THE STRUCTURE AND SYNTHESIS OF METABOLITES FROM VIRGILIA OROBOIDES AND CHLOROPHORA EXCELSA (IROKO)

by

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ABSTRACT

In the present study the acetone extract of the heartwood of two trees, Virgilia oroboides and Chlorophora excelsa, were investigated.

The heartwood of Virgilia oroboides afforded a variety of known flavonoids, as well as a new pterocarpene and a new α-hydroxydihydrochalcone, viz.;
3-hydroxy-8,9-methylenedioxy-6a,11a-dihydropterocarpan (αS), 2',4'-trihydroxy-4-methoxydihydrochalcone.

A series comprising substituted hydroxygeranylstilbenes, substituted benzenoid compounds and quercitin-type flavones were isolated from the acetone extract of the heartwood of Chlorophora excelsa. The new compounds isolated from this tree are:
3,5-dihydroxy-4-geranylbenzaldehyde
3',4,5'-trihydroxy-4'-geranylstilbene
2'-methoxy-3,4',7-tri-O-methylquercitin.

A combination of solvent extraction, Craig countercurrent, column (LH 20 and silica gel) and thin layer chromatography procedures were used to isolate and purify the compounds mentioned.

Structures were elucidated by high resolution (300 MHz) ^{1}HNMR spectroscopy (including NOE and spin-spin decoupling experiments) and mass spectrometry.

The proposed structural assignments of the following compounds were confirmed by synthesis:

3,5-dihydroxy-4-geranylbenzaldehyde

3',4,5'-trihydroxy-4'-geranylstilbene

2,3',4,5'-tetrahydroxy-4'-geranylstilbene (chlorophorin)

The modified Wittig reation was used to synthesize 3',4,5'-trihydroxystilbene.

U.V. irradiation experiments were performed on chlorophorin in an attempt to synthesize the cis-isomer and a phenanthrene-type compound.

Biosynthetic pathways showing the structural relationships of the identified compounds in Virgilia oroboides and Chlorophora excelsa were proposed.

An attempt to synthesize (+)-catechin lignoid involved the coupling of (+)-catechin to sinapyl alcohol, with the latter synthesized from 2,6-dimethoxyphenol via a vinyl quinone methide. Further investigations on lignoid synthesis are currently in progress.

Publications arising from this investigation:

A. Metabolites from Chlorophora excelsa : Possible intermediates in the biogenesis of a pentasubstituted stilbene.
Phytochemistry, 27, 2309 (1988).

B. Quercitin derivative from Chlorophora excelsa.
Phytochemistry. In press.

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CHAPTER 1

THE FLAVONOIDS

1.1 INTRODUCTION

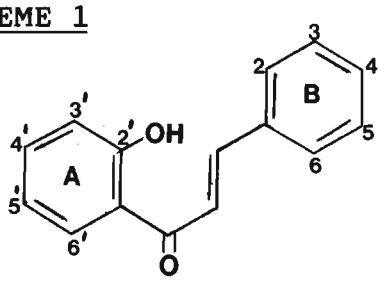
The flavonoids are one of the most widespread and numerous natural products found in plants, having many diverse properties and applications. The attractive colours [1] of flowers, leaves, fruit, fruit-juices and wines are mainly due to the water-soluble anthocyanins and tannins. The flavonoid family has widespread use; Formononetin displays oestrogenic activity[2], Daidzein is an antispasmodic agent[3], condensed tannins are used in the preservation of leather and in wood adhesives[4].

The major flavonoids occurring in plants are the anthocyanins, the flavones and the flavonols, together with related derivatives called the minor flavonoids. These minor (so called because of restricted distribution and contribution to plant colour) flavonoids include the chalcones [1], flavanones [2], flavan —3,4-diols [3], isoflavonoids and biflavonyls.

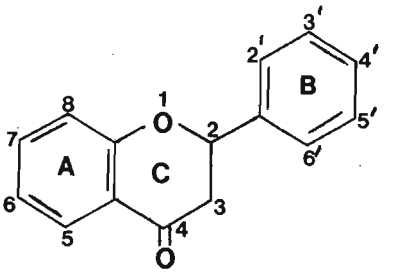
The substituted chromone skeleton, (Scheme 1), forms the nucleus of the wide variety of flavonoid compounds. The main criterion which distinguishes one flavonoid structural

type from another is the oxidation level of the heterocylic ring (C-ring). The flavones [4] and flavonols [5] represent the one extreme of the oxidation state, whereas the catechins [6] are on the other end of the scale. The flavanones and anthocyanins represent intermediate oxidation states.

SCHEME 1



[1]



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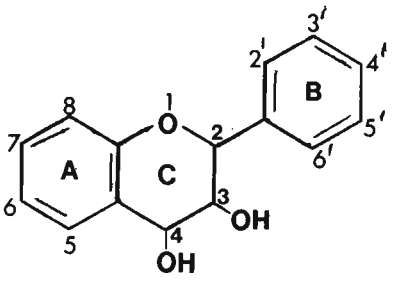
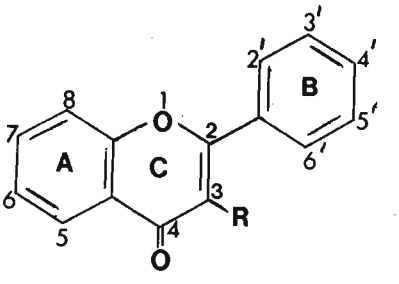
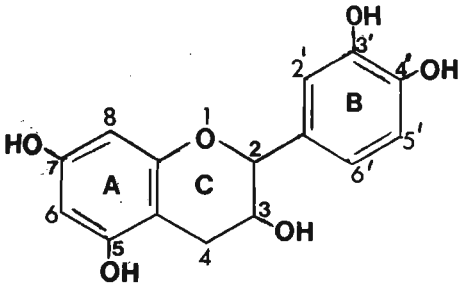


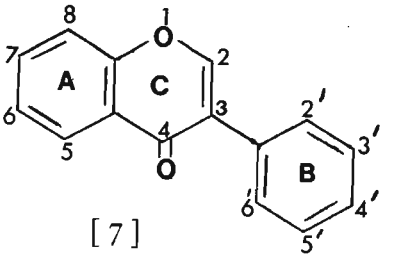
[3]



[4] R = H
[5] R = OH



[6]



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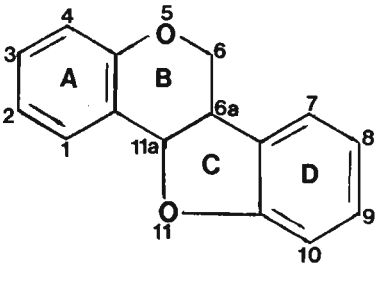


[8]

1.2 FLAVONES

von Kostanecki and Tambor[5] suggested the name flavone [4], to represent the simplest member of the flavones.

Chevreul[6] isolated the first flavone luteolin (3',4',5,7-tetrahydroxyflavone). Hlasimetz and Pfaunder[7] assigned the correct empirical formula, Perkin[8] determined the structure, and von Kostanecki[9] confirmed its structure by synthesis.

Frequent substitution at positions 5 and 7 of most flavones indicates that the A-ring is derived from phloroglucinol[10]. Positions 6 and 8 may also be substituted and are in many cases methoxylated. As expected from the acetate - shikimate pathway[11], the B-ring is mostly 4'-; 3',4'- or 3',4',5'- oxygenated with 2' and 6' substitutions very rare[12,13].

Flavones with mono- to octa- hydroxylated and methoxylated substitution patterns and some with unusual formyl[14], methylenedioxy[15], isoprene and pyrano[16] substituents have been isolated and identified as shown in the comprehensive summary given by Wollenweber[17a].

1.3 ISOFLAVONOIDS

Due to the variation in oxidation levels and presence of additional heterocylic rings, the isoflavonoid group may be subdivided into the isoflavones [7], pterocarpans [8], isoflavans, isoflavanones and rotenoids. The isoflavonoids have a limited taxonomic distribution and are mainly confined to the sub-family Lotoideae of the Leguminoseae, but also occur to a lesser extent in the sub-families Caesalpinioideae and other families (Rosaceae, Moraceae, Amaranthaceae, Podocarpaceae, Chenopodiaceae, Cupressaceae, Iridaceae, Myristicaceae, Stomonaceae)[17b].

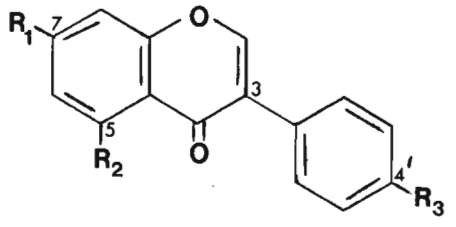
1.4 ISOFLAVONES

The isoflavones [7] are the most common of the natural isoflavonoids and occur in the free state or as glycosides.

Ononin (formononetin -7-glucoside) was the first isoflavone to be isolated[18], followed by Genistein [9][19,20]. The structure of the latter was synthetically confirmed by Baker and Robinson[21].

Many naturally occurring isoflavones with different oxygenation and substitution patterns have since been isolated and identified[17c]. The simple isoflavones are hydroxylated and/or methoxylated compounds containing two

or more substituents. Positions 5 and 7 are also most frequently substituted with positions 6 and 8 rarely substituted, but showing a greater frequency of methoxylation. In ring B the positions 4' and 2',3' are most frequently substituted. Substitutions at positions 5' and 6' are rare. Genistein [9][20], Formononetin [10][22], Daidzein [11][3] and Biochanin A [12][23] are the most common of the simple isoflavones.



[9] $R_1 = R_2 = R_3 = OH$

[10] $R_2 = H$; $R_1 = OH$; $R_3 = OMe$

[11] $R_2 = H$; $R_1 = R_3 = OH$

[12] $R_1 = R_2 = OH$; $R_3 = OMe$

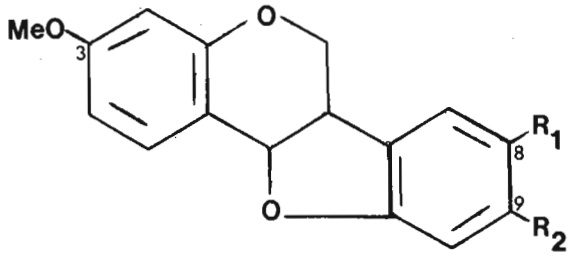
Isoflavones lacking B-ring substitution[24] are rare. This is thought-provoking because the accepted current theories (See section 1.8.3.) for aryl migration during the biosynthesis of isoflavones require the presence of a 4' -(or 2') oxygen function.

Corylinal (3'-formyldaidzein) is a novel 3'-aldehydic isoflavone which was found co-occurring with neobaisoflavone and could possibly be a result of dimethylallyl oxidation[25]. Methylenedioxy substituents were found on positions 3',4'[26], 4',5'[27], 6,7[28] of the isoflavonoid skeleton.

Isoprenoid substituents may sometimes be cyclized with a hydroxyl group to give a variation in pattern[29]. Bezuidenhout and co-workers[30] isolated an isoflavone - isoflavan dimer from Dalbergia nitidula. Maakiaasin[31] is an isoflavonostilbene extracted from the heartwood of Maakia amurenis. The 4'-OMe isoflavone is attached at positions 7,8 via two ether links to the α and β carbons of the 3,3', 4',5 -tetrahydroxystilbene.

1.5 PTEROCARPANS

In 1874 Cazeneuve[32] isolated and separated two colourless optically active constituents, pterocarpin [13] and homopterocarpin [14] from red sandalwood. Although other researchers [33,34] had tried, the structure was not satisfactorily elucidated until Robertson and co-workers[35] concluded that homopterocarpin had two methoxyl groups and no hydroxyl or carbonyl groups, whereas pterocarpin contained a methylenedioxy group.



[13] $R_1 - R_2 = O - CH_2 - O$

[14] $R_1 = H$; $R_2 = OMe$

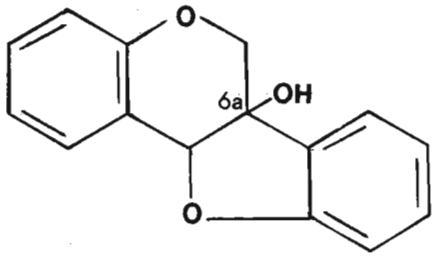
Subsequently the name pterocarpan [8] has been given to the coumaranochroman ring system (systematic name of 6a, 11a - dihydro -6-H-benzofuro[3,2-c][1]benzopyran).

(-)-Pterocarpin and (-)-homopterocarpin were for many years the only known pterocarpans, but recently more pterocarpans have been isolated from the heartwood and bark of many leguminous plants. Many of the pterocarpans act as phytoalexins[36] and are present in young tissue which is susceptible to attack by microorganisms.

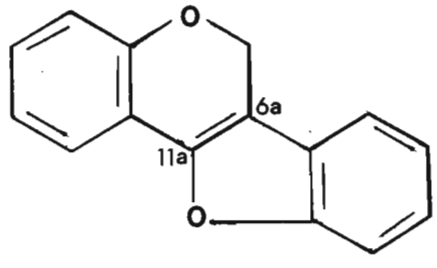
It is apparent from Dewick's[17d] review that there is a wide variety of substitution patterns but the 3,9-[37] and 3,8,9-[38] oxygenation patterns predominate, methylenedioxy

substitutents are commonly found on positions 8,9[38] and 1-oxygenated compounds are rare[39]. Geranyl substituents were also found on positions 4 and 10[40]. In nitiducarpin[40] the geranyl group on positon 4 was cyclized with the 3-OH substituent. Dimethylallyl units also occurred on positions 2, 4 and 10 but very rarely on 6a as in lespein[41].

Variations to the basic pterocapan structure [8] include the 6a-hydroxypterocarpans [15][42] and pterocarpenes (6a, 11a-dehydropterocarpans) [16][43]



[15]



[16]

The first 6a-hydroxypterocarpan[42] discovered from the Pisum spp, Pisatin, acted as a phytoalexin on fungal infection. Ferreira and co-workers[43] have isolated five pterocarpenes from the heartwood of Brya ebenus.

1.6 THE CHALCONES

The Chalcones [1 ; Scheme 1] are brightly yellow coloured compounds appearing most conspicuously in flowers[44], thereby contributing significantly to the corolla pigmentation.

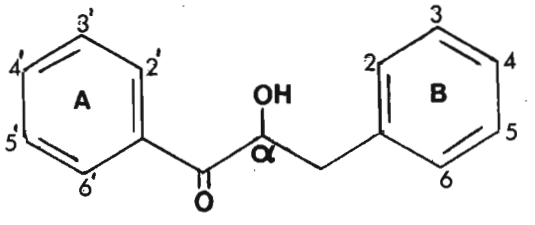
The frequent co-occurrence of chalcones, flavanones, flavones and flavonols and the results obtained from tracer studies has led to the current acceptance that chalcones are the central intermediates in flavonoid biosynthesis (see section 1.8.5).

The structure of the first chalcone isolated, Carthamin, was described by Seshadri[45] as being 2'-glycosidoxy-3',4,4',6'-tetrahydroxychalcone. A variety of chalcones has since been isolated, a comprehensive summary of which is given by Bohm[17e].

O- or C-prenylation of the resorcinol (2',4')[46] or phlorogluicinol (2',4',6')[47] type A-ring, and rare furano[48], geranyl[49] and aldehydic[50] substitutents are responsible for some of the variety. Chalcones with tetra-oxygenated A-rings[51] occur more frequently than the penta-oxygenated[52] compounds.

1.6.1 The α-Substituted Chalcones

The α-hydroxydihydrochalcones [17] represent a rare group of natural products whose importance is recognized in their close biogenetic relationship to the isoflavonoids (Sec. 1.8.5)



[17]

Nubigenol (α,2′,4′,6′,4 -pentahydroxydihydrochalone)[53] was the first of these compounds to be isolated and identified. A summary of other α-hydroxydihydrochalcones which have since been identified appears in Table 1.

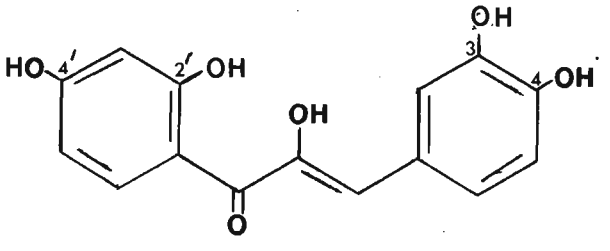
TABLE 1 SUMMARY OF KNOWN α-HYDROXYDIHYDROCHALCONES

A-RING	B-RING	SOURCE
1) 2',4',6'-OH	4-OH	Podocarpus nubigena[53]
2) 2',4'-OMe	4-OH	Lyona formosa[54]
3) 2'-OH,4'-OMe	4-OMe	Zollernia paraensis[55]
4) 3'-C-β-gluco-pyranosyl, 2'-,4'-OH	4-OH	Eysenhardtia polystachia[56]
5) 3'-C-β-gluco-pyranosyl-2',4'-OH	3,-4-OH	Eysenhardtia polystachia[56]
6) 2'-OH,4'-OMe	4-OMe	Pterocarpus angolensis[57]
7) 2',-4'-OH	4-OMe	Pericopsis elata[58]

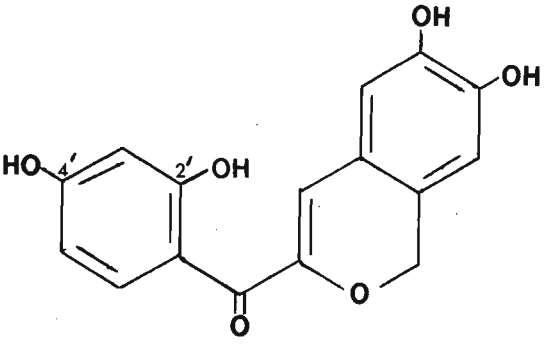
Structure elucidation of these compounds was accomplished by spectroscopic (See section 1.7.1) and synthetic means[57,58] (See section 1.7.2). The (αR) absolute configuration was determined[57] by comparison of the CD spectra of the reduced α-hydroxydihydrochalcone against that of 3-(3,4-dimethoxyphenol)-1-(2-hydroxy-4,6-dimethoxy-phenyl) propan-2-ol. More recently, Bezuidenhout

and co-workers[58], in their efforts towards enantioselective synthesis and definition of the absolute stereochemistry at C-α, have proposed an (αR) configuration for 4-methoxy-α,2',4'-trihydroxydihydrochalcone isolated from Pericopsis elata.

A further variation to the chalcone skeleton was evident in the occurrence of α, 2',4',3,4-penta-hydroxy-chalcone [18] in Peltogyne spp, [59] thereby suggesting a possible biosynthetic relationship with the peltogynoid flavonoids represented below by the "chalcone" [19].



[18]



[19]

1.7 STRUCTURE ELUCIDATION OF FLAVONOIDS

1.7.1 Spectroscopic Methods

1.7.1.1 Proton Magnetic Resonance (^{1}HNMR) Spectroscopy.

1.7.1.1.1 The Aromatic Protons

The coupling patterns for the flavonoid aromatic protons (Scheme 1) are typical for the benzenoid system with combinations of ABX, AB, AX and AA'BB'. The chemical shifts of these protons occur from 6,0 ppm to 8,0 ppm, with J-values of 8-,2- and 1,0 Hz for the ortho, meta and para coupled protons respectively.

A detailed discussion of chemical shift and coupling constant values is given by Mabry, Markham and Thomas[60]. In 5,7- dihydroxyflavonoids the protons at C-6 and C-8 appear separately as doublets (meta-coupled), with the H-6 doublet occurring at higher field than the H-8 doublet. When a 7-hydroxyflavonoid (ABX system) has a C-4 keto group, the C-5 proton is deshielded and appears as a doublet due to ortho coupling with H-6. In 4' oxygenated flavonoids, the H-3', H-5' doublet appears upfield from the H-2', H-6' doublet due to the deshielding effect of the oxygens of the C-ring functions on H-2' and H-6'. In

3',4',5' -oxygenated flavonoids H-2' and H-6' appear as a two proton singlet because of magnetic equivalence. O-Methylation of the 3' or 5' hydroxyl may lead to equivalence of H-2' and H-6'resulting in distinct doublets (J = 2 Hz).

1.7.1.1.2 Methoxyl and Acetoxyl Protons

Methoxyl proton signals appear in the range 3,5-4,1 ppm while aromatic acetoxyl proton signals occur in the range 2,25-2,50 ppm[60]. Protons ortho and para to acetoxyl groups are shifted downfield by about 0,3 pm and 0,5 ppm respectively while meta protons are shifted very slightly[61]. Comparison[62] of spectra of flavonoid derivatives, run first in $CDCl_3$ and then in benzene-d_6 showed that the signals of the methoxyls adjacent to protons are shifted upfield by approximately 0,3 ppm. Wilson and co-workers[63] observed that methoxyl groups at C-2', C-4', C-5 and C-7 in flavones show large upfield benzene induced shifts (0,5-0,8 ppm) in the absence of ortho-methoxyl or hydroxyl substituents. In contrast, a C-3 methoxyl group and a methoxyl group which has substituents on either side of it show little or no shifts. These workers also observed that a C-6 methoxyl adjacent to a C-5 methoxyl forces the C-5 group into the sphere of influence of the carbonyl function which causes a downfield solvent shift of the C-5 group. Due to hydrogen bonding

with the pyrone carbonyl, the C-5 hydroxy group signal appears between 12-15 ppm[64].

1.7.1.1.3 Heterocyclic Ring Protons

The chemical shifts (ppm) and coupling constants of the heterocyclic ring protons of the flavonoids (Scheme 1) may be summarized as follows:

a)	Flavones[65]	H_3 : 6.3 (s)
b)	Isoflavones[22]	H_2 : 7.6 - 7.9 (s)
c)	Flavanones[66]	H_2 : 5.0 - 5.5 (q, J=11$_{trans}$, 5$_{cis}$ Hz)
		H_3 : 2.8 (qq, J = 17 Hz)
d)	Dihydroflavonol[67]	H_2 : 4.8-5.0 (d, J = 11 Hz)
		H_3 : 4.1-4.3 (d, J = 11 Hz)
e)	Pterocarpans[68]	H_{11a} : 5.45 (d, J = 6.8 Hz)
		H_{6eq} : 4.23 (dd, J = 4.9 11.0 Hz)
		H_{6ax} : 3.64(dd, J = 10.5, 11.0 Hz)
		H_{6a} : 3.50 (ddd, J = 4.9, 6.8, 10.5 Hz)

1.7.1.1.4 α-and β-Hydrogens of Chalcones

The chemical shifts (ppm) and coupling constants of the above are summarized as follows.

a) Chalcones[69] Hα : 6.7-7.4(d, J = 17 Hz)
Hβ : 7.3-7.7(d, J = 17 Hz)

b) α-hydroxydihydrochalcone[58] Hα : 5.20 (ddd, J 4.0, 7.0, 7.0 Hz)

α-OH : 3.61(d,J 7.0 Hz)

β-CH_2 : 3.13(dd,J 4.0, 14.0 Hz)
and
2,90(dd,J 7.0, 14.0 Hz)

1.7.1.2. Mass Spectrometry

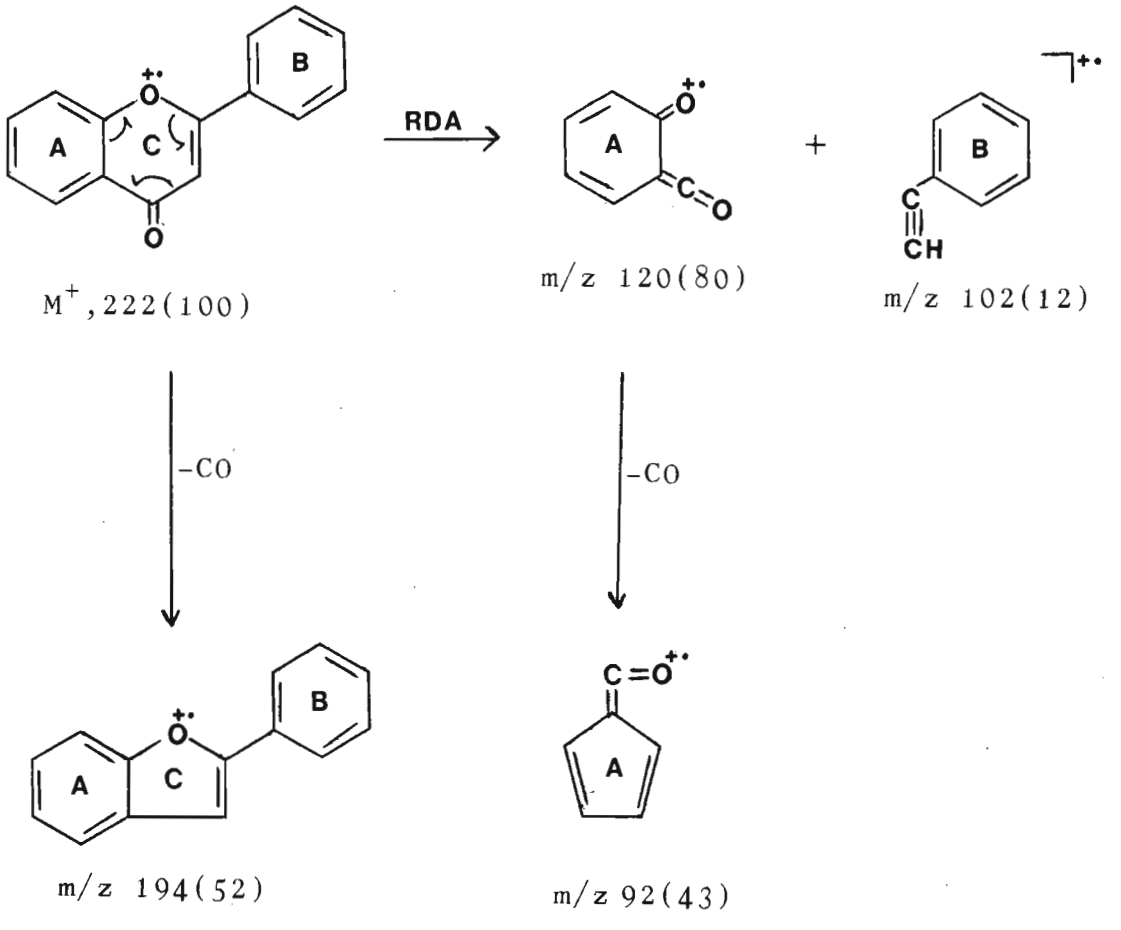
Mass spectrometry has been applied successfully in the structure determination[70] of all classes of flavonoids.

1.7.1.2.1 Flavones

Most flavones produce a molecular ion (M^+) with an intense peak. Peaks of moderate intensity due to

Retro-Diels-Alder (RDA) reaction[71,72] are obtained for flavones with less than four substituents, as shown in Scheme 2. Other molecular ion peaks obtained for simple flavones are the $[M-CO]^+$, $[M-1]^+$, and benzoyl cation.

SCHEME 2



M^+,222(100)

m/z 120(80)

m/z 102(12)

m/z 194(52)

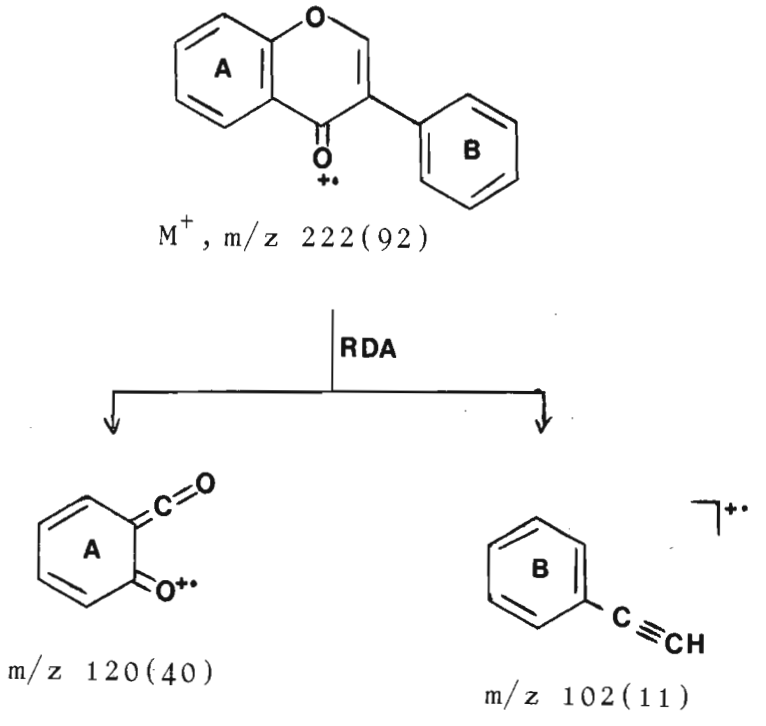
m/z 92(43)

Flavones with four or more hydroxyl or methoxyl groups give only weak RDA fragments which are of little diagnostic value[73].

1.7.1.2.2 Isoflavones

As with the flavones, diagnostic RDA fragments are obtained for isoflavones. Isoflavones are characterized by an intense $[M-1]^+$ ion. Other ions of moderate intensity are $[M-CH_3]^+$ and $M^{+\cdot}$[72]. Unlike the $[M-CO]^+$ ion of flavones, the $[M-CO]^+$ ion of isoflavones is very weak. A doubly charged [M-CO] ion is therefore not observed; a doubly charged parent ion is however prominent[74].

SCHEME 3



M^+, m/z 222(92)

m/z 120(40)

m/z 102(11)

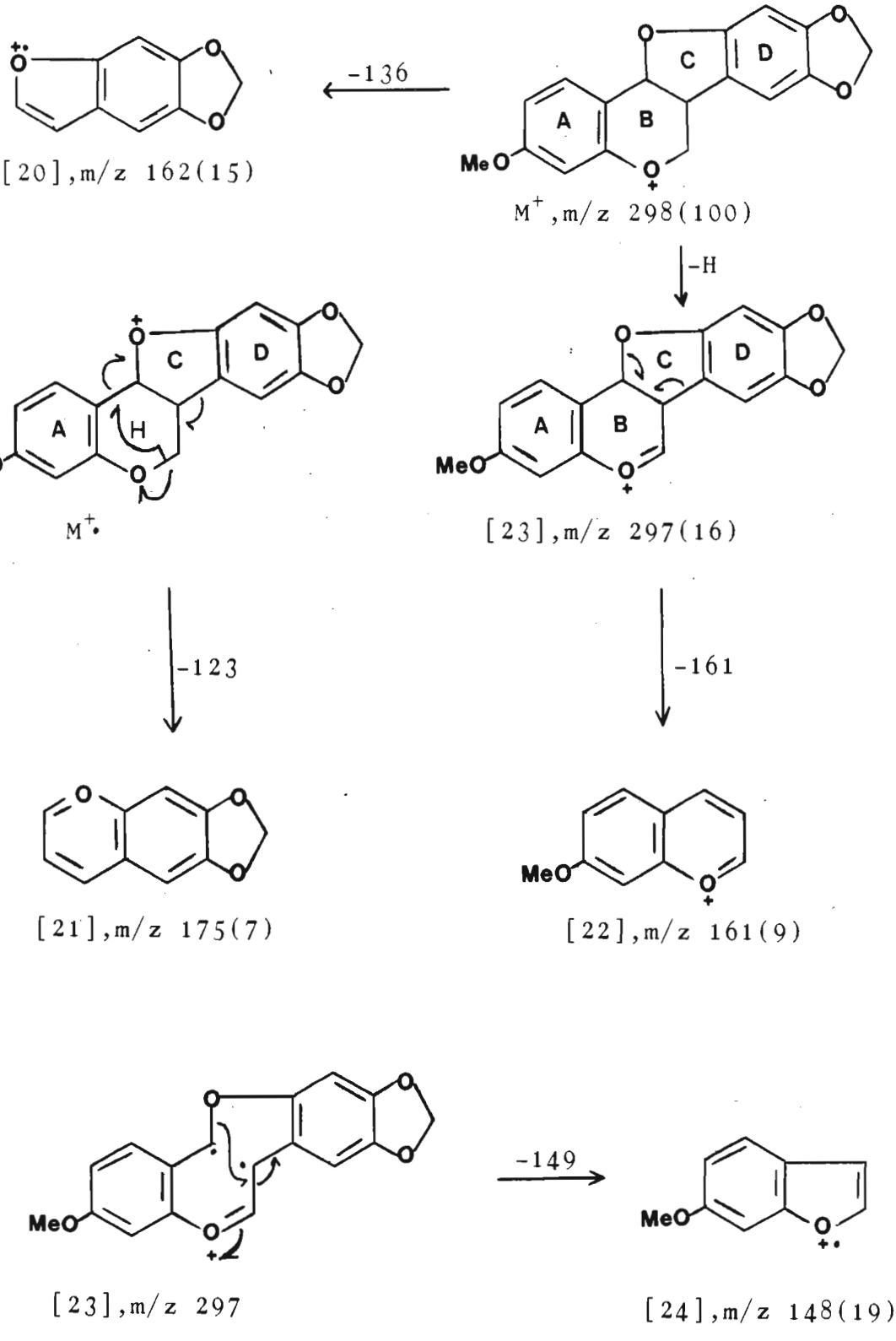
The more highly substituted isoflavones only give the molecular ion[75].

1.7.1.2.3 Pterocarpans

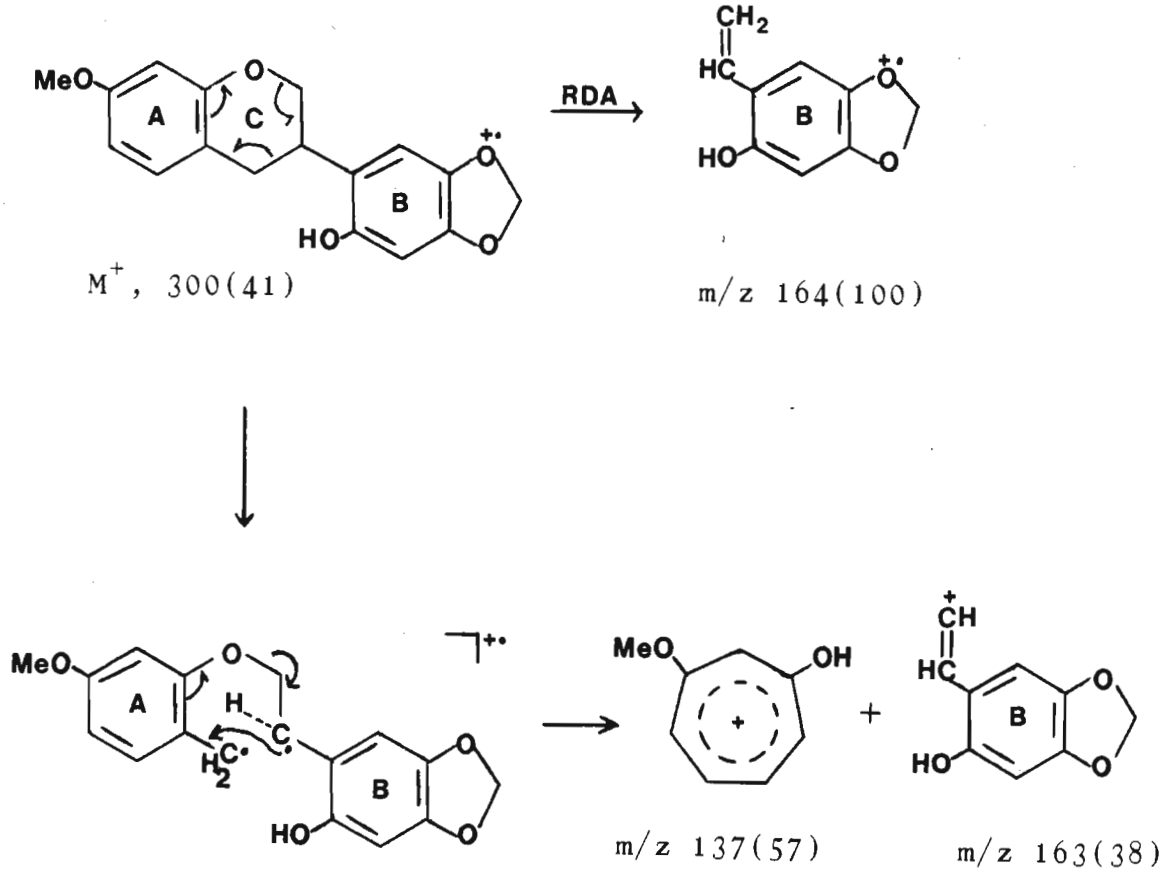
In pterocarpans, the RDA reaction does not lead to fragmentation, and this is shown by the intensity of the molecular ion and the small degree of fragmentation observed in the spectra of pterocarpin[76]. Benzofuran radical ions such as [20] and chromenyl ions such as [21] and [22], Scheme 4, are of general importance in the spectra of pterocarpans. Other ions of importance are the $[M-1]^+$ ion [23], the $[M-CH_3]^+$ (9%) ion, and an ion [24] at m/z 148 which probably arises from the $[M-1]^+$ ion.

In their work Pelter and Amanechi[77,78] stated that due to the lack of RDA fragmentation it was difficult to distinguish between the A-ring and B-ring. The argument was that every fragment could be suspected as arising from either ring. Mention was made that if the pterocarpan was converted to the parent isoflavan typical diagnostic RDA fragmentation pattens would be obtained. Audier[79] has published the mass spectrum of an isoflavan (Scheme 5).

SCHEME 4


-136
[20],m/z 162(15)
M+,m/z 298(100)
-H
M+•
[23],m/z 297(16)
-123
-161
[21],m/z 175(7)
[22],m/z 161(9)
-149
[23],m/z 297
[24],m/z 148(19)

SCHEME 5



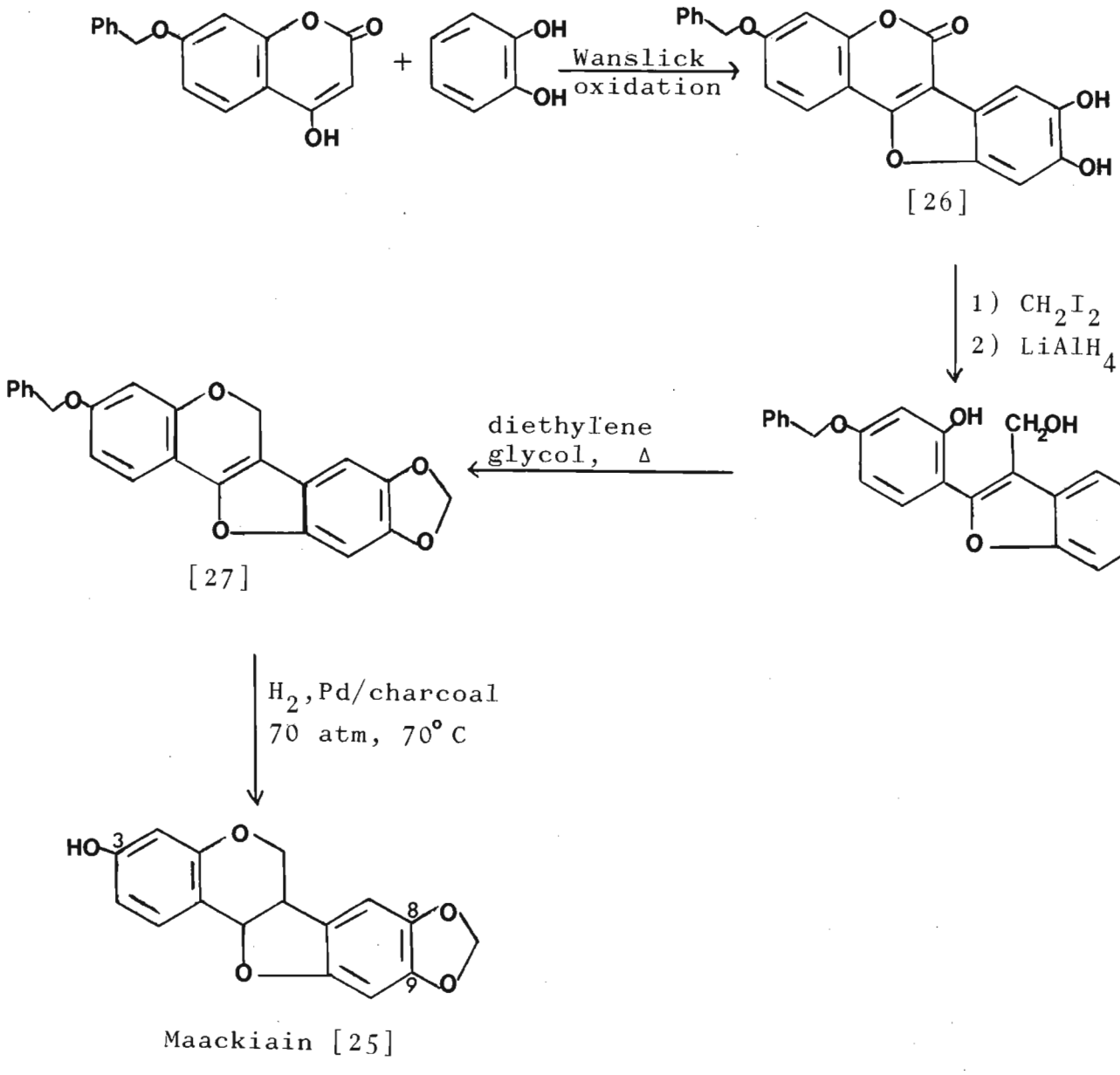
1.7.2 Synthetic Methods

1.7.2.1 Synthesis of Pterocarpans

Fukui and co-workers[80] have synthesized Maackiain (3-hydroxy-8,9-methylenedioxy pterocarpan; [25]), Scheme 6, via a coumestan derivative [26], the latter being

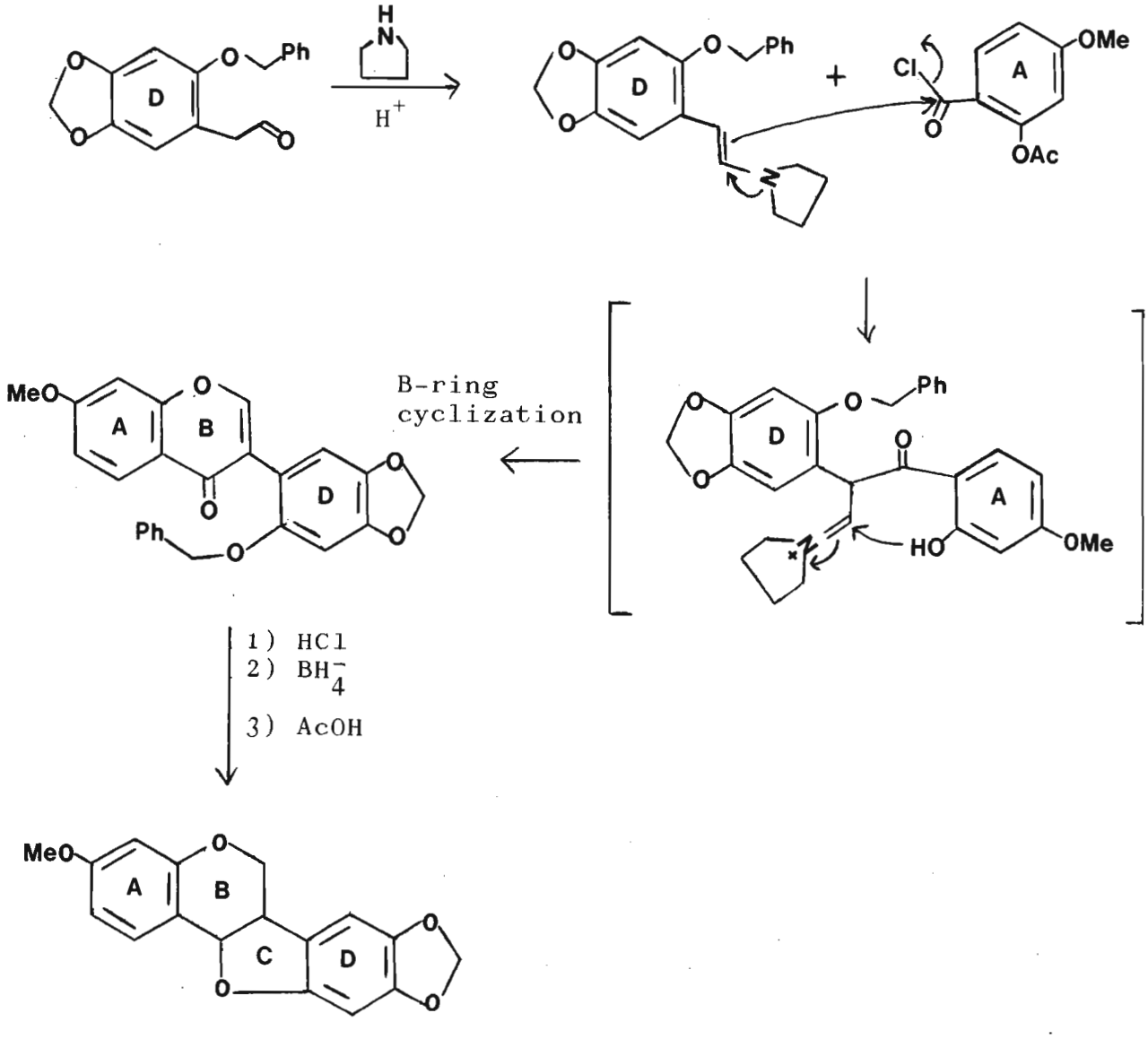
obtained utilizing a Wanslick oxidation[81]. This method produces very satisfactory yields of the dehydropterocarpan [27] which can in turn be hydrogenated to give a racemic mixture of the pterocarpan.

SCHEME 6



Pterocarpin(3-methoxy-8,9-methylenedioxypterocarpan;[13]) was synthesized (Scheme 7) via a method of obtaining isoflavones from enamine condensations[82]. Protective techniques are commonly used and combined with this synthetic method they form a flexible alternative in pterocarpan synthesis.

SCHEME 7

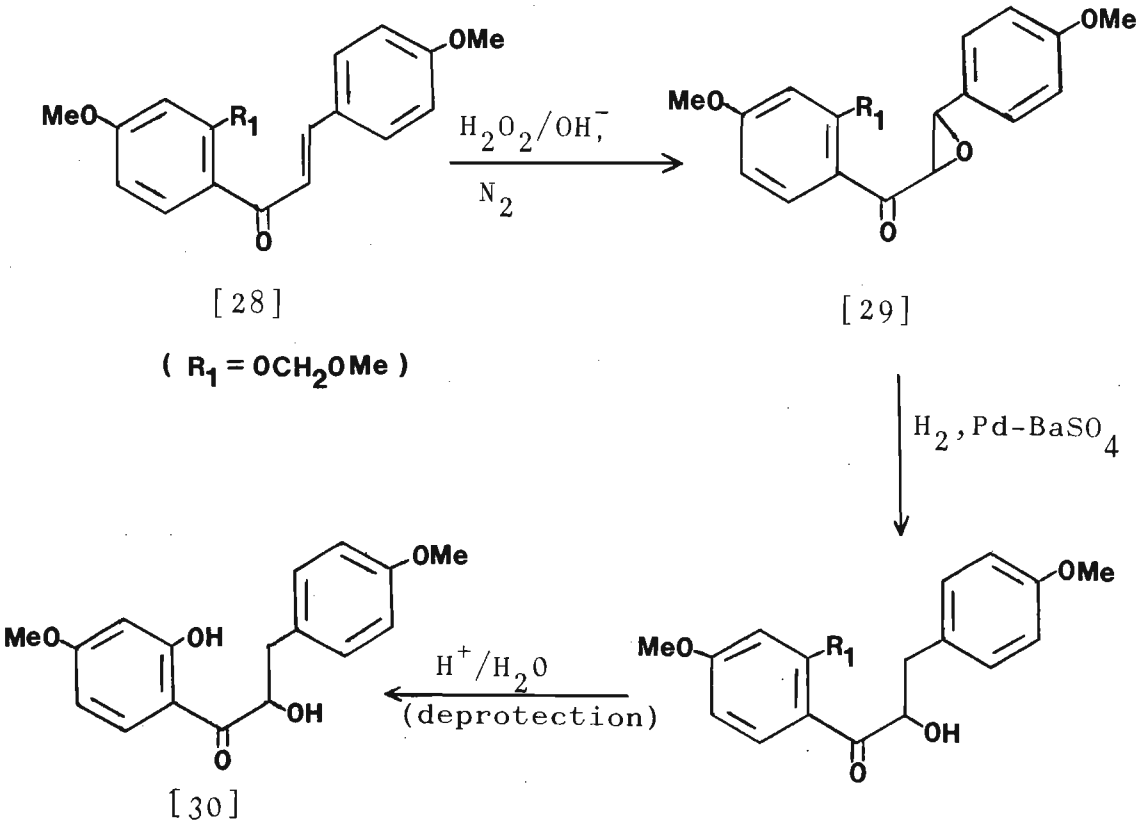


Pterocarpin [13]

1.7.2.2 Synthesis of α- Hydroxydihydrochalcones

The only existing method (Scheme 8) entails the epoxidation of an intermediate chalcone [28] followed by hydrogenation of the oxirane [29] to yield the racemic mixture of the α-hydroxydihydrochalcone [30][57].

SCHEME 8



More recently Bezuidenhout and co-workers[58] have epoxidized 4-methoxy-2',4'-dimethoxymethyl-(E)-chalcone with H_2O_2 in the triphase system aqueous NaOH, poly-L-alanine and CCl_4.

1.8 BIOSYNTHESIS OF FLAVONOIDS

1.8.1 General Phenylpropanoid Metabolism

Reviews[17f] covering feeding experiments with radioactively labelled precursors indicated that the carbon skeleton of the flavonoids was derived from acetate and phenylalanine. The A-ring was formed by a head-to-tail condensation of three acetate units and the B-ring as well as carbons 2,3 and 4 of the heterocylic C-ring were from phenylalanine, which was itself derived from the shikimic acid pathway[11].

The term "general phenylpropanoid metabolism"[83] describes the sequence of reactions in the conversion of phenylalanine [31] to CoA ester derivatives of substituted cinnamic acid [32], (Scheme 9). The known enzymes related to this pathway are phenylalanine ammonia-lyase[84], cinnamate-4-hydroxylase[85] and 4-coumarate - CoA ligase[86]

1.8.2 The Chalcone/Flavanone Intermediate

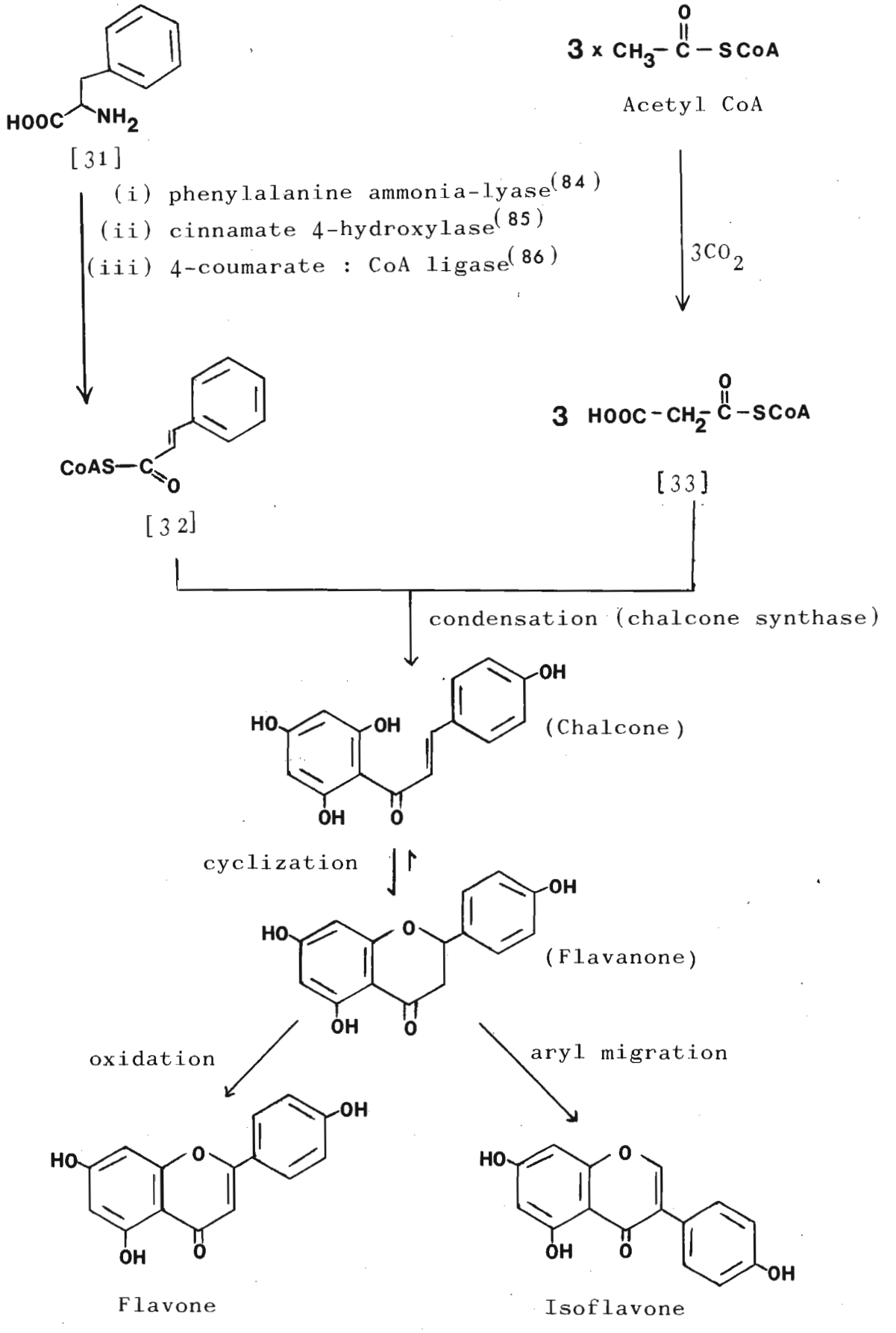
Grisebach[17f,87], in 1962, postulated that the first specific reaction in flavonoid biosynthesis was the enzyme-mediated condensation of the acyl residues from one molecule of 4-coumaroyl-CoA [32] and three molecules of

malonyl CoA [33].

The frequent co-occurrence of chalcones, flavanones, flavones and flavonols has led to the suggestion that the chalcone/flavanone isomers were the central intermediates in the synthesis of all flavonoids[17f]. The enzyme essential for the formation of the chalcone was chalcone synthase[88]. Chalcone isomerase[89] catalysed the conversion of chalcone to form the 6-membered heterocylic ring of flavanones, (Scheme 9).

In their review, Hahlbrock and Grisebach[17f], stated that chalcones with a phloroglucinol type A-ring were exclusively incorporated into 5,7-dihydroxyflavonoids, while chalcones with a resorcinol-type A-ring were selectively converted to 7-hydroxy flavonoids. No such specificity for ring-B substituted patterns was observed but several plants are known to contain flavonoid specific 3'-O-methyltrans-ferases[90] and 3'-hydroxylases[91]. This suggested [91,92] that introduction of 3'-hydroxyl and 3'-O-methyl groups occurred after formation of the flavonoid ring structure.

SCHEME 9

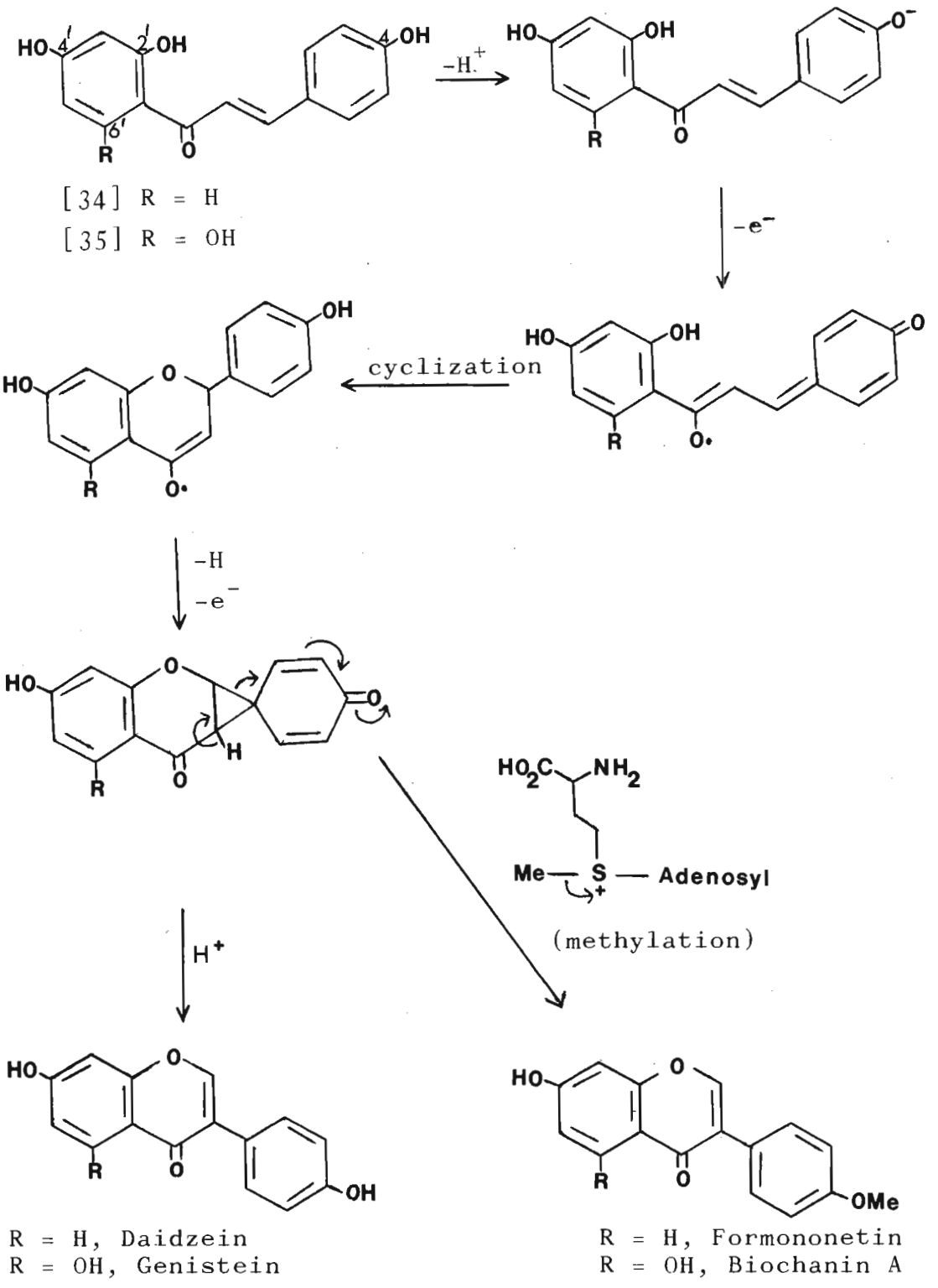

HOOC
NH2
[31]
3 x CH3–C(=O)–SCoA
Acetyl CoA
(i) phenylalanine ammonia-lyase(84)
(ii) cinnamate 4-hydroxylase(85)
(iii) 4-coumarate : CoA ligase(86)
3CO2
CoAS–C(=O)
[32]
3 HOOC–CH2–C(=O)–SCoA
[33]
condensation (chalcone synthase)
HO
OH
OH
O
(Chalcone)
cyclization
(Flavanone)
oxidation
aryl migration
Flavone
Isoflavone

1.8.3 Biosynthesis of Isoflavonoids

The isoflavonoids have a common biosynthetic pathway with the flavonoids up to the chalcone intermediate, after the formation of which a 1,2 aryl migration of the B-ring occurs to give the isoflavonoid skeleton[93]. On the basis of feeding experiments[94], in which daidzein and formononetin were isolated as end products, Dewick[95] proposed that two chalcones, 2',4',4-trihydroxychalcone [34] and 2',4',6',4-tetra-hydroxychalcone [35] acted as substrates for aryl migration.

Pelter and co-workers[96], and later Crombie and co-workers[97], suggested a mechanism, (Scheme 10), whereby the chalcone was oxidized to a spirodienone [36] intermediate. Subsequent protonation or methylation would give four isoflavonoids which could then act as precursors, on further substitution, of all other natural isoflavonoids.

SCHEME 10



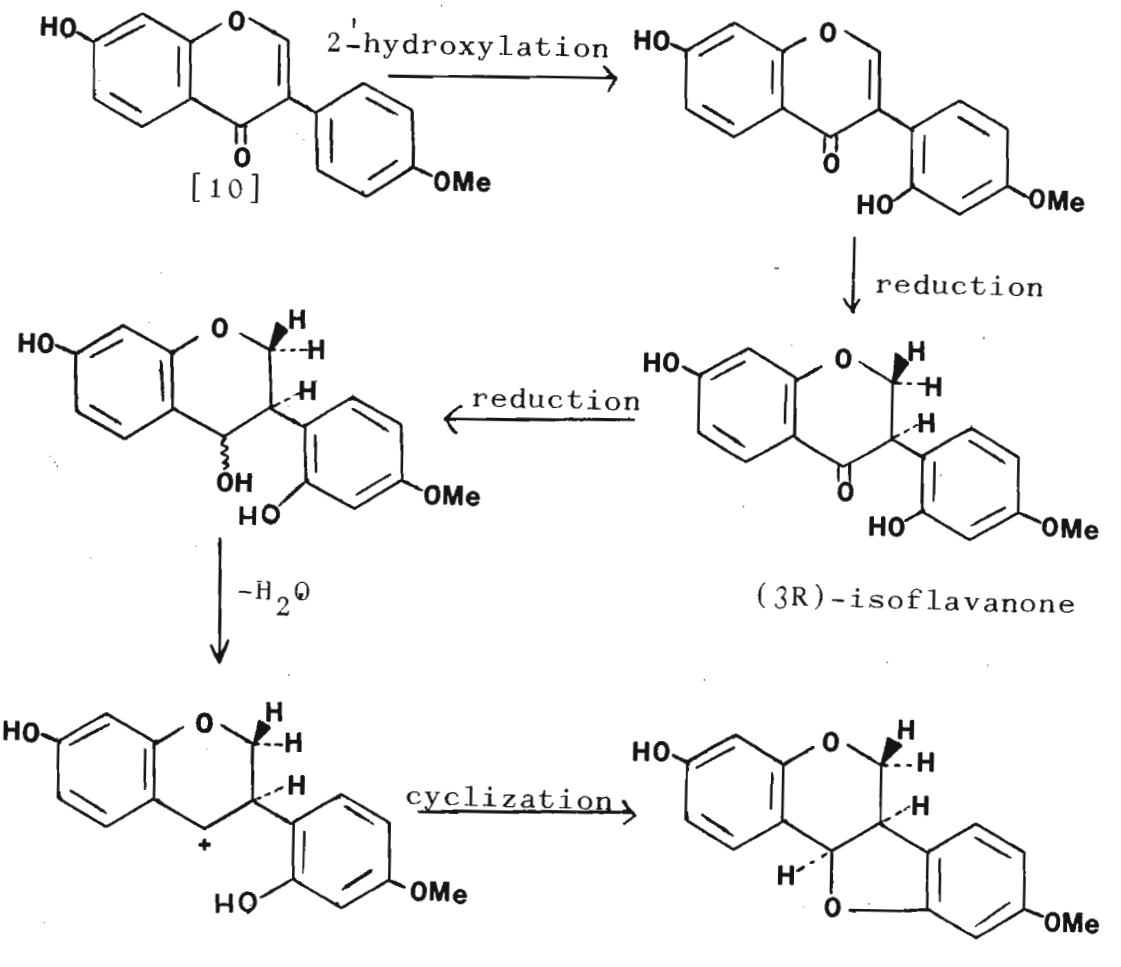
[34] R = H
[35] R = OH

R = H, Daidzein
R = OH, Genistein

R = H, Formononetin
R = OH, Biochanin A

Dewick[98], investigating $CuCl_2$-treated seedlings of red clover, found that labelled 2',4',4-trihydroxychalcone and formononetin[10] were good precursors of the pterocarpans (-)-medicarpan [37] and (-)-maackiain, whereas 2',4'-dihydroxy-4-methoxychalcone and ^3H-labelled daidzein were poor precursors. The interpretation was that 4'-methylation accompanied the aryl migration step. Later results[99] indicated that the pathway, (Scheme 11), possibly involved 2'-hydroxylation, reduction and cyclization.

