STRUCTURE AND SYNTHESIS OF POLYPHENOLS FROM HONEYBUSH TEA (*CYCLOPIA INTERMEDIA*) AND THE POTENTIAL OF FLAVONOIDS AS ACTIVE OXYGEN SCAVENGERS.

Thesis submitted in fulfilment of the requirements for the degree

DOCTOR OF PHILOSOPHY

in the

Department of Chemistry
Faculty of Natural Sciences

at the
University of the Free State
Bloemfontein

by

BUKIRWA IRENE KAMARA

Supervisor: Prof. E. V. Brandt
Co-supervisor: Prof. D. Ferreira

November 1999

Acknowledgements

I thank the almighty God for making it possible, by providing me with good health, courage, wisdom, guidance and a supportive family.

I also wish to express my sincere gratitude to the following people:

Prof. E. V. Brandt as a supervisor and for recording my NMR spectra and Prof. D. Ferreira as a co-supervisor for their guidance, assistance, perseverance and invaluable advice;

Prof. D. Ferreira for believing in me, his friendship, encouragement, and enormous support

Dr. L. Fourie of the Univ. of Potchefstroom for recording my mass spectra;

Dr. J.Coetzee, for being a wonderful friend, mentor and encourager and for always being their for me.;

Dr H. van Rensburg, co-students and colleagues for their constant encouragement and companionship;

my friends especially Jacqueline, Khosi, Maureen and Osei Abunyewa for their support and encouragement;

my parents Wilson and Joyce Bugembe for their life time love, support and encouragement throught my life and the educational background they gave me;

The most important people in my life to whom I dedicate this thesis, my wonderful loving children Edmond, J ce, Samuel, Edward jnr and my husband, Edward, as a token of my appreciation for their unwavering love, patience, encouragement, support and sacrifices under difficult circumstances, without which completion of this thesis would have been impossible;

B I. Kamara

A part of this study resulted in the following publications:

1. *Phenolic Compounds from Cyclopia intermedia (Honeybush tea). 1*

   D Ferreira, BI Kamara, EV Brandt

   J.Agric. and Food Chem., **1998**, *46* 3407

2. *The first Direct Transformation of 2,2'-Dihydroxychalcones into Coumestans.*

   BI Kamara, EV Brandt and D Ferreira

   Tetrahedron, **1999**, *55*, 861

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Key words: *Cyclopia intermedia;* Fabaceae; Honeybush tea; flavonoid; isoflavonoids; glycosides; tannin cleavage; superoxide scavengers; deoxygenation; coumestan synthesis

Cyclopia intermedia E. Mey (Fabaceae), from which Honeybush tea is brewed, is one of approximately 24 *Cyclopia* species of woody legumes endemic to the Cape fynbos (Cape macchia) region of South Africa. Supported by results from our initial investigations on the plant, demonstrating the presence of phenolic compounds including coumestans, isoflavones, flavanones, xanthones, a flavone, pinnitol and *p*-coumaric acid, the tea is gaining popularity as a health beverage. Presence of these compounds that are claimed to have interesting pharmacological properties, supported by belief that the tea contains very little, if any, caffeine and a low tannin content, as well as its usage as a medicinal concoction by the people of the Western and Eastern Cape, prompted further investigations of the plant.

A series of enriched fractions of the methanol extracts of the fermented shoots of *C. intermedia* followed by chromatographic (column Sephadex LH-20 and preparative thin layer) separations afforded flavones and glycosylated flavonols, flavanones, isoflavones and $C_6.C_2$- and $C_6.C_1$-type compounds. Determination of the tannin content and the average degree of polymerisation was accomplished by cleavage of the crude tannin fraction in dilute acid with benzyl mercaptan and phloroglucinol as capture nucleophiles. Stuctures of the compounds were elucidated and characterised as their full acetate derivatives by high resolution (300 MHz) ^{1}H NMR spectometry (including COSY and NOESY experiments), Circular Dichroism and Electron Impact Mass spectrometry.

Along with tyrosol and its 3-methyl ether analogue, two new $C_6.C_2$ and $C_6.C_1$ monoaryls with the same *β*-apiofuranosyl-4-*O*-*β*-D-glucopyranosyl unit were isolated. These were accompanied by the known flavonoids, 3',4',7-trihydroxy- and 3',5,7-trihydroxy-4'-methoxy flavones, 6-*C*-*β*-D- and 5-*O*-*α*-D-glucopyranosylkaempferol, the flavanones 7-*O*-*β*-D-glucopyranosylnaringenin and eriodictyol, 5-*O*-*β*-D-glucopyranosyleriodictyol and the isoflavone, 7-*O*-*β*-D-glucopyranosyl-4',6-di-*O*-methylafrormosin. The new compounds isolated were 5-*O*-*α*-D-rutinosylnaringenin, 8-*C*-*β*-D-glucopyranosyl- and 3-*O*-,6-*C*-di-*β*-D-glucopyranosylkaempferol, 6"-*O*-*β*-apiofuranosyl-7-*O*-*β*-D-glucopyranosyl-4'-*O*-

methylisoflavone and 6"-*O*-*β*-apiofuranosyl-6-*O*-*β*-D-glucopyranosyl-3'-4'-methylenedioxyflavonol.

While cleavage of the tannin fraction afforded three new 4-arylflavans, 4-(2,4,6-trihydroxyphenyl)-4',5,7-trihydroxyflavan, its 5-*O*-*β*-D-glucopyranosyl analogue and 4-(2,4,6-trihydroxyphenyl)-3',4',5,7-tetrahydroxyflavan from the phloroglucinol reaction, 4-thiobenzyl-4',7-dihydroxy-5-*O*-*β*-D-glucopyranosylflavan and 4-thiobenzyl-4',7-dihydroxy-5-*O*-*β*-D-rutinosylflavan were the new 4-thiobenzylflavans isolated the benzyl mercaptan reaction.

Absence of the heterocyclic protons of the C-ring in the coumestan structure precluded structural elucidation by NMR alone and necessitated confirmation by synthesis. The three coumestans were, therefore, synthesized *via* a novel route by directly transforming the appropriate 2,2'-dihydroxychalcones into coumestans.

Since the flavonoids are presumed to contribute significantly towards the scavenging effects of the active oxygen species, flavonols (quercetin, myricetin, fisetin, and robinetin) and flavan-3-ols (catechin and epicatechin), with similar hydroxylation pattern were selected and reacted with super oxide. Besides expected products resulting from cleavage of the molecule, a unique deoxygenation of the 3'-OH on B-ring occurred with some of the compounds.

These results clearly indicate that the tentative claimed health promoting properties of Honeybush tea may at least, in part, be attributed to the presence of these and other phenolics in *C.intermedia.*

LITERATURE SURVEY

CHAPTER 1

POLYPHENOLS FROM HONEYBUSH TEA

1.1 Introduction

In an initial survey on Honeybush tea[1], a herbal tea indigenous to South Africa[2,3], phenolic compounds claimed to have interesting pharmacological properties were isolated and characterised as flavonoids.

Flavonoids, a group of polyphenols of which flavones and flavonols are representatives, are one of the most numerous and widespread group of natural products that are of importance and interest to man due to their physiological activity in humans. As a group, they are universally distributed among vascular plants and at least 4000 different structures have been reported[4]. In marked contrast to flavones and flavonols the isoflavonoids have a very limited distribution in the plant kingdom[5]. Coumestans, on the other hand, are occasionally found in nature accompanying other isoflavonoid types[6]. While flavones are known to form the largest family of naturally occurring heterocyclic compounds[7], flavanones are classified as 'minor flavonoids'[8].

The presence of the flavonoids in the extracts from the tea[1], supported by the belief that the tea contains very little, if any, caffeine and a considerably lower content of tannins that are common in the oriental tea, prompted further investigations on the plant. The claimed

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[6] K. R. Markham and T. J. Mabry in *The Flavonoids,* (ed. J. B. Harborne, T. J. Mabry and H. Mabry), **1975**, 63
[7]E. Wollenweber, in *The Flavonoids: Advances in Research since 1986* (eds J. B. Harborne), Chapman and Hall, London, **1993**, 259
[8]B. A. Bohm, in *The Flavonoids: Advances in Research since 1980* (eds J. B. Harborne), Chapman and Hall, London, **1988**, 329

antioxidant properties of the flavonoids[9] and reported usage of the beverage by the people in the Western Cape as a medicine for the treatment of asthma, as a diuretic and a restorative for coughs[2] also supported the proposal.

In the survey that follows the second investigation on the concise chemistry of the phenolic compounds and related compounds that were encountered in Honeybush tea is presented.

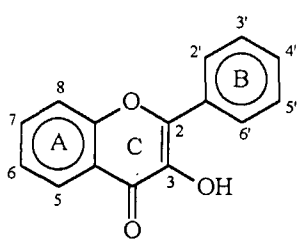
[9] E. Middleton and C. Kandaswami, in *The Flavonoids: Advances in Research since 1986,* (ed. J. B. Harborne), Chapman and Hall, London, **1993,** 619 and the references there in

CHAPTER 2

NOMENCLATURE AND OCCURRENCE

2.1 Flavones and Flavonols

Flavonols **(1),** with carbon structure $C_6.C_3.C_6$, only differ from flavones **(2)** with respect to the presence of a 3-hydroxyl group, i.e they are flavon-3-ols. Though the properties of the two classes are fairly similar, this structural difference is of considerable biosynthetic, physiological, chemosystematic, pharmacological and analytical significance[7]. The known flavonols (380) are greater in number than flavones (300). The substitution is dominated by *O*-substitution (hydroxyl or methoxyl) as compared to *C*-methyl, isoprenyl, *O*-glycosyl and *C*-glycosyl substituents.



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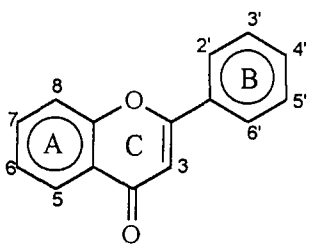
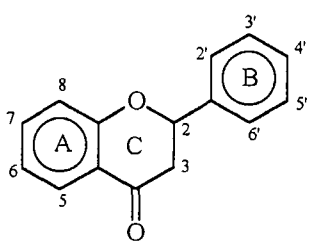


(2)

2.2 Flavanones

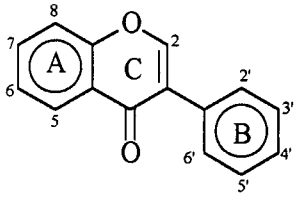


(3)

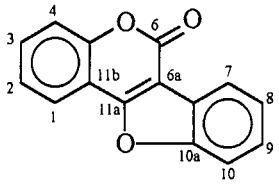
The flavanones **(3)** are 2,3-dihydroflavones with an asymmetric centre at C-2, thus, capable of existing as two enantiomers. Apparently most if not all naturally occurring flavanones are laevorotatory and probably belong to the (2S) series[10]. Flavanones are of fairly general distribution but occur most abundantly in angiosperm families such as *Rosaceae, Rutaceae, Leguminosae, Ericaceae* and *Citrus*[11,12]. Recently, microbial sources such as *streptomyces*[13] have been identified to produce flavanones. Flavones occur very characteristically in higher plants[14] (e.g. *Eucalyptus, Baccharis, Miyrica, and Citrus*), but they have also been detected in ferns[15].

2.3 Isoflavones

Isoflavones **(4)**, on the other hand constitute the largest group of natural isoflavonoid analogues, and are a distinctive subclass of the flavonoids also possessing a $C_6.C_3.C_6$ carbon backbone[10,16]. However, isoflavonoids have a very limited distribution in nature, being essentially confined to the subfamily *Papilionideae* of the *Leguminosae*[17]. Several non-legume sources including the dicotyledons[18], and recently microbial sources[19] are known to produce isoflavonoid derivatives. Though isoflavonoid distribution in the plant kingdom is very limited, they have a large structural variation[16].



(4)



(5)

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2.4 Coumestans

Coumestans (3-phenylcoumarin derivatives) **(5)** represent the highest oxidation level possible for the isoflavanoids. Though they are occasionally found in heartwood[6], coumestans have been isolated from various leguminous plants (many of them occurring in roots)[20]. A number of the new analogues have been obtained from species of *Pueraria*[21]. Substitution patterns of the coumestans are very similar to those of the isoflavanoids[16]. The rare 7-oxygenation such as in repensol and trifoliol have been encountered[20].

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CHAPTER 3

STRUCTURE

3.1 Introduction

Chemical analyses of naturally occurring flavonoids include cleavage reactions i.e alkaline, oxidative and reductive cleavage reactions, while the derivatisation of functional groups are common during structural elucidation. The instrumental methods used constitute UV spectroscopy, IR, Mass and ^{1}H NMR spectrometry. Comparable UV spectra of flavonols and flavanones exhibit two major absorption peaks band I (328-385 nm) and band II (240-280)[22]. While Markham and Mabry[23] comprehensively discuss ^{1}H NMR spectroscopy of the flavonoids, Markham *et al.*[24] treat ^{13}C NMR in detail. Nuclear magnetic resonance (NMR) spectroscopic techniques continue to be the methods of choice for of structural elucidation[23,25].

Detailed data of flavonoids have been obtained from application of two-dimensional techniques such as homonuclear correlation spectroscopy (COSY), and nuclear Overhauser effect correlation spectroscopy (NOESY) which are typically used to determine the position *e.g.* of the prenyl functionality at C-3' of an isoflavone through observed interactions between the prenyl methylene protons and both H-2' and a 4' methoxyl groups. NOE association of the protons on the aromatic rings with the substituent groups, (to establish the oxygenation patterns) and the 2D heteronuclear correlation experiments [heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple-bond coherence (HMBC)] are now routine and provide structural data to a level that almost obviates the need for other information[26].

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3.2 Flavanones

Flavanones are easily and unequivocally characterised by the typical coupling patterns of their C-ring protons in their ^{1}H NMR[23] spectra which display the C-2 proton at δ5.2 (J_{cis} = 5.0 Hz and J_{trans} = 11.0 Hz) as quartets. The C-3 protons are each displayed as quartets between δ2.5 and 3.0. The tertiary and quaternary carbons are assigned with certainty *via* the ^{13}C NMR long-range coupling with deuterium isotopes[27]. A greater downfield shift of the hydrogen-bonded hydroxyl signal is observed in the 6-prenylated flavanones in comparison to those which are 8-prenylated[28].

3.3 Flavones

The characteristic sharp singlet of the vinylic 3-H near δ 6.3 ppm in the ^{1}H NMR[23] and ^{13}C NMR spectra [24,29] of flavones is used to characterise their structures.

3.4 Flavonols

Characterisation of the flavonols is based on the absence of the C-ring protons in their ^{1}H NMR spectra in conjunction with other spectroscopic methods *e.g.* ^{13}C NMR, UV and IR[23,24].

3.5 Isoflavonoids

The isoflavonoid structure is characteristically elucidated specifically on the chemical shifts of the 'H NMR singlet at ca δ8.0–8.5, which is attributed to H-2[23,24].

3.5 Coumestans

Coumestans are easily recognised in solution or on chromatograms from their intense bright-blue or violet fluorescence under UV light[21]. The structures of these compounds have been elucidated by UV, IR, ^{1}H NMR, NOE experiments and X-ray crystallography has been used for confirmation in some instances[30,31,32].

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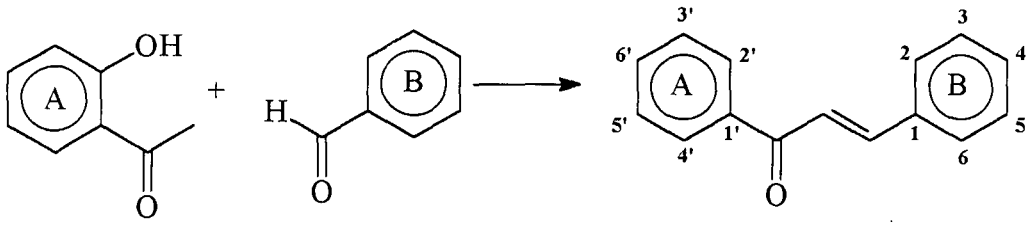
CHAPTER 4

SYNTHESIS

4.1 Introduction

The favoured synthetic route to the $C_6C_3C_6$ flavonoid structure in the laboratory is based on the condensation of a C_6C_2 unit **(6)** (2-hydroxyacetophenone) with a C_6C_1 unit **(7)** (a benzaldehyde) resulting in an intermediate (the chalcone) **(8)** [**Scheme (1)**][33].



(6) **(7)** **(8)**

Scheme 1

4.2 Flavanones, flavones, flavonols and isoflavones

Most frequently flavanones, which are isomeric with the chalcones, are obtained from the latter by acid- or alkali catalysed ring closure[34,35]. The flavones, on the other hand are synthesised from chalcones[36], by dehydrogenation of flavanones[37,38,39] as well as from simpler precursors by

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the condensation methods of Baker and Venkataraman[19] and Allan and Robinson[23,40]. Oxidative conversion of 2'-dihydroxychalcones into flavonols according to Algar, Flynn and Oyamada[41] is most frequently used in the preparation of flavonols. Although new synthetic routes for the synthesis of isoflavones such as the facile biomimetic one step conversion of flavanones into isoflavones by use of thallium trinitrate[42] and, the excellent yielding synthesis of isoflavones by palladium-catalysed cross-coupling reaction of bromochromones with arylboronic acids[43] are in use, more frequently isoflavone synthesis is achieved by the oxidative rearrangement of the chalcone skeleton[44,45].

4.3 Coumestans

Synthesis of coumestans has been conveniently achieved by methods including oxidation of pterocarpans by DDQ[46], oxidation of 2'-hydroxy-3-arylcoumarins[47], the coupling of 4-hydroxycoumarins with an *ortho*-quinone[48] and the novel direct transformation of 2,2'-dihydroxychalcones into coumestans[49].

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CHAPTER 5

GLYCOSIDES

5.1 Occurrence

5.1.1 Flavones and flavonol glycosides

The term 'glycosides' includes all flavonoids carrying sugar moieties and their acylated and sulphated derivatives. A range of at least 400 different flavone and flavonol glycosides has been reported with the most common flavonols, quercetin, kaempferol and myricetin each having over seventy glycosidic combinations while numerous derivatives of the two most common flavones, apigenin and luteolin[50], exist. 36 Glycosides of isoprenylated flavonols have been reported. Variations of flavones in which a glucosylated acylating acid is directly linked to the flavone for example apigenin 7-(2"-glucosyllactate)[51] as well as those with the acylating acid linked *via* a glycosidic residue [7-(6"-crotonyl glucoside)][52] have been isolated. Flavonol glycosides with all the hydroxyl groups of the glucose unit substituted by acyl groups change the solubility properties of the flavonol glycoside, converting it into a lipophilic substance[53]. Such glycosides, likely to occur in the cytoplasm or on the leaf surface have fungitoxic properties [54,55].

Rare glycosides for example 6,8-dimethoxyluteolin-3'-methyl ether (sudachiin D), linked to 3-hydroxy-3-methyl glutaric acid, through glucose units attached to the 7- and 4'- positions has been isolated from the green peel of *Citrus sidachi* [56]. Although flavones and flavonols become more lipophilic and less prone to glycosylation with increasing *O*-methylation, it is

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evident that polyhydroxylated flavones and flavonols occur as glycosides rather than in the free state[22].

5.1.2 Isoflavone glycosides

The number of known isoflavonoid glycosides are extremely small compared to the vast range of known flavonoid glycosides and isoflavone glycosides account for the majority of these. Though the number of *C*-glycosides known is increasing, the *O*-glycosides predominate. Of the *O*-glycosides, the majority are 7-glucosides or 7-rhamnosylglucosides, the 4'-glucosides and 4'-rhamnosylglucosides are much less frequently encountered. Both *O*- and *C*- glycosides are present in *Pueraria lobata* [57]. Pueraria glycoside-6 is an example of the di (8-*C*, 4-*O*)-glucosides of daidzein[58]. Acylated isoflavone glycosides with acetyl or malonyl and with p-coumaric and p-hydroxy benzoic acid as acyl groups[59] have been reported.

5.2 Structure

Structural variation among the flavanoid glycosides lie both in the nature of the sugar residue as well as the position of attachement *via* the hydroxyl groups to the flavone or flavonol aglycone. Due to the vast difference in the concentrations of these glycosides (ranging from 0.001% to 20%) of the plant dry weight, the minor consitituents often have to be ignored due to insufficient material for full identification.

Methods including paper[50], thin layer[50] and column chromatography[50,60] have been employed in the separation and purification of the glycosides. Although identification of glycosides has been successful via traditional chemical methods such as acid and enzyme hydrolysis[61], from R_f values and colour properties, selective methylation of phenolic hydroxyl groups and periodate oxidation[23], spectral methods including UV, IR, MS and NMR have played the major role in glycoside identification. UV spectral analysis is of primary importance in the determination of

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[58] Y. Ohshima, T. Okuyama, K. Takahashi, T. Takizawa and S. Shibata, *Planta Med.*, **1988,** *54*, 250
[59] V. K. Saxena and A. K. Jain, *Phytochemistry*, **1986,** *25*, 2686
[60] K. M. Johnston, D. J. Stern and A. C. Waiss, *J. Chromatog.* **1968**, *33*, 539
[61] C. W. Glennie and J. B. Harborne, *Phytochemistry*, **1971**, *10*, 1325

the position of substitution of the sugars in the flavanoid nucleus and, combined with IR,[62,63] are also criteria used when very small amounts of material are available.

Though separation and purification has been achieved *via* novel techiniques such as centrifugal partition chromatography (CPC) in conjuction with HPLC, flavonol glycosides are often purified by gel filtration on Sephadex LH 20, before spectral analysis. Hiermann[64] claims better results if Fractogel PGM 2000 is used instead of Sephadex with ethanol as eluent.

The advances in methods of separation e.g. the excellent resolution of closely related structures by HPLC and the wider use of proton and ^{13}C NMR spectroscopy for glycoside identification has led to the increase in the number of new glycoside reports. Mass spectrometry has played a fundamental role and continues to be explored as means of structural elucidation. While most researchers use fast atom bombardment mass spectrometry (FAB-MS) for the strong molecular-ion peak, which indicates clearly the number and type of sugar units present, Sakushima *et al*[65] have proposed desorption chemical ionisation mass spectrometry (DCI-MS) as an alternative for analysing the sugar moieties as well as the presence of 1→6 linked diglycosides such as robinobiosides, gentiobiosides and rutinosides. ^{1}H NMR spectroscopy has been applied to the structural analysis and is valuable for the flavone and flavonol glycosides in the elucidation of more complex derivatives[66] such as trimethyl silyl[60] and methyl ethers and acetals.

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[64]A. Hiermann, *J. Chromatogr*, **1986,** *362*, 152

[65]A. Sakushima, S. Nishibe and H. Brandenberger, *Biomed. Environ. Mass. Spectrom.*, **1989**, *18*, 809

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CHAPTER 6

SUGAR UNITS OF THE FLAVANOIDS

6.1 Introduction

Generally the identification of the monosaccharides are based on paper chromatography and gas chromatography of trimethylsilyl derivatives as well as ^{1}H and ^{13}C NMR spectroscopy. FAB-MS and ^{13}C NMR spectroscopy can be regarded as standard techniques for determining the linkages in the oligasaccharides[9].

6.2 Monosaccharides

Ten monosaccharides (**Table 1**) are commonly found in O-glycosidic combination with flavones and flavonols.

Table 1 Monosaccharides of Flavone and Flavonol glycosides

Pentoses	Hexoses	Uronic acids
D-Apiose	D-Allose	D-Galacturonic acid
L-Arabinose	D-Galactose	D-glucoronic acid
L-Rhamnose	D-Glucose	
D-Xylose	D-Mannose	

The monosaccharides are usually present in the expected pyranose form[67] although occasionally the less stable furanose forms have been reported[68]. Glucose, galactose,

[67]H. El. Khadam and Y. S. Mohammed, *J. Chem. Soc.*, **1958**, 3320
[68]Z. P. Pakudina and A. S. Sadykov, *Khim. Prir. Soedin*, **1970**, *6*, 27

glucuronic acid and xylose are D-sugars and are usually β-linked to the hydroxyl group of the aglycone while rhamnose and arabinose are L-sugars and are normally α-linked. α- And β-linked 3-arabinosides of quercetin have, however, been reported[69,70]. Both kaempferol 3-α- and 3-β- glucosides are present in flowers of *Alcea nudiflora*[71]. Apiose, a branched chain pentose, was first discovered in glycosidic form as apigenin 7-apiosylglucoside, apiin, being the only rare sugar associated with flavones.

6.3 Disaccharides

Harborne *et. al*[9] describes the combination of the disaccharide units as pentose-pentose, hexose-pentose, hexose-hexose, pentose-uroglucoronic acid and uroglucoronic acid-uroglucoronic acid. Of the disaccharides containing two different sugars, the most wide spread in plants is rutinose (6-*O*-α-L-rhamnosyl-D-glucose) *e.g* quercetin-3-rutinoside[52]. Although disaccharides with two rhamnoside residues such as rhamnetin (3-rhamnosyl-(1→4)-rhamnoside)[72] and 8-prenylkaempferol-4'-methylether-3-rhamnosyl-(1→3)-rhamnoside-7-glucoside[73] have long been identified, it is noteworthy to mention the disaccharide of the three newly identified isomers of rhamnosylrhamnose, *i.e* *O*-α-L-rhamnosyl-(1→2)-rhamnose, *O*-α-L-rhamnosyl-(1→3)-rhamnose and *O*-α-L-rhamnosyl-(1→4)-rhamnose.

6.4 Trisaccharides

The trisaccharides of flavones and flavonols have been characterised under two groups (linear and branched) mainly by FAB-MS and ^{13}C NMR spectroscopy. Linear trisaccharides are encountered more frequently than branched sugars. An example of the trisaccharides glucosyl (1→3)-rhamnosyl- (1→6)-glucose, has been found linked to the 3-position of both quercetin and kaempferol in the leaves of the tea plant Teacceace (*Camellia sinensis*)[74]. Among the novel branched trisaccharides are apiosyl-(1→2)-[rhamnosyl-(1→6)-galactose] linked to the 3-

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[70]V. I. Glyzin, A. I. Bankoviskii, *Khim. Prir. Soedin*, **1971,** *7*, 662
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position of kaempferol[75,76], and glucosyl-(1→6) -[apiosyl-(1→2)-glucose] attached to the 3-hydroxy of palutetin[77]. Other glucose combination are based on further glucose units, galactose and rhamnose[78,79,80]

6.5 Tetrasaccharides.

Although no linear tetrasaccharides have been reported so far, a branched tetrasaccharide, rhamnosyl-(1→4)-glucosyl-(1→6)saphorase, has been found attached to the 7-hydroxy of tacocetin and acetylated at the 6'''-position of the saphorase[81]. Characterisation was based on UV and ^{1}H NMR analyses, following acid hydrolysis to yield the sugar moiety, and the sugar linkage determined by ^{13}C NMR measurements.

6.6 Acylated derivatives

Both flavone and flavonol glycosides occur in acylated form with acids such as *p*-coumaric[82], caffeic[83], sinapic[84], ferulic[85], gallic[86], benzoic[87], acetic[88] and malonic[89] acid, with the *p*-coumaric[82] and ferulic acids[85] occurring most frequently. Novel acylated derivatives (42 flavones and 99 flavonols) have been reported in literature between 1986 and 1991[9]. However, most new reports concern acetic acid as acylating agent of the sugar units (16 flavones and 44 new flavonol derivatives)[9]. As a result of successful application of FAB-MS and ^{13}C NMR techniques, the difficulties encountered with the traditional PC and TLC procedures to detect the acetic acid which is volatile and the acetyl groups which are labile by

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mild acid hydrolysis has been overcome. Hence, new acylated flavanoids for example a tri-acetate, kaempferol-3-(2''', 3''', 5'''-triacetyl) arabinofuranosyl-(1→6)- glucoside, from flowers of *Calluna vulgaris* (Ericaceae)[90] and two tetra-acylated glucosides of kaempferol, which have two acetyl and two *p*-coumaroyl substituents on the same glucose residue[55], have been characterised.

Five flavones and two flavonol malonate derivatives have been identified, including the 5-(6''-malonylglucosides) of apigenin, genkwanin and luteolin[91] and kaempferol-3-apiosylmalonyl glucoside[92].

6.7 Sulphate conjugates

The number of known flavone and flavonol sulphate conjugates approximately numbers 80[63]. Among the known flavone sulphate conjugates are the 6-hydroxyluteolin and the 6,7-disulphates of 6-hydroxy luteolin and nodiflorentin[93] while 3'-Sulphate and 3-glucoronide-3'-sulphate quercetin [94] and the 3,3'-disulphates of quercetin and patuletin[95] are examples of flavanols. Confirmation of 23 structures have been accomplished by synthesis[96]. The new method using N, N'-dicyclohexyl-carbodiimide (DCC) and tetrabutylammonium hydrogensulphate (TBAHS) in dimethylformamide has allowed the synthesis of specifically sulphated flavanoids in good yield.

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[91]M. Viet, H. Greiger, F. -C. Czygan and K. R. Markham, *Phytochemistry*, **1990**, *29,* 2555
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[93]F. A. Thomäs-Barberän, J. B. Harborne and R. Self, *Phytochemistry,* **1987,** *26*, 2281
[94]R. M. Seabra and C. Elves, *Phytochemisrty,* **1991,** *30*, 1344
[95]D. Barron and R. K. Ibrahim *Phytochemistry,* **1987,** *26,* 1181
[96]D. Barron and R. K. Ibrahim *Phytochemistry,* **1988,** *27,* 2362

CHAPTER 7

BIOLOGICAL SIGNIFICANCE

7.1 Introduction

Flavanoids exhibit important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors, precursors of toxic substances, pigments and light screens[97,98] and are actively involved in photosensitization and energy transfer. Numerous physiological activities have been attributed to flavanoids which have been recognised to possess antiallergic, anti-inflammatory, antiviral, anti proliferative and anticarcinoganic activities as well as the ability to affect some aspects of mammalian metabolism[9]. They possess important enzyme-inducing activities for a large variety of enzymes, free-radical scavenging activity, the potential to chelate certain metal cations, antioxidant properties and, notably affect cellular protein phosphorylation[9].

7.2 Flavonones

Interesting physiological properties have been attributed to both flavanones and flavones. Of historical importance is the observation of the vitamin-like activity of citrin[9], a mixture of eriodictyol and hesperidin. Many reports including antimicrobial[99], antiviral[100], anti-inflammatory[90], spasmolytic, and enzyme inhibiting actions[93] of various flavones and flavanones exist. Highly hydroxylated flavones act as diuretics[91]. The remarkable efficacy of these highly hydroxylated flavones in acting as antioxidants[101] is now well established. Since these

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[98] J. W. McClure, in *Plant Flavonoid in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships* **1986,** (eds. V. Cody, E. Middleton and J. B. Harborne), Alan R. Liss, New York, pp.77-85

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[100] Y. Inouye. K. Yamaguchi. Y. Take and S. Nakamura, *J. Antibiot.*, **1989,** *XL11,* 1523

[101] S. N. Onyeneho and N. S. Hittiarachy, *J. Agric. Food Chem.*, **1992,** *40,* 1496

compounds are found in fruits, vegetables, tea, wine, and seeds, they are important constituents of the human diet[9].

7.3 Isoflavones

Isoflavonoids are important because of their physiological activity in humans. The polyhydroxy isoflavonoids have been investigated and anticancer activities[102,103], as well as oestrogenic activities have been established[104]. Some of the isoflavanoids have been found to have strong antimicrobial activities[105,106]. One of the characteristic features is their ability to act as phytoalexins which play a key role in defence against fungal infection[107]. The presence of a methoxy group plays a decisive role in conferring such activity[108]. Thus, these substances may be an alternative to conventional fungicides in the control of fungi which cause great losses in the storage of grain.

7.4 Flavonols

Kaempferol and quercetin have been found to act competitively with hyaluronidases, enzymes possibly involved in tumor cell invasiveness and, hence display antitumor activity[109]. Besides having anticancer[110], antioxidant[9], antiallergic[9], antiviral[111], antitoxic[112] and antihistamine[113] activities, flavonols inhibit the production of lipid peroxidation and oxyradical production which are implicated in pathological conditions such as ageing, atherioscelerosis, anti-

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[102] H. Adlercreutz, T. Fotsis, C. Bannwart, K. Mäkelä, K. Wähälä, G. Brunow and T. Hase, *J. Steroid Biochem.*, **1986**, *25*, 791

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[108] J. B. Harborne and R. J. Grayer, in *The Flavonoids: Advances in Research since 1986.* (ed. J. B. Harborne), Chapman and Hall, London, **1993**, 589

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[113] W. Sieghart, T. C. Theoharides, W. W. Douglas and P. Greengard, *Biochem. Pharmacol.*, **1981**, *30*, 2737

inflammatory, hepatotoxicity and iron toxicity[114,115]. Among other physiological properties, flavonols also have the ability to inhibit platelet aggregation, adhesion and secretion[116].

7.5 Glycosides

Although their aglycones have been recognised to possess important physiological and pharmacological properties, not much information is available on glycosylated flavanoids. However, the active consitituents are usually the *O*-glycosides rather than the aglycones. In general glycosides have not been allocated any specific role in human metabolism. To some extent, their role in plants has been investigated and several interesting properties have been reported. Among the reported properties, are their ability to act as insect feeding stimulants, insect feeding deterrents and signalling the availability of the flower for pollination[117]. Quercetin glycosides in the leaves makes them resistant against defoliation and isoquercetin (quercetin-3-*O*-glucoside) is used by the silkworm in the selection of its food plant[118].

[114] J. Torel, J. Gigillard and P. Gillard, *Phytochemistry*, **1986,** *25,* 383
[115] A. K. Ratty and N. P. Das, *Biochem. Med. Metab. Biol.*, **1988,** *39*, 69
[116] R. J. Grygleqski, R. Korbut, J. robak and J. Swies, *Biochem. Pharmacol.*, **1987**, *36*, 317
[117] W. R. Thompson, J. Meinwald, D. Aneshansly and T. Eisner *Science*, **1972,** *171,* 528
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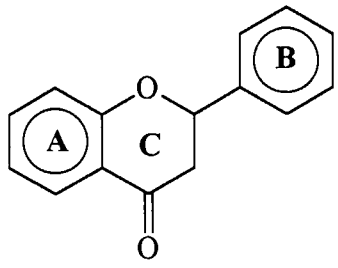
CHAPTER 8

BIOSYNTHESIS

8.1 Flavonones, flavones, flavonols and isoflavonoids

Means for comprehensive studies at molecular level for the flavonoid formation and regulation have been improved as a result of improved protein purification procedures, successful preparation of antibodies, and rapid isolation of flavonoid-specific genes[119]. All the flavonoids derive their carbon skeletons from two basic compounds, malonyl-CoA (coenzyme A) and 4-Coumaroyl-CoA[120,121]. The steps leading to the flavonoid precursors and various flavanoid classes with their respective enzymes are outlined in **Scheme 2** which illustrates the general structural relationship of different types of compounds in the biosynthesis of flavonoids. The flavonoid $C_6.C_3.C_6$ (**9**) carbon back bone consists of the A-, B- and C-rings.



(9)

While the B-ring and part of the heterocyclic ring of the flavonoid skeleton are provided most frequently by 4-coumaroyl-CoA, the A-ring originates from three acetate units *via* malonyl CoA (from acetyl -CoA and CO_2). Chalcones (**13**), the immediate precursors for all flavonoid compounds and the key step to the flavonoid biosynthesis result from the chalcone synthetase (**i**) catalysed condensation of three acetate units from malonyl-CoA with 4-coumaroyl CoA.

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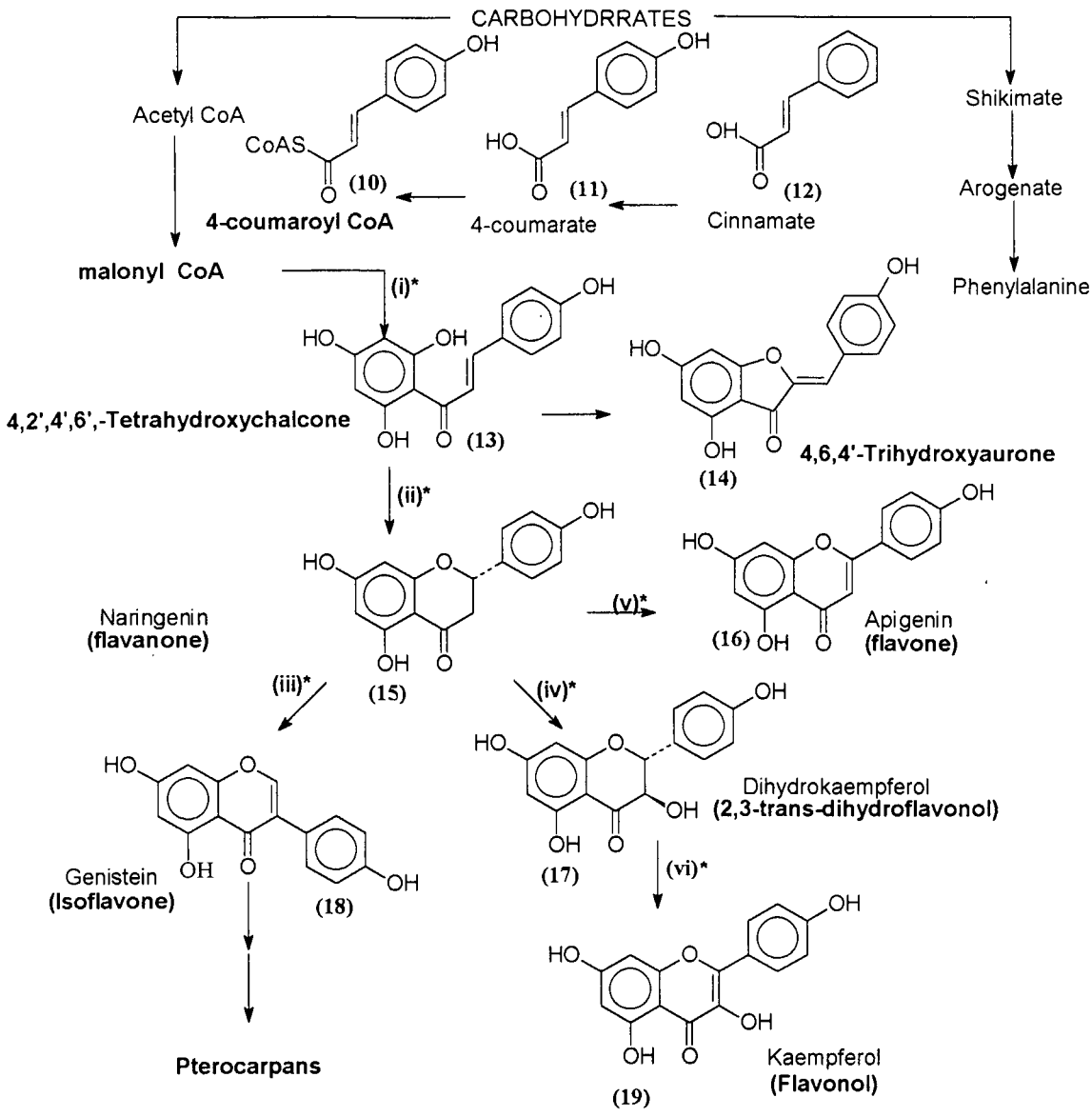
[119] W. Heller and G. Forkmann, in *The Flavonoids:, Advances in Research 1980,* (eds. J. B. Harborne), Chapman and Hall, London, **1988,** 399

[120] J. D. Bu'Lock, *The Biosynthesis of Natural Products; An Introduction to Secondary Metabolism.* McGraw-Hill, London, **1965,** 13

[121] U. Weiss and J. M. Edwards, *The Biosynthesis of Aromatic Compounds.* John Wiley & Sons, New York, **1980,** 326



Scheme 2

*** Enzyme systems:**

(I)- Chalcone synthase

(ii)- Chalcone isomerase

(iii)- 2-Hydroxyisoflavanone synthase

(iv)- (2*S*)-Flavanone 3-hydroxylase

(v)- Flavone synthase

(vi)-Flavonol synthase

Chalcones with phloroglucinol-type substitution in the A-ring are exclusive intermediates in the formation of 5,7-dihydroxyflavanoids, while those with a resorcinol-type A-ring are selectively converted into 7-hydroxy flavanoids[122].

The stereo-specific cyclization of the chalcone, catalysed by chalcone isomerase (**ii**), provides a 2*S*-flavanone [*e.g.*naringenin(**15**)]. Flavanones are the direct precursors for the large class of flavanoids, the flavones [*e.g.*apigenin(**16**)].

The flavones are synthesised from flavanones by introduction of a double bond between C-2 and C-3 with the enzyme flavone synthase (**v**).

Formation of isoflavones [*e.g.* genistein (**18**)] from flavanones, catalysed by 2-hydroxyisoflavone synthetase (**iii**) involves an oxidative rearrangement of the flavanone, comprising a shift of the aryl ring from position 2 to 3.

(2*S*)-flavanone 3-hydroxylase (**iv**) catalyses the formation of dihydroflavonols [*e.g.* dihydrokaempferol (**17**)].

Introduction of a double bond between C-2 and C-3, catalysed by flavonol synthetase (**vi**) gives the flavonols [*e.g.* kaempferol (**19**)].

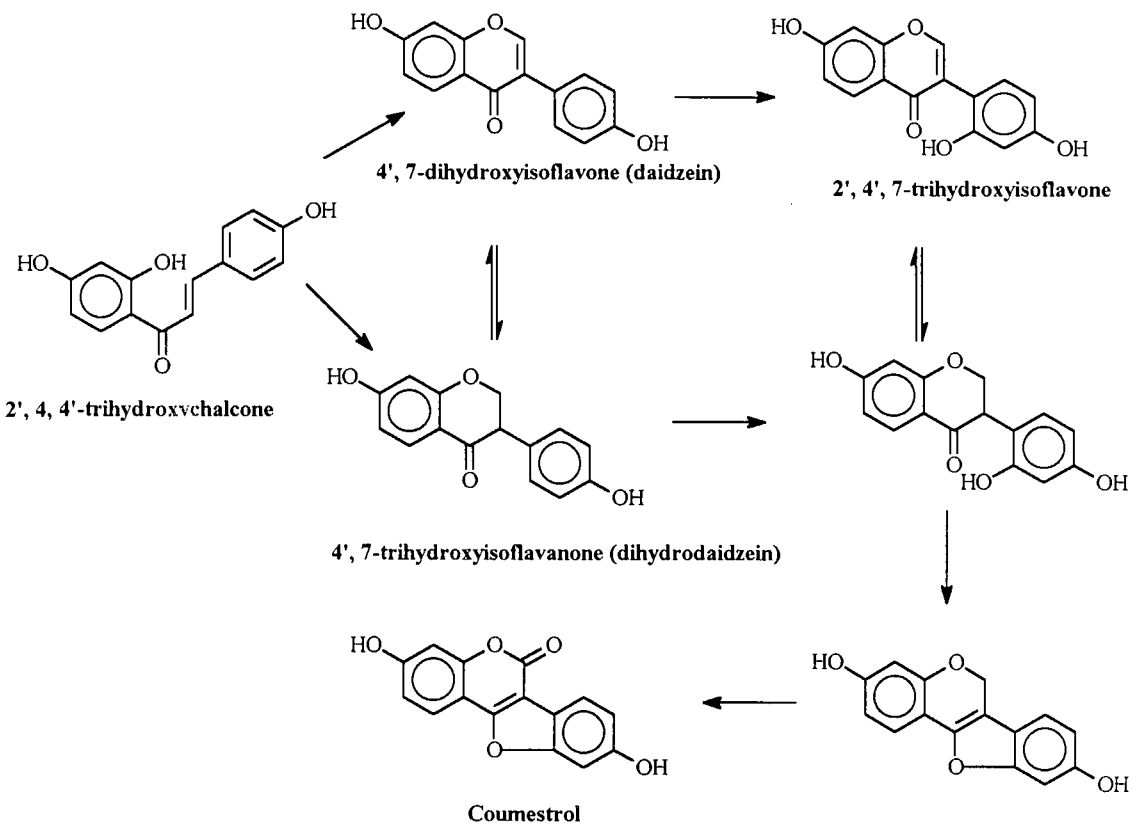
While the 5-deoxyisoflavone pathway is well established, the pathway for the dihydroflavonols and flavonols lacking a 5-hydroxy function still has to be demonstrated.

8.2 Coumestans

The coumestans are unique in that they have a benzopyrone nucleus derived in part *via* the shikimate-chorismate pathway like the A-ring in flavonoids, and in part *via* the polyketide pathway[123,124,125], where acetate and cinnamic acid[123] are precursors. Biosynthetically the

[122]K. Hahlbrock and H. Grisebach, in *The Flavonoids*, (eds. J. B. Harborne, J. B. Mabry and H. Mabry), Chapman and Hall, London, **1975,** 876
[123] H. Grisebach and W. Barz, *Chem. Ind.* (London), **1963,** 690
[124] H. Zilg and H. Grisebach, *Phytochemistry*, **1968**, *7*, 1765

isoflavonoid framework is transformed into coumestans *via* a series of oxidation's as is depicted in **Scheme 3**.



Scheme 3

8.3 Glycosylation

The abundance of flavonoid glycosides found in nature suggests the occurrence of a great range of glycosyltransferases[126] with varying substrate specificities. Indeed, many well-known glycosyltransferases[126,127], have been characterised. Glycosylation is a necessary flavonoid modification that increases their water solubility[119], and it may also serve as a recognition signal for transmembrane metabolite transport. The reaction is also a prerequisite for acylation of the carbohydrate moiety with carboxylic acids.

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[125] R. D.H. Murray, J. Méndez and S. A. Brown, *The Natural Coumarins: Occurrence, Chemistry and Biochemistry,* **1982,** 179

[126] W. Hösel, in *The Biochemistry of Plants* (eds. P. K. Stumpf and E. E. Conn), Academic Press, New York, **1981**, *7*, 725

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Among the characterised glucosyltransferases are flavonoid 7-*O*-glucosyltransferases (FGT) which are specific for glucosylating flavonoids hydroxy groups in position 7[119], and glucosyltransferases specific for 2'. Isoflavone 4'-*O*-glucosyltransferases was characterised from *Prunus* x *yedoensis* leaves[119,128]

[128]N. Ishikura and K. Yamamoto, *Plant Cell Physiol*, **1990**, *31*, 1109

CHAPTER 9

TANNINS

9.1 Introduction

Owing to the complexity of the material handled, much of the accumulated knowledge regarding tannin behaviour during penetration of hide or skin (collagen fibers) is based on empiricism[129]. Today the composition of many plant extracts can usually be adequately defined in terms of their polyphenolic (tannin) and simple phenolic consitituents[129].

9.2 Properties and Characterisation

Polyphenols are secondary metabolites widely distributed in various sectors of the higher plant kingdom. Characterisation of plant polyphenols is based upon three structural themes namely, the condensed tannins (proanthocyanidins)[130,131,132], hydrolysable tannins[133,134] (gallotannins and ellagitannins) and complex tannins[134,135] which are the most recently found tannins in plants.

Condensed tannins are now more properly referred to as proanthocyanidins, which possess the general polymeric flavan-3-ol structure in which the interflavanyl bonds are most commonly C-4 to C-8, but in some cases may be C-4 to C-6, *e.g* **(20)**, and some branching may occur in the polymer chain. The proanthocyanidins are, therefore, irregular polymers[135]. A particularly interesting example of irregular polymers, termed "angular' proanthocyanidins ***e.g*** **(21)**, occurs in the Leguminoseae and Anacardiacaea where catechin and gallocatechin commonly represent terminal units of the chains, and fisetinidol or robinetinidol constitute extender units

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[129] D. G. Roux in *Plant Polyphenols - Synthesis, Properties, Significance* (eds.R. W. Hemingway and P. E. Laks), Plenum Press, New York **1992**, 7

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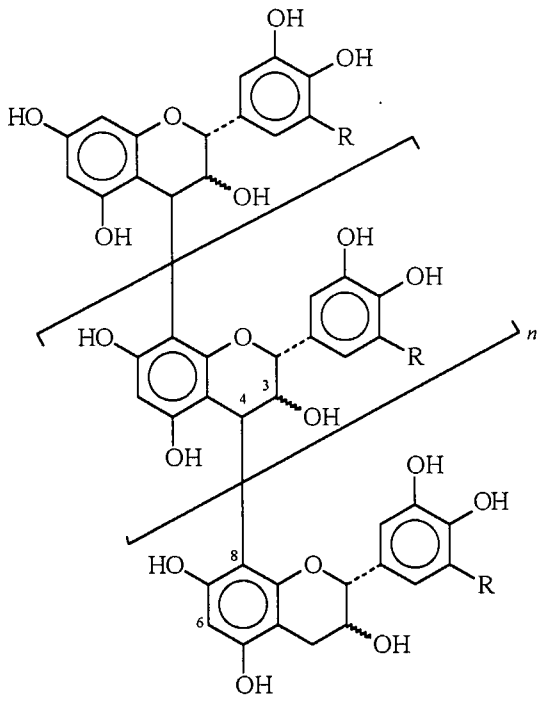
[135] L. J. Porter, in *Plant Polyphenols - Synthesis, Properties, Significance* (eds. R. W. Hemingway and P. E. Laks), Plenum Press, New York **1992**, 245



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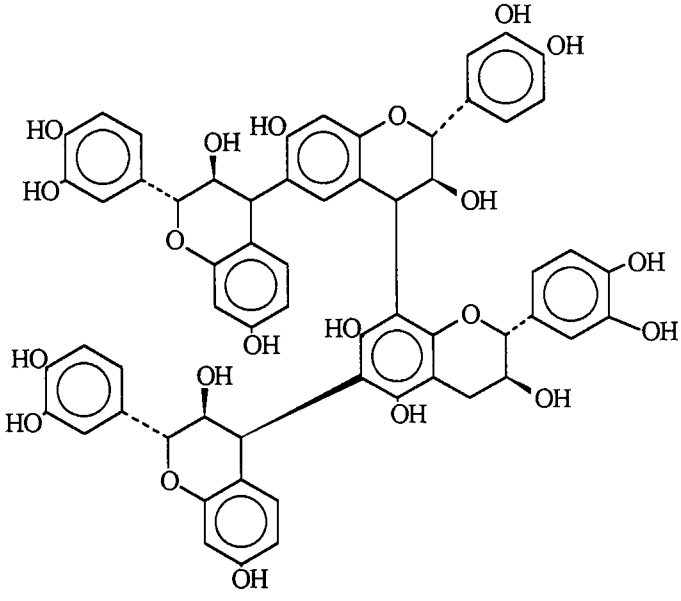

(21)

Hydrolyzable tannins are generally glucose esters of gallic acid **(22)** and derivatives such as ellagic acid **(23)** or more precisely hexahydroxy diphenic acid (HHDP) acid **(24)**. Other more highly condensed structures such as flavogallonic acid **(25)** and valoneic acid derivatives **(26)** are also known. The hydrolysable tannins are often subdivided according to the type of polyphenolic acids which are produced on hydrolysis.

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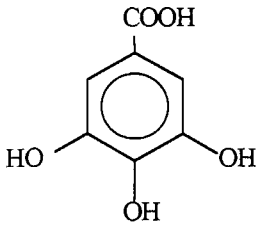

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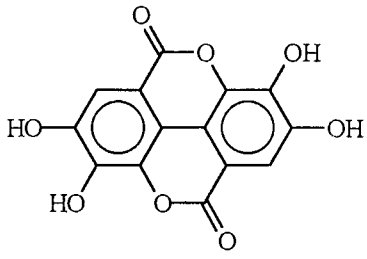

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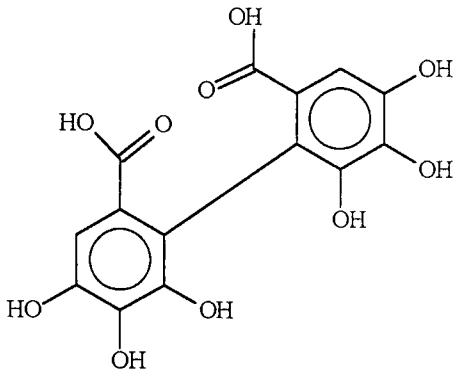

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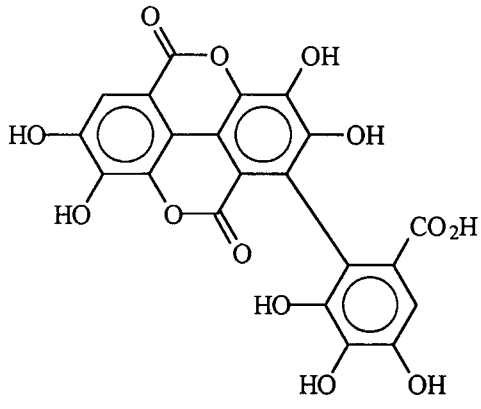

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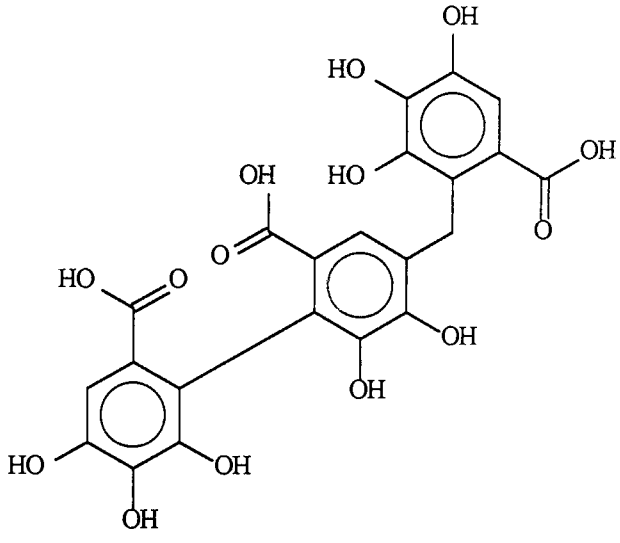
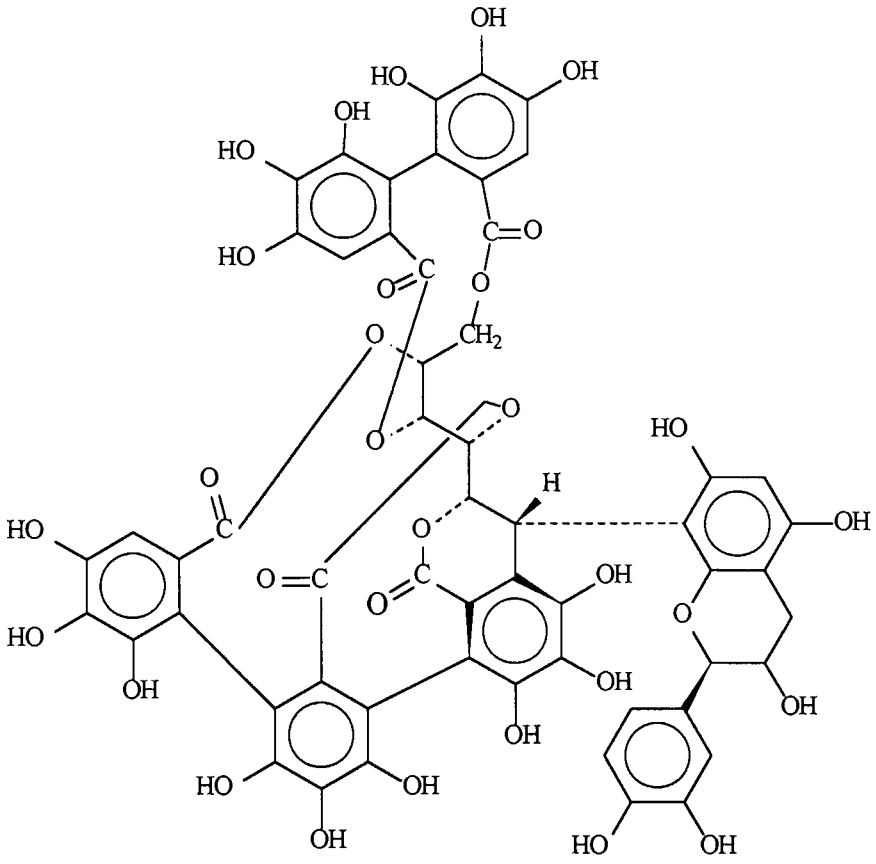


(26)

The most recently found tannins, referred to as complex tannins possess both condensed and hydrolyzable tannins moieties linked *via* a carbon-carbon bond. An example of this kind is acutissim A **(27)** which was isolated from *Castanca crenate*[134].



(27)

9.3 Isolation and Structure

Substantial progress in the isolation has been made possible by application of new techniques of isolation and analysis *e.g.* MPLC and HPLC using Sephadex gels and Mitsubishi MCI gel CHP-20P[130,135].

Fast atom bombardment Mass Spectrometry (FAB-MS) is necessary to obtain high-quality mass spectra of molecules which are polar, thermally unstable and of relatively high molecular weight *e.g* polyphenols and polysaccharides[136,137]. Nuclear magnetic resonance spectroscopic methods, ^{1}H and ^{13}C-NMR[136,137,138,139], provide the most basic information needed for the

[136] R. Self, *Biomed. Mass, Spec.*, **1986**, *13*, 449
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[138] L. J. Porter in *The Flavonoids: Advances in Research*, (ed. J. B. Harborne), Chapman and Hall, London, **1988**, 21-62
[139] W. L. Mattice and L. J. Porter, *Pytochemistry*, **1983**, *22*, 569

detailed elucidation of the structure of tannins[140,141]. Two-dimensional NMR experiments *e.g* COSY and NOESY provide for a complete structural analysis for many hydrolyzable tannins having either a D-glucopyranose core or C-glucosidic structures with the glucose residue in an open chair form[142].

9.4 Biological significance

The biological action of tannins needs to be considered not only on astrigency alone but in terms of their molecular structure. Various phenolic compounds have been isolated from a variety of medicinal plants. Notable biological and pharmacological activities have been found for several of these polyphenolic compounds[143]. The free radical -scavenging activity of tannins is an important property underlying their various biological and pharmacological activities[15,144], *e.g* protective effects on oxidative damage in the ocular lens[143,145], anti-hepatotoxic activity[146] and suppression of the super oxide anionic radical and the 1,1-diphenyl-2-picrylhydrazyl radical[143,147]. The antitumor promoting activity of (-)-epigallocatechin gallate (EGCG) on skin cancer and on duodenal cancer[148] is another important activity. Native Americans have used polyphenols for purposes such as reducing sinus swelling[151], colds, sore and swollen throat[151], diabetes[151], headache[151], and as a laxative[149]. Antiviral activities include the treatment of viral infections such as HIV[150], herpes simplex[151] and influenza[152].

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[140] J. P. Steynberg, J. F. W. Burger, D. A. Young, E. V. Brandt, J. A. Steenkamp and D. Ferreira, *J. Chem. Soc., Perkins Trans. 1*, **1989**, 671

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[150] H. Nakane and K. Ono, *Biochemistry*, **1990**, *29*, 2841

[151] C. J. M. Kane, J. H. Menna, C.-C. Sung and Y.-C. Yeh, *Bioscience Reports*, **1988,** *8*, 95

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CHAPTER 10

THE SUPEROXIDE ($O_2^{\bullet -}$): 'AN ACTIVE OXYGEN SPECIE'

10.1 Introduction

The importance of oxygen in sustaining life forms has been the subject of intensive studies by biologists and chemists. Recent evidence indicates that the superoxide anion is present in all aerobic organisms and is believed to be involved in several enzyme oxidation processes[153]. Superoxide can result from the reduction of molecular oxygen [**equation (1)**], from oxidation of peroxide [**equation (2)**] as well as the reduction of oxygen to water [**equation (3)**] which requires the transfer of four electrons and involves several intermediates including the hydroperoxy radical ($HO_2^{\bullet}$) or its ionised form, the super oxide species ($O_2^{\bullet -}$). Although both the superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide are relatively stable, their reactivity manifests in the ability to form the highly reactive hydroxy radical (·OH). This radical reacts with biological material and causes the so called oxygen poisoning[154].

$$O_2 + e \rightarrow O_2^{\bullet -}$$

EQUATION (1)

$$2O^{2-} \rightarrow 2O_2^{\bullet -} + 2e$$

EQUATION (2)

$$O_2 \xrightarrow[+H^+]{+e'} HO_2\cdot \xrightarrow[+H^+]{+e'} H_2O_2 \xrightarrow[+H^+, -H_2O]{+e'} HO\cdot \xrightarrow[+H^+]{+e'} H_2O$$

EQUATION 3

[153] I. Fridovich, *Adv. Enzymol.*, **1974**, *41*, 35
[154] M. Namiki, *Rev. Food Sci. Nutr.*, **1990**, *29*, 273

Normally, reactions of oxygen with organic compounds are in general highly exothermic, however, such reactions are usually slow at physiological temperatures. This is due to the fact that the electron spins of oxygen (triplet) and most stable organic substrates (singlet) are incongruent, hence oxygen does not behave as a random oxidising agent capable of destroying complex organic material[155]. However, a number of interesting mechanisms and intermediates for the interaction of molecular oxygen with specific biological substrates have been suggested[156]. The involvement of the superoxide ion ($O_2^{\bullet -}$) in biological oxidations has been investigated by studying its reactions with simple substrates with functionalities similar to those encountered in the more complex living systems[131].

10.1 Effects of superoxides on human life

Although molecular oxygen is essential for life, its normal metabolism results in the formation of free radicals, *e.g.* superoxide and peroxides as well as singlet oxygen that are detrimental to human health[157,158]. The highly reactive hydroxyl radical which is formed from superoxide and hydrogen peroxide is believed to indiscriminately attack the lipids, proteins and DNA, manifesting in lipid peroxidation, protein denaturation and DNA mutation[159]. Singlet oxygen that is formed in the lens and retina of the mammalian eye can attack lipids to cause lipid peroxidation.

As a result of age, life style, fat intake, smoking, alcohol intake, infections, pollution, occupation etc. that influence oxidative stress levels, superoxides are produced in abnormally high concentrations, and this is believed to be a contributing factor in a broad spectrum of diseases including atheriosclerosis, apoplexy, inflammatory diseases such as arthritis, heart disease, Alzheimer's disease and various cancers.

10.3 Quenching of the 'active oxygen species'

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[155]E. Lee-Ruff, *Chem. Soc. Rev.*, **1977**, *6*, 195
[156]G. A. Hamilton in 'Chemical Models and Mechanisms for Oxygens in Molecular Machanisms of Oxygen Activation', ed. O. Hayaishi, Academic Press, New York, 1975, 405
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[158]B. Halliwell, M. A. Murcia, S. Chirico and O. O. Auroma, *Crit. Rev. Food Sci. Nutr.*, **1995**, *7*, 35
[159]B. Halliwell, *FASEB J.* **1987**, *1*, 358

The natural way the human body is protected against 'active oxygen species' is through superoxide dismutase (SOD), an 'enzyme' that is capable of quenching an excess of superoxides [**equation (4)**]. In the past, research on antioxidants was focused on β-carotene, vitamin C and vitamin E. However, scientists are progressively beginning to realise the potential of flavonoids as antioxidants and the effect they have on biological systems and more specifically on human life.

$$2\,O_2 \xrightarrow{SOD} H_2O_2 + O_2$$

EQUATION (4)

The flavonoids act as potent primary antioxidants[160] with the ability to scavenge superoxides[161], hydroxyl[162] and peroxyl-radical[163]. Flavanoids also display secondary antioxidant activity due to their metal chelating ability[164] and quenching of singlet oxygen[165].

Vitamin E (**28**), one of the best quenchers of singlet oxygen ($^1O_2$)[166], also reacts with superoxide ($O_2^{\bullet -}$), while vitamin C (ascorbic acid) (**29**) is not only capable of reducing two equivalents of superoxide ($\mathbf{O_2^{\bullet -}}$), but can also quench both peroxy- ($HO_2\cdot$) and hydroxyl ($\cdot OH$) radicals as well as singlet oxygen[130].

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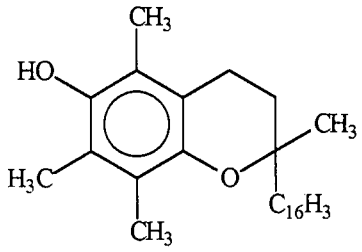
[160]D. D. Pratt and B. J. F. Hudson, *in Food Antioxidants,* ed. B. J. F. Hudson, Elseveir Applied Science, London, **1990**, 17.1
[161]H. Ogawaram, T. Akiyama, S. Watanable, N. Ito, M. Kobori and Y. Seoda, *J. Antibiot,* **1989**, *XL11*, 340
[162]S. R. Husain, J. Cillard and P. Cillard, *Phytochemistry,* **1987**, *26*, 2489
[163]J. Torel, J. Cillard and P. Cillard, *Phytochemistry,* **1986**, *25*, 383
[164]B. J. F. Hudson and J. I. Lewis, *Food Chem,* **1983**, *10,* 47
[165]Y. Sorata, U. Takahama and M. Kimura, *Biochim. Biophys. Acta,* **1984**, 313
[166]M. B. Korycka-Dahl and T. Richardson, *Crit. Rev. in Food Sci. and Nutr.,* **1978,** *10,* 209



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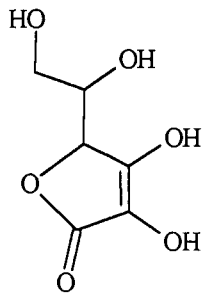

(29)

10.4 Reactivity of superoxide

10.4.1 $O_2^{\bullet-}$ as an oxdising agent

In view of the resemblance between the action of oxygenases (enzymes known to catalyse the activation of molecular oxygen and its subsquent fixation in various substrates) and photosensitized oxygenations, the photosensitized oxygenation of quercetin, a 3- hydroxyflavonol, was investigated[167]. The discovery of new superoxide reagents and the preparation of stable solutions of $O_2^{\bullet-}$ in aprotic organic solvents have made such investigations relatively easy[155]. The results (**Scheme 4**) indicated a decarboxylation giving the depside (**35**) as a major product in the photosensitized oxygenation which apparently proceeds *via* one of the two peroxides (**32** or **33**) followed by cleavage of the C-ring[167]. The depside (**35**) is then hydrolyzed to the acids (**40**) and (**41**) indicated in **Scheme 5**. A similar depside is also a product in the microbiological oxidation[168]. Similarly other 3-hydroxyflavones behaved as expected from the quercetin model. However, photosensitized oxygenation of the 3-methoxyflavones proceeded very slowly and, if persued for a long time, induced ring closure giving rise to compounds of type (**36**) and (**37**)[169]. The results clearly indicated that the presence of the 3-hydroxyl group is a prerequisite for the formation of the formation of the depside (**34**).

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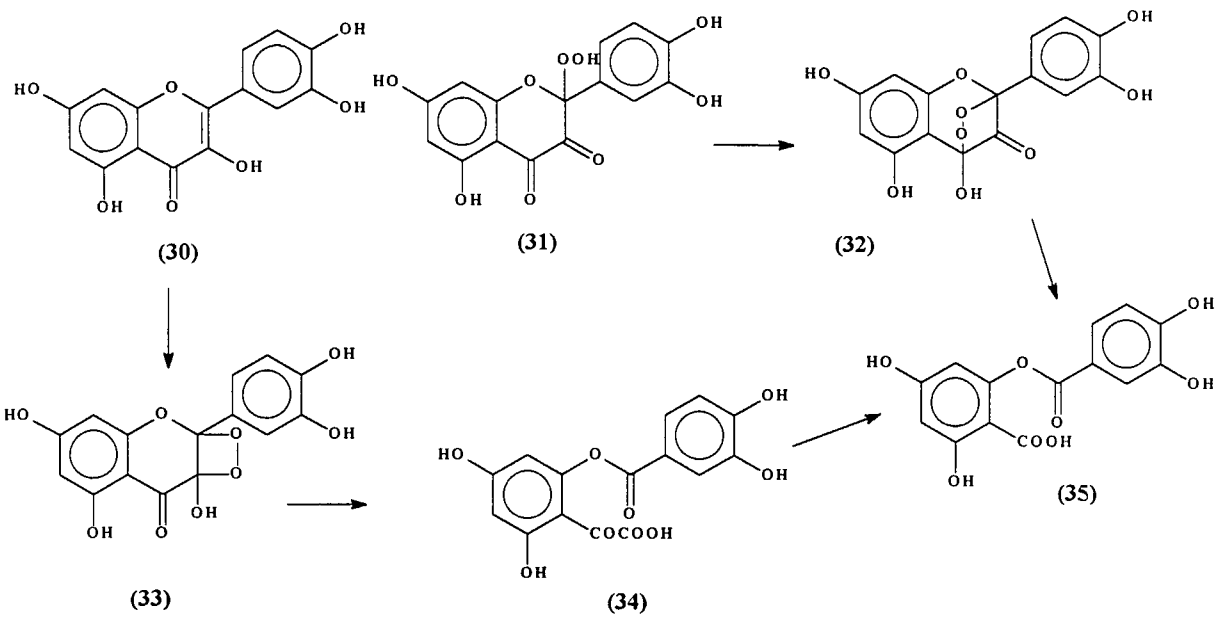
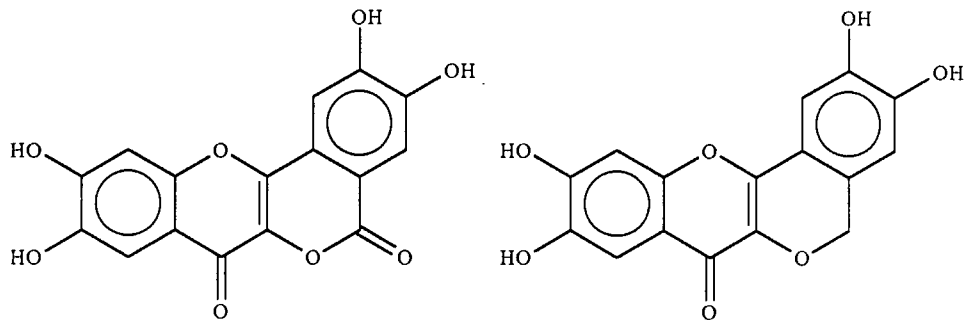
[167]T. Matsuura, H. Matsushima and R. Nakashima, *Tetrahedron,* **1970**, *26,* 435
[168]T. Oka, F. J. Simpson, J. J. Child and C. Mills, *Can. J. Microbiol.*, **1971,** *17,* 111
[169]T. Matsuura andH. Matsushima, *Tetrahedron,* **1968**, *24,* 6615



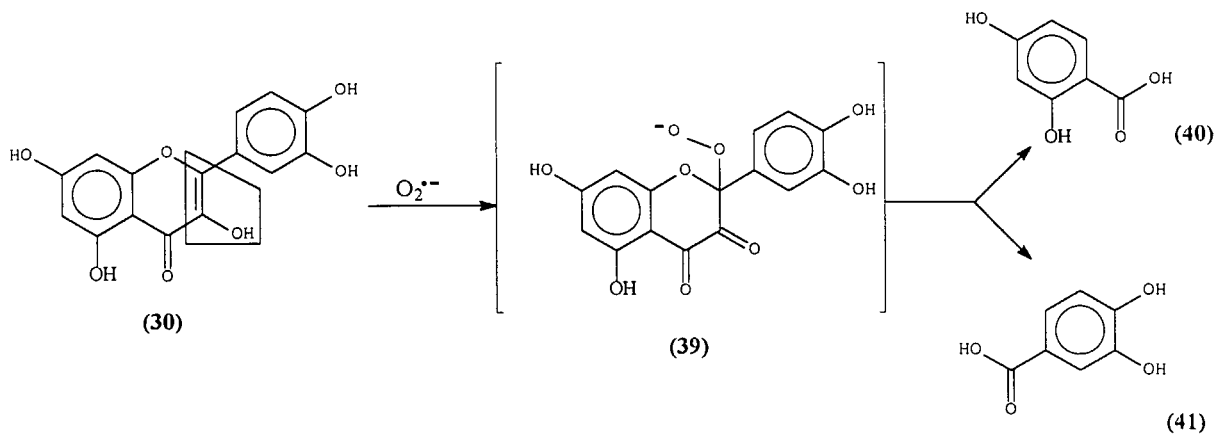
Scheme 4



(36) **(37)**

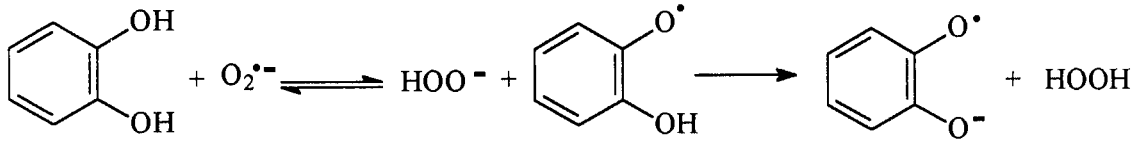
Recently, it was demonstrated[170] that flavonols, *e.g.* quercetin (**30**), with the enol functionality of the C-ring boxed in structure (**38**) are oxidised by superoxide ($O_2^{\bullet -}$), (the $O_2^{\bullet -}$ was generated from potassium superoxide, KO_2) in heterogenous aprotic media to the carboxylic acids (**40**) and (**41**) *via* the peroxy intermediate (**39**) in **Scheme 5.**

[170]C. Tournaire, M. Hocquaux, I. Beck, E. Oliveros and M-T. Mauretta, *Tetrahedron*, **1994**, *50*, 9303



Scheme 5

On the other hand, the aromatic ring of dihydric phenols has been cleaved by photo-oxidation[171], as examples providing models for the enzymatic cleavage of the aromatic ring, as well as with oxygenase enzyme in the presence of molecular oxygen[172,173] to give a dicarboxylic acid[174]. The oxidising potential of the superoxide displayed when the anion abstracts a proton from the diphenols to produce semiquinones has been reported for a number of catechols **Scheme 6** [175,176,177], hydroquinones[151,178], and ene-1,2-diols such as ascorbic acid (**29**) [179].



(42) **(43)** **(44)**

Scheme 6

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[171] T. Matsuura, A. Nishinaga, N. Yoshimura, T. Azarai, K. Omura, H. Matsushima, S. Kato and I. Saito, *Tetrahedron*, **1969**, *21*, 1673

[172]K. Block, O. Hayaishi, "Biological and Chemical aspects of Oxygenases", **1966,** Maruzen, Tokyo

[173]O. Hayaishi, ed., "Oxygenases", **1962,** Academic Press, New York

[174]J. E. Baldwin, H. H. Basson, H. Krauss, *Chem. Comm.*, **1968**, 984

[175]E. Lee-Ruff, A. B. P. Lever and J. Rigaudy, *Canad. J. Chem.*, **1976,** *54*, 1873

[176]R. W. Miller and. U. Rapp, *J. Biol. Chem.*, **1973,** *248*, 6084

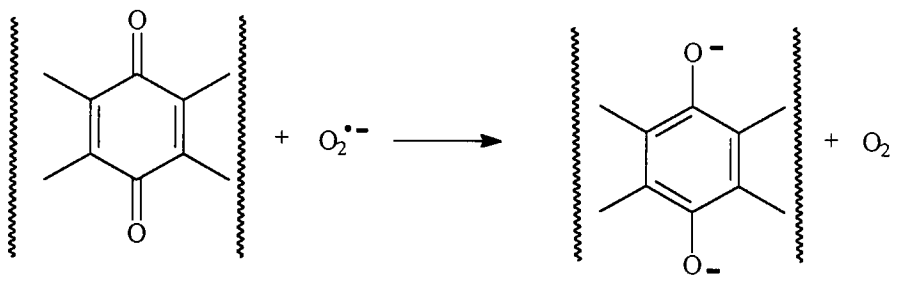
[177]Y. Moro-Oka and C. S. Foote, *J. Amer. Chem. Soc.*, **1976,** *96*, 1510

[178]P. S. Rao and E. Hayon, *J. Phys. hem.*, **1973,** *77*, 2274

[179]M. Nishikimi, *J. Biochem. Biophys. Res. Comm.*, **1975**, *63*, 463

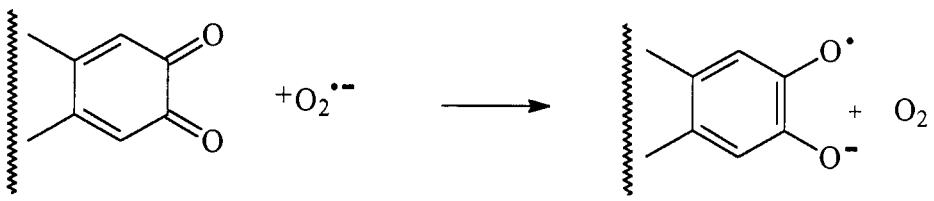
10.4.2 $O_2^{\bullet-}$ as a reducing agent

Reactions involving electron transfer from $O_2^{\bullet-}$ to metallic ions in their higher oxidation states as well as organic substrates as assays for $O_2^{\bullet-}$ have been reported[131]. $O_2^{\bullet-}$ transfer of electrons to *ortho*-or *para*- quinones leads to semiquinone radicals **Scheme 7** and **(8)**][141,151,180,181].



(45) **(46)**

Scheme 7



(47) **(48)**

Scheme 8

In addition to quinones a number of other organic compounds will oxidise $O_2^{\bullet-}$. The reaction in which an extremely reactive oxidant, the hydroxyl radical is generated, is believed to be

[180]R. L. Willson, *Chem. Comm.*, **1970,** 1005
[181]K. B. Patel and R. L. Willson, *J. C. S. Farady I*, **1973,** *69,* 816

responsible for a number of aromatic hydroxylation's occurring in living organisms[182] [**equation (5)**]. Organic peroxides and hydroperoxides are reduced by $O_2^{\bullet -}$ according to [**equation (6)** and (7)][183,184].

$$O_2^{\bullet -} + H_2O_2 \rightarrow OH^- + {}^{\bullet}OH + O_2$$

EQUATION (5)

$$ROOR + O_2^{\bullet -} \rightarrow RO^- + RO^{\bullet} + O_2^{\bullet -}$$

EQUATION (6)

$$ROOR + O_2^{\bullet -} \rightarrow RO^{\bullet} + OH^-$$

EQUATION (7)

[182]F. Haber and J. Weiss, *Proc. Roy. Soc.*, **1934**, *A147*, 332
[183]A. LeBerre and Y. Berguer, *Bull. Soc. chim. France*, **1966,** 2363
[184]J. W. Peters and C. S. Foote, *J. Amer. Chem. Soc.*, **1976,** 2363

DISCUSSION

CHAPTER 11

PHENOLIC COMPOUNDS FROM *C. INTERMEDIA* (Honeybush Tea)[1,185]

11.1 Introduction

Cyclopia intermedia E. Mey (Fabaceae) is one of the approximately 24 *Cyclopia* species of woody legumes endemic to the Cape fynbos (Cape macchia) region of South Africa. The leaves and stems of several species are used to brew a traditional herbal tea with a pleasant taste and a characteristic honey flavour[186]. This commodity, known as honeybush tea, is manufactured from mainly two species, *C. intermedia* and *C. subternata* Vogel, both of which are being developed as commercial crop plants[187]. The only known reports of chemical analyses of the aerial parts of *Cyclopia* species date back to the late 19th century[1]; the presence of unidentified compounds in the leaves of a variety of species revealed the occurrence of the xanthone *C*-glycoside, mangiferin, and *O*-glycosides of the two flavanones, hesperitin and isosakuranetin[188]. To support the establishment of the honeybush tea industry as a viable agricultural enterprise, we reinvestigated the phenolic consituents of *C. intermedia*, which is currently the main natural resource for a limited export program (~25 tons/annum) of this potential health beverage.

11.2 Results and Discussion

The methanol extract of the fermented leaves and stems of *C. intermedia*, that is, the commercial product, afforded a complex mixture of phenolic compounds which was resolvable only after extensive enrichment and fractionation procedures. Owing to the complexity of the mixture, the fractions had to be derivatized to attain an acceptable level of purity. This invariably led to substantial losses, hence prohibiting reliable quantification of the constituents.

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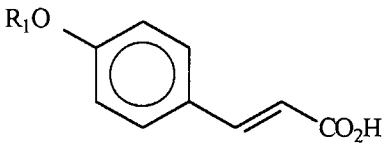
[185] B. I. Kamara. E. V. Brandt. D. Ferreira, *Structure and Synthesis of Phenolic Metabolites from Honeybush Tea (Cyclopia Intermedia)*, UFS **1997**

[186] J.M.Watt and M. G. Breyer-Brandwijk, *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd ed.; Livingstone: Edinburgh, **1962**, 590.

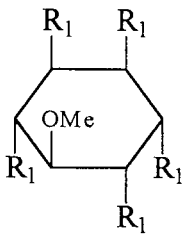
[187] J. H. De Lange, National Botanical Insititute, Claremont, South Africa, personal communication, **1996**

The mixture comprised the hydroxy-cinnamic acid, 4-coumaric acid **(49),** the inostol, (+)-pinitol **(51),** and a variety of $C_6.C_3.C_6$ polyphenols as well as two xanthones *C*-glucosides. 4-Coumaric acid **(49)**and (+)-pinitol **(51)** were identified by comparison of the ^1H NMR data of their *O*-acetyl derivatives (**50** and **52)** with those of the same derivatives of commercially available reference compounds. Structure elucidation of the single flavone, (luteolin **53)**, with its known anti-spasmodic properties[189] was similary performed by comparison of the ^1H NMR data of its tetra-*O*-acetyl derivative **54** with those of authentic tetra-*O*-acetylluteolin[15].



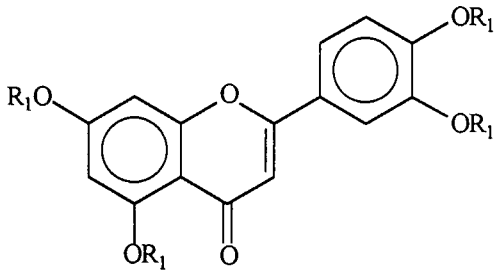
49, R_1=H

50, R_1=Ac



51, R_1=OH

52, R_1=OAc



53, R_1=H

54, R_1=Ac

11.3 Isoflavones.

A single fraction resulting from consecutive enrichment via a Craig countercurrent distribution and column chromatography on Sephadex LH-20 in ethanol afforded the five known flavones 7-hydroxy-4'-methoxy-(**55**, formononentin)[190], 7-hydroxy-6,4'-dimethoxy- (**57**, afrormosin), 7,3'-dihydroxy-4'-methoxy- (**59** calycosin)[191], 7-hydroxy-3',4'-methylenedioxy- (**61**,

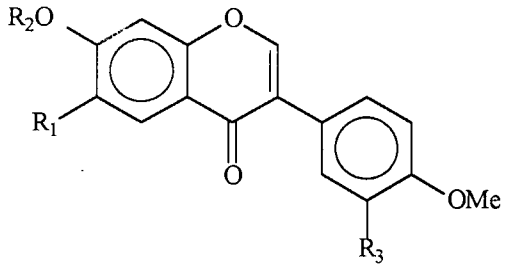
[188] A. M. De Nysschen, B. E. Van Wyk, F. R. Van Heerden and A. L. Schutte, *Biochem. Syst. Ecol.* **1996**, *24*, 243

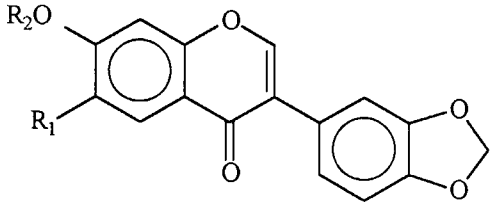
[189] Y. Shirataki, A. Manaka, I. Yokoe and M. Komatsu, *Phytocmemistry,* **1982**, *21*, 2959

[190] B. C. B. Bezuidenhoudt, E. V. Brandt and D. Ferreira, *Phytochemistry,* **1987**, *2*, 531

[191] M. Arisawa, Y. Kyozuka, T. Hayashi, M. Shimizu and N. Morita, *Chem. Pharm. Bull.* **1980**, *28*, 3686

pseudobaptigen)[192], and 7-hydroxy-6-methoxy-3'4'-methylenedioxyisoflavone (**63**, fujikinetin)[193]. Identification was performed on the respective *O*-acetyl derivatives (**56, 58, 60, 62** and **64**). Their ^{1}H NMR spectra in $CDCl_3$ invariably displayed the one proton singlet at δ 7.98-8.02 reminiscent of the vinylic H-2 resonanse of isoflavones[194]., whereas those of the pseudobaptigen and fujikinetin derivatives (**62** and **64**), respectively, additionally displayed a two-proton singlet at δ 6.02, characteristic of the methylenedioxy functionalities. The oxygenation patterns were simply established from the multiplicity and chemical shifts of the aromatic spin systems, the positions of the *O*-methyl and -O-CH_2-O substituents then being confirmed by the appropriate correlation ^{1}H NMR spectrometric experiments, for example, nuclear Overhauser effect spectrometry (NOE) or correlation spectrometry (COSY).





55, R_1=R_2=R_3=H

56, R_1=R_3=H, R_2=Ac

57, R_1=OMeR_2=Ac, R_3=H

58, R_1=OMe, R_2=Ac, R_3=H

59, R_1=R_2=H, R_3=OH

60, R_1=H, R_2=Ac, R_3=OAc

61, R_1=R_2=H

62, R_1=H, R_2=Ac

63, R_1=OMe, R_2H

64, R_1=OMe, R_2=Ac

In addition to their significant natural functions in plants, for example, as phytoalexins in the defense against fungal infection[195], the isoflavones also have important physiological effects in humans, that is; anticancer[196], estrogenic[197] and antimicrobial[198] activities.

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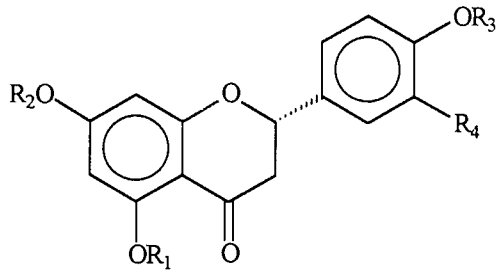
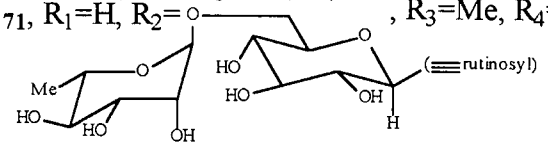
[192] D. Adinarayana, K. V. Syamasundar, O. Seligmann and H. Wagner, *Z. Naturforsh.* **1982**, *37C*, 145.
[193] K. N. Rao and G. Srimannarayana, *Phytochemistry* **1984**, *23*, 927
[194] R. R. Markham and H. Geiger, In *The Flavonoids-Advances in Research since 1986*; Harborne, J. B. Ed.; Chapman and Hall: London, 1993; p 441
[195] P. E. Laks and M. S. Pruner, *Phytochemistry*, **1989**, *28*, 87
[196] H. Adlercreutz, T. Fotsis, C. Bannwart, K. Mäkelä, K. Wähälä, G. Brunow and T. Hase, *J. Steroid Biochem.*, **1986**, *25*, 791
[197] S. Smolenski, A. D. Kinghorn and M. F. Belandrin, *Econ. Bot.*, **1981**, *35*, 321
[198] D. R. Perrin and I. A. M. Cruickshank, *Phytochemistry*, **1969**, *8*, 971

11.4 Flavanones

Four known flavanones, 5,7,4'-trihydroxy-(**65**, naringenin)[199], 5,7,3',4'-tetrahydroxy-(**67**, eriodictyol)[200]., 5,7,3'-trihydroxy-4'-methoxy-(**69**, hesperitin)[199] and 5,3'-dihydroxy-4'-methoxy-7-*O*-rutinosylflavanone(**71**, hesperedin)[201] were obtained in a relatively pure state.



65, R_1=R_2=R_3=R_4=H
66, R_1=R_2=R_3=Ac, R_4=H
67, R_1=R_2=R_3=H, R_4=OH
68, R_1=R_2=R_3=Ac, R_4=OAc
69, R_1=R_2=H, R_3=Me, R_4=OH
70, R_1=R_2=Ac, R_3=Me, R_4=OAc
71, R_1=H, R_2=  , R_3=Me, R_4=OH

72, R_1=Ac, R_2=hexa-*O*-acetylrutinosyl, R_3=Me, R_4=OAc

These compounds were identified by comparison of the ^{1}H NMR data of their *O*-acetyl derivatives (**66, 68, 70** and **72)** with those of the authentic samples from our collection of reference compounds. Their flavanone character was immediately apparent from the typical three-spin system of the protons of the heterocyclic ring, for example, H-2(C), δ 5.50 (dd, J = 2.5, 8.5 Hz), H-3β-(C), δ 2.81 (dd, J = 2.5, 12.0 Hz), and H-3α(C), δ 3.02 (dd, J = 8.5, 12.0 Hz) for tetra-*O*-acetyleriodictyol (**68**). The circular dichroism(CD) spectra of these derivatives exhibited the anticipated synchronous Cotton effects (negative for the π→π* transition at ~290 nm and positive for the n→π* transition at ~340 nm) that were compatible with flavanones possessing 2*S* absolute configuration[202]

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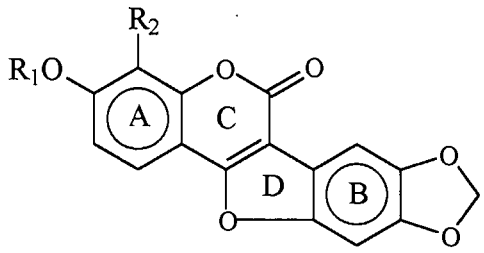
[1] Bohlman, C. Zdero, M. Grenz, A. K. Dhar, H. Robinson and R. M. King, *Phytochemistry,* **1981,** *20,* 281
[200] F. O. Snyckers and G. Salemi, *S. Afr. J. Chem.* **1974**, *27,* 5
[201] G. A. Barthe, P. S. Jourdan, C. A. MacIntosh and R. L. Mansell, *Phytochemistry,* **1988**, *27,* 249
[202] W. Gaffield, *Tetrahedron,* **1970,** *26,* 4093

Interesting physiological properties have been attributed to the flavanones, for example, the vitamin-like activity of citrin (a mixture of eriodictyol and hesperidin)[203,204] and antimicrobial, antiviral and anti-inflammatory[210] properties.

11.5 Coumestans

The three coumestans representative of the fully oxidised state of the heterocyclic C-ring of isoflavonoids, comprised 3-hydroxy-8,9-methylenedioxy-(**73**, medicagol)[197,198], 3-methoxy-8,9-methylenedioxy- (**75**, flemichapparin)[194] and 3-hydroxy-4-methoxy-8,9-methylenedioxy coumestan (**76**, sophoracoumestan B)[193] Flemichapparin C (**75**) and the *O*-acetyl derivatives (**74** and **77**) of medicagol **73** and sophoracoumestan B **76** all displayed the intense bright blue fluorescence on TLC underUV irradiation characteristic of coumestans[205].



73, R_1=R_2=H

74, R_1=Ac, R_2=H

75, R_1=Me, R_2=H

76, R_1=H, R_2=OMe

77, R_1=Ac, R_2=OMe

Despite the relatively simple spin system displayed in the ^{1}H NMR spectra ($CDCl_3$) of compounds (**74, 75** and **77**), the absence of protons associated with heterocyclic rings rendered structure elucidation more complicated. Crucial aspects relevant to the definition of structure are hence discussed for the medicagol derivative **74**. The molecular formula, $C_{18}H_{10}O_7$, was confirmed by MS analysis (M^+, *m/z* 326). The ^{1}H NMR spectrum ($CDCl_3$) displayed an aromatic ABX system (1-H, δ 7.98, d, J = 8.5 Hz; 2-H, δ 7.20, dd, J = 2.5, 8.5 Hz; 4-H, δ

[203] R. E. Hughes and H. K. Wilson, *Prog. Med. Chem.*, **1977**, *14*, 285

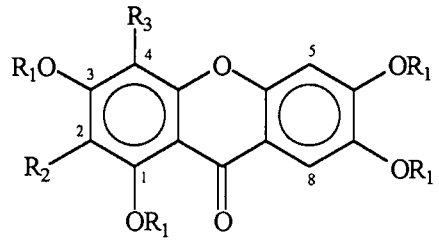
[204] E. Middleton and C. Kandaswami, *The Flavonoids-Advances in research since 1986*; J. B. Harborne, Ed.;Chapman and Hall: London, 1983; p619

7.30, d, J = 2.5 Hz), two aromatic singlets (7-H, δ 7.51; 10-H, δ 7.17), the two-proton singlets (δ 6.12) reminiscent of the methylenedioxy functionality, and a single aromatic *O*-acetyl resonance (δ 2.39). A carbon resonance at δ 158.0 in the ^{13}C NMR spectrum of derivative (**74**) (**Table 2**) strongly suggested a lactone-type carbonyl group[206] and thus the coumarin arrangement typical of coumestans. Such an assumption was supported by the conspicuous deshielded C-11a signal (158.5 ppm) resulting from the inherent electron deficiency at the β-carbon of α,β-unsaturated esters and which is enhanced by the 11a-O function in the coumestan framework. This resonance was then utilized as the reference signal to access the spin system and hence substitution pattern of the A-ring via the appropriate C-H correlation experiments, for example, HMBC and HMQC. These techniques similarly also facilitated definition of the 8,9-methylenedioxy-substituted B-ring. A comparable approach also led to full structural assignment for compounds (**75**) and (**77**) (cf. **Table 2**).

The coumestans share many of the physiological properties of the isoflavones (*vide supra*), with arguably the most important their phytoestrogenic characteristics[207,208].

11.6 Xanthones

Two known xanthones, 2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy- (**78**, mangiferin)[202] and 4-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9*H*-xanthen-9-one (**80**, isomangifrein)[202], were purified and identified as their per-*O*-acetyl derivatives (**79**)



78, R_1=R_3=H, R_2= 2-*C*-*β*-D-glucopyranosyl

79, R_1=Ac, R_3=H, R_2=tetra-*O*-acetyl-2-*C*-*β*-D-glucopyranosyl

80, R_1=R_2=H, R_3=2-*C*-*β*-D-glucopyranosyl

81, R_1=Ac, R_2=H, R_3=tetra-*O*-acetyl-2-*C*-*β*-D-glucopyranosyl

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[205] J. L. Ingham, S. Tahara and S. Dzedzic, *Z. Naturforsch.*, **1988,** *44C,* 5

[206] H Kalinowski and S. Braun, *Carbon-13 NMR Spectroscopy;* Wiley: New York, **1984,** pp375, 377

[207] E. M. Bickhoff, R. R. Spencer, S. C. Witt and B. E. Knuckles, *Studies on Chemical and Biological Properties of Coumesterol and Related Compounds;* Technical. Bulletin; U.S. Department of Agriculture: Washington, DC 1969.

[208] P. M. Martin, K. B. Horowitz, D. S. Ryan and W. L. MacGuire,*Endocrinology,* **1978,** *103,* 1860

and (**81**), respectively. At ambient temperature (23 °C) the spectra of both (**79** and **81**) displayed the typical adverse effects of dynamic rotational isomerism about the xanthenyl-glucosyl bond. The relatively low energy barrier to free rotation was overcome at 60 °C , at which sharp resonances were evident in both the ^{1}H and ^{13}C NMR spectra. Both compounds (**79** and **81**) displayed relatively simple splitting patterns, that is, three one-proton singlets [δ 6.88 (H-4), 7.50 (H-5), 8.05 (H-8); δ 7.28 (H-2), 7.40 (H-5), 8.03 (H-8) for (**79** and **81**), respectively] in the aromatic region, four three-proton singlets for the aromatic *O*-acetyl resonances, and four three-proton singlets reminiscent of the acetoxy signals of a C-C linked β-D-glucopyranosyl moiety [δ 5.27, 4.90 (both d, both J = 6.0 Hz), anomeric protons for (**79** and **81**), respectively]. The substitution pattens of the aromatic rings of each compound were unequivocally established by the extensive utilization of heteronuclear multiple quantum correlation (HMQC) for direct C-H couplings and heteronuclear multiple bond correlation (HMBC) for couplings over two to four bonds. When taken in conjunction with the characteristic xanthone carbonyl chemical shifts[206] [(δ 174.0 for both compounds **79** and **81**)] **(Table 3),** these data confirmed the structures of the xanthone derivatives as the per-*O*-acetates of mangiferin (**78**) and isomangiferin (**80**), respectively.

Xanthones exhibit a variety of pharmacological properties, for example, the antviral, antifungal, anti-inflammatory, and lysosomal membrane stabilization effects of mangiferin[209].

Our current efforts further aimed at unraveling the phenolic profile in the metabolic pool of *C. intermedia*, as well as the potential of the various phenolic compounds to act as antioxidants and synthesis of a group of flavonoids.

[209] . C. W. W. Beecher, N. R. Farnsworth and C. Gylenhaal, in the *Natural Products of Woody Plants II*; (ed, J. W. Rowe), Springer-Verlag, Berlin, **1990**, p 1059 and the references cited therein

CHAPTER 12

$C_6.C_2$- AND $C_6.C_1$-TYPE PHENOLS.

12.1 Introduction

Owing to the claimed physiological properties associated with the different phenolics revealed in our initial investigation[1], a continuation of the study on honeybush tea *Cyclopia intermedia* has resulted in the isolation of $C_6.C_2$, as well as $C_6.C_1$, monoaryl compounds in addition to the common $C_6.C_3.C_6$ -type flavonoids, and their glycoside analogues.

2-(*p*-hydroxyphenyl)-ethanol (tyrosol) [see acetate derivative **(82)**] is a $C_6.C_2$-type monoaryl compound previously isolated[210], and its methyl and ethyl ethers, as well as its glucosyl analogue (salidrosid) have been reported. In this survey we have isolated two additional compounds with a unique apiofuranosyl-4-*O*-glucopyranosyl substituent. The compounds have been characterised as:

2-(6'-*O*-β-apiofuranosyl-4-*O*-β-D-glucopyranosyl-phenyl)ethanol **(84)**

2'-*O*-β-apiofuranosyl-4-*O*-β-D-glucopyranosylbenzaldehyde **(85)**

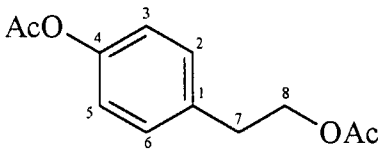
Following PLC chromatography, acetylation of fractions C.4 and C.5 of the methanol extract of *C. intermedia* afforded the monoaryl derivatives **(82)** and **(83)** as white armophous solids and their glycosidic analogues **(84 and 85)** as colourless oils.

12.2 Aglycones

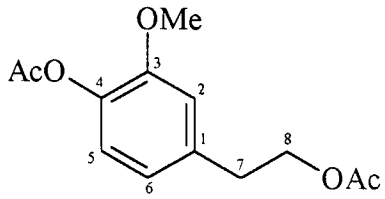
Following acetylation, the simple 4-*O*-acetyl (δ 2,32) substituted phenylethanol **(82)** gave a ^{1}H NMR spectrum [plate 1 ($CDCl_3$ - 296K)] displaying two aromatic doublets (δ 7.05, d, 8.5 Hz, 3,5-H and δ 7.25, d, 8.5 Hz, 2,6-H) attributed to a 1-4-disubstituted phenyl ring. In the same

[210] R. T. LaLonde, C. Wong and A. I. Tsai, *J. Am. Chem. Soc.*, **1976**, *98:10*, 3007

spectrum also appeared an aliphatic acetoxy group (δ 2.06) and two aliphatic triplets (δ 2.95, t, 7.0 Hz, and δ 4.30, t, 7.0, Hz), assigned to two methylene protons.



(82)

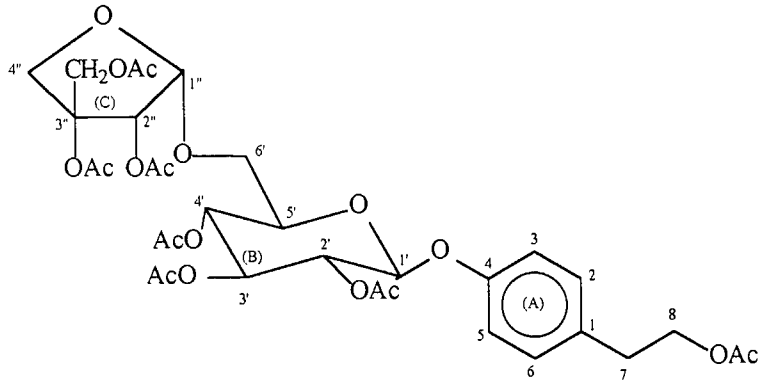


(83)

The ^{1}H NMR spectrum [plate 2 ($CDCl_3$ - 296K)] of the 2-(4-acetoxy-3-methoxyphenyl)-ethanol **(83)**, a 3-methoxy analogue of compound **(82),** displayed two analogous pairs of aliphatic methylene protons at δ 2.95, t, 7.0 Hz and δ 4.30, t, 7.0 Hz, an aliphatic acetoxy group (δ 2.07) and a deshielded acetoxy group (δ 2.33), identical to those of **(82)**. The AA'BB' system in the ^{1}H NMR spectrum of **(82)** is however, replaced by a 3-methoxy group (δ 3.84) and an ABX system (δ 6.98, d, 8.5 Hz, H-5; δ 6.81, dd, 2.5 and 8.5 Hz, H-6 and δ 6.83, d, 2.5 Hz, H-2) in **(83)**.

