STRUCTURE AND SYNTHESIS OF POLYPHENOLS FROM CYCLOPIA SUBTERNATA

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by

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D. J. Brand

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Key words: *Cyclopia subternata;* Fabaceae; Honeybush tea; flavonoid; isoflavonoid; glycosides; flavan glycoside; glycosylation ; flavanone; flavan synthesis

Cyclopia subternata (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 *C. intermedia*, demonstrating the presence of phenolic compounds including coumestans, isoflavones, flavanones, xanthones, a flavone, pinitol, *p*-coumaric acid and flavonoid glycosides, 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 low tannin content, as well as its usage as a medicinal plant by the people of the Western and Eastern Cape prompted investigations on the *subternata* species.

The acetone and methanol extracts of the unfermented shoots and stems of *C. subternata* were subjected to chromatographic separations (Shephadex LH-20 column and preparative thin layer) which afforded a novel flavan, flavonols, flavanones, flavones, isoflavone and $C_6.C_2$- and $C_6.C_1$-type compounds. Their full acetate or methylated derivatives were elucidated and characterized by high resolution (300 MHz) ^{1}H NMR spectrometry which included COSY, NOESY, HMQC, HMBC, DEPT 135° experiments and ^{13}C NMR spectroscopy, Molecular Modeling and Circular Dichroism.

Along with (+)-Pinitol a novel apiofuranosyl (1"→6')-glucopyranosyl carboxylic acid was isolated as a non-flavonoid compound. The flavonoid aglycones isolated included epicatechin-3-*O*-gallate (flavanol), luteolin (flavone) and orobol (isoflavone). The non-flavonoid glycosides included a 4-*O*-glucopyranosyl tyrosol, apiofuranosyl (1"→6')-glucopyranosyl benzaldehyde and the acetate derivatives of two new *O*-glycosides namely 1-[β-D-2',3',4'-tri-*O*-acetylglucopyranosyloxy]-2-(3,5-diacetoxyphenyl)ethane and 1-acetoxy-2-(β-D-2',3',4',6'-tetra-*O*-acetylglucopyranosyloxy)ethane.
The flavonoid *O*-glycosides comprised a novel 3',4',7-trihydroxy-5-(glucopyranosyloxy) flavan, the flavone, scolymoside and the flavanones, hesperedin, narirutin and eriocitrin. *C*-glycosides isolated includes the xanthone, mangiferin, a *C*-6-glycosylated kaempferol

as well as two new *C*-glycosides, 3,4',6,7-tetrahydroxy-5-(β-D-glucopyranosyl) flavonol and 3',4',5,5',7-pentahydroxy-8-(β-D-glucopyranosyl) flavanone.

Due to the novelty and the many proposed health properties of flavans, the relatively unexplored routes to flavonoid *O*-glycosylation and the effects of glycosylation on the aglycone solubility and general behaviour, including the absorption in tissues of man, prompted the synthesis of the isolated flavan glycoside. The uncertainty of the point of sugar attachment to the aglycone of some glycosides, especially with limited material available, further inspired the exploration of synthetic routes to glycosylated flavonoids.

In an attempt to obtain the 5-*O*-glycosylated flavan, the selected glycosylation procedure was attempted on the analogous 5,7-dihydroxy-3',4'-dimethoxyflavanone, but proved unsuccessful because of the acidity of the 3-protons leading to ring opening. The same procedure was attempted on the analogous chalcone with an unprotected A-ring. Glycosylations was successfully done on resacetophenone, phloroacetophenone and 4-methoxyphloroacetophenone as precursors to the formation of the glycosylated chalcone. However, the condensation with the appropriately protected benzaldehyde to obtain the glycosylated chalcone as precursor to the 5-*O*-glycosylated flavan was unsuccessful.

The proposals that phenolic metabolites have physiological and therapeutic properties may also be associated with the compounds isolated from the tea. Luteolin and eriodictyol constitute part of the phenolic metabolic pool with a 3',4'-diol functionality which is claimed to have antioxidant activity. The antimicrobial activity of flavonoids in plants is also well documented and so is the antiviral activity of flavonoids and their ability to inhibit key enzymes in mitochondrial respiration. It was found that a C-2, C-3-double bond, a 4-keto group and a 3',4',5' –trihydroxylation of the B-ring are significant features of those flavonoids which show strong inhibition of NADH-oxidase. Coronary heart disease is also reported to be reduced in humans with high flavonoid intake. The anti-inflammatory and antitumor activity along with the ever-increasing interest in plant flavonoids for treating human diseases and especially for controlling the immunodeficiency virus, the cause of AIDS, also attract interest in this herbal tea. These results clearly indicate that the claims of the health promoting properties of Honeybush tea may at least, in part, be attributed to the presence of these and other polyphenols in *C. subternata.*

CHAPTER 1

HONEYBUSH TEA

1.1 Overview

Honeybush is a unique plant growing mainly in areas ranging from the Southern to Eastern Cape, although some species also grow in parts of the Cederberg mountains. In the wild, 23 species of honeybush tea are found of which two, *Cyclopia intermedia & Cyclopia subternata,* are the most popular cultured species. The former grows in the high mountainous areas of the Eastern Cape and the latter in the Southern Cape lowlands.
The leguminous bush from which the herbal tea is brewed has bright yellow flowers that are heavily honey scented, grows to about 1.5 metres and has an unusual root structure allowing the plant to flourish in fairly dry conditions. Honeybush tea is caffeine free and low in tannin content and it has a pleasant sweet tasting honey flavour with several alleged health properties[1]. Honeybush tea was initially used by the early Bushmen population and has a recorded history dating back to the 18th century. The claims around Honeybush tea recently attained some scientific backing with research done on the tea and reports published.[2] Each tea-maker has a unique method of creating the richest flavour and deepest colour of tea. In principle the tea is processed, subjected to an oxidation process, and sun dried.

Attempts to grow Honeybush commercially have had variable success and thus most of the tea is still harvested from the wilderness areas. This makes the tea precious and rare and uniquely South African.[3]

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[1] C. A. Smith, *Common Names of South African Plants*, The Government Printers, Pretoria, **1966**, 94.
[2] D. Ferreira, B. I. Kamara, E. V. Brandt, E. Joubert, *J. Agric. Food Chem.*, **1998**, *46*, 3406.
[3] http://www.herbafrica.com/production.htm.

1.2 Polyphenols from Honeybush Tea

The presence of flavonoids in the extracts of *Cyclopia Intermedia*[2], 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 continued investigations on *Cyclopia Subternata*. The claimed antioxidant properties of the flavonoids[4] 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[1] also supported the proposal.

Although earlier investigations of the mammalian metabolism of flavonoid compounds were largely concerned with the identification of their urinary metabolites[5] more recent studies have centred on the role of the intestinal micro flora in the catabolism of flavonoid molecules, on the significance of the biliary-enteric route of excretion and on the disposition of flavonoids in mammalian tissues following oral and parenteral administration. Additionally the introduction of chemically modified flavonoids and of synthetic phenylchromones as therapeutic agents has promoted metabolic and pharmacokinetic investigations on compounds showing close structural relationships with the naturally occurring flavonoids.

The reported biological activity of certain flavonoids in specific mammalian systems has also resulted in the initiation of studies on their interaction with isolated enzymes, cell constituents and membranes, which may be of importance in the mediation of pharmacological effects. Interest in the metabolism of other flavonoids has been stimulated by the finding that the aglycones of certain naturally occurring flavonol glycosides are mutagenic [6] and that the aglycone quercetin may, following oral ingestion, give rise to neoplasm's of the gastrointestinal tract. [7]

Flavonoids may have existed in nature for over one billion years and thus may have interacted with evolving organisms over the eons. Clearly the flavonoids possess some important functions in nature, having survived in vascular plants throughout evolution.

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[4] 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.
[5] F. De Eds in *Comprehensive Biochemistry* (ed. Florkin), Vol. 20, Elsevier, London.
[6] Bjeldanes and Chang, **1977**; Sugimura *et al.*, **1977**; MacGregor and Jurd, **1978**.
[7] Pamukcu *et al., Cancer Res.*, **1980**, *40*, 3468.

The ancient flavonoid protection of plants against various animal herbivore species and other plant eating organisms throughout evolution may account for the extraordinary range of biochemical and pharmacological activities of these molecules in mammalian and other cell systems.[8] A unique example is the inhibition of gamete membrane fusion in sea urchins caused by quercetin during egg fertilization.[9]

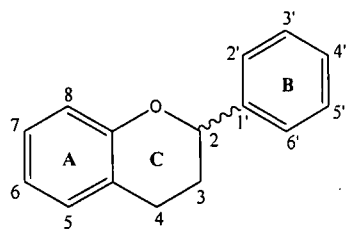
[8] J. B. Harborne, C. A. Williams, Advances in flavonoid research since 1992 Review, *Phytochemistry,* **2000**, *55*, 481.
[9] W. R. Eckberg, M.E. Perotti, *Biol. Bull.*, **1983**, *164*, 62.

CHAPTER 2

NOMENCLATURE AND OCCURRENCE

2.1 Flavans and proanthocyanidins

The system of nomenclature for flavans **(1)**, flavan-3-ols **(2)** and proanthocyanidins in general employs trivial names for the basic units (Table 2.1.1 and 2.1.2). All Flavan-3-ols in Table 2.1.2 are of the (2*R*,3*S*) configuration and those with a (2*R*,3*R*) configuration are prefixed with 'epi', e.g. epicatechin[10]. The flavan-3-ol units with a 2*S* configuration are distinguished by the enantio (*ent*) prefix (Hemingway)[10]. The flavanoid skeleton is drawn and numbered as shown below.



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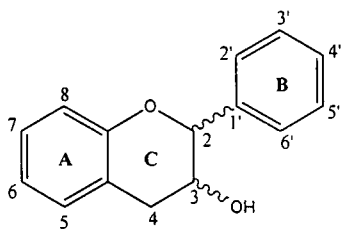

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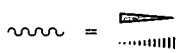


Table 2.1.1

Monomer	3	5	7	8	3'	4'	5'
Cassiaflavan	H	H	OH	H	H	OH	H
Apigeniflavan	H	OH	OH	H	H	OH	H
Luteoliflavan	H	OH	OH	H	OH	OH	H
Tricetiflavan	H	OH	OH	H	OH	OH	OH

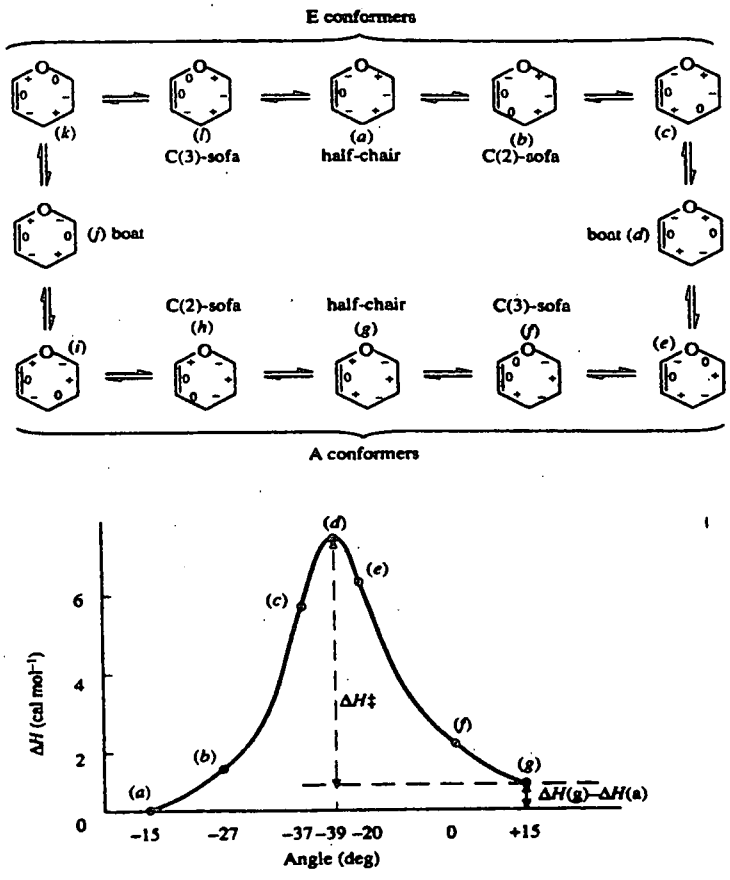
[10] L. J. Porter, in *The Flavonoids: Advances in Research since 1986*, (ed. J. B. Harborne), Chapman and Hall, London, **1993**, p. 23. and references therein.

Table 2.1.2

Monomer	3	5	7	8	3'	4'	5'
Afzelechin	OH	OH	OH	H	H	OH	H
Catechin	OH	OH	OH	H	OH	OH	H
Gallocatechin	OH	OH	OH	H	OH	OH	OH
Guibourtinidol	OH	H	OH	H	H	OH	H
Fisetinidol	OH	H	OH	H	OH	OH	H
Prosopin	OH	H	OH	OH	OH	OH	H
Oritin	OH	H	OH	OH	H	OH	H

The C-ring of the flavan is conformationally labile and can adopt a number of conformations shown in Figure 2.1. Here the E and A conformers are those with the orientation of the B-ring equatorial and axial respectively. MM2 molecular calculations[11] on the conformation of the C –ring shows that it preferentially adopts the E-conformer in a half-chair with various degrees of distortion, most frequently towards a C (2)-sofa[12].

Figure 2.1.

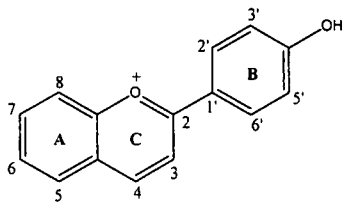


[11] V. N. Viswanadhan, W. L Mattice, *J. Comput. Chem.*, **1986**, *7*, 711.
[12] V. N. Viswanadhan, W. L Mattice, *J. Chem Soc., Perkin Trans. II*, **1987**, 739.

Flavans substituted on the heterocyclic ring (3 and 4-positions, e.g. catechin) are frequently encountered in nature, but the unsubstituted flavans have rarely been found due, presumably, to their instability in solution leading to polymeric products[13]. Flavan glycosides also rarely occur in the plant kingdom.[14]

2.2 Anthocyanidins

Anthocyanins are water-soluble glycosides and acylglycosides of anthocyanidins, which are polyhydroxy and polymethoxy derivatives of 2-phenylbenzo-pyrylium (flavylium cation)[15] (e.g. 3). They belong to the phenolic class of flavonoids with the typical A-ring benzoyl and B-ring hydroxycinnamoyl systems, with the carbon numbering system shown in **(3)**. There are almost 300 known naturally occurring structures.



(3)

Besides the basic flavylium cation **(3)**, the 'primary structure', anthocyanins occur in aqueous acidic solution as 'secondary structures', a mixture of the quinonoidal base(s), the carbinol pseudobase and the chalcone pseudobase[16]. In addition, there are four possible stabilization mechanisms leading to 'tertiary structures', such as self-association, inter- and intramolecular copigmentation, and metal complex formation.[17]

With a few exceptions, e.g. the betalain, anthocyanins are the most important group of water-soluble plant pigments visible to the human eye. They are universal plant colorants and largely responsible for the cyanic colors of flower petals and fruits[18]. They may also occur in roots, stems, leaves and bracts, accumulating in the vacuoles[19] of epidermal or

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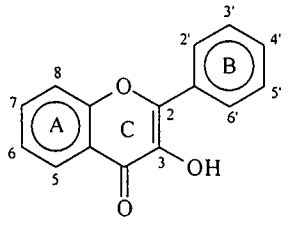
[13] S. Kulwant, S. Ghosal, *Phytochemistry* **1984,** *23 no.11*, 2415.
[14] I. Kubo, M Kim, *Tetrahedron Letters*, **1987**, *28 no. 9*, 921.
[15] D. Strack, V Wray in *The Flavonoids: Advances in Research since 1986*, (ed. J.B. Harborne), Chapman and Hall, London, **1993**, p.6.
[16] R. Brouillard, in *Anthocyanins as Food Colors*, ed. P. Marakakis, Academic Press, N.Y., pp. 1-40.
[17] D. Strack, V. Wray in *The Flavonoids: Advances in Research since 1986*, (ed. J. B. Harborne), Chapman and Hall, London, **1993**, p.7.
[18] D. Strack, V Wray in *The Flavonoids: Advances in Research since 1986*, (ed. J. B. Harborne), Chapman and Hall, London, **1993**, p.1.
[19] Wagner, G. J., in *Cellular and Subcellular Localization in Plant Metabolism* (eds L. L. Creasy and G. Hrazdina), Recent Advances in *Phytochemistry*, vol 16, Plenum Press, N.Y., **1982**, pp. 1-45.

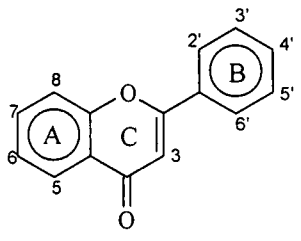
sub-epidermal cells. The anthocyanins are usually in solution within the vacuole, although they may sometimes be located in spherical vesicles, called 'anthocyanoplasts'[20]

2.3 Flavones and Flavonols

Flavonols (flavon-3-ols) **(4),** only differ from flavones **(5)** with respect to the presence of a 3-hydroxy group. Though the properties of the two classes are fairly similar, this small structural difference is of considerable biosynthetic, physiological, chemosystematic, pharmacological and analytical significance[21]. Respectively 380 and 300 flavonols and flavones with various hydroxy and / or methoxy substitution are known and also that more selective (methyl or monoglycoside) *O*-substitution exist in flavones than in flavonols[22]. Flavones occur as anthocyanin co-pigments to produce the characteristic purple-blue colour found mostly in higher plant species, responsible for bee attraction and pollination[23]. To increase solubility polyhydroxylated flavones and flavonols occur as glycosides rather than aglycones.[22]



(4)



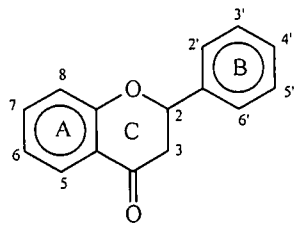
(5)

2.4 Flavanones

Flavanones are one of the minor types of flavonoids. The flavanones (2,3-dihydroflavones) **(6)** have a stereo centre at C-2, and therefore can assume the 2(*R*) or

[20] R. C. Pecket, C. J. Small, *Phytochemistry*, **1980**, *19*, 2571.
[21] E. Wollenheber, in *The Flavonoids: Advances in Research since 1986,* (ed. J. B. Harborne), Chapman and Hall, London, **1993,** 259 and the references there in.
[22] E. Wollenheber, in *The Flavonoids: Advances in Research since 1986,* (ed. J. B. Harborne), Chapman and Hall, London, **1993,** 260 and the references there in.
[23] J. B. Harborne, C. A. Williams, Advances in flavonoid research since 1992 Review, *Phytochemistry* 55, **2000**, 482/3.

2(*S*) configuration, although almost all flavanones exist as the 2(*S*) enantiomer[24]. Flavanones display a fairly general distribution but occur most abundantly in angiosperm families such as *Rosaceae, Rutaceae, Leguminosae, Ericaceae* and *Citrus*[25,26]. Recently, microbial sources such as *streptomyces*[27] have been found to produce flavanones.
Again flavanones are commonly associated with the presence of sugars / methoxy groups to facilitate solubility in an aqueous environment[28].



(6)

2.5 Isoflavones

The isoflavonoids are biogenetically related to the flavonoids but constitute a distinctly separate class in that they contain a rearranged skeleton and may be regarded as derivatives of 3-phenylchroman (**7**).[29] The enzyme(s) responsible for this biochemical rearrangement would appear to be rather specialized, since isoflavonoids have a very limited distribution, being confined essentially to the subfamily Papilionoideae (Lotoideae) of the Leguminosae.[27] Other sources include monocotyledons (Iridaceae family), *Iris* species, two gymnosperm genera and a moss *(Bryum capillare)*. Non-plant sources include a marine coral *(Echinopora lamellosa),* and several microbial cultures, although in most cases the presence of the isoflavonoid can be traced to the food source (in microorganisms and mammals)[30]. Though isoflavonoid distribution in the plant

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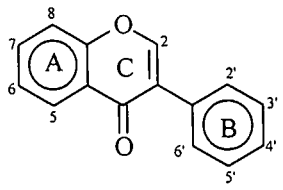
[24] B. A. Bohm, in *The Flavonoids,* (eds. J. B. Harborne, T. J. Mabry, and H. Mabry), Chapman and Hall, London, **1975,** 561.
[25] R. F. Albach and G. H. Redman, *Phytochemistry,* **1969,** *8*, 127.
[26] M. Nishura, S. Kamiya, S. Esaki and F. Ito, *Agric, Biol, Chem.*, **1971,** *35*, 1683.
[27] O. Nakayama, M. Yagi, M. Tanaka, S. Kiyoto, I. Uchida, M. Hashimoto, M. Okuhara and M. Kohsaka, *J. Antibiot.*, **1990,** *43*, 1394.
[28] B.A. Bohm in *The Flavonoid: Advances in Research since 1986*, (ed. J.B. Harborne), Chapman and Hall, London, **1993**, p.406-418.
[29] P. M. Dewick, in *The Flavonoids: Advances in Research* (ed. J. B. Harborne, T. J. Mabry), Chapman and Hall, London, **1982,** 535.
[30] P. M. Dewick, in *The Flavonoids: Advances in Research since 1986* (ed. J. B. Harborne), Chapman and Hall, London, **1993,** 117.

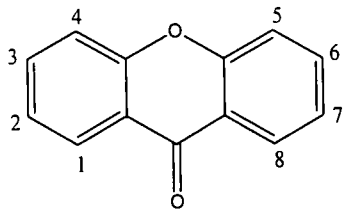
kingdom is very limited, they have a large structural variation[31] based on various oxygenation patterns of the aromatic rings and the state of oxidation of the heterocyclic C-ring.



(7)

2.6 Xanthones

The term xanthone refers to dibenzo-γ-pyrone-type compounds **(8)** with a $C_6C_1C_6$ carbon skeleton. All xanthones have a hydroxy group at position 1 or 8 and a resorcinol or phloroglucinol nucleus as one component.



(8)

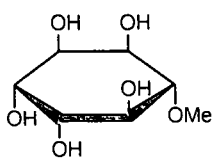
As the other aromatic component, the majority of xanthones have a quinol or hydroxyquinol nucleus and thereby differ markedly from all the related groups of pyrones, e.g. coumarins or flavones[31]. Although the xanthone structure is fairly simple, a large variation of oxygenated derivatives, including methyl ethers occur in nature[32]. While xanthones have been found in plants and in fungi - (one in a lichen) - they are not

[31] H. Grisebach, in *Recent Developments in the Chemistry of Natural Phenolic Compounds*, (ed. W. D. Ollis), Pergamon Press, Oxford, **1961**, 59.
[32] J. C. Roberts, *Chem. Rev.*, **1961**, *61*, 591.

numerous, and the parent pyrans, the xanthenes, are not as yet known to occur in nature. With the exception of mangostin, which carries two isoprenoid side chains, jacareubin, which is a chromene, and sterigma tocystin, which has a unique structure, the xanthones are not complex and vary only in the number and disposition of hydroxy or methoxy substituents[33]. These rare plant metabolites have been found in higher plants such as mangiferin from the mango tree *Mangifera indica,* and mangostin, the major pigment of the mangosteen tree, *Garcinia mangostana L.* (Family *Guttiferae,)*[34]. Their presence in the fungi (Ravenelin produced by *Helminthosporium ravenelli* Curtius and *H. Turcicum* Passerini)[35] has been established. Lichexanthone has been isolated from the Lichen, *Parnielia Fornzosana*[36].

2.7. Pinitol

Pinitol belongs to the acyclic polyalcohols known as cyclitols. (+)-Pinitol ("sennite or matezite") has long been known as the monomethyl ether of D-inositol with structural formula **(9)**[37,35]. It has been thought of as a secondary plant source because of the presence of a methoxy group. Inositol glucosides that are known to occur naturally include gallactinol, mannositose, and other inositol mannosides[34]. Among all the cyclitols, inositols (hexahydroxycyclohexanes) and their methyl ethers are the most abundant. Nine stereoisomeric forms **(10-19)** of inositols are known to exist. Seven of the inositols have a plane of symmetry. The two without a center of symmetry are (+)-inositol and (-)-inositol **(17,18)**, occur naturally.



(9)

Naturally occurring cyclitols have the generic name *"inositol"*. Eight of these are

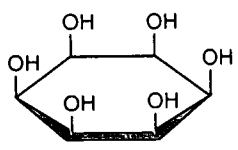
[33] F. M. Dean, *Naturally occurring oxygen ring compounds*, **1963**, 266.
[34] M. Sumb, H. J. Idris, A. Jefferson and F. Scheinmann, *J. Chem. Soc., Perkin Trans. 1*, **1977**, 2158.
[35] H. Raistrick, R. Robinson and D. E. White, *Biochem. J.*, **1936**, *30*, 1303.
[36] Y. Asahina and H. Nogami, *Bull. Chem. Soc. Japan*, **1942**, *17*, 202.

[37] T. Posternak, *The Cyclitols*, Holden-Day, Inc., Publishers, U.S.A. **1965**.

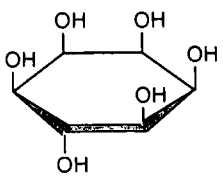
[35] C. D. Foxall and J. W. W. Morgan, *J.Chem, Soc.*, **1963**, 5573.

distinguishable by the prefixes, *allo, epi, myo, muco, cis, neo, dextro* and *laevo,* the ninth being named scyllitol[37].

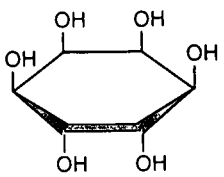
O-methyl derivatives of the inositols are frequently encountered in plants, with D-pinitol as the most widely distributed inositol ether. Berthelot's first discovery of the compound in the gymnosperm family has led to isolations from various species, *inter alia Picea abies, Pinus nigra, Pinus halepensis* and *Schinus molle.* Pinitol is also found among angiosperms, e.g. *Acacia mearnsii.* The wide distribution of pinitol in plants has been demonstrated by the work of Plouvier[37].



