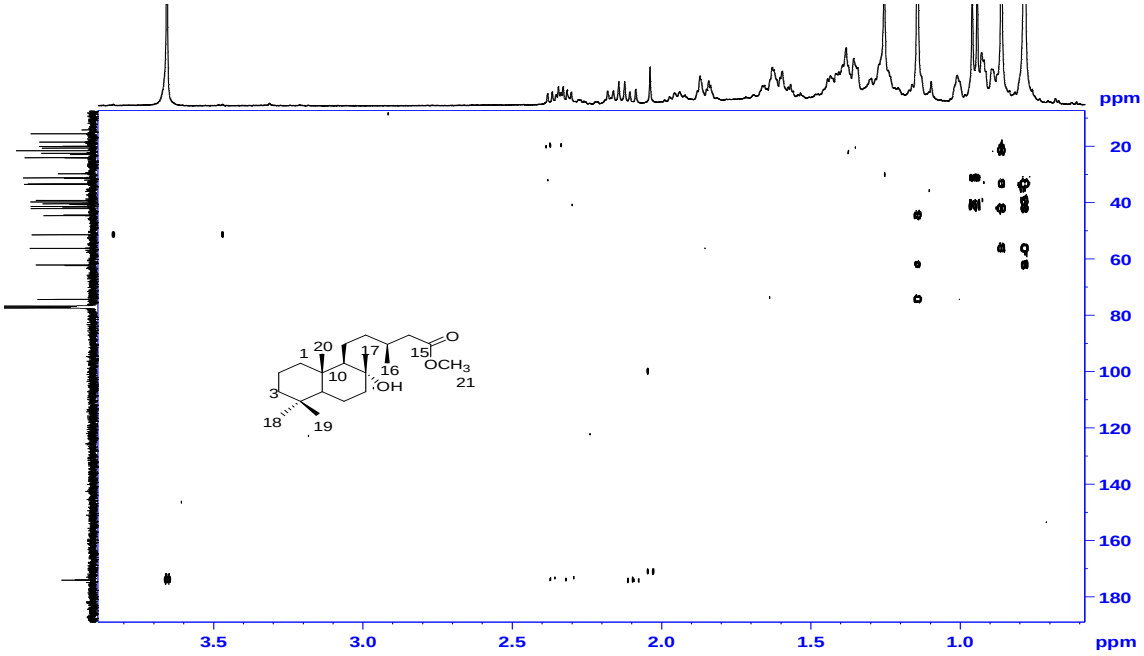


**Plate 92:** $^{1}$H NMR spectrum of scoparone (6,7-dimethoxycoumarin) **(3.15)**

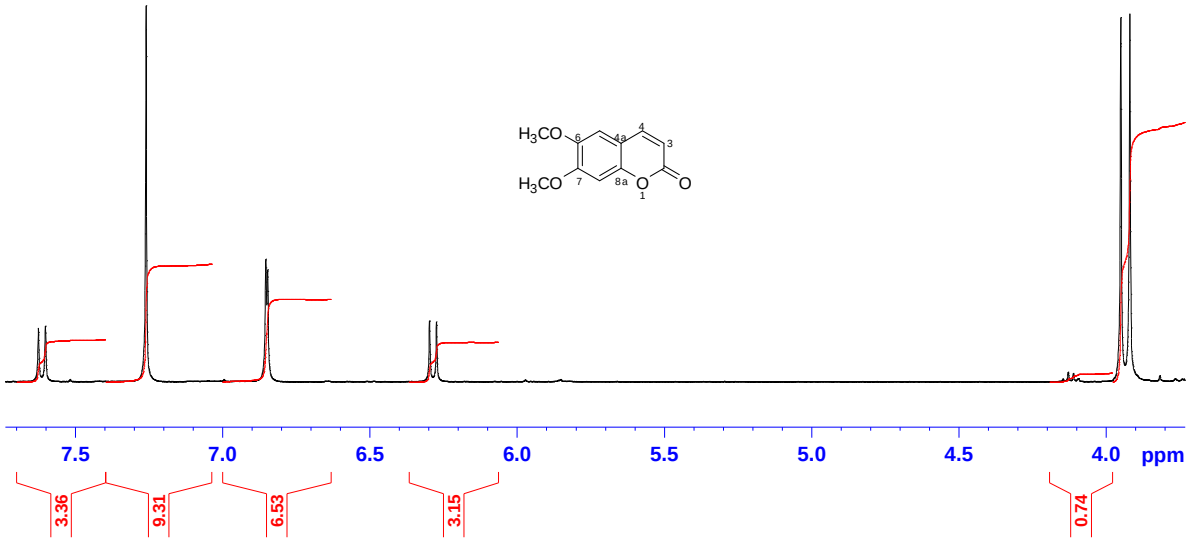