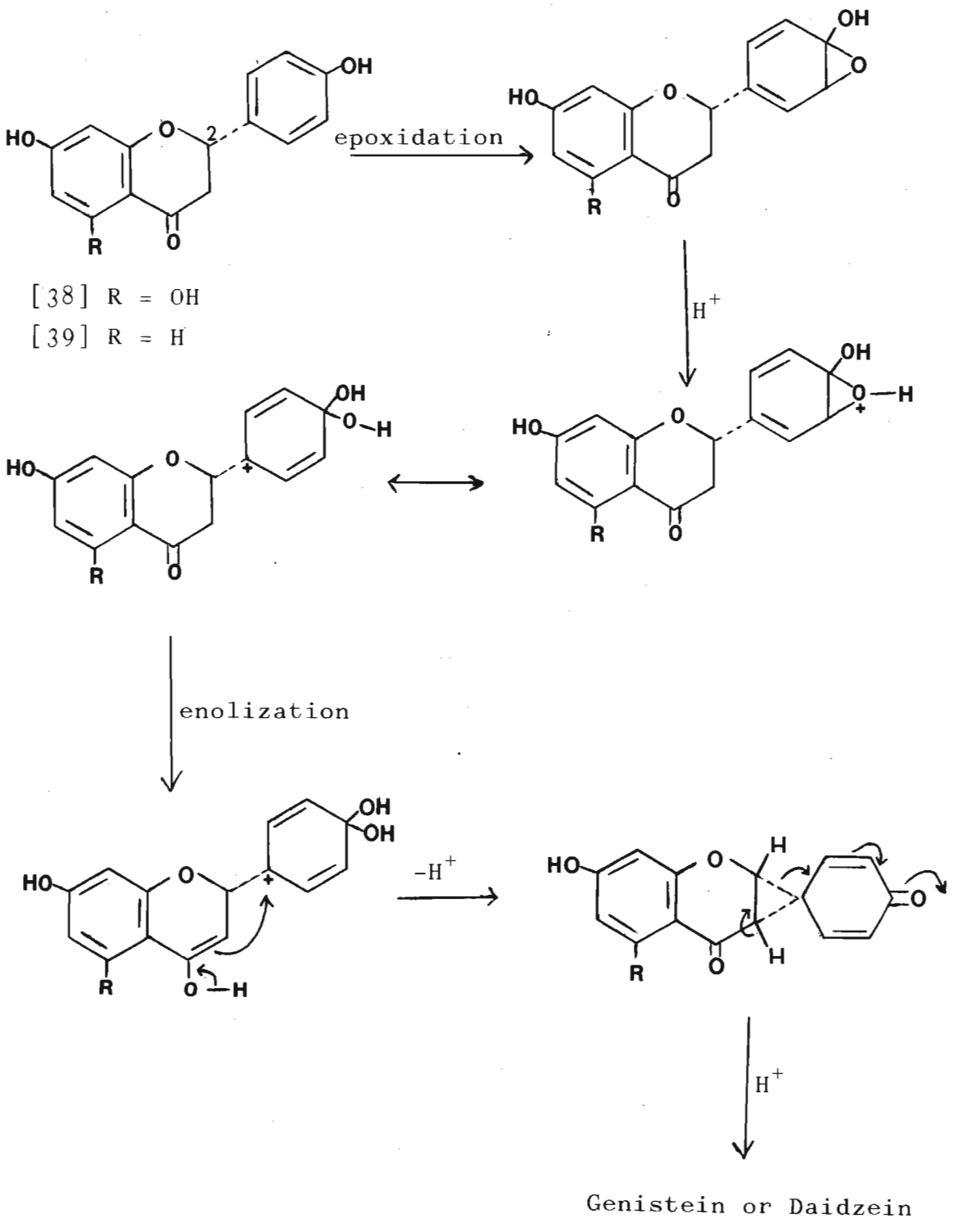
SCHEME 11



Interpretation[17a] of feeding studies on phytoalexins indicated that the B-ring oxygenation pattern was generally built up at the isoflavone level by the sequences 4'→2',4' and 4'→4',5'(≡ 3',4') 2',4',5'. The A-ring pattern of 7 or 5,7 was determined at the chalcone level but could be modified (7→6,7) at the isoflavone level.

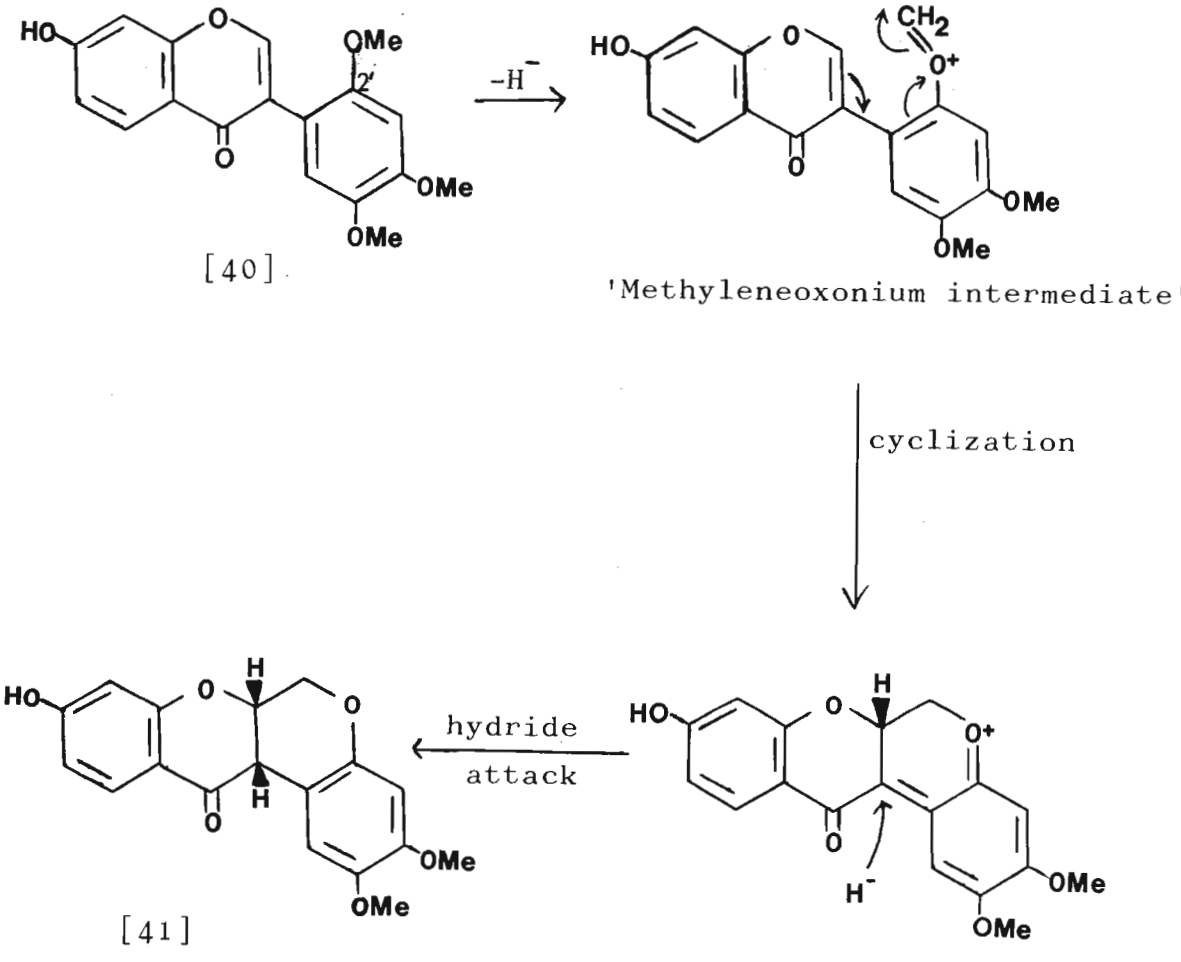
An isoflavone synthase activity that was capable of effecting the aryl migration was recently detected[100] in a microsomal preparation from cell suspension cultures of soybean. It was shown to convert the flavanone substrates (2S)-naringenin[38] or (2S)-liquiritigenin [39] into genistein or daidzein respectively. A mechanism, Scheme 12, in which the migrating aryl ring was epoxidized, rather than the heterocylic ring, was proposed by Hagmann and Grisebach[100].

SCHEME 12


epoxidation
[38] R = OH
[39] R = H
enolization
$-H^+$
H^+
H^+
Genistein or Daidzein

Crombie and co-workers[101] have done biosynthetic studies on Derris elliptica and Amorpha fructicosa. A hypothesis, Scheme 13, was put forward for the conversion of a 2'-methoxyisoflavone [40] into the rotenoid, dimethylmundeserone [41]

SCHEME 13

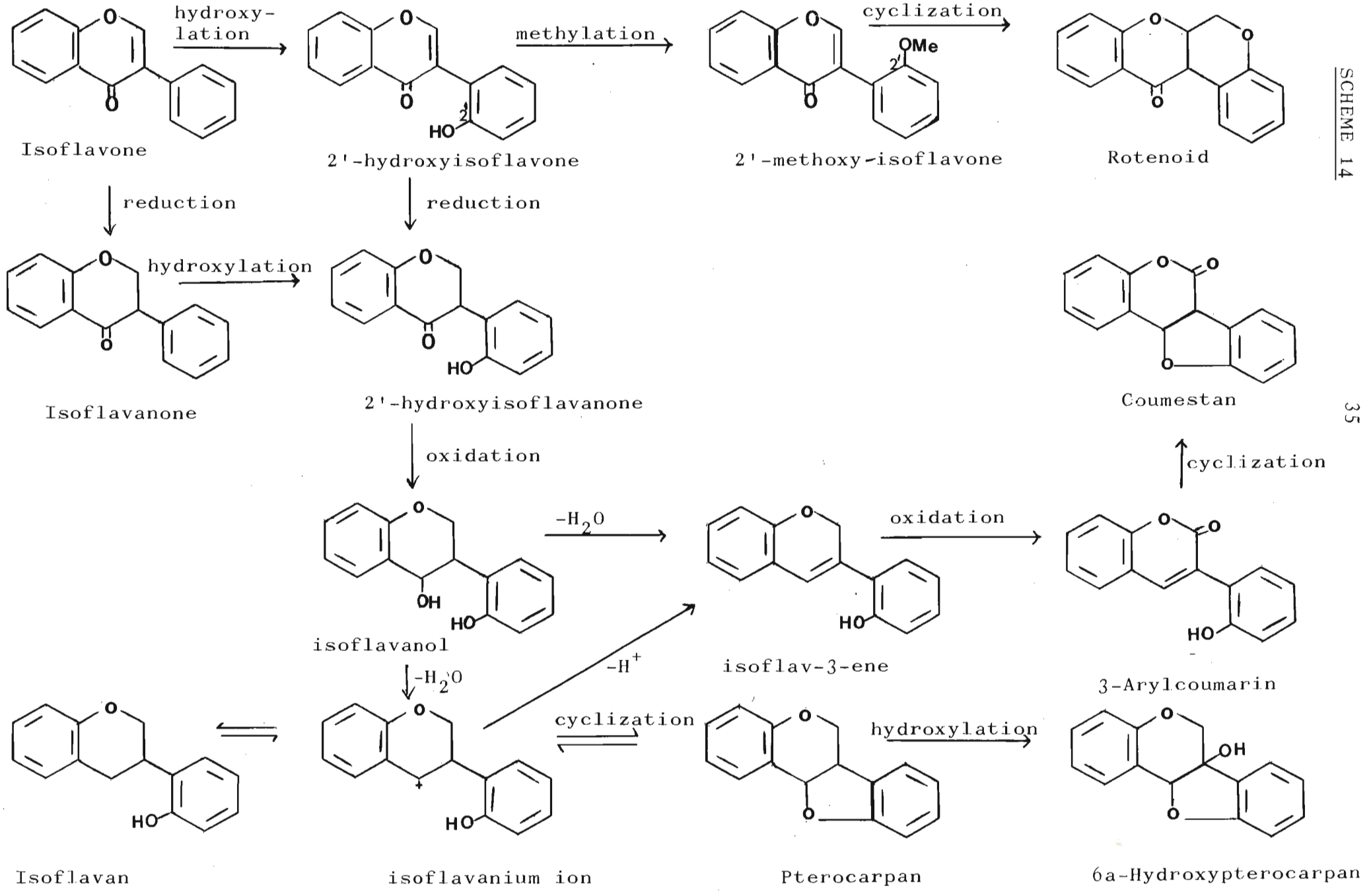


1.8.4 ISOFLAVONOID BIOGENETIC RELATIONSHIPS

Earlier reports[17h] of biogenetic relationships relied heavily on information about natural co-occurrence of various isoflavonoid classes and chemical interconversion of these classes. With increasing biosynthetic evidence it was possible to produce[17h] a scheme, (14), based on experimental data.

Natural co-occurrence of the isoflavonoids is still however an important aid. The rotenoid dolineone co-occurred with the isoflavonoid neotenone in Neorantaneuia pseudo-achyrrhiza[102] and toxicarol co-occurred with toxicarol isoflavone in Derris malaccensis[103]. Woodward's investigations on Phaseolus vulgaris[104] showed how a whole range of isolated 5-oxy- and 5-deoxy-isoflavonoids were linked to form a plausible biosynthetic sequence.

The presence of 2'-oxygenation is a striking feature of the isoflavonoids which after O-methylation is essential for the formation of rotenoids as the cyclization of the 2'-methoxyisoflavone is the critical rotenoid-forming step[101].


hydroxy-
lation
methylation
cyclization
OMe
2'
Isoflavone
2'-hydroxyisoflavone
2'-methoxy-isoflavone
Rotenoid
reduction
reduction
hydroxylation
Isoflavanone
2'-hydroxyisoflavanone
Coumestan
oxidation
cyclization
$-H_2O$
oxidation
isoflavanol
isoflav-3-ene
3-Arylcoumarin
$-H_2O$
$-H^+$
cyclization
hydroxylation
Isoflavan
isoflavanium ion
Pterocarpan
6a-Hydroxypterocarpan

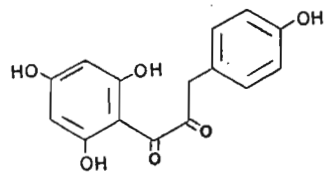
SCHEME 14

1.8.5 α-HYDROXYCHALCONES AS BIOGENETIC INTERMEDIATES PRECURSORS)

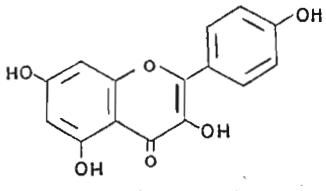
The suggestion that α-hydroxychalcones [42] could be the immediate biogenetic precursors of mopanols and peltogynols was first substantiated by the co-occurrence of α, 2', 3, 4, 4'-pentahydroxychalcone, (+)-peltogynol and (+)-mopanol in the heartwood of Trachylobium verrucosum[105].

The existence of α-hydroxychalcones in other heartwoods later supported their incorporation into a flavonoid biogenetic scheme, in which cyclization involving the β-position of the trans-enolic isomer of α-hydroxychalcones and subsequent reduction of the 2,3-cis- and 2,3-trans-dihydroflavonol could lead to the 2,3 cis- and 2,3-trans-diastereomers of flavan - 3,4-diols [43] and flavan -3-ols [44], as shown in scheme 15[106].

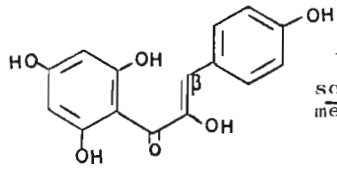
Scheme 15



keto-enol tautomerism of α-hydroxyketone

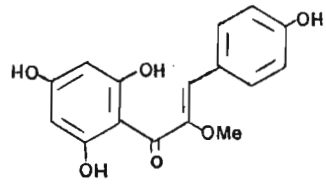


Flavonols



[42]

selective (107) methylation



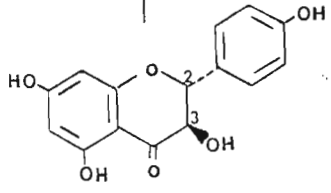
α-methoxychalcone

cyclization

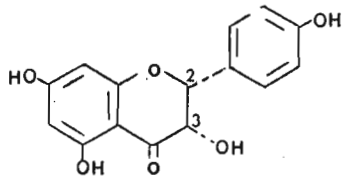
oxidation

cyclization

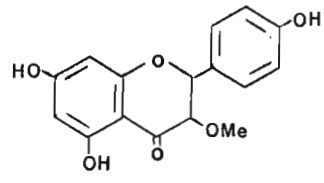
C-ring cyclization



2,3-trans-dihydroflavonols



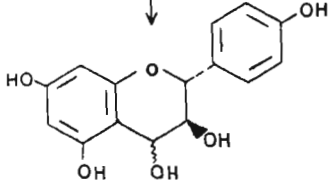
2,3-cis-dihydroflavonols



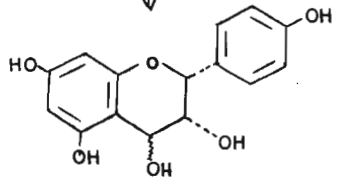
2,3-cis- and 2,3-trans-3-O-methyldihydroflavonol

reduction

reduction



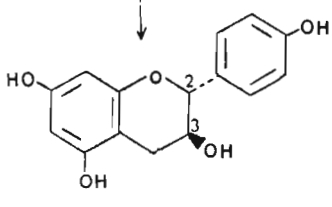
2,3-trans-flavan-3,4-diols



2,3-cis-flavan-3,4-diols

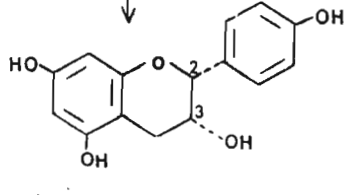
reduction

reduction



2,3-trans-flavan-3-ols

[43]



2,3-cis-flavan-3-ols

[44]

CHAPTER 2

THE STILBENES

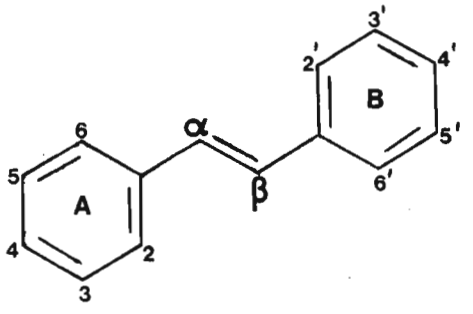
2.1 INTRODUCTION

The stilbenes and their derivatives have been found throughout the plant kingdom from algae and liverworts to conifers, having been isolated from the heartwood, sapwood, bark, leaves and roots.

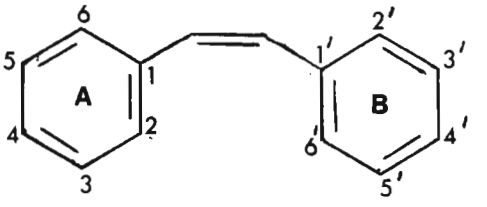
Hydroxylated stilbene derivatives are known to display a wide range of properties including estrone-like[108], antihypertensive and coronary vasodilatory[109], plant growth inhibitory[110] and antifungal activities[111].

Woods containing stilbenes exhibit a darkening in colour on exposure to sunlight, a phenomenon which is of importance in the furniture industry. Morgan and Orsler[112] found that the ethanolic solutions of some selected hydroxylated stilbenes all discoloured on exposure to sunlight, whereas their methyl ethers, being more stable, did not discolour.

The structure of the naturally occurring stilbenes range from the unsubstituted to the polysubstituted trans- [45] and cis- [46] parent hydrocarbon.



[45]



[46]

2.2 TRANS - STILBENES

Asakawa[113] obtained the simplest trans-stilbene [45] from Alnus firma.

A variety of mono- to penta-substituted (-OH and $-OCH_3$) natural trans-stilbenes have been isolated and identified (Table 2). Most of the substituted natural stilbenes occur in the more stable trans-rather than the less stable cis-form.

TABLE 2 SUMMARY OF SOME NATURAL TRANS-STILBENES

SUBSTITUTION PATTERN		SOURCE
A-RING	B-RING	
1. 4-OH		Pinus griffithi[114]
2. 4-OCH_3		Pinus griffithi[114]
3. 3,5-OH		Pinus sylvestris[115]
4. 3-OH,5-OCH_3		Pinus sylvestris[115]
5. 3,5-OCH_3		Pinus spp.[116,123]
6. 3,5-OH	4'-OH	Veratrum grandiflorum[117]
7. 3,5-OCH_3	4'-OH	Vitis vinifera[111]
8. 3,4-OH	3',5'-OH	Vouacapoua macropetala[118]
		Schotia brachypetala[121]
9. 3-OCH_3, 4-OH	3',5'-OH	Picea spp[119]
10. 3,4,5-OH	3',5'-OH	Vouacapoua macropetala[118]
		Schotia brachypetala[121]
11. 2,4-OH	3',5'-OH	Veratrum grandiflorum[117]
12. 2,3-OH, 4-OCH_3	3',4', 5'-OCH_3	Combretum caffrum[120]

2.3 CIS-STILBENES

During chromatographic work done on Eucalyptus wandoo, Hathway[122] found that the main spots were accompanied by weaker, higher R_f secondary spots, the main spots having the reported R_f of the trans stilbene. The shorter length of the conjugated system in the cis- configuration and the decrease in coplanarity owing to steric interference between the O-hydrogen atoms of the two phenyl groups was responsible for the decrease in λ max and E max of the absorption band of cis- stilbene compared to that of the trans isomer.

Rowe and co-workers[123], in investigating the bark of Pinus banksiana, separated the 3,5-dimethoxy-cis-stilbene from the 3,5-dimethoxy-trans-stilbene. It was suspected that the cis-stilbene was an artefact formed by natural irradiation of the bark. However isomerization studies proved that the cis-stilbene was a naturally occurring compound.

Traces of 3,3',4,5,5'-pentahydroxy -cis- stilbene were found[121] in the heartwood of Schotia brachypatela. Irradiation of the trans-pentahydroxystilbene gave a cis-isomer identical to the one extracted.

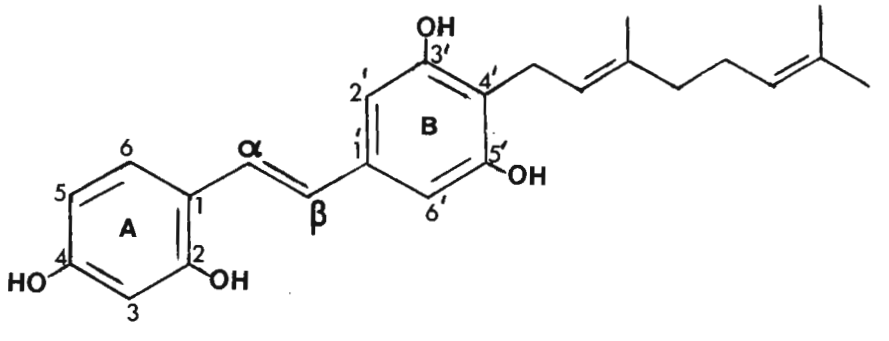
Manners and co-workers[124] showed that eight trans-stilbenes in Picea spp. occurred with minor amounts of their cis-isomers.

Five novel cis-stilbenes together with fourteen other trans-stilbenes were isolated from Rhubarb by Kashivada and co-workers[125].

3,4',5-trimethoxy-cis-stilbene and it's trans-isomer were isolated and identified by Gonzalez and co-workers[126].

2.4 SOME COMPLEX AND MISCELLANEOUS STILBENES

Chlorophorin [47] (2,3',4,5'-tetrahydroxy-4'-geranylstilbene) was first isolated from the heartwood of Chlorophora excelsa (Iroko) by King and co-workers[127].



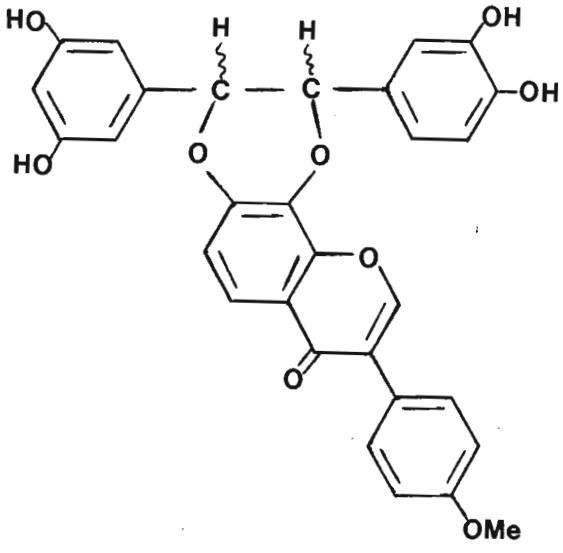
[47]

The phenolic nature of chlorophorin was confirmed by the preparation of the tetra-O-acetate, tetra-O-methyl and tetra-O-ethyl ethers by the alkyliodide-potassium carbonate-acetone method. Hydrogenation in the presence of Raney Nickel or palladised charcoal indicated the presence of two easily hydrogenated double bonds. Further reduction did not readily occur, but the existence of a third and less reactive double bond was later detected by ozonolysis.

$KMnO_4$ oxidation of tetra-O-methylchlorophorin produced 2,4-dimethoxybenzoic acid and 4-carboxy-2,6-dimethoxyphenyl-acetic acid. The structure of these two products was proved by synthesis.

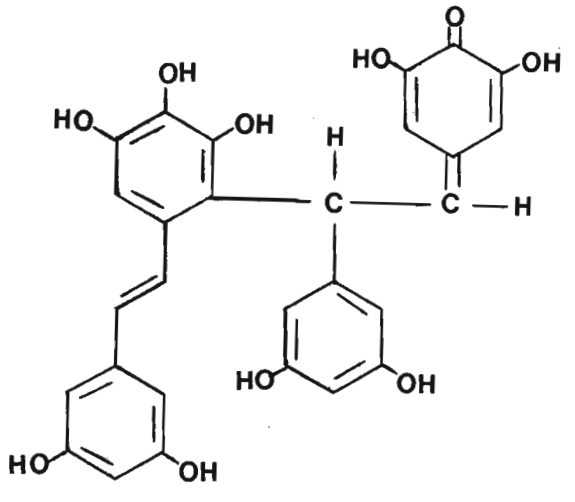
The nature of the aliphatic (geranyl) side-chain was left unsettled. Oxidation of chlorophorin in alkaline H_2O_2 destroyed the aromatic consitituent leaving an unsaturated acid $C_{11}H_{18}O_2$. Ozonolysis of this acid yielded acetone, isolated as the 2,4-dinitrophenyl-hydrazone and laevulal-dehyde. The acid was identified as 4,8-dimethylnona-3,7-dienoic acid (homogeranic acid). A synthesis[128] from citral further demonstrated the structure of the acid. No ^{1}HNMR or mass spectroscopy studies were carried out on chlorophorin.

Maakiasin [48] is a novel isoflavonostilbene obtained[31] from the heartwood of Maackia amurensis.

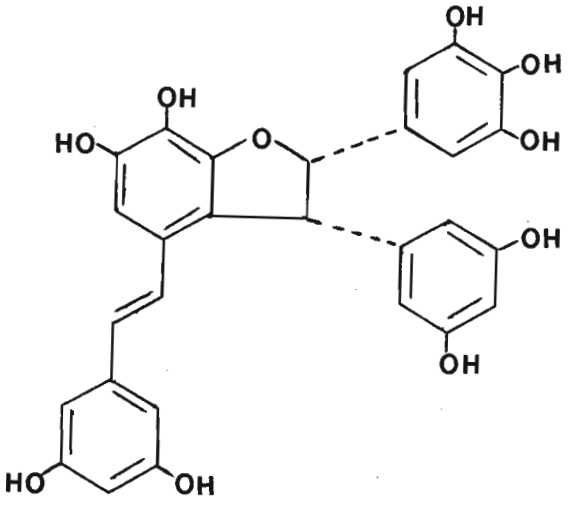


[48]

The heartwood of Schotia latifolia[129] yielded two complex stilbenes [49] and [50].

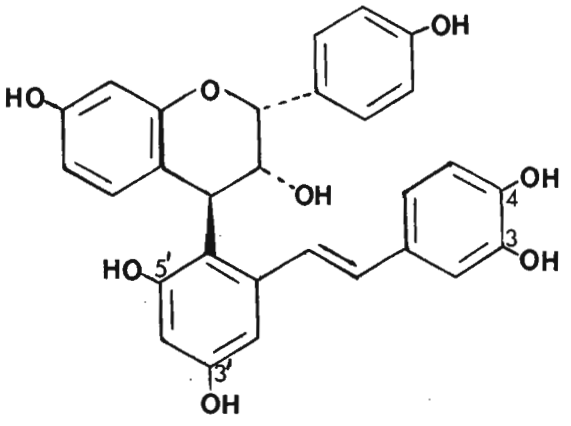


[49]

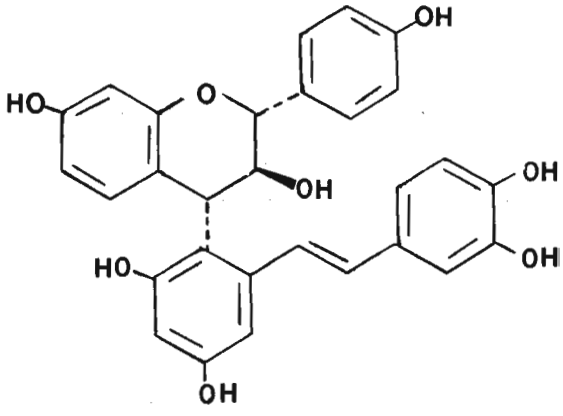


[50]

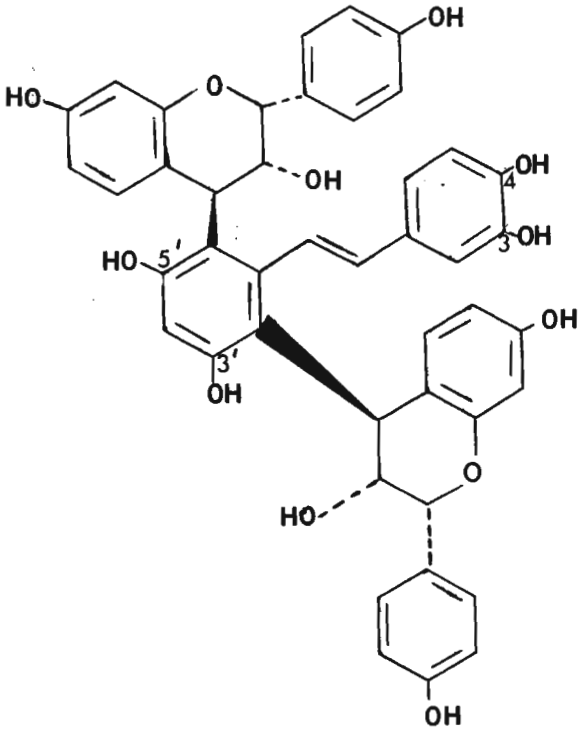
The first stilbene (trans-3,3′,4 ,5′-tetrahydroxystilbene) based condensed tannins were obtained as dimers [51,52] and trimers [53,54] from Guibourtia coleosperma by Roux and co-workers[130]



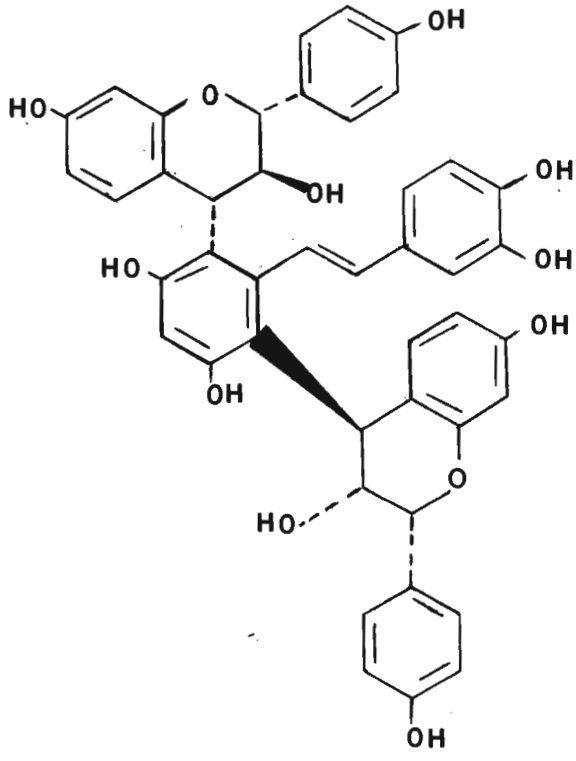
[51]



[52]



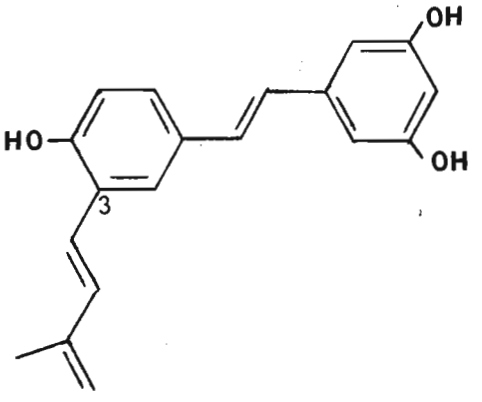
[53]



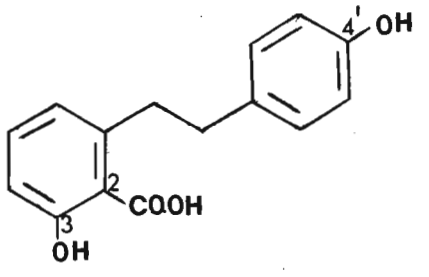
[54]

Novel stilbene glycosides are also known to occur[131], [132].

Cooksey and co-workers[133] characterized an antifungal stilbene [55], with a 3-isoprenyl substituent, from the kernels of Arachis hypogaea.



[55]



[56]

Lunularic acid [56], a dihydrostilbene carboxylic acid derivative (3,4'-OH,2-COOH) displaying dormancy inducing activities, was found in liverworts and algae[134]. Hashimoto and co-workers[132] have synthesized lunularic acid and also investigated the biological activities of stilbene and dihydrostilbene derivatives.

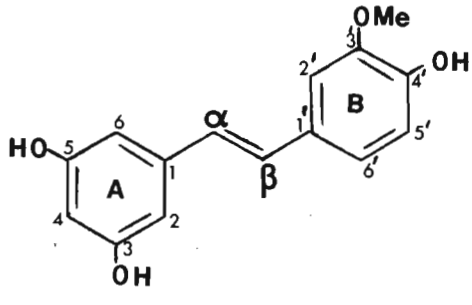
2.5 STRUCTURE ELUCIDATION OF STILBENES

2.5.1 Spectroscopic Methods

2.5.1.1 ^{1}HNMR Spectroscopy of Stilbenes

Güsten and Salzwedel[135] recorded the ^{1}HMNR spectra of a number of mono- and di-substituted trans- and cis- stilbenes. The chemical shifts of the olefinic and the aryl protons were found to be greater in the cis-form than in the trans-form. It was suggested that the non-coplanar structure of the cis- form caused a stronger shielding of the olefinic protons. This effect was however not quite as strong in the case of the aryl protons. The coupling constants for the cis- and trans-olefinic protons (α and β) were respectively J = 12,0-12,5 Hz and J = 16,0-16,8 Hz.

The spectrum of Rhapontigenin [57][125] showed typical stilbene characteristics.



[57]

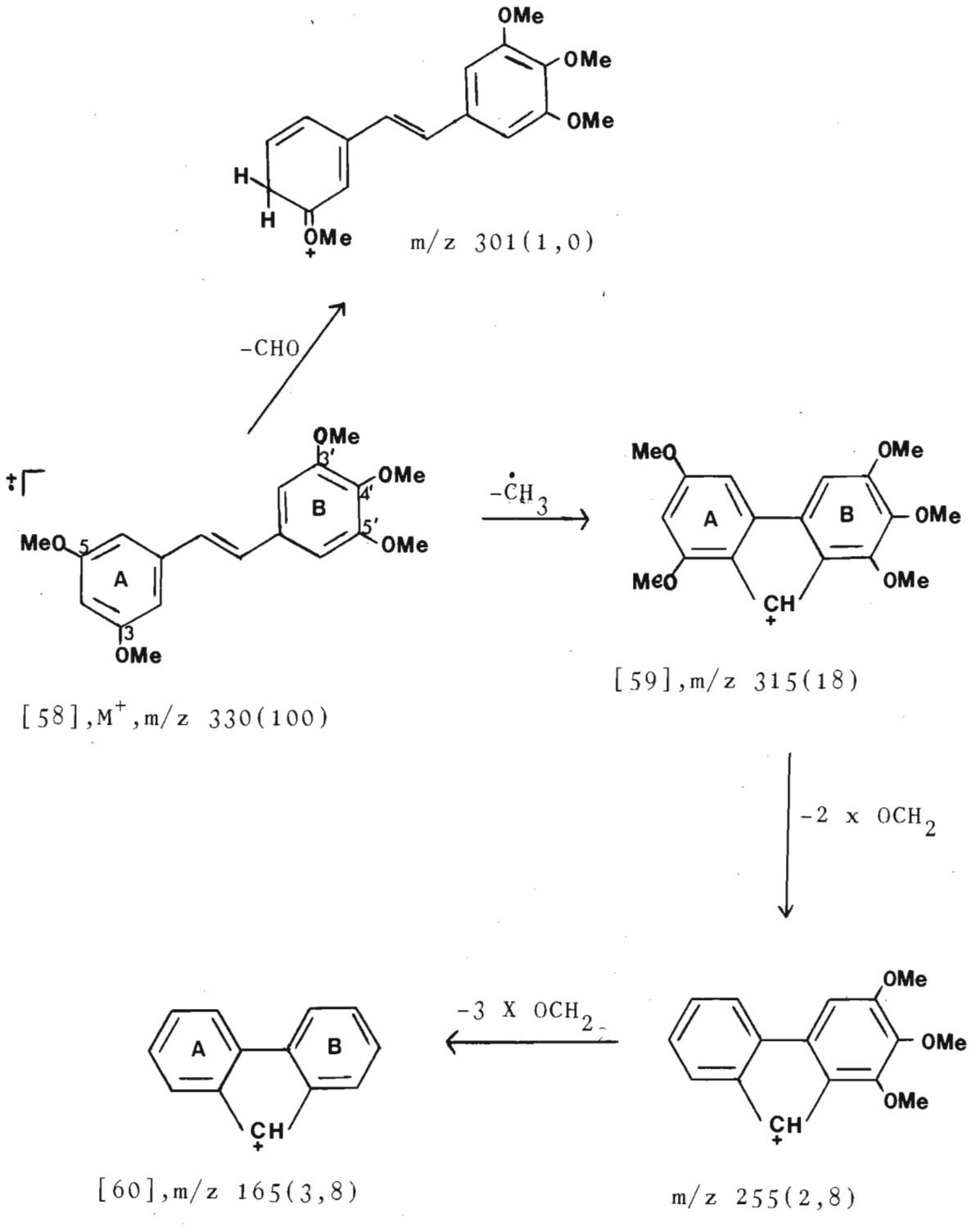
The trans-olefinic protons (α and β) appeared as two doublets (δ6.84, 7.02, each 1H, d, J = 16 Hz). The A-ring exhibited an A_2X system (δ6.29, 1H, t, J = 2 Hz; δ6.56, 2H, d, J = 2 Hz). The B-ring protons formed an ABX pattern (δ6.88, 1H, d, J = 8 Hz; δ7.02, 1H, dd, J = 2 and 8Hz; δ7.09, 1H, d, J = 2 Hz), and the methoxyl protons appeared at δ3.84.

2.5.1.2 Mass Spectrometry

The fragmentation pattern (Scheme 16) of trans-3,3',4',5,5'-pentamethoxystilbene[129] showed the stable molecular ion [58] M^+330, (100%) as the base peak. The loss of a methyl radical [59], m/z 315(18,5) and the formation of the fluorene cation [60], m/z 165(8,5) are diagnostic for stilbenes.

Hydrogen randomization of the stilbene molecular ion precedes the formation of $[M-H]^+$ and $[M-CH_3]^+$, while the carbon atom involved in the methyl radical elimination originates randomly from the whole molecule[136].

SCHEME 16


OMe
OMe
OMe
H
H
OMe
+
m/z 301(1,0)
-CHO
OMe
3'
OMe
4'
B
5'
OMe
MeO
5
A
3
OMe
[58],M+,m/z 330(100)
-CH3
MeO
OMe
A
B
OMe
MeO
OMe
CH
+
[59],m/z 315(18)
-2 x OCH2
OMe
OMe
OMe
CH
+
m/z 255(2,8)
-3 X OCH2
A
B
CH
+
[60],m/z 165(3,8)

2.5.2 Synthesis of Stilbenes

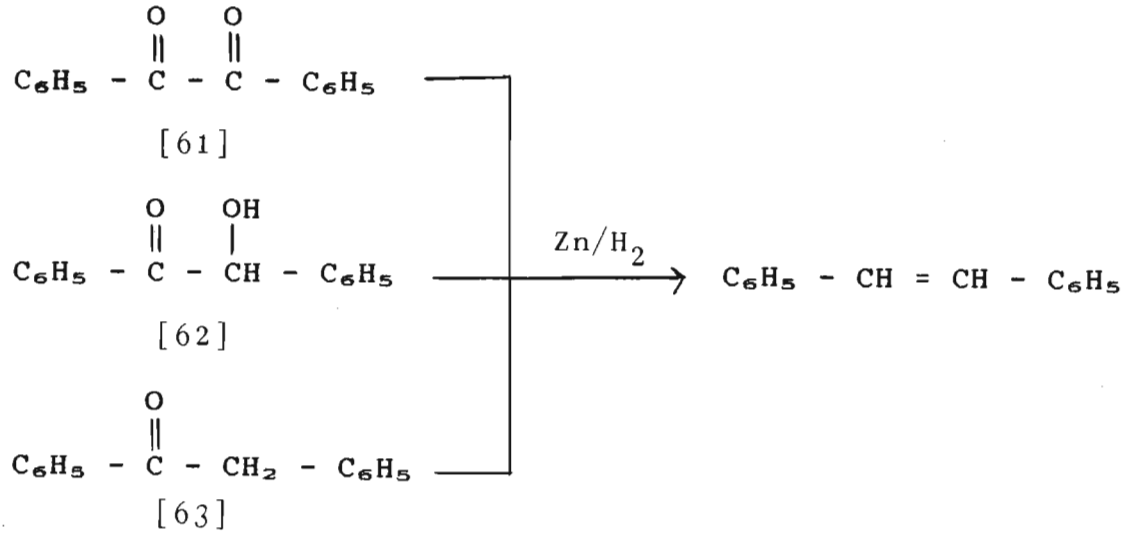
Many reports on stilbene synthesis have appeared in the literature. The applicability of the methods is dependant upon the isomer (cis, trans) and substituents required in the final product. A few of the more well-known and successful reported methods will be discussed.

2.5.2.1 Reduction of, and Elimination from Diphenyl Compounds

2.5.2.1.1 Reduction of Benzil, Benzoin and Deoxybenzoin Derivatives

Benzil [61], Benzoin [62] and Deoxybenzoin [63] are reduced to cis-stilbene by zinc in hydrogen atmosphere[137] (Scheme 17).

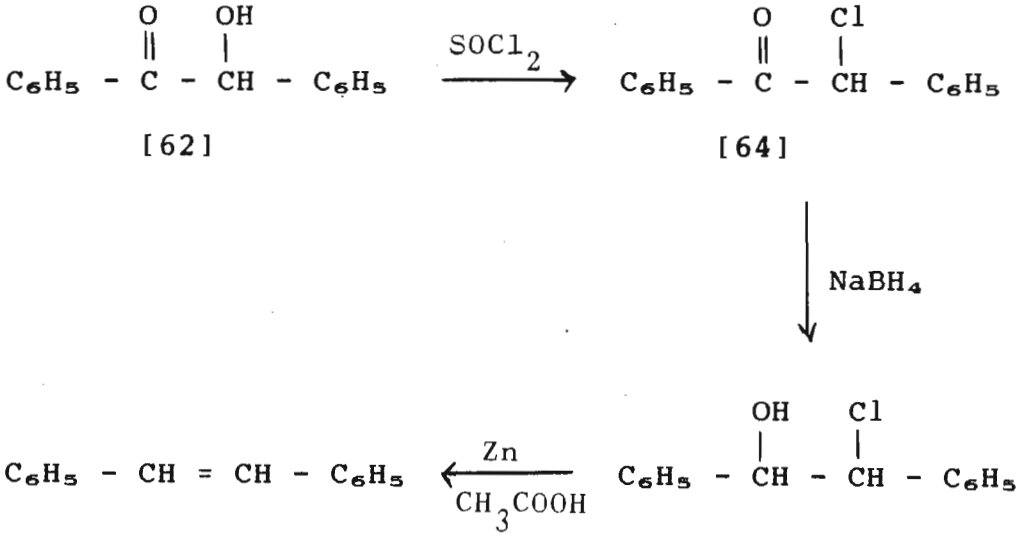
SCHEME 17



Benzoin [62] is also reduced to trans-stilbene in 53-57% yield, using the Clemmensen reduction[138].

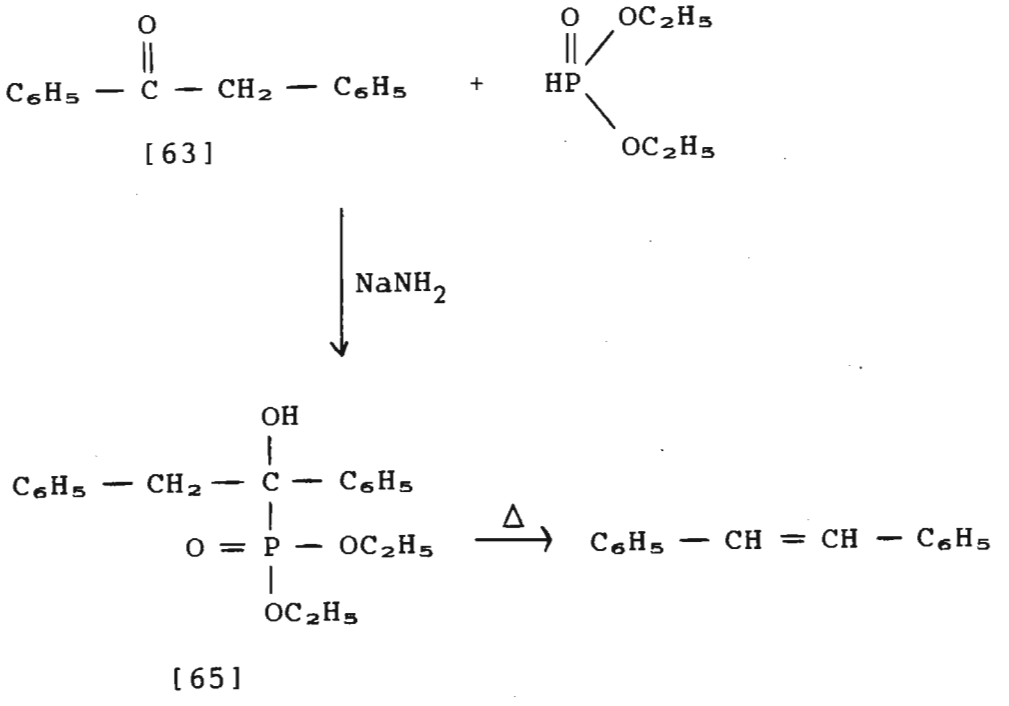
When benzoin [62] is treated with thionyl chloride, reduction of the resulting desyl chloride [64] with $NaBH_4$ and subsequent treatment with zinc in acetic acid produces trans-stilbene in 53-65% yield[139], (Scheme 18).

SCHEME 18



In the presence of sodium amide, Deoxybenzoin [63] and diethyl phosphite may condense to form 1-hydroxyethane-phosphonate [65], which on warming rearranges and eliminates diethyl phosphate. The result is trans-stilbene in 81% yield[140], (Scheme 19).

SCHEME 19

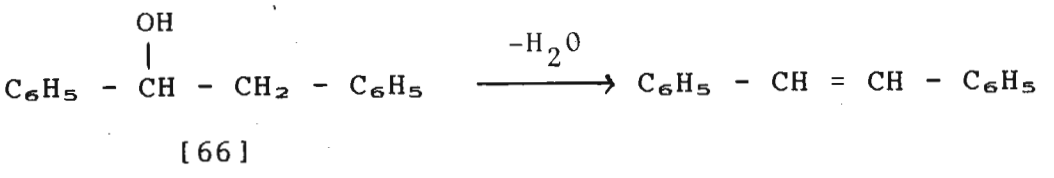


The deoxybenzoins are useful reagents because they are readily available by Friedel-Crafts reactions of phenylacetic acid chlorides with a variety of arenes. In general, the above methods are suitable for the preparation of trans-stilbene but are of limited use in the synthesis of substituted trans-stilbenes.

2.5.2.1.2 Elimination of Water from 1,2-Diphenyl-Ethanol

The elimination of water from 1,2-diphenylethanol [66] may be accomplished by sodium hydrogen sulphate[141], (scheme20).

SCHEME 20

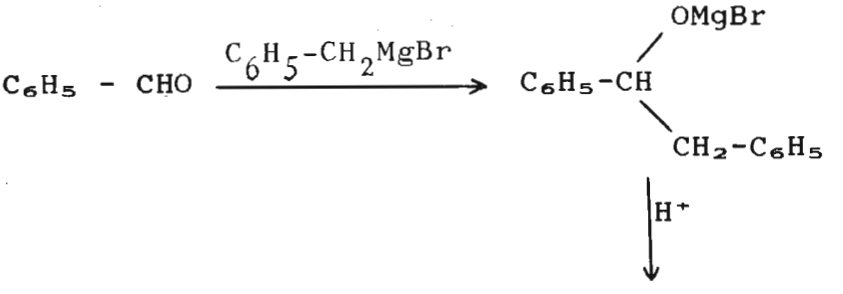


[66]

Trans-stilbene was produced in 93% yield[142].

1,2-Diphenylethanol [67] may be formed by the reaction of benzaldehyde with benzylmagnesium halides[143],(scheme 21).

SCHEME 21



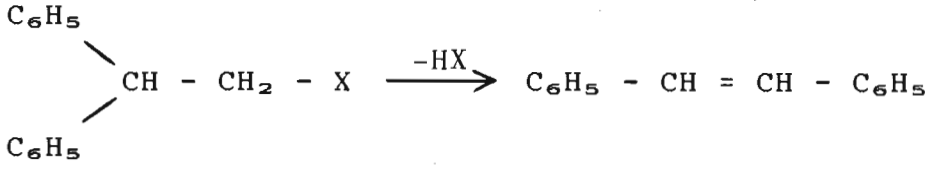
[67]

This method is limited to the benzyl halides that form Grignard derivatives and to those benzaldehydes with substituents that do not react with the Grignard reagent. However combined with the ease of formation of 1,2-diphenylethanols (Scheme 21), this elimination constitutes a simple access to a variety of substituted trans-stilbenes.

2.5.2.1.3 Rearrangement of 2,2-Diphenylethyl Halides

2,2-Diphenylethyl halides [68] experience a Wagner-Meerwein type of rearrangement when heated in a polar solvent such as ethylene glycol. Elimination of hydrogen halide forms the trans-stilbene[144], (Scheme 22).

SCHEME 22



(X = Cl,Br)

[68]

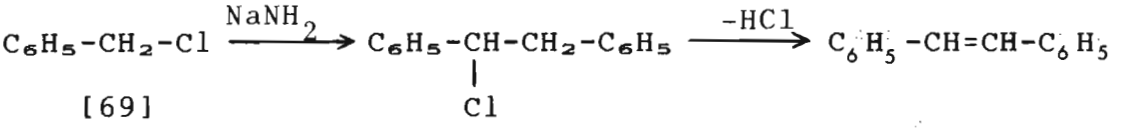
Sieber[145] obtained 4,4'-dihydroxystilbene in 42% by employing this method while stilbenes with electron withdrawing substituents are not accessible.

2.5.2.2 Dimerization (Coupling) Reactions

2.5.2.2.1 Eliminative Dimerization of Arylmethyl Halides

Benzyl chloride [69] yields stilbene when treated with a strong base ($NaNH_2$)[146], (Scheme 23).

SCHEME 23



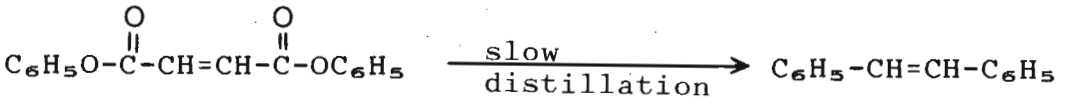
Trans-stilbenes bearing methyl- or chloro-substituents may be obtained by this method.

2.5.2.3 Coupling of Aromatic Compounds with Styrenes

2.5.2.3.1 Pyrolysis of Aryl Fumarates

Symmetric alkyl-, halo- and alkoxy- substituted trans-stilbenes are obtained (in low yield) by slow distillation of diaryl fumarates [70][147], as shown in Scheme 24.

SCHEME 24



2.5.2.3.2 Reaction of Arenediazonium Salts with Cinnamic Acids or Styrenes in the Presence of Copper Salts - Meerwein Reaction

In the Meerwein arylation reaction[148], cinnamic acids [71] are arylated and decarboxylated by arenediazonium salts in the presence of copper(I) or copper(II) chloride in acetone/water. The yields are not high due to competing Sandmeyer reaction (aryl halide formation) and polymerization reactions.

SCHEME 25

$$C_6H_5 - CH = CH - COOH \xrightarrow[CuCl_2]{C_6H_5-\overset{+}{N}_2\overset{-}{Cl}} C_6H_5 - CH = CH - C_6H_5$$

[71]

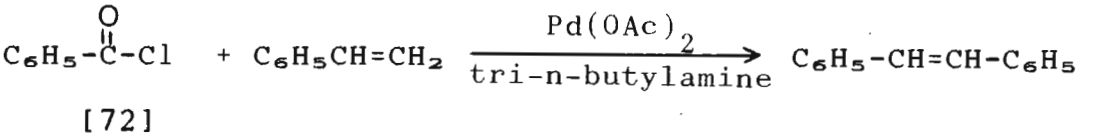
The reaction is often applied because the starting materials are readily available and many substituents can be tolerated. Various meta-, ortho- and disubstituted anilines have been reacted with cinnamic acid[149] or ortho-, meta-, or para-substituted cinnamic aicds[150] to form trans-stilbenes. The Meerwein arylation may also be conducted with styrenes in place of cinnamic acid[151].

Palladium salts may be used in place of the copper salts resulting in a 40 - 75% yield[152].

2.5.2.3.3 Reaction of Arylpalladium Compounds with Styrenes

Aroylchlorides [72] react with styrenes and a tertiary amine to produce trans-stilbenes[153] with concurrent decarbonylation, (Scheme 26).

SCHEME 26



[72]

A large number of different substituents (-CHO, -Cl, $-CH_3$, OCH_3) are tolerated and the starting materials are readily available.

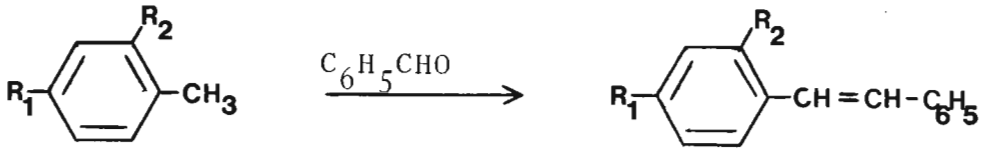
2.5.2.4 Condensation of a Nucleophilic with an Electrophilic Arylmethyl Compound

Suitable nucleophiles for condensation are benzyl compounds with substituents that are able to stabilize the negative charge of a benzyl carbanion, whereas the electrophile may be an aryl aldehyde or benzyl halide.

2.5.2.4.1 Condensation of Activated Methylarenes with Aryl Halides

Methylarenes [73] activated by a strong electron-withdrawing group in the ortho-or para-position (R_1 and R_2) react with aryl aldehydes[154] in the presence of base (piperidine) to give the corresponding trans-stilbene in a type of Knoevenagel reaction, (Scheme 27).

SCHEME 27



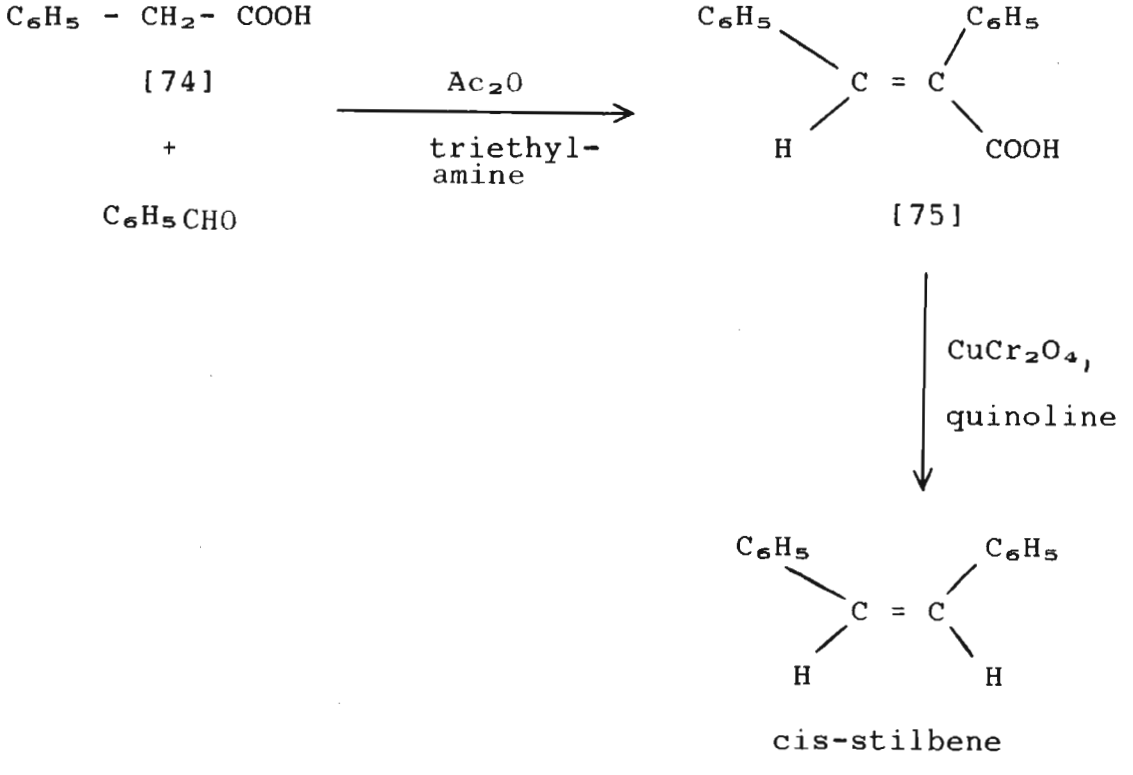
[73]

This condensation has been applied to many substituted nitrotoluenes and aryl aldehydes.

2.5.2.4.2 Condensation of Arylacetic Acids and Related Compounds with Aryl Aldehydes

In the presence of triethylamine and acetic anhydride, Phenylacetic acid [74] and benzaldehyde condense, producing trans-α-phenylcinnamic acid [75] in 54-59% yield[155]. Decarboxylation of [75] with copper chromite in quinoline results in a 62-65% yield of cis-stilbene[156], (Scheme 28).

SCHEME 28



This condensation reaction constitutes a special case of the Perkin condensation[157,158] or the Knoevenagel condensation[159].

The arylacetic acid may be introduced in the form of the potassium or sodium salt or one equivalent of an amine (triethylamine, pyridine, piperidine) may be added. Acetic anhydride is the usual Lewis acid of choice.

The decarboxylation of [75] is best performed in quinoline. Pure cis-stilbene isomerizes at 180°C to trans-stilbene, however in quinoline this isomerization is slow[160].

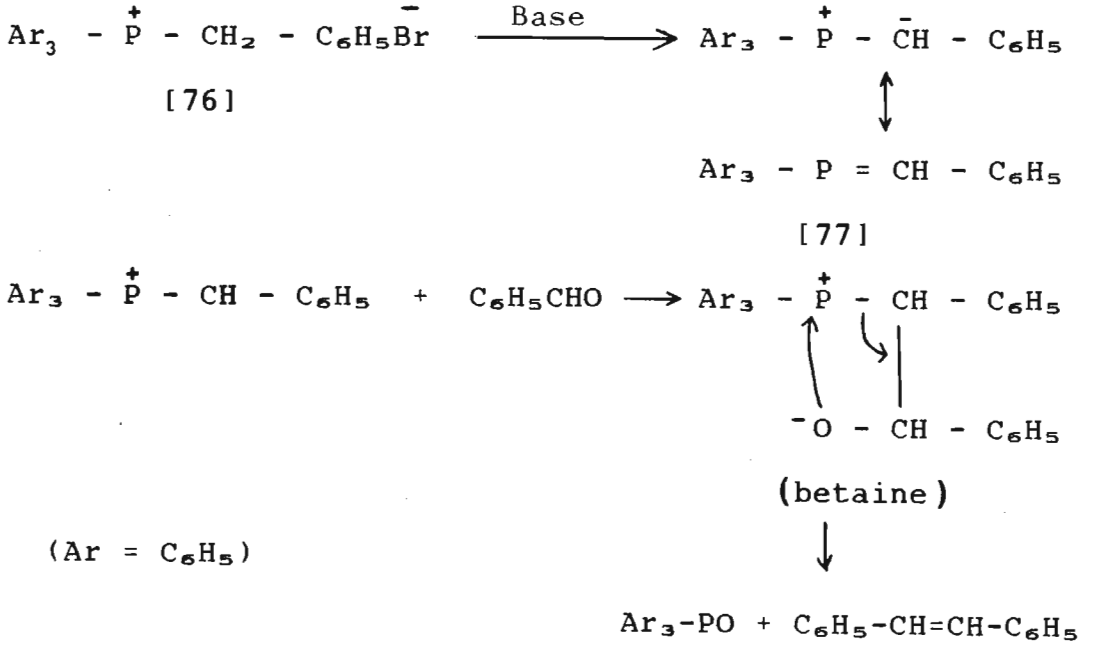
This condensation reaction has been used to synthesize a great number[161] of stilbenes and is probably the best synthetic method for cis-stilbenes. A large number of substituents may be tolerated and the starting materials are readily available. Heterocylic cis-stilbenes are also obtained[162].

In a one pot procedure, 4-nitrostilbene was formed directly from p-nitrophenylacetic acid and benzaldehyde in the presence of piperidine[163]. 4-Hydroxystilbenes are also formed in one step from p-hydroxybenzaldehyde and arylacetic acids[164]. In both cases the product is the trans-stilbene. The decarboxylation of trans-α-phenylcinnamic acid to the trans-stilbene was shown by Jambotkar and co-workers[163] to proceed via a carbanion mechanism.

2.5.2.4.3 Condensation of Phosphorus-Stabilized Carbanions with Aryl Aldehydes - Wittig Reaction

The Wittig[165] reaction is probably the most popular method for the synthesis of substituted stilbenes. The reaction involves treating an arylmethyl halide with triphenylphosphine, resulting in the arylmethyltriphenylphosphonium halide [76]. Deprotonation of [76] with base forms the corresponding phosphorus ylid [77]. The ylid reacts with the aryl aldehyde, followed by elimination of a phosphine oxide from the intermediate betaine to form the stilbene, as shown in Scheme 29a.

SCHEME 29a



The strength of the base depends on the acidity of the α-hydrogen. Common bases and solvents used include butyl- and phenyllithium in ether, benzene or tetrahydrofuran, and sodium or lithium alkoxides in the corresponding alcohol or dimethylformamide[166].