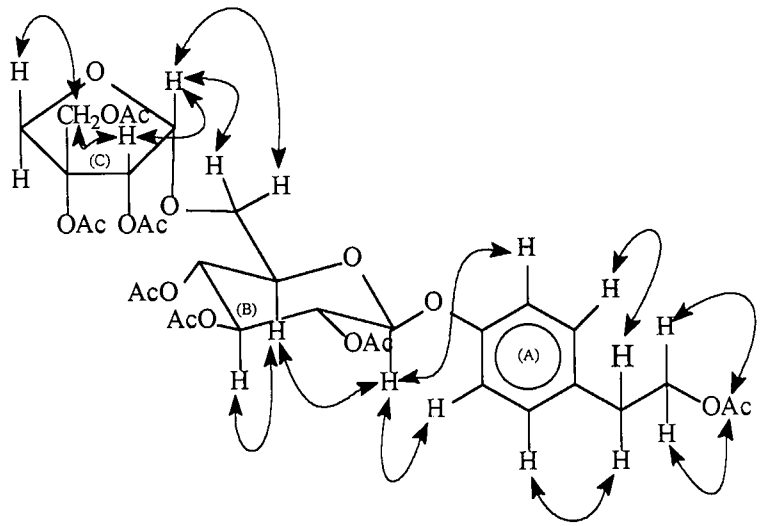
12.3 Glycosides of $C_6.C_2$- type phenols

The ^{1}H NMR spectrum [plate3 ($CDCl_3$ - 296K)] of 2-(6'-*O*-β-apiofuranosyl-4-*O*-β-D-glucopyranosyl-phenyl)-ethanol, a 4-*O*-glycosyl analogue of **(82)** revealed two methylene doublets of doublets at δ 2.95 (H-7) and δ 4.26 (H-8), common to **(82)** and **(83).** These were defined by a COSY experiment showing coupling [plate 3b-2 ($CDCl_3$ - 296K)] of H-8 with the acetoxy group (δ 2.04), and a strong association in a NOESY spectrum [plate 3a-1 ($CDCl_3$ - 296K)] of H-7 with the aromatic doublet H-2,6 (δ 7.15, d, 8.5 Hz) and a weak association with H-3,5 (δ 6.93, d, 8.5 Hz) **(84a),** of the AA'BB' spin system, analogous to those in **(82).**



(84)

When compared with compound **(82),** the less deshielded 2,6-H and 3,5-H protons, in the spectrum of **(84)**, in conjunction with the presence of two glycosyl units, suggested the 4-*O*-glycosyl linkage to the aromatic ring. This connectivity was substantiated by the association of the anomeric proton, H-1" (δ 5.05, d, 8.0 Hz) with the aromatic H-3,5 doublet observed in the NOESY spectrum [plate 3a-1 ($CDCl_3$ - 296K)].



(84a)

Linkage of the apiofuranosyl unit (C) to C-6' of the glucopyranosyl moiety is evident from the NOE association [plate 3a-1 ($CDCl_3$ - 296K)] between the anomeric proton of the apiofuranosyl and H-6' of the glucosyl moiety. An NOE association between H-1' (δ 5.05, d,

8.0)[211] of the glucosyl unit (B) and the diaxial H-3' and 5' glucosyl protons indicated the β-D-glucopyranosyl moiety in compound **(84)** . Small couplings, J = 1.0 Hz[212] for H-1" and H-2" (H-1", δ 5.37, d, 1.0 Hz and H-2", δ 5.00, d, 1.0 Hz) confirm a *cis* diaxial-β-D-apiofuranosyl moiety (C)[212,213]

The remaining glycosidic protons of moiety (B) were assigned from a COSY spectrum as: H-2', δ 5.23, t, 9.0 Hz; H-3', δ 5.28, t, 10.0 Hz; H-4', δ 5.03, t, 10.0 Hz; H-5',H-6', δ 3.73-3.85 and H-6', δ 3.62, dd, 7.0, 12.0 Hz. The two sets of doublets δ 4.79 and δ 4.52 (12.0 Hz) and δ 4.21 and δ 4.13 (10.0) Hz were respectively assigned to 4"- and -CH_2 methylene protons of the apiofuranosyl ring (C). Confirmation of the assigned glucosyl protons of unit (B) was substantiated by long range coupling of H-5' to H-1' in the COSY spectrum [plate 3b ($CDCl_3$ - 296K)].

Strong coupling in the same COSY spectrum from H-1" to H-2" on the apiofuranosyl unit (C) but only a very weak coupling of the latter to the 3"-CH_2 OAc protons (δ 4.21 and δ 4.13) methylene protons notably establishes the absence of the H-3" proton on moiety (C). However, coupling from 3"-CH_2 to H-4" (δ 4.79 and δ 4.52) methylene protons confirm the allocation of the apiofuranosyl protons. The structure is confirmed by MS analysis (M^+, *m/z* 599) in accordance with the formula $C_{33}H_{43}O_{10}$.

Seven aliphatic acetoxy groups **(Table 4)** are displayed in the ^1H NMR spectrum of compound **(84).**

12.4 Glycosides of $C_6.C_1$- type phenols

The ^1H NMR data [plate 4 ($CDCl_3$ - 296K) of compound **(85)** revealed an AA'BB' system similar to that of compound **(84)**. In comparison to compound **(84), (85)** lacks the set of methylene protons (δ 2.95, and 4.28), which are replaced by deshielded singlet at δ 9.95, suggesting the presence of an aldehyde or a hydroxyl group as well as broadened signals.

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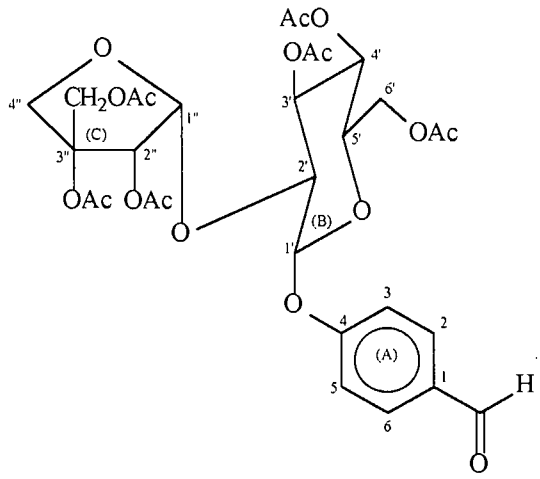
[211] T. J. Mabry, K. R. Markham and M. B. Thomas, *The Systematic Identification of Flavonoids*, Springer-Ver-lag, Berlin, **1970**

[212] T. J. Mabry, J. Kagan and H. Rösler, *Phytochemistry*, **1965b**, *4*, 487

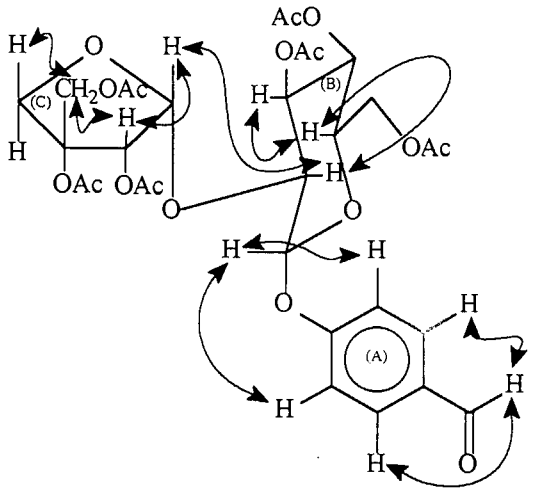
[213] K. R. Markham, J. W. Wallace, Y. N. Babu, V. K. Marty and M. G. Rao, *Phytochemistry*, 1989, *42c*. 1039



(85)

Since this proton does not under go D_2O exchange, the presence of an aldehyde functionality in compound **(85)** was strongly suggested. A positive DNPH test on the com ·und confirmed presence of the carbonyl group in the structure and hence the assigned CHO (δ 9.95, s). Association in NOESY and COSY spectra [plates 4a and 4b $CDCl_3$ - 296K) of the aldehydic singlet and 2-,6-H protons substantiated the allocation **(85a)**. Owing to insufficient material ^{13}C NMR experiment was not done.

The two doublets δ 7.15, d, 8.5 Hz and δ 7.88, d, 8.5 Hz displayed in the ^1H NMR spectrum, respectively allocated to H-3,5 and H-2,6 showed association of the former with the anomeric proton (δ 5.14, d, 7.5 Hz) and the latter with the aldehyde (δ 9.95, s).



(85a)

The presence of a glucopyranosyl and an apiofuranosyl unit were evident from the protons resonating between δ 3.80 and 5.5, the conspicuous H-4" and 3"-CH_2 methylene protons and the corresponding acetoxy groups. A coupling sequence from the H-5' multiplet to H-1' in the COSY spectrum [plate 4b ($CDCl_3$ - 296K)], defined the protons as: H-1' δ 5.14, d, 7.5 Hz; H-2', δ 4.01, t, 7.5 Hz; H-3', δ 5.32, t, 10.0 Hz; H-4', δ 5.08, t, 10.0 Hz; H-5', δ 3.91, m and the overlapping H-6' protons δ 4.23, d, 12.0 Hz and H-6', δ 4.16, dd, 2.5, 12.0 Hz for the glucosyl moiety. The remaining protons in the ^1H NMR spectrum were allocated to H-4", δ 4.60 and δ 4.50, 2 x d, 12.0 Hz; and 3"-CH_2, δ 4.30 and δ 4.15, 2 x d, 10.0 Hz of the apiofuranosyl unit. Allocation of the acetoxy groups is given in **Table 4**. MS analysis (M+, m/z 597), in the agreement with the molecular formular of $C_{32}H_{37}O_{11}$, substantiated the structure.

Linkage of the glucosyl residue to 4-*O* position of the phenyl ring (A) was again confirmed by NOE association of the glucosyl anomeric proton (H-1', δ 5.14) with 3,5-H(A), [plate 4a-2 ($CDCl_3$ - 296K)]. C-2'-O-C-1'' connectivity of the sugar units was unambiguously confirmed by the association between H-2'of (B) and H-1" of (C) in the NOESY spectrum [plate 4a-3 ($CDCl_3$ - 296K)]. NOE association of the anomeric proton H-1' of glucosyl (B) with the di-axial H-3' and H-5'' [plate 4a-4 ($CDCl_3$ - 296K)] of the same unit assisted in the establishement of the configuration as β-D-glucopyranosyl and a β-apiofuranosyl moieties.

CHAPTER 13

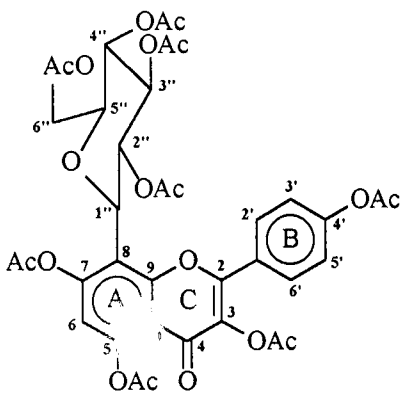
FLAVONOLS

13.1 Introduction

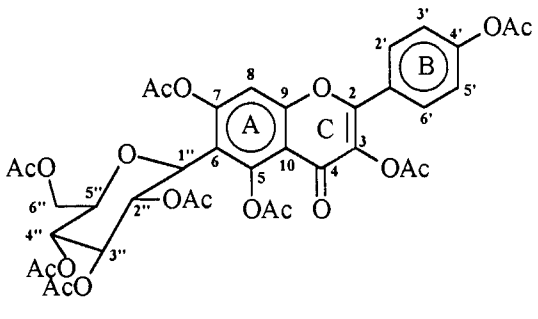
Following acetylation, fractions 8 and 9 of the methanol extract of *C. intermedia* afforded flavonol glycosides **(86-90)** as colourless oils. All displayed the absence of heterocyclic protons of the flavonoid C-ring in the ^{1}H NMR spectra [plates 5 and 6 ($CDCl_3$-296K)], characteristic of flavonols possessing an enolic functionality of the C-ring.

13.2 *C*-Glycosylated flavonols

The ^{1}H NMR spectrum of compounds **(86)** and **(87)** [plates 5 and 6 ($CDCl_3$-296K)] displayed one aromatic singlet, an aromatic AA'BB' system, resonances reminiscent of a glucosyl unit, three aromatic- and four aliphatic acetoxy groups. Allocation of coupling via the 6- or 8-position to the glycosyl unit in the *C*-glucosyl flavonols is based on the absence of the H-6 or H-8 proton on the A-ring in their ^{1}H NMR spectrum[214], thus yielding a residual singlet. Considering absence of the heterocyclic C-ring protons, the evidence suggested two possible structures, **(86)** and **(87).**



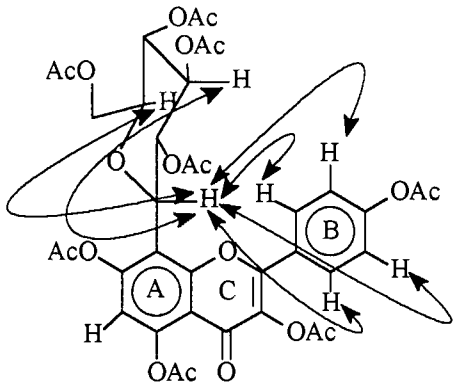
(86)



(87)

[214]M. K. Seikel and T. J. Mabry, *Tetrahedron Lett.* **1965**, 1105

A NOESY experiment on compound **(86)** [plate 5a-1 ($CDCl_3$ - 296K)] showed NOE association (**see 86a**) between H-1" and H-2',6' and H-3',5' thus strongly indicating the position of the glucosyl group at C-8. This is confirmed by restricted rotation about the C-8-C-1" bond, due to steric interaction of the 2"- and 6"-acetoxy groups and with 7-OAc and the B-ring[215]. This is manifested in the broadening of some of the aromatic and acetoxy protons[216] in the ^1H NMR spectrum [plate 5 ($CDCl_3$ - 296K)]. For compound **(86)** the singlet at δ 6.53 is thus attributed to H-6. Association of H-1" of the glucosyl unit to the di-axial H-3" and H-5" in the NOESY spectrum [plate 5a-2 ($CDCl_3$ - 296K)] confirms a *β*-D-glucopyranoside moiety.



(86a)

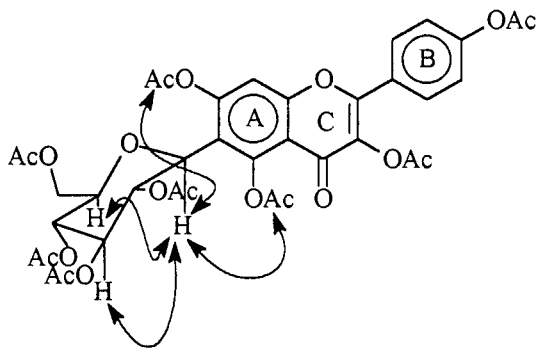
In the same ^1H NMR spectrum [plate 5 ($CDCl_3$ - 296K)] the 4'-*O*- acetyl substituted B-ring yields the AA'BB'system (δ 7.63, d, 8.5 Hz, H-2',6' and δ 7.18, d, 8.5 Hz, H-3',5'). The four acetoxy groups (δ- 2.36, 2.17, 2.07, 1.80) were allocated to the flavonol unit and the remaining four acetoxy groups [δ 1.59 (x2), 1.56, 1.51] to the glucosyl moiety.

The glucosyl unit further displays a multiplet for H-5" (δ 3.90) resulting from the couplings of 2x H-6" (δ 4.35, dd, 2.5, 12.0 Hz and δ 4.12, br.d, 12.0 Hz) and H-4" (δ 5.27, t, 9.0 Hz). The strong coupling of H-5" to both H-6" and H-4" was observed in the COSY spectrum [plate 5b

[215]R. A. Eade, W. E. Hillis, D. H. S. Horn and J. J. H. Simes, *Aust. J. Chem.* **1965**, *18*, 715

($CDCl_3$ - 296K)] of compound **(86)**. Similar strong couplings established the sequence from H-4" to H-3" (δ 5.27, br.t, 9.0 Hz) to H-2" (δ 5.40, br.t, 10.0 Hz) and to H-1" δ 4.86, d, 10.0 Hz) in the same spectrum.

In comparison with compound **(86)**, the effect of broadening of the acetyl groups disappears in the ^1H NMR spectrum of compound **(87)** [plate 6, ($CDCl_3$ - 296K)], and is attributed to the C-6 regioisomer of **(86)**. The NOESY experiment [plate 6a-1 ($CDCl_3$ - 296K)], where association between H-1" and H-2',6' or H-3'5' is absent but indicating association between the anomeric proton and/or 5-and 7-OAc of the A-ring **(87a)** supports the conjecture. Association between the anomeric proton (H-1") and the axial H-3" and H-5" observed in the NOESY spectrum [plate 6a-1 ($CDCl_3$ - 296K)] again confirmed the presence of the *β*-anomer.



(87a)

The remainder of the ^1H NMR spectrum [plate 6, ($CDCl_3$ - 296K)] of compound **(87)** displays B-ring protons (δ 7.76, d, 8.5 Hz, 2',6'-H and δ 7.16, d, 8.5 Hz, 3',5'-H) similar to those displayed by compound **(86),** and a residual singlet (δ 6.71) which was assigned to H-8 on the A-ring. Four deshielded acetoxy groups (δ- 2.32, 2.16, 2x 2.08) and four acetoxy groups which are aliphatic in nature (δ- 2.03, 1.88, 1.74 and 1.65), are respectively allocated to the flavonol and glucosyl unit.

Assignment of the glucosyl protons was on the same principle as in compound **(86)** and a COSY experiment [plate 6b, ($CDCl_3$ - 296K)] confirmed the assignment as follows: H-1" (δ 4.93, d, 10.0 Hz); H-2" (δ 5.42, t, 9.0, Hz); H-3" (δ 5.35, t, 9.0 Hz); H-4" (δ 5.29, t, 9.0, Hz); H-5" (δ 3.92, m); H-6" (δ 4.36, dd, 3.5, 12.0 Hz and δ 4.18, dd, 2.5, 12.0 Hz). The C-

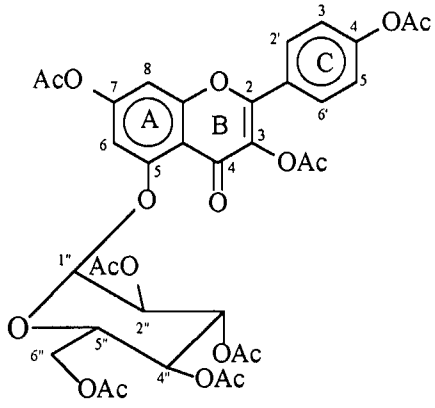
[216]J. Chopin and M. L. Bouillant, in *The Flavonoids*, (eds. J. B. Harborne, T. J. Mabry, H. Mabry), Chapmah and Hall, London, **1975**, 673

glucosyl linkage is verified by the large coupling constant (10.0 Hz) of the anomeric H-1" proton due to the restricted rotation of the glucosyl moiety with respect to the flavonoid nucleus[217,218].

Compounds **(87)** and **(86)** were therefore identified as 4,5,7,4'-tetraacetoxy-6-β-D-glucopyranosylflavonol and its 8-β-D-glucopyranosyl analogue, respectively, and hence are the peracetates of 6-*C*-glucopyranosylkaempferol and 8-*C*-glucopyranosylkaempferol. Identical MS analysis (M+, *m/z* 717), in agreement with molecular formula $C_{42}H_{37}O_{11}$ for both compounds **(86)** and **(87)** confirmed the two isomers.

13.3 *O*-Glycosylated flavonols

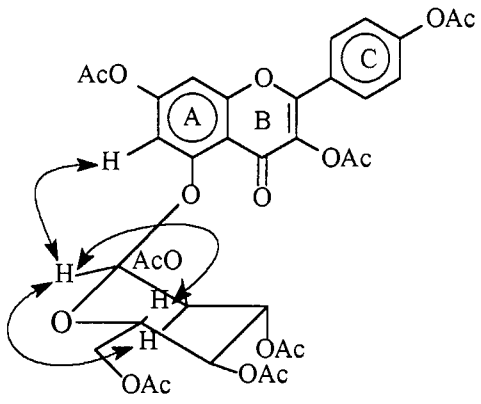
The ^{1}H NMR spectrum [plate 7 ($CDCl_3$ - 296K] of the hepta-*O*-acetyl derivative of flavonol **(88)** exhibits an AA'BB' spin system (δ 7.83, d, 8.5 Hz, 2',6'-H and δ 7.20, d, 8.5 Hz, 3',5'-H) similar to that of compound **(87),** which is attributed to the B-ring. The singlet in compound **(87)** is replaced, however, by two *meta* doublets (δ 6.92, d, 2.5 Hz, H-6 and δ 6.86 , d, 2.5 H.z, H-8), assigned to the A-ring protons, while the presence of only three deshielded acetoxy groups (δ- 2.34, 2.33, 2.12), as opposed to four in compounds **(86)** and **(87)** , suggested *O*-glycosylation.



(88)

[217]B. H. Koeppen, *Ztsch Naturf*, **1964**, *19b*, 173
[218]R. M. Horowitz and B. Gentili, *Chem. Ind.*, **1964**, 498

Following the same protocol as discussed for compounds **(86)** and **(87)**, the glucosyl protons were assigned as: H-1", δ 5.01, d, 2.0 Hz; H-2", δ 5.20, t, 9.0 Hz; H-3", δ 5.01, t, 10.0 Hz; H-4", δ 5 .05, t, 10.0 Hz; H-5", δ 3.86, m; H-6", δ 4.26, d, 5.5, 12.0 Hz and δ 4.17, d, 2.5, 12.0 Hz. A COSY experiment [plate 7b ($CDCl_3$ - 296K] showing consecutive couplings from H-6" to H-1" confirmed the positions of the glucosyl protons. A NOESY experiment [plate 7a ($CDCl_3$ - 296K] showing dipolar coupling between H-1" and H-6(A) only **(88a)**, establishes the 5-*O*-glucosyl linkage, which is verified by the small coupling constant of H-1", 2.0 Hz[219]. The un usual small coupling constant of the anomeric proton (J = 2.0 Hz), indicative of the α-linked glucosyl to the flavonol, is evident in the NOESY spectrum [plate 7a-2 ($CDCl_3$ - 296K], which showed an association between H-1" and H-2" and H-5", thus, a di-equatorial coupling of H-1" and H-2"[212]. Compound **(88)** therefore was identified as the peracetate of α-D-5-*O*-glucopyranosylkaempferol.



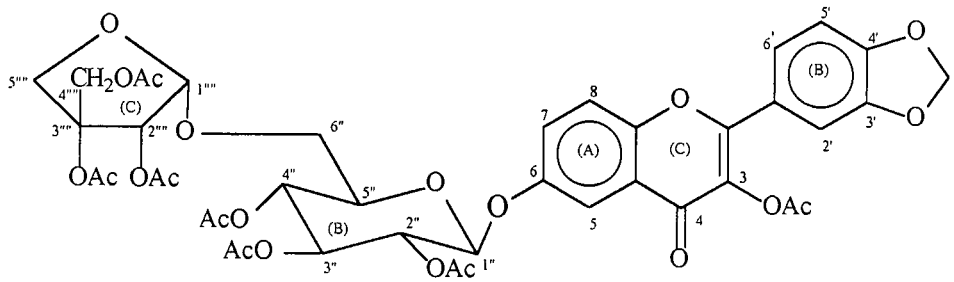
(88a)

The ^1H NMR spectral data of the peracetate derivatives of flavonol **(89)** [plate 8 ($CDCl_3$ - 296K)] showed a 6-*O*-β-D-glycosyl [NOESY association of H-5(A) to H-3" and H-7(A) , plate 8a-2 ($CDCl_3$ - 296K)] substituted ABX system (δ 7.05, d, 8.5 Hz, 8-H; δ 7.22, dd, 2.5, 8.5 Hz, 7-H; 7.67, d, 2.5 Hz, 5-H) allocated to the A-ring and a second ABX system attributed to the 3',4'-methylenedioxy substituted B-ring (δ 7.38-7.44, 2',5',6',-H). A COSY spectrum [plate 8b-1 ($CDCl_3$ - 296K)] distinguished between the A- and B-ring ABXsystems.

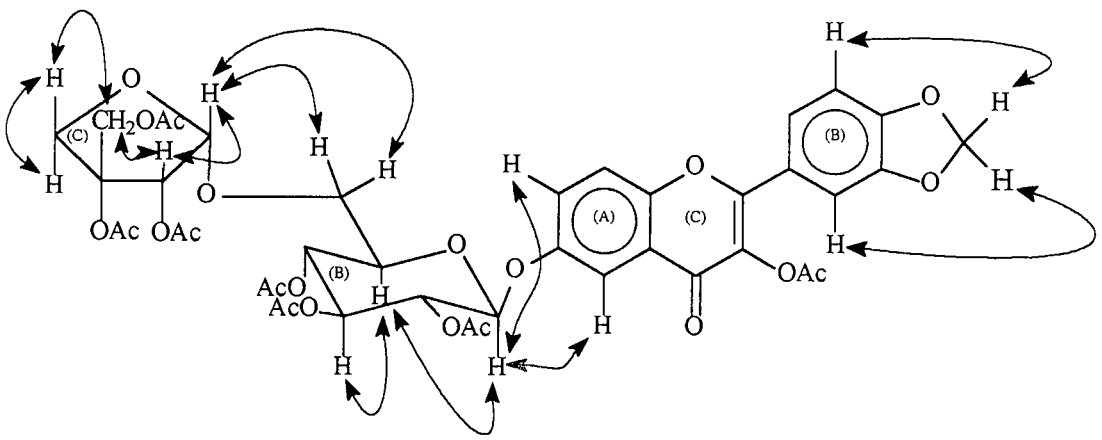
[219]H. Rösler, T. J. Mabry, M. Cranmer and J. Kagan, *J. Org. Chem.*, **1965**, *30*, 4346

Owing to its peri-position to the carbonyl, H-5 resonates at the lowest field (δ 7.67) as a *m*-doublet, suggesting a 6-O-glycosyl linkage on the A-ring. Respective NOE associations [plate 8a-2 ($CDCl_3$ - 296K)] of the 3',4'-OCH_2O with 2'-H(B) and 5'-H(B) and of the anomeric proton H-1''' with 5-H(A) and 7-H(A) [indicated in **(89a)**] facilitated the differentiation between the spin systems of the A- and B-rings, and confirmed the allocation of the methylenedioxy functionality (δ 5.31) to the B-ring as well as the 6-*O*-glycosyl substitution on the A-ring.

These are accompanied by protons of an additional apiofuranosyl unit, one deshielded- and six aliphatic acetoxy groups **Table 5** which suggested compound **(89)** to be a disaccharide (cf.**84**) derivative of a flavonol.



(89)



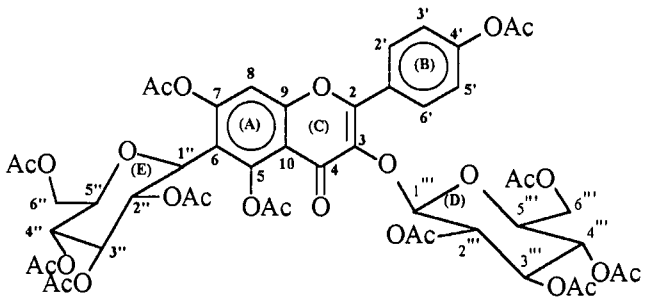
(89a)

The NOESY experiment [plate 8a-1 ($CDCl_3$ - 296K] showing association of 2x H-6" protons of the glucosyl moiety (D) with the anomeric proton (H-1"') of the apiofuranosyl unit (E) confirmed C-6"-O-C-1"' connectivity of the sugar units. The consecutive couplings in the COSY spectrum [plate 8b ($CDCl_3$ - 296K] from H-1" to H-6" confirmed the allocation of the glucopyranosyl protons of unit (D) as: H-1", 5.07, d, 8.5 Hz; H-2", H-3", δ 5.27, t, 8.5 Hz; H-4", δ 5.06; overlapping H-1"; H-5, δ 3.83, m: H-6", δ 3.72, dd, 2.5, 12.0 Hz and 3.59, dd, 4.0, 12.0 Hz

Confirmation of the second unit (E) of the disaccharide was substantiated by similar couplings observed in the COSY spectrum [plate 8b ($CDCl_3$ - 296K]. The protons were allocated as follows: H-1"', δ 4.99, d, 1.0 Hz; H-2"', δ 5.37, d, 1.0 Hz; 3"'-CH_2, δ 4.14, d, 10.0 Hz and 4.23, d, 10.0 Hz; 4-H"', δ 4.58, d, 12.0 Hz and 4.77, d, 12.0 Hz. MS analysis of compound **(89)** (M+, *m/z* 759), was in line with the empirical formula, $C_{41}H_{43}O_{14}$.

13.4 C-*O*-glycosylated flavonols

The ^1H NMR spectrum [plate 9 ($CDCl_3$ - 296K)] of the acetate derivative **(90)**, exhibited A- and B-ring proton substitution patterns similar to those of compound **(87)**. An AA'BB' spin system (δ 8.08, d, 8.5Hz, 2'6'-H and δ 7.40, d, 8.5Hz, 3'5'-H) was attributed to the B-ring protons.



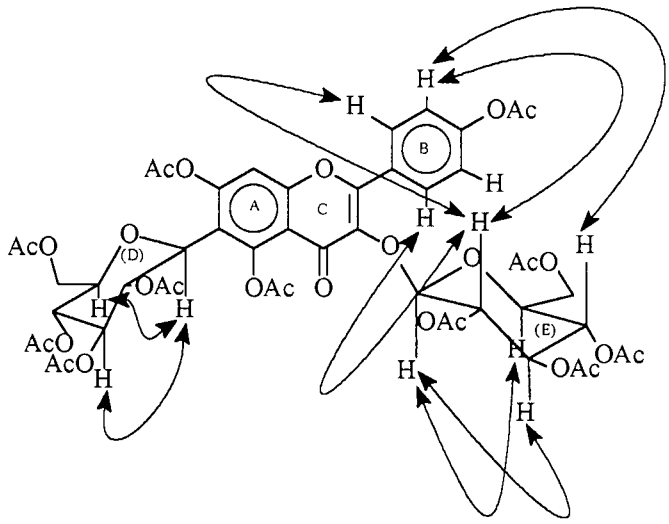
(90)

The absence of broadening of the peaks in the ^{1}H NMR spectrum [plate 9 ($CDCl_3$ - 296K)], and of dipolar coupling between H-1'' and 2',6'-H and 3',5'-H in the NOESY spectrum [plate 9a-1 ($CDCl_3$ - 296K] indicated 6-C-glucosylation. This evidence taken in conjunction with the coupling constant (10.0 Hz) of the anomeric proton H-1" of glucosyl unit (D), confirms the assignment of the singlet at δ.6.67 to H-8 and defines the C-1"-C-5 linkage of the glucosyl unit (D) to the A-ring. Association of H-1"(D) with H-3'']- H-5"(D) in the NOESY spectrum [plate 9a-1 ($CDCl_3$ - 296K)], confirms the β-D-configuration of unit (D). A similar association is observed for unit (E).

The presence of three deshielded acetoxy groups (δ 2.56, 2.51, 2.37), eight aliphatic acetoxy groups (δ 2.11, 2.07, 2.06, 2.04, 2.01, 1.94, 1.89, 1.76) and two hexose units, were also displayed in the spectrum [plate 9a ($CDCl_3$ - 296K].

Thus, protons of the glucosyl unit (D) were assigned as: H-1", δ 4.82, d, 10 Hz; H-2", δ 5.71, t, 10.0 Hz; H-3", δ 5.32, t, 9.0 Hz; H-4", δ 5.16, t, 9.0 Hz; H-5", δ 3.77, m; H-6", δ 4.30, dd, 5.0, 12.0 Hz and δ 4.47, dd, 5.0, 12.0 Hz. This was based on sequential, strong couplings from H-6" to H-1" in the COSY spectrum [plate 9b ($CDCl_3$ - 296K)].

The C-3 - O - C-1''' glucosyl connectivity of the glucosyl moiety (E) to the C-ring was confirmed by the association between H-2''' and H-4''' and 2',6' [see **(90a)**] in the NOESY spectrum [plate 9a-2 ($CDCl_3$ - 296K]. A β-D-glucosyl configuration was confirmed in the manner discussed for the glucosyl unit (D). Thus, compound **(90)** is a 3,7-di-*O*-glucopyranosylkaempferol. MS analysis (M+, *m/z* 599) with the molecular formular $C_{33}H_{43}O_{10}$ substantiated the structure.



(90a)

Assignment of glucosyl protons of unit (E) was as follows: H-1''' (δ 4.59, d, 10.0 Hz); H-2''' (δ 5.95, dd, 2.0, 10.0 Hz), H-3''' (δ 5.43, t, 9.0 Hz), H-4''' (δ 5.50, t, 9.0 Hz), H-6''' (δ 4.47, dd, 4.20, 12.5 Hz and δ 4.30, dd, 2.5, 12.5 Hz), and the multiplet resonating at δ 3.82 was attributed to H-5''' according to both the ^{1}H NMR and COSY spectra.

CHAPTER 14

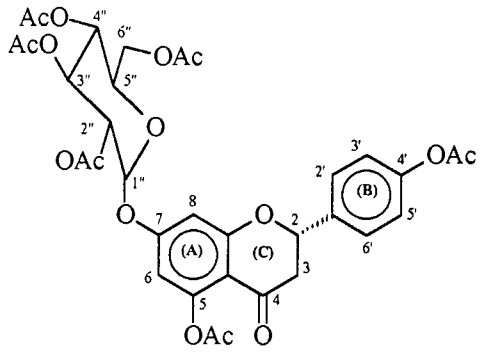
FLAVANONES

14.1 Introduction

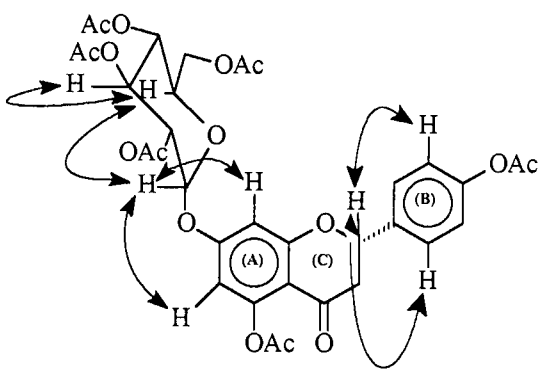
Flavanones **(91, 92, 93, 94)** were isolated after acetylation of fractions 8 and 9 of the methanol extract by means of PLC chromatography. Characteristic of this group of compounds is the presence of the 3-CH_2 [two doublet of doublets (~δ 2.75-3.00)] and the 2-H [doublet of doublets (~δ 5.5)] in their ^{1}H NMR spectrum [plates 10-13 ($CDCl_3$ - 296K)]. Owing to insufficient material, hydrolysis and ^{13}C NMR experiments were not done.

The CD spectra (plate 34, 35, 36 and 37) of compounds (**91, 92, 93** and **94**) were in line with the anticipated[220] synchronous positive Cotton effect due to the n → π* transition (~340 nm), and were compatible with flavanones possesing 2*S* absolute configuration[221,222,223].

14.2. Monoglycosylated flavanones



(91)



(91a)

[220] J. W. Nel, B. C. B. Bezuidenhout, E. V. Brandt and D. Ferreira, *Die Eerste Oligomeriese Neoflavonoied. Strukuur en Sintese van Modelverbindings. PhD Thesis*, UOFS, **1993,** 43
[221] W. Gaffield, *Tetrahedron*, **1970**, *26*, 4039
[222] J. H. van der Westhuizen, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, **1981**, 1220
[223] W. Gaffield and A. C. Waiss, *Chem. Commun*, **1968**, 29

Two doublets in the ^{1}H NMR spectral data [plate 10 ($CDCl_3$ - 296K)] of compound **(91)**, allocated to 2',6'-H (δ 7.46, d, 8.5 Hz) and 3',5'-H (δ 7.16, d, 8.5 Hz) on the B-ring according to the COSY spectrum [plate 10 ($CDCl_3$ - 296K)][224,225], were confirmed by an association in the NOESY spectrum [plate 10a-1 ($CDCl_3$ - 296K)] between 2-H(C) and 2',6'-H. The remaining two *meta*-doublets were respectively assigned to the A-ring protons, 6-H (δ 6.59, d, 2.5 Hz) and 8-H (δ 6.61, d, 2.5 Hz). Assignment of the aromatic protons was, therefore, in agreement with the presence of the two deshielded acetoxy groups (δ 2.33, 2.31) and a glucosyl unit. In the same spectrum the overlapping H-3β and H-3α resonate at (δ 2.90) protons.[220, 221].

The four aliphatic acetoxy groups (δ 2.12, 2.10, 2.07, 2.06) in conjunction with the aliphatic protons displayed in the region between ~δ 3.8 and δ 5.5 suggested a 7-*O*-glucosyl substitution. Strong association of the glucosyl anomeric proton H-1" to the *meta*-coupled doublets H-6(A) and H-8(A) **(91a)** from a NOESY experiment [plate 10a-1 ($CDCl_3$ - 296K)] confirmed the substitution.

Assignment of the glucosyl protons was established from the ^{1}H NMR spectrum [plate 10 ($CDCl_3$ - 296K)] and a COSY experiment [plate 6b-2 ($CDCl_3$ - 296K)] in which the H-5" multiplet (δ 3.93) resulting from the coupling with H-6" and H-4" in the ^{1}H NMR spectrum [plate 10 ($CDCl_3$ - 296K)], of compound **(91)** was observed.

Strong couplings established the sequence H-4", H-3", H-2" and H-1", and hence, allocation of the glucosyl protons as: H-1" (δ 5.10 , d, 7.5 Hz); H-2" (δ 5.45, dd, 2.5, 9.0 Hz); H-3" (δ 5.31, t, 9.0 Hz); H-4" (δ 5.18, t, 10.0 Hz); 2x H-6" at δ 4.25, and the multiplet resonating at δ 3.93 to H-5". Association of the diaxial H-3" and 5" protons with the anomeric proton (H-1"), **(see 91a)** in the NOESY spectrum [plate 10a-2 ($CDCl_3$ - 296K)] confirmed the allocation of the proton resonances, and thus, a 7-*O*-β-D-glucopyranosylflavanone.

The ^{1}H NMR spectrum [plate 11 ($CDCl_3$ - 296K)] of flavanone **(92)**, displayed the characteristic heterocyclic spin system [H-2 (δ 5.40); H-3-α (δ 2.90); H-3β (δ 2.85)][222, 223].

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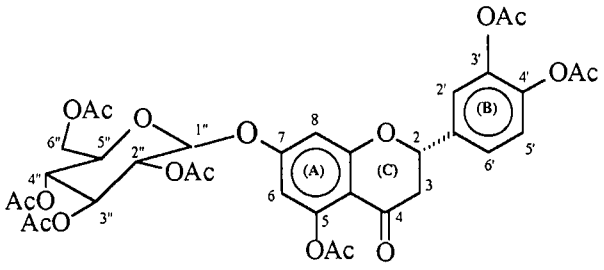
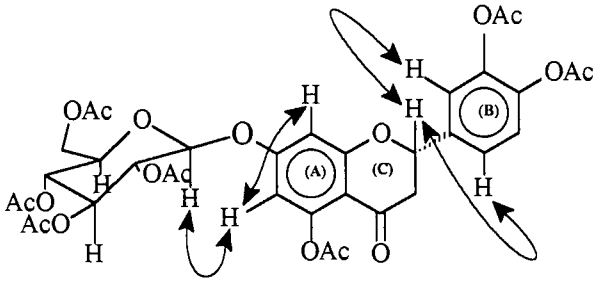
[224] B. A. Bohm, in *The Flavonoids: Advances in Research since 1980* (ed. J. B. Harborne), Chapman and Hall, **1988**, 348-372, 399

[225] E. Wollenweber and M. Jay, in *The Flavonoids: Advances in Research since 1980* (ed. J. B. Harborne), Chapman and Hall, **1988**, 233

An aromatic AA'BB' pattern of compound **(91)** attributed to the B-ring is, however, replaced by an ABX spin system (δ 7.25, d, 8.5 Hz, H-5' and the overlapping, H-6' and H-2' at δ 7.31) in compound **(92),** where the 3' proton is substituted by an acetoxy group[220, 221]. Evidence of association between 2-H(C) and 2'-H(B) and 6'-H(B) from the NOESY spectrum [plate 11a-1 ($CDCl_3$ - 296K)] confirmed the ABX spin system. The AB system δ 6.59, d, 2.5 Hz, H-6 and δ 6.61, d, 2.5 Hz, H-8) of the 7-*O*-glucosyl substituted A-ring analogous to that of flavanone **(91)** was confirmed by NOE association in the NOESY spectrum [plate 11a-1 ($CDCl_3$ - 296K)] between the anomeric proton of the glucosyl (δ 5.09, d, 8.0 Hz) and 6-and 8-H doublets of the A-ring is thus, unequivocal confirmation of the C-1"-O-C-7 connectivity.



(92)



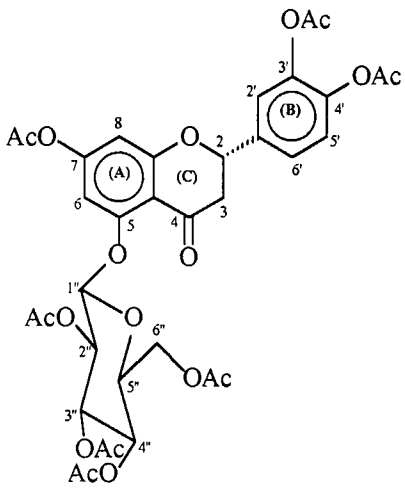
(92a)

Both compound **(92)** and **(91)** exhibited in their ^1H NMR spectra a similar proton pattern for the glucosyl unit and four aliphatic acetoxy groups.

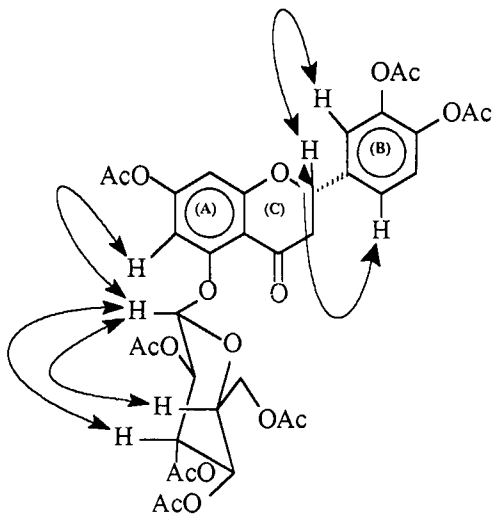
Following the same protocol as for compound **(91)** the glucosyl protons of compound **(92)** assigned from the ^1H NMR spectrum were confirmed by sequential couplings from H-1" to H-

6" in the COSY spectrum [plate 11b-1 ($CDCl_3$ - 296K)] as: H-1" (δ 5.09, d, 8.0 Hz); H-2" (δ 5.45, dd, 2.0, 9.0 Hz); H-3" (δ 5.30, t, 9.0 Hz); H-4" (δ 5.17, t, 9.0 Hz); H-5" (δ 3.93, m); 2x H-6" (δ 4.25) and four aliphatic acetoxy groups (δ 2.12, 2.09, 2.07, 2.06). Once again strong association of the H-3" and H-5" protons to H-1" in the NOESY spectrum [plate 11a-2 ($CDCl_3$ - 296K)] confirmed a β-D-glucopyranosyl configuration.

Flavanone **(93),** ^{1}H NMR spectrum [plate 12 ($CDCl_3$ - 296K), a 5-*O*-glucosyl isomer of compound **(92)** displays flavanone the characteristic flavanone system [H-2 and H-3, overlapping (δ 2.73-2.92)][220,221], an ABX system attributed to 3',4'-di-acetoxy substituted B-ring analogous to the B-ring of **(92)**, and an AB system (H-6, δ 6.36 , d, 2.5 Hz and H-8, δ 6.19, d, 2.5 Hz) whose chemical shifts differ significantly from those of compound **(92).** Coupling of the glucosyl unit *via* 5-OH(A) follows from the NOE association [plate 12a $CDCl_3$ - 296K)], between the *m*-doublet 6-H(A)(δ 6.37) and the anomeric proton H-1" of the glucosyl unit (see **93a**). Three deshielded and four aliphatic acetoxy groups displayed in ^{1}H NMR spectrum [plate 12 $CDCl_3$ - 296K) of compound **(93)** were also analogous to those of compound **(92)**. Arrangement of the glucosyl protons is the same as in compound **(92)**, NOESY spectrum [plate 12a $CDCl_3$ - 296K)], **(92a).** Thus, compound **(92)** is a 5-*O*-glucosylflavanone.



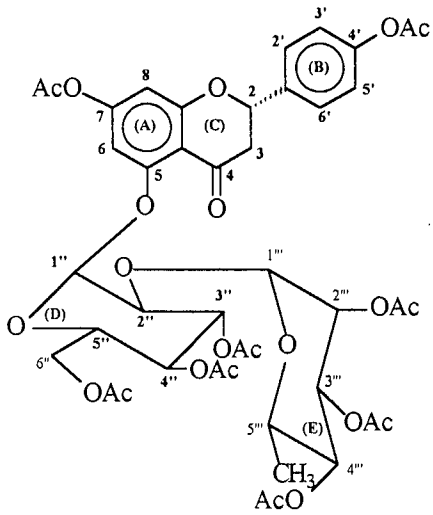
(93)



(93a)

14.3. Di-glycosylated flavanones

Flavanone **(94)**, the ^{1}H NMR spectrum [plate 13 ($CDCl_3$ - 296K)] of which exhibited the flavanone C-ring protons [H-2 (δ 5.45); H-3α (δ 3.01); H-3β (δ 2.78)], and an aromatic AA'BB' pattern (δ 7.48, d, 8.5 Hz, H-2',6' and δ 7.17, d, 8.5 Hz, H-3',5') attributed to the B-ring[220,221], also displayed the *meta*-coupled doublets respectively assigned to the A-ring protons, 6-H (δ 6.45, d, 2.5 Hz) and H-8 (δ 6.57, d, 2.5 Hz)[220,221]. Once again confirmation of the B-ring pattern was by the association of 2-H-(C) with H-2' and H-6' in the NOESY spectrum [plate 13a-1 ($CDCl_3$ - 296K)]. The 5-*O*-glucosyl substitution is evident from the association of anomeric H-1" and H-6(A) (δ 6.45, d, 2.5 Hz) in the same NOESY spectrum.



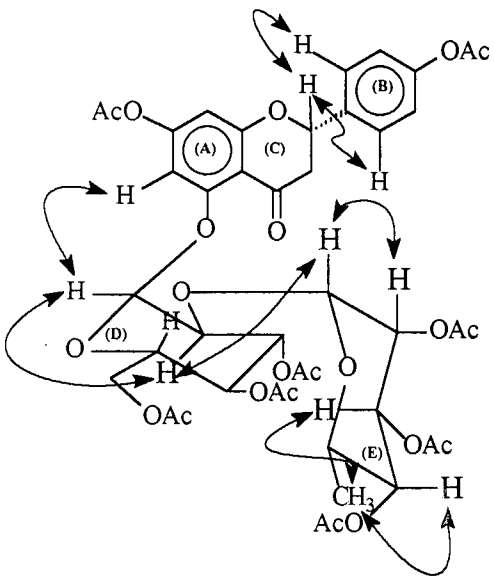
(94)

The ^{1}H NMR data further suggested the substituted 5-O-glycosyl moiety to be comprised of α-L rhamnosyl and β-D-glucopyranosyl units. While the rhamnosyl unit is evident from the NOE assocation, [plate 13a-2 ($CDCl_3$ - 296K)] of a CH_3 (δ 0.93, d, 6.0 Hz) with H-4"' and H-5"', the β-D-glucosyl (δ 5.15, d, 4.0 Hz, anomeric H) was confimed by association of the anomeric proton with the diaxial H-3'' and H-5".

The shielded doublet of doublets (δ 4.12, dd, 4.0, 7.0 Hz) assigned to H-2" of the glucosyl unit, established by couplings in the COSY spectrum [plate 13b ($CDCl_3$ - 296K)], H-1" to H-2" to H-3" suggested the C-1"'-O-C2" linkage of the sugar units. NOE association of the anomeric hydrogen, H-1'" (δ 5.13, d, 4.0 Hz) of the rhamnosyl unit (E) with the H-2" (δ 4.12,) of the glucopyranosyl unit (D) in the NOESY spectrum [plate 13a-3 ($CDCl_3$ - 296K)] confirms

a C-1'''-O-C-2" linkage of the sugar units. Weak association of H-1" (δ 5.15) with H-1''' (δ 5.13) further confirms the connectivity of the units [indicated in (**94a**)]. In addition to the above evidence, weak association of H-3" and H-1''' evident in the NOESY spectrum [plate 13a-3 ($CDCl_3$ - 296K)] substantiated the arrangement.

Two deshielded acetoxy groups (δ 2.34, 2.32) were allocated to the A- and B-rings and seven aliphatic acetoxy groups (δ 2.18, 2.13, 3x 2.07, 2.01, 1.98) and a CH_3 (0.925) were assigned to the two glycosyl moieties.



(94a)

The remaining glycosidic protons were allocated to the glucosyl and rhamnosyl units according to the strong consecutive couplings established in the COSY spectrum [plate 13b ($CDCl_3$ - 296K)]: H-1" (δ 5.15, d, 3.0 Hz); H-2" (δ 4.12, dd, 2.0, 7.0 Hz); H-3" (δ 5.31, t, 10.0 Hz); H-4" (δ 5.19, overlapping H-2'''); H-5" (δ 3.86, m); H-6" (δ 4.27, dd, 5.0, 12.0 Hz), H-6" (δ 4.18, dd, 3.0, 12.0 Hz) for the glucopyranosyl and; H-1''' (δ 5.13, d, 4.0 Hz); H-2''', 4''' overlapping at δ 5.19; H-4''' (δ 4.99, t, 10.0 Hz); H-5''' (δ 3.92, m) and a CH_3 (δ 0,93) for the rhamnosyl unit. The α-D-configuration was evident from the NOE association [plate 13a-4 ($CDCl_3$ - 296K)], of H-1" to H-2" and 5" of the glucosyl unit. The small coupling constant of the anomeric proton (J = 2.0 Hz) [cf. **(88)**], indicative of the α-configuration is evident in the NOESY spectrum [plate 13a-4 ($CDCl_3$ - 296K)], where H-1" shows an association with H-2".

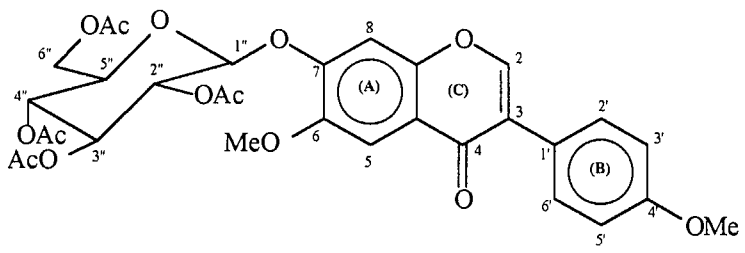
CHAPTER 15

ISOFLAVONES

15.1 Introduction

Isoflavones (**62, 63**) were isolated after the acetylation of fractions **C-8** and **9** of the methanol extract by means of PLC chromatography as colourless oils. The ^1H NMR spectra [plates 14 and 15 ($CDCl_3$ - 296K)] displayed singlets between δ 7.8 and 8.2 which is a characteristic of the 2-H resonances of isoflavones[6].

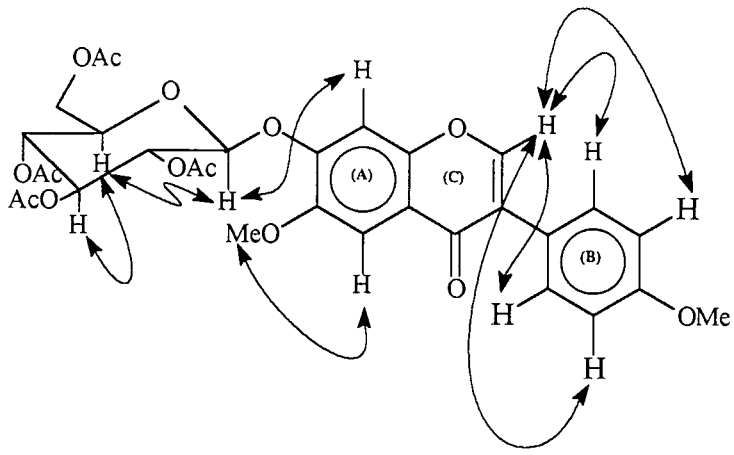
15.2. Isoflavones with a monosaccharide unit



(95)

The ^1H NMR spectrum of isoflavone **(95)** [plate 14 ($CDCl_3$ - 296K)] displayed the such a 2-H(C)[7,1] singlet at δ 7.97. An AA'BB' spin system (δ 7.52, d, 8.5 Hz, 2'-,6'-H and δ 7.00, d, 8.5 Hz, 3'-,5'-H) was attributed to the B-ring based on an association observed between 2-H(C) and 2',6'-H(B) **(95a)** in the NOESY spectrum [plate 14a-1 ($CDCl_3$ - 296K)]. Coupling of 4'-OMe (δ3.87) coupling to the 3',5'-H(B) in the COSY spectrum [plate 14b-2 ($CDCl_3$ - 296K)] confirmed the B-ring allocations.

Confirmation of the A-ring methoxy substitution was by the same COSY spectrum [plate 14b-2 ($CDCl_3$ - 296K)] which showed the coupling between the 6-OMe (δ 3.95) and 5-H(A) (δ 7.69), which is anisotrophically desheilded owing to its peri-position to the carbonyl group.



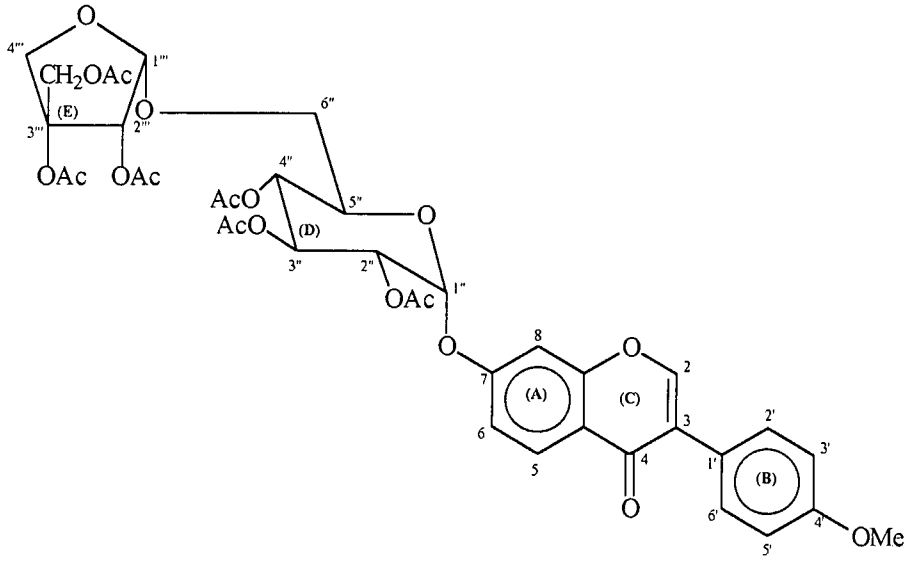
(95a)

In the ^1H NMR spectrum [plate 14 ($CDCl_3$ - 296K)], four aliphatic acetoxy groups (δ 2.14, 2.11, 2.06, 2.07) were assigned to the glucosyl unit, whose protons were assigned as follows: H-1", δ 5.14, d, 8.0 Hz; H-2",H-3" δ 5.37, m; H-4", 5.20, t, 8.0, Hz; H-5", δ 3.90, m, and the overlapping 2x H-6" (δ 4.35-4.20). Subsequent couplings indicated in the COSY spectrum [plate 14b-1 ($CDCl_3$ - 296K)] confirmed the assignment. NOE association of *cis*-diaxial association of H-1" to both H-3" and H-5" [plate 14a-2 ($CDCl_3$ - 296K)] once again confirms a β-D-glucopyranosyl configuration. Compound **(95)** was thus found to be 7-O-*β*-D glucopyranosyl-4',6-dimethoxyisoflavone, based on the above information in conjunction with H-2, whose chemical shift (δ 7.97).is in line with those of isoflavones and not with H-3 of flavones [plate 4a-2 $CDCl_3$ - 296K)].

15.3 Isoflavones with a disaccharide unit

The ^1H NMR spectrum of isoflavone **(96)** revealed the same characteristic singlet at δ 8.06 [2H(C)][6]. Its spectrum [plate 15 ($CDCl_3$ - 296K)] showed a 4'-*O*-methoxy (δ 3.86) substituted B-ring similar to the B-ring of **(95)** with an AA'BB' spin system δ 7.54, d, 8.5 Hz, 2'-, 6'-H and δ

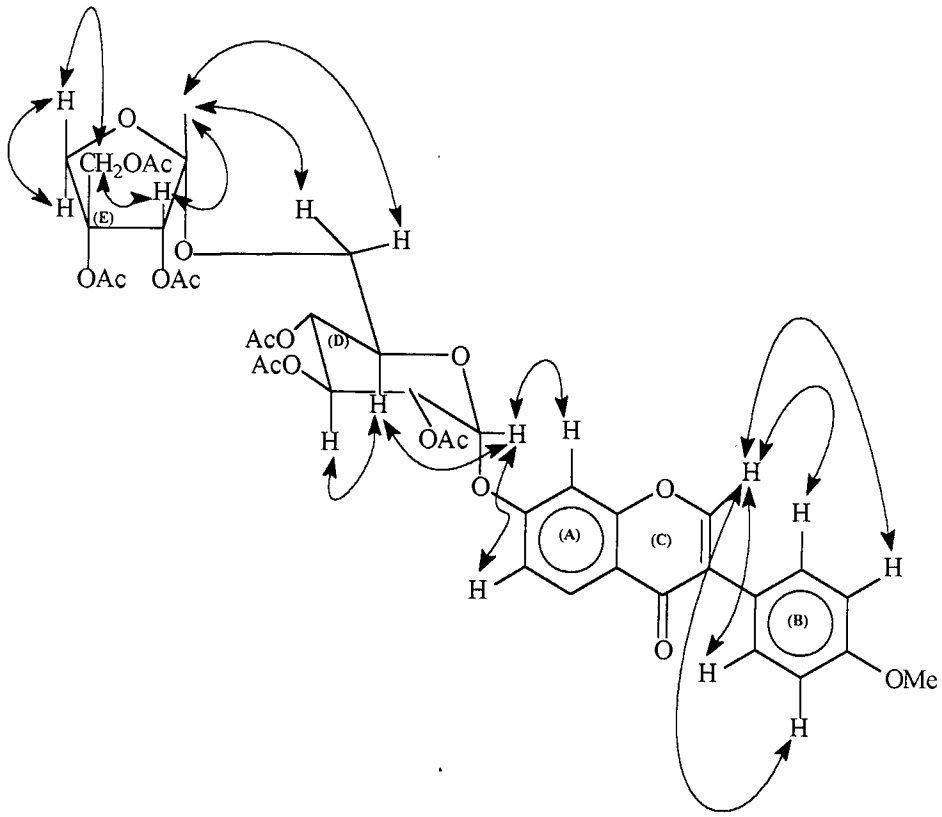
6.99 d, 8.5 Hz, 3'-,5'-H assigned to the B-ring. The NOESY experiment [plates 15a-1 and 15a-2 ($CDCl_3$ - 296K)] which showed association of 2-H(C) to 2'-,6'-H(B) and of the 4'-OMe to 3'-,5'-H in **(96a)** confirmed the B-ring allocations. The remaining ABX pattern is attributed to the 7-*O*-glucosyl substituted A-ring (δ 8.25, d, 8.5 Hz, 5-H; δ 7.05, dd, 2.5, 8.5 Hz, 6-H; δ 7.12, d, 2.5 Hz, 8-H). The substitution was confirmed by means of NOE association [plates 15a-2 ($CDCl_3$ - 296K)] between the anomeric proton H-1" (δ 5.23, d, 8.0 Hz) and the 6- and 8-H(A) protons. H-5 is characteristically deshielded by anisotropy of the carbonyl group (δ 8.25).



(96)

Six aliphatic acetoxy groups in conjunction with aliphatic protons displayed in the region δ 3.5-5.5 in the ^1H NMR spectrum [plate 15 ($CDCl_3$ - 296K)] establish the presence of a glucosyl and a furanosyl ring in compound **(96)**. The very close resemblance of the sugar protons in the ^1H NMR spectral data [plate 15 ($CDCl_3$ - 296K)] of compound **(96)** to that of compound **(84** and **89)** (discussed in **12.3** and **13.3)** confirmed the suggested *β*-D-glucopyranosyl and the apiofuranosyl units. MS analysis (M+, *m/z* 788), in agreement with the molecular formula of $C_{43}H_{48}O_{14}$, confirmed the structure.

The conspicuous methylene groups δ 4.84 and δ 4.67, d, 12 0 Hz attributed to H-4"', and δ 4.275 and δ 4.16, d, 10 0 Hz to 3"'-CH_2 displayed in the ^1H NMR spectrum [plate 15 ($CDCl_3$ - 296K)] characterising the apiofuranosyl ring were confirmed by the couplings in the COSY spectrum [plate 6b ($CDCl_3$ - 296K)].



(96a)

Assignment of the glucosyl protons as: (δ 5.23, d, 8.0 Hz, H-1"; δ, 5.34, m, H-2", H-3", δ 5.06, t, 10.0 Hz, H-4"; δ 3.93, m, H-5"; δ 3.63, dd, 2.5 and 11.0 Hz and δ 3.80, dd, 7.5 and 11 0 Hz, H-6" was confirmed by couplings (H-6" to H-5", H-4" to H-3" and H-3" to H-2" to H-1") observed in the COSY spectrum [plate 15b ($CDCl_3$ - 296K)].

The apiofuranosyl protons were assigned as H-1"', δ 5.02, d, 1.0 Hz; H-2"', δ 5.45, d, 1.0 Hz; 3"'-CH_2, δ 4.28, d, 10.0 Hz and δ 4.16, d, 10.0Hz; H-4"', d, 4.83, d, 12.0 Hz and 4.67, d, 12.0 Hz and confirmed by the same COSY spectrum.

Linkage of C-1" of the glucosyl moiety to C-7(O) of the A-ring was defined by the association in the NOESY spectrum [plate 15a-3 ($CDCl_3$ - 296K)] of H-1" with 6-H(A) and 8-H(A) (see **96a**), thus, confirming the proposed C-1"-O-C-7 connectivity, while association of H-1" with H-3" and H-5" confirmed the β-anomer. Linkage and arrangement of the sugar units were evident from the association of the anomeric proton of the apiofuranosyl unit (E) with the C-6" methylene protons of the glucosyl moiety (D) in the NOESY spectrum [plate 15a-3 ($CDCl_3$ - 296K)].

CHAPTER 16

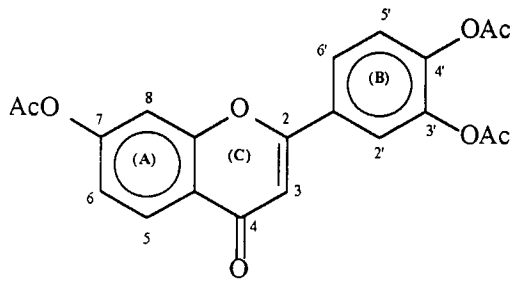
FLAVONES

16.1. Introduction

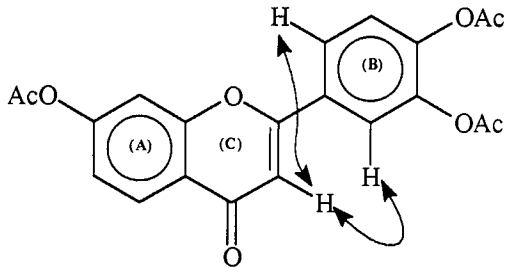
The characteristic sharp singlet of the 3-H near δ 6.3 ppm in the ^{1}H NMR and the ^{13}C NMR spectra of flavones **(97)** and **(98)** were used to characterise their structures. Acetylation followed by PLC chromatography of fraction C.8 of the methanol extract of *C.intermedia* afforded the two flavones **(97)** and **(98)** as white armophous solids.

16.2. Flavones

The ^{1}H NMR of flavone **(97)** [plate 16 ($CDCl_3$ - 296K)] displayed a characteristic residual singlet at δ 7.79, two ABX systems and three acetoxy singlets. The 7-*O*-acetoxy substituted A-ring resulted in an ABX spin system (δ 8.26, d, 8.5 Hz, H-5; δ 7.19, dd, 2.5, 8.5 Hz, H-6; δ7.44, d, 2.5 Hz, H-2). The remaining ABX pattern (δ 7.40, d, 8.5 Hz, H-5'; δ 7.81, dd, 2.5, 8.5 Hz, H-6'; δ 7.78, d, 2.5 Hz, H-2') is attributed to the 3'-4'-diacetoxy substituted B-ring. Couplings between 3-H(C) and H-2' and H-6' of the B-ring evident in the NOE experiment, [plate 16a ($CDCl_3$ - 296K)] distinguished between the A- and B-ring ABX systems.

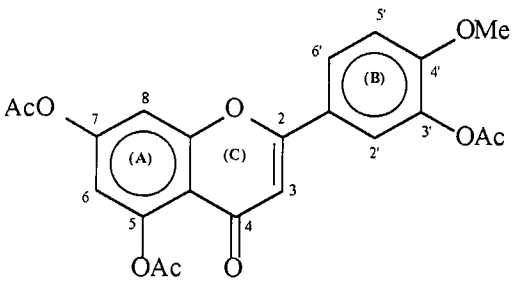


(97)

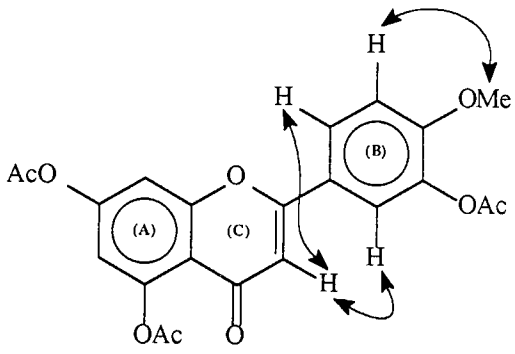


(97a)

Compound **(98)** [δ 5.75, 3-H(C)] was identified as a 5-oxygenated flavone with a ^{1}H NMR spectrum [plate 17 ($CDCl_3$ - 296K)] comprising *meta*-coupled doublets (δ 7.36, d, 2.5 Hz, H-6 and δ 7.58, d, 2.5Hz, H-8) allocated to the A-ring, an ABX system (δ 7.09, d, 8.5 Hz, H-5'; δ 7.75, dd, 2.5, 8.5 Hz, H-6'; δ 6.85, d, 2.5 Hz, H-2') attributed to the B-ring, a methoxy group (δ 3.93) and three deshielded acetoxy groups (δ 2.46, 2.38, 2.37). The B-ring substitution is defined by couplings from the 4'-OMe group (δ 3.93) to H-5'(B) in the COSY spectrum [plate 17a ($CDCl_3$ - 296K)].



(98)



(98a)

CHAPTER 17

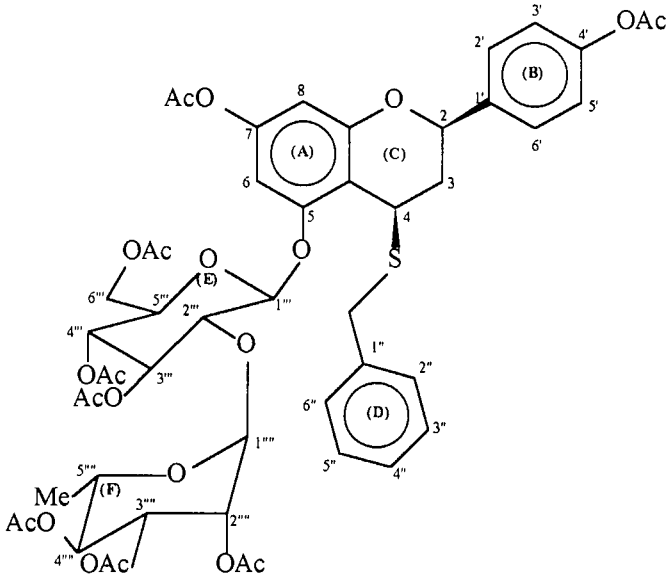
TANNIN STRUCTURE

17.1. Introduction

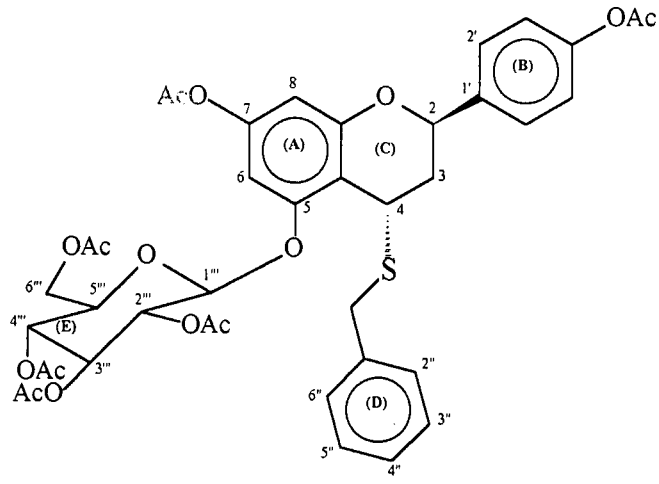
In order to determine the nature of the tannin content in Honeybush tea and the average degree of polymerisation, the crude extract was cleaved in dilute acidic medium with benzyl mercaptan and phloroglucinol as capture nucleophiles. The aromatic monomers were identified by spectrometric methods after appropriate separation and derivatisation. The most significant products with benzyl mercaptan as nucleophile were **(99)** and **(100)** which constitute flavonoid-benzyl mercaptain adducts possessing sugar units.

17.2. Benzyl mercaptan as the nucleophile

The AA'BB' and AB systems as well as the protons of the thiobenzyl unit evident in both the ^{1}H NMR spectra [plates 18 and 19 ($CDCl_3$ -296K)] of **(99)** and **(100)** indicate that a thiobenzyl moiety had been added at C-4 of the flavan unit. Association of 4-H(C) and 2-H(C) with the methylene protons of this unit observed in the NOESY spectra [plates 18a-1 and 19a-1 ($CDCl_3$ -296K)] confirmed the substitutions. Couplings of the heterocyclic protons *i.e* H-2 with two H-3 and one H-4 proton in their COSY spectra [plates 18b and 19b ($CDCl_3$ -296K)] was in line with a flavan-type C-ring.



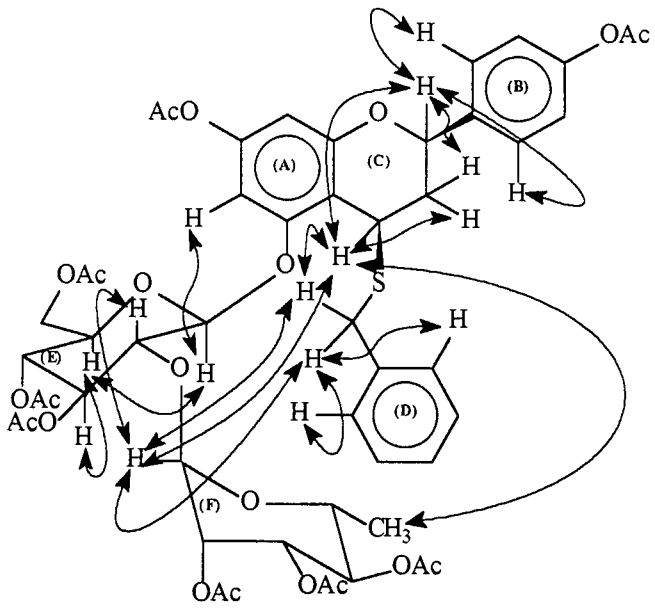
(99)



(100)

The almost identical AA'BB' spin systems, *m*-doublets, thiobenzyl groups, heterocyclic protons (2-H, 3-H, 4-H) and the two deshielded acetoxy groups displayed in both the ^{1}H NMR spectra [plates 18 and 19 ($CDCl_3$ -296K)]of compounds **(99)** and **(100)** indicate that compound **(99)** and **(100)** are analogues, only differing with respect to the glycosidic substituents. While compound **(100)** has a monosaccharide (glucose unit), compound **(99)** possessrs a disaccharide (rutinoside) unit. For proof of the absolute configuration cf. paragraph **17.4.**

The ^{1}H NMR spectrum [plate 18 ($CDCl_3$ -296K)] of **(99)** displayed an AA'BB' spin system (δ 7.35, d, 8.5 Hz, H-2',6' and δ 7.10, d, 8.5 Hz, H-3',5'), confirmed by association in a NOESY spectrum [plate 18a-2 ($CDCl_3$ -296K)] between 2-H(C) at (δ 5.52) and 2',6'-H(B). The 5-*O*-glucosyl substitution was unambiguously determined by the selective association of the anomeric proton (δ 5.13) with 6-H(A) (δ 6.33, d, 2.5 Hz), [plate 18a-1 ($CDCl_3$ -296K)] indicated in **(99a).** The remaining *m*-doublet δ 6.40 (d, 2.5 Hz) was, therefore, assigned to 8-H(A). A singlet (δ 3.86) intergrating for two protons was allocated to the two methylene protons of the thiobenzyl group, and confirmed by their association with 2",6"-H(D) [plate 18a-1 ($CDCl_3$ -296K)].

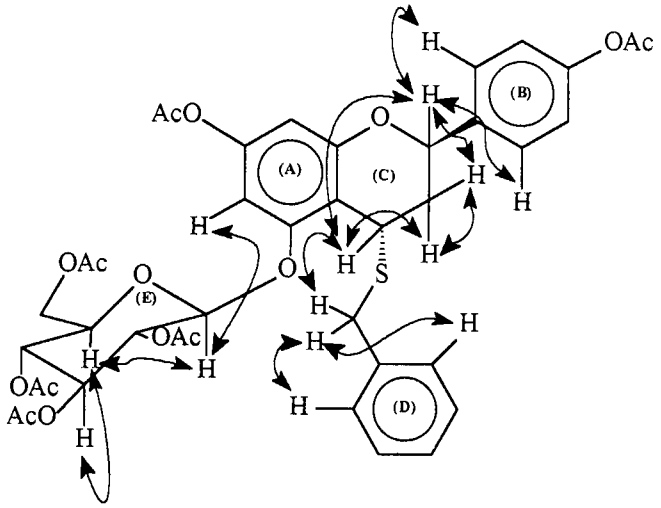


(99a)

The glycosidic protons of **(99)** were allocated from the ^{1}H NMR and COSY spectra [plate 18 and 18b ($CDCl_3$ -296K)] as: H-1''', δ 5.14, d, 7.0 Hz; H-2''', δ 3.59, dd, 2.5, 9.0 Hz; H-3''', δ 5.30, t, 9.0 Hz; H-4''', δ 5.13, t, 9.0 Hz; H-5''', δ 3.90, m; H-6''', δ 4.27, dd, 5,5, 12.0 Hz and 4.19, dd, 2.5, 12.0 Hz, to the glucosyl, and H-1'''', δ 4.69, d, 2.0 Hz; H-2'''' and H-3'''' overlapping δ 5.07; H-4'''', δ 4.96, t, 10.0 Hz; H-5'''', δ 3.71, m and the Me protons at δ 0.88 d, 6.0 Hz to the rhamnosyl. The β-D-glucosyl configuration is evident from the large coupling constant (J = 7.5 Hz) of the anomeric proton, and its association with the *cis*-diaxial

H-3" and H-5" in the NOESY spectrum [plate 18a-1 ($CDCl_3$ -296K)]. The structure is confirmed by MS analysis (M^+, *m/z* 901) in accordance with the formula $C_{50}H_{61}SO_{13}$.

The connectivity of the sugar units was confirmed by the strong association of the anomeric proton (H-1"", δ 4.69) of the rhamnosyl unit with H-2"' (δ 3.59) of the glucosyl unit in the NOESY spectrum [plate 8a-3 ($CDCl_3$ -296K)]. The spectrum further displayed association of the Me group (δ 0.88) of the rhamnosyl moiety with H-4 (δ 4.35) on the C-ring, thus confirming the proposed arrangement of the glycosidic units.



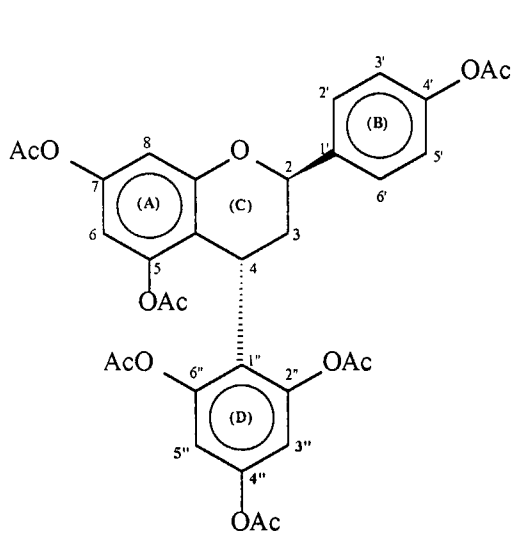
(100a)

The ^1H NMR spectrum of compound **(100)** displayed the aromatic ring (D) and methylene protons of the thiobenzyl group suggesting it to be analogous to compound **(99)**. A NOESY experiment [plate 19a-1 ($CDCl_3$ -296K)] showed similar association between 4-H(C) and -CH_2-S and confirmed the sustitution of the adduct.

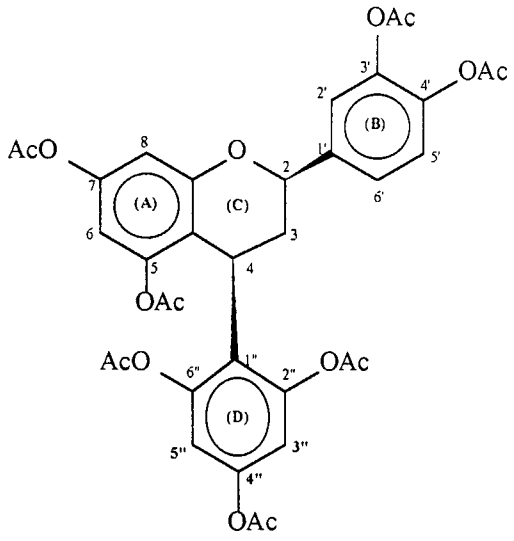
The protons of compound **(100)** were assigned in the same manner as for **(99)** see **(Table 9).** The COSY [plates 19b-1 and 19b-2 ($CDCl_3$ -296K)] and NOESY [plate 19a-1and 19a-2 ($CDCl_3$ -296K)] spectra assisted in these assignments.

17.3. Phloroglucinol as the nucleophile

In order to establish the significance of the aforementioned results, the cleavage was repeated with phloroglucinol as an alternative capture nucleophile. As expected, the flavonoid-phloroglucinol adducts **(101)** and **(102)** as well as the glucoside analogue **(103)** were identified from the reaction. The ^{1}H NMR spectra [plates 20 and 21 ($CDCl_3$ -296K)] of both compounds **(101)** and **(102)** displayed almost identical heterocyclic protons [H-2(C), H-3(C), and H-4(C)] and a two proton singlet at δ 6.83 reminiscent of the phloroglucinol D-ring protons. The spectra additionally showed an ABX spin system and an AA'BB' respectively for **(101)** and **(102)**, thus suggesting that they only differ as far as the B-rings are concerned.



(101)

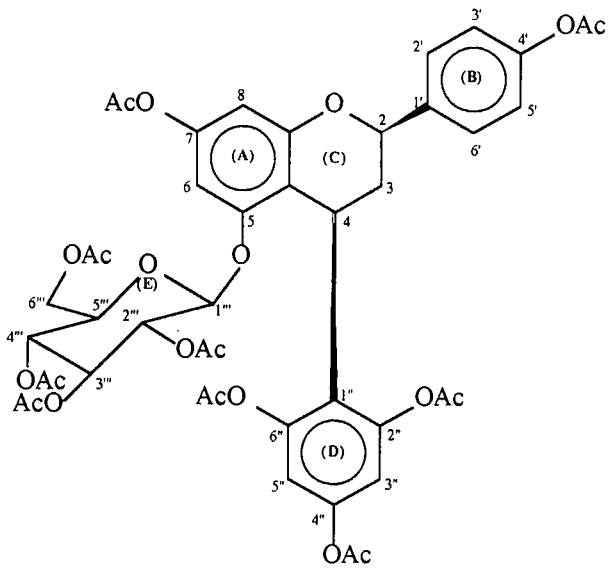


(102)

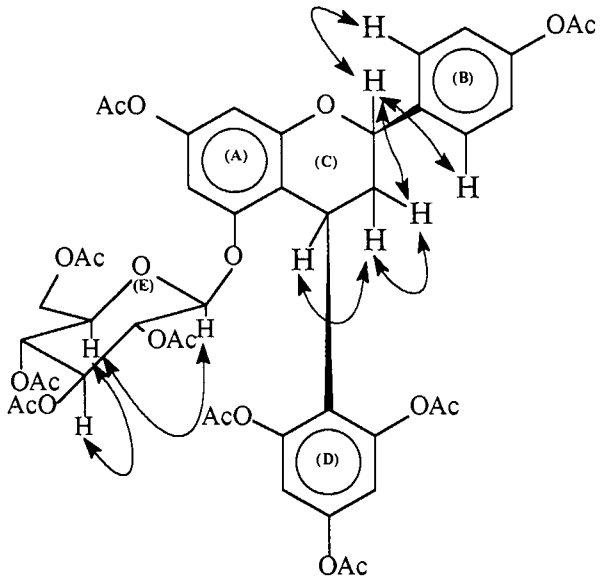
The ^{1}H NMR spectrum of compound **(101)** [plate 20 ($CDCl_3$ -296K)] displayed the heterocyclic spin system [H-2 (δ 5.1, dd, 2.5, 12.0 Hz); H-3α (δ 2.70, m); H-3β (δ 2.03, m); H-4 (δ 4.33, br. d, 5.0 Hz)], similar to those for compounds **(101)** and **(102)**. In the same spectrum, a two proton singlet at δ 6.83 was attributed to the phloroglucinol D-ring protons, and the AA'BB' pattern (δ 7.12, d, 8.5 Hz, H-2',6' and δ 7.05, d, 8.5 Hz, H-3'.5') to the B ring as in **(101)** and **(102)**. The remaining broadened two proton singlet δ 6.60, was assigned to H-6 and H-8, while five deshielded acetoxy groups (see **Table 10**) were in line with the substitution pattern.

Compound **(102)** displayed the same heterocyclic protons [2-H(C), (δ 5.12, dd, 2.5, 12.0 Hz); 3-H(C), (δ 2.69 and δ 2.02, m); 4-H(C), (δ 4.33, br. d, 5.0 H)], an [δ 6.60, d, 2.5 Hz, 6-H(A) and δ, 6.61, d, 2.5 Hz, 8-H(A)], assigned to the A-ring, two proton singlet (δ 6.83) for the D-ring and an ABX system [2'-H(B), δ 6.91, d, 2.5 Hz; 5'-H(B), δ 7.17, 8.5 Hz; 6'-H(B), δ 7.02, 2.5, 8.5 Hz] in its ^{1}H NMR spectrum [plate 21 ($CDCl_3$ -296K)]. Six deshielded acetoxyl groups **(Table 10)** were in agreement with the substitution pattern. MS analysis (M+, m/z 539), in the agreement with the molecular formular of $C_{33}H_{31}O_7$, substantiated the structure.

The ^{1}H NMR spectrum of compound **(103)** [plates 22 ($CDCl_3$ -296K)] showed the same heterocyclic protons [2-H(C), (δ 5.10, dd, 2.5, 11.0 Hz); 3-H(C), (2x δ 2.28, m); 4-H(C), (δ 4.52, dd, 3.0, 8.0, Hz)], an AA'BB' spin system [δ 7.46, d, 8.5 Hz, 2',6'-H(B) and δ 7.12, d, 8.5 Hz, 3',5'-H(B)], two sets of meta-doublets, glucosyl protons, four aliphatic- and five deshielded acetoxy groups which suggested compound **(103)** to be a glucosylated analogue of **(101)**. The 5-*O*-glucosyl was confirmed by association of the anomeric proton (δ 4.18, d, 7.0 Hz) with H-6(A) only, (δ 6.27, d, 2.5 Hz) in the NOESY spectrum [plate 23a-1 ($CDCl_3$ -296K)], which also served to distinguished the *m*-doublets of the A-ring from those of the phloroglucinol unit (D), (δ 6.85, d, 2.5 Hz and δ 6.72, d, 2.5 Hz). The aromatic protons were confirmed by the couplings in the COSY spectrum [plate 23b-1 ($CDCl_3$ -296K)]. Association of H-2',6'(B) with H-2(C) confirmed the B-ring substitution.



(103)



(103a)

The glucosyl protons were allocated from the ^{1}H NMR and COSY spectra [plate 23b-2 ($CDCl_3$ -296K)] as: H-1"', δ 5.10, d, 7.0 Hz; H-2"', δ 4.76, dd, 7.0, 8.0 Hz; H-3"', δ 5.22, dd, 8.0 9.0 Hz; H-4"', δ 5.14, dd, 9.0, 10.0 Hz; H-5"', δ 3.78, m; H-6"', δ 4.32, dd, 7.0, 12.0 and H-6"', δ 3.90, dd, 2.5, 12.0 Hz. Four aliphatic- and five aromatic acetoxy groups were allocated to the glucosyl and aromatic rings respectively, **(Table 10)**.

17.4 Absolute configuration of the adducts.

CD spectra for compounds **(102)** and **(103),** (plates 41 and 42) respectively showed positive inflextion at 245 nm and a positive Cotton effect at 237.1nm, which are in accordance with a 4-β configuration and, thus, a 4*R* absolute configuration[226,227,228]. Compound **(101)** with a negative Cotton effect at 235 nm, (CD spectrum plate 40) reminiscent of a 4α-configuration was assigned as 4*S* absolute configuration. A phase NOESY experiment of **(103)** [plate 22a-2 ($CDCl_3$ -296K)] showed association between 2- and 4-H(C), hence indicating 2,4-*cis* relative

[226] H. van Rensburg, P. J. Steynberg, J. F. W. Burger P. S. van Heerden and D. Ferreira, *J. Chem. Res. (s)*, **1999**, 451

[227] A. L. Tökés, M. Rárosi and R. Bognár, *Tetrahedron*, **1973**, *29*, 909

[228] J. J. Botha, D. A. Young, D. Ferreira and D. G. Roux, J. Chem. Soc. Perkin Trans. 1, 1980, 1214

NOE association between 2- and 4-H(C) in compound **(101)** was interpreted as a 2,4-*trans* relative configuration of its C-ring[229].

Thus, compounds **(101), (102), and (103)** were respectively assigned 2*R*,4*S*, 2*R*,4*R*, and 2*R*,4*R* absolute configuration.

NOE association of H-2 with H-4 [plate 18a-2 ($CDCl_3$ -296K)] for compound **(99)** was interpreted as 2,4-*cis* relative configuration, while absence of the same effect in the compound **(100)** was interpreted as a 2,4-*trans* relative configuration. In the absence of established CD correlations, (plates 38 and 39) for the thiobenzyl adducts, absence of absolute configuration for compounds **(99** and **100)** could be concluded tentatively, based on the above assignments. Compound **(99)** was assigned a 2*R*,4*R* absolute configuration,and **(100)** a 2*R*,4*S* configuration.

Although no 'chain terminating units' were identified hence precluding a proposal of a possible structure for the 'tannin', the aforementioned results indicate that we are dealing with a unique tannin-type in *Cyclopia intermedia*. These deficiencies will be addressed in a follow up investigstion. To our knowledge, the 4-arylflavans **(101, 102**, and **103)** as well as the 4-thiobenzylflavans (**99** and **100**) are new compounds. This remains, however, to be substantiated, possibly by reductive cleavage (*e.g.* with Rancy-Nickel) of the thioester bond to yield the flavan which could be compared to known reference compounds.

[229] J. Coetzee, Lmciteka, E. Malan and D. Ferreira, *Phytochemistry*, **1999**, *52*, 737

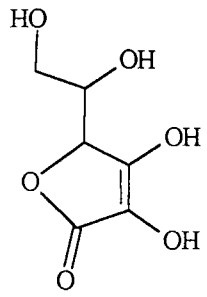
CHAPTER 18

FLAVONOIDS AS 'SCAVENGERS OF ACTIVE OXYGEN SPECIES'

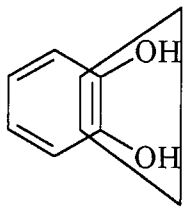
18.1. Introduction

Honeybush herbal tea is rapidly gaining popularity as a health beverage. In our investigations on the plant we have demonstrated the presence of phenolic compounds such as flavonols, flavanones, and flavones as well as their glycosides. These compounds presumably contribute significantly towards the scavenging effects of the 'active oxygen spiecies'[155,170,174]. However, the role and mechanism of flavonoids, with 3',4'-dihydroxy- functionality on their B-ring, acting as scavengers of 'active oxygen spiecies' are not yet fully understood.

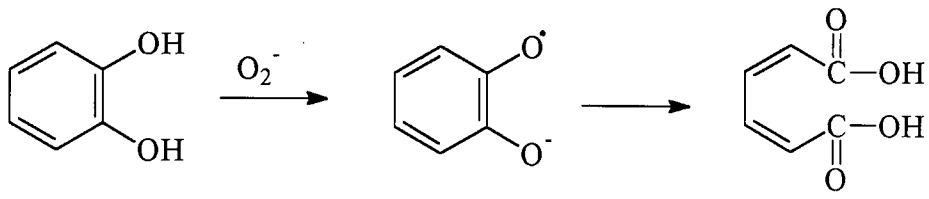
In previous investigations, the reaction between 3',4'-dihydroxyflavonoids, *e.g* quercetin (a flavonol) and super oxide ($O_2^{\bullet -}$) was envisaged to proceed *via* two routes[155,175]. A recent investigation demonstrated that flavonols with their enolic functionality of the C-ring are oxidised through a series of steps to aromatic acids as the final products[170]. The 3-OH is a prerequisite for this transformation. The second approach to the study of super oxide neccessitated the presence of the enediol functionality on the B-ring[174]. Catechol **(105)** with the enediol functionality has been used to mimic the course of the reaction in relation to the naturally occurring powerful O_2 scavenger, vitamin C **(104)** which exhibits the same enediol functionality. The mechanism involves the formation of semiquinone which yields mucoic acid through oxidative ring opening (**Scheme 9**). As a parallel, it has been claimed that all flavonoids with 3',4'-dihydroxy functionality of their B-rings quench'active oxygen species'.



(104)



(105)

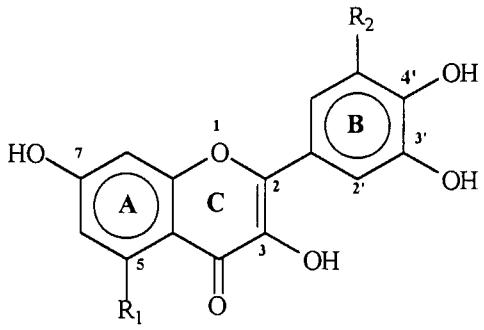


(105) (106) (106)

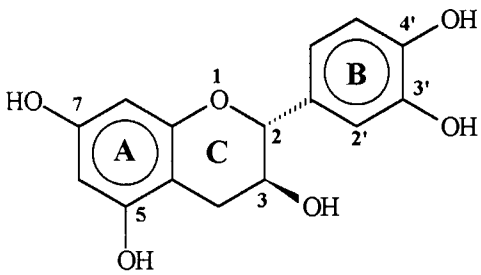
Scheme 9

18.2. Flavonoids with 3',4'-dihydroxy functionality

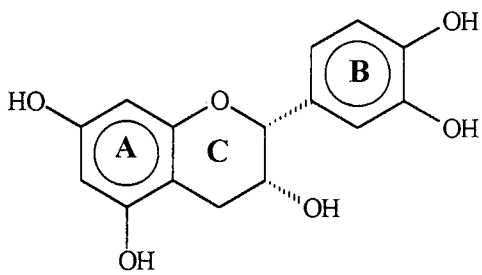
In an attempt to verify the claims and to explore the radical nature of $O_2^{\bullet-}$, we carried out a series of reactions of super oxide (KO_2) with the flavanoids of varied hydroxylation patterns but with the 3',4'-dihydroxy functionality as a common denominator. These included 5-hydroxylated flavonols [quercetin **(109)** and myricetin **(110)**] and 5-deoxy flavonols fisetin **(111)** and robinetin **(112)**]. Catechin **(113)** and epicatechin **(114)** were selected as representative flavan-3-ols.



$R_1 = OH, R_2 = H$	quercetin	**(109)**
$R_1 = R_2 = OH$	myricetin	**(110)**
$R_1 = R_2 = H$	fisetin	**(111)**
$R_1 = H, R_2 = OH$	robinetin	**(112)**



(113)



(114)

18.2.1 Dimethyl sufoxide as a solvent

Super oxide ($O_2^{\bullet -}$) was conveniently generated by stirring finely powdered KO_2 in dry dimethyl sulfoxide at room temperature for 30-45 minutes[230]. Treatment of selected flavonoids with a suspension of KO_2 in DMSO at room temperature resulted *inter alia* in the unique process of C-3' deoxygenation of the B-ring [plates 23a and 27a ($CDCl_3$ - 296K)]. Other products included an aldehyde, [plate 23b ($CDCl_3$ - 296K)] a carboxylic acid [plate 23c ($CDCl_3$ - 296K)] and a ring methylated analogue [plate 23d ($CDCl_3$ - 296K)], **Table 11**. In cases where the flavanoids were optically active, racemization also occurred, **Table 12**.

[230] E. J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, **1965**, *87*, 1354

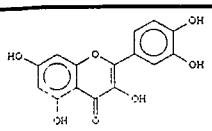
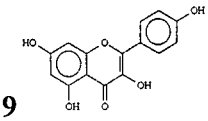
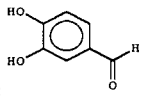
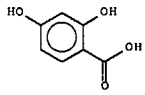
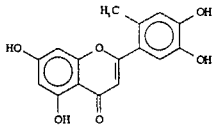
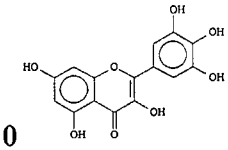
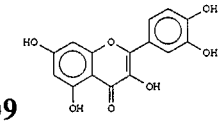
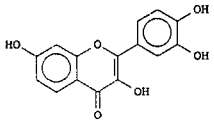
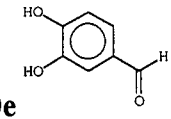
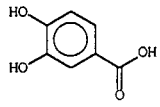
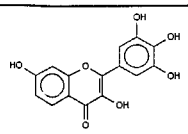
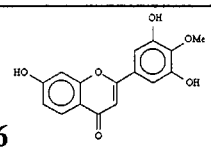
Flavonol	Results	Plates of peracetate derivatives	Yield %
 **109**	 **119**	**119-** Kaempferol **plate 23a ($CDCl_3$ - 296K)**	15.0
	 **120e**	**109b-** 3,4-Dihydroxybenzaldehyde **plate 23b ($CDCl_3$ - 296K)**	10.4
	 **120g**	**109c** 2,4-Dihydroxybenzoic acid **plate 23c ($CDCl_3$ - 296K)**	1.8 6.4
	 **125**	**109d** 2'-Methylquercetin **plate 23d ($CDCl_3$ - 296K)** Starting material **plate 23 ($CDCl_3$ - 296K)**	47.6
 **110**	 **109**	**110**-Quercetin **plate 24a ($CDCl_3$ - 296K)** Starting material **plate 24 ($CDCl_3$ - 296K)**	19.6 56.0
 **111**	 **120e**	**109b** 3,4-Dihydroxybenzaldehyde **plate 23a ($CDCl_3$ - 296K)**	6.0
	 **120g**	**109c** 3,4-Dihydroxybenzoic acid **plate 23b ($CDCl_3$ - 296K)** Starting material	3.0 16.0
 **112**	 **126**	**112a** 4'-*O*-Methylrobinetin **plate 25a ($CDCl_3$ - 296K)** Starting material **plate 25 ($CDCl_3$ - 296K)**	3.6 47.8

Table 11: Products from the super oxide reactions of the flavonols

Since it has been found that $O_2^{\bullet -}$ may act as a reducing agent[131], it is believed that methylsulfinyl carbanion ($CH_3SOCH_2^-$) can be generated from DMSO in the presence of $O_2^{\bullet -1}$. As anticipated, the reaction of finely powdered KO_2 with dry DMSO at room temperature yielded a methylsulfinyl carbanion. This methylsulfinyl carbanion is renowned for its strong basic and nucleophilic abilities.

$$O_2^{\bullet -} + CH_3SOCH_3 \longrightarrow HO_2^{\bullet} + CH_3SOCH_2^-$$

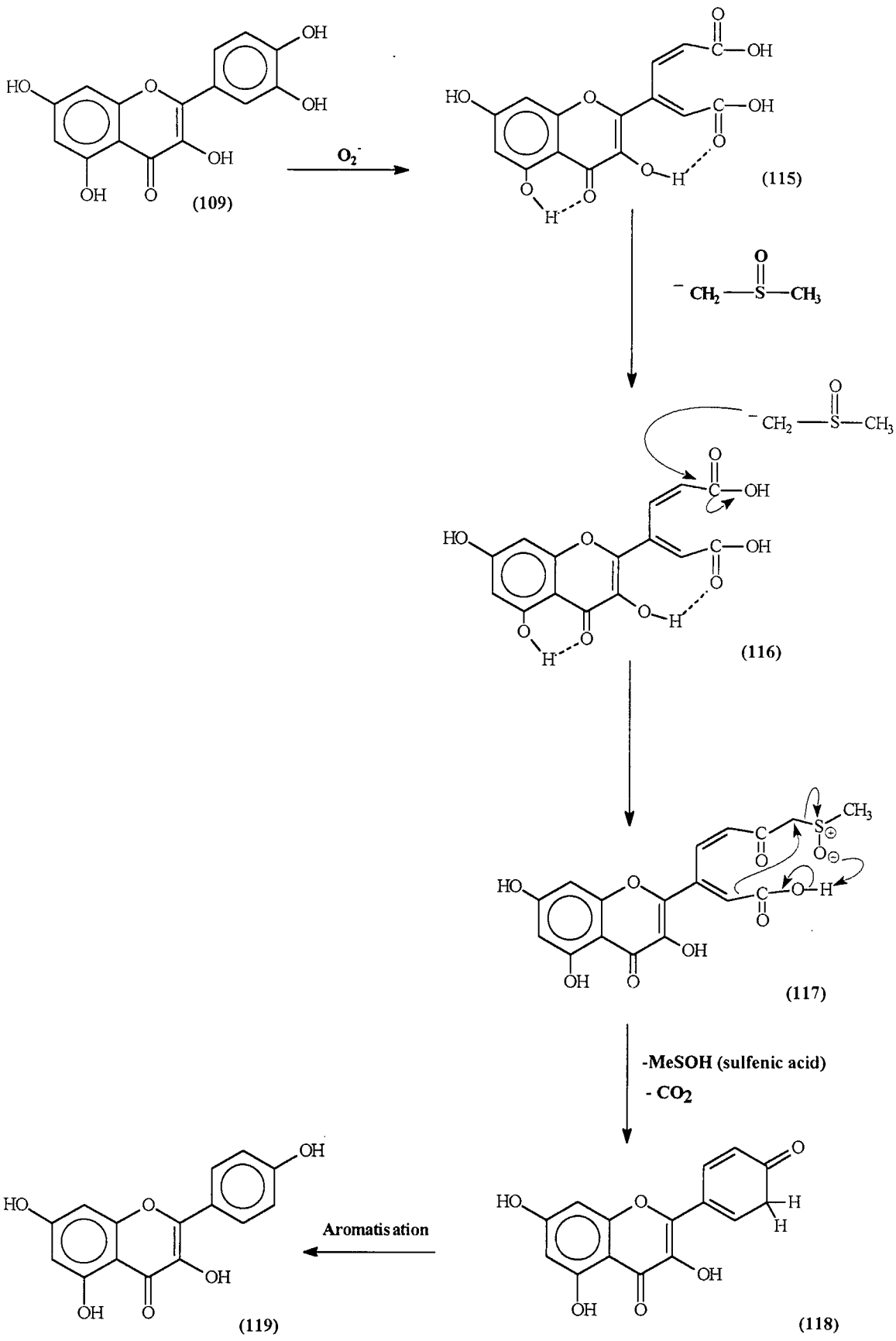
EQUATION 8

$$HO_2^{\bullet} + O_2^{\bullet -} \longrightarrow HOO^- + O_2^{\bullet -}$$

EQUATION 9

Based on the existing information, two possible routes for the course of the reaction were proposed.