**Plate 93:** $^{13}$C NMR spectrum of scoparone (6,7-dimethoxycoumarin) **(3.15)**

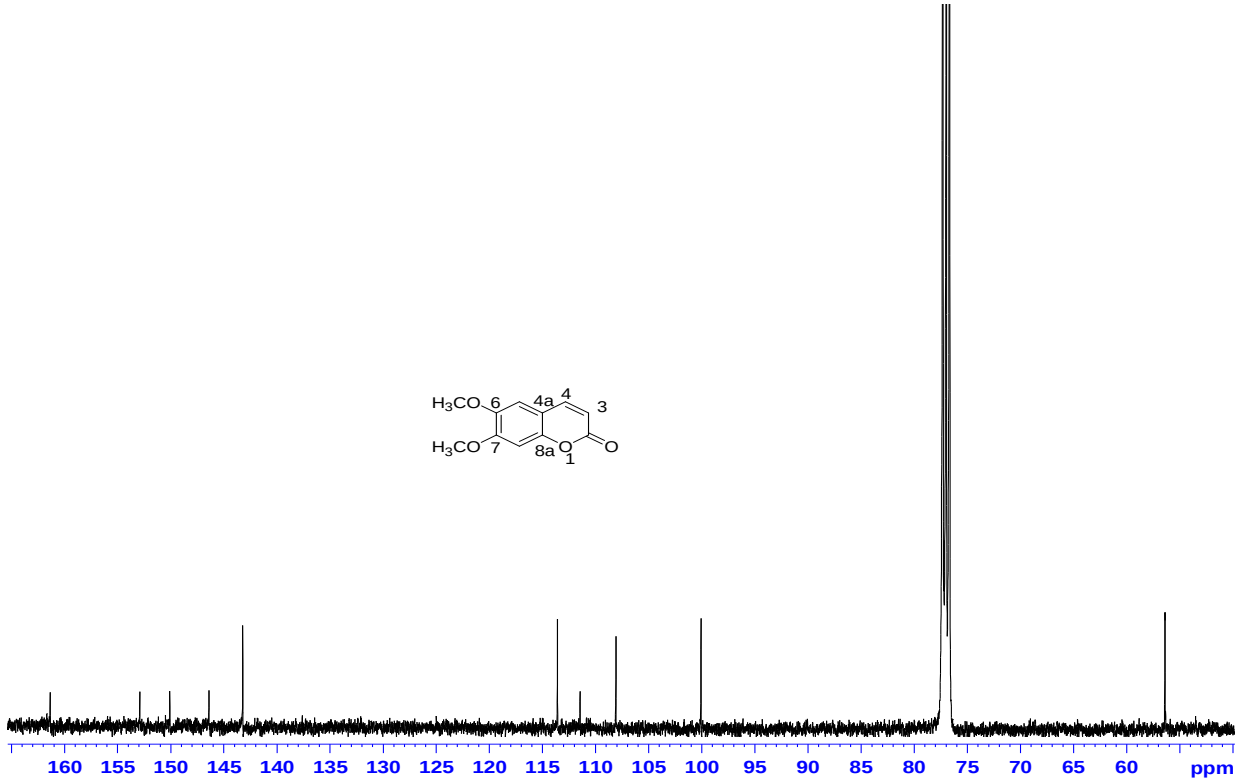


**Plate 94:** DEPT135 spectrum of scoparone (6,7-dimethoxycoumarin) **(3.15)**

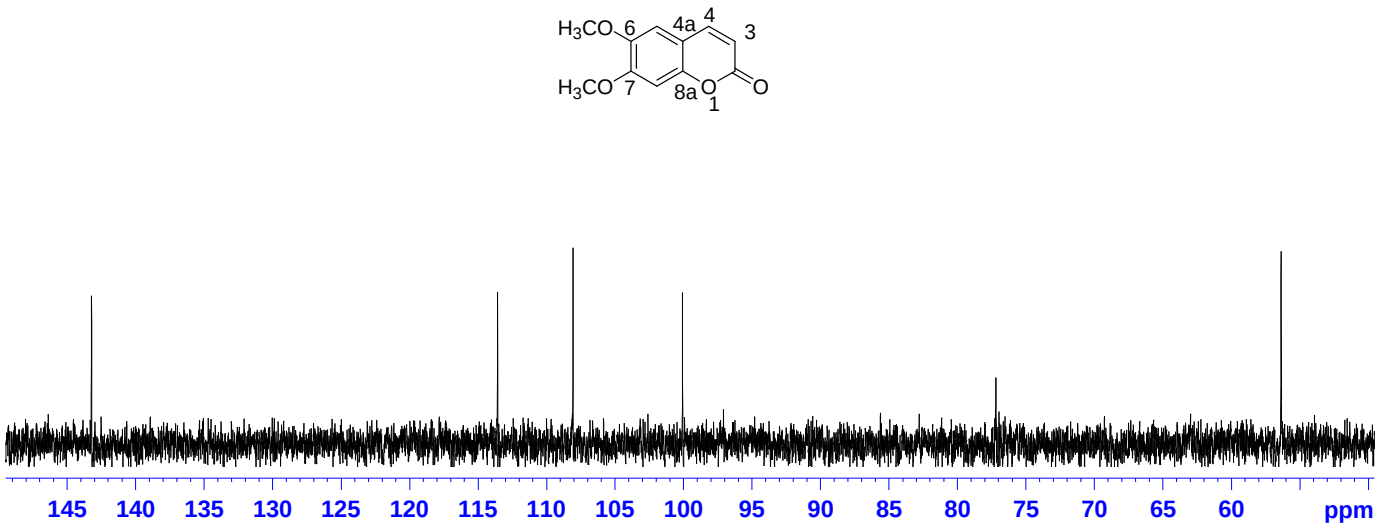