In a report by Wheeler[167], benzyltriphenylphosphonium chloride treated with sodium ethoxide in ethanol and benzaldehyde, produced a quantitative yield of the parent stilbene in an isomer ratio (trans : cis) = 55 : 45. Potassium t-butoxide in t-butyl alcohol reduced the cis-stilbene proportion to 25%.

The trans isomer is formed exclusively in the Wittig reaction of 4-methoxy- and 4-nitrobenzaldehydes with sodium methoxide and lithium bromide in dimethylformamide as well as in the reaction of 4-methoxy- and 2-chlorobenzaldehydes with sodium ethoxide[167]. The trans isomer is the only product in the reaction of 4-chloro- and 2,4-dichlorobenzaldehyde with t-butoxide in t-butyl alcohol[167].

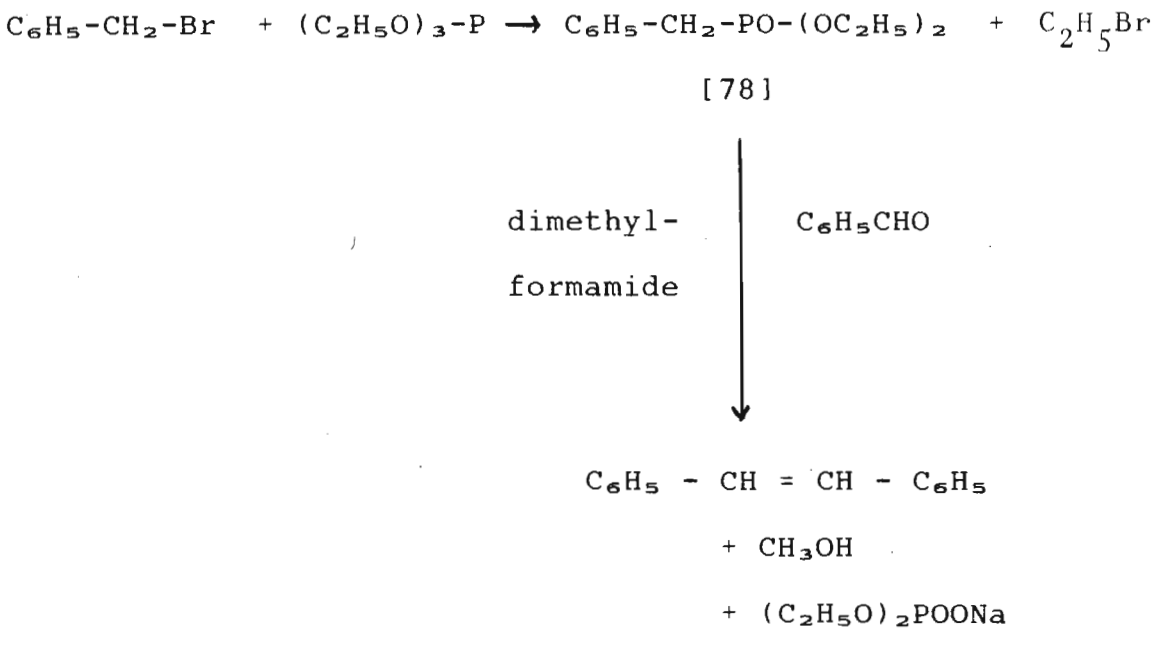
4-Nitrobenzaldehyde treated with 4-methoxy-phenyl-methylenetriphenylphosphorane yielded stilbene in a isomer ratio, (cis : trans) of 48 : 52. If 4-methoxybenzaldehyde is treated with 4-phenylmethylenetriphenylphosphorane, only the trans isomer is formed.

It is apparent that the formation of the cis-isomer is favoured by the presence of electron donating substituents on the phenylmethylenephosphorane and electron withdrawing substituents on the aldehyde. The formation of the trans-isomer is favoured by the presence of electron withdrawing substituents on the phenylmethylenephosphorane and electron donating subsubstuents on the aldehyde[168].

It seems clear therefore that the (cis/trans) ratio may be influenced by the choice of base and solvent, added salts, reaction conditions and substituents on the phenyl groups.

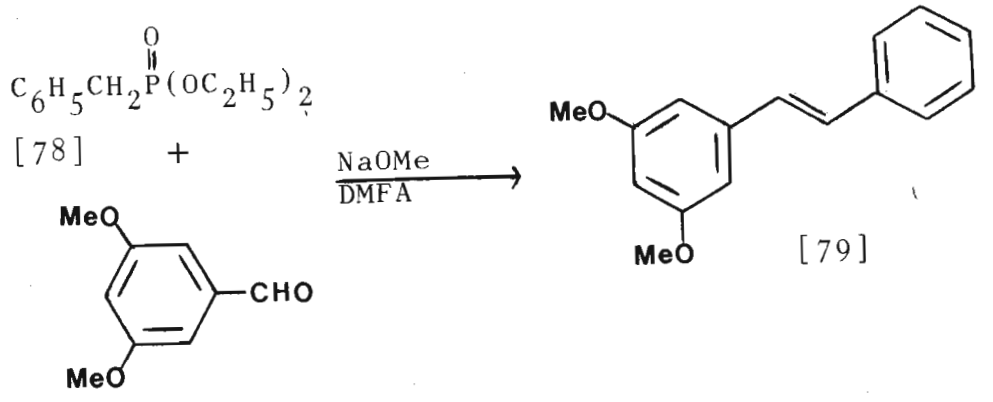
In 1941 Späth reported multi-step syntheses for pinosylvin[169] and pinosylvin monomethyl ether[170] based on Perkin condensations and decarboxylation of the α-phenyl-cinnamic acids formed. Low yields of 6% were obtained. Aulin-Erdtman and Erdtman reported a similar synthesis of pinosylvin dimethyl ether[171]. Bachelor and co-workers[172] made an unsuccessful attempt to prepare pinosylvin dimethyl ether via a Wittig reaction between triphenylbenzylphosphonium bromide and 3,5-dimethoxy-benzaldehyde. Attention was then focussed on the modified Wittig reaction [173,174]. In the modified Wittig reaction, the phosphonates [78] obtained from trialkyl phosphites via the Michaelis-Arbuzov reaction[175] undergo reaction with aldehydes and ketones in the presence of a strong base producing good yields of stilbenes,(scheme 29b).

SCHEME 29b



Using the modified Wittig reaction, Bachelor and co-workers[172] reported that diethyl benzylphosphonate [78], prepared from benzyl bromide and triethyl phosphite, reacted with 3,5-dimethoxybenzaldehyde in dimethylformamide. Pinosylvin dimethyl ether [79] was obtained in good yield,(scheme 30).

SCHEME 30



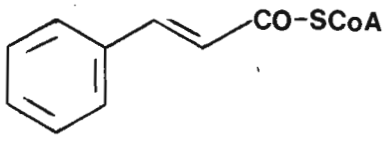
In contrast to the phosphonium ylids which give mixtures of ylids[176], the phosphonate esters form the trans isomer exclusively[177].

Although many methods for the synthesis of stilbenes have been reported, the modified Wittig reaction seems to be most successful for the synthesis of substituted trans-stilbenes while substituted cis-stilbenes are best synthesized by decarboxylation of α-phenyl cinnamic acids.

2.6 BIOSYNTHESIS OF STILBENES

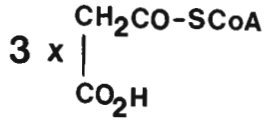
On investigating the biosynthesis of pinosylvin [82][178], Birch and Donavon proposed (Scheme 31) that the phenylpropanoid precursor [83] linked with three malonyl CoA units [32] to form a linear β-triketo acid [80]. Cyclization of [80] produced a stilbene carboxylic acid [81] which on decarboxylation yielded pinosylvin [82].

SCHEME 31

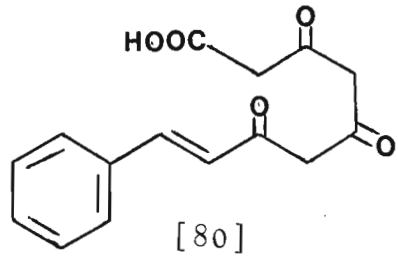


cinnamoyl-CoA

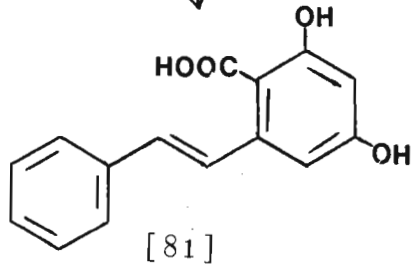
[83]



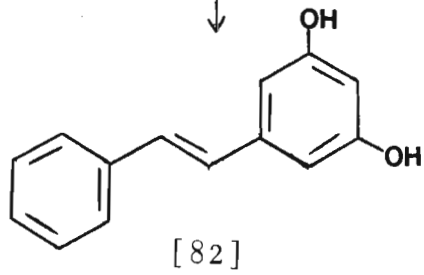
[33]



[80]



[81]



[82]

EXPERIMENTAL

CHAPTER 3

STANDARD EXPERIMENTAL PROCEDURE

The following standard experimental techniques as detailed below will be briefly referred to in subsequent chapters.

3.1 CHROMATOGRAPHIC METHODS

3.1.1. Column Chromatography

Three sizes of glass columns were available, with dimensions of 33 x 900, 50 x 300 and 50 x 1200mm. These will be referred to as Type 1,2 and 3 columns respectively.

3.1.1.1 Silica gel as adsorbent

A column was packed by adding a slurry of Merk Kieselgel Art 7734 (170-230mesh) to the column. The slurry was prepared by using the same solvent as used for the eluent. The column was vibrated with its tap open to ensure dense, efficient packing free of air bubbles. The ratio of material-to-be-separated to silica gel was 1:20. The material was first absorbed onto a small quantity of silica gel and then transferred to the top of the column. An elution rate of 30 cm^3 per 30 minute was used, and fractions were collected in

100 cm^3 beakers or in test tubes.

3.1.1.2 LH-20 as absorbent

An ethanol slurry of LH-20 was allowed to stand for 24 hours. The slurry was then transferred into the column, the tap being left open to ensure compact packing. The ratio of material-to-be-separated to LH-20 resin was 1:25. The material to be separated was dissolved in a minimum amount of ethanol and then transferred to the column. An elution rate of 30 cm^3 per 30min. was used.

3.1.1.3 Flash column

A dry mixture of silica gel (100g of Merk Kieselgel 7734/20g of Merk Kieselgel 7747) was poured onto a 5mm layer of dry sand at the bottom of a type 2 column. A further 5mm layer of dry sand was placed ontop of the silica gel layer. The column was then flushed with the eluting solvent under applied pressure. The material to be separated (maximum of 1,0g) was dissolved in a minimum amount of the eluting solvent and then transferred to the column. The separation was carried out under applied pressure to give an elution rate of 30cm^3 per 10 minutes.

3.1.2 Thin layer Chromatography (tlc)

Preparative thin layer chromatography (plc) plates were prepared by using Merck Kieselgel (Art 7747) on 200mm x 200mm glass plates. A slurry of silica gel in water (200g/475 cm^3) was uniformly spread (2mm) over the plates. The plates were air dried and then heated at 80°C for 24 hours before being used. The loading of material-to-be-separated was 10 to 25mg per plate. After developing the chromatogram in a separating tank with solvent, the plates were air dried and examined under ultraviolet light (λ 254nm and 360nm). The relevant bands of compounds were marked and scraped off. The silica gel was then extracted with acetone. The acetone was removed under reduced pressure and the residue dried in a vacuum oven. Micro-separations were carried out on commerically available aluminium backed silica gel plates (Merk Art 5554) with a loading of 3-5mg per plate.

3.1.2.1 Spray-reagent

Thin-layer chromatograms were sprayed lightly with a mixed solution of 5% p-anisaldehyde / 5% conc. H_2SO_4 / 90% ethanol, (v/v) then heated at 110° until maximum colour was developed.

3.2 MELTING POINTS

Melting points (which are uncorrected) were determined (°C) on a Koffler hot stage melting point apparatus.

3.3 SPECTROSCOPIC METHODS

3.3.1 Proton Magnetic Resonance Spectroscopy (^{1}HNMR)

^{1}HNMR spectra were recorded on a 300 MHz Bruker Spectrometer.

Chemical shifts are given on the delta (δ) scale and coupling constants (J) are accurate to 0,1 Hz. The abbreviations s,d,dd,t,q,m and br are used to denote singlet, doublet, doublet of doublets, triplet, quartet, multiplet and broad, respectively.

3.3.2 Mass Spectrometry

Mass spectra were recorded on a Kratos MS 80 RF spectrometer.

3.3.3. Infrared Spectroscopy (IR)

Infrared spectra were recorded on a Beckman Acculab 2 spectrophotometer.

3.4 CHEMICAL METHODS

3.4.1 Acetylation with Acetic Anhydride[179]

The dried material was dissolved in the minimum amount of pyridine and an excess of acetic anhydride was added. Heating the solution at about 50°C for 8 hours and then pouring it over crushed ice precipitated the acetate derivative on standing. The precipitate was filtered, washed free of excess pyridine and acetic anhydride with ice water, and dried in a vacuum oven.

3.4.2 Methylation with dimethyl sulphate[180]

A mixture of the phenolic compound (1 mmol) and anhydrous K_2CO_3 (4 mmol per phenolic group to be alkylated) in anhydrous acetone was refluxed. The dimethyl sulphate (1,2 mmol per phenolic group) was added to the refluxing solution with continuous stirring. The course of the reaction was followed by tlc. On completion of the reaction, the hot mixture was filtered under suction, and the filter washed with warm anhydrous acetone (x3). After removal of the acetone by distillation (vacuum), concentrated ammonia solution was added to the residue to destroy the excess dimethyl sulphate. An appropriate solvent system was used to recover the methylated product.

3.4.3 Methylation with diazomethane[181]

Dried phenolic material (150-200mg) was dissolved in methanol (50cm³) and the solution cooled to -10°C in an ice-salt bath. Diazomethane, generated by the reaction of KOH (8g) in ethanol (48cm³) and water (2cm³) with N-methyl-N-nitroso-p-toluenesulfonamide (diazald, 10g) in ether under mild refluxing, was transferred directly (by distillation) into the pre-prepared phenolic solution. The mixture was left in a deep-freeze at -10°C for 48 hours, after which the excess diazomethane was evaporated in a fume cupboard at room temperature.

3.4.4 Generation of Hydrogen Bromide[182]

Bromine was dropped at a steady rate into dry tetrahydronaphthalene. The hydrogen bromide formed was carried through the assembled train in a stream of air directly into the solution to be brominated.

3.4.5 Abbbreviations

The following abbreviations are used in describing the solvent systems employed for chromatographic separations.

A = acetone EA = ethyl acetate

B = benzene

CHAPTER 4

ISOLATION OF METABOLITES FROM VIRGILIA OROBOIDES

A log of Virgilia oroboides was kindly supplied by Mr. W.H. Strydom, Die Staatsbosbouer, Witfontein, Posbus 7, GEORGE.

4.1 EXTRACTION OF THE HEARTWOOD

The air-dried heartwood chips (25,5kg) of Virgilia oroboides were defatted with hexane (48 hours) followed by extraction with acetone (72 hours). The acetone was removed under reduced pressure at 45°C to give a light brown friable solid extract (1250g).

4.2 SEPARATION

The extract (25g) was subjected to Craig countercurrent separation using a solvent system of water : 2-butanol : hexane (5 : 3 : 2, v/v). Evaporation of the combined secondary butanol layers under reduced pressure at 50°C yielded a brown sticky extract (21,5g). The extract was subjected to column chromatography (Type 3 column). The column was eluted consecutively with B : A mixtures of 9 : 1 - 400 cm^3; 8 : 2 - 400 cm^3; 8 : 3 - 400 cm^3; 8 : 4 - 200 cm^3; 8 : 6 - 200 cm^3 and the fractions collected (as summarized in Table

3) in test tubes on a fraction collector.

Table 3 Summary of fractions

FRACTION	TEST TUBES	MASS (mg)
1	1-10	210
2	11-29	362
3	30-36	989
4	37-45	979
5	46-54	581
6	55-57	350
7	58-66	2191
8	67-70	553
9	71-75	1806
10	76-105	942
11	106-120	560
12	121-123	210

4.3 PLC separation of 120mg of fraction 1 (Table 3) using B : A, 9 : 1, (v/v) produced a main band with R_f 0,61.

4.3.1 3-Methoxy-8,9-methylenedioxypterocarpan (Pterocarpin)[32,183] [13]

The band with R_f 0,61 yielded the pterocarpan[13] (10mg) as a light brown crystalline solid.

M.P. 158-160°C [lit 163-164°[183]]

^{1}HNMR : δ[$CDCl_3$, 300MHz, 298K, plate 1]

7.38 (1H,d,J = 8.5Hz, H-1), 6.71 (1H, s, H -7),

6.61 (1H,dd,J = 8.5Hz and = 2.5Hz, H-2),

6.45(1H,d,J = 2.5Hz, H-4), 6.42(1H, s, H-10),

5.89(2H,q,J = 1.0Hz and 9.0Hz, O—CH_2-O),

5.47(1H,d, J 11a,6a = 6.9Hz, H-11a),

4.21(1H,dd,J6eq,6a = 5.1Hz, J6eq,6ax = 10.8Hz, H-6eq), 3.64(1H,dd,J6ax,6eq = 10.8Hz; J6ax,6a = 10.5Hz; H-6ax), 3.46(1H; ddd; J6a,6ax = 10.5Hz; J6a,6eq = 5.1Hz; J6a,11a = 6.9Hz; H-6a)

3.78(3H, s, -OCH_3)

4.4 PLC separation of 150mg of fraction 2 (Table 3) using, B :A, 9:1, (v/v) resulted in two bands with R_f 0,35 and 0,33.

4.4.1 3-Hydroxy-8,9-methylenedioxy-6a,11a-dihydropterocarpan [85]

The band with R_f 0,35 yielded white dendritic crystals (20mg) from an acetone-hexane mixture. The crystals decomposed at 210°C without melting.

Found : m/z 282.0524 $C_{16}H_{10}O_5$ requires 282.0527

^{1}HNMR : δ[$(CD_3)_2CO$, 300MHz, 297K, plate 3]
8.73(1H, br, 3-OH), 7.28(1H, d, J = 8.5Hz, H-1), 7.12(1H, s, H-7), 6.95(1H, s, H-10), 6.49(1H, dd, J = 2.0 and 8.5Hz, H-2), 6.41(1H, d, J = 2.0Hz, H-4), 6.02(s, O-CH_2-O), 5.51(2H, s, 6-CH_2).

MS, Scheme B : m/z 282(M^+, 96%), 281 (100), 253 (10.7), 223 (5.6), 195 (5.5)

4.4.2 3-Acetoxy-8,9-methylenedioxy -6a, 11a - dihydropterocarpan[85a]

Acetylation of 10mg of 3-hydroxy-8,9-methylenedioxy-6a,11a-dihydropterocarpan produced 6mg of the acetate as white needles, from an acetone-hexane mixture, with R_f 0,81 in B:A, 9:1, (v/v)

M.P. 191 - 193°

^{1}HNMR : δ[$CDCl_3$, 300MHz, 297K, plate 4]

7.41(1H;d;J = 8.5 Hz; H-1), 7.10(1H, s, H-7), 6.73(1H,s, H-10), 6.68(1H; dd; J = 8.5Hz and 2.0 Hz; H-2), 6.64(1H; d; J = 2.0 Hz; H-4) 5.99 (2H, s, 6-CH_2), 5.54(2H,s, O-CH_2-O), 2.28(3H, s, -OAc)

^{1}HNMR : δ[$(CD_3)_2$ CO, 300MHz, 296K]

7.43(1H, d, J = 8.5Hz, H-1), 7.16(1H, s, H - 7), 7.10(1H, s, H-10), 6.76(1H, dd, J = 2.0 and 8.5Hz, H-2), 6.69(1H, d, J = 2.0Hz, H-4), 6.05(2H, s, O-CH_2-O), 5.62(2H, s, 6-CH_2), 2.24(3H, s, 3-OAc).

4.4.3 (6aR, 11aR)-3-Hydroxy-8,9-methylenedioxy-pterocarpan (Maackiain)[183] [25]

The pterocarpan [25] was obtained as colourless plates (95mg) from the band with R_f 0,33.

M.P. : 176-179° [Lit[183] 175 - 177°]

^{1}HNMR : δ[$(CD_3)_2CO$, 300MHz, 297K, plate 2]

8.68(1H, s, 3-OH), 7.28(1H, d, J = 8.5Hz, H-1), 6.87(1H, s, H-7), 6.54(1H, dd, J = 8.5Hz and 2.5Hz, H-2), 6.38(1H, s, H-10), 6.34(1H, d, J = 2.5Hz, H-4) 5.90(2H, q, J = 1.3Hz and 9.0Hz, O-CH_2-O), 5.46(1H, d, J 11a,6a = 7.0Hz, H-11a), 4.25(1H, dd, J 6eq, 6ax = 10.7, J 6eq, 6a = 5.1Hz; H-6eq), 3.60(1H, dd,

J 6ax,6eq = 0.7, J 6ax,6a = 10.5Hz, H-6ax), 3.53(1H, ddd, J 6a,6ax = 10.5, J 6a,6eq = 5.1, J 6a,11a = 7.0Hz, H-6a).

MS, Scheme A : m/z 284 (M^+,100%), 283(25), 175(14), 162(32), 147(19), 134(33).

4.4.3.1 (6aR,11aR)-3-Acetoxy-8,9-methylenedioxy-pterocarpan[183] [25a]

Acetylation of Maackiain (20mg) gave 24mg of the acetate as colourless needles with an R_f 0,54 in B:A,9:1, (v/v).

M.P. 171-174° [Lit.[183] 176-177°]

^{1}HNMR : δ[$CDCl_3$, 300 MHz, 297K]
7.49(1H, d, J = 8.5 Hz, H-1), 6.77(1H, dd, J = 8.5Hz and 2.5 Hz, H-2), 6.70(1H, s, H-7), 6.68(1H, d, J = 2.5 Hz, H-4), 6.41(1H,s,H-10), 5.89(2H,q, J = 1.3Hz and 9.0Hz, O-CH_2-O), 5.47 (1H, d, J 11a,6a = 7.0Hz, H-11a), 4.23(1H, dd, J 6eq,6ax = 11.1Hz, J 6eq, 6a = 5.2Hz, H-6eq), 3.62(1H, dd, J 6ax, 6eq = 11.1Hz, J 6ax,6a = 10.7Hz, H-6ax), 3.48(1H, ddd, J 6a,6ax = 10.7Hz, J 6a,6eq = 5.2Hz, J 6a,11a = 7.0Hz, H-6a), 2.28(3H, s, -OAc).

4.4.3.2 (6aR,11aR)-3-Methoxy-8,9-methylenedioxy-pterocarpan[32,183][13]

Methylation of Maackiain (30mg) with dimethyl sulphate afforded the methyl ether as light brown crystals.

M.P. 159-162° [Lit. [183] 163 - 164°]

^{1}HNMR : Identical spectrum to that of natural pterocarpin [13], (^{1}HNMR Plate 1).

4.5 Fraction 3 (Table 3) was separated (200mg : B : EA : A 7 : 2 : 1, v/v) by plc and two bands of R_f 0,55 and 0,50 were obtained.

4.5.1 (2R)-7-Hydroxyflavanone[185][87]

Creamy white needles (52mg) of the flavanone were obtained from the band with R_f 0,55.

M.P. 188-190° [Lit. [185] 190 - 191°]

^{1}HNMR : δ[$(CD_3)_2CO$, 300MHz, 297K, plate 5]
7.73(1H, d, J = 9.0Hz, H-5), 7.35-7.59(6H, m, H-2', 6',3',4',5'), 6.58(1H, dd, J = 9.0Hz and 2.5Hz, H-6), 6.45(1H, d, J = 2.5Hz, H-8), 5.56(1H, dd, J = 3.0 and 13.0Hz, H-2), 3.04(1H, dd, J = 13.0

and 17.0Hz, H-3ax), 2.74(1H, dd, J = 3.0 and 17.0Hz H-3eq).

4.5.1.1 (2R)-7-Acetoxyflavanone[185] [87a]

Acetylation of (2R)-7-hydroxyflavanone (10mg), produced the acetate as white needles (11mg) with an of R_f 0,51 (B : A, 9 : 1, v/v)

M.P. 90 - 92° [Lit.[185] 93 - 94°]

^{1}HNMR δ[$CDCl_3$, 300MHz, 297K].
7.95(1H, d, J = 8.6Hz, H-5), 7.35 - 7.49(6H, m, H-2',3',4',5',6'), 6.82(1H, d, J = 2.5Hz, H-8), 6.79(1H, d, J = 2.5 and 9.0Hz, H-6), 5.49(1H, dd, J = 3.0 and 13.0Hz, H-2), 3.07(1H, dd, J = 13.0 and 17.0Hz, H-3ax), 2.87(1H, dd, J = 3.0 and 17.0Hz, H-3eq), 2.31(3H, s, -OAc).

4.5.2 (±)-7-Hydroxy-4'-methoxyisoflavanone[186] [88]

The band with R_f 0,50 produced a light brown amorphous solid (19mg).

M.P. 187 - 189° [Lit[186] 185 - 188°)

^{1}HNMR : δ[$(CD_3)_2CO$, 300MHz, 297K, plate 7]

7.73(1H, d, J = 8.5Hz, H-5), 7.22(2H, d, J = 9.0Hz, H-2',6'), 6.88(2H, d, J = 9.0Hz, H-3',5'), 6.57(1H, dd, J = 2.5 and 8.5Hz, H-6), 6.40(1H, d, J= 2.5 Hz, H-8), 4.63(2H, m, H-2eq, H-2ax), 3.89(1H, dd, J = 6.0 and 8.0Hz, H-3), 3.76(3H, s, $-OCH_3$).

4.5.2.1 7,4'-Dimethoxy-isoflavanone[186] [88a]

Methylation of 7-hydroxy-4'-methoxy-isoflavanone afforded the dimethyl ether (6mg) with R_f 0,50 (B : A, 9 : 1, v/v).

M.P. 131 - 132° [Lit.[186] 129 - 130°]

^{1}HNMR : δ[$CDCl_3$, 300MHz, 298K].

7.87(1H, d, J = 9.0Hz, H-5), 7.18(2H, dd, J = 9.0Hz, H-2',6'), 6.86(2H, dd, J = 9.0Hz, H-3',5'), 6.59(1H, dd, J = 2.5 and 9.0Hz, H-6), 6.42(1H, d, J = 2.5Hz, H-8), 4.65(1H, dd, J = 6.0 and 11.5Hz, H-2eq), 4.57(1H, dd, J = 8.0 and 11.5Hz, H-2ax), 3.86(1H, dd, J = 6.0 and 8.0Hz, H-3), 3.77(3H, s, $-OCH_3$), 3.83(3H, s, $-OCH_3$).

4.6 PLC separation of fraction 5 [Table 3] (150mg, B:EA:A, 7 : 2 : 1, v/v) produced three bands with R_f 0,45 (20mg); 0,42 (50mg) and 0,39 (60mg).

4.6.1 (2R, 3R) - 3,7-Diacetoxy-dihydroflavonol [90]

Acetylation of the band with R_f 0,45 followed by plc (B : A, 9 : 1, v/v) afforded the dihydroflavonol as a gummy compound (5mg).

^{1}HNMR : δ[$CDCl_3$, 300MHz, 296K, plate 12]

7.93(1H, d, J = 8.0Hz, H-5), 7.49-7.40(6H, m, H-2', 3',4',5',6'), 6.84(1H, dd, J = 2.0 and 8.0Hz, H-6), 6.82(1H, d, J = 2.0Hz, H-8), 5.80(1H, d, J = 12.0Hz, H-2), 5.43(1H, d, J = 12.0Hz, H-3), 2.31(3H, s, -OAc), 2.0(3H, s, -OAc).

CD : (c 0.3, CH_3OH)

$[\theta]_{364}$ 0,0 ; $[\theta]_{328}$ +4,4 x 10^{-4} ; $[\theta]_{316}$ 0,0 ; $[\theta]_{297}$ -7,4 x 10^{-4} ; $[\theta]_{262}$ -2,2 x 10^{-4} ; $[\theta]_{250}$ +3,5 x 10^{-4} ; $[\theta]_{236}$ 0,0 ; $[\theta]_{228}$ +2,9 x 10^{-4} ; $[\theta]_{225}$ 0,0.

4.6.2 (αS), 2',4'-Trihydroxy-4-methoxydihydrochalcone [91]

The band with R_f 0,42 afforded 20mg of the dihydrochalcone, as a non-crystalline gummy compound.

^{1}HNMR : δ[$CDCl_3$, 300MHz, 296K, plate 13]

10.29(1H, s, 2'-OH), 7.56(1H, d, J = 9.0Hz, H-6'), 7.03(2H, d, J = 9.0Hz, H-2,6), 6.79(2H, d, J = 9.0Hz, H-3,5), 6.41(1H, d, J = 2.5Hz, H-3'), 6.41(1H, dd, J = 2.5 and 9.0Hz, H-5'), 5.19(1H, dd, J = 4.0 and 7.0Hz, H-α), 3.12(1H, dd, J = 4.0 and 14.0Hz, H-β), 2.89(1H, dd, J = 7.0 and 14.0Hz, H-β), 3.76(3H, s, -OMe).

MS, Scheme D : m/z 288(M^+,0%), 270(M^+-18, 5.1%),
137(45), 134(3.4), 152(3.0), 121(100)

4.6.3. (αS), 2',4'-Triacetoxy-4-methoxydihydrochalcone [91a]

Acetylation of (αS), 2',4'-trihydroxy-4-methoxydihydrochalcone (15mg) yielded 11mg of the triacetate derivative (R_f 0,51) after plc, (B : A, 9 : 1, v/v), together with a minor band with R_f 0,64. None crystalline.

^{1}HNMR : δ[$CDCl_3$, 300MHz, 296K, plate 14]
7.76(1H, d, J = 9.0Hz, H-6'), 7.09(1H, dd, J = 2.5 and 9.0Hz, H-5'), 7.08(2H, d, J = 9.0Hz, H-2,6), 6.99(1H, d, J = 2.5Hz, H-3'), 6.79(2H, d, J = 9.0Hz, H-3,5), 5.89(1H, dd, J = 4.0 and 7.0Hz, H-α), 3.05(1H, dd, J = 4.0 and 14.0Hz, H-β), 2.91(1H, dd, J = 7.0 and 14.0Hz, H-β), 2.28 - 2.00(3 x -OAc), 3.76(3H, s, -OMe).

MS : m/z 414(M^+, 1.1%), 354(34), 312(29), 270(18), 221(17), 179(51), 137(78), 134(12), 121(100).

CD : (c 0.0600, CH_3OH, figure 1)
$[\theta]_{210}$ 0, $[\theta]_{233}$ +2,0 x 10^{-4}, $[\theta]_{258}$ 0, $[\theta]_{267}$ -0,3 x 10^{-4}, $[\theta]_{281}$ 0, $[\theta]_{295}$ +0,2 x 10^{-4}, $[\theta]_{343}$ 0.

4.6.4 (αS),4'-diacetoxy-2'-hydroxy-4-methoxydihydrochalcone [91b]

The minor band with R_f 0,64 (par. 4.6.3) afforded the diacetate derivative. None crystalline.

Found m/z 372.1201 $C_{20}H_{20}O_7$ requires 372,1207

^{1}HNMR : δ[$CDCl_3$, 300MHz, 296K, plate 15]
10.25(1H, s, 2'-OH), 7.73(1H, d, J = 9.0Hz, H-6'), 7.12(2H, d, J = 9.0Hz, H-2,6), 6.81(2H, d, J = 9.0 Hz, H-3,5), 6.75(1H, d, J = 2.5Hz, H-3'), 6.67(1H, dd, J = 2.5 and 9.0Hz, H-5'), 5.93(1H, dd, J = 4.0 and 7.0Hz, H-α), 3.15(1H, dd, J = 4.0 and 14.0Hz, H-β), 3.05(1H, dd, J = 7.0 and 14.0Hz, H-β), 2.30 and 2.10(-OAc), 3.77(3H, s, -OMe).

MS, Scheme E : m/z, 372(M^+, 3.02%), 312(37.58), 270(17.2), 179(25.89), 137(100), 134(11.32), 109(3.19).

4.6.5 7-Hydroxy-4'-methoxyisoflavone (Formononetin)[187,188] [10]

Formononetin[10] was obtained (60mg) as white nodules from the band with R_f 0,39 .

M.P. 259 - 261° [Lit.[187] 260 - 261°]

^{1}HNMR : δ[$(CD_3)_2CO$, 300MHz, 298K, plate 8]
8.17(1H, s, H-2),, 8.05(1H, d, J = 8.5Hz, H-5), 7.55(2H, d, J = 9.0Hz, H-2',6'), 6.99(1H, dd, J = 2.0 and 8.5Hz, H-6), 6.97(2H, d, J = 9.0Hz, H-3',5'), 6.89(1H, d, J = 2.0Hz, H-8), 3.82(3H, s, $-OCH_3$).

4.6.5.1 7-Acetoxy-4'-methoxyisoflavone[189] [10a]

Acetylation of 7-hydroxy-4'-methoxyisoflavone (20mg) yielded 22mg of the acetate as white needles with an R_f 0,51 (B : A, 9 : 1, v/v).

M.P. 165 - 169° [Lit.[189] 169°]

^{1}HNMR : δ[$CDCl_3$, 300MHz, 297K].
8.31(1H, d, J = 8.5Hz, H-5), 7.96(1H, s, H-2), 7.48(2H, d, J = 9.0Hz, H-2',6'), 7.28(1H, d, J = 2.0Hz, H-8), 7.15(1H, dd, J = 2.0 and 9.0Hz,

H-6), 6.96(2H, d, J = 9.0Hz, H-3',5'), 3.83(3H, s, $-OCH_3$), 2.35(3H, s, -OAc).

MS, Scheme C : m/z 310(M^+, 77.8%), 268(100), 267(52.7), 137(2), 132(86.5)

4.7 Fraction 9 (Table 3) was separated by plc (250mg , B : EA : A, 7 : 2 : 1, v/v) and two main bands with R_f 0,32 and 0,23 were obtained.

4.7.1 (±)-7,4'-Dihydroxyflavanone [(±)-Liquiritigenin][185,190] [93]

The band with R_f 0,32 yielded a white non-crystalline solid (40mg).

M.P. 195 - 197° [Lit.[185] 197 - 198°]

^{1}HNMR : δ[$CDCl_3$, 300MHz, 298K, plate 6].
7.74(1H, d, J = 8.3Hz, H-5), 7.25(1H, d, J = 8.3Hz, H-2',6'), 6.84(1H, d, J = 8.3Hz, H-3',5'), 6.50(1H, dd, J = 2.4 and 8.3, H-6), 6.39(1H, d, J = 2.4Hz, H-8), 5.29(1H, dd, J = 3 and 13.0Hz, H-2), 2.96(1H, dd, J = 13.0 and 17.0, H-3ax), 2.68(1H, dd, J = 3.0 and 17.0, H-3eq).

4.7.1.1 (±)-7,4'-Dimethoxyflavanone[93a]

Methylation of (±)-7,4'-dihydroxyflavanone (10mg) with diazomethane afforded the dimethyl ether (9mg) with Rf 0,49 (B : A, 9 : 1, v/v). None crystalline.

^{1}HNMR : δ[$CDCl_3$, 300MHz, 298K,]
7.85(1H, d, J = 8.4Hz, H-5), 7.39(1H, dd, J = 9.0Hz, H-2',6'), 6.94(1H, dd, J = 9.0Hz, H-3',5'), 6.59(1H, dd, J = 2.5 and 8.4Hz, H-6), 6.46(1H, d, J = 2.5Hz, H-8), 5.40(1H, dd, J = 3.0 and 13.0Hz, H-2), 3.82(6H 2 x s, 7,4'-OMe), 3.05(1H, dd, J = 13 and 17.0 Hz, H-3ax), 2.78(1H, dd, J = 3 and 17.0, H-3eq).

4.7.2 3',7-Dihydroxy-4'-methoxyisoflavone (Calycosin)[191,192] [94]

The band with R_f 0,32 afforded a colourless crystalline solid (65mg).

M.P. 236 - 238° [Lit.[191] 240 - 242°]

^{1}HNMR : δ[$(CD_3)_2CO$, 300MHz, 296K, plate 9]
8.15(1H, s, H-2), 8.05(1H, d, J = 8.5Hz, H-5), 7.15(1H, d, J= 3.0Hz, H-2'), 7.06(1H, dd, J = 3.0 and 8.5Hz, H-6'), 6.98(1H, dd, J = 3.0Hz and 8.5Hz,

H-6), 6.96(1H, d, J = 8.5Hz, H-5'), 6.89(1H, d, J = 3.0Hz, H-8), 3.86(3H, s, $-OCH_3$).

MS : m/z 284(M^+, 100%), 283(100), 269(18), 241(13), 213(13), 137(28), 126(18), 112(26), 105(20).

4.7.2.1 3',7-Diacetoxy-4'-methoxyisoflavone[191,192] [94a]

Acetylation of 3'7-dihydroxy-4'-methoxyisoflavone (30mg) yielded a white crystalline solid (18mg) with R_f 0,29 after plc separation (B : A, 9 : 1, v/v).

M.P. 198 - 200°C

^{1}HNMR : δ[$CDCl_3$, 300MHz, 296K]
8.29(1H, d, J = 8.5Hz, H-5), 7.98(1H, s, H-2), 7.42(1H, dd, J = 3.0Hz and 8.5Hz, H-6'), 7.29(1H, d, J = 3.0Hz, H-8), 7.28(1H, d, J = 3.0Hz, H-2'), 7.15(1H, dd, J = 3.0 and 8.5Hz, H-6), 7.06(1H, d, J = 8.5Hz, H-5'), 3.86(3H, s, $-OCH_3$), 2.35(3H, s, -OAc), 2.31(3H, s, -OAc).

4.8 Acetylation of fraction 12, [Table 3] (50mg), afforded two main bands with R_f 0,26 and 0,20 (B : A, 9 :1, v/v).

4.8.1 (2R,3R)-3,3',4',7-Tetra-acetoxy-dihydroflavanol (+)-Fustin[193,195] [95]

(+)Fustin (5mg) was obtained as a non-crystalline solid from the band with R_f 0,26 , B : A, 9 : 1, v/v).

M.P. 148 - 151° [Lit.[195] 150 - 151°]

^{1}HNMR : δ[$CDCl_3$, 297K, 300MHz, plate 10]
7.93(1H, d, J = 8.5Hz, H-5), 7.40(1H, dd, J = 2.0 and 8.5Hz, H-6'), 7.31(1H, d, J = 2.0Hz, H-2'), 7.26(1H, d, J = 8.5Hz, H-5'), 6.86(1H, dd, J = 2.0. and 8.5Hz, H-6), 6.82(1H, d, J = 2.0Hz, H-8), 5.70(1H, d, J = 12Hz, H-2), 5.43(1H, d, J = 12.0Hz, H-3), 2.29 - 2.31(12H, 3 x s, 4 x -OAc).

4.8.2 3,3',4',7-Tetra-acetoxy-flavone[194,195] (Fisetin) [96]

Fisetin (3mg) was obtained as a non-crystalline solid from the band with R_f 0,20 (B : A, 9 : 1, v/v).

M.P. 199 - 200° [Lit.[195] 201.5°]

^{1}HNMR : δ[$CDCl_3$, 300MHz, 297K, plate 11]

8.25(1H, d, J = 10.0Hz, H-5), 7.75(1H, dd, J = 2.0 and 8.70Hz, H-6'), 7.72(1H, d, J = 2.0Hz, H-2'), 7.38(1H, d, J = 2.0Hz, H-8), 7.34(1H, d, J = 8.7Hz, H-5'), 7.17(1H, dd, J = 2.0 and 10.0Hz, H-6), 2.36(3H, s, -OAc), 2.35(3H, s, -OAc), 2.33(6H, s, -OAc).

CHAPTER 5

ISOLATION OF METABOLITES FROM CHLOROPHORA EXCELSA

The heartwood material was kindly supplied by Mr. J. Vorster of the Department of Indian Education Inspectorate in Durban. A specimen of the heartwood was kindly confirmed by Dr. B.J. Ter Welle University of Utrecht, Institute for Systematic Botany, Utrecht, Netherlands.

5.1 EXTRACTION OF THE HEARTWOOD

The air-dried milled heartwood (15kg) of Chlorophora excelsa was defatted with hexane (48 hours) followed by extraction with acetone (48 hours), and the extract evaporated under reduced pressure to give a dry dark brown material (350g).

The extract(77g) was separated by repeated column chromatography - (Silica gel - Type 3 columns x 7g each). Fractions of 50cm^3 were collected in beakers by eluting each column consecutively with B : A mixtures of (9:1 - 300 cm^3); (8:2 - 250 cm^3), (8:4 - 250 cm^3) and (1:1 - 300 cm^3).

The fractions were surveyed over tlc (B : EA : A; 7:2:1 v/v) and combinations were made to give enriched fractions as summarized in Table 4.

Table 4 Summary of fractions

FRACTION	MASS(mg)
1.	1071
2.	200
3.	495
4.	945
5.	288
6.	675
7.	37458
8.	6753
9.	11610
10.	1809
11.	7092

5.2 PLC separation of fraction 2 ,[Table 4] (200mg), produced four bands with R_f values of 0.33, 0.32, 0.34 (B:A, 9:1, v/v) and 0.54 (B:A, 8:3, v/v).

5.2.1 3,5-Dihydroxybenzaldehyde[196] [97]

The band with R_f 0,33 yielded the aldehyde as colourless needles (15 mg).

M.P. 156-157° [Lit[196] 156 - 157°]

5.2.2 2,4-Dihydroxybenzaldehyde[197] [98]

This aldehyde was obtained as pale needles (60 mg) from the band with R_f 0.32.

M.P. 199-202° [Lit[197] 201 - 202°]

5.2.3 3,4',7-tri-O-methylquercitin (Ayanin)[198,199] [99]

Ayanin was obtained as a crystalline solid (35 mg) from the band with R_f 0,34 .

M.P. 172-174° [Lit[198] 172 - 173°]

^{1}HNMR : δ[$(CD_3)_2CO$, 300MHz, 296K, plate 29]
δ8.72 (1H, s, -OH), 8.10 (1H, br, -OH),
7.69(1H, dd, J = 2.0 and 9.0Hz, H-6'), 7.64(1H, d, J = 2.0Hz, H-2'), 7.12(1H, d, J = 9.0Hz. H-5'),
6.68(1H, d, J = 2Hz, H-8), 6.31(1H, d, J = 2.0Hz, H-6), 3.94(3H, s, $-OCH_3$), 3.92(3H, s, $-OCH_3$),
3.87(3H, s, $-OCH_3$)

5.2.4 2'-methoxy -3,4',7-tri-O-methylquercitin [100]

The band with R_f 0.54 produced 20mg of the non-crystalline substituted quercitin .

Found m/z 374.0997 $C_{19}H_{18}O_8$ requires 374.0999

M.P. 160 - 163°

^{1}HNMR : δ [$(CD_3)_2CO$, 300MHz, 296K, plate 30]
8.70(1H, s, -OH), 7.61(1H, br, -OH), 6.98(1H, s, H-6'), 6.86 (1H, s, H-3'), 6.50(1H, d, J = 2.0Hz, H-8), 6.31(1H, d, J = 2.0Hz, H-6), 3.95 (3H, s, $-OCH_3$), 3.89(3H, s, $-OCH_3$), 3.84(3H, s, $-OCH_3$), 3.78(3H, s, $-OCH_3$).

MS : m/z 374 (M^+,100%), 373 (42.9), 344 (20.9), 343 (86.6), 167 (38.5).

5.2.5 5,5'-Diacetoxy-2',3,4',7-tetramethoxyflavone [100a]

Acetylation of (12mg) of 2',-methoxy-3,4',7-tri-O-methyl-quercitin [100] produced 10mg of the diacetate derivative as long white needles from acetone (R_f 0.22; B:A, 9:1, v/v).

M.P. 188 - 190°C.

^{1}HNMR : δ[$(CD_3)_2CO$, 300MHz, 296K, plate 31]

7.21(1H, s, H-6'), 6.93(1H, s, H-3'), 6.91(1H, d, J = 2.0Hz, H-8), 6.65(1H, d, J = 2.0Hz, H-6), 3.94(3H, s, $-OCH_3$), 3.93(3H, s, $-OCH_3$), 3.91(3H, s, $-OCH_3$), 3.71(3H, s, $-OCH_3$), 2.33(3H, s, -OAc), 2.23(3H, s, -OAc).

MS, Scheme I ; m/z 458(M^+, 34%), 416 (24), 415 (17), 374 (65), 373 (54), 359 (15), 344 (23), 343 (100), 167 (32).

5.3 Separation of fraction 4 ,[Table 4] (600mg), on a Sephadex LH20 column produced fractions as summarized in Table 5.

Table 5 Summary of fractions

Fractions	Beakers (40 c.c. fractions)	Mass(mg)
1	1 - 14	97
2	15 - 18	195
3	19 - 24	248

5.4 Two bands with R_f 0,33 and 0,26 were obtained by plc separation (B:A , 9:1 v/v) of 195mg of fraction 2, [Table 5].

5.4.1 3,5-Dihydroxy-4-geranylbenzaldehyde[200] [101]

The band with R_f 0,26 produced a non-crystalline colourless solid (30mg) which turned into a brown residue when it was not kept under constant vacuum.

Found : m/z 274.1547 $C_{17}H_{22}O_3$ requires 274,1568

M.P. 80 - 81°

^{1}HNMR : δ[$(CD_3)_2CO$, 300MHz, 296K, plate 16]
9.75(1H, s, CHO), 8.76(2H, s, OH-3,5). 6.93(2H, s, H-2,6).
Geranyl group protons:
Refer to Table 7, par. 5.8.

MS, Scheme F : m/z 274(M^+,12.1%), 151(M^+-123, 100), 205(7.5), 191(20), 189(51.4).

5.5 Fractions 3(495mg), 4(345mg) and 5(288mg), [Table 4], were combined and chromatographed on a flash column (B : A , 9 : 1, v/v). Fractions were collected in test tubes as

shown in Table 6.

Table 6 Summary of fractions

Fractions	Test Tubes	Mass (mg)
1	1-12	50
2	13-14	491
3	15-16	198
4	17-20	179

5.5.1 Fraction 3, [Table 6] (100mg), was methylated with dimethyl sulphate and separated by plc (B : A, 9 : 1 v/v) to produce three bands with R_f 0,50: 0,45 and 0,40.

5.5.2 3,5-Dimethoxy-4-geranylbenzaldehyde[200] [101a]

The band with R_f 0.45 yielded the dimethyl ether as a non-crystalline gummy compound (25mg).

Found : C, 75.34%; H, 8.75% $C_{19}H_{26}O_3$ requires C, 75.46%; H, 8.67%

Found : m/z 302.1879 ($C_{19}H_{26}O_3$ requires 302.1880)

IR : $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1700

^{1}HNMR : δ[$CDCl_3$, 300MHz. 296K, plate 17]

10.08 (s, 1H, CHO), 7.24(2H, s, H-2,6),

4.10(6H, s, 2 x OMe)

Geranyl protons:

Refer to Table 7, par.5.8.)

MS, m/z 302(M^+,14.3%),233(39),219(10.5) 217(8.5), 179(100)

5.5.3 3,5-Dimethoxy-4-geranylbenzyl alcohol[200] [101b]

3,5-Dimethoxy-4-geranylbenzaldehyde (25mg) was reduced with excess $LiAlH_4$ (50mg) in dry ether (5 cm^3) at 0°C with stirring for 10 minutes. Cold water (3 cm^3) was added followed by 6M HCl (2 cm^3). The mixture was extracted with ether. After drying the organic layer with anhydrous Na_2SO_4, the ether was evaporated under reduced pressure to afford a product (20mg), which was separated by PLC (B : A, 9 : 1, v/v) to give a band with R_f 0,69. The alcohol (6mg) was obtained as a non-crystalline gummy compound from this band.

^{1}HNMR : δ[$CDCl_3$, 300MHz, 297K, plate 18]

6.54(2H, s, H-2,6), 4.63(2H, s, $-CH_2-$) 3.80(6H, s, 2x-OMe)

Geranyl protons

Refer to Table 7, par.5.8 .

5.6 PLC separation of fraction 3 , [Table 5] (200mg, B : A, 9 : 1, v/v) resulted in two bands with R_f 0,26 and 0,23.

5.6.1 3',4,5'-Trihydroxy-4'-geranylstilbene(200) [102]

The band with R_f 0,23 yielded the substituted stilbene as a non-crystalline brown solid (160mg).

M.P.: 138-140°

Found: C, 79.113%; H, 7.673% $C_{24}H_{28}O_3$ requires C, 79.077%; H, 7.748%

m/z 364.2045 requires 364.2036

m/z 241.0866 requires 241.0863

^{1}HNMR : δ[$(CD_3)_2CO$, 300 MHz, 296K, plate 19]

7.37(2H, d, J = 8.5Hz, H-2,6), 6.91(1H, d, J = 16.5, Hz, H-α), 6.81(1H, d, J = 16.5Hz, H-β), 6.81(2H, d, J = 8.5Hz, H-3,5), 6.58(2H, s, H-2',6').

Geranyl protons:

Refer to Table 7, par. 5.8 .

MS: Scheme G ; m/z 364 (M^+,25%), 295(18), 281(16), 279(34), 241(100), 123(14), 107(22.4)

5.6.1.1 3',4,5'-Trimethoxy-4'-geranylstilbene[200] [102a]

Methylation of 3',4,5'-trihydroxy-4'-geranylstilbene (50mg) with dimethyl sulphate and subsequent plc separation (B : A, 9 : 1, v/v) afforded the trimethyl ether as a non-crystalline solid (51mg) with an R_f of 0,40.

Found : m/z 406.2504 $C_{27}H_{34}O_3$ requires 406.2505

M.P. 74 - 75°

^{1}HNMR : δ[($CDCl_3$, 300MHz, 296K, plate 20]

7.38(2H, d, J = 8.5Hz, H-2,6), 6.95(1H, d, J = 16.5Hz, H-α), 6.86(1H, d, J = 16.5Hz, H-β), 6.83(2H, d, J = 8.5Hz, H-3,5), 6.61(2H, s, H-2',6'), 3.84 - 3.81(9H, 3 x OMe).

Geranyl protons

Refer to Table 7, par. 5.8 .

5.7 2,3',4,5'-Tetrahydroxy-4'-geranylstilbene (Chlorophorin)[127,200] [47]

Fraction 7, [Table 4], yielded a non-crystalline light brown solid (R_f 0.05 , B : A, 9 : 1, v/v) in large quantity which required no further purification.

M.P. 154 - 156° [Lit.[127] 157 - 159°]

^{1}HNMR : δ[$(CD_3)_2CO$, 300MHz, 296K, plate 23]

7.37(1H, d, J = 8.5Hz, H-6), 7.25(1H, d, J = 16.5Hz, H-α), 6.80(1H, d, J = 16.5Hz, H-β), 6.56(2H, s, H-2',6'), 6.41(1H, d, J = 2.5Hz, H-3), 6.35(1H, dd, J = 2.5Hz and 8.5Hz, H-5).

Geranyl protons

Refer to Table 7, par. 5.8 .

MS, Scheme H ; m/z 380 (M^+, 22%), 311 (19), 297 (15), 295 (32), 257 (100), 123 (20 and 12 %).

5.7.1 2,3',4,5'-Tetramethoxy-4'-geranylstilbene[127,200] [47a]

Methylation of 2,3',4,5'-tetramethoxy-4'-geranylstilbene (500mg) with dimethyl sulphate afforded the tetramethyl ether (47a; 391mg; R_f 0,68) after plc separation (B : A; 9 : 1, v/v).

M.P. 73-74° [Lit 73-74°[127]]

^{1}HNMR : δ [($CDCl_3$, 300MHz, 296K, plate 24]

7.49(1H, d, J = 8.5Hz, H-6), 7.30(1H,d, J = 16.5Hz, H-α), 6.96(1H, d, J = 16.5Hz, H-β), 6.68(2H, s, H-2',6'), 6.50(1H, dd, J = 2.5Hz and 8.5Hz, H-5), 6.46(1H, d, J = 2.5Hz, H-3), 3.86-3.82(12H, 4xs, 4x -OMe)

Geranyl protons

Refer to Table 7, par. 5.8 .

MS, m/z 436 (M^+, 21%), 313 (M^+ - 123, 100%), 367 (44), 353 (14), 351 (22) .

5.8 The chemical shifts of the geranyl protons of the compounds [101], [101a] , [101b], [102], [102a], [47] and [47a] are summarized in Table 7 .

TABLE 7 CHEMICAL SHIFTS(δ) OF GERANYL GROUP PROTONS

Compound	Protons						
	1	2	4	5	6	8	1'
[101]	3.42d*	5.29m	1.94m	2.02m	5.06m	1.59d†	1.77d†
						1.54d†	
[101a]	3.58d*	5.33m	2.13m	2.22m	5.23m	1.82d†	1.95d†
						1.75d†	
[101b]	3.31d*	5.14m	1.95m	2.04m	5.03m	1.61d†	1.74d†
						1.55d†	
[102]	3.35d*	5.31m	1.94m	2.02m	5.07m	1.61d†	1.77d†
						1.55d†	
[102a]	3.27d*	5.12m	1.88m	1.97m	5.00m	1.58d†	1.69d†
						1.50d†	
[47]	3.34d*	5.31m	1.94m	2.02m	5.07m	1.61d†	1.77d†
						1.55d†	
[47a]	3.32d*	5.17m	1.94m	2.03m	5.06m	1.64d†	1.75d†
						1.56d†	
	*(d,J=7Hz)					†(d, J= 1 Hz)	

5.9 2,3',4,5'- Tetrahydroxystilbene[117,200,201] [103]

Without further purification of fraction 9, [Table 4] the stilbene was obtained in bulk quantity as a light brown crystalline solid (R_f 0.03 , B : A, 9 : 1, v/v).

M.P. 197-199° [Lit.[201] 203 - 208°]

^{1}HNMR : δ[$(CD_3)_2CO$, 300MHz, 296K, plate 25]
8.40(2H, br, 2x-OH), 7.39(1H, d, J = 8.5Hz, H-6), 7.32(1H, d, J = 16.5Hz, H-α), 6.88(1H, d, J = 16.5Hz, H-β), 6.52(2H, d, J = 2.5Hz, H-2',6'), 6.43(1H, d, J = 8.5 and 2.5Hz, H-3), 6.38(1H, d, H-5), 6.23(1H, t, J = 2.5Hz, H-4'), 3.60(2H, br, 2 x -OH).

MS : m/z 244 (M^+, 100%), 226(49.1), 225(64.8), 197(60.6), 186(18).

5.9.1 2,3',4,5'-Tetramethoxystilbene[117,200,201] [103a]

Methylation of 2,3',4,5'-tetramethoxystilbene (150mg) with dimethyl sulphate afforded the methoxylated stilbene (98mg) as a brown crystalline solid after plc separation (B : A, 9 : 1, v/v), with R_f 0,56.

M.P. 82-83° [Lit[201] 83 -84°]

^{1}HNMR : δ [$CDCl_3$, 300MHz, 296K, plate 26]

7.49(1H, d, J = 8.5Hz, H-6), 7.38(1H, d, J = 16.5Hz, H-α), 6.94(1H, d, J = 16.5Hz, H-β), 6.68 (2H, d, J = 2.5Hz, H-2',6'), 6.51(1H, dd, J = 8.5 and 2.5Hz, H-5), 6.46(1H, d, J = 2.5Hz, H-3), 6.37(1H, t, J = 2.5Hz, H-4'), 3.83(12H, 4 x OMe).

5.10 PLC separation of fraction 10 , [Table 4] (200mg : B : A, 8 : 6, v/v) produced a minor band with R_f 0,37.

5.10.1 3',4,5'-Trihydroxystilbene[117,120,125,200,202] [104]

The stilbene was obtained as light brown needles (15mg) from the band with R_f 0,37 .

M.P. 260° [Lit[202] 261°]

^{1}HNMR : δ [$(CD_3)_2CO$, 300MHz, 296K, plate 21]

7.40(2H, d, J = 9.0Hz, H-2,6), 6.82(2H, d, J = 9.0Hz, H-3,5), 6.56(2H, d, J = 2.0Hz, H-2',6'), 6.27 (1H, t, J = 2.0Hz, H-4'), 7.01(1H, d, J = 16.0Hz, H-α), 6.87(1H, d, J = 16.0Hz, H-β).

MS : m/z 228(M^+, 100%), 227(12), 211(5), 181(8)

5.10.1.1 3',4,5'-Trimethoxystilbene[200,203] [104a]

Methylation of 3',4,5'-trihydroxystilbene (8mg) with dimethyl sulphate yielded the trimethyl ether (7mg) as light yellow needles.

M.P. 55-57° [Lit[203]56 - 57°]

^{1}HNMR : [$CDCl_3$, 300MHz, 296K, plate 22]
7.44(2H, d, J=9.0 Hz, H-2,6), 6.88(2H, d, J=9.0 Hz, H-3,5), 6.64(2H, d, J=2.0 Hz, H-2',6') 6.37(1H, t, J=2.0 Hz, H-4'), 7.03(1H, d, J=16.0 Hz, H-α), 6.89 (1H, d, J=16.0 Hz, H-β), 3.84(9H, s,3×OMe)

MS , m/z 270 (M^+, 100%), 269(6), 255(3)

5.11 Qualitative tlc (B : EA : A, 7 : 2 : 1, v/v) of fraction 8, [Table 4], showed low concentration compounds appearing between [47] and [103]. Several attempts to isolate these compounds (in appreciable concentrations) failed. It was therefore decided to isolate them as their methyl ethers. A further 84g of the heartwood acetone extract was chromatographed (silica gel Type 3 columns). Fractions of 100 cm^3 were collected using the gradient eluting procedure described in section 5.1. Relevant combinations were made to give one enriched fraction (1741mg) containing the required compounds, this was

separated on a flash column (B : EA : A, 7 : 2 : 1, v/v), the results of which are summarized in Table 8.

Table 8 Summary of fractions

Fractions	Beakers (20 c.c)	Mass (mg)
1	1 - 4	90
2	5 - 7	154
3	8 - 12	178
4	13 - 22	179
5	23 - 26	71
6	27 - 40	188
7	41 - 50	74

5.12 Fraction 5 (71mg), [Table 8], was methylated with diazomethane and separated by plc (B : A, 9 : 1, v/v) to produce a band with R_f 0,19.

5.12.1 2',4',5,7,-Tetramethoxyflavone[204] [105]

The band with R_f 0,19 yielded the flavone as a crystalline solid (12mg).

M.P. 176-178° [Lit.[204] 179 - 180°]

^{1}HNMR : δ[$CDCl_3$, 300MHz, 296K , plate 28]
7.94(1H, d, J=9.5 Hz, H-6'), 7.14(1H, s, H-3), 6.57 (1H, dd, J=9.5 and 2.5 Hz , H-5'), 6.51(1H, d, J= 2.5 Hz, H-3'), 6.43(1H, d, J=2.5 Hz, H-8), 6.41 (1H, d, J=2.5Hz, H-6), 3.96(3H,s,-OMe), 3.92(3H,s,-OMe),3.90(3H,s, -OMe), 3.84(3H, s, -OMe)

5.13 Fraction 7(74mg), [Table 8], was methylated with diazomethane. Subsequent separation by plc (B : A, 9 : 1, v/v) produced a band with R_f 0,49.

5.13.1 3,4,3',5'-Tetramethoxystilbene[118,121] [106]

The stilbene (7mg) was obtained as a non-crystalline brown solid from the band of R_f 0,49 .

M.P. 66-68° [Lit.[121] 68 - 69°]

^{1}HNMR : δ[$CDCl_3$, 300 MHz, 298K, plate 27]
7.05(1H, d, J=2.0Hz, H-2), 7.03(1H, dd, J=2.0 and 8.0Hz, H-6), 7.02(1H, d, J=16.0Hz, H-α), 6.88(1H, d, J=16.0Hz, H-β), 6.84(1H, d, J=8.0Hz, H-5), 6.64 (2H, d, J=2.0Hz, H-2',6'), 6.36(1H, t, J=2.0Hz,H-4') 3.81-3.93(12H, 4 x s,4 x OMe)

CHAPTER 6

SYNTHESIS AND REACTIONS OF SOME IROKO METABOLITES

6.1 SYNTHESIS OF 3',4,5'-TRIHYDROXY-4'-GERANYLSTILBENE[102]

The stilbene [102] was synthesized[200] as shown in scheme 35, par. 10.1, from 3,5-dimethoxybenzoic acid [111] via the modified Wittig[174,175] reaction. An earlier attempt starting from 3,5-dihydroxybenzoic [108] acid was abandoned after attempted bromination of the 3,5-dihydroxybenzyl alcohol [110] produced a tarry residue.

6.1.1 Methyl 3,5-dihydroxybenzoate [109]

A methanol (140cm^3) solution containing 3,5-dihydroxybenzoic acid (30g) and concentrated H_2SO_4 (5cm^3) was refluxed for 4,5 hours[205]. The excess methanol was distilled off and the residue extracted with ether (3 x 100 cm^3). The ether extract was neutralized with $NaHCO_3$ solution (4 x 15cm^3), washed with distilled water (3 x 20cm^3) and dried over anhydrous Na_2SO_4. Evaporation of the ether under reduced pressure gave a white crystalline product, which was dried in a vacuum oven to give 27,39g of the ester [109].

6.1.2 3,5-Dihydroxybenzyl alcohol [110]

An ether solution (100cm³) of methyl 3,5-dihydroxybenzoate (15,0g) was added dropwise to 3,0g $LiAlH_4$ dissolved in 300cm₃ dry ether[206].The reaction mixture was stirred for 4hrs. after which the excess $LiAlH_4$ was decomposed with wet ether(80cm³) and the suspension acidified with 3M HCl. The layers were separated and the ether layer washed with water and dilute $NaHCO_3$ solution. The ether solution was dried (anhydrous $MgSO_4$) and the solvent removed under reduced pressure leaving a solid crystalline residue (8,9g) which was separated on a flash column (B A, 8 : 6, v/v). Fractions of 60 cm₃ were collected in beakers as summarized in Table 9.

Table 9 Summary of fractions

Fraction	Beakers (60cm³)	Mass (mg)
1	1 - 4	500
2	5 - 8	1021
3	9 - 14	5100

Fraction 1, (Table 9), contained the pure unreduced ester (R_f 0,48). Fraction 2 (Table 9) was separated by PLC (B : A, 8 : 6, v/v) to give two bands with R_f 0,48 and 0,25. The band with R_f 0,25 afforded the alcohol (540mg) as square

plates, which when combined with fraction 3, (Table 9) gave 5,64g of the alcohol [110].

^{1}HNMR : δ[$(CD_3)_2CO$, 300MHz, 296K, plate 32]

6.1.3 Attempted Bromination of 3,5-dihydroxybenzyl alcohol

3,5-dihydroxybenzyl alcohol [110] (5,0g) was dissolved in Na-dried distilled benzene (200cm^3). Excess dry HBr[182] was bubbled into the solution with stirring over a 20 minute period after which the solution was refluxed for 2 hours. A thick black tarry mass was produced which was extracted with chloroform. The extract (1,0g) was chromatographed on a column (B : A, 8 : 6, v/v) to obtain fractions which showed little prospect as no significant spots were obtained on tlc.

6.1.4 3,5-Dimethoxybenzyl alcohol [112][172]

An ether (25cm^3) solution of *3,5-dimethoxybenzoic acid [111] (7,85g) was stirred with lithium aluminium hydride (5,0g in 100cm$_3$ ether) for 24 hours at room temperature. After this time the excess $LiAlH_4$ was decomposed with wet ether and the suspension acidified with dilute HCl. The layers were separated and the ether layer washed with water and dilute $NaHCO_3$. The ether solution was dried ($MgSO_4$ anhydrous) and the solvent evaporated off under reduced pressure leaving white crystals (5,64g) of 3,5-

dimethoxybenzyl alcohol [112].