The mechanism of the reaction **(Scheme 10)** is based upon the fact that under strong oxidising conditions, the B-ring cleaves to yield a dicarboxylic acid (in a similar manner to catechol) **(Scheme 9).** The dicarboxylic acid is susceptible to nucleophillic attack to give an intermediate sulfoxide **(117).** Concerted loss of carbon dioxide and methyl sulfenic acid would then lead to intermediate **(118)** which would undergo aromatization leading to the formation of kaempferol (**119**), [plate 23a ($CDCl_3$ - 296K)] with its C-3'deoxygenated B-ring as compared to that of quercetin. The consecutive steps of decarboxylation, desulfenylation and aromatisation would provide a powerful driving force for the conversion of **(117)** → **(119)**.


(109)
$O_2^{\cdot}$
(115)
$^{-}CH_2—S(=O)—CH_3$
(116)
(117)
-MeSOH (sulfenic acid)
- CO_2
(118)
Aromatisation
(119)

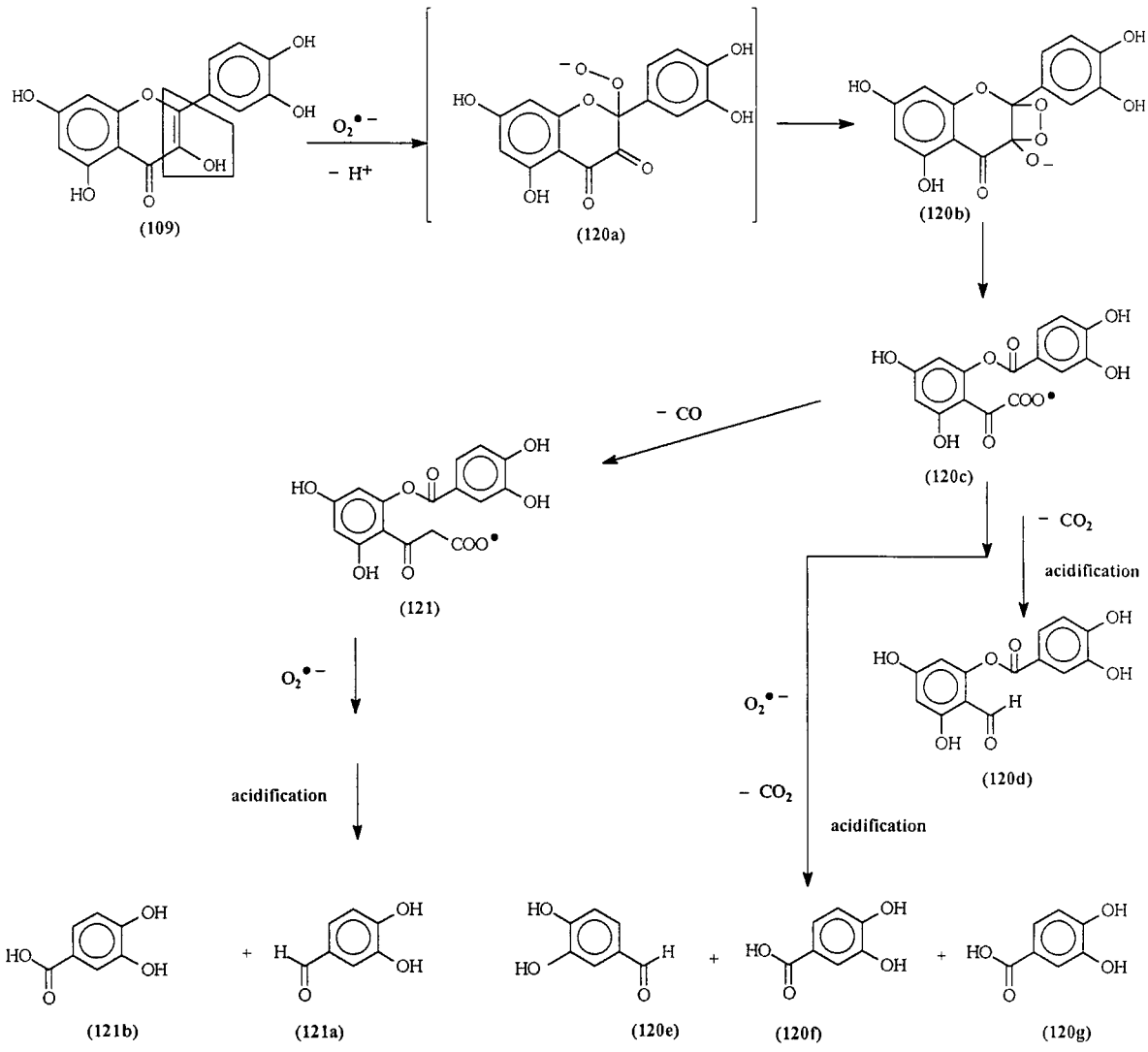
Scheme 10

Quercetin when treated with methylsulfinyl carbanion (prepared from NaH and DMSO) undergoes deoxygenation of the B-ring, to yield kaempferol [plate 23a ($CDCl_3$ - 296K)], thus, confirming the proposed mechanism in **Scheme 10**, as well as **Equation 8**.

18.2.2 Flavonoids with the C-ring enolic functionality

It is anticipated that the rest of the products *i.e.* aromatic acids (**120f**) and (**121a**) [plate 23c ($CDCl_3$ - 296)], and aldehydes **(120e)** and **(121b)** [plate 23b ($CDCl_3$ - 296)] were formed *via* the already established mechanism **(Scheme 11).** Since the products from mechanisms in **Schemes 10** and **11** are isolated from a single reaction, it is evident that the deoxygenation on the B-ring takes place concurrently with the oxidation at the enol functionality on the C-ring.

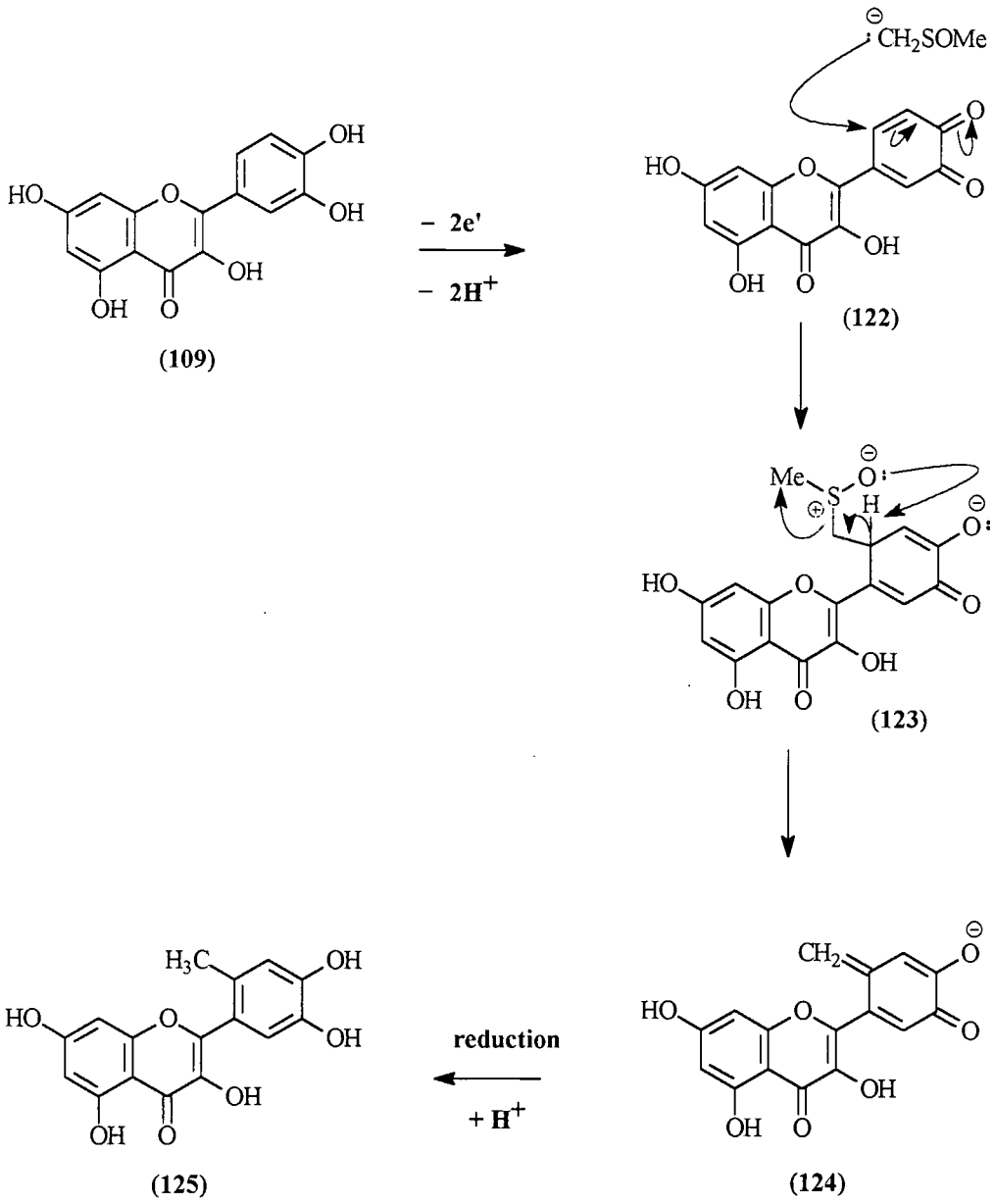
While the first mechanism **(Scheme 10)** explains the results from the reaction of super oxide with the 5-hydroxy flavonols posessing a 3',4'-dihydroxy- functionality [quercetin (**109**) and myricetin (**110**)], the remainder of the products from quercetin **(109),** *i.e* an aldehyde [plate 23b ($CDCl_3$ - 296)] and a carboxylic acid [plate 23c ($CDCl_3$ - 296)], identical to those from fisetin **(111)**, are mechanistically explained in **Scheme 11**. Ring methylation for quercetin [plate 23d ($CDCl_3$ - 296)] is explained in **Scheme 12,** (see **Table 11**).



Scheme 11

18.2.3 Selective methylation

The nature of the reactants and products in the methylation reaction suggested the possibility of a nucleophilic aromatic substitution. In exploring the mechanistic possibilities for the reaction, methylation is attributed to the strong nucleophillic abilities of the methylsulfinyl carbanion, presumably on an intermediate α-diketone (**122**) (**Scheme 12**). This 1,4-Michael - type of addition would then lead to the intermediate sulfoxide which would eliminate methane sulfenic acid to give the *p*-quinone methide (**123**). Reduction would then lead to the 'methylated' product (**124**) *via* a mechanism that is not fully understood.



Scheme 12

Of even greater significance is the findings that flavonols lacking the 5-OH did not show dehydroxylation at C-3' on their B-ring but, underwent selective methylation on the B-ring in the case of robinetin (**112**) to give 4'-*O*-methylrobinetin [**(126), Table 11**] *via* a mechanism which is yet to be sorted out. On the other hand, fisetin (**111**) gave, an aldehyde [plate 23b ($CDCl_3$ - 296)], and a carboxylic acid [plate 23c ($CDCl_3$ - 296)] as in **Scheme 11.** This clearly reflects the importance of the hydrogen bonds involving both the 3- and 5-OH functions depicted in **Scheme 10**.

18.3 Reaction of superoxide with the flavan-3-ol epimers catechin and epicatechin

Deoxygenation of the B-ring was further investigated with the 3',4'-dihydroxyflavan-3-ols. In comparison with the flavonols, reactions of the flavan-3-ols with super oxide in dry DMSO, where a C-4 carbonyl group is absent, may depend on the stereochemistry of the substrate. Epicatechin, a 2,3-*cis* isomer, afforded deoxygenation on the B-ring [plate 27a ($CDCl_3$ - 296)] while catechin, a 2,3-*trans*, isomer resisted deoxygenation. Both catechin and epicatechin afforded corresponding epimers see **Table 13**. Formation of the epimers is attributed to the lability of the O_1-C_2 bond under basic conditions. The reaction involves the opening of the heterocyclic ring to give an intermediate quinone methide which can undergo recyclisation to give the starting material or the product in which epimerisation has occurred **(Scheme 13)**.

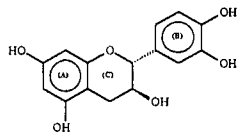
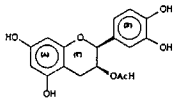
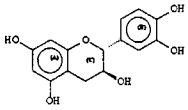
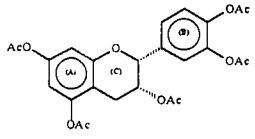
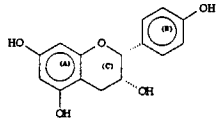
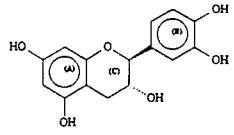
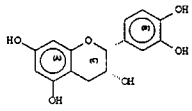
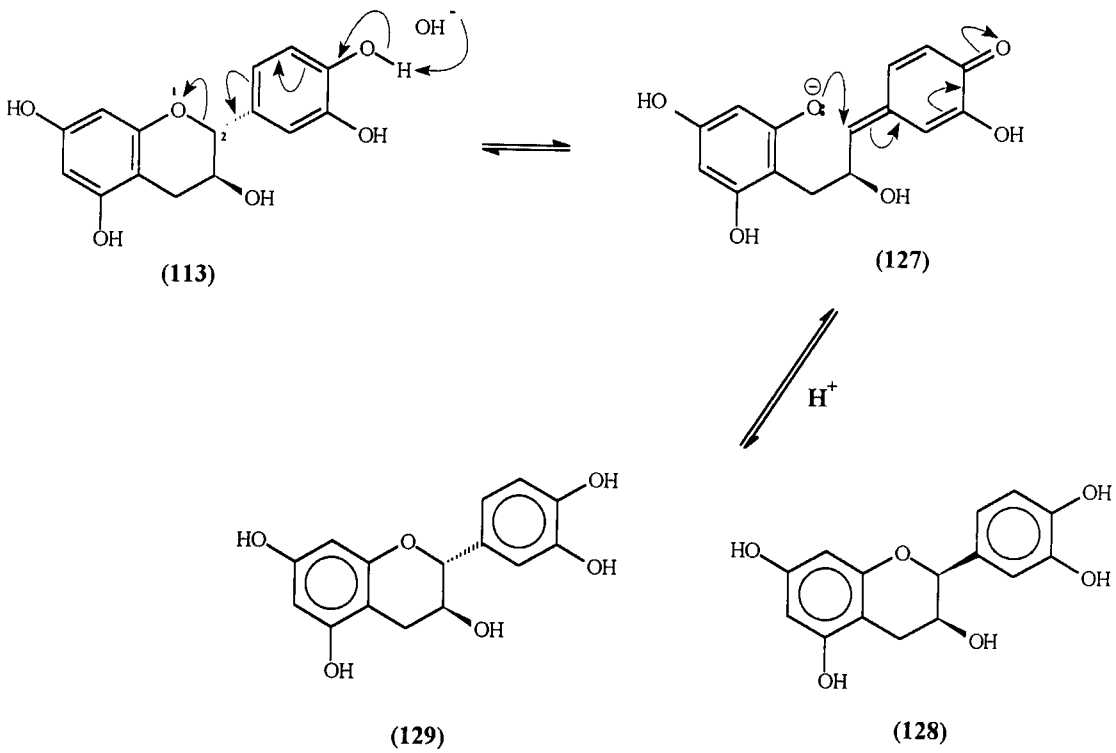
Starting material	Results	Plates of the peracetate derivatives	Yield %
 **113**	 **113a**  **113**	**113a** *ent*-Epicatechin **plate 26a ($CDCl_3$ - 296K)** **113** Starting material **plate 26 ($CDCl_3$ - 296K)**	6.1 28.6
 **114**	 **114a**  **114b**  **114**	**114a** Epiafzelechin **plate 27a ($CDCl_3$ - 296K)** **114b** *ent*-Catechin **114** Starting material **plate 27 ($CDCl_3$ - 296K)**	5.8 18.5 12.7

Table 12: Products from treatment of flavan-3-ols with super oxide



Scheme 13

Following cleavage of the B-ring **(Scheme 10)** of the flavan-3-ols a hydrogen bond is presumably formed between the carbonyl of the carboxylic acid and the 3-OH in the *cis* isomer, allowing the reaction to proceed according to the proposed mechanism to afford afzelechin [plate 27a ($CDCl_3$ - 296K)] as the 3'-deoxygenated analogue of epicatechin. This is not possible in the *trans* isomer where the hydrogen bond is not formed due to the increased distance between the 3-OH and the carboxylic acid. The C-3 OH which is cofacial with the B-ring in epicatechin **(114)** may additionally assist in stablisation of the electron deficient diene system originating from the B-ring.

The stuctures of the products were established by comparison with authentic samples. The positive Cotton effect at ~278 nm in the CD spectra (plates 44 and 47) of compounds (**113** and **114b**) was is in agreement with 2*S* configuration *cis*-, and *trans*- relative configurations being indicated respectively by ^1H NMR [cf. Plate 26 ($CDCl_3$ - 296)] ($^3J_{2,3}$= 2 0 Hz and $^3J_{2,3}$=12.0

Hz). The negative Cotton effect in the same region of the CD spectra (plates 43, 45 and 46) was in agreement with 2*R* configuration[226,231,232], *cis*- and *trans*- relative configurations once again indicated respectively in the ^{1}H NMR spectra [plates 27a ($CDCl_3$ - 296)] ($^3J_{2,3}$= 3.0 Hz and $^3J_{2,3}$= 7.0 Hz) for compounds **113a, 114** and **114a**.

It appears that the relative order of reactivity in the above reactions may be controlled by the presence of hydrogen bonding. In addition the presence of 3- and 5-hydroxy groups are prerequisites for these reactions to occur. We have thus demonstrated amongest others, a novel, versatile single step approach towards the deoxygenation of cetain di- and tri-oxygenated rings of the flavanoids.

The low yields are attributed to the many products formed in the reaction and to the highly exothermic nature of the reaction. Optimisation of conditions may probably lead to improved yields.

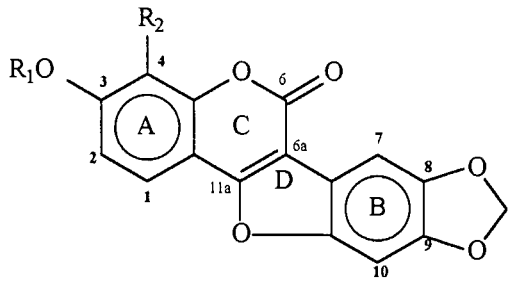
[231] O. Korver and C. K. Wilkins, *Tetrahedron*, **1971**, *27*, 5459
[232] G. Snatzke and F, Znatzke, *Tetrahedron*, **1973**, *29*, 909

CHAPTER 19

SYNTHESIS OF COUMESTANS

19.1 $Tl(NO_3)_3$ catalyzed rearrangement of chalcones

Syntheses of the three coumestans, flemichaparrin **(147)**[233], medicagol **(148)**[234] and sophoracoumestan B **(149)**[235], [plate 31-33 ($CDCl_3$ - 296) were conceived to proceed *via* a procedure commencing with the analogous chalcones **(134, 135, 136)** [plate 28-29 ($CDCl_3$ - 296). These were prepared by base catalyzed aldol-type condensation[236] of the appropriate acetophenones **(130, 131, 132)** with the benzaldehyde **(133)**, which is common to all three chalcones and accessible by formylation[237] of the methylenedioxyphenol, sesamol, followed by protection of the 2-OH by methoxymethylation.



147, R_1=Me, R_2=H

148, R_1=H, R_2=H

149, R_1=H, R_2=OMe

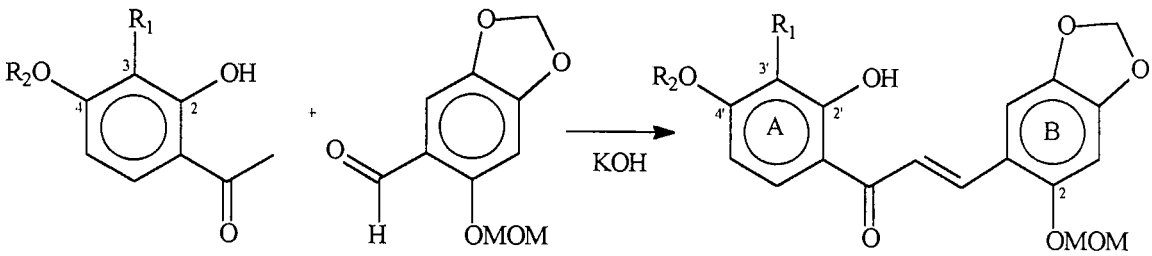
153, R_1=Ac, R_2=H

154, R_1=Ac, R_2=OMe

The acetophenones **(130)** and **(131)** were prepared by selective methylation or methoxymethylation, respectively, of resacetophenone while the third **(132)** required the

[233] D. T. Burns, B. G. Dalgrano, P. E. Gragan and J. Grimshaw, *Phytochemistry*, **1984**, *23*, 167
[234] Y. Komatsu Y. Shirataki, I. Yokoe and A. Manaka, *Chem. Pharm. Bull.*, **1981**, *29*, 532
[235] M. Komatsu, I. Yokoe and Y. Shirataki, *Chem. Pharm. Bull.*, **1981**, *29*, 2069
[236] M. Shimokoriyama, in the *Chemistry of Flavonoid Compunds*, (ed. T.A. Geissman), Pergamon Press, London , **1962**, p.308, and references therein
[237] H. Wynberg, *Chem. Rev.*, **1960**, *60*, 169

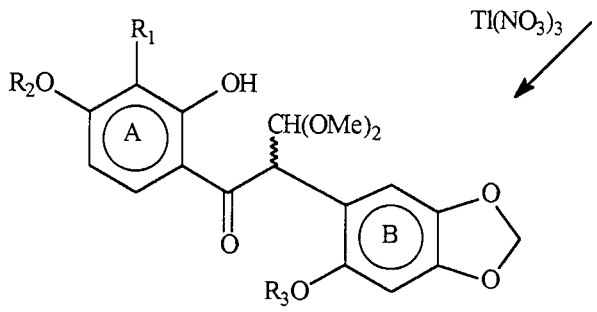
selective methylation of pyrogallol[238], Friedel-Crafts acylation of the product with $ZnCl_2/AcOH$[239] and selective protection of the acetophenone at 2-OH by methoxymethylation. The ensuing chalcones **(134, 135, 136)** were treated with $Tl(NO_3)_3/MeOH$[240] to yield respectively the intermediate acetal-type diaryl-propanones **(137, 138, 139)** by oxidative rearrangement **(Scheme 14)**.



130, R_1 = H ; R_2 = Me
131, R_1 = H ; R_2 = MOM
132, R_1 = OMe ; R_2 = MOM

(133)

134, R_1 = H ; R_2 = Me
135, R_1 = H ; R_2 = MOM
136, R_1 = OMe ; R_2 = MOM

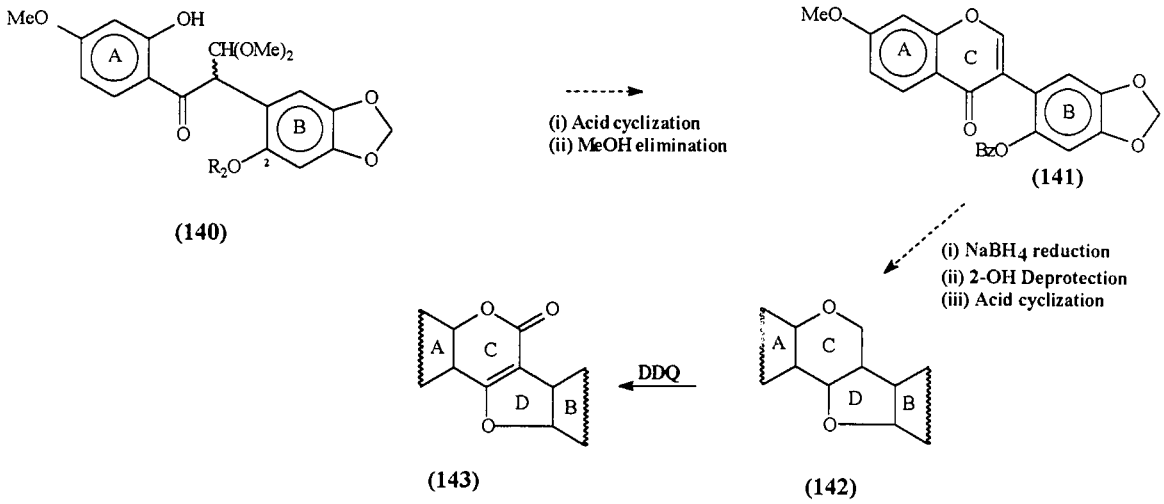


137, R_1 = H ; R_2 = Me ; R_3 = MOM
138, R_1 = H ; R_2 = MOM ; R_3 = MOM
139, R_1 = OMe ; R_2 = MOM ; R_3 = MOM

Scheme 14

[238] T. A. Geissman and W. J. Moje, *J. Am. Chem. Soc.*, **1951**, *73*, 5765
[239] R. Robinson, and R. C. Shah, *J. Chem. Soc.*, **1934**, 1491
[240] A. McKillop, B. P. Sw n and E. C. Taylor, *Tetrahedron Lett.*, **1970**, 5281

In the classic approach[241] **(Scheme 15)**, the 2-OH is protected by benzylation **(141)** and the masked aldehydes **(140)** serve as direct precursors to the isoflavones via acid-catalyzed cyclization followed by elimination of MeOH. These are then reduced by $NaBH_4$ in EtOH, deprotected at 2-OH and cyclized to form the D-ring[242] of the pterocarpan **(142)**. The pterocarpan is oxidized by DDQ[243] in benzene to the corresponding coumestan[240] **(143).**



Scheme 15

19.2 Direct chalcone - coumestan conversion

In an attempt, however, to reduce the number of steps, we anticipated that a sequence involving an acetal with free 2-OH, generated for example by the hydrolysis of a methoxymethyl group in **(144)**, should directly give the corresponding coumestan. Indeed consecutive treatment of the chalcone with $Tl(NO_3)_3$/MeOH and *aq.* acid led to a one step conversion to the coumestan. **(Scheme 16)**.

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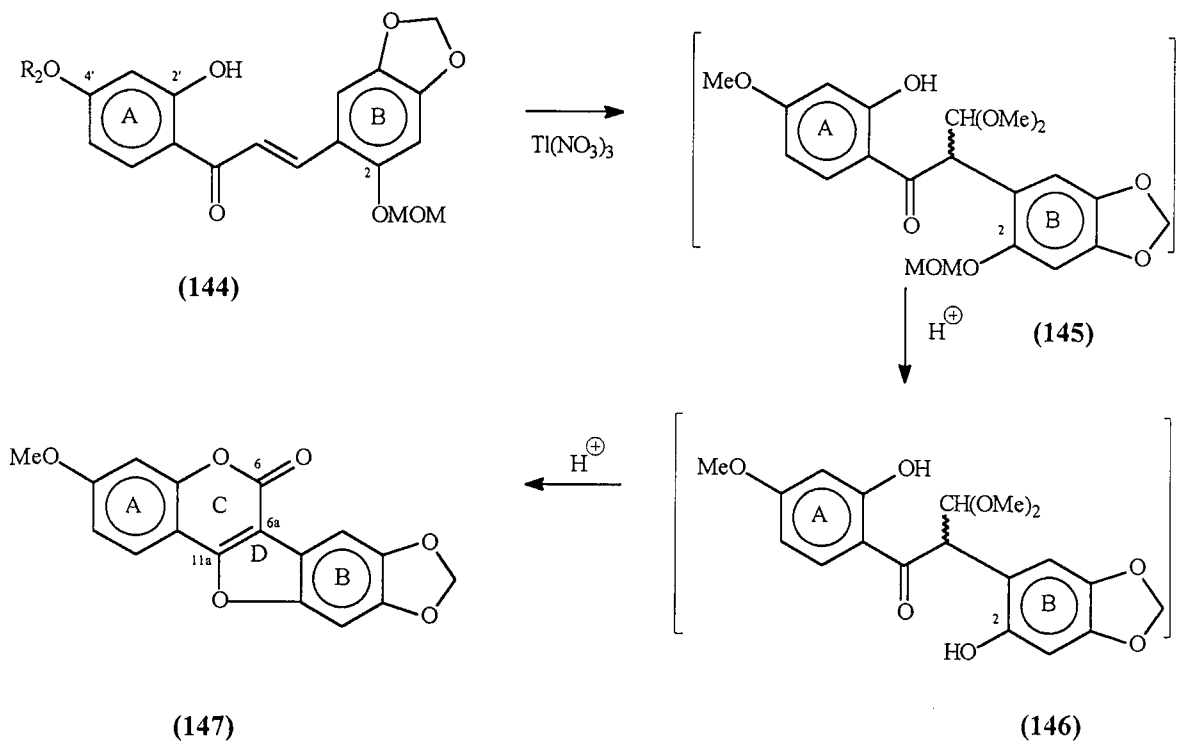
[241] L. Farkas, A. Gotsegen, M. Nogradi and S. J. Antus, *Chem. Soc., Perkin Trans. I*, **1974,** 305

[242] Z. Rappoport, L. K. Dyall, S. Winstein and W. G. Young, *Tetrahedron Lett.*, **1970**, 3483

[243] P. J. M. Gunning , P.J. Kavangh, M. J.Meegan and D. M. X. Donnelly, *J. Chem. Soc., Perkin Trans. I,* 1977, 691



(144)

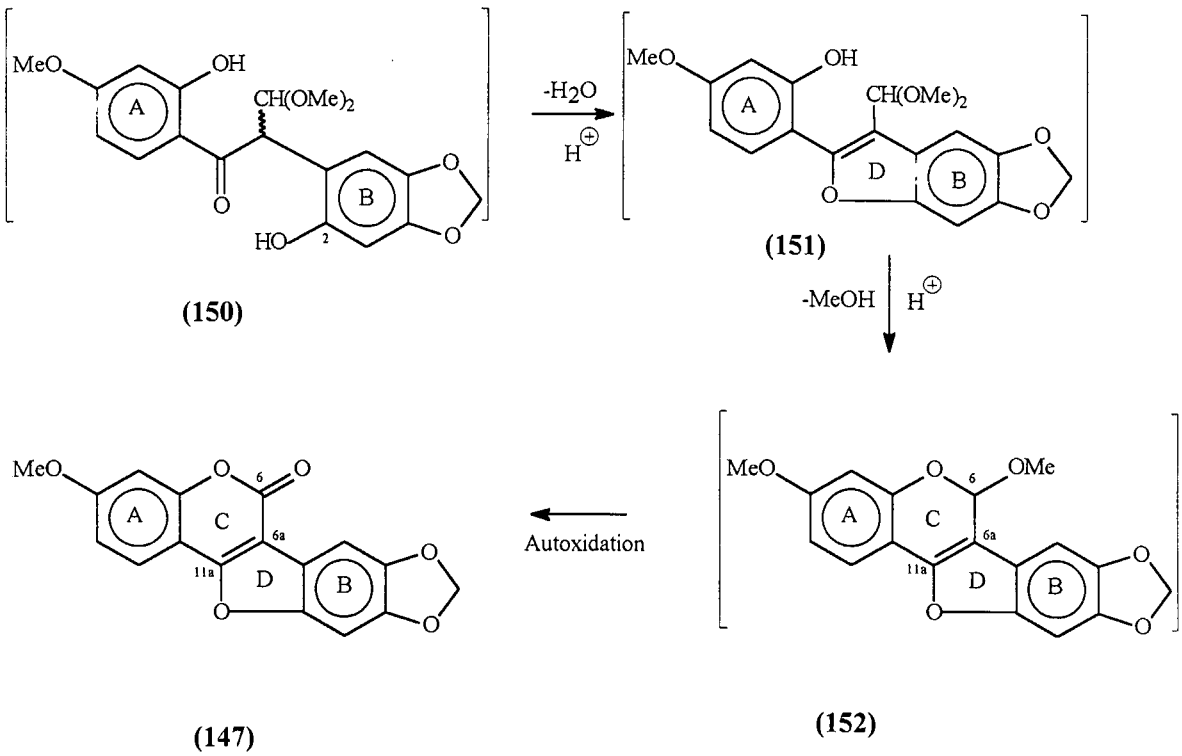
(147)

(146)

Scheme 16

The anticipated course of the reaction is attributed to the acid lability of the methoxymethyl protective group on the B-ring **(144)**. This is hydrolyzed during treatment of the acetal with dilute HCl to liberate the 2-OH on the B-ring affording an intermediate activated acetal **(150)**. Under these conditions acid -catalyzed D-ring formation followed by dehydration is presumably the principle step which leads to a 6-methoxy-dehydropterocarpan **(152)**. This compound would be extremely susceptible to allylic autoxidation[244] involving the 6-H to yield the coumestan **(Scheme 17)**..

[244] M. A. Ferreira, M. Moir and R. H. Thomson, *J. Chem. Soc., Perkin Trans. 1*, **1974**, 2429



Scheme 17

An alternative mechanism could involve the reverse order, *i.e* initial closure of the C-ring followed by D-ring formation, dehydration and autoxidation, or possibly a combination of the two mechanisms. The mechanistic conclusion is substantiated by the absence of similar conversions in acetals bearing an acid-resistent 2-O-benzyl protective group which exclusively gives the C-ring formation by transacetalation followed by the elimination of MeOH to yield the corresponding coumestan. ^{1}H NMR spectra [plate 32a and 33a ($CDCl_3$ - 296) of compounds **(153 and 144),** the respective acetate derivatives of **(148 and 149),** were identical to the naturally occuring coumestans[245,246] isolated from Honeybush tea, thus confirming elucidation by spectroscopic methods of the structures.

Analogous sequences of reactions proceeded similarly for the three different coumestans, albeit in modest unoptimized yield. Because yields in the classic multistep approach are however often not recorded, we suspect that our isolated yields compare favourably with the overall yield in a typical multi-step conversion of chalcones and we have therefore demonstrated a versatile and simplified approach to the synthesis of coumestans.

[245]P. M. Dewick, in *The Flavonoids- Advances in Reasearch since 1980*, (ed. J.B. Harborne), Chapman & Hall, London, **1988,** p.125

EXPERIMENTAL

CHAPTER 20

STANDARD EXPERIMENTAL TECHNIQUES

Unless specified to the contrary the following experimental techniques were used during this project.

20.1. Chromatographic techniques.

20.1.1. Thin layer chromatography.

Qualitative thin layer chromatography (TLC) was performed on pre-coated Merck plastic sheets (silica gel PF_{254}, 0.25 mm). After development the plates were sprayed with H_2SO_4-HCHO (40:1, v/v) and R_f values were determined on these.

Preparative scale thin layer chromatography (PLC) was conducted on glass plates (20×20 cm) coated with Kieselgel PF_{254} (1.0 mm), which were air-dried overnight at room temperature. The plates (loaded with 10-15 mg of material per plate) were developed in an appropriate eluent and dried in a stream of air. The bands were distinguished by UV (254 nm) light and scraped off. Compounds were eluted from the adsorbent with acetone which was removed on a rotary evaporator under reduced pressure at *ca.* 40^0C. Separations of material (less than 3 mg) were conducted on Merck "Pre-coated (0.25 mm) PLC plates (silica gel 60 PF_{254})".

The following abbreviations were used in the description of the solvent systems used during the development of TLC plates:

A = acetone

B = benzene

H = hexane

M = methanol

C = chloroform

20.1.2. Paper chromatography.

Two-dimensional paper chromatograms were conducted on Whatman nr.1 paper (28.5×46 cm) using water saturated butan-2-ol in the first direction and 2% (v/v) acetic acid in the second direction. After development the chromatograms were air dried and sprayed with benzidine spraying reagent.

20.1.3. Column chromatography (CC).

Separations on Sephadex LH-20 were carried out on various column sizes and at differing flow rates (to be specified in each instance) in ethanol and ethanol/water mixtures. Fractions of 10 ml were collected with an ISCO (model 273) automatic fraction collector.

20.2. Spraying agents.

20.2.1. Formaldehyde-sulphuric acid[246].

All TLC plates were sprayed lightly with a 2% (v/v) solution of formaldehyde (40%) in concentrated sulphuric acid and subsequently heated to ensure optimum colour development.

10.2.2. Benzidine spraying agent[247,248].

All paper chromatograms were sprayed with benzidine reagent prepared by mixing (just before use) benzidine solution [benzidine (5g) in HCl (14 ml) made up to 1litre with H_2O] and $NaNO_2$ (10% w/v) in the ratio 3:2.

20.3. Chemical methods.

20.3.1. Acetylation[249].

Dry phenolic material was dissolved in a minimum volume of pyridine and twice the amount of acetic anhydride was added. After 8-12 hours at ambient temperatures, crushed ice was added to precipitate the acetylated material which was filtered and excess pyridine washed out with cold water.

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[246] H. M. Saayman and D. G. Roux, *Biochem J.*, **1965**, *96*, 36
[247] D. G. Roux and E. A. Maihs, *J Chromatoq.*, **1960**, *4*, 65
[248] G. Linstedt, *Acta. Chem. Scand.*, **1960**, *4*, 65
[249] T. Kametani and S. Kano, *J. Pharm. Soc.*, Japan, **1962**, *82*, 1059

20.3.2. Methylation with diazomethane[250].

Methylations were performed with an excess of diazomethane prepared by the reaction of cold (-10°C) potassium hydroxide [5 g in a 95% (v/v) ethanol solution] (55 ml) with N-methyl-N-nitroso-*p*-toluene sulphonamide (22 g) in cold ether (150ml) and distilled directly into a solution of dry phenolic material (250 mg) in ethanol (5-10 ml)] at -10°C. After 48 hours at -15°C the excess diazomethane and solvents were evaporated at room temperature.

20.4. Spectroscopic methods.

20.4.1. Nuclear magnetic resonance spectrometry (NMR).

NMR-spectrometry was performed on a Bruker 300MHz DRX 300 spectrometer at 296K (23°C) with tetramethylsilane as the internal standard. The solvents used were deuteriochloroform ($CDCl_3$), or deuterioacetone [$(CD_3)_2CO$] as indicated. Chemical shifts are reported in parts per million (ppm) on the δ-scale and coupling constants are given in Hz.

The following abbreviations are used:

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

20.4.2. Fast atom bombardment (FAB) mass spectrometry

All FAB mass spectra were recorded on a VG 70 - 70E double-focusing mass spectrometer.

20.4.3. Circular dichroism (CD).

CD spectra were recorded on a Jasco J-710 spectropolarimeter with methanol as solvent.

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[250] A. I. Vogel, *Vogel's Textbook of Practical Organic Chemistry*, Fifth Edition, Longman. Scientific and Techinical, **1989**, 433

20.5. Freeze-drying.

Phenolic material in aqueous solution was freeze-dried using a Virtis Freezemobil 12SL at 40 millitorr.

CHAPTER 21

ISOLATION OF PHENOLIC COMPOUNDS FROM HONEYBUSH TEA (*Cyclopia intermedia)*

21 Extractions

21.1 *Cyclopia Intermedia* Shoots

Dried fermented shoots of *C. intermedia* were pulverised and the chlorophyl was extracted consecutively with chloroform (2 x 6.0 L, 24 h each) and acetone (2 x 5.0 L, 24 h each) at ~25^{0}C to yield dark green solids (155.5 and 86.8 g respectively) on evaporation of the solvents. Subsequent extraction with methanol (4 x 5.0 L, 24 h each, ~25^{0}C) and 70% acetone/water (5 x 5.0 L, 24 h each, 25^{0}C) gave brown solids following evaporation of the solvents. These were redissolved in water and freeze-dried (459.5 and 702.5 g, respectively). The methanol and acetone/water extracts were respectively investigated for phenolic metabolites and tannin content.

21.2 Phenolic metabolites from the methanol extract.

The methanol extract (4 x 95.0 g) was subjected to a Craig (20 tubes) counter current distribution with water/*n*-butanol/hexane (5:4:1) as mobile phase (200mL of organic and 200mL of aqueous phase per tube).

Following paper chromatographic analysis, fractions from the four Craig separations were combined as follows:

Fraction	tubes	yield
C-1	1-3	103.5 g
C-2	4-8	42.0 g
C-3	9-14	27.9 g
C-4	15-20	53.0 g

21.3 Separation of fraction C.2

Fraction C-2 (21.0 g) was separated on Sephadex LH-20/EtOH column (5 x 160 cm) with a flow rate of 1ml/min, collecting 32 min fractions.

Following TLC on the collected volumes, the following combinations were made:

Tubes	fraction	yield
0-57	C2.1	1.25 g
58-93	C2.2	0.20 g
94-135	C2.3	2.98 g
136-162	C2.4	1.78 g
163-190	C2.5	2.90 g
202-269	C2.6	1.25 g
270-313	C2.7	2.92 g
314-330	C2.8	2.55 g
332- 418	C2.10	1.04 g

21.4 Isolation of compounds from C2.1, C2.2, C2.3 and C2.10

Fractions C2.1-3 and C2.10 did not contain compounds of interest pertaining to this investigation.

21.5 Isolation of compounds from fraction C2.4

Acetylation of fraction C2.4 (100 mg) followed by PLC [B:A:M, 90:8:2 (v/v)] gave 3 bands, C2.4.1 (3.1 mg, R_f 0.84), C2.4.2 (4.6 mg, R_f 0.79) and C2.4.3 (3.5 mg, R_f 0.74).

21.5.1 2-(4-*O*-Acetyl-3-methoxyphenyl)-ethanol (83)

PLC purification of fraction C2.4.1 [B:A:M, 90:8:2 (v/v)] yielded compound (**83**) as a white amorphous solid (2.8 mg, R_f 0.84)

^{1}H NMR plate 2

Table 4

21.5.2 2-(4-*O*-Acetylphenyl)-ethanol, (Tyrosol) (82)

Fraction C2.4.2 was further purified by PLC [B:A:M, 90:8:2 (v/v)] to afford compound (**82**) as a white amorphous solid (4.2 mg, R_f 0.79).

^{1}H NMR plate 1

Table 4

21.5.3 2'-*O*-β-Apiofuranosyl-4-*O*-β-D-glucopyranosyl benzaldehyde (85)

Fraction C2.4.3 was further purified [B:A:M, 90:8:2 (v/v)] by PLC to afford compound (**85**) as a *white amorphous* solid (0.74, 3.1 mg)

^{1}H NMR plate 4

NOESY plates 4a-1, 4a-2, 4a-3 and 4a-4

COSY plates 4b-1 and 4b-2

Table 4

21.6 Isolation of compounds from fraction C2.5

Acetylation of fraction C2.5 (100 mg) followed by PLC separation [B:A:M, 90:8:2 (v/v)] gave one band, C2.5.1 (8.5 mg, R_f 0.62).

21.6.1 2-(6'-*O*-β-Apiofuranosyl-4-*O*-β-D-glucopyranosyl-phenyl)-*O*-acetylethanol (84)

PLC purification [B:A:M, 90:8:2 (v/v)x2] of C2.4.1 yielded compound (**84**) (6.7 mg, R_f 0.62) as *colourless oil.*

^{1}H NMR plate 3

NOESY plates 3a-1 and 3a-1

COSY plates 3b-1 and 3b-2

Table 4

21.7 Isolation of compounds from C2.6

Fraction C2.6 (100 mg) was acetylated and separated [B:A:M, 90:8:2 (v/v)] on PLC to yield two bands, C2.6.1 (3.6 mg, R_f 0.70) and C2.6.2 (8.8. mg, R_f 0.66).

21.7.1 3',4',7-Triacetoxyflavone (97)[251]

PLC purification [B:A:M, 90:8:2 (v/v)] of the C2.6.1 fraction yielded the title compound (**97**) as a *white* amorphous solid (R_f 0.70, 3.1 mg).

^{1}H NMR plate 16

NOESY plate 16a

COSY plate 16b

Table 8

21.7.2 3',5,7-Tri-*O*-acetyl-4' -methoxyluteolin (98)

Fraction C2.6.2 was further purified [B:A:M, 90:8:2 (v/v)] by PLC to afford compound (**98**) as a yellowish amorphous solid (R_f, 0.66, 7.8 mg)

^{1}H NMR plate 17

COSY plate 17b-1, 17b-2

Table 8

21.8 Isolation of compounds from fraction C2.7

Acetylation of fraction C2.7 (100 mg) followed by PLC [B:A:M, 90:8:2 (v/v)] gave 3 bands, C2.7.1 (3.0 mg, R_f 0.67), C2.7.2 (14.5 mg, R_f 0.64) and C2.7.3 (6.8 mg, R_f 0.41)

21.8.1 4',5-Di-*O*-acetyl-7-*O*-*β*-D-glucopyranosylnaringenin, (Prunin) (91)[252]

PLC purification of fraction C2.7.1 [B:A:M, 90:8:2 (v/v)] yielded compound (**91**) as a colourless oil (2.8 mg, R_f 0.67)

^{1}H NMR plate 10

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[251] J. B. Harborne, *Comparative Biochemistry of the Flavonoids*, **1967**, Academic Press, London

[252] J. S. Choi, T. Yokozawa and H. Oura, J. Nat. Prod., **1991**, *54*, 507

NOESY plates 10a-1, 10a-2

COSY plate 10b-1, 10b-2

CD spectrum, plate 34

Table 6

21.8.2 3',4',5-Tri-*O*-acetyl-7-*O*-*β*-D-glucopyranosyleriodictyol (92)[253]

Fraction C2.6.2 was further purified by PLC [B:A:M, 90:8:2 (v/v)] to afford compound (**92**) as a colourless oil (14.1 mg, R_f 0.64).

^{1}H NMR plate 11

NOESY plates 11a-1, 11a-2

COSY plates 11b

CD spectrum, plate 35

Table 6

21.8.3 3',4',7-Tri-*O*-acetyl-5-*O*-*β*-D-glucopyranosyleriodictyol (93)[254]

PLC purification of fraction C2.7.3 [B:A:M, 90:8:2 (v/v)x2] yielded compound (**93**) as a colourless oil (5.9 mg, R_f 0.41)

^{1}H NMR plate 12

NOESY plates 12a-1, 12a-2

COSY plate 12b-1, 12b-1

CD spectrum, plate 36

Table 6

21.9 Isolation of compounds from fraction C2.8

Acetylation of fraction C2.8 (100 mg) followed by PLC separation [B:A:M, 90:8:2 (v/v)] gave 4 bands, C2.8.1 (3.4 mg, R_f 0.64), C2.8.2 (4.2 mg, R_f 0.42), C2.8.3 (6.3 mg, R_f 0.38) and C2.8.2 (5.9 mg, R_f.0.33).

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21.9.1 5-*O*-α-D-Glucopyranosylkaempferol (88)[255]

PLC purification [B:A:M, 90:8:2 (v/v)] of the C2.8.1 fraction yielded compound (**88**) as colourless oil (R_f 0.64, 3.0 mg).

^{1}H NMR plate 7

NOESY plate 7a

COSY plate 7b

Table 5

21.9.2 7-*O*-β-D-Glucopyranosyl-4',6-di-*O*-methylafrormosin (wistin) (95)[256]

PLC purification [B:A:M, 90:8:2 (v/v)] of the C2.8.2 fraction yielded compound (**95**) colourless oil (R_f 0.42, 3.8 mg).

^{1}H NMR plate 14

NOESY plates 14a-1, 14a-2

COSY plates 14b-1, 14b-2

Table 7

21.9.3 6-*C*-β-D-Glucopyranosylkaempferol (87)[257]

PLC purification of fraction C2.8.3 [B:A:M, 90:8:2 (v/v)x2] yielded compound (**87**) as a colourless oil(R_f 0.38, 6.1 mg).

^{1}H NMR plate 6

NOESY plates 6a-1, 6a-2

COSY plate 6b

Table 5

21.9.4 8-*C*-β-D-Glucopyranosylkaempferol (86)

PLC purification [B:A:M, 90:8:2 (v/v)x2] of the C2.8.4 [B:A:M, 90:8:2 (v/v)] fraction yielded the title compound (**86**) as a *colourless oil* (R_f 0.33, 4.6 mg).

^{1}H NMR plate 5

NOESY plates 5a-1, 5a-2

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COSY plate 5b

Table 5

21.10 Isolation of compounds from C2.9

Fraction C2.9 (100 mg) was acetylated and separated [B:A:M, 80:15:5 (v/v)x2] on PLC to yield 4 bands, C2.9.1 (3.4 mg, R_f 0.38), C2.9.2 (5.7 mg, R_f 0.30), C2.9.3 (4.2 mg, R_f 0.26) and C2.9.4 (5.1 mg, R_f 0.16).

21.10.1 3-Acetoxy-6"-*O*-*β*-apiofuranosyl-6-*O*-β-D-glucopyranosyl-3',4'-methylenedioxyflavonol (89)

PLC purification [B:A:M, 80:15:5 (v/v)x2] of the C2.9.1 fraction yielded compound (**89**) as a *colourless oil* (R_f,38, 3.2 mg).

^{1}H NMR plate 8

NOESY plates 8a-1, 8a-2

COSY plate 8b-1, 18b-2

Table 5

21.10.2 4'-7-Di-*O*-acetyl-5-*O*-*α*-D-rutinosylnaringenin (94)

Purification of fraction C2.9.2 [B:A:M, 90:8:2 (v/v)x2] yielded the title compound (**94**) as a *colourless oil* (R_f 30, 5.0 mg).

^{1}H NMR plate 13

NOESY plates 13a-1, 13a-2, 13a-3, 13a-4

COSY plate 13b

CD spectrum, plate 34

Table 6

21.10.3 6''-*O*-β-Apiofuranosyl-7-*O*-β-D-glucopyranosylisoflavone (96)

PLC purification [B:A:M, 90:8:2 (v/v)x2] of the C2.9.3 fraction yielded compound (**96**) as a *colourless oil* (R_f 0.16, 4.2 mg).

^{1}H NMR plate 15

NOESY plates 15a-1, 15a-2, 15a-3

COSY plates 15b-1, 15b-2

Table 8

21.10.4 3-*O*-6-*C*-*β*-D-Diglucopyranosylkaempferol (90)

PLC purification [B:A:M, 80:15:5 (v/v)x2] of the C2.9.4 fraction yielded the title compound (**90**) as a *colourless oil* (R_f 0.16, 4.9 mg).

^{1}H NMR plate 9

NOESY plates 9a-1, 9a-2,

COSY plates 9b

Table 5

CHAPTER 22

TANNIN CLEAVAGE

22.2 'Purification of the tannin'.

The acetone water extract (70g) was exhaustively extracted with methanol (5x 500ml) to remove the sugars as well as the organic soluble compounds. The extractions were carried out by continuous stirring of the mixture at room temperature over a period of 24h. The mixture was periodically filtered under suction with sintered glass. Following acetylation of a portion (50mg) of the extract, TLC chromatography and ^{1}H NMR did not indicate non-polymeric compounds.

22.2 Reaction of the tannin with phloroglucinol and benzyl mercaptain.

The nucleophile (4 g), phloroglucinol or benzyl mercaptan, was added separately to a solution of the extract (4g) ethanol (30ml), containing 15% HCl, iand the mixture was stirred under nitrogen for 5h. Water (50ml) was added and the aqueous solution was extracted with EtOAc (5x 100ml). The combined organic layers were dried (Na_2SO_4), and evaporated to afford 4g of the residue.

22.3 Separation of the reaction mixtures.

To increase the concentration of the compounds, the recovered organic layer extract from **22.2** (4g) was separated on a Sephadex LH-20/EtOH column (3.5x80 cm) with a flow rate of 1ml/min, collecting 32min fractions with ethanol/water (80:20, v/v) as an eluent.

The tubes were combined as indicated below;

22.3.1. Phloroglucinol as nucleophile.

Tubes	Fraction	Yield (g)
0-58	P1	2.085
59-80	P2	0.334
81-120	P3	0.277

Fraction P1 comprised phloroglucinol and fraction P3 was still a complex mixture which was not further investigated..

22.3.2. Benzyl mercaptan as nucleophile.

Tubes	Fraction	Yield (g)
0-50	B1	0.785
51-120	B2	1.267

Fraction B1 was mainly composed of sugar fragments, which were not identifiable due to their very low concentrations.

22.4 Isolation of compounds from fraction P2

Acetylation of fraction P2 (100 mg) followed by PLC [B:A:M, 90:8:2 (v/v)] gave three bands, P2.1 (6.0 mg, R_f 0.79), P2.2 (4.5 mg, R_f 0.74) and P2.3 (6.8 mg, R_f 0.67)

22.4.1 4-(2,4,6-Triacetoxyphenyl)-4',5,7-triacetoxyflavan (101)

PLC purification of fraction P2.1 [B:A:M, 90:8:2 (v/v)] yielded compound (**101**) as a colourless oil 4.8 mg, R_f 0.79)

^{1}H NMR plate 20

Table 9

22.4.2 4-(2,4,6-Triacetoxyphenyl)-3'4',5,7-tetra-acetoxyflavan (102)

Fraction P2.2 was further purified by PLC [B:A:M, 90:8:2 (v/v)] to afford compound (**102**) as a *colourless oil* (4.1 mg, R_f 0.74).

^{1}H NMR plate 21

Table 9

22.4.3 4-(2,4,6-Triacetoxyphenyl)-4',5,7-triacetoxy-5-*O*-β-D-glucopyranosylflavan (103)

PLC purification of fraction P3.3 [B:A:M, 90:8:2 (v/v)x2] yielded compound (**103**) as a *colourless oil* (5.9 mg, R_f 0.67)

^{1}H NMR plate 22

NOESY plates 22a-1, 22a-2

COSY plate 22b-1, 22b-2

Table 9

22.5 Isolation of compounds from fraction B2

Acetylation of fraction B2 (100 mg) followed by PLC [B:A:M, 90:8:2 (v/v)] gave two bands, B2.1 (11.4 mg, R_f 0.59) and B2.2 (4.5 mg, R_f 0.79)

22.5.1 4',7-Diacetoxy-4-thiobenzyl-5-*O*-glucopyranosylflavan (100)

Fraction B2.1 was further purified by PLC [B:A:M, 90:8:2 (v/v)] to afford compound (**100**) as a *colourless oil* (4.1 mg, R_f 0.79).

^{1}H NMR plate 19

NOESY plates 19a-1, 19a-2

COSY plate 19b-1, 19b-2

Table 8

22.5.2 4',7-Diacetoxy-4-thiobenzyl-5-*O*-β-D-rutinosylflavan (99)

PLC purification of fraction B2.2 [B:A:M, 90:8:2 (v/v)] yielded compound (**99**) as a *colourless oil* (9.5 mg, R_f 0.59)

^{1}H NMR plate 18

NOESY plates 18a-1, 18a-2, 18a-3

COSY plate 18b

Table 8

CHAPTER 23

REACTION OF SUPEROXIDE (KO_2) WITH FLAVONOLS AND FLAVAN-3-OLS

23.1 General procedure

To powdered KO_2 (10g) was added dry DMSO (10ml) and the mixture stirred for 30min to make a suspension which was used in all the reactions. The suspension (20ml) was added to the free phenolic flavonol or flavan-3-ol (200 or 100 mg respectively) dissolved in a minimum of DMSO at 5-10 ^{0}C. The mixture of each substrate was stirred individually for different times as will be specified. To the cooled reaction mixture saturated NaCl (20ml at O^{0}C) was added, followed by acidification (10% HCl) and extraction with EtOAc (200ml x 5). The extract was washed with water (300ml x 3) to remove the DMSO, dried (Na_2SO_4), the organic layers combined, evaporated and the residue acetylated by conventional methods. Following purification by PLC, [B:A:M (90:8:2)], ^{1}H NMR and COSY experiments were used to identify the products.

23.2 Flavonols

23.2.1 Quercetin

Quercetin afforded four peracetate derivatives from the reaction (15-20 min):

(i) Tetra -*O*-acetylkaempferol (**119**) (3.75%, 7.5 mg, R_f 0.55) plate 23a ($CDCl_3$ -296K), as a white amorphous solid,

(ii) 2,3-Di-acetoxybenzaldehyde (**120e**) (0,5%, 0.95 mg, 0.70), plate 23b ($CDCl_3$ -296K) as white amorphous solid,

(ii) 2,4-Di-acetoxybenzoic acid (**120g**) (1.6%, 3.2 mg, R_f, 0.60), plate 23c ($CDCl_3$ - 296K), as a white armophous solid,

(iv) Penta-*O*-acetyl-2'-methylquercetin (**125**) (2.6%, 5.2m g, R_f 0.32) plate 23d ($CDCl_3$ - 296K) as a white amorphous solid,

and 11.5% of the starting material (**109**) was recovered.

23.2.2 Myricetin

Only the quercetin peracetate derivative (**110**), (4.9%, 9.8 mg, R_f 0.45), plate 24a ($CDCl_3$ -296K), and the starting material (14%), plate 24 ($CDCl_3$ -296K), were recovered from the reaction (5min).

23.2.3 Fisetin

Following PLC separation 2,3-di-acetoxybenzaldehyde (**120e**) (1.5%), 2,4-di-acetoxybenzoic acid (**120g**) (0.8%) (cf. **23.3.1**) and the peracetate of the starting material (**111**), (4.0%) were isolated from the reaction (10-15min) as white amorphous solids

23.2.4 Robinetin

From the reaction (10-15min), Tetra-*O*-acetyl-4'-methoxyrobinetin, (**126**), plate 25a ($CDCl_3$ -296K), (0.9%, 1.8 mg, R_f 0.57) was isolated in addition to the peracetate of the starting material (12%), plate 25 ($CDCl_3$ -296K),.

23.3 Flavan-3-ols

23.3.1 Catechin

ent-Epicatechin (**113a**) plate 26a ($CDCl_3$ -296K) and the starting material plate (**114**) 28.6%), 26 ($CDCl_3$ -296K), were isolated as the peracetates from the reaction (15-25min) mixture.

23.3.2 Epicatechin

ent-Catechin (**114b**) , (18.5%), *epi*-afzelechin (**114a**) (5.8%, 5.8, R_f 0.64), plate 27a ($CDCl_3$ -296K), and the starting material, plate (**114**) (12%), 27 ($CDCl_3$ -296K), were isolated as peracetates from the reaction (5 min) mixture.

CHAPTER 24

SYNTHESIS OF THE COUMESTANS

24.1 2-*O*-Methoxymethyl-4,5-methylenedioxybenzaldehyde (133)

NaOH (15.0 g) in H_2O (20 ml) was added to a stirred solution of sesamol (5.0 g) in ethanol (15 ml). The solution was heated to 80 ^{0}C, $CHCl_3$ (10 ml) added dropwise over 10-15 min and refluxed gently with stirring for 6 h. Excess solvents were evaporated on a water bath and conc. HCl (9 ml) was added dropwise to produce a dark oil. Sufficient H_2O was added to dissolve the precipitated NaCl and the oil was extracted with EtOAc (3x100 ml), dried (Na_2SO4) and the solvent evaporated. Following purification by flash CC (tubes 28-55) with hexane-EtOAc (8 : 2) and methoxymethylation the product **(133)** was obtained as a yellow armophous solid (1.5 g, 30%).

^{1}H NMR ($CDCl_3$): δ 13.0 (*s*, CHO), 7.28 (s, H-6), 6.8 (*s*, H-3), 6.02 (s, OCH_2O), 5.25 (s, OCH_2OMe), 3.25 (s,)

Found : M^+, 210 0531. $C_{10}H_{10}O$ requires M^+ , 210.0528).

24.2 2-Hydroxy-4-methoxyacetophenone (130).

2,4-Dihydroxyacetophenone (1.0 g) was methylated to yield the methyl ether **(130)** as white needles (from EtOH), m.p. 51-52 °C, lit. m.p., 52-53 °C[243] (0.96 g, 95%). ^{1}H NMR ($CDCl_3$): δ 7.44 (d, *J*=9.0 Hz, H-6), 6,51 (*dd*, *J*=2.5, 9.0 Hz, H-5), 6.54 (*d*, *J*=2.5 Hz, H-3), 3.99 (s, OMe), 2.57 (s, $COCH_3$).

24.3 2-Hydroxy-4-*O*-methoxymethylacetophenone (131).

2,4-Dihydroxyacetophenone (1.0 g) was methoxymethylated to give the product **(131)** as a colourless oil (0.93 g, 91%). ^{1}H NMR ($CDCl_3$): δ 7.53 (*d*, *J*=9.0 Hz H-6), 6.52 (*d*, *J*=2.5 Hz H-3), 6.48 (*dd*,

J=2.5, 9.0 Hz, H-5), 5.12 (s, OC*H*$_2$OMe), 3.40 (s, OCH$_2$O*Me*), 2.48 (s, COCH$_3$) (Found: M^+, 196.0731. $C_{10}H_{12}O_4$ requires M^+, 196.0736).

24.4 2-*O*-Methylpyrogallol.

To a solution of pyrogallol (8.0 g) in H_2O (12 ml) was added dimethylsulfate (6ml) and 10% (w/v) *aq* NaOH (28 ml) and the mixture was stirred under N_2 for 10 min . The solution was heated on a water bath for 2 h., cooled, acidified with 3M HCl, saturated with NaCl, and extracted with EtOAc (5 x 100 ml). Flash CC with hexane-EtOAc (7 : 3) gave a mixture (*ca.* 1 : 1) of 1-*O*-and 2-*O*-methylpyrogallol (tubes 36 - 68, 4.9 g, 30% total) which was not further resolved.

24.5 2-Hydroxy-3-methoxy-4-*O*-methoxymethylacetophenone (132).

To a mixture of anhydrous $ZnCl_2$ (1.65 g) in glacial AcOH (20 ml) at 140 °C was added the mixture of 1-*O*- and 2-*O*-methyl-pyrogallol (1.1 g) with constant stirring. The mixture was refluxed for 6 h., cooled and extracted with EtOAc (4x100 ml). The combined extracts were dried (Na_2SO_4), and the solvent evaporated. Following purification by PLC in C_6H_6-Me_2CO (95:5) (R_f 0.42) and methoxymethylation the product **(132)** was obtained as a colourless oil (0.33 g, 31%). ^{1}H NMR ($CDCl_3$): δ7.46 (*d*, *J*=9.0 Hz, H-6), 6.53 (*d*, *J*=9.0 Hz, H-5), 5.12 (s, OC*H*$_2$OMe), 4.01 (s, OMe), 3.90 (s, OCH$_2$O*Me*), 2.59 (s, COCH$_3$) (Found: M^+, 226.0838. $C_{11}H_{14}O_5$ requires M^+, 226.0841).

24.6 General procedure for the preparation of chalcones.

50% (m/v) *aq* KOH (2.5 ml) was mixed with a solution of the appropriate acetophenone (0.7 g) in EtOH (10 ml), stirred at room temperature for 30 min and and excess of 2-hydroxy-4,5-methylenedioxybenzaldehyde **(133)** (0.5 g) in EtOH (5 ml) added dropwise. After depletion of the acetophenone (18-24 h.), H_2O (10 ml) was added, the mixture acidified with 10% (v/v) H_2SO_4 and extracted with EtOAc (4x20 ml). Drying of the extract (Na_2SO_4) followed by evaporation of the solvent and flash CC gave the pure chalcone.

24.6.1 2'-Hydroxy-4'-methoxy-2-*O*-mehtoxymethyl-4,5-methylenedioxychalcone (134).

Flash CC of the reaction product with hexane-EtOAc (7 : 3) gave the chalcone **(134)** (tubes 56 - 70) as a yellow amorphous solid (0.60 g, 50%). IR ($CHCl_3$): 1628, 1576, 1506, 1484, 1470, 1380, 1346 cm^{-1}; ^1H NMR [plate 28 ($CDCl_3$)]: δ 8.27 (*d*, *J*=10.5 Hz, H-α), 7.82 (*d*, *J*=9.0 Hz, H-6'), 7.43 (*d*, *J*=10.5 Hz, H-β), 7.15 (s, H-6), 6.5 (*d*, *J*=2.5 Hz, H-3'), 6.48 (*dd*, *J*=2.5 and 9.0 Hz, H-5'), 6.80 (s, H-3), 6.10 (s, OCH_2O), 5.22 (s, OCH_2OMe), 3.87 (s,OMe), 3.53 (s, OCH_2OMe) (Found: M^+ ,358.1048. $C_{19}H_{18}O_7$ requires M^+ ,358.1053).

24.6.2 2'-Hydroxy-2,4'-di-*O*-Methoxymethyl-4,5-methylenedioxychalcone (135).

Flash CC of the reaction product with C_6H_6-hexane-EtOAc (5 : 4 :1) yielded the chalcone **(135)** (tubes 32 - 57) as a yellow amorphous solid (0.72 g, 60%). IR ($CHCl_3$): 1632, 1574, 1506, 1484, 1410, 1370, 1346 cm^{-1}; ^1H NMR [plate 29 ($CDCl_3$)]: δ 8.28 (*d*, *J*=10.5 Hz, H-α), 7.85 (*d*, *J*=9.0 Hz, H-6'), 7.42 (*d*, *J*=10.5 Hz, H-β), 7.16 (s, H-6), 6.82 (*d*, *J*=2.5 Hz, H-3'), 6.67(*dd*, *J*=2.5, 9.0 Hz, H-5'), 6.62 (s, H-3), 6.02 (s, OCH_2O), 5.25 and 5.23 (2xs, OCH_2OMe), 3.54 and 3.51 (2xs, OCH_2OMe) (Found: M^+ , 388.1319. $C_{20}H_{20}O_8$ requires M^+ ,388.1315).

24.6.3 2'-Hydroxy-3'-methoxy-2,4'-di-O-methoxymethyl-4,5-methylenedioxychalcone (136).

Flash CC of the reaction product with C_6H_6-hexane-EtOAc (5 : 4 :1) gave the chalcone **(136)** (tubes 45 - 63) as a yellow amorphous solid (0.56 g, 47 %). IR ($CHCl_3$): 1632, 1574, 1506, 1484, 1452, 1338 cm^{-1}, ^1H NMR [plate 30 ($CDCl_3$)]: δ 8.30 (*d*, *J*=10.5 Hz, H-α), 7.66 (*d*, *J*=9.0 Hz, H-6'), 7.44 (*d*, *J*=10.5 Hz, H-β), 716 (s, H-6), 6.84 (s, H-3), 6.75 (*d*, *J*=9.0 Hz, H-5'), 6.02 (s, OCH_2O), 5.34 and 5.23 (each s, OCH_2OMe), 3.95 (s, OMe), 3.535 (each s, OCH_2OMe) (Found: M^+, 418.1266. $C_{21}H_{22}O_9$ requires M^+, 418.1264).

24.7 General procedure for the preparation of coumestans.

$Tl(NO_3)_3.3H_2O$ (38 mg) was added to a vigorously stirred suspension of the chalcone (28 mg) in MeOH (1.0 ml) and the stirring continued for 24 h. The mixture was filtered, satd. *aq* NaCl (0.4 ml) and satd. *aq* $NaHCO_3$ (0.2 ml) was evaporated and the residue refluxed with MeOH/10%HCL (10:1, 0.5 ml) for 1 h. Water (30 ml) was evaporated and the mixture and the products were extracted with EtOAc (3x20 ml). Purification by PLC in C_6H_6-Me_2CO (95:5) afforded the coumestan.

24.7.1 3-Methoxy-8,9-methylenedioxycoumestan (147) (flemichapparin).

Obtained (R_f 0.81) as white needles (from EtOH), m.p. 178-180 °C, lit. m.p., 179-180 °C[258] (8.4 mg, 31%). IR ($CHCl_3$): 1744, 1634, 1604, 1504, 1430, 1360 cm^{-1} ; ^1H NMR [plate 31 ($CDCl_3$)]: δ 7.87 (*d*, *J*=9.0 Hz, H-1), 7.49 (s, H-7), 7.14 (s, H-10), 7.01 (*d*, *J*=2.5 Hz, H-4), 6.99 (*dd*, *J*=2.5, 9.0 Hz, H-2), 6.1 (s, OCH_2O), 3.93 (s, OMe).

24.7.2 3-Hydroxy-8,9-methylenedioxycoumestan (148) (medicagol).

Obtained (R_f 0.35) as white needles (from MeOH), m.p. 324-326 °C, lit. m.p. 324-325 °C[259] (7.0 mg, 25%). IR ($CHCl_3$): 1732, 1668, 1626, 1504, 1464, 1360 cm^{-1} ; ^1H NMR [Plate 32 $(CDC_3)_2CO$]: δ 7.86 (*d*, *J*=9.0 Hz, H-1), 7.35 (s, H-7), 7.13 (s, H-10), 7.0 (*dd*, *J*=2.5 9.0 Hz, H-2), 6.94 (*d*, *J*=2.5 Hz, H-4), 6.13 (s, OCH_2O).

24.7.3 3-Hydroxy-4-methoxy-8,9-methylenedioxycoumestan (149) (sophoracoumestan B

Obtained (R_f 0.44) as white needles (from MeOH), m.p. >300 °C, lit. m.p. >300 °C[234] (5.0 mg, 21%). IR ($CHCl_3$): 1744, 1636, 1604, 1540, 1466, 1426, 1360 cm^{-1} ; ^1H NMR [Plate 33 $(CDC_3)_2CO$]: δ 7.62 (*d*, *J*=9.0 Hz, H-1), 7.35 (s, H-7), 7.34 (s, H-10), 7.06 (*d*, *J*=2.5 Hz, H-2), 6.13 (s, OCH_2O), 4.0 (s, OMe).

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24.7.4 3-*O*-Acetyl-8,9-methylenedioxycoumestan (153).

Acetylation of the coumestan **(148)** (5.0 mg) gave the monoacetate **(153)** as white needles (from EtOH), m.p. 260-262 °C, lit., m.p. 262-263 °C (4.9 mg). ^{1}H NMR [plate 32a ($CDCl_3$)]: δ 7.98 (*d*, *J*=9.0 Hz, H-1), 7.51 (s, H-7), 7.29 (*d*, *J*=2.5 Hz, H-4), 7.20 (*dd*. *J*=2.5, 9.0 Hz, H-2), 7.17 (s, H-10), 6.12 (s, OCH_2O), 2.38 (s, OAc).

24.7.5 3-*O*-Acetyl-4-methoxy-8,9-methylenedioxycoumestan (154).

Acetylation of the coumestan **(149)** (4.5 mg) gave the monoacetate **(154)** as a white amorhous solid (4.0 mg). ^{1}H NMR [plate 33a ($CDCl_3$)]: δ 7.70 (*d*, *J*=9.0 Hz, H-1), 7.51 (s, H-7), 7.16 (s, H-10), 7.13 (*d*, *J*=9.0 Hz, H-2), 6.12 (s,OCH_2O), 4.13 (s, OMe), 2.41 (s, OAc) (Found : M^+, 368.0529. $C_{19}H_{12}O_8$ requires M^+,368.0532).

APPENDIX A

Carbon	74	75	77
C-1	122.4	122.5	119.8
C-2	119.2	114.0	116.0
C-3	153.0	156.0	146.0
C-4	111.5	102.0	108.0
C-4a	153.9	155.5	145.9
C-6	158.0	163.0	158.0
C-6a	117.0	116.5	117.2
C-6b	107.0	105.0	106.0
C-7	100.0	100.5	100.7
C-8	146.5	147.0	146.8
C-9	148.5	147.5	148.5
C-10	94.5	96.5	94.5
C10a	152.0	151.0	151.5
C-11a	158.5	161.0	159.5
C-11b	111.0	107.0	112.6
OCH_3		57.0	62.0
OCH_2O	102.5	102.5	102.5
CH_3CO	21.6		21.1
CH_3CO	169.2		170.0

Table 2 ^{13}C NMR of the coumestan cerivatives, **74, 75**, and 77 in $CDCl_3$ at 23^0C

Carbon	79	81
C-1	157.5	158.0
C-2	155.8	112.1
C-3	151.6	154.9
C-4	113.2	152.0
C-4a	152.8	150.0
C-4b	151.1	153.0
C-5	116.2	113.0
C-6	148.1	148.0
C-7	139.9	139.7
C-8	121.2	121.2
C-8a	114.5	118.5
C-9	174.0	174.0
C-9a	115.4	120.5

Table 3 ^{13}C NMR of the aglycone moieties of xanthone derivatives, **79**, and **81** in $CDCl_3$ at 23^0C

Protons	(82) $CDCl_3$ 296K	(83) $CDCl_3$ 296K	(84) $CDCl_3$ 296K	(85) $CDCl_3$ 296K
2-H		6.83 (d, 2.5)		
5-H		6.98 (d, 8.5)		
6-H		6.81 (dd, 2.5, 8.5)		
2'6'-H	7.25 (d, 8.5)		7.15 (d, 8.5)	7.88 (d, 8.5)
3'5'-H	7.05 (d, 8.5		6.93 (d, 8.5)	7.15 (d, 8.5)
OAc (arom.)	2.32	2.33		
OAc (aliph.)	2.06	2.07	2.12, 2.16, 2x 2.04	2.20, 2.14, 2x 2.07
			2.03, 2.02	2.04
CH_2	2,95 (t, 7.0)	2,95 (t, 7.0)	2.89 (t, 7.0)	
	4.30 (t, 7.0)	4.30 (t, 7.0)	4.26 (t, 7.0)	
OMe		3,84 (s)		
CHO				9.95 (s)
H-1'			5.05 (d, 8.0)	5.14 (d, 7.5)
H-2'			5.23 (t, 9.0)	4.01 (t, 7.5)
H-3'			5.28 (t, 10.0)	5.32 (t, 10.0)
H-4'			5.03 (t, 10.0)	5.08 (t, 10.0)
H-5'				3.91 (m)
H-5, H-6'			3.73-3.85	
H-6'			3.62 (dd, 7.0, 12.0)	4.23 (d,, 12.0)
				4.16 (dd, 2.5, 12.0)
H-1"			5.37 (d, 1.0)	5.21 (d, 1.0)
H-2"			5.00 (d, 1.0)	5.18 (d1.0)
3"-CH_2			4.21 (d, 10.0	4.30 (d, 10.0)
			4,13 (d, 10.0)	4.15 (d, 10.0)
H-4"			4.79 (d, 12.0)	4.60 (d, 12.0)
			4.52 (d, 12.0)	4.50 (d, 12.0)

Table 4: ^1H NMR peaks of $C_6.C_2$- and $C_6.C_1$-type phenols derivatives (methanol extract) **(82), (83), (84)** and **(85)** from *C.intermedia* at 300 MHz (23^0C in $CDCl_3$). Splitting patterns and *J* (Hz) values are given in parentheses

Protons	(85) $CDCl_3$ 296K	(87) $CDCl_3$ 296K	(88) $CDCl_3$ 296K	(89) $CDCl_3$ 296K	(90) $CDCl_3$ 296K
5-H(A)				7.67 (d, 2.5)	
6-H(A)	6.54 (s)		6.92 (s)		
7-H(A)				7.22 (dd, 2.5, 8.5)	
8-H(A)		6.71 (s)	6.86 (s)	7.05 (d, 8.5)	6.67 (s)
2,5,6 (B)				7.38-7.44	
2'6'-H(B)	7.63 (br. d,, 8.5)	7.76 (d, 8.5)	7.83 (d, 8.5)		8.08 (d, 8.5)
3'5'-H(B)	7.18 (d, 8.5)	7.16 (d, 8.5)	7.20 (d, 8.5)		7.40 (d, 8.5)
OAc (arom.)	2.36, 2.17	2.33, 2.16	2.34, 2.33		2.56, 2.37
	2.07	2x 2.08 ,	2.12		
OAc (aliph.)	1.81, 2x 1.59	2.03, 1.88	2.04, 1.98	2.11, 2.08, 4x 2.05	2.11, 2.07, 2.06
	1.56, 1.27	1.74, 1.65	1.97, 1.93	2.03	2.04, 2.01, 1.94
					1.89, 1.76
H-1"	4.86 (d, 10,0)	4.93 (d, 10.0)	5.01 (d, 2.0)	5.07 (d, 8.5)	4.82 (d, 10.0)
H-2"	5.40 (dd, 9.0, 10.0)	5.42 (dd, 9.0, 10.0)	5.20 (t, 9.0)	5.27 (t, 8.5)	5.71 (t, 10.0)
H-3"	5.27 (br.t, 9.0)	5.35 (t, 9.0)	5.01 (t, 10.0)	5.27 (t, 8.5)	5.32 (t, 9.0)
H-4"	5.27 (br.t, 9.0)	5.29 (t, 10.0)	5.10 (t, 10.0)	5.06 (t, 8.5)	5.16 (t, 9.0)
H-5"	3.90 (m)	3.92 (m)	3.86 (m)	3.83 (m)	3.77 (m)
H-6"	4.35 (d, 2.5)	4.36 (dd, 3.5, 12.0)	4.26 (dd, 5.5, 12.5)	4.72 (d, 2.5, 12.0)	4.30 (dd, 4.0, 12.5)
	4.21 (d, 12.0)	4.18 (dd, 2.5, 12.0)	4.17 (dd, 2.5, 12.5)	4.59 (d, 4.0, 12.0)	4.47 (dd, 5.5, 12.5)
H-1"'				4.99 (d, 1.0)	4.5 9 (d, 10.0)
H-2"'				5.37 (d, 1.0)	5.95 (dd, 2.0, 10.0)
H-3"'				4.77 (d, 12.0)	5.43 (t, 9.0)
H-4"'				4.58 (d, 12.0)	5.50 (t, 9.0)
H-5"'					3.82 (m)
H-6"'					4.20 (dd, 4.0, 12.5)
					4.30 (dd, 2.5, 12.5)
3"'-CH_2				4.14 (d, 10.0)	
				4.23 (d, 10.0)	
OCH_2				5.31 (s)	

Table 5: ^{1}H NMR peaks of flavonol acetate derivatives (methanol extract) **(85), (86), (87), (89)** and **(90)** from *C.intermedia* at 300 MHz (23^0C in $CDCl_3$). Splitting patterns and *J* (Hz) values are given in parentheses

Protons	(91) $CDCl_3$ 296K	(92) $CDCl_3$ 296K	(93) $CDCl_3$ 296K	(94) $CDCl_3$ 296K
2-H(C)	5.42 (dd, 3.0, 12.0)	5.40 (dd, 4.0, 12.0)	5.320 (dd, 4.0, 12.0)	5.45 (dd, 3.0, 13.0)
H-3β	2.90 (overlaps H-3α)	2.85 (dd, 4.0, 12,0)	2.73-2.92 (overlaps H-3β)	2.78 (dd, 3.0, 16.0)
H-3α	2.90 (overlaps H-3β)	2.90 (d, 12.0)	2.73-2.92 (overlaps H-3α)	3.01 (dd, 13.0, 16.0)
6-H(A)	6.59 (d, 2.5)	6.59 (d, 2.5)	6.66 (d, 2.5)	6.45 (d, 2.5)
8-H(A)	6.61 (d, 2.5)	6.61 (d, 2.5)	6.19 (d, 2.5)	6.57 (d, 2.5)
2-H(B),6- H(B)		7.31 (m)	7.30 (m)	
5-H(B)		7.25 (d, 8.5)	7.24 (d, 8.5)	
2'6'-H(B)	7.46 (d, 8.5, 8.5)			7.48 (d, 8.5)
3'5'-H(B)	7.16 (d, 8.5, 8.5)			7.17 (d, 8.5)
OAc (arom.)	2.33, 2.31	2.33, 2.32, 2.31	3x 2.33	2.34, 2.32
OAc (aliph.)	2.12, 2.10, 2.07	2.12, 2.09, 2.07,	2.13, 2.12, 2.07	2.18, 2.13, 3x 2.07
	2.06	2.06	2.06	2.01, 1.97
H-1"	5.10 (d, 7.5)	5.09 (d, 8.0)	5.05 (dd, 8.0)	5.15 (d, 4.0)
H-2"	5.45 (dd, 2.0, 9.0)	5.45 (dd, 2.0 , 9.0)	5.42 (dd, 8.0, 9.5)	4.12 (dd, 2.0, 7.0)
H-3"	5.31 (t, 9.0)	5.30 (t, 9.0)	5.31 (t, 9.5)	5.31 (t, 10.0)
H-4"	5.18 (t, 10.0)	5.17 (t, 9.0)	5.16 (t, 10.0)	5.19 (m)
H-5"	3.93 (m)	3.93 (m)	3.87 (m)	3.86 (m)
H-6"	4.25 x2	4.20 x2	4.26 x2	4.27 (dd, 4.0, 12.0)
				4.18 (dd, 3.0, 12.0)
H-1"'				5.13 (dd, 4.0)
H-2"', H-3"'				5.19 (m)
H-3"'				5.31 (t, 10.0)
H-4"'				4.99 (t, 10.0)
H-5"'				3.92 (m)
CH_3				0,93 (d, 6.0)

Table 6: ^1H NMR peaks of flavanone acetate derivatives (methanol extract) **(91), (92), (93),** and **(94)** from *C.intermedia at 300 Mhz* (23^0C in $CDCl_3$). Splitting patterns and *J* (Hz) values are given in parentheses

Protons	(95) $CDCl_3$ 296K	(96) $CDCl_3$ 296K
5-H(A)	7.69 (s)	6.99 (d, 8.5)
6-H(A)		7.05 (dd, 2.5, 8.5)
8-H(A)	7.22 (s)	7.12 (d, 2.5)
2'6'-H(B)	7.52 (d, 8.5)	8.25 (d, 8.5)
3'5'-H(B)	7.00 (d, 8.5)	7.54 (d, 8.5)
2-H(C)	7.97 (s)	8.06 (s)
OAc (aliph.)	2.138, 2.112, 2.68, 2.07	2.12, 2x 2.10, 2.06, 2.05, 2.30
OMe	3.87 (s) 3.95 (s)	3.86 (s)
H-1"	5.14 (d, 8.0)	5.23 (d, 8.0)
H-2",H-3"	5.36 (m)	5.34 (m)
H-4"	5.20 (t, 10.0)	5.06 (t, 10.0)
H-5"	3.90 (m)	5.93 (m)
H-6"	4.265-4.280 2x	3.80 (dd, 7.5, 11.0) 3.63 (dd, 2.5, 11.0)
H-1"'		5.02 (d, 1.0)
H-2"'		5.45 (d, 1.0)
3"'-CH_2		4.25 (d, 10.0) 4.16 (d, 10.0
H-4"'		4.84 (d, 12.0) 4.67 (d, 12.0)

Table 7: ^{1}H NMR peaks of isoflavone acetate derivatives (methanol extract) **(95)** and **(96)** from *C.intermedia* at 300 MHz (23^{0}C in $CDCl_3$). Splitting patterns and *J* (Hz) values are given in parentheses

Protons	(97) $CDCl_3$ 296K	(98) $CDCl_3$ 296K
3-H(C)	6.79 (s)	6.57 (s)
5-H(A)	8.26 (d, 8.5)	
6-H(A)	7.19 (dd, 2.5, 8.5)	7.36 (d, 2.5)
8-H(A)	7.44 (d, 2.5)	7.58 (d, 2.5)
2'-H(B)	7.78 (d, 2.5)	6.85 (d, 2.5)
5'-H(B)	7.40 (d, 8.5)	7.09 (d, 8.5
6'-H(B)	7.81 (dd, 2.5, 8.5)	7.75 (dd, 2.5, 8.5)
OAc (arom.)	2.39, 2.38, 2.36	2.46, 2.38, 2.37
OMe		3.93

Table 8: ^{1}H NMR peaks of flavones acetate derivatives (methanol extract) **(97)** and **(98)** from *C.intermedia* at 300 MHz (23^0C in $CDCl_3$).
Splitting patterns and *J* (Hz) values are given in parentheses

Protons	(99) $CDCl_3$ 296K	(100) $CDCl_3$ 296K
2-H(C)	5.52 (dd, 2.5, 11.0)	5.47 (dd, 2.5, 11.0)
3-Hα,β(C)	overlapped by acetoxy groups	2.04(m)
4-H(C)	4.37 (m)	4.09 (m)
6-H(A)	6.40 (d, 2.5)	6.40 (d, 2.5)
8-H(A)	6.33 (d, 2.5)	6.36 (d, 2.5)
2'6'-H(B)	7.35 (d, 8.5)	7.32 (d, 8.5)
3'5'-H(B)	7.10 (d, 8.5)	6.08 (d, 8.5)
2",6"-H(D)	7.43 (m)	7.45 (m)
2",6"-H(D	7.34 (m)	7.33 (m)
OAc (arom.)	2.33, 1.29	2.32, 2.29
OAc (aliph.)	2.19, 2.14, 2.08, 2.00, 1.97	2.10, 2x 2.09 2.08
H-1"'	5.13 (d, 7.0)	5.17 (d, 7.5)
H-2"'	3.59 (dd, 2.5, 9.0)	5.43 (t, 7.5)
H-3"'	5.30 (t, 9.0)	5.35 (t, 9.0)
H-4"'	5.14 (t, 9.0)	5.24 (t, 10.0)
H-5"'	3.90 (m)	3.97 (m)
H-6"'	4.27 (dd, 5.5, 12.0)	4.22 (dd, 2.5, 12.0)
	4.19 (dd, 2.5, 12.0)	4.30 (dd, 5.5, 12.0)
H-1""	4.69 (d, 2.0)	
H-2"",H-3""	5.07 (m)	
H-4""	4.96 (t, 10)	
H-5""	3.73 (m)	
CH_3	0.88 (6.0)	
2x CH_2S	3.86 (br.s)	

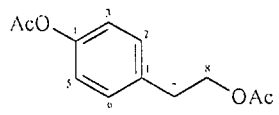
Table 9: ^{1}H NMR peaks of acetate derivatives **(99)** and **(100)** from cleavage reaction of the 'tannin' extract with phloroglucinol as the capture nucleophile at 300 MHz (23^{0}C in $CDCl_3$). Splitting patterns and *J* (Hz) values are given in parentheses

Protons	(101) $CDCl_3$ 296K	(102) $CDCl_3$ 296K	(103) $CDCl_3$ 296K
2-H(C)	5.10 (dd, 2.5, 11.0)	5.12 (dd, 2.5, 12.0)	5.10 (dd, 2.5, 11.0)
3-Hα,β(C)	2.70, 2.03 (m)	2.69, 2.02 (m)	2x 2.28 overlapped
4-H(C)	4.33 (br.d, 5.0)	4.33 (d, 5.0)	4.52 (dd, 3.0, 8.0)
6-H(A),8-H(A)	2x 6.60 (s)	6.60, 6.61 (d, 2.5)	6.27, 6.48 (d, 2.5)
2'6'-H(B)	7.12 (d, 8.5)		7.46 (d, 8.5)
3'5'-H(B)	7.05 (d, 8.5)		7.12 (d, 8.5)
2'-H(B)		6.91 (d, 2.5)	
5'-H(B)		7.17 (d, 8.5)	
6'-H(B)		7.02 (dd, 2.5, 8.5)	
3",5"-H(D)	2x 6.83 (s)	2x 6.83 (s)	6.85, 6.72 (d. 2.5)
OAc (arom.)	2x 2.30, 2.28	2.29, 2x 2.23, 2x 1.94	2.46, 2.32, 2.28, 2.26
	2x 1.90, 1.89	1.92	2.05, 2.03, 1.98, 1.88,
OAc (aliph.)			1.86
H-1"'			5.18 (d, 7.0)
H-2"'			4.76 (dd, 2.0, 8.0)
H-3"'			5.22 (t, 9.0)
H-4"'			5.14 (t, 10.0)
H-5"'			3.78 (m)
H-6"'			4.32 (dd, 7.0, 12.0)
			3.90 (dd, 2.5, 12.0)

Table 10: ^{1}H NMR peaks of acetate derivatives **(101) (102) (103)** from the cleavage reaction of the 'tannin' extract with phloroglucinol as the capture nucleophile at 300 MHz (23^{0}C in $CDCl_3$). Splitting patterns and *J* (Hz) values are given in parentheses

APPENDIX B

Plate 1 ($CDCl_3$ - 296K)
(^1H NMR)



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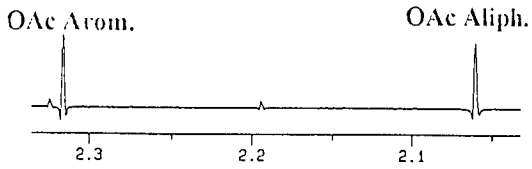
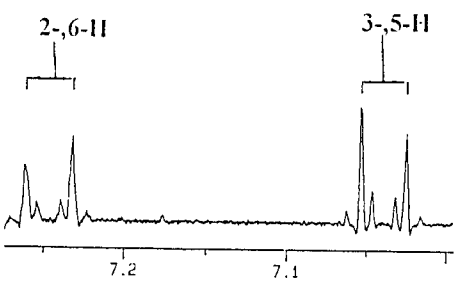
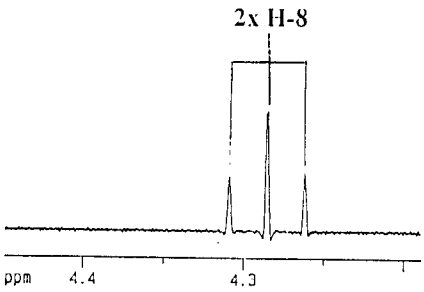
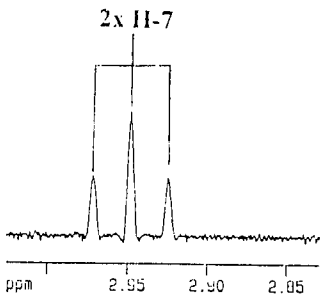








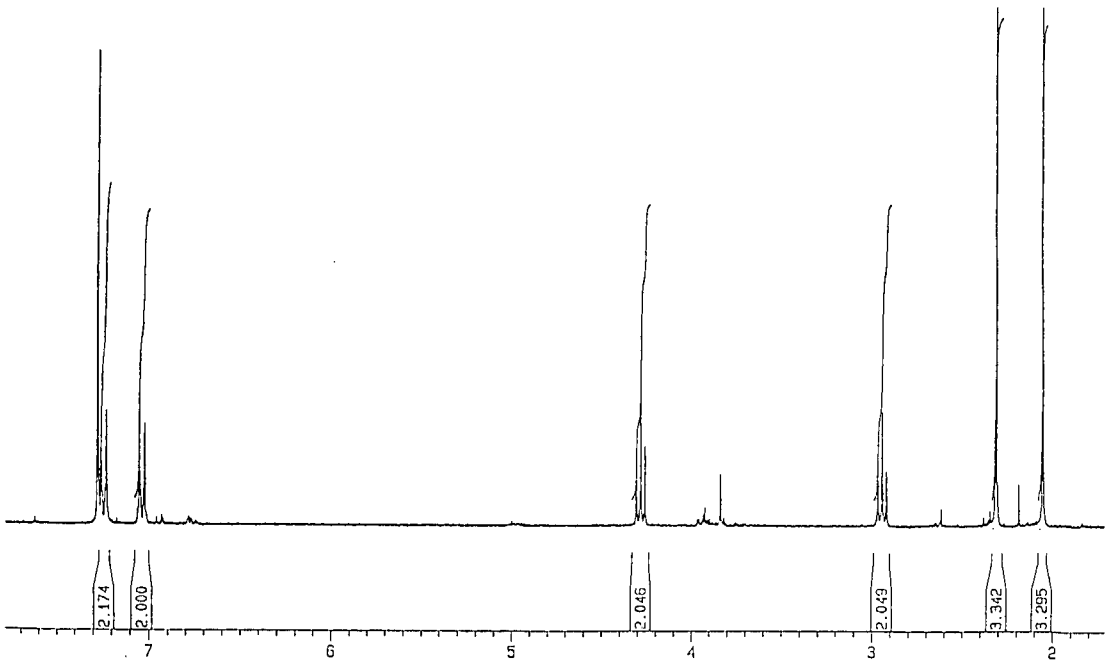
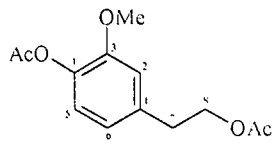


Plate 2 ($CDCl_3$ - 296K)
(^1H NMR)



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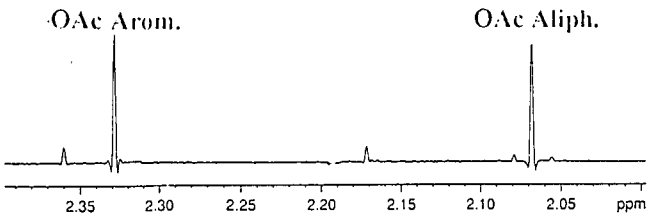
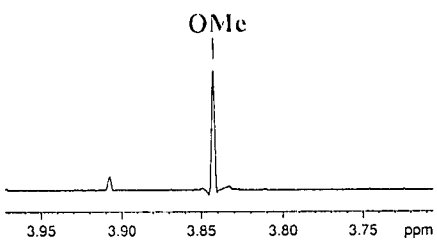
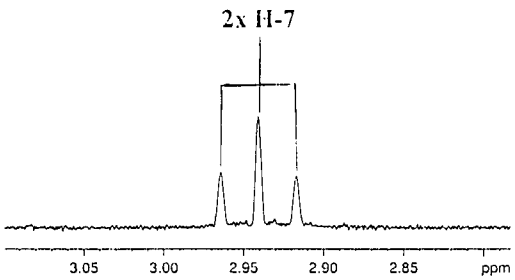
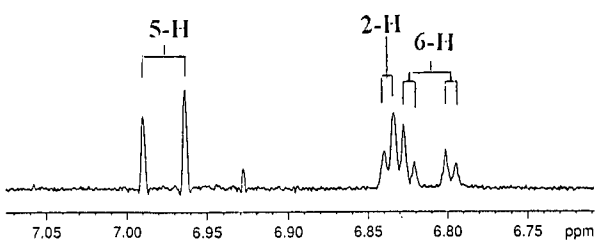
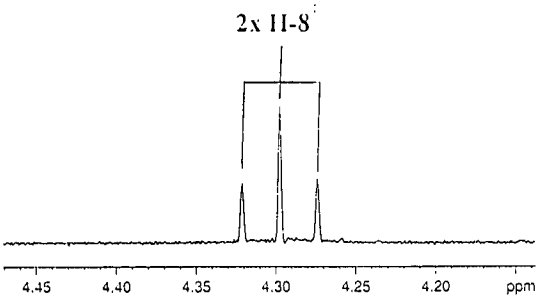










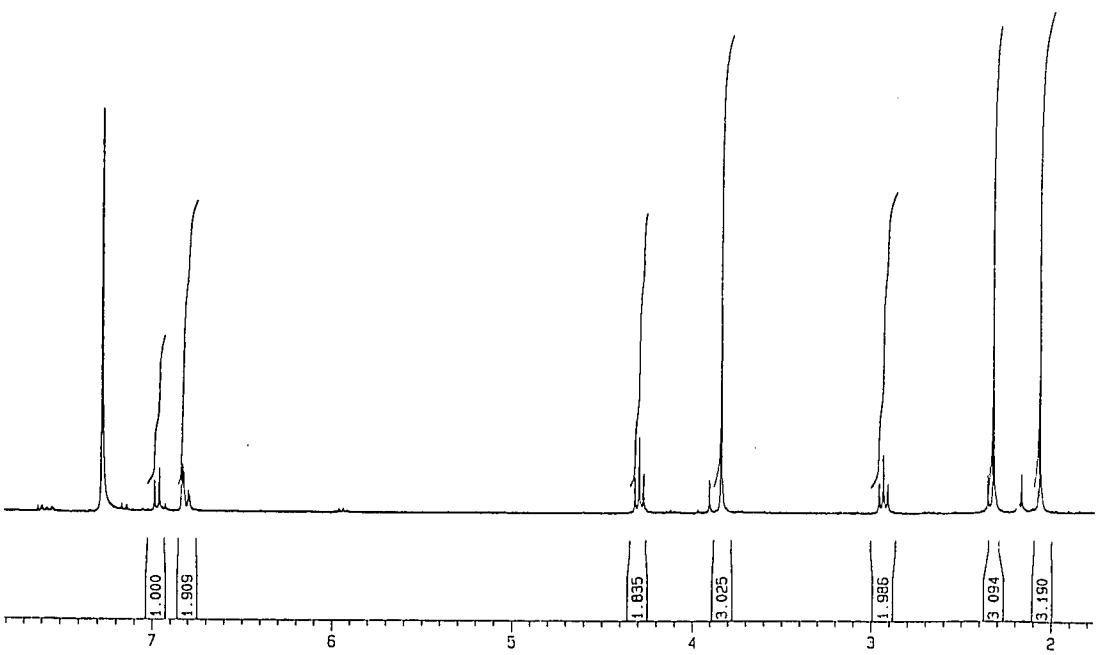
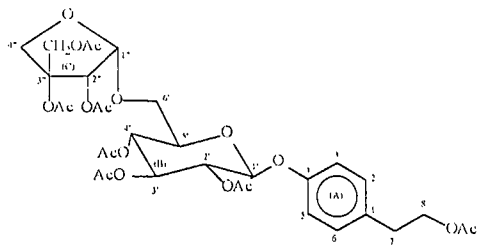


Plate 3 ($CDCl_3$ - 296K)
(^1H NMR)



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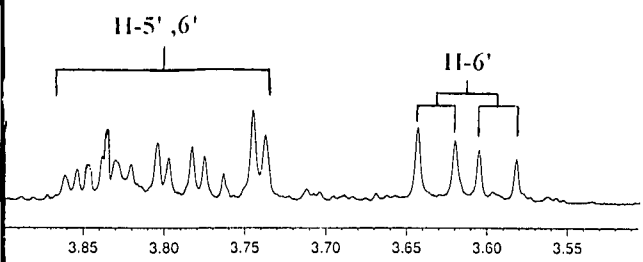
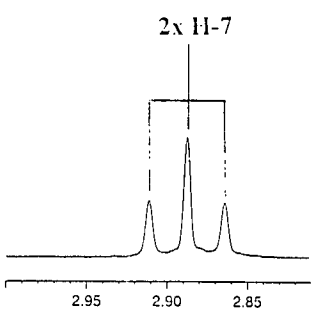
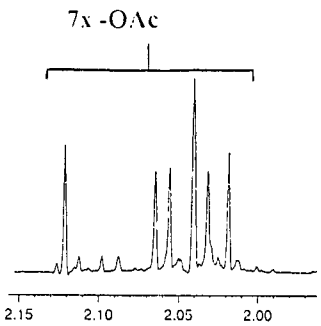
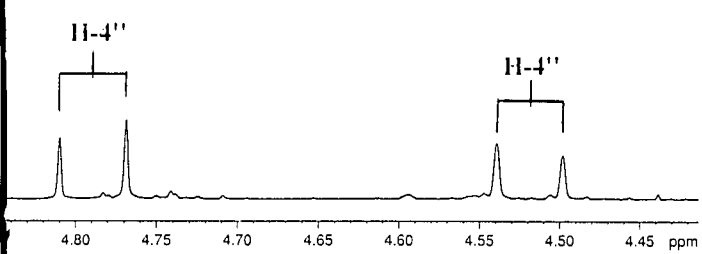
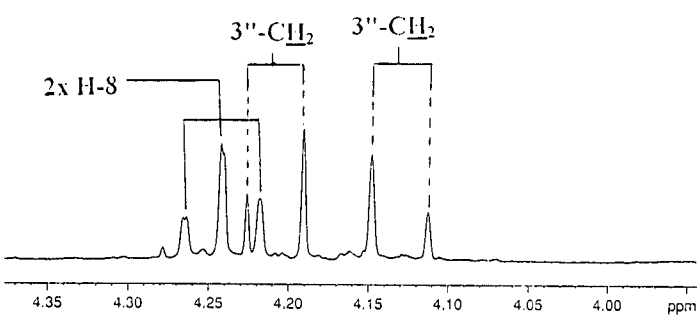
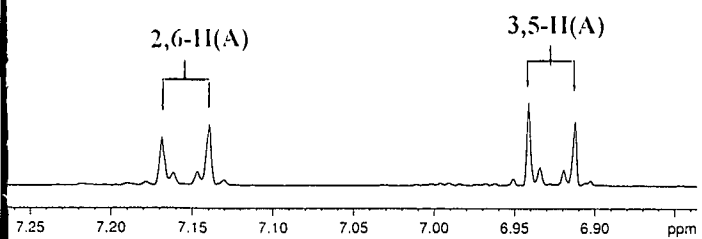