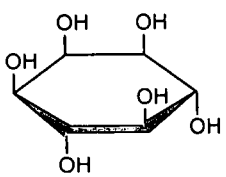
(10) Cisinositol



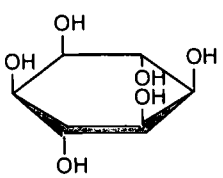
(11) Epinositol



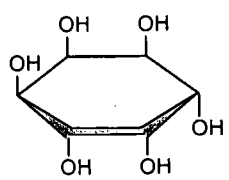
(12) Alloinositol



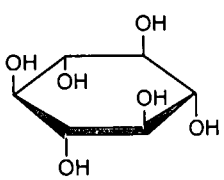
(13) Myolnositol



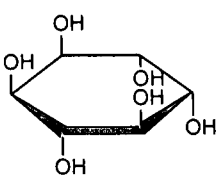
(14) Mucoinositol



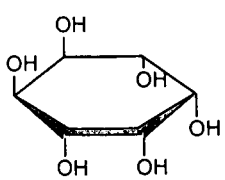
(15) Neoinositol



(16) Scyllitol



(17) (+)-Inositol



(18) (-)-Inositol

CHAPTER 3

O- Glycosides

3.1 Introduction

Flavonoids, which are found abundantly in plants, may play a role in reducing the risk of chronic diseases such as cardiovascular disease and cancer[38, 39, 40]. They exist in nature almost exclusively as β-glycosides. The flavonols are found mainly as the 3-*O*-glycoside, although the 7- and 4-positions may also be glycosylated in some plants, e.g. onions[41]. Other classes of flavonoids, such as the flavones, flavanones and isoflavones, are found mainly glycosylated in the 7-position[42]. Structural variation among the flavonoid glycosides lie both in the nature of the sugar residue (glucose, fructose, etc.) as well as the position and orientation (α,β) of attachment *via* the hydroxy groups to the aglycone. Due to the vast difference in the concentrations (0.001% to 20%) of these glycosides of the plant dry weight, the minor constituents are often overlooked due to insufficient material for full identification.

3.2 Structure and occurrence

3.2.1 Flavone and flavonol glycosides

Flavonoids occur mostly in *O*-glycosidic combinations with a number of sugars such as glucose, galactose, rhamnose, arabinose, xylose and rutinose[43]. Flavonoids carrying sugar moieties and their acylated and sulphated derivatives are all termed 'glycosides'. At least

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[38] Middleton, E. and Kandaswami, C. in *The Flavonoids: Advances in Research since 1986*, (ed. J. B. Harborne), Chapman and Hall, London, **1994**, 619.
[39] M-T. Huang, and T. Ferraro, in Phenolic Compounds in Foods and their Effect on Health II, (Eds. M-T. Huang, C. Ho and C.Y. Lee), *American Chemical Society*, Washington, DC, **1992**, 8.
[40] N. Salah, N.J. Miller, G. Paganga, L. Tijburg, G.P. Bolwell, and Rice-Evans, *C. Arch. Biochem. Biophys.* **1995**, *322*, 339-346.
[41] Fossen, T., Pedersen, A. T. and Anderson, O.M., *Phytochemistry* **1998**, *47*, 281.
[42] Harborne, J.B., Mabry, T .J. and Mabry, H. (1975) *The Flavonoids*, Chapman and Hall, London.
[43] U. Justesen , P. Knuthsen, T. Leth, *Journal of Chromatography A*, **1998**, 101

2500 different flavone and flavonol glycosides have been reported[44] 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[45], exist. 36 Glycosides of isoprenylated flavonols have been reported[36]. Flavonol glucosides with all the hydroxy groups of the glucose unit substituted by acyl groups change the solubility properties of the flavonol glucoside, converting it into a hydrophobic substance[46]. These glucosides occur in the cytoplasm or epidermal cells of the leaf and are known to have fungitoxic properties[47,48]. Variations of flavones in which a glycosylated acylating group is directly linked to the flavone for example apigenin 7-(2"-glucosyllactate)[49] as well as with the acylating acid linked *via* a glycosidic unit [7-(6"-crotonylglucoside)][50] have been isolated.

Rare glycosides, for example 6,8-dimethoxyluteolin-3'-methyl ether (sudachiin D), linked to 3-hydroxy-3-methyl glutaric acid, *via* glucose units attached to the 7- and 4'-positions has been isolated from the green peel of *Citrus sidachi* [51]. Glycosylation and *O*-methylation of flavones and flavonols increase the lipophilic character and polyhydroxylated flavones and flavonols occur as such glycosides rather than the aglycone[52].

3.2.2 Flavan Glycosides

Three natural flavan *O*-glycosides are known as viscutin-1,2 and 3 **(21,22,23)**[53], and are found in twigs of *Viscum tuberculatum*[54].

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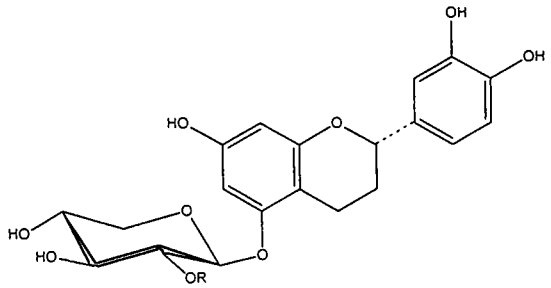
[44] C.A. Williams, J.B Harborne, in *The Flavonoids: Advances in Research since 1986* (ed. J. B. Harborne), Chapman and Hall, London, **1993,** 337.
[45] J. B. Harborne and C. A. Williams, in *The Flavonoids*, **1975,** (eds J. B. Harborne, T. J. Mabry and H. Mabry), Chapman and Hall, London, 376.
[46] G. Romussi, G. Bignardi, C. Pizza and N. De Tommasi, *Arch. Pharm.*, **1991**, *324*, 519.
[47] K. R. Markham, A. Franke, B. P. J. Molloy and R. F. Webby, *Phytochemistry*, **1990**, *29*, 501.
[48] B. L. Cui, J. Kinjo, M. Nakamura and T. Nohara, *Tetrahedron Lett.*, **1991**, *32*, 6135.
[49] M. A. M. Nawwar, H. I. El-Sissi and H. B. Barakat, *Phytochemistry*, **1984,** *23*, 2937.
[50] M. P. Yuldashev, E. Kh. Batirov, A. D. Vdovin, V. M. Malikov and M. R. Yagudaev, *Khim. Prir. Soedin*, **1989**, 352.
[51] T. Horie, M. Tsukayama, Y. Toshihide, I. Miura and M. Nakayama, *Phytochemistry*, **1986,** *25*, 2621.
[52] O. R. Gottlieb, in *The Flavonoids*, **1975,** (eds J. B. Harborne, T. J. Mabry and H. Mabry), Chapman and Hall, London, 297.
[53] L.J. Porter, in *The Flavonoids: Advances in Research since 1986* (eds J. B. Harborne), Chapman and Hall, London, **1993**, 27.
[54] I. Kubo et.al., *Tett. Lett.*, **1987**, *28 no. 9*, 921.



(21) R= p- Hydroxybenzoyl
(22) R= Caffeoyl
(23) R= H

3.3 Identification

In the separation and purification of glycosides, paper[50], thin layer[50] and column chromatography[50,55] have been employed. Spectral methods such as UV, IR, MS and NMR have played a prominent role in glycoside identification, although traditional chemical methods such as acid and enzyme hydrolysis[56], R_f values and colour properties, selective methylation of phenolic hydroxy groups and periodate oxidation[23] have been successful in the identification of glycosides. UV spectral analysis is of primary importance in the determination of the position of substitution of the sugars on the aglycone. When very small amounts of material are available, IR,[57,58] is also used.

Novel techniques such as centrifugal partition chromatography (CPC) in conjunction with HPLC have been used in purification. Before spectral analysis flavonol glycosides are often purified by gel filtration on Sephadex LH 20. Hiermann[59] claims better results if Fractogel PGM 2000 is used instead of Sephadex.

The increase in the number of reports of new glycosides, is largely due to the advances in methods of separation e.g. the excellent resolution of closely related structures by HPLC and the more prominent use of ^{1}H and ^{13}C NMR spectroscopy for glycoside identification. Mass spectrometry has played an important role and continues to be explored as a means of structural elucidation. While fast atom bombardment mass

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[55]K. M. Johnston, D. J. Stern and A. C. Waiss, *J. Chromatogr.*, **1968**, *33*, 539.
[56]C. W. Glennie and J. B. Harborne, *Phytochemistry*, **1971**, *10*, 1325.
[57]L. Jurd, in *The Chemistry of Flavonoid Compounds*, (ed. T. A. Geissman), Pergamon Press, Oxford, **1962**, 107-155.
[58]H. Wagner, in *Methods in Polyphenol Chemistry*, (ed. J. B. Pridham), Pergamon, Oxford, **1963**, 37-48.
[59]A. Hiermann, *J. Chromatogr.*, **1986**, *362*, 152.

spectrometry (FAB-MS) is used by most researchers to obtain a strong molecular-ion peak which clearly indicates the number and type of sugar units present, Sakushima *et al.*[60] have proposed desorption chemical ionisation mass spectrometry (DCI-MS) as an alternative for analysing the sugar units as well as the presence of 1→6 linked diglycosides such as robinobiosides, gentiobiosides and rutinosides. ^{1}H NMR spectroscopy is widely used for structural analysis and is valuable for the identification of more complex derivatives[61] such as trimethyl silyl[60] and methyl ethers or acetals.

[60]A. Sakushima, S. Nishibe and H. Brandenberger, *Biomed. Environ. Mass. Spectrom.*, **1989**, *18*, 809.
[61]T. J. Mabry, K. R. Markham and M. B. Thomas, *The Systematic Identification of Flavonoids*, **1970**, Springer-Verlag, Berlin .

CHAPTER 4

FLAVONOID *O*-GLYCOSIDIC UNITS

4.1 Introduction

Paper chromatography and gas chromatography of trimethylsilyl derivatives as well as ^{1}H and ^{13}C NMR spectroscopy are commonly used for the identification of the monosaccharides of flavonoid-*O*-glycosides. Oligosaccharide linkages are commonly detected with FAB-MS and ^{13}C NMR spectroscopy[62].

4.2 Monosaccharides

The monosaccharides (**Table 4.1**) are most commonly found in *O*-glycosidic combination with flavone and flavonol aglycones.

Table 4.1 Monosaccharides of Flavone and Flavonols

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 usually assume the pyranose form,[63] although the less stable furanose form has been reported occasionally [64]. The D-sugars, glucose, galactose,

[62] 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.
[63]H. E. Khadam and Y. S. Mohammed, *J. Chem. Soc.*, **1958,** 3320.
[64]Z. P. Pakudina and A. S. Sadykov, *Khim. Prir. Soedin,* **1970,** *6,* 27.

glucuronic acid and xylose are usually β-linked to the hydroxy group of the aglycone while the L-sugars, rhamnose and arabinose are normally α-linked. However α- and β-linked 3-arabinosides of quercetin have been reported[65,66]. Both kaempferol 3-α- and 3-β-glycosides are present in the flowers of *Alcea nudiflora*[67]. The most uncommon sugar associated with flavones is apiose, a branched chain pentose.

4.3 Disaccharides

Harborne *et.al* describes the combination of the disaccharide units as pentose-pentose, hexose-pentose, hexose-hexose, pentose-uroglucoronic acid and uroglucoronic acid-uroglucoronic acid. Rutinose (6-*O*-α-L-rhamnosyl-D-glucose) *e.g* quercetin-3-rutinoside[52], is the most common disaccharide in plants with two different sugar units. The number of known allose-containing glycosides have increased in recent years with flavones bearing allosyl-(1$\rightarrow$2) glycosides fairly common in the family Labiatae. They have also been found in *Teucrium, Sideritis,* and *Stachys* genus[68].

4.4 Trisaccharides

The trisaccharides of flavones and flavonols have been assigned to two groups, linear and branched, mainly by FAB-MS and ^{13}C NMR spectroscopy. More linear trisaccharides are known than the branched sugars. The trisaccharide, glucosyl-(1$\rightarrow$3)-rhamnosyl-(1$\rightarrow$6)-glucose, has been found attached to the 3-position of quercetin and kaempferol in the leaves of the tea plant Teacceace (*Camellia sinensis*)[69]. Some of the novel branched trisaccharides are apiosyl-(1$\rightarrow$2)-[rhamnosyl-(1$\rightarrow$6)-galactose] attached to the 3-position of kaempferol[70,71], and glucosyl-(1$\rightarrow$6)-[apiosyl-(1$\rightarrow$2)-glucose] attached to palutetin in the same position[72]. Other glucose combinations are based on the glucose units of, galactose and rhamnose[73, 74, 75].

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[65]T. A. Geissman, in *The Chemistry of Flavanoid Compounds*, **1962,** Pergamon Press, Oxford.
[66]V. I. Glyzin and A. I. Bankoviskii, *Khim. Prir. Soedin.*, **1971,** *7*, 662.
[67]Z. P. Pakudina, V. B. Leontiev and F. G. Kamaev, *Khim. Prir. Soedin*, **1970**, *6*, 555.
[68] J. B. Harborne and C. A. Williams in *The Flavonoids: Advances in Research 1980,* (eds. J. B. Harborne), Chapman and Hall, London, **1988,** 306.
[69]A. Finger, U. H. Engelhardt and V. Wray, *Phytochemistry*, **1991**, *30*, 2057.
[70]F. De Simone, A. Dini, C. Pizza, P. Saturnino and O. Schettino, *Phytochemistry,* **1990,** 3690.
[71]A. Bashir, M. Hamburger, M. P. Gupta, P. N. Solis and K. Hostettmann, *Phytochemistry,* **1991,** *30,* 3781.
[72]M. Aritomi, T. Komori and T. Kawasaki, *Phytochemistry*, **1986,** *25,* 231.
[73]M. A. M. Nawwar, A. M. D. El-Mousallamy and H. H. Barakat, *Phytochemistry*, **1989,** *28,* 1755.
[74]J. A. Marco, J. Adell, O. Barbera. D. Strack and V. Wray, *Phytochemistry*, **1989**, *28,* 1513.

4.5 Tetrasaccharides.

Although no linear tetrasaccharides have been reported so far, a branched tetrasaccharide acetylated at the 6'''-position of the saphorase, rhamnosyl-(1→4)-glucosyl-(1→6)saphorase, was found attached *via* the 7-hydroxy of tacocetin [76]. UV and ^{1}H NMR analyses were used for structure elucidation, following acid hydrolysis to yield the free sugar moiety, and the position of the sugar linkage determined by ^{13}C NMR spectroscopy.

4.6 Acylated derivatives

Both flavone and flavonol glycosides occur in acylated form with acids such as *p*-coumaric[77], caffeic[78], sinapic[79], ferulic[80], gallic[81], benzoic[82], acetic[83] and malonic[84] 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[4]. Most new reports view acetic acid as acylating agent of the sugar units (16 flavones and 44 new flavonol derivatives) [4]. The difficulties encountered with PC and TLC procedures to detect the acetic acid which is volatile and the acetyl groups which are labile by mild acid hydrolysis have been overcome by the application of FAB-MS and ^{13}C NMR techniques. Hence, new acylated flavonoids such as a tri-acetate, kaempferol-3-(2''',3''',5'''-triacetyl)-arabinofuranosyl-(1→6)-glucoside, from flowers of *Calluna vulgaris* (Ericaceae)[85] and two tetra-acylated glycosides of kaempferol with two acetyl and two *p*-coumaroyl units on the same glucose residue have been characterized[55].

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[75]T. Sekine, J. Arita, A. Yamaguchi, K. Saito, S. Okonogi, N. Morisaki, S. Iwasaki and I. Murakoshi, *Phytochemistry,* **1991,** *30,* 991.
[76]A. A. Ahmed and N. A. M. Saleh, *J. Nat. Prod.*, **1987,** *50,* 256.
[77]C. Karl, G. Muller and P. A. Pedersen, *Phytochemistry*, **1976**, *15*, 1084.
[78]E. V. Gella, G. V. Makarova and T. G. Borisyuk *Farmatsert. Zh. (Kiev)*, **1967**, *22,* 80.
[79]B. Stengel and H. Geiger, *Z. Naturforsch.*, **1976**, *31*, 622.
[80]K. R. Markham, H. D. Zinsmeister and R. Mues, *Phytochemistry*, **1978**, *17*, 1601.
[81]F. W. Collins, B. A. Bohm and C. K. Wilkins, *Phytochemistry*, **1975,** *14*, 1099.
[82]I. Sconsiegel, K. Egger and M. Keil, *Z. Naturforsch.*, **1969**, *24*, 1213.
[83]C. Radaelli, L. Fotmentin and E. Santaniello, *Phytochemistry*, **1980**, *19,* 985.
[84]M. Woeldecke and K. Herrmann, *Z. Naturforsch.*, **1974,** *29,* 355.
[85]D. P. Allias, A. Simon, B. Bennini, A. J. Chulia, M. Kaouadji and C. Delage, *Phytochemistry*, **1991,** *30,* 3099.

The malonate derivatives (five flavones and two flavonols) identified, include the 5-(6''-malonylglycosides) of apigenin, genkwanin and luteolin[86] and kaempferol-3-apiosylmalonyl glycosides[87].

4.7 Sulphate conjugates

The number of known flavone and flavonol sulphates add up to approximately 80[48]. The known flavone sulphate conjugates include the 6-hydroxyluteolin and the 6,7-disulphates of 6-hydroxy luteolin and nodiflorentin[88], while examples of flavonols include the 3'-sulphate and 3-glucoronide-3'-sulphate quercetin [89] and the 3,3'-disulphates of quercetin and patuletin[90]. The syntheses of 23 structures have been used for their structural elucidation[91]. In the syntheses of specifically sulphated flavonoids a novel method using *N,N'*-dicyclohexyl-carbodiimide (DCC) and tetrabutylammonium hydrogensulphate (TBAHS) in dimethylformamide have been used.

[86]M. Viet, H. Greiger, F. -C. Czygan and K. R. Markham, *Phytochemistry*, **1990**, *29*, 2555.
[87]B. Wald, V. Wray, R. Galensa and K. Herrmann, *Phytochemistry*, **1989**, *28*, 663.
[88]F. A. Thomäs-Barberän, J. B. Harborne and R. Self, *Phytochemistry*, **1987**, *26*, 2281.
[89]R. M. Seabra and C. Elves, *Phytochemistry*, **1991**, *30*, 1344.
[90]D. Barron and R. K. Ibrahim *Phytochemistry*, **1987**, *26*, 1181.
[91]D. Barron and R. K. Ibrahim *Phytochemistry*, **1988**, *27*, 2362.

CHAPTER 5

Biosynthesis

5.1 Flavones, Flavanones, Flavan-3-ols, and other flavonoids

Both flavonoid precursors (4-coumaroyl-CoA **(32)** and malonyl-CoA) are derived from carbohydrates. Malonyl-CoA is synthesized from the glycolysis intermediate, acetyl-CoA, and carbon dioxide, by acetyl-CoA carboxylase. The formation of 4-coumaroyl-CoA involves the shikimate/arogenate pathway, the main route to the aromatic amino acids phenylalanine and tyrosine in higher plants[92]. Subsequent transformation of phenylalanine to trans-cinnamate is catalyzed by phenylalanine ammonia-lyase, which provides the link between primary metabolism and the phenylpropanoid pathway. Aromatic hydroxylation of cinnamate by cinnamate 4-hydroxylase leads to 4-coumarate, which is further transformed to 4-coumaroyl-CoA by the action of 4-coumarate CoA ligase[93]. (**Scheme 5.1**)

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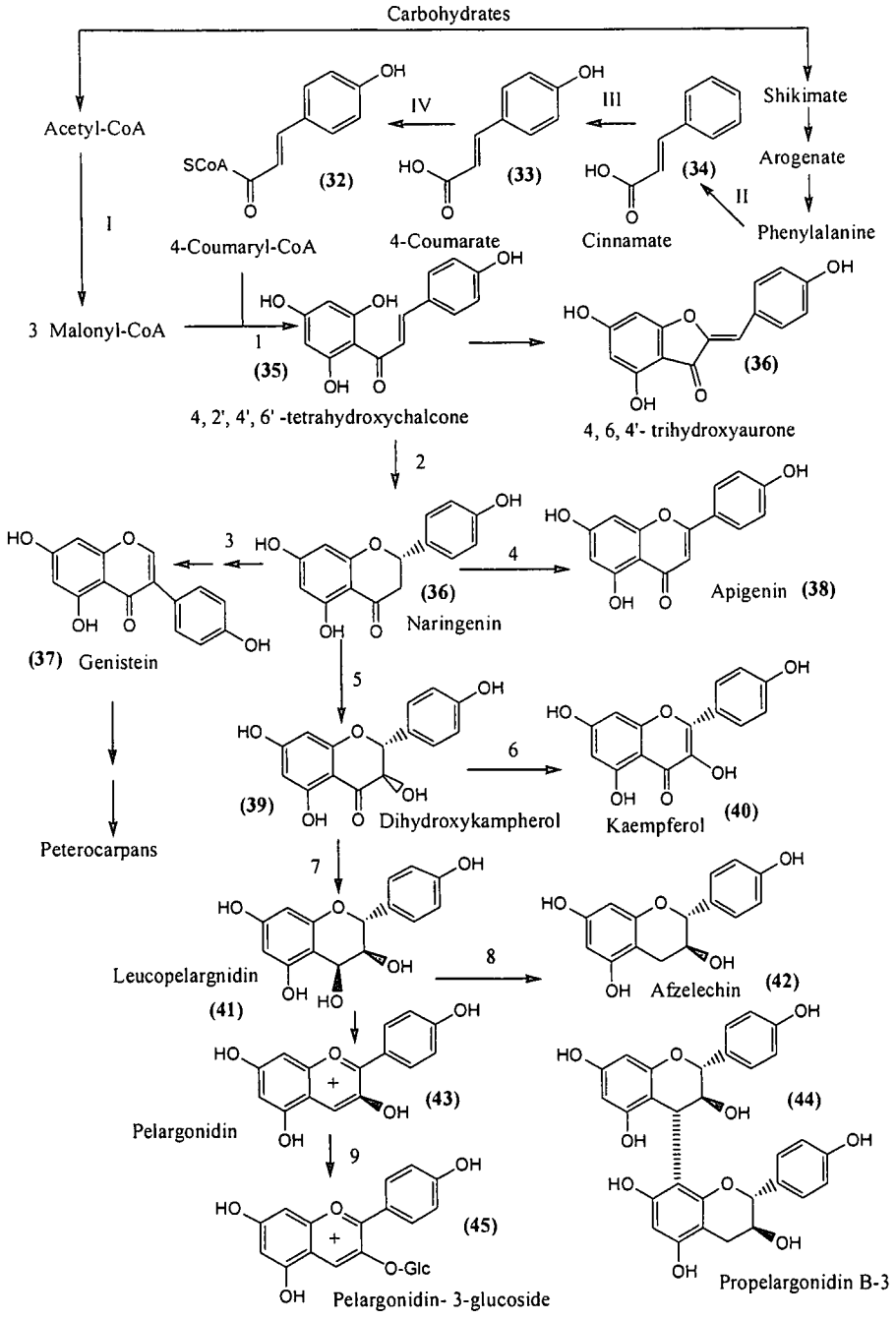
[93] W. Heller and G. Forkmann, in *The Flavonoids: Advances in Research 1980,* (eds. J. B. Harborne), Chapman and Hall, London, **1988,** 399

Scheme 5.1



Enzymes for reactions in Scheme 5.1

Non-flavonoid precursors

I Acetyl-CoA carboxylase

II Phenylalanine ammonia-lyase

III Cinnamate 4-hydroxylase

IV 4-Coumarate: CoA ligase

Flavonoid Enzymes

1 Chalcone synthase
2 Chalcone isomerase
3 2-Hydroxyisoflavone synthase
4 Flavone synthase
5 (2*S*)-Flavanone 3-hydroxylase
6 Flavonol synthase
7 Dihydroflavonol 4-reductase
8 Flavan-3, 4-cis-diol 4-reductase
9 Anthocyanidin/ flavonol 3-*O*-glucosyltransferase

The tetrahydroxychalcone intermediate **(35)** is formed by the condensation of three molecules of malonyl-CoA with a suitable hydroxycinnamic acid CoA ester, normally 4-coumaroyl-CoA, and is catalysed by chalcone synthase. Flavonoids, aurones and other diphenylpropanoids are derived from the chalcone intermediate. Transformation by stereo selective action of chalcone isomerase provides the flavonoid, (2*S*)-flavanone (naringenin) **(36)**. Oxidative rearrangement of the flavanone, involving a 2,3-aryl shift, which is catalyzed by 'isoflavone synthase' yields an isoflavone (genistein) **(37)**. The oxidation of the flavanone leads to the abundant flavones (apigenin) **(38)**, and is catalyzed by two enzymes, a dioxygenase and a mixed-function mono-oxygenase[94]. Dihydroflavonols (dihydrokaempferol) **(39)** are formed by α-hydroxylation of flavanones. This reaction is catalyzed by flavanone 3-hydroxylase. Dihydroflavonols are intermediates in the formation of flavonols, catechins, proanthocyanidins and anthocyanidins[99]. The large class of flavonoids, the flavonols (e.g. kaempferol)**(40)** are formed by the oxidation of the C-2,3 bond of dihydroflavonols and is catalyzed by flavonol synthase. Reduction of the carbonyl group of dihydroflavonols in the 4-position gives rise to flavan-2,3-*trans*-3,4-*cis-diols* (leucopelargonidin) **(41)**. Leucoanthocyanidins, are the immediate precursors for flavan-3-ols and proanthocyanidins. These e.g. **(42)** are synthesized from leucoanthocyanidins by action of flavan 3,4-*cis-diol* reductase. Proanthocyanidins (propelargonidin B-3) **(44)** are formed by the condensation of flavan-3-ols and leucoanthocyanidins. The reaction steps from leucoanthocyanidins to anthocyanidins (pelargonidin) **(43)** are still unknown but an essential reaction in the sequence is glycosylation, usually glucosylation, in the 3-position

[94] W. Heller and G. Forkmann, in *The Flavonoids: Advances in Research 1980,* (eds. J. B. Harborne), Chapman and Hall, London, **1988,** 401.

of the anthocyanidin or of a suitable intermediate. This reaction leads to the first stable anthocyanin (e.g. pelargonidin-3-glucoside)**(45)**[99]. Hydroxylation and methylation of the A- and in particular the B-ring hydroxy groups, as well as glycosylation and acylation reactions result in the great diversity of flavonoids found in nature. Numerous enzymes catalyzing these modifications have been described, some of which can act on both intermediates (flavanone or dihydroflavonol) and end products (flavone, isoflavone, flavonol or anthocyanidin-3-glucoside), others only on the end products.