M.p. 45 - 47° [Lit[172] 44 - 47°]

^{1}HNMR : δ[($CDCl_3$, 300MHz, 296K, plate 33]

*3,5-dimethoxybenzoic acid was kindly supplied by Prof.E.Malan.

6.1.5 3,5-Dimethoxybenzyl bromide [113][172]

3,5-dimethoxybenzyl alcohol [112] (8,5g) was dissolved in Na dried distilled benzene (200cm^3). Excess dry HBr[182] was bubbled into the solution with stirring over a 20 min period and the solution was refluxed for 2 hours. Evaporation of the benzene gave light mauve-coloured crystals (11.48g) of 3,5-dimethoxybenzyl bromide [113].

M.P. 70 - 71° [Lit[172] 71 - 72°].

^{1}HNMR : δ [$CDCl_3$, 300MHz, 296K, plate 34]

6.1.6 3',4,5'-Trimethoxystilbene[200] [104a]

3,5-dimethoxbenzyl bromide (3,1g) was heated with excess triethyl phosphite (5cm^3) to 130°C until the evolution of ethyl bromide had ceased[172]. Excess triethyl phosphite was removed by distillation under reduced pressure and the residual diethyl benzylphosphonate was added to 25cm^3 of dry dimethylformamide containing 1g of sodium methoxide and

cooled to 0°C. To this was added 4-methoxybenzaldehyde (2,0g) and the mixture allowed to stand at room temperature for 1 hour. The reaction mixture was then heated on a steam bath for 1 hour and allowed to stand overnight at room temperature. Evaporation of the ether extract (dried with $MgSO_4$ anhydrous) gave a product (2,4g), with m.p. 53 - 55° [Lit[203] 56 - 57°]. The ^{1}HNMR spectrum was identical to that of [104a, ^{1}HNMR plate 22] discussed in 5.10.1.1.

6.1.7 3',4,5'-Trihydroxystilbene[125,202] [104]

3',4,5',-trimethoxystilbene (1,4g) and pyridine hydrochloride (5,5g) were heated together at 180° (sand bath) for 2 hours[172]. The melt was poured into 25cm^3 of cold 2M HCl and the reaction mixture extracted with ether. The ether solution was dried (anhydrous $MgSO_4$) and the solvent removed under reduced press to give a product (1,018g) showing more than one spot on tlc. The product was purified by plc (B : A, 8 : 6, v/v) to give pure 3',4,5'-trihydroxystilbene [104] (218mg), with R_f 0.43 and m.p 255-258° [Lit[202] 261°C] The ^{1}HNMR spectrum obtained was identical to that of natural 3',4,5'-trihydroxystilbene [104] [^{1}HNMR plate 21], isolated from the Iroko heartwood.

6.1.8 3',4,5'-Trihydroxy-4'-geranylstilbene[200] [102]

3',4,5'-trihyroxystilbene (0,015 mol dissolved in 20cm³ ethanol) and geraniol (0,030mol dissolved in 20cm³ EtOH) were added almost simultaneously (1,0ml each at a time over 30 minutes) into aqueous ethanol (10cm³) at a strictly controlled (with HCl) pH of 1[207]. The reaction mixture was stirred at 60°C for 2 hours. Evaporation of the ethanol under reduced pressure produced a brown residue which was separated by plc (B : A, 9 : 1, v/v) to afford a band with R_f 0,23. This band afforded the geranylstilbene [102] (8% yield) as a non-crystalline compound, melting at 138-140°C. The ^{1}HNMR spectrum obtained was identical to that of natural 3',4,5'-trihydroxy-4'-geranylstilbene [102], [^{1}HNMR plate 19], isolated from the Iroko heartwood.

6.2 SYNTHESIS OF 2,3',4,5'-TETRAHYDROXY -4'-GERANYLSTILBENE (CHLOROPHORIN) [47]

6.2.1 Acid Condensation

Coupling reactions of geraniol [123] (200mg) to 2,3',4,5'-tetrahydroxystilbene [103] (100mg) were attempted in ethanol under a variety of acidic (HCl[207] and p-toluenesulphonic acid[208]) conditions.

The reaction mixture was stirred initially at 50°C for 1

hour and thereafter for 24 hours at room temperature(25°C). The course of the reaction was monitored by qualitative tlc (B : A, 8: 4, v/v). Only one reaction out of several attempts showed positive results. In this reaction, 1 cm^3 of concentrated HCl was added to an ethanolic(10 cm^3) solution of the stilbene and the geraniol. The solution was extracted (after 24 hrs.) with ethyl acetate (3 x 15 cm^3). Drying of the organic layer (Na_2SO_4) and subsequent evaporation of the solvent gave a brown residue (196mg) which after plc separation (B : A, 8 : 6, v/v) produced a band with R_f 0,45. This band afforded 5mg of 2,3',4,5'-tetrahydroxy-4'-geranylstilbene whose ^{1}HNMR spectrum was identical to that of natural chlorophorin [47], ^{1}HNMR plate 23, isolated from Iroko heartwood.

6.2.2 Friedel-Crafts acylation

Another attempt at coupling geraniol to 2,3',4,5'-tetrahydroxystilbene [102] employed a Friedel – Crafts acylation[209] between geranoyl chloride (obtained from geranic acid) and the stilbene. Citral was first oxidized to geranic acid [125].

6.2.2.1 Geranic Acid [125]

Citral (2,5g) was added to a suspension of 4,0g Ag_2O in 6,0cm^3 of 10% NaOH and 10cm^3 H_2O in a round bottom flask[210]. The mixture was shaken using a mechanical shaker for 24 hours. The silver and unreacted silver oxide were removed by filtration and the filtrate acidified with 3M HCl to pH 2. Ether extraction (3 x 15cm_3) followd by drying (anhydrous Na_2SO_4) and evaporation of the solvent resulted in a light yellow oil (2,2g). The above procedure was repeated another three times to give a total yield of 9,0g of impure product. The oil was purified on a flash column (B : A, 9 : 1, v/v) and 30cm^3 fractions obtained as summarized in Table 10.

Table 10 Summary of fractions

Fraction	Beakers	Mass (mg)
1	1 - 2	300
2	3	131
3	4 - 8	5641
4	9 - 13	549

Fraction 3 (Table 10) afforded geranic acid [125] as a colourless oil with an R_f of 0,51 (B : A, 9 : 1, v/v)

^{1}HNMR : δ[$CDCl_3$, 300MHz, 296K, plate 35]

6.2.2.2 Attempted Acylation

Excess oxalyl chloride was added with stirring to 500mg geranic acid at 0°C. The resultant solution was added dropwise at 0°C to a mixture of 0,976g 2,3',4,5'-tetrahydroxystilbene (dissolved in 0,5 cm^3 dry THF), nitrobenzene (60mg) and anhydrous $AlCl_3$ (799mg)[209]. The reaction mixture was stirred for 24 hours at room temperature. A thick brown mixture was obtained. Ice/dil HCl (6M) were added and the mixture extractd with ether (3 x 30cm^3). Drying (Na_2SO_4) and evaporation of the ether produced a brown solid (1,1g) which on tlc analysis (B : A, 9 : 1) showed the unreacted stilbene but none of the expected chlorophorin. The above procedure was repeated four times with no success.

6.3 ATTEMPT TO SYNTHESIZE CIS-CHLOROPHORIN

6.3.1 U.V. irradiation of Chlorophorin [47]

400mg chlorophorin and 40mg benzophenone were dissolved in 200cm^3 MeOH and the solution, surrounded by a cooling jacket, irradiated with a U.V. lamp (300nm) under an argon

atmosphere for 2 hours. The progress of the reaction was followed by tlc. Irradiation for longer than 2 hours caused the formation of a tarry substance.

6.4 ATTEMPT TO CYCLIZE CHLOROPHORIN

6.4.1 Reaction of Chlorophorin [47] with DDQ

Chlorophorin (50mg) was dissolved in 10cm^3 dry solvent, excess DDQ was added and the mixture stirred under nitrogen for 24 hours. A variety of solvents (benzene, THF, dioxane, acetone, methanol, ether) were tried, but tlc analysis showed no positive reaction. Different temperatures (25°, 40°, 60°, reflux) were also tried with no success.

CHAPTER 7

SYNTHESIS OF A LIGNOID : (+)-CATECHIN-LIGNOID

Sinapyl alcohol was synthesized from 2,6-dimethoxyphenol via a vinyl quinone methide intermediate[211]. The product obtained from this reaction was immediately reacted with (+)-catechin with the idea of forming a lignoid.

7.1 2,6-Dimethoxy -1-0-allyl phenol[211][126]

Allyl bromide (1,7cm^3) and anhydrous K_2CO_3 (7,0g) were added to a solution of 2,6-dimethoxyphenol (3,0g) in dry acetone (100cm^3) and refluxed for 10 hours under nitrogen. The K_2CO_3 was filtered off and the solvent removed under vacuum. The product [126; R_f 0,42] was obtained as a colourless oil (3,48g) using flash chromatography (B : A, 9 : 1, v/v).

^{1}HNMR : δ[$CDCl_3$, 300MHz, 296K, plate 36].

6.97(1H, t, J = 8.2Hz, H-4), 6.55(1H, d, J = 8.2Hz, H-3,5), 6.10(1H, m, J = 6.0, 10.4 and 17.2Hz, H-2'), 5.33 - 5.24 and 5.29 - 5.13 each(2H, m, 3'-CH_2), 4.52 - 4.48(2H, m, 1'-CH_2), 3.83(6H, s, 2x -OMe).

7.2 2,6-Dimethoxy-4-allyl phenol[211][127]

2,6-dimethoxy-1-0-allyl phenol [126 ; 3,03g] and N,N-dimethylaniline (3cm³) were refluxed for 6 hours under a nitrogen atmosphere. Thereafter 4 M HCl (6cm³) was added and the solution was stirred for a further 20 minutes. The reaction mixture was extracted with chloroform (3 x 15cm³). The chloroform layer was washed repeatedly with 4N HCl (3 x 50cm³) and water (3 x 50cm³), dried over anhydrous Na_2SO_4 and evaporated under vacuum to give the product [127; 2,10g; B : A, 9 : 1, v/v, R_f 0,35]

^{1}HNMR : δ[$CDCl_3$, 300MHz, 296K, plate 37].
6.39(2H, s, H-3,5), 5.93(m, J = 6.8, 10.1 and 16.5Hz, H-2'), 5.38(1H, s, OH), 5.11 - 5.02(2H, m, 3'-CH_2), 3.86(6H, s, 2 x OMe), 3.32 - 3.27(2H, m, 1'-CH_2).

7.3 Sinapyl Acetate[211][128]

Ag_2O (2,4g) was added to a vigorous stirring solution of 2,6-dimethoxy-4-allyl phenol (1,0g) in dry benzene (20cm³) at room temperature. After 20 minutes the benzene solution was filtered directly into a flask containing anhydrous sodium acetate (2,64g) and dry acetic acid (16 cm³). After

stirring at room temperature for 3 hours the sodium acetate was filtered off and the excess acetic acid was removed under high vacuum. The residual oil was quickly filtered through 30g of silica gel by eluting with hexane/ethyl acetate (1 : 1, v/v). Evaporation of the solvent under reduced pressure afforded an oil [129, 573mg; R_f = 0,25, B : A, 9 : 1, v/v]. The above procedure was carried out in duplicate.

^{1}HNMR : δ[$CDCl_3$, 300MHz, 296K, plate 38].
6.61(2H, s, H-3,5), 6.54(1H, m, H-1'), 6.13(m, J = 6.8 and 15.8Hz, H-2'), 5.57(1H, s, OH), 4.69(dd, J = 1.4 and 6.8Hz, 3'-CH_2), 3.88(6H, s, 2 x OMe), 2.08(3H, s, 3'-OAc).

7.4 (+)-Catechin Lignoid [129]

A solution of sinapyl acetate [128;170mg] in dry THF (3cm^3) was added dropwise to a stirred suspension of $LiAlH_4$ (45mg) in dry THF (4ml) under a nitrogen atmosphere at 10°C. After stirring at the same temperature for 3 hours, ethylacetate (3cm^3), aq. THF (3cm^3), cold water (40cm^3) and dichloromethane (20cm^3) were added. The water phase was neutralized with a $KHSO_4$ solution then extracted twice with

dichloromethane. The organic layers were dried over Na_2SO_4, and the dichloromethane removed under high vacuum (N_2-atmosphere) to give 30mg of a product (sinapyl alcohol). Due to its instability the product (alcohol) was reacted immediately with (+)-catechin by adding Ag_2O (15mg) to a solution of (+)-catechin (30mg) and sinapyl alcohol (25mg) in dry benzene (7cm^3) and dry spectroscopic methanol (3cm^3), after which the mixture was stirred for 24 hours. With filtration (celite), evaporation of the solvent and plc separation (B : A, 9 : 1, v/v), a band of R_f 0,89 afforded a colourless non-crystalline solid (4mg), which was suspected to be the lignoid.

DISCUSSION

CHAPTER 8

METABOLITES FROM VIRGILIA OROBOIDES

Virgilia oroboides, commonly known as the Keurboom, is a medium sized tree rarely exceeding 10m and occurs on forest edges and river valleys on the coastal strip from the South-Western to Southern Cape [212]. The smooth pale-brownish-grey bark and attractive profusion of flowers from August to September make this one of the most beautiful of indigenous trees. This tree is endemic to the Knysna area alone.

The tree was classified[212] as:

Family : Papilionoideae

Genus : Virgilia

Species : oroboides

The present study of the acetone extract of Virgilia oroboides heartwood has revealed the presence of a variety of flavonoid compounds, with the following being isolated in their free phenolic form:

a) (6aR,11aR)-3-methoxy-8,9-methylenedioxypterocarpan (Pterocarpin)[32,183, 184] [13].

b) (6aR,11aR)-3-hydroxy-8,9-methylenedioxypterocarpan (Maackiain)[183,184] [25].

c) 3-hydroxy-8,9-methylenedioxy-6a,11a-dihydropterocarpan [85].

d) (2R)-7-hydroxyflavanone[185] [87].

e) (±)-7,4'-dihydroxyflavanone (Liquiritigenin)[185,190] [93].

f) 7-hydroxy-4'-methoxyisoflavone (Formononetin)[187,188,189] [10].

g) 3',7-dihydroxy-4'-methoxyisoflavone (Calycosin)[191,192] [94].

h) (±)-7-hydroxy-4'-methoxyisoflavanone [88][186].

i) (αS),2',4'-trihydroxy-4-methoxydihydrochalcone [91].

The following compounds were isolated as their acetates:

j) 3,3',4',7-tetra-acetoxyflavone (Fisetin)[193,195] [96].

k) (2R,3R)-3,3',4',7-tetra-acetoxydihydroflavonol [(+)-Fustin][194,195] [95].

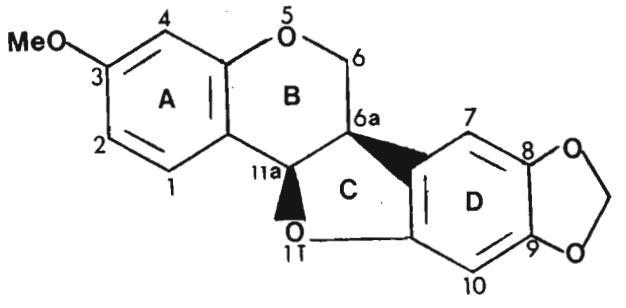
l) (2R,3R)-3,7-diacetoxy-dihydroflavonol [90].

All the above compounds except [85] and [91] have previously been isolated and identified from other plant species. The (αR)-enantiomer of [91] has been isolated from Pericopsis elata[58].

8.1 PTEROCARPANS

8.1.1 [6aR,11aR)-3-Methoxy-8,9-methylenedioxypterocarpan (Pterocarpin)[32,183,184] [13]

Pterocarpin [13] was obtained as light brown crystals as described in 4.3.1.



[13]

The ^{1}HNMR spectrum (plate 1) of [13] showed an ABMX substitution pattern which indicated the protons of the heterocyclic B-ring. A doublet at δ5.47 (J = 6.9Hz) was associated with the deshielded H-11a. The high field proton H-6a, δ3.46 (ddd, J = 5.1, 6.9 and 10.5Hz) was identified by its relatively strong coupling (J = 6.9Hz) to the low field proton H-11a. The magnitude of the vicinal coupling constant (J 6ax,6eq = 10.8Hz) identified the protons on C-6, H-6ax being nearer δ3.64 (dd, J 6ax,6a = 10.5Hz) to H-6a than H-6eq, δ4.21 (dd, J 6eq,6a = 5.1 Hz).The aromatic region in the spectrum showed an ABX system, attributable to

an ortho-meta-coupled doublet of doublets (δ6.61, J = 2.5 and 8.5Hz,H-2), a meta coupled doublet (δ6.45, J = 2.5Hz,H-4) and an ortho-doublet (δ7.38, J = 8.5Hz, H-1). Singlets at δ6.71 and δ6.42 were assigned to the para-coupled protons H-7 and H-10. A methylenedioxy group attached to positions 8,9 was responsible for a narrow quartet at δ5.89(J = 1.0 and 9.0Hz). A high-field singlet at δ3.78 was attributed to a methoxy group at C-3.

Spin-spin decoupling experiment results on the heterocyclic ring protons of [13] are summarized in Table 11. Irradiation of H-11a caused a sharpening of H-6a, to form a doublet of doublets and a sharpening of the H-6eq doublet of doublets. The sharpening of the H-11a doublet, the H-6ax doublet of doublets and the sharpening of H-6a (ddd) to a doublet of doublets, occurred on irradiation of H-6eq. These decoupling experiments confirmed the assignment and coupling of the B-ring protons.

Comparison of the ^{1}HNMR spectrum (plate 1) with an authentic spectrum[184] confirmed the proposed structure of pterocarpin.

The 6aR,11aR absolute configuration of [13] was obtained by comparison of the CD curve of [13] against an authentic curve supplied by Professor D. Ferreira, University of Orange Free State, Bloemfontein.

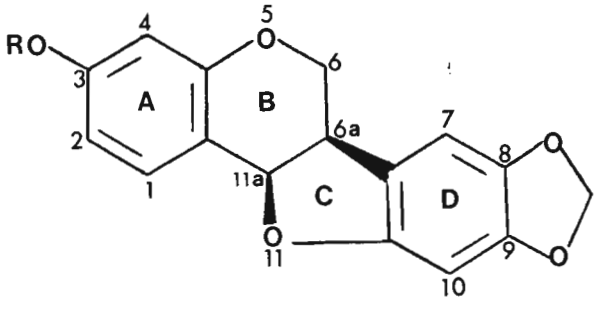
The melting point of pterocarpin [13] is in good agreement with that reported[182].

Table 11 : Decoupling experiments on [13]

Irradiation point	Change observed
d, δ5.47, H-11a	ddd, δ3.46, H-6a to dd. dd, δ4.21, H-6eq sharpening.
dd, δ4.21, H-6eq	d, δ5.47, H-11a sharpening dd, δ3.64, H-6ax sharpening ddd, δ3.46, H-6a to dd.
dd, δ3.64, H-6ax	dd, 4.21, H-6eq sharpening ddd, δ3.46, H-6a sharpening

8.1.2 (6aR,11aR)-3-Hydroxy-8,9-methylenedioxypterocarpan (Maackiain)[183,184] [25]

Maackiain is a well known pterocarpan and was obtained as indicated in 4.4.3.



[25] R = H

[13] R = Me

[25a] R = Ac

The ^{1}HNMR spectrum (plate 2) of [25] revealed the characteristic[183] pterocarpan chemical shifts and coupling constants which were very similar to those of the previously discussed 3-methoxy-8,9-methylenedioxypterocarpan [13]. The only difference was the presence of an hydroxyl group at C-3 which appeared as a singlet at δ8.68.

This ^{1}HNMR spectrum was similar to an authentic spectrum[184] of Maackiain. Methylation of [25] to form Pterocarpin [13] produced a spectrum identical to that of natural pterocarpin [13], showing a methoxyl at δ3.78 and the disappearance of 3-OH. Acetylation of [25][182],

confirmed the presence of the hydroxyl group (δ2.28, 3-OAc) and caused a downfield shift (deshielding) of H-2 and H-4.

Further confirmation of the aromatic proton couplings in [25] were shown by the results of spin-spin decoupling experiments on [25]. Table 12 gives a summary of decoupling experiments on the A-ring. Irradiation of H-4 caused H-2 to show only an ortho-doublet while irradiation of H-1 caused H-2 to show only a meta-doublet. The sharpening of H-4 and H-1 was noticed on irradiation of H-2. These observations confirmed the ABX proton coupling of the A-ring.

Table 12 : Decoupling experiments on [25]

Irradiation Point	Change observed
d, δ6.34, H-4	dd, δ6.54, H-2 to d (ortho)
dd, δ6.54, H-2	d, δ6.34, H-4 sharpening (s) d, δ7.28, H-1 sharpening (s)
d, δ7.28, H-1	dd, δ6.54, H-2 to d (meta)

The decoupling observations on the B-ring were the same as those made for the B-ring of [13], thereby confirming the characteristic pterocarpan structure.

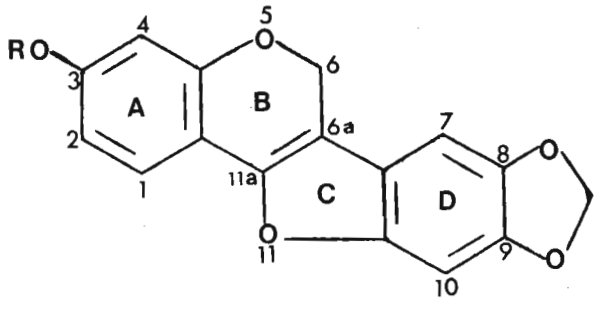
The 6aR,11aR absolute configuration of [25] was obtained by comparison against an authentic specimen supplied by Professor D. Ferreira.

Mass spectral analysis (Scheme A) showed fragments m/z 162(32) and m/z 147(19) together with a prominent peak at m/z 284 (M^+, 100%). This supported the above proposed structure of [25].

The melting point for [25] was in agreement with that previously reported[183].

8.1.3 3-Hydroxy-8,9-methylenedioxy-6a,11a-dihydropterocarpan [85]

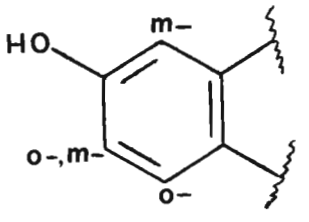
This pterocarpene was obtained as discussed in 4.4.1.



[85] R = H

[85a] R = Ac

The ^{1}HNMR spectrum (plate 3, acetone-d_6) of [85] showed an ABX system (δ7.28, o-doublet; δ6.49 o-,m-doublet of doublets, δ6.41 m-doublet; J = 2.0 and 8.5Hz), indicating a trisubstituted aromatic ring. A broad singlet (δ8.73), integrating for a single proton and indicating a free hydroxyl group, disappeared on acetylation of [85], resulting in the appearance of an acetoxyl singlet at δ2.28 (^{1}HNMR plate 4, $CDCl_3$). Acetylation of [85] also resulted in a downfield shift (deshielding) of the ortho-doublet (0,15ppm), the ortho-meta-doublet (0,27ppm) and the meta-doublet (0,28ppm) in acetone -d_6. The above observations indicated the fragment [85b].



[85b]

Also present in the ^{1}HNMR spectrum (plate 3) of [85] were two singlets (each 2H) at δ5.51 and δ6.02 and an aromatic AB system (2H, δ7.12 and 6.95). Comparison of this spectrum against spectra of known compounds[43] indicated that [85] was a pterocarpene. The two-proton singlet at δ5.51 could then be assigned to the methylene group at C-6 and the AB system to H-7 and H-10, thereby allowing the assignment of the singlet (2H) at δ6.02 to a methylenedioxy group at position 8,9 and the ABX system (fragment 85b) to the A-ring.

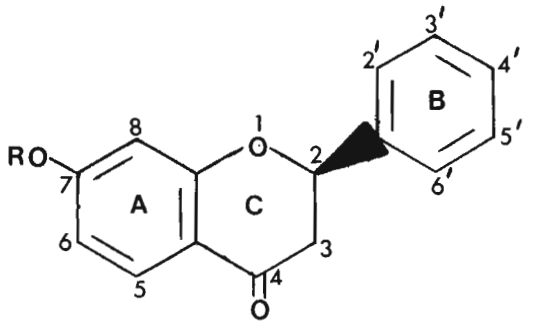
The mass spectrum (Scheme B) of [85] confirmed the above proton assignments. A typical pterocarpene fragmentation pattern[214] with the most prominent fragments being those of a molecular ion at m/z 282 (96%) and M^{+}-1 ion at m/z (100%) was obtained. The M^{+}-1 fragment corresponded to loss of hydrogen from 3-OH to give the stable quinonoid ion. Loss of 30(m/z 223, 5.6%) was indicative of a methylenedioxy group at positions 8 and 9.

This is the first report of the isolation of this natural compound in the literature.

8.2 FLAVANONES

The flavanones [87] and [93] were obtained as described in 4.5.1 and 4.7.1.

8.2.1 (2R)-7-Hydroxyflavanone[185] [87]



[87] R = H

[87a] R = Ac

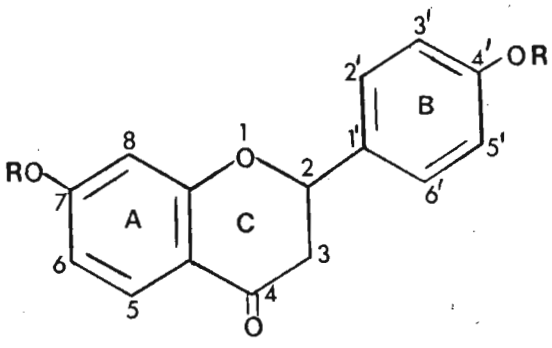
The ^{1}HNMR spectrum (plate 5) of [87] showed an aromatic ABX pattern whch was attributed to the A-ring protons, δ7.73 (d, J= 9.0Hz, H-5), 6.58 (dd, J = 2.5 and 9.0Hz, H-6), 6.45 (d, J = 2.5Hz, H-8). The 7-OH was confirmed by acetylation of [87] to form [87a], subsequently producing a singlet (δ2.31,-OAc), and causing a downfield shift (deshielding) of the A-ring ortho and meta-protons, H-6 and H-8. A multiplet in the aromatic region (δ7.59-7.35), integrating for five protons, indicated an unsubstituted B-ring. A non-aromatic ABX system was typical of the flavanones with H-2 appearing as a doublet of doublets at δ5.56 with characteristic Jcis = 3.0Hz and Jtrans = 13.0Hz. H-3ax and H-3eq appeared as two doublet of doublets at δ3.04 and δ2.74 respectively, J = 17.0Hz. The ^{1}HNMR spectrum of [87] was similar to an authentic spectrum reported in the literature[185].

Comparison of the CD curve of 7-hydroxyflavanone against that of an authentic sample of (2R) -4',7-diacetoxy-flavanone[215] confirmed a (2R-) absolute configuration.

The melting points of [87] and [87a] are similar to those reported[185].

8.2.2 (±)-7,4'-Dihydroxyflavanone

[(±)-Liquiritigenin][185,190] [93]



[93] R = H

[93a] R = Me

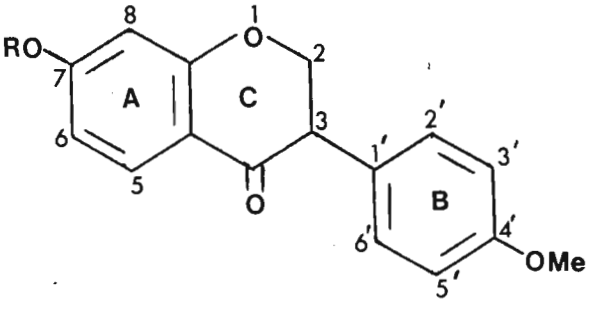
The ^{1}HNMR spectrum (plate 6) of [93] revealed an ABX system; δ7.74 (d, J = 8.3Hz, H-5), 6.50 (dd, J = 2.4 and 8.3Hz,H-6), 6.39 (d,J = 2.4Hz, H-8). An AA'BB' pattern was allocated to the B-ring, δ7.25 (d,J = 8.3Hz, H-2',6') and δ6.84 (d,J = 8.3Hz, H-3',5'). Methylation of [93] to form [93a] indicated a singlet at δ3.82 integrating for two methoxyls. An hydroxyl group was therefore assigned to C-7 and C-4' of [93]. A doublet of doublets at δ5.29, J cis = 3.0Hz and J trans = 13.0Hz, was attributed to H-2 which was coupled to H-3ax and H-3eq. H-3ax and H-3eq appeared as two doublet of doublets at δ2.96 and δ2.68 respectively, J = 17.0Hz.

The ^{1}HNMR observations and melting point of [93] were consistent with those reported in the literature[185,190].

8.3 ISOFLAVANONES

8.3.1 (±)-7-Hydroxy-4'-methoxyisoflavanone[186] [88]

The isoflavanone was obtained as indicated in 4.5.2.



[88] R = H

[88a] R = Me

[88b] R = Ac

An aromatic ABX system (δ7.73, 6.57 and 6.40; J = 2.5 and 9.0Hz) together with an aromatic AA'BB' system (δ7.22, 6.88; J = 9.0Hz), indicating a 7,4'-disubstitution pattern, appeared in the ^{1}HNMR spectrum (plate 7) of [88]. A singlet at δ3.76 integrated for one methoxyl group which could either be at C-7 or C-4'.

Methylation of [88] to form [88a][186] produced an additional singlet at δ3.83, integrating for three methoxyl protons. This spectrum clearly showed, in addition to an aromatic ABX and an AA'BB' system, a non-aromatic ABX system

with chemical shifts and coupling constants typical of the heterocyclic ring of isoflavanones, δ4.65 (dd, J = 6.0 and 11.5 Hz, H-2eq), 4.57 (dd, J = 8.0 and 11.5 Hz, H-2ax), 3.86 (dd, J = 6.0 and 8.0Hz, H-3).

Acetylation of [88] produced a singlet (δ2.35) integrating for three protons. The deshielding of the A-ring protons (δ7.15, H-6, and δ6.77, H-8) was evidence for the acetate group at position 7.

The methoxyl group on the B-ring was also confirmed by a NOE experiment showing an association of 12.2% with H-3' and H-5'.

Spin-spin decoupling experiment results on [88] are summarized in Table 13. Irradiaton of H-5 confirmed the coupling of H-5 to H-6 while a sharpening of the H-2',6', signal occurred on irradiation of H-3',5'. The isoflavanone moeity was confirmed by a sharpening of H-2eq,H-2ax on irradiation of H-3.

Table 13 : Decoupling experiments on [88]

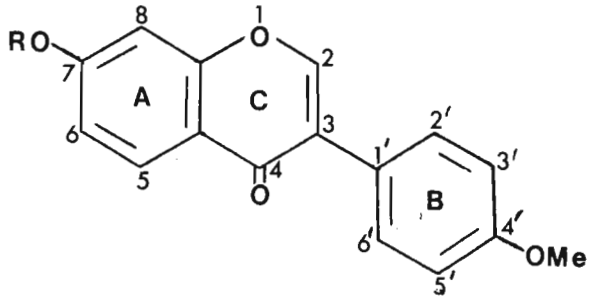
Irradiation point	Change observed
d, δ7.73, H-5	dd, δ6.57, H-6 to d
d, δ7.22, H-2',6'	d, δ6.88,H-3',5' sharpening (s)
d, δ6.88, H-3,5'	d, δ7.22,H-2',6' sharpening (s)
d, δ4.63, H-2eq,H-2ax	dd, δ3.89, H-3 to d
dd, δ3.89, H-3	dd, δ4.63, H-2eq,H-2ax sharpening

Comparison of the above features against those reported for[186] 7-hydroxy-4'-methoxyisoflavanone confirmed the structure.

8.4 ISOFLAVONES

Formononetin [10], and Calycosin [94] were obtained as indicated in 4.6.5 and 4.7.2 respectively.

8.4.1 7-Hydroxy-4'-methoxyisoflavone (Formononetin)[187,188,189] [10]



[10] R = H

[10a] R = Ac

An ABX system (δ8.05, d; δ6.99, dd; and δ6.89, d; J = 2.0 and 8.5 Hz) and an AA'BB' system (δ7.55 and δ6.97, J = 9.0Hz) in the ^{1}HNMR spectrum (plate 8) of [10], suggested a 7,4'-disubstitution pattern. A singlet at δ3.82 integrated for three protons, these being assigned to an aromatic methoxyl. NOE correlations (Table 14) indicated that the methoxyl was closely associated (7.89%) with the H-3',5' doublet of the AA'BB' system and therefore situated at position 4'.

Table 14 : NOE associations for isoflavone [10]

Decoupling Applied	NOE effect observed
B-ring: 4'-OMe (δ3.82)	H-3',5' (δ6.97, 7.89%)

Acetylation of [10] to form [10a][189] produced a singlet at δ2.35(- OAc) integrating for three protons. The downfield shift (deshielding) of the A-ring meta-doublet and ortho-, meta-doublet of doublets confirmed the 7-acetoxy group. A low field singlet (δ8.17) in the spectrum of [10] was assigned to the characteristic vinylic H-2 of isoflavones.

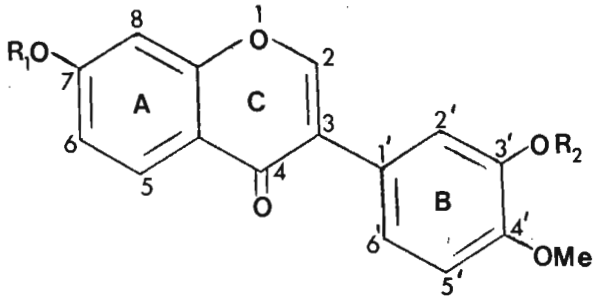
The results of spin-spin decoupling experiments performed on [10a] are summarized in Table 15. Irradiation of H-2',6' caused a sharpening of the H-3',5' signal. Irradiation of H-8 resulted in a doublet (H-6) while a sharpening of the H-5 and H-8 signal occurred on irradiation of H-6.

Table 15 : Decoupling experiments on [10a]

Irradiation Point	Change observed
d, δ7.48, H-2',6'	d. δ6.96, H-3',5' sharpening (s)
d, δ7.28, H-8	dd, δ7.15, H-6 to d
dd, δ7.15, H-6	d, δ7.28, H-8 sharpening (s)
	d, δ8.31, H-5 sharpening (s)
d, δ6.96, H-3',5'	d, 7.48, H-2',6' sharpening (s)

The mass spectrum (Scheme C) of [10a] produced a molecular ion m/z 310 (77.8%) together with the RDA fragments m/z 137(2%) and m/z 132(86.5%) which supported the proposed structure of [10]. Formononetin is a widely occurring isoflavone and the above proposed structural assignment is in agreement with the literature[187,188,189].

8.4.2 7,3'-Dihydroxy-4'-methoxyisoflavone (Calycosin)[191,192] [94]



[94] $R_1 = R_2 = H$

[94a] $R_1 = R_2 = Ac$

A characteristic low-field singlet, δ8.15, in the ^{1}HNMR spectrum (plate 9) of [94] was assigned to an isoflavone vinylic H-2. An aromatic ABX system (δ8.05 o-doublet; δ6.89 m-doublet and δ6.98 o-,m-doublet of doublets; J = 3.0 and 8.5Hz) implied a 7-substituted A-ring while a 'higher field' aromatic ABX system (δ7.06, o-,m-doublet of doublets, δ7.15 m-doublet and δ6.96 o-doublet; J = 3.0 and 8.5Hz) indicated a 3',4' substitution pattern. A singlet at δ3.86 integrated for 3 protons suggesting a methoxyl group. Acetylation,to form [94a][192], produced two singlets in the acetate region δ2.35 and δ2.31 implying two free hydroxyls in [94]. On acetylation the protons in both aromatic rings showed a marked downfield shift (deshielding). One hydroxyl was assigned to C-7. The A-ring pattern was confirmed by

spin-spin decoupling experiments results of which are summarized in Table 16. Irradiation and subsequent decoupling of the ortho-doublet (δ8.05, J = 8.5Hz, H-5) caused a relaxation of the ortho-coupling signal at δ6.98 (H-6), leaving only the meta couplings (J = 3.0Hz) at H-6 and H-8(δ6.89). Irradiation and decoupling of the meta-doublet (δ6.89, J = 3.0Hz, H-8) caused a relaxation of the meta coupling signal at δ6.98 (H-6), leaving only the ortho couplings at δ6.98 (J = 8.5Hz) and δ8.05 (H-5).

Table 16 : Decoupling experiments on [94]

Irradiation point	Change observed
d, δ8.05, H-5	dd, δ6.98, H-6 to d (meta)
d, δ6.89, H-8	dd, δ6.98, H-6 to d (ortho)

NOE association (Table 17) of the methoxyl protons with the H-5' doublet (9.7%) indicated a 3'-OH, 4'-OMe substitution for the B-ring.

Table 17 : NOE associations for isoflavone [94]

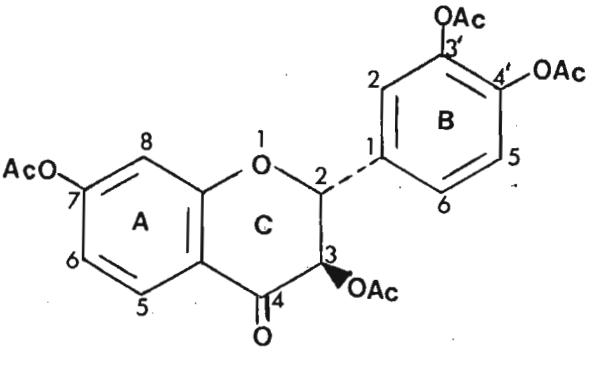
Decoupling applied	NOE effect observed
B-ring: 4'-OMe (δ3.86)	H-5' (δ6.96 ; 9.7%)

The isolation and identification of [94] has been reported in the literature[191,192] and the above proposed structure is in agreement with that reported.

8.5 DIHYDROFLAVONOLS AND FLAVONOLS

Compounds [95], [96] and [90] were obtained as indicated in 4.8.1; 4.8.2 and 4.6.1 respectively.

8.5.1 (2R,3R)-3,3',4',7-Tetra-acetoxy-dihydroflavonol [(+)-Fustin)[194,195] [95]

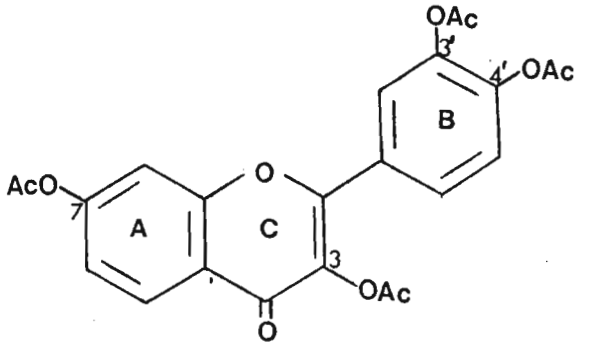


[95]

Trans-diaxial coupling, J = 12.0Hz, between H-2 (δ5.7,d) and

H-3 (δ5.43,d) as shown in the ^{1}HNMR spectrum (plate 10) suggested a 2R,3R absolute and relative 2,3-trans-configuration (half-chair conformation) for [95]. The absolute configuration (2R,3R) was confirmed by comparison of the CD-curve of [95] against that of an authentic sample of (+)-Fustin. An ABX system (δ7.93 o-doublet, δ6.86 o-m-doublet of doublets and δ6.82 m-doublet; J = 2.0 and 8.5Hz) for the A-ring and an ABX system for the B-ring (δ7.40 o-,m-doublet of doublets, 7.31 m-doublet and 7.26 o-doublet; J = 2.0 and 8.5Hz) together with acetoxyl singlets at δ2.31-2.29 reveàled an ^{1}HNMR pattern similar to that of 3-hydroxy-3',4',7-triacetoxyfustin isolated from Trachylobium verrucosum[195].

8.5.2 3,3',4',7-Tetra-acetoxyflavone[193,195](Fisetin) [96]

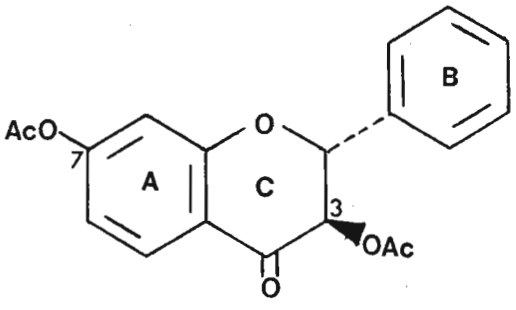


[96]

The ^{1}HNMR spectrum (plate 11) of [96] showed an ABX system (δ8.25, 7.38 and 7.17; J = 2.0 and 10.0Hz) which indicated a

7-substituted A-ring and, another aromatic ABX system (δ7.75, 7.72 and 7.34; J = 2.0 and 8.7Hz) implying a 1',3',4'-sub-stitution pattern. Singlets at δ2.36-2.33 integrated for four acetoxy groups. This spectrum showed a similar pattern to that of tetra-O-methylfisetin isolated from Trachylobium verrucosum[195].

8.5.3 (2R,3R)-3,7-diacetoxy-dihydroflavonol [90]



[90]

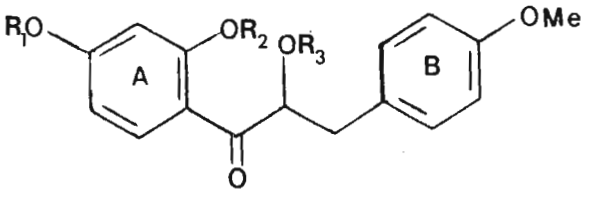
The ^{1}HNMR spectrum (plate 12) of [90] showed prominent trans-diaxial coupling J = 12.0Hz (with half-chair conformation) between protons H-2 (δ5.80,d) and H-3 (δ5.43,d). A relative 2,3-trans and absolute 2R,3R configuration (with half-chair conformation) was therefore expected. Comparison of the CD curve of [90] against that of an authentic sample of (+)-Fustin confirmed the 2R,3R absolute configuration. An ABX system (δ7.93 o-doublet, δ6.84 o-,m-doublet of doublets, δ6.82 m-doublet; J = 2.0 and

8.0Hz) indicated a 7-substituted aromatic A-ring. An aromatic multiplet at δ7.49-7.40 was assigned to an unsubstituted B-ring. The two acetoxy groups at δ2.31 and δ2.00 were assigned to C-7 and C-3. The above spectrum is similar to that reported in the literature[185] for (2S, 3S)-3,7-diacetoxy-dihydroflavonol.

8.6 α-HYDROXYDIHYDROCHALCONES

8.6.1 (αS), 2',4'-trihydroxy-4-methoxydihydrochalcone [91]

Compound [91] was isolated as discussed in 4.6.2



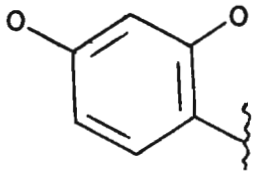
[91] $R_1 = R_2 = R_3 = H$

[91a] $R_1 = R_2 = R_3 = Ac$

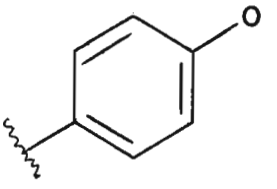
[91b] $R_1 = R_3 = Ac; R_2 = H$

The presence of an aromatic ABX system (δ7.56, o-doublet; δ6.41, m-doublet; and δ6.41, o-m-doublet of doublets; J = 2.0 and 9.0Hz) together with an AA'BB' system (δ7.03, d and

δ6.79, d ; J = 8.5Hz) in the 'HNMR spectrum (plate 13) of [91] could be associated with the aromatic fragments [91c] and [91d] respectively.



[91c]



[91d]

The presence of another ABX system (δ5.19, J = 4.0, 7.0 and 7.0Hz, H-α ; δ3.12 and 2.89, J = 4.0, 7.0 and 14.0Hz, β-CH_2) a deshielded free hydroxyl at δ10.29, and a methoxyl group (δ3.76) suggested that [91] was an α-hydroxy-dihydrochalcone on comparison of this spectrum against authentic spectra[55,56,57] of α-hydroxydihydrochalcones. Acetylation of [91] to form [91a] produced three acetoxyl signals (^{1}HMR plate 14) indicating the presence of three free hydroxyl groups in [91]. Acetylation also resulted in a downfield shift (deshielding) of the ortho-, ortho-meta- and meta-doublets of the fragment [91c], whereas no change was observed for the fragment [91d]. This suggested that two of the three hydroxyl groups were attached to fragment [91c] and the methoxyl group was associated with fragment [91d]. Comparison of the ^{1}HNMR spectrum (plate 13) of [91] against an authentic spectrum of (αR),2',4'-trihydroxy-dihydrochalcone isolated from Pericopsis elata[187] indicated that [91c] and [91d] were the 2',4'-dihydroxy A-ring and 4-methoxy B-ring respectively, with the third hydroxyl group being assigned to C-α.

It is noteworthy that acetylation of α,2',4'-trihydroxy-4-methoxydihydrochalcone [91] also produced a minor quantity of α,4'-diacetoxy-2'-hydroxy-4-methoxydihydrochalcone [91b] (^{1}HNMR plate 15).

Re-acetylation of the diacetate [91b] produced the tri-acetate [91a], which on mild acid hydrolysis (1% HCl) de-acetylated to the α,4-diacetoxy-2'-hydroxy derivative [91b].

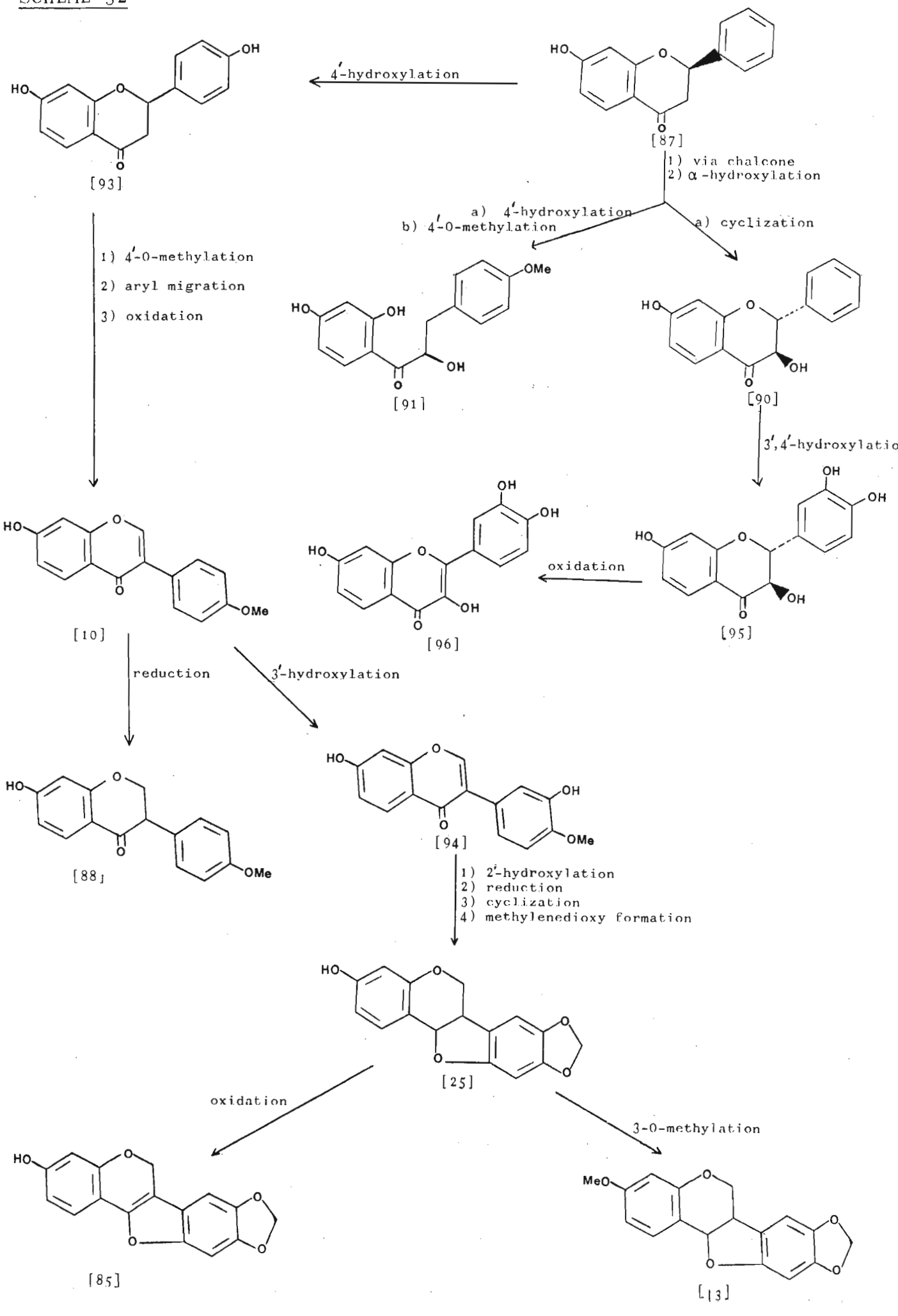
The mass spectrum (Scheme E) of [91b] showed a molecular ion at m/z 372 (3.02%) and RDA fragments m/z 134 (11.32%), 137 (100%) and 179 (25.89%), which supported the proposed structure for [91].

The CD curve (Fig. 1) of [91a], when compared with the curve of an authentic sample[58], showed identical but opposite Cotton effects and on this basis the αS configuration was assigned.

8.7 POSSIBLE BIOGENETIC RELATIONSHIPS

The presence of this wide variety of flavonoids in the heartwood of Virgilia oroboides could suggest a biogenetic pathway as illustrated in Scheme 32 .

SCHEME 32


4'-hydroxylation
[87]
1) via chalcone
2) α-hydroxylation
[93]
a) 4'-hydroxylation
b) 4'-O-methylation
a) cyclization
[91]
[90]
1) 4'-O-methylation
2) aryl migration
3) oxidation
3',4'-hydroxylatio
[10]
[96]
oxidation
[95]
reduction
3'-hydroxylation
[88]
[94]
1) 2'-hydroxylation
2) reduction
3) cyclization
4) methylenedioxy formation
[25]
oxidation
3-O-methylation
[85]
[13]

CHAPTER 9

METABOLITES FROM CHLOROPHORA EXCELSA

The tree Chlorophora excelsa occurs in low altitude evergreen forests in central and eastern tropical regions of Africa, reaching a height of 50m[213]. The timber, generally known as Iroko, is resistant to fungus and insect attack and its durability and attractive appearance make it a popular choice in the building and furniture (laboratory benches) industries.

The tree was classified[213] as:

Family	:	Moraceae
Genus	:	Chlorophora
Species	:	excelsa

Davidson[216] commented on the irritant effects of the wood dust but there is no record of an attempted isolation of the irritant substance. King and Grundon[127] isolated and identified 2,3',4,5'-tetrahydroxy-4'-geranylstilbene (Chlorophorin) from the ether extract of the heartwood.

This present re-investigation of Iroko for minor metabolites afforded an interesting series of potential biogenetic precursors of chlorophorin. The acetone extract of the heartwood produced:

a) 2,4-dihydroxybenzaldehyde[197,200] [98]

b) 3,5-dihydroxybenzaldehyde[196,200] [97]

c) 3,5-dihydroxy-4-geranylbenzaldehyde[200] [101]

d) 3',4,5'-trihydroxy-4'-geranylstilbene[200] [102]

e) 2,3',4,5'-tetrahydroxy-4'-geranylstilbene (Chlorophorin)[127,128,200] [47]

f) 3,3',4,5'-tetramethoxystilbene[118,121] [106]

g) 2,3',4,5'-tetrahydroxystilbene (Oxyresveratrol)[117,200,201] [103]

h) 3',4,5'-trihydroxystilbene (Resveratrol)[117,120,125,200,202] [104]

i) 2',4',5,7-tetramethoxyflavone[204] [105]

j) 3,4',7-tri-O-methylquercitin (Ayanin)[198,200] [99]

k) 2'-methoxy-3,4',7-tri-O-methylquercitin [100]

On analysis, chlorophorin[47] constituted the largest relative abundance in the heartwood to the other metabolites characterized. The two simple dihydroxybenzaldehydes were isolated, together with the two quercitin derivatives from the fraction with highest R_f, with 3,5-dihydroxybenzaldehyde [97] being present in a much lower quantity than the 2,4-isomer [98].

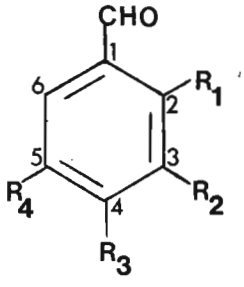
3,5-dihydroxy-4-geranylbenzaldehyde [101] was very sensitive to the atmosphere and heat. It had to be kept under constant vacuum once purified by plc, otherwise it turned into a brown residue. When the aldehyde, however, was in a mixture with 2,4-dihydroxybenzaldehyde [98] in an approximate ratio of 1:2, it was not so sensitive to the factors mentioned earlier, which could suggest a type of hydrogen bonding protection.

Several attempts to isolate 3,3'4,5'-tetramethoxystilbene [106] and 2',4',5,7-tetramethoxyflavone [105] in their free phenolic form were unsuccessful. Spots were seen on tlc, but after separation very small (almost negligible) quantities were obtained. The fraction containing these components was therefore methylated with diazomethane and [106] and [105] isolated as their methyl ethers.

The structures of 3,5-dihydroxy-4-geranylbenzaldehyde [101 and 3',4,5'-trihydroxy-4'-geranylstilbene [102] were confirmed by synthesis[200]. An attempt at the synthesis of chlorophorin [47], by condensing ethanolic geraniol with 2,3',4,5'-tetrahydroxystilbene [103] in acid medium yielded only 5%, with only one out of several attempts being successful. An attempted Friedel-Crafts acylation of geranoyl chloride to the stilbene [103] failed.

9.1 2,4-Dihydroxybenzaldehyde [98] and 3,5-Dihydroxybenzaldehyde [97]

These two compounds [98] and [97] were obtained as indicated in 5.2.2 and 5.2.1 respectively.



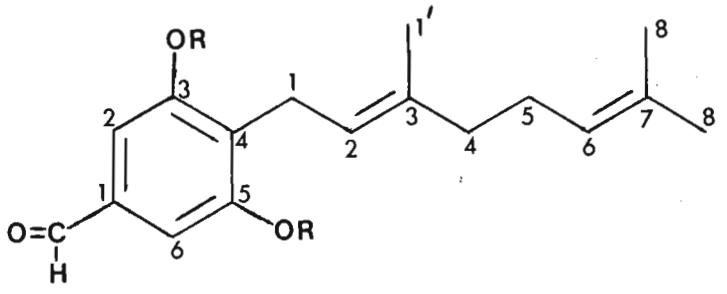
[98] $R_1 = R_3 = OH$; $R_2 = R_4 = H$

[97] $R_2 = R_4 = OH$; $R_1 = R_3 = H$

Comparison of the ^{1}HMNR spectra and melting points[196,197] of the above aldehydes with authentic reference compounds, commercially available, confirmed the structures.

9.2 3,5-Dihydroxy-4-geranylbenzaldehyde[200] [101]

The aldehyde [101] was isolated as discussed in 5.4.1.

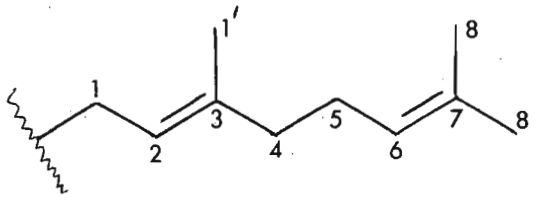


[101] R=H

[101a] R=Me

The ^{1}HNMR spectrum (plate 16) of [101] showed a singlet at δ6.93, integrating for two protons, indicating the equivalent protons H-2 and H-6. A broadened singlet, integrating for two protons at δ8.76 represented the 3- and 5-hydroxyls. Typical of a deshielded aldehydic proton was a singlet at δ9.75. Methylation of [101] with dimethyl sulphate to form [101a] produced a singlet at δ4.10 (^{1}HNMR plate 17), integrating for six hydrogens (methoxyls at C-3 and C-5), a singlet at δ7.24 (H-2, H-6) and a low field aldehydic singlet at δ10,08(1H) with the aldehyde carbonyl being confirmed by an IR absorption at 1700 cm^{-1}.

The structure of the geranyl group was elucidated as follows:



4-geranyl group

Two narrow doublets at δ1.59 and δ1.54(J=1.0Hz) in the ^{1}HNMR spectrum of [101] were assigned to the two methyl groups, 8-CH_3, whose protons were coupled to H-6(δ5.06,m). This was confirmed by spin-spin decoupling experiments whereby irradiation of H-6 caused a sharpening of the two methyl peaks (δ1.59 and δ1.54) and the methylene proton peaks on C-5(δ2.02,m). As a result of the coupling to H-2 (δ5.29,m) the two methylene protons on C-1 appeared as a doublet (δ3.42, J=7.0Hz). H-2 was also coupled to the 1'-methyl group (δ1.77,d,J = 1.0Hz). Confirmation of this coupling was given by a spin-spin decoupling experiment in which irradiation of H-2 caused a sharpening of the 1'-methyl signal. A mild sharpening of the multiplet at C-4 (δ1.94, -CH_2-) also indicated a coupling between H-2 and the C-4 methylene protons. The multiplets at C-4 (δ1.94) and C-5 (δ2.02) each integrated for two protons confirming the presence of the two methylene groups. The results of spin-spin decoupling experiments are summarized in Table 18.

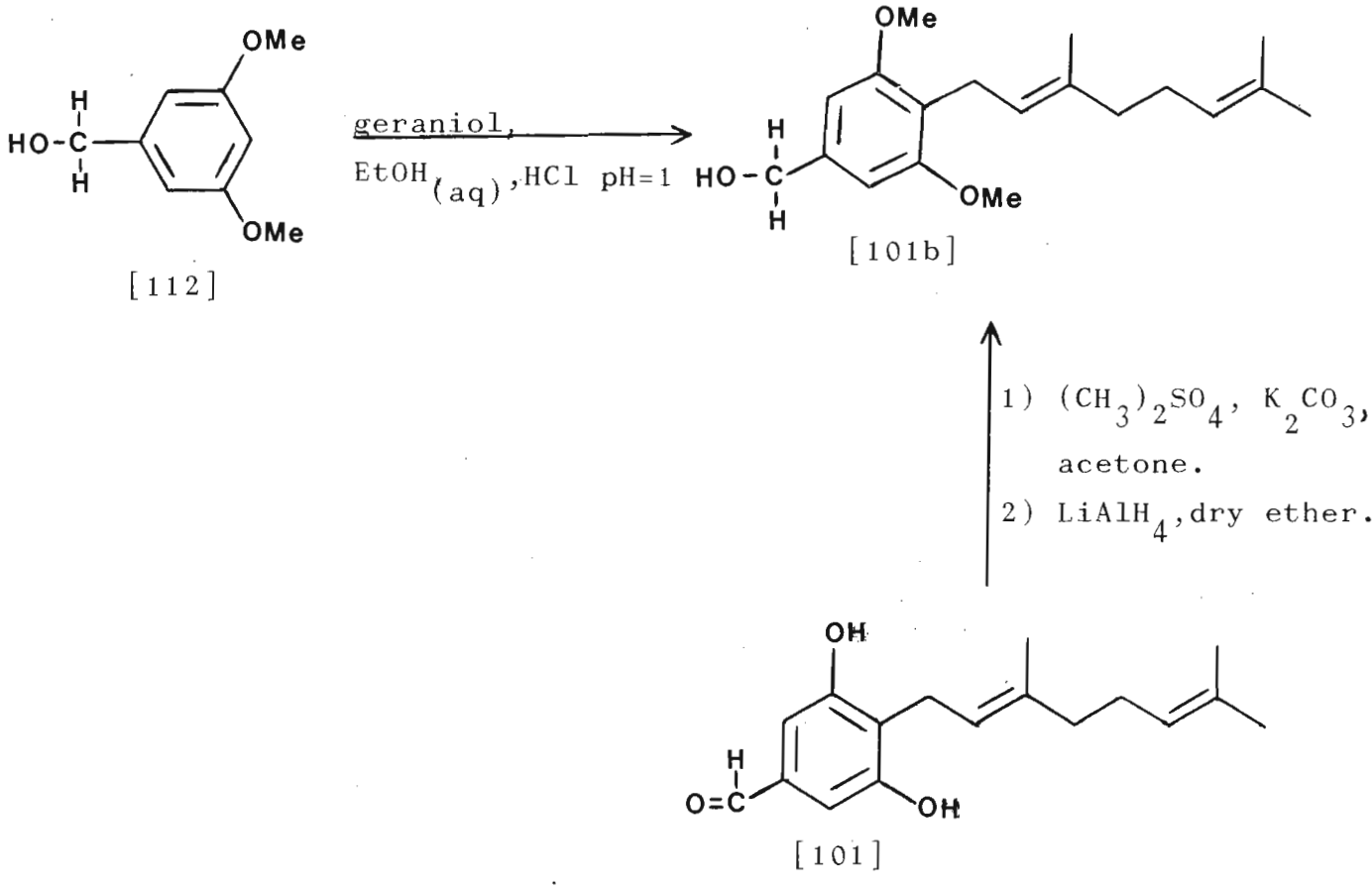
Table 18 : Decoupling experiments on 4'-geranyl group of [101]

Irradiation point	Observed change
m, δ5.06, H-6	d, δ1.59, 8-CH_3 sharpening (s)
	d, δ1.54, 8-CH_3 sharpening (s)
	m, δ2,02, 5-CH_2 sharpening.
m, δ5.29, H-2	d, δ1.77, 1'-CH_3 sharpening
	m, δ1.94, 4-CH_2 sharpening.

The molecular mass of [101] was confirmed to be 274 by high resolution mass spectrometry. The loss of 123 mass units to yield the base peak at m/z 151, confirmed the presence of the geranyl group, as shown in Scheme F.

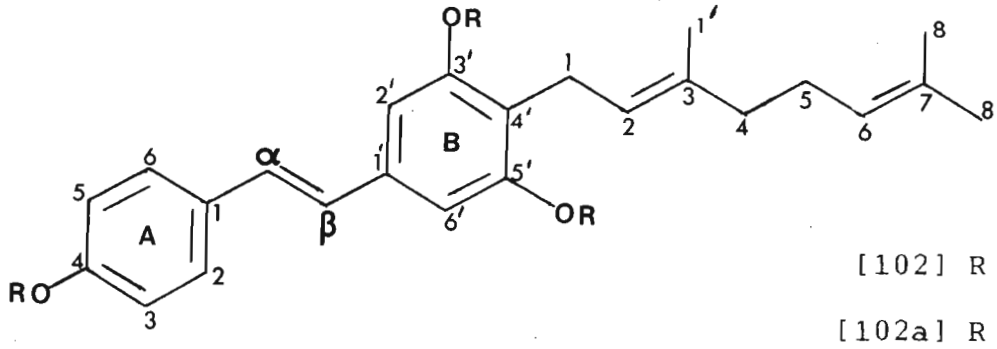
The structure of [101] was confirmed by synthesis[200] as shown in Scheme 33. Initially an ethanolic geraniol solution was carefully added to an aqueous ethanolic (pH=1)[207] solution of 3,5-dimethoxybenzyl alcohol [112]. Attempts to oxidize the resulting 3,5-dimethoxy-4-geranylbenzyl alcohol (^{1}HNMR plate 18) to the aldehyde always resulted in a dark brown residue. A change of approach involved reducing the dimethyl ether of the isolated aldehyde [101] with $LiAlH_4$ to give the required 3,5-dimethoxy-4-geranylbenzyl alcohol [101b]. The ^{1}HNMR spectrum (plate 18) of [101b] showed a distinct singlet at δ4.63 (2H, -CH_2-), indicating the benzylic protons.

SCHEME 33



9.3 3',4,5'-Trihydroxy-4'-geranylstilbene[200] [102]

This stilbene was obtained as indicated in 5.6.1.