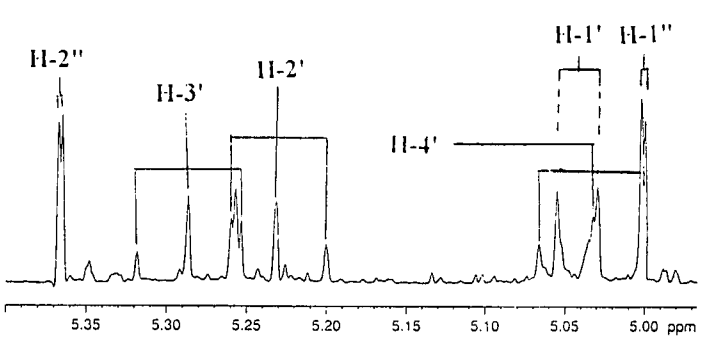












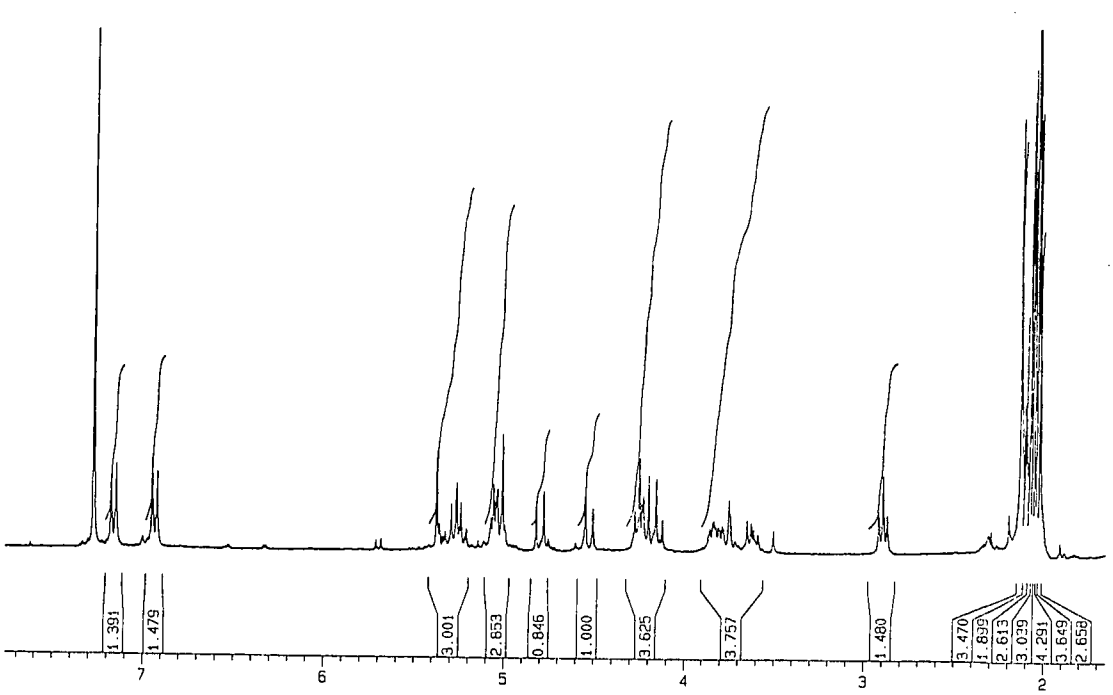
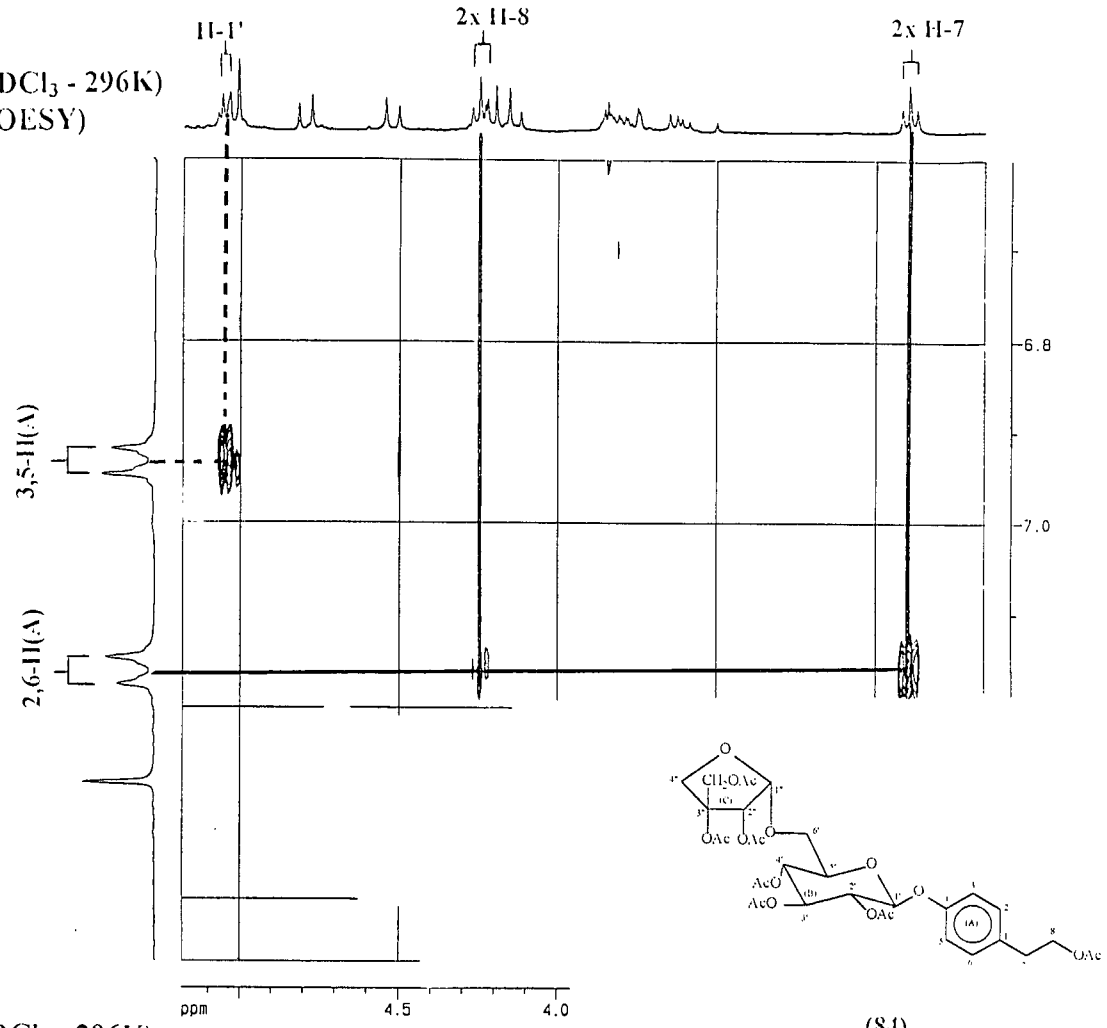


Plate 3a-1 ($CDCl_3$ - 296K)
(NOESY)



(84)

Plate 3a-2 ($CDCl_3$ - 296K)
(NOESY)

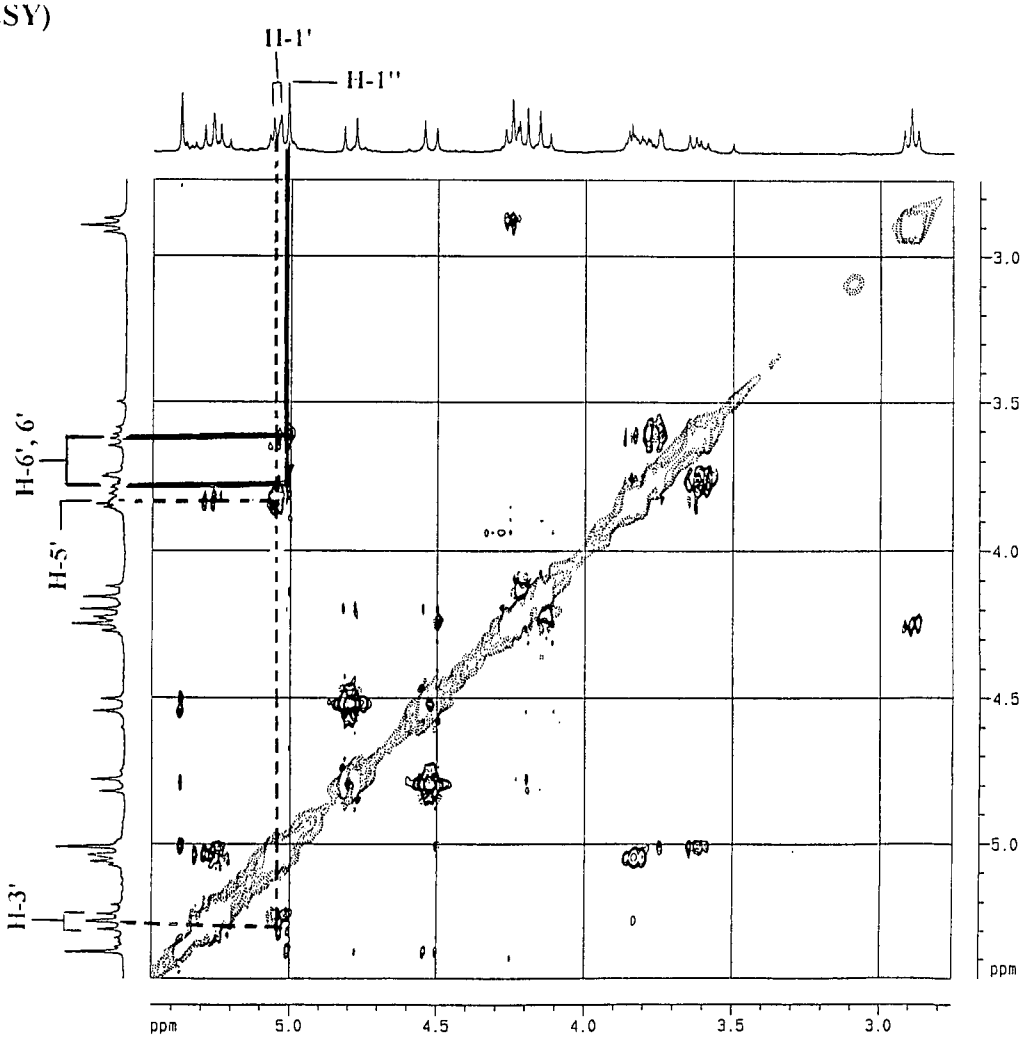
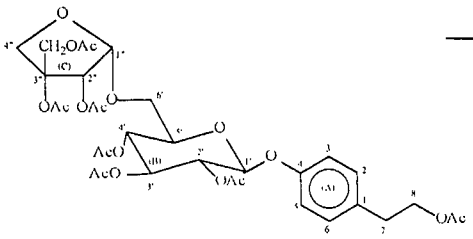


Plate 3b-1 ($CDCl_3$ - 296K)
(COSY)



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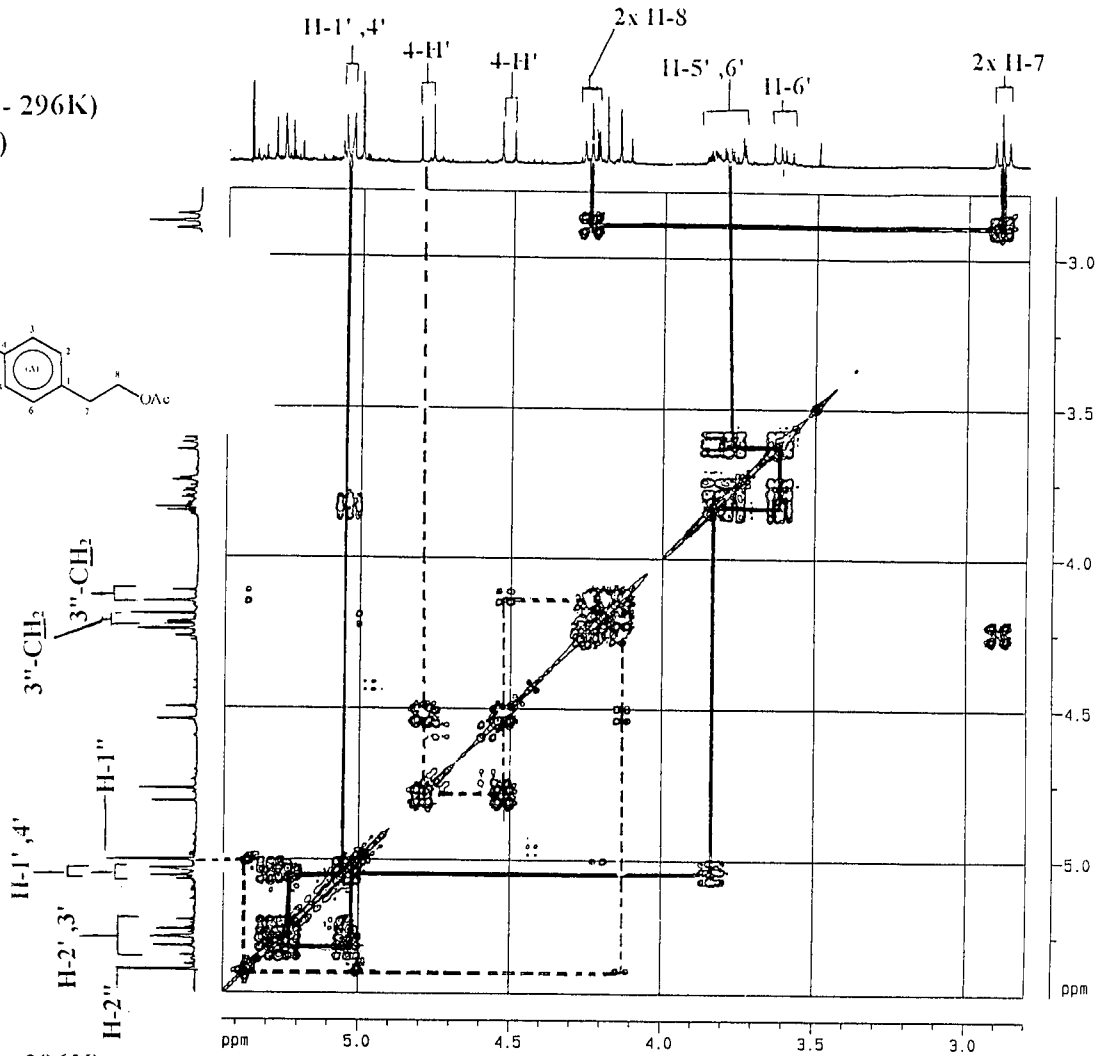


Plate 3b-2 ($CDCl_3$ - 296K)
(COSY)

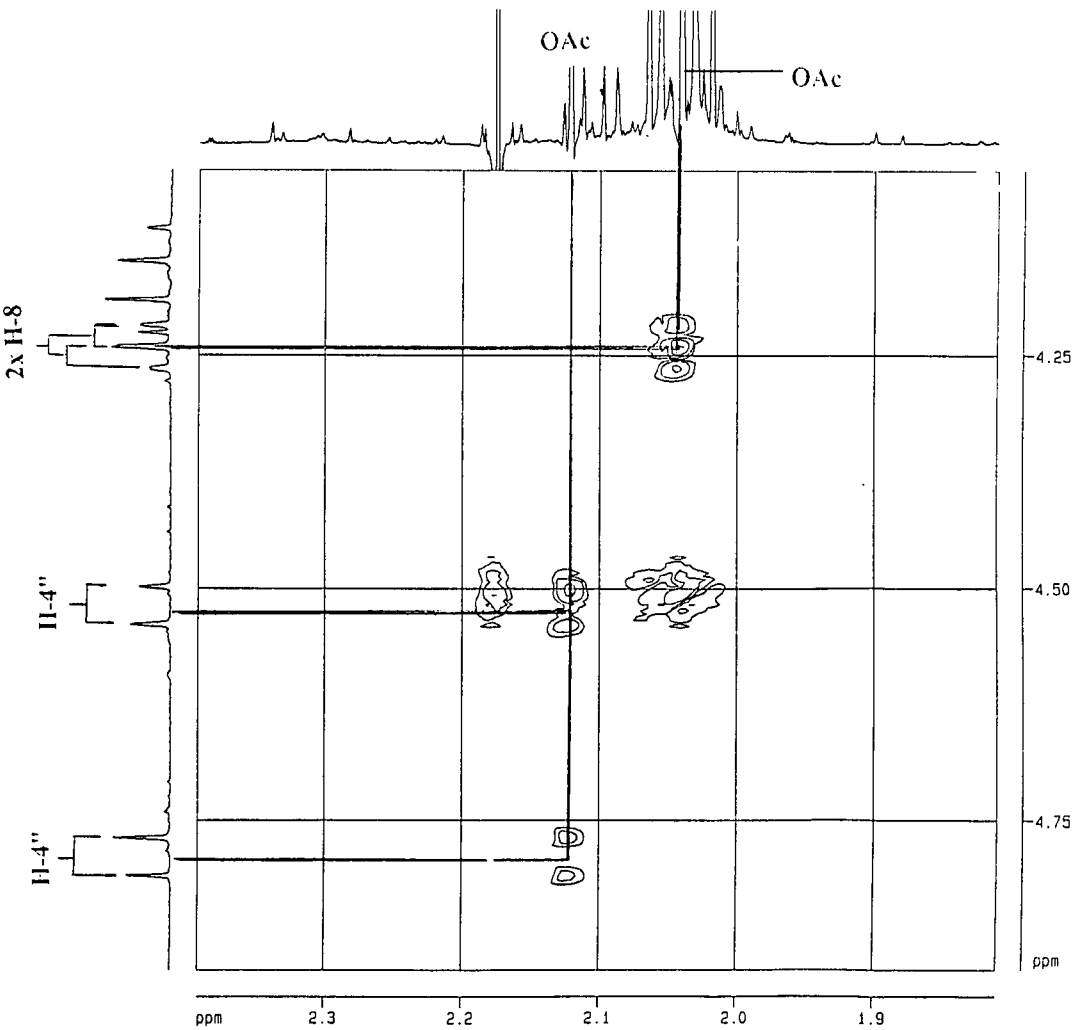
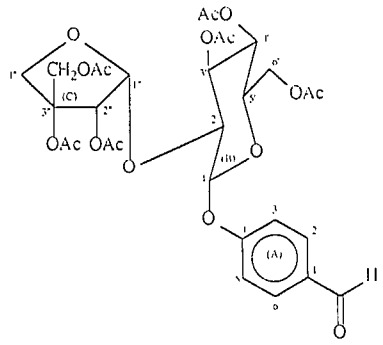


Plate 4 ($CDCl_3$ - 296K)
(^1H NMR)



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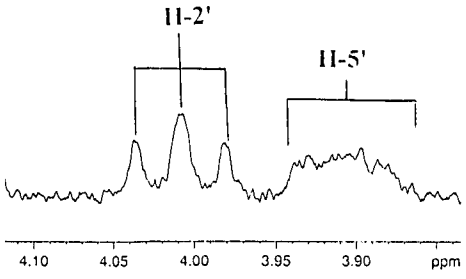
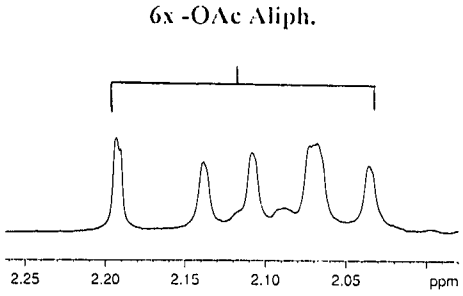
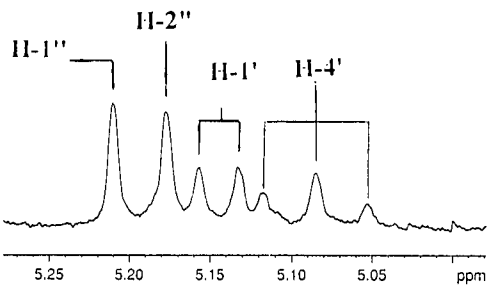
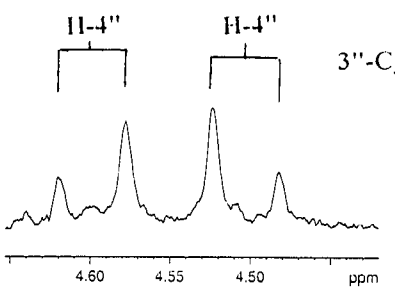
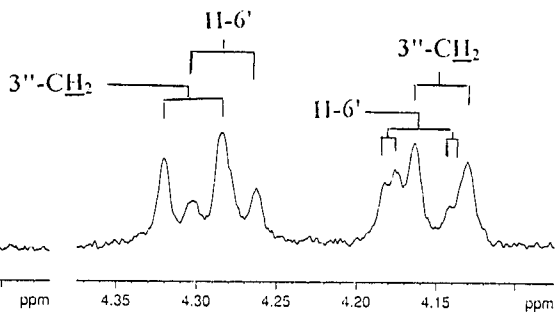
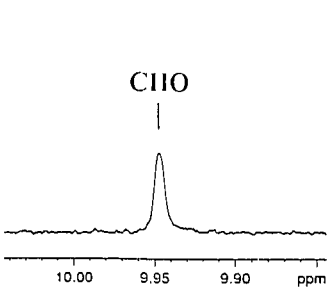


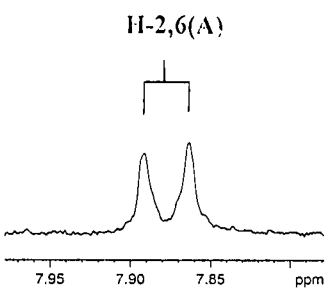


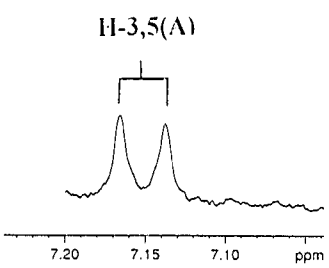


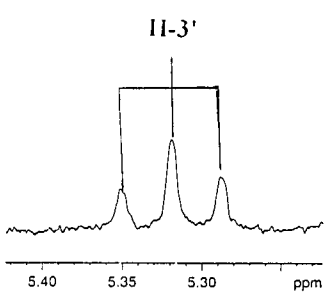












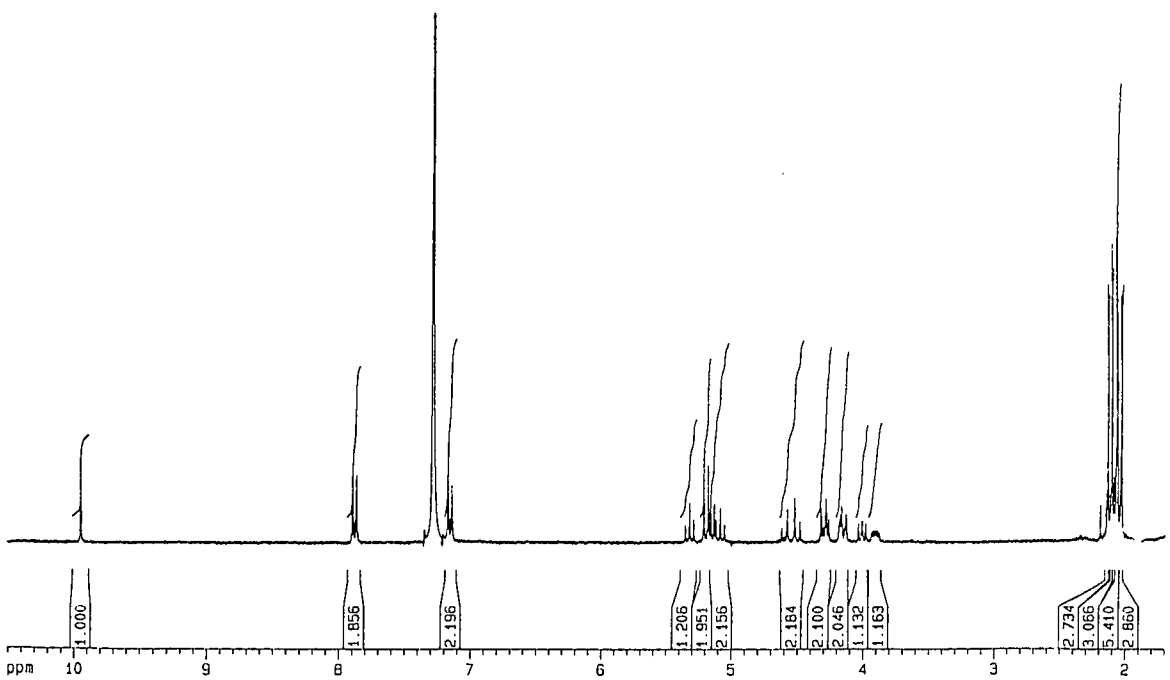
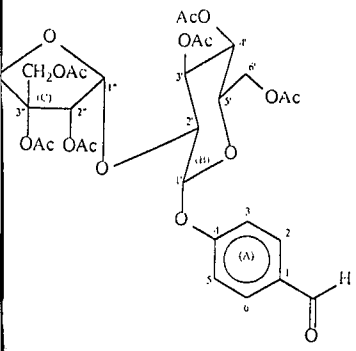


Plate 4a-1 ($CDCl_3$ - 296K)
(NOESY)



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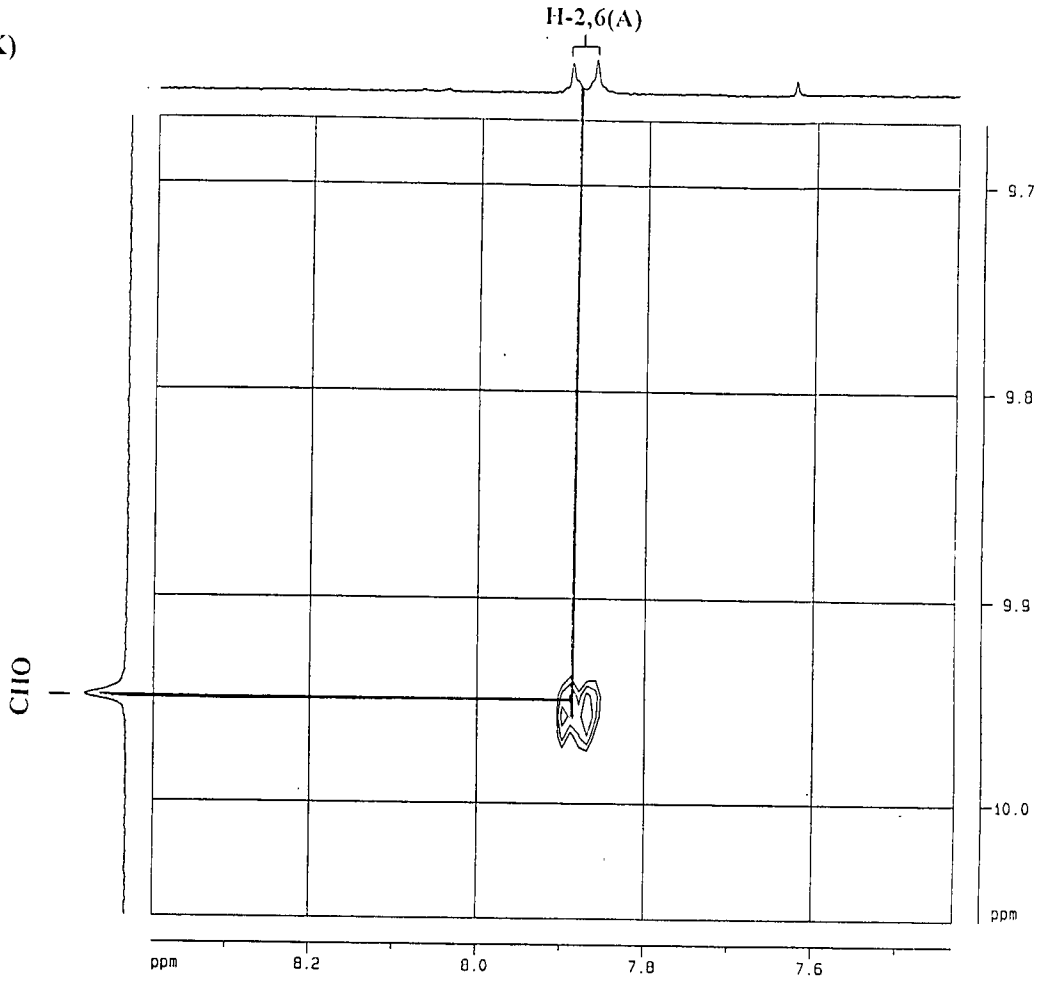


Plate 4a-2 ($CDCl_3$ - 296K)
(NOESY)

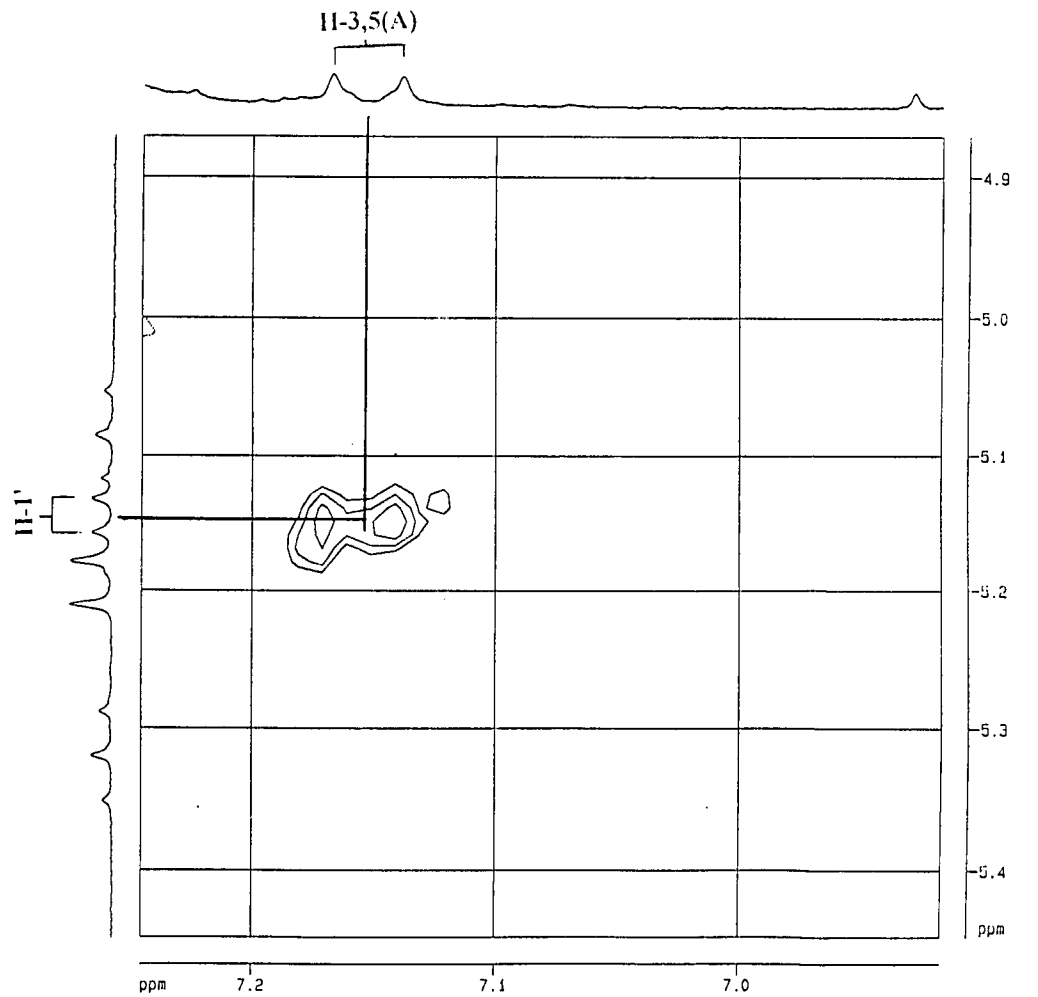
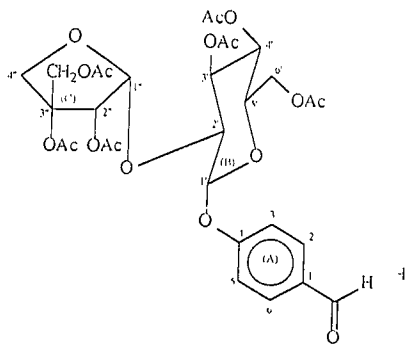


Plate 4a-3 ($CDCl_3$ - 296K)
(NOESY)



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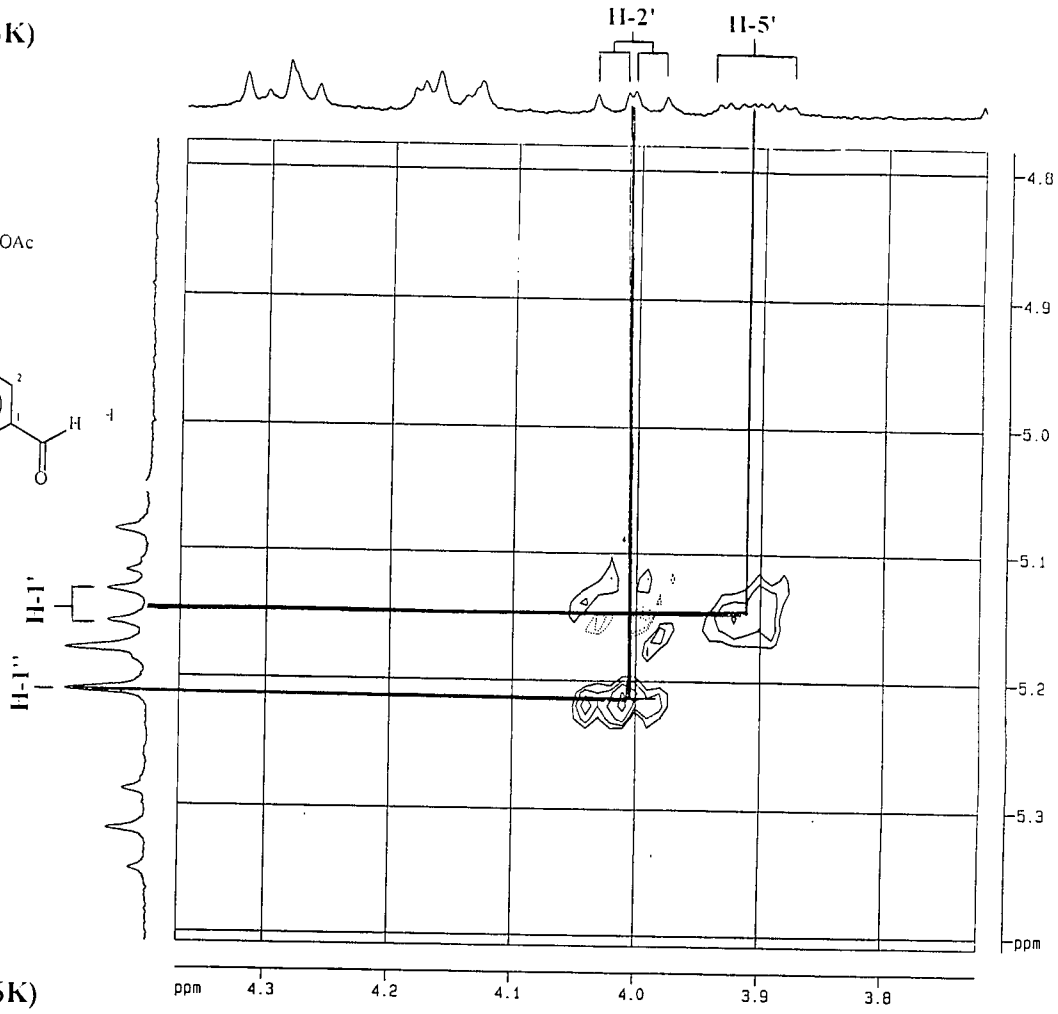

Plate 4a-4 ($CDCl_3$ - 296K)
(NOESY)

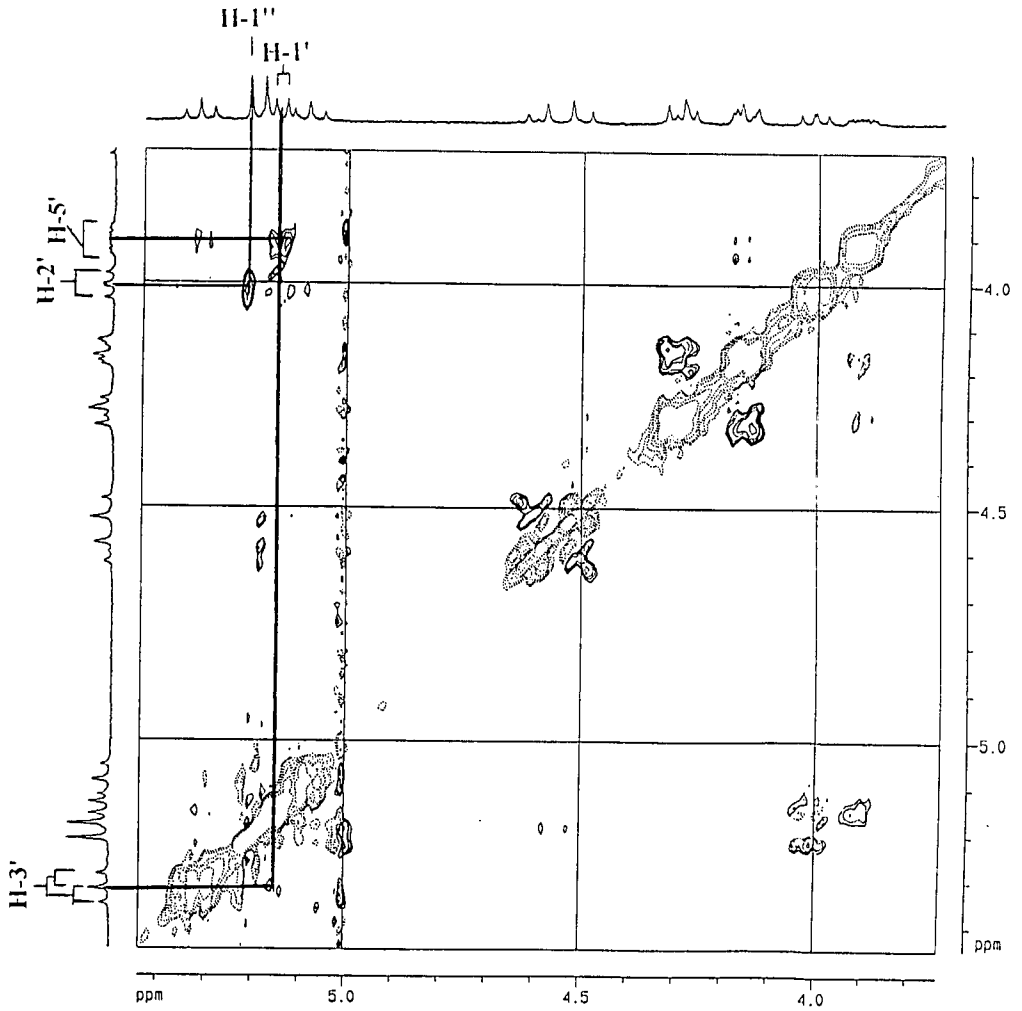

Plate 4b-1 ($CDCl_3$ - 296K)
(COSY)

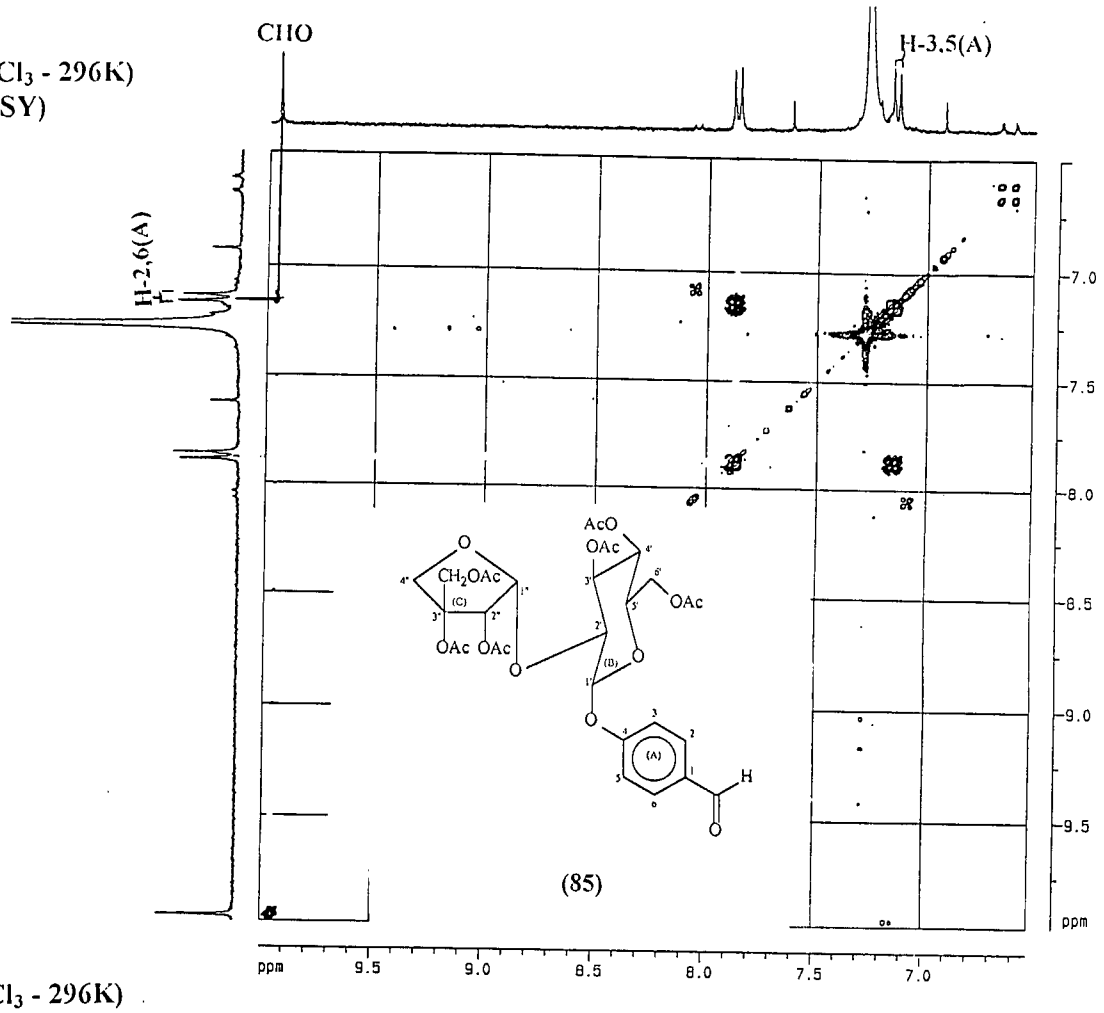

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Plate 4b-2 ($CDCl_3$ - 296K)
(COSY)

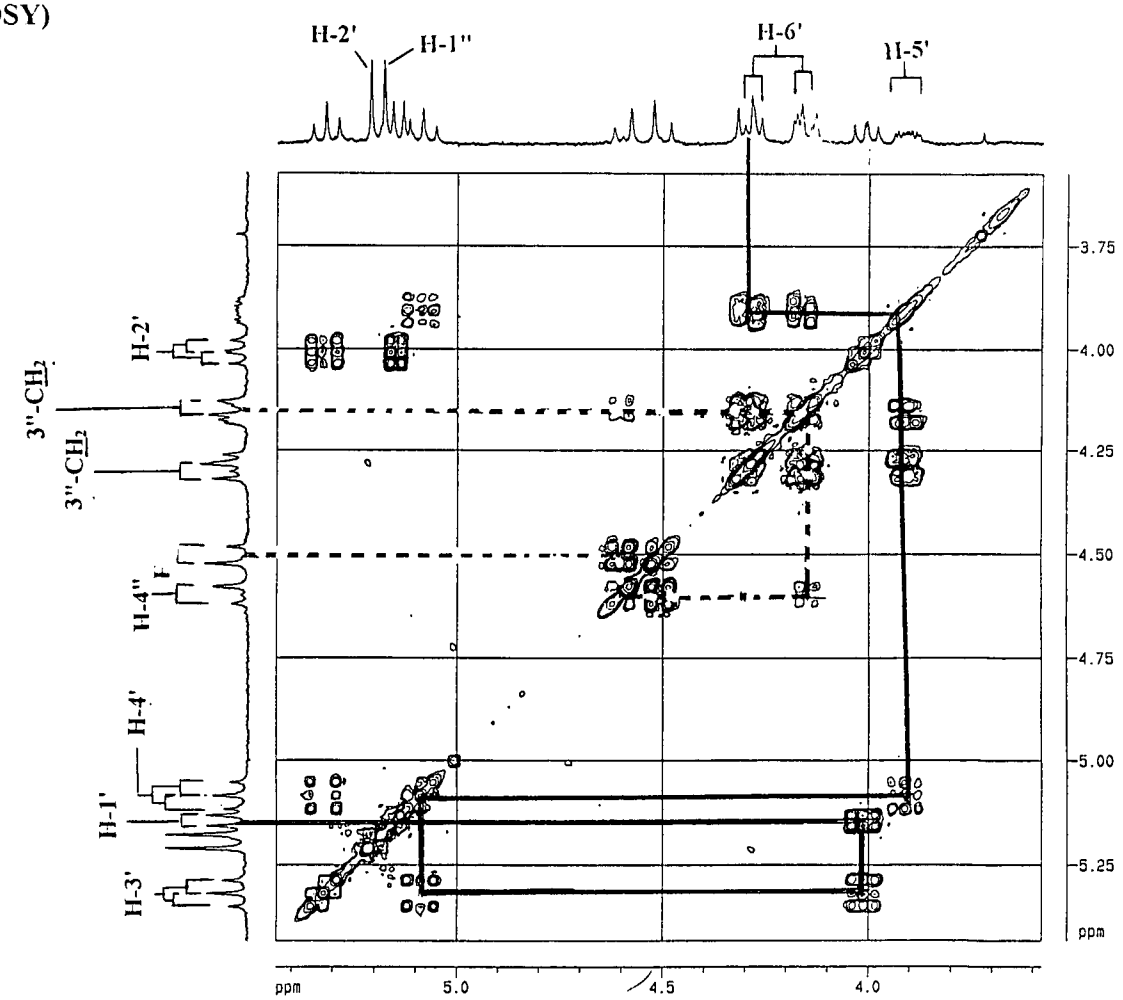
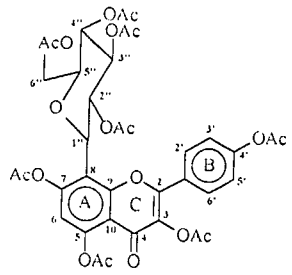


Plate 5 ($CDCl_3$ - 296K)
(^1H NMR)



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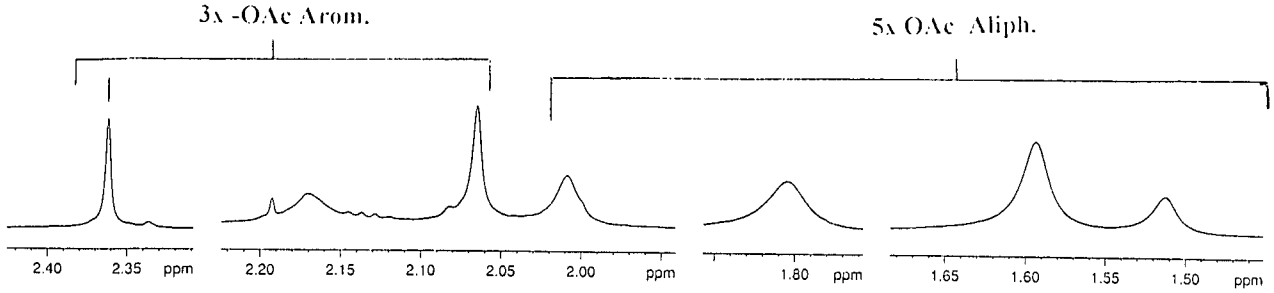

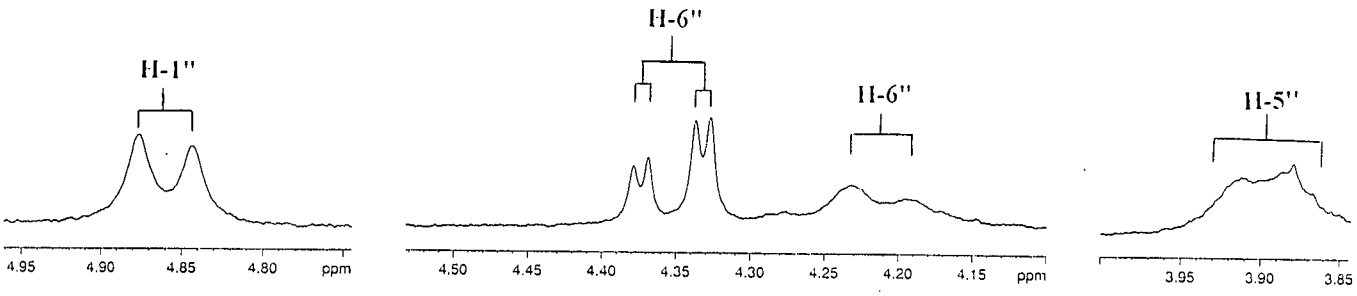

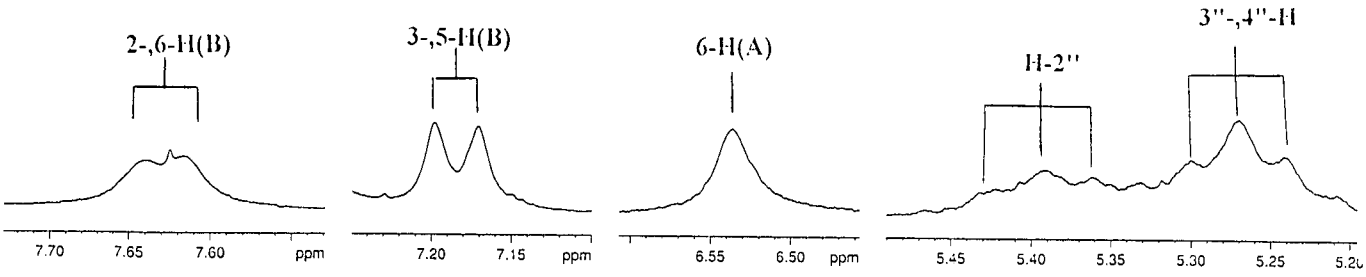

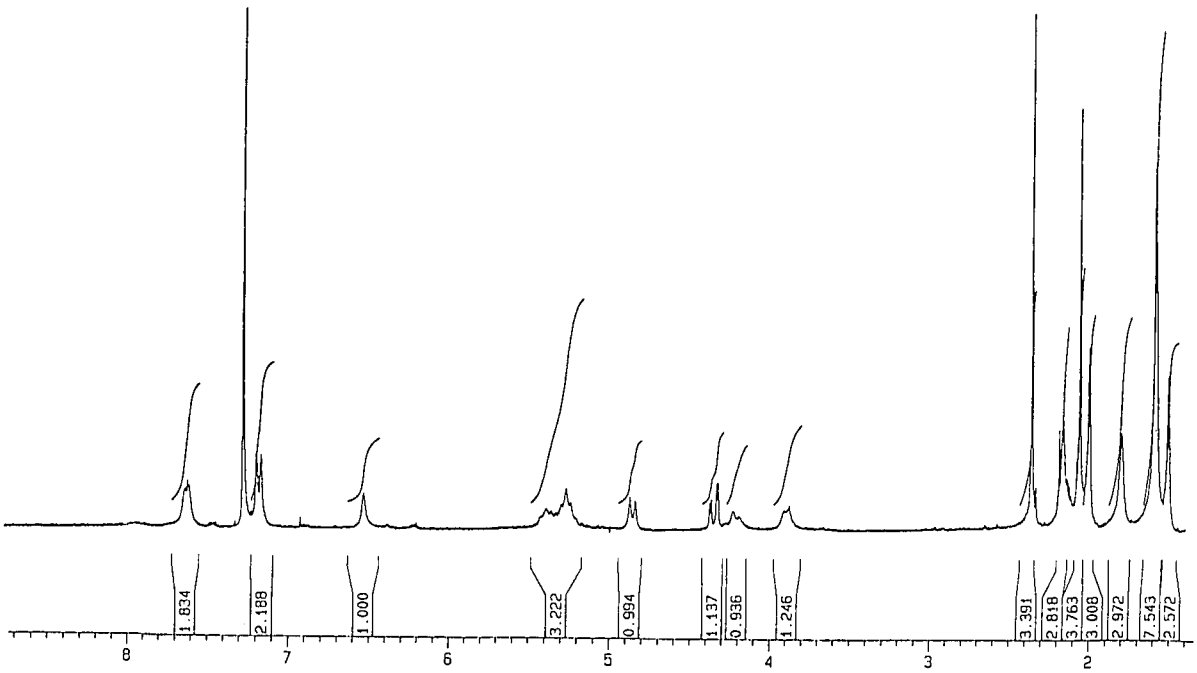

Plate 5a-1 ($CDCl_3$ - 296K)
(NOESY)

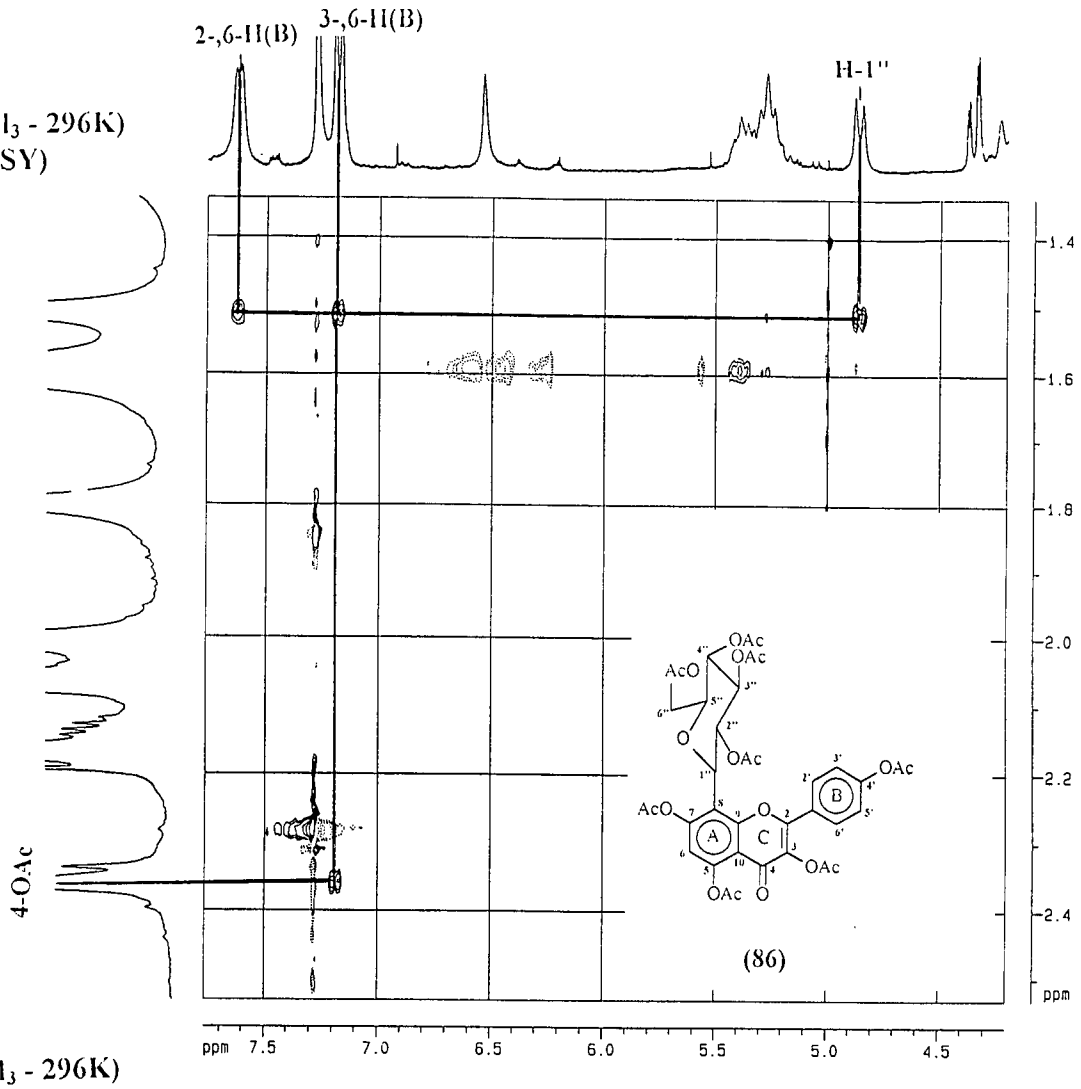

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Plate 5a-2 ($CDCl_3$ - 296K)
(NOESY)

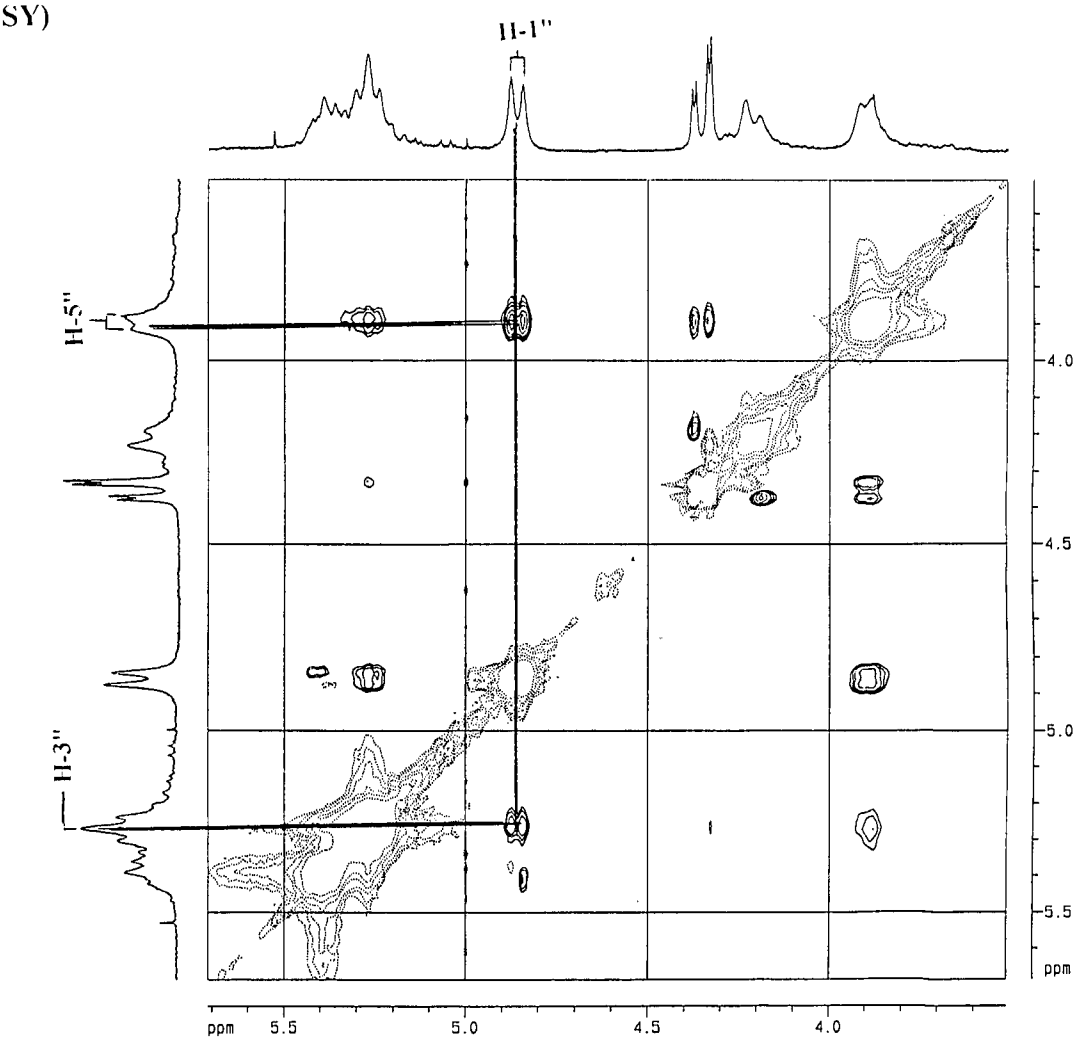
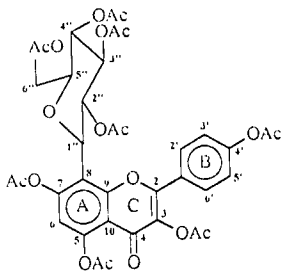


Plate 5b ($CDCl_3$ - 296K)
(COSY)



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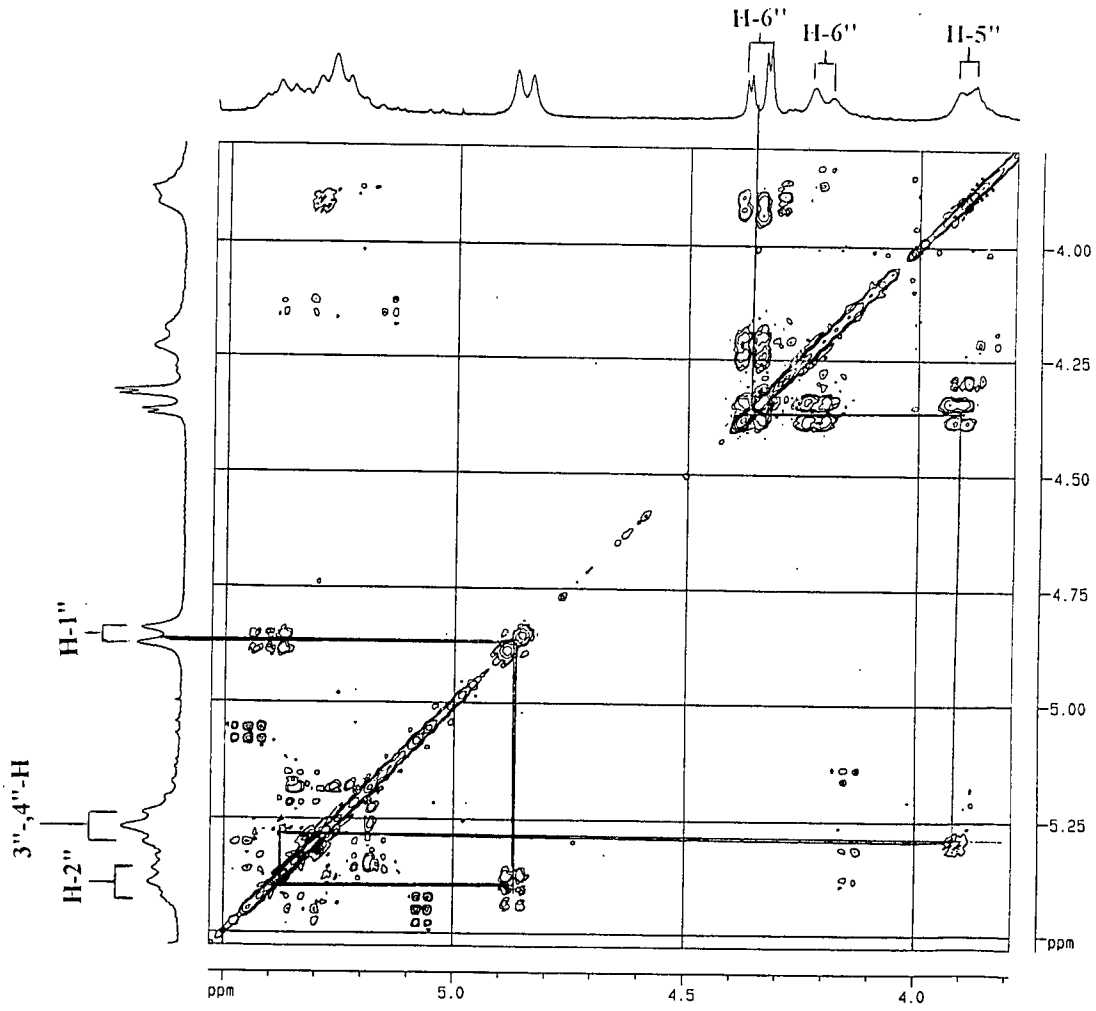
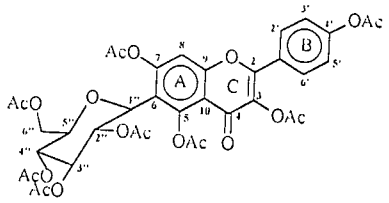


Plate 6 ($CDCl_3$ - 296K)
(^1H NMR)



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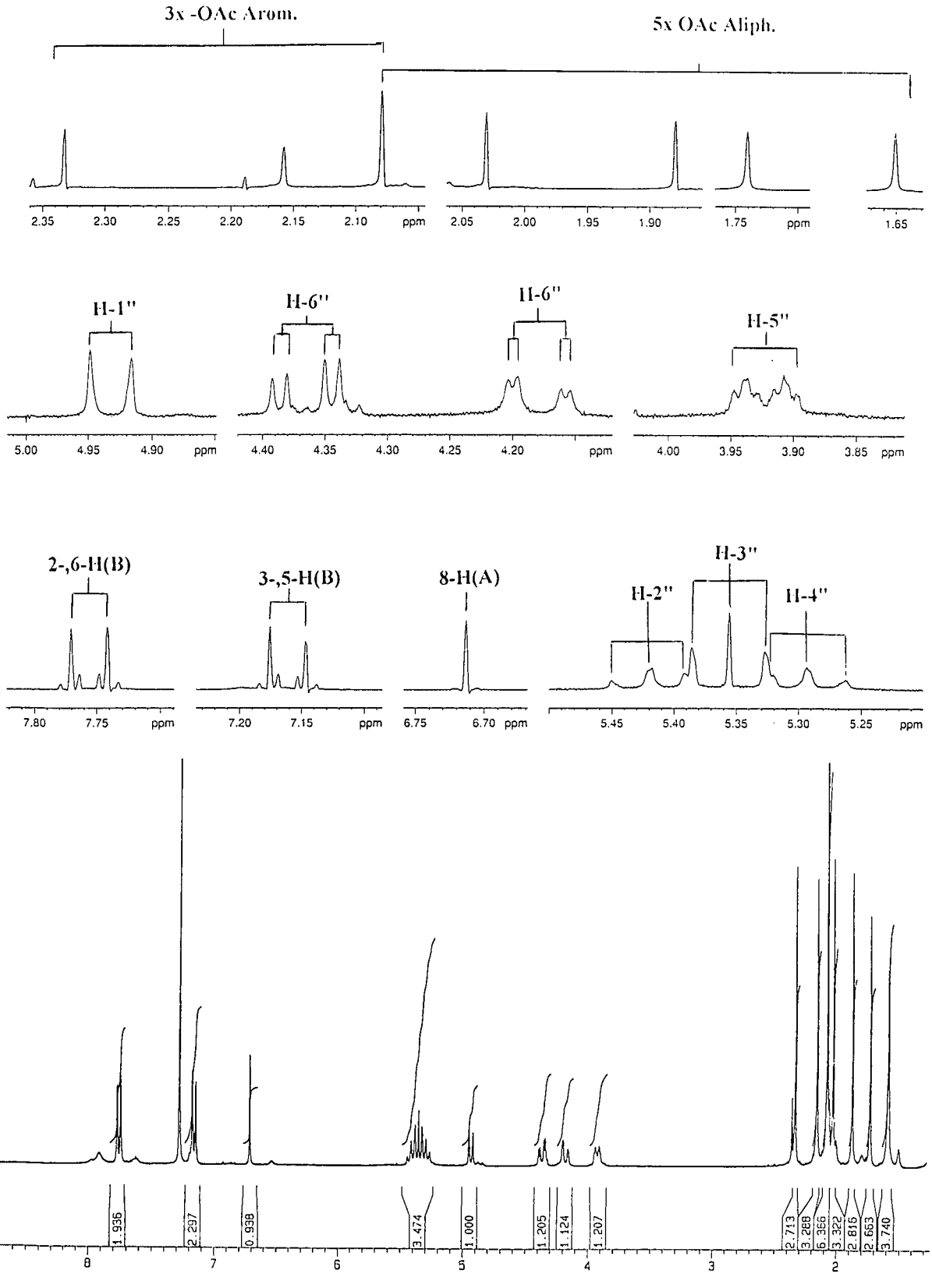

Plate 6a-1 ($CDCl_3$ - 296K)
(NOESY)

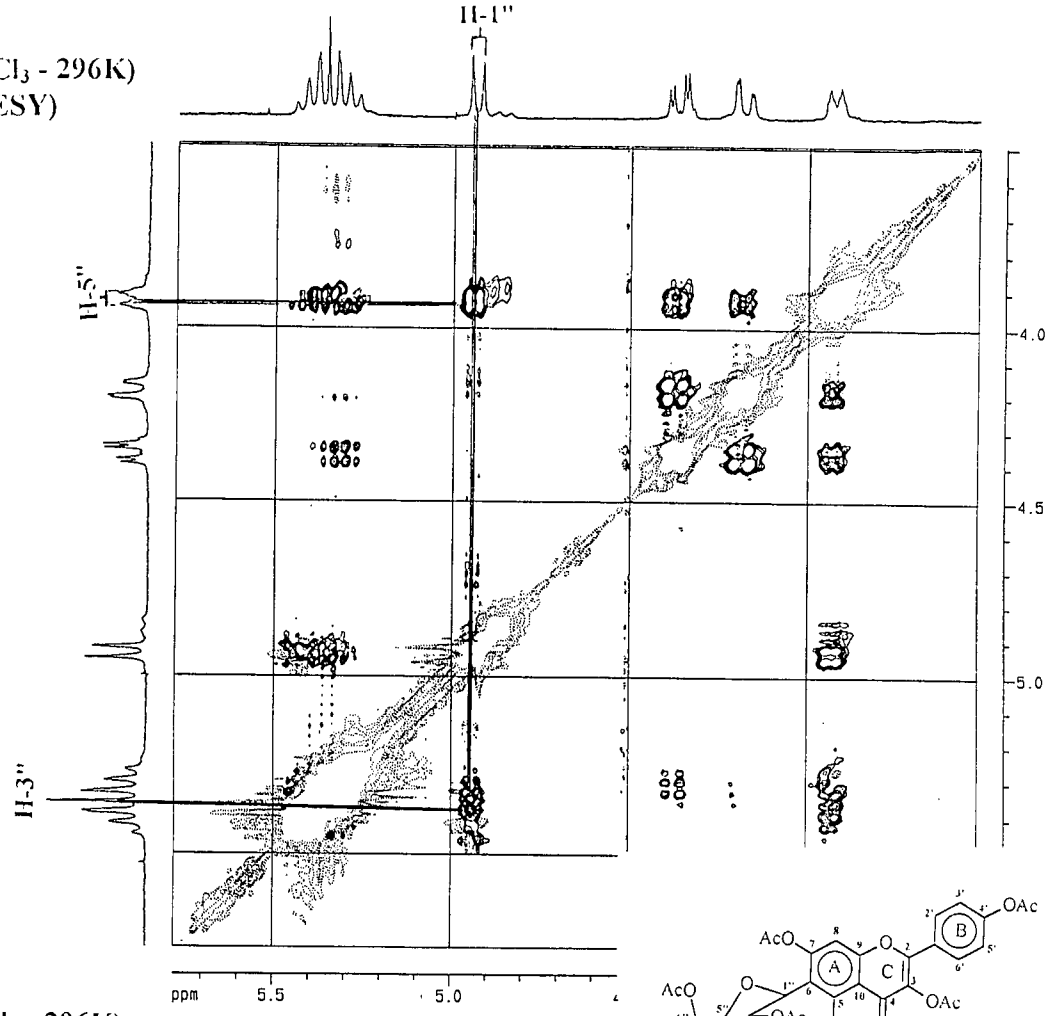

Plate 6a-2 ($CDCl_3$ - 296K)
(NOESY)

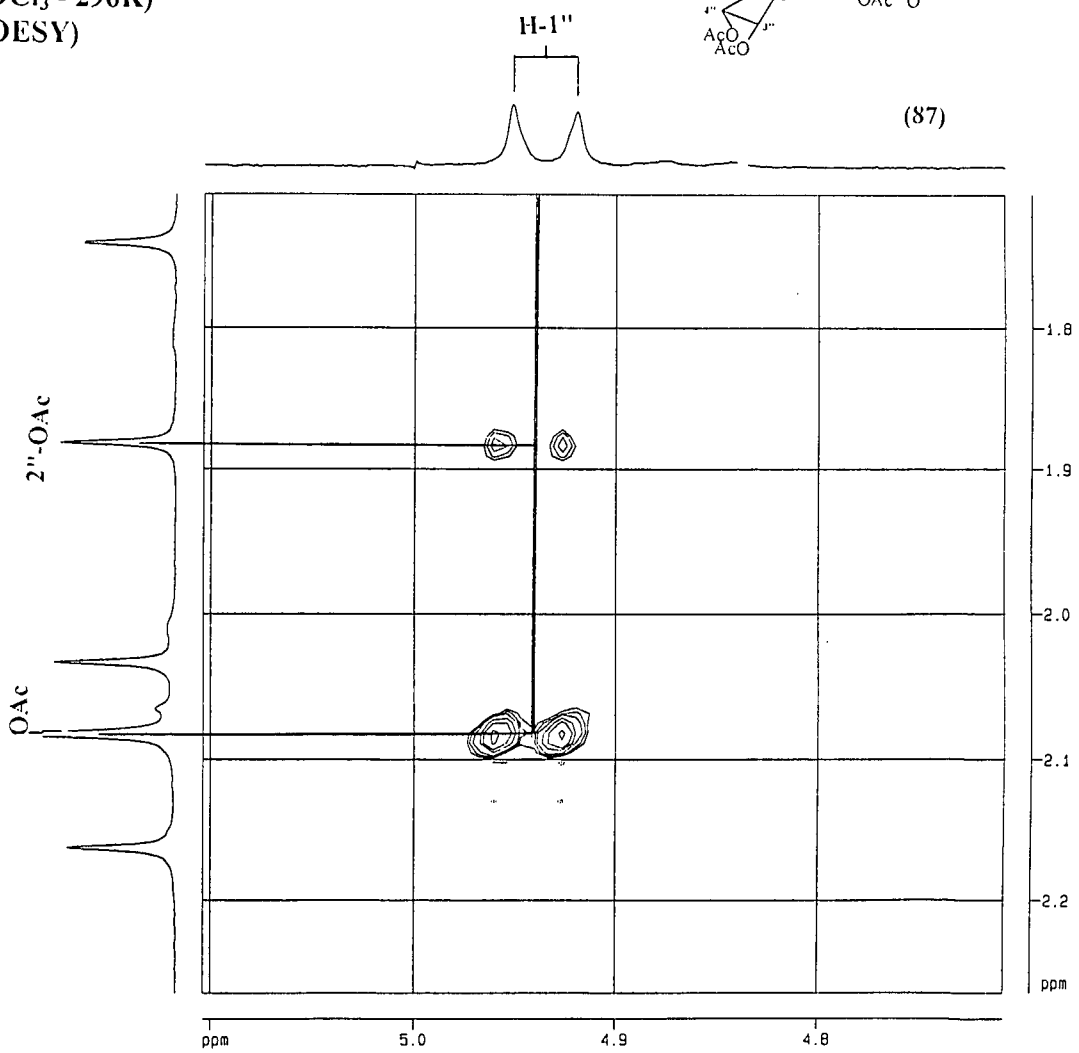

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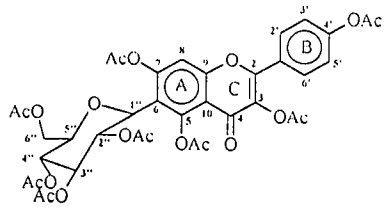
Plate 6b ($CDCl_3$ - 296K)
(COSY)



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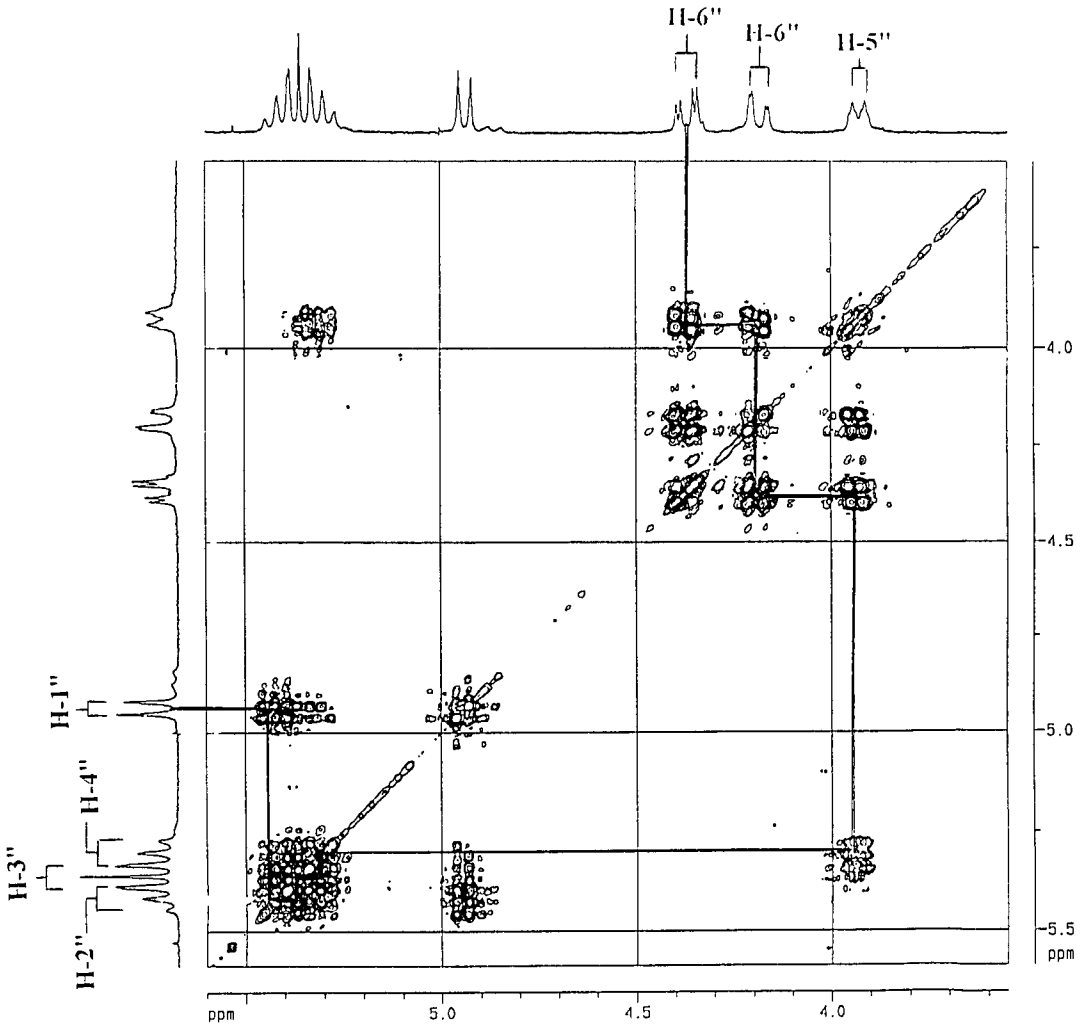
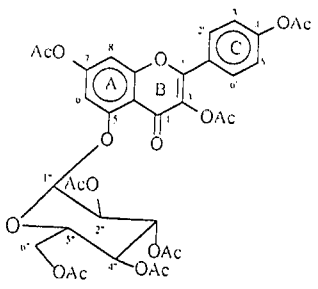


Plate 7 ($CDCl_3$ - 296K)
(^1H NMR)



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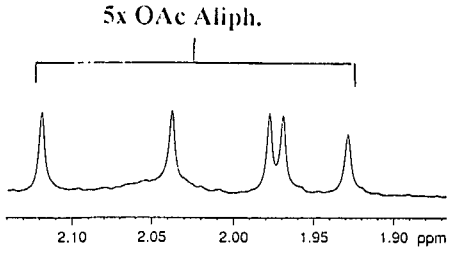
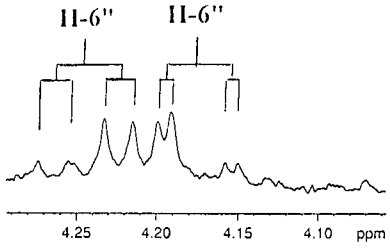
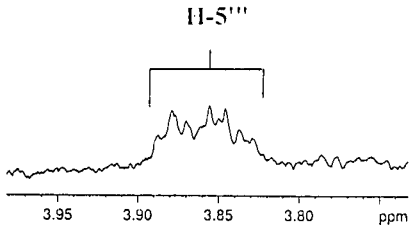
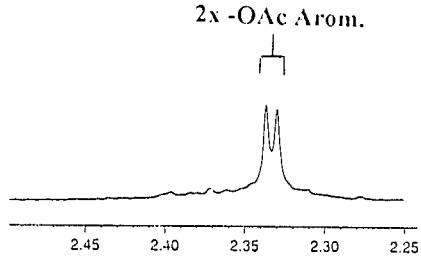
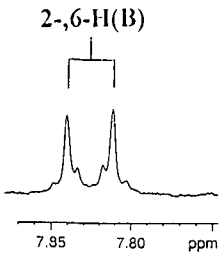
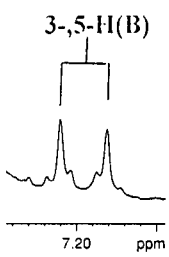


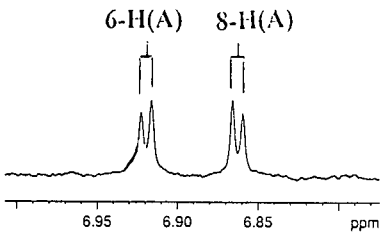


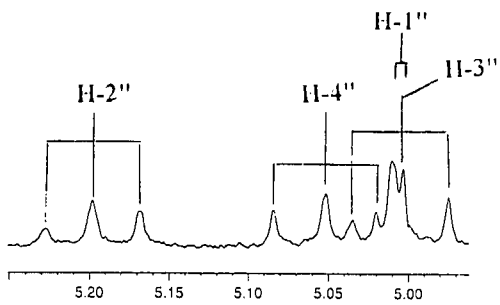












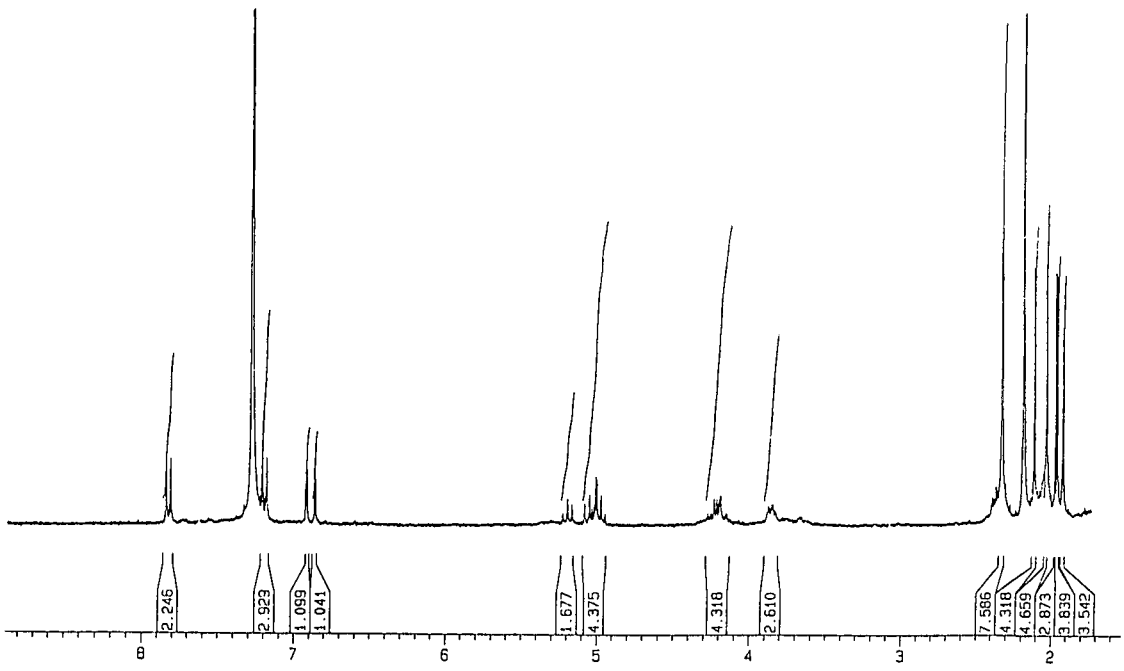


Plate 7a-1 ($CDCl_3$ - 296K)
(NOESY)

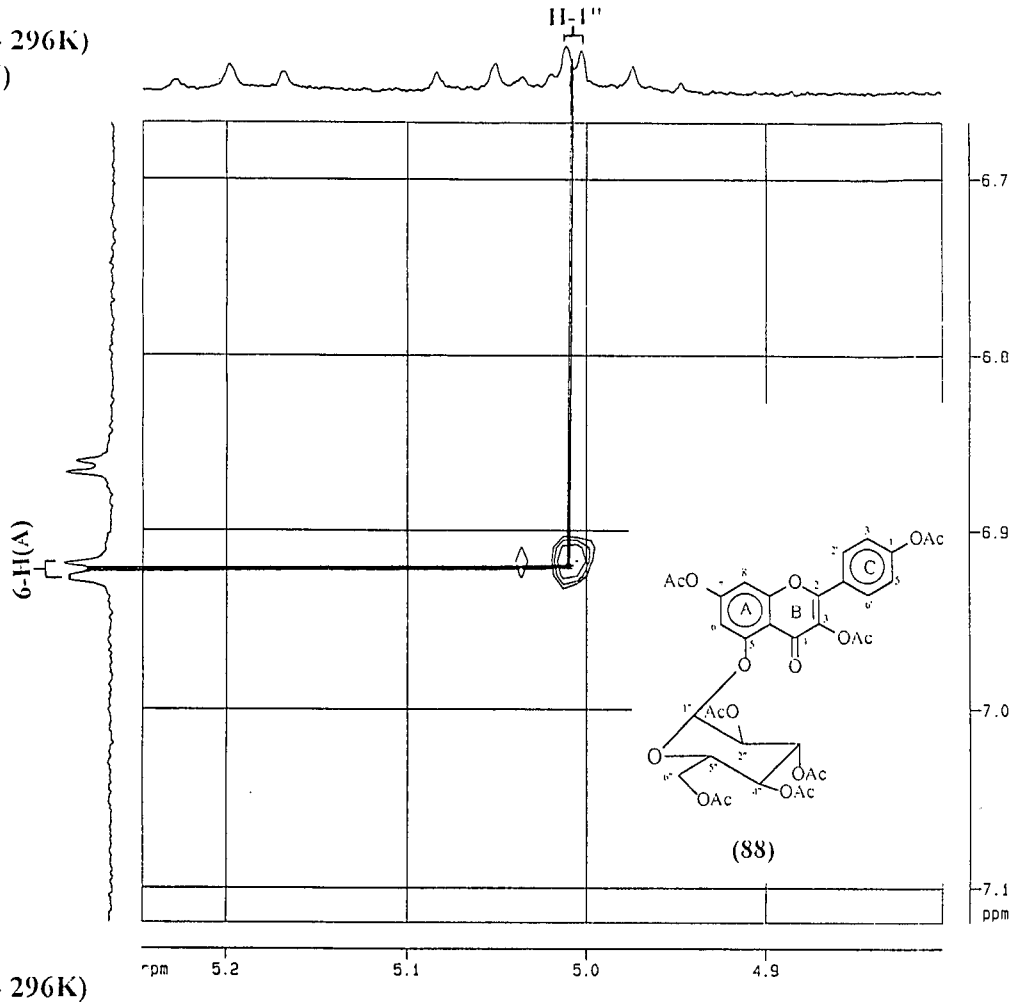


Plate 7a-2 ($CDCl_3$ - 296K)
(NOESY)

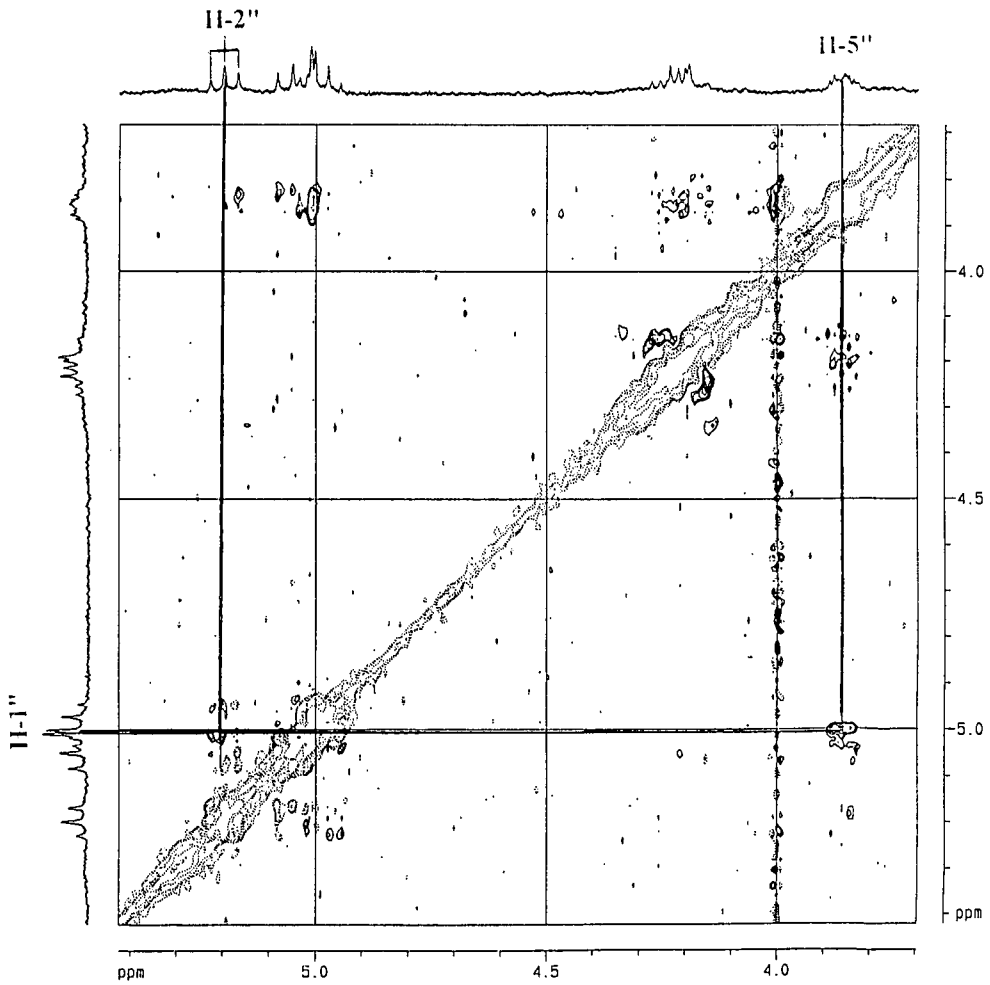
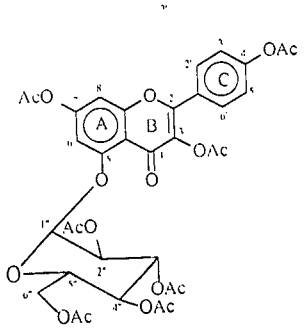


Plate 7b ($CDCl_3$ - 296K)
(COSY)



(88)

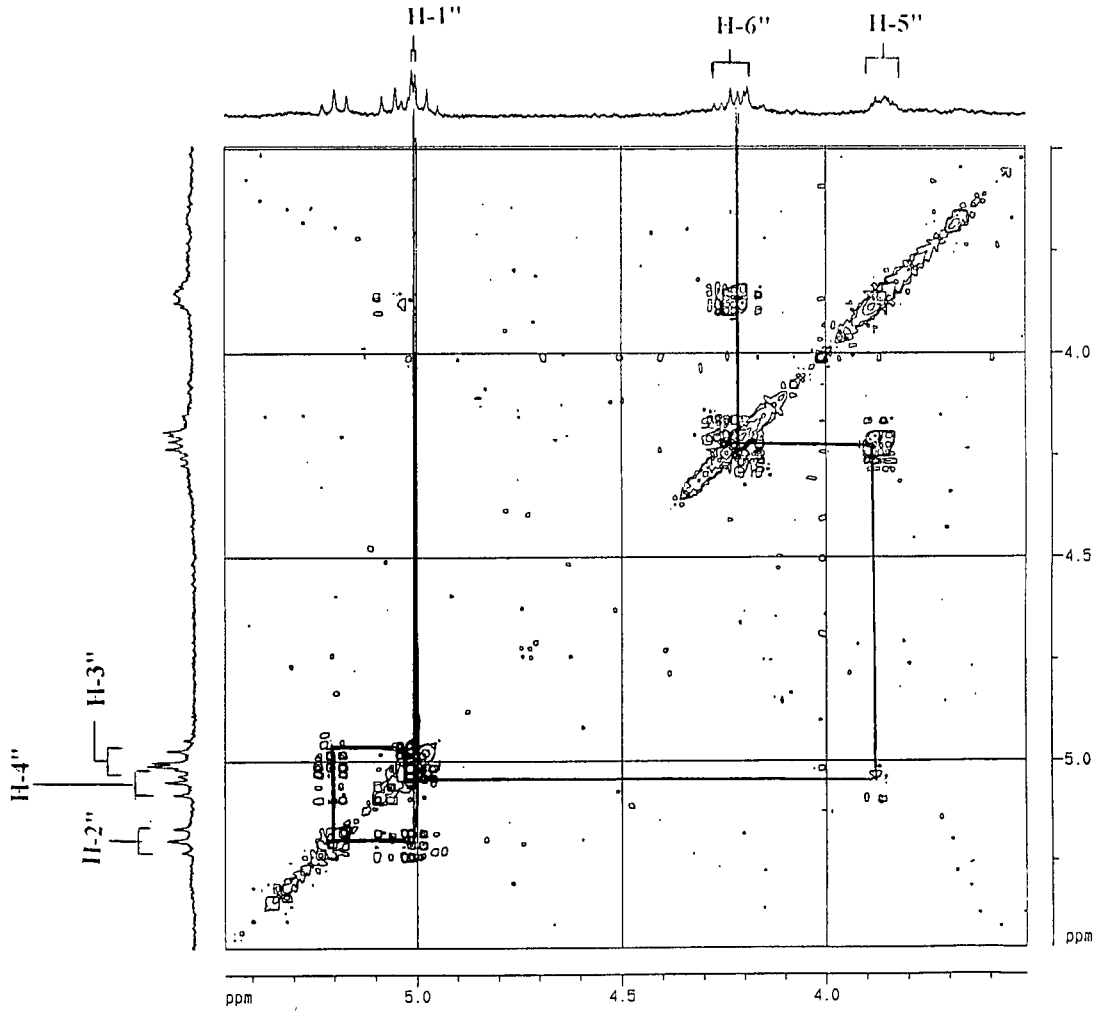
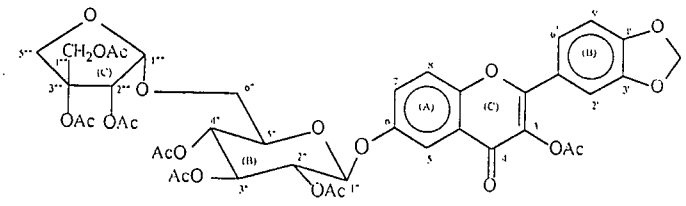
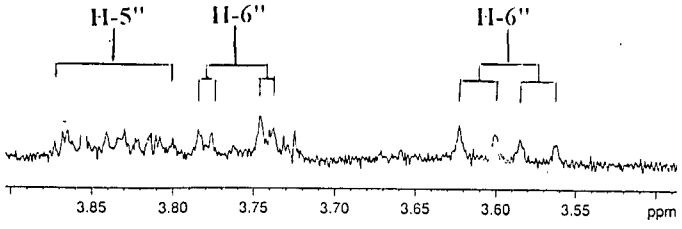
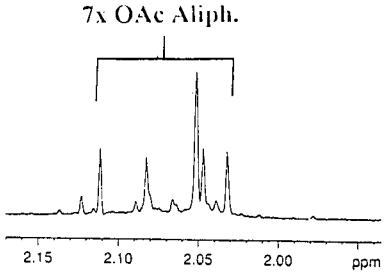


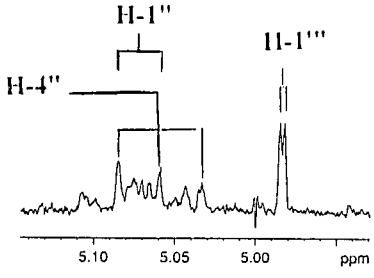
Plate 8 ($CDCl_3$ - 296K)
(^1H NMR)

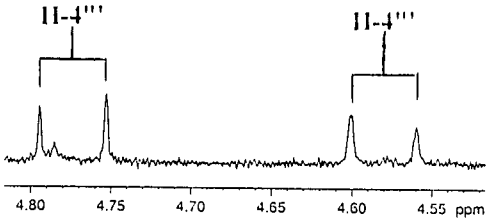


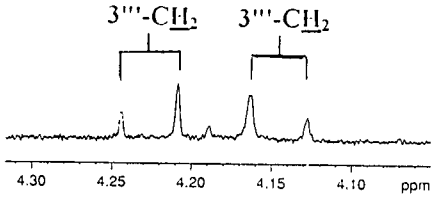
(89)

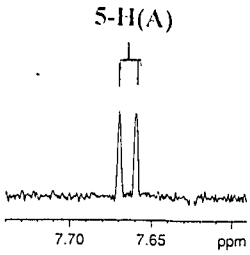


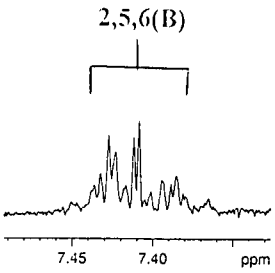


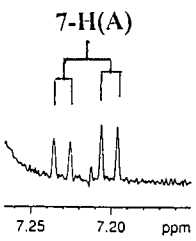


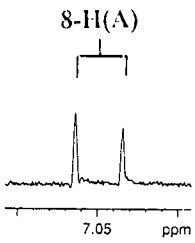


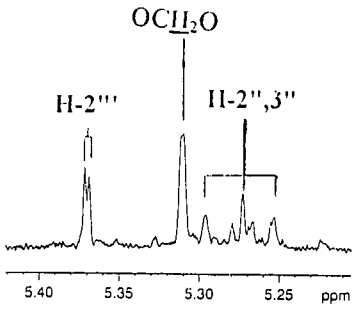












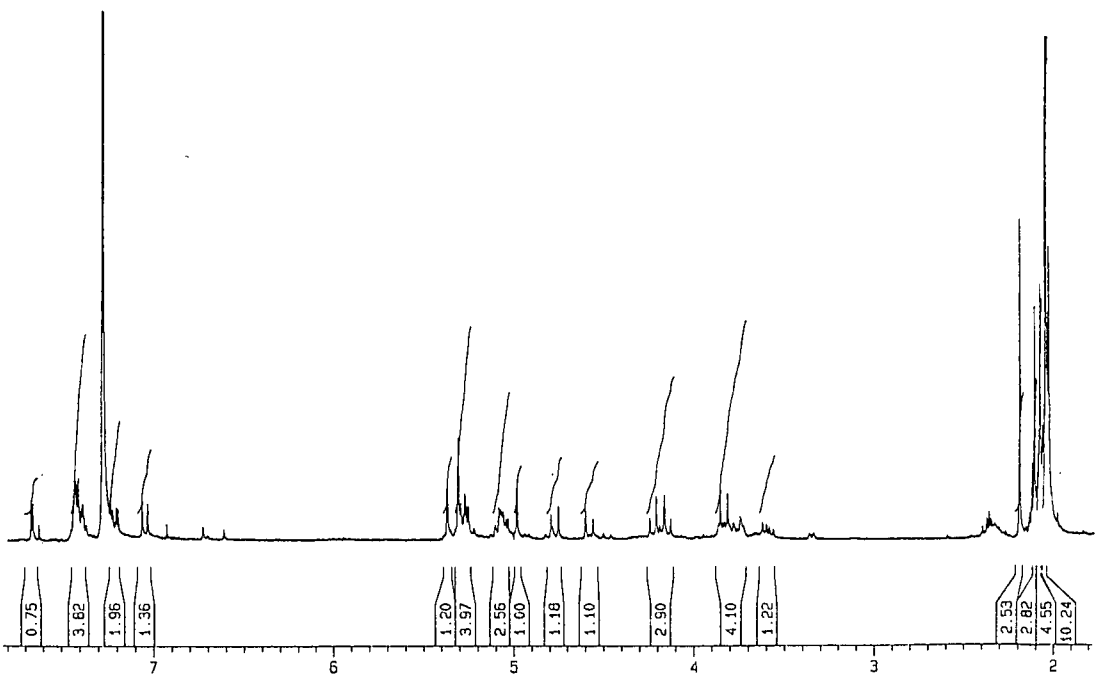


Plate 8a-1 ($CDCl_3$ - 296K)
(NOESY)