**Plate 95:** DEPT90 spectrum of scoparone (6,7-dimethoxycoumarin) **(3.15)**

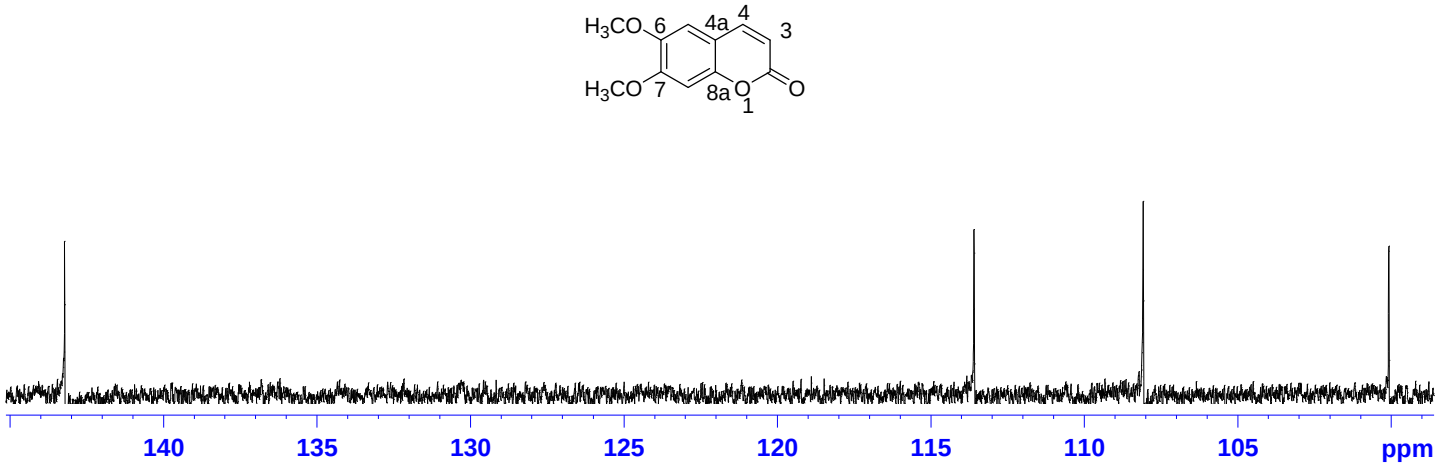


**Plate 96:** COSY spectrum of scoparone (6,7-dimethoxycoumarin) **(3.15)**

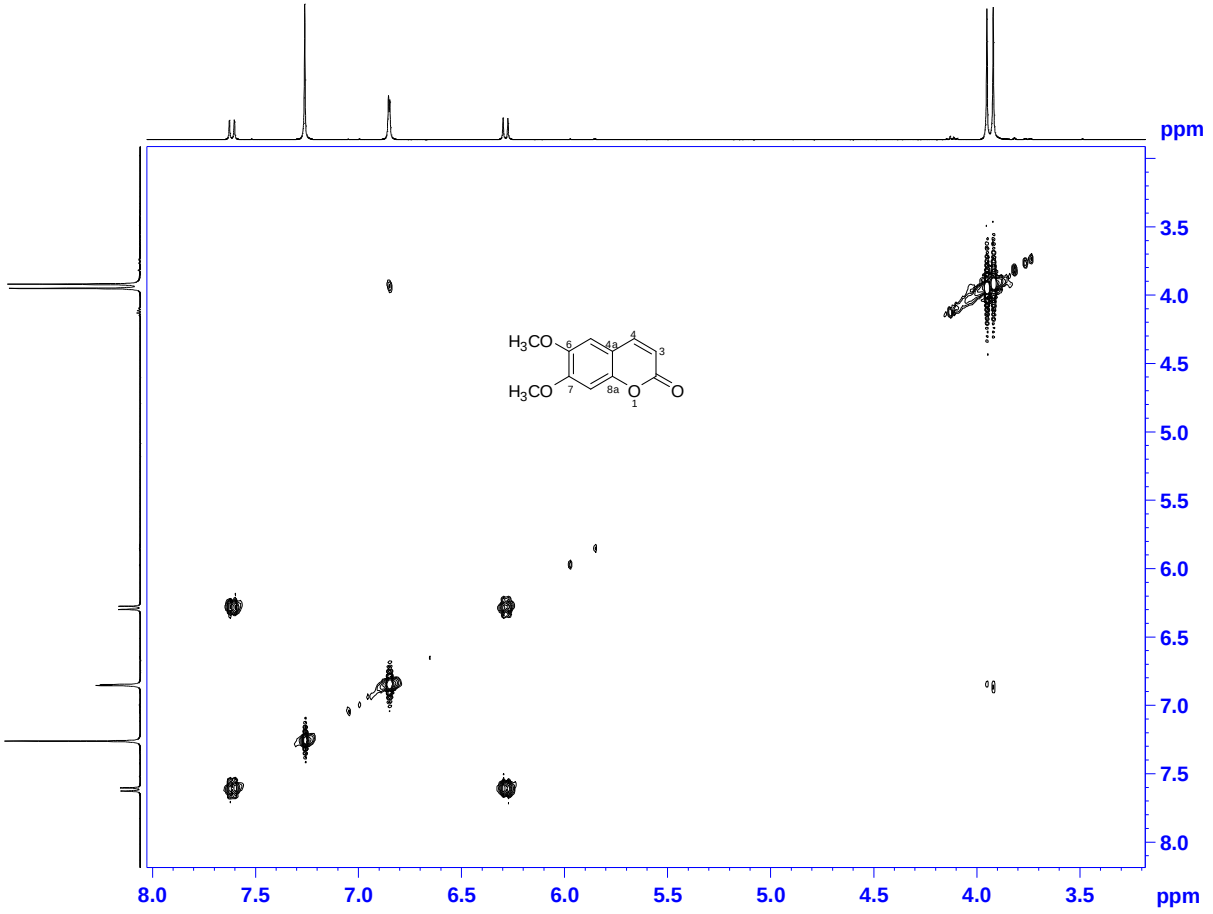


**Plate 97:** HSQC spectrum of scoparone (6,7-dimethoxycoumarin) **(3.15)**

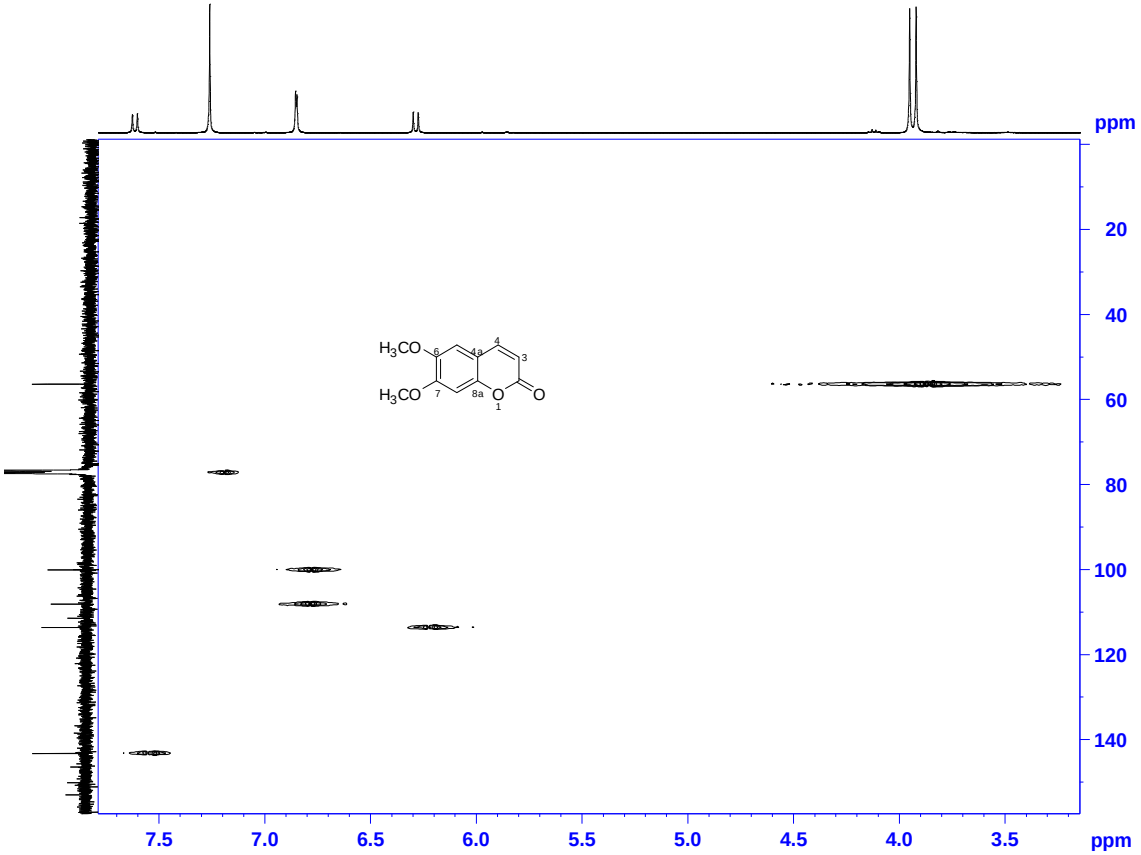