[102] R = H

[102a] R = Me

The ^{1}HNMR spectrum (plate 19) of [102] showed, two typical

trans-olefinic proton doublets (δ6.91, δ6.81, each 1H, J=16.5Hz). The one aromatic ring showed an AA' (δ7.37,d,2H) BB' (δ6.81,d,2H) system, while a singlet (2H) at δ6.58 accounted for two magnetically equivalent protons of the second ring. The above observations suggested a 4,3',4',5'- substituted stilbene. The presence of three free hydroxyl groups was indicated by methylation of [102] with dimethyl sulphate, producing methoxy singlets at δ3.81 - δ3.84 (9H), plate 20.

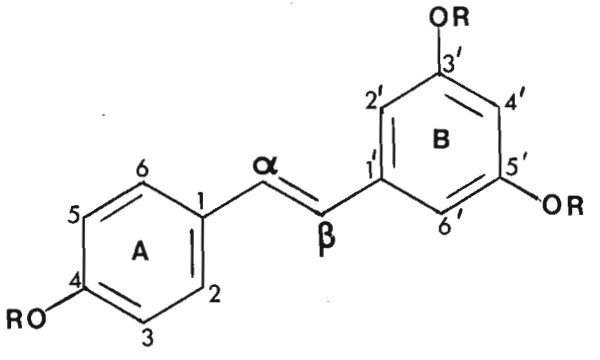
Very similar chemical shifts and coupling constants, as summarized in Table 7, to those obtained for the geranyl group in compound [101] were obtained in the ^{1}HNMR spectrum of [102]. Homodecoupling experiments also produced similar results to those obtained for [101]. The structure of the 4'-geranyl group was therefore confirmed.

The mass spectrum of [102] (Scheme G) gave a molecular ion at m/z 364 (25%). The 4'-geranyl group was further confirmed by the fragments m/z 295(M^{+}-69;18%), 281(M^{+}-83;16%) 279(M^{+}-85;34%) together with the base peak at m/z 241 (M^{+}-123;100%). The A and B aromatic rings were confirmed by fragments m/z 107(M^{+}-257;22.4%) and 123(M^{+}-241;14%)

The proposed structure of [102] was therefore confirmed on the basis of the above evidence. Synthesis[200] of [102], Scheme 35, Sect. 10.1 also substantiated this confirmation.

9.4 3'4,5'-Trihydroxystilbene (Resveratrol)[117,120,125,200,202] [104]

Resveratrol was obtained as described in 5.10.1.



[104] R = H

[104a] R = Me

The aromatic region in the ^{1}HNMR spectrum (plate 21) of [104] showed the characteristic two-proton doublet (δ6.56, J=2.0Hz) and single-proton triplet (δ6.27, J=2.0Hz) of a 1,3,5-trisubstituted benzene ring, an additional ring with an AA'BB' system (δ6.82 and δ7.40,J=9.0Hz) and the presence of two trans-olefinic protons (δ7.01 and δ6.87, d,d, J=16.0Hz). Methylation of [104] with dimethyl sulphate to form [104a] produced a singlet (δ3.84,9H) (^{1}HNMR plate 22) indicating the presence of three free hydroxyl groups in [104].
Consideration of the above data led to the proposed structure of [104].

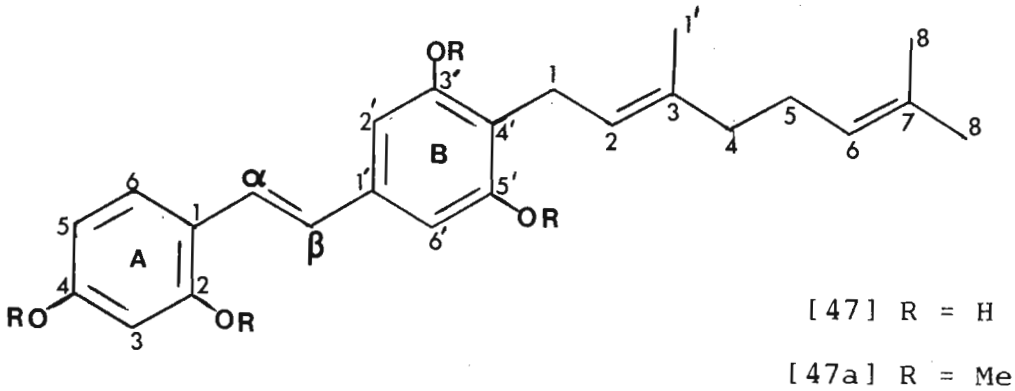
Further confirmation of the above structure was given by the presence of the molecular ion [M^+] at m/z 228 (100%) for [104] and [M^+] at m/z 270 (100%) for [104a].

The structure of [104] was also proved by synthesis as shown in Scheme 35, par. 10.1.2.

The above proposed structure is in agreement to that for resveratrol reported in the literature[117,120,125,202].

9.5 2,3',4,5'-Tetrahydroxy-4'-geranylstilbene (Chlorophorin)[127,128,200] [47]

Chlorophorin was present as a major constituent in the heartwood extract and was obtained as discussed in 5.7.



[47] R = H

[47a] R = Me

An aromatic ABX system (δ7.37,d; δ6.41,d; δ6.35,dd; J=2.5 and 8.5Hz), in the ^{1}HNMR spectrum (plate 23) of [47], together with two doublets of a trans-olefinic system

(J=16.5 Hz) at 7.25 and 6.80, and a two-proton singlet (δ6.56) of an additional aromatic ring, indicated a 2,3',4,4',5'-pentasubstituted stilbene. Methylation of [47] with dimethyl sulphate to form [47a] produced characteristic methoxyl peaks δ3.86-3.82 (12H), (^{1}HNMR plate 24), which were assigned to the 2,4,3'5'-substitution pattern.

Chemical shifts and coupling constants, as summarized in Table 7, Sect. 5.8 which were very similar and comparable to those of the geranyl group in compound [101] were obtained in the ^{1}HNMR spectrum of [47]. Homodecoupling experiments on the geranyl substituent also produced similar results as those obtained for [101]. This evidence served to confirm the structure of the 4'-geranyl group.

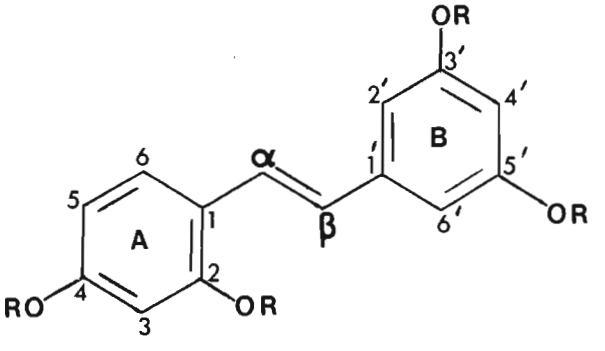
The mass spectrum of [47] (Scheme H) showed a molecular ion at m/z 380 (22%) and the fragments m/z 311(M^+-69,19%), 297(M^+-83,15%) and 295 (M^+-85,32%) together with the base peak at m/z 257 (M^+-123,100%) further confirmed the 4'-geranyl group. The A and B aromatic rings were confirmed by fragments m/z 123 (12 and 20%).

The molecular ion of the tetramethyl ether [47a] appeared at m/z 436(31%), and the fragment m/z 313 (100%) indicated the loss of the geranyl group.

The proposed structure of [47], based on the above evidence, is in agreement with the structure of chlorophorin elucidated, using chemical methods only , by King and Grundon [127,128].

9.6 2,3',4,5'-Tetrahydroxystilbene (Oxyresveratrol)[117,200,201] [103]

Oxyresveratrol was obtained as indicated in 5.9.



[103] R = H

[103a] R = Me

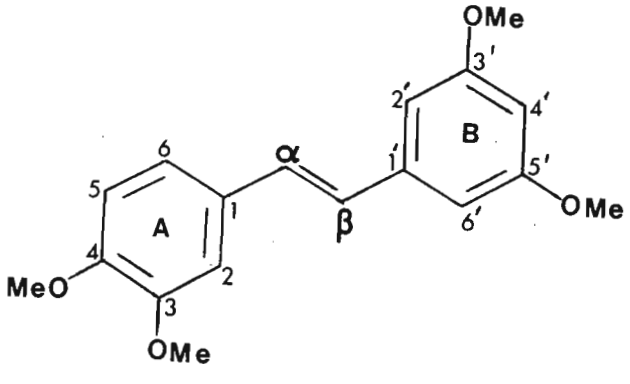
The ^{1}HNMR spectrum (plate 25) of [103] showed, together with signals due to trans-olefinic protons (δ7.32 and δ6.88, each 1H, d, J = 16.5Hz), the occurrence of two independant aromatic rings, each with three protons. One ring showed an ABX-system (δ7.39, 1H, d, J = 8.5Hz; δ6.43, 1H, d, J = 8.5Hz and 2.5Hz; δ6.38,1H, d) assignable to protons on a 1,2,4-trisubstituted aromatic ring, while the other ring showed an AX_2-system (δ6.52, 2H, d, J = 2.5Hz;

and δ6.23, 1H, t, J = 2.5Hz) assignable to protons on a 1,3,5-trisubstituted system. Two broad singlets at δ8.40 and δ3.60 disappeared on addition of D_2O. This indicated the presence of free hydroxyl groups. Methylation of [103] to form [103a] produced, (^{1}HNMR plate 26) aromatic methoxyl signals (δ3.82-3.86) integrating for a total of twelve protons, and caused the disappearance of the hydroxyl signals.

The above evidence together with the occurrence of a molecular ion at m/z 244 (100%) in the mass spectrum, led to the above proposed structure of [103], which is in accordance with the structure of oxyresveratrol previously reported[117,200,201].

9.7 3,3'4,5'-Tetramethoxystilbene[118,121] [106].

This tetramethyl ether of astringenin was obtained as was indicated in 5.13.1.



[106]

Trans-olefinic proton signals (δ7.02 and δ6.88, each 1H,

d, J = 16.0 Hz) together with the peaks of two aromatic rings each with three protons, in the ^{1}HNMR spectrum (plate 27) of [106], indicated a stilbene moiety. ABX-type signals (δ7.05, 1H,d,J=2.0Hz; δ7.03, 1H, dd,J=2.0 and 8.0 Hz; δ6.84, 1H, d, J = 8.0Hz) assignable to the protons on a 1,3,4-substituted aromatic ring and an AX_2 system (δ6.64, 2H, d, J = 2.0Hz and δ6.36, 1H, t, J = 2.0Hz) assignable to the protons on a 1,3,5-trisubstituted aromatic ring, together with aromatic methoxyl signals at δ3.81-3.93 (12H) led to the proposed structure for [106].

NOE association results, summarized in Table 19, indicated association of the methoxyl protons with the adjacent protons. An association of H-2(4.0%) and H-5(3.5%) with the methoxyl protons at δ3.93 and δ3.89 indicated a 3-OMe and 4-OMe substitution respectively. On the B-ring an association of H-4' (12.4%) and H-2',6'(5.5%) with the methoxyl protons at δ3.81 indicated a 3'-OMe and 5'-OMe substitution respectively.

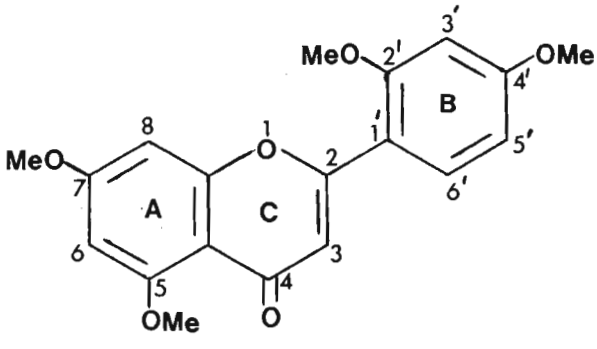
The structure of [106] was confirmed by comparison against authentic ^{1}HNMR data[121] and melting point[118].

Table 19 : NOE associations for stilbene [106]

Decoupling applied	NOE effect observed
A-ring:	
3-OMe (δ3.93)	H-2(δ7.05 , 4.0%)
4-OMe (δ3.89)	H-5(δ6.84 , 3.5%)
B-ring:	
3',5'-OMe (δ3.81)	H-4'(δ6.36 , 12.4%)
	H-2',6'(δ6.64, 5.5%)

9.8 2',4',5,7-Tetramethoxyflavone[204] [105]

This flavone was obtained as discussed in 5.12.1.



[105]

Two single-proton doublets (δ6.43, J = 2.5 Hz and δ6.41,

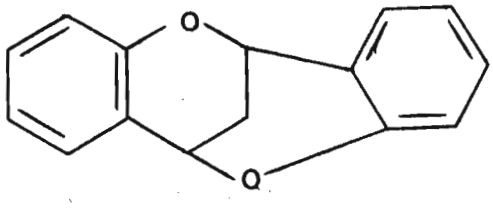
J = 2.5Hz) indicating meta-coupled H-8 and H-6 respectively and an ABX system (δ7.94, d, J = 9.5Hz; δ6.57, dd, J = 2.5 and 9.5 Hz; δ6.51, d, J = 2.5Hz) assignable to H-6', H-5' and H-3' of the C-ring respectively were apparent in the ^{1}HNMR spectrum (plate 28) of [105]. A singlet at δ7.14(1H) was typical of a flavone C-3 proton. Four singlets, integrating for three protons each (δ3.96-3,84) indicated four methoxyls. Table 20 summarizes NOE associations of the methoxyl protons with the adjacent protons. An association of H-6 (3.8%) and H-8(7%) with the methoxyl protons at δ3.90 implied a 7-OMe, while a 9.3% association of H-6 with the methoxyl protons at δ3.96 indicated a 5-OMe. On the B-ring H-3' and H-5' showed an association of 4.4% and 7.6% with the methoxyl protons at δ3.84, indicating a 4'-OMe, while an association (11.7%) of H-3' with the methoxyl protons at δ3.92 indicated a 2'-OMe.

The melting point and ^{1}HNMR spectrum of [105] is similar to that of naturally occurring 2',4',5,7- tetramethoxy-flavone previously isolated by Nagar and co-workers[204].

Table 20: NOE association for flavone [105]

Decoupling applied	NOE effect observed
A-ring:	
7-OMe (δ3.90)	H-6 (δ6.41 , 3,8%)
	H-8 (δ6.43 , 7.0%)
5-OMe (δ3.96)	H-6 (δ6.41 , 9.3%)
B-ring:	
4'-OMe (δ3.84)	H-3'(δ6.51 , 4.4%)
	H-5'(δ6.57 , 7.6%)
2'-OMe (δ3.92)	H-3'(δ6.51 , 11.7%)

This 2'-substituted flavone could represent a possible intermediate in the biosynthesis of the cyanomaclurin-type structures[237] .

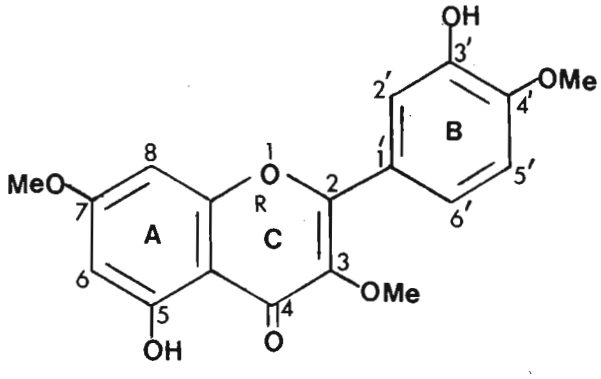


'cyanomaclurin nucleus'

9.9 FLAVONOLS

Two flavonols 3,4',7-tri-O-methylquercitin (ayanin)[99] and 2'-methoxy-3,4,7-tri-O-methylquercitin [100] were obtained as was discussed in 5.2.3 and 5.2.4 respectively.

9.9.1 3,4',7-Tri-O-methylquercitin (Ayanin)[198,199] [99]

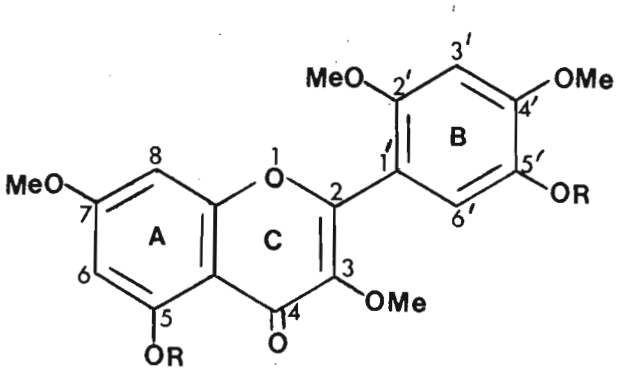


[99]

The ^{1}HNMR spectrum (plate 29) of [99] showed two meta-coupled doublets (J=2.0Hz) at δ6.31 and δ6.68. These were assigned to H-6 and H-8 respectively. A meta-coupled doublet (δ7.64, J=2.0Hz), an ortho-meta-coupled doublet of doublets (δ7.69, J=2.0 and 9.0Hz) and an ortho-coupled doublet (δ7.12, J=9.0Hz) were observed, indicating a 1,3,4-trisubstituted B-ring. Three singlets in the aromatic methoxyl range were observed. The appearance of a down-field singlet (δ8.72) indicated a 5-OH hydrogen bonded to the C-4 carbonyl group. A broad 'hump' at δ8.10 indicated a second hydroxyl.

Comparison of the ^{1}HNMR spectrum of [99] against that reported in the literature[199] indicated the structure to to be similar to that of ayanin discovered by King and co-workers[198].

9.9.2 2'-Methoxy-3,4',7-tri-O-methylquercitin [100]



[100] R=H

[100a] R=Ac

The ^{1}HNMR spectrum (plate 30) of [100] showed two meta-coupled doublets (δ6.31 and δ6.50, J = 2.0Hz) and two singlets (δ6.98 and δ6.86) which could represent the A-ring (H-6 and H-8) and B-ring para-coupled protons respectively.

A sharp downfield singlet at δ8.70 indicated a typical deshielded 5-OH hydrogen bonded to the C-4 carbonyl oxygen[199]. A broad 'hump'at δ7.61 indicated a second hydroxyl group.

The ^{1}HNMR spectrum of the acetylated derivative (100a) compared with that of the natural phenol (100) showed deshielding (downfield shift of 0.34 ppm) of the m-doublet at δ6.31 indicating a 5-OH closely associated with H-6. Acetylation also caused a deshielding of the para-coupled B-ring protons with the proton at δ6.98 showing a greater downfield shift (0.23 ppm) than the proton at δ6.86 (0.07 ppm) which indicated that there was a B-ring hydroxyl group closely associated with the proton at δ6.98.

Four methoxyl signals at δ3.95, 3.89, 3.83 and 3.77 were observed. Table 21 summarizes NOE association of these methoxyl protons with the adjacent protons. An association of H-6 (5.8%) and H-8 (8.1%) with the methoxyl protons at δ3.89 indicated a 7-OMe, while a 8.8% and 8.2% association of the proton at δ6.86 with the methoxyl protons at δ3.95 and δ3.84 implied a 2'-OMe and 4'-OMe respectively. This indicated therefore that the para-coupled B-ring protons were H-3' and H-6'. On the C-ring a 0.8% association of H-6' with the methoxyl protons at δ3.78 indicated a 3-OMe.

Table 21 : NOE associations for flavone [100]

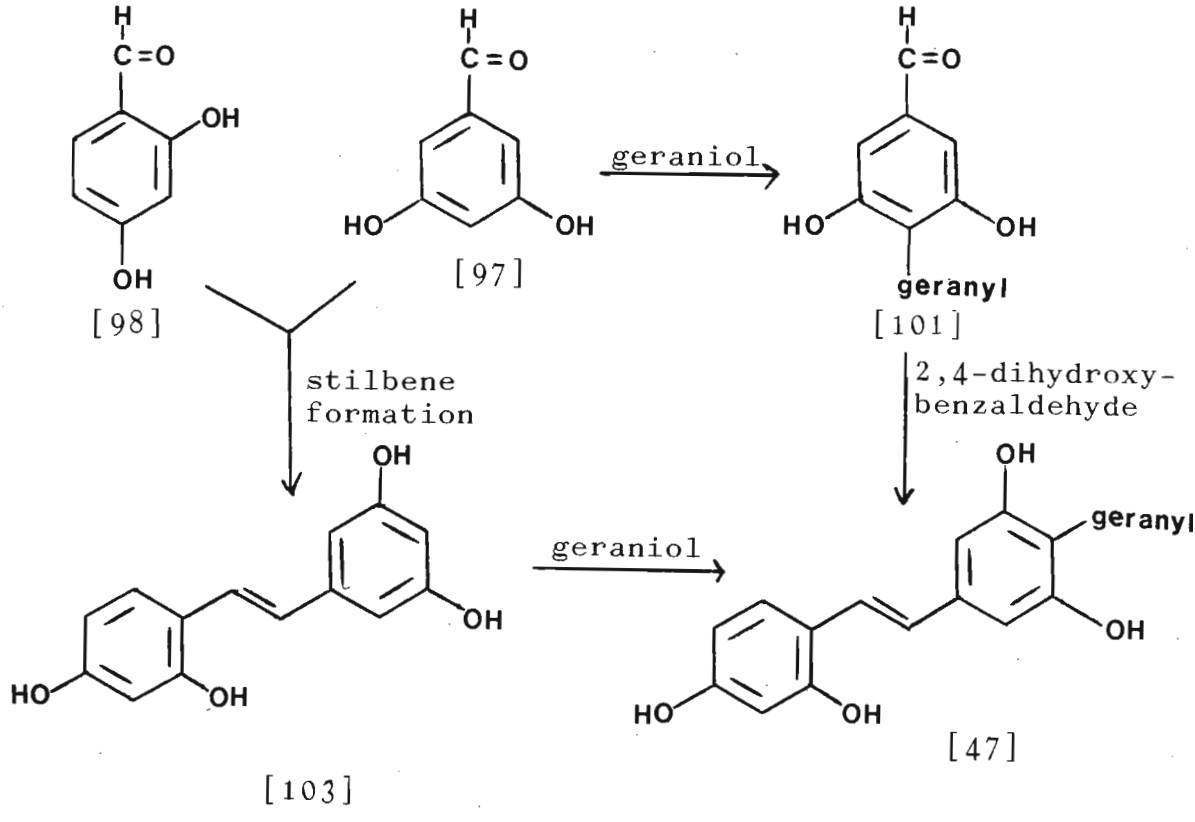
Decoupling applied	NOE effect observed
A-ring:	
7-OMe (δ3.89)	H-6 (δ6.31 , 5,8%)
	H-8 (δ6.50 , 8.1%)
B-ring:	
2'-OMe (δ3.95)	H-3' (δ6.86 , 8.8%)
4'-OMe (δ3.84)	H-3' (δ6.86 , 8.2%)
C-ring:	
3-OMe (δ3.78)	H-6' (δ6.98 , 0.8%)

Mass spectral analysis (Scheme I) of the acetate derivative [100a] showed a fragment at m/z 374 (M^+-84, 65%) indicating the loss of two acetate units. Loss of 30 (m/z 344, 23%) and 31 (m/z 343, 100%) mass units from M^+-84 confirmed a methoxyl group at the 2' position. Only one RDA fragment (m/z 167, 32%) was obtained, which confirmed the A-ring substitution pattern. Bowie and Cameron[73] stated that flavonols with four or more oxygen substituents do not give meaningful RDA fragments. Malan and Roux[199] however reported that 2',6-dihydroxy-3,4',5', 5,7-pentamethoxy-flavone was an exception in that RDA fragments representing rings A(m/z 197, 29%) and B(m/z 181, 14%) were obtained.

9.10 POSSIBLE BIOGENETIC RELATIONSHIPS

A possible biogenetic relationship between some of the isolated metabolites of Iroko is illustrated in Scheme 34.

SCHEME 34



CHAPTER 10

SYNTHESIS AND REACTIONS OF SOME IROKO METABOLITES

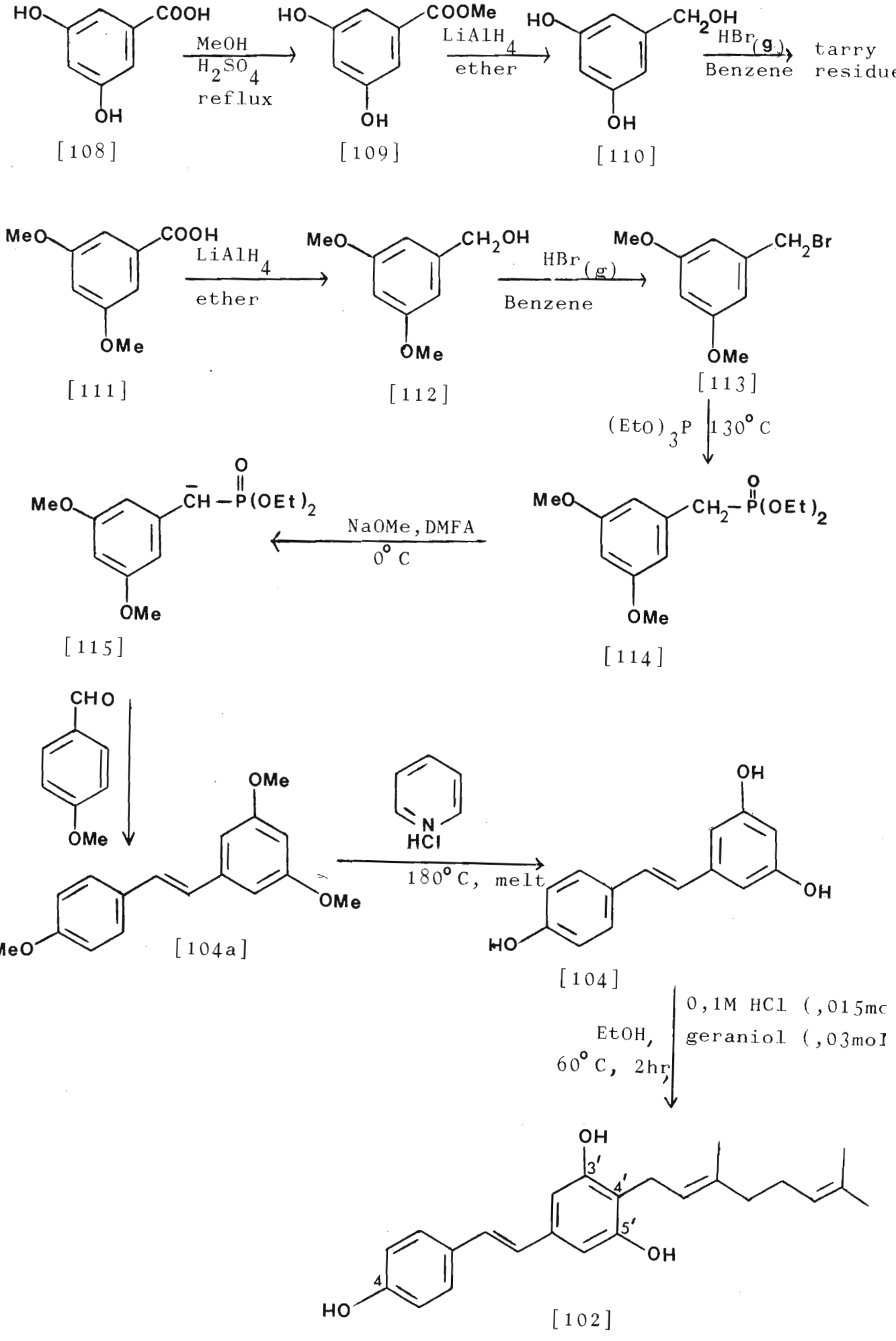
10.1 SYNTHESIS OF 3',4,5'-TRIHYDROXY-4'-GERANYL-STILBENE[200] [102]

The synthesis of 3',4,5'-trihydroxy-4'-geranylstilbene [102] was attempted in several steps as outlined in Scheme 35.

3,5-dihydroxybenzoic acid [108] was esterified to form methyl 3,5-dihydroxybenzoate [109]. The ester was reduced with lithium aluminium hydride to give a mixture containing 3,5-dihydroxybenzyl alcohol [110] and the original starting material (ester). The alcohol was purified by chromatography (column and plc) and treated with HBr. The result was a thick tarry product which was then extracted with chloroform and chromatographed on a column. The fractions obtained showed little prospect as no significant spots of the expected product were obtained on tlc.

The use of 3,5-dimethoxybenzoic acid [111] instead of 3,5-dihydroxybenzoic acid [108] as starting material proved to be more successful.

SCHEME 35

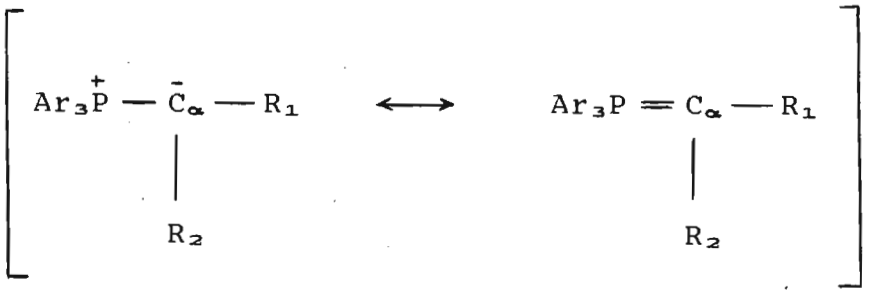

HO
COOH
OH
[108]
MeOH
H_2SO_4
reflux
COOMe
[109]
$LiAlH_4$
ether
CH_2OH
[110]
$HBr_{(g)}$
Benzene
tarry
residue
MeO
OMe
[111]
[112]
CH_2Br
[113]
$(EtO)_3P$
130° C
$CH_2-P(OEt)_2$
[114]
NaOMe, DMFA
0° C
CH—P(OEt)$_2$
[115]
CHO
[104a]
N
HCl
180° C, melt
[104]
EtOH,
60° C, 2hr
0,1M HCl (,015mc
geraniol (,03mol
3′
4′
5′
4
[102]

The modified Wittig reaction was employed to synthesize 3',4,5'-trimethoxystilbene [104a] which was demethylated and subsequently reacted with geraniol, under acidic conditions, to yield the required geranylstilbene [102].

10.1.1 The Wittig[165] and Modified Wittig[174,175] Reaction

In the Wittig reaction[165] a phosphorus ylid (phosphorane) may be reacted with an aldehyde to give a stilbene (a mixture of cis- and trans-isomers) together with triphenylphosphine oxide.

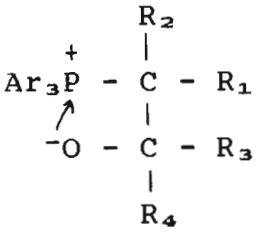
The phosphorus ylid is a resonance hybrid of two canonical forms [116] and [117].



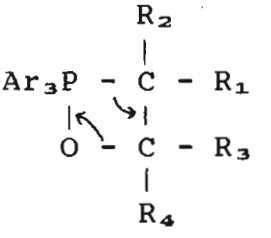
[116] [117]

ylid

A betaine [118] and a cyclic alkoxyphosphorane [119] are intermediates in the two-stage stilbene-forming process.



[118]



[119]

Because betaine formation involves the nucleophilic attack of the α-carbon of the ylid on the carbonyl carbon of the aldehyde, substituents on the phosphorus that decrease its positive character will stabilize the dipolar form [116], thereby increasing the reactivity of the phosphorane, whereas delocalization of the negative charge on the α-carbon increases the reactivity[166].

Substituents on the phosphorus which decrease its positive character, and thereby its oxygen affinity, retard the betaine decomposition[166].

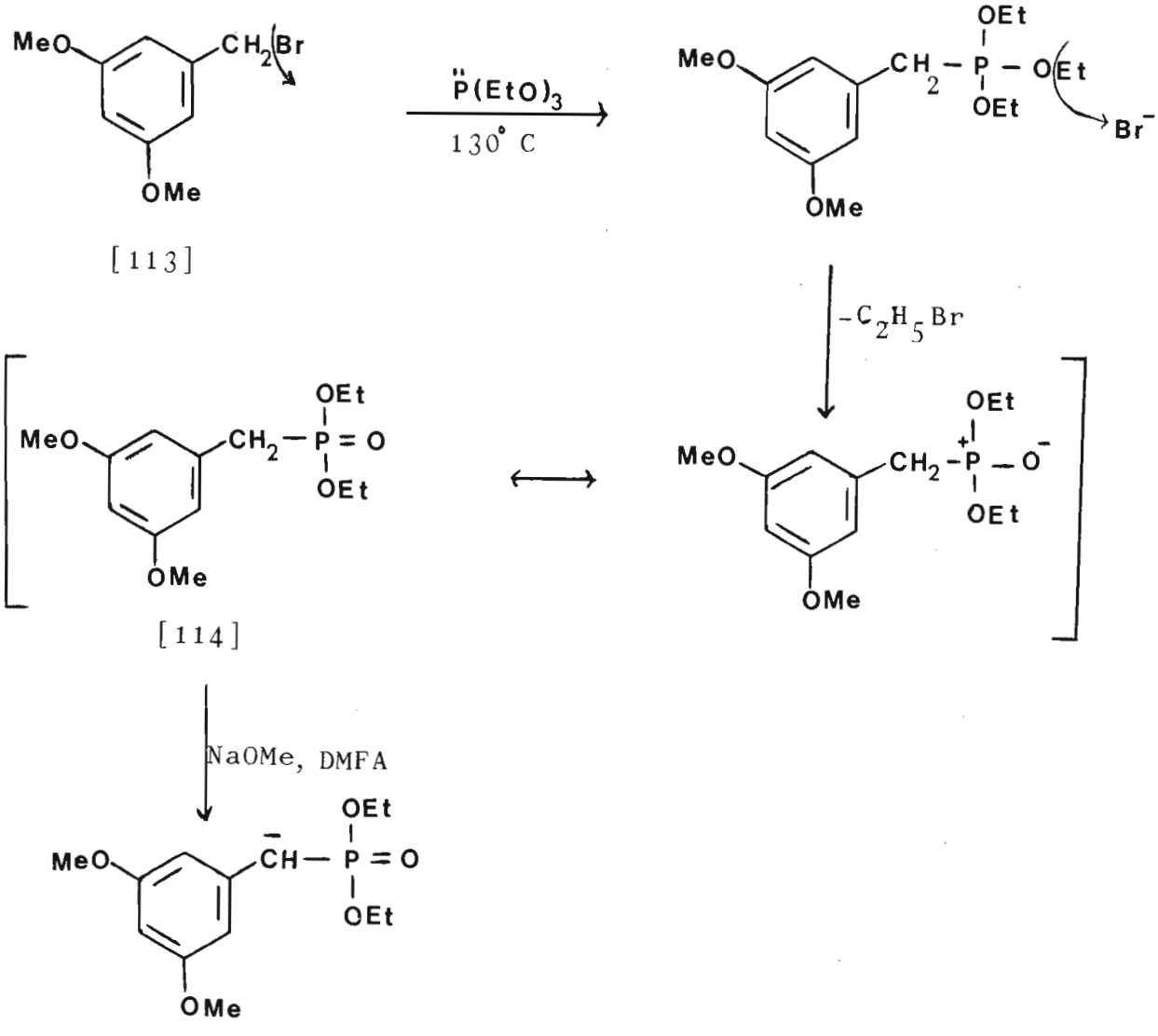
Johnson and Kyllingstad[217] concluded that formation of trans-stilbenes was strongly favoured by the use of phosphonium ylids carrying election donating phosphorus substituents while formation of cis-stilbenes was favoured by use of ylids carrying election-withdrawing phosphorus substituents.

In the modified Wittig reaction[174], aryl methyl halides

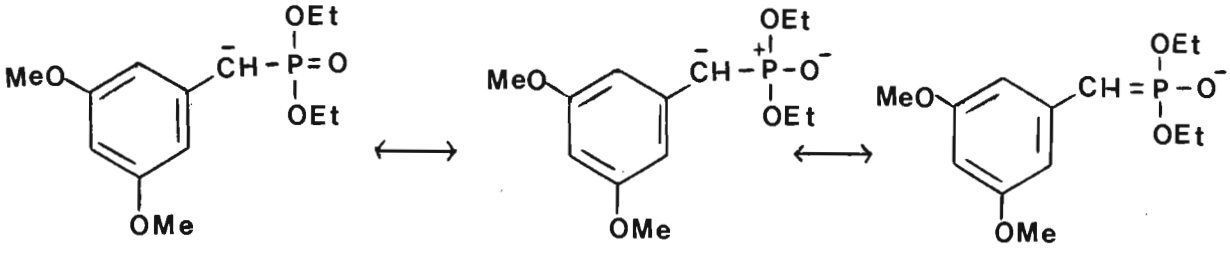
are heated with trialkyl phosphites to give the phosphonates (Arbusov reaction)[175]. The reaction of aryl aldehydes with the phosphonate anion, formed in situ with an alkoxide, gives trans-stilbenes exclusively.

In the present study 3,5-dimethoxybenzyl bromide [113] was heated with excess triethyl phosphite. The resulting diethylbenzyl phosphonate [114] was treated with sodium methoxide to form the phosphonate anion [115]. The suggested mechanism is as shown in Scheme 36.

SCHEME 36



The phosphonate anion could be regarded as a resonance hybrid of three contributing structures[177].

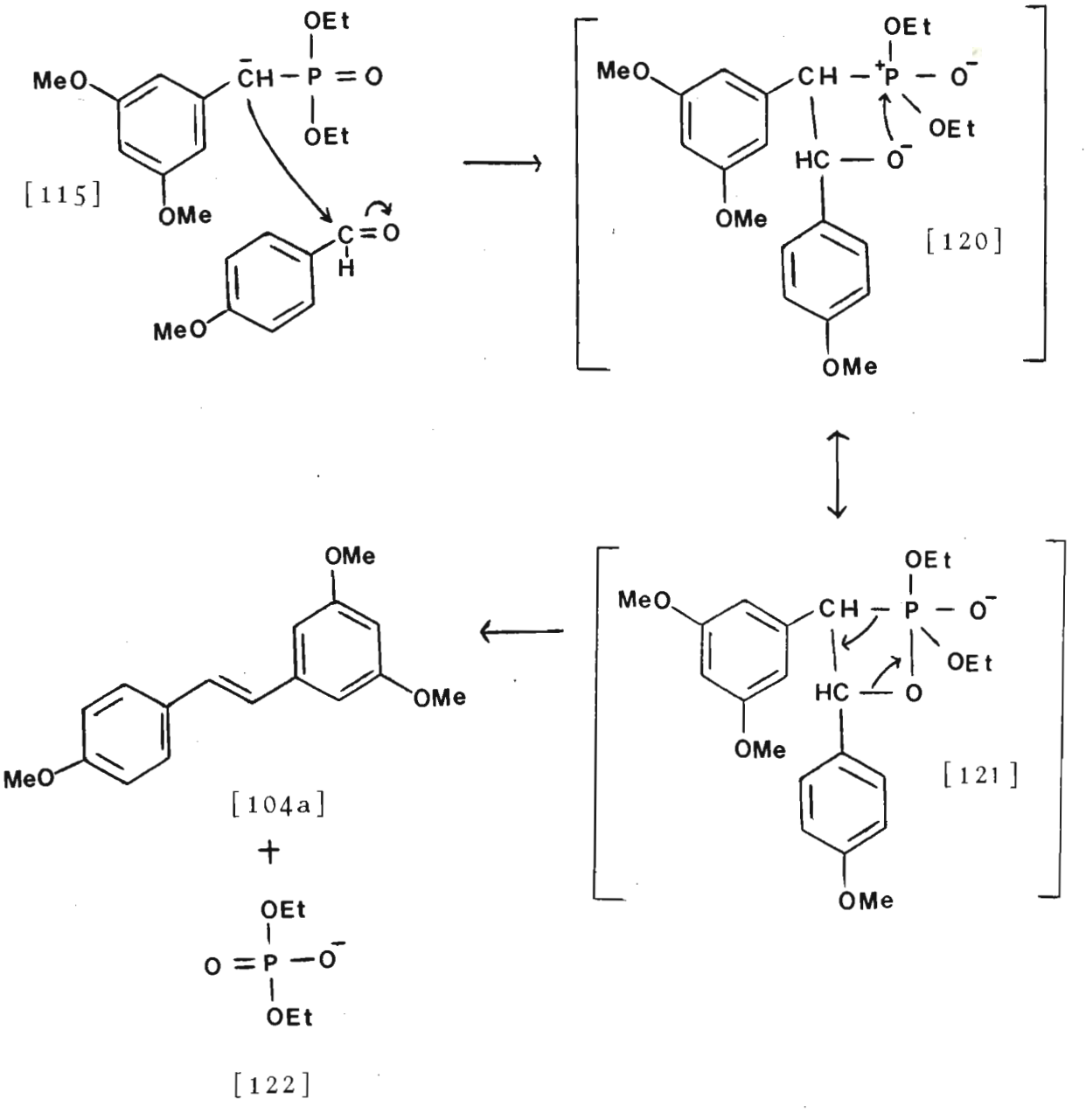


[115a] [115b] [115c]

The structures [115a] and [115b] ulitized a phosphorus d-orbital in a manner similar to the phosphoranes. The P=O bond also had some single bond character which enabled structures [115b] and [115c] to contribute.

The phosphonate anion [115] reacted with the carbonyl group of 4-methoxybenzaldehyde (as shown in Scheme 37) to form the intermediate [120], which Wadsworth and Emmons[177] suggested could be in equilibrium with a four-membered ring [121] which then fragmented to yield 3',4,5'-trimethoxy-stilbene [104a] and the phosphate anion [122].

SCHEME 37



The driving force for the reaction was provided by the formation of the stable phosphate anion [122] which was a more thermodynamically stable species than the phosphonate anion. Wadsworth[177] also suggested that the greater reactivity of a phosphonate anion over a comparable phosphorus ylid may reflect the fact that the phosphonate intermediate is a saturated carbanion having a high degree of charge separation while the triarylphosphoranes have relatively little charge separation and are therefore less reactive.

10.1.2 Demethylation of Stilbenes

There are a number of methods reported in the literature for the demethylation of stilbenes.

Bachelor and co-workers[172] attempted to demethylate pinosylvin dimethyl ether with boron tribromide and obtained a 90% yield of the monodemethylated product. Further attempts to cleave the second ether with boron tribromide gave only good yields of the recovered ether. Rieman[218] also attempted unsuccessfully to demethylate 2,4,3',5'-tetramethoxystilbene using boron tribromide. Although examples of selective cleavage of polyethers are known [219,220], these have always involved the participation of a neighbouring carbonyl group.

Bachelor and co-workers[172] suggested that the complete demethylation of polymethoxy aromatics by BBr_3 was obtained in good yield only when the methoxyl groups were adjacent or in different aromatic rings[221,222,223]. When the methoxyls were separated and in the same ring poor yields were obtained.

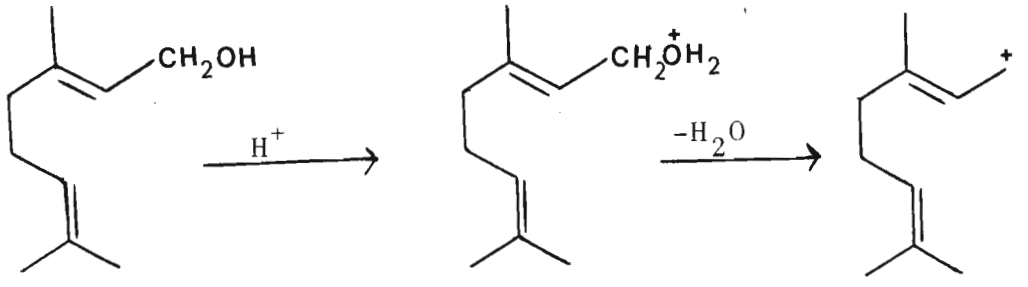
Riemann[218] and Bachelor[172] obtained complete demethylation of pinosylvin dimethyl ether by the use of pyridine hydrochloride[223]. Lindberg[224] was also successful in the demethylation of pinosylvin monomethyl ether using pyridine hydrochloride.

In the present study, after attempting all the above methods, the pyridine hydrochloride procedure was successfully used to demethylate 3',4,5'-trimethoxystilbene, thereby producing 3',4,5'-trihydroxystilbene [104]. The high temperature (180°;2hours) needed for the melt was however a disadvantage as it most certainly caused considerable decomposition which could account for the low yield of [104].

10.1.3 Acid catalyzed condensation of 3',4,5'-trihydroxystilbene [104] with geraniol[200]

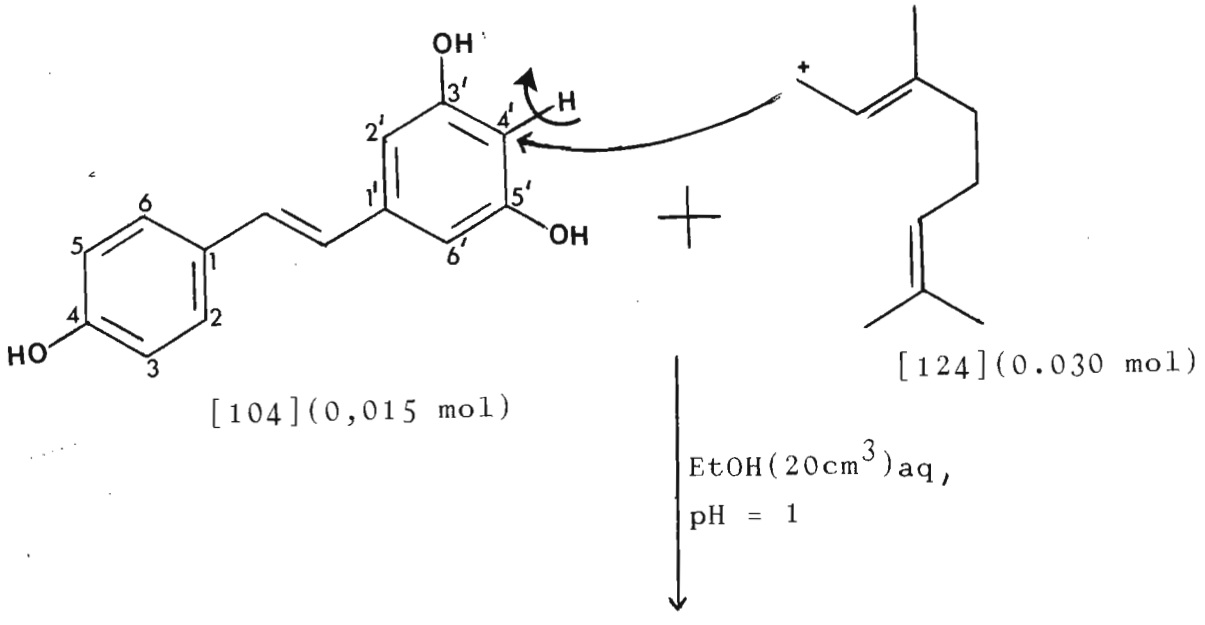
The acid-catalysed[207] condensation of geraniol and 3',4,5'-trihydroxystilbene [104] resulted in a 8.0% yield of

3',4,5'-trihydroxy-4'-geranylstilbene [102]. The attempt at this reaction was based on the assumption that geraniol [123] would form a carbocation [124], which would attack the site of greatest nucleophilicity (4') on the stilbene.



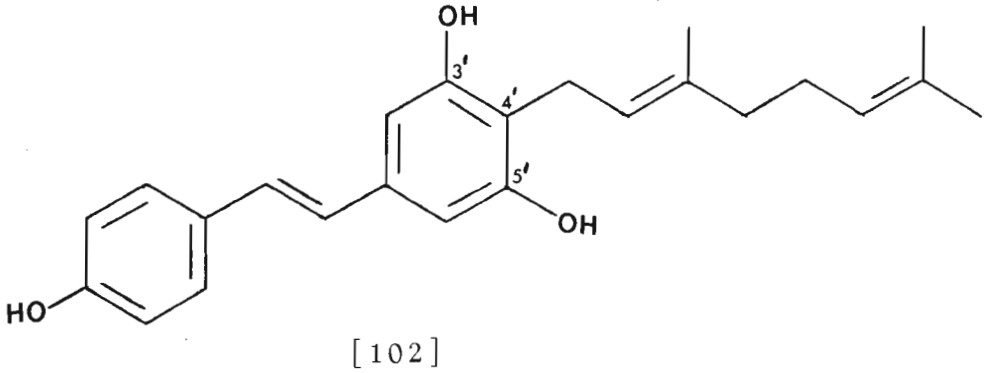
[123] [124]

The 4'-positon of the stilbene was the most activated nucleophilic site because of the stable conjugated system present. Therefore attack, if any, of the geranyl carbocation was most likely to be at this position.



[104](0,015 mol) [124](0.030 mol)

EtOH($20cm^3$)aq,
pH = 1

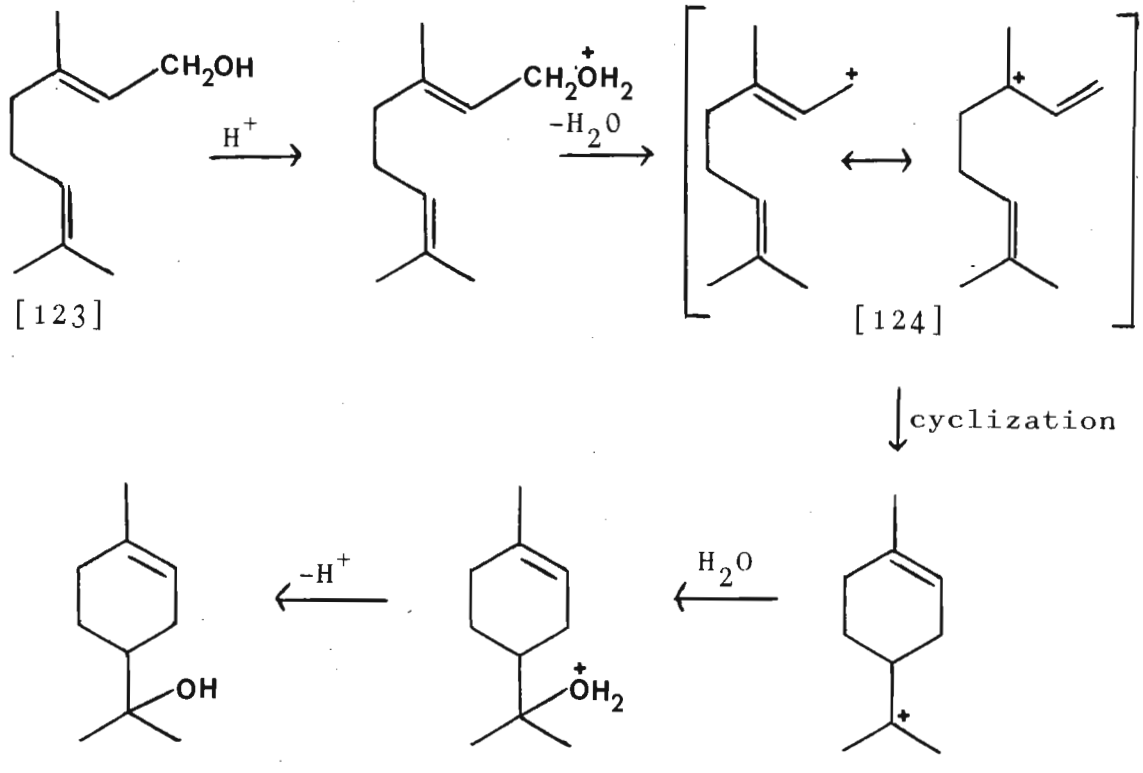


[102]

The resulting stilbene [102] had an ^{1}HNMR spectral pattern and a melting point identical to that of natural 3,4,5'-trihydroxy-4'-geranylstilbene isolated from the Iroko heartwood. This synthesis was a confirmation of the structure of [102].

The low yield could be partly attributed to the expected rearrangement of geraniol and its carbocation in acid medium[225] as shown in scheme 38.

SCHEME 38



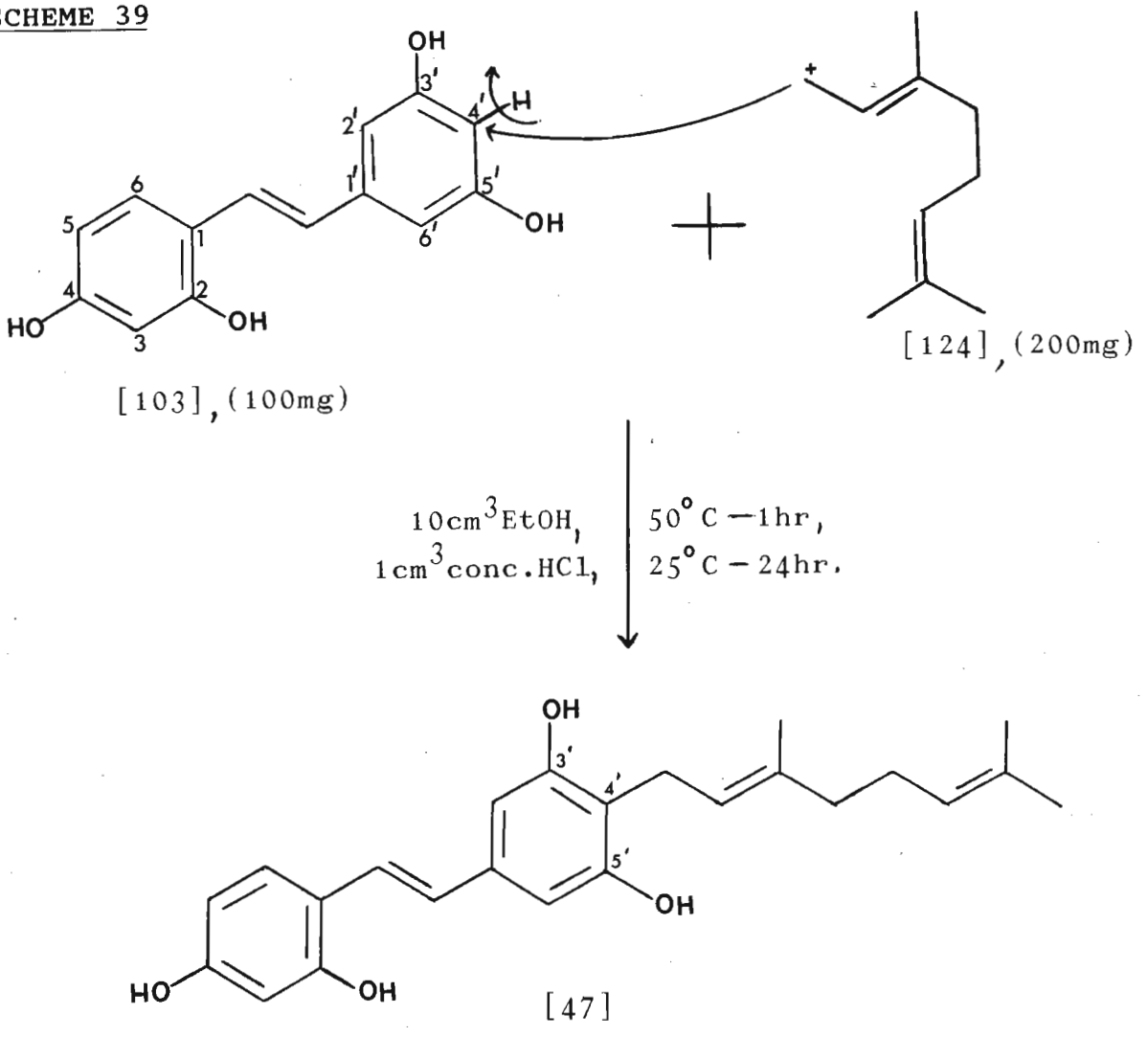
It seems that the carbocation was short-lived. A very strictly controlled pH medium was therefore necessary to ensure formation of the carbocation and its immediate attack on the nucleophilic site of the stilbene.

The low yield may also be explained by steric hindrance in view of the bulkiness of the geraniol carbocation. The nucleophilic 4'-position on the stilbene was surrounded by the adjacent 3'-and 5'-hydroxyls and attack at this position was therefore hindered.

10.2 SYNTHESIS OF 2,3',4,5'-TETRAHYDROXY-4'-GERANYL-STILBENE (CHLOROPHORIN) [47]

Several attempts were made to couple geraniol to natural 2,3',4,5'-tetrahydroxystilbene [103] (isolated from Iroko) under a variety of acidic conditions[207] in ethanol medium. Only one positive result (5% yield), using an acidic ethanolic medium (9% conc. HCl, v/v) was obtained, as shown in Scheme 39, producing 2,3',4,5'-tetrahydroxy-4'-geranyl-stilbene [47]. This resulting stilbene was identical to natural chlorophorin with respect to ^{1}HNMR, melting point and R_f.

SCHEME 39

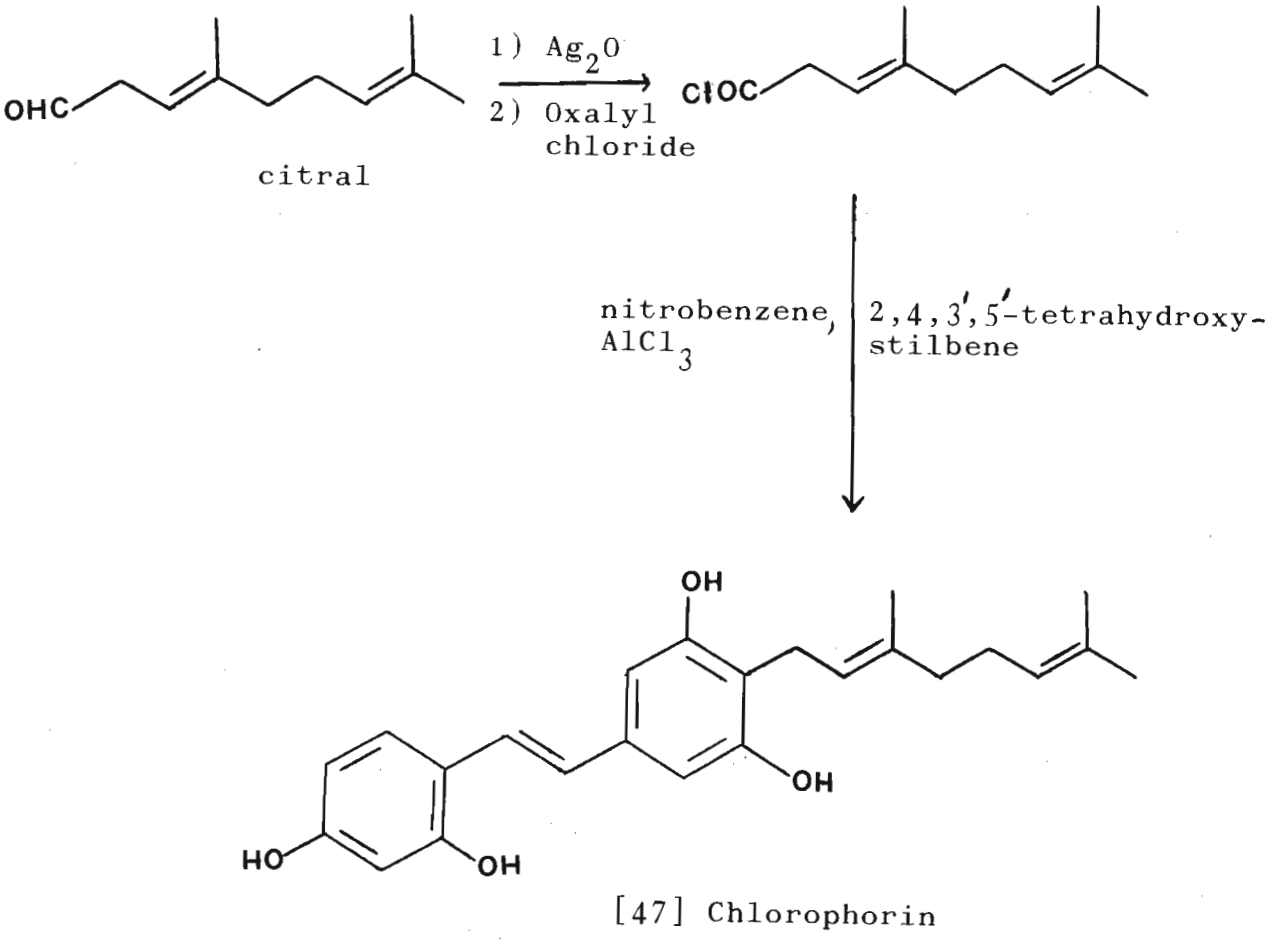


Although positions 3 and 4' of [103] were both nucleophilic sites, it is suggested that the 4'-position was more favoured than the 3-position.

Morimoto and co-workers[208] prenylated (+)-catechin in the presence of p-toluenesulphonic acid. On this evidence, different concentrations of p-toluenesulphonic acid in ethanol were also tried as coupling medium in the reaction between geraniol [123] and 2,3',4,5'-tetrahydroxystilbene [103]. No positive results were obtained.

An alternative attempt at the coupling envisaged a Friedel Crafts[209] acylation (Scheme 40) of geranoyl chloride to the stilbene.

SCHEME 40

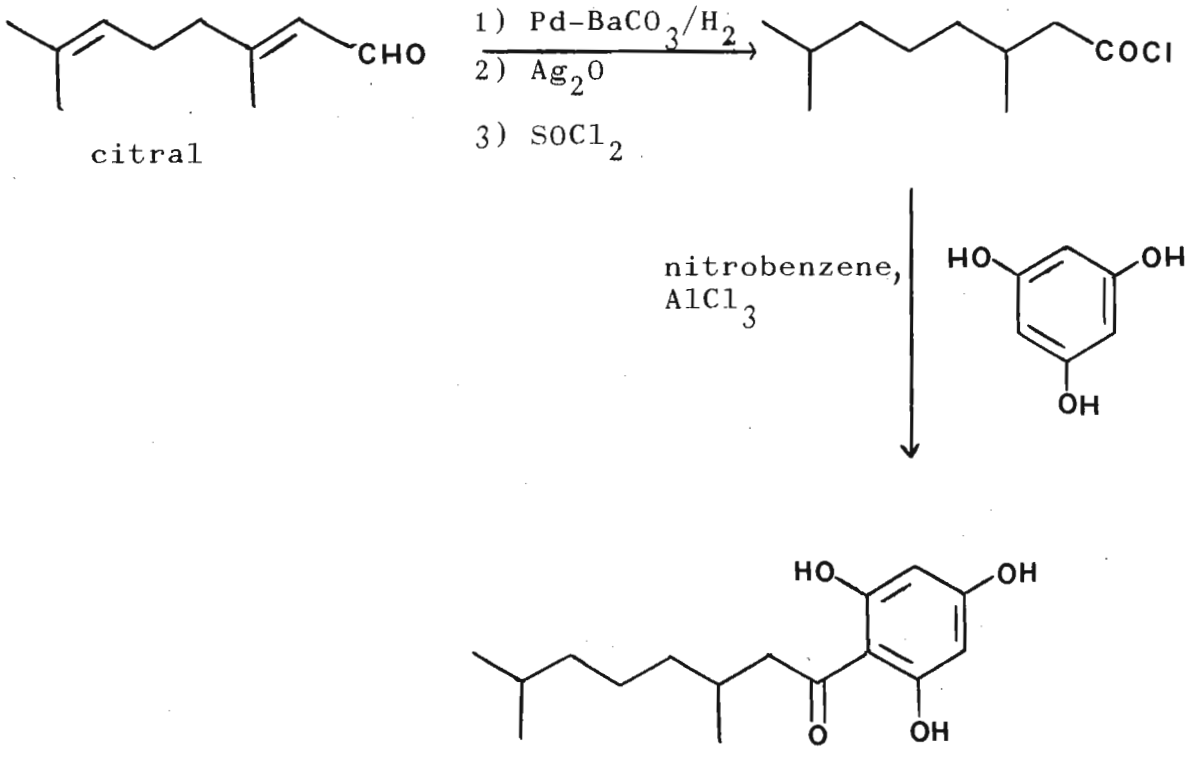


[47] Chlorophorin

Although ^{1}HNMR spectroscopy clearly indicated the formation of geranic acid (^{1}HNMR plate 35) on silver oxide oxidation of citral[210], no further positive results were obtained.