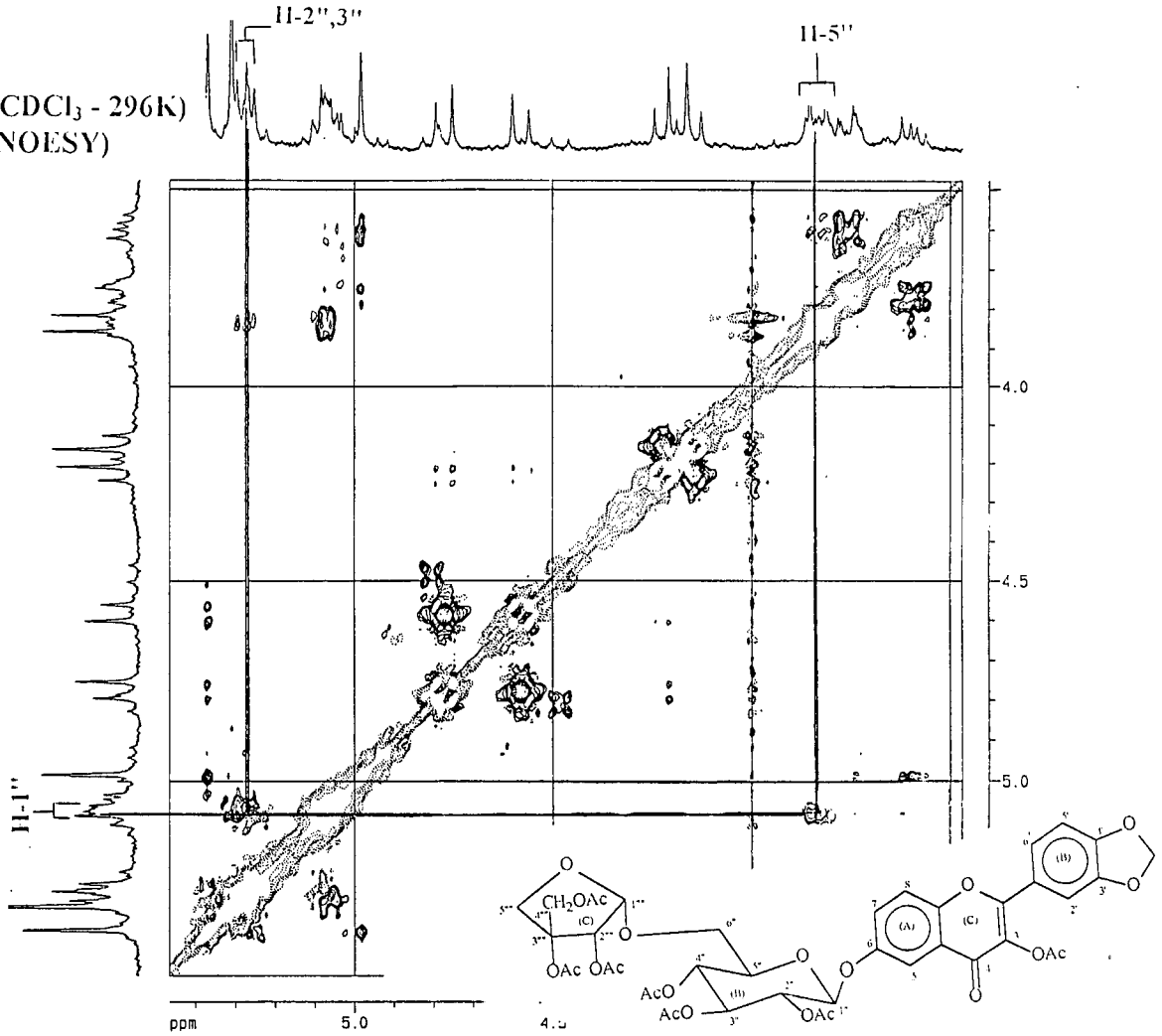


Plate 8a-2 ($CDCl_3$ - 296K)
(NOESY)

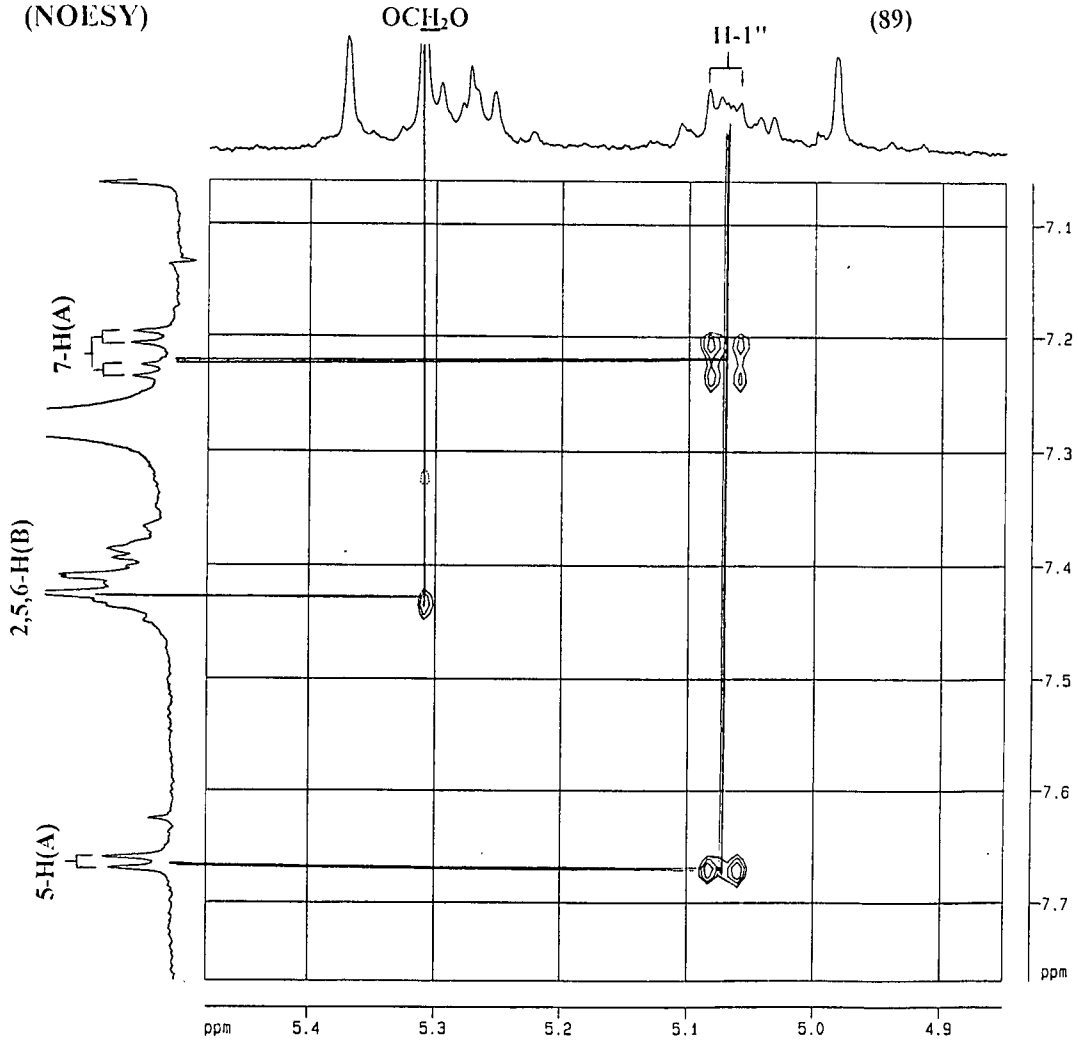


Plate 8b-1 ($CDCl_3$ - 296K)
(COSY)

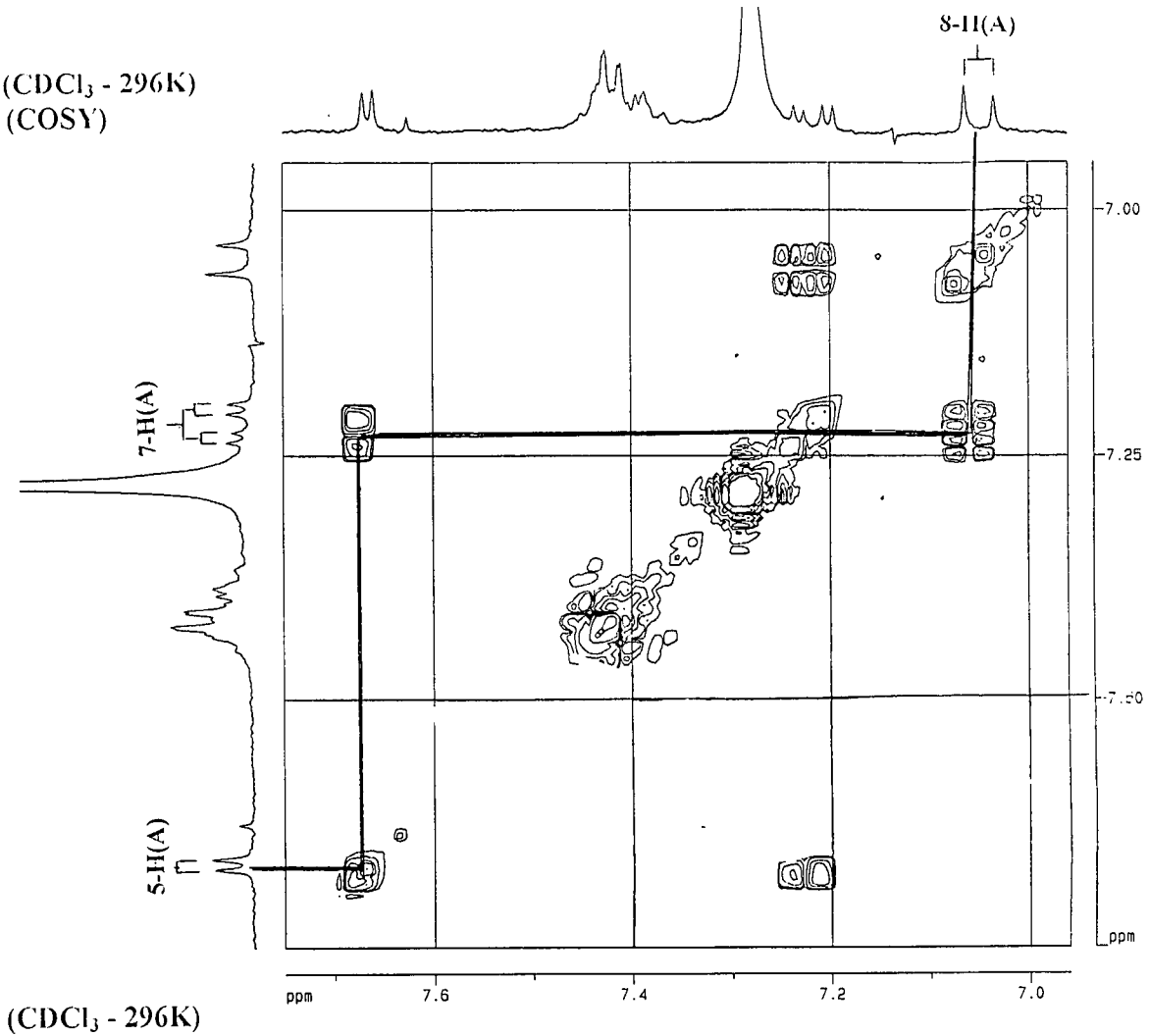


Plate 8b-2 ($CDCl_3$ - 296K)
(COSY)

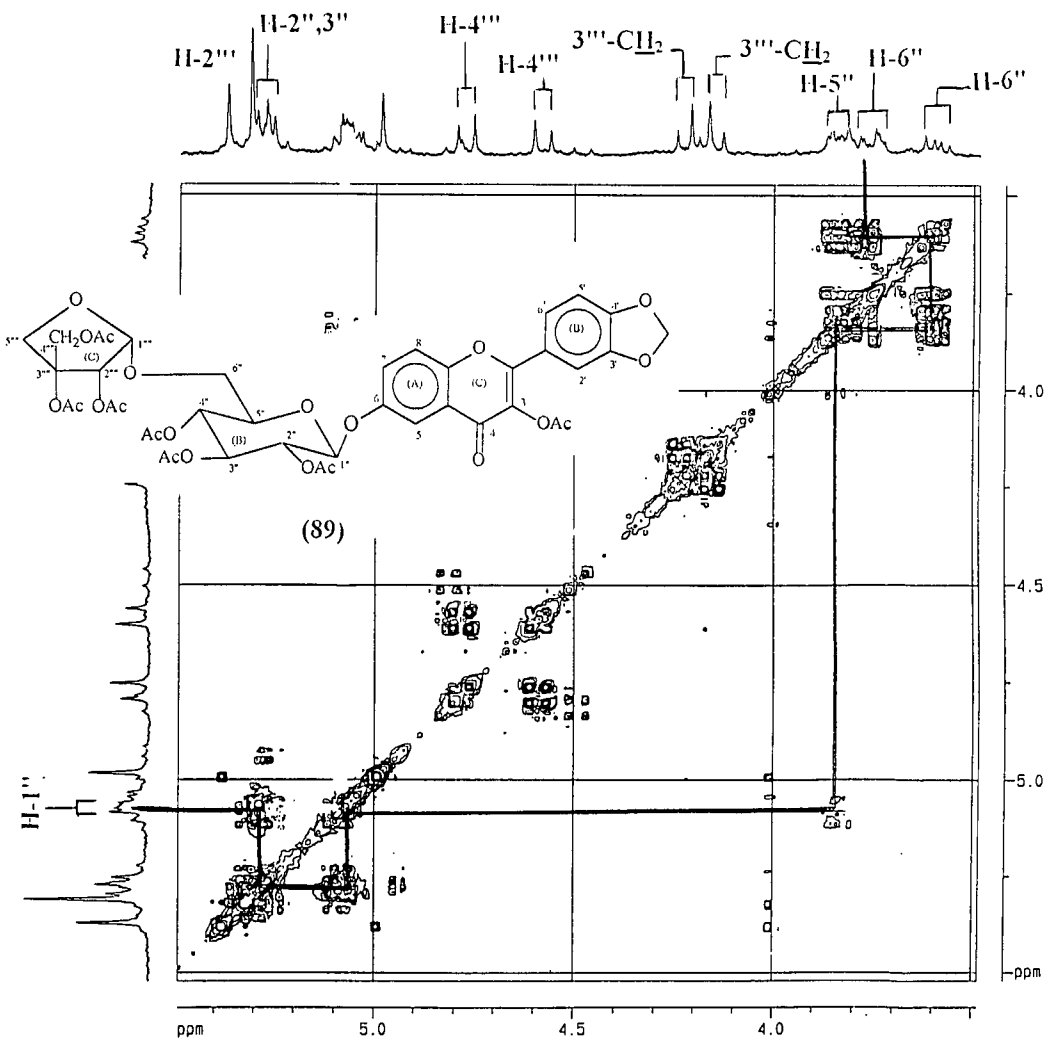
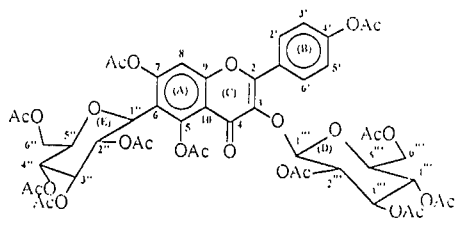


Plate 9 ($CDCl_3$ - 296K)
(^1H NMR)



(90)

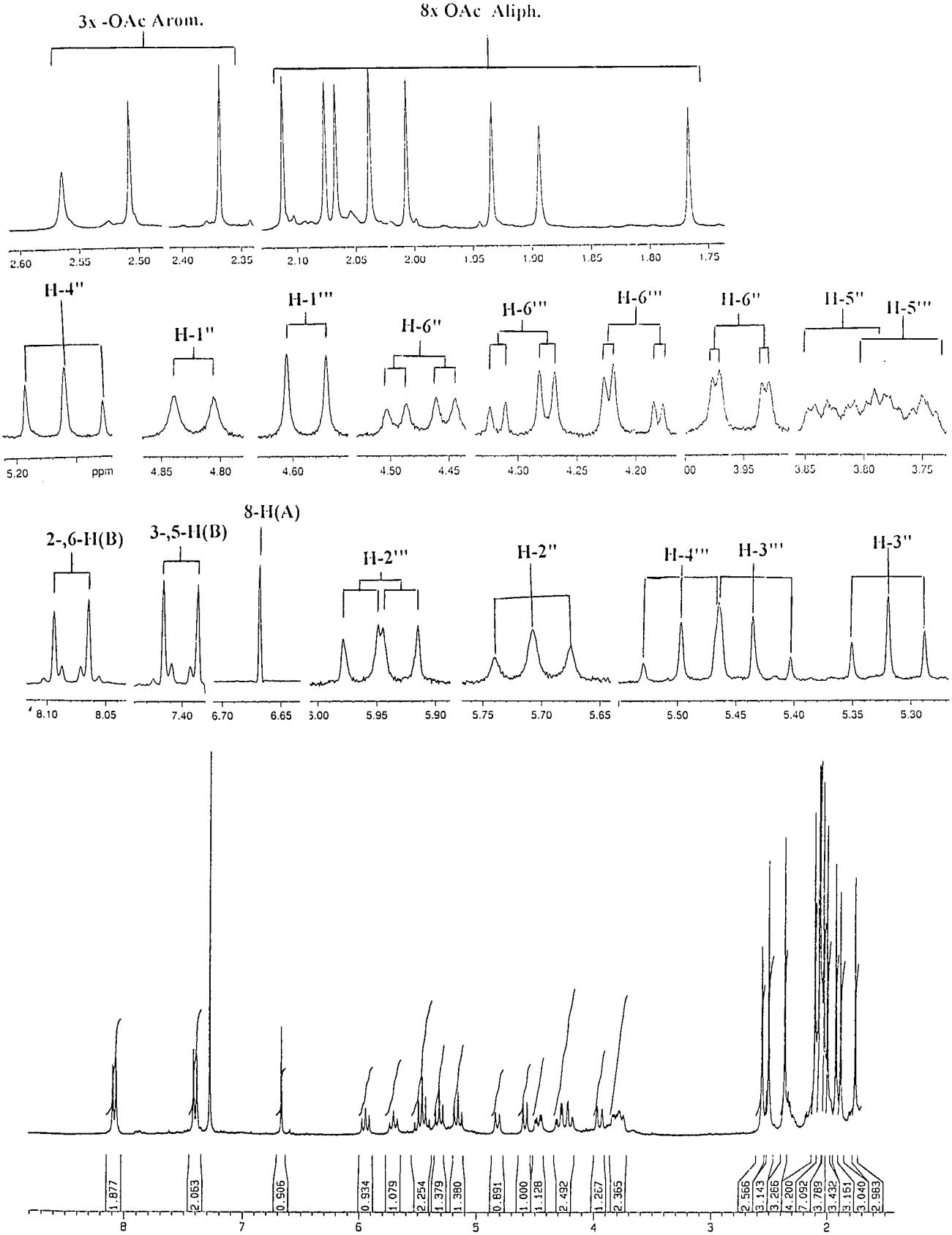


Plate 9a-1 ($CDCl_3$ - 296K)
(NOESY)

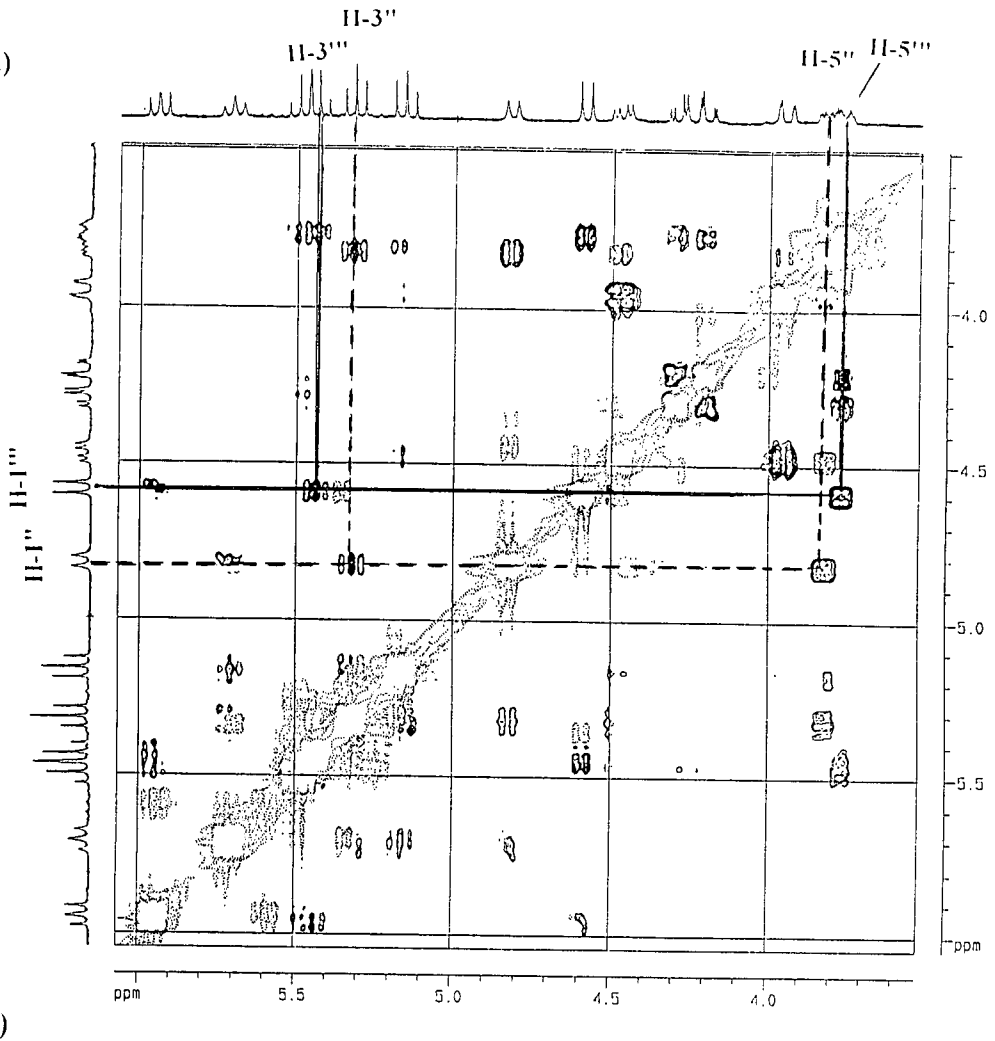


Plate 9a-2 ($CDCl_3$ - 296K)
(NOESY)

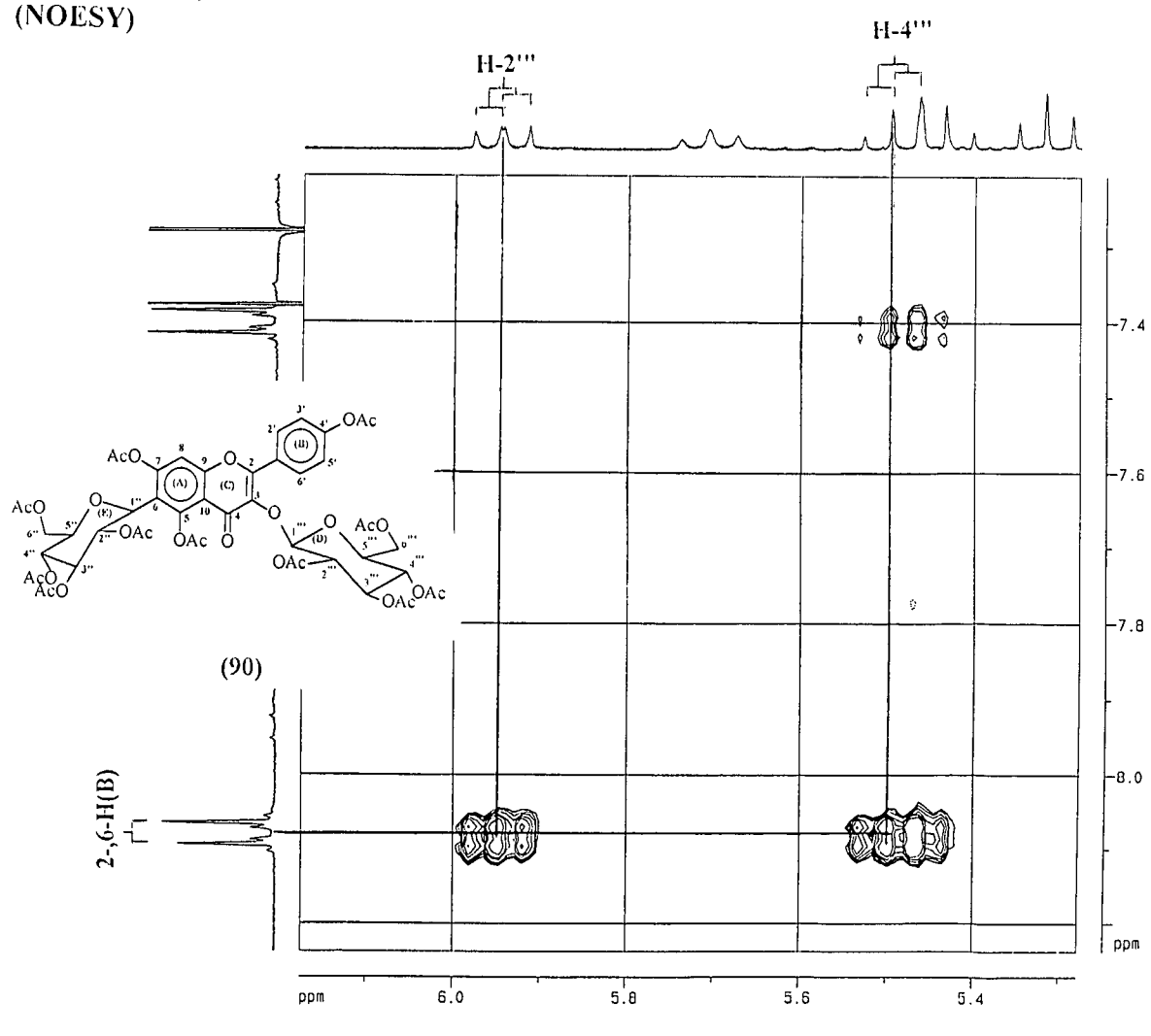
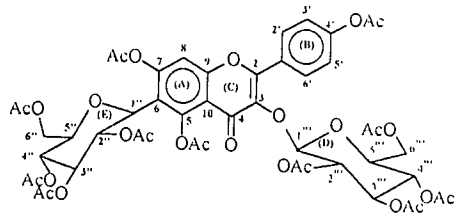


Plate 9b ($CDCl_3$ - 296K)
(COSY)



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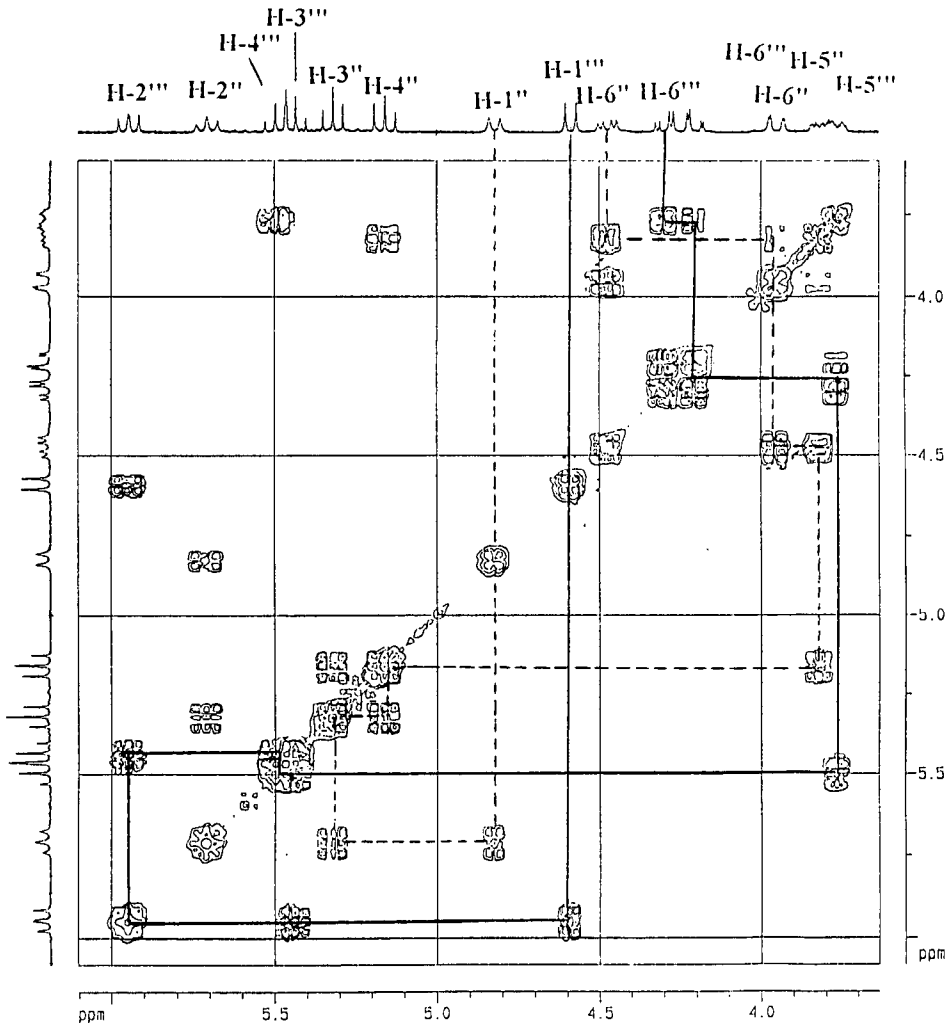
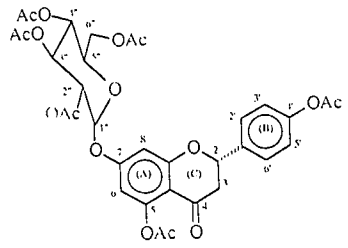


Plate 10 ($CDCl_3$ - 296K)
(^1H NMR)



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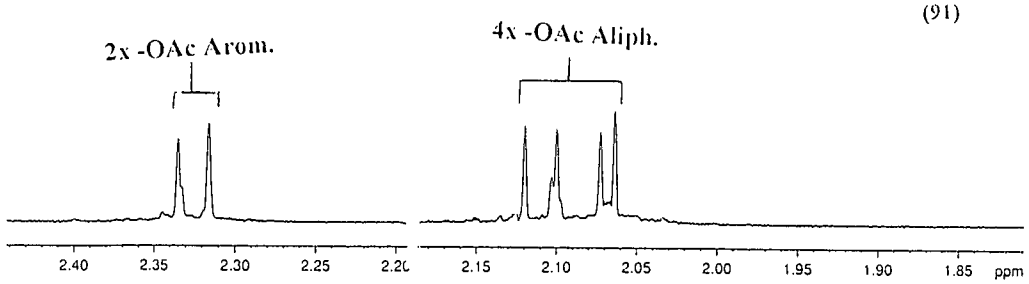
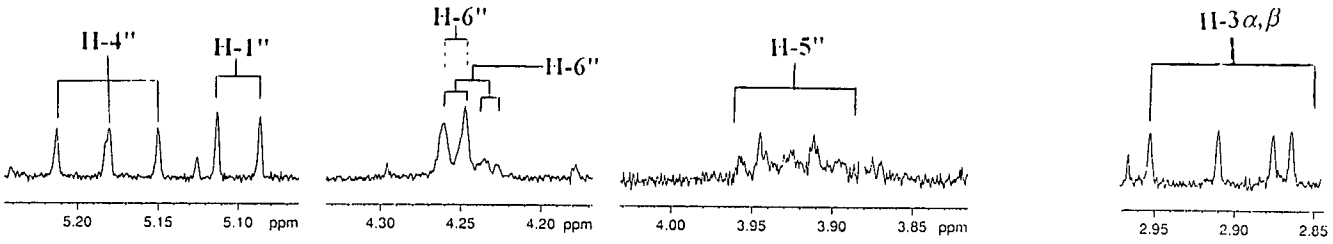
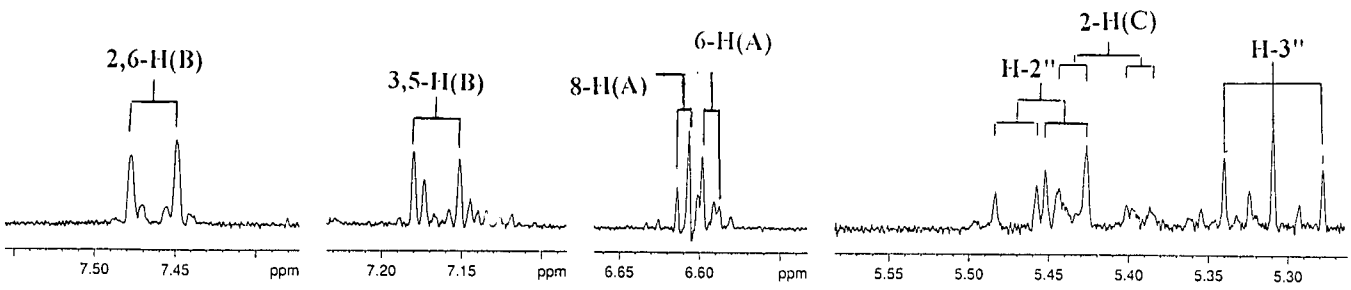






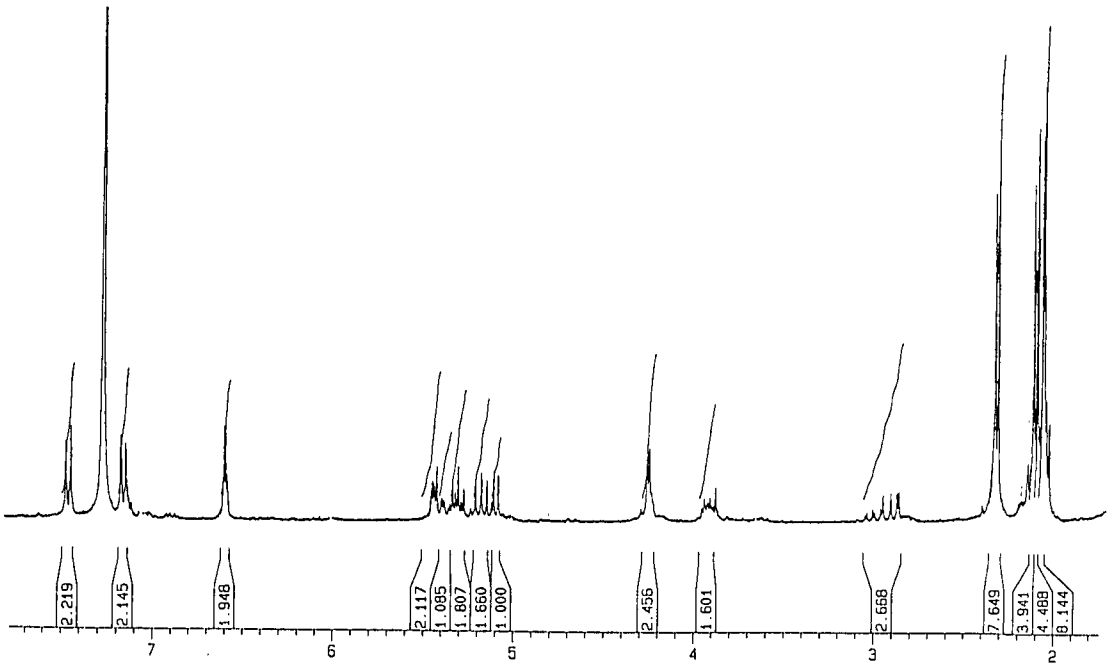


Plate 10a-1 ($CDCl_3$ - 296K)
(NOESY)

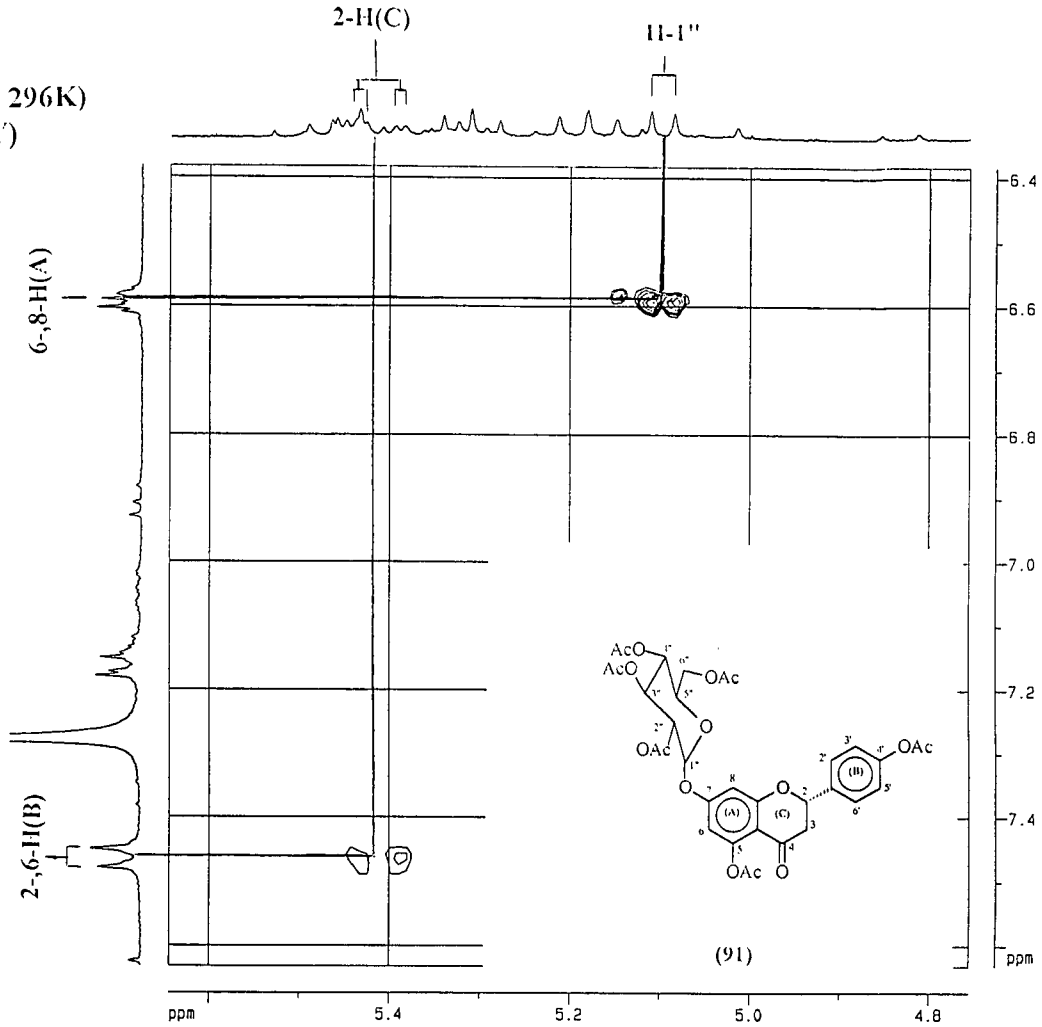


Plate 10a-2 ($CDCl_3$ - 296K)
(NOESY)

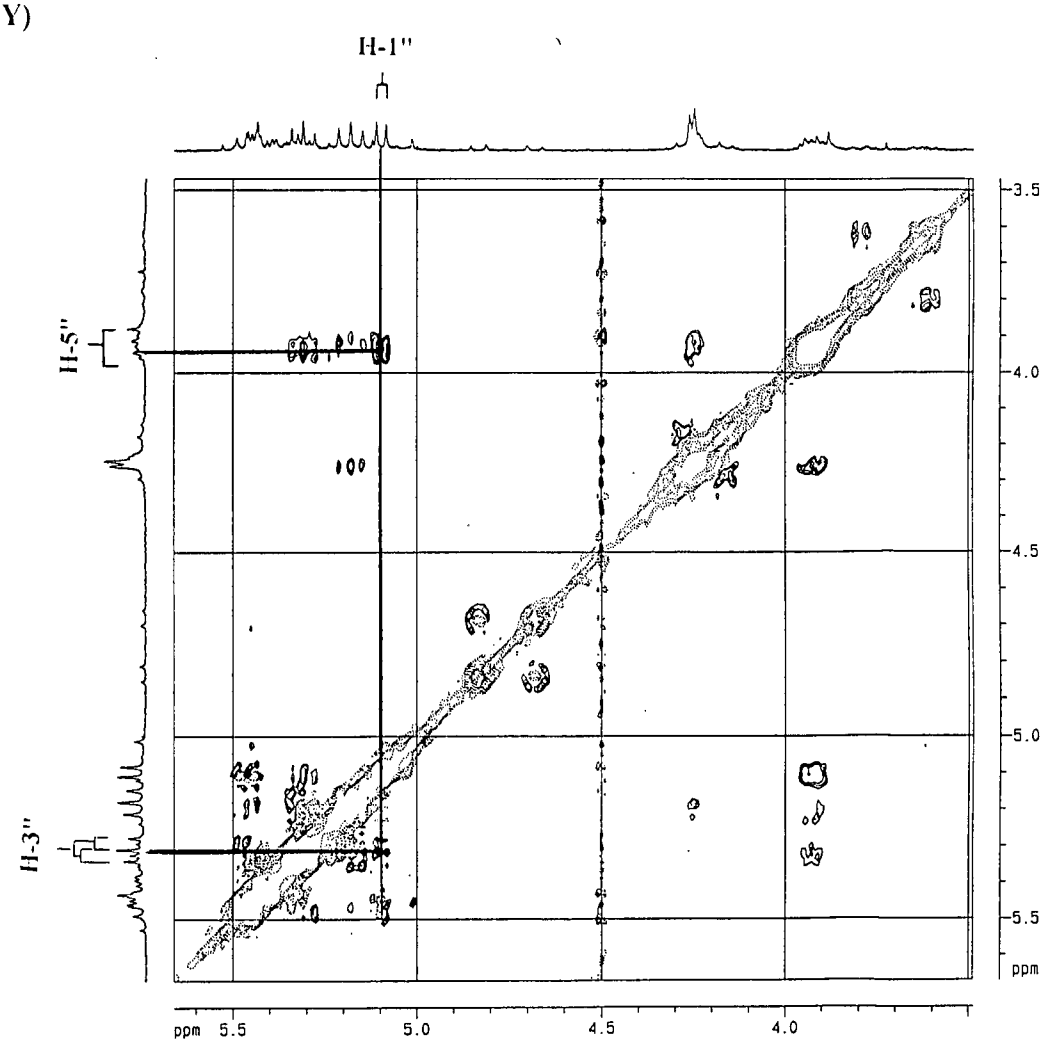


Plate 10b-1 ($CDCl_3$ - 296K)
(COSY)

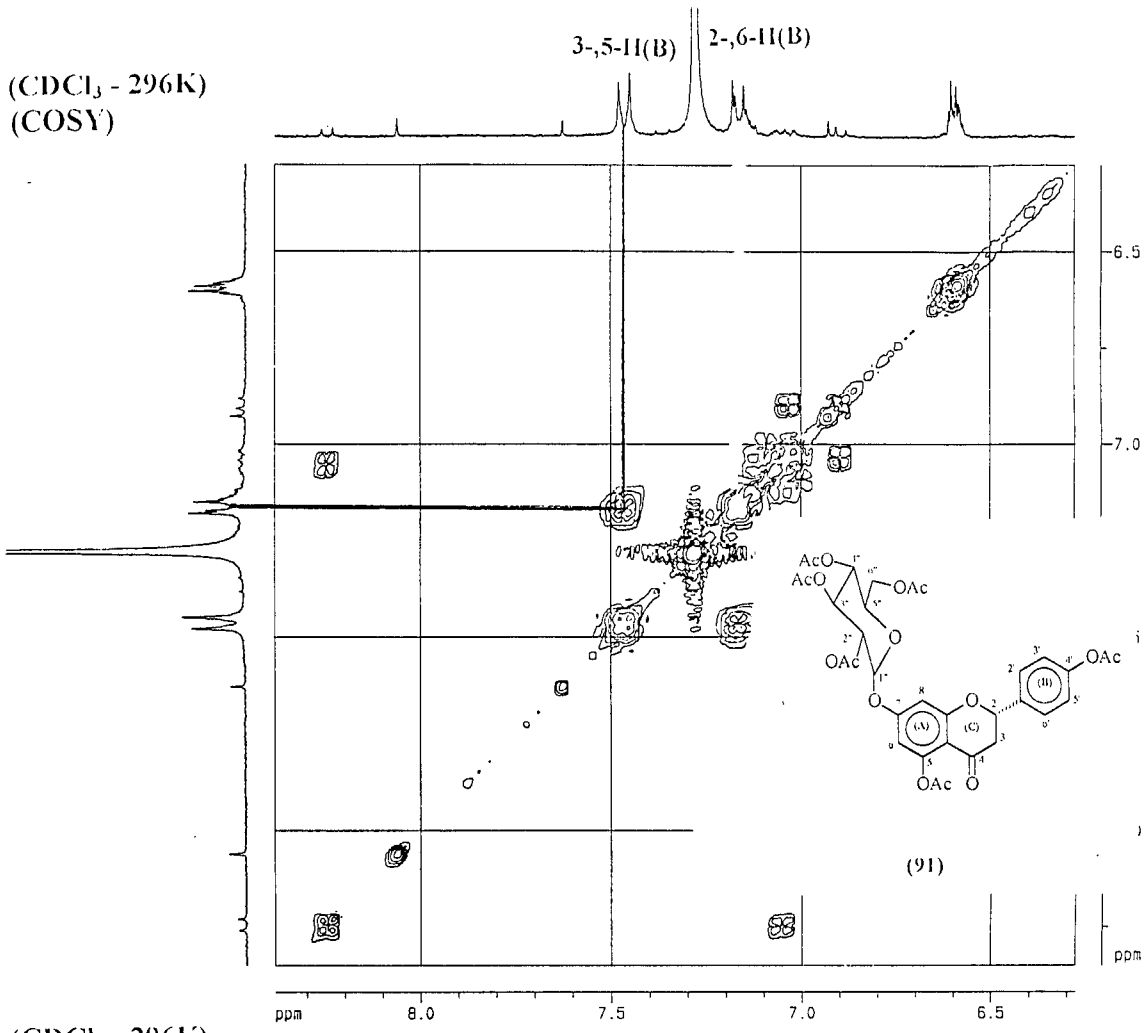


Plate 10b-2 ($CDCl_3$ - 296K)
(COSY)

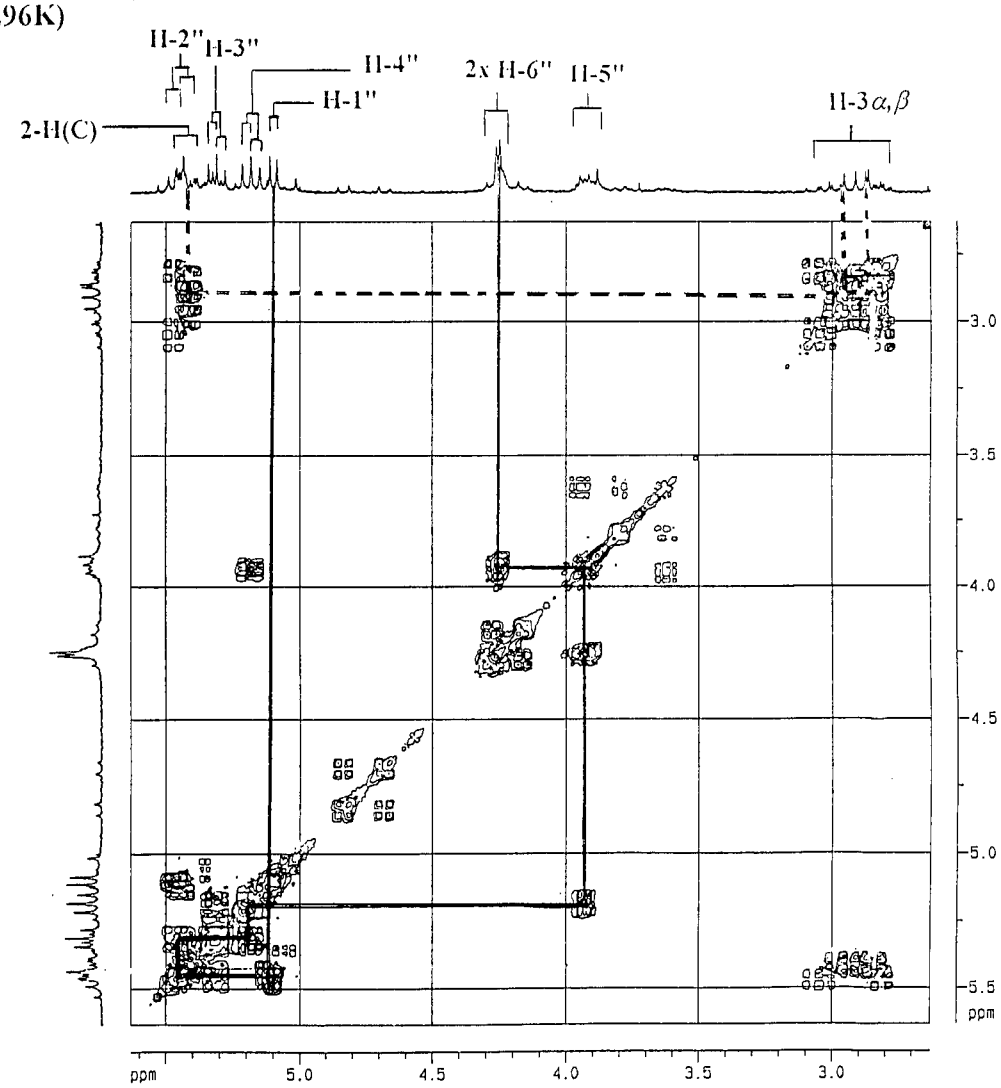
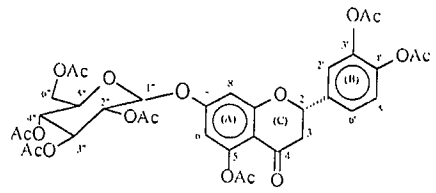
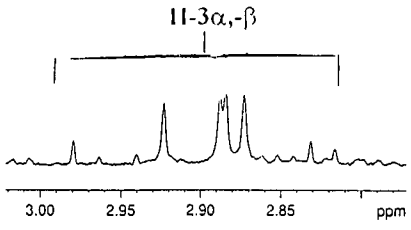
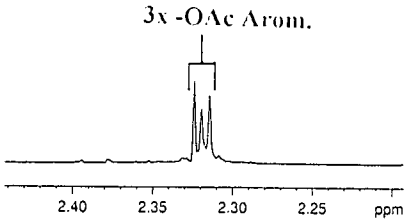


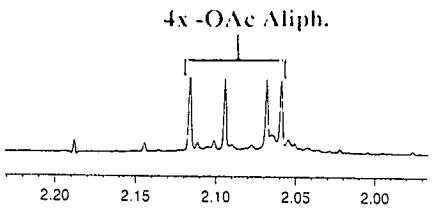
Plate 11 ($CDCl_3$ - 296K)
(^1H NMR)

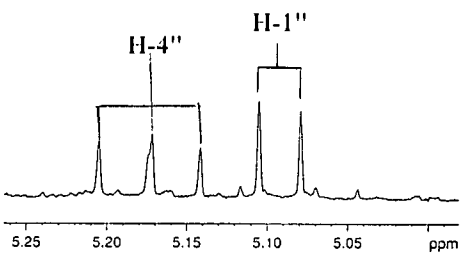


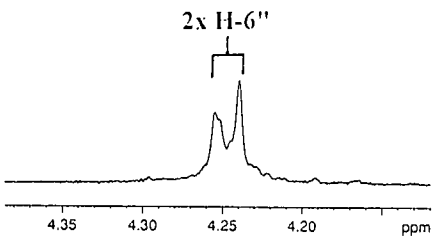
(92)

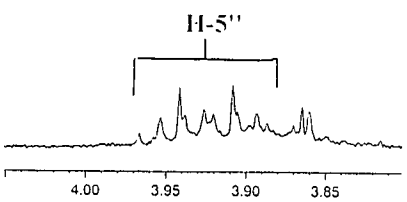


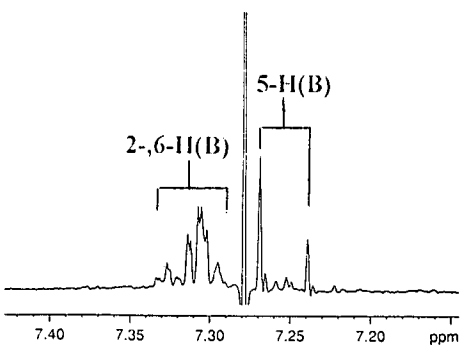


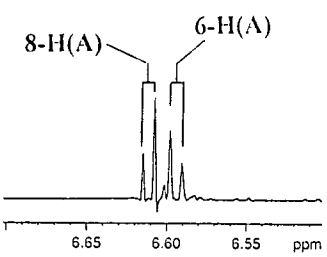


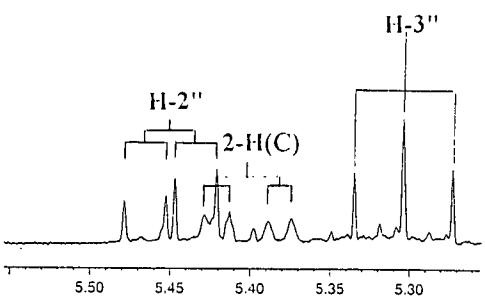












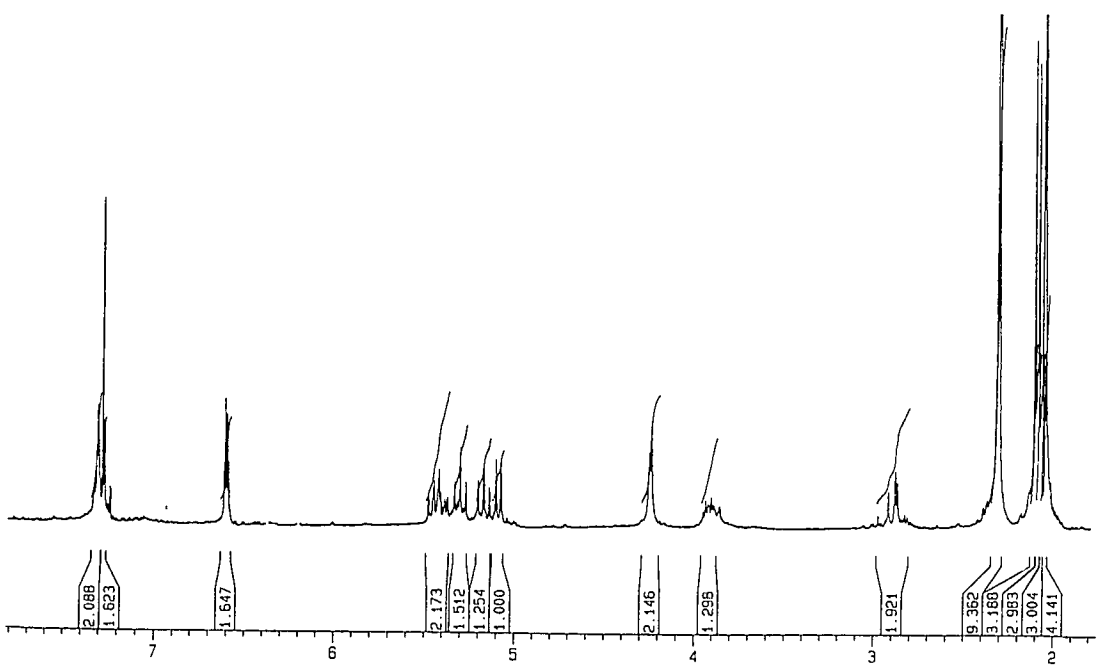


Plate 11a-1 ($CDCl_3$ - 296K)
(NOESY)

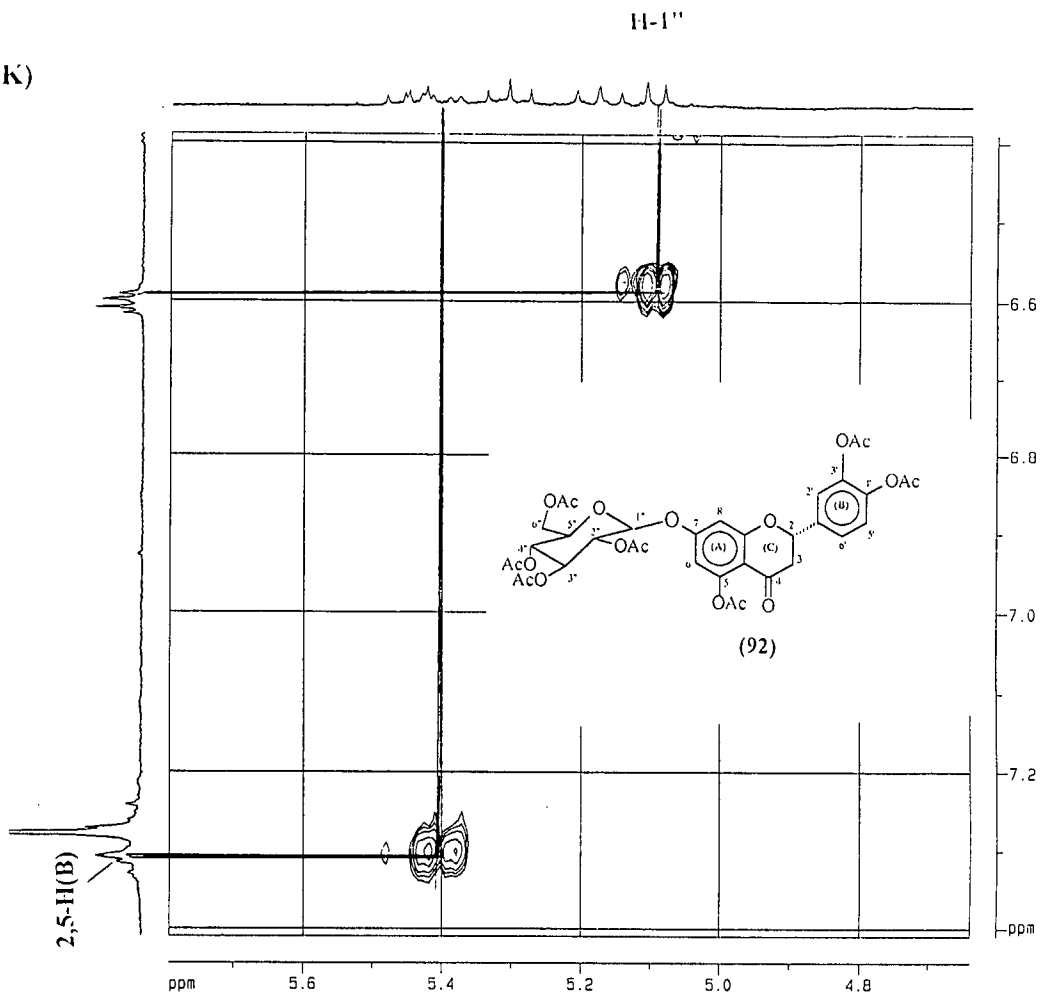


Plate 11a-2 ($CDCl_3$ - 296K)
(NOESY)

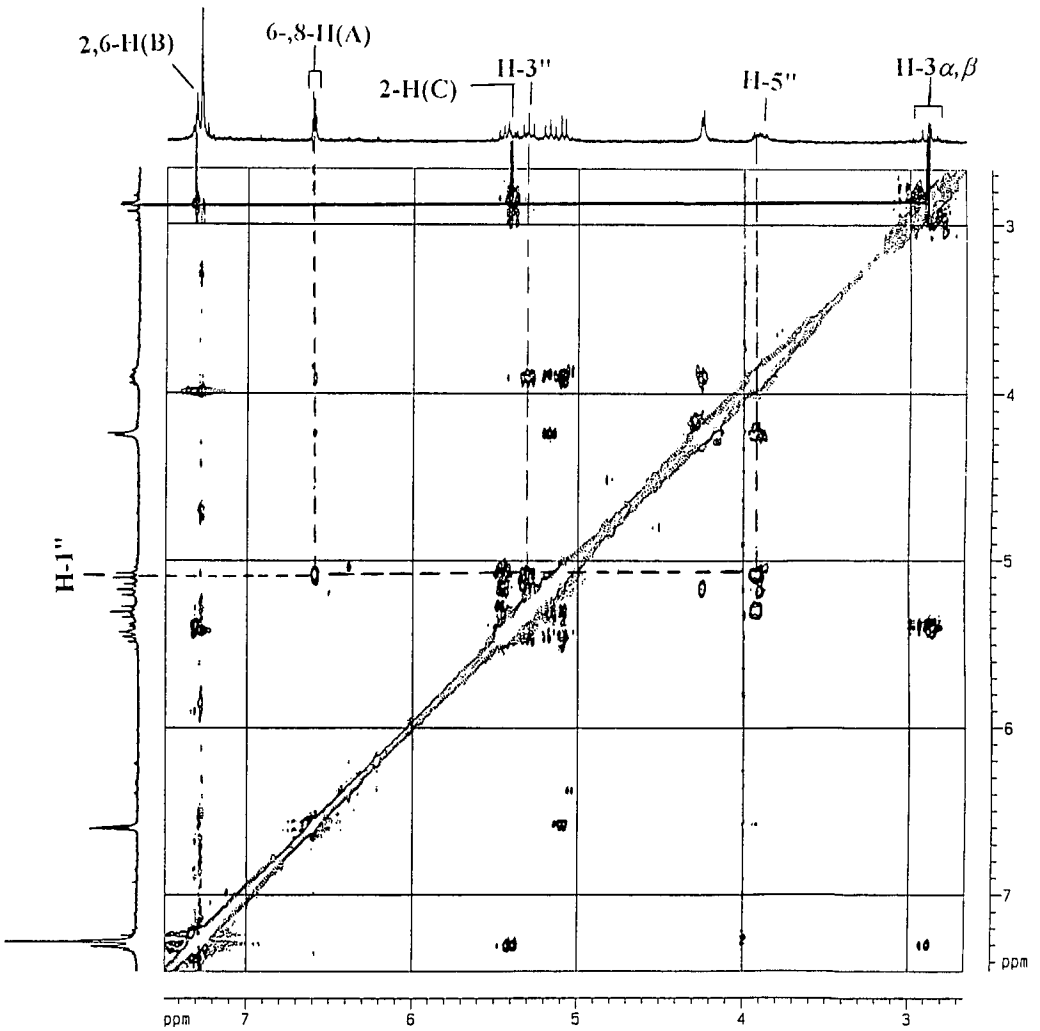
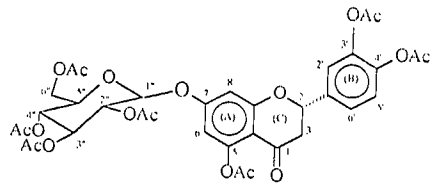


Plate 11b-1 ($CDCl_3$ - 296K)
(COSY)



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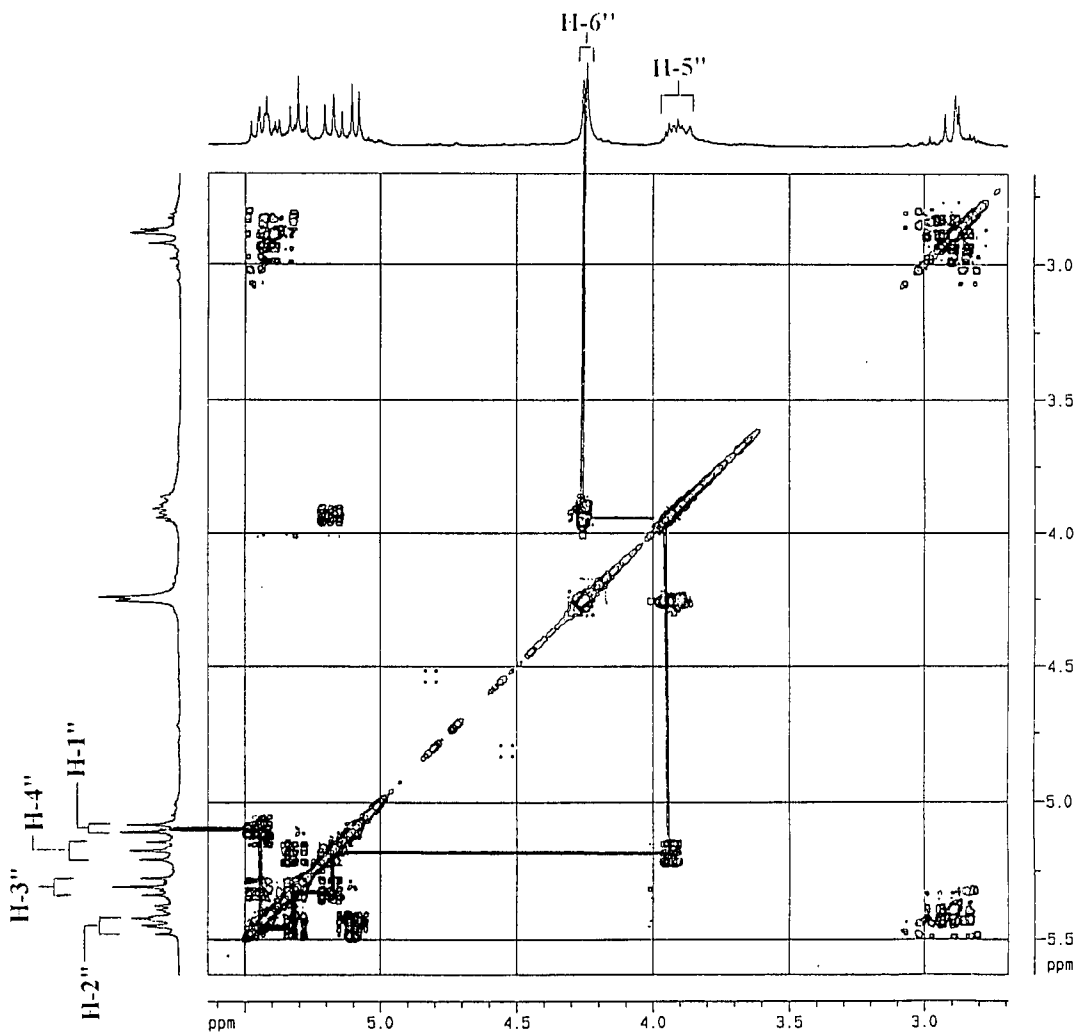
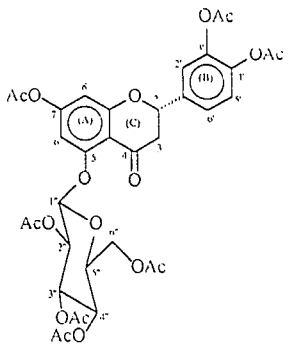


Plate 12 ($CDCl_3$ - 296K)
(^1H NMR)



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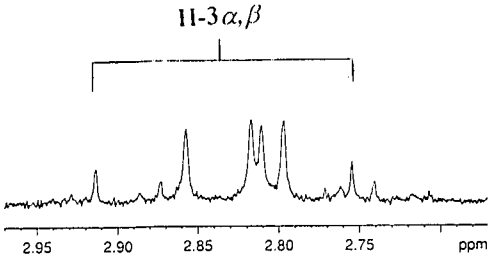
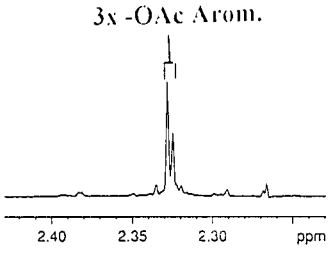
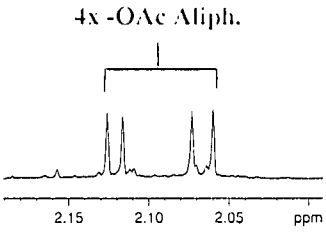
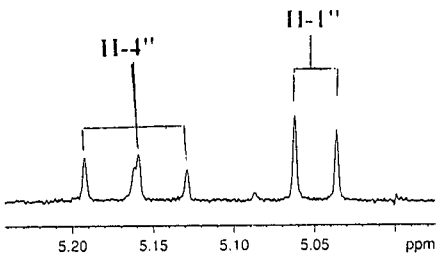
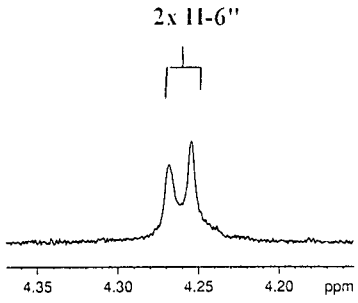
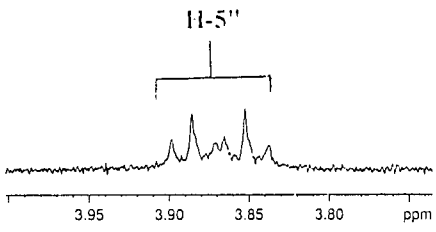


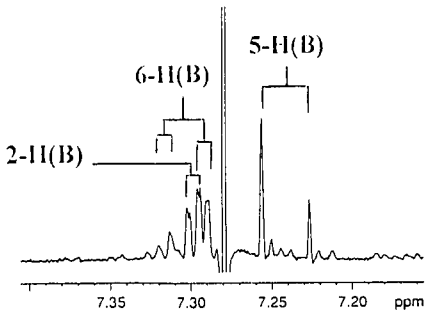


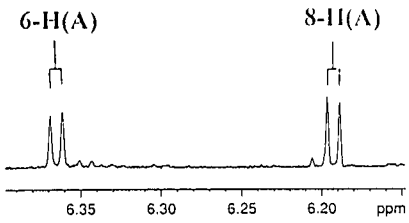


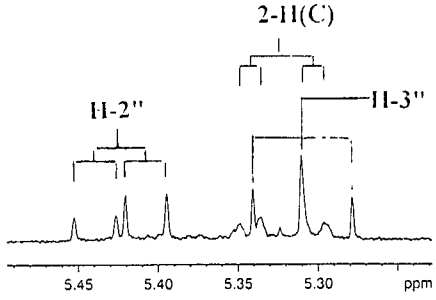












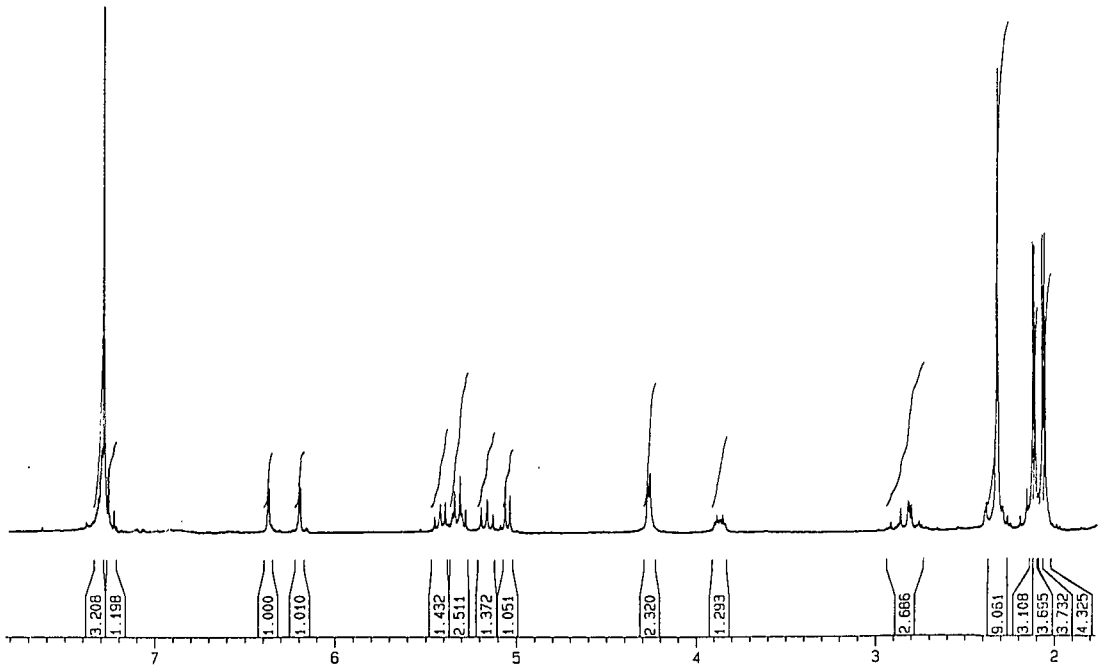


Plate 12a-1 ($CDCl_3$ - 296K)
(NOESY)

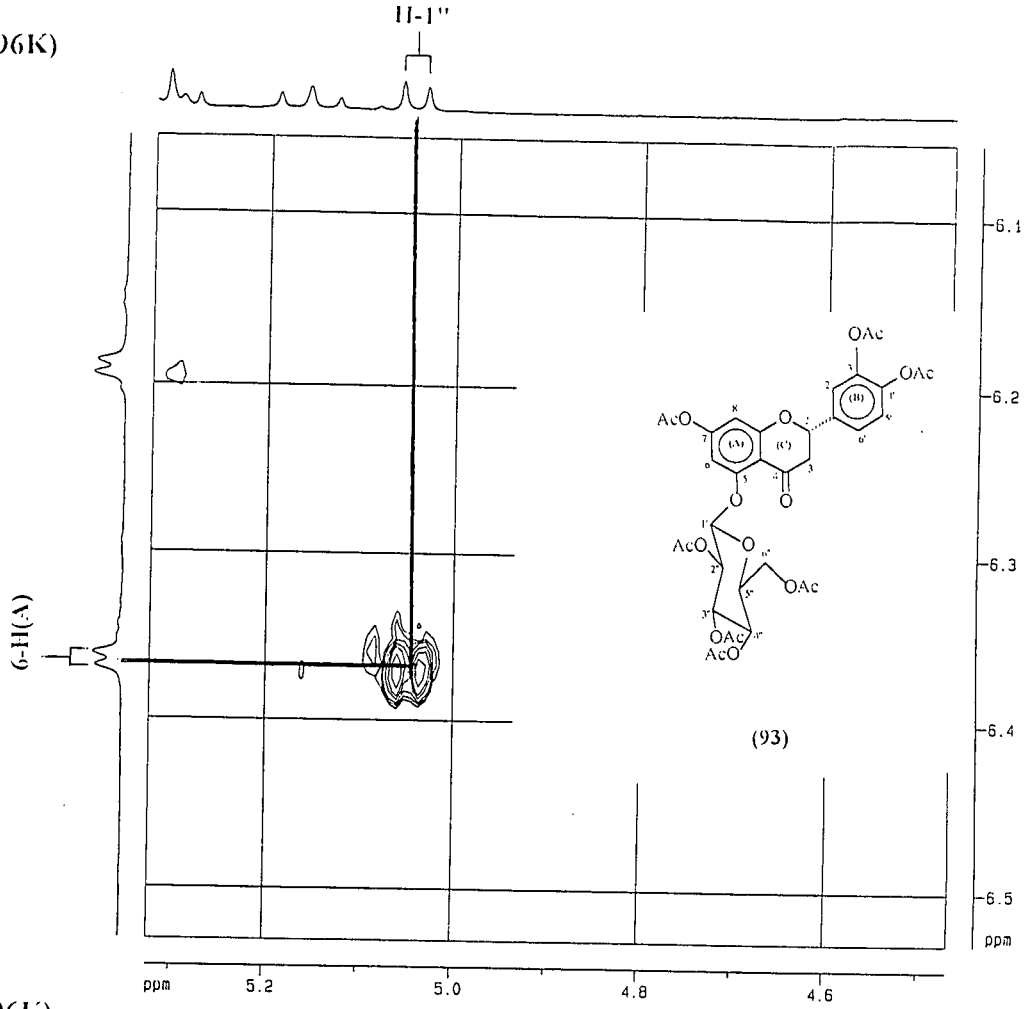


Plate 12a-2 ($CDCl_3$ - 296K)
(NOESY)

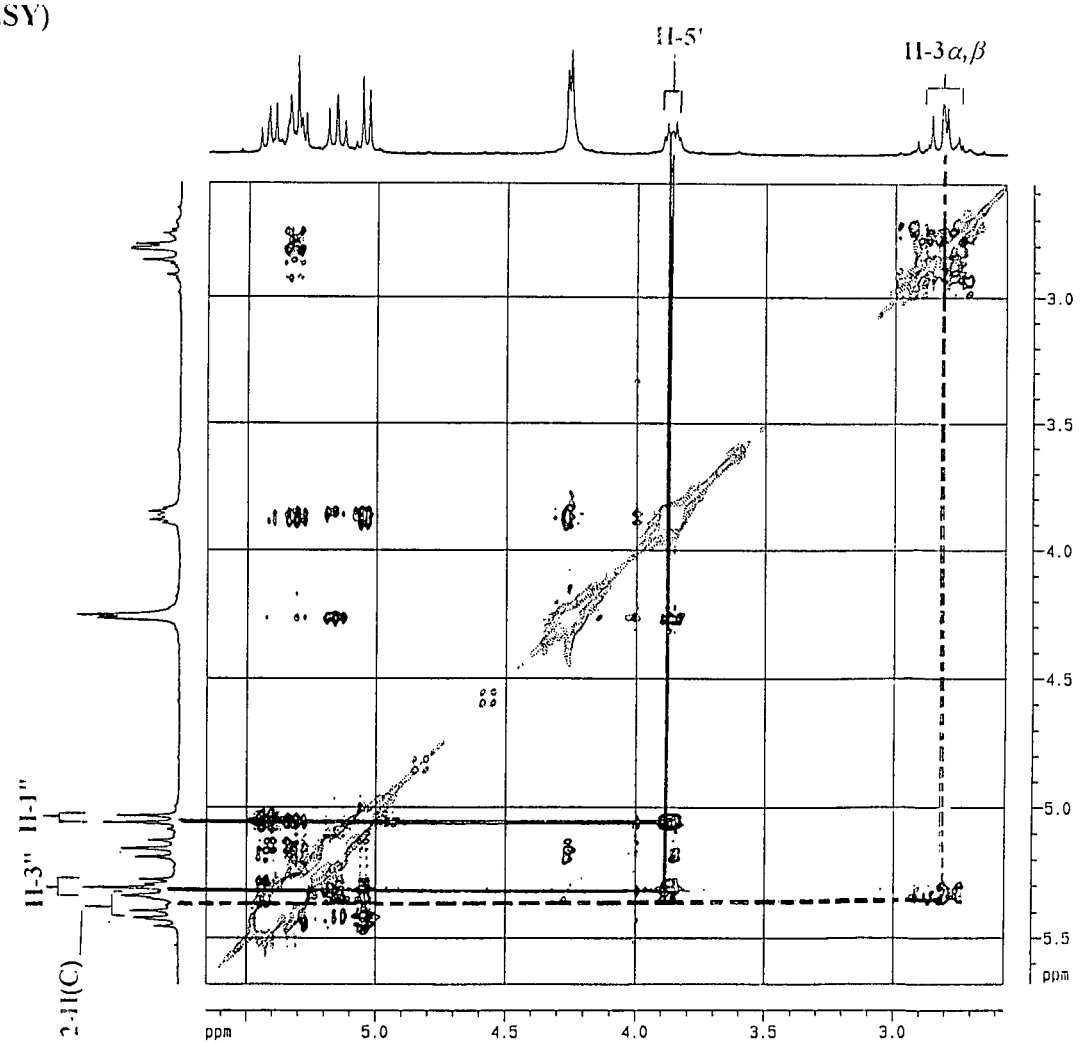
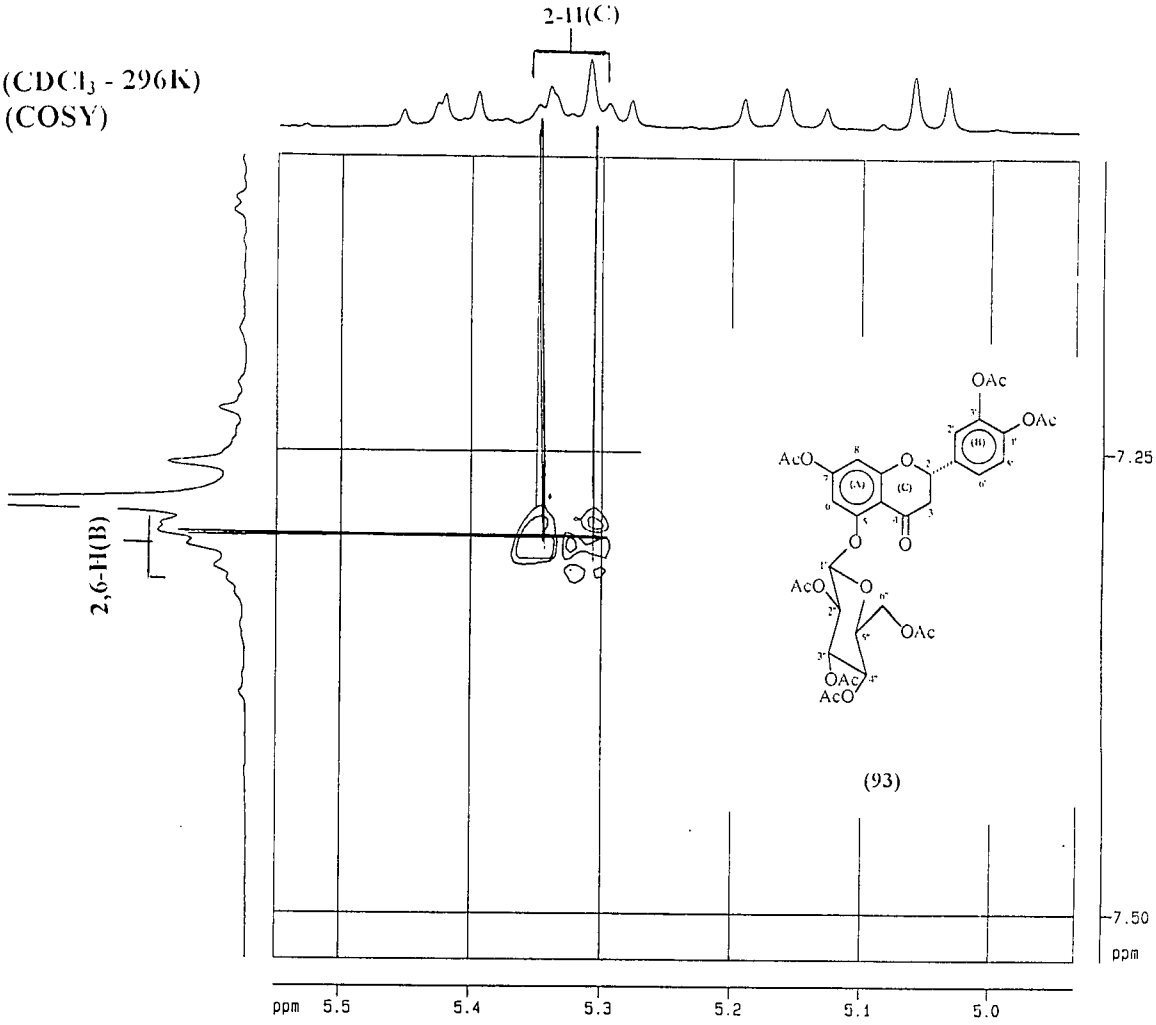


Plate 12b-1 ($CDCl_3$ - 296K)
(COSY)



(93)

Plate 12b-2 ($CDCl_3$ - 296K)
(COSY)

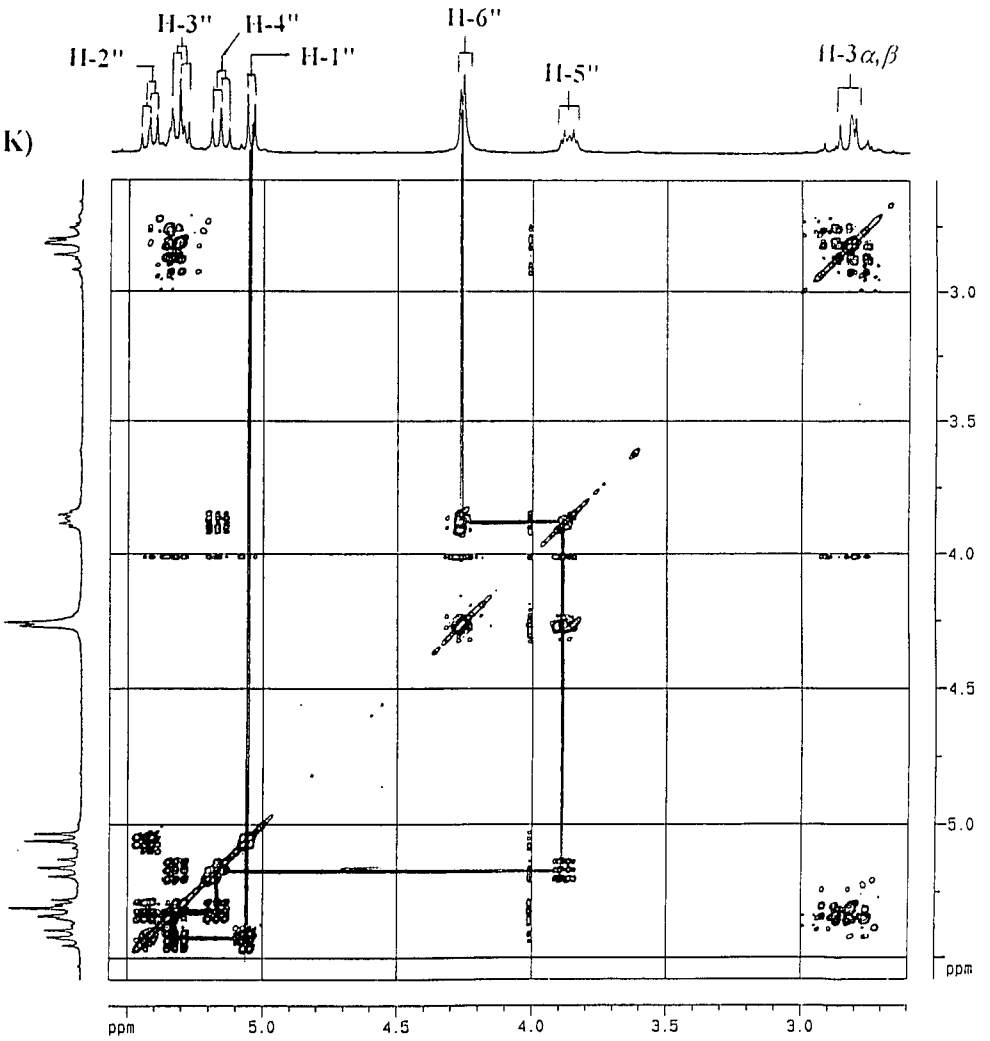
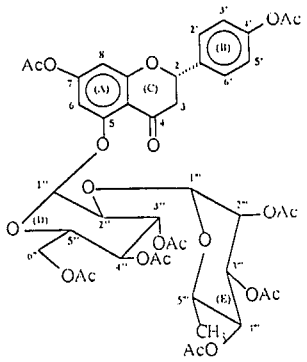
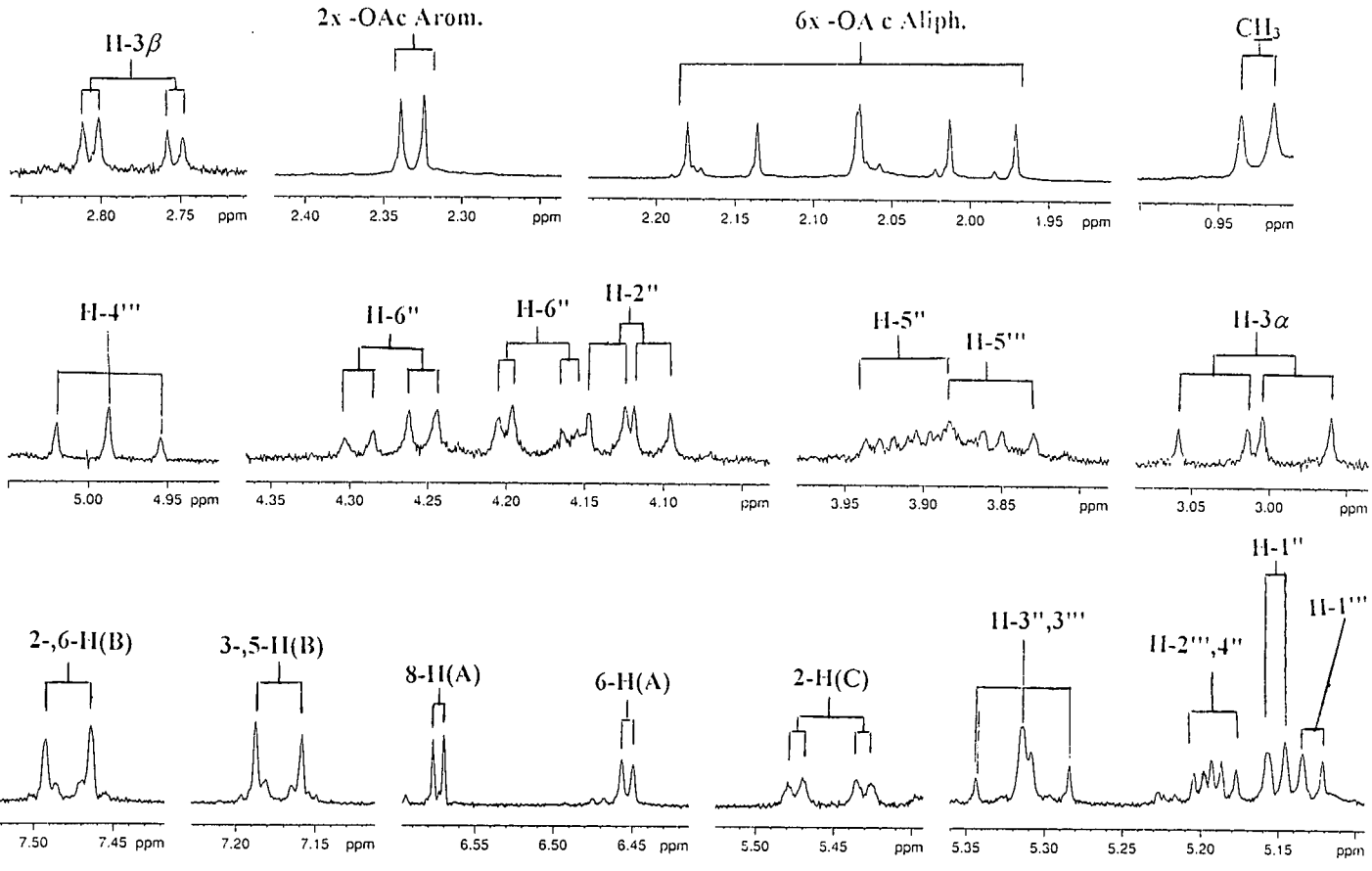


Plate 13 ($CDCl_3$ - 296K)
(^1H NMR)



(94)



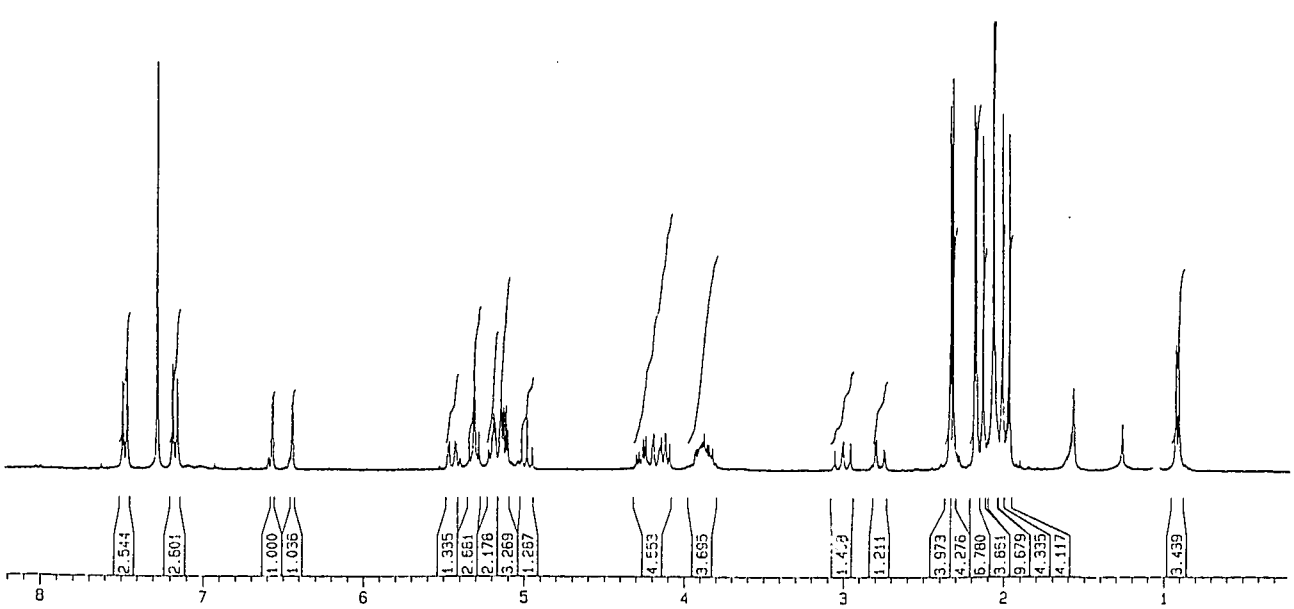


Plate 13a-1 ($CDCl_3$ - 296K)
(NOESY)

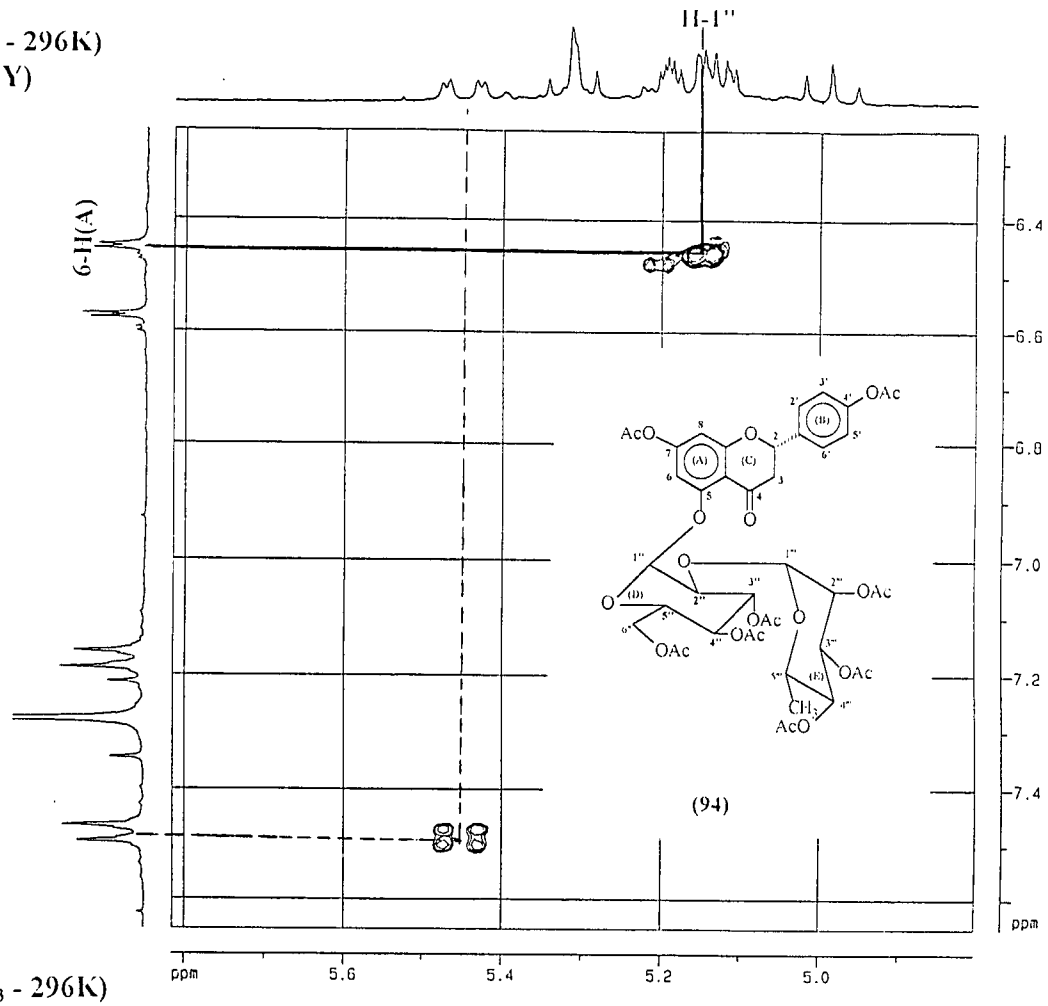


Plate 13a-2 ($CDCl_3$ - 296K)
(NOESY)

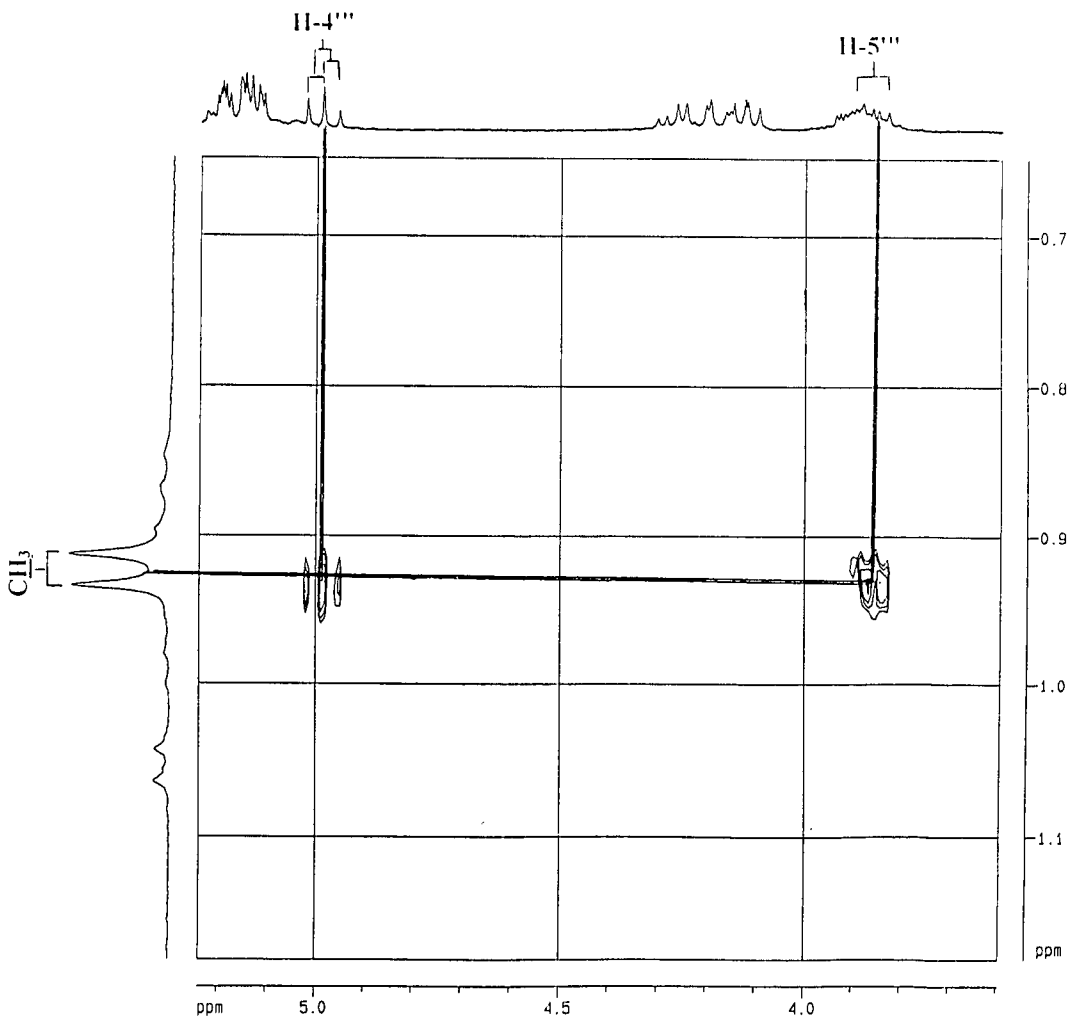


Plate 13a-3 ($CDCl_3$ - 296K)
(NOESY)