**Plate 98:** HMBC spectrum of scoparone (6,7-dimethoxycoumarin) **(3.15)**

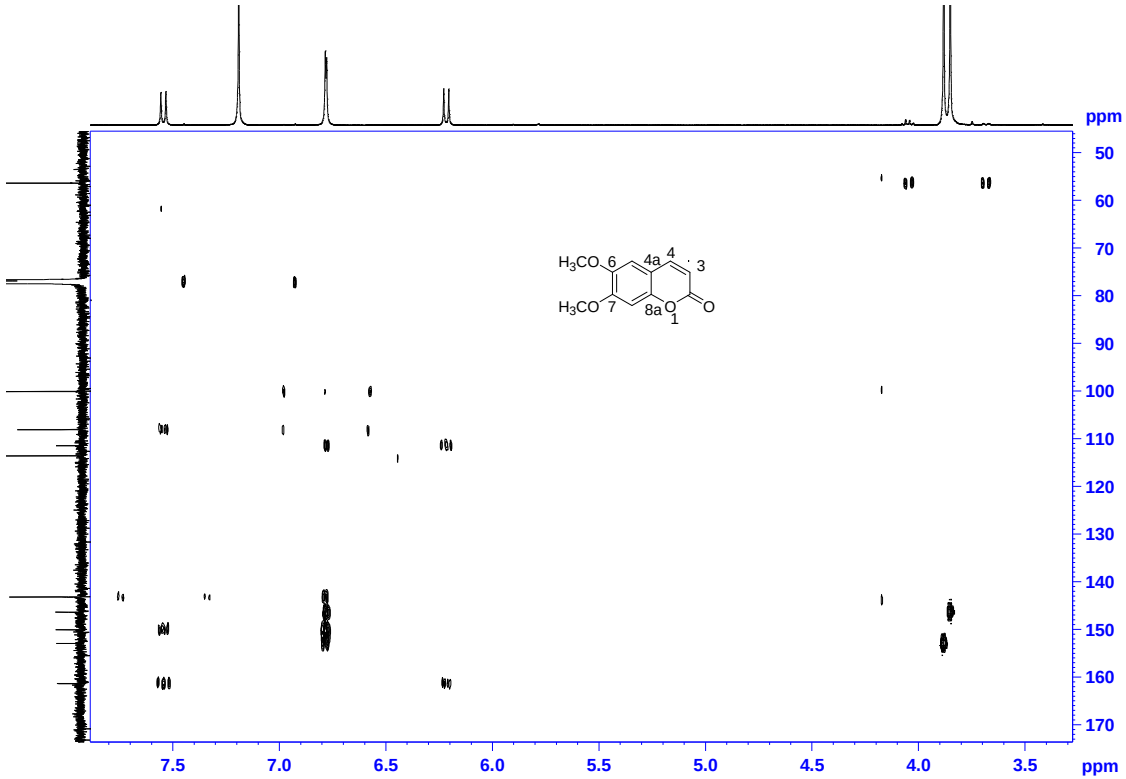


**Plate 99:** $^{1}$H NMR spectrum of viridiflorol **(3.16)**

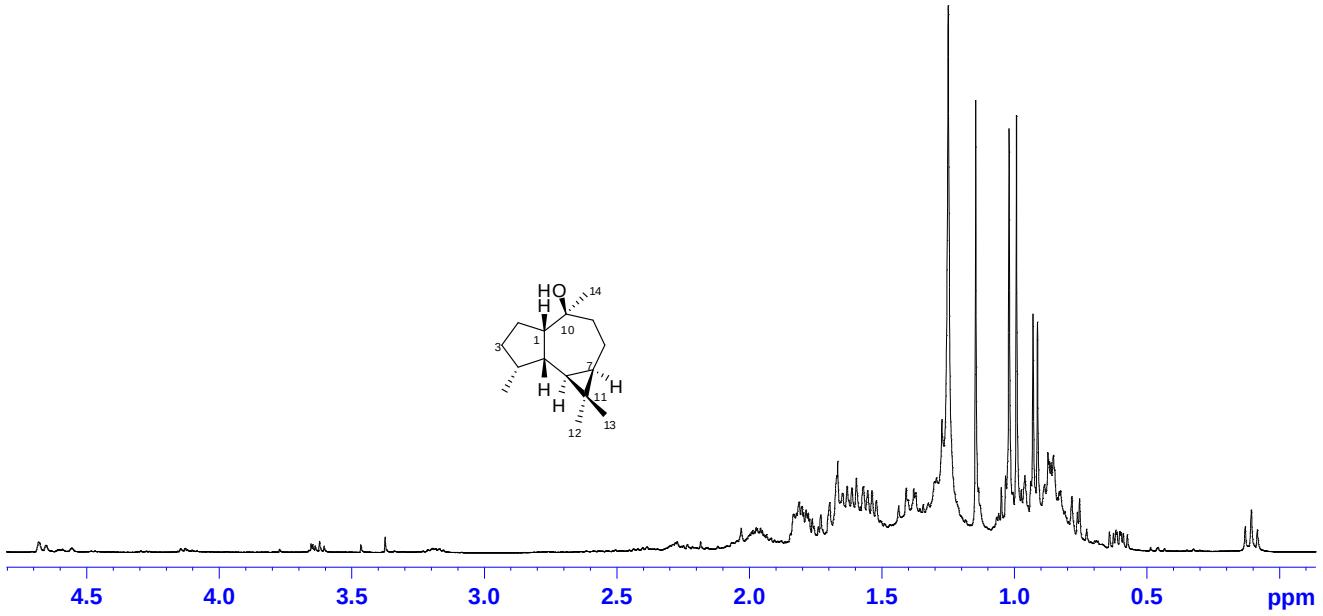