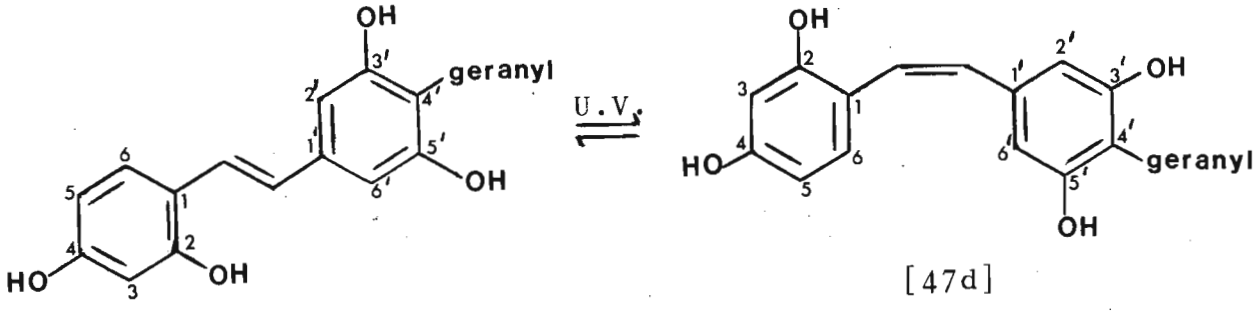
The above set of reactions was based on the work done, shown in Scheme 41, by Ueno and co-workers[209].

SCHEME 41



10.3 U.V. IRRADIATION OF CHLOROPHORIN [47]

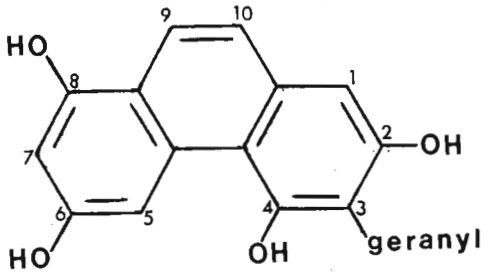
U.V. irradiation (300nm, 3 hrs) of a solution of chlorophorin in methanol, using benzophenone as initiator, produced an equilibrium mixture of the trans- and the expected cis-isomer [47d].



[47] [47d]

On tlc (B : A, 8 : 6, v/v) the cis-stilbene showed up as a distinct spot (R_f 0,53) slightly above the spot of the trans-chlorophorin (R_f0,46). ^{1}HNMR analysis of the reaction product showed that there was a mixture of the cis-($J\alpha,\beta$ = 12,0 Hz) and trans-chlorophorin ($J\alpha,\beta$ = 16,0Hz). The isolation of the cis-isomer was not successful due to its speedy reconversion to the trans-isomer.

It was thought that further irradiation (>2 hrs) would cause cyclization to form a phenanthrene type of compound [47e].



[47e]

The phenanthrene compound did not form possibly due to steric effects. The A- and B-rings of chlorophorin were substituted in positions which possibly made the ring closure difficult.

In their attempts to photocyclize 3,3'-dimethoxystilbene, Hügelschofer and co-workers[226] isolated 2,5- and 2,7- but not 4,5-dimethoxyphenanthrene. They sugested that the steric effects between the methoxyl groups in the 4- and 5-positions prevented ring closure to the phenanthrene.

Mallory, Wood and Gordon[227] attempted to explain failure of 9,10-diphenylphenanthrene to photocyclise. They suggested that without olefinic unsaturation the electron distribution in the excited state might have been such that there was insufficient electron availability at the two ortho-positions between which the new bond would be expected to form.

Kasha[228] has showed that stilbenes bearing acetyl and nitro-groups failed to photocyclize. The suggest-

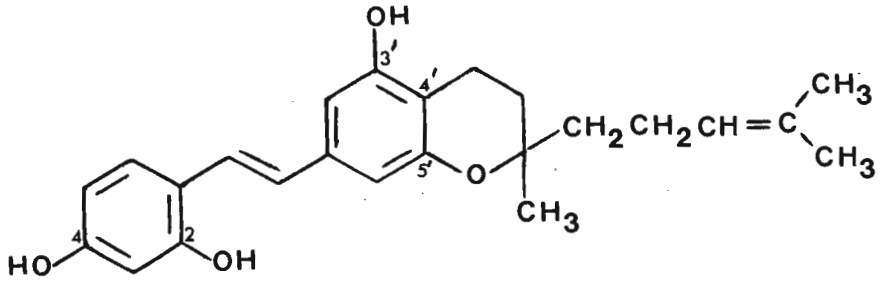
ion[228,229] was that substituents that promoted a high degree of intersystem crossing retarded photocyclyization.

Blackburn and Timmons[229], investigating stilbene analogues which failed to photocyclize, stated that if photocyclization took place then cyclodehydrogenation would also occur on electron impact to give a cyclized ion of relative abundance greater than 5%.

10.4 REACTION OF CHLOROPHORIN WITH DDQ

DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) may be used to dehydrogenate hydroaromatic compounds[230], selectively oxidize allylic[231] and benzylic[232] alcohols, promote oxidative cyclization[233], and oxidize phenols[234].

It was expected that DDQ would cause cyclization of chlorophorin [47], with either the geranyl group being cyclized to form [47f] or, alternatively, the formation of the phenanthrene derivative [47e].



[47f]

However no distinct product was obtained. Instead there seemed to be a large decomposition of chlorophorin.

10.5 REACTION OF CHLOROPHORIN WITH DCC

DCC (dicyclohexylcarbodiimide)[235] is a powerful dehydrating agent which by its action could possibly promote cyclization of chlorophorin.

Treatment of chlorophorin with DCC in different dry solvents (benzene, acetone and ether) and different temperatures did not form any new products.

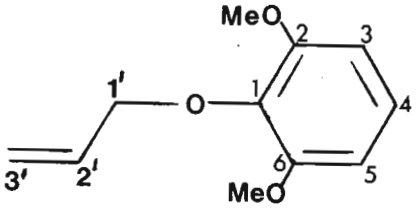
CHAPTER 11

SYNTHESIS OF (+)-CATECHIN LIGNOID

Studies on the synthesis of lignoids are currently in progress in these laboratories. An initial experiment has involved the synthesis of sinapyl alcohol[211] (as outlined in Scheme 42) via a vinyl quinone methide intermediate, and the attempted coupling of the alcohol to (+)-catechin. The intermediates and reaction products were identified by their ^{1}HNMR spectral patterns and are discussed below.

11.1 2,6-Dimethoxy-1-O-allylphenol [126] (^{1}HNMR plate 36)

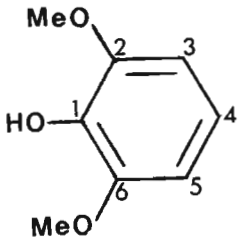
2,6-dimethoxy-1-O-allyl phenol was synthesized as discussed in 7.1



[126]

11.1 Continued/Page 193

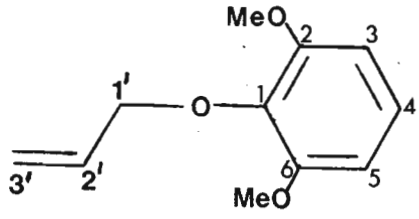
SCHEME 42



(2,6-dimethoxy-phenol)

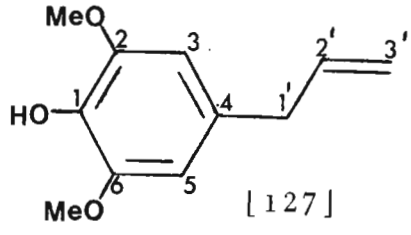
allyl bromide

K_2CO_3



[126]

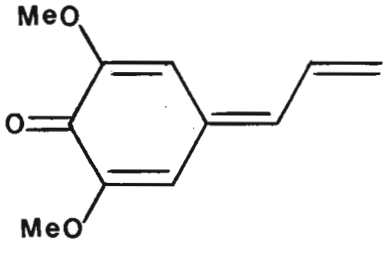
N,N-dimethyl-aniline, $N_{2(g)}$



[127]

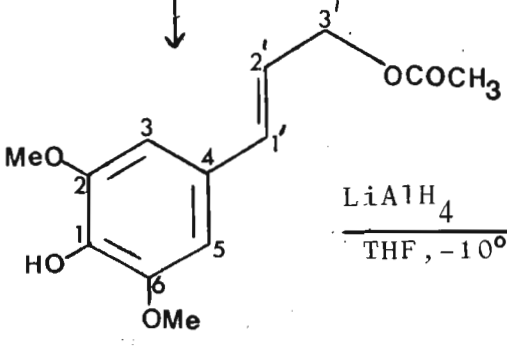
Ag_2O

benzene



vinyl quinone methide

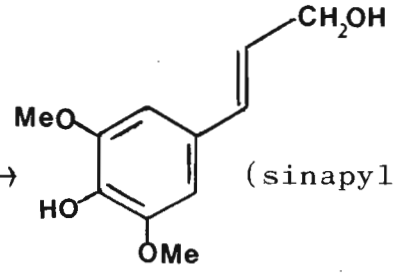
CH_3COOH, CH_3COONa



[128]

$LiAlH_4$

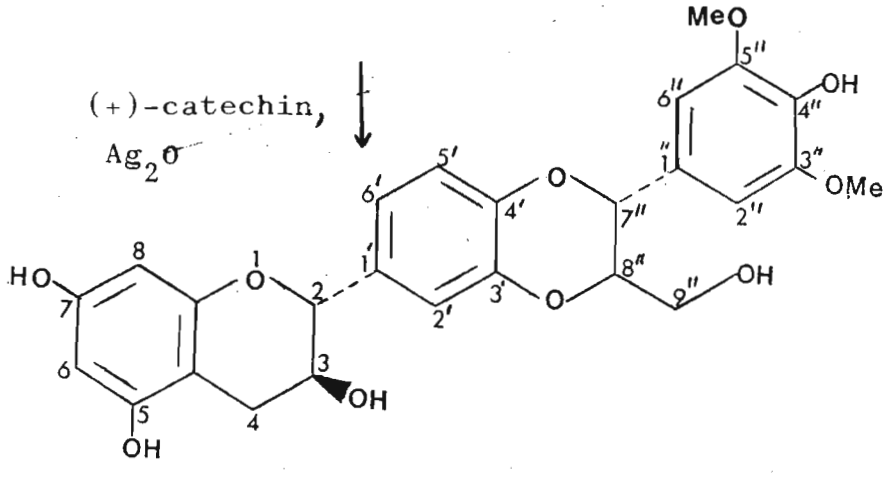
THF, -10° C



(sinapyl alcohol)

(+)-catechin,

Ag_2O



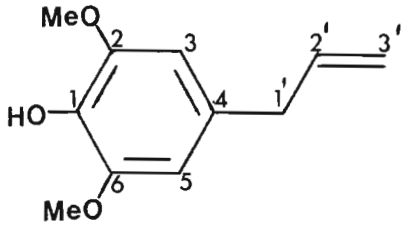
Lignoid[129]

11.1 (Continued/from Page 191)

A triplet at δ6.97, integrating for a single proton was assigned to H-4 with H-2', 3'-CH_2 and 1'-CH_2 appearing as multiplets at δ6.10; δ5.33-5.24 and 5.29-5.13; δ4.52-4.48 respectively in the ^{1}HNMR spectrum of [126]. H-3 and H-5 apeared as a doublet (δ6.55, J = 8.2Hz) with the two methoxyls at positions 2 and 6 appearing as a singlet (δ3.83).

11.2 2,6-Dimethoxy-4-allyl phenol [127] (^{1}HNMR plate 37)

2,6-dimethoxy-4-allyl phenol was synthesized as discussed in 7.2



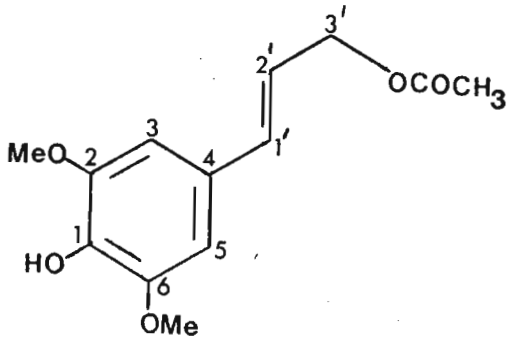
[127]

When 2,6-dimethoxy-1-O-allyl phenol was refluxed in N,N-dimethylaniline, it resulted in a typical Claisen rearrangement[236]. The ^{1}HNMR spectrum of [127] showed multiplets at δ5.11-5.02 and δ3.32-3.27, each multiplet integrating for two protons, these being assigned as 1'-CH_2 and 3'-CH_2. A

multiplet at δ5.93, integrating for a single proton indicated H-2'. A singlet at δ6.39 integrating for two protons indicated H-3 and H-5. The above data confirmed the allyl substituent on 2,6-dimethoxyphenol.

11.3 Sinapyl acetate [128]

Sinapyl acetate was synthesized as discussed in 7.3.

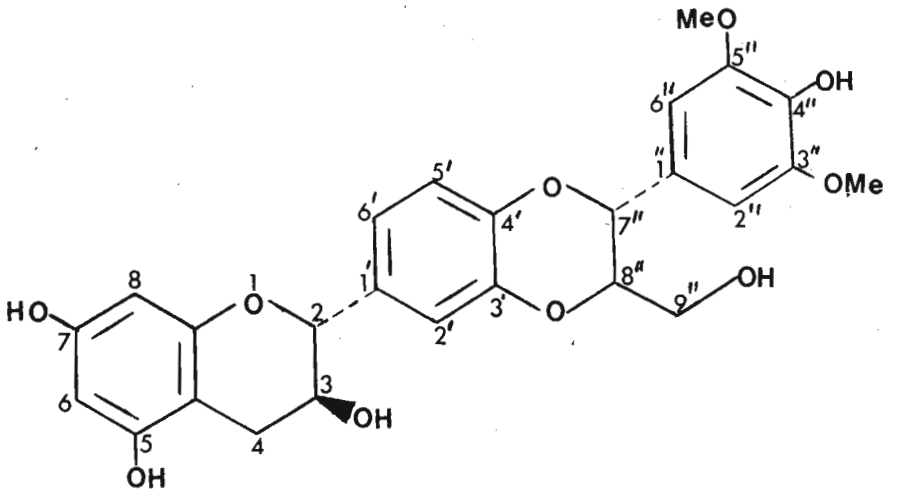


[128]

A singlet (δ2.08) in the ^{1}HNMR spectrum of [128] identified the 3'-acetate group. H-1' and H-2' appeared as multiplets at δ6.54 and δ6.13 while 3'-CH_2 appeared as a doublet of doublets at δ4.69.

Although the ^{1}HNMR spectrum run in $CDCl_3$ showed rapid decomposition of the product, the above signals served to confirm the formation of sinapyl acetate.

11.4 (+)-Catechin lignoid [129]



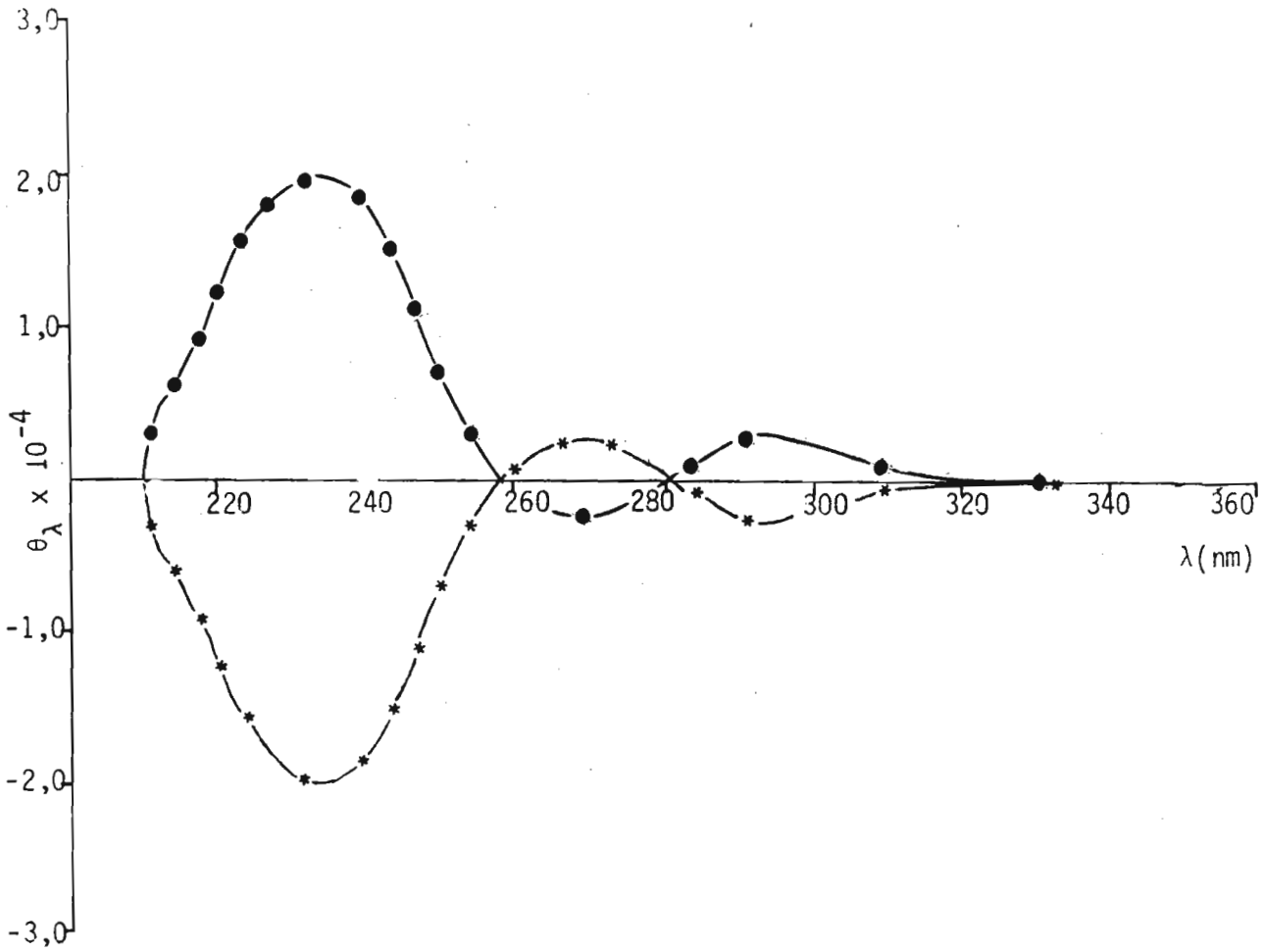
[129]

It was hoped that the reaction between sinapyl alcohol and (+)-catechin as discussed in 7.4 would produce the lignoid [129]. The ^{1}HNMR spectrum of the final product (4mg obtained after plc separation), however, showed traces of the (+)-catechin and allylphenol entities but because of the instability of the product there was no clear evidence to substantiate the formation of the lignoid. The very low stability of sinapyl alcohol, which was obtained in very low yield, could also have been a contributing factor.

Further investigations on lignoid synthesis are currently in progress.

CIRCULAR DICHROISM

FIG. 1



CD curve of :

• (αS)-2',4'-Tri-acetoxy-4-methoxydihydrochalcone [91a], compared against an authentic sample (supplied by Professor D. Ferreira, U.O.F.S.) of:

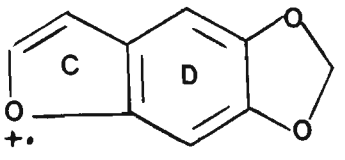
* (αR)-2',4'-Tri-acetoxy-4-methoxydihydrochalcone

MASS SPECTROMETRY

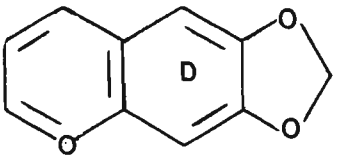
INDEX - MASS SPECTRA

SCHEME	COMPOUND
A	(6aR,11aR)-3-Hydroxy-8,9-methylenedioxypterocarpan (Maackiain) [25].
B	3-Hydroxy-8,9-methylenedioxy-6a,11a-dihydropterocarpan [85].
C	7-Acetoxy-4'-methoxyisoflavone (Formononetin) [10].
D	(αS),2',4'-Trihydroxy-4-methoxydihydrochalcone [91].
E	(αS),4'-Diacetoxy-2'-hydroxy-4-methoxydihydrochalcone [91b].
F	3,5-Dihydroxy-4-geranylbenzaldehyde [101] 3,5-Dimethoxy-4-geranylbenzaldehyde [101a].
G	3',4,5'-Trihydroxy-4'-geranylstilbene [102].
H	2,3',4,5'-Tetrahydroxy-4'-geranylstilbene (Chlorophorin) [47].
I	5,5'-Diacetoxy-2',3,4',7-tetramethoxyflavone [100a].

Scheme A



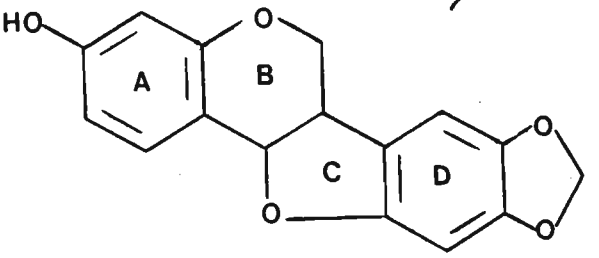
m/z 162(32)



m/z 175(14)

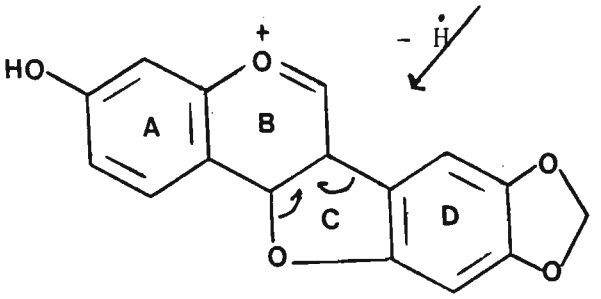
- 122

-109



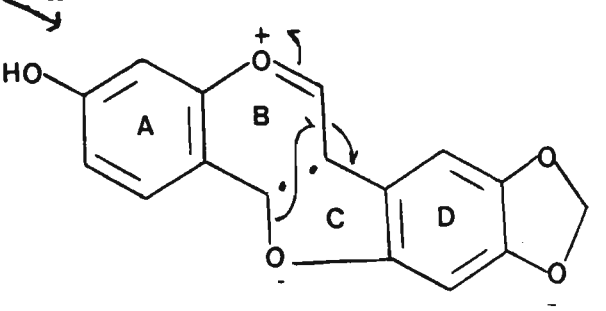
M^+, m/z 284(100%)

- H•



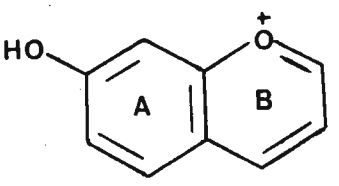
m/z 283(25)

- H•



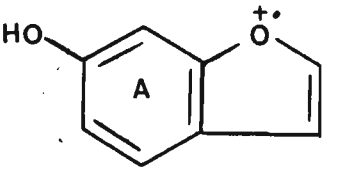
m/z 283

-136



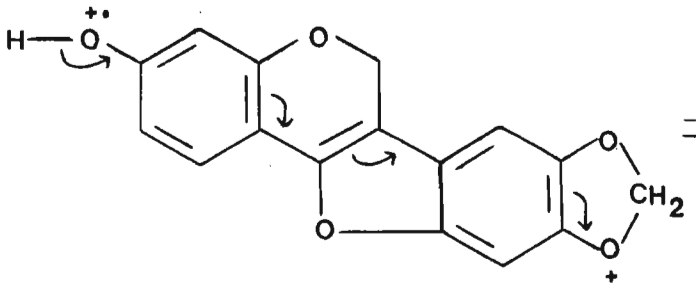
147(19)

-149

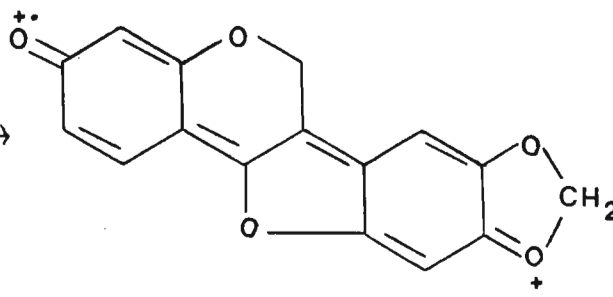


m/z 134(33)

SCHEME B



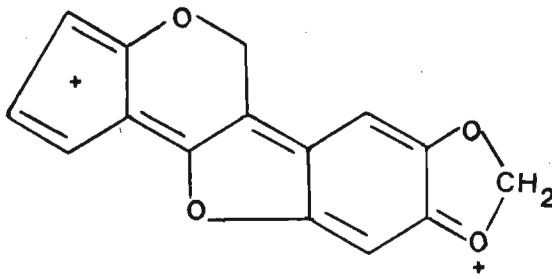
M^+, m/z 282 (96%)



m/z 281 (100)

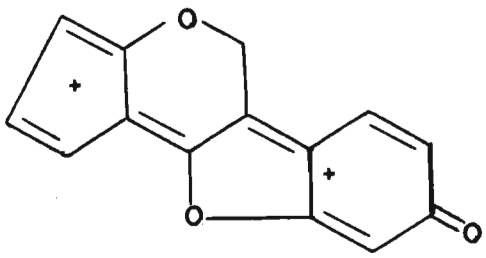
-H·

-CO



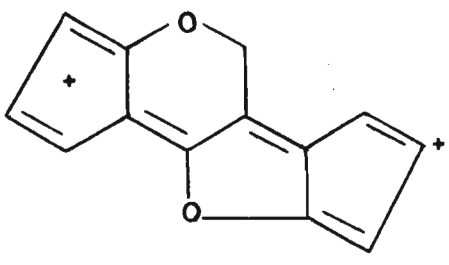
m/z 253 (10.7)

-CH_2O



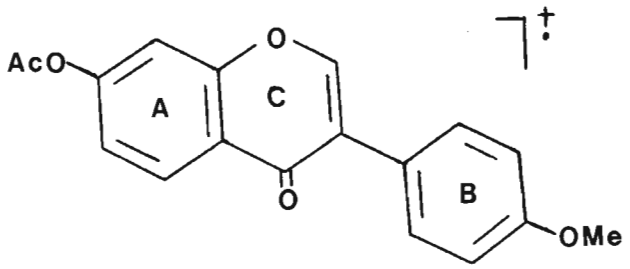
m/z 223 (5.6)

-CO

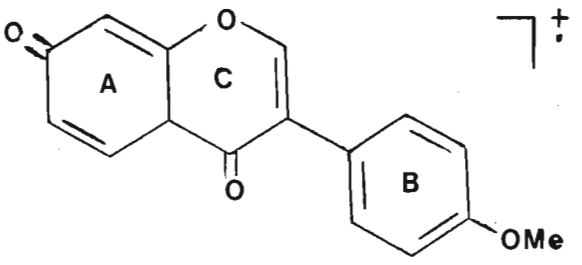


m/z 195 (5.5)

Scheme C



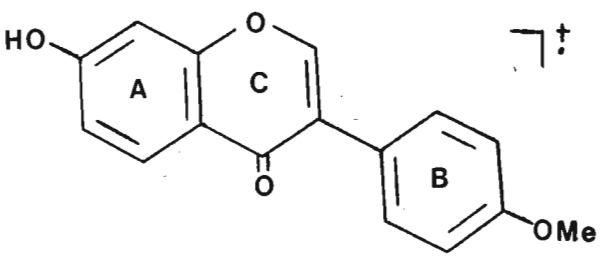
M^+, m/z 310(77.8%)



m/z 267(52.7)

$-CH_2CO$

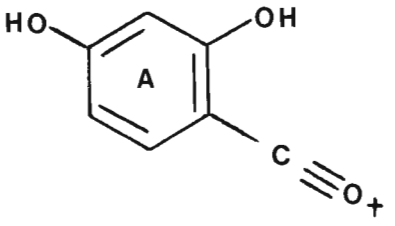
$-H^{\cdot}$



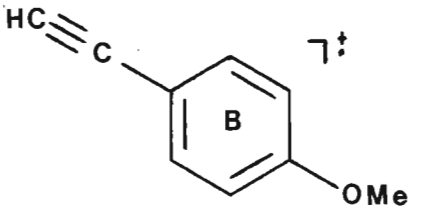
m/z 268(100)

RDA

$+H^{\cdot}$

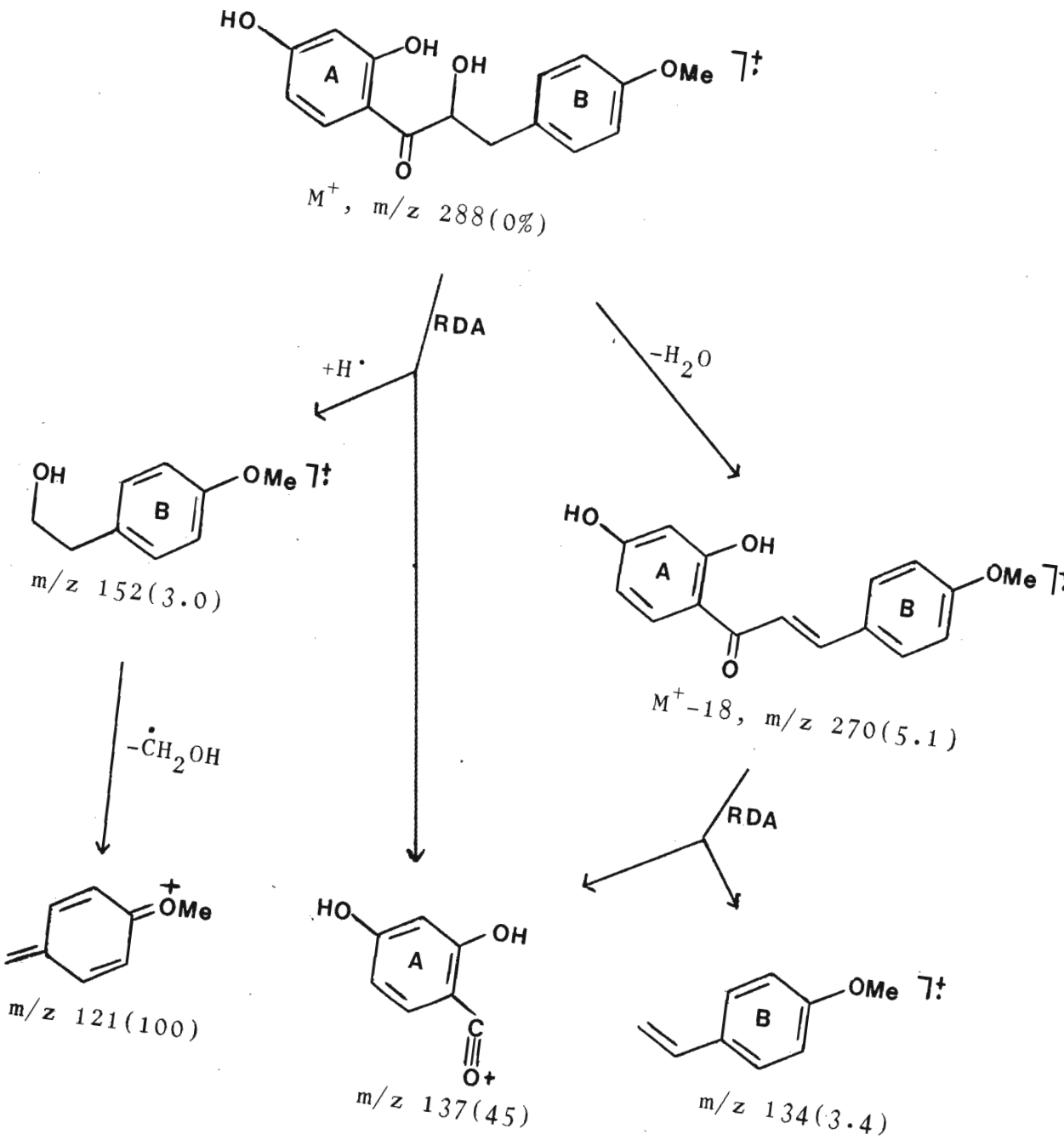


m/z 137(2)

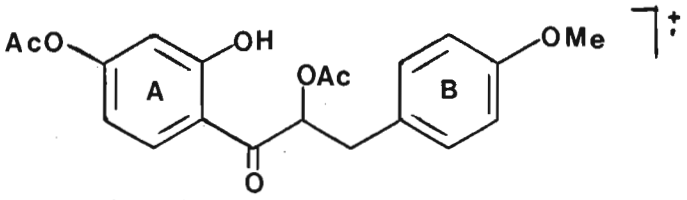


m/z 132(86.5)

Scheme D

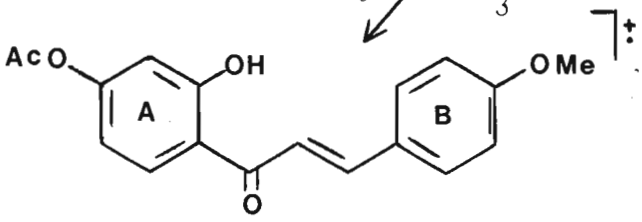

HO
OH
OH
A
B
OMe
O
M+, m/z 288(0%)
RDA
+H·
-H2O
OH
B
OMe
m/z 152(3.0)
HO
OH
A
B
OMe
O
M+-18, m/z 270(5.1)
-·CH2OH
+
OMe
m/z 121(100)
HO
OH
A
C
O+
m/z 137(45)
RDA
B
OMe
m/z 134(3.4)

SCHEME E



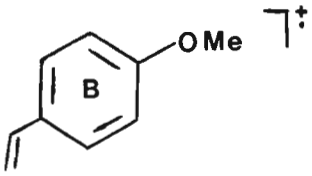
M^+, m/z 372 (3,02%)

McLafferty $-CH_3COOH$

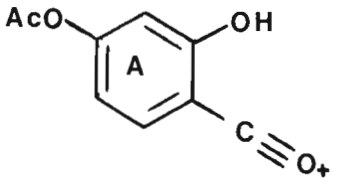


m/z 312 (37,58)

RDA

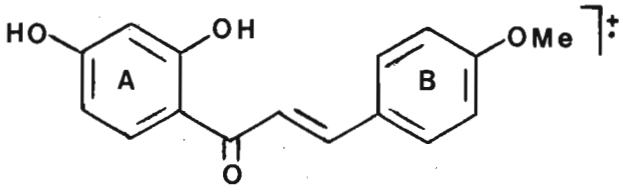


m/z 134 (11,32%)



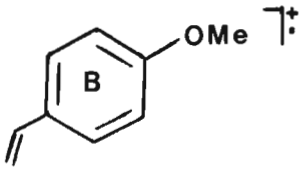
m/z 179 (25,89%)

$-\mathcal{C}H_2CO$

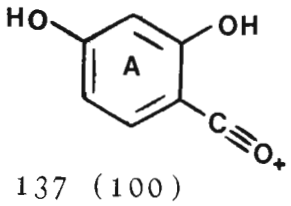


m/z 270 (17,2)

RDA

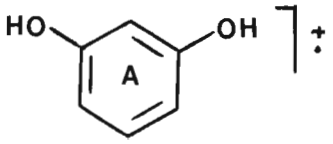


m/z 134 (11,32)



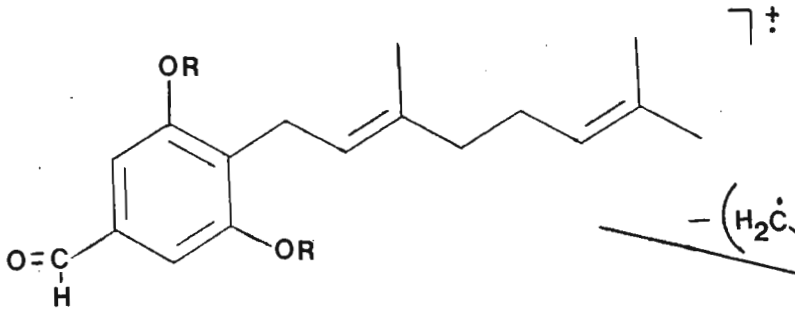
m/z 137 (100)

$-CO$

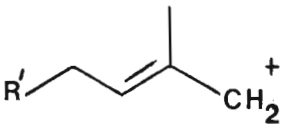


m/z 109 (3,19)

Scheme F

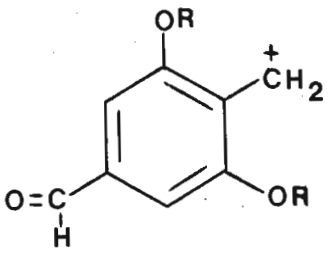


R	m/z(rel. int.)
H	274(M^+, 12.1%)
Me	302(M^+, 14.3)



R'	m/z (rel. int.)
R'_1	205(7.5%)
R'_2	233(39)

- 123



R	m/z (rel.int.)
H	151(100)
Me	179(100)

- 14

$R'-CH_2-CH=CH-CH_2^+$

R'	m/z (rel.int.)
R'_1	191(20)
R'_2	219(10.5)

- 2

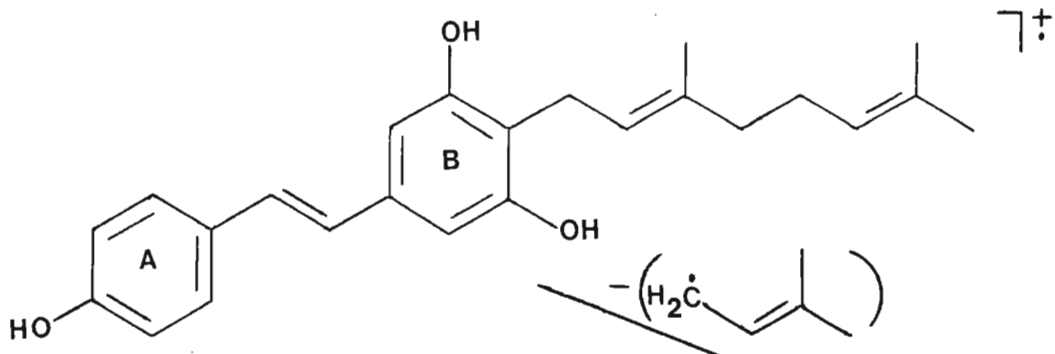
$R'-CH_2-C\equiv C-CH_2^+$

R'	m/z (rel.int.)
R'_1	189(51.4)
R'_2	217(8.5)

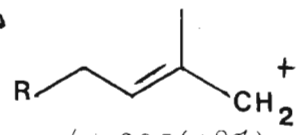
R'_1 = 3,5-dihydroxybenzaldehyde

R'_2 = 3,5-dimethoxybenzaldehyde

Scheme G



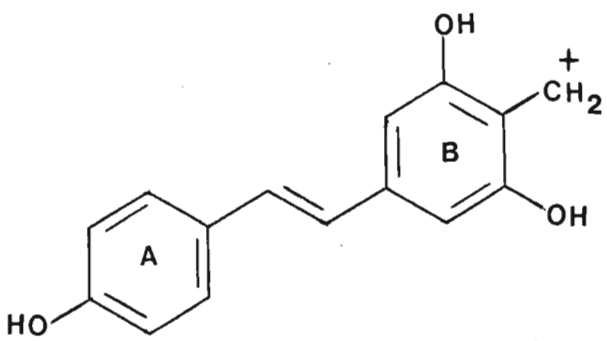
m/z 364(M^+, 25%)



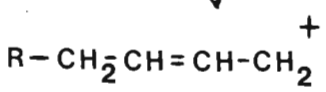
m/z 295(18%)

- 123

- 14

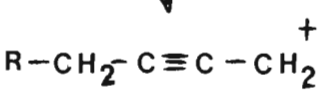


m/z 241(100)



m/z 281(16)

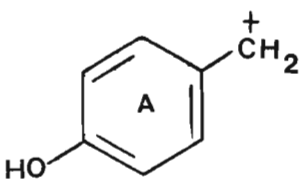
- 2



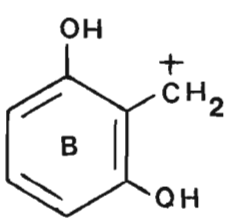
m/z 279(34)

-143

-118



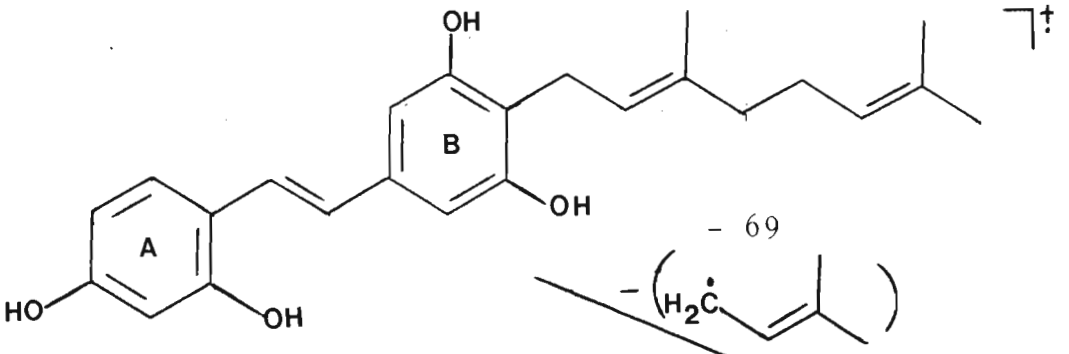
107 (22.4)



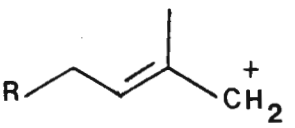
123 (14)

(R = 3,4,5 - trihydroxystilbene group)

Scheme H



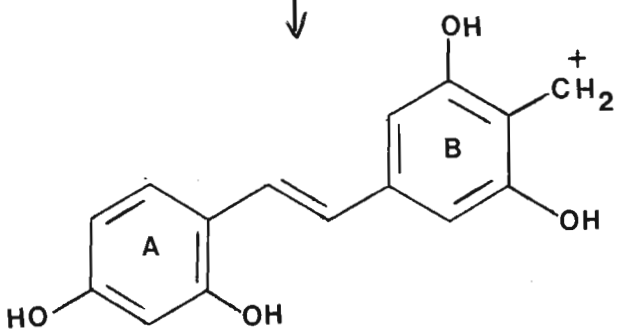
M^+, 380 (22%)

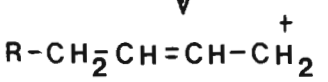


m/z 311 (19%)

- 123

- 14

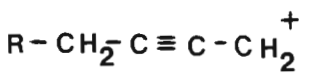




m/z 297 (15)

- 2

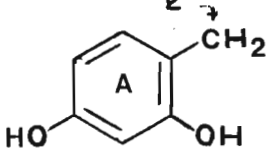
m/z 257 (100)



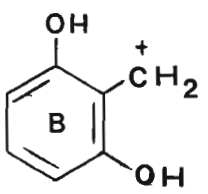
m/z 295 (32)

-134

-134



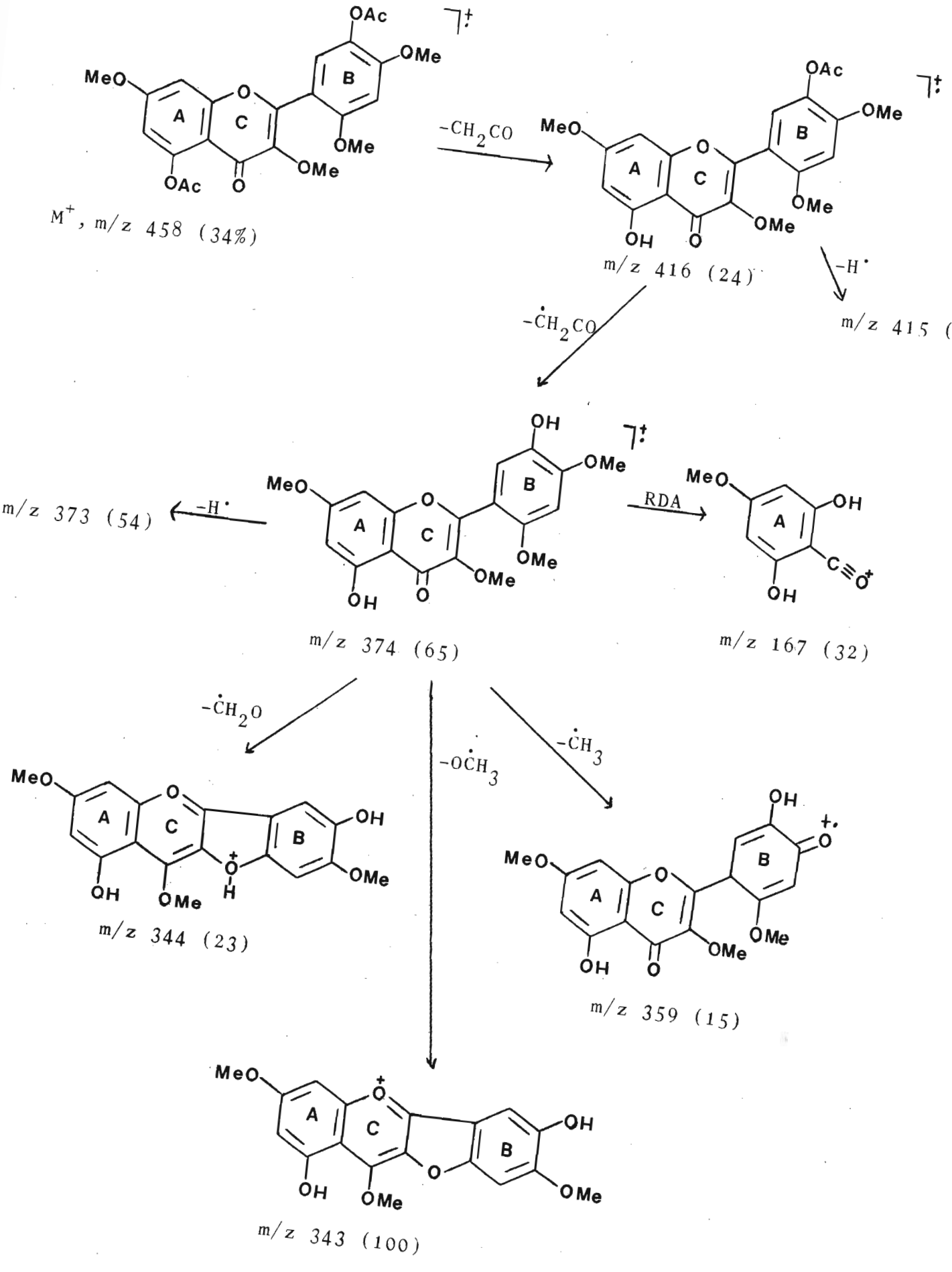
123 (20)



123 (12)

(R = 2,4,3′,5′ - tetrahydroxystilbene)

SCHEME I


OAc
MeO
OMe
A
C
B
O
OMe
OMe
OAc
O
M+, m/z 458 (34%)
-CH2CO
OAc
MeO
OMe
A
C
B
O
OMe
OMe
OH
O
m/z 416 (24)
-H·
m/z 415
-·CH2CO
OH
MeO
OMe
A
C
B
O
OMe
OMe
OH
O
m/z 374 (65)
-H·
m/z 373 (54)
RDA
MeO
OH
A
C≡O+
OH
m/z 167 (32)
-·CH2O
MeO
A
C
B
OH
O+
H
OH
OMe
OMe
m/z 344 (23)
-·OCH3
-·CH3
MeO
A
C
B
OH
+·
O
OMe
OMe
OH
O
m/z 359 (15)
MeO
A
O+
C
B
OH
OH
OMe
O
OMe
m/z 343 (100)

^{1}H NMR

INDEX - ^{1}HNMR SPECTRA

PLATE	COMPOUND
23.	2,3',4,5'-Tetrahydroxy-4'-geranylstilbene (Chlorophorin) [47].
24.	2,3',4,5'-Tetramethoxy-4'-geranylstilbene [47a].
25.	2,3',4,5'-Tetrahydroxystilbene (Oxyresveratrol) [103].
26.	2,3',4,5'-Tetramethoxystilbene [103a].
27.	3,3',4,5'-Tetramethoxystilbene [106].
28.	2',4',5,7-Tetramethoxyflavone [105].
29.	3,4',7-Tri-O-methylquercitin (Ayanin) [99].
30.	2'-Methoxy-3,4',7-tri-O-methylquercitin [100].
31.	5,5'-Diacetoxy-2',3,4'7-tetramethoxyflavone [100a].
32.	3,5-Dihydroxybenzyl alcohol [110].
33.	3,5-Dimethoxybenzyl alcohol [112].
34.	3,5-Dimethoxybenzyl bromide [113].
35.	Geranic acid [125].
36.	2,6-Dimethoxy-1-O-allyl phenol [126].
37.	2,6-Dimethoxy-4-allyl phenol [127].

PLATE 1 : (6aR, 11aR)-3-Methoxy-8,9-methylenedioxypterocarpan (Pterocarpin) [13].

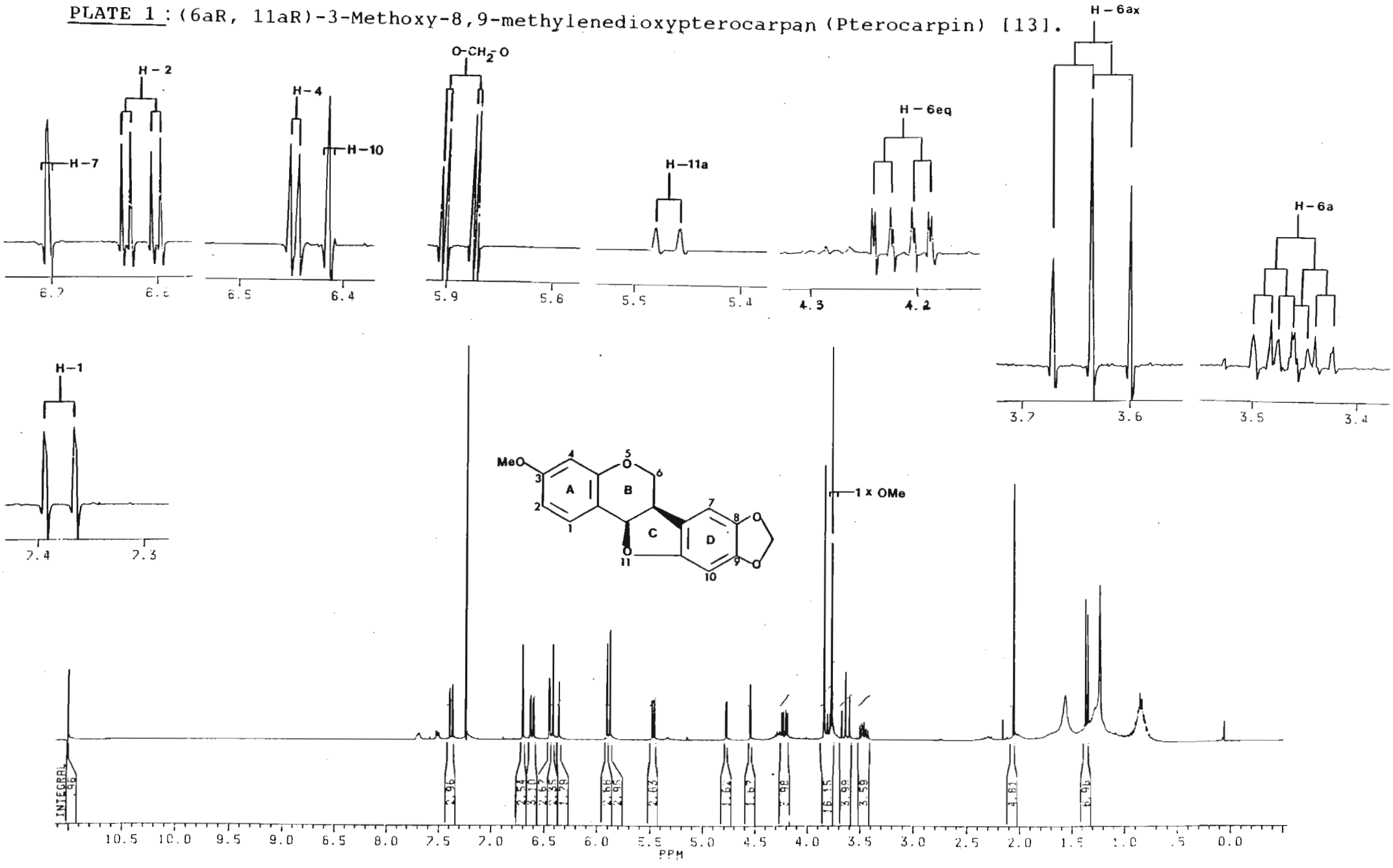


PLATE 2 : (6aR, 11aR)-3-Hydroxy-8,9-methylenedioxypterocarpan (Maackiain) [25].

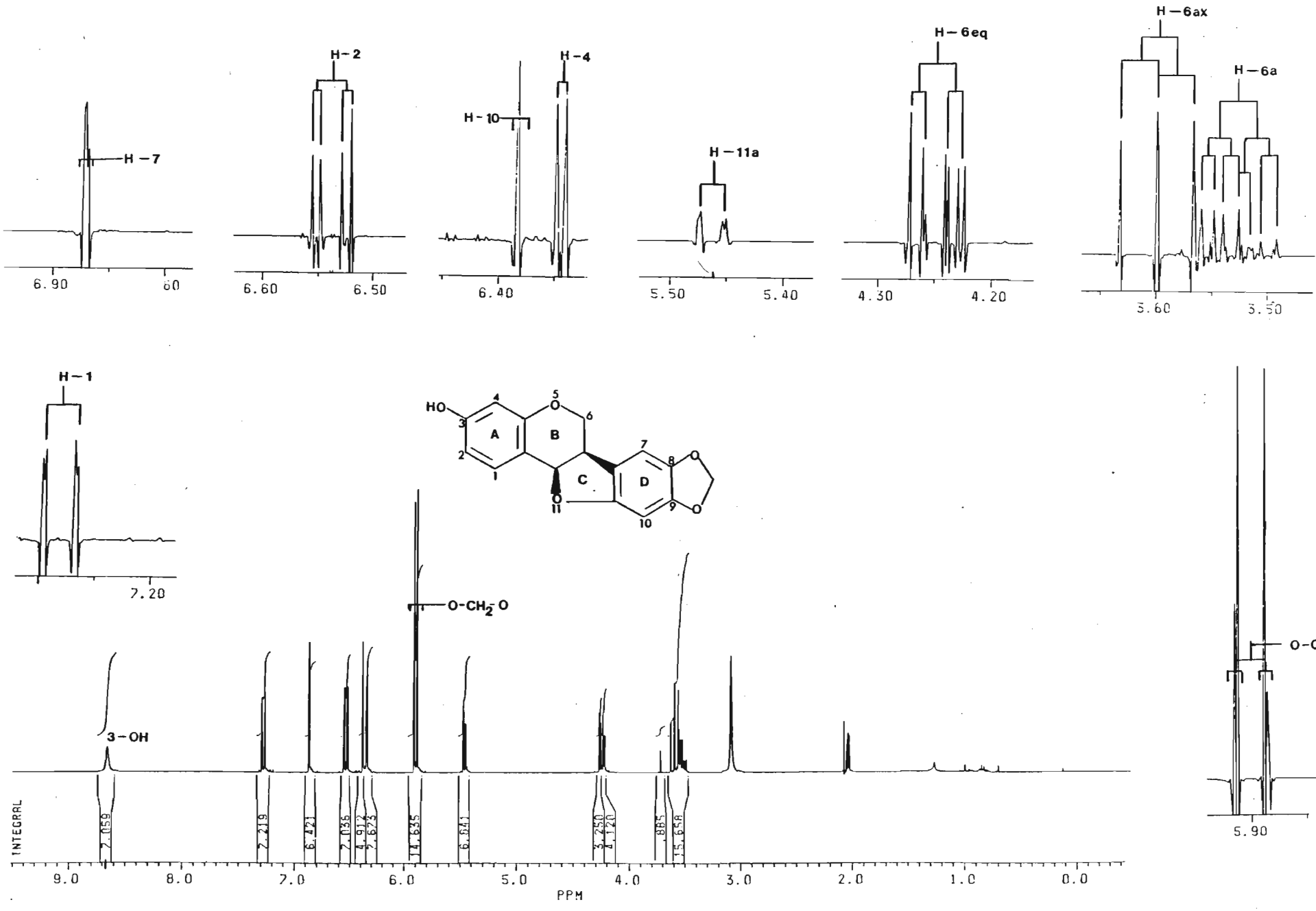
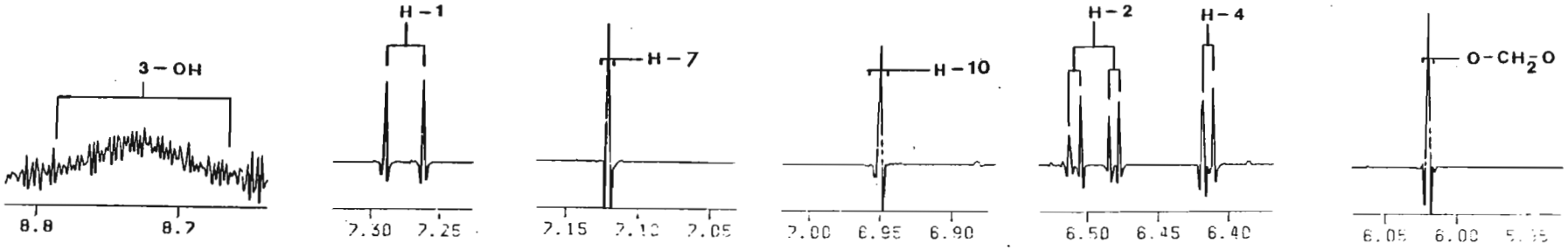


PLATE 3: 3-Hydroxy-8,9-Methylenedioxy-6a,11a-dihydropterocarpan [85].



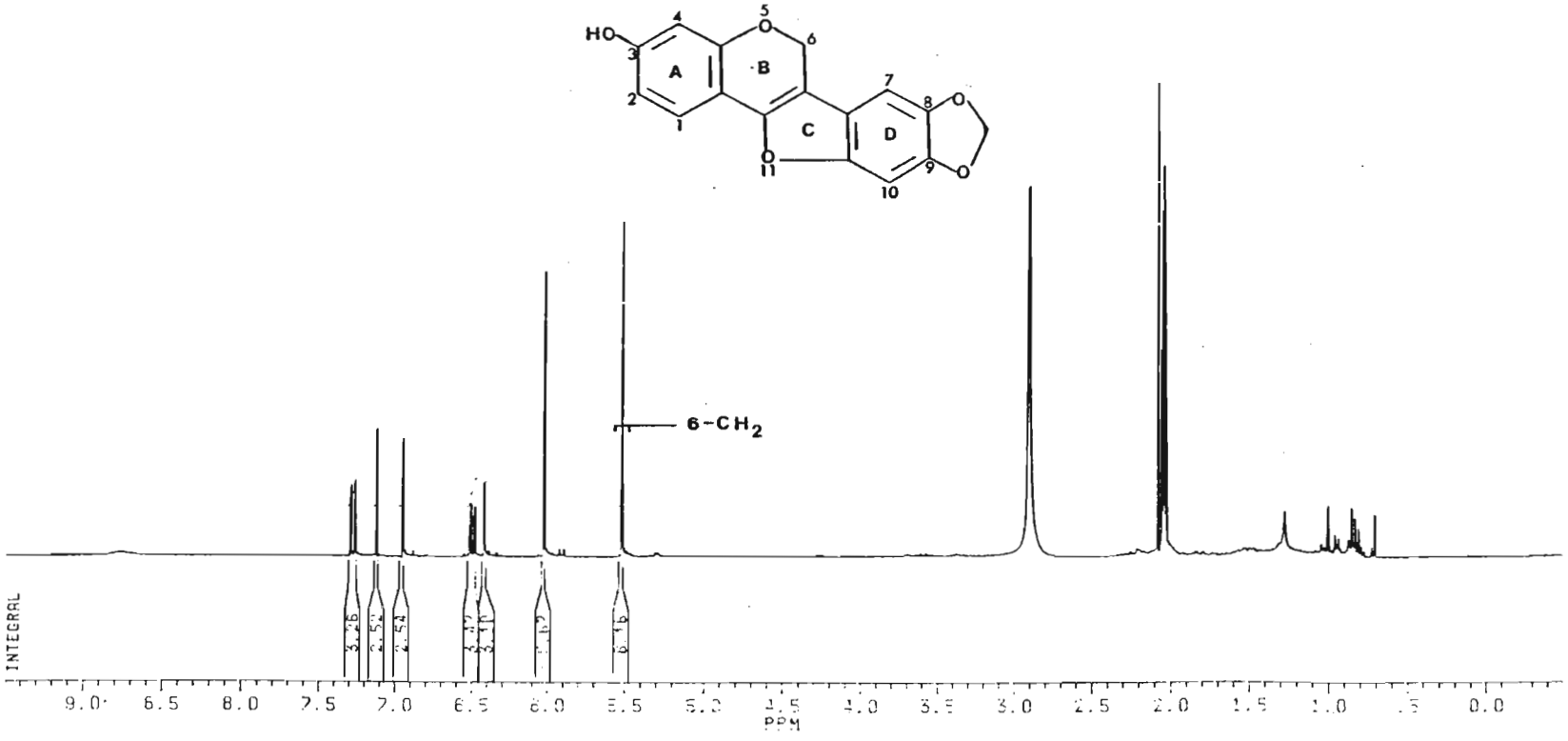


PLATE 4 : 3-Acetoxy-8,9-methylenedioxy-6a,11a-dihydropterocarpin [85a].

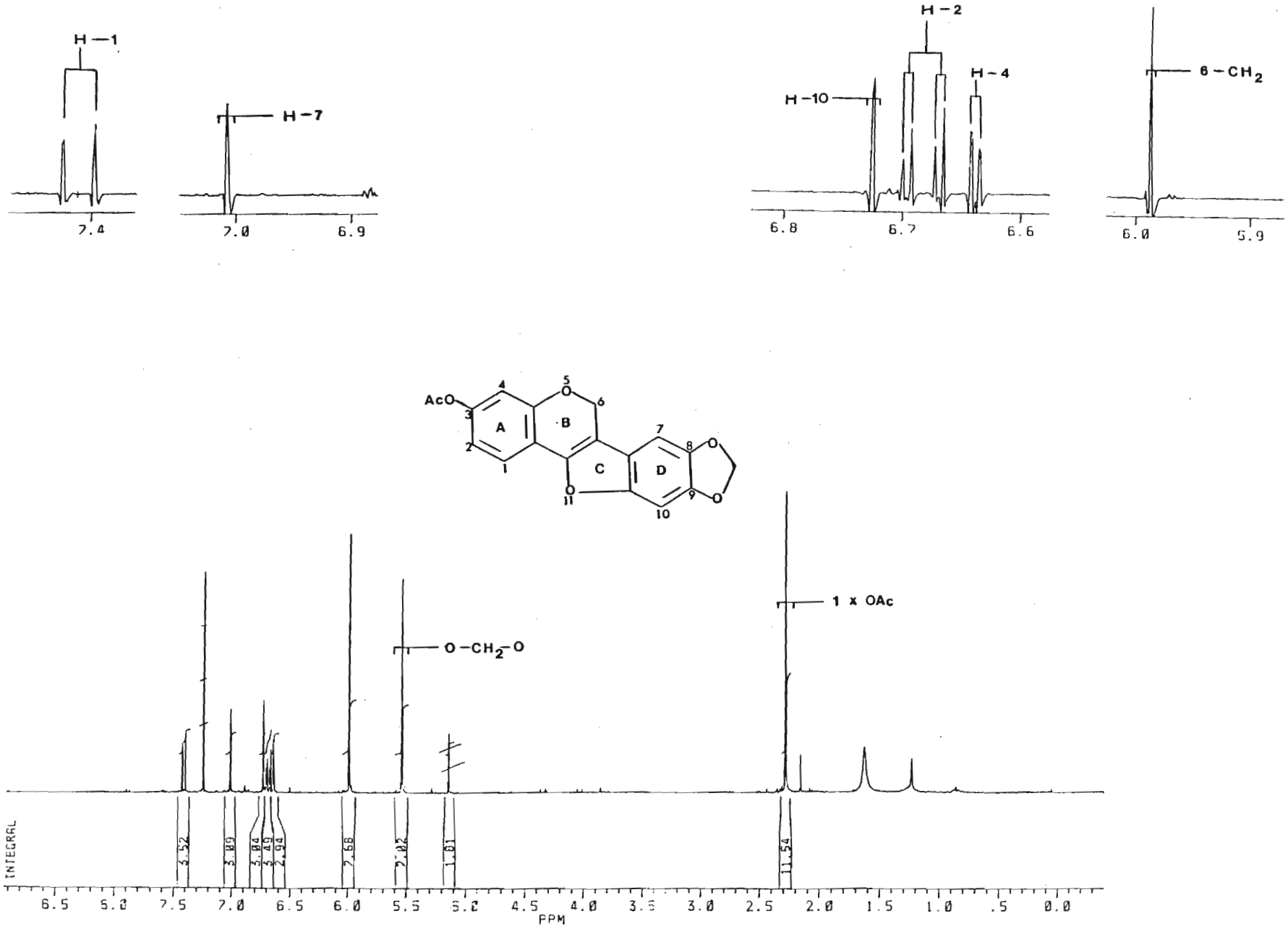


PLATE 5: (2R)-7-Hydroxyflavanone [87].