CHAPTER 6

C-Glycosylflavonoids

6.1 Introduction

The *C*-glycosylflavonoids are quite common in plants and more than 300 have been described. Glycosyl residues include β-,α-D-glucopyranosyl, β-D-galactopyranosyl, β-D-xylopyranosyl, α-,β-L-arbinopyranosyl, α-L-rhamnopyranosyl[95], 6-deoxy-*xylo*-hexos-4-ulosyl, β-L-fucopyranosyl, α-D-mannopyranosyl, β-D-oliopyranosyl, β-L boivinopyranosyl, β-D-chinovopyranosyl and D-apiopyranosyl[96]. They occur in 4 groups; mono-*C*-glycosyl-, di-*C*-glycosyl-, *O*-glycosyl-*C*-glycosyl- or as the *O*-acyl-*C*-glycosyl-flavonoid derivatives[97].

Sources of flavone-*C*-glycosides are *V. lucens* (heartwood), *Castanospermum australe* (wood)[98], and *Zelkowa serrata* (wood)[99]. Chalcone-*C*-glycosides have been isolated from *Cladrastis shikokiana* (leaf), isoflavone-*C*-glucosides from *Dalbergia paniculata* (seed, bark), isoflavanone-*C*-glycosides from *Dalbergia paniculata* (flower) and flavanol-*C*-glycosides from *Cinnamomum cassia* (bark)[100].

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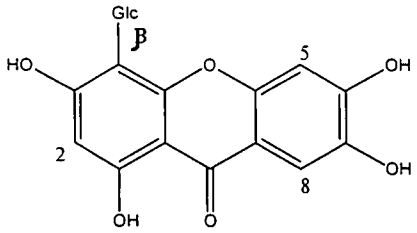
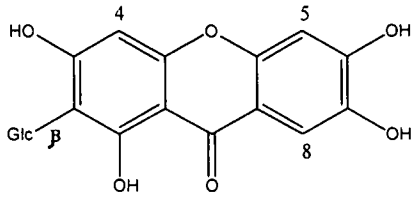
[95] J. Chopin, G. Dellamonica, in *The Flavonoids: Advances in Research since 1980* (ed. J. B. Harborne), Chapman and Hall, London, **1988,** 63.
[96] M. Jay, in *The Flavonoids: Advances in Research since 1986* (eds J. B. Harborne), Chapman and Hall, London, **1993**, 64.
[97] M. Jay, in *The Flavonoids: Advances in Research since 1986* (eds J. B. Harborne), Chapman and Hall, London, **1993**, 63.
[98] J.B Harborne, in the *Natural Products of Woody Plants I*; (ed, J. W. Rowe), Springer-Verlag, Berlin, **1990**, 537.
[99] J.B Harborne, in the *Natural Products of Woody Plants I*; (ed, J. W. Rowe), Springer-Verlag, Berlin, **1990**, 541.
[100] J. Chopin, G. Dellamonica, in *The Flavonoids: Advances in Research since 1980* (ed. J. B. Harborne), Chapman and Hall, London, **1988,** 70-71 and references there in.

Few xanthone glycosides are known and all are difficult to hydrolyse to the aglycones[101]. More *O*-glycosylated xanthones are known than *C*-glycosylated analogues. Two examples of naturally occurring *C*-glycosides include mangiferin and isomangiferin **(20)**[102]



(19)



(20)

Sources of Mangiferin include *Gyrinops walla*[103] and *Mangifera indica*[104].

6.2 Synthesis of *C*-glycosylflavonoids

The high-yield *C*-glucosylation of 1,3,5-trimethoxy benzene with tetra-*O*-acetyl-α-D-glucosyl bromide in the synthesis of 4,5,7,-tri-*O*-methylvitexin[105] has not yet been repeated in the synthesis of other 4,5,7-tri-O-methyl-8-*C*-glycosylapigenins. However, the reaction of 1,3,5-trimethoxybenzene with tetra-*O*-acetyl-α-D-galactopyranosyl bromide, triacetyl-α-D-xylopyranosyl bromide, tri-*O*-acetyl-β-L-arabinopyranosyl bromide and tri-*O*-acetyl-α-L-rhamnopyranosyl bromide has successfully been employed by Chari (unpublished) to synthesize the corresponding l-(2',4',6'-trimethoxyphenyl)-1,5-anhydroalditols for ^{13}C NMR spectroscopy.[106] A synthesis of 7,4'-di-*O*-methylisobayin (6-*C*-β-D-glucopyranosyl-7,4'-dimethoxyflavone) has been described[107] (Scheme 6.1) and involves the reaction between 2,4-dimethoxyphenylmagnesium bromide **(46)** and 2,3,4,6-tetra-*O*-benzylglucopyranosyl chloride **(47)** to yield 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl-2,4-dimethoxybenzene **(48)** which was converted to the tetra-acetate **(49)** after debenzylation.

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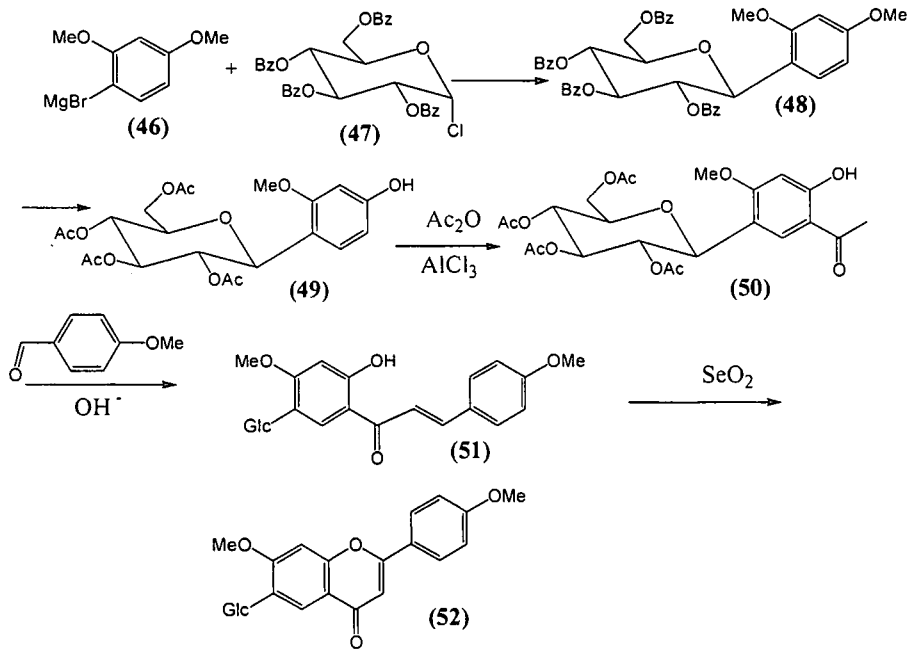
[101] F. M. Dean, *Naturally occurring oxygen ring compounds*, **1963**, 268.
[102] F. M. Dean, *Naturally occurring oxygen ring compounds*, **1963**, 275.
[103] Y. Schun and G. A. Cordell, *J. of Nat. Prod.*, **1985**.*48*, 684.
[104] N. A.M Saleh and M.A.I El-Ansari, *Planta Med.*, **1975**, *28*, 124.
[105] R. A. Eade and H. P. Pham, *Aust. J. Chem.*, **1979**, *32*, 2483.
[106] K. R. Markham and V. M. Chari, in *The Flavonoids: Advances in Research* (eds. J. B. Harborne, T. J. Mabry), Chapman and Hall, London, **1982**, 19.
[107] R. Tschesche and W. Widera, *Liebigs. Ann. Chem.*, **1982**, 902.

Scheme 6.1



The acylation of the tetra-acetate gave 5-β-D-glucopyranosyl-2-hydroxy-4-methoxyacetophenone tetra-acetate **(50)**. Condensation of the latter with 4-methoxybenzaldehyde in alkaline medium gave 5'-β-D-glucopyranosyl-2'-hydroxy-4,4'-dimethoxychalcone **(51)** which reacts with selenium dioxide to give 7,4', di-*O*-methylisobayin **(52)**.

Many different methodologies exist for *C*-glycosylation and considerable progress has been made in this regard (table 6.1).

Table 6.1.

6-*C*-Glycosylflavones	
6-*C*-α-D-Arabinopyranosylapigenin	Dubois, unpublished
6-*C*-α-L-Arabinopyranosylacacetin	Besson and Chopin (1983)
6-*C*-α-L-Arabinofuranosylacacetin	Besson and Chopin (1983)
6-*C*-β-D-Glucopyranosyl-4-*O*-methyltricin	Lardy *et al.* (1983)
6-*C*-β-D-Galactopyranosyl-4-*O*-methyltricin	Lardy *et al.* (1983)
6-G-α-D-Xylopyranosyl-4-*O*-methyltricin	Lardy *et al.* (1983)
6-*C*-α-L-Arabinopyranosyl-4-*O*-methyltricin	Lardy *et al.* (1983)
6-*C*-α-L-Rhamnopyranosyl-4-*O*-methyltricin	Lardy *et al.* (1983)
6-*C*-Glycosylflavonols	
6-*C*-β-D-Galactopyranosylquercetin	Rasolojaona and Mastagli (1985)
6-*C*-β-D-Xylopyranosylquercetin	Rasoiojaona and Mastagli (1985)
6-*C*-α-L-Arabinopyranosylquercetin	Rasolojaona and Mastagli (1985)
C-Glycosylflavanols	
6-*C*-β-D-Glucopyranosyl-(-)-epicatechin	Morimoto *et al.* (1986)
8-*C*-β-D-Glucopyranosyl-(-)-epicatechin	Morimoto *et al.* (1986)
6,8-di-*C*-glycosylflavones	
6,8-Di-*C*-β-D-Glucopyranosyl-4-*O*-methyltricin	Lardy *et al.* (1983)
6,8-Di-*C*-β-D-Xylopyranosyl-4-*O*-methyltricin	Lardy *et al.* (1983)
6,8-Di-*C*-α-L-Arabinopyranosyl-4-*O*-methyltricin	Lardy *et al.* (1983)
6,8-Di-*C*-α-L-Rhamnopyranosyl-4-*O*-methyltricin	Lardy *et al.* (1983)
6-*C*-β-D-Galactopyranosylvitexin	Dubois *et al.* (1984)
6-*C*-β-L-Arabinofuranosylcytisoside	Besson and Chopin, unpublished
6-*C*-Diglycosyl-8-*C*-glycosylflavone	
6-*C*-Cellobiosyl-8-*C*-glucosylacacetin	Bouillant *et al.* (1984)

References

E. Besson, J. Chopin, *Phytochemistry*, **1983**, 22, 2051.
M. L. Bouillant, M. L. Ferreres, *et al.*, *Phytochemistry*, **1984**, 23, 2653.
M. A. Dubois. A. Zoll, *et al.*, *Phytochemistry*, **1984**, 23, 706.
C. Lardy, J. Chopin, *et al, Phytochemistry*, **1983**, 22, 2571.
S. Morimoto, G. Nonaka, *et al.*, *Chem. Pharm. Bull.*, **1986**, 34, 633.
L. Rasolojaona, P. Mastagli, *Carbohydr. Res.*, **1985**, 143, 246.

6.3 Identification

Nuclear magnetic resonance spectroscopy

^{1}H and ^{13}C NMR spectroscopy are classically used to assign the *C*-glycosyl, *O*-glycosyl or *O*-acyl to the 6- or 8-position, and to indicate the configuration of the glycosidic linkage, with respect to the anomeric proton. The distinction between *C*-6 and *C*-8 for the position of the sugar moiety has been made, partly on the basis of chromatographic comparison, partly on the chemical shift changes induced in aromatic protons when phenolic hydroxy groups are acetylated, and, finally, partly on the fragmentation patterns of the methylated derivative in EI-MS[108].

Since the 5-methoxy group occurs at the most downfield position in a polymethoxylated flavone, 6-*C*-boivinosyl-chrysoeriol (alternanthin)[109] this signal could easily be irradiated, resulting in a highly significant NOE association between the methoxyl protons and anomeric proton (1"-H) when the sugar residue is attached at C-6.

^{1}H and ^{13}C NMR spectra for certain 8-*C*-glycosylflavones exhibit extensive doubling of signals as in the case of tricetin-6,8-di-*C*-glycoside where two signals are noted for 2-C (164.3, 164.9), 5-C (158.9, 160.2), 3-H (6.57, 6.54) and 5-OH (13.77, 13.69)[110]. This phenomenon was observed in almost all compounds containing an R-*C*-hexosyl substituent (vitexin, vitexin-7-*O*-glucoside, tricetin-6,8-di-*C*-glucoside, lucenin-2, tricetin-6-*C*-arabinosyl-8-*C*-glucoside and stellarin-2). In contrast, no doubling of the signals were found in compounds without an 8-*C*-hexosyl residue. The spectra of vitexin-2"-*O*-rhamnoside and orientin-2"-*O*-glucoside showed only doubtful doubling. These observations suggested that in flavones, interaction occurs between a *C*-linked monohexose at C-8 and the B-ring. Since the 8-*C*-pentopyranosides do not exhibit this feature, the primary hydroxy group of the hexose would appear to be the functional group interacting with the B-ring. This would result in restricted rotation of the B-ring and/or the hexose, giving rise to a mixture of two rotamers, which are distinguishable by NMR[111]. An additional sugar at the 2"-position complicates this situation by apparently locking the 8-*C*-hexosyl unit in a position that hinders its interaction with the B-ring. Likewise, signal doubling was not observed in the spectra of 8-*C*-hexosides in which the

[108] M. Jay, in *The Flavonoids: Advances in Research since 1986* (eds J. B. Harborne), Chapman and Hall, London, **1993**, 85.
[109] B. N Zhou, G. Blasko, *et al.*, *Phytochemistry*, **1988**, *27*, 3633.
[110] K. R. Markham, R. Mues, *et al.*, *Z. Naturforsch*, **1987**, *42c*, 562.
[111] M. Jay, in *The Flavonoid: Advances in Research since 1986*; Harborne, J. B. Ed.; Chapman and Hall: London, **1993**; p 85.

B-ring is moved away from possible steric interaction with the sugar moiety, as in the 8-*C*-glycosyl-isoflavones.

The existence of such rotamers was confirmed for lucenin-2 and stallarin-2 where duplicated signals were observed at 25°C, which disappeared completely at 90°C[112].

6.4 Biological Properties

Co-pigmentation

It was shown that the color of delphinidin 3-*O*-(p-coumaroyl)-rhamnosylgalactosyl-5-*O*-glycoside was significantly affected by *C*-glycosylflavones as co-pigments[112]. Flavone co-pigmentation increased the absorption wavelength and was responsible for the purple color of the flower petals.[113]

C-Glycosylflavones and ultraviolet light screening

A study were carried out on 17 species of the pondweed genus *Potamogeton* [114] several *C*-glycosylflavones were found in the floating foliage of species with both submerged and floating foliage. With respect to a hypothesis regarding the potentially important evolutionary role of flavonoids as a UV light screen, *C*-glycosylflavones would be synthesized in floating leaves because of their filtering ability; the lack of these compounds in submerged leaves would be attributable to the ability of naturally colored water to absorb UV radiation significantly. These results seem to support an earlier hypothesis suggesting the importance of flavonoid evolution in the conquest by plants of exposed terrestrial habitats[113].

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[112] S. Asen, *et al.*, *Phytochemistry*, **1986**, *25*, 2509.
[113] M. Jay, in *The Flavonoids: Advances in Research since 1986*; Harborne, J. B. Ed.; Chapman and Hall: London, **1993**, 86.
[114] D. Les, D. J. Sheridan, *Am. J. Bot.*, **1990**, *77*, 453.

C-Clycosylflavones and medicinal properties

Flavones isolated from *Citrus*[115,116] have been actively studied for their hypotensive effects. Four compounds were tested, 3,8-di-*C*-glucosylapigenin, 3,8-di-*C*-glucosyldiosmetin, 2"-*O*-xylosylvitexin and vicenin-2. The results showed that the latter two compounds were strongly hypotensive, while the first two were inactive.

[115] H. Kumamoto, Y. Matsubara, *et al.*, *Agric. Biol. Chem.*, **1986**, *50*, 781.
[116] Y. Matsubara, *et al.*, *Yoshishu*, **1985**, *27*, 702.

CHAPTER 7

Biological significance of Flavonoids

7.1 Introduction

Since flavonoids have survived evolution it might be justified to assume that they perform essential physiological functions, at least in plants. Szent Györgyi argued that flavonoids might also be essential for man, similar to vitamins. This suggestion could not be substantiated, but the investigations of Szent Györgyi performed on vitamins at the same time initiated and promoted the use of flavonoids as drugs. One major reason for the skepticism in accepting bioflavonoids as drugs might be their ubiquitous occurrence in the plant kingdom and their presence in vegetables, fruits, spices, e.g. in our daily nutrition[117].

Thus the question was posed whether a class of compounds of which large amounts are ingested daily in food could be recognized as a drug. A second reason might be due to the polyphenolic character of many flavonoids, which means the possibility of multiple interactions with proteins on the cell surface, receptors and enzymes[118]. Such multiple interactions suggest unspecific reactions with various body functions in line with the numerous biological activities described for flavonoids.

Another characteristic property of most phenolic compounds after oral intake is their rapid conjugation with glucuronic acid or sulfuric acid, which results in a very fast inactivation and elimination rate. As a consequence the amount of flavonoids to be ingested has to be extremely high (grams/diet) to have them in sufficient blood concentration for their bioavailability. Nevertheless the average daily diet of humans contains about 1 g of flavonoids, which is high enough to bring the flavonoid concentration to a pharmacological significant level in tissues[119].

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[117] E. Middleton, *Pharmaceutical News*, **1994**, *1*, 6-8.
[118] C.M. Spencer, Y. Cai, R. Martin, *et al, Phytochemistry*, **1988**, *27*, 2397-2409.
[119] D. K. Das, *Methods Enzymol*, **1994**, *234*, 410-420.

7.2. Antioxidant activity of flavonoids

Flavonoids have been shown to act as scavengers of various oxidizing species i.e. superoxide anion (O_2^-), hydroxyl radical or peroxy radicals. They may also act as quenchers of singlet oxygen. Often an overall antioxidant effect is observed. However, an improved method has been developed to compare the antioxidant activity of selected flavonoids[117] from different classes by measuring the quantum yields of sensitized photo-oxidation of individual flavonoids. This was coupled with the determination of photo-oxidation based on measuring the singlet oxygen luminescence. It was concluded that the presence of a catechol moiety in the B-ring is the main factor controlling the efficiency of O_2^- physical quenching. The presence of a 3-OH likewise contributes to the efficiency of their chemical reactivity with O_2^- , but the catechol moiety is generally more prominent[118].

A carbonyl group at C-4 and a double bond between C-2 and C-3 are also important features for high antioxidant activity in flavonoids[119]. Butein and other 3,4-dihydroxychalcones are more active than analogous flavones because of their ability to achieve greater electron delocalisation[120]. Similarly, isoflavones are often more active than flavones due to the stabilizing effects of the 4-carbonyl and 5-OH in the former[123]. In the antioxidant action of *ortho*-dihydroxyflavonoids metal chelation becomes an important factor[121].

7.3 Antimicrobial activity of flavonoids

One of the functions of flavonoids and related polyphenols is their role in protecting plants against microbial invasion. This not only involves their presence in plants as essential agents but also their function as phytoalexins in response to microbial attack[122,123]. Because of their widespread ability to inhibit spore germination of plant pathogens, they have also been proposed for use against fungal pathogens of man. There

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[117] C. Tournaire and S. Croux, *Journal of Photochemistry and Photobiology*, **1993**, *19*, 205.
[118] J. B. Harborne, C. A. Williams, *Phytochemistry*, **2000**, 55, 490.
[119] N. P. Das, T. A. Pereira, *Journal of American Oil Chemists Society*, **1990**, *67*, 255.
[120] S. Z. Dziedzic, B. J. F. Hudson, *Food Chemistry*, 1983, *11*, 161.

[121] F. Shahidi, P. Wanasundara, C. Hong, *American Chemical Society*, **1991**, 214
[122] R. J. Grayer, J. B. Harborne, E. M. Kimmins, F.C. Stevenson, H. N. P. Wijayagunasekera, *Acta Horticulturae*, **1994**, *381*, 691.
[123] J. B. Harborne, *Biochemical Systematics and Ecology*, **1999**, *27*, 335.

is an ever-increasing interest in plant flavonoids for treating human diseases and especially for controlling the immunodeficiency virus, the cause of AIDS[124].

7.4 Inhibition of enzymes by Flavonoids.

Flavonoids have been tested for their ability to inhibit key enzymes in mitochondrial respiration. It was found that a C-2,3-double bond, a C-4-keto group and a 3',4',5'-trihydroxy B-ring are significant features of those flavonoids which show strong inhibition of NADH-oxidase[125]. The order of inhibition for the molecules tested was, robinetin, rhamnetin, eupatorin, baicalein, 7,8-dihydroxyflavone and norwogonin.

It was also shown that flavonoids with adjacent trihydroxy or *para*-dihydroxy groups exhibited a substantial rate of auto-oxidation, which was accelerated by the addition of cyanide[128].

Some flavonoids also inhibit the enzyme xanthine oxidase, which catalyses the oxidation of xanthine and hypoxanthine to uric acid. During the re-oxidation of xanthine oxidase both superoxide radicals and hydrogen peroxide are produced. It was found that flavones showed higher inhibitory activity than flavonols and that hydroxy groups at both C-3 and C-3' were essential for high superoxide scavenging activity[126].

7.5 Dietary antioxidant flavonoids and coronary heart disease

Flavonoids are naturally present in fruits, vegetables, tea and wine and was shown to inhibit oxidation of low-density-lipo protein (LDL) *in vitro*. In such studies it was found that the phenolic constituents of red wine inhibits the copper-catalyzed oxidation of LDL. It was shown that 10 mol/l of quercetin has the same antioxidant activity as red wine diluted 1000 times (10 mol/l of phenolics), during the inhibition of LDL oxidation[127]. Catechin is the major flavonoid constituent of red wine with a concentration of 190 mg/l. Others include: gallic acid (95 mg/l), epicatechin (82 mg/l), malvidin 3-glucoside (24

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[124] J. B. Harborne, C. A. Williams, *Phytochemistry*, **2000**, *55*, 487.
[125] W. F. Hodnick, D. L. Duval, R. S. Pardini, *Biochemical Pharmacology*, **1994**, *47*, 573.

[126] P. Cos, L. Ying, M. Calomme, J. P. Hu, K. K. Cimanga, B. Van Poel, L. Pieters, A. J. Vlietinck, D. Van den, Berghe, *Journal of Natural Products*, **1998**, *61*, 71.
[127] E. N. Frankel, J. Kanner, J. B. German, E. Parks and J. E. Kinsella,, *The Lancet*, **1993** *341*, 454.

mg/l), rutin (9 mg/l), myricetin (8 mg/l), quercetin (8 mg/l), caffeic acid (7 mg/l), cyanidin (3 mg/l) and resveratrol (1.5 mg/l)[128]. The three major inhibitors of LDL-oxidation were epicatechin, quercetin and resveratrol. However, epicatechin and quercetin had twice the antioxidant potency of resveratrol[131], but the latter was also found in much lower concentrations. Another study using pure quercetin glucosides indicated that the presence of a glucose moiety was important in increasing the rate and extent of absorption in man[129]. The role of dietary antioxidant flavonoids in protection against coronary heart disease has been widely reviewed by Leake (1997)[130].

7.6 Flavonoids with anti-inflammatory activity

Kaempferol showed good activity against Croton oil-induced dermatitis in mouse ear but, this was dramatically reduced by glucosylation at the 3-hydroxy (astragalin). The addition of a *p*-coumaroyl group to the sugar at 6'' increased the activity 8 times, while addition of another *p*-coumaroyl group at 2'' gave an activity 30 times greater than that of astragalin. Astragalin-2'',4''-di-*p*-coumarate had a potency between that of indomethacin and hydrocortisone[131].

Three anthocyanins and their aglycone, cyanidin, were tested for their ability to inhibit prostaglandin endoperoxide hydrogen synthase-1 and -2 (PGHS-1 and -2), because of their association with the alleviation of arthritic pain and gout[132]. The glycosides showed little or no activity at a concentration of 300 mM and higher concentrations actually increased the activity of the enzymes. However, the aglycone, cyanidin, showed significant inhibitory activity against both enzymes with IC_{50} values of 90 and 60 mM, respectively compared with 1050 mM for aspirin. Ulcerogenic and adverse properties of non-steroidal anti-inflammatory drugs are attributable to the inhibition of PGHS-1, whereas the beneficial therapeutic effects result from the inhibition of PGHS-2. Thus, a

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[128] E. N. Frankel, A. L. Waterhouse and J.E. Kinsella, *The Lancet*, **1993**, *342*, 1103.

[129] P. C. H. Hollman, M. N. C. P. Buijsman, Y. van Gameren, P. J. Cnossen, J. H. M. de Vries and M. B. Katan, *Free Radical Research*, **1999**, *31*, Iss. 6, 569.

[130] D. S. Leake, (Eds.) F. A. Tomas-Bar-beran and R. J. Robins, *Phytochemistry of Fruit and Vegetables*, **1997**, 287.

[131] J. B. Harborne and C. A. Williams, *Phytochemistry*, **2000**, *55*, 493.