**Plate 100:** $^{13}$C NMR spectrum of viridiflorol **(3.16)**

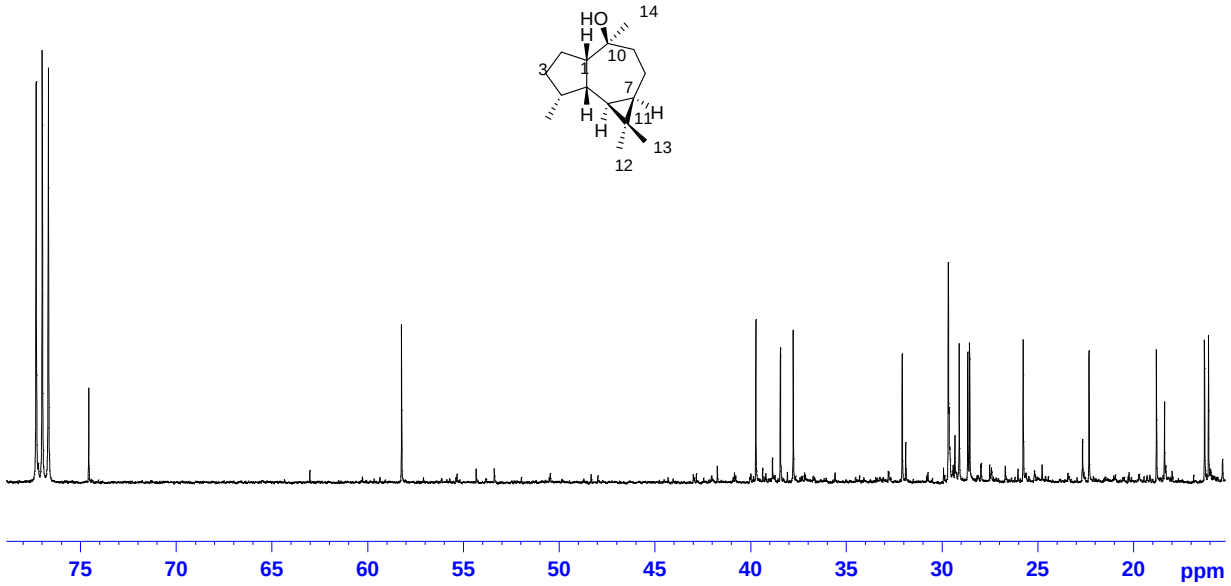


**Plate 101:** DEPT135 spectrum of viridiflorol **(3.16)**