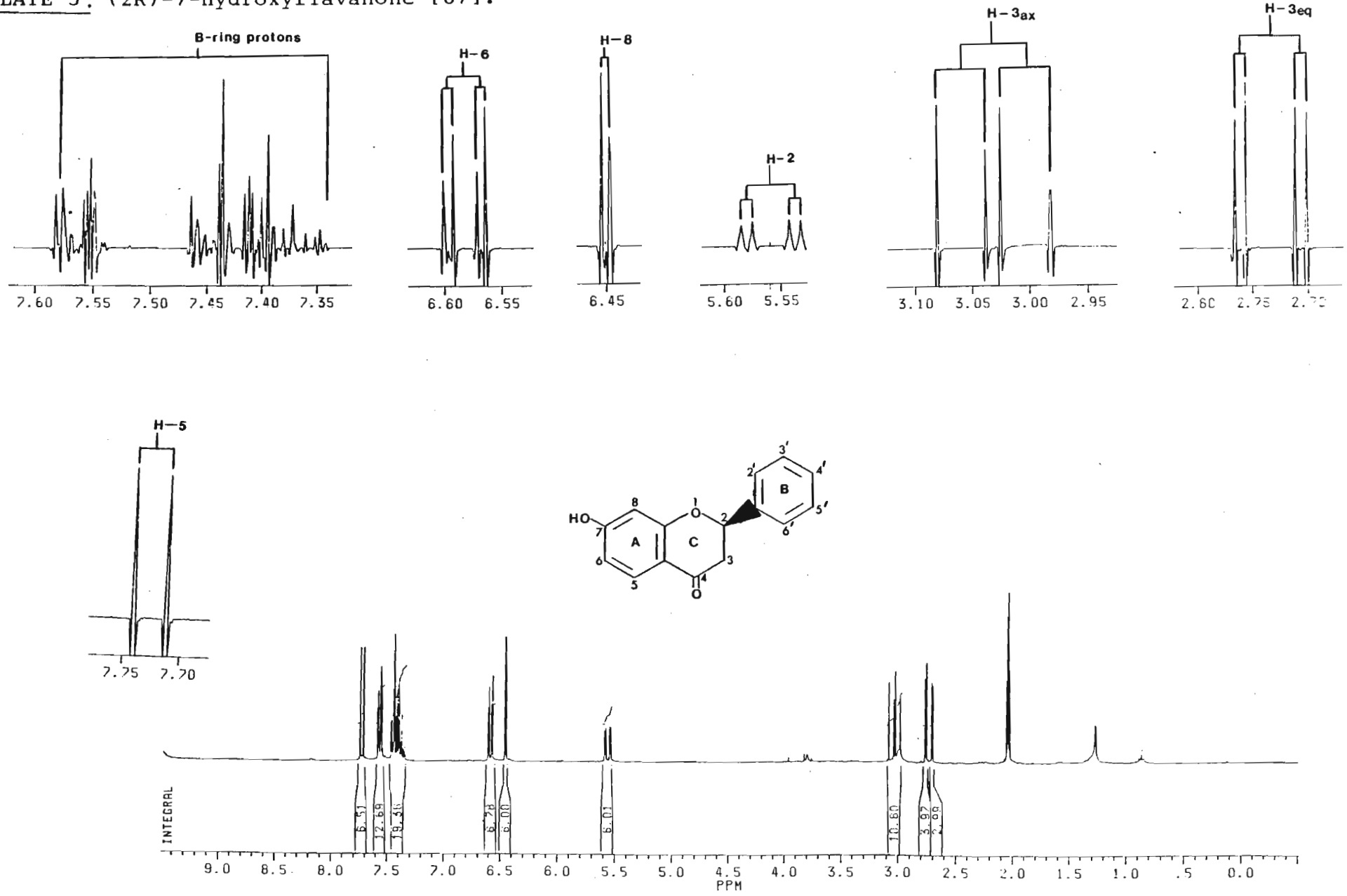
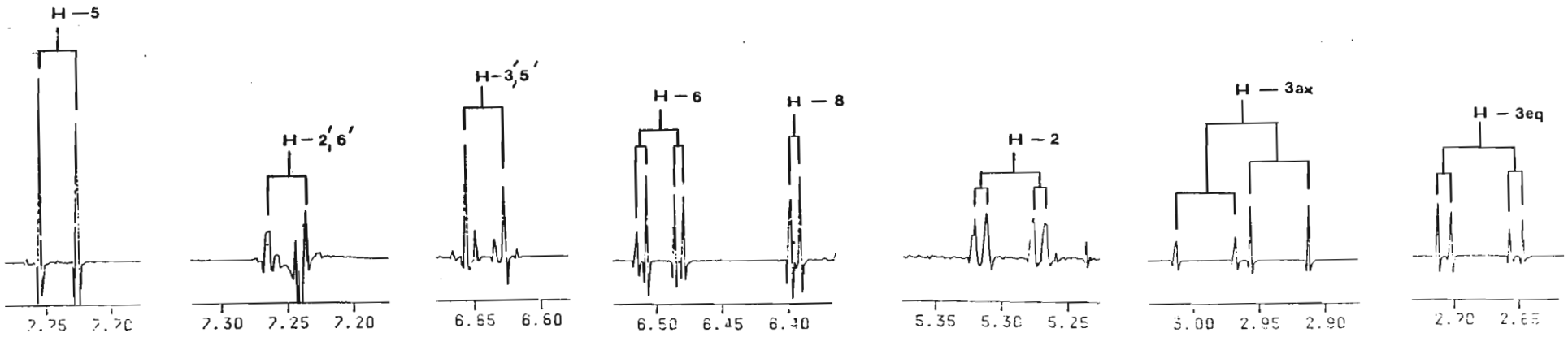


PLATE 6 : (±)-7,4'-Dihydroxyflavanone (Liquiritigenin) [93].



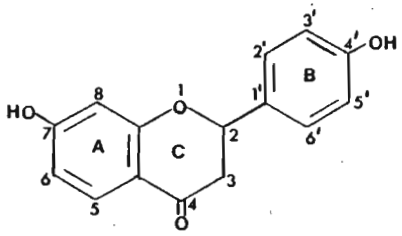


PLATE 7 : (±)-7-Hydroxy-4'-methoxyisoflavanone [88].

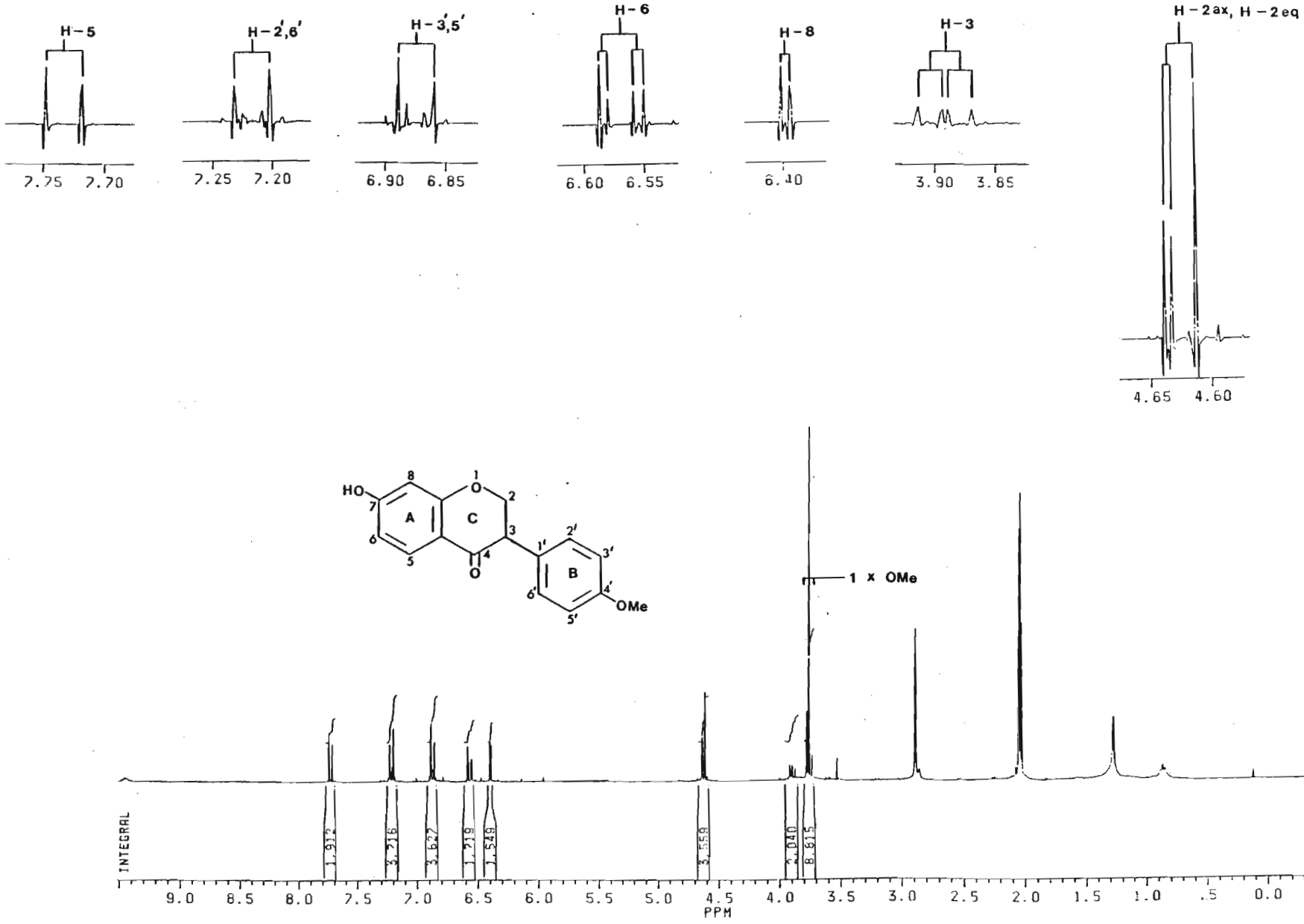


PLATE 8: 7-Hydroxy-4'-methoxyisoflavone (Formononetin) [10].

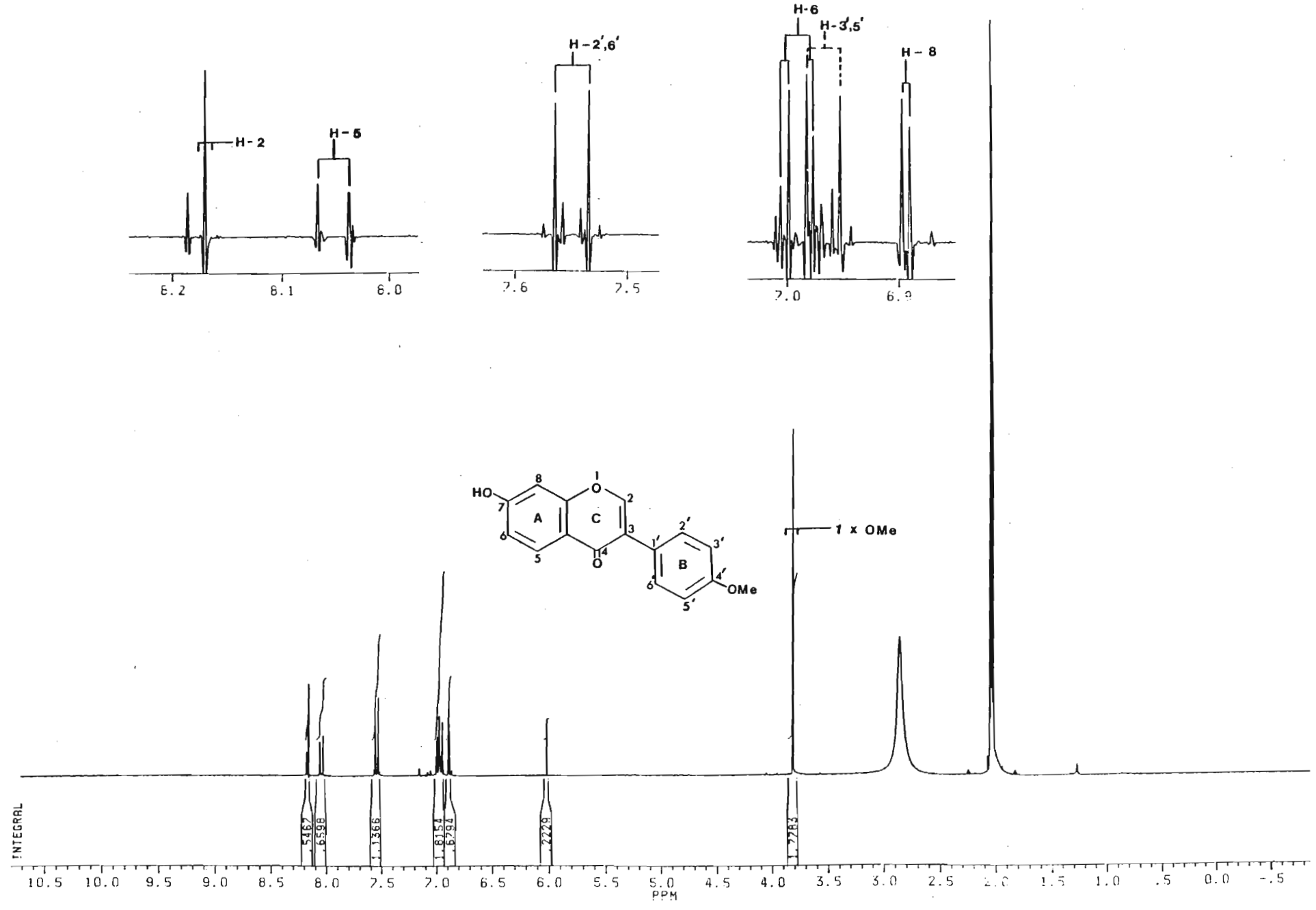


PLATE 9 : 3',7-Dihydroxy-4'-methoxyisoflavone (Calycosin) [94].

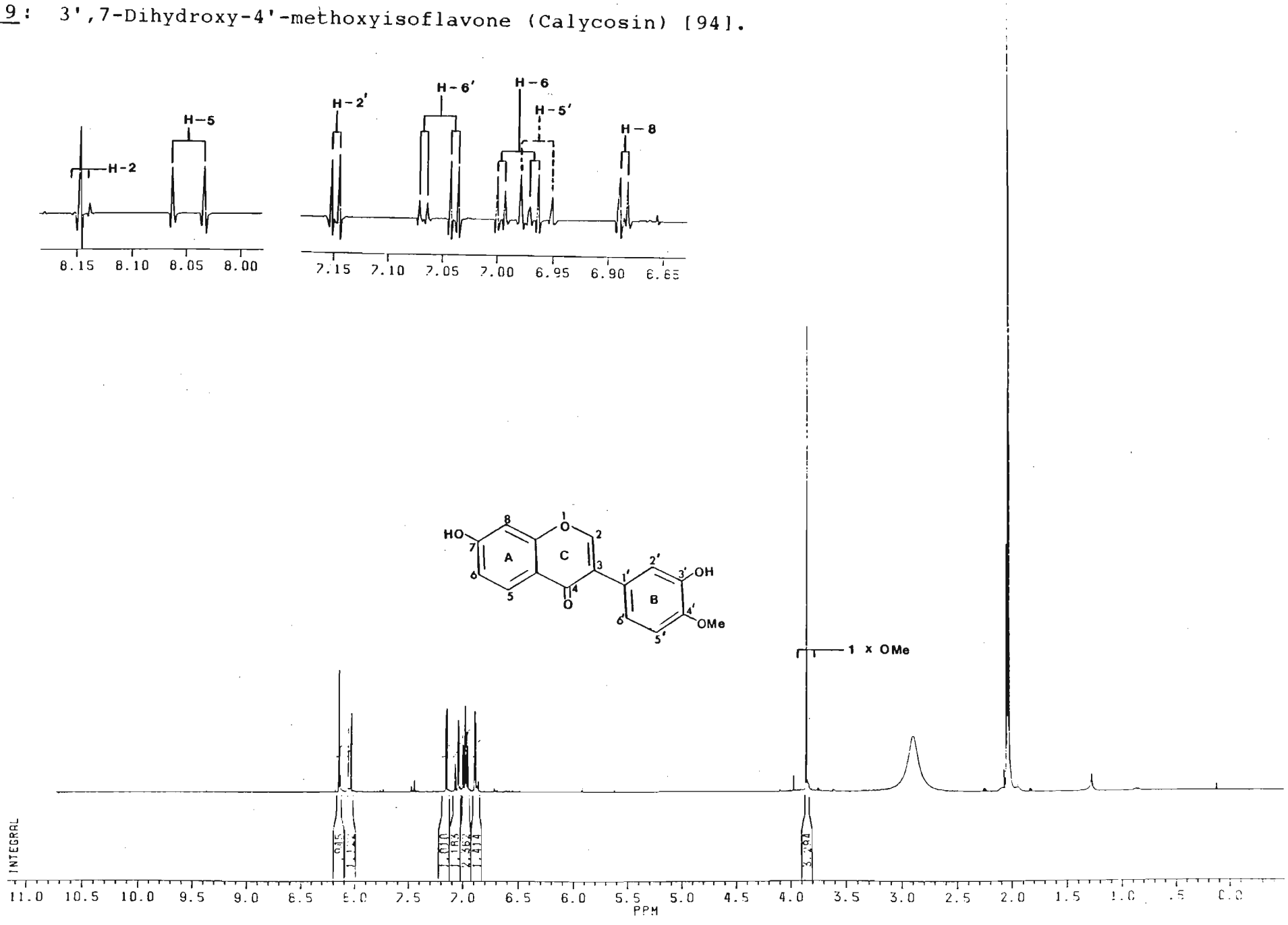
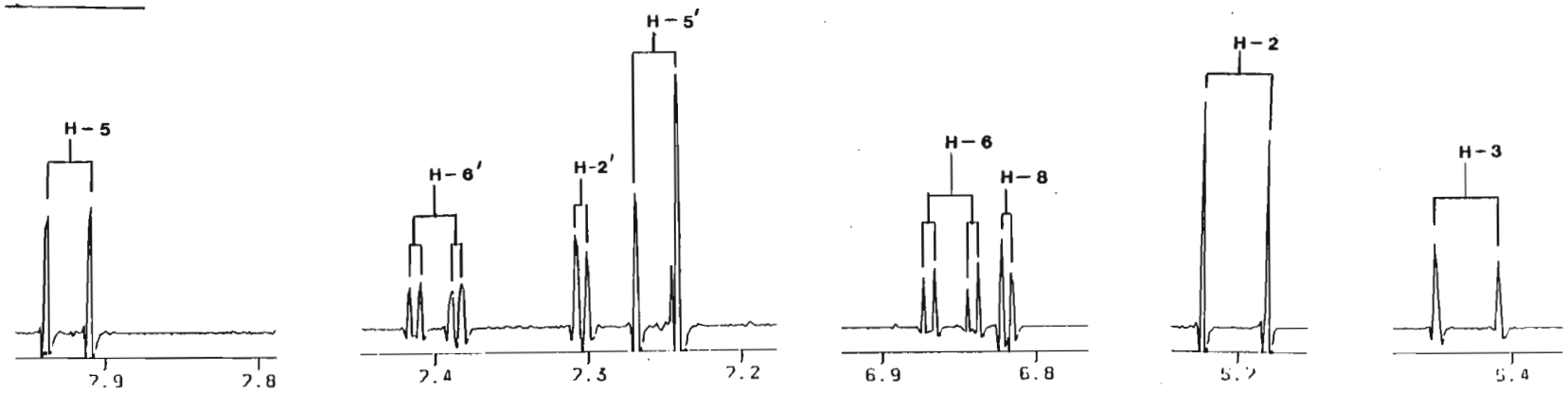


PLATE 10 : (2R,3R)-3,3',4',7-Tetraacetoxydihydroflavonol [(+)-Fustin] [95].



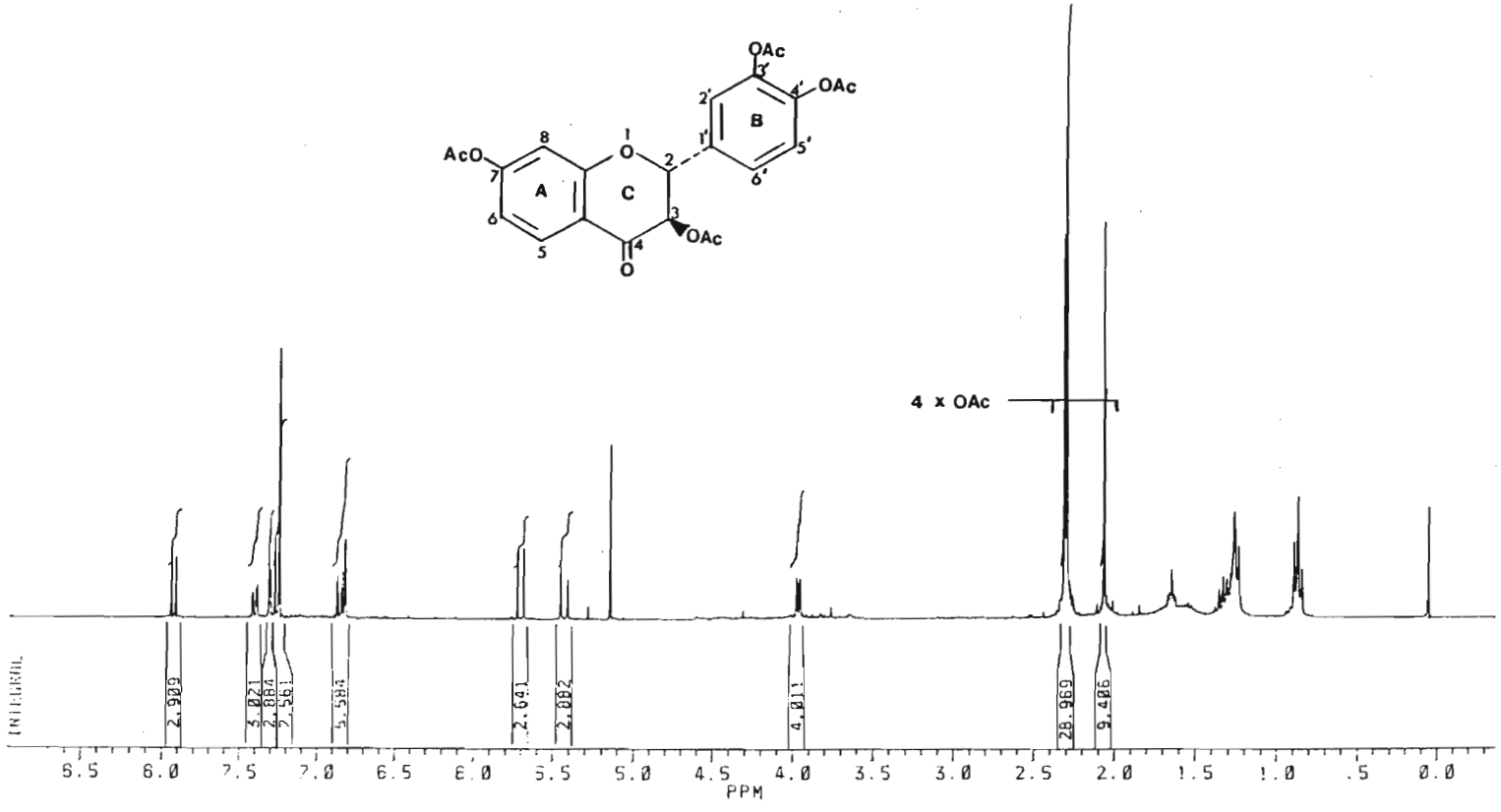
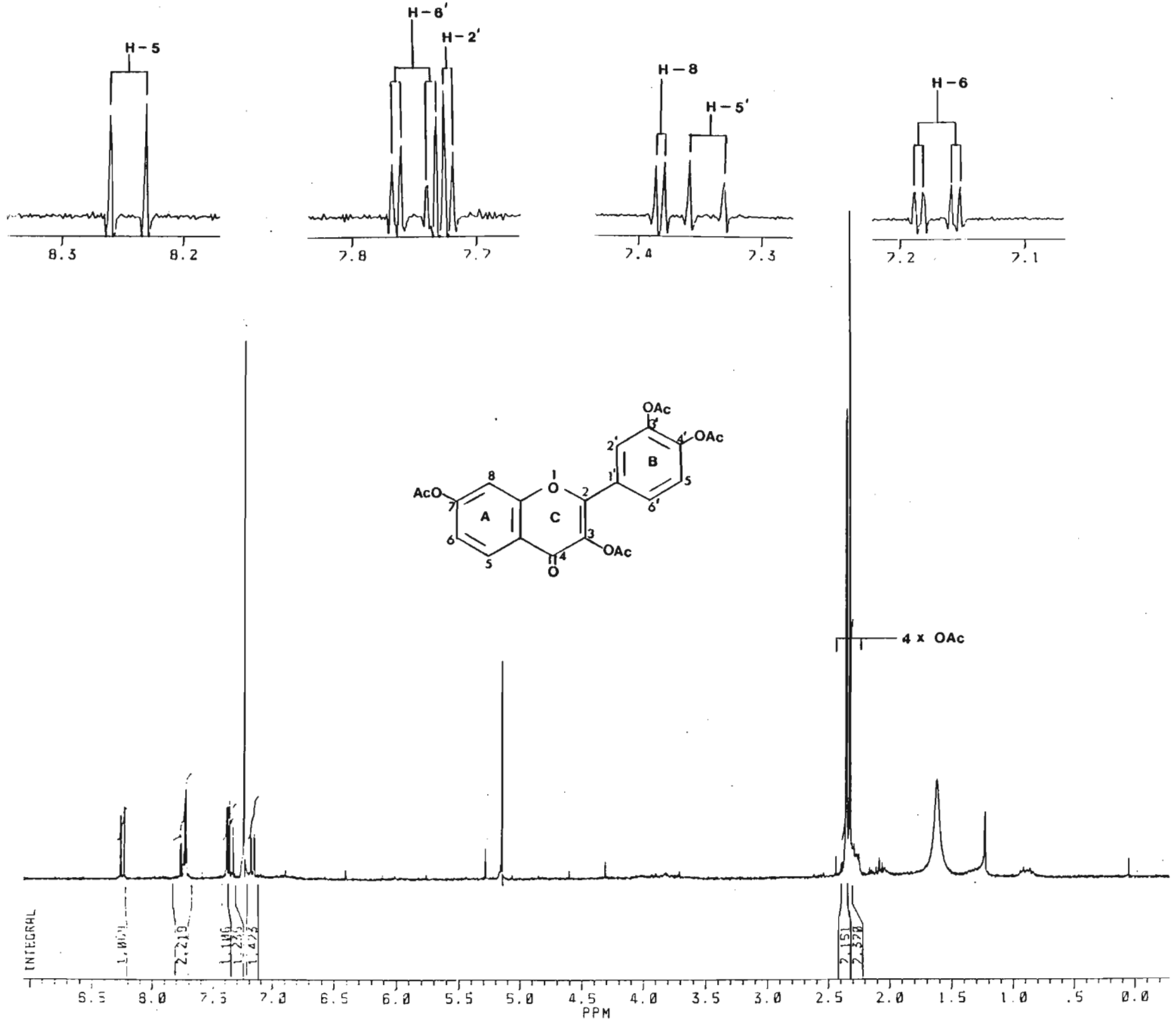


PLATE 11 : 3,3',4',7-Tetraacetoxyflavone (Fisetin) [96].



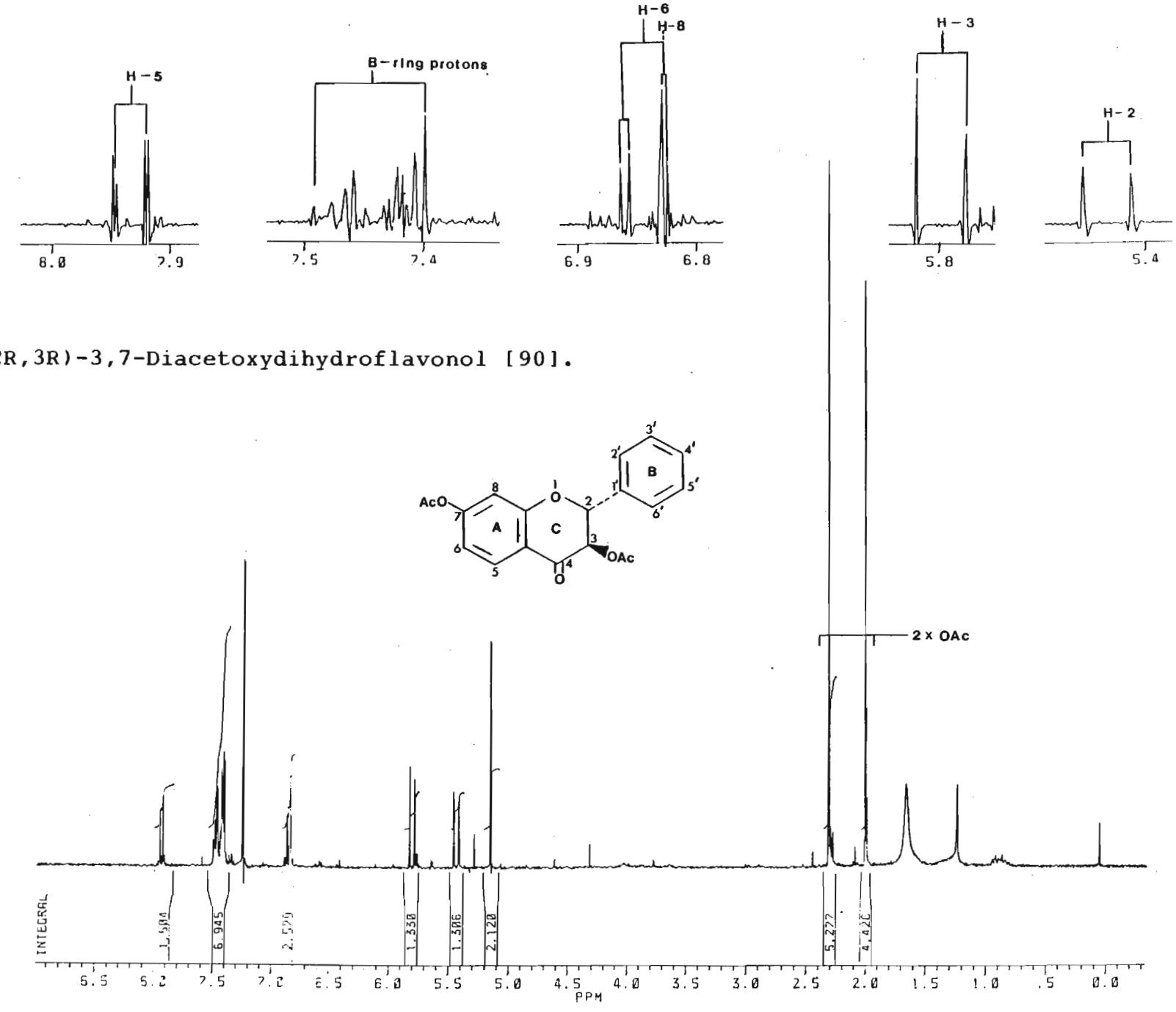


PLATE 12: (2R,3R)-3,7-Diacetoxydihydroflavonol [90].

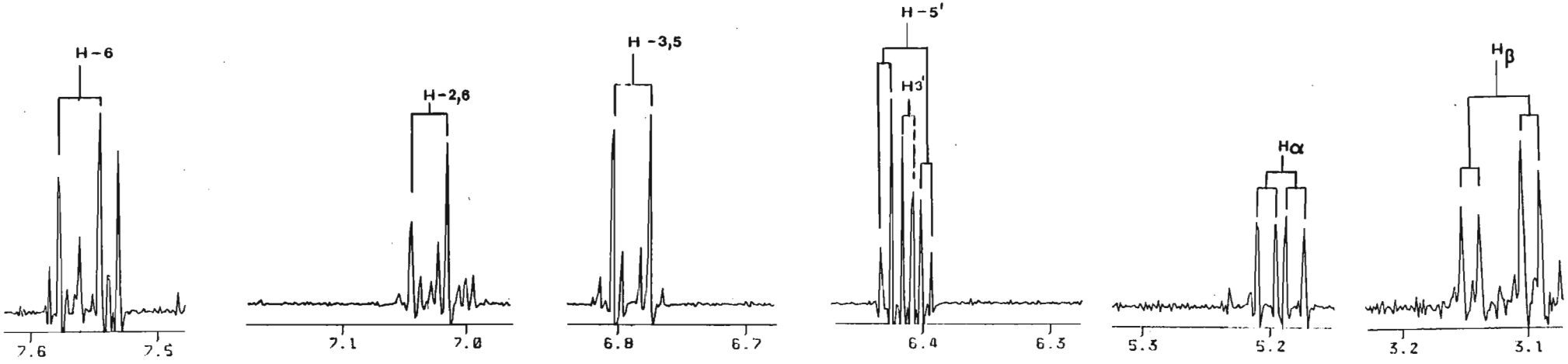


PLATE 13 : (αS),2',4'-Trihydroxy-4-methoxydihydrochalcone [91].

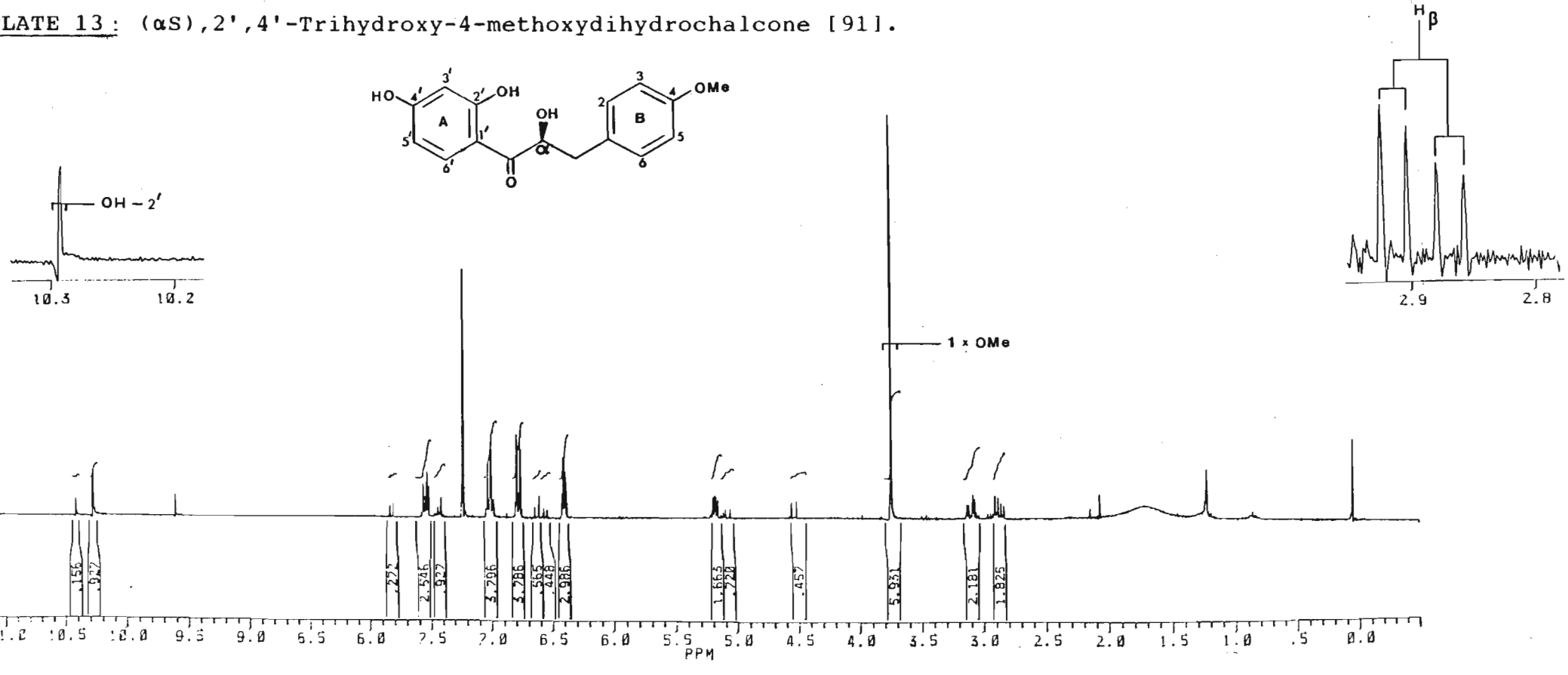


PLATE 14 : (αS),2',4'-Triacetoxy-4-methoxydihydrochalcone [91a].

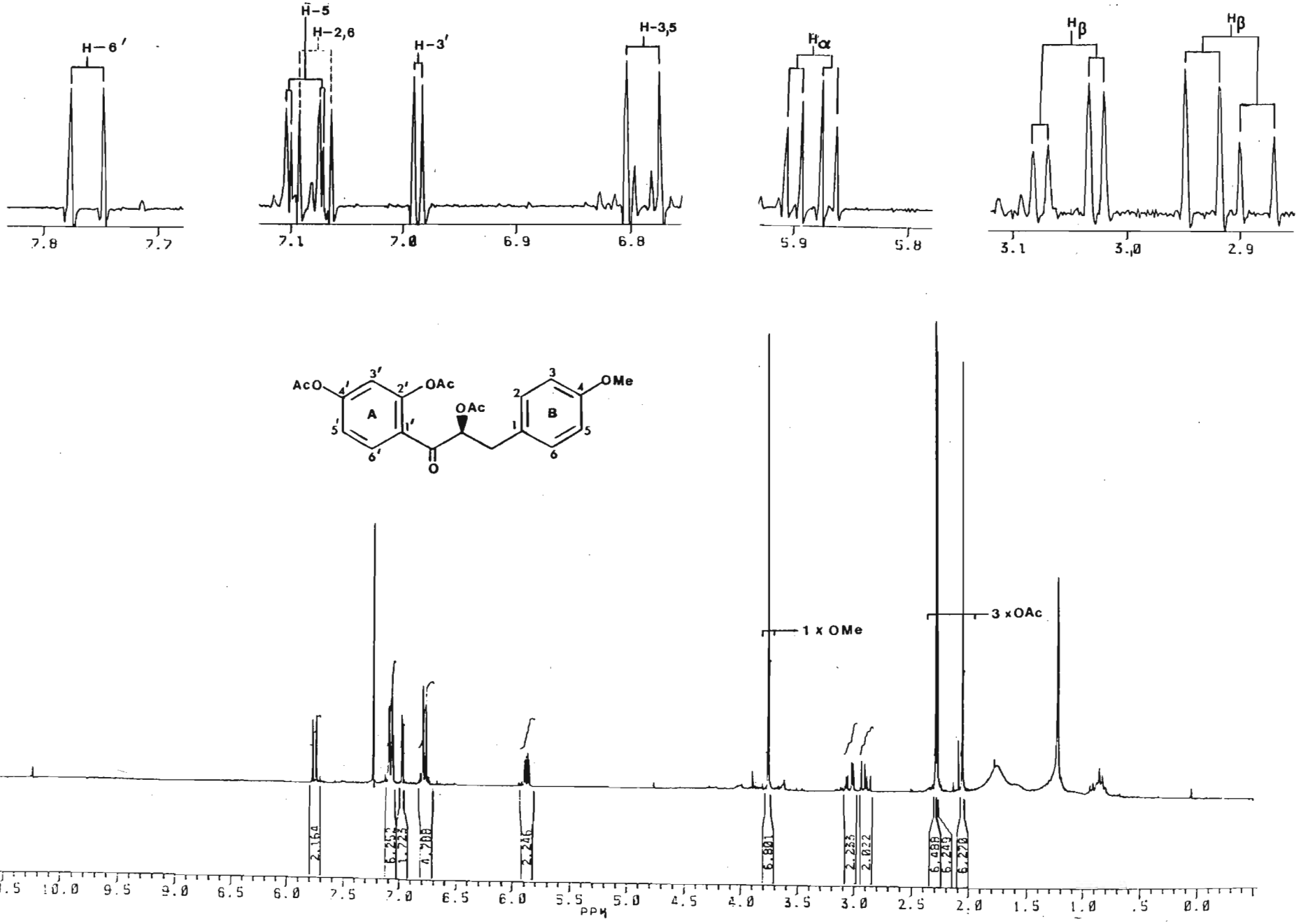


PLATE 15: (αS),4'-Diacetoxy-2'-hydroxy-4-methoxydihydrochalcone [91b].

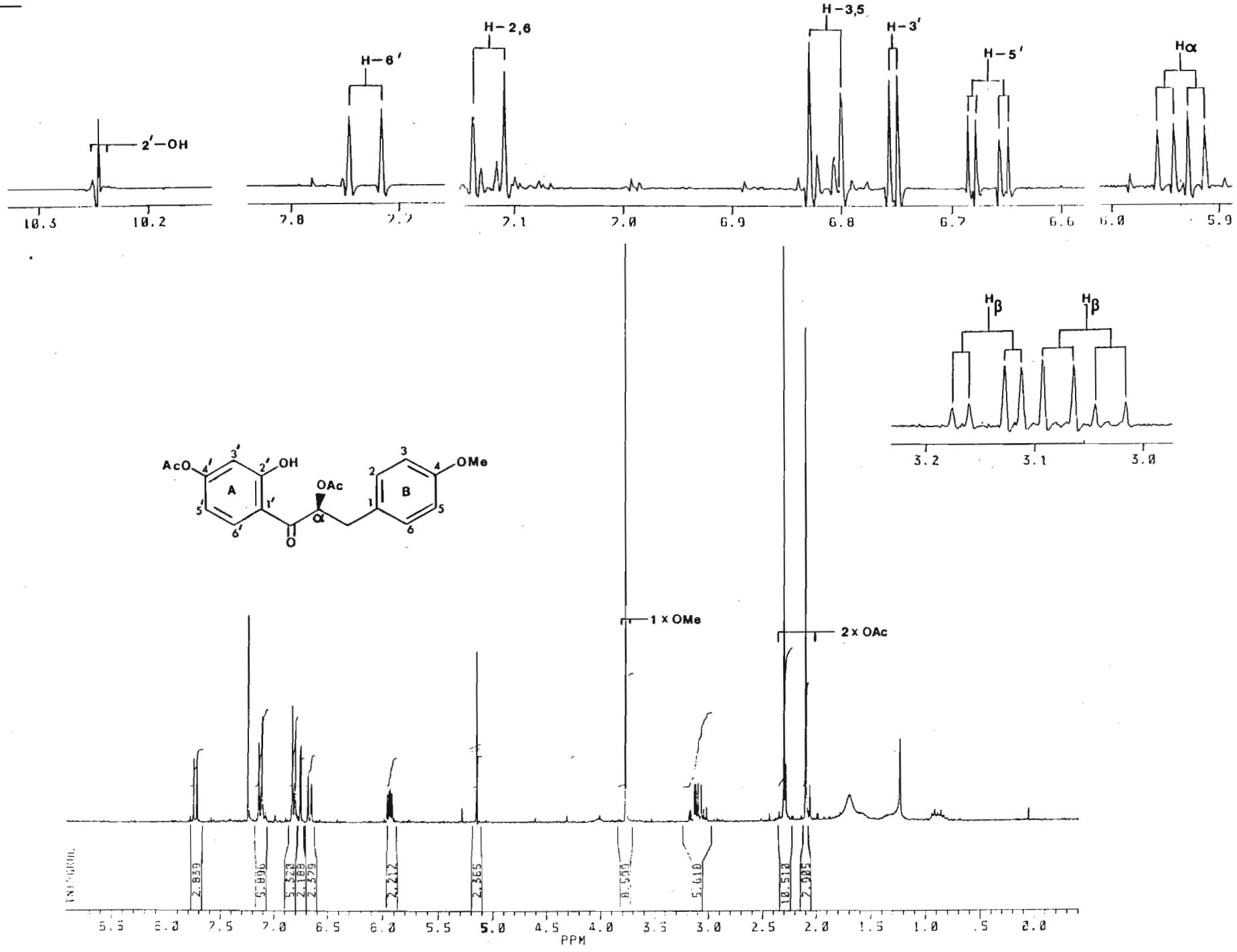


PLATE 16: 3,5-Dihydroxy-4-geranylbenzaldehyde [101].

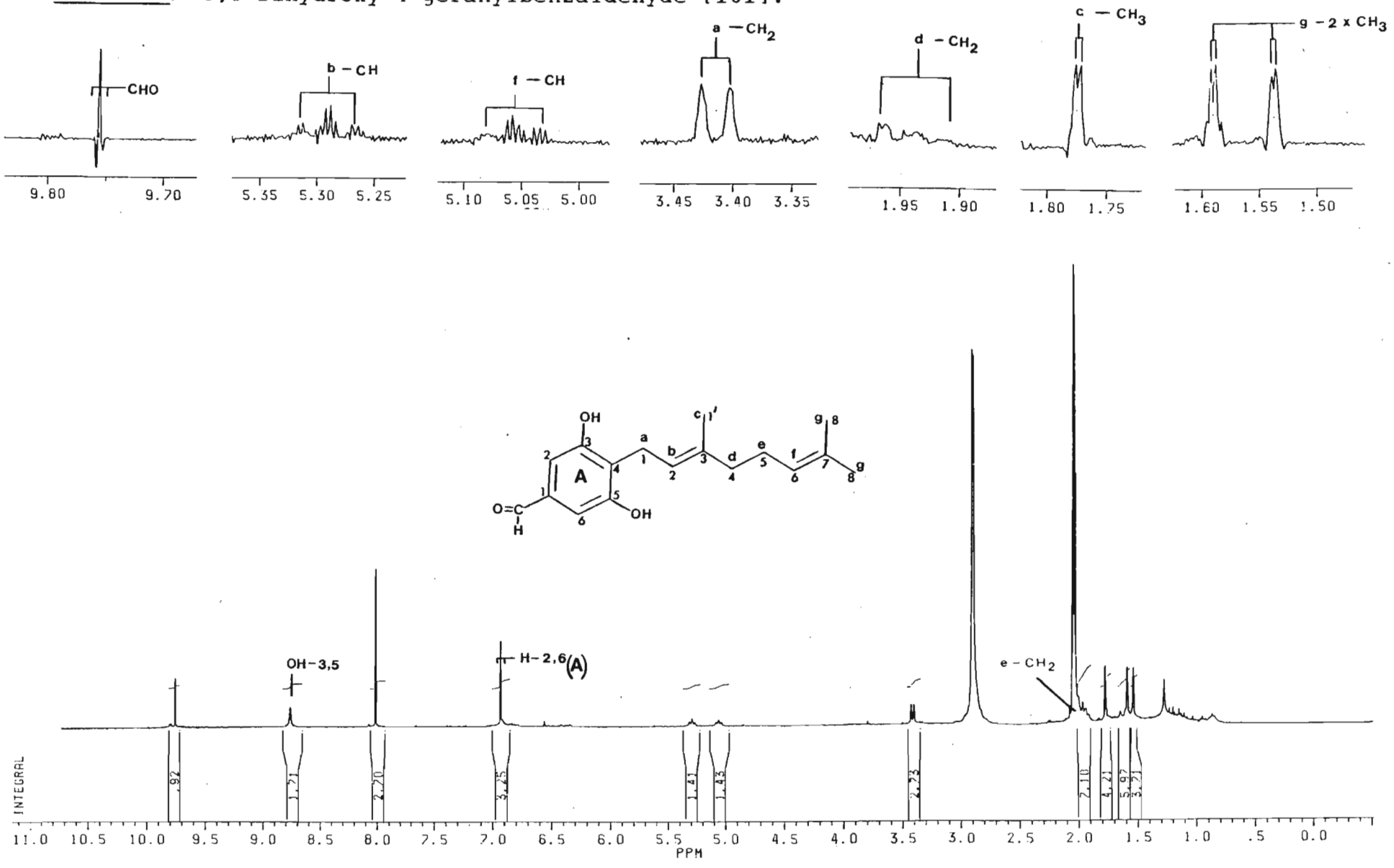


PLATE 17 : 3,5-Dimethoxy-4-geranylbenzaldehyde [101a].

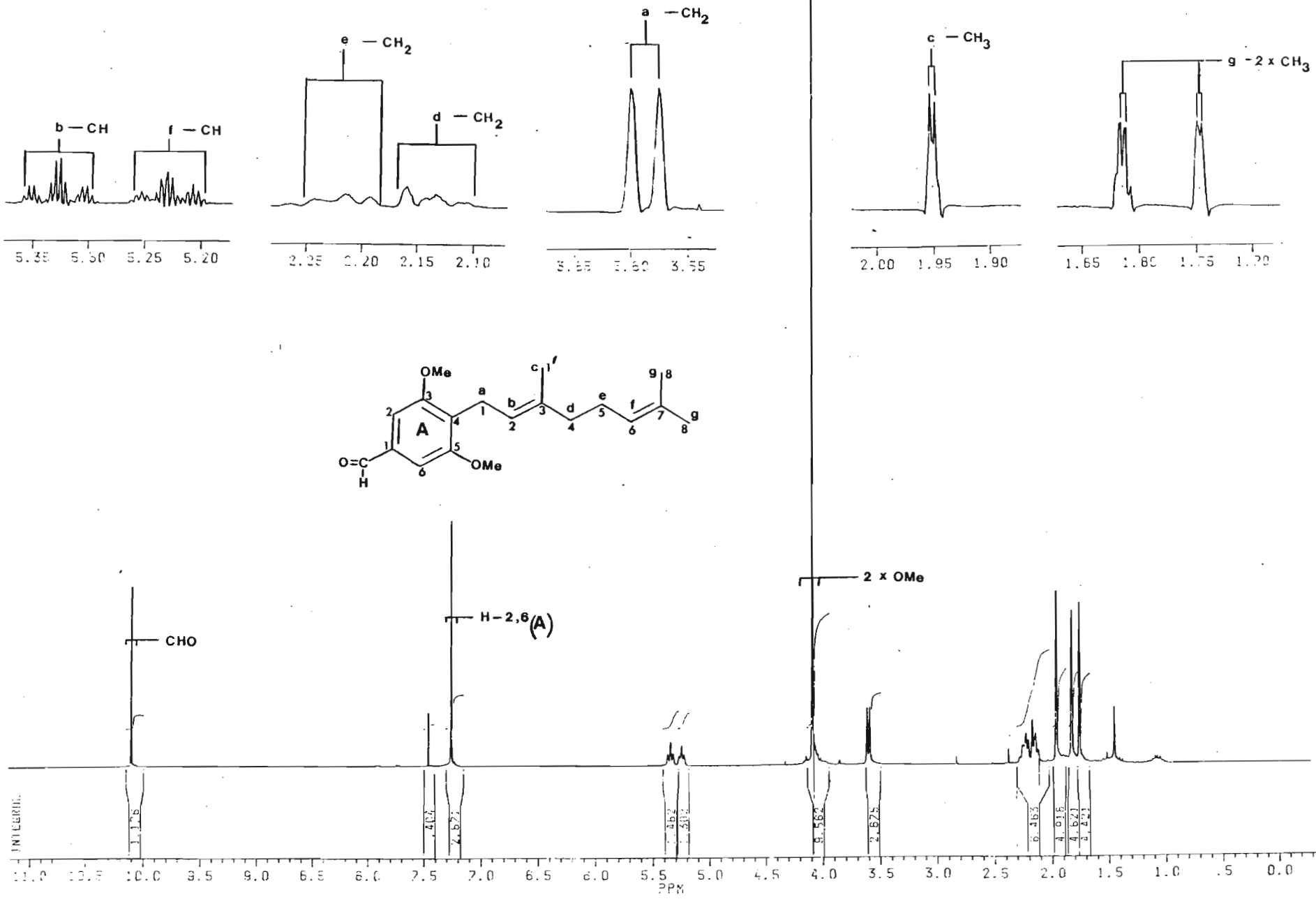


PLATE 18 : 3,5-Dimethoxy-4-geranylbenzyl alcohol [101b].

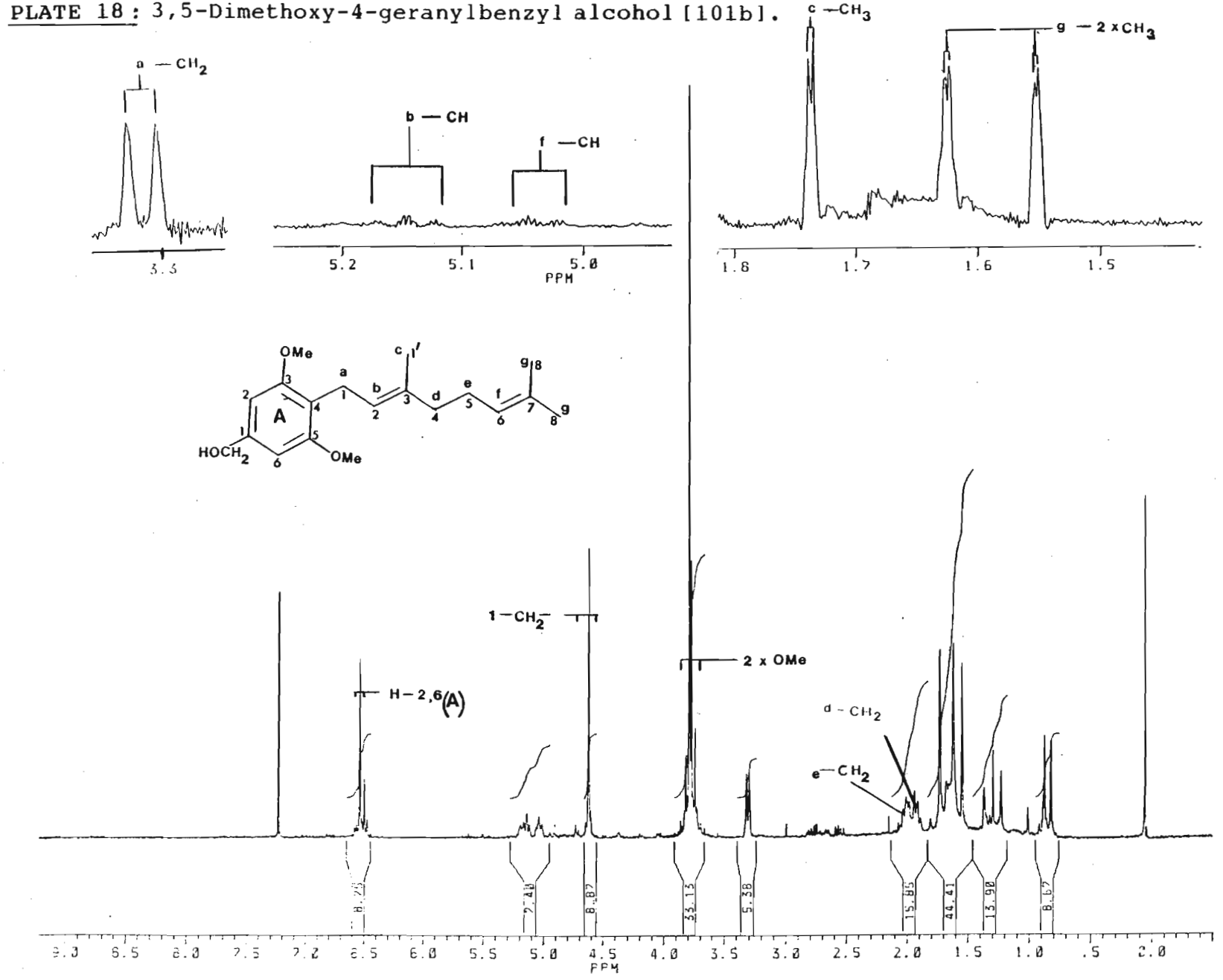
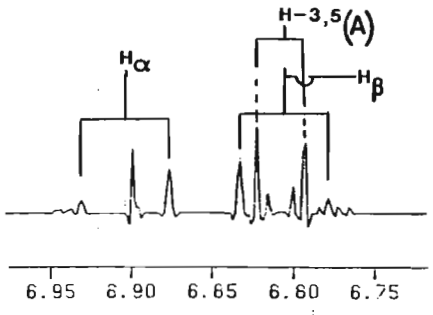
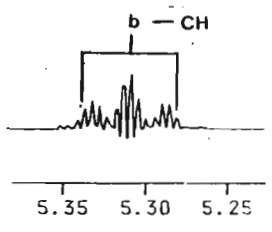
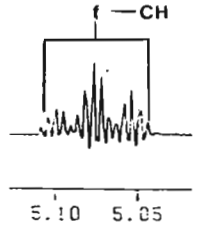
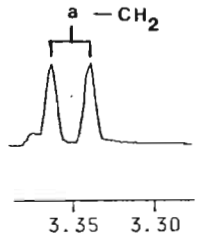


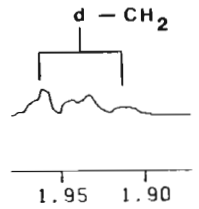
PLATE 19 : 3',4,5'-Trihydroxy-4'-geranylstilbene [102].

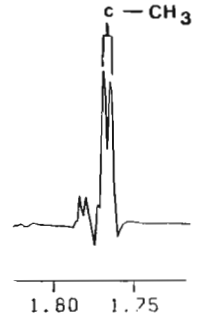


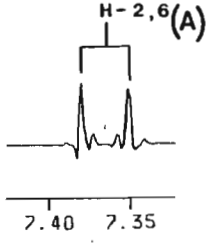


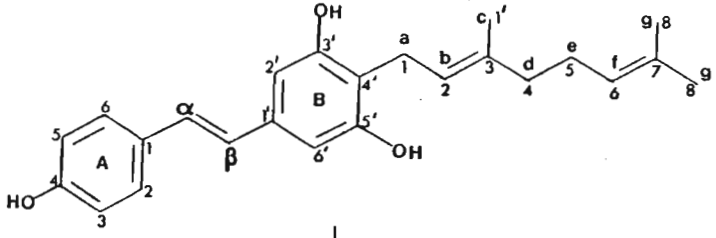


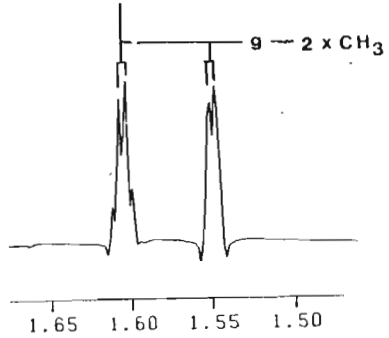












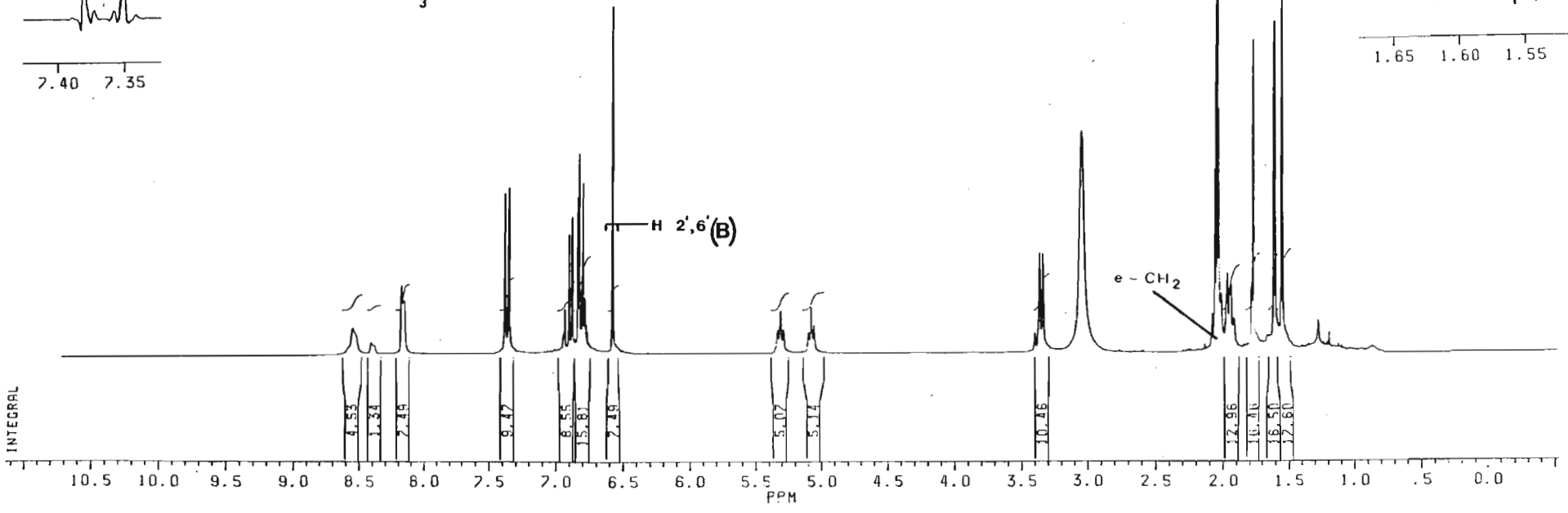


PLATE 20 : 3',4,5'-Trimethoxy-4'-geranylstilbene [102a].