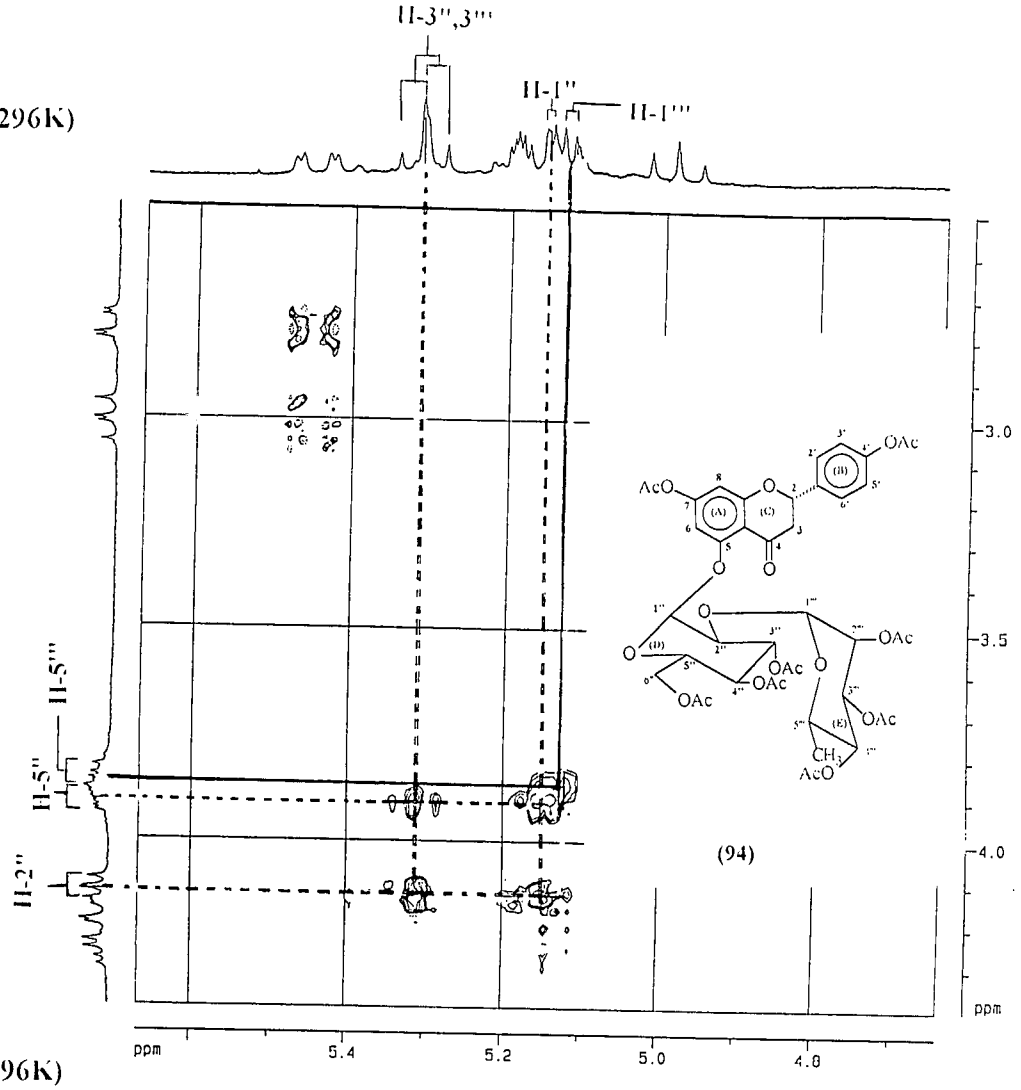


Plate 13a-4 ($CDCl_3$ - 296K)
(NOESY)

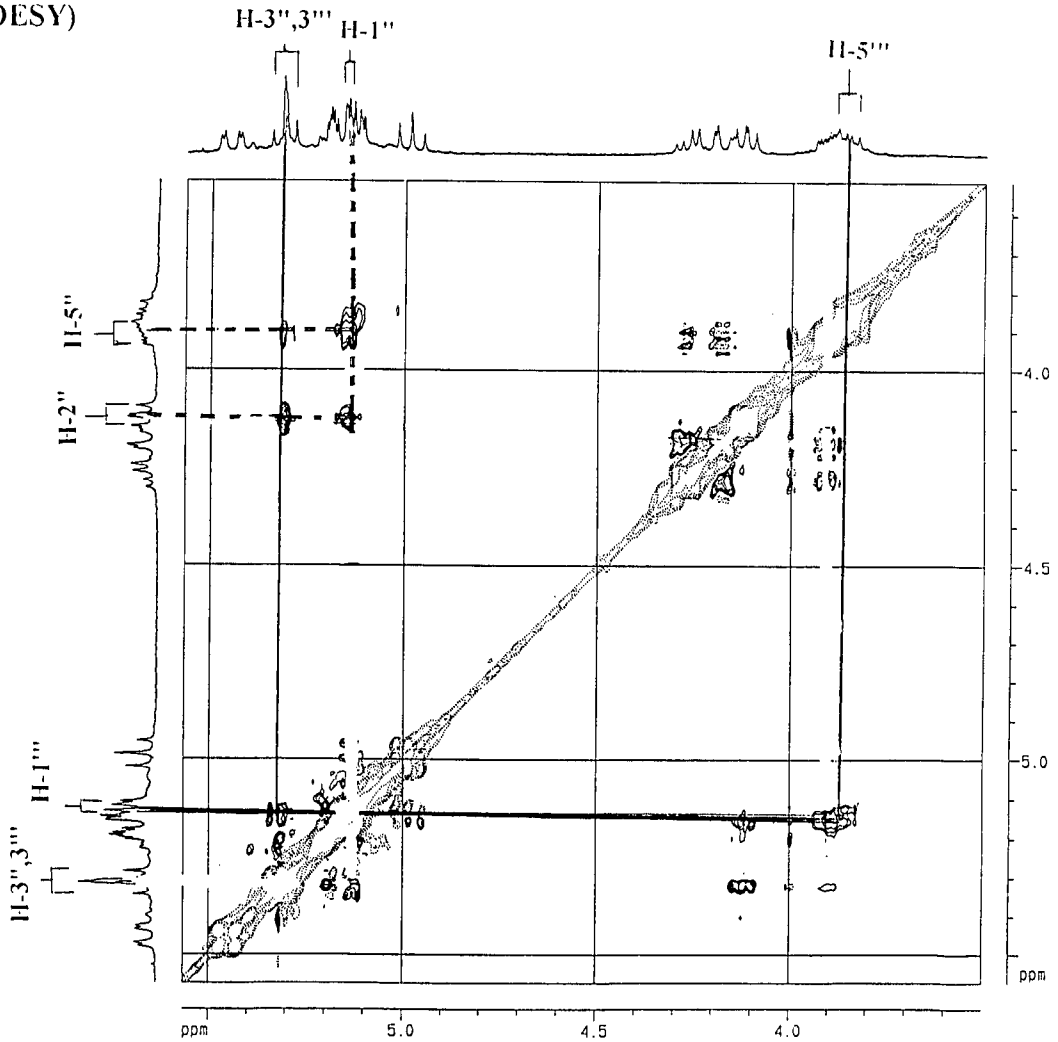
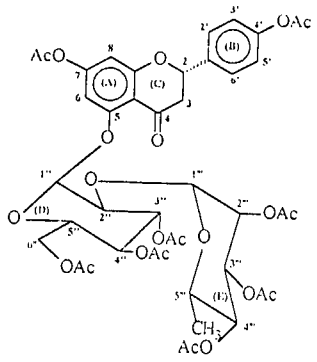


Plate 13b ($CDCl_3$ - 296K)
(COSY)



(94)

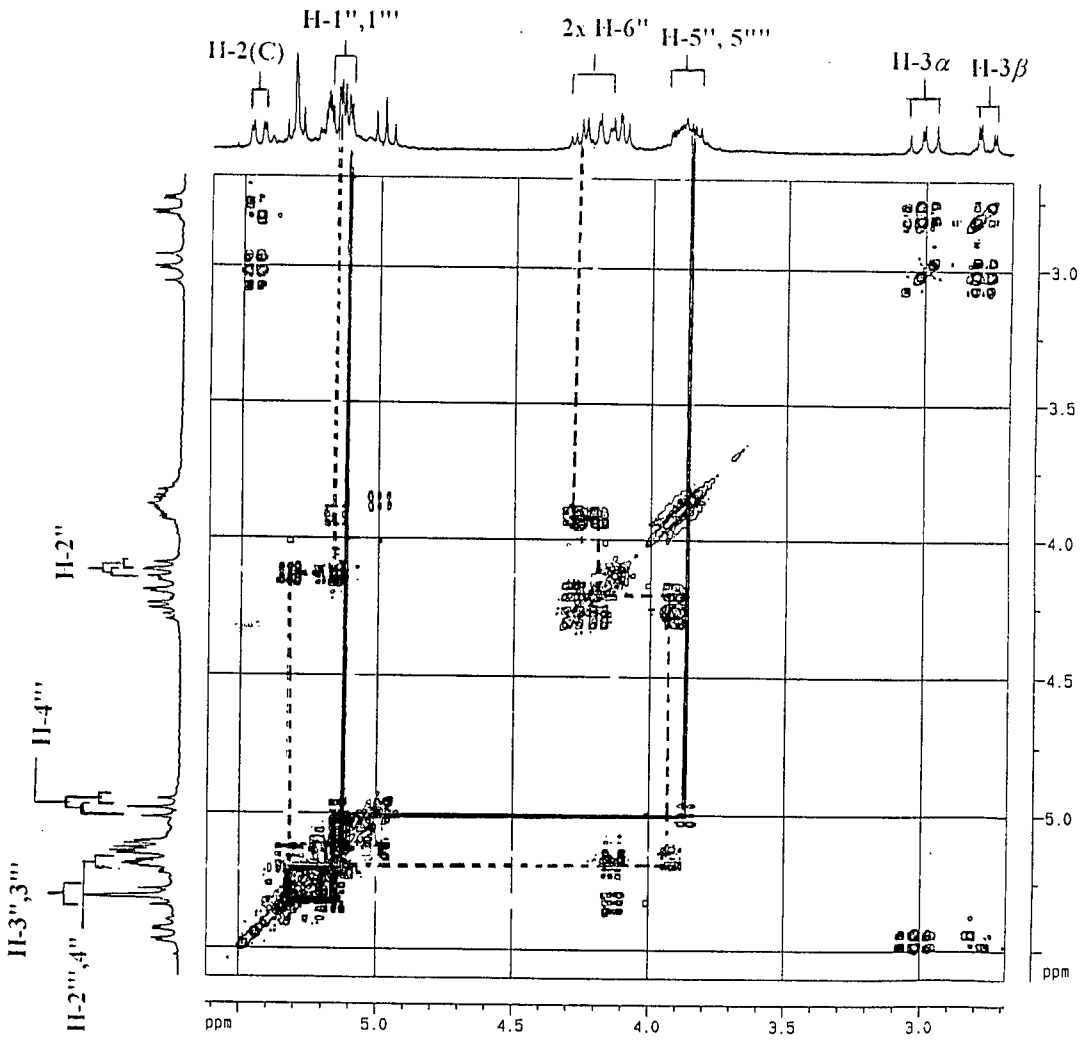
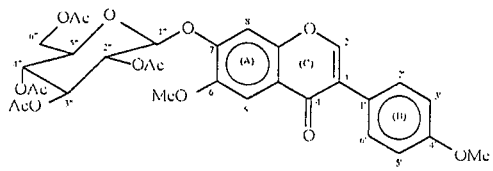


Plate 14 ($CDCl_3$ - 296K)
(^1H NMR)



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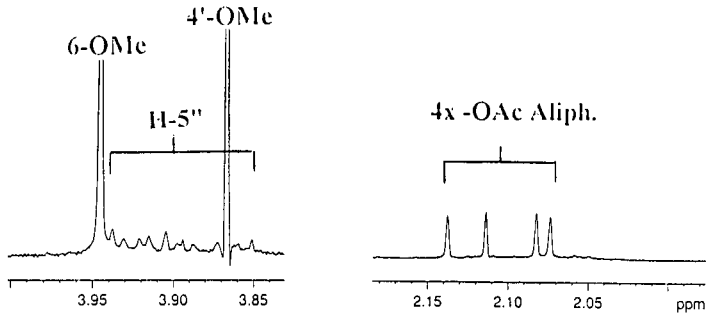
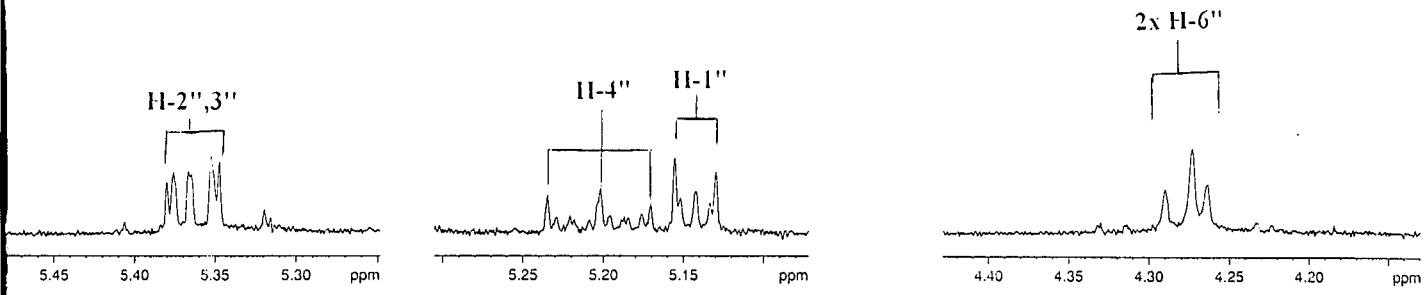
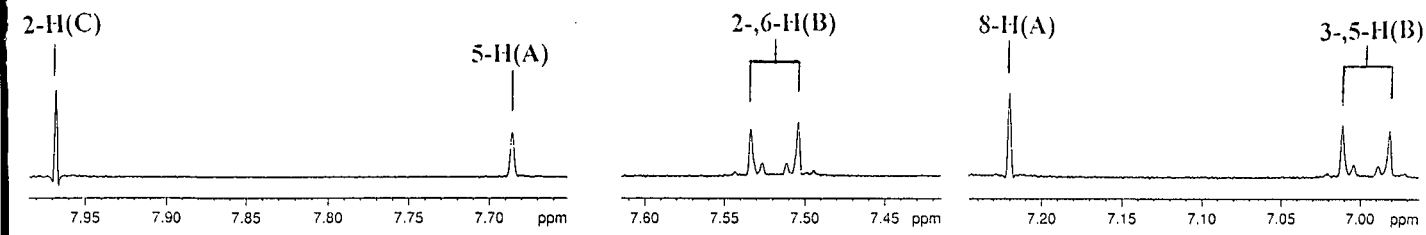






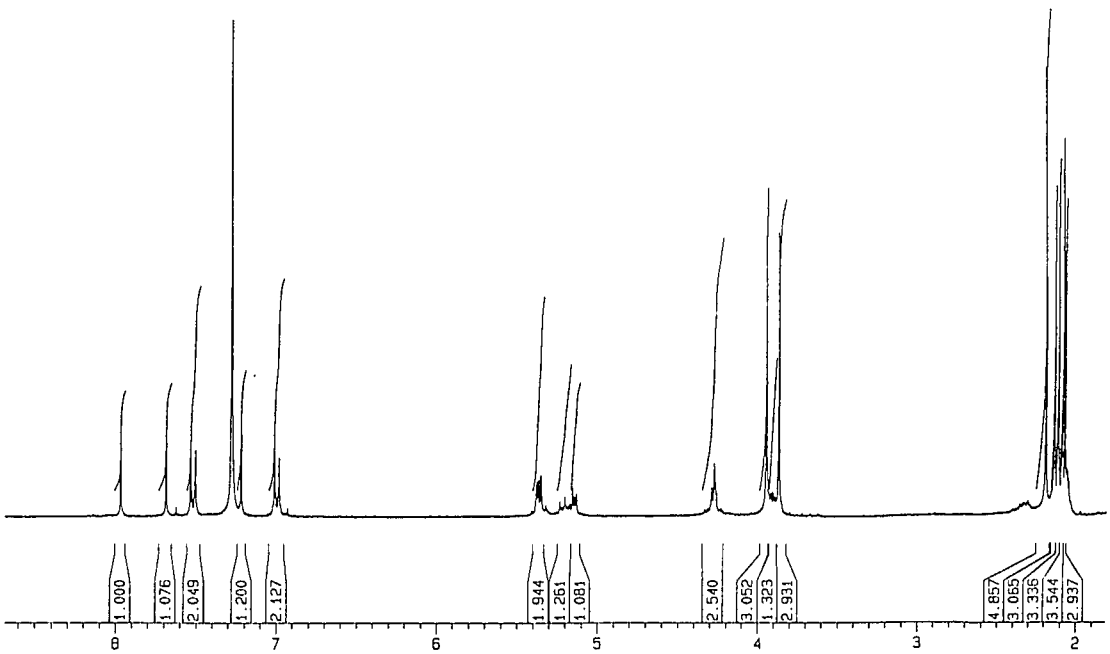
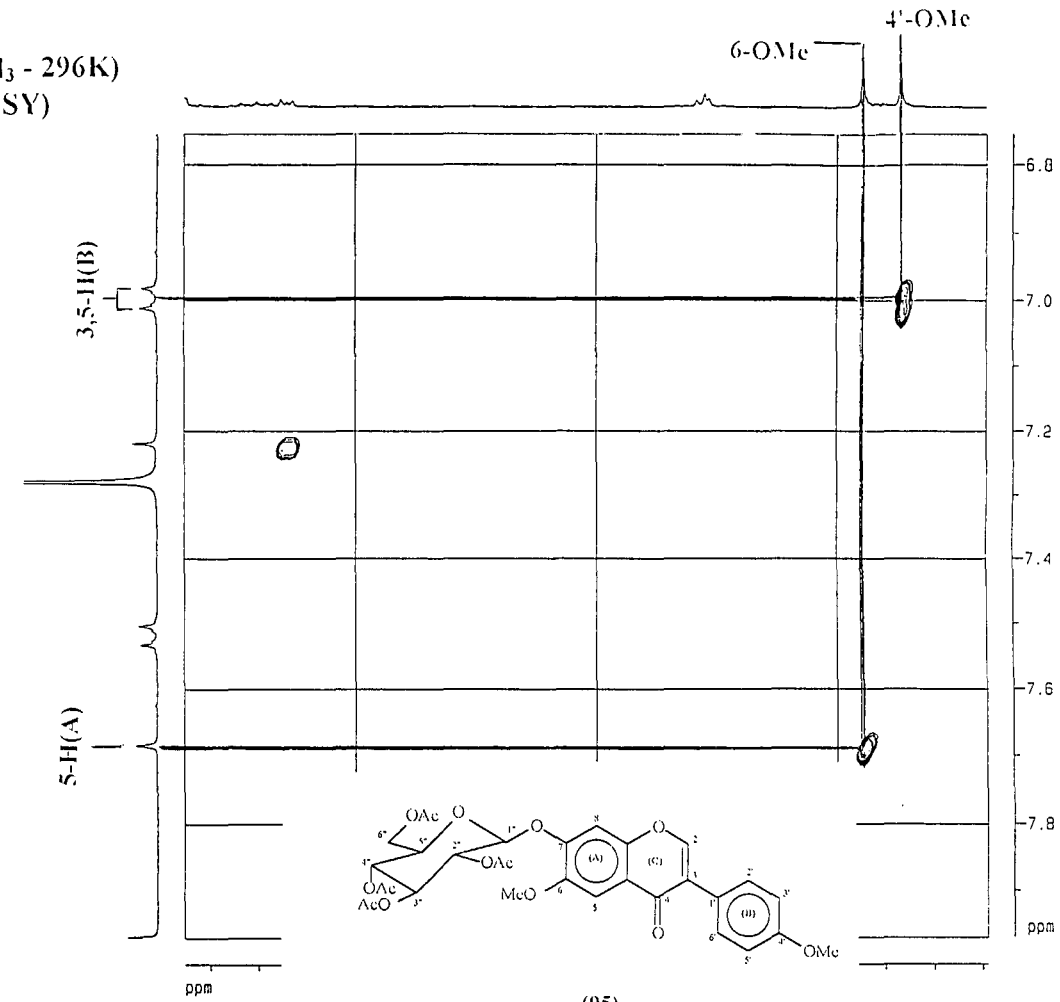


Plate 14a-1 ($CDCl_3$ - 296K)
(NOESY)



(95)

Plate 14a-2 ($CDCl_3$ - 296K)
(NOESY)

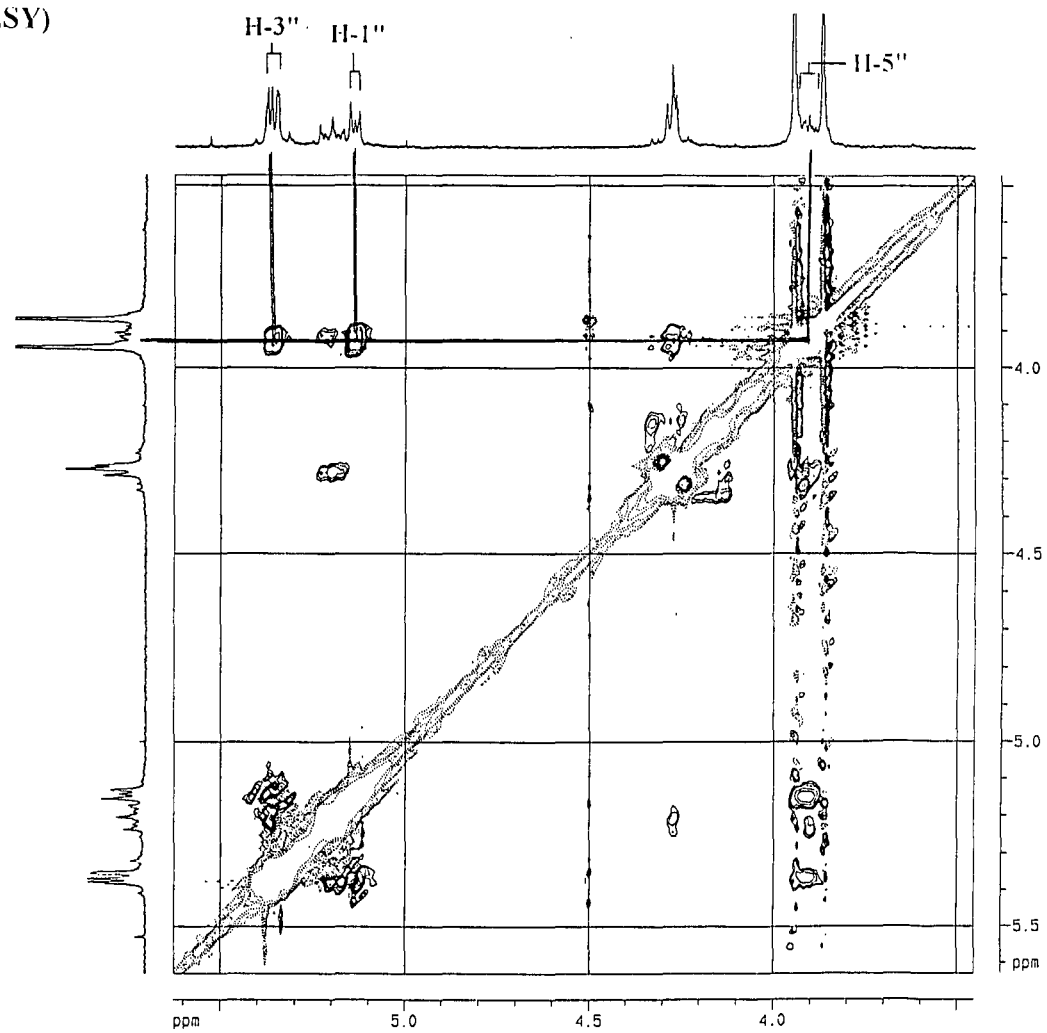


Plate 14b-1 ($CDCl_3$ - 296K)
(COSY)

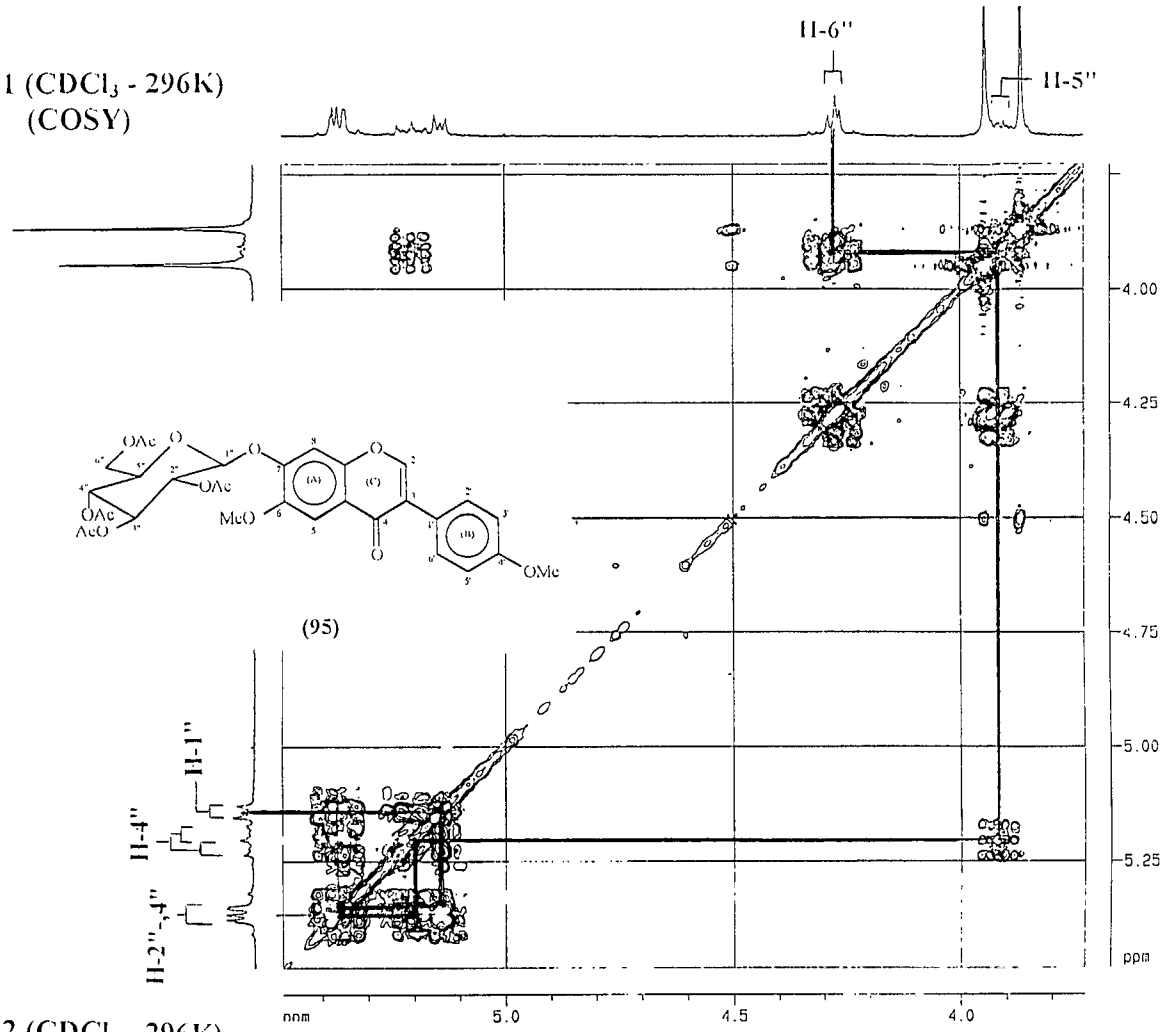


Plate 14b-2 ($CDCl_3$ - 296K)
(COSY)

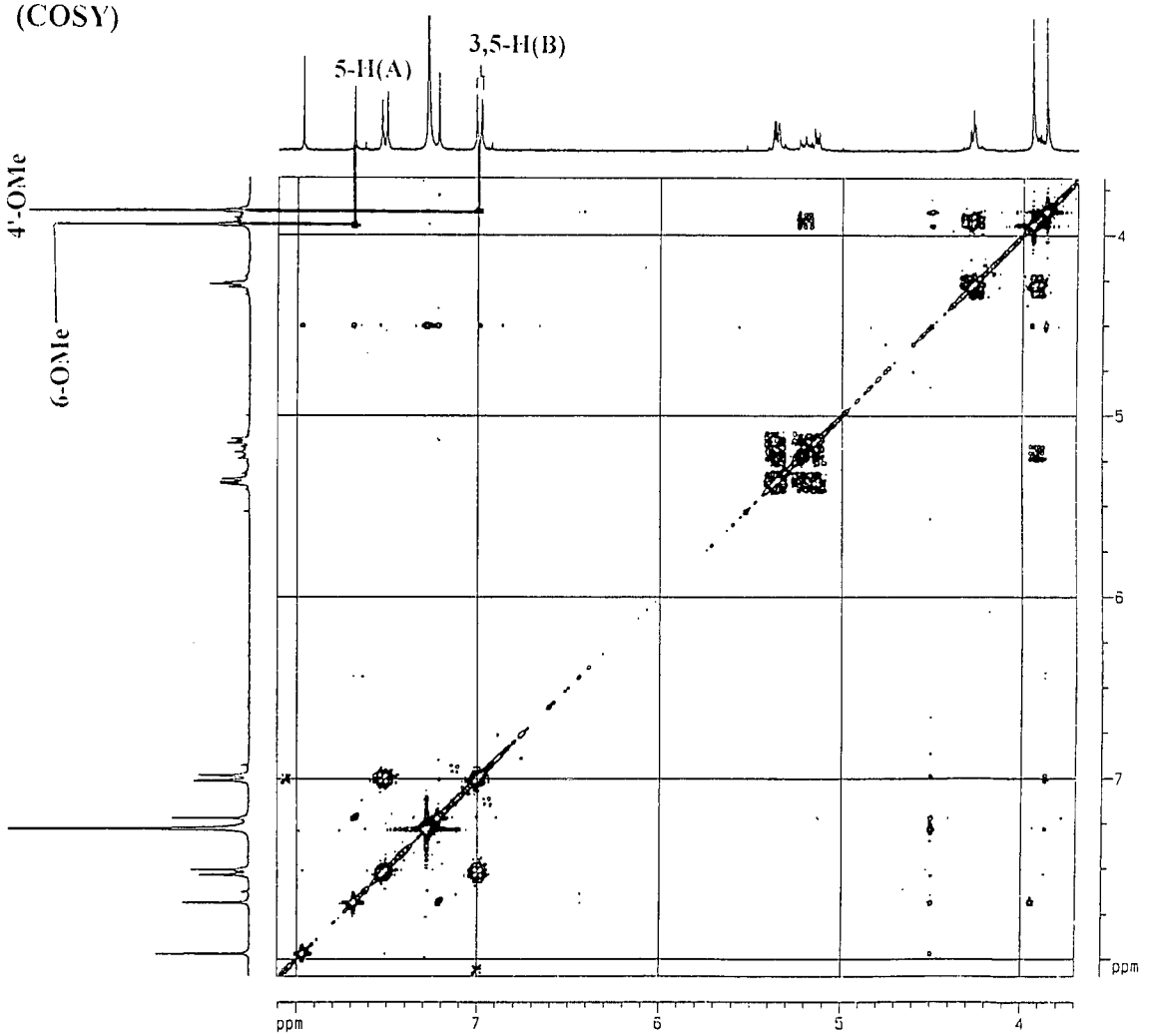
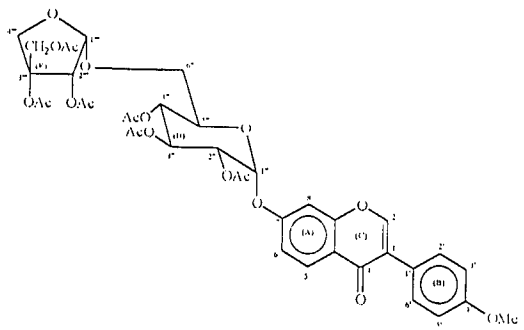


Plate 15 ($CDCl_3$ - 296K)
(^1H NMR)



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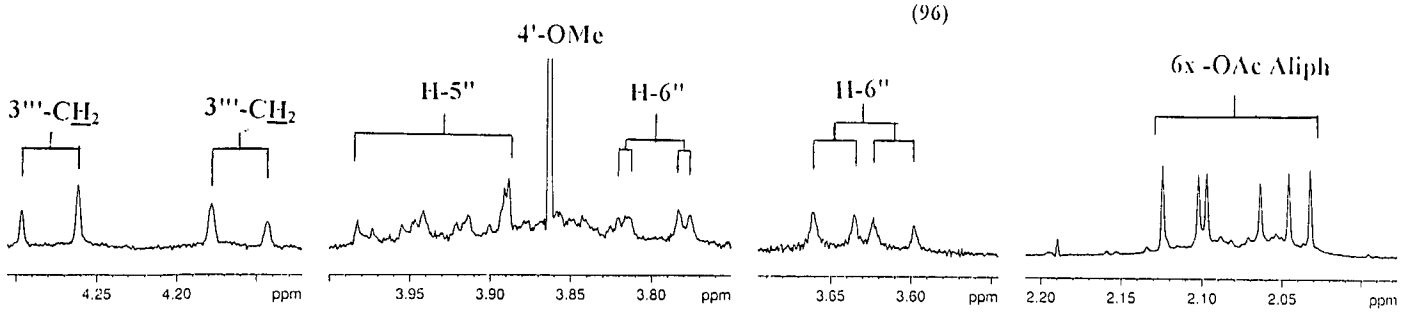
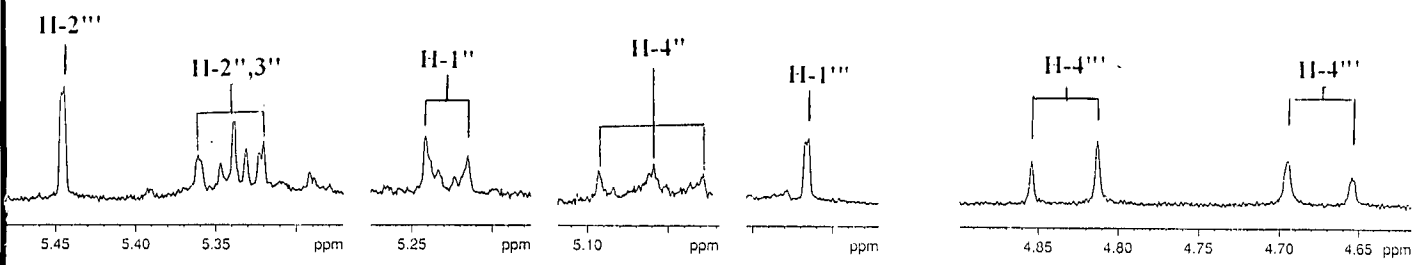
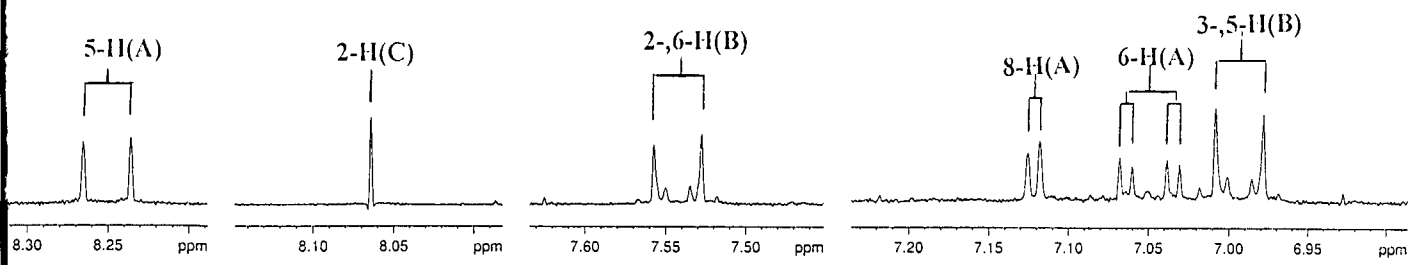






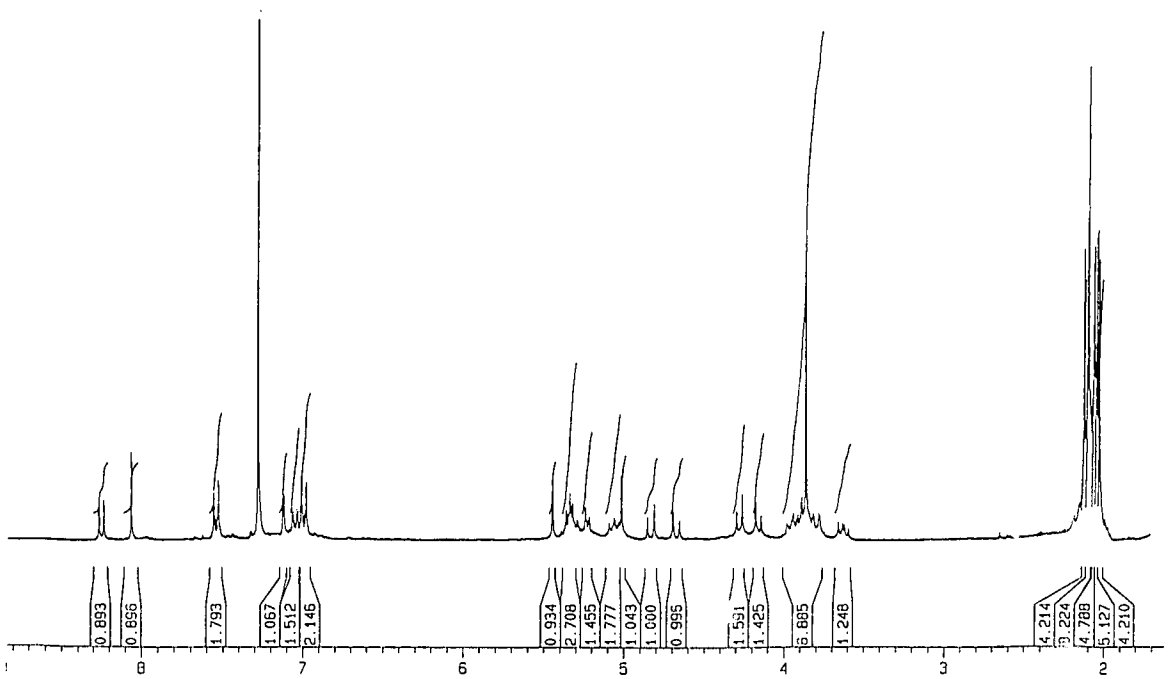


Plate 15a-1 ($CDCl_3$ - 296K)
(NOESY)

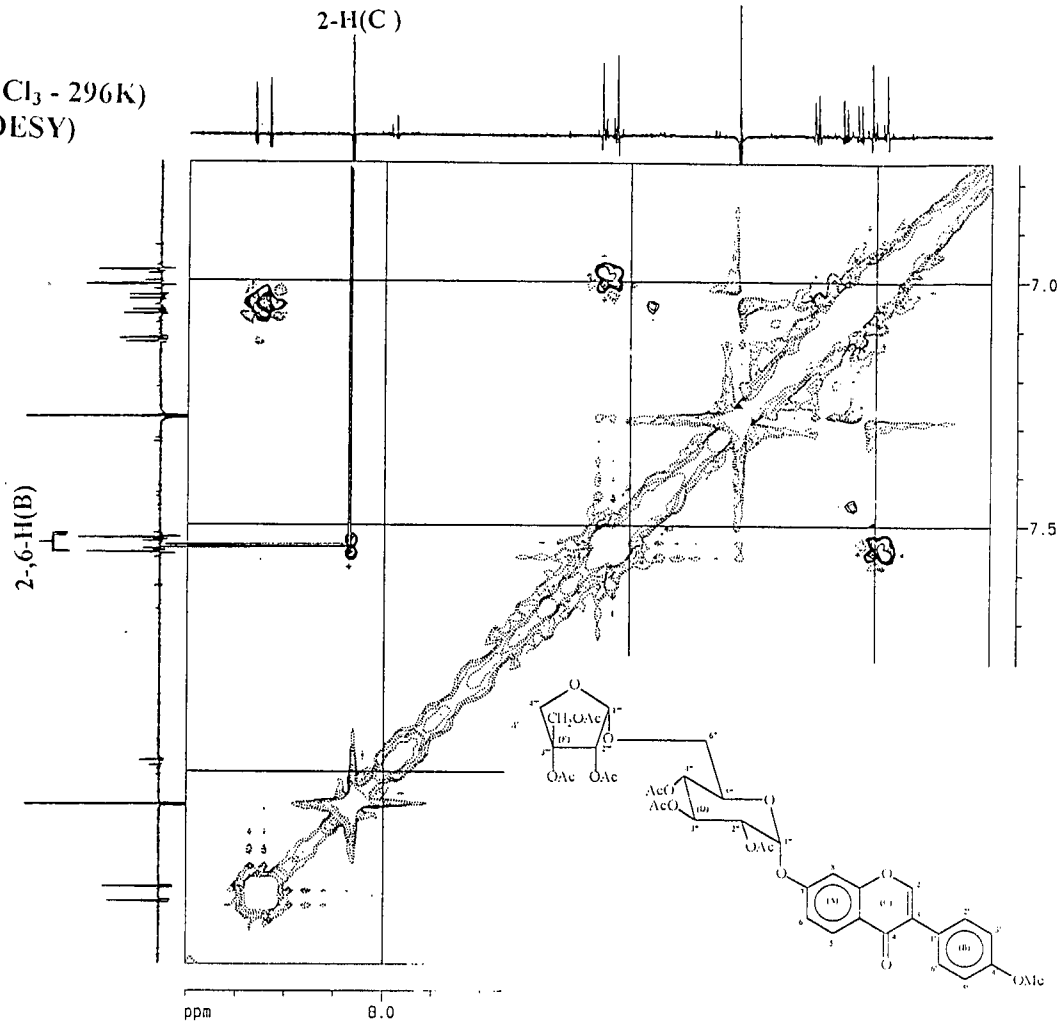


Plate 15a-2 ($CDCl_3$ - 296K)
(NOESY)

(96)

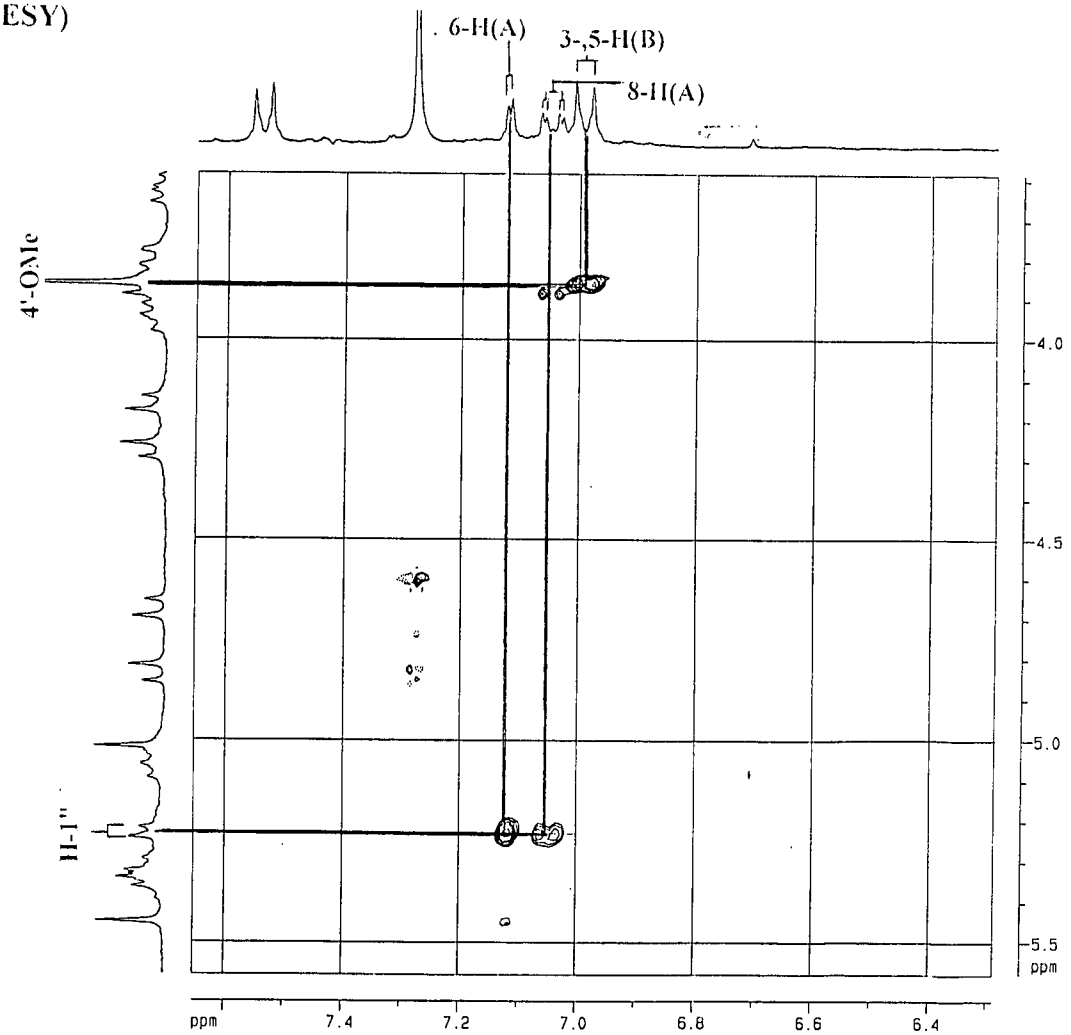


Plate 15a-3 ($CDCl_3$ - 296K)
(NOESY)

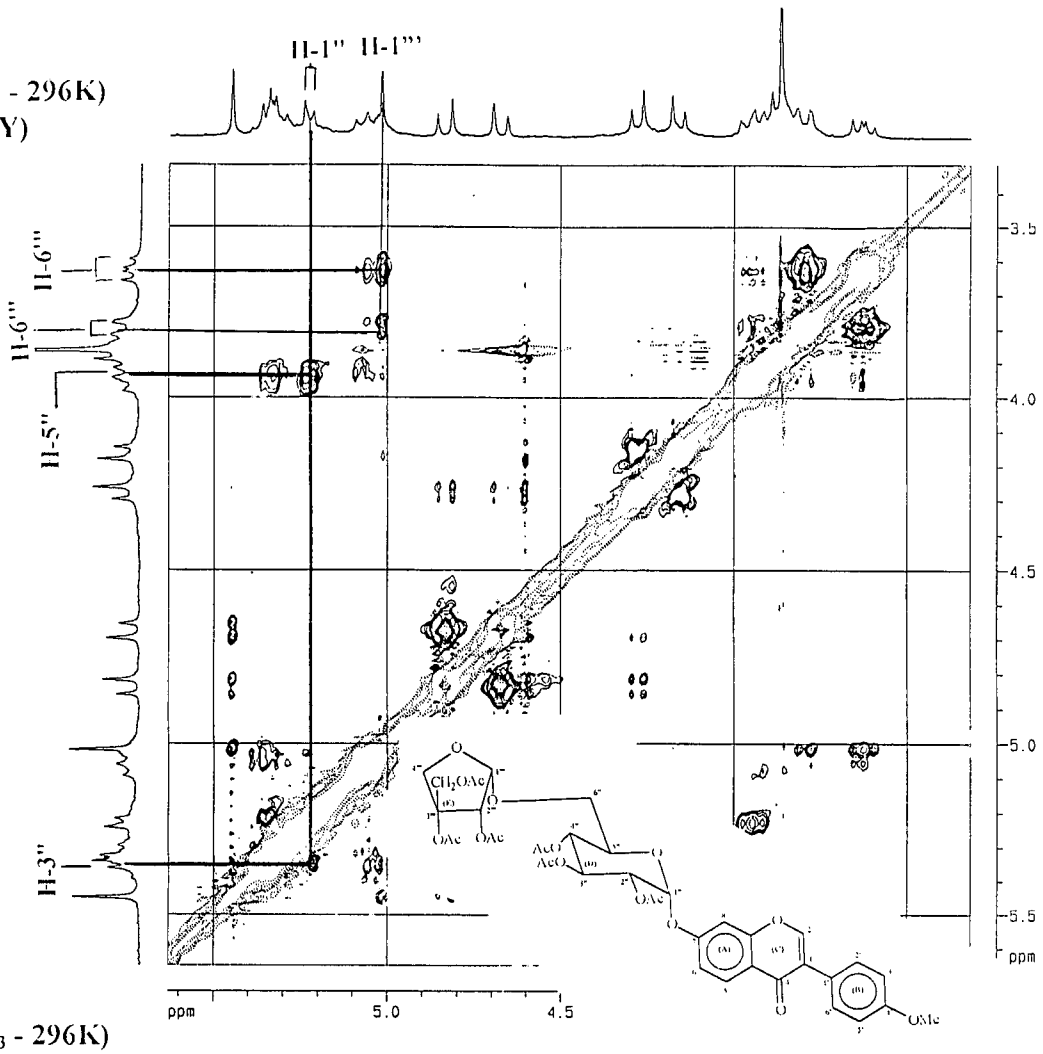


Plate 15a-4 ($CDCl_3$ - 296K)
(NOESY)

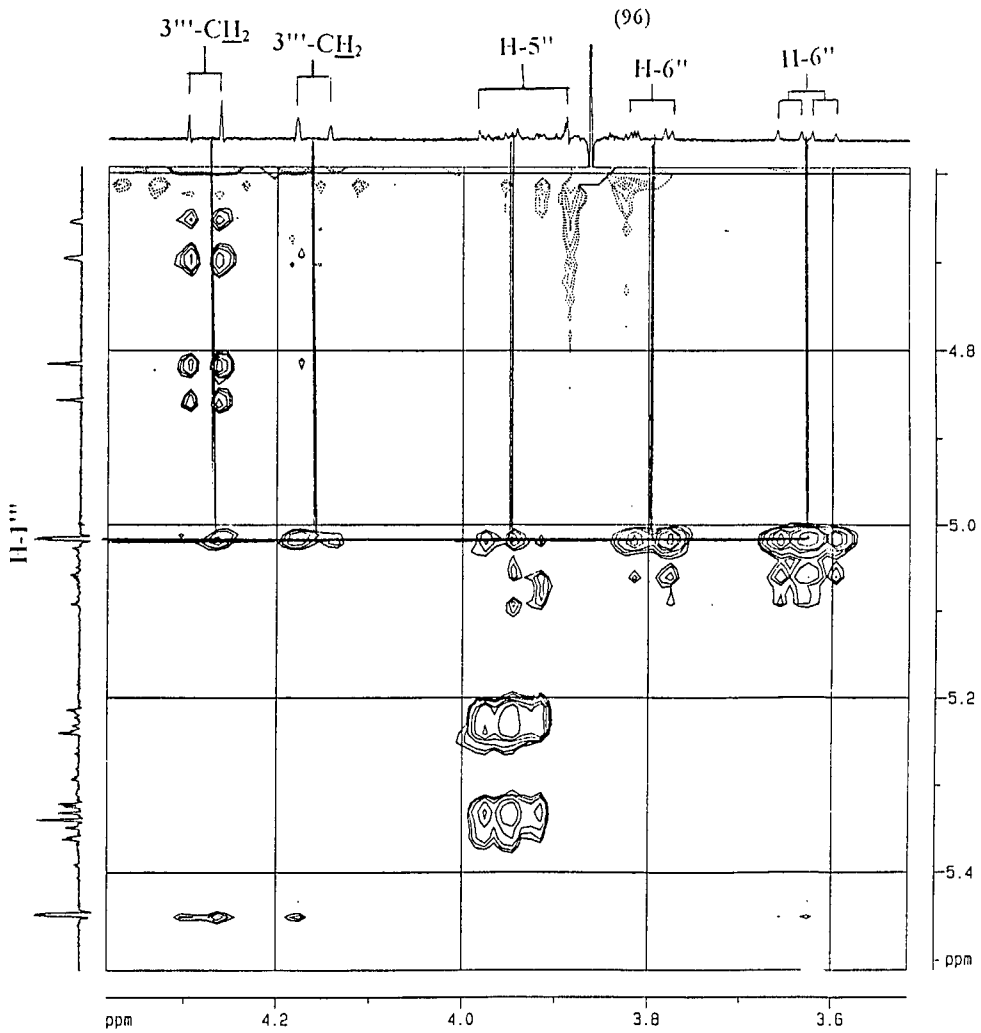
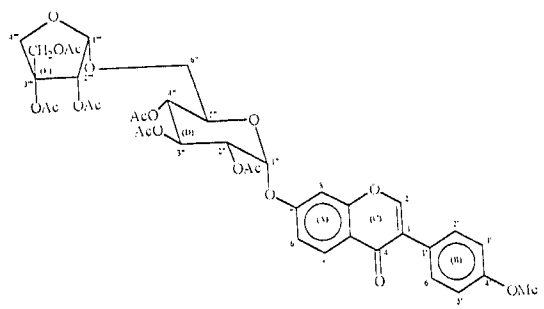


Plate 15b ($CDCl_3$ - 296K)
(COSY)



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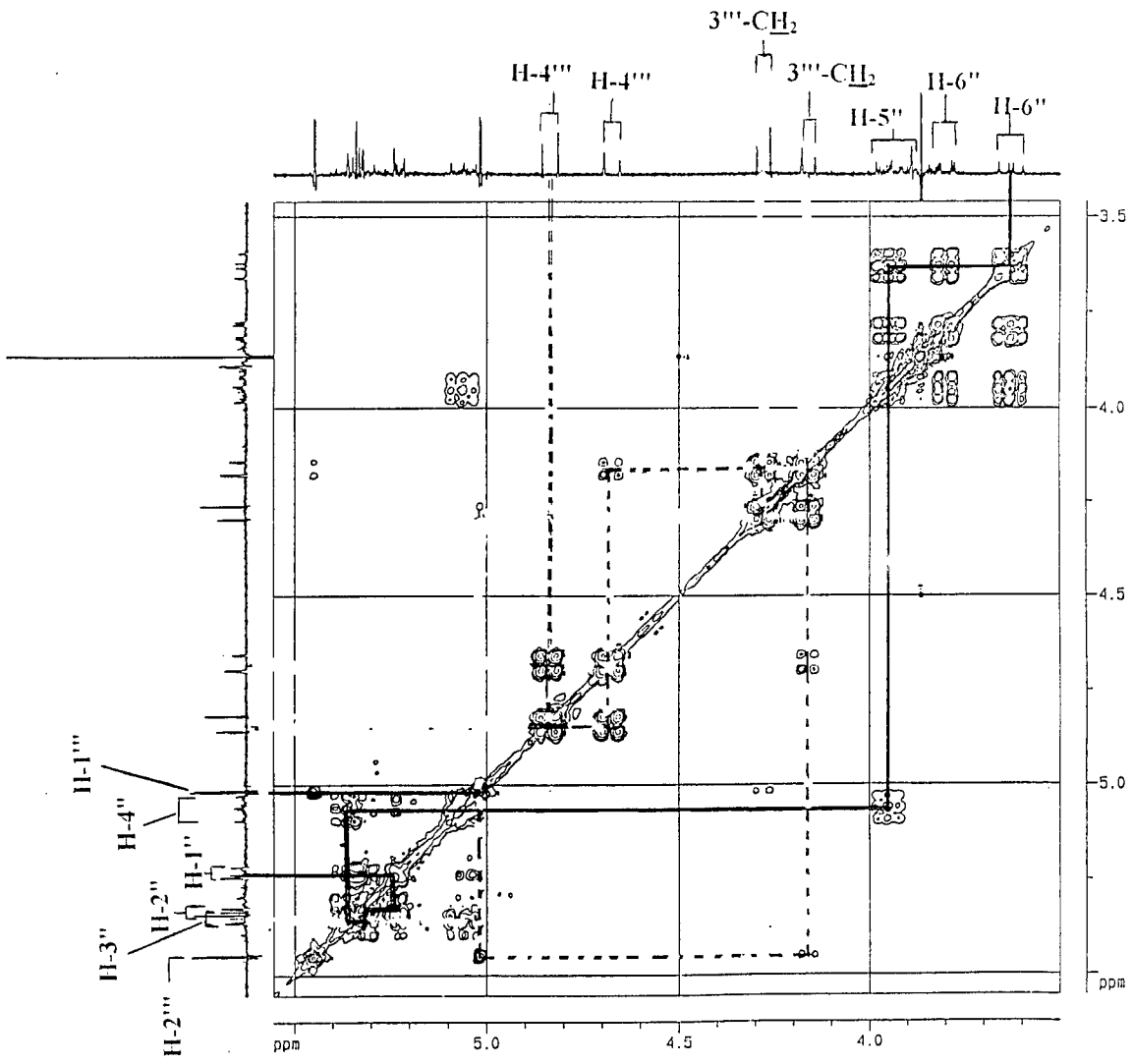
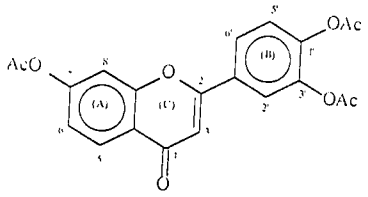


Plate 16 ($CDCl_3$ - 296K)
(^1H NMR)



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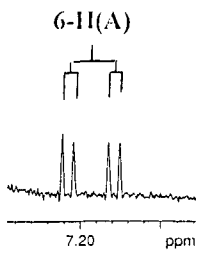
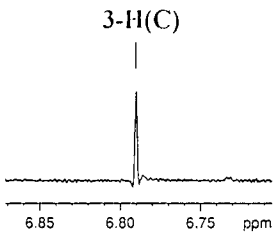
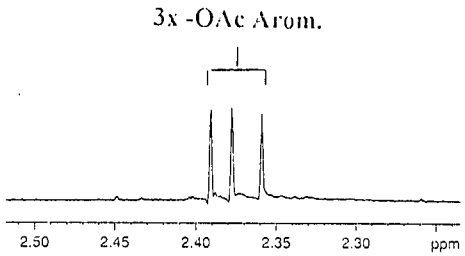
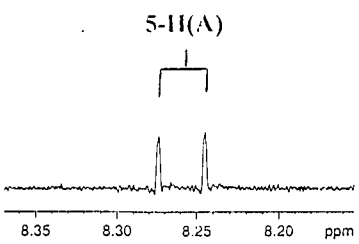
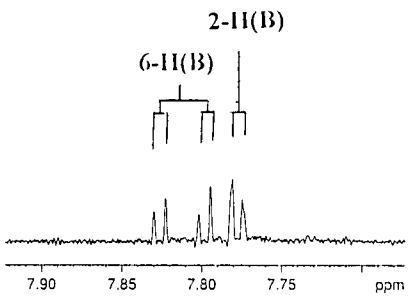
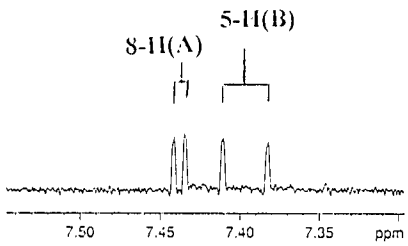












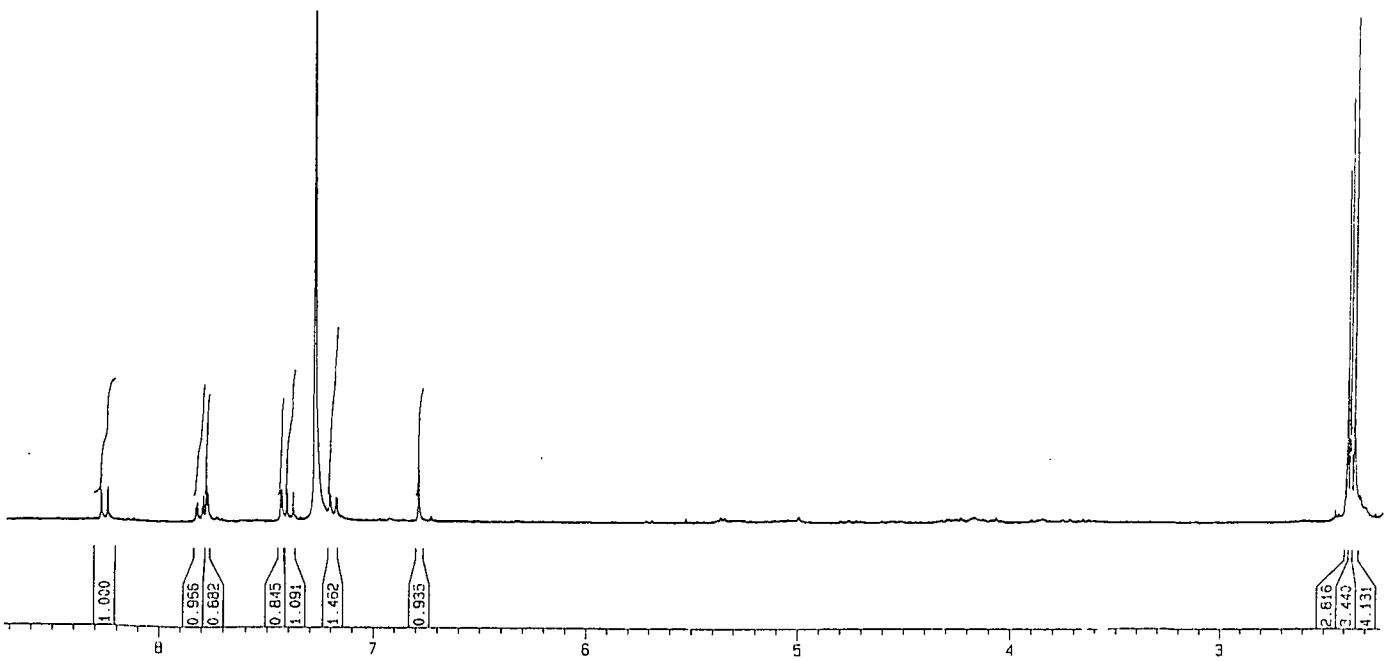
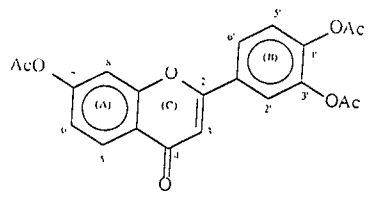


Plate 16a (CDCl$_3$ - 296K)
(NOESY)



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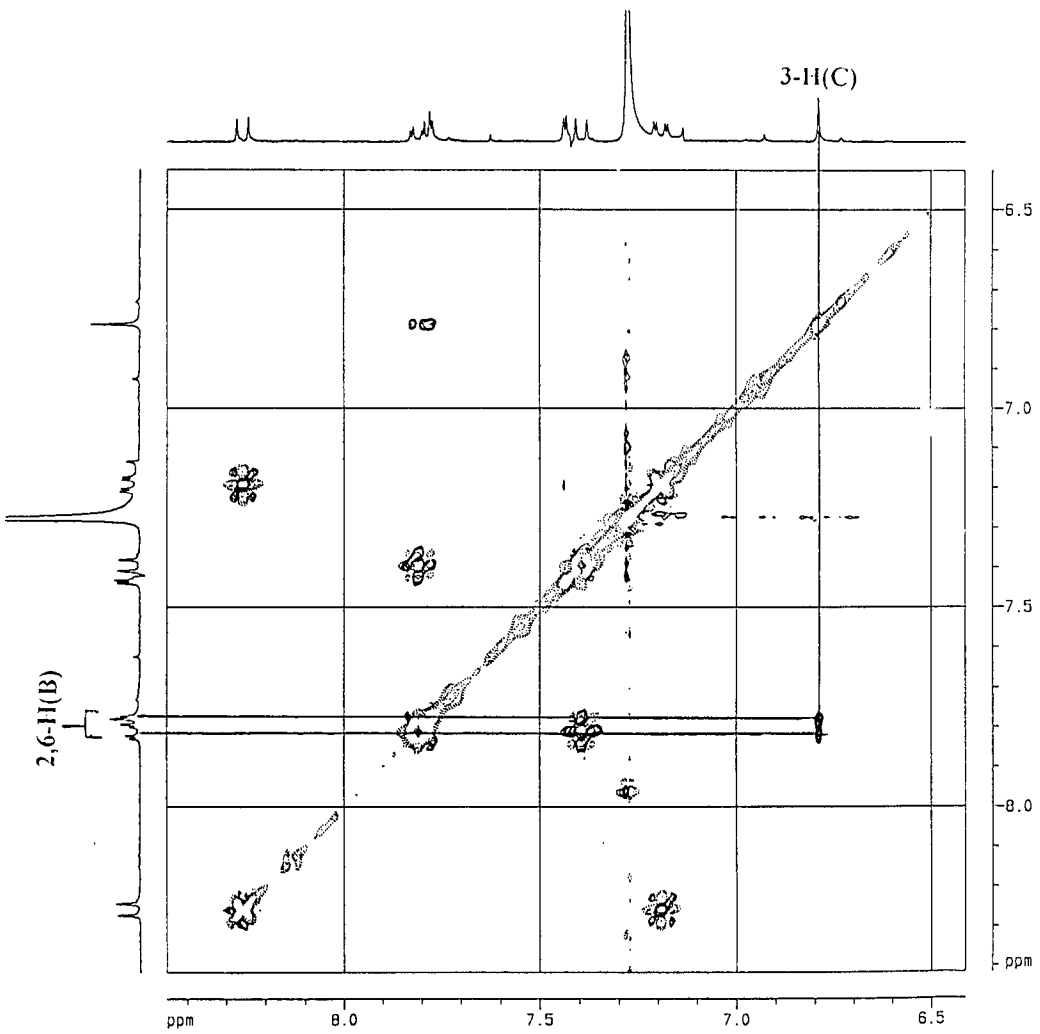
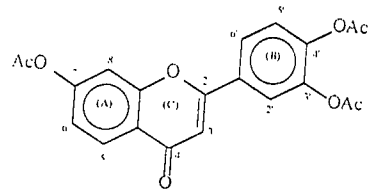


Plate 16b ($CDCl_3$ - 296K)

(COSY)



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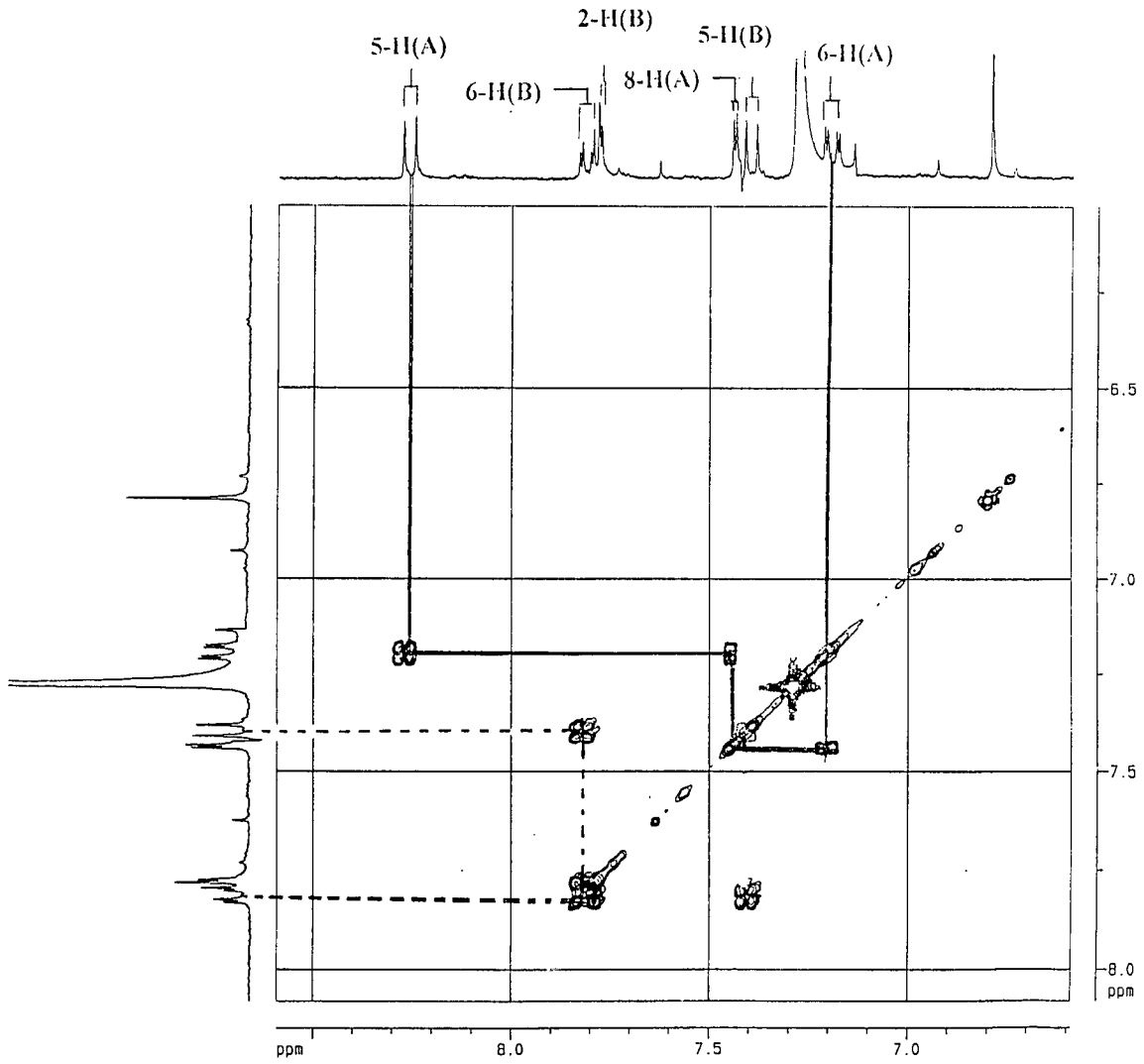
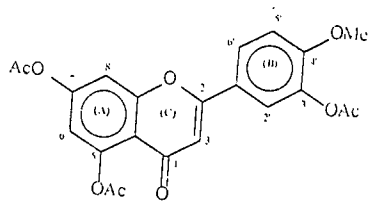
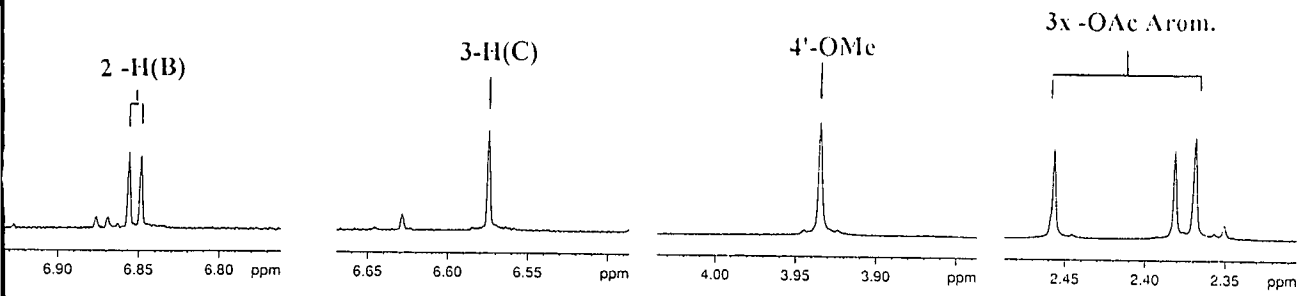
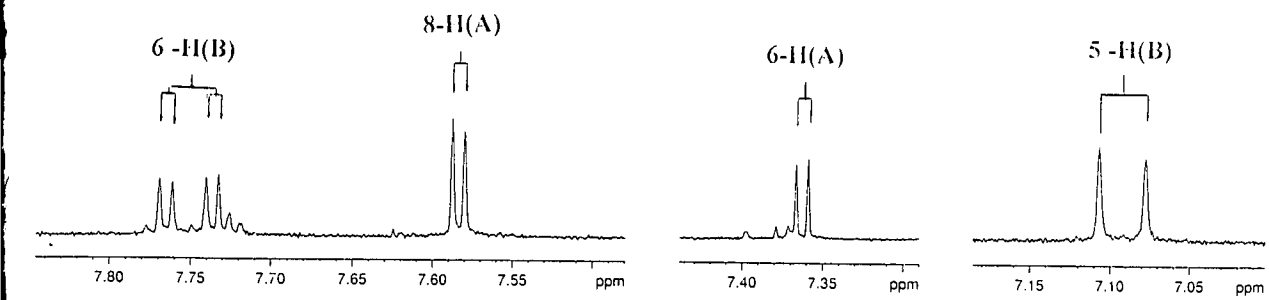


Plate 17 ($CDCl_3$ - 296K)
(^1H NMR)



(98)





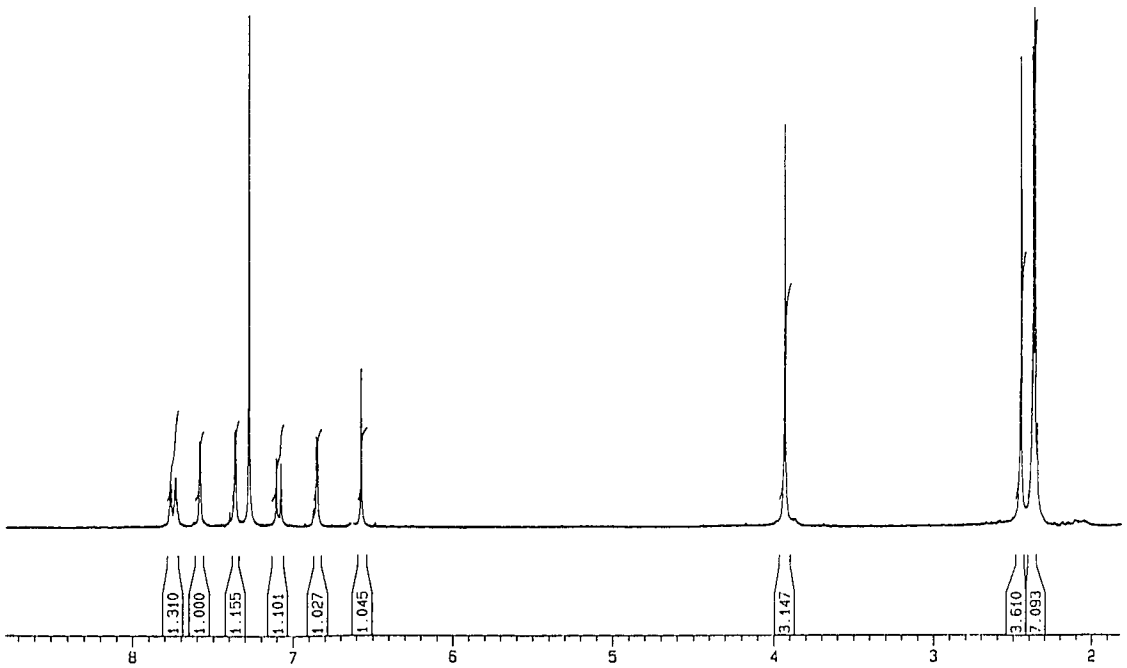


Plate 17b-1 ($CDCl_3$ - 296K)
(COSY)

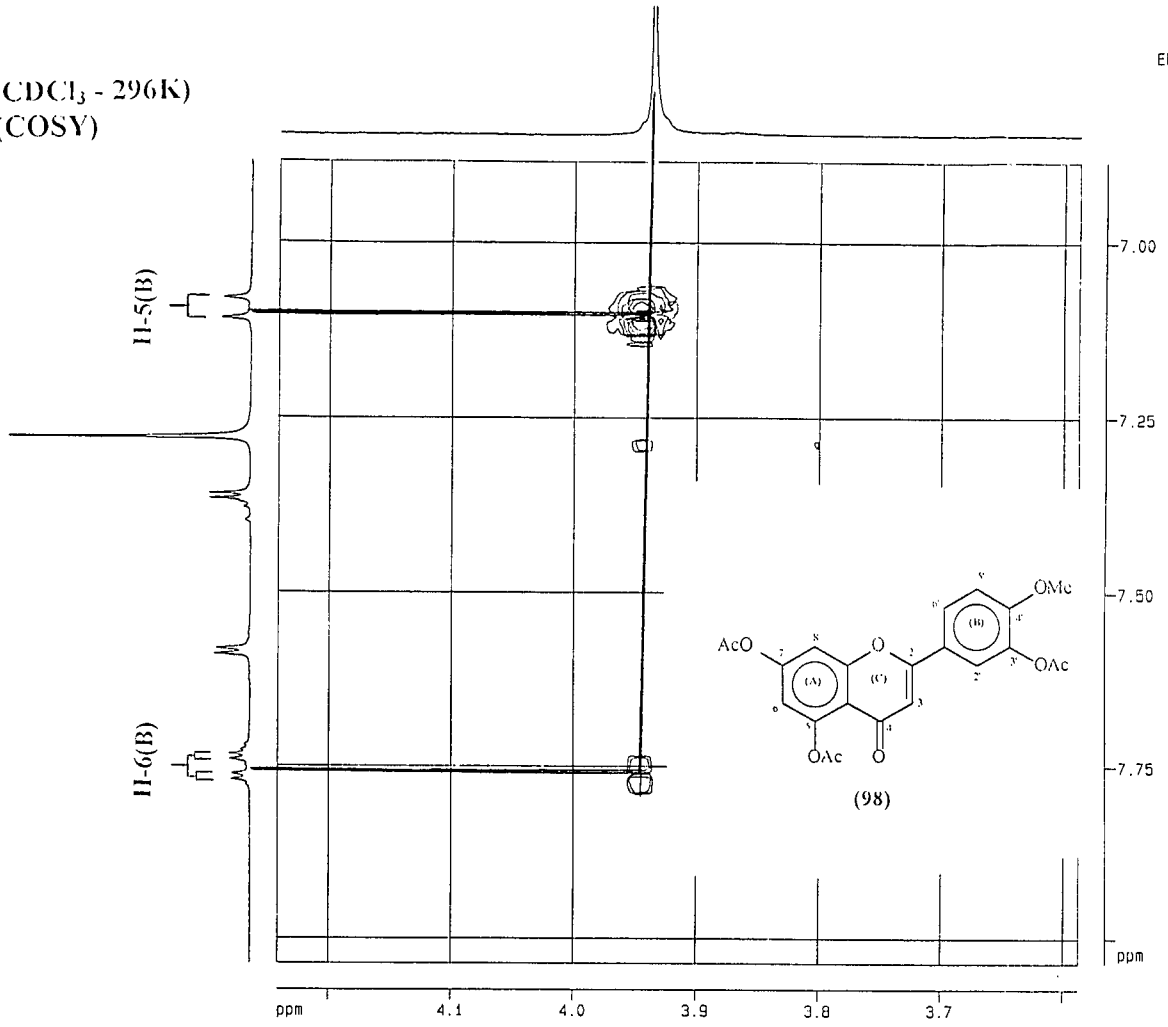


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Plate 17b-2 ($CDCl_3$ - 296K)
(COSY)

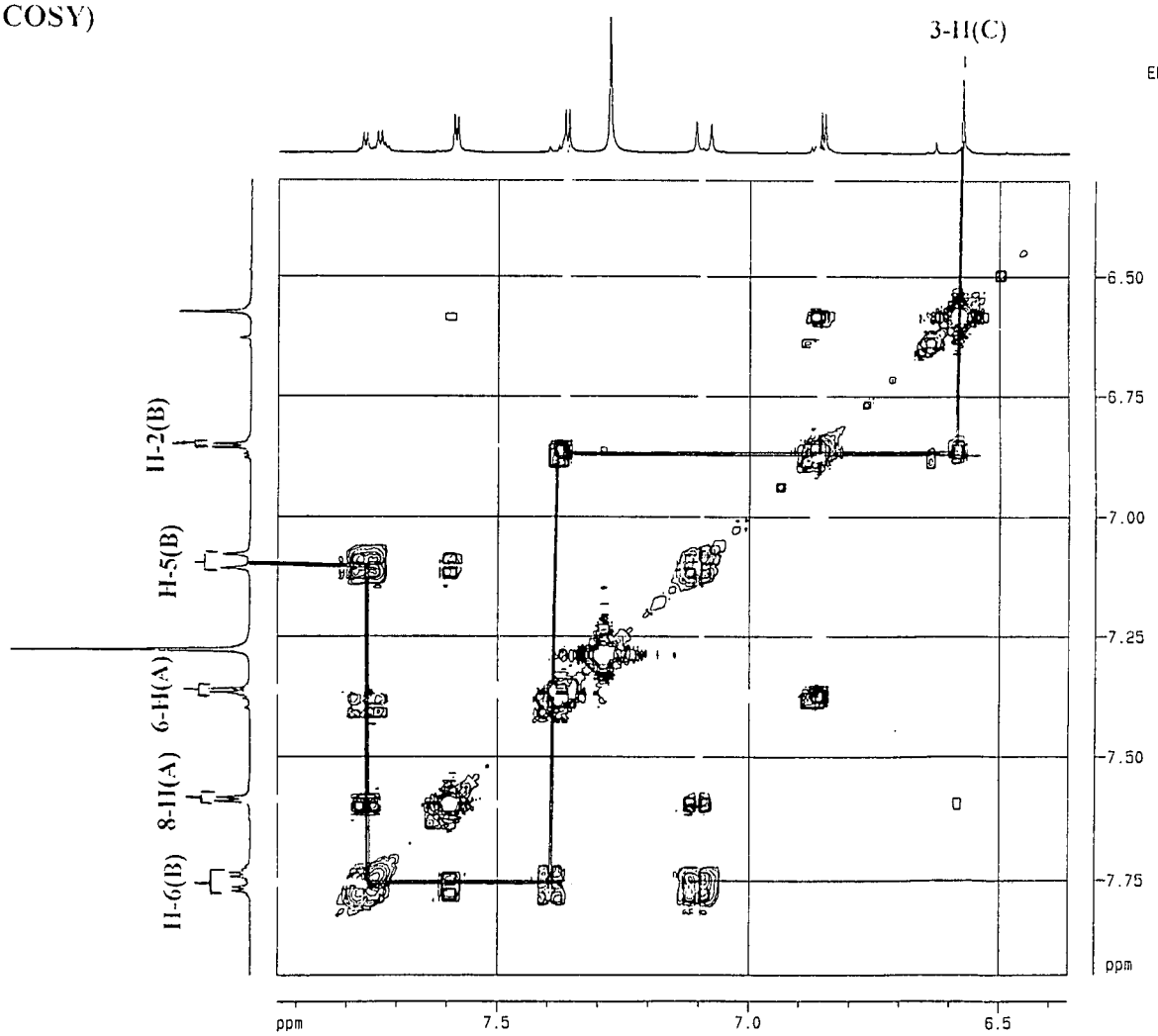
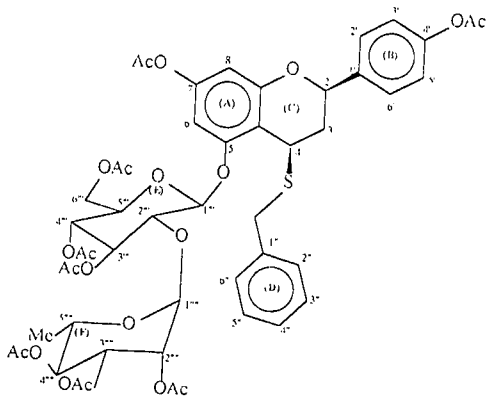
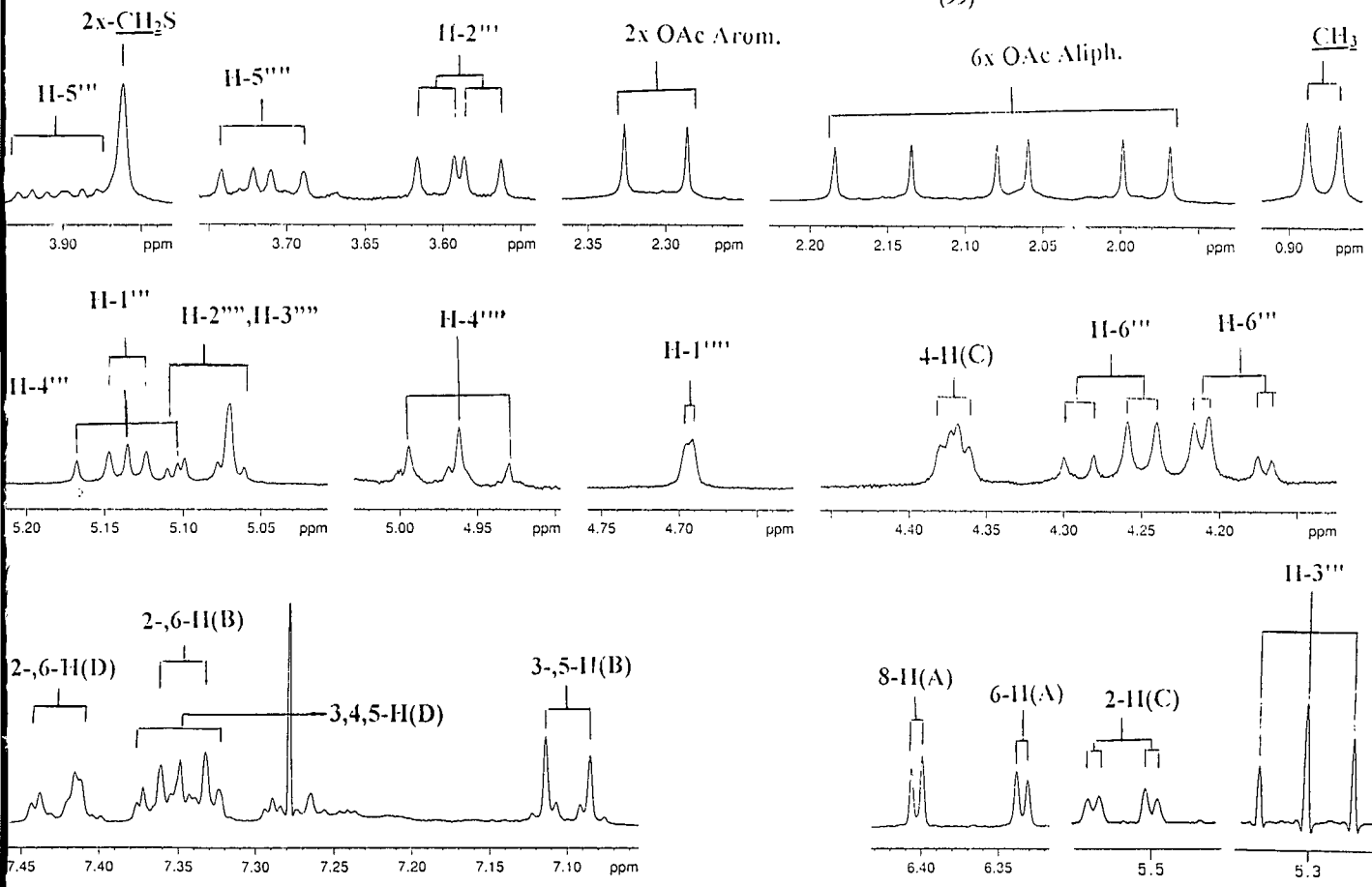


Plate 18 ($CDCl_3$ - 296K)

(^1H NMR)



(99)



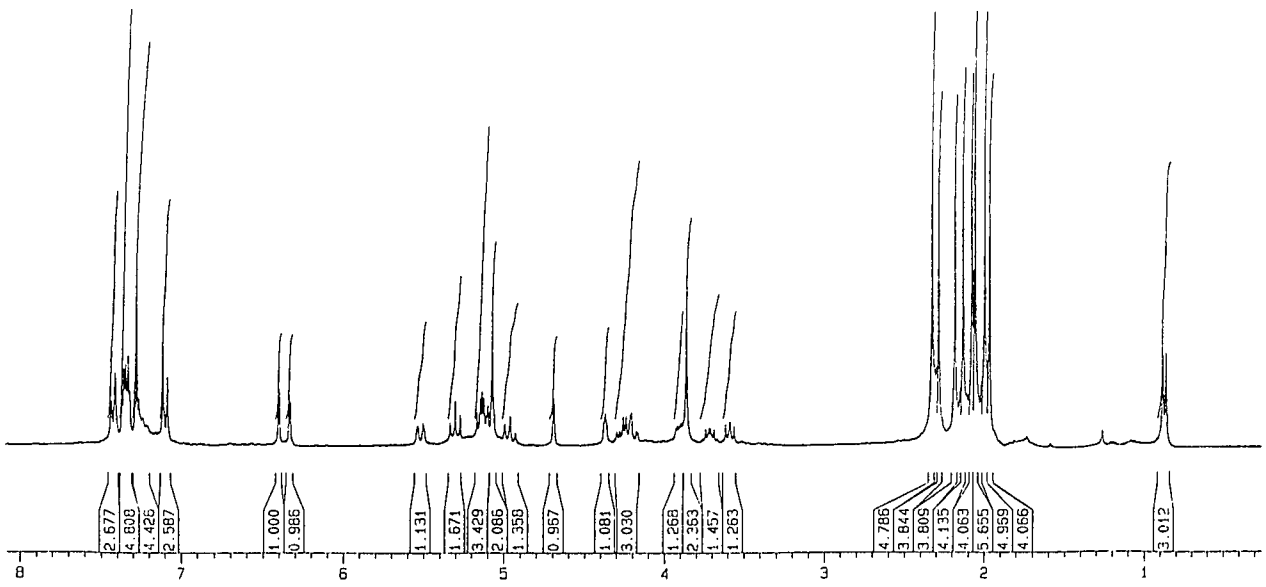


Plate 18a-1 ($CDCl_3$ - 296K)

(NOESY)

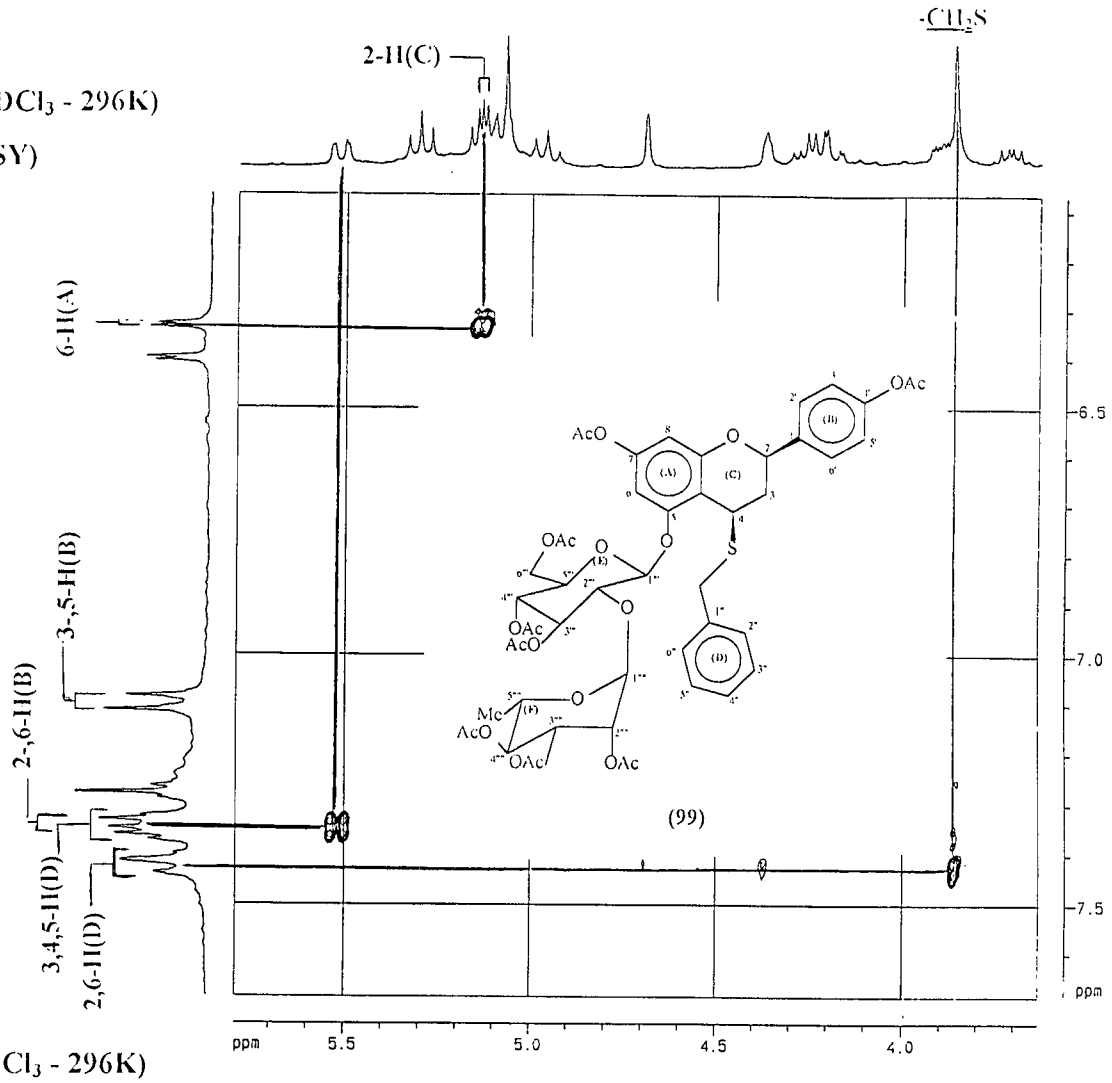


Plate 18a-2 ($CDCl_3$ - 296K)

(NOESY)

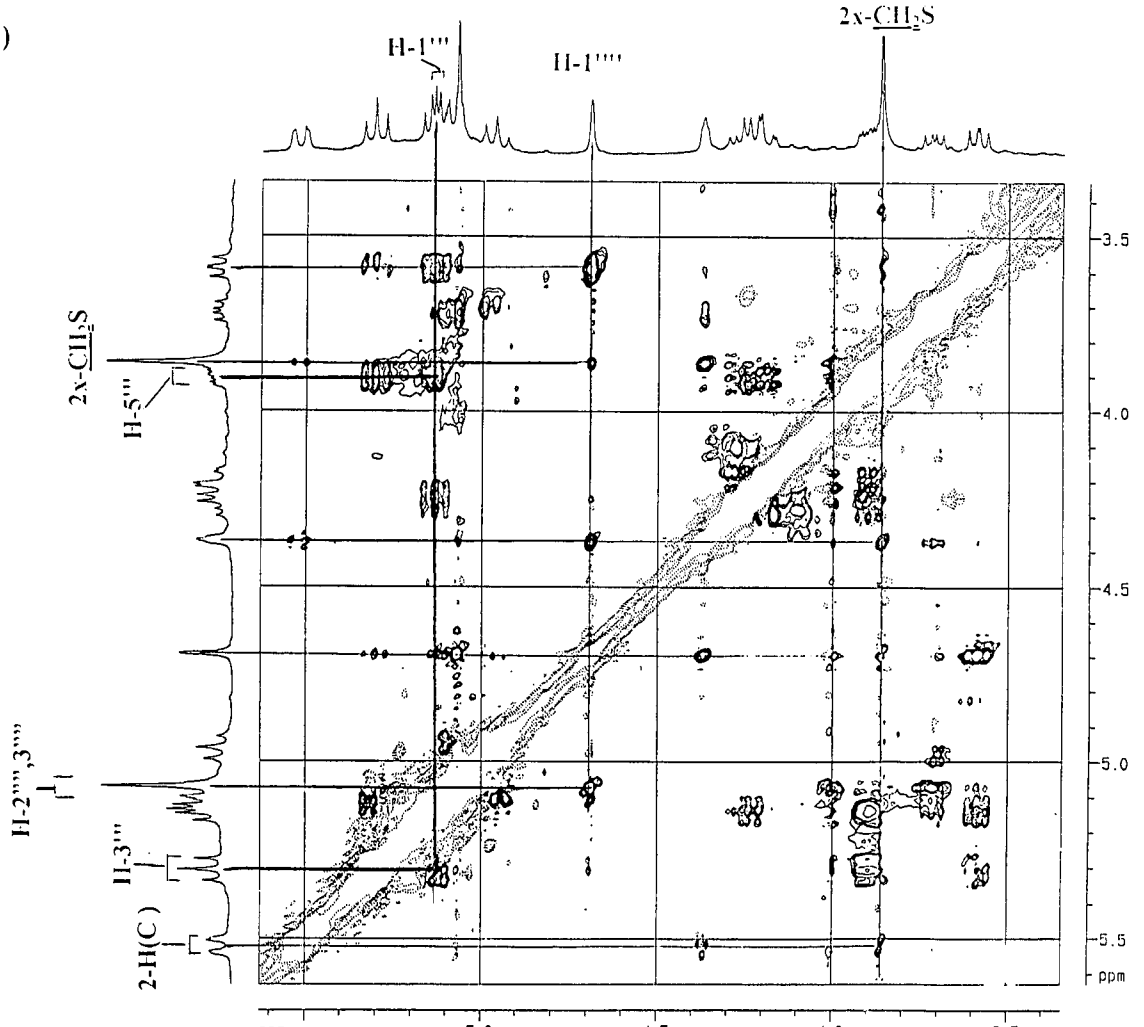
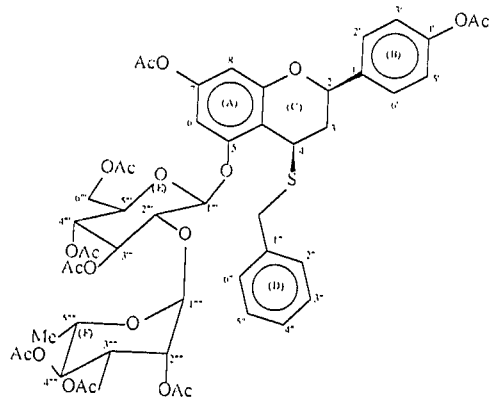


Plate 18a-3 ($CDCl_3$ - 296K)

(NOESY)



(99)

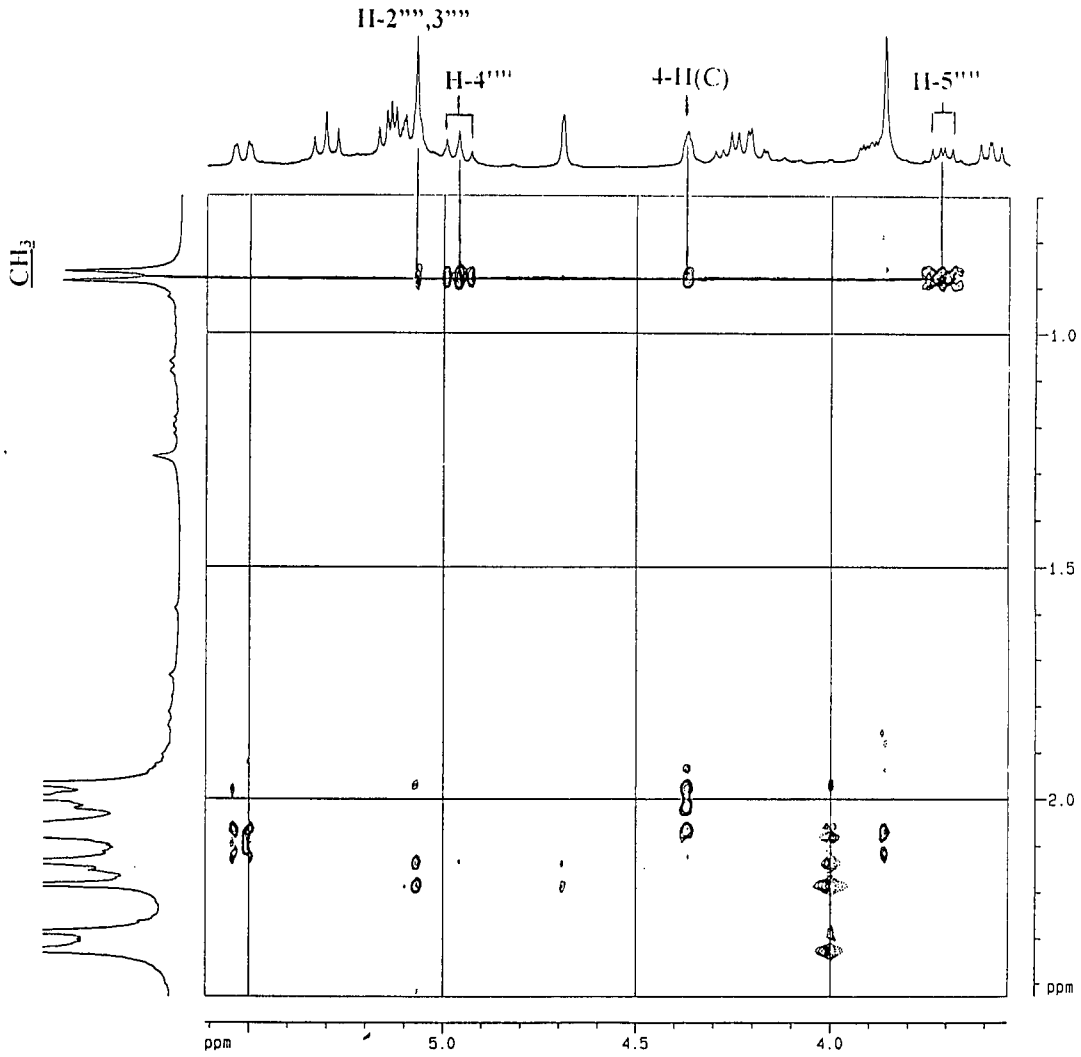
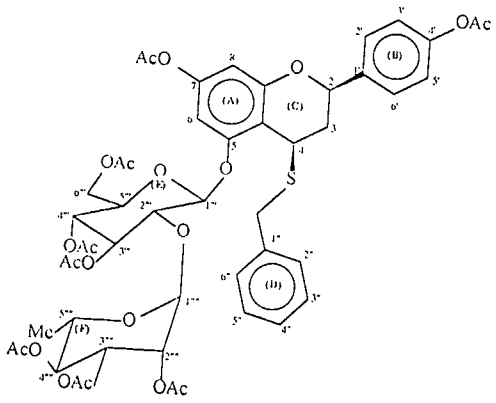


Plate 18b ($CDCl_3$ - 296K)

(COSY)