[132] H. Wang, M.G. Nair, G. M. Strasburg, Y. C. Chang, A. M. Booren, J. I. Gray and D. L. Dewitt, *Journal of Natural Products*, **1999**, *62*, 294.

strong preferential inhibition of PGHS-2, as exhibited by cyanidin, is desirable to reduce the adverse effects of the inhibition of PGHS-1[135].

7.7 Cytotoxic antitumor activities of flavonoids

From *Ononis natrix* ssp. *ramosissima* (Leguminosae)[133] 4,2',6'-trihydroxy-4'-methoxydihydrochalcone, 2',6'-dihydroxy-4'-methoxydihydrochalcone and 2',4'-diacetoxychalcone were identified as having moderate activity against murine leukemia, human non-small cell lung cancer and human colon cancer. However, the most active compound was 2',6'-diacetoxy-4,4'-dimethoxydihydrochalcone, which showed selective activity for the cell line murine leukemia. The chalcone, pedicin (2',5'-dihydroxy-3',4', 6'-trimethoxychalcone), from leaves of *Fissistigma languinosum* (Annonaceae), was found to inhibit tubulin assembly into microtubules[134].

The isoflavone, genistein, a plant oestrogen in Soya bean, was shown to block the action of a transcription factor, known as CCAAT binding factor, neutralizing it before it is activated, so that the cancer cell starves and dies. Genistein, commonly consumed as a component of Soya bean, is a flavonoid, which stops cancer growth and angiogenesis. It has no harmful effects on normal healthy cells[135]. Other studies on the flavonoids of tea draw attention to the relatively large concentrations of catechins (flavan-3-ols) and especially of epigallocatechin 3-gallate in tea. Human cancers need proteolytic enzymes to invade cells and form metastases. One such enzyme is urokinase. Inhibition of urokinase in mice decreases tumor size and can even lead to complete cancer remission. Epigallocatechin 3-gallate acts by binding to urokinase blocking histidine 57 and serine 195 at the catalytic site. Although it is a weaker urokinase inhibitor than the synthetic drug amiloride, epigallocatechin 3-gallate is normally consumed by man in a relatively high concentration. A single cup of tea contains about 150 mg epigallocatechin 3-gallate whereas the maximum tolerated dose of amiloride is 20 mg a day. Hence epigallocatechin 3-gallate in tea through its inhibitory action on urokinase could be an important dietary

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[133] A. F. Barrero, M. M. Herrador, P. Arteaga, E. Cabrera, I. Rodriguez-Garcia, M. Garcia-Moreno, D. G. Gravalos, *Fitoterapia*, **1997**, *68*, 281.
[134] Y. Alias, K. Awang, H. A. Hadi, O. Thoison, *et al*, *Journal of Natural Products*, **1995**, *58*, 1160.
[135] A. Coghlan, *New Scientist*, 14 March, **1998**, 14.

constituent for reducing human cancers[136]. Epigallocatechin 3-gallate in tea is capable of suppressing angiogenesis, a key process of blood vessel growth required for tumor growth and metastasis. The growth of all solid tumors depends on angiogenesis, and thus may explain why drinking tea is a useful preventative for avoiding the growth of many human cancers[137].

[136] J. Jankun, S. H. Selman, R. Swieroz and E. S. Jankun, *Nature*, **1997**, *387*, 561.
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DISCUSSION

CHAPTER 8

PHENOLIC COMPOUNDS FROM *CYCLOPIA SUBTERNATA* (Honeybush Tea)

8.1 Introduction

Epidemiological data suggest that more than 80% of cancers are attributable to lifestyle, of which at least one third is diet-related[138]. Diets containing substances that can inhibit or prevent cancer may, thus, play a role in the general health of humans, especially if they are inexpensive and easily attainable. Tea is a widely consumed beverage throughout the world while the popularity of herbal health teas have increased significantly during the past 20 years[139]. The antitumor properties of tea are well known, and the tumor inhibition potential of certain polyphenolic compounds from green and black tea has been well documented[140, 141, 142, 143, 144]. Both rooibos tea *(Aspalathus linearis)* and honey-bush tea *(Cyclopia intermedia)*, two South African herbal teas, have been shown to contain a complex mixture of polyphenolic compounds of which aspalathin, a dihydrochalcone, is unique to rooibos tea while luteolin is one of the most important flavones in honeybush tea[145, 146, 147].

It can be argued that the herbal teas could protect against the activity of diverse mutagenic and possible carcinogenic compounds *in vivo* and their effectiveness, as potential chemo-preventive drugs, will depend on the mechanism of cancer development in a specific organ. It would, thus, appear that the herbal teas may not only be a good

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[140] M. Hirose, T. Hoshiya, K. Akagi, S. Takasi, Y. Hara, N. Ito, *Carcinogenesis*, **1993**, *14*, 1549.
[141] H. Mukhtar, S.K. Katiyar, R. Agarwal, *J. Invest. Dermatol.*, **1994**, *102*, 3.
[142] Z.Y. Wang, M.T. Huang, Y.R. Lou, J.G. Xie, K.R. Reuhl, H.L. Newmark, C.T. Ho, C.S. Yang, A.H. Conney, *Cancer Res.*, **1994**, *54*, 3428.
[143] C. Han, Y. Xu., *Biomed. Environ. Sci.*, **1990**, *3*, 35.
[144] Y. Xu, C. Han, *Biomed. Environ. Sci.*, **1990**, *3*, 406.
[145] D. Ferreira, B. I. Kamara, E. V. Brandt, E. Joubert, *J. Agric. Food Chem.*, **1998**, *46*, 3407.
[146] A. Von Gadow, E. Joubert, C. F. Hansmann, J. *Agric. Food Chem.*, **1997**, *45*, 632.
[147] C. Rabe, J.A. Steenkamp, E. Joubert, J.F.W. Burger, D.Ferreira, *Phytochemistry*, **1994**, *35*, 1559.

dietary source of natural antioxidants to counteract the damaging effects of free hydroxy, superoxide and peroxy radicals in vitro[148,149], but may also protect against mutagens[150]. This study accordingly presents the comprehensive isolation and structural elucidation of phenolic metabolites from *Cyclopia subternata* and has revealed the occurrence of isoflavones, flavanones, xanthones, flavones and a flavan among others.

Taxonomy of *C. subternata*:

Kingdom: Plantae

Division: Anthophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae (Leguminosae)

Genus: *Cyclopia*

Species: *subternata*

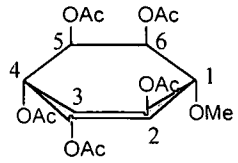
Common name: Honeybush tea

The leaves and stems of *Cyclopia subternata* were collected from the farm Biên Donne in the Western Cape, South Africa, and harvested and dried in January 2000. The dry, unfermented pulverized leaves and stems were extracted with various solvents to afford complex mixtures of polyphenolic compounds that were resolvable only after extensive enrichment and fractionation procedures. Due to the complexity of the mixtures the different fractions were derivatized to achieve an acceptable purity level. These fractionation and derivatization procedures prevented, however, reliable quantification of the constituents.

[148] V.L. Singleton, J.A. Rossi, *Am. J. Enol. Vitic.*, **1965**, *16*, 144.
[149] M.E. Hubbe, E. Joubert, *Food and Cancer Prevention III Conference*, Norwich, UK, September **1999**, 5.
[150] J.L. Marnewick *et al. Mutation Research*, **2000**, *471*, 157.

8.2 Non-aromatic compounds

Due to the high concentration of some compounds in the extracts they precipitated from solution at various stages before fractionation.

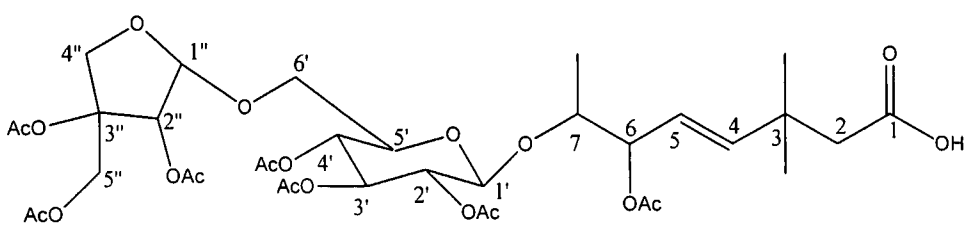


(53)

Penta-*O*-acetyl-(+)-pinitol (53), a cyclitol, precipitated from an ethanolic solution of the acetone extract and was identified as a white crystalline substance following acetylation and purification. ^{1}H NMR spectral data [plate 1 ($CDCl_3$) (298K)] are identical to that from the literature[151]. The multiplet at δ5.34 is assigned to H-1,4,6, two doublet of doublets at δ5.17 and δ5.12 are attributed to H-2,5 while the remaining doublet of doublets at δ3.64 is assigned to H-3. The methoxy group is correlated with the singlet at δ3.49 and five acetoxy groups with those at δ2.80, 2.82, 2.11, 2.65 and 2.09.

6-acetoxy-3,3-dimethyl-7-[*O*-α-2",3",4"-tri-*O*-acetylapiofuranosyl-(1"→6')-β-D-2',3',4'-tri-*O*-acetylglucopyranosyloxy]oct-4-enoic acid (54)

Fraction B3 of the methanol extract yielded **(54)** as a yellowish oily substance after acetylation and purification by PLC. ^{1}H NMR spectrum [plate 2 ($CDCl_3$) (298K)] shows a range of aliphatic protons between δ3.3 and δ6.0 with no aromatic protons, expected



(54)

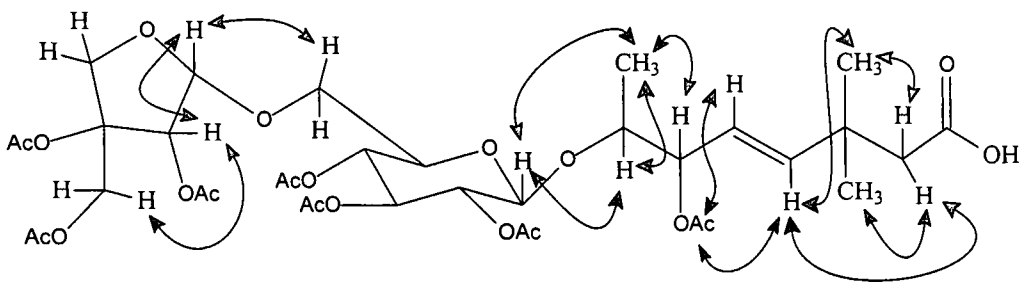
between δ6.0 and δ8.0. The seven acetoxy singlets (δ1.89, δ2.12, δ2.10, 3 x δ2.05 and δ2.01) together with the number of aliphatic protons suggest two sugar moieties and an

[151] C. Marais, J.A. Steenkamp, E. V. Brandt, *Struktuur en Sintese van Metaboliete uit Rooibostee (Aspalathus linearis). Fisiologiese Aktiwiteit en Biomimetiese Model vir die Fermentasieproses.***1995.** UOVS.

aliphatic chain. Three well defined up-field methyl groups resonate as singlets at δ1.02 and δ1.09 and a doublet (J = 6.5Hz) at δ 1.24. The glycosyl unit comprises of an apiofuranosyl ring with a C-1''-O-C-6' linkage to a glucopyranosyl ring, similar to an authentic sample[159]. The apiofuranosyl moiety is defined by the conspicuous 5-CH_2 (δ4.62 and δ4.72 d, J = 12Hz) and 4-CH_2 (δ4.15 and δ4.23, d, J = 12Hz) along with the *cis* coupling (J = 0.5Hz) between the H-1'' (δ5.38, d, J = 0.5Hz) and the H-2'' (δ5.01, d, J = 0.5Hz) in accord with the expected configuration of the ring. NOE experiment [plate 2b-1 (298K) ($CDCl_3$)] showing an association between H-1'' and H-2''and weak association between H-2'' and 5''-CH_2, further affirms the apiofuranosyl ring along with COSY experiment [plate2a-1 (298) ($CDCl_3$)]. The CH and CH_2 of H-1'', H-2'' and H-4'', H-5''are also confirmed by HMQC experiment [plate 2d-1 (298K) ($CDCl_3$)], and by DEPT 135° experiment [plate 2f-1 (298K) ($CDCl_3$)].

The C-1''-O-C-6'linkage of the apiofuranosyl to the glucopyranosyl ring is unambiguously confirmed by NOE association between H-1'' and 2 x H-6' (δ3.71, dd, J = 2HZ, 11Hz and δ3.55, dd, J = 6Hz, 11Hz) [plate 2b-1 (298K) ($CDCl_3$)], as well as long range coupling between the protons in COSY experiment [plate2a-1 (298) ($CDCl_3$)]. The glucopyranosyl moiety is defined by H-5' (δ3.60, m) overlapping 1 x H-6' (δ3.54), H-4', (δ5.02, t, J = 9.5Hz), H-3',2' (δ4.95, m) and H-1' (δ4.58, d, J = 8Hz (β-coupled to aliphatic chain).

The connectivity of the aliphatic chain is established by NOE [plate 2b-2 (298K) ($CDCl_3$)] between the anomeric proton H-1', H-7 (δ4.31,t, J = 6Hz), and the C-7-CH_3 (δ1.24, d, J = 6.5Hz).



(54b)

The connectivity of H-6,7 is confirmed by COSY experiment [plate2a-1 (298) ($CDCl_3$)] that shows correlation between H-7 and H-6 (δ 5.84, m overlapping H-4,5), which is affirmed by NOE between C-7-$C\underline{H}_3$ and H-6. HMQC experiment [plate 2d-2 (298K)

($CDCl_3$)] together with COSY experiment [plate 2a-2 (298K) ($CDCl_3$)] confirms the connectivity between H-7, H-6 and H-5,4 (δ5.78, m). HMBC experiment [plate2e-1 (298K) ($CDCl_3$)] also shows the correlation between H-7 and H-6. COSY experiment [plate 2a-3 (298K) ($CDCl_3$)] shows long distance coupling between C-6-OAc (δ1.89, s) and H-6, which designates the up-field acetoxy group to C-6. The same acetoxy group shows NOE to H-5 and H-4 [plate 2b-2 (298K) ($CDCl_3$)] and the latter two to each other. DEPT 135° confirms C-4,5,6,7 as C-H carbons [plate 2f-1(298K) ($CDCl_3$)].

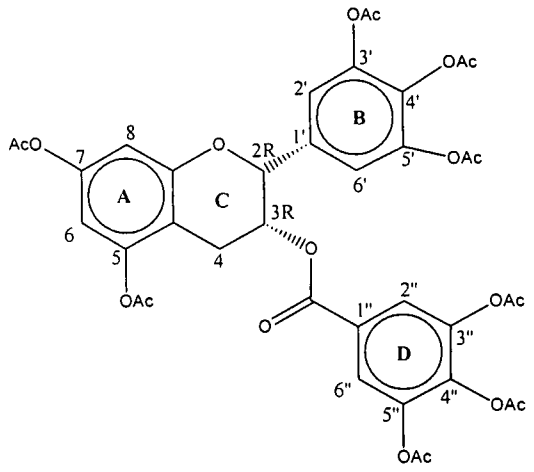
^1H NMR spectrum [plate 2 ($CDCl_3$) (298K)] shows no protons for C-3, but its position between C-4 and C-2 is shown by NOE between a C-3-$C\underline{H}_3$ (δ1.02, s) and H-4 as well as between 1 x H-2 (δ2.44, d, 17Hz) and H-4 [plate 2b-2 (298K) ($CDCl_3$)]. COSY experiment [plate 2a-4 (298K) ($CDCl_3$)] assigns the other 1 x H-2 (δ2.25, d, J = 17Hz) and also shows correlation to a C-3-$C\underline{H}_3$ (δ1.09, s). A deshielded carbonyl carbon at δ198 observed in ^{13}C NMR experiment [plate 2c-1 (298K) ($CDCl_3$)] and HMBC [plate2e-2 (298K) ($CDCl_3$)] showing strong correlation between the 2 x H-2 protons and the carbonyl carbon (C-1), suggests the position of the carboxylic group. Synthesis of (**54**) is envisaged to confirm the proposed structure.

8.3 Aglycones

8.3.1 Flavanol

The acetylation and purification by PLC of fraction A14 from the methanol extract, yielded **3',3",4',4",5,5',5",7-octa-*O*-acetyl-*epi*-catechin-3-*O*-gallate (55)** as a light-yellow powdery substance. The ^1H NMR spectral data [plate 3 ($CDCl_3$) (298K)] are identical to those in the literature[152]. The two-proton singlet at δ7.64 is assigned to H-2"(D) and H-6"(D), and that at δ7.25 is attributed to the H-2'(B) and H-6'(B). The doublets at δ6.75 and δ6.63 represents H-8(A) and H-6(A) respectively. The broadened multiplet at δ5.65 is assigned to H-3(C) of the flavonol unit. H-2(C) appears at δ5.20 as a broadened singlet due to the *cis*-coupling with H-3(C) (J = 2.0Hz) and benzylic coupling with the H-2',6'(B) protons. 4-CH_2(C) appears as a multiplet at δ3.05. The eight aliphatic acetoxy singlets occur between δ2.25 and δ2.31.

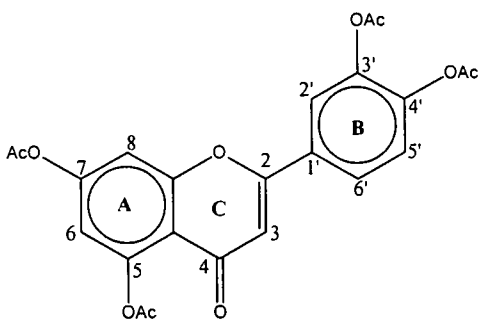
[152] A. E. Bradfield, M. Penny, *J. Chem. Soc.*, **1948**, 2249.



(55)

8.3.2 Flavone

Fraction A6 of the acetone extract yielded the flavone, **3',4',5,7-tetra-*O*-acetyl-luteolin (56)**, after acetylation and purification. The ^{1}H NMR of the compound [plate 4 ($CDCl_3$) (298K)] is identical to an authentic sample[153], and shows the characteristic heterocyclic proton, H-3(C), as a singlet at δ6.63. The ABX system of the B-ring is defined by the doublet of doublets [δ7.76, J = 2.0 and J = 8.0 Hz, H-6'(B)], and the associated doublets [δ7.72, J = 2.0 Hz, H-2'(B) and δ7.38, J = 8.0 Hz, H-5'(B)]. The AB system (J = 2.0 Hz) of the A-ring resonates at δ7.38 (H-6) and δ6.88 (H-8) respectively. The four acetoxy signals occur at δ2.35, δ2.37 x 2 and at δ2.46.

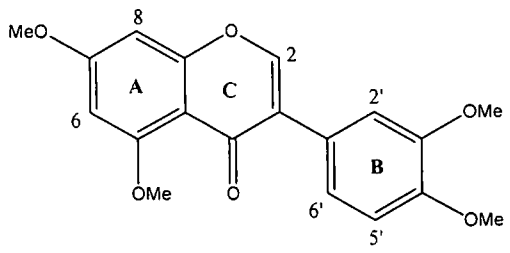


(56)

[153] J. B. Harborne, C. A. Williams and K. L. Wilson, *Phytochemistry*, **1985**, *24*, 751.

8.3.3 Isoflavone

Methylation followed by PLC purification of fraction A8 of the acetone extract yielded **3',4',5,7-tetra-*O*-methyl-orobol (57).**



(57)

The ^{1}H NMR spectra [plate 5 ($CDCl_3$) (298K)] of compound **(57)** notably show a sharp singlet, [δ7.81, H-2 (C)] characteristic of the isoflavone. The doublet of doublets [δ7.24, J = 2.0 and 8.0 Hz, H-6'(B)] and the associated doublets [δ7.15, J = 2.0 Hz, H-2'(B) and δ6.92, J = 8.0 Hz, H-5'(B)] define the ABX system of the B-ring.

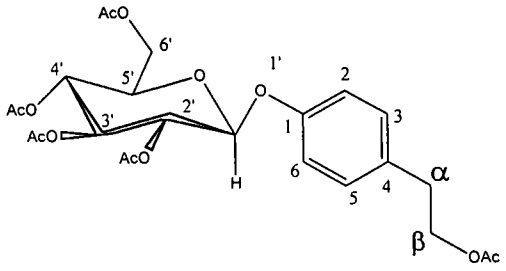
The AB-system of the A-ring, H-8 and H-6, appear at δ6.14 and δ5.99 (J = 2.0 Hz) respectively. Four singlets at δ3.96, δ3.95, δ3.94 and δ3.86 represent the methoxy groups. The spectrum is identical to that in the literature[154].

8.4 *O*-glucosides

4-(*O*-β-D-2',3',4',6'-tetra-*O*-acetylglucopyranosyl) tyrosol (58)

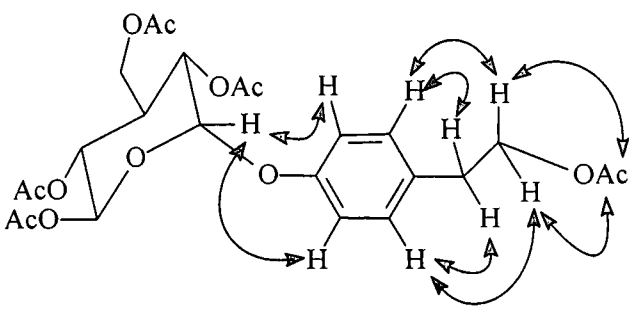
The free phenolic fraction A4 of the acetone extract was acetylated and purified by chromatographic methods (PLC) to yield compound **(58)**. The ^{1}H NMR spectrum [plate 6 ($CDCl_3$ - 298K)] displays two aromatic two proton doublets for the *ortho*-oriented pairs, H-2,6 and (δ 6.95, d, J = 8.0Hz) and H-3,5 (δ 7.16, d, J = 8.0Hz) attributed to a 1-4-di-substituted phenyl ring. The spectrum also shows two distinct upfield aliphatic triplets (δ 2.95, t, J = 7.0 Hz, and δ 4.25, t, J = 7.0, Hz), assigned to the two methylene groups (α-CH_2 and β-CH_2) respectively, which were identical with an authentic sample of the aglycone[158].

[154] K. Asres, *et al., Z. Naturforsch.*, **1985**, *40c*, 617.



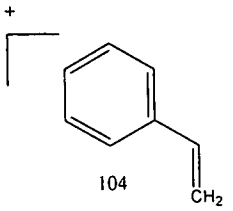
(58)

A COSY experiment [plate 6a-1 ($CDCl_3$ - 298K)] shows strong long distance coupling between the β-CH_2 and the CH_3 of the acetoxy group. NOESY experiments [plate 6b-1 ($CDCl_3$ - 298K)] confirm the position of attachment of the aliphatic chain to the phenyl ring by strong association between the aromatic protons H-3,5 and the α-CH_2 protons. A weak association between the same H-3,5 and the β-CH_2 is in line with the α- and β-positions of the two methylene groups. A strong association between the anomeric proton H-1' [δ5.07, d, 8.0Hz (β-coupled)] and the H-2,6 aromatic protons indicates the position of attachment of the sugar unit and also confirms the AA'BB' spin system of the phenyl ring.



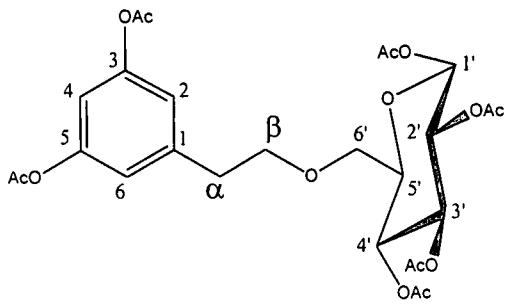
(58a)

A COSY spectrum [plate 6a-2 ($CDCl_3$ - 298K)] designates the remaining glucoside protons of H-2', 3' as the overlapping multiplet at δ5.30, H-4' (δ5.18, m), H-5' (δ3.87, m) and the 6-CH_2 as doublet of doublets at δ4.31 (J = 5.0, 12.0Hz) and at δ4.18 (J = 2.0, 12.0Hz). The five aliphatic acetoxy groups resonate as singlets at δ2.19, δ2.10, δ2.08, δ2.07, and δ2.05. The duplication of the aromatic and the sugar proton signals indicates slow rotation on the ^1H NMR time scale observed in ^1H NMR spectrum [plate 6 ($CDCl_3$ - 298K)]. Electron ionization (EI) mass spectra produced the following recognizable fragment ion: m/z = 104 [plate 6c] which are consistent with the proposed aglycone structure.



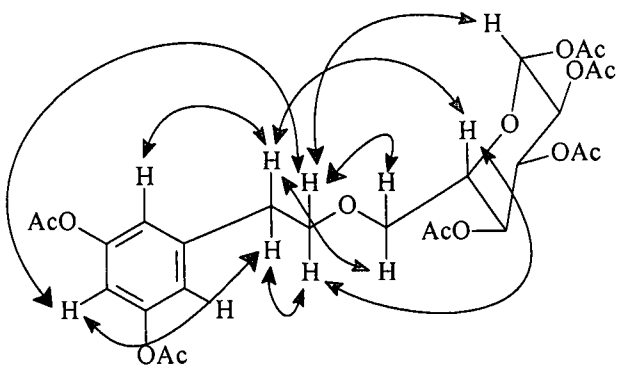
1-[β-D-2',3',4'-tri-*O*-acetylglucopyranosyloxy]-2-(3,5-diacetoxyphenyl)ethane(59)

Compound **(59)** was obtained from fraction A5 as the acetylated derivative after derivatization and purification by PLC chromatography. Similar to the above **(58)** and related compounds,[155] the ethylene moiety α- and β-CH_2 (δ2.90, m and δ3.69, m) (^{1}H NMR spectrum [plate 7 ($CDCl_3$ - 298K)]) display NOE association with the aromatic H-2,6 (δ7.10, d, 2.0Hz). H-4 resonated at δ7.04 (t, 2.0Hz) [plate 7b-1 ($CDCl_3$ - 298K)].



(59)

NOE association of the same moiety with 6'-CH_2 (δ4.28, dd, J = 4.5Hz, 12.0Hz and δ4.15, dd, J = 2.0Hz, 12.0Hz) indicates the linkage of the aliphatic chain to the glucoside.