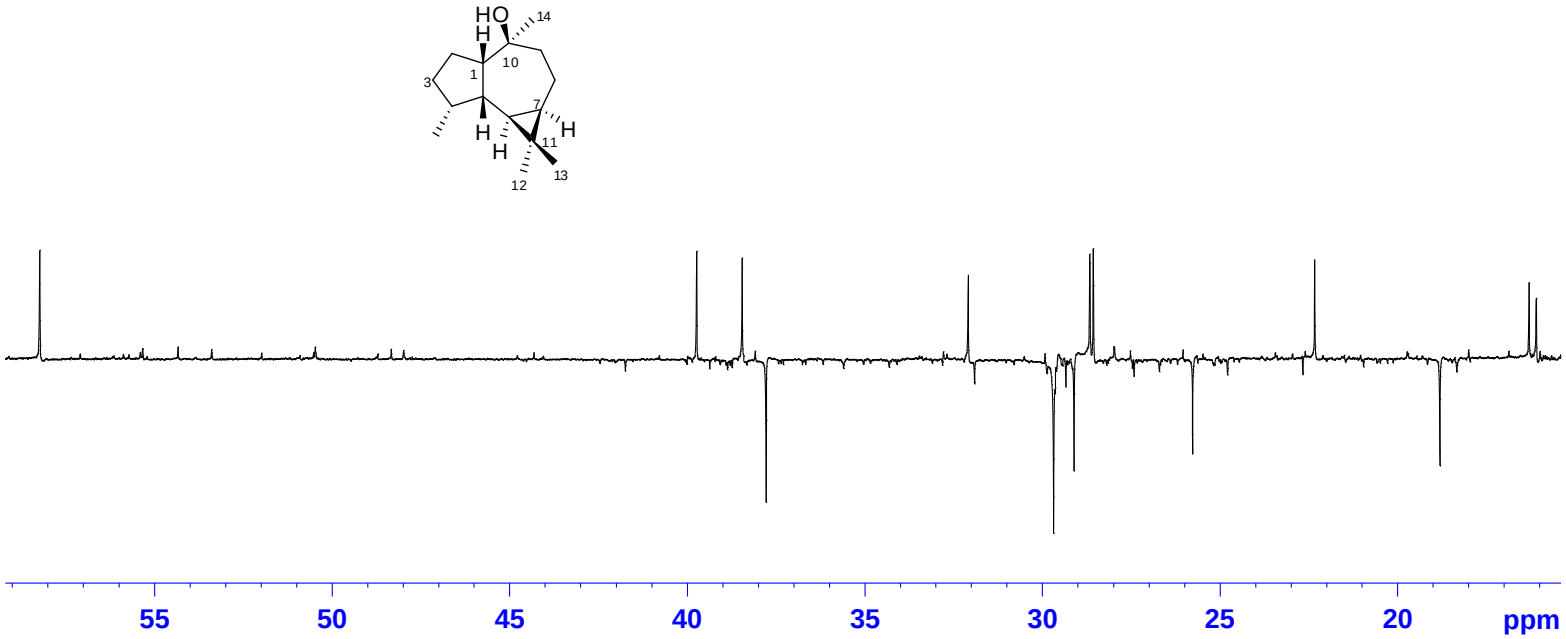


**Plate 102:** DEPT90 spectrum of viridiflorol **(3.16)**

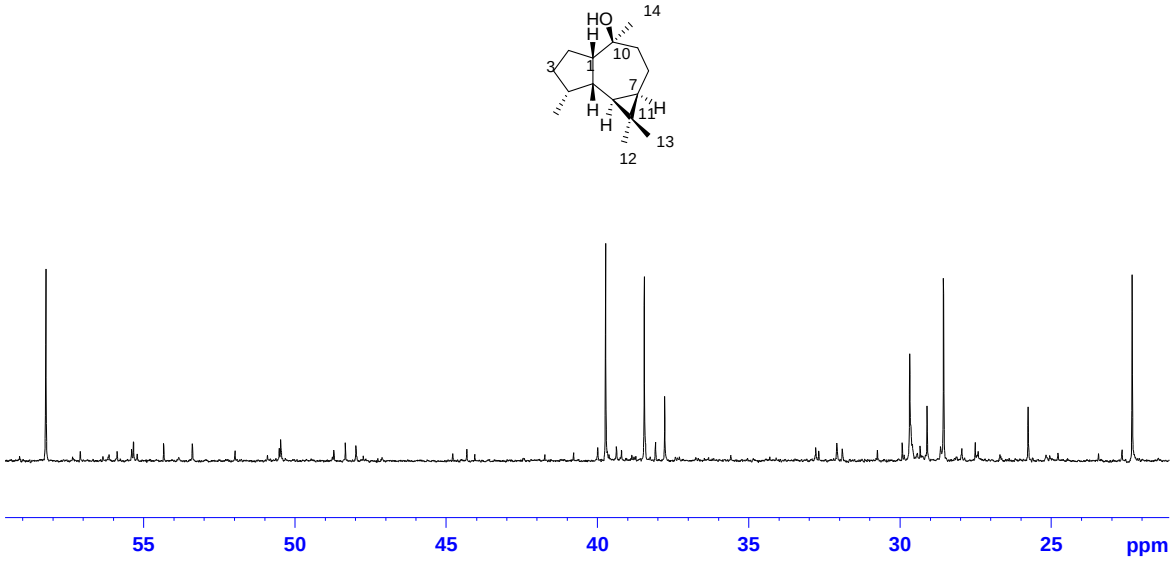


**Plate 103:** COSY spectrum of viridiflorol **(3.16)**