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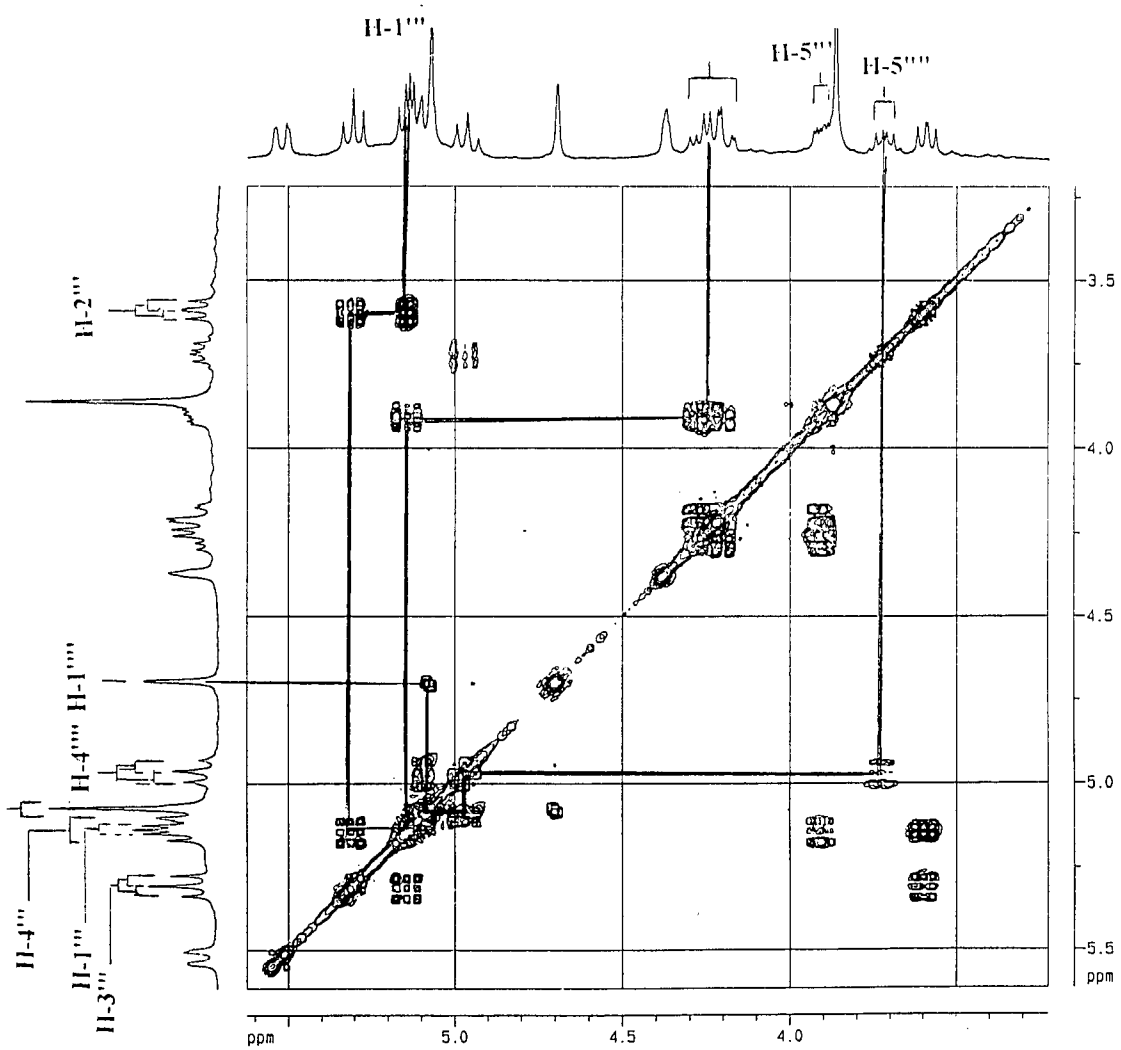
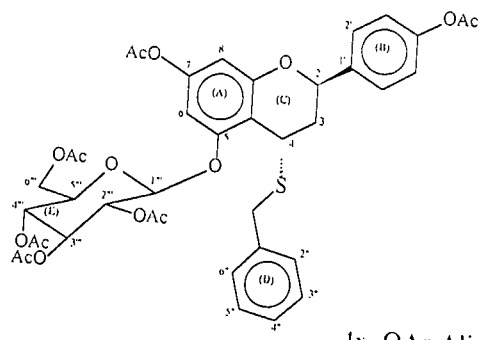
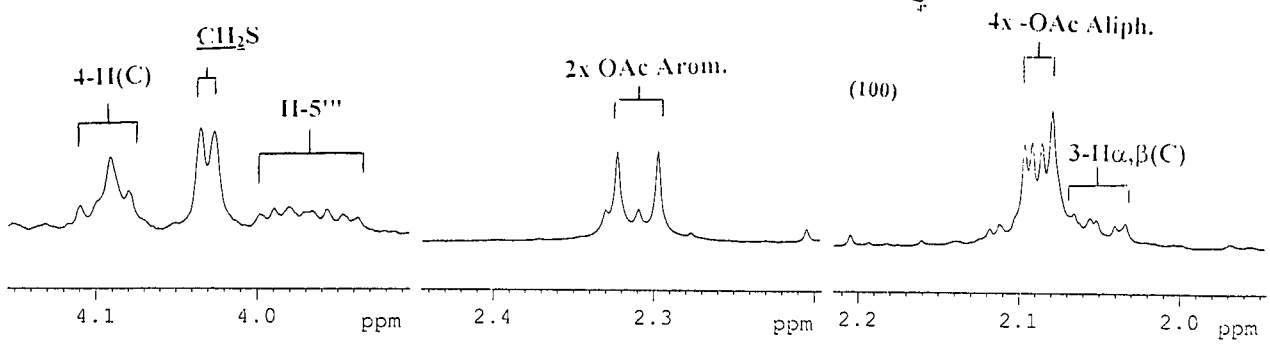
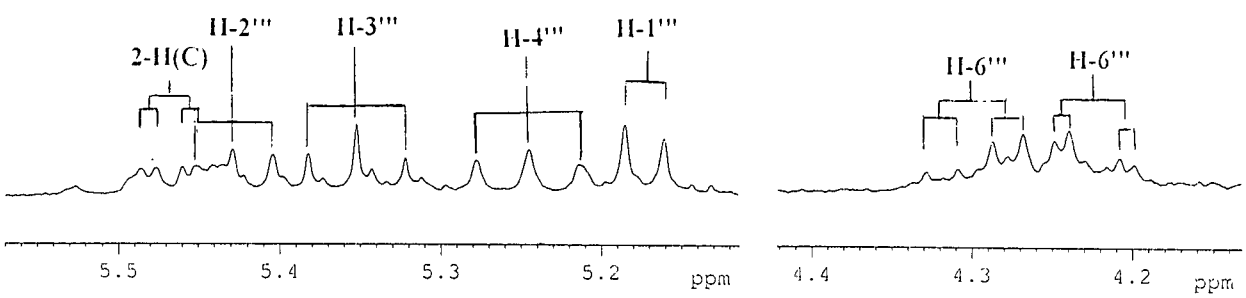


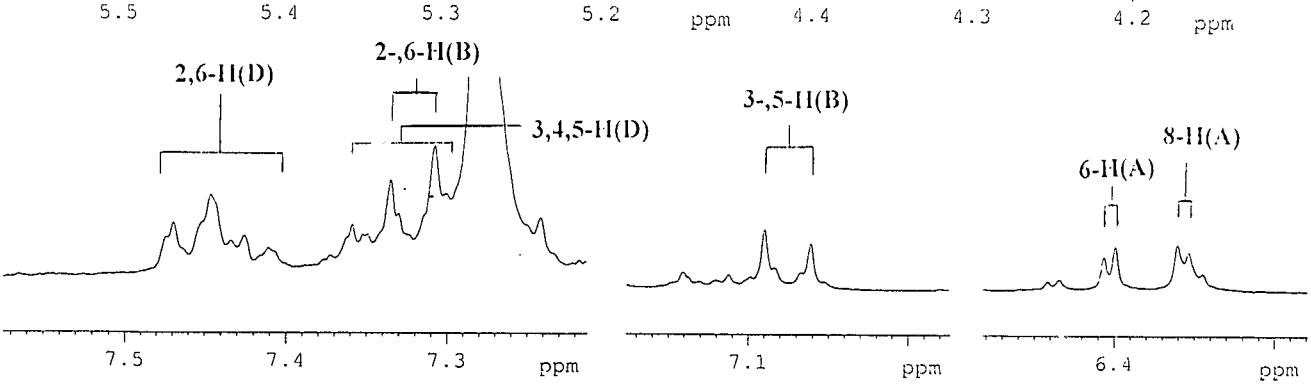
Plate 19 ($CDCl_3$ - 296K)

(^1H NMR)









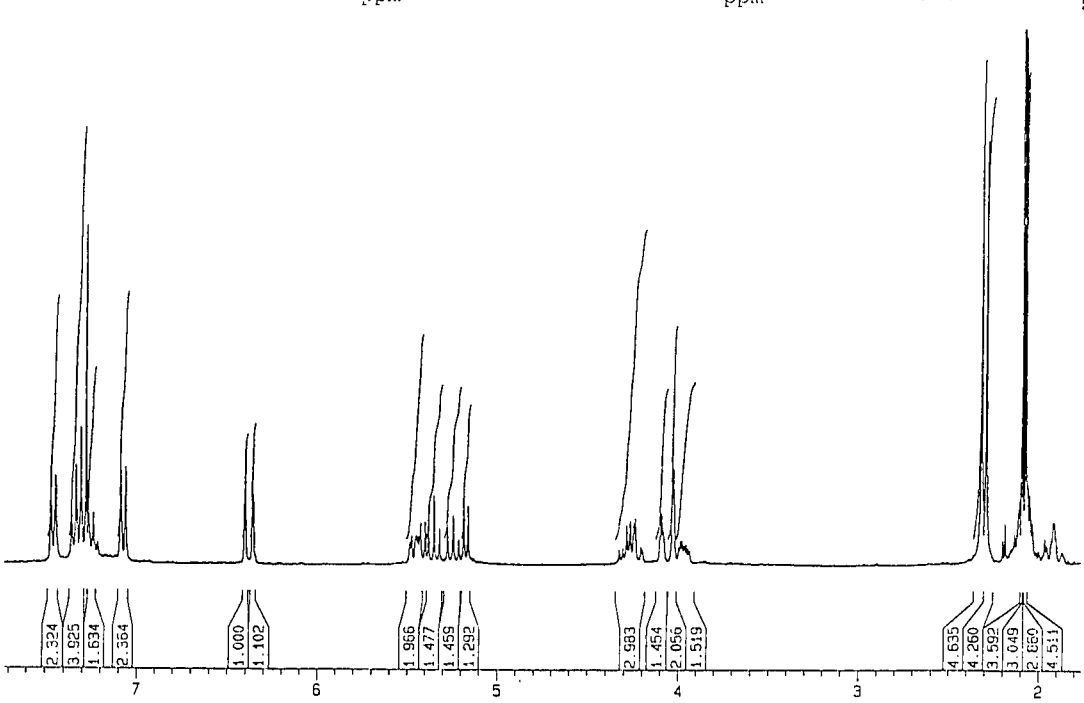


Plate 19a-1 ($CDCl_3$ - 296K)

(NOESY)

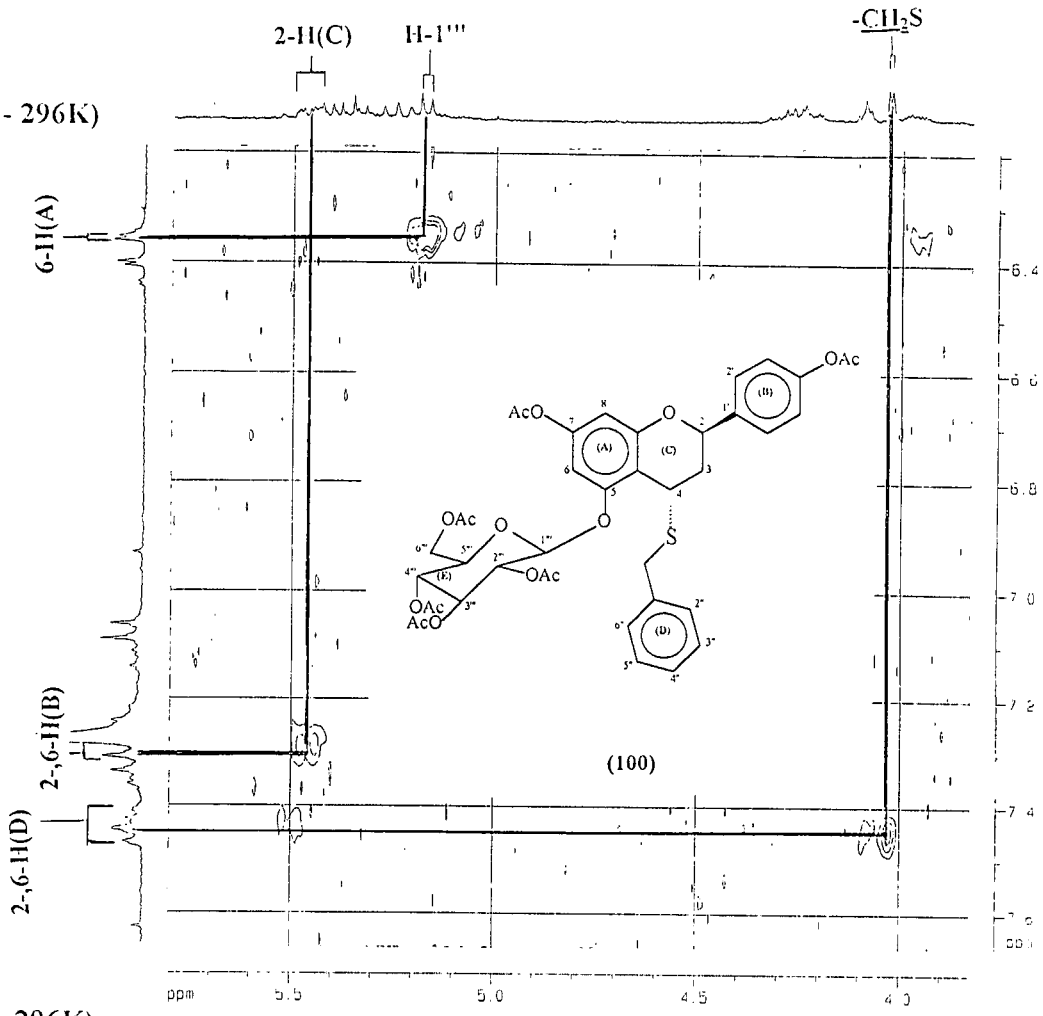


Plate 19a-2 ($CDCl_3$ - 296K)

(NOESY)

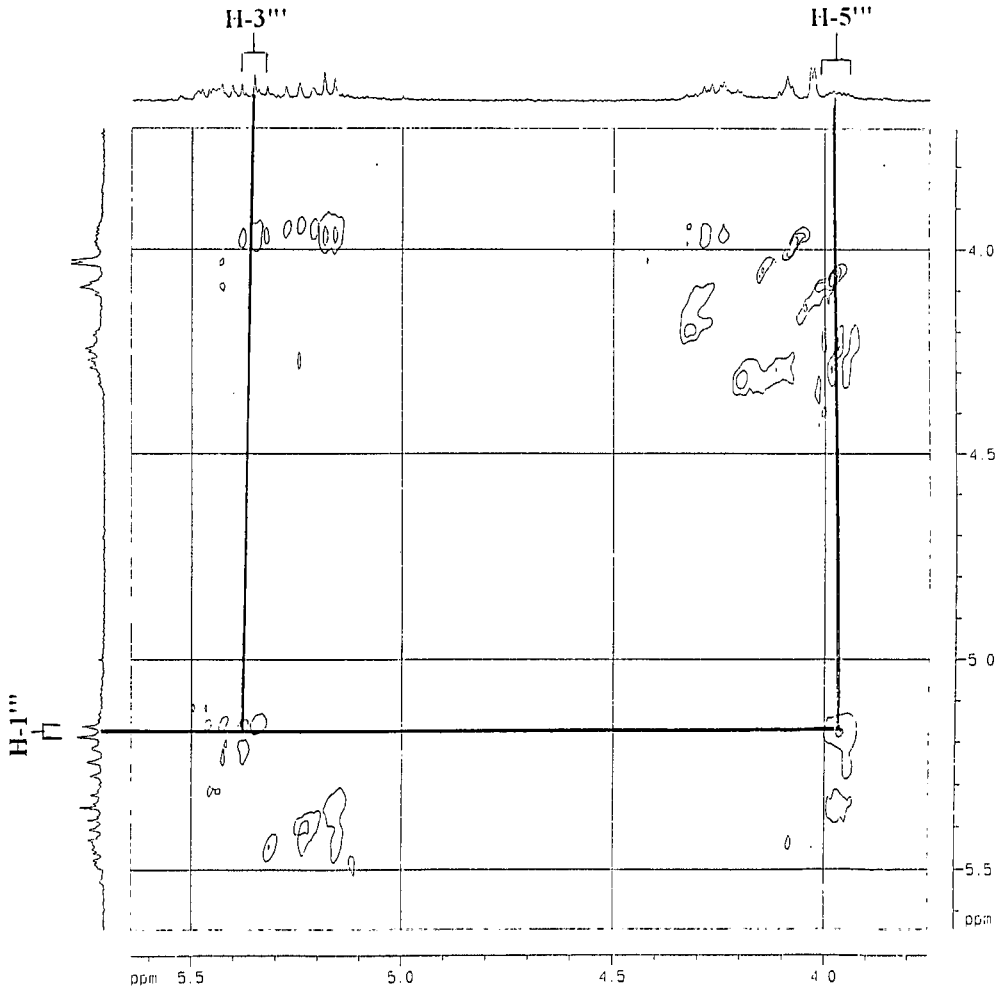


Plate 19b-1 ($CDCl_3$ - 296K)

(COSY)

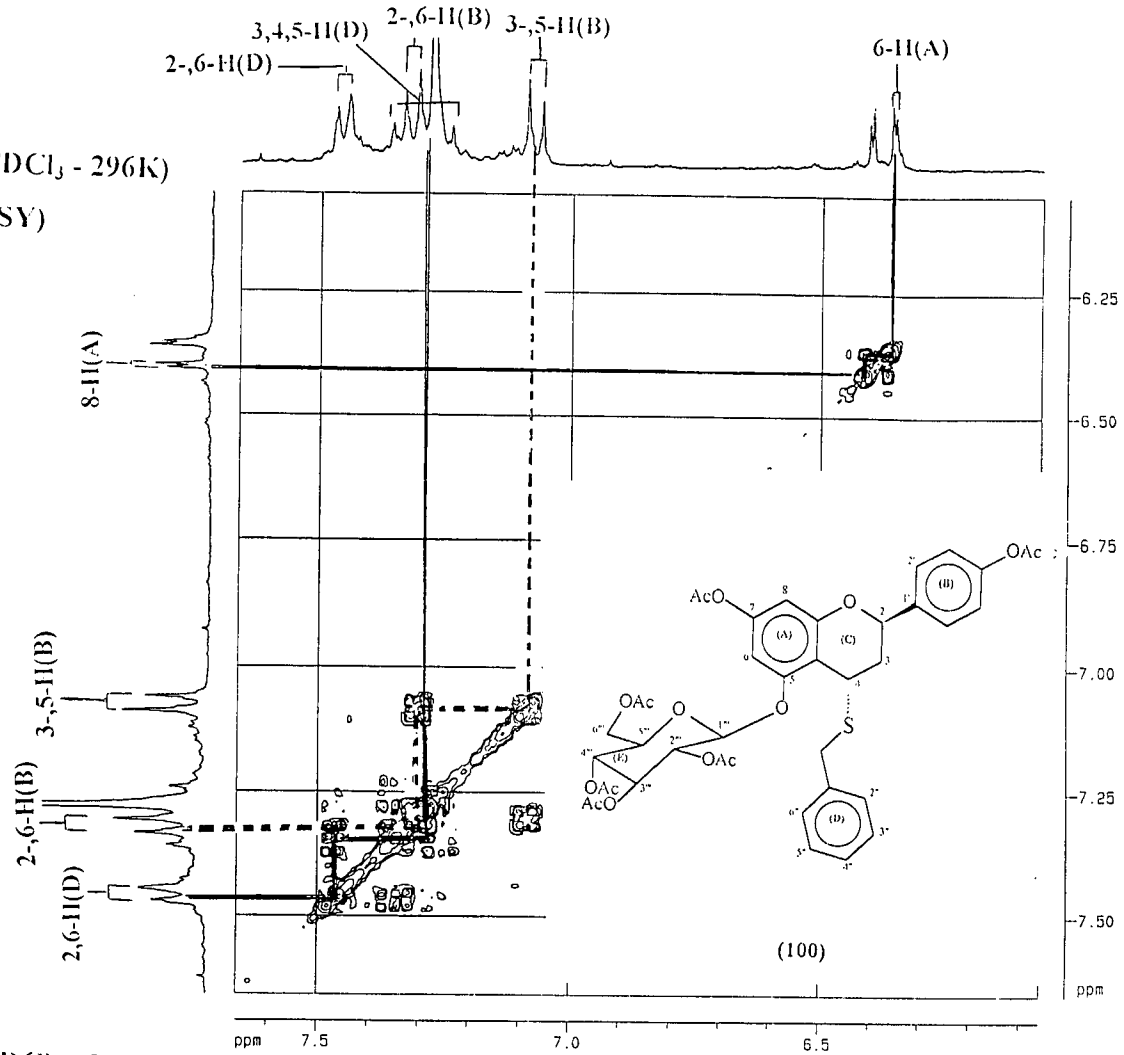


Plate 19b-2 ($CDCl_3$ - 296K)

(COSY)

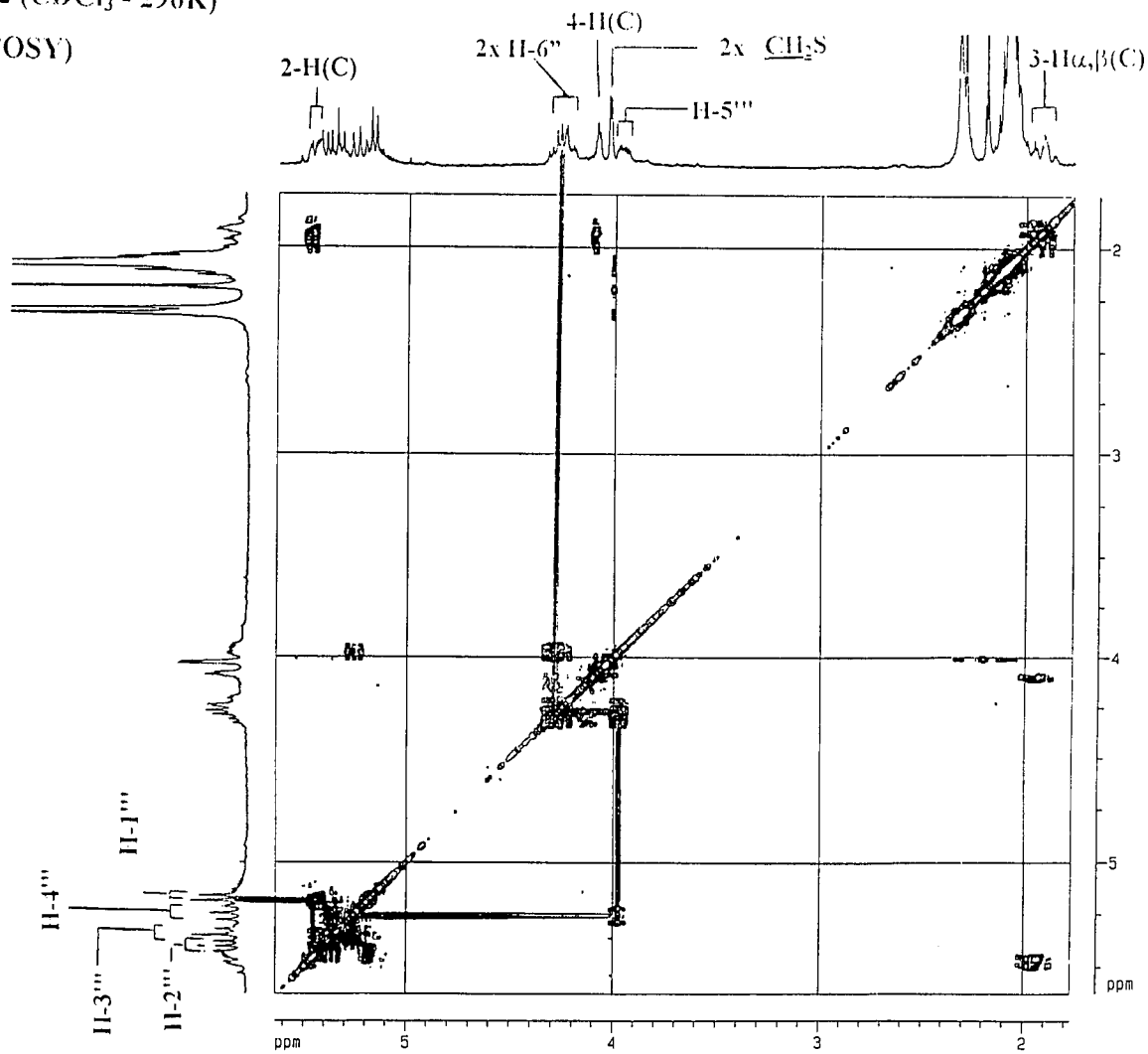
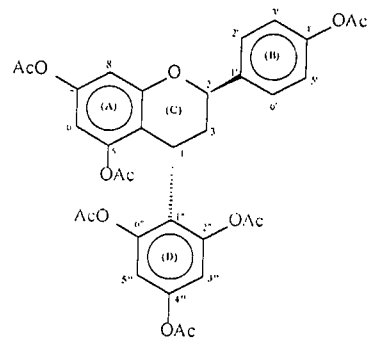
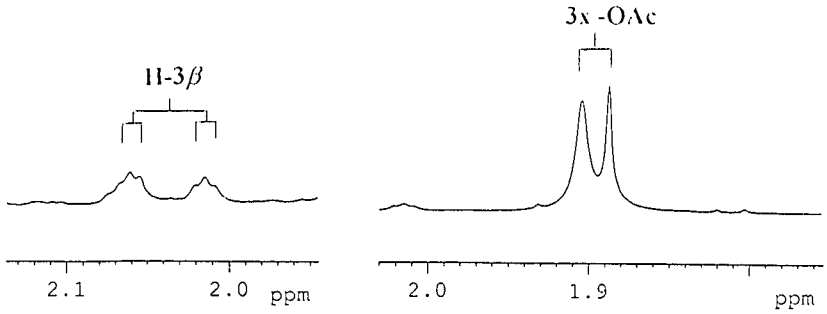


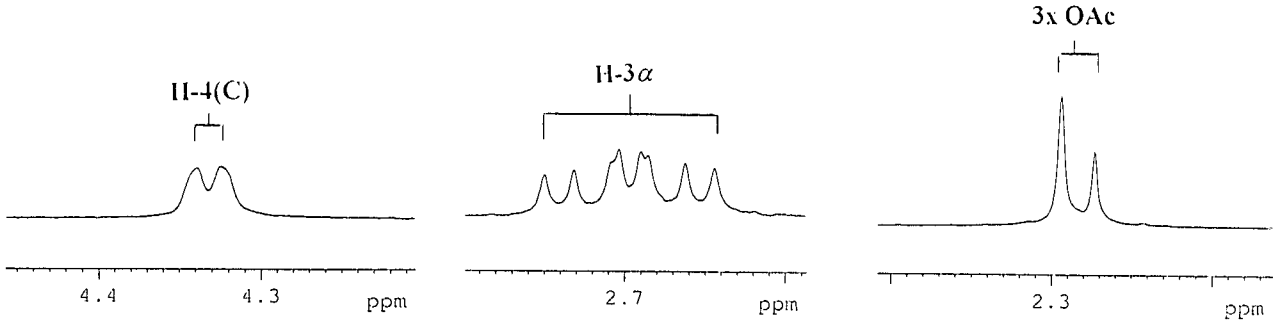
Plate 20 ($CDCl_3$ - 296K)

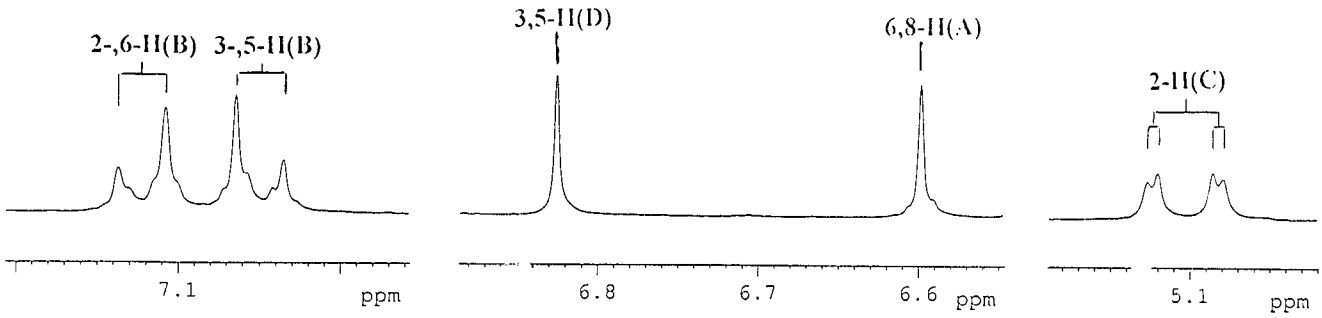
(^1H NMR)



(101)







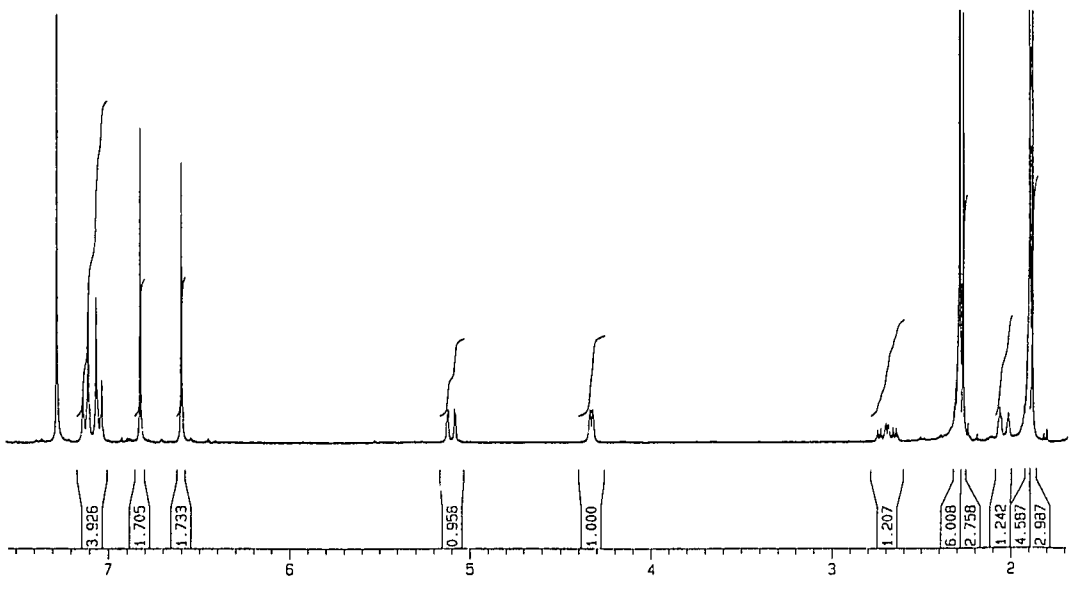
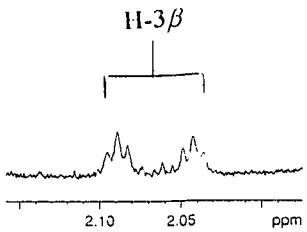
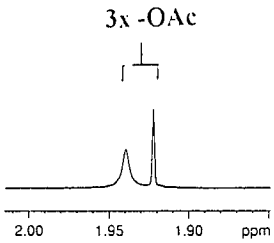
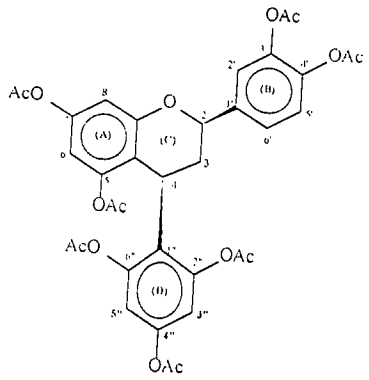


Plate 21 ($CDCl_3$ - 296K)

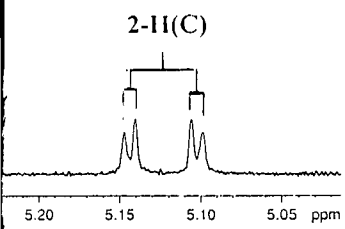
(^1H NMR)

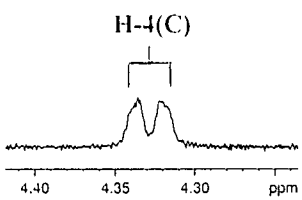


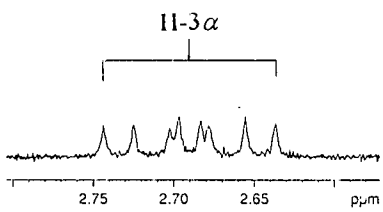


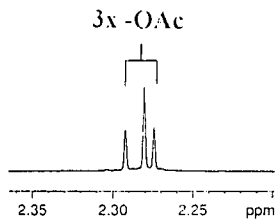


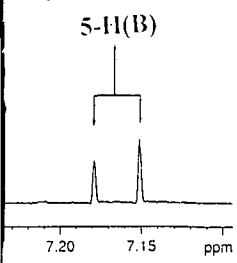
(102)

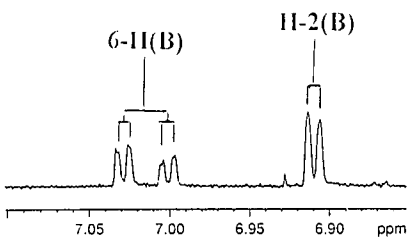


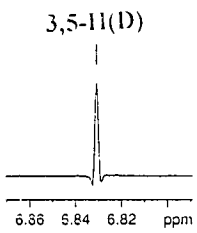


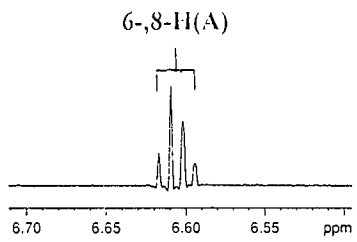












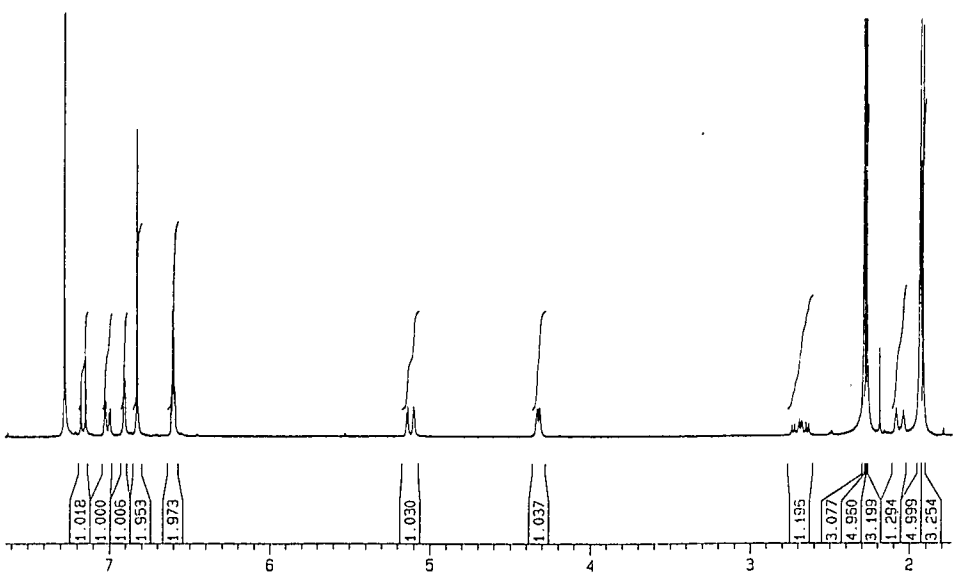
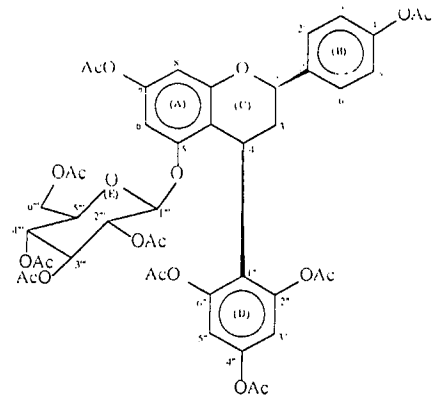
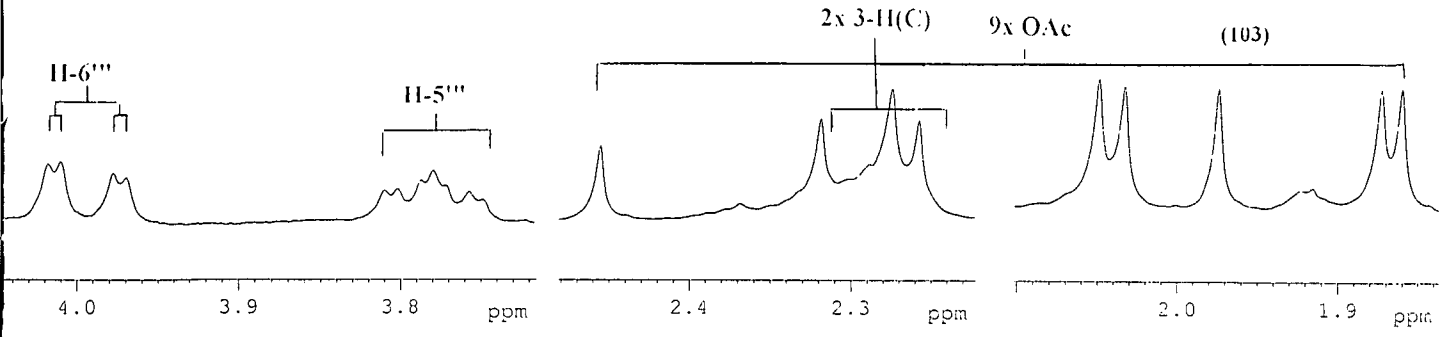
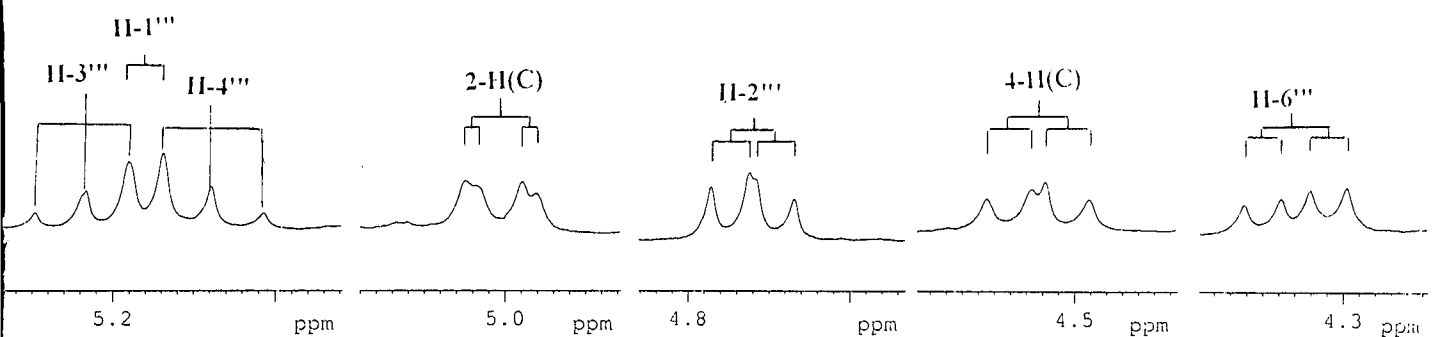


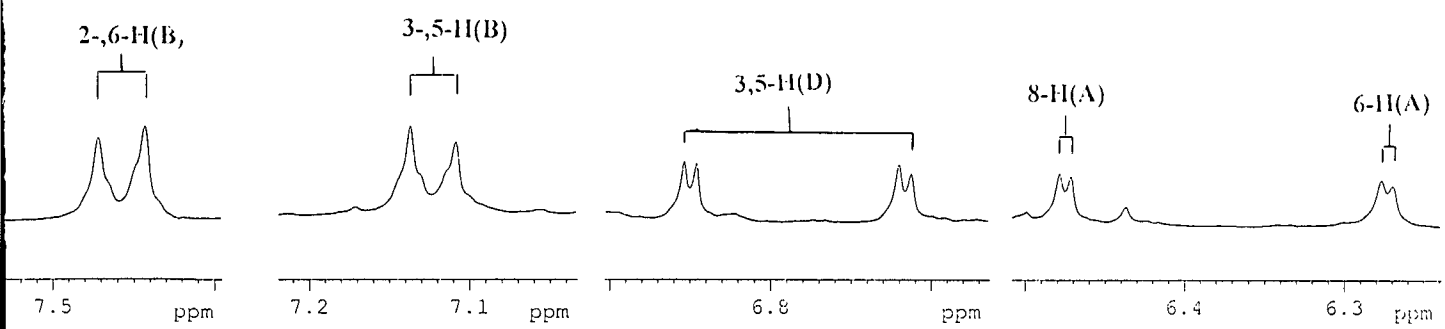
Plate 22 ($CDCl_3$ - 296K)

(^1H NMR)









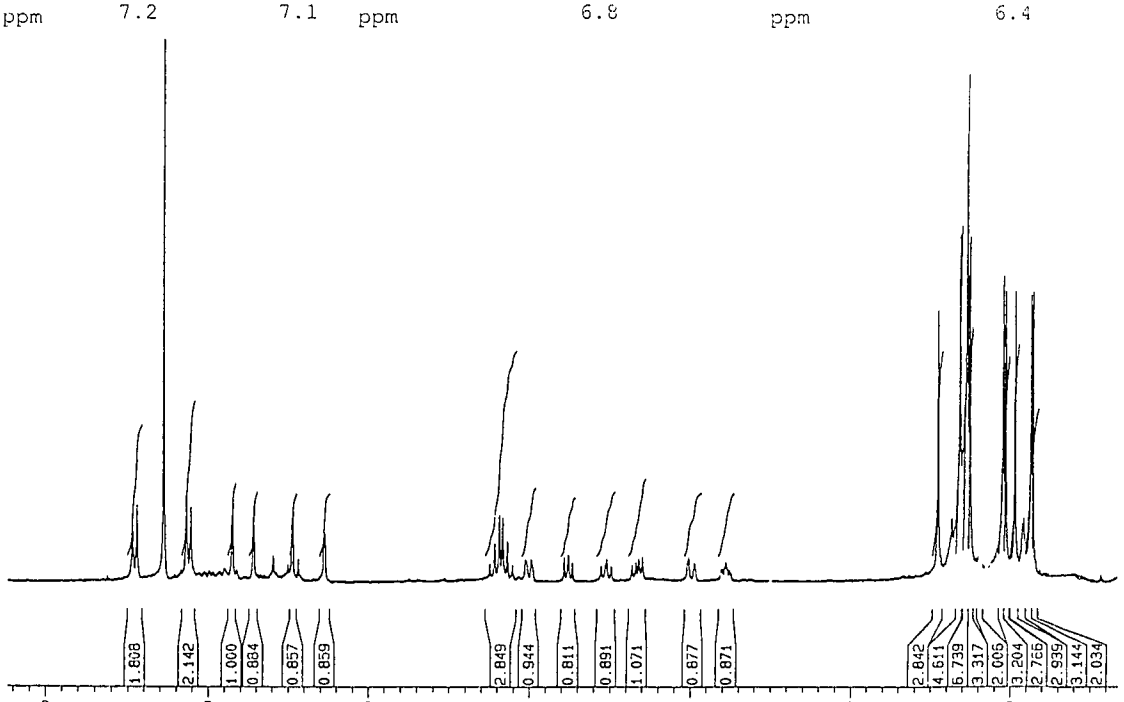


Plate 22a-1 ($CDCl_3$ - 296K)

(NOESY)

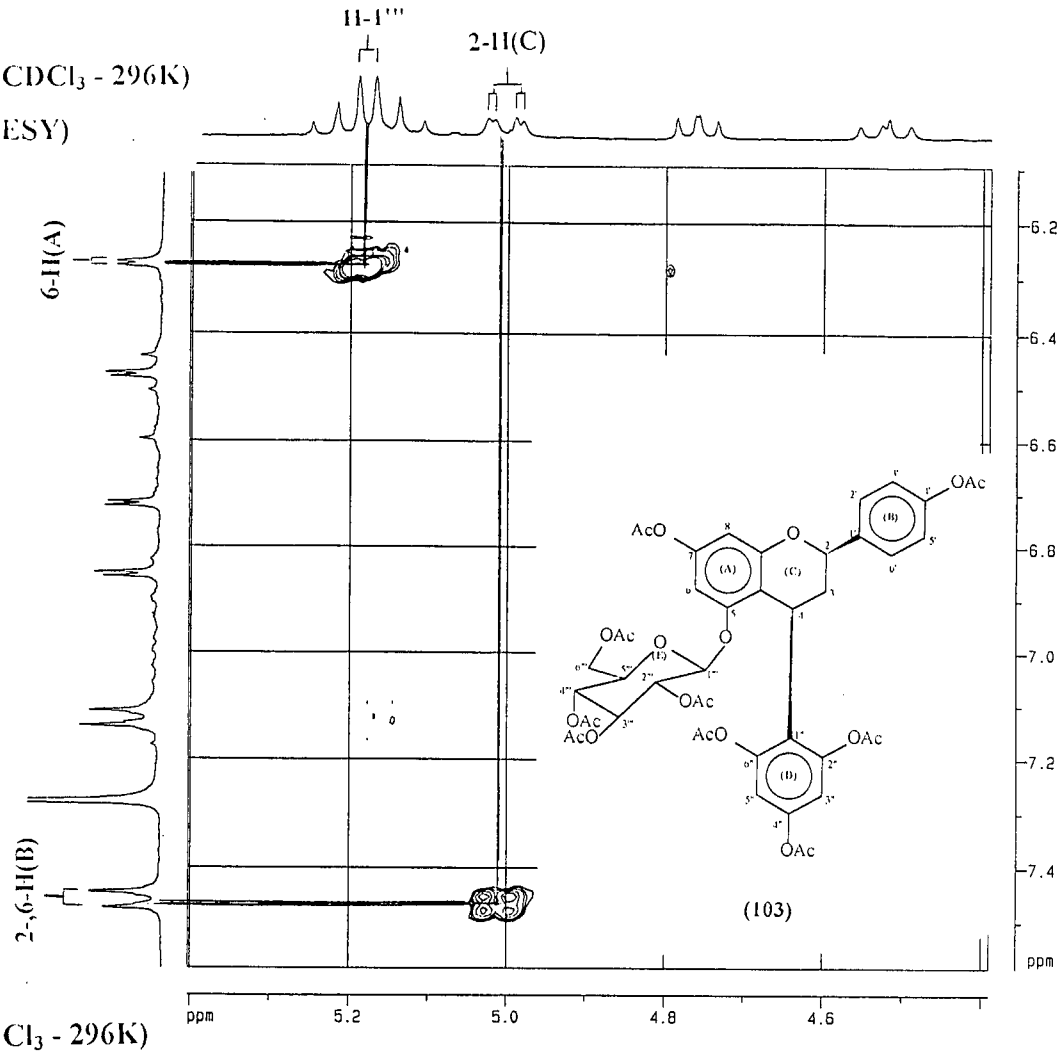


Plate 22a-2 ($CDCl_3$ - 296K)

(NOESY)

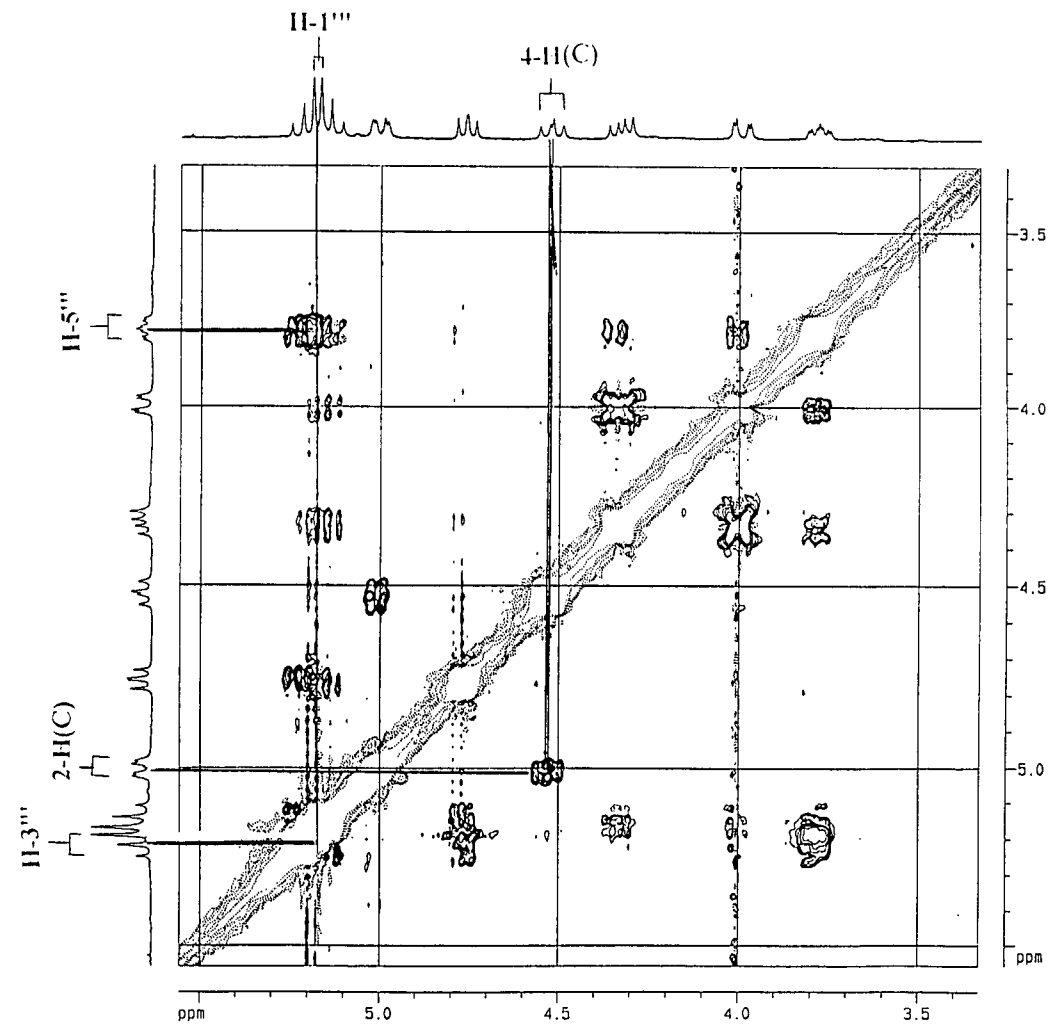
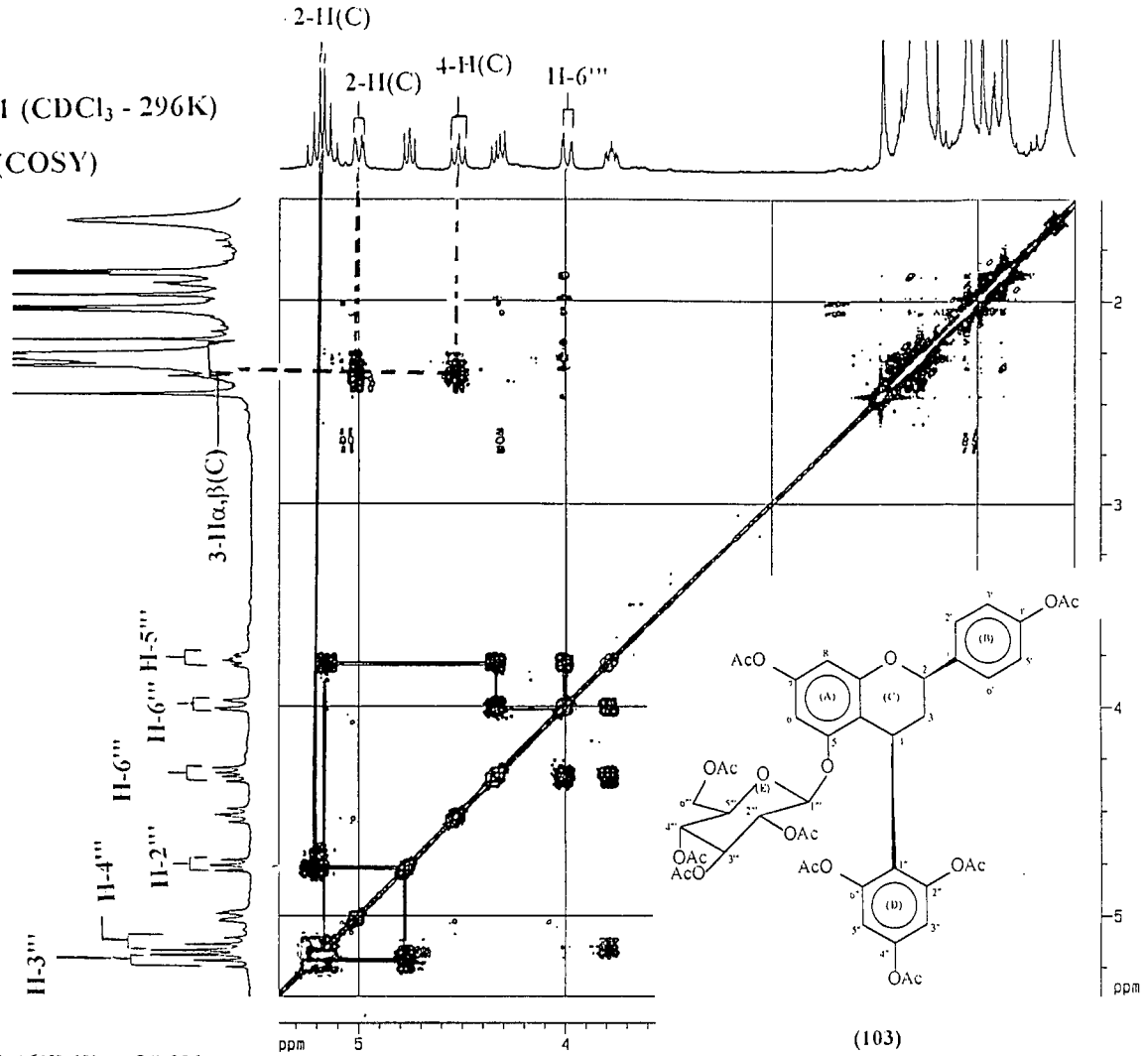


Plate 22b-1 ($CDCl_3$ - 296K)

(COSY)



(103)

Plate 22b-2 ($CDCl_3$ - 296K)

(COSY)

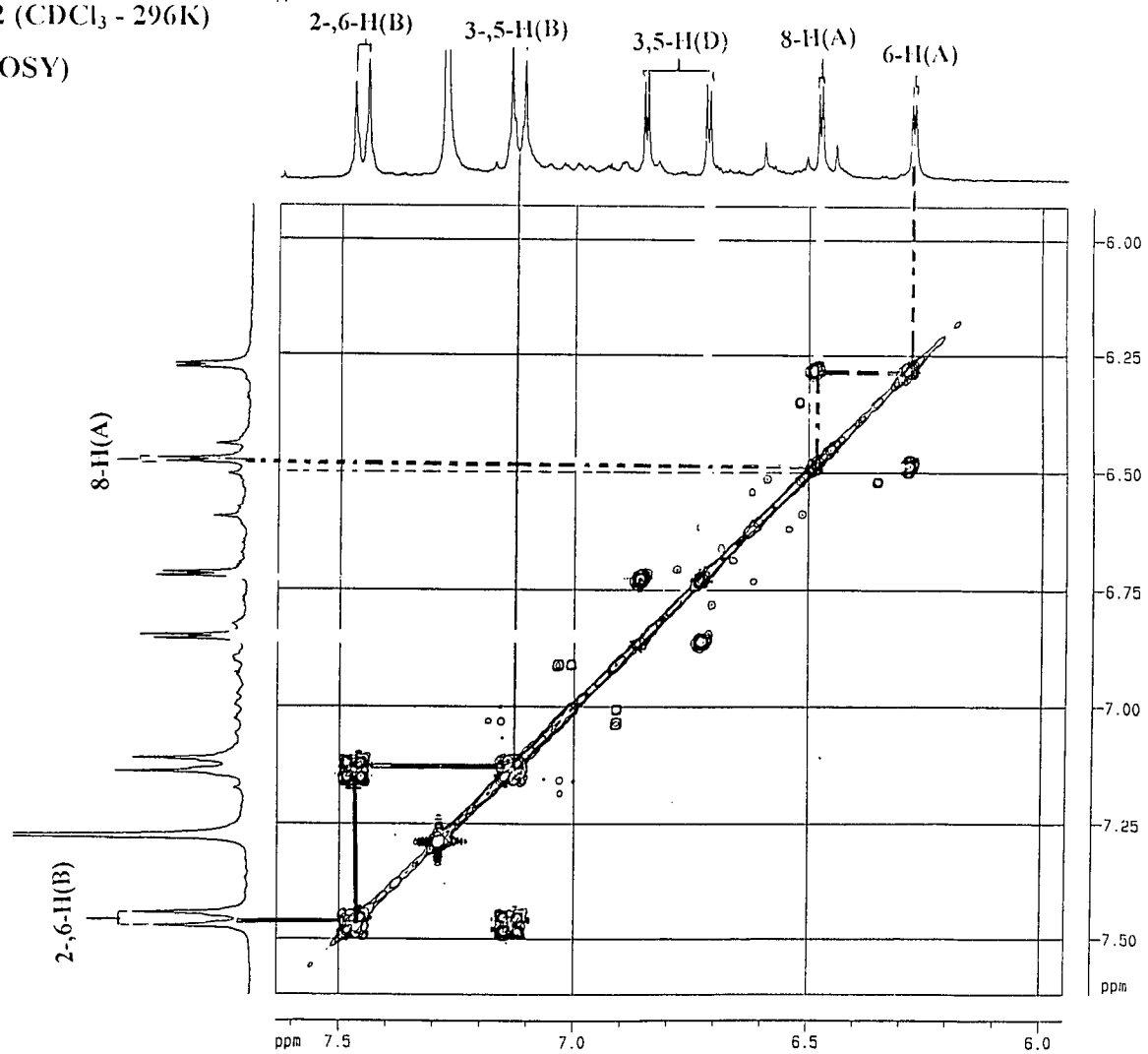
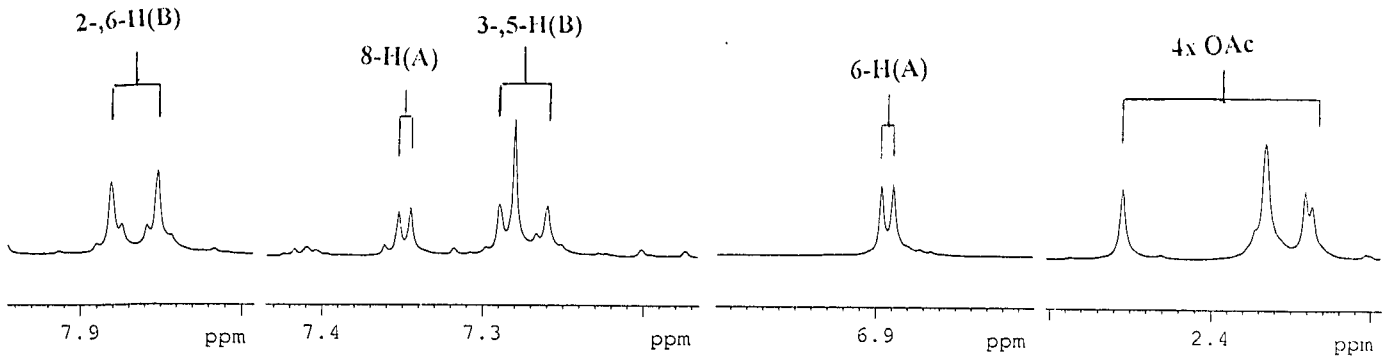
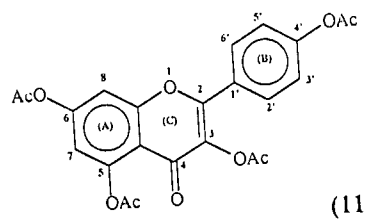


Plate 23a ($CDCl_3$ - 296K)

(^1H NMR)

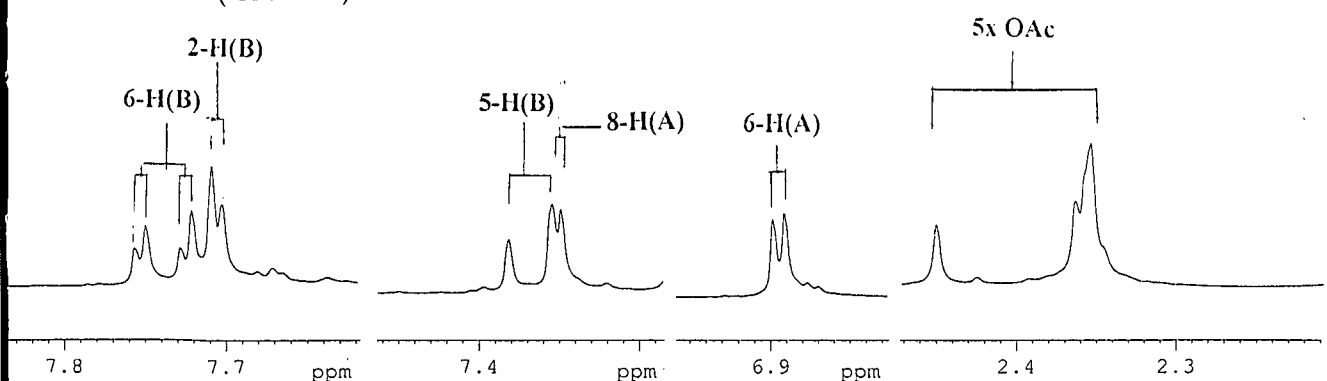


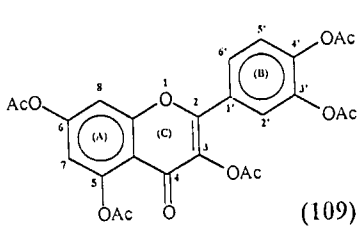


(119)

Plate 23 ($CDCl_3$ - 296K)

(^1H NMR)





(109)

Plate 23b ($CDCl_3$ - 296K)

(^1H NMR)

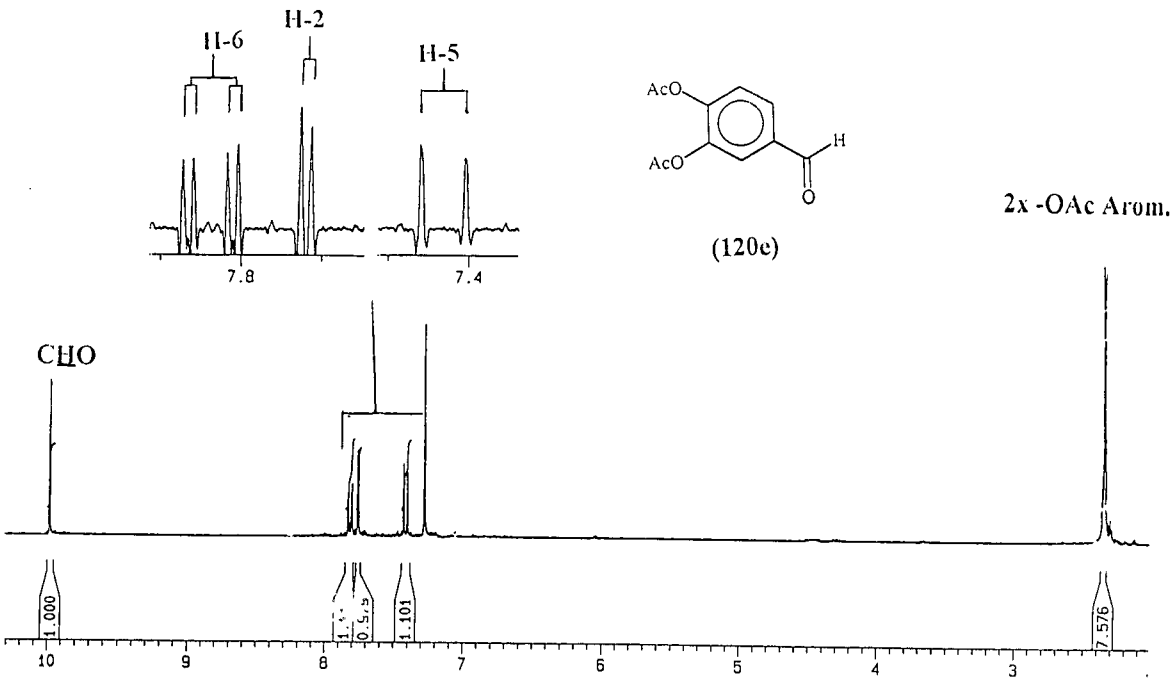


Plate 23c ($CDCl_3$ - 296K)

(^1H NMR)

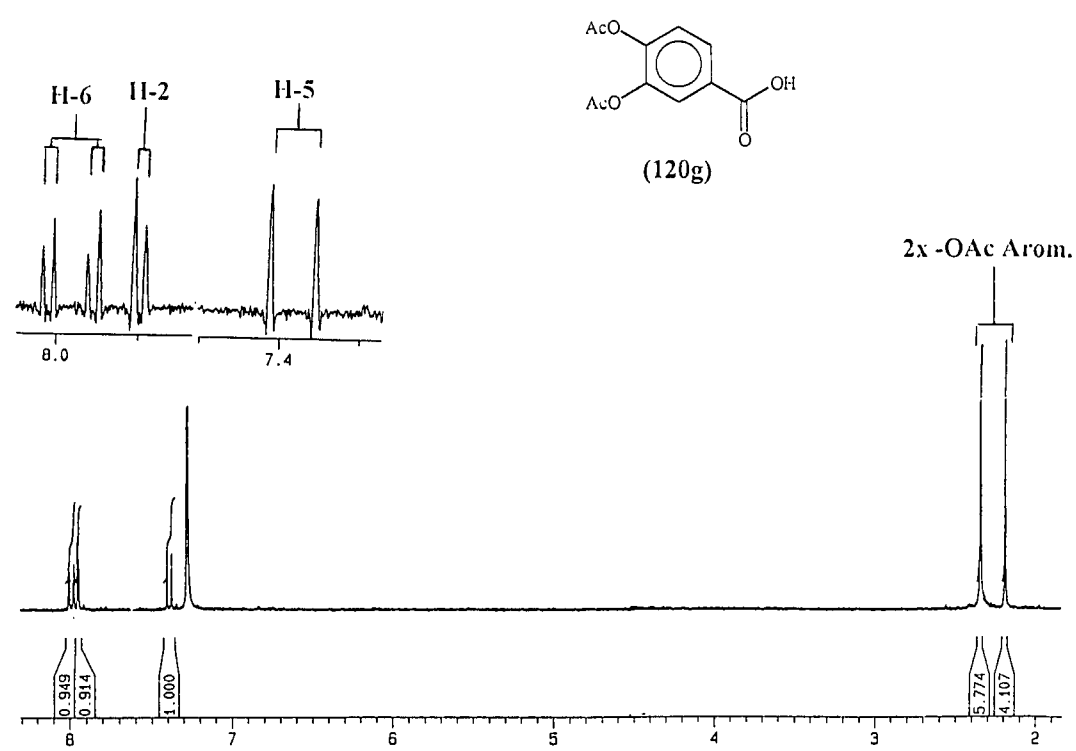
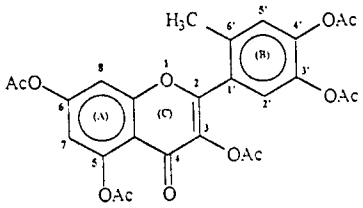


Plate 23d ($CDCl_3$ - 296K)

(^1H NMR)



(125)

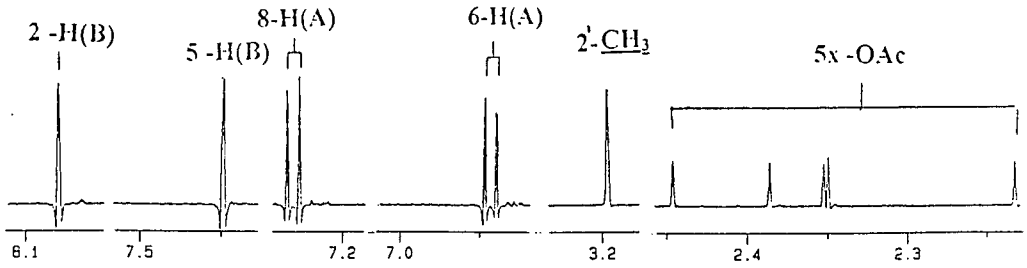
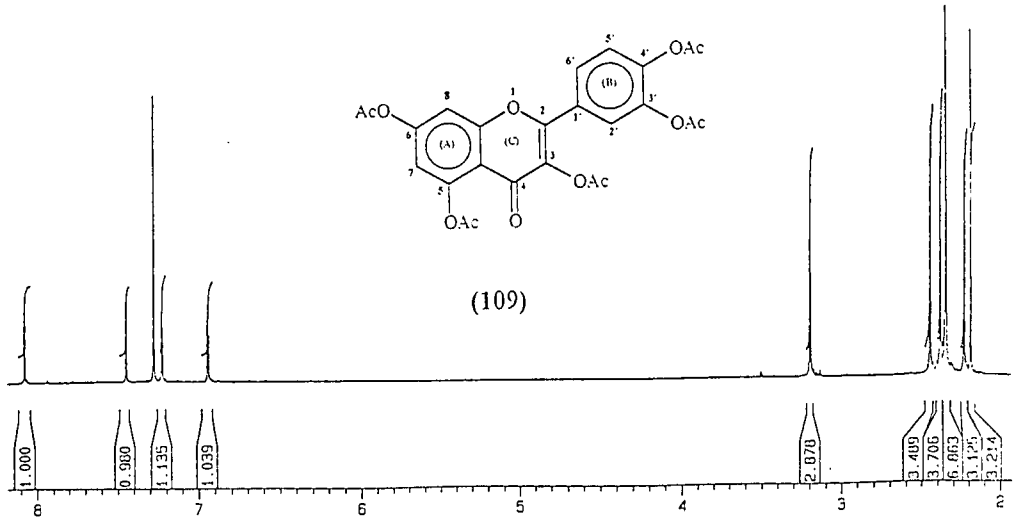


Plate 23 ($CDCl_3$ - 296K)

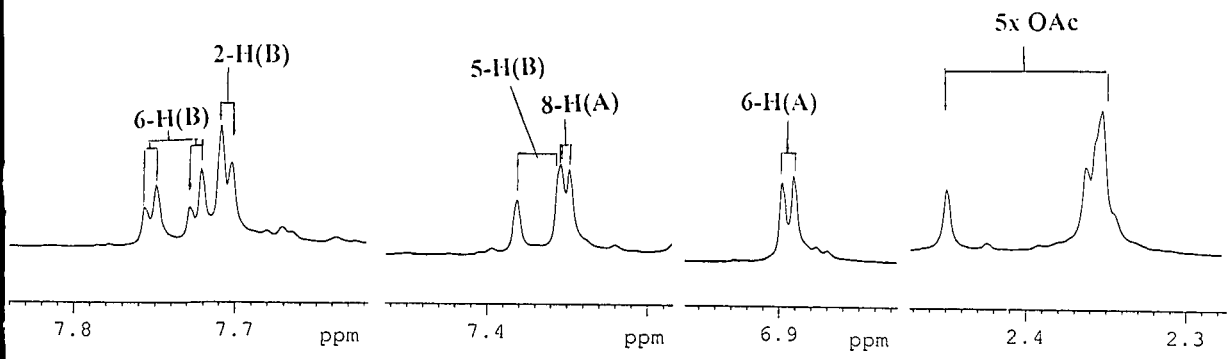
(^1H NMR)



(109)

Plate 24a ($CDCl_3$ - 296K)

(^1H NMR)



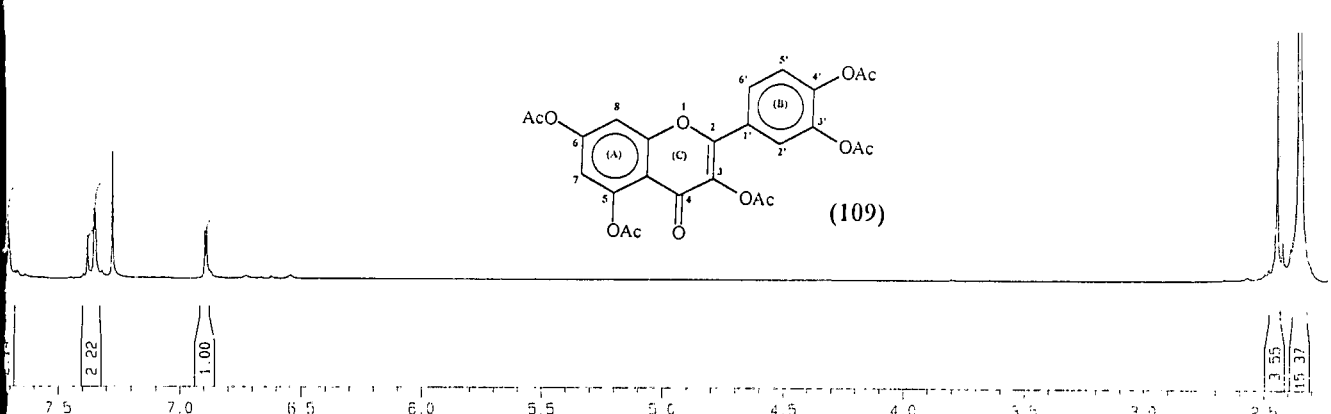
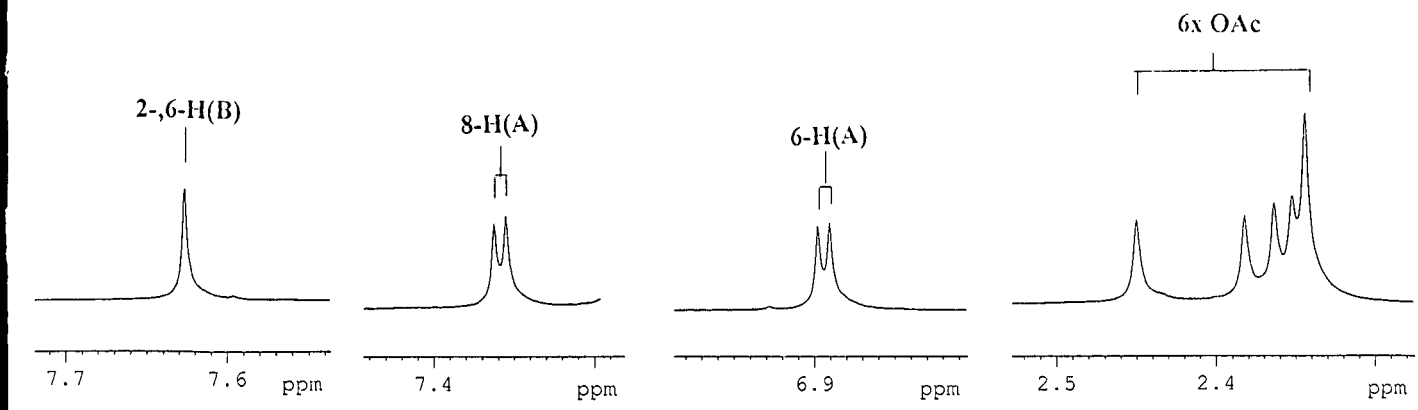


Plate 24 ($CDCl_3$ - 296K)

(^1H NMR)



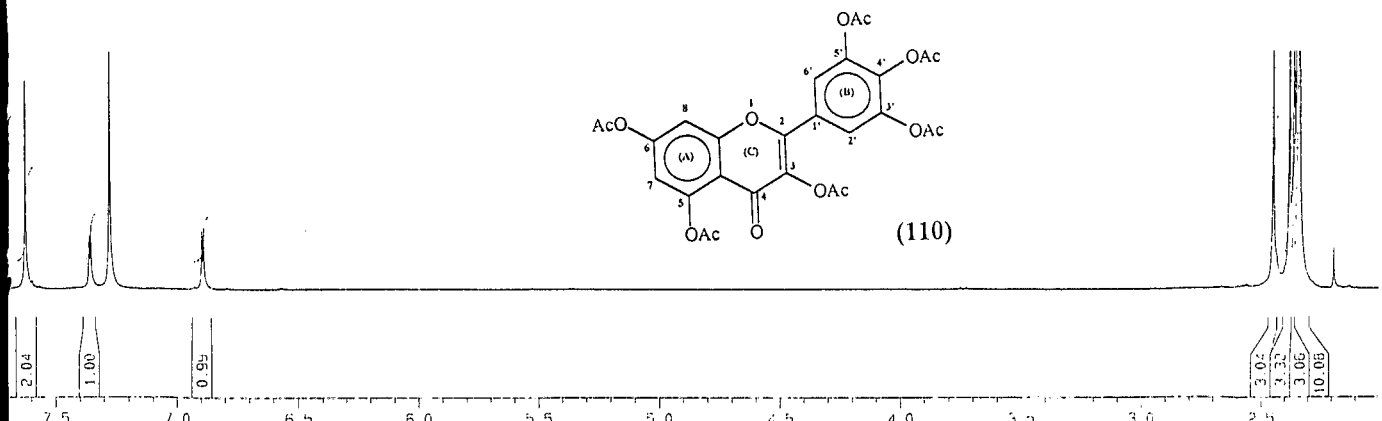
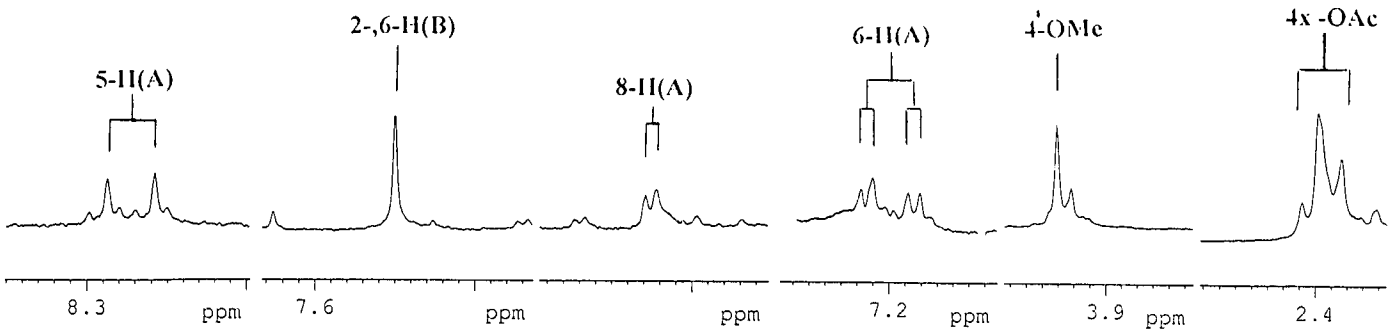


Plate 25a ($CDCl_3$ - 296K)

(^1H NMR)



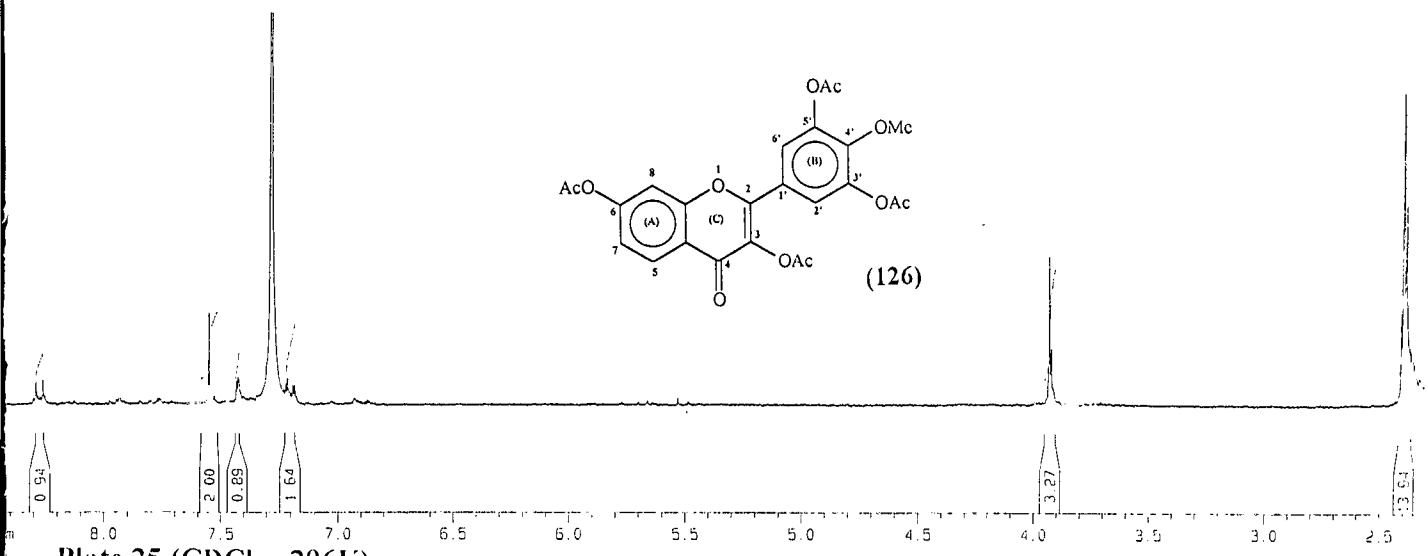


Plate 25 ($CDCl_3$ - 296K)

(^1H NMR)

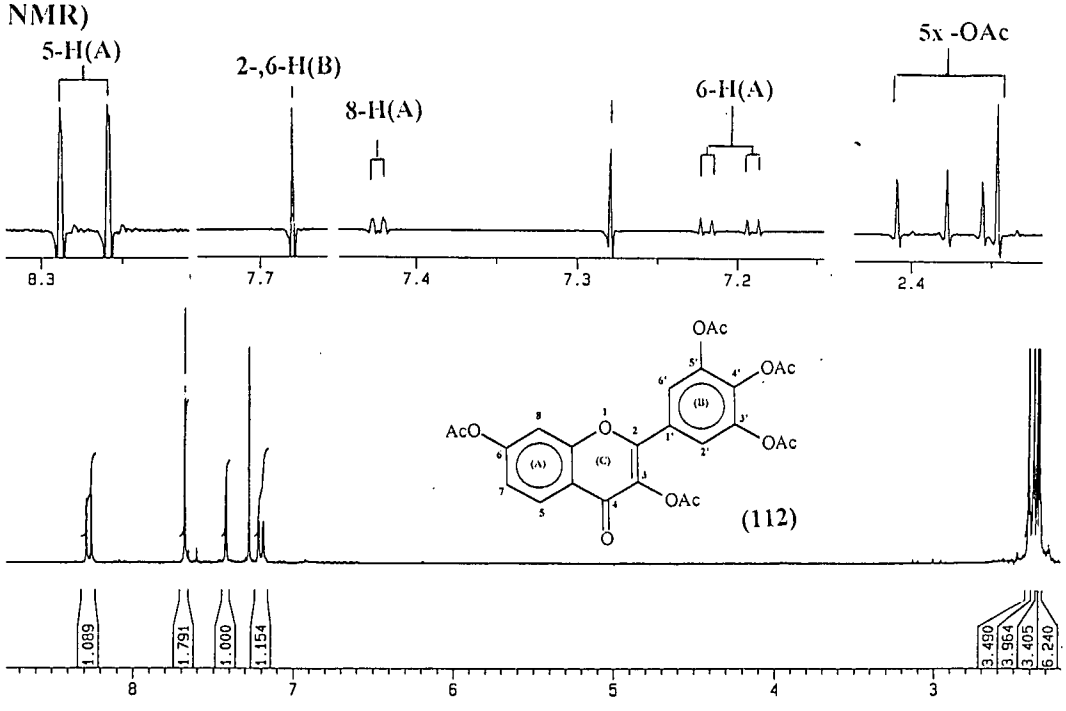
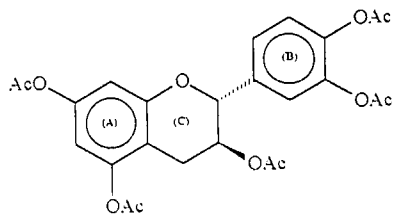
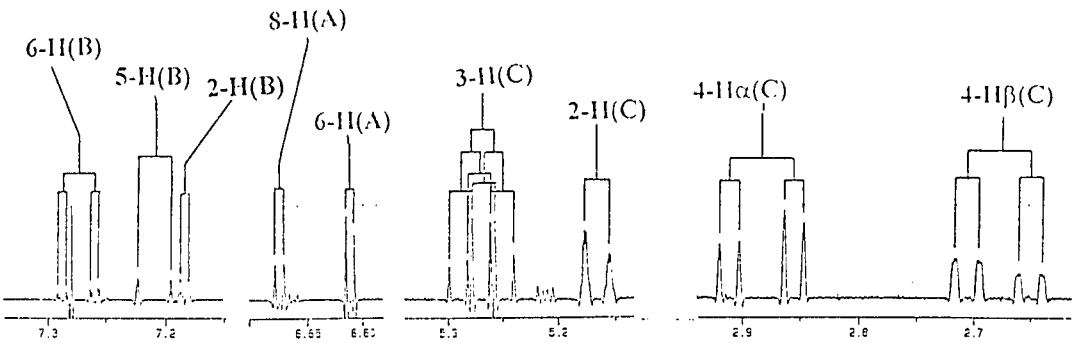


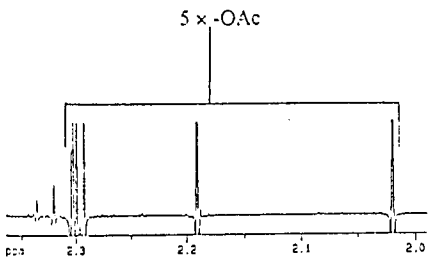
Plate 26 ($CDCl_3$ - 296K)

(^1H NMR)



113





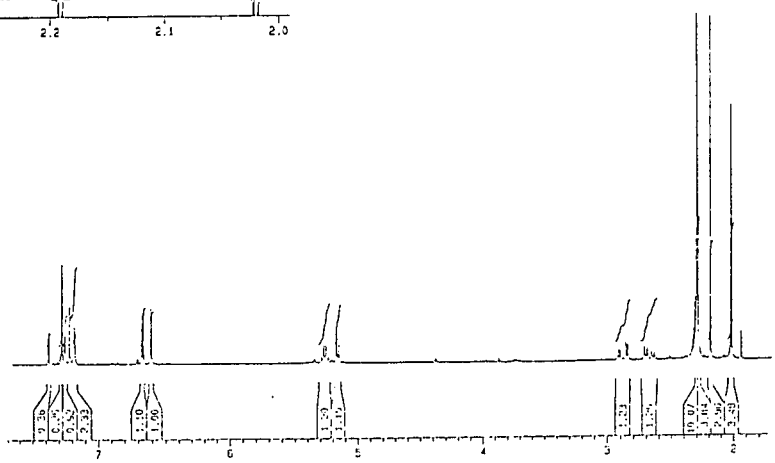
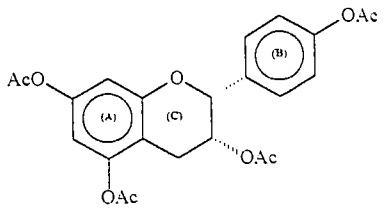


Plate 27 ($CDCl_3$ - 296K)

(^1H NMR)



114a

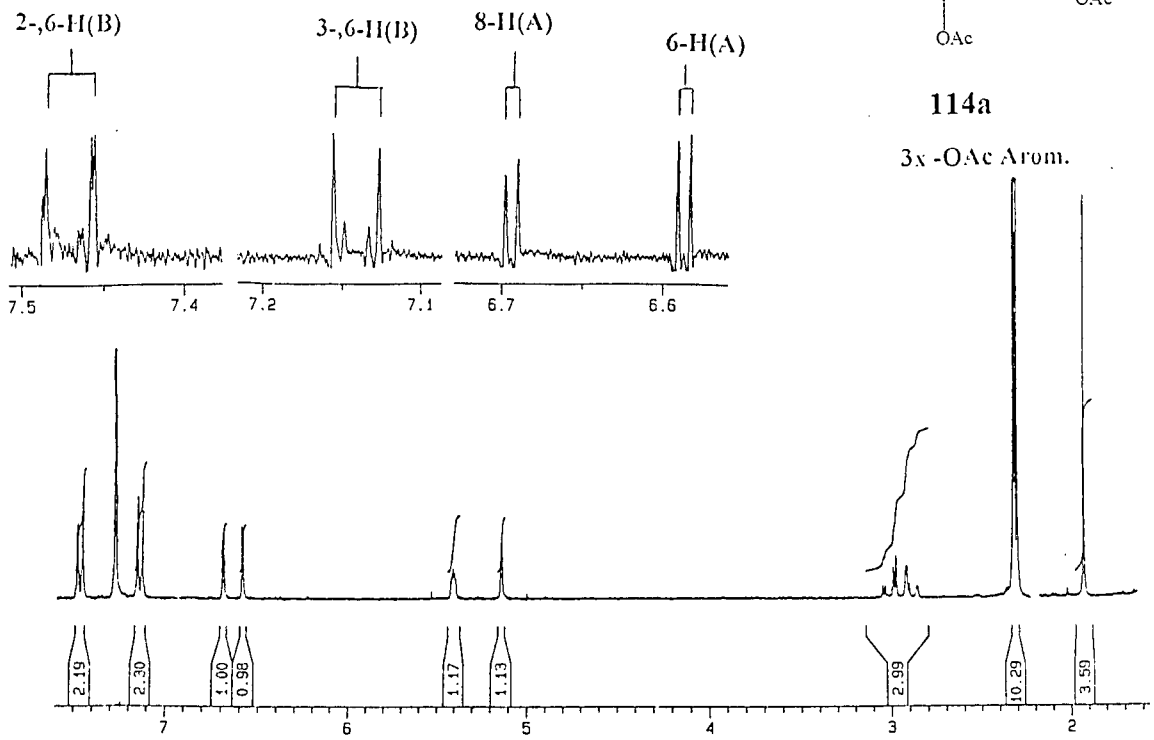
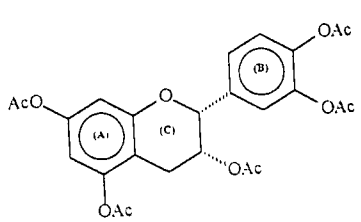
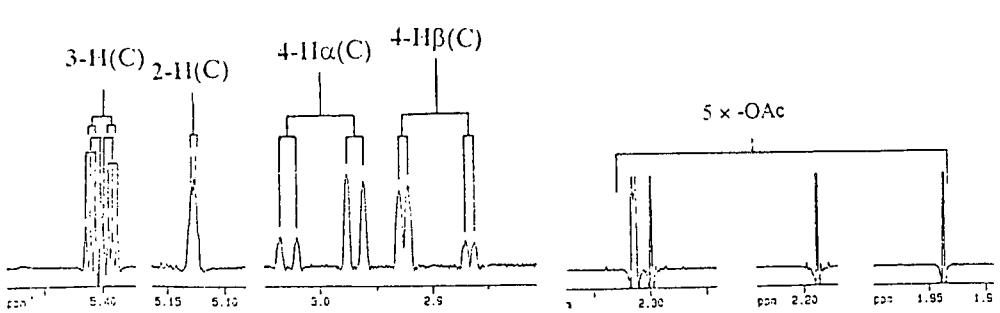


Plate 27a ($CDCl_3$ - 296K)

(^1H NMR)



114



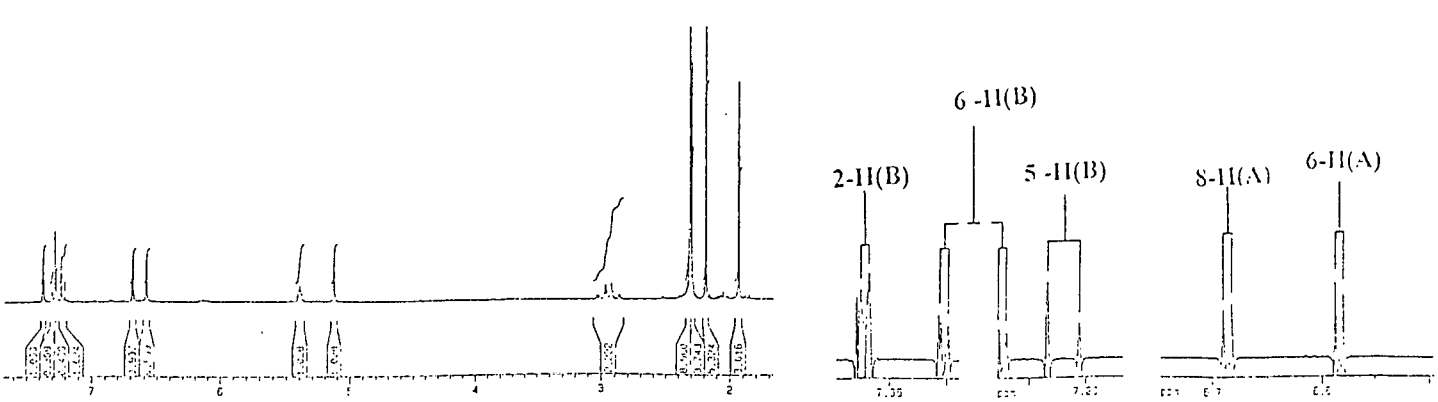
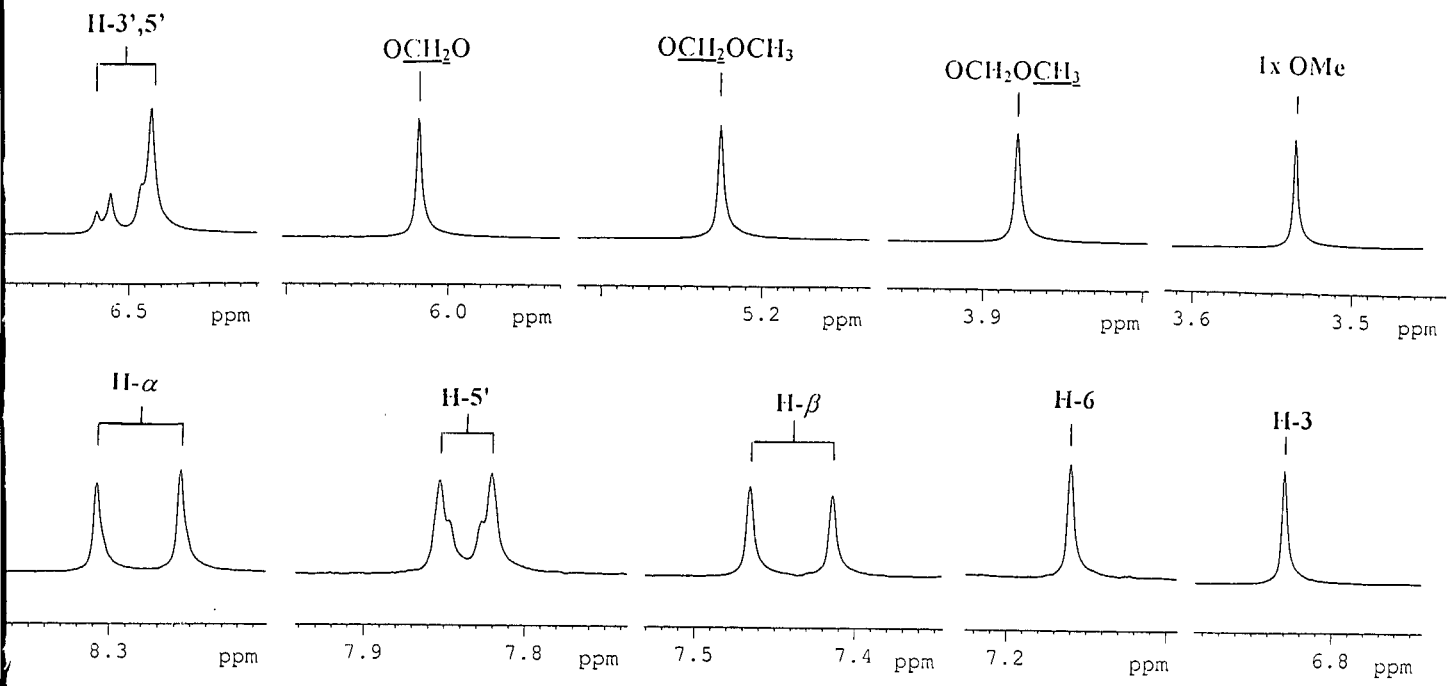
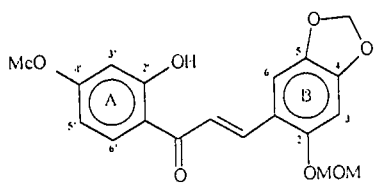


Plate 28 ($CDCl_3$ - 296K)

(^1H NMR)





(134)

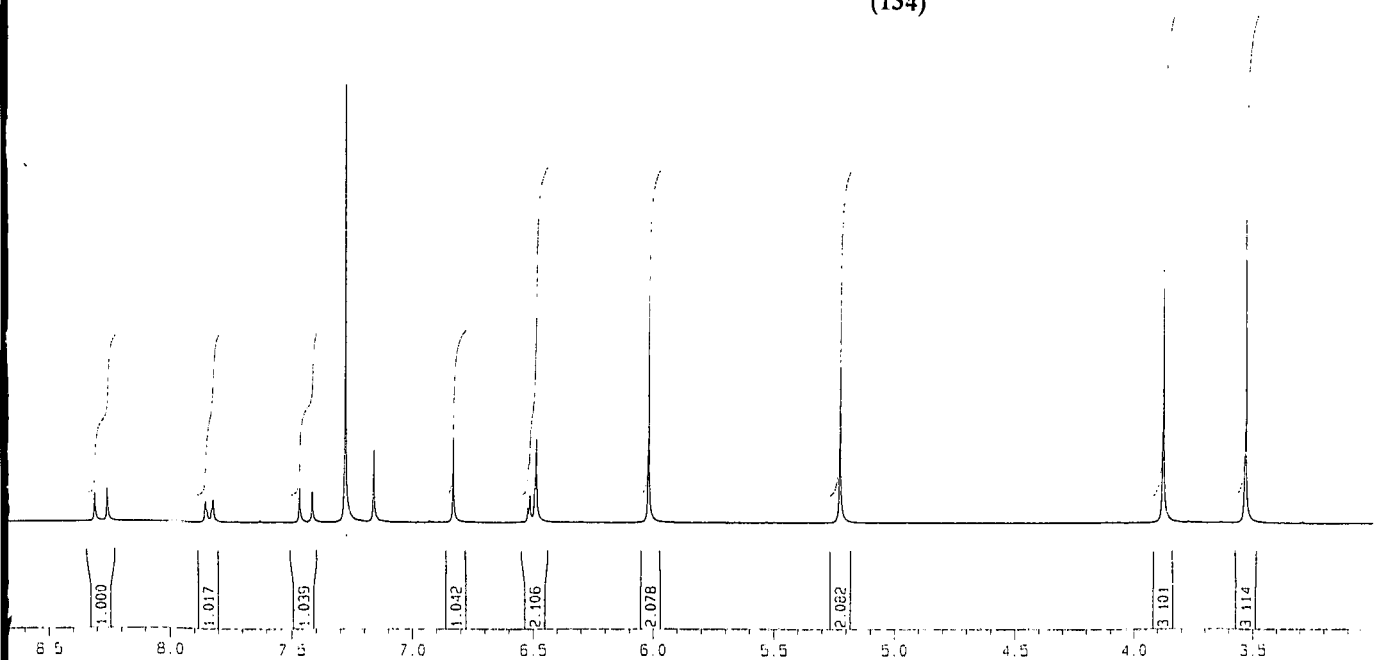
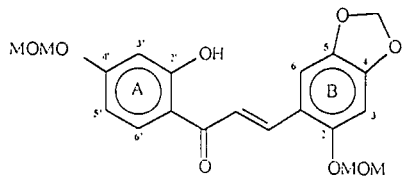
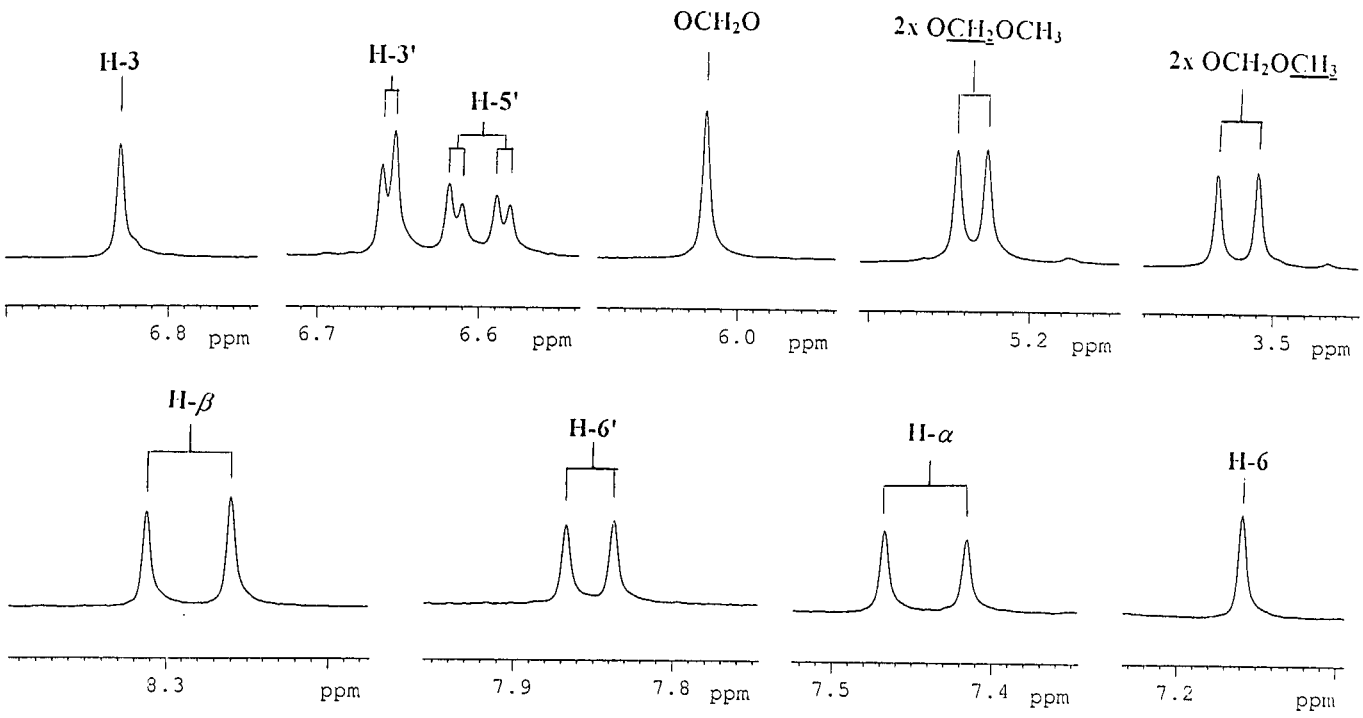