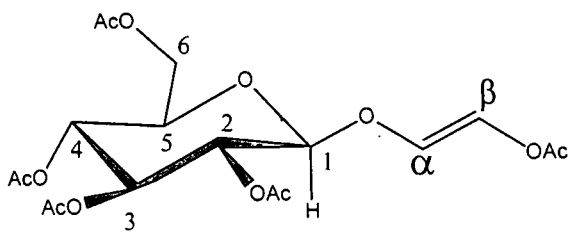
(59a)

[155] J. F. W. Burger, D. Ferreira, D. G. Roux, Nuwe Inhoudstowwe van 'n medisinale plant, *Harpagophytum procumbens* DC, University of the Free State, Bloemfontein, South Africa, **1985**

The anomeric proton H-1' shows NOE association with only the β-CH_2 and H-2' [plate 7b-2 ($CDCl_3$ - 298K)]. COSY experiments [plate 7a-1 ($CDCl_3$ - 298K)] confirms the linkage of the glucoside unit by showing long distance coupling between the β-CH_2 and the two 6'-CH_2. The COSY experiment is consistent with the remainder of the glycosidic protons as: H-2' (δ5.01, t, 10Hz), H-3' (δ5.20, t, 9Hz), H-4' (δ5.10, t, 9.5Hz) and H-5' (δ4.15, m) overlapping one of the 6'-CH_2. The two aromatic acetoxy signals resonate at δ2.32 and the aliphatic acetoxy signals at δ2.11, δ2.04, δ2.01, and δ1.94.

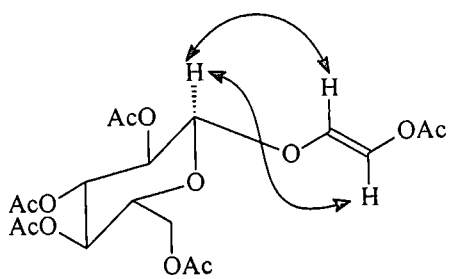
1-acetoxy-2-(β-D-2',3',4',6'-tetra-*O*-acetylglucopyranosyloxy)ethene (60)

Fraction A5 of the acetone extract yielded compound **(60)** upon acetylation and PLC purification. ^{1}H NMR spectrum [plate 8 ($CDCl_3$ – 333K)] displays four aliphatic acetoxy singlets (δ2.08, δ2.07, δ2.06, δ2.04), which suggested the presence of a glycosidic unit and a deshielded acetoxy singlet at δ2.17. Due to slow rotation on the ^{1}H NMR time scale, at ambient temperatures, the compound was heated to 333K to minimize the duplication of signals during the recording of the ^{1}H NMR spectra.



(60)

Two ethylene protons H-α and H-β resonated as a two proton singlet at δ6.70, the



(60a)

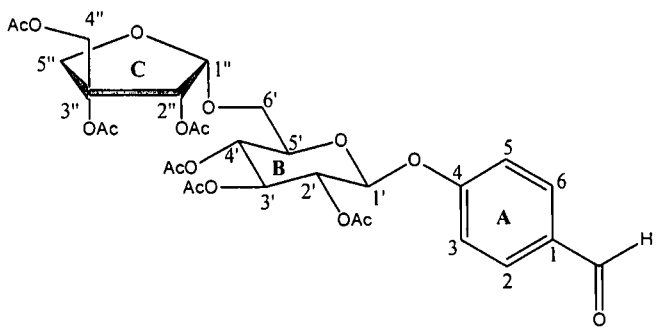
double bond affirmed by the discoloration of bromine water, and shows a NOE association [plate 8b-1 ($CDCl_3$ – 333K)] to the anomeric proton H-1' (δ5.10, d, 7.0Hz) of

the glucosidic unit.

The remaining protons of the glucopyranosyl unit were assigned by COSY experiment [plate 8a-1 ($CDCl_3$ – 333K)] as H-2' (δ5.27, t, 8.0Hz), H-3', H-1' (β-coupled) overlapping (δ5.10, m), H-4' (δ5.18, t, 10Hz), H-5' (δ3.78, m) and 2 x H-6 (δ4.25, m). Synthesis of (**60**) is envisaged to confirm the structure.

4-[*O*-α-2",3",4"-tri-*O*-acetylapiofuranosyl-(1"→6')-β-D-2',3',4'-tri-*O*-acetylglucopyranosyloxy] benzaldehyde (61).

Acetylation and PLC purification of fraction A4 of the acetone extract yielded 4-[*O*-α-2",3",4"-triacetoxyapiofuranosyl-(1"→6')-β-D-2',3',4'-triacetoxyglucopyranosyloxy] benzaldehyde (**61**). ^1H NMR data of authentic samples [168] with a C-1"-*O*-C-2' linked apiofuranose-glucoside moiety and another with the same C-1"-*O*-C-6' linkage but different aglycone unit are consistent with the proposed structure.



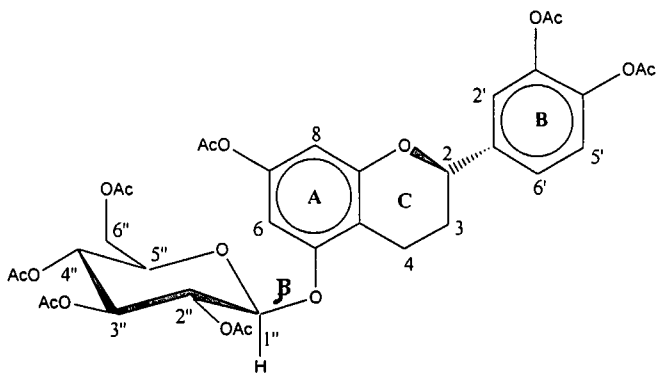
(61)

The ^1H NMR spectrum [plate 9 ($CDCl_3$ - 298K)] reflects the 4-*O*-substituted benzaldehyde moiety as the deshielded aromatic H-2,6 (δ7.88, d, 8.0Hz) and H-3,5 (δ7.12, d, 8.0Hz), and the aldehydic proton as a singlet at δ9.50.

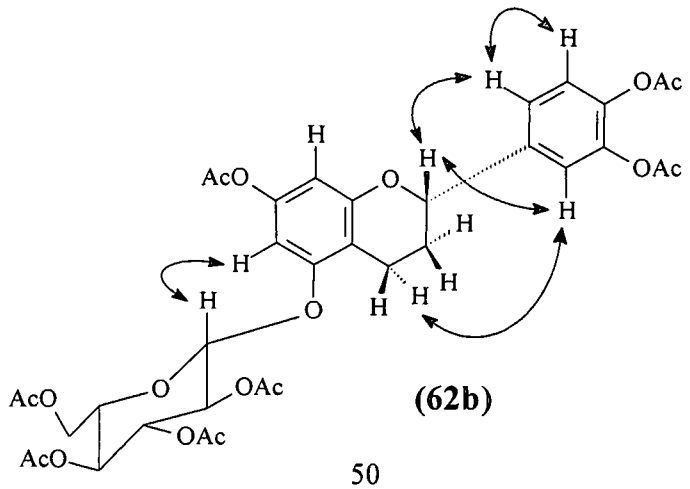
Flavan.

Acetylation of fraction A7 of the acetone extract afforded the acetylated flavan glucoside,**3',4',7-tri-acetoxy-5-(β-D-2'',3'',4'',6''-tetra-*O*-acetylglucopyranosyloxy)flavan (62)**. The ^{1}H NMR spectrum [plate 10 (C_6D_6) (298K)] shows two methylene multiplets [δ2.60, H-3a,b] and [δ1.65, H-4a,b] consistent with the flavan moiety. A negative DNPH test confirms the absence of a carbonyl functionality in the molecule. The doublet of doublets at δ4.58 (J = 3.0, 9.0Hz) defines H-2(C) which shows NOE association with H-2',6'(B), and thus designates the ABX spin system of the B-ring [plate 10b-1 (C_6D_6) (298K)]. The NOE association of H-3(C) with H-2',6'(B) is in line with the presence of the flavan unit **(62b)** [plate 10b-3 (C_6D_6) (298K)].

The ABX spin system of the B-ring is indicated by the doublet of doublets [δ7.01, J = 2.0 and 8.0 Hz, H-6'(B)] and its associated doublets [δ7.33, J = 2.0, H-2'(B)] and [δ7.15, J= 8.0Hz, H-5'(B)]. The AB spin system of the A-ring is represented by the doublets [δ6.79, J = 2.0Hz, H-6(A)] and [δ6.87, J = 2.0Hz, H-8(A)].



(62)



(62b)

The glycosyl protons[156] manifest as the doublet [δ4.87, J = 8.0Hz (β-coupled), H-1''], the doublet of doublets [δ5.69, J = 8.0, 10Hz, H-2''], the two triplets [δ5.50, J = 9.0Hz, H-3''], and [δ5.35, J = 9.0Hz, H-4''], the multiplet [δ3.25, H-5''] and the two methylene protons H-6" [δ4.28, J = 6.0, 12Hz, and δ4.11, J = 3.0, 12Hz], and are confirmed by COSY experiment [plate 10a-1 (C_6D_6) (298K)] and NOE data [plate 10b-2 (C_6D_6) (298K)]. COSY experiment [plate 10a-2 (C_6D_6) (298K)] also shows long range coupling between the anomeric proton (H-1'') and H-6(A), which confirms the attachment of the sugar to C-5 of the aglycone. The COSY experiment [plate 10a-3 (C_6D_6) (298K)] furthermore confirms the spin systems of the A and B-ring respectively. NOE data [plate 10b-1 (C_6D_6) (298K)] show association between H-1'' and H-6(A), which suggests the predominance of conformer 1 over conformer 2, (**Table 8.1**) suggested by Molecular Modeling calculations, also showing a lower molecular energy for conformer 1 [plate 10c].

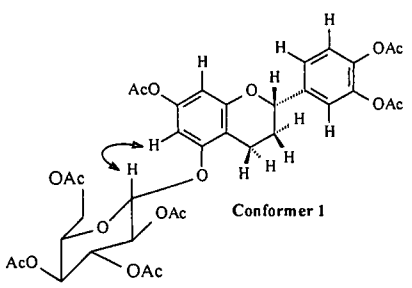
Table 8.1

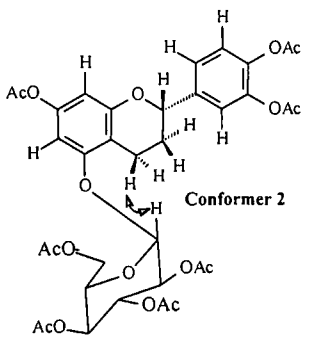
Conformer1

Energy		141.42kcal/mol
H-6	H-1"	2.515 Å
H-2	H-2',6'	2.359 Å
H-3a	H-2',6'	2.674 Å
H-2	H-4b	2.612 Å

Comformer 2

Energy		147.09kcal/mol
H-4	H-1"	2.441 Å
H-2	H-2',6'	2.338 Å
H-3a	H-2',6'	2.558 Å





The experimental NOE spectral data is also supported by the MM calculations and distances between different protons are shown in angstrom units (Å) [plate 10c]

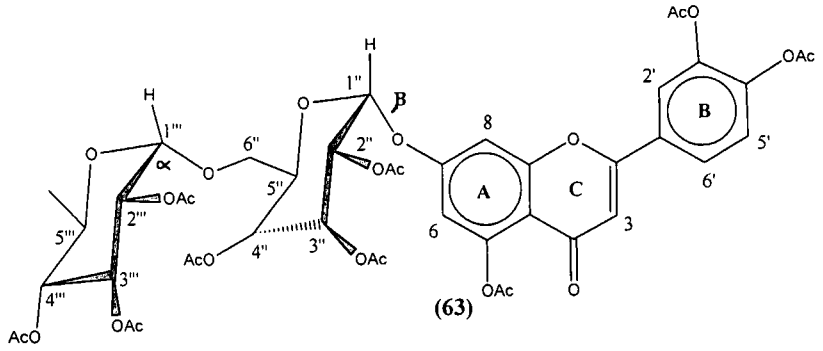
The seven acetoxy groups resonate as singlets at δ1.89, δ1.87 x 3, δ1.86, δ1.81, δ1.76. The absence of a carbonyl functionality in this structure is concluded by a ^{13}C spectrum

[156] I. Kubo and M. Kim, *Tetrahedron Lett.*, **1987**, *28* no. 9, 921.

which indicated the absence of a signal beyond 170ppm[157]

Flavones

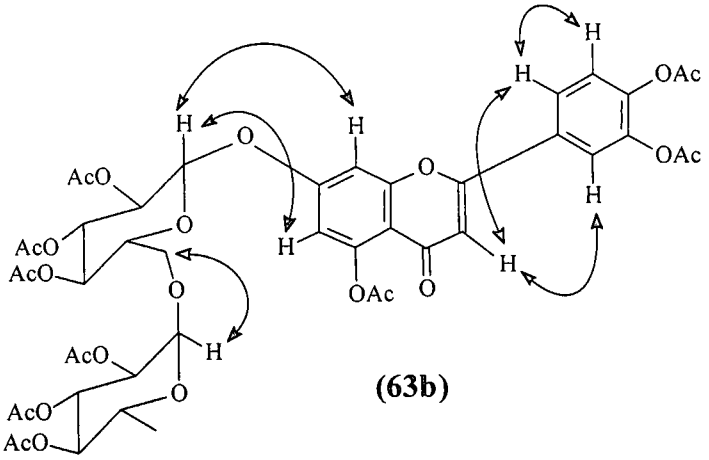
The acetylated flavone diglycoside, **3', 4', 5-triacetoxy-7-(β-2'',2''', 3'',3''', 4'', 4'''-hexa-*O*-acetyl-rutinosideoxy) flavone** (Scolymoside)[158] **(63)**, is obtained after



(63)

precipitation from an ethanolic solution of the acetone extract.

^{1}H NMR spectra [Plate 11 (C_6D_6) (298K)] displayed the characteristic sharp singlet of the vinylic H-3 proton[159] that resonates at δ6.58. The *meta* protons of the AB spin system of



(63b)

the A-ring are displayed as two doublets [δ6.81, J = 2.0Hz, H-6(A)], and [δ6.77, J = 2.0Hz, H-8(A)], with the anomeric proton [δ5.05, J = 8.0Hz, H-1''](β-coupled) showing NOE association [plate 11b-1, ($CDCl_3$) (298K)] to both protons, thus confirming the spin system and the point of attachment [7-OH(A)] of the rutinoside unit to the flavone. The

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[157] S. Kulwant, S. Ghosal, *Phytochemistry* **1984,** 23 no.11, 2419.
[158] M. A. Makabul, *et al. Khim. Prir. Soedin.*, **1979**, *15*, 725 ; Chem. Nat. Compd. (Engl. Transl.), 640.
[159] K. R. Markham and T. J. Mabry, in *The Flavonoids,* (eds. J. B. Harborne, T. J. Mabry, and H. Mabry), Chapman and Hall, London, **1975,** 67.

COSY experiment is in line with this point of attachment by displaying long range coupling between H-1'' and the H-6, H-8 protons [plate 11a-1, (C_6D_6) (298K)].

The ABX spin system of the B-ring is indicated by the doublet of doublets [δ7.34, J = 2.0 and 8.0 Hz, H-6' (B)] and its associated doublets [δ7.75, J = 2.0Hz, H-2' (B)] and [δ7.22, J= 8.0Hz, H-5' (B)]. This spin system and the flavone moiety is further supported by NOE association between the H-2' and H-6' of the B-ring protons and the H-3(C) [plate 11b-2, ($CDCl_3$) (298K)]

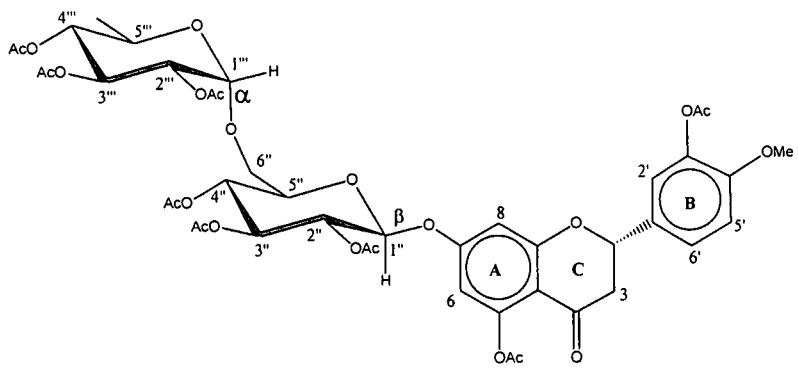
Coupling of the rhamnoside to the 6''-OH of the glucoside is displayed by NOE association between the anomeric proton [δ4.88, J = 2.0Hz, H-1''' (α-coupled)] of the rhamnoside and the two H-6'' of the glucoside [plate 11b-3, ($CDCl_3$) (298K)].

The glycosidic protons are allocated to the glucosyl and rhamnosyl units according to the consecutive couplings established in the COSY spectrum [plate 11a-2 (C_6D_6) (298K)]: H-2", H-3" overlapping (δ 5.58, m); H-4" (δ 5.16, t, J = 10.0Hz); H-5" (δ 3.36, m); α and β-H-6" (δ 3.63, m) for the glucopyranosyl and; H-2"'(δ5.66, m); H-3''' (δ5.72, dd, 3.0Hz, J = 10.0Hz); H-4''' (δ 5.53, d, J = 10.0Hz); H-5''' (δ4.02, m) and a CH_3 (δ1.28, d, J = 6.0Hz) for the rhamnosyl unit.

The 9 acetoxy groups are displayed as singlets at [δ1.99, δ1.90, δ1.86 (arom.)] and [δ1.86, δ1.83, δ1.80, δ1.72, δ1.69, δ1.66 (aliph.)]

Flavanone

The flavanone, hesperedin, precipitated from an ethanolic solution of the acetone

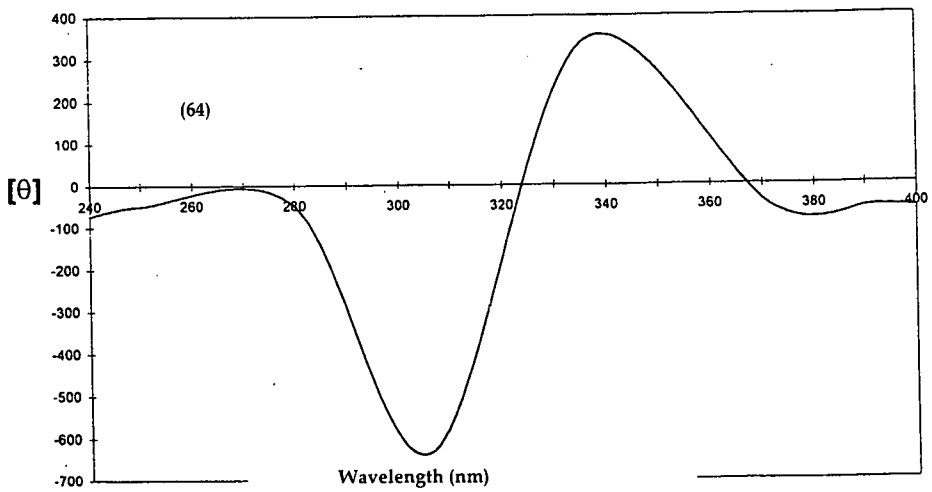


(64)

extract and was derivatized and purified (PLC) to yield **3', 5, -diacetoxy-7-(β-2'',2''', 3'',3''', 4'',4'''-hexa-*O*-acetyl-rutinosideoxy)-4'-methoxyflavanone (64)**, as a white amorphous substance. The ^1H NMR spectrum [plate 12 ($CDCl_3$ - 298K)] exhibited the heterocyclic C-ring protons [H-2 (δ 5.46, dd, J = 3.0, 12.5Hz); H-3α (δ 3.01, dd, J = 12.5, 16.5Hz); H-3β (δ 2.78 dd, J = 3.0, 16.5Hz)], and an aromatic ABX pattern of H-6' (δ

7.28, dd, J = 8.0 Hz, 2.0Hz), H-2' (δ 7.17, d, J = 2.0Hz,) and H-5' (δ7.03, d, J = 8.0Hz) for the B-ring, as well as the *meta*-coupled protons assigned to the A-ring, respectively, H-6 (δ 6.49, d, J = 2.0 Hz) and H-8 (δ 6.33, d, J = 2.0 Hz). The rutinoside moiety is β-coupled to the aglycone following the coupling constant of the H-1" [(δ5.18, d, J = 7.5Hz (β-coupled)]. All protons appear identical to those in the literature. [160]

The CD spectra of flavanone **(64)** were in line with the anticipated[161] synchronous positive Cotton effect due to the n → π* transition (~340 nm), and were compatible with flavanones possessing 2*S* absolute configuration in nature[162, 163, 164].



CD spectrum of flavanone (64)

The flavanone **4',5-diacetoxy-7-(β-2'',2''',3'',3''',4'',4'''-hexa-O-acetyl-rutinosideoxy) flavanone (Narirutin) (65)**[165] was isolated from fraction B10 of the methanol extract after acetylation and PLC purification.

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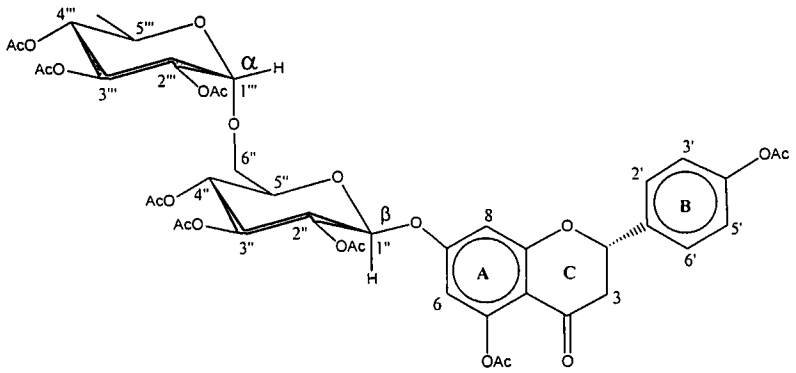
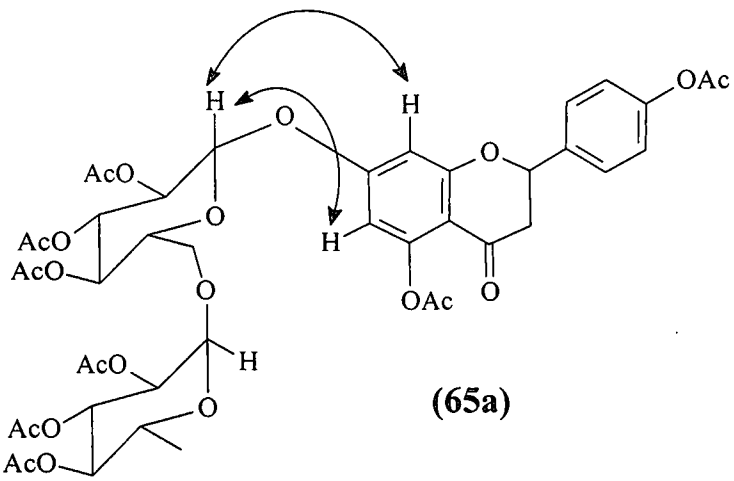
[160] B. I Kamara, E. V. Brandt, D. Ferriera, *Structure and Synthesis of Phenolic Metabplites from Honeybush Tea (C. intermedia)*, MSc, University of the Freestate, Bloemfontein, South Africa, **1997**.
[161] J. W. Nel, B. C. B. Bezuidenhout, E. V. Brandt and D. Ferreira, *Die Eerste Oligomeriese Neoflavonoied. Strukuur en Sintese van Modelverbindings. PhD Thesis,* UOFS, **1993,** 43.
[162] W. Gaffield, *Tetrahedron*, **1970**, *26*, 4039.
[163] J. H. van der Westhuizen, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1,* **1981**, 1220.
[164] W. Gaffield and A. C. Waiss, *Chem. Commun*, **1968**, 29.
[165] B. A. Bohm, in *The Flavonoids,* (eds. J. B. Harborne, T. J. Mabry, and H. Mabry), Chapman and Hall, London, **1975**, 564.



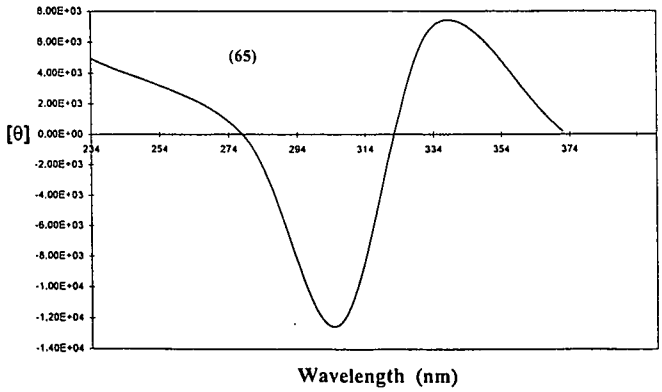
(65)

The ^{1}H NMR spectrum [plate 13 ($CDCl_3$ - 298K)] displayed the same sugar unit as **(64)**, ether linked to the C-7 as confirmed by NOESY experiment [plate 13b ($CDCl_3$ - 298K)] which shows NOE from H-1'' to both H-6 (δ6.50, d, 2.0 Hz) and H-8 (δ 6.34, d, 2.0 Hz) **(figure 65a)**. The only difference is displayed by the aglycone B-ring where an AA'BB' spin system contrasts with the ABX spin system of Hesperedin **(64)**, which confirms the C-4' hydroxylated B-ring.