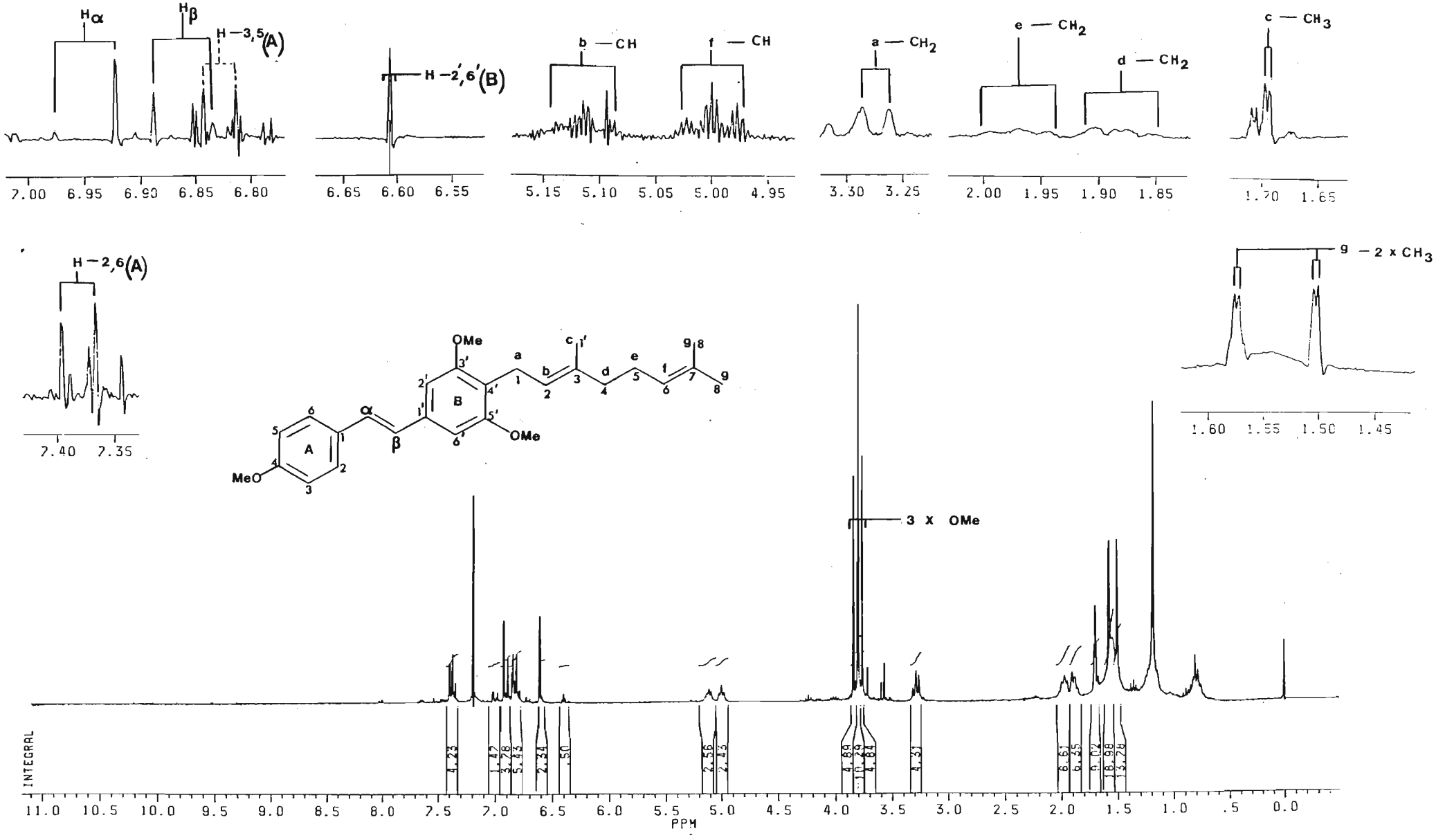


PLATE 21 : 3',4,5'-Trihydroxystilbene (Resveratrol) [104].

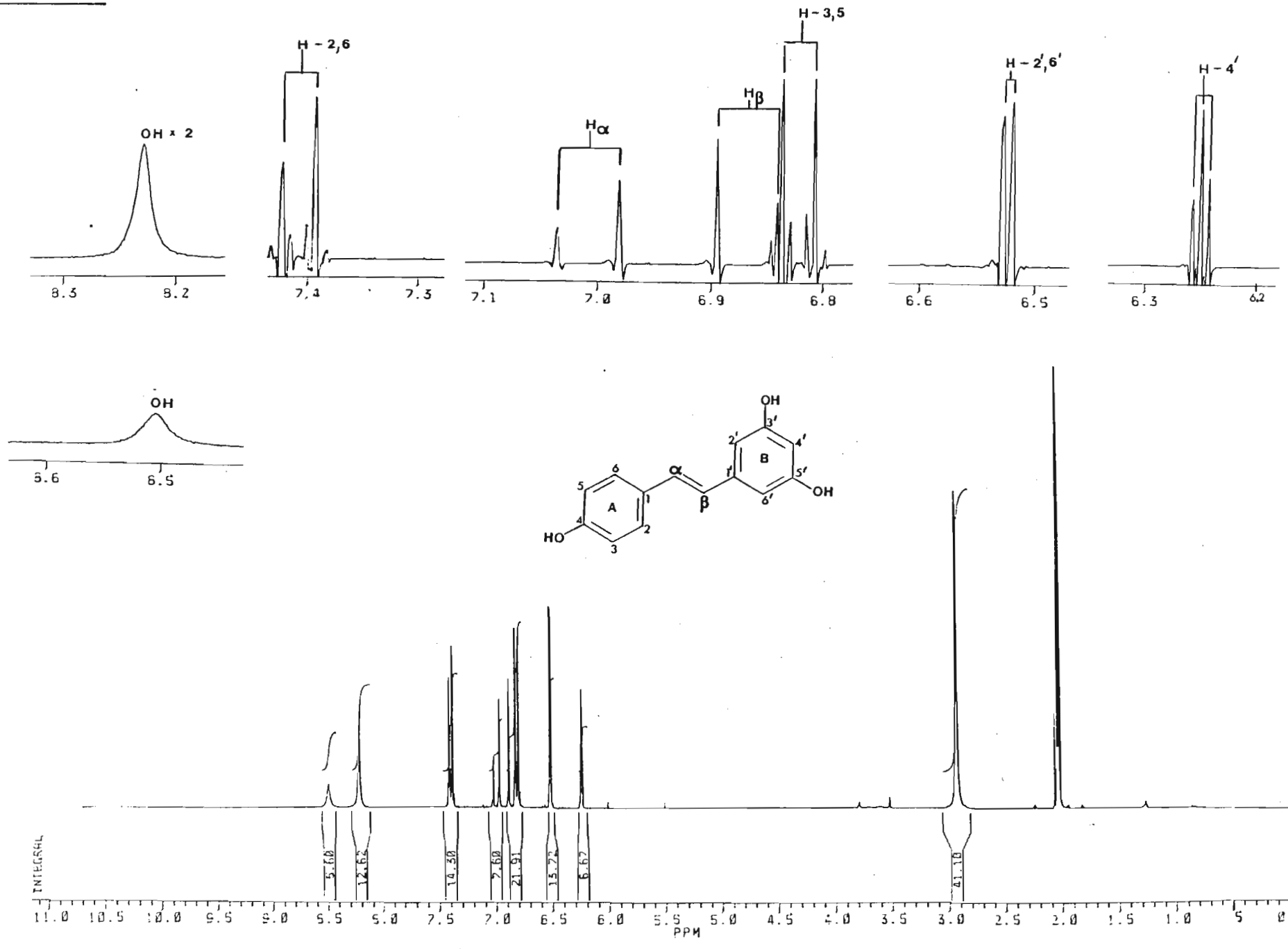


PLATE 22 : 3',4,5'-Trimethoxystilbene [104a].

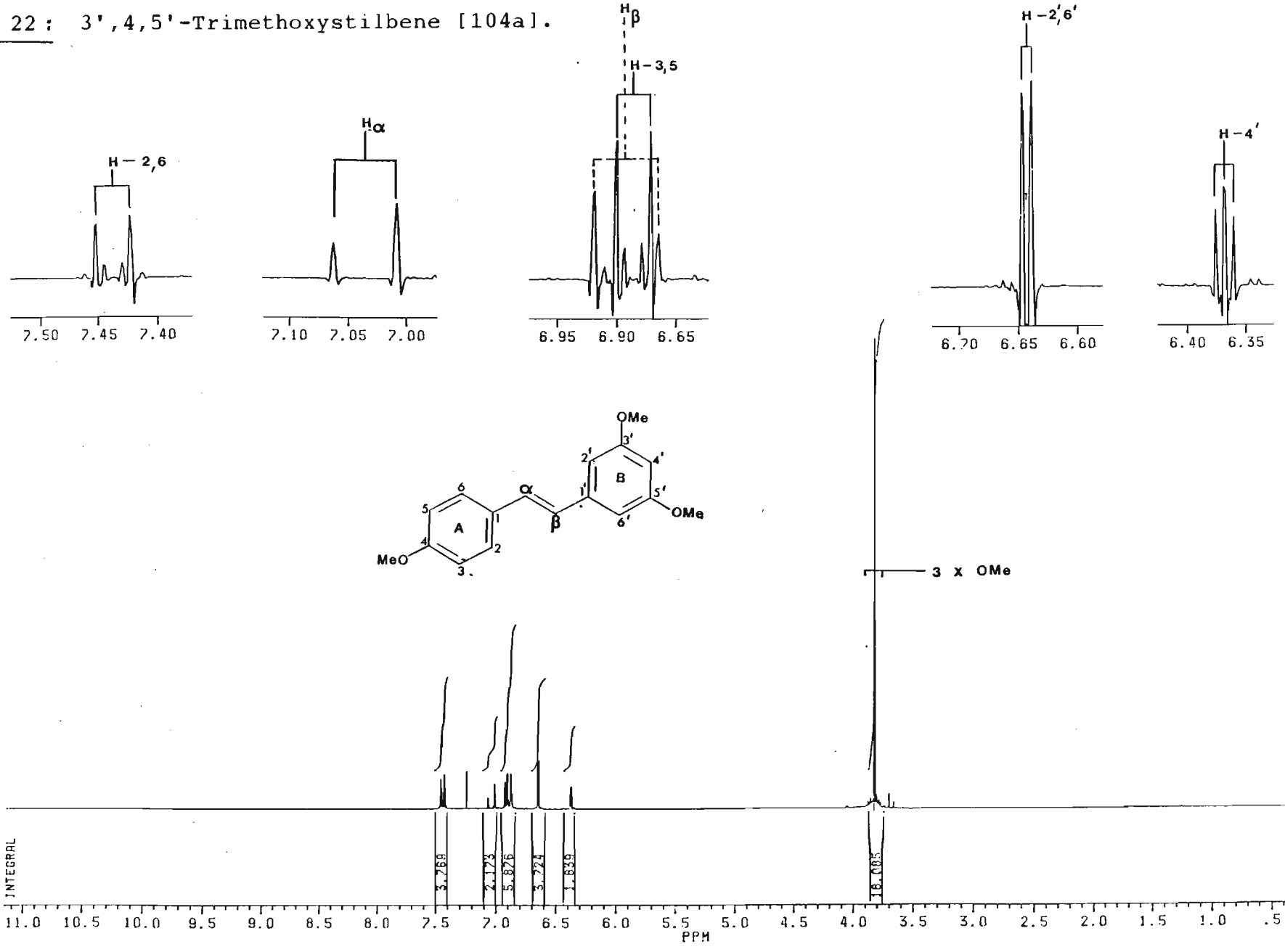


PLATE 23 : 2,3',4,5'-Tetrahydroxy-4'-geranylstilbene (Chlorophorin) [47].

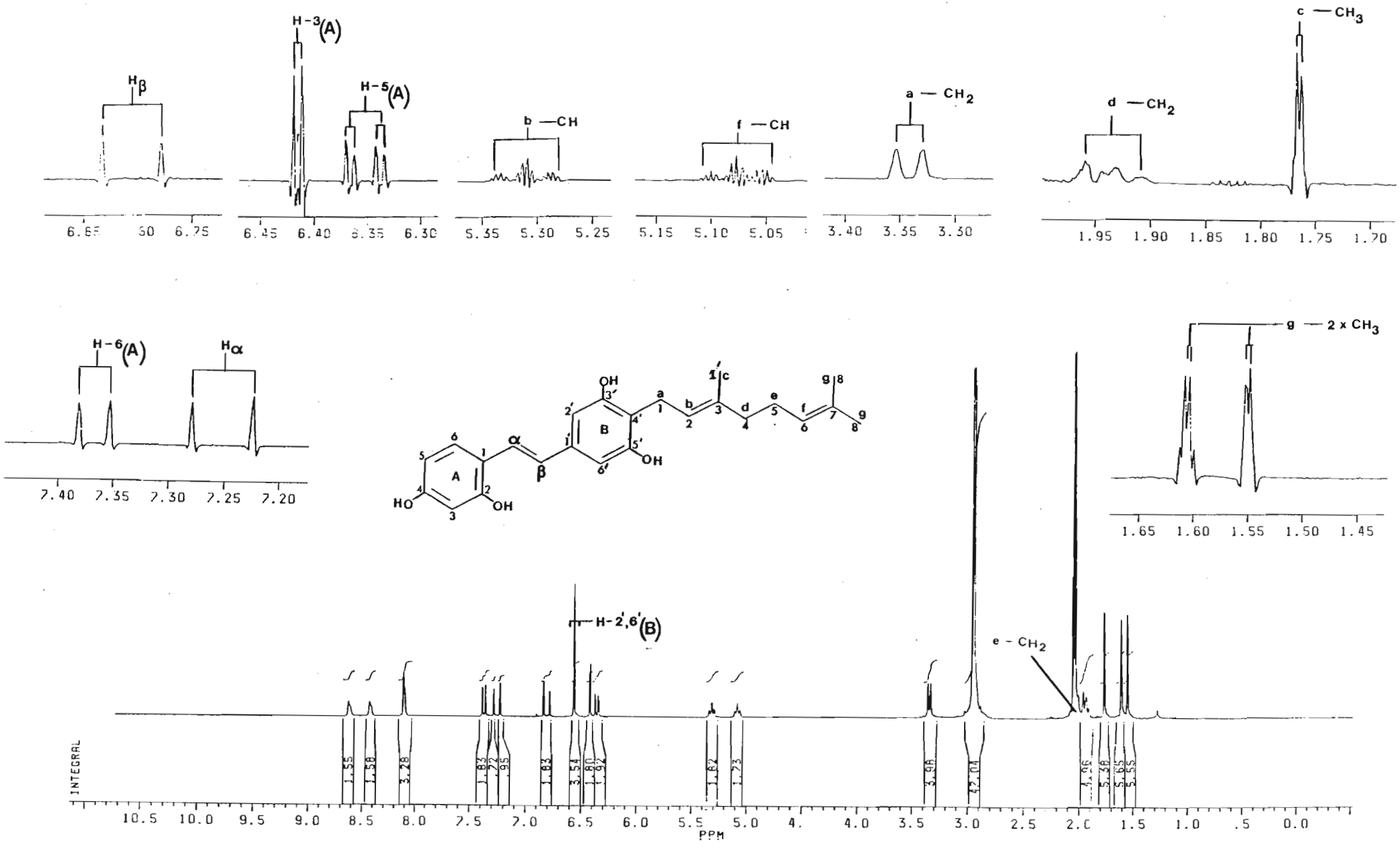


PLATE 24: 2,3',4,5'-Tetramethoxy-4'-geranylstilbene [47a].

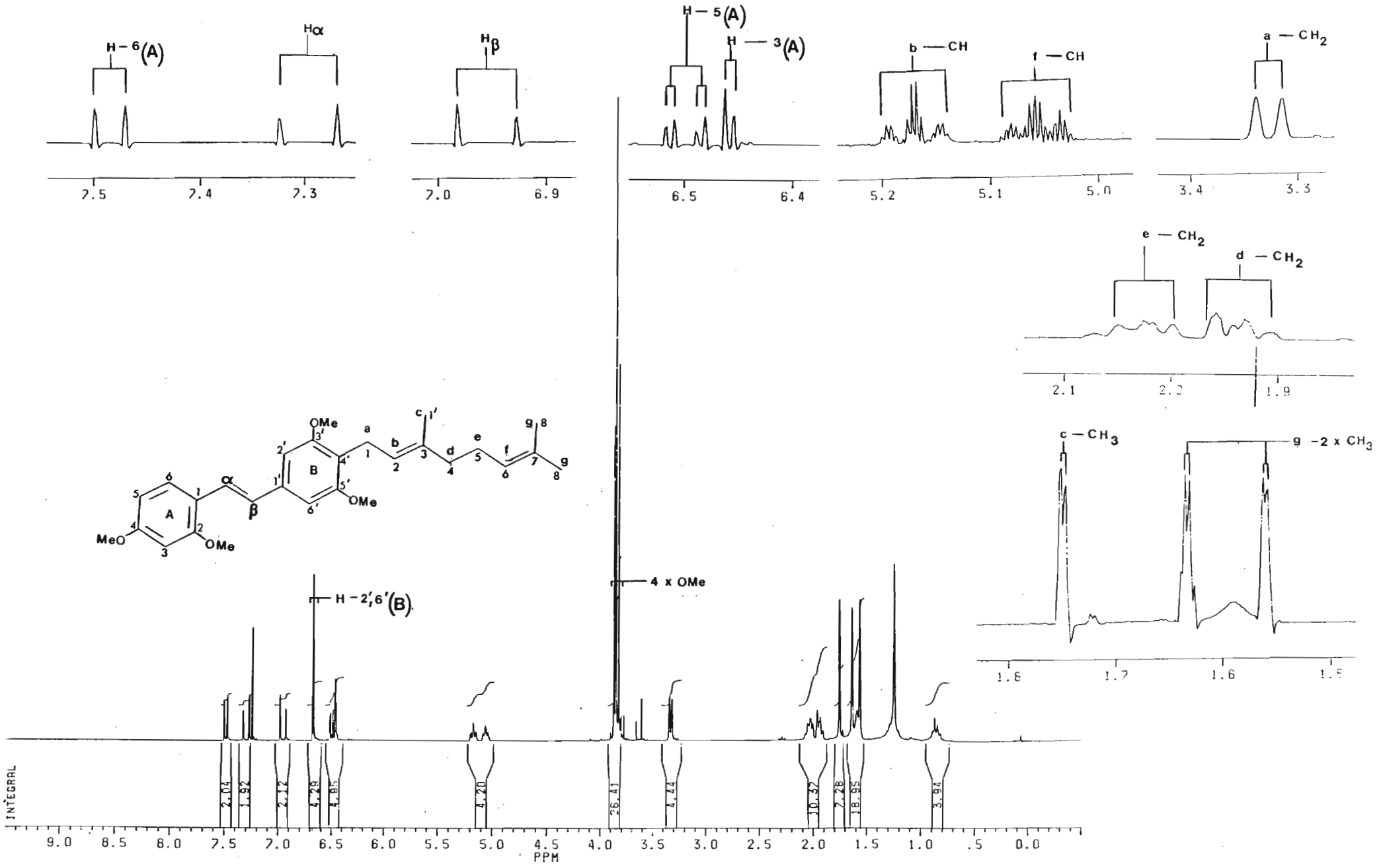
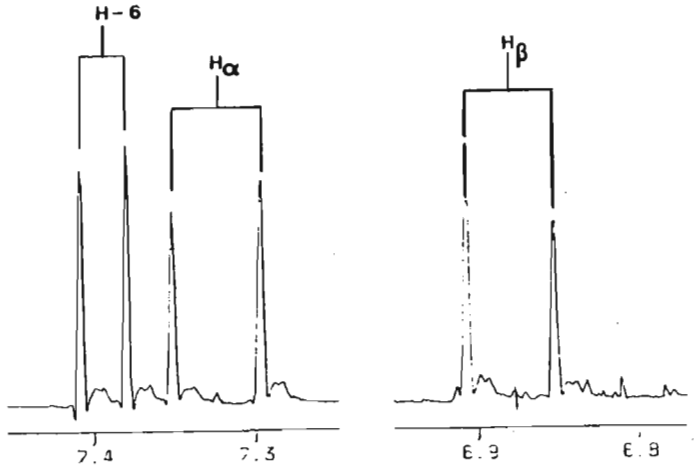
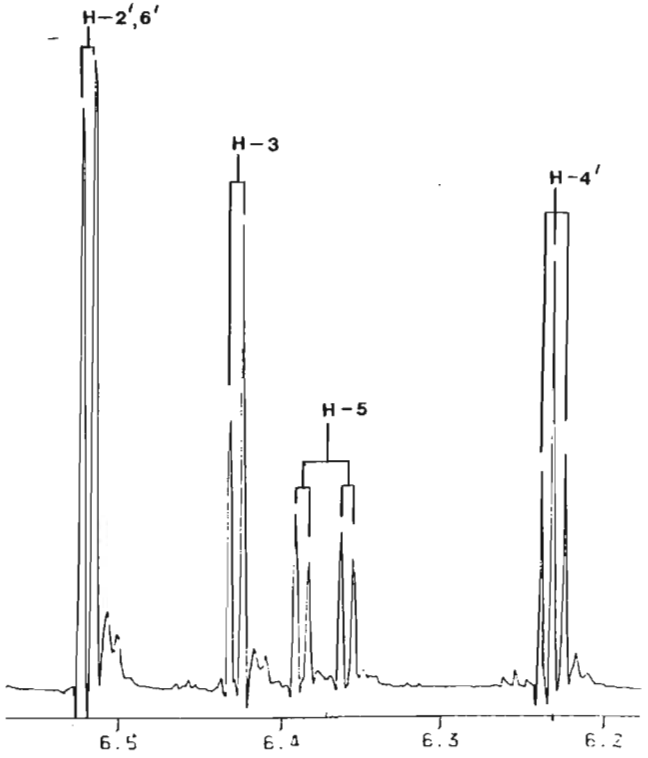


PLATE 25: 2,3',4,5'-Tetrahydroxystilbene (Oxyresveratrol) [103].





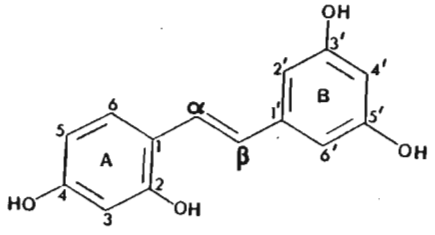


PLATE 26 : 2,3',4,5'-Tetramethoxystilbene [103a].

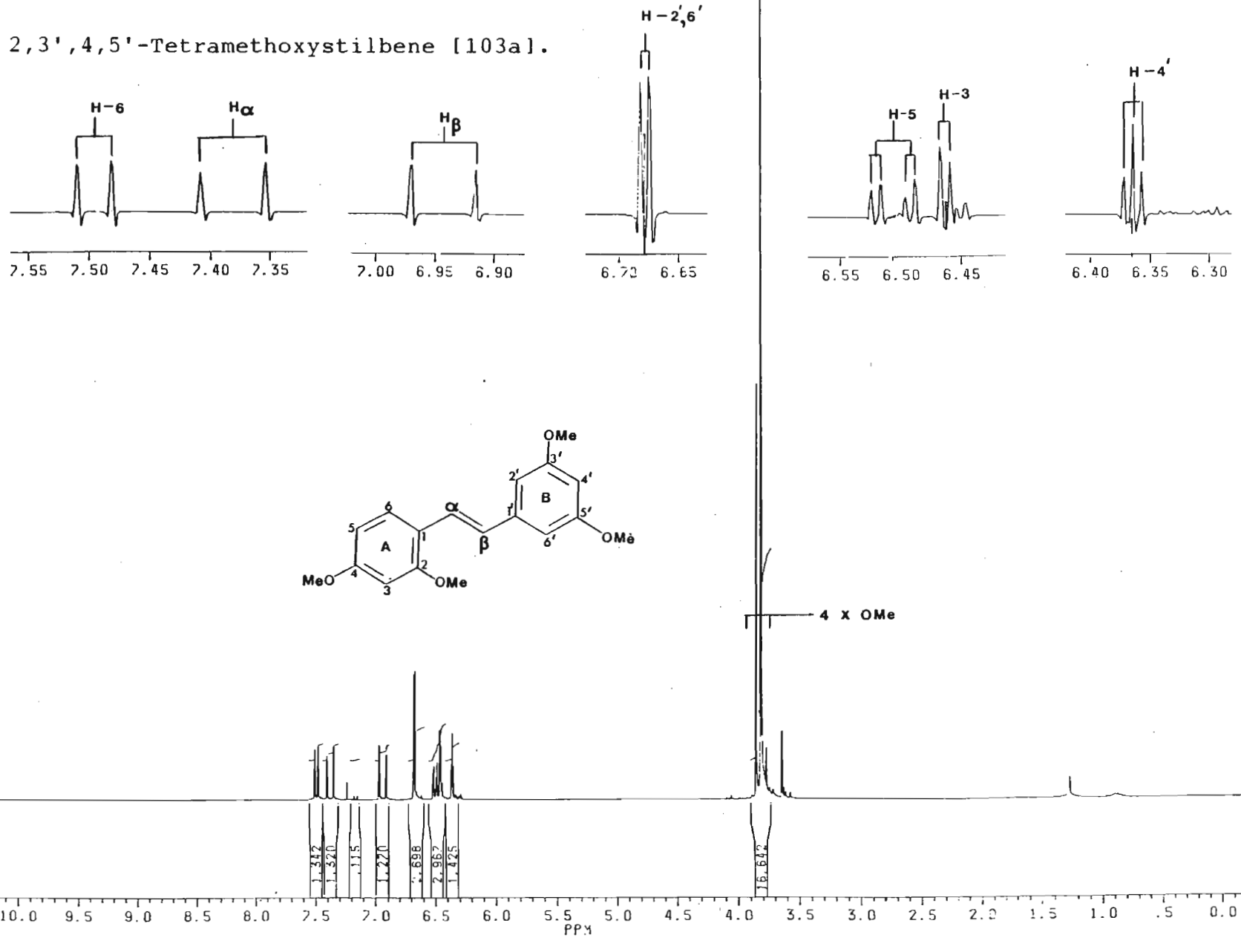


PLATE 27 : 3,3',4,5'-Tetramethoxystilbene [106].

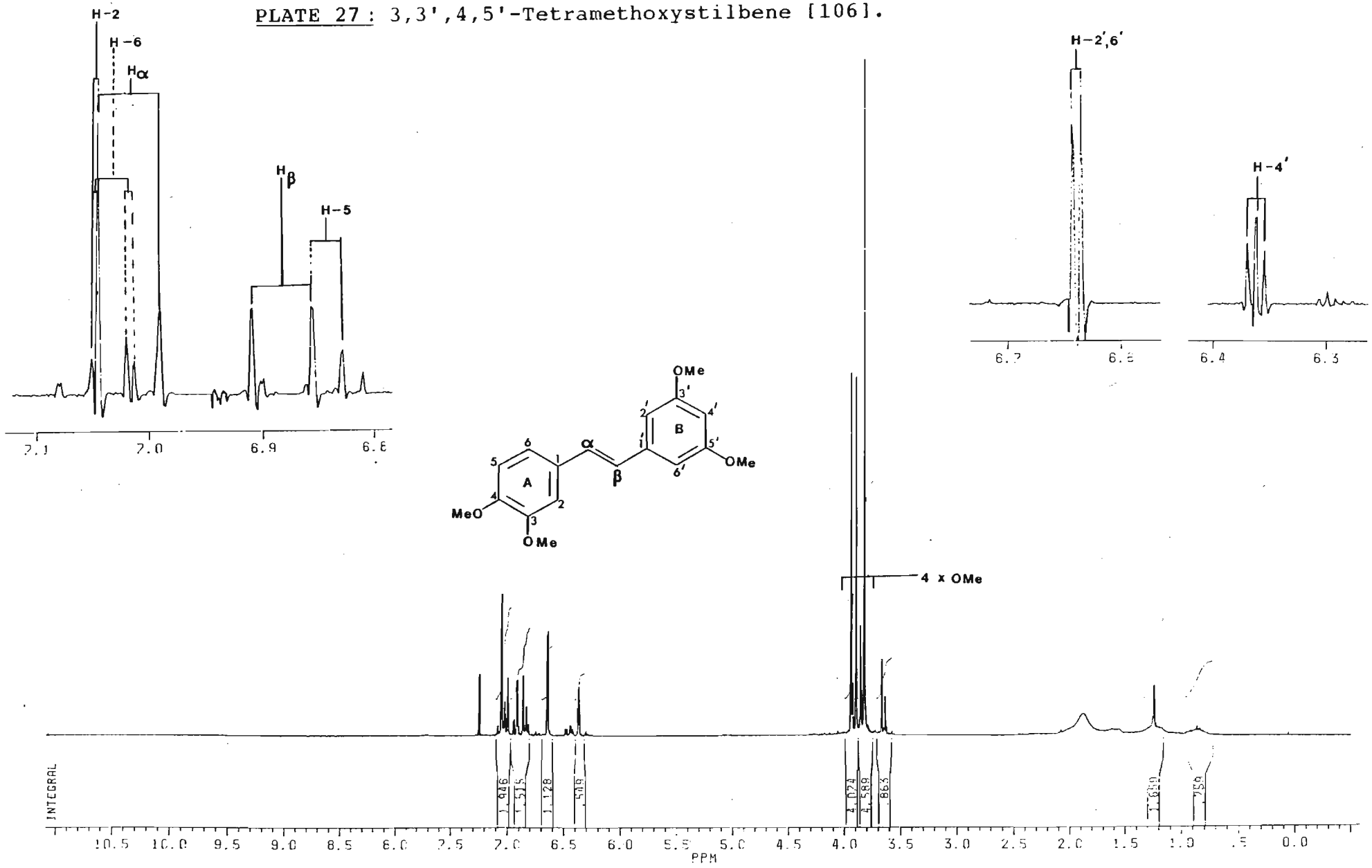


PLATE 28 : 2',4',5,7-Tetramethoxyflavone [105].

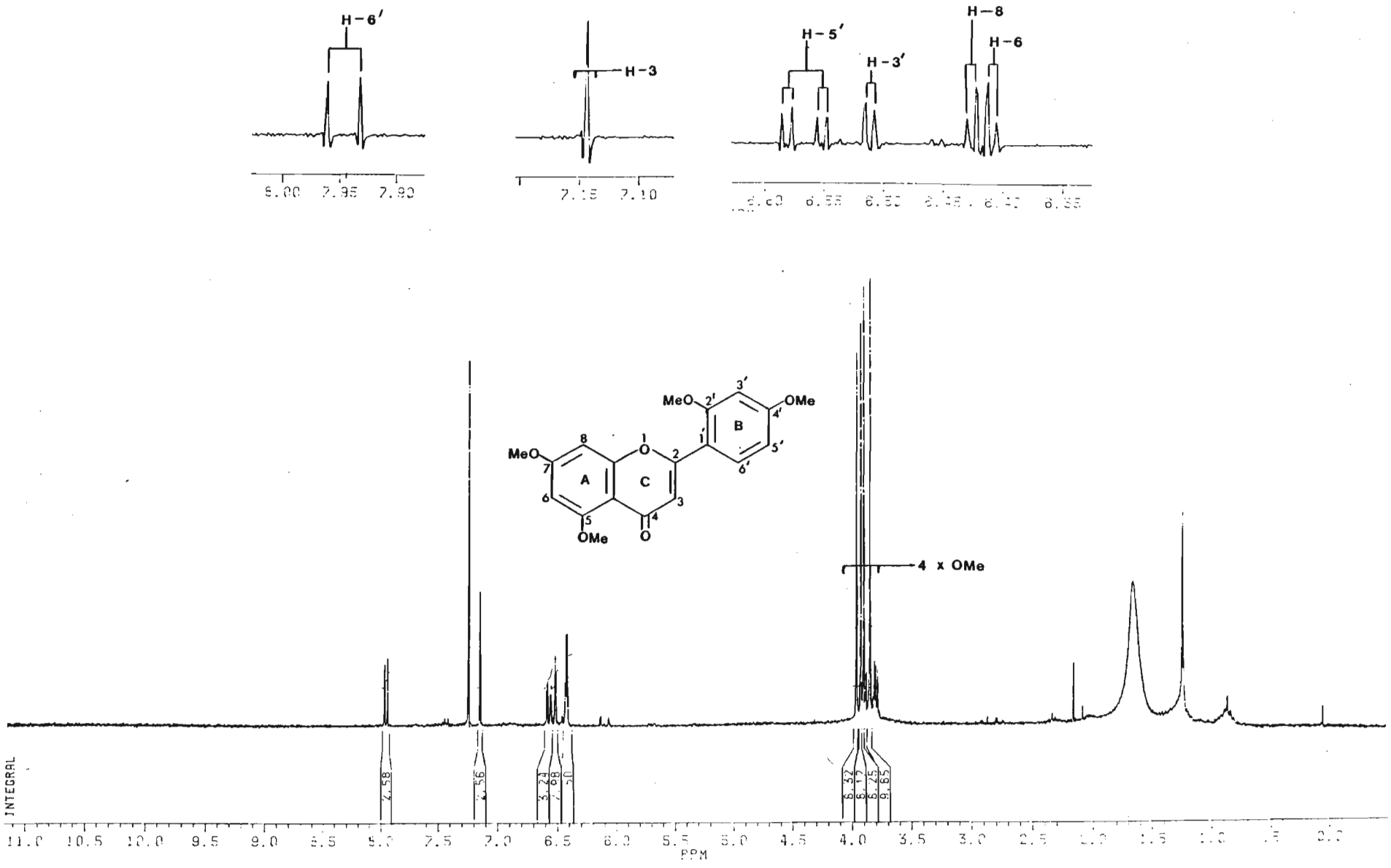


PLATE 29 : 3,4',7-Tri-O-methylquercitin (Ayanin) [99].

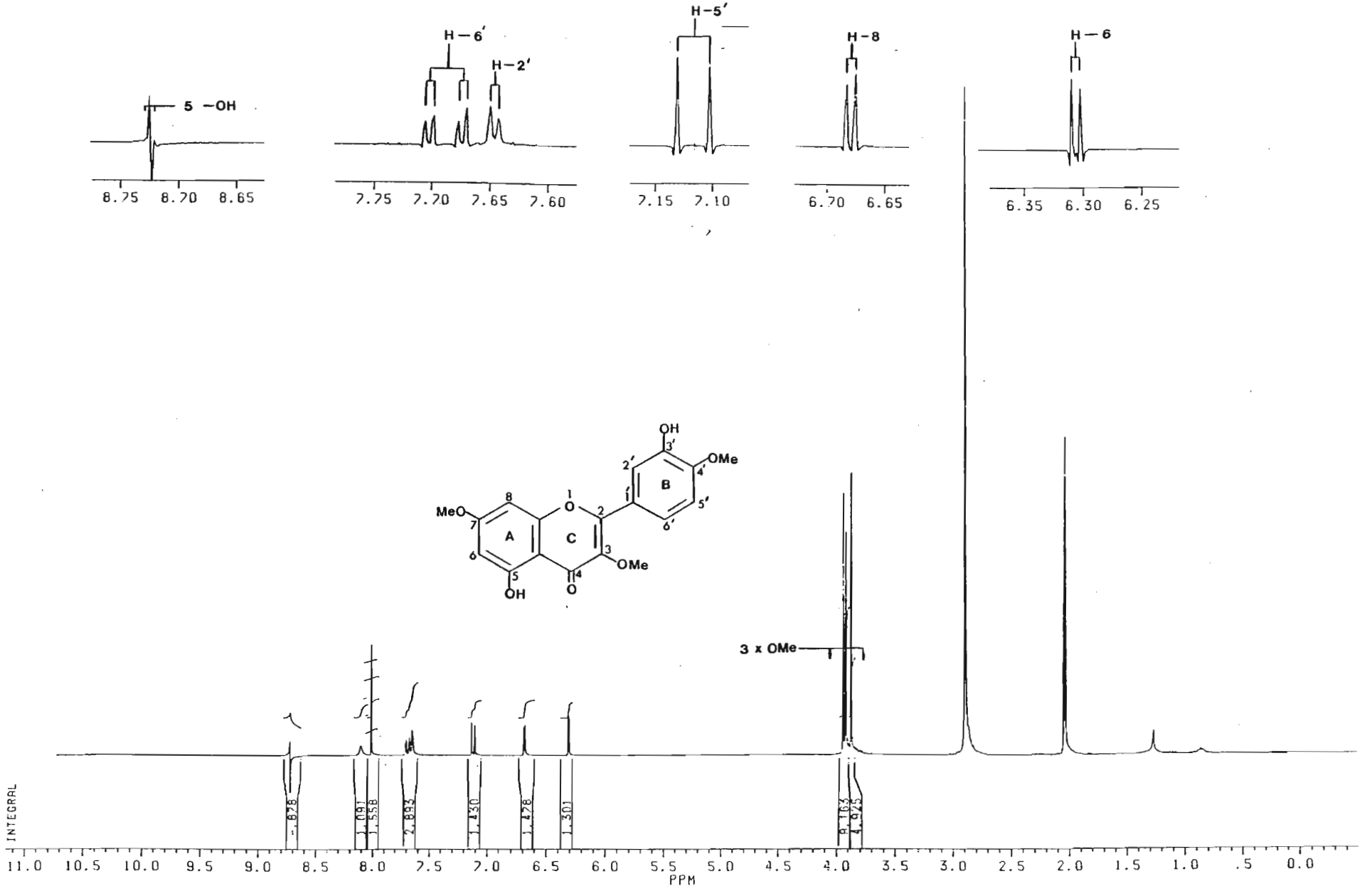
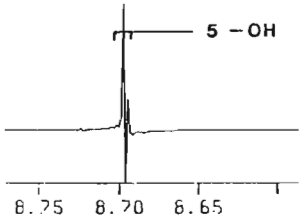
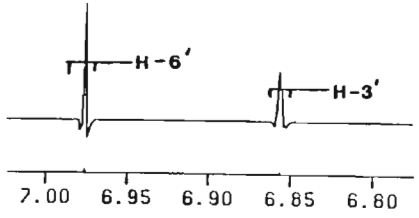
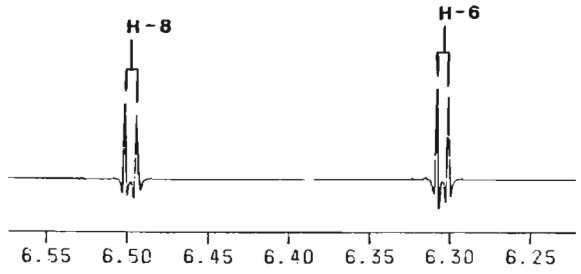


PLATE 30: 2'-Methoxy-3,4',7-tri-O-methylquercitin [100].







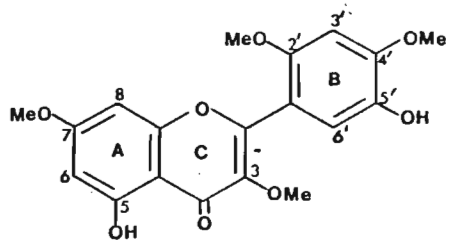


PLATE 31: 5,5'-Diacetoxy-2',3,4'7-tetramethoxyflavone [100a].

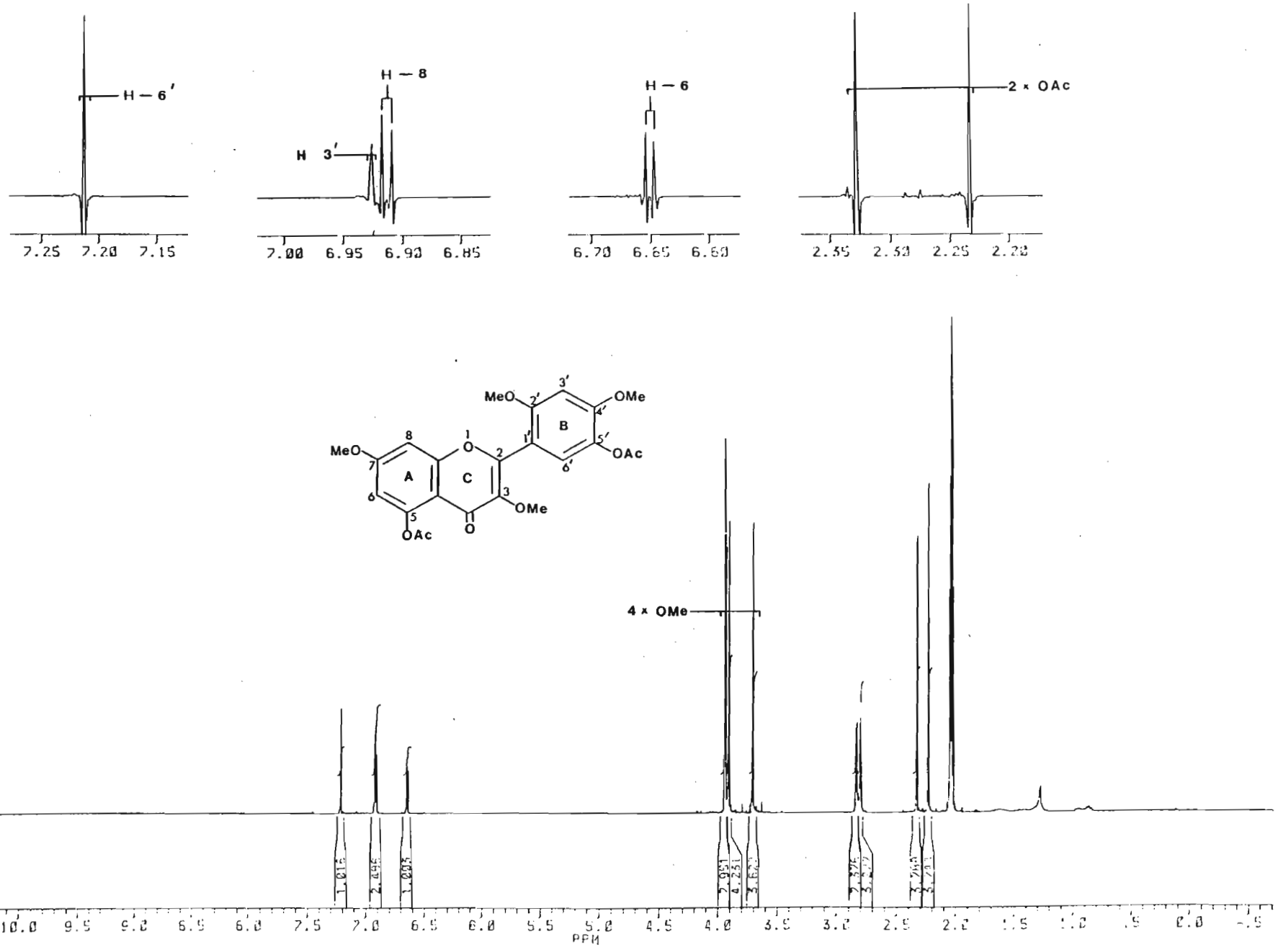
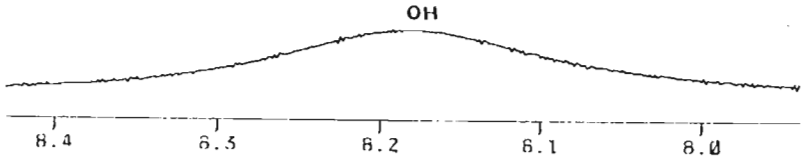
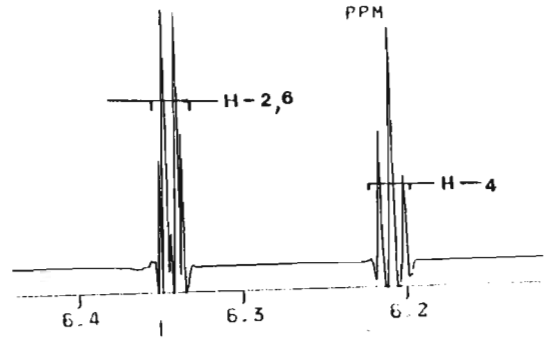
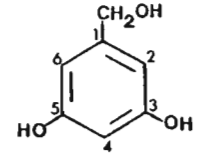


PLATE 32 : 3,5-Dihydroxybenzyl alcohol [110].







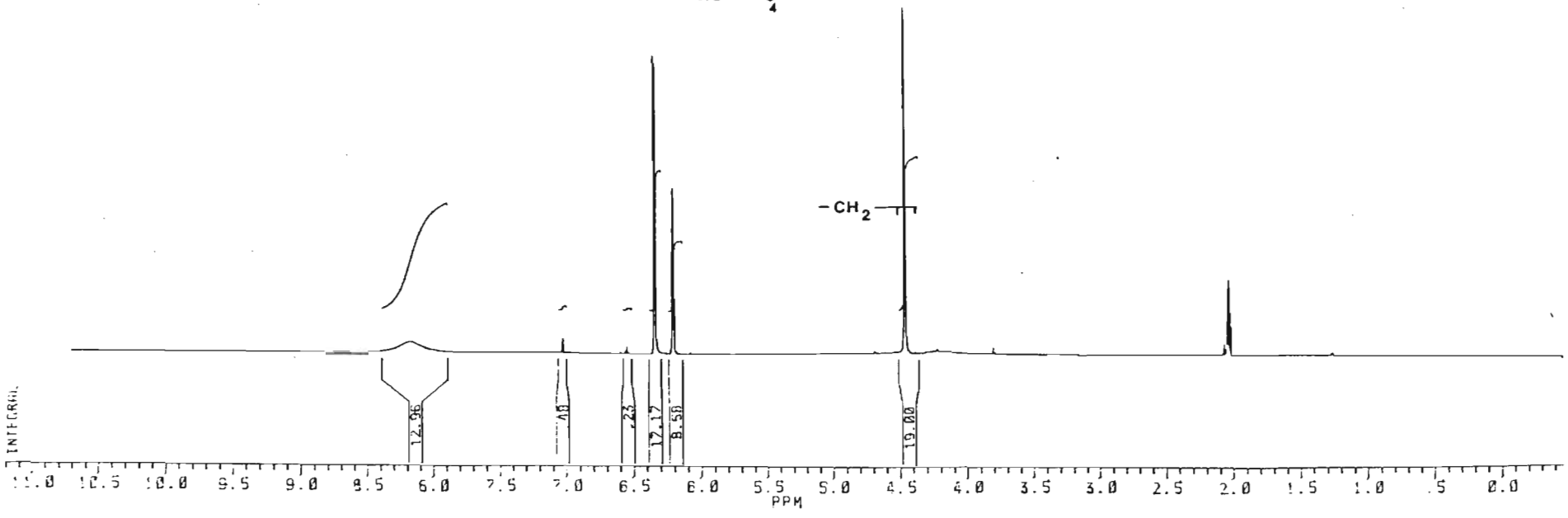
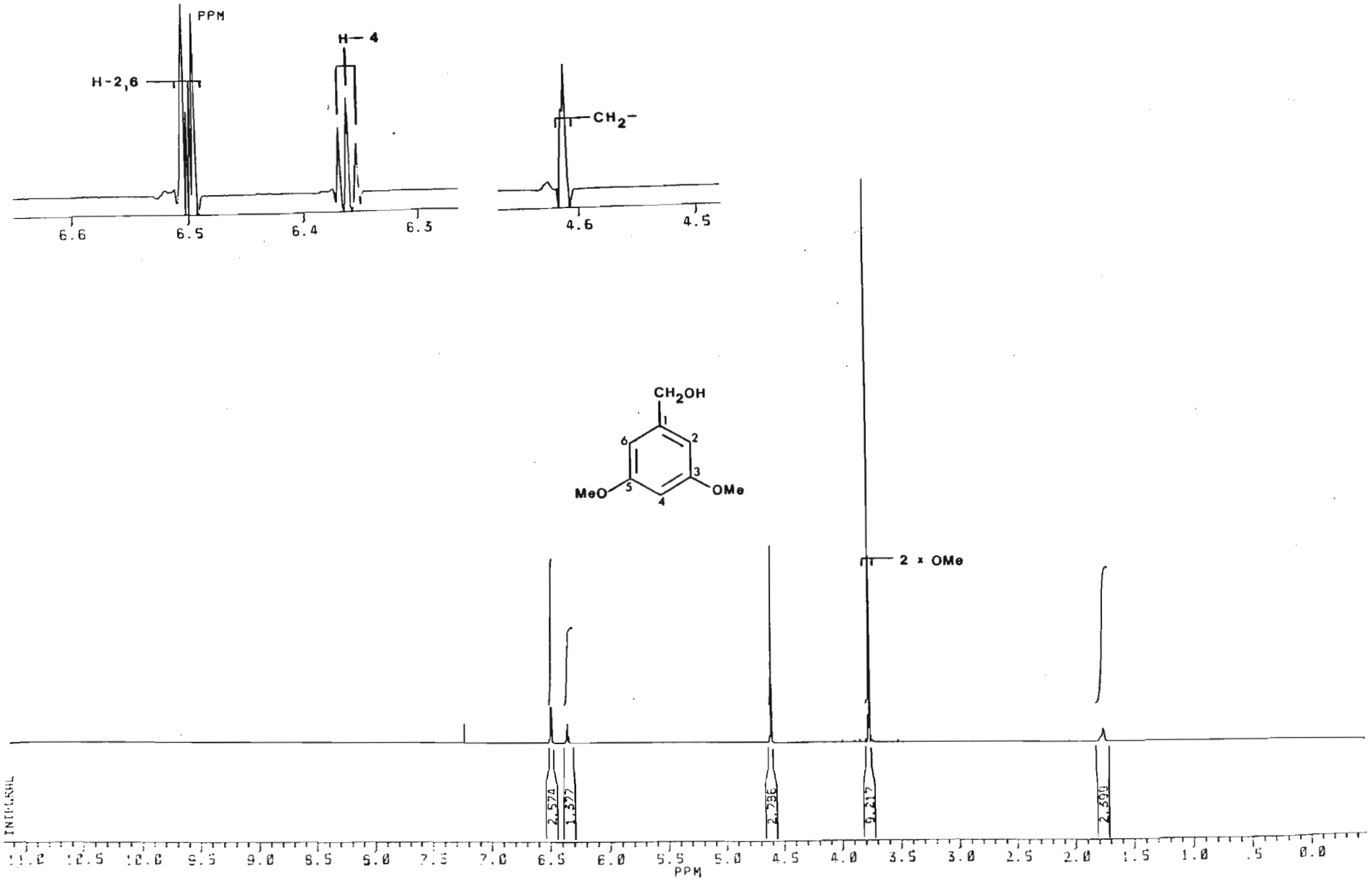


PLATE 33 : 3,5-Dimethoxybenzyl alcohol [112].



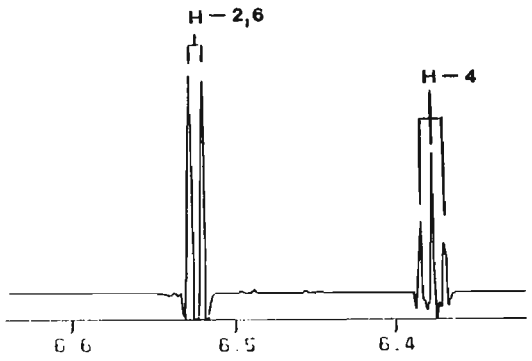
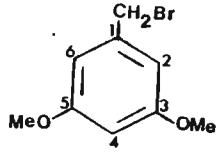


PLATE 34: 3,5-Dimethoxybenzyl bromide [113].



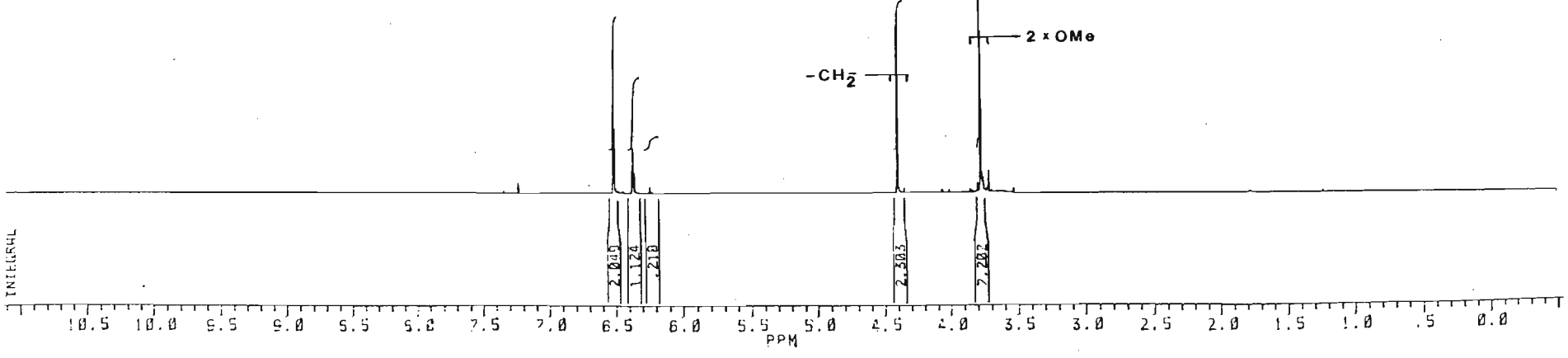


PLATE 35 : Geranic acid [125].

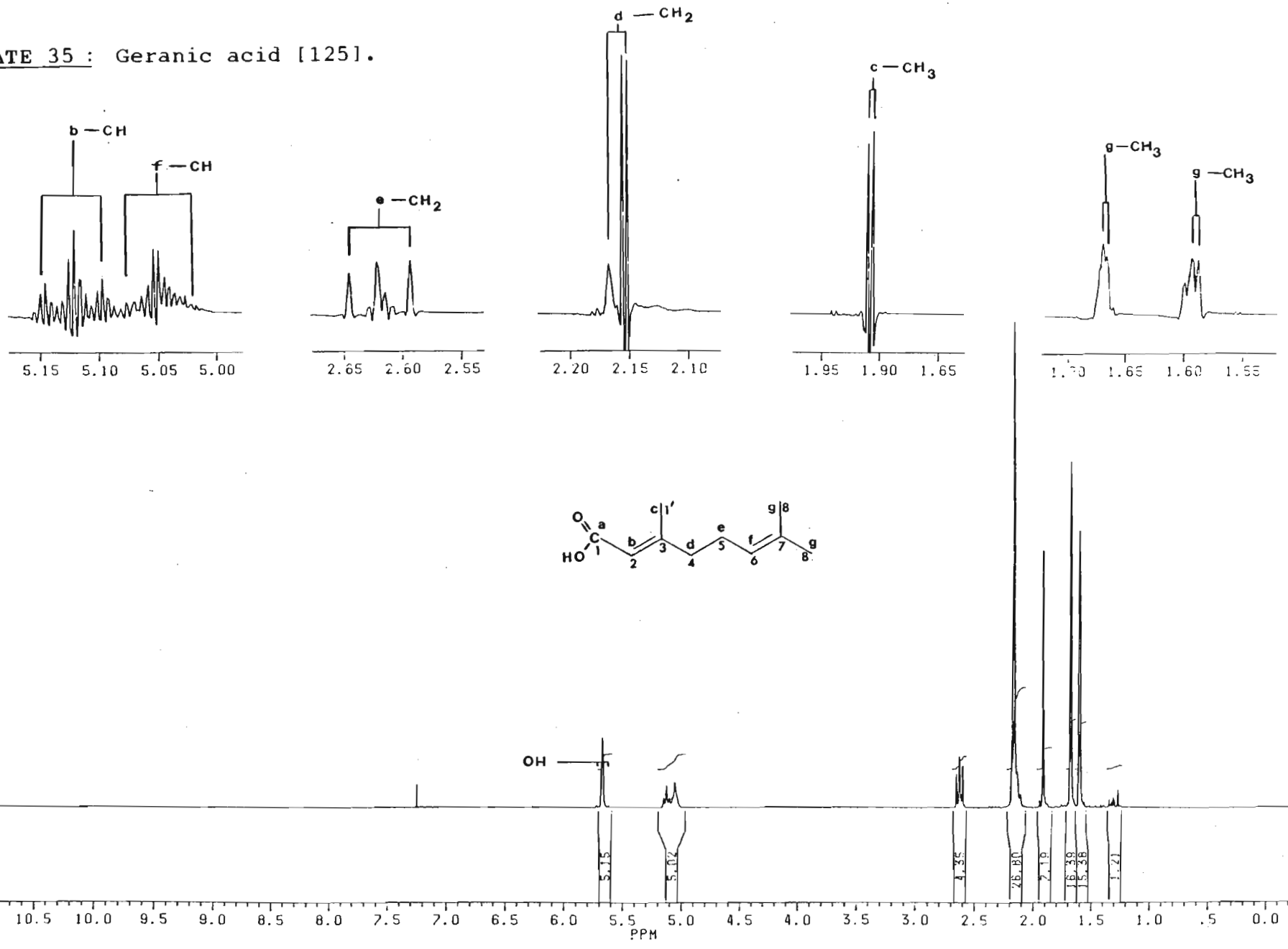


PLATE 36 : 2,6-Dimethoxy-1-O-allyl phenol [126].

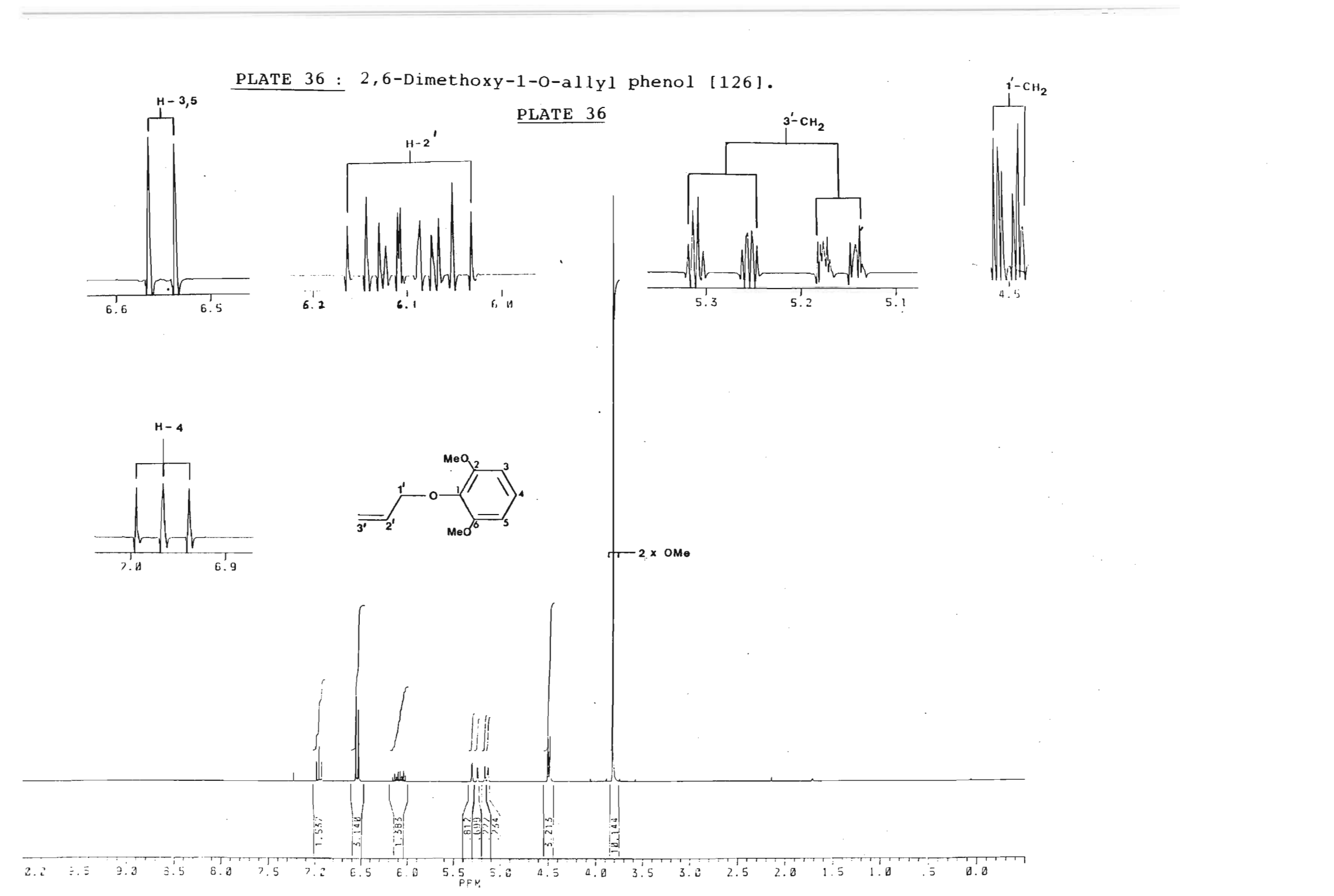
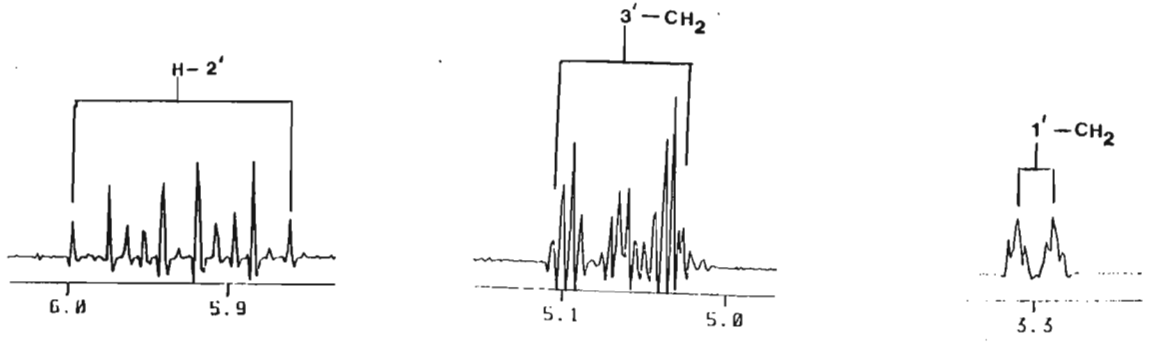
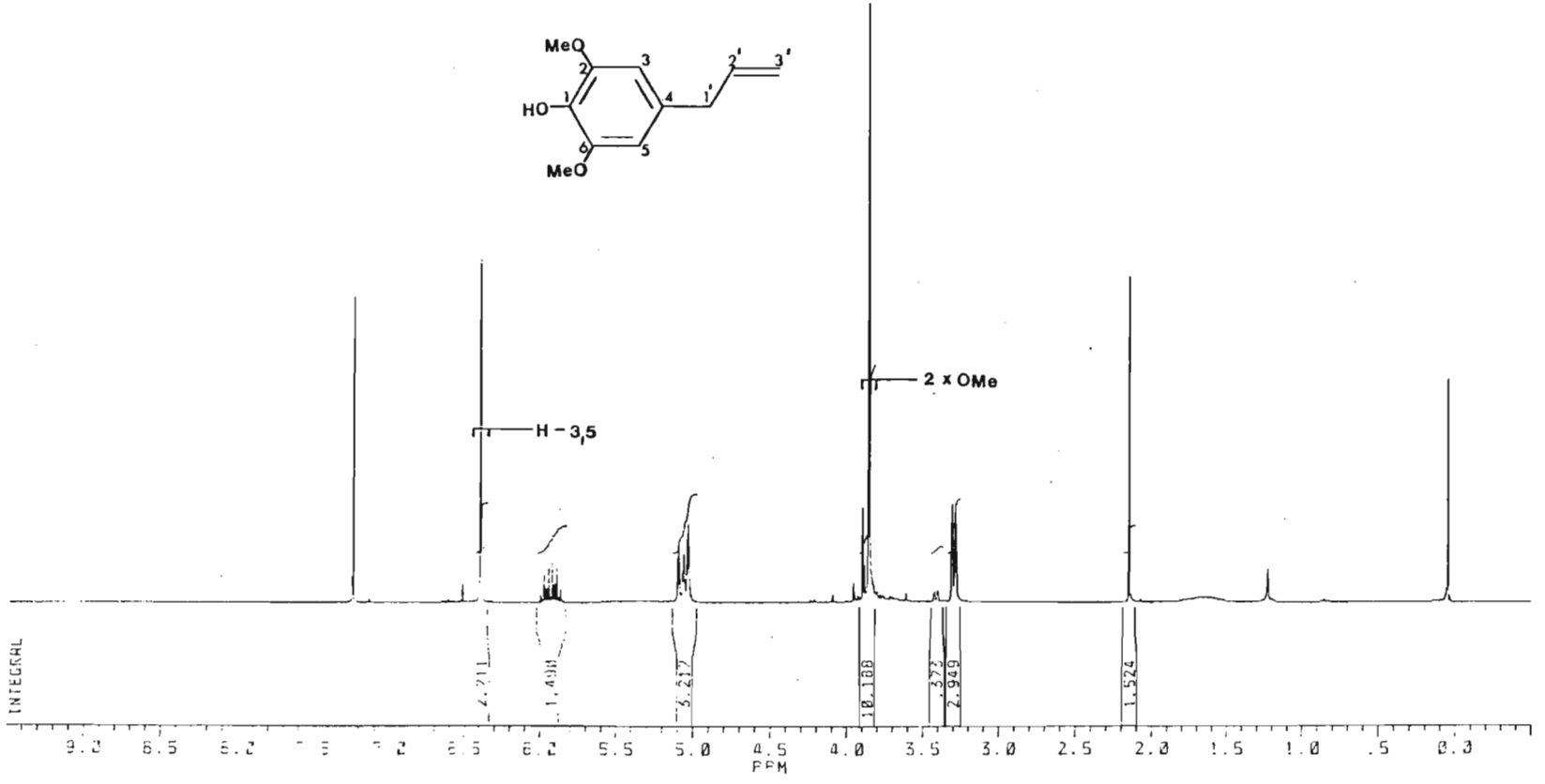


PLATE 37 : 2,6-Dimethoxy-4-allyl phenol [127].





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