Plate 29 ($CDCl_3$ - 296K)

(^1H NMR)



(135)



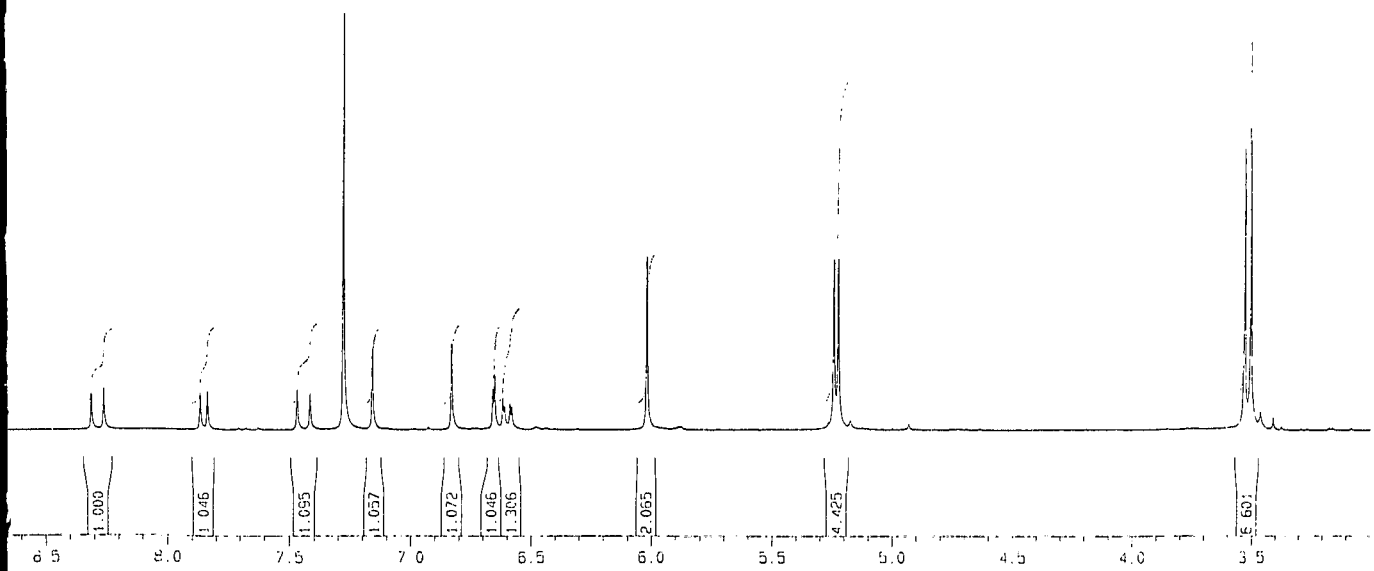
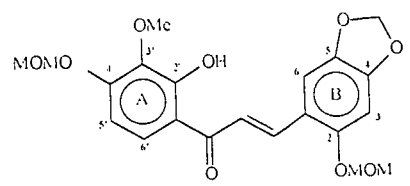
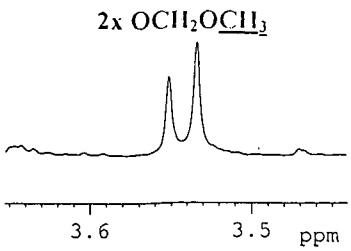


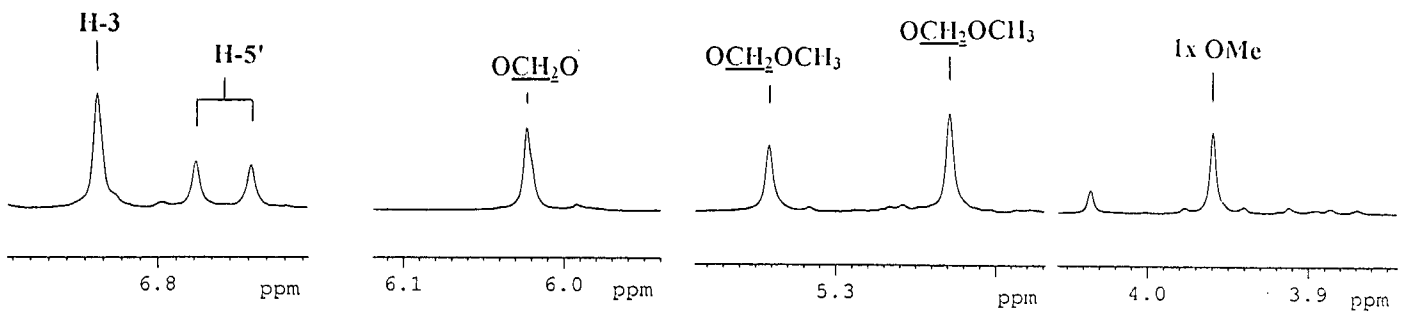
Plate 30 ($CDCl_3$ - 296K)

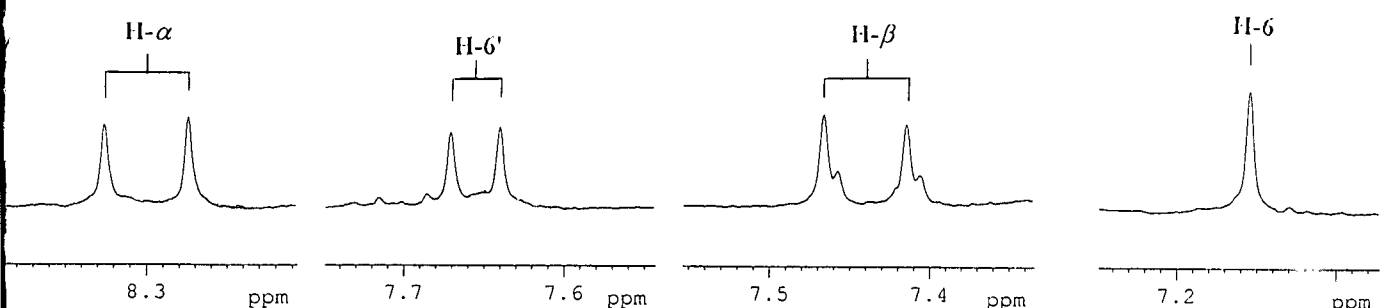
(^1H NMR)



(136)







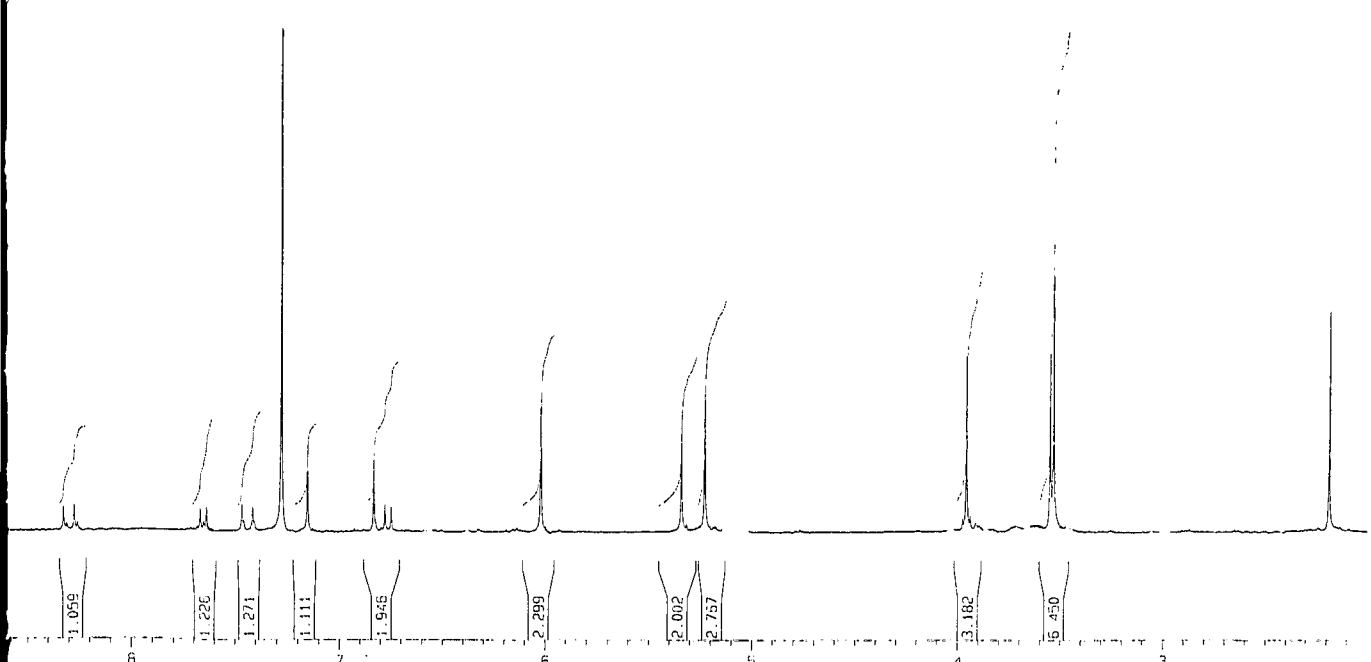
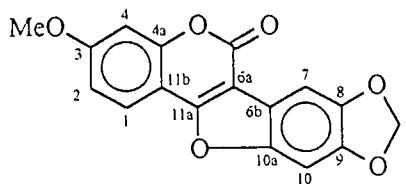
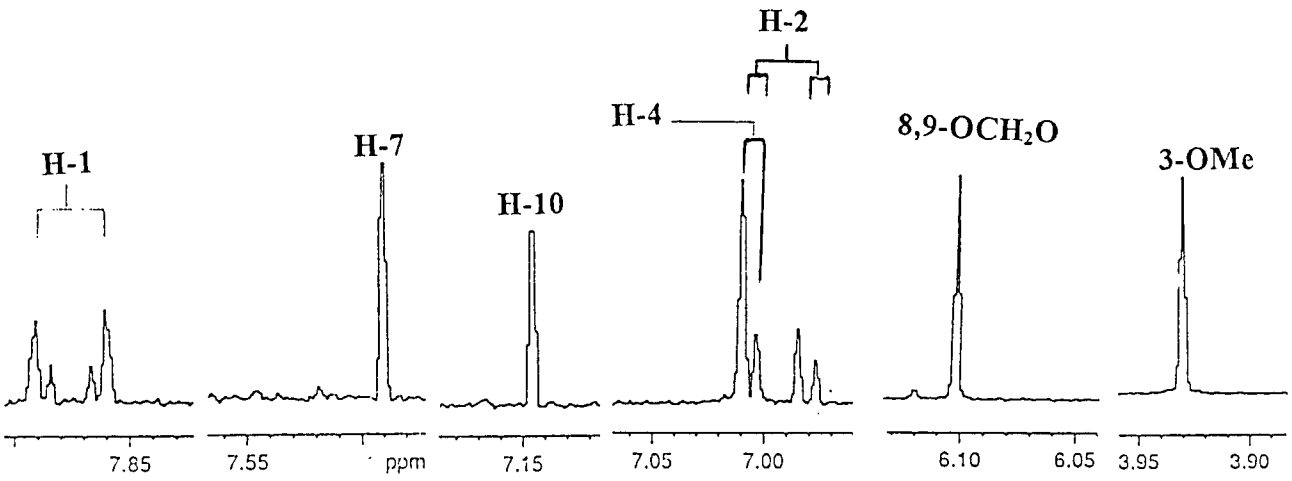


Plate 31 ($CDCl_3$ -296K)
(^1H NMR)



(144)



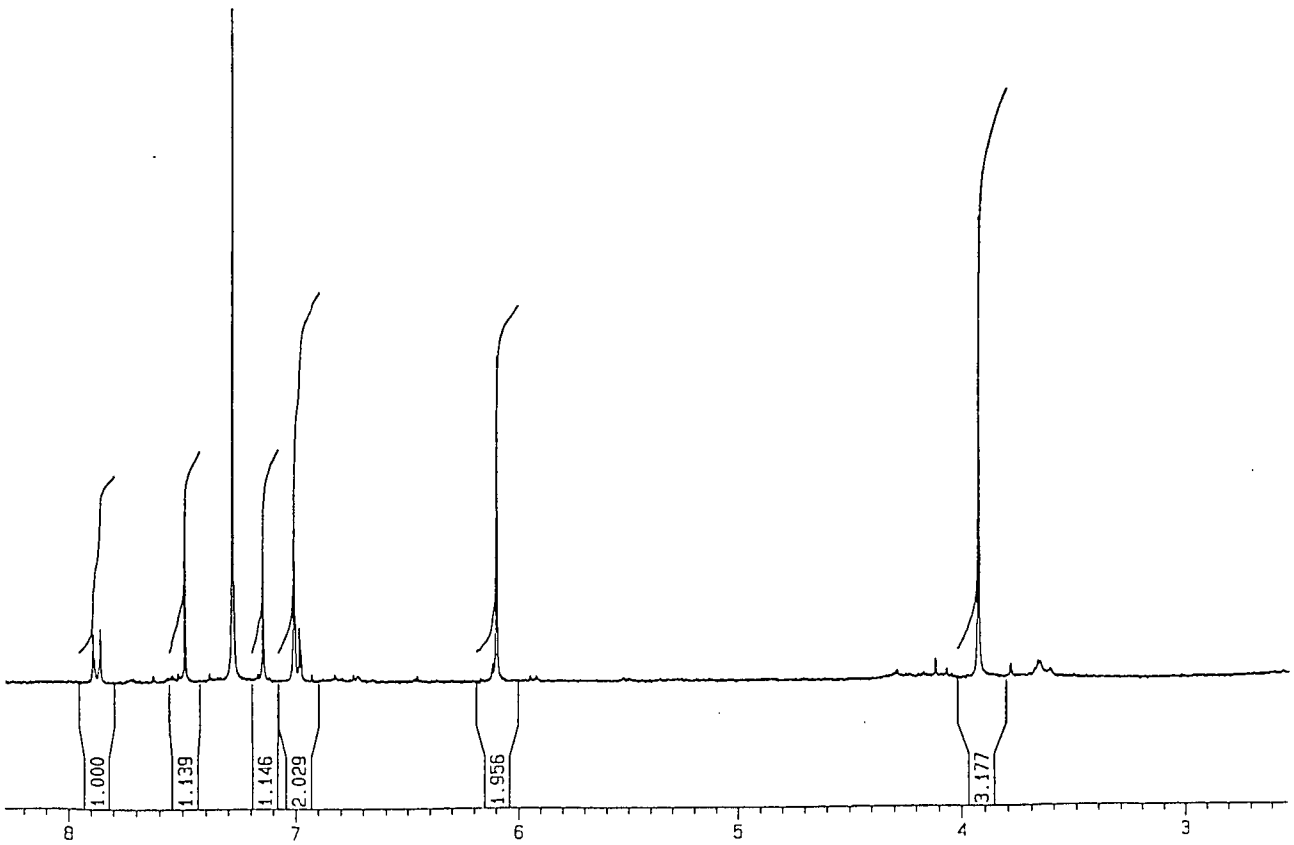
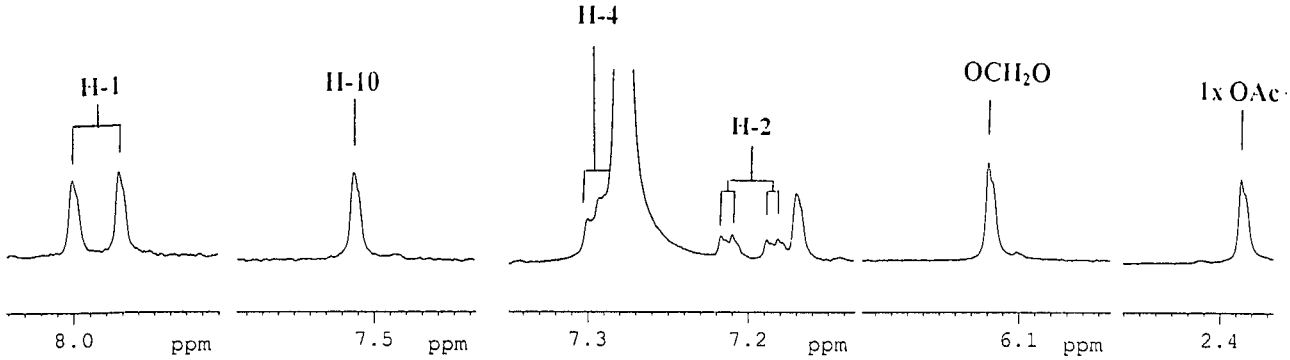
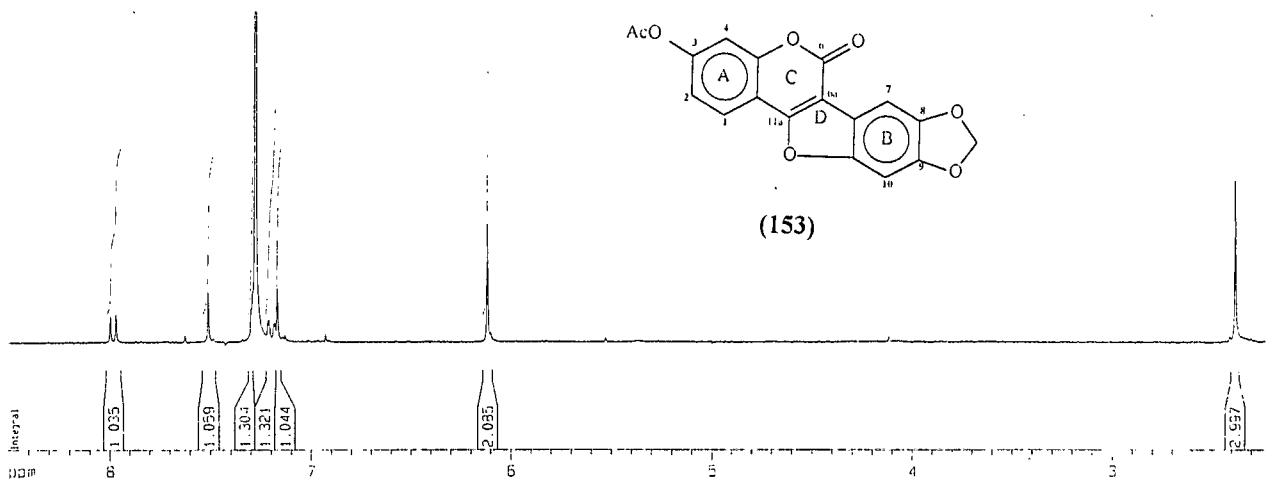


Plate 32a ($CDCl_3$ - 296K)

(^1H NMR)

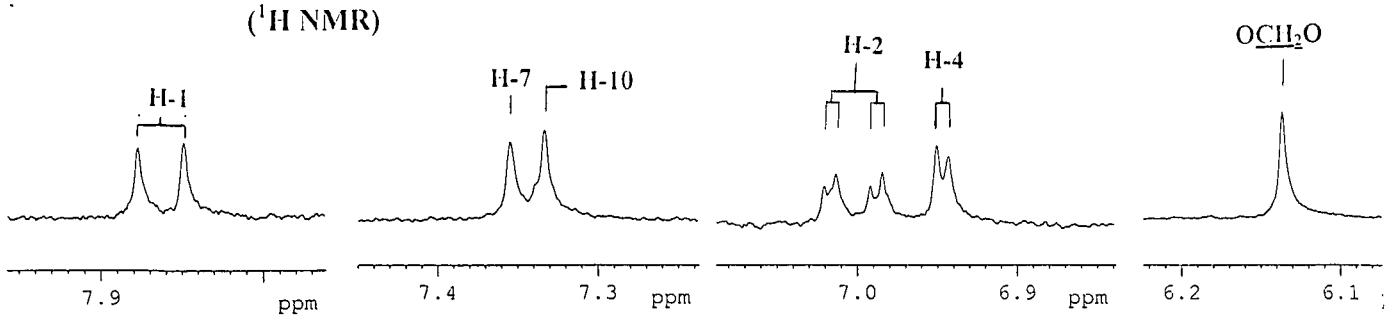


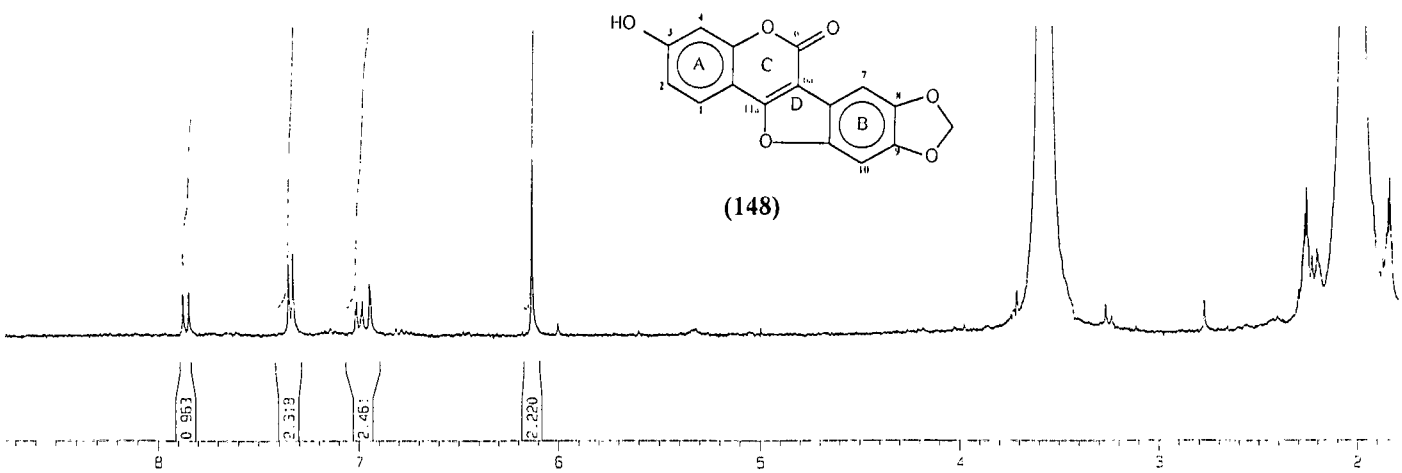


(153)

Plate 32 ($CDC_3)_2CO$ - 296K

(^1H NMR)

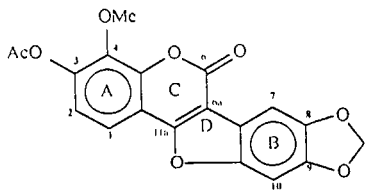




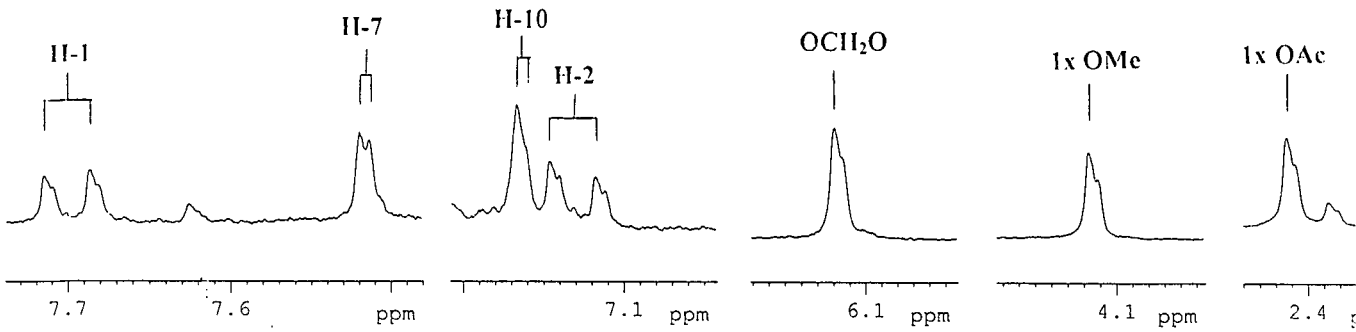
(148)

Plate 33a ($CDCl_3$ - 296)

(^1H NMR)



(154)



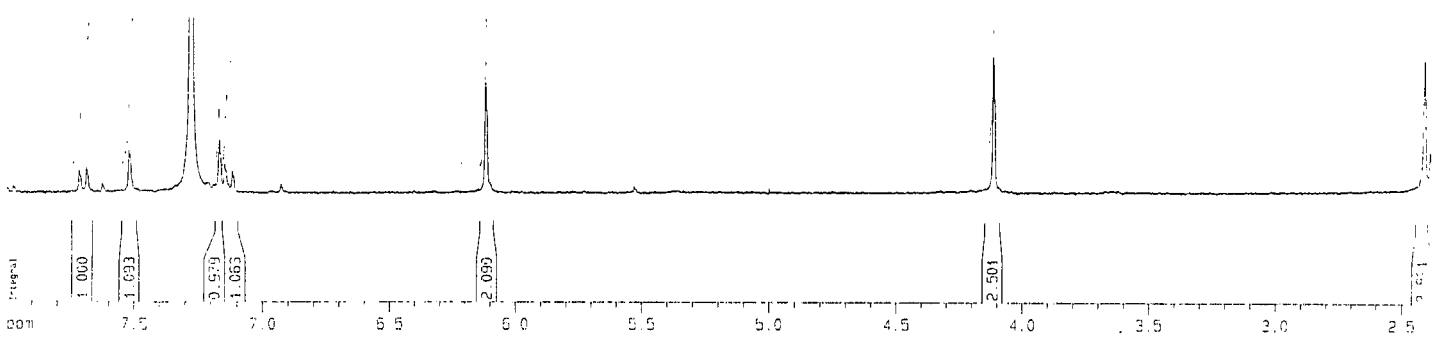
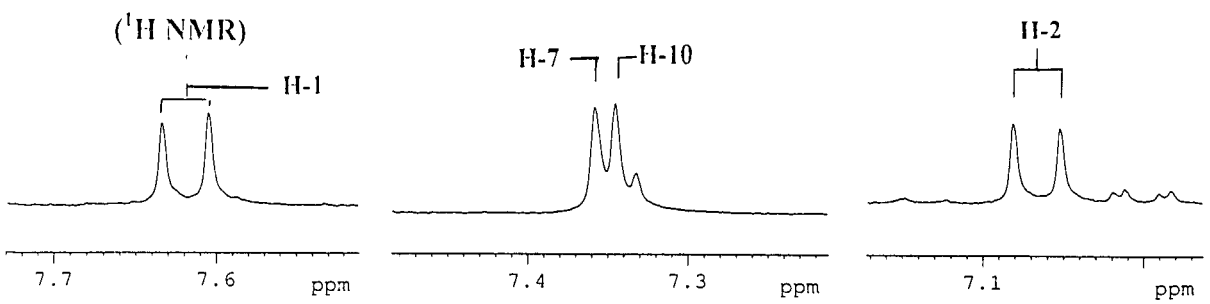
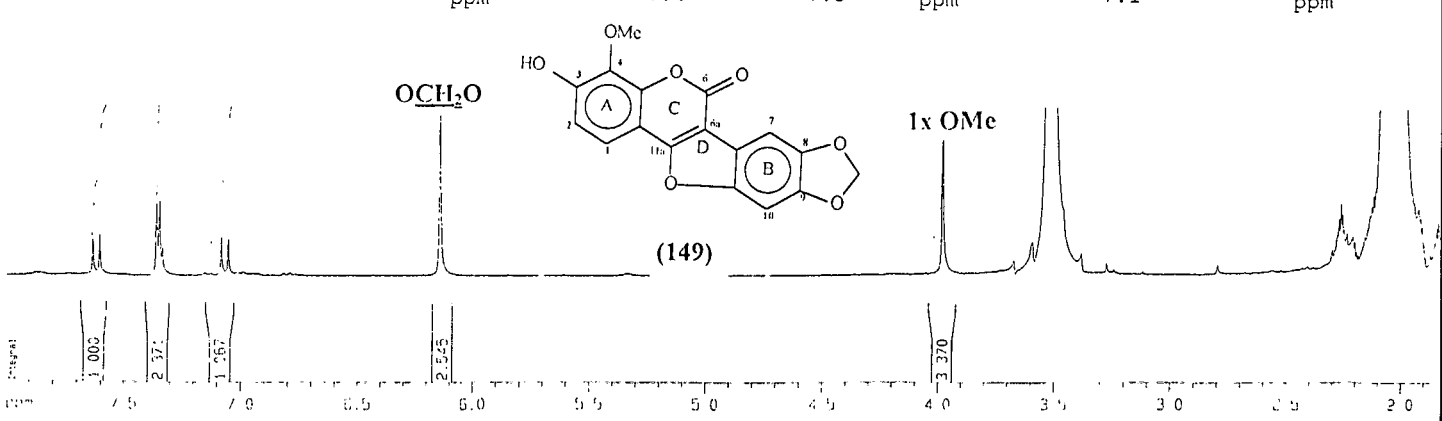


Plate 33 ($CDC_3)_2CO$ - 296K

(^1H NMR)

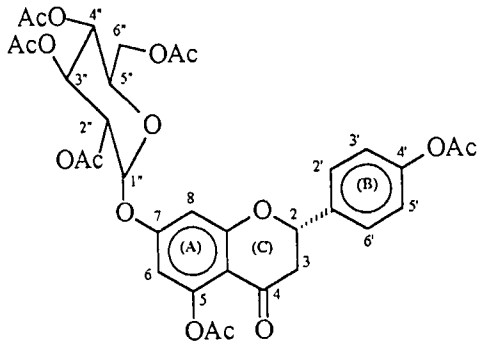




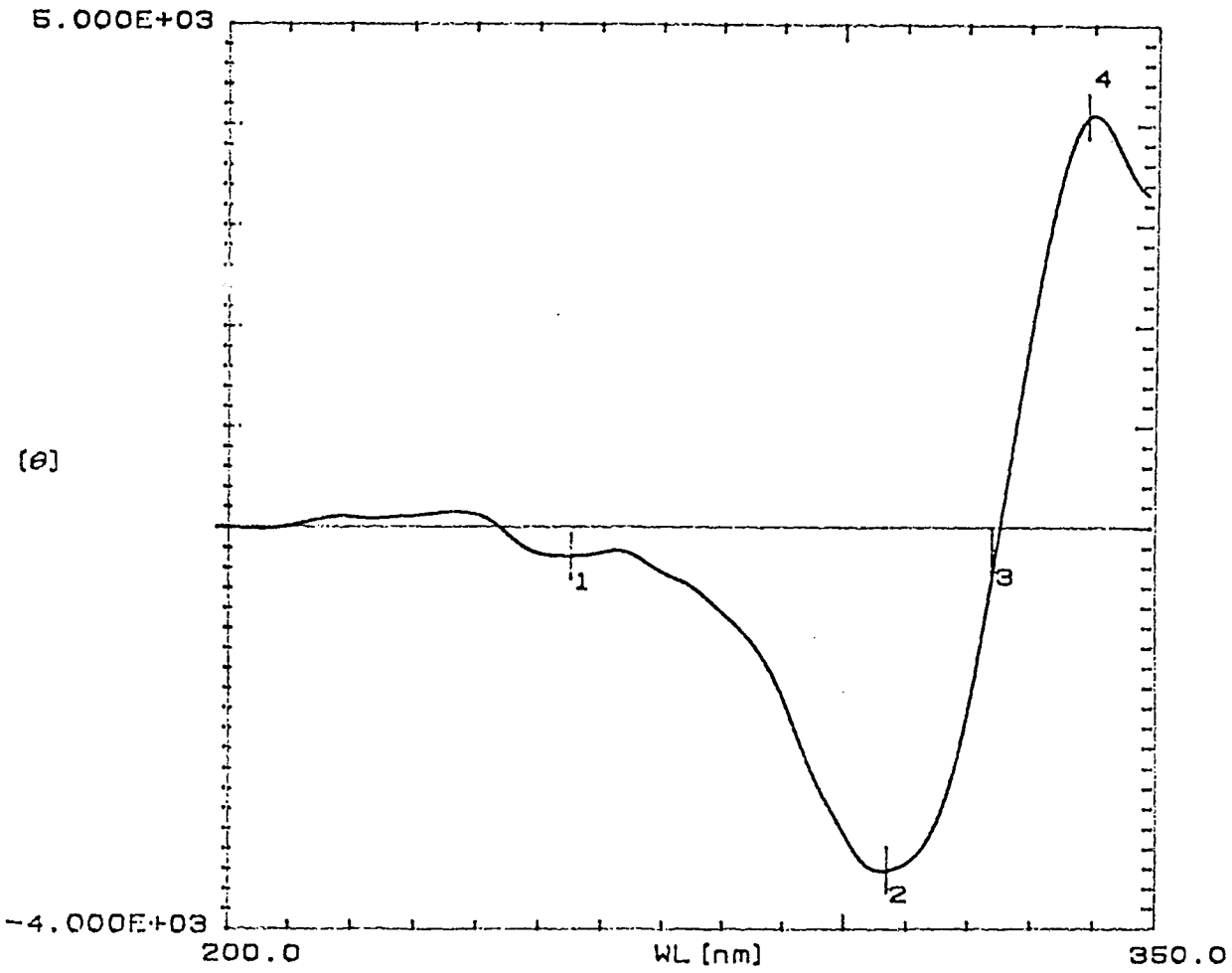
(149)

APPENDIX C

CD Spectrum [plate 34 (1.01mg/10ml in MeOH)]

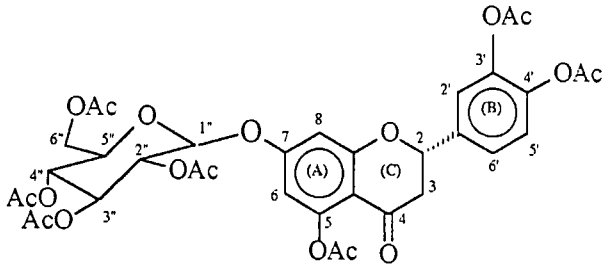


(91)

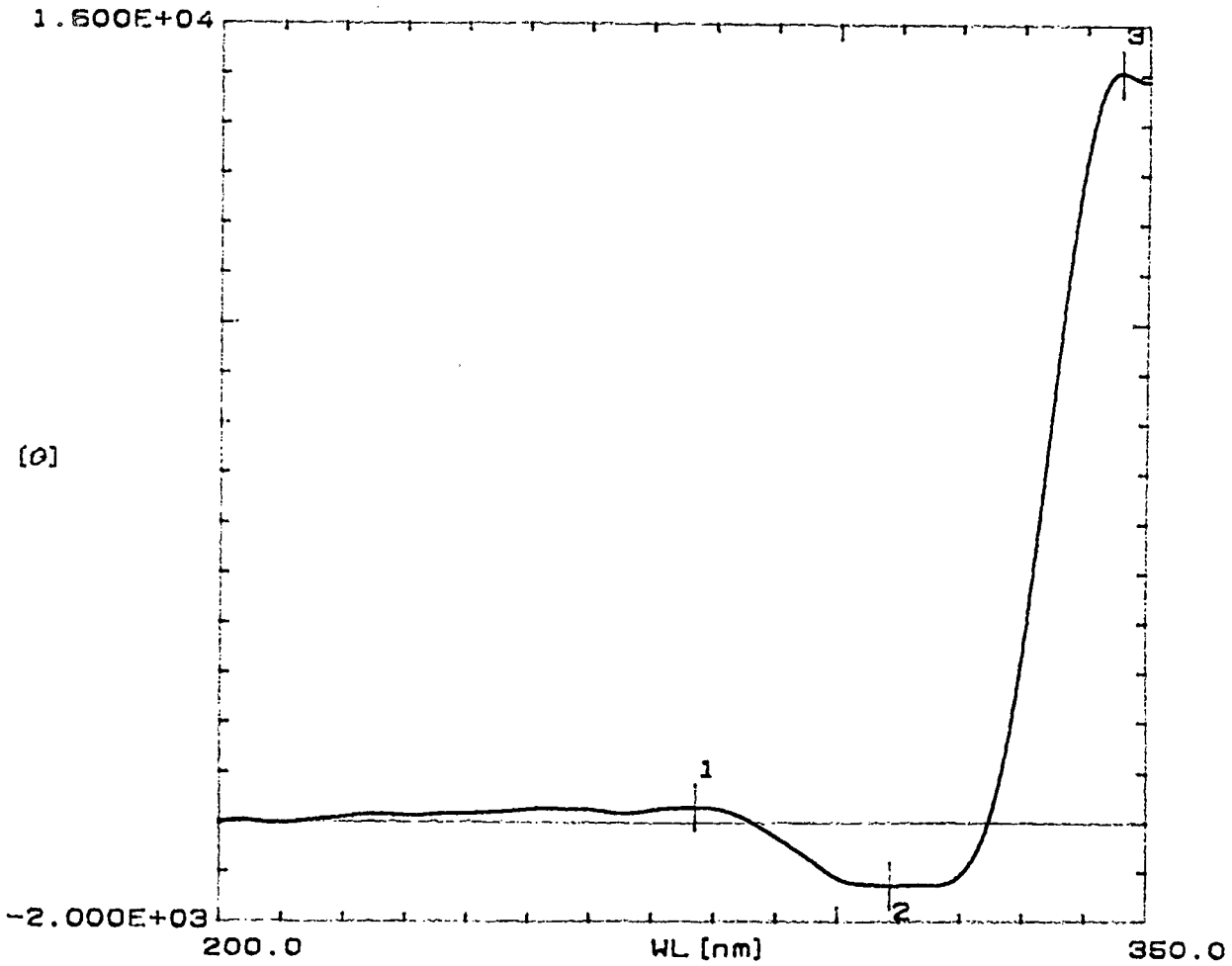


No.	Wavelength	Value
1	257.00 nm	-2.983E+02
2	308.00 nm	-3.419E+03
3	326.00 nm	-2.430E+02
4	341.00 nm	4.098E+03

CD Spectrum [plate 35 (1.00mg/10ml in MeOH)]

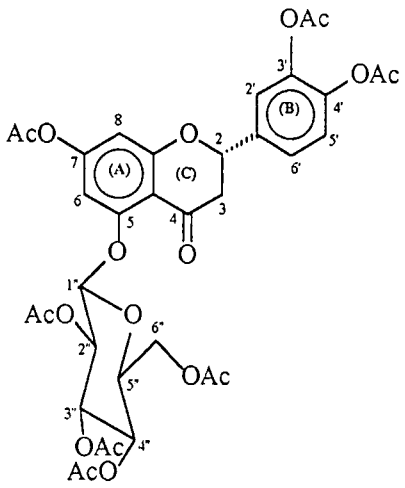


(92)

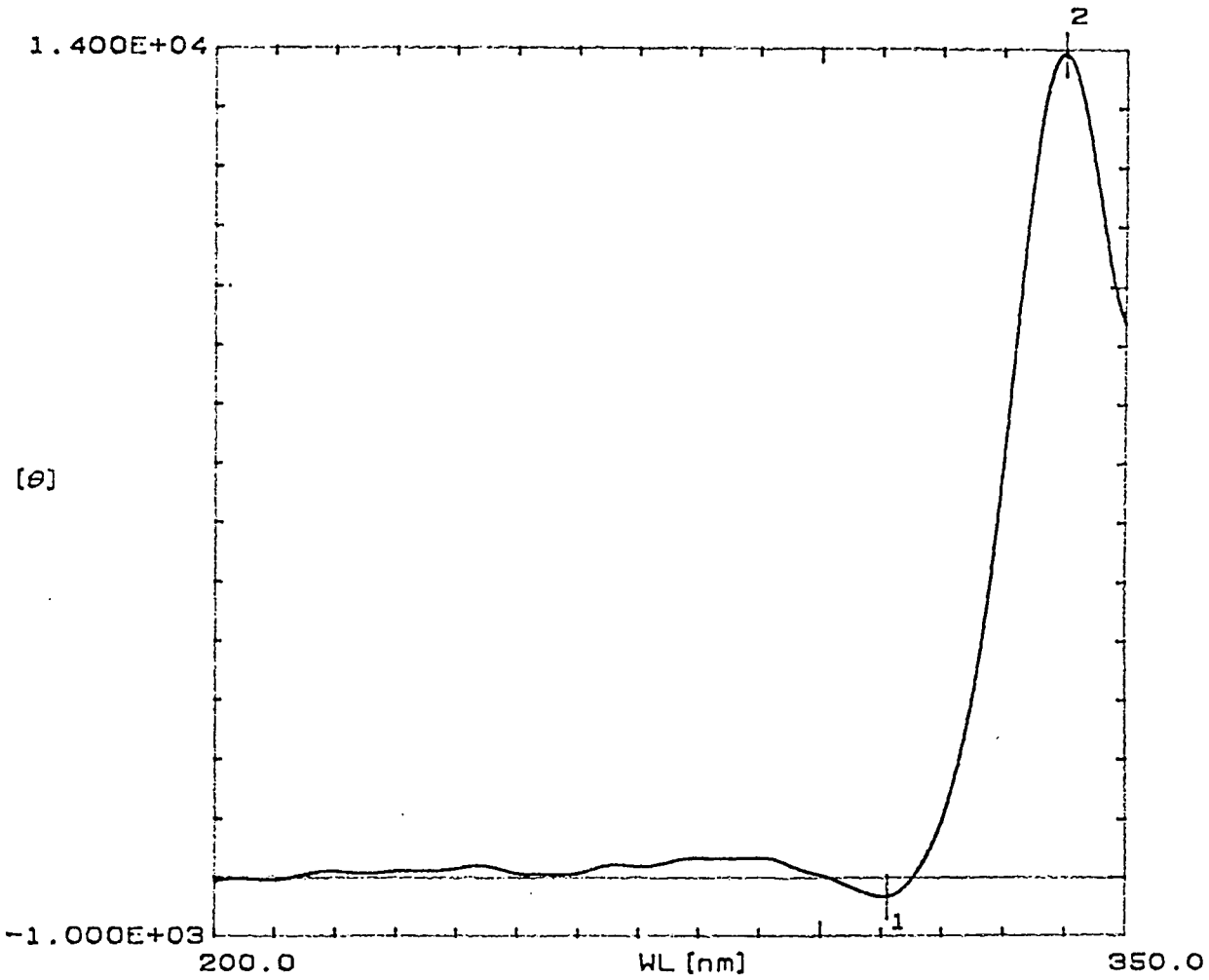


No.	Wavelength	Value
1	277.00 nm	3.103E+02
2	308.60 nm	-1.273E+03
3	345.60 nm	1.505E+04

CD Spectrum [plate 36 (1.00mg/10ml in MeOH)]

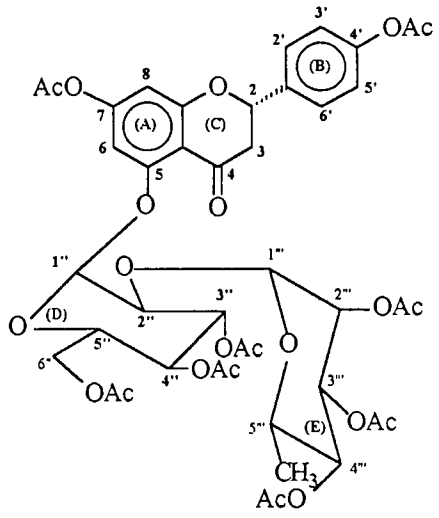


(93)

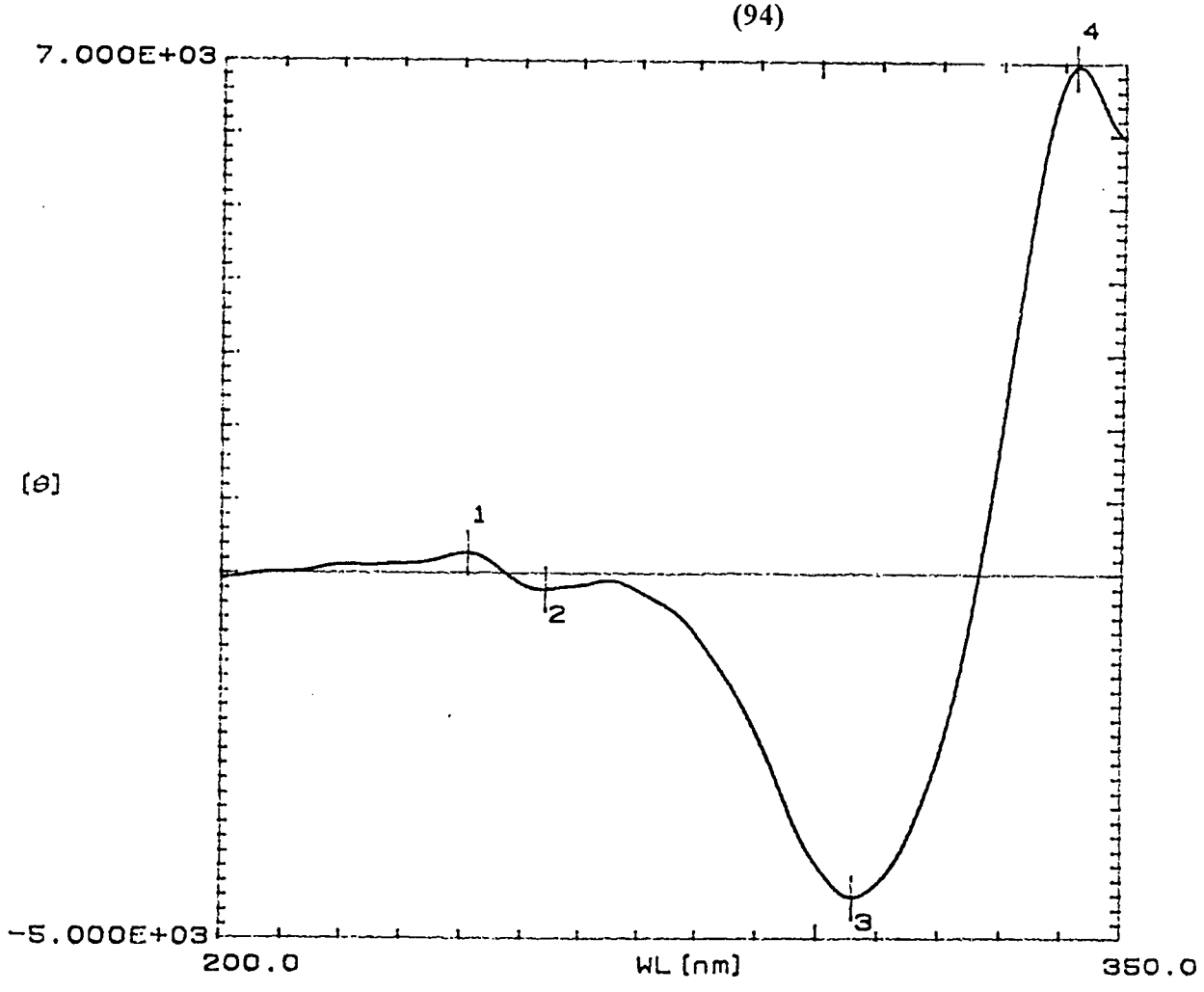


No.	Wavelength	Value
1	311.00 nm	-3.208E+02
2	340.00 nm	1.392E+04

CD Spectrum [plate 37 (1.01mg/10ml in MeOH)]

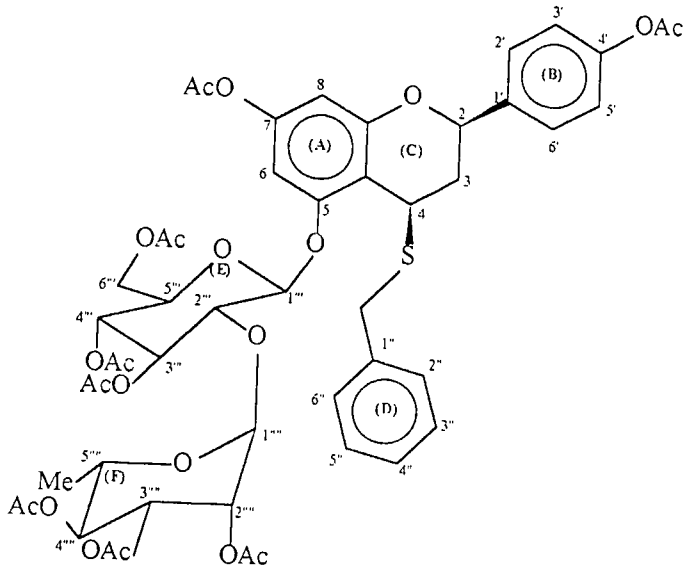


(94)

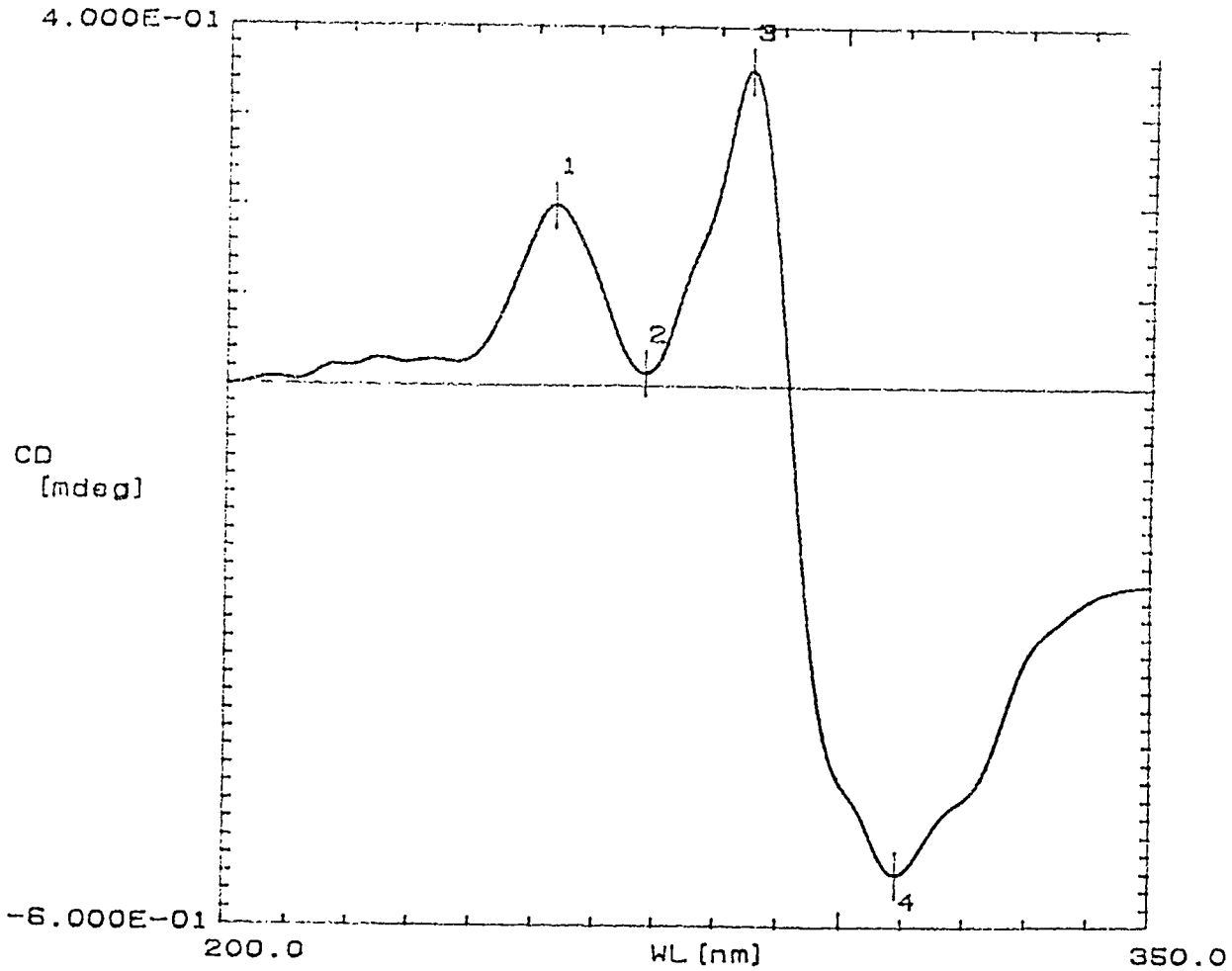


No.	Wavelength	Value
1	241.00 nm	2.774E+02
2	254.00 nm	-2.203E+02
3	306.00 nm	-4.425E+03
4	342.00 nm	6.946E+03

CD Spectrum [plate 38 (1.01mg/10ml in MeOH)]

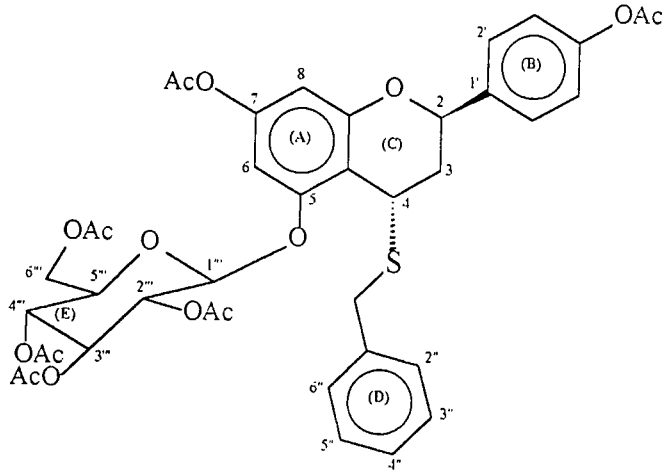


(99)

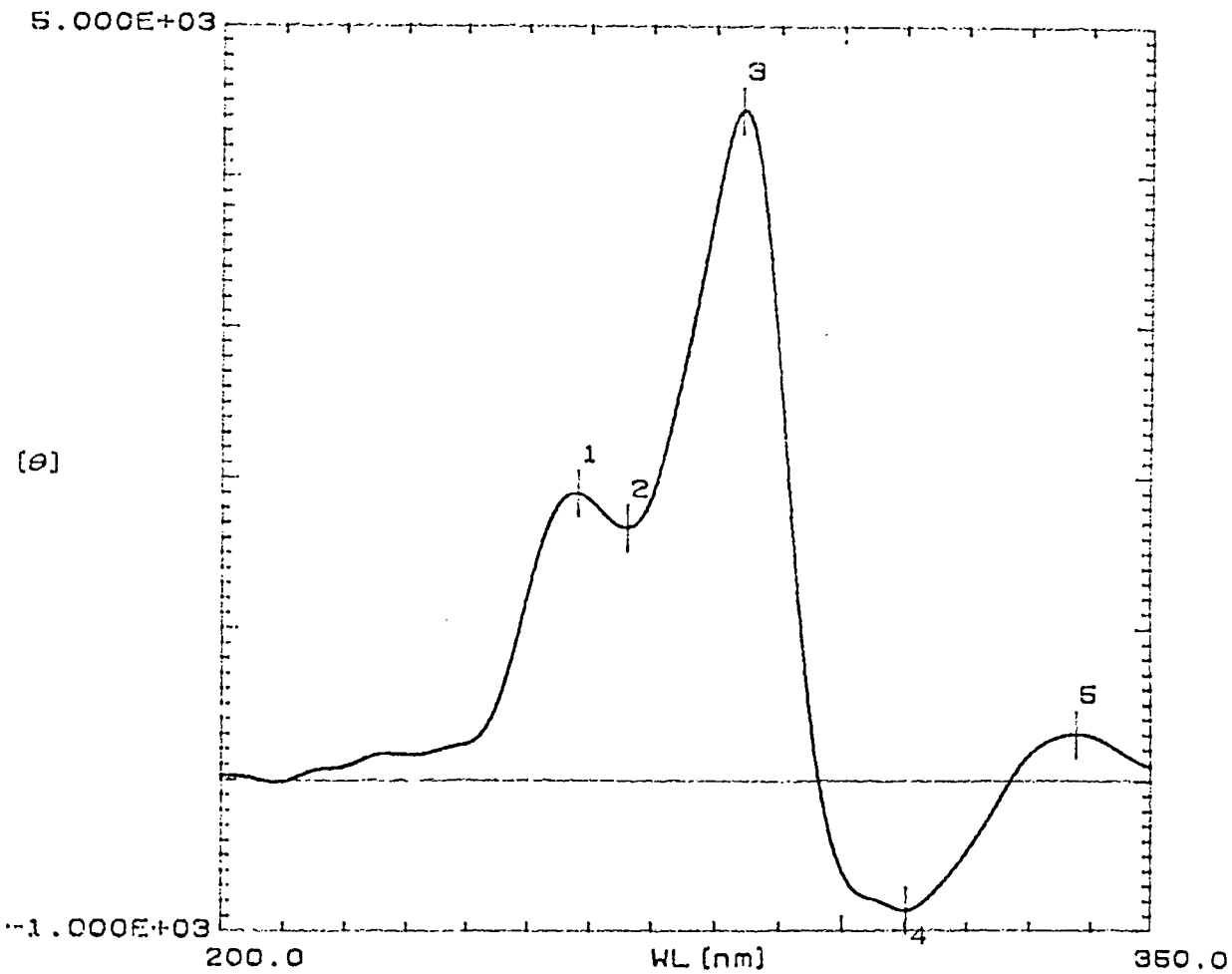


No.	Wavelength	Value
1	253.00 nm	2.008E-01
2	268.00 nm	1.386E-02
3	285.00 nm	3.516E-01
4	309.00 nm	-5.415E-01

CD Spectrum [plate 39 (1.01mg/10ml in MeOH)]

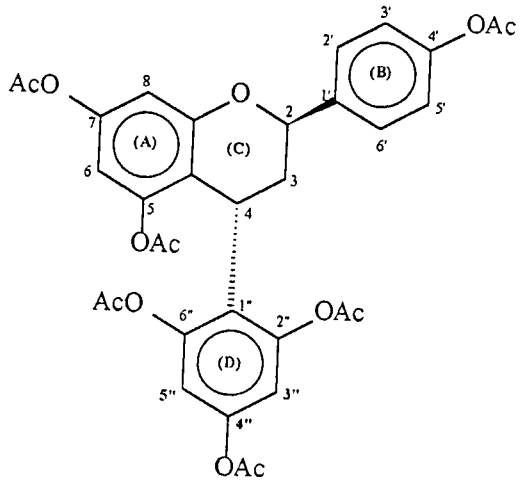


(100)

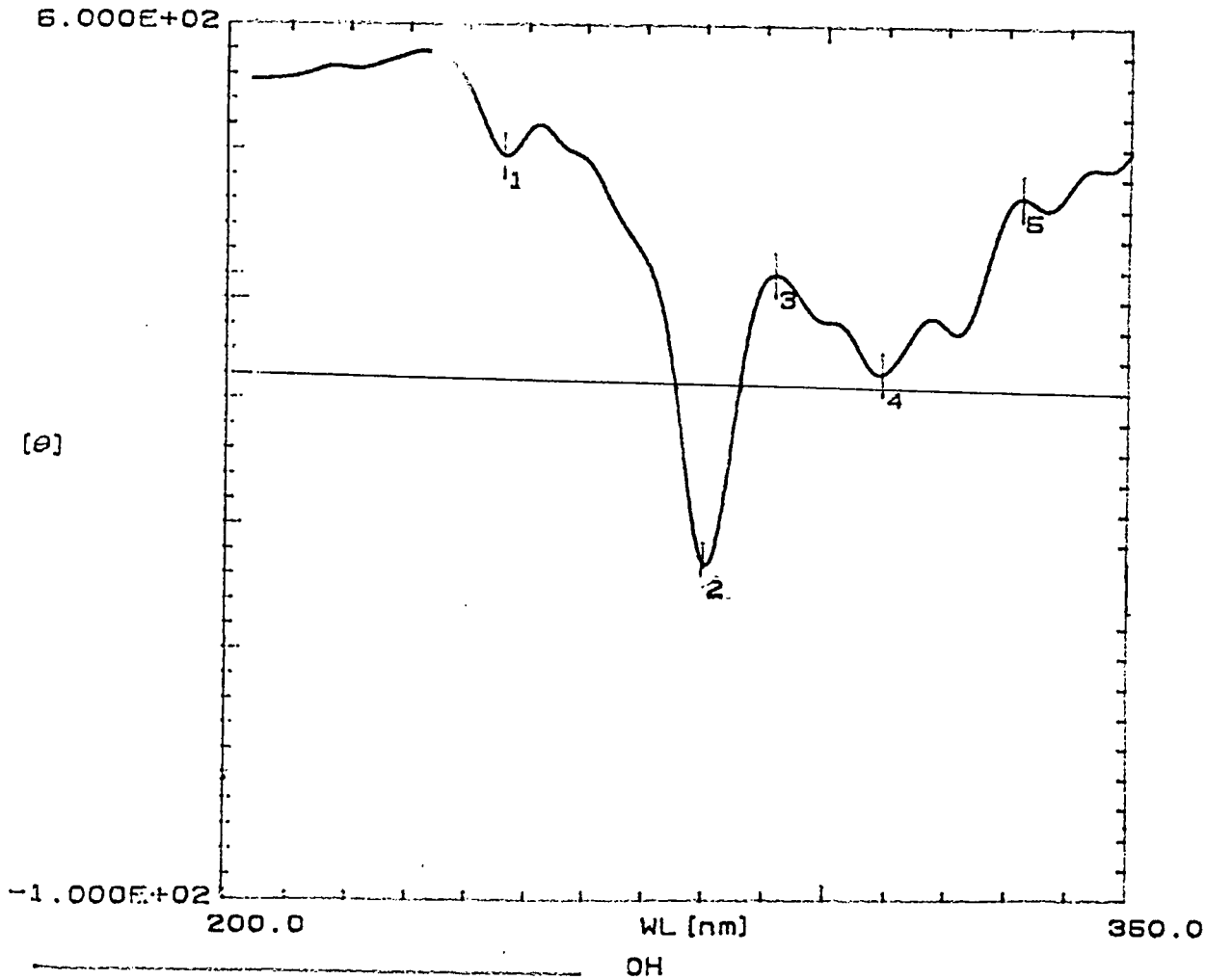


No.	Wavelength	Value
1	258.00 nm	1.900E+03
2	266.00 nm	1.672E+03
3	284.00 nm	4.442E+03
4	310.30 nm	-8.598E+02
5	337.50 nm	3.048E+02

CD Spectrum [plate 40 (1.01mg/10ml in MeOH)]



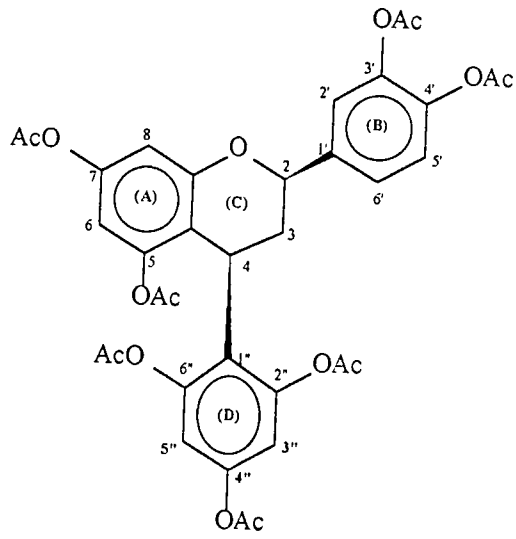
(101)



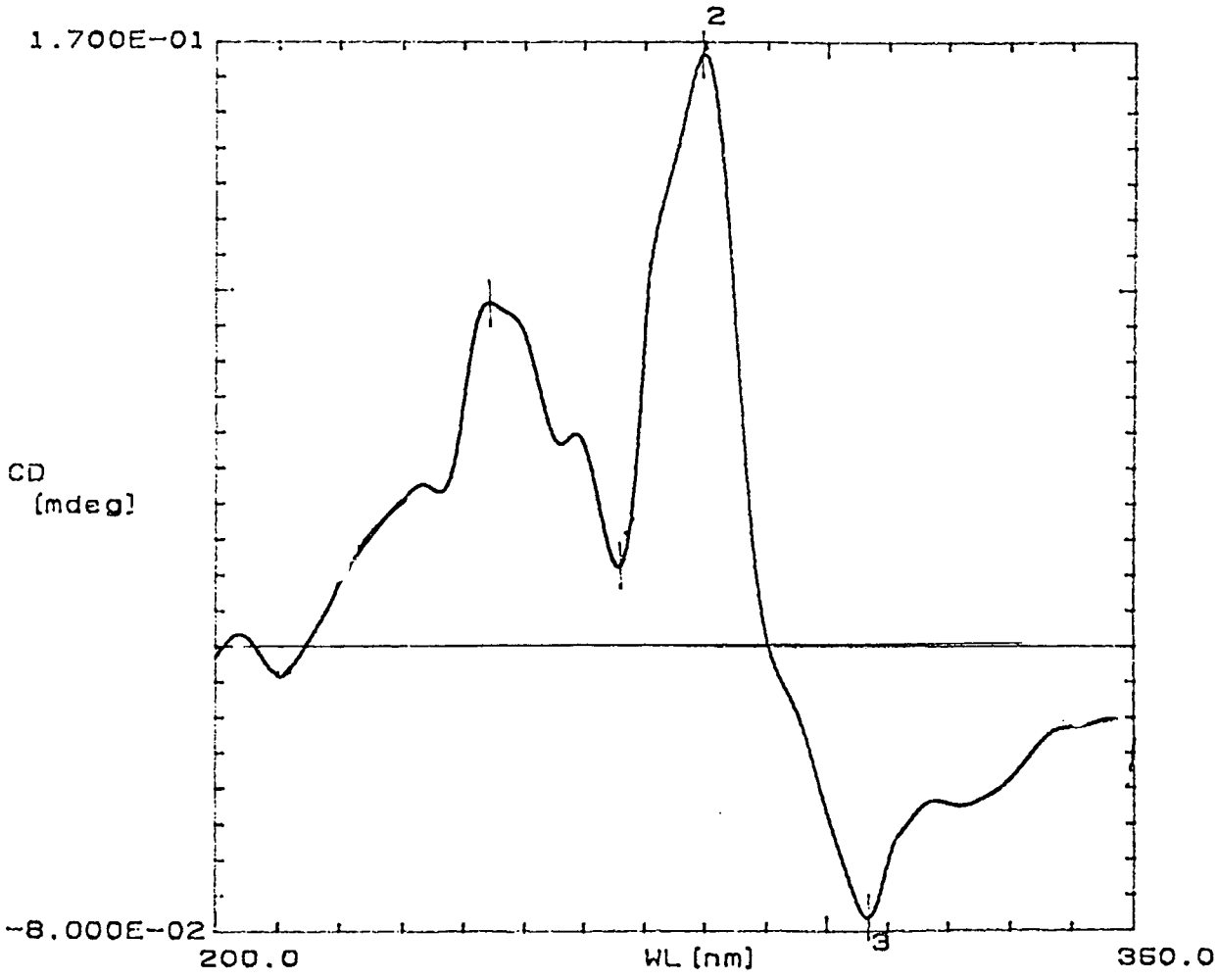
OH

No.	Wavelength	Value
1	246.00 nm	-2.291E+02
2	280.00 nm	-1.561E+03
3	291.60 nm	-6.147E+02
4	309.30 nm	-9.370E+02
5	332.30 nm	-3.579E+02

CD Spectrum [plate 41 (1.01mg/10ml in MeOH)]

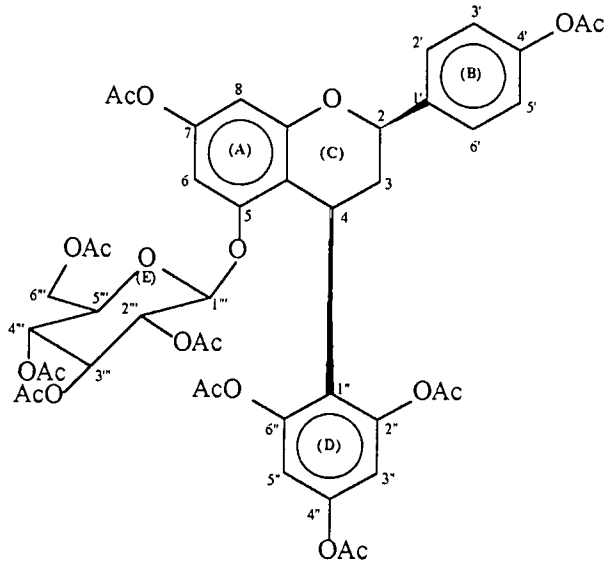


(102)

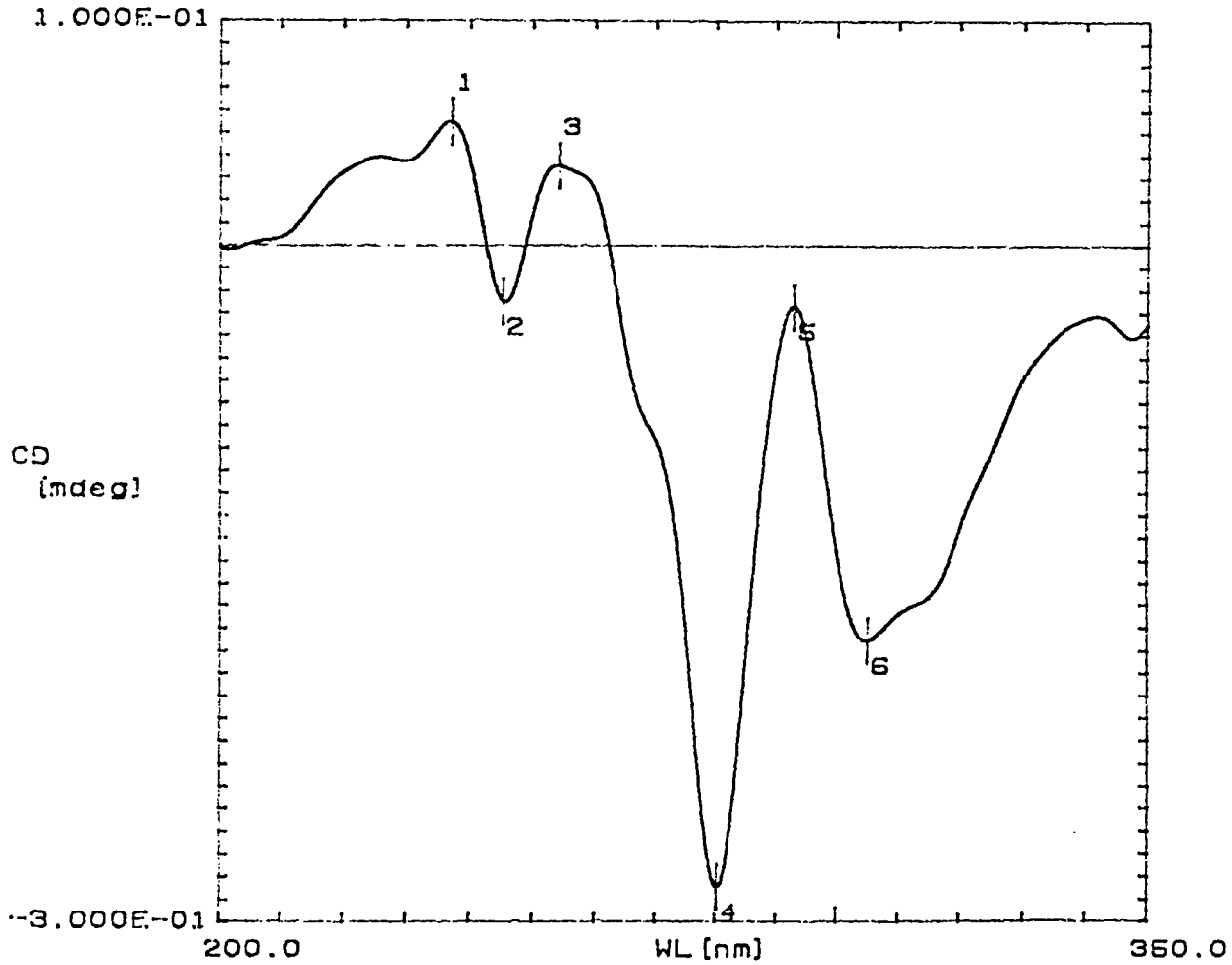


No.	Wavelength	Value
1	266.00 nm	6.642E-02
2	279.50 nm	1.663E-01
3	307.00 nm	-7.591E-02

CD Spectrum [plate 42 (1.01mg/10ml in MeOH)]

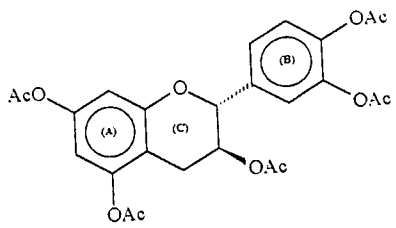


(103)

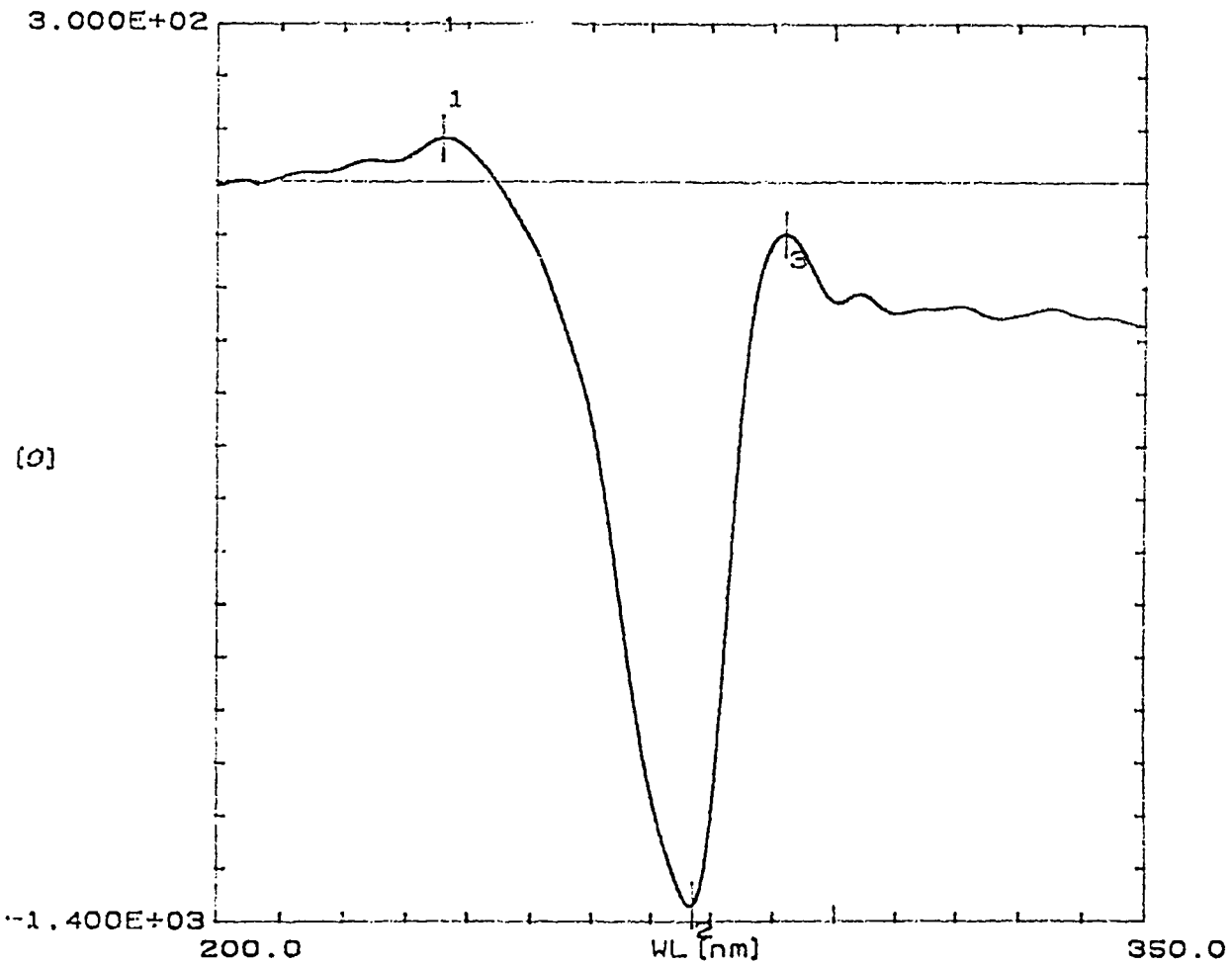


No.	Wavelength	Value
1	237.20 nm	5.496E-02
2	245.20 nm	-2.481E-02
3	254.20 nm	3.544E-02
4	280.20 nm	-2.841E-01
5	293.00 nm	-2.699E-02
6	305.30 nm	-1.753E-01

CD Spectrum [plate 43 (1.01mg/10ml in MeOH)]-Catechin

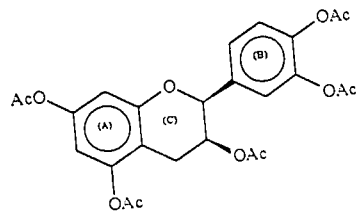


113

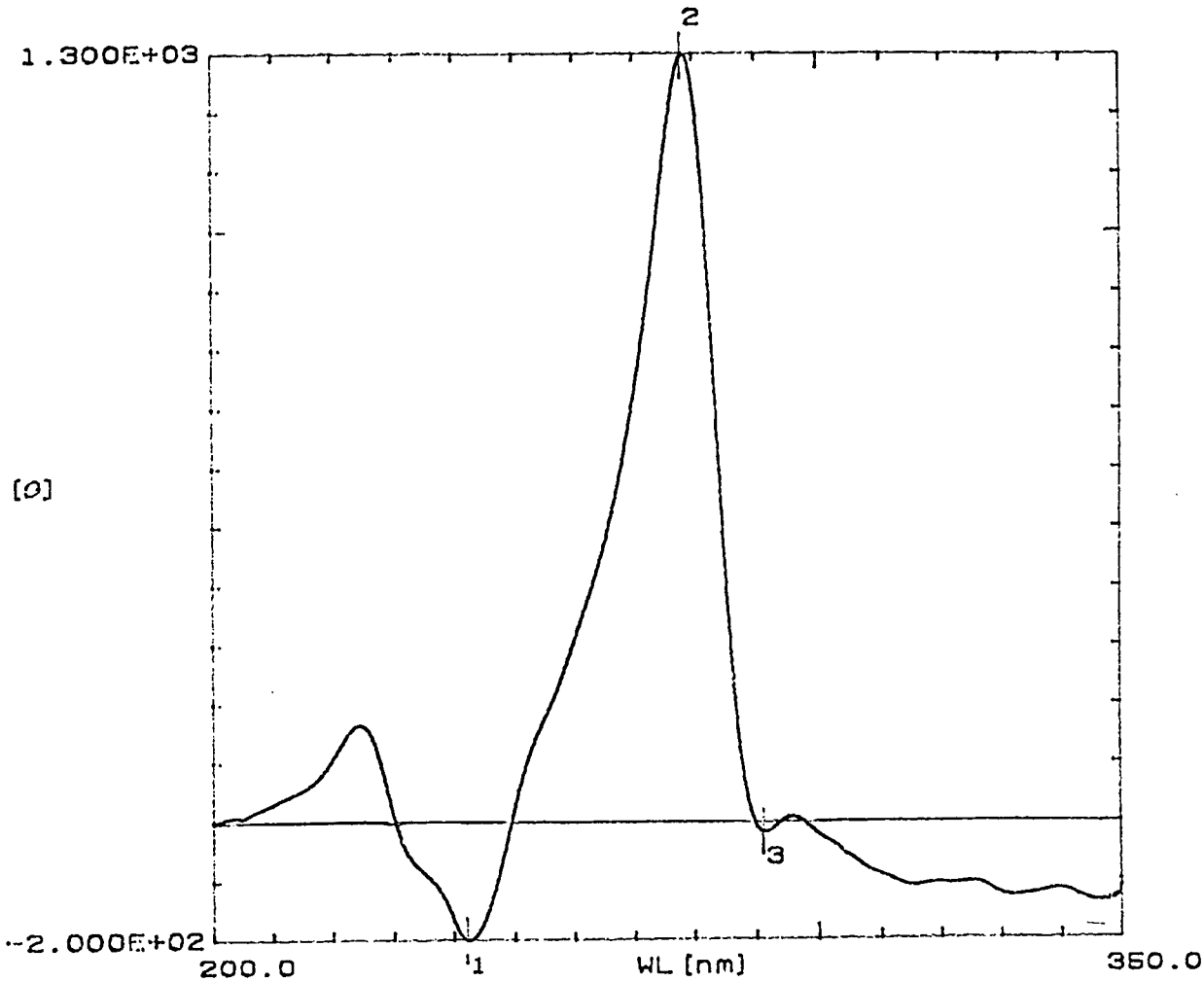


No.	Wavelength	Value
1	235.00 nm	2.214E+02
2	277.00 nm	-1.367E+03
3	292.00 nm	-9.890E+01

CD Spectrum [plate 44 (1.01mg/10ml in MeOH)]-*ent*-Epicatechin

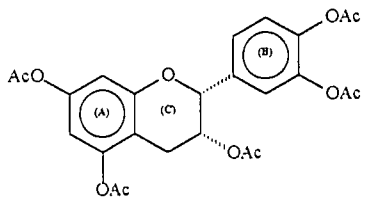


113a

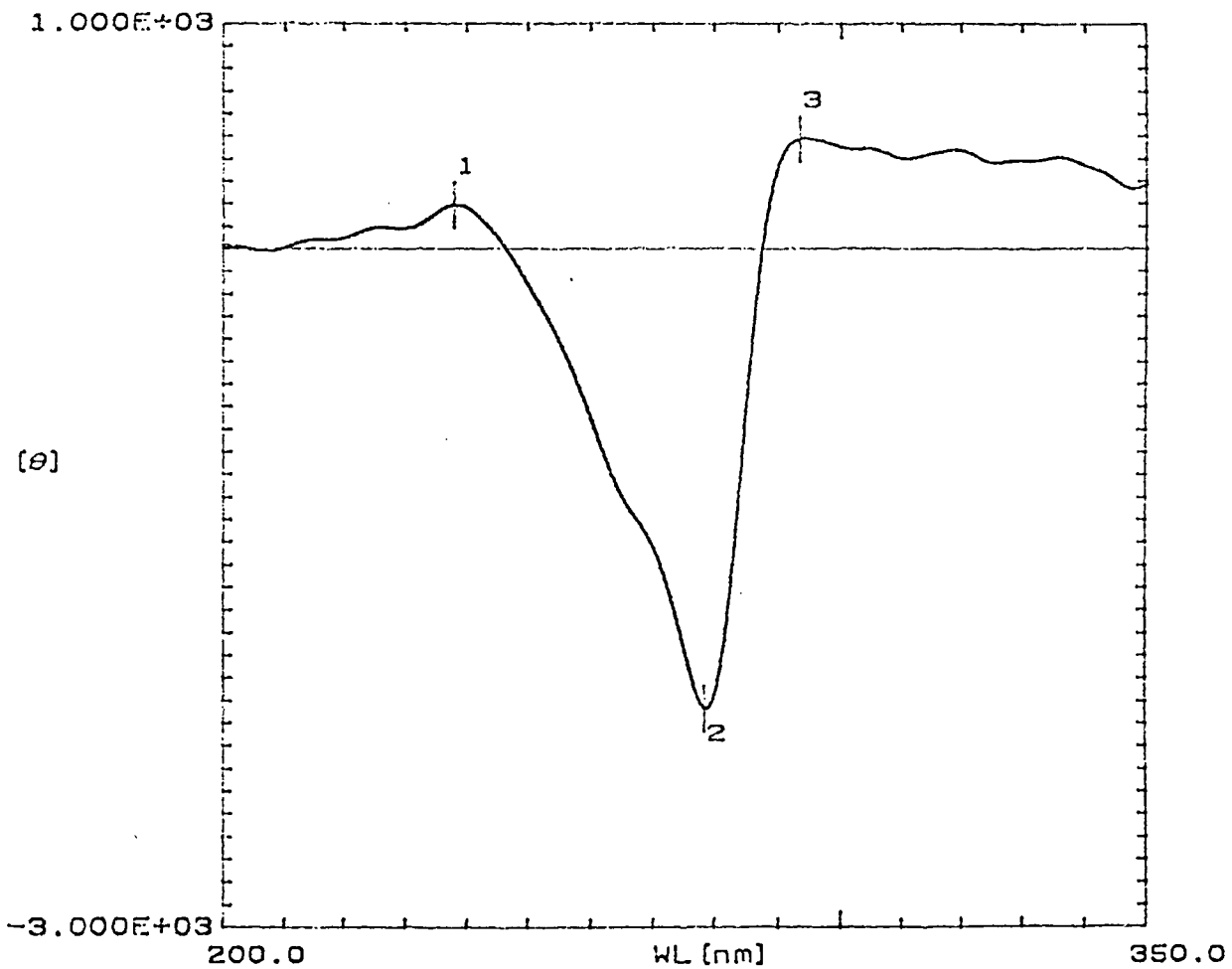


No.	Wavelength	Value
1	242.00 nm	-1.993E+02
2	278.00 nm	1.296E+03
3	291.00 nm	-1.675E+01

CD Spectrum [plate 45 (1.01mg/10ml in MeOH)]-Epicatechin

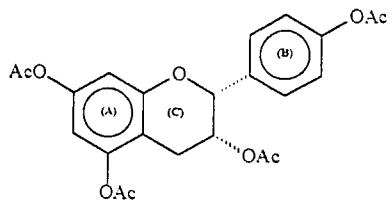


114

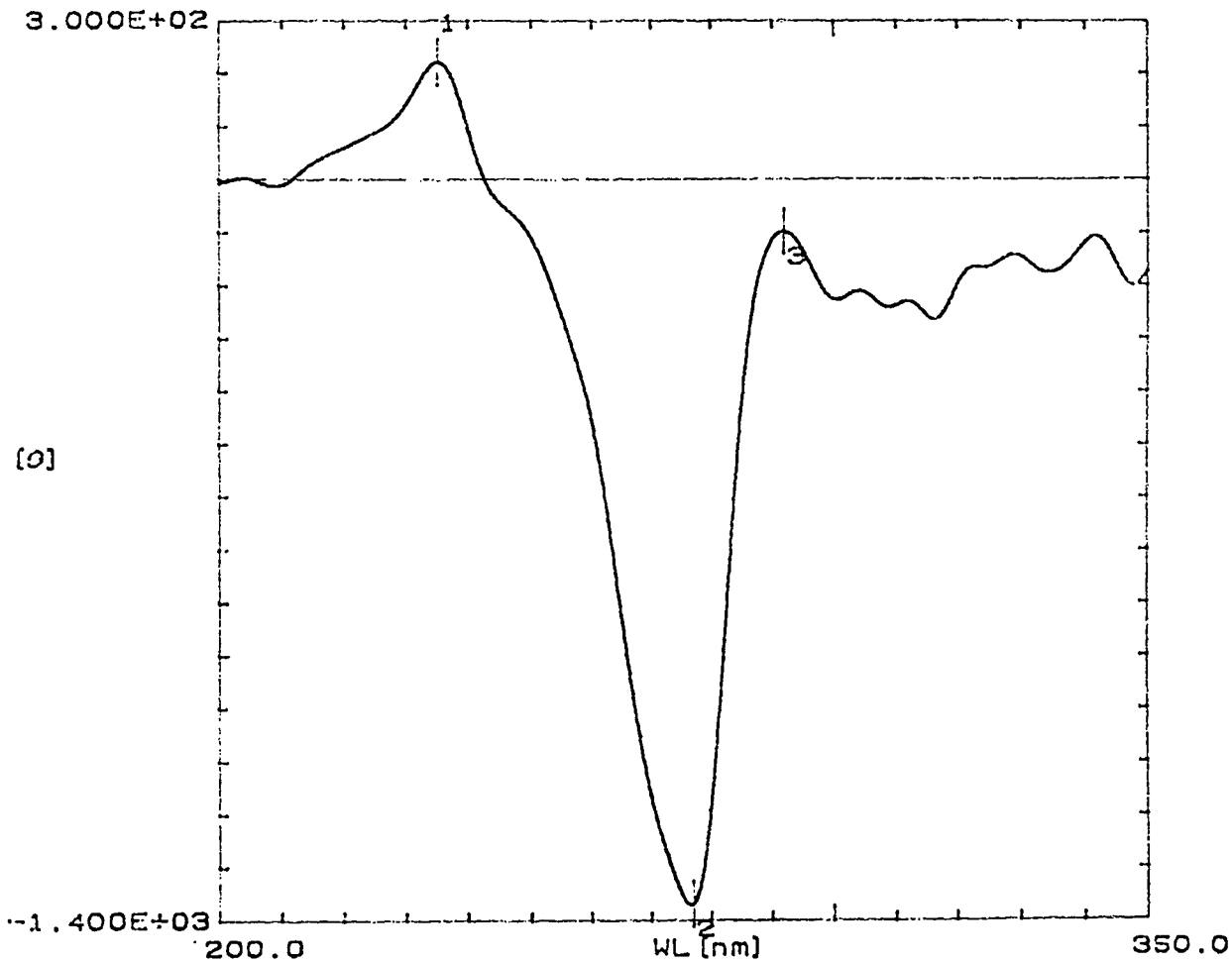


No.	Wavelength	Value
1	237.90 nm	1.947E+02
2	278.40 nm	-2.033E+03
3	293.40 nm	4.870E+02

CD spectrum [plate 46 (101mg/10ml in MeOH)]-Epiafzelechin

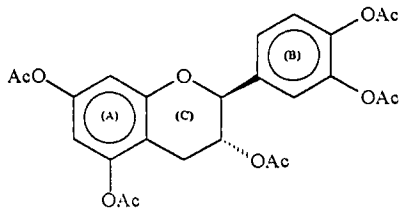


114a

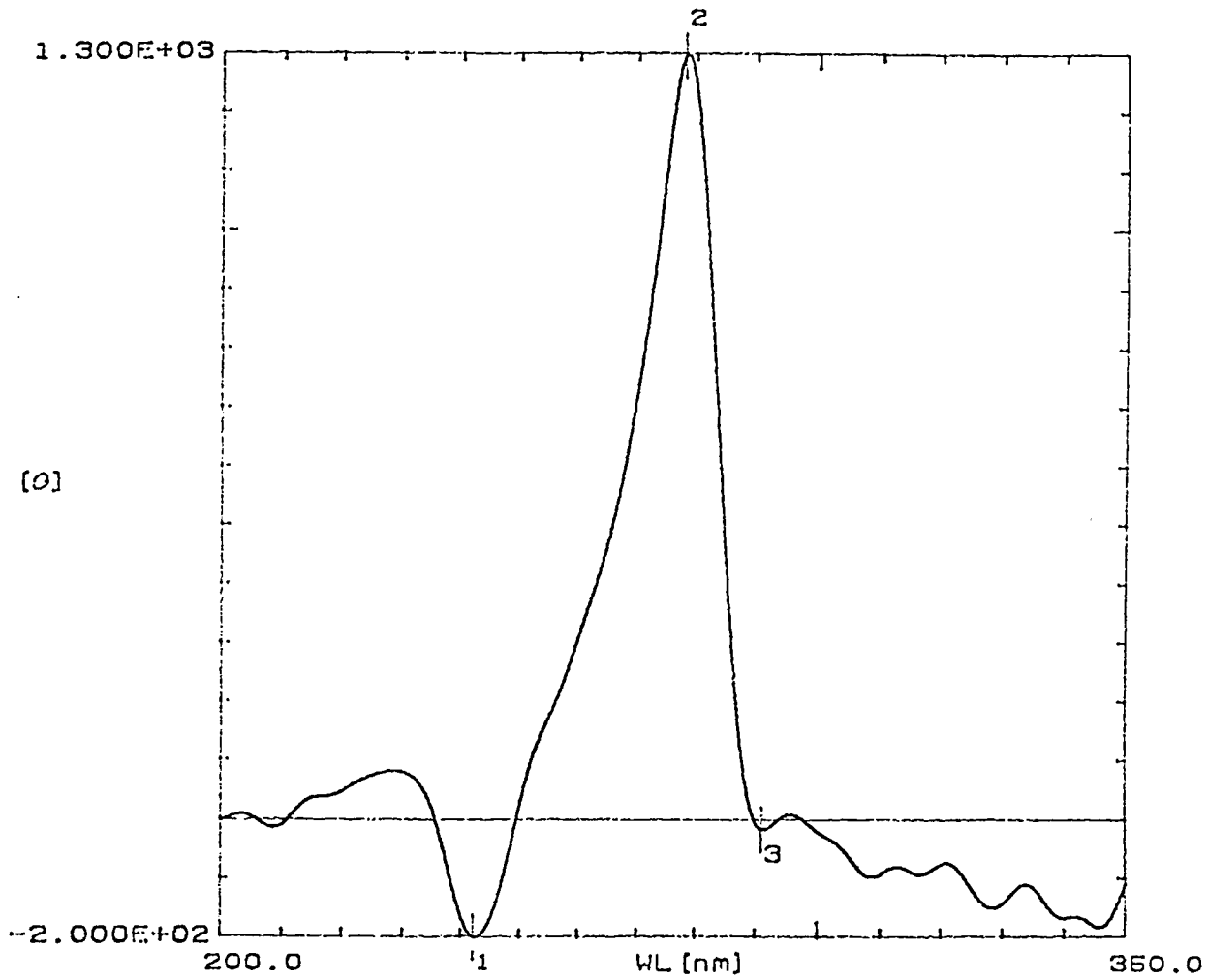


No.	Wavelength	Value
1	235.00 nm	2.214E+02
2	277.00 nm	-1.367E+03
3	292.00 nm	-9.890E+01

CD Spectrum [plate 47 (1.01mg/10ml in MeOH)]-*ent*-Catechin



114b



No.	Wavelength	Value
1	242.00 nm	-1.993E+02
2	278.00 nm	1.296E+03
3	291.00 nm	-1.675E+01

OPSOMMING

Cyclopia intermedia E. Mey (fabaceae), algemeen bekend as Heuningbostee, is een van nagenoeg 24 *Cyclopia* species endemies tot die Kaapse fynbosgebied van Suid-Afrika. Met die ondersteuning van ons aanvanklike ondersoek wat die teenwoordigheid van 'n verskeidenheid fenoliese verbindings, insluitend kumestane, isoflavone, flavanone, xantone, flavone, pinnitol en p-kumaarsuur, aangedui het, geniet die tee huidiglik toenemende populariteit as gesondheidsdrankie. Die teenwoordigheid van hierdie verbindings, waaraan interessante farmakologiese eienskappe toegeskryf word, ondersteun deur die feit dat die tee baie min, indien enige, kaffeïn en 'n lae tannieninhoud bevat, asook die medisinale gebruik van die tee deur die plaaslike bevolking in die Wes- en Oos-Kaap, het gelei tot hierdie voortgesette ondersoek na die inhoud van die plant.

'n Reeks verrykte fraksies uit die metanolekstrak van die gefermenteerde lote van *C. intermedia* het na chromatografiese skeidings (kolom met Sephadex LH 20 en preperatiewe dunlaag) 'n verdere verskeidenheid flavone asook glikosiede van flavanone, isoflavone en eenvoudige $C_6.C_1$ en $C_6.C_2$-tipe verbindings gelewer. Die ru-tannienekstrak is onder suurmedium gesplyt met bensielmerkaptaam en floroglusinol as nukleofiele om die tannieninhoud en die gemiddelde graad van polimerisasie te bepaal. Die strukture van die verbindings, as die asetaatderivate, is met behulp van hoë-resolusie (300 MHz) ^{1}H KMR spektrometrie (insluitend COSY en NOESY eksperimente), Sirkulêre dichroïsme en Elektron Impak Massaspektrometrie bepaal.

Tesame met tirosol en die 3-*O*-metieleter daarvan, is twee nuwe $C_6.C_1$ en $C_6.C_2$-monoarielverbindings met dieselfde *β*-apiofuranosiel-4-*O*-*β*-D-glukopiranosiel-eenheid geïsoleer. Dié word vergesel van die bekende flavanoïde, 3',4'7-trihidroksie- en 3',5',7-trihidroksi-4'-metoksiflavone, 6-*C*-*β*-D- en 5-*O*-*α*-D-glukopiranosielkaemferol, die flavone, 7-*O*-*β*-D-glukopiranosielnaringenin en -eriodiktoïl asook 5-*O*-*β*-D-glukopiranosieleriodiktoïl en die isoflavoon, 7-*O*-*β*-D-glukopiranosiel-4',6-di-O-metielafrormosin. Nuwe verbindings wat geïsoleer is sluit 5-*O*-*α*-D-rutinosielnaringenin, 8-*C*-*β*-D-glukopiranosiel- en 3-*O*-,6-*C*-di-*β*-D-

glukopiranosielkaemferol, 6"-*O*-*β*-apiofuranosiel-7-*O*-*β*-D-glukopiranosiel-4'-*O*-metielisoflavoon en 6"-*O*-*β*-apiofuranosiel-6-*O*-*β*-D-glukopiranosiel-3-hidroksi – 3',4'-metileendioksiflavonol in.

Splyting van die tannienfraksie met floroglusinol as nukleofiel het 4-(2,4,6,-trihidroksifeniel)-4',5,7-trihidroksiflavaan, die 5-*O*-*β*-D-glukopiranosielanaloog daarvan en 4-(2,4,6,-trihidroksifeniel)-3',4',5,7-tetrahidroksiflavaan gelewer, terwyl die reaksie met bensielmerkaptaam 4-tiobensiel-5-*O*-*β*-D-glukopiranosiel-4',7-dihidroksiflavaan en die 5-*O*-*β*-D-rutinosielanaloog gee.

Afwesigheid van heterosikliese protone in die C-ring van die kumestane het struktuuropklaring met KMR alleen nie bo twyfel gelaat nie en die strukture is deur sintese bevestig. Die drie kumestane is gevolglik *via* 'n nuwe roete gesintetiseer deur die direkte omskakeling van 2,2'-dihidroksichalkoonanaloë na die ooreenstemmende kumestane.

Aangesien flavanoïde 'n noemenswaardige rol mag vertolk by die blussing van aktiewe suurstofspesies, is hierdie effek nageboots deur die reaksie van 'n verskeidenheid verbindings, insluitend kwersitien, myrisitien, fisetien, robinetien, katesjien en epikatesjien, met superoksied te ondersoek. Buiten die verwagte splyting van die molekuul is 'n buitengewone deoksigenering (3'-OH) van die B-ring vir sommige van hierdie verbindings waargeneem.

Die resultate van hierdie ondersoek as geheel ondersteun die vermoede dat die beweerde gesondheidsbevorderlike eienskappe van Heuningbostee ten minste gedeeltelik verband mag hou met die fenoliese inhoud van die plant.