(65a)

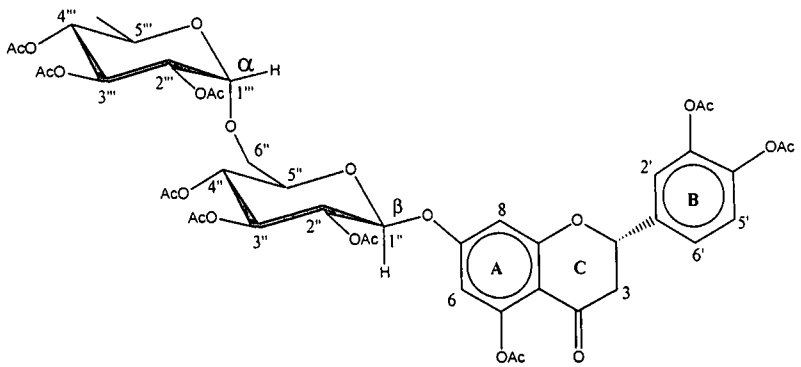
The CD spectra of flavanone **(65)** were in line with the anticipated positive Cotton effect due to the n → π* transition (~340 nm), and were compatible with flavanones possessing 2*S* absolute configuration in nature.



CD spectrum of compound (65)

The flavanone, **Eriocitrin**[166], was isolated from the acetone extract, fraction A7.

The compound was acetylated and purified (PLC) as **3', 4', 5, -triacetoxy-7-(β-2'',2''', 3'',3''', 4'', 4'''- hexa-*O*-acetyl-rutinosideoxy) flavanone (66).**



(66)

The ^{1}H NMR spectrum similar to **(64)** [plate 14 (C_2D_6CO - 298K)] displayed protons [H-2 (δ 5.64); H-3α (δ 3.15); H-3β (δ 2.75)], of the C-ring and an aromatic ABX pattern of H-6'and H-2' (δ 7.53, m) and H-5' (δ7.35, d, 8.0Hz) attributed to the B-ring, and also displayed the *meta*-coupled protons respectively assigned to the A-ring, 6-H (δ 6.45, d, 2.0 Hz) and H-8 (δ 6.69, d, 2.0 Hz), both affirmed by COSY experiment. [plate 14a-2 (C_2D_6CO - 298K)].

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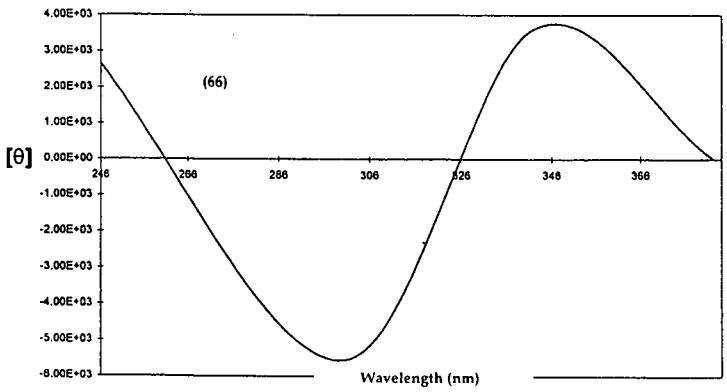
[166] F. Tomas, F. A. T. Barberian, F. Ferreres, J. L. Nieto, *Journal of Natural products-Lloydia*, **1985**, *48*, Iss 3, 506.

The rutinoside proton signals are confirmed by COSY [plate 14a-1 (C_2D_6CO - 298K)] experiment as the glucoside moiety, H-1'' [(δ5.70, d, 8.0Hz (β-coupled)], and protons H-2'',2''',3''', 4'' (δ5.20, m), H-3'' (δ5.42, t, 10.0Hz), H-5'' (δ4.33, m), 1 x H-6'' (δ3.73, m), 1 x H-6'' (δ3.90, m) overlapping H-5'''(δ3.91, m) and the rhamnoside anomeric proton, H-1''' (δ4.77, d, 2.0Hz), which is α-coupled to the C-6''-O of the glycosidic unit. The CD spectra of flavanone (**66**) were in line with the anticipated positive Cotton effect due to the n → π* transition (~340 nm), and were compatible with flavanones possessing 2*S* absolute configuration in nature.

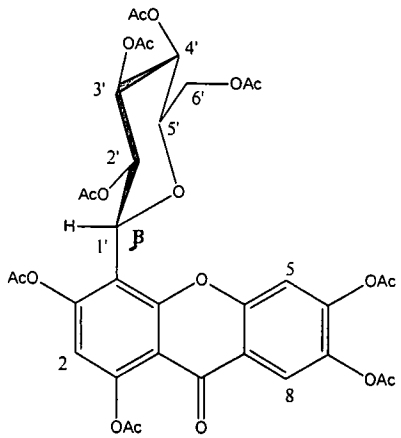


CD spectrum of compound (66)

8.5 *C*- glucosides

Xanthone

The xanthone, mangiferin, precipitated from fraction A3 of the acetone extract and was purified and derivatized to yield **1,3,6,7-tetraacetoxy-4-(β-D-2'', 3'', 4'', 6''-tetra-*O*-acetyl-glucopyranosyl) xanthone (67)** as a yellow amorphous solid.



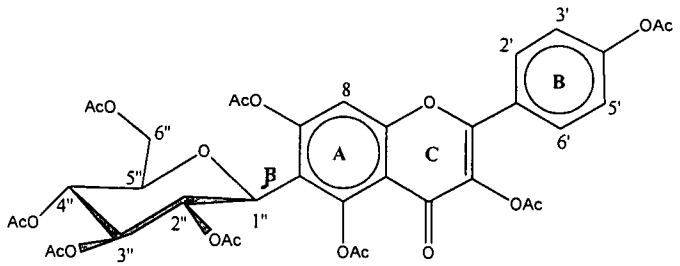
(67)

The ^{1}H NMR spectrum identical to an authentic sample[160] [plate 15 ($CDCl_3$ - 343K)] characteristically displayed the singlets of H-8 (δ8.10, s) and H-5 (δ7.37, s). The broadened signal of H-2 (δ7.25, s) at room temperature is indicative of intermediate rotation on the NMR timescale, most likely about the carbon-carbon bond between the sugar unit and the aglycone. Heating to 343K affected pronounced sharpening of the signals.

The assignment of the glucoside protons are; H-1' (δ4.87, d, 10Hz (β-coupled)), H-2' (δ5.17, t, 9.5Hz), H-3' (δ5.30, t, 9.5Hz), H-4' (δ5.70, t, 9.5Hz), H-5' (δ3.80, m) and the two C-6' protons H-6' (δ4.04, dd, 12.5Hz, 2Hz and δ4.37, dd, 12.5Hz, 4.5Hz).

Flavonol

3,4',5,7-tetra-*O*-acetyl-6-(β-D-2'', 3'', 4'', 6''-tetra-*O*-acetyl-glucopyranosyl) kaempferol (68) was obtained after acetylation and purification (PLC) of fraction A5 of the acetone extract.



(68)

An absence of heterocyclic protons on the ^{1}H NMR spectrum [plate 16 ($CDCl_3$ – 298K)] of the molecule is in line with a flavonol moiety. The AA'BB' spin system of the 4'-*O*-acetyl substituted B-ring is represented by the two doublets H-2',6' (δ7.76, d, 8.5Hz) and H-3',5' (δ7.16, d, 8.5Hz). A sharp singlet of H-8 (A) at δ6.72, and only 3 aromatic acetoxy signals (δ2.34, δ2.19, δ2.16) indicates the *C*-glycosylated A-ring[167]. Absence of the broadened signals present in the C-8 glucoside [164] due to B-ring interaction, further confirms the *C*-6 glucoside. The ^{1}H NMR data is identical to an authentic sample[168].

3,4',6,7-tetraacetoxy-5-(β-D-2'', 3'', 4'', 6''-tetra-*O*-acetyl-glucopyranosyl) flavonol (69)

Acetylation and purification of fraction A5 of the acetone extract yielded 3,4',6,7-tetraacetoxy-5-(β-D-2'', 3'', 4'', 6''-tetra-*O*-acetyl-glucopyranosyl) flavonol **(69)** as a white amorphous solid.

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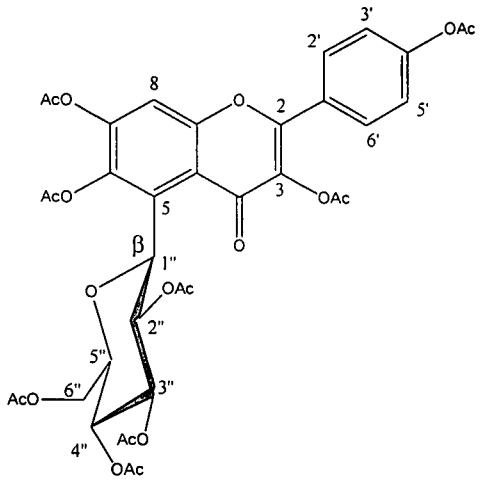
[167] M.K Seikel and T. J. Mabry, *Tetrahedron Letters.*, **1965**, 1105.

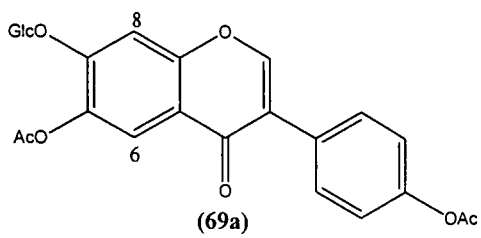
[168] B.I Kamara, E.V. Brandt, D. Ferriera, *Structure and Synthesis of Polyphenols from Honeybush Tea (C. intermedia) and the Potential of Flavonoids as Active Oxygen Scavengers*, PhD, University of the Freestate, Bloemfontein, South Africa, **1999**.



(69)

The ^{1}H NMR and COSY spectra [plate 17a-1 ($CDCl_3$ – 333K)] of **(69)** displayed seven aliphatic protons between δ3.50 and 5.80 reminiscent of a glycoside moiety, but with no heterocyclic protons, thus, indicating a possible flavonol aglycone. The AA'BB' spin system of the B-ring is represented by H-3',5' (δ7.19, d, 9.0Hz) and H-2',6' (δ7.82, d, 9.0Hz). The aromatic singlet of the A-ring (δ7.07, s), which indicates a tri-substituted ring, was assigned to H-8 for the following reasons.

An authentic sample **(69a)**[167] with the same A-ring oxygenation pattern displays H-8 at δ7.22, which contrasts with a 5,7-hydroxylation pattern where the H-6 and H-8 occur between δ5.7 and δ6.9.[169] Also the carbonyl induced deshielded H-5 resonates near δ8.0.[168]



(69a)

Furthermore H-8 of an authentic kaempferol diglycoside **(69b)**[167] resonates at δ6.67 in line with the 6,7-diol hydroxylation pattern.

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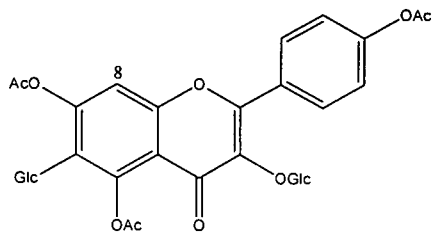
[169] K. R. Markham and T. J. Mabry, in *The Flavonoids*, (eds. J. B. Harborne, T. J. Mabry and H. Mabry), Chapman and Hall, London, **1975**, 63.

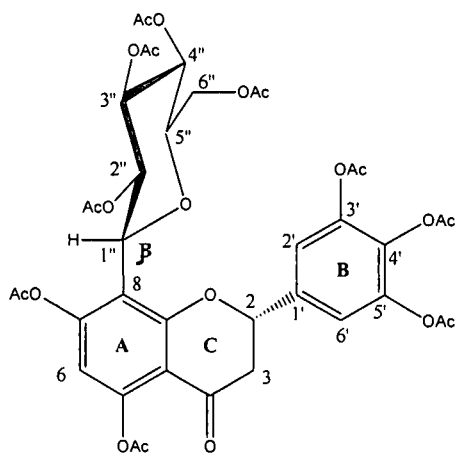


(69b)

The carbon linkage of the glycoside moiety is confirmed by HMQC experiment [plate 17d-1 (DMSO-d_6 – 298K)] were the C-1" resonates at δ72, which is consistent with C-linked flavonoid glycosides.[170] The C-3-*O*-C-1" linkage of the glycoside is ruled out on the grounds of H-1" of C-3-*O*-C-1" linked glycosides which resonate between δ5.7 and δ6.0[171] whereas this anomeric proton (d, J = 10.0Hz) resonates at δ4.77 (β-linked). A COSY experiment [plate 17a-2 ($CDCl_3$) (333K)] also shows long range coupling between the 6 and 7 acetoxy singlets (δ2.32 and δ2.17) and the anomeric proton, which are in line with the proposed structure. Synthesis of **(69)** is envisaged to confirm the structure.

Flavanone

Acetylation and purification of fraction B13 of the methanol extract yielded **3',4',5,5',7-pentaacetoxy 8-(β-D-2'', 3'', 4'', 6''tetra-*O*-acetyl-glucopyranosyl) flavanone (70)** as a white amorphous solid.



(70)

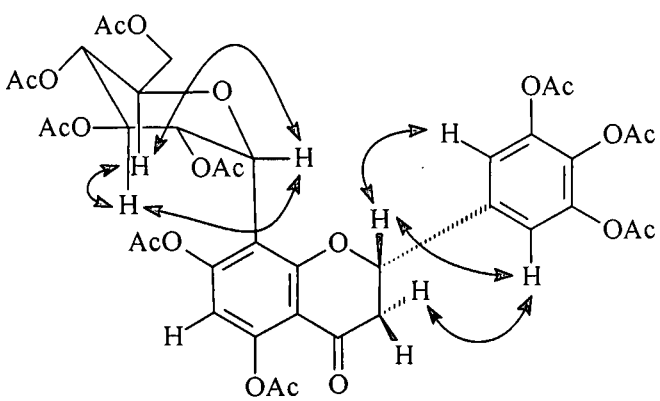
^{1}H NMR spectrum [plate 18 ($CDCl_3$ – 343K)] was obtained at 343K to reduce the duplication of signals observed at room temperature due to slow rotation on the NMR time scale. As a result of the duplication the integration of the signals is not a reliable

[170] K. R. Markham, *Techniques of Flavonoid Identification* (ed. J. E. Treherne and P. H. Rubery), **1982**, 81.
[171] K. R. Markham and T. J. Mabry, in *The Flavonoids*, (eds. J. B. Harborne, T. J. Mabry, and H. Mabry), Chapman and Hall, London, **1975**, 69.

indication of the amount of protons present, even at the elevated temperatures. The ^1H NMR spectrum of the heated compound indicated a flavanone glucoside with H-2 (δ5.60, t, 7.5Hz) and 2 x H-3 (δ3.38, d, 7.5Hz) as well as glucoside protons between δ3.3 and δ5.8. NOE of H-3 and H-2 [plate 18b-1 ($CDCl_3$ – 298K)] to both the protons of the B-ring, H-2' and H-6' (δ7.11, d, 2.0Hz and δ6.98, d, 2.0Hz) confirms a pyrogallol B-ring and the flavanone aglycone **(70a)**. COSY experiment affirmed the flavanone with correlation between H-3 and the B-ring protons, [plate 18a-1 ($CDCl_3$ – 298K)].

Although NOE between the anomeric H-1'' [δ4.47, d, 8.0Hz (β-coupled)] proton and the B-ring protons are absent, which would indicate a C-8 coupled sugar moiety, the C-6 coupled isomer is highly unlikely because of the amount of signal duplication observed. Other C-6 flavonoid glucosides in this section and other sources[167] show very little if any restrictive rotation. Thus, the singlet at δ7.13 is assigned to H-6.

NOE [plate 18b-2 ($CDCl_3$ – 298K)] is observed between H-1'', H-3'' (δ5.24, t, 9.5Hz) and H-5'' (δ3.68, m) and between the H-4'' (δ5.12, t, 9.5Hz) and H-2'' (δ5.68, t, 9.5Hz) indicating the sugar unit to be the β-D-glucoside. NOE from 2 x H-6'' to H-5'' and a COSY experiment [plate 18a-2 ($CDCl_3$ – 298K)] confirms the conclusion.



(70a)

CHAPTER 9

Synthesis of 3',4',7-triacetoxy-5-(β-D-2'',3'',4'',6''-tetra-*O*-acetylglucopyranosyloxy)flavan.

9.1 Introduction

Owing to the novelty of the flavan glucoside, 3',4',7-triacetoxy-5-(β-D-2'',3'',4'',6''-tetra-*O*-acetyl-glucopyranosyloxy)flavan **(62)**, isolated from *C. subternata* and the relatively unexplored synthetic methods of flavonoid *O*-glycosylation, we explored three routes in an attempt to synthesize compound **(62)**. The synthesis was aimed at final structure confirmation of the flavan glycoside and other flavonoid glycosides isolated, and to unambiguously confirm the position of the sugar linkage to the aglycone moieties. Another attraction to synthesize this compound lay in the claims that naturally occurring flavans exhibit a number of important biological activities which, if explored properly, may lead to valuable new drugs or agro-chemicals[172]. The antimicrobial activity of flavans include antibacterial action and the defensive role played by these compounds against microorganisms[173], activity against the fungus *Botrytis cinerea*[174], bactericidal activity against gram-positive bacteria[175] as well as anti-viral activity[176]. Pharmacological activity includes a mild central nervous system (CNS) depressant[177] and pronounced adaptogenic (anti-stress/anti-anxiety)[178, 179] activity.

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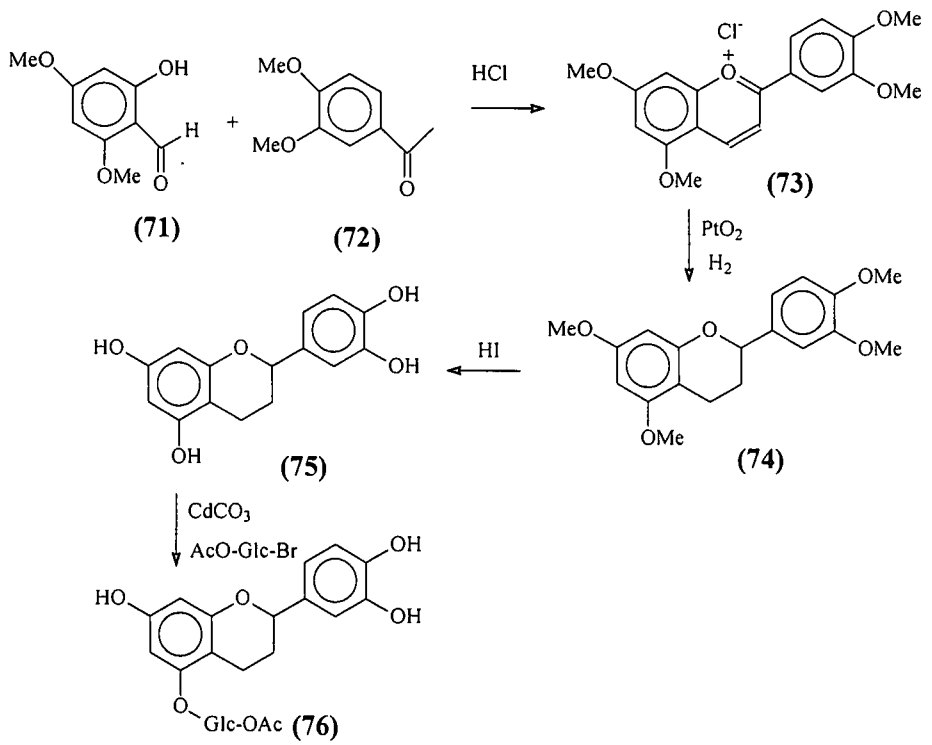
[172] S. Kulwant, S. Ghosal, *Phytochemistry*, **1984,** 23 no.11, 2420.
[173] M. Takasugi, Y, Kumagai, S. Nagao, T. Masamune, A. Shirata and K Takahashi, *Chem. Letters*, **1980,** 1459.
[174] D. T. Coxon, T. M. O'Neill, J. W. Mansfield and A. E. A. Porter, *Phytochemistry*, **1980**, *19*, 889.
[175] S. S. Gnanamanickam and J. W. Mansfield, *Phytochemistry*, **1981**, *20*, 997.
[176] J. F. Batchelor, D. J. Bauer H. F. Hodson, J. W. T. Selway and D. A. B. Young, *Eur.Pat. Appl.*, **1979**, *4*, 579.
[177] R. Sahai, S. K. Agarwal and R. P. Rastogi, *Phytochemistry*, **1980**, *19*, 1560.
[178] S. Ghosal, K. S. Saini and B. N. Sinha, *J. Chem. Res.*, 1983, pp. 330, 2601.
[179] S. Ghosal, K. S. Saini, *Abstracts, XVI Annual Scientific Conference on Indian Medicine*, Varanasi, India, pp. 1.

9.2. Synthesis of the title flavan glycoside (62) using the corresponding flavylium salt as intermediate *via* the Robinson Condensation.

In the exploration of this route the Robinson condensation[180] was employed to obtain the appropriate flavylium salt (**73**) (**Scheme 9.1**). The Robinson condensation employs the condensation of a benzaldehyde (e.g. 4,6-dimethoxy-2-hydroxybenzaldehyde (**71**)) and an acetophenone (e.g. 3,4-dimethoxyacetophenone (**72**)) under acidic conditions to the corresponding flavylium salt (**73**). It was expected that catalytic hydrogenation (Adams catalyst) of the latter would yield the corresponding racemic flavans (5,7,3',4'-tetramethoxyflavan) (**74**), which on demethylation[181] would yield the free phenolic product (**75**). Glycosylation with tetra-*O*-acetyl-bromoglucose and cadmium carbonate as the catalyst[182], would then give the unprotected flavan glycoside (**76**). The poor yields (≈ 11%) during selective methylation (dimethylsulfate) of the 2,4,6-trihydroxybenzaldehyde (**71**) and probably the instability of the flavylium salt (**73**) in solution[13] led to the abandoning of the proposed method.

Scheme 9.1

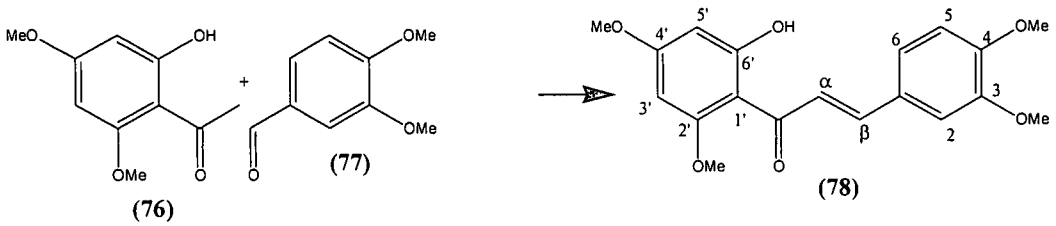


[180] D. T. Coxon, T. M. O'Neill, J. W. Mansfield and A. E. A. Porter, *Phytochemistry*, **1980**, *19*, 890.
[181] T. W. Greene, *Protective Groups in organic synthesis*, **1981**, 91
[182] K. Tsujihara, M. Hongu, K. Saito, M. Inamasu, K. Arakawa, A. Oku and M. Matsumoto, *Chem. Pharm. Bull.*, **1996**, *44(6)*, 1180.

9.3. Selective demethylation of the flavanone as a key step in the synthesis

The classic synthetic route to the $C_6C_3C_6$ flavonoid skeleton is based on an Aldol type condensation, under basic conditions, of a C_6C_2 unit of the appropriate acetophenone (2-hydroxy-4,6-dimethoxyacetophenone) **(76)** [plate 19] with a C_6C_1 unit of the appropriate aldehyde (3,4-dimethoxybenzaldehyde) **(77)** [plate 21] resulting in the corresponding intermediate chalcone, in this case, 2'-hydroxy-3,4,5',7'-tetramethoxy chalcone **(78)** [plate 22] [**Scheme 9.2**][183].

Scheme 9.2

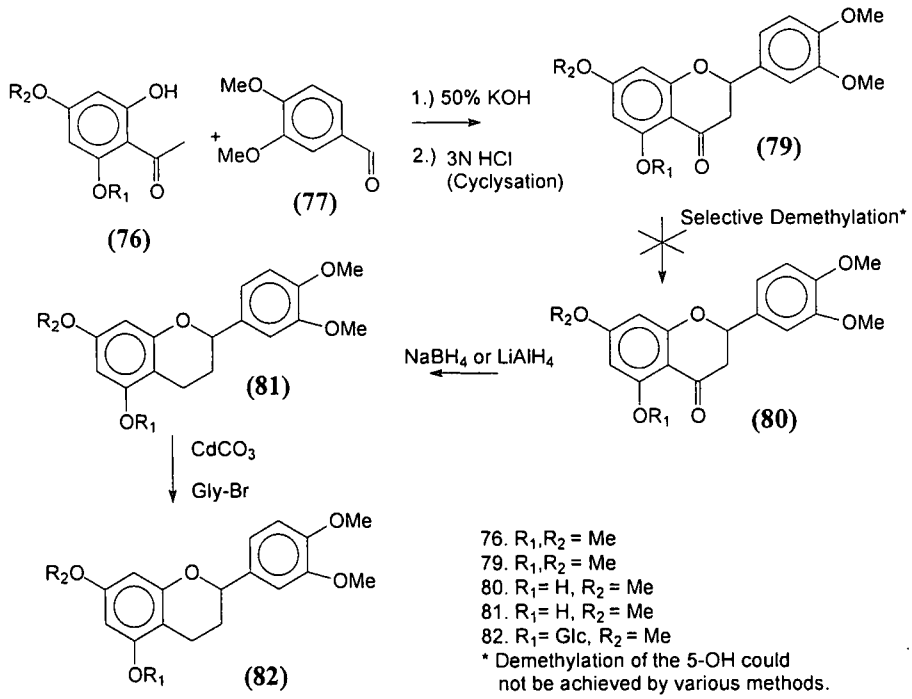


The corresponding flavanone **(79)** [plate 25], which is isomeric with the chalcone, is obtained from the latter by acid- or alkali catalysed ring closure[184, 185].