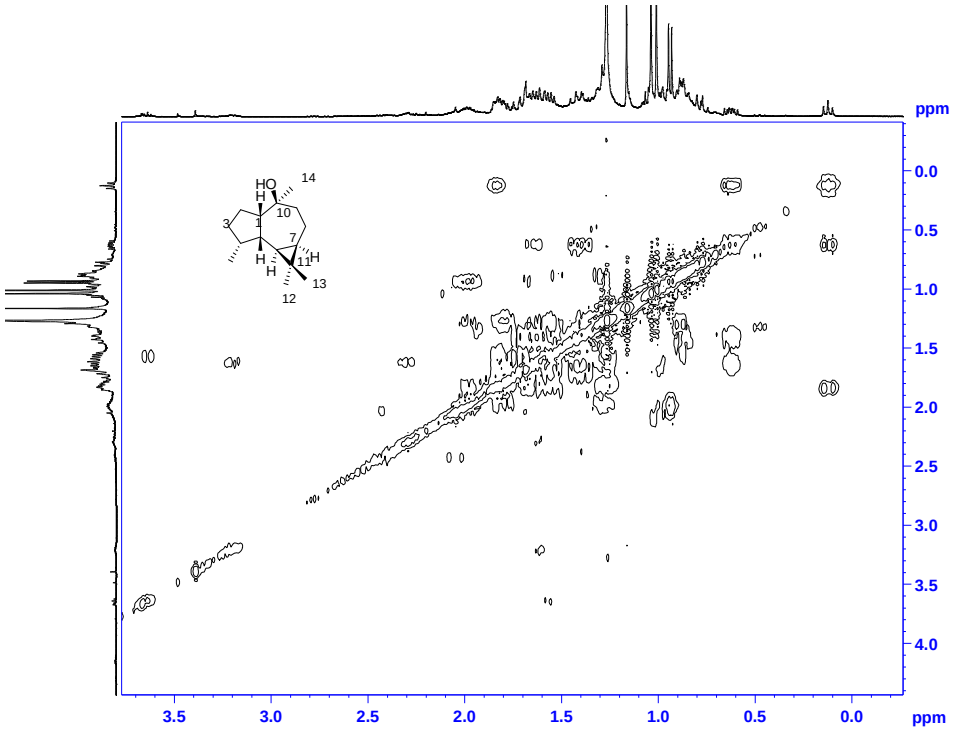


**Plate104:** HSQC spectrum of viridiflorol **(3.16)**

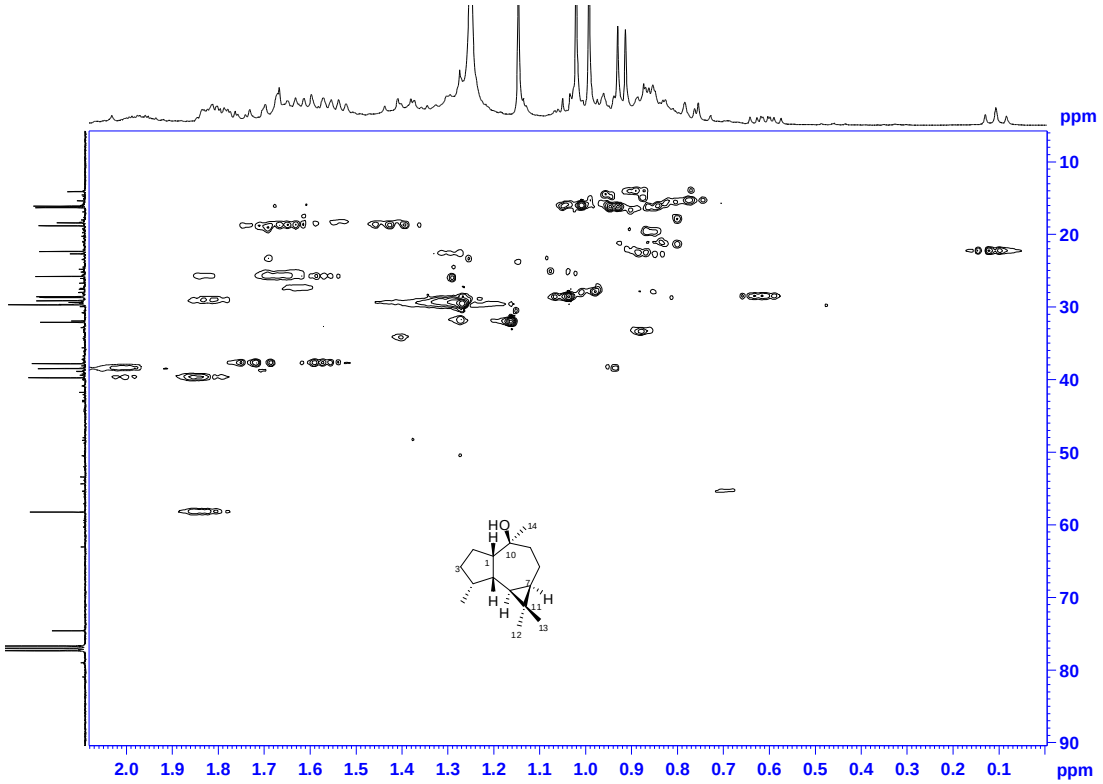


**Plate 105:** HMBC spectrum of viridiflorol **(3.16)**