Selective methylation of commercial 2,4,6-trihydroxyacetophenone yielded 2-hydroxy-4,6-dimethoxyacetophenone **(76)** [plate 19], which on condensation with commercial 3,4,-dimethoxybenzaldehyde **(77)** [plate 21] under basic conditions yielded the corresponding chalcone **(78)** [plate 22]. The aldol condensation with the free phenolic 2,4,6-trihydroxyacetophenone as substrate could not be used due to the presence of too many reactive unprotected hydroxy groups, which led to polymerization. However, the demethylation of the chalcone or the cyclized flavanone was a prerequisite for the proposed 5-OH flavanone glycosylation (**Scheme 9.3**). The hydrolysis of the sugar moiety made the acid/base catalyzed cyclization of 2-hydroxy-4-methoxy-6-glucopyranosyl chalcone unfeasible.

[183]H. Wagner and L. Farkas, in *The Flavonoids*, (eds J. B. Harborne, T. J. Marbry and H. Mabry), Chapman and Hall, London, and references there in, **1975**, 127.
[184]S. C. Bhrara, R. N. Goel, A. C. Jain and T. R. Seshadri, *Indian J. Chem.*, **1964**, *2*, 399.
[185]S. A. Kagal, P. M. Nair and K. Venkataraman, *Tetrahedron*, **1962**, 593.

Scheme 9.3



The demethylation was attempted on the flavanone (5-OMe) in analogy to an existing methodology used on flavones,[186] and because the cyclization of the chalcone is only viable with one unprotected hydroxy group. The selective demethylation of the flavanone 5-OMe was also attempted by various methods which included BCl_3[187], $AlCl_3$[188, 189] and LiCl[190] without success. A possible explanation could be similar to that of the 5-methoxy groups in flavones with 6-oxygenation which are cleaved more easily than those in flavones with no substituent at the 6-position.[191, 192, 193] Furthermore the existence of 7-oxygenation slightly reduces the electron density of the 5-methoxy group making the formation of the metal complex between the 5-oxygen and the carbonyl oxygen less probable[186] (figure 9.1).

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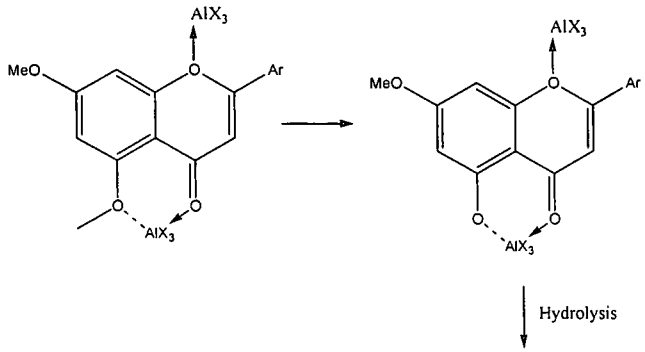
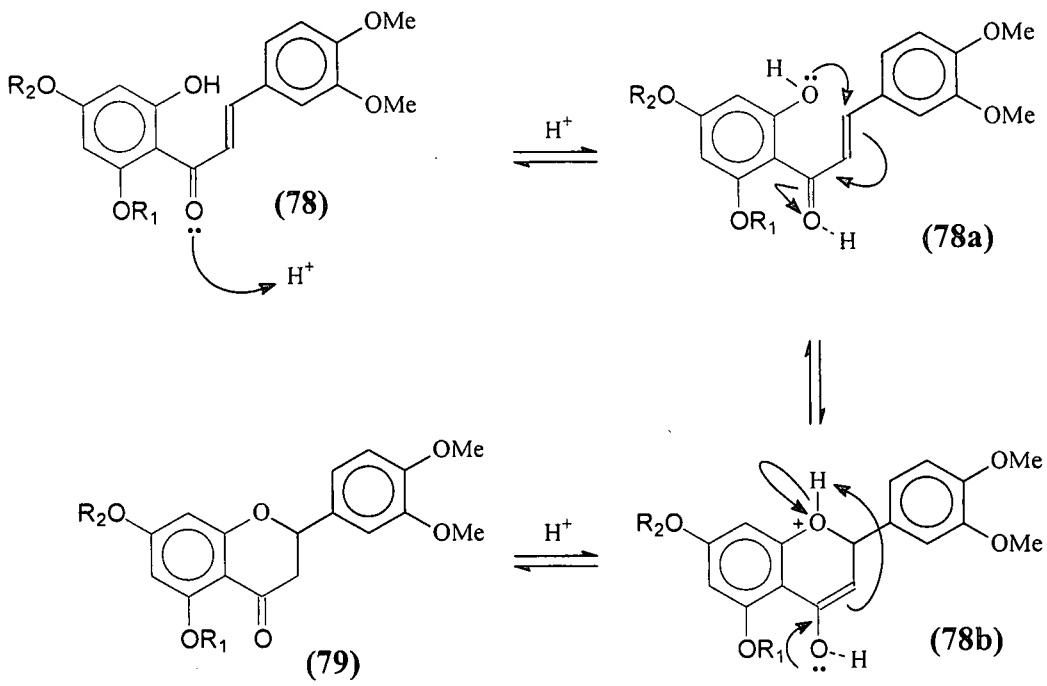
[186] T. Horie, M. Tsukayama, Y. Kawamura, and M. Seno, *J. Org. Chem.*, **1987**, *52*, 4702.
[187] H. Nagaoka, G. Schmid, H. Lio, and Y. Kishi, *Tetrahedron Lett.*, **1981**, *22*, 899.
[188] K. A. Parker and J. J. petraitis, *Tetrahedron Lett.*, **1981**, *22*, 397.
[189] T. –t. Li, and Y. L. Wu, *J. Am. Chem. Soc.* **1981**, *103*, 7007.
[190] A. M. Bernard, M. R. Ghiani, P. P. Piras, and A. Rivoldini, *Synthesis*, **1989**, 287.
[191] T. Horie, M. Tsukayama, H. Kourai, M. Masumura, M. Nakayama, *Zasshi*, **1984**,*105*, 232.
[192] T. Horie, H. Kourai, N. Fujita, *Bull. Chem. Soc. Jpn.* **1983**, *59*, 3773.
[193] T. Horie, H. Kourai, M. Nakayama, M. Tsukayama, M. Masumura, *Nippon Kagaku Kaishi*, **1980**, 1397.

Figure 9.1



The mechanism of the acid catalyzed ring closure to the β-position relative to the carbonyl proceeds by a 1,4-Michael addition, which is the well known chalcone ↔ flavanone equilibrium[194], and which course is dependent on the presence of a 5-hydroxy function in, and the acidity of the 3-protons of the resulting flavanone **(Scheme 9.4)**.[195] The chalcone ↔ flavanone equilibrium shifts in favour of the flavanone with a stronger catalyzing acid and in favour of the chalcone with stronger base.[194]

Scheme 9.4 Cyclization

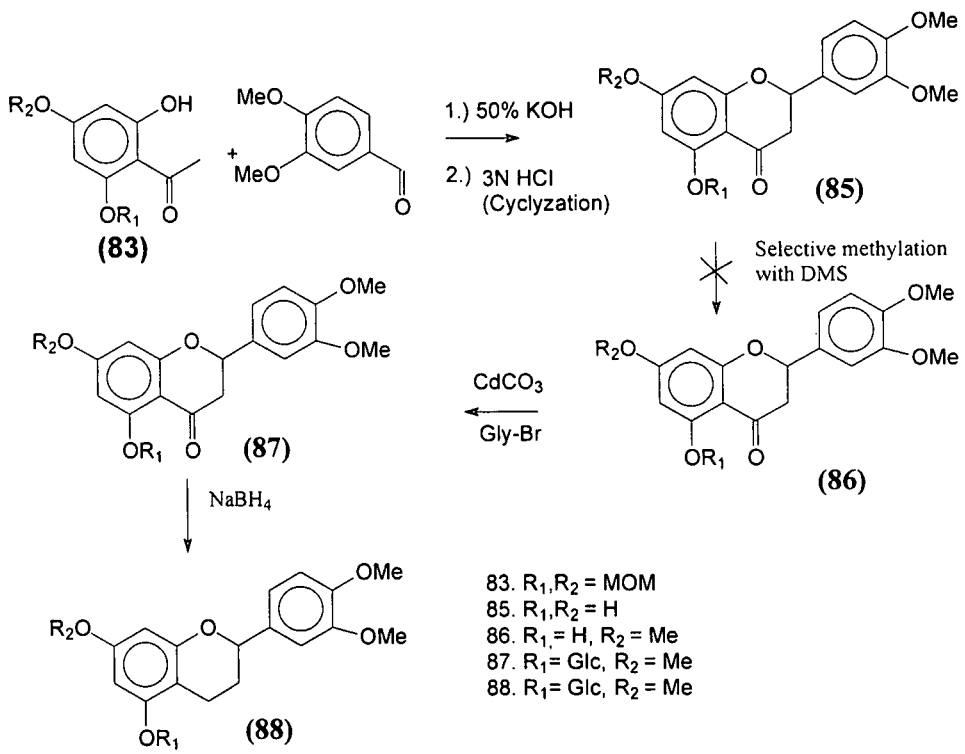


[194] S. von Konstanecki, V. Lampe and J. Tambor, *Ber.*, **1904**, *37*, 786.
[195] D. Ferreira, E. V. Brandt, F. du R. Volsteedt and D. G. Roux, *J. Chem. Soc., Perkin I*, **1975**, 1437.

Scheme 9.3 further shows the anticipated route after selective demethylation of the flavanone, which is followed by reduction of the carbonyl and catalytic glycosylation of the 5-OH.

In an alternative route (**scheme 9.5**) the commercially obtained 2,4,6-trihydroxyacetophenone selectively methoxy-methylated to yield 2-hydroxy-4,6-di-(methoxymethyl)acetophenone **(83)** [plate 20], was condensed with commercial 3,4-dimethoxybenzaldehyde to yield 2'-hydroxy-3,4-dimethoxy-4',6'-dimethoxymethylchalcone **(84)** [plate 23]. The latter was cyclized to give the deprotected 5,7-dihydroxy-3',4'-dimethoxyflavanone **(85)** [plate 24]. Because of the deprotection of 2'-hydroxy-3,4-dimethoxy-4',6'-dimethoxymethylchalcone **(84)** [plate 23] during the aldol condensation reaction, the yields were significantly reduced. The deprotected chalcone would not undergo cyclization or re-methoxymethylation.

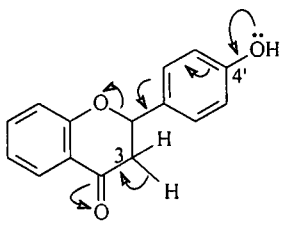
Scheme 9.5



Owing to strong hydrogen bonding between the adjacent carbonyl oxygen and the 5-OH of the flavanone, glycosylation of the 7-OH was favoured. The 5,7-diglycosylated flavanone was also a possibility. In order to target glycosylation only at the 5-OH of the flavanone, selective methylation of the 7-OH was a necessity. The selective methylation

of 5,7-dihydroxy-3',4'-dimethoxyflavanone **(85)** [plate 24] was unsuccessful, probably due to the acidity of the 3-protons of the flavanone in weak basic conditions (K_2CO_3) leading to opening of the heterocyclic ring within 5-10 min. to form the chalcone. The acidity of the α-protons are further increased by the presence of a 7-OH group together with the negative inductive effect of the 4'-OH[196] **(Figure 9.2)**.

Figure 9.2



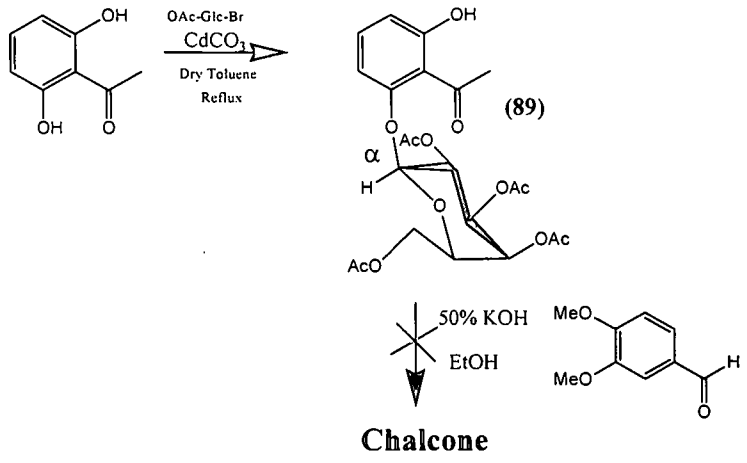
Glycosylation of 5-OH was also attempted on the flavanone **(85)** in basic conditions ($CdCO_3$) at reflux temperatures (393K) without success, probably due to the same reasons stated above.

9.4. Glycosylation of phloroacetophenone in the attempted synthesis of the flavan glycoside (62).

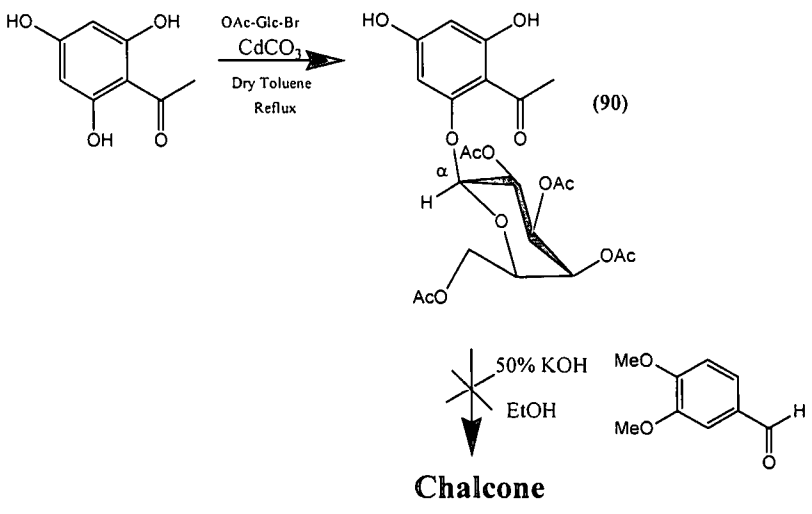
Since the glycosylation of the flavanone **(85)** was not feasible, glycosylation of the appropriate acetophenone as a precursor for the chalcone, based on existing methodology of glycosylation of phenolic acetophenones **(Scheme 9.6 and 9.7)** was attempted[182]. Glycosylations of resacetophenone and phloroacetophenone yielded the α-coupled glycoside analogues **(89 and 90)** (plate 28 and 29). In an attempt to obtain the glycosylated chalcone, both the glycosylated acetophenones were unsuccessfully subjected to the aldol condensation. The total lack of reactivity and recovery of the substrate is probably due to steric hindrance of the benzaldehyde to gain access to the glycosylated acetophenone enolate during the condensation reaction. **(Scheme 9.6 and 9.7)**

[196] R. M. Horowitz, and L. Lurd, *J. Org. Chem.*, **1961**, *26*, 2446.

Scheme 9.6



Scheme 9.7



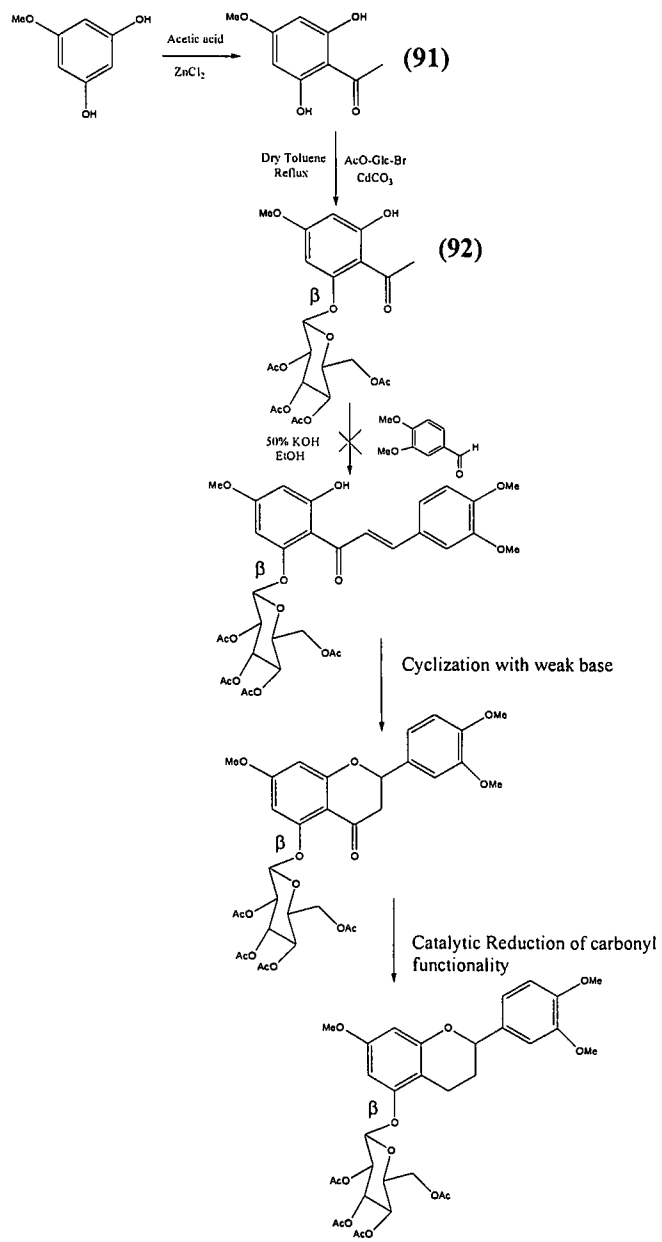
The third attempt was aimed at trying to synthesize the appropriate chalcone with the 4-OH of the phloroacetophenone precursor protected **(Scheme 9.8)**. Thus, the acetophenone substrate was obtained from 3,5-dihydroxyanisole which was acylated with $ZnCl_2$ and acetic acid[197] to obtain a mixture of 2,6-dihydroxy-4-methoxyacetophenone and 2,4-dihydroxy-6-methoxyacetophenone **(91)** [plate 26]. The protected acetophenone was subjected to the glycosylation procedure[182] to yield 6-(β-D-2',3',4',6'-tetra-*O*-acetyl glucopyranosyloxy)-2-hydroxy-4-methoxyacetophenone **(92)** [plate 27]. The fact that the

[197] Pearson, Bueler, *Synthesis*, **1972**, 533

4-methylated acetophenone yields the β-coupled glucoside instead of previously encountered α-coupling in the unprotected phloroacetophenone, suggests that the sugar is stericly hindered by the added methyl-group. This indicates that the glycosylation reaction proceeds via an S_N1 mechanism, which was positive since the target flavan's glycoside is β-coupled.

However, Aldol condensation with 3,4,-dimethoxybenzaldehyde gave no results. Due to time constraints these protocols were not investigated further at present, but we envisage further development of the topic in future.

Scheme 9.8



EXPERIMENTAL

CHAPTER 10

Standard experimental techniques

Unless specified otherwise the following techniques were applied throughout the course of this study.

10.1 Chromatographic Techniques.

10.1.1 Thin layer chromatography

Qualitative thin layer chromatography (TLC) was performed on Merck TLC-plastic sheets (3 x 8cm): Silica Gel F_{254} (0.2 mm layer). After development the plates were sprayed with H_2SO_4-HCHO (40:1, v/v) and R_f values reported are those observed in these qualitative TLC assessments.

Preparative scale thin layer chromatography (PLC) was conducted on glass plates (20 x 20 cm) coated with a layer (1.0 mm) of Merck Kieselgel 60 PF_{254} and air-dried overnight at room temperature. 10 - 15 mg of crude mixture was applied on each plate. Small-scale separations were conducted on Merck Pre-coated (0.25 mm) TLC Plates (20 x 20) Silica Gel 60 F_{254} and each plate charged with 3-5 mg of the crude product. After development in the appropriate eluent the plates were dried in a stream of air and the bands distinguished by either UV-light (254 nm) or appropriate spraying reagent. The bands were scraped off, eluted with acetone and removed under reduced pressure on a water bath at *ca.* 40°C.

10.1.2 Paper chromatography

Two-dimensional paper chromatograms on Whatman no. 1 paper (28.5 x 46 cm) were developed in two directions with water saturated butan-2-ol in the first and a 2% (v/v) acetic acid solution in the second direction. After development the chromatograms were air dried and sprayed with benzidine spraying reagent or inspected under UV light.

10.1.3 Column chromatography

Separations on **Sephadex** LH-20 were performed on various column sizes and differing flow rates as specified. The Sephadex LH-20 was prepared by soaking it in the eluent (ethanol or ethanol/water solution) for 24 hours. The glass column was filled with this suspension of Sephadex LH-20 and the crude extract, dissolved in a minimum of the eluent, was slowly applied to the column. Columns were run at a flow rate of approximately 0.5 ml/min. and fractions of 15 ml were collected with an ISCO (model 273) automatic fraction collector.

Flash column chromatography (FCC) was performed on a glass column (5 cm diameter) charged with 100 g of Merck Kieselgel 60 (230-400 mesh) for every 1 g of crude mixture. Air was expelled by elution with the appropriate solvent under N_2-pressure (*ca.* 50 kPa). The crude mixture was dissolved in a minimum amount of the appropriate solvent and slowly applied to the column. The column was developed under N_2-pressure with the appropriate solvent system and 15 ml fractions were collected.

10.2 Spray reagents

10.2.1 Formaldehyde-sulphuric acid[198]

Thin layer chromatograms were gently sprayed with a 2% v/v solution of formaldehyde (37 wt. % solution in water) and concentrated sulfuric acid and gently heated to *ca.* 120°C to effect maximum development of colour.

10.2.2 Bis-diazotated benzidine[199,200]

Benzidine (5g) was dissolved in 14 ml of concentrated HCl and made up to 1 litre with distilled water just before use. This benzidine solution was then mixed at a ratio of 3:2 with a 10% (m/v) sodium nitrite solution. The paper chromatograms were gently sprayed with this freshly prepared mixture and were subsequently rinsed for 1 hour under flowing tap water. (The lifetime of the solutions in the dark is approximately 3 weeks.)

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[198] H. M. Saayman and D. G. Roux, *Biochem. J.*, **1965**, *96*, 36.
[199] D.G. Roux, and E. A. Maihs, *J. Chromatoq.*, **1960**, *4*, 65.
[200] G. Linstedt, *Acta. Chem. Scand.*, **1960**, *4*, 65.

10.3 Spectroscopical methods

10.3.1 Nuclear magnetic resonance spectroscopy (NMR)

NMR spectra were recorded at 300 MHz (^1H) on a Bruker AVANCE DPX_{300} spectrometer with tetramethylsilane as internal standard. The solvents used were deuteriochloroform ($CDCl_3$, δ7.28), deuteriodimethylsulfoxide (C_2D_6OS), deuteriobenzene (C_6D_6, δ7.15) and deuterioacetone [$(CD_3)_2CO$/acetone-d_6, δ2.04]. Chemical shifts are reported in parts per million (ppm) on the δ-scale and coupling constants were measured in Hz.

The following abbreviations are used:

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

10.3.2 Circular dichroism (CD)

CD spectra were recorded on a Jasco J-710 spectropolarimeter in spectrophotometric grade methanol (~ 1 mg/10 ml methanol). The formula used to calculate the molecular ellipticity [θ] was:

$$[\theta] = \frac{(L)(\text{scale})(\text{molecular weight})(100)}{[\text{length of tube (cm)}][\text{concentration (g/l)}]}$$

where L is the difference (at any given wavelength) between the reading (in cm) of the compound in solution and the reading (in cm) of pure solvent.

10.4 Anhydrous solvents and reagents

Acetone was dried over dry K_2CO_3 (oven-dried, 24 hours, 300°C) for 24 hours. The K_2CO_3 was filtered off and the solvent distilled over 3Å molecular sieves and stored under N_2.

Benzene and **THF**, were refluxed over sodium/benzophenone under N_2 until a dark blue colour persisted with subsequent fresh distillation under N_2 prior to use.

Acetonitrile, **dichloromethane** and **DMF** were refluxed over CaH_2 under N_2 for 12 hours with subsequent fresh distillation under N_2 before use.

10.5 Chemical methods

10.5.1 Standard work-up procedure

Unless specified to the contrary, the appropriate amount of water was added to the reaction mixture and extracted with diethyl ether or ethyl acetate. The organic extract was washed with water, dried (Na_2SO_4) and the ether or ethyl acetate removed on a water bath under reduced pressure at *ca.* 40 °C.

10.5.2 Selective methylation with dimethylsulfate[201]

Phenolic material was dissolved in dry acetone, dry K_2CO_3 (8 *eq.*) was added and dimethylsulfate (3-10 *eq.*) was subsequently added drop wise over a period of 30 min. under N_2. The reaction mixture was refluxed for 8 hours after which the mixture was filtrated and the acetone removed under reduced pressure. The excess dimethylsulfate was destroyed by addition of dilute ammonia and extraction with ethyl acetate afforded the methylated product (s).

10.5.2 Methylation with diazomethane[202]

Methylations were performed with an excess of diazomethane. The latter was generated 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 methanol (5-10 ml)] at

[201] Grundy; James; Pattenden, *Tetrahedron Lett.*, **1972**, 757.
[202] A. I. Vogel, *Vogel's Textbook of practical Organic Chemistry*, Fifth Edition, Longman. Scientific and Technical, **1989**, 433

-10°C. After 48 hours at -15°C the excess diazomethane and solvents were evaporated at room temperature.

10.5.3 Acetylation[203]

Dry phenolic material was dissolved in the minimum volume of pyridine and twice the amount of acetic anhydride was added. After 8-12 hours at ambient temperature the reaction was quenched with crushed ice and the excess pyridine removed by repetitive washing with cold water.

10.5.4 Methoxymethylation[204]

A solution of the phenolic compound (1.0 equiv.) in dry THF was added to a stirred suspension of sodium hydride (1.4-2.4 equiv.) in dry THF at 0 °C. After the suspension was stirred for 30 minutes, freshly distilled chloromethyl ether (1.2 equiv.) was added while stirring continued until completion of the reaction (1-2 hrs. /TLC) Crushed ice was added to quench the reaction and the aqueous phase extracted with diethyl ether. The organic extracts were washed with water, dried (Na_2SO_4) and the ether removed under reduced pressure at *ca.* 40 °C.

10.6 Freeze-drying

Phenolic material in aqueous solution was freeze-dried using a Virtis Freezemobile 12 SL (40 millitorr).

[203] T. Kametani and S. Kano, *J. Pharm. Soc.*, Japan, **1962**, *82*, 1059.
[204] A. McKillop, J. D. Fiond, R. P. Hug., *Tetrahedron*, **1974**, *30*, 1379.

10.7 Abbreviations

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

A	=	acetone
B	=	benzene
C	=	chloroform
DCM	=	dichloromethane
DMF	=	*N*,*N*-dimethylformamide
Et_2O	=	diethyl ether
EOAc	=	ethyl acetate
EtOH	=	ethanol
H	=	hexane
M	=	methanol
THF	=	tetrahydrofuran

CHAPTER 11

PHENOLIC COMPOUNDS FROM *CYCLOPIA SUBTERNATA* (Honeybush Tea)

Extractions

11.1 *Cyclopia subternata* shoots and stems

Dried unfermented shoots and stems of *C. subternata* (3kg.) were pulverised and the chlorophyll (181g) was extracted with chloroform (5 x 5.0 L, 24 h each) The phenolic compounds were extracted with acetone (3 x 5.0 L, 24 h each), methanol (5 x 8.0 L, 24 h each) and acetone/water (7:3) at ~25^{0}C to yield a dark green solid and two brownish syrups respectively on evaporation of the solvents. These were re-dissolved in water and freeze-dried to respectively afford 84g, 510.83g and 361.92g.

After inspection of the different extracts by paper chromatographic methods, the acetone and part of the methanol extracts were respectively investigated for phenolic metabolites. Owing to time constraints the acetone/water extract and part of the methanol extract were not investigated. Investigations on those fractions will be addressed in a follow up investigation.

11.2 Separation and enrichment of the phenolic metabolites from the acetone extract (Column A).

The acetone extract (49g) was separated on Sephadex LH-20/EtOH column with a flow rate of 1ml/min, collecting 32 min fractions. Following TLC inspection the collected tubes were combined as follows:

Tubes	Fraction	Yield
0 - 48	A1	3.23g
49 – 106	A2	4.05g

107 - 128	A3	4.12g
129 - 204	A4	14.64g
205 – 270	A5	2.56g
271 - 350	A6	4.98g
351 - 470	A7	4.83g
471 - 520	A8	3.74g
Precipitates from EtOH solution	4.69g	
Total phenolic compounds	46.84g	
Column residue	2.15g	

11.2.1 Isolation of compounds from fractions A1 and A2

Fractions A1 and A2 did not contain compounds of interest pertaining to this investigation.

11.2.2 Compounds precipitated from ethanolic solution.

Acetylation or methylation (when specified) with diazomethane, of the precipitates (100mg) followed by PLC yielded the purified compounds.

11.2.2.1 Penta-*O*-acetyl (+)-pinitol (53)[151]

PLC purification of the precipitate [B:A, 8:2 (v/v)] yielded **(53)** as a white amorphous solid (15mg, R_f 0.35).

^{1}H NMR plate 1

Appendix I

11.2.2.2 3', 4', 5-Triacetoxy-7-(β-2'',2''', 3'',3''', 4'', 4'''-hexa-*O*-acetyl-rutinosideoxy) flavone (Scolymoside)[158](63).

PLC purification of the precipitate [B:A, 8:2 (v/v)] yielded **(63)** as a white amorphous solid (11mg, R_f 0.30).

^{1}H NMR plate 11
COSY 11a-1
NOESY 11b-1, 2, 3
Appendix I

11.2.2.3 3', 5, -Diacetoxy-7-(β-2'',2''', 3'',3''', 4'',4'''-hexa-*O*-acetyl-rutinosideoxy)-4'-methoxyflavanone (Hesperedin)[160] (64)

PLC purification of the precipitate [B:A, 8:2 (v/v)] yielded **(64)** as a white amorphous solid (9mg, R_f 0.43).

^{1}H NMR plate 12
Appendix I

11.2.3 Isolation of compounds from fraction A3

11.2.3.1 1,3,6,7-Tetraacetoxy-4-(β-D-2'', 3'', 4'', 6''-tetra-*O*-acetyl-glucopyranosyl) xanthone (Mangiferin)[160] (67)

The precipitate from a ethanolic solution of fraction A3 [B:A, 8:2 (v/v)] yielded **(67)** as a white amorphous solid (5mg, R_f 0.45).

^{1}H NMR plate 13
Appendix I

11.2.4 Isolation of compounds from fraction A4

11.2.4.1 *O*-Acetyl-4-(*O*-β-D-2',3',4',6'-tetra-*O*-acetylglucopyranosyl) tyrosol (58)

PLC separation of fraction A4 [B:A, 7:3 (v/v)] yielded **(58)** as a white amorphous solid (3.3mg, R_f 0.21).

^{1}H NMR plate 6
COSY 6a-1, 2
NOESY 6b-1
Appendix I

11.2.4.2 4-[*O*-α-2",3",4"-Tri-*O*-acetylapiofuranosyl-(1"→6')-β-D-2',3',4'-tri-*O*-acetylglucopyranosyloxy] benzaldehyde (61)

PLC separation of fraction A4 [B:A, 7:3 (v/v)] yielded **(61)** as a white amorphous solid (0.4mg, R_f 0.40).

^{1}H NMR plate 6
Appendix I

11.2.5 Isolation of compounds from fraction A5

11.2.5.1 1-[β-D-2',3',4'-Tri-*O*-acetylglucopyranosyloxy]-2-(3,5-diacetoxyphenyl)ethane(59)

PLC separation of fraction A5 [B:A, 8:2 (v/v)] yielded **(59)** as a white amorphous solid (3.1mg, R_f 0.45).

^{1}H NMR plate 7
COSY 7a-1
NOESY 7b-1, 2
Appendix I

11.2.5.2 1-Acetoxy-2-(β-D-2',3',4',6'-tetra-*O*-acetylglucopyranosyloxy)ethene (60)

PLC separation of fraction A5 [B:A, 8:2 (v/v)] yielded **(60)** as a white amorphous solid (3.5mg, R_f 0.52).

^{1}H NMR plate 8
COSY 8a-1
NOESY 8b-1
Appendix I

11.2.5.3 3,4',5,7-Tetra-*O*-acetyl-6-(β-D-2'', 3'', 4'', 6''-tetra-*O*-acetyl-glucopyranosyl) kaempferol (68)[168]

PLC separation of fraction A5 [B:A:M, 90:8:2 (v/v)] yielded **(68)** as a white amorphous solid (0.5mg, R_f 0.42).

^{1}H NMR plate 16
Appendix I

11.2.5.4 3,4',6,7-Tetraacetoxy-5-(β-D-2'', 3'', 4'', 6''-tetra-*O*-acetyl-glucopyranosyl) flavonol (69)

PLC separation of fraction A5 [B:A 8:2 (v/v)] yielded **(69)** as a white amorphous solid (8mg, R_f 0.40).

^{1}H NMR plate 17
COSY 17a-1
HMQC 17d-1
Appendix I

11.2.6 Isolation of compounds from fraction A6

11.2.6.1 3',4',5,7-Tetra-*O*-acetyl-luteolin (56)[153]

PLC separation of fraction A6 [B:A 8:2 (v/v)] yielded **(56)** as a white amorphous solid (4.4mg, R_f 0.46).

^{1}H NMR plate 4

Appendix I

11.2.7 Isolation of compounds from fraction A7

11.2.7.1 3',4',7-Tri-acetoxy-5-(β-D-2'',3'',4'',6''-tetra-*O*-acetylglucopyranosyloxy)flavan (62)

PLC separation of fraction A7 [B:A 8:2 (v/v)] yielded **(62)** as a white amorphous solid (9.4mg, R_f 0.50).

^{1}H NMR plate 10

COSY 10a-1, 2, 3

NOESY 10b-1, 2, 3

Molecular Mechanics Model 10c

Appendix I

11.2.7.1 3', 4', 5, -Triacetoxy-7-(β-2'',2''', 3'',3''', 4'', 4'''- hexa-*O*-acetyl-rutinosideoxy) flavanone (Eriocitrin)[166] (66)

PLC separation of fraction A7 [B:A 8:2 (v/v)] yielded **(66)** as a white amorphous solid (14.6mg, R_f 0.42).

^{1}H NMR plate 14

COSY 14a-1, 2

Appendix I

11.2.8 Isolation of compounds from fraction A8

11.2.8 3',4',5,7-Tetra-O-methyl-orobol (57)[154]

PLC separation of fraction A7 [B:A 8:2 (v/v)] yielded (**57**) as a white amorphous solid (10mg, R_f 0.76).

^{1}H NMR plate 5

Appendix I

11.3 Separation and enrichment of the phenolic metabolites from the methanol extract (Column B).

An aqueous suspension of the methanol extract was exhaustively extracted with ethyl acetate (32g) and separated on Sephadex LH-20/EtOH column with a flow rate of 1ml/min, collecting 32 min fractions. Following TLC inspection the collected tubes were combined as follows:

Tubes	Fraction	Yield
0 - 30	B1	1.28g
31 – 54	B2	0.94g
55 - 86	B3	4.218g
87 - 104	B4	2.71g
105 – 130	B5	2.23g
131 – 168	B6	1.52g
169 – 198	B7	0.91g
199 – 230	B8	0.74g
231 – 258	B9	2.23g
259 – 290	B10	1.52g
291 – 320	B11	1.27g
321 – 358	B12	1.39g
359 – 445	B13	1.68g
446 - 545	B14	0.60g
Total phenolic compounds	23.23g	
Precipitate1	1.36g	

Precipitate 2	4.67g	
Precipitate 3	2.24g	
Total	31.54g	
Column residue	0.46g	

11.3.1 Isolation of compounds from fraction B3

11.3.1 6-Acetoxy-3,3-dimethyl-7-[*O*-α-2",3",4"-tri-*O*-acetylapiofuranosyl-(1"→6')-β-D-2',3',4'-tri-*O*-acetylglucopyranosyloxy] oct-4-enoic acid (54)

PLC separation of fraction B3 [B:A 8:2 (v/v) x 2] yielded **(54)** as a white amorphous solid (34mg, R_f 0.57).

^{1}H NMR plate 2
COSY 2a-1, 2, 4
NOESY 2b-1, 2
^{13}C 2c-1
HMQC 2d-1, 2
HMBC 2e-1, 6
DEPT 135° 2f-1
Appendix I

11.3.2 Isolation of compounds from fraction B13

11.3.2.1 3',4',5,5',7-Pentaacetoxy 8-(β-D-2'', 3'', 4'', 6''tetra-*O*-acetyl-glucopyranosyl) flavanone (70)

PLC separation of fraction B13 [B:A:M 90:8:2 (v/v) x 2] yielded **(70)** as a white amorphous solid (3.0mg, R_f 0.33).

^{1}H NMR plate 18
^{1}H NMR plate 18-1 ambient temp
COSY 18a-1,2
NOESY 18b-1, 2
Appendix I

11.3.3 Isolation of compounds from fraction C14

11.3.3.1 3',3",4',4",5,5',5",7-Octa-*O*-acetyl-epicatechin-3-*O*-gallate (55)[152]

PLC separation of fraction B14 [B:A 8:2 (v/v)] yielded **(55)** as a white amorphous solid (33.6mg, R_f 0.27).

^{1}H NMR plate 3

Appendix I

11.3.4 Isolation of compounds from fraction C10

11.3.4.1 4',5-Diacetoxy-7-(β-2'',2''',3'',3''',4'',4'''-hexa-O-acetyl-rutinosideoxy) flavanone (Narirutin)(65)[165]

PLC separation of fraction B10 [B:A 8:2 (v/v)] yielded **(65)** as a white amorphous solid (8mg, R_f).

^{1}H NMR plate 13

NOESY 13-b-1

Appendix I

CHAPTER 12

Synthesis of 3', 4', 7-triacetoxy-5-(β-D-2'', 3'', 4'', 6''-tetra-*O*-acetyl-glucopyranosyloxy) flavan.

4,6-Dimetoxy-2-hydroxyacetophenone (76)

Commercial phloroacetophenone (500 mg, 2.97 mmol) was selectively methylated with dimethylsulfate to yield 4,6-dimetoxy-2-hydroxy acetophenone **(76)** (204 mg, 35%) (R_f = 0.86, B:A 8:2) after FCC. ^{1}H NMR [plate 19 ($CDCl_3$)]: H-5 (δ6.08, d, J = 2.0Hz), H-3(δ5.94, d, J = 2.0Hz), OMe (δ3.87, s), OMe (δ3.84, s), $C\underline{H}_3$ (δ2.63, s).

4,6-Dimethoxymethyl-2-hydroxyacetophenone (83)

Commercial phloroacetophenone (500 mg, 2.97 mmol) was selectively metoxymethylated and purified by FCC (R_f = 0.84, B:A 8:2) to yield 4,6-dimethoxymethyl-2-hydroxyacetophenone **(83)** (116 mg, 15.2%) (R_f = 0.86, B:A 8:2) after FCC. ^{1}H NMR [plate 20 ($CDCl_3$)]: H-5(δ6.26, d, J = 2.0Hz), H-3(δ6.24, d, J = 2.0Hz), $C\underline{H}_2$-O (5.26, s), $C\underline{H}_2$-O (5.17, s), O-$C\underline{H}_3$ (δ3.57, s), O-$C\underline{H}_3$ (δ3.47, s), -$COC\underline{H}_3$(δ2.66, s).

2-Hydroxy-4,6-dimethoxybenzaldehyde (77)

Commercial 2,4,6-trihydroxy benzaldehyde (500mg, 3.24 mmol) was selectively methylated with dimethylsulfate and purified by FCC (R_f = 0.84, B:A 8:2) to yield the dimethylated product **(77)** (66.2 mg, 11.2%).. ^{1}H NMR [plate21 ($CDCl_3$)]: H-5(δ6.04, d, J = 2.0Hz), H-3(δ5.90, d, J = 2.0Hz), OMe(δ3.82, s), OMe(δ3.84, s), $C\underline{H}$O(δ10.10, s).

12.1 Robison condensation

Commercial 3,4-dimetoxy acetophenone (100mg) and 2-hydroxy-4,6-dimethoxybenzaldehyde **(77)** (1 *eq.*) were dissolved at 1°C in EtOAc (5ml) saturated with HCl gas and kept at 1°C for 3 days. The mixture was then poured into dry diethyl ether (20ml) and the crystalline flavylium salt collected.

12.2 General procedure for the preparation of chalcones.

To a solution of the appropriate acetophenone (3.0 mmol) in ethanol (20 ml / 1g acetophenone) was added 50% (m/v) *aq.* KOH (0.4 ml /mmol acetophenone) and the mixture was stirred at room temperature for 30 min. Excess benzaldehyde (1.2 *eq.*) was added to the mixture. After exhaustion of the acetophenone (5-24h) (TLC), water was added to the solution which was then extracted with Et_2O (4 x 20ml). Drying of the extracts (Na_2SO_4) followed by evaporation of the solvent and FCC and / or crystallisation from ethanol, gave the chalcones.

2'-Hydroxy-,3,4,4',6'-tetramethoxy chalcone (78)

4,6-dimethoxy-2-hydroxy acetophenone **(76)** (200mg, 1.02 mmol) and commercial 3,4-dimethoxy benzaldehyde (203mg, 1.22 mmol, 1.2 *eq.*) was condensed under basic conditions to yield 2'-hydroxy-,3,4,4',6'-tetramethoxy chalcone **(78)** (91mg, 26%) after recrystallization from ethanol (Rf = 0.78, B:A 8:2). ^{1}H NMR [plate 22 ($CDCl_3$)]: H-α(δ7.77, d, J = 16.0Hz), H-β (δ7.83, d, J = 16.0Hz), H-6(δ7.24, dd, J = 2.0, 8.0Hz), H-2(δ7.15, d, J = 2.0Hz), H-5(δ6.92, d, H = 8.0Hz), H-5'(δ6.13, d, J = 2.0Hz), H-3'(δ5.98, d, J = 2.0Hz), OMe(δ3.97, s), OMe(δ3.96, s), OMe(δ3.95, s) and OMe(δ3.86, s).

2'-Hydroxy-4',6'-dimethoxymetyl-3,4-dimethoxychalcone (84)

2-hydroxy -4,6-dimethoxymethylacetophenone **(83)** (200mg, 0.78 mmol) and commercial 3,4-dimethoxybenzaldehyde (1.2 *eq.*) was condensed under basic conditions to yield 2'-hydroxy-4',6'-dimethoxymetyl-3,4-dimethoxychalcone **(84)** (44mg, 14%) after FCC (Rf = 0.76, B:A 8:2).. ^{1}H NMR [plate 23 ($CDCl_3$):] H-β(δ7.85, d, J = 16Hz), H-α(δ7.75, d, J = 16Hz), H-6 (δ7.20, dd, J = 2.0, 8.0Hz), H-2(δ7.14, d, J = 2.0Hz), H-5(δ6.90, d, H = 8.0Hz), H-5'(δ6.31, d, J = 2.0Hz), H-3'(δ6,21 d, J = 2.0Hz), C$\underline{H}_2$ (5.29, s), C$\underline{H}_2$ (5.18, s), 2 x OMe (δ3.93, s), C$\underline{H}_3$ (δ3.54, s), C$\underline{H}_3$ (δ3.48, s).

12.3 β-Cyclization of 2'-hydroxy-,3,4,4',6'-tetramethoxy- (78) and 2'-hydroxy-4',6'-dimethoxymetyl-3,4-dimetoxychalcone (84).

5,7-Dihydroxy-3',4'-dimethoxyflavanone (85)

2'-hydroxy-4',6'-dimethoxymetyl-3,4-dimetoxychalcone **(84)** (100mg, 247 mmol) in ethanol (10ml) was refluxed for 24h on a waterbath after addition of 3N HCl (5ml). After completion of the reaction (t.l.c.) the chalcone and the isomerization product, 5,7-dihydroxy-3',4'-dimethoxyflavanone **(85)** (10mg, 10%) was purified by chromatographic methods (FCC or PLC) (Rf = 0.58, B:A 8:2). ^{1}H NMR [plate 24 ($CDCl_3$)]: H-5'(δ7.21, d, J = 2.0Hz), H-6'(δ7.03, dd, J = 2.0, 8.0Hz), H-2'(δ7.00, d, J = 8.0Hz), H-6(δ5.98, d, J = 2.0Hz), H-8(δ.96, d, J = 2.0Hz), H-2(δ5.47, dd, J = 3.0, 12.5Hz), OMe(δ3.86, s), OMe(δ3.84, s), H-3α(δ3.24, dd, J = 12.5, 17.0Hz), H-3β(δ2.75, dd, J = 3.0, 17.0Hz).

5,7,3',4'-Tetramethoxyflavanone (79)

2'-hydroxy-,3,4,4',6'-tetramethoxychalcone **(78)** (100mg, 290 mmol) in ethanol (10ml) was refluxed for 24h on a water-bath after addition of 3N HCl (5ml). After completion of the reaction (TLC) the chalcone and the isomerization product, 5,7,3',4'-tetramethoxyflavanone **(79)** (15mg, 15%) was separated by chromatographic methods (FCC or PLC) (Rf = 0.38, B:A 8:2). ^{1}H NMR [plate 25 ($CDCl_3$)]: H-5',6'(δ7.00, m), H-2'(δ6.90, d, J = 8.5Hz), H-6(δ6.16, d, J = 2.0Hz), H-8(δ6.10, d, J = 2.0Hz), H-2(δ5.35, dd, J = 2.5, 13Hz), OMe (δ3.93, s), 2 x OMe(δ3.90, s), OMe(δ3.83, s), H-3α(δ3.05, dd, J = 13.0, 16.5Hz), H-3β(δ2.77, dd, J = 2.5, 16.5Hz).

Attempted demethylation of 5,7,3',4'-tetramethoxyflavanone (79) with anhydrous aluminum chloride in acetonitrile.

The title flavanone **(79)** (20 mg, 5.44 x 10^{-2} mmol) was dissolved in a solution of anhydrous aluminum chloride (0.2 g) in acetonitrile (2 ml) and refluxed at 70 °C for 10 h. The solution was poured into 2% hydrochloric acid (4 ml), refluxed at 80 °C for 30 min, diluted with water (4 ml), and concentrated to 5 ml under reduced pressure. The product was separated by PLC chromatography.

Attempted demethylation of 5,7,3',4'-tetramethoxyflavanone (79) with LiCI.

5,7,3',4'-tetramethoxyflavanone (20 mg, 5.44 x 10^{-2} mmol) and LiCl (15.2 mg, 0.36 mmol) were heated in boiling DMF (2 ml) and the reaction monitored by TLC. When the starting material was exhausted (22 h), 10% aqueous NaOH (1 ml) was added, the solution washed with Et_2O (2 x 5 ml), acidified with 10% aqueous HC1 (1 ml), and extracted with Et_2O (2 x 5 ml). The organic phase was washed with brine (2ml), dried (Na_2SO_4) and concentrated on a rotavapor. Product was separated by PLC.

Attempted demethylation of 5,7,3',4'-tetramethoxyflavanone (79) with BCl_3

Boron trichloride (15.9 mg, 1.36 x 10^{-1} mmol) in dichloromethane (1 ml) was added to an ice-cold solution of the substrate (50 mg, 1.36 x 10^{-1} mmol) in dichloromethane (1 ml). The cooling bath was removed, the crimson solution was stirred at room temperature for 20 h, and then poured into ice and water. The aqueous suspension was extracted with Et_2O (2 x 5 ml). The ethereal extract was washed with 10% aqueous sodium hydroxide and acidification and isolation with ether. Evaporation of the above ethereal extract gave the product which was separated by PLC.

2,6-Dihydroxy-4-methoxyacetophenone (91)

To a solution of anhydrous $ZnCl_2$ (1.65 g, 12.1 mmol) in glacial AcOH (20 ml) at 140 °C, 3,5-dihydroxyanisol (1.69g, 12.1 mmol) was added 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. Purification by PLC with C_6H_6-Me_2CO (8:2) as solvent afforded a mixture of both 2,6-dihydroxy-4-methoxyacetophenone **(91)** and 4,6-dihydroxy-2-metoxyacetophenone [plate 26] as white needles (R_f 0.7) in 34% yield. ^1H NMR [plate 26 (CD_3CO)]: H-5(δ6.04, d, J = 2.0Hz), H-3(δ5.96, d, J = 2.0Hz), H-3,5(δ5.99, s), OMe(δ3.91, s), OMe(δ3.80, s), CH_3(δ2.63, s), CH_3(δ2.55, s).

12.4 Glycosylations

6-(β-D-2',3',4',6'-Tetra-*O*-acetyl-glucopyranosyloxy)-2-hydroxy-4-methoxyacetophenone (92)

A solution of the mixture 2,6-dihydroxy-4-methoxyacetophenone / 4,6-dihydroxy-2-methoxyacetophenone (50mg, 2.74 x 10^{-1} mmol) in dry toluene (3.0 ml) containing $CdCO_3$ (172 mg, 1.09 mmol) was refluxed for 4h with removal of the generated water through molecular sieves 4Å in a dropping funnel. The tetra-*O*-acetylbromoglucose (409mg, 1.09 mmol) was added and the mixture was refluxed for a further 21h. The hot reaction mixture was immediately filtered through a Celite pad, and the solid was washed with hot $CDCl_3$ (10 ml). The filtrate and the washings were combined and evaporated *in vacuo*. The residue was purified by preparative TLC (B:A / 8:2) to give 6-(β-D-2',3',4',6'-tetra-*O*-acetylglucopyranosyloxy)-2-hydroxy-4-methoxyacetophenone **(92)** (R_f 0.67) as colorless oil (34.5 mg, 24.5%). ^{1}H NMR [plate 27 ($CDCl_3$)]: H-5(δ6.18, d, J = 2.0Hz), H-3(δ6.06, d, J = 2.0Hz), H-1'(δ5.35, d, J = 8.0Hz, β-coupled), H-2'[δ5.35, m, (overlapping H-1')], H-3'(δ5.27, t, J = 8.0Hz), H-4'(δ5.18, t, J = 10.0Hz), 1 x H-6(δ4.27, dd, J = 6.0, 12.0Hz), 1 x H-6(δ4.19, dd, J = 2.0, 12.0Hz), H-5'(δ3.92, m), OMe(δ3.83, s), C<u>H</u>$_3$(δ2.56, s) and four acetoxy singlets at δ2.11, δ2.08, δ2.07, δ2.05.

6-(β-D-2',3',4',6'-Tetra-*O*-acetylglucopyranosyloxy)-2,4-dihydroxyacetophenone (90)

Commercial phloroacetophenone (50 mg, 297 mmol) was glycosylated and separated by PLC to yield the glycosylated product **(90)** (37mg, 25%) (Rf = 0.45, B:A 8:2) as colorless oil. ^{1}H NMR [plate 28 ($CDCl_3$)]: H-3 (δ6.32, d, J = 2.0Hz), H-5(δ6.13, d, J = 2.0Hz), H-1'(δ5.71, d, J = 3.5Hz) (α-coupled), H-3' (δ5.68, dd, J = 9.0, 10.0Hz), H-4'(δ5.21, dd, J = 9.0, 10.0Hz), H-2'(δ5.14, dd, J = 3.5, 10.0Hz), 1 x H-6'(δ4.27, dd, J = 4.0, 12.0Hz), 1 x H-6'(δ4.20, dd, J = 2.0, 12.0Hz), H-5'(δ4.07, m), C<u>H</u>$_3$(δ2.79, s) and 4 x OAc at δ2.20, δ2.10, δ2.07 and δ2.06.

6-(β-D-2',3',4',6'-Tetra-*O*-acetylglucopyranosyloxy)-2-hydroxyacetophenone (89)

Commercial resacetophenone (50 mg, 329 mmol) was glycosylated and separated by PLC to yield the glycosylated product **(89)** (55mg, 35%) (Rf = 0.7, B:A 8:2) as a colorless oil. . ^{1}H NMR [plate 29 ($CDCl_3$)]: H-4(δ7.35, t, J = 8.0Hz), H-5(δ6.75, dd, J = 1.0, 8.0Hz), H-3(δ6.71, dd, J = 1.0, 8.0Hz), H-1'(δ5.81, d, J = 3.5Hz), H-3'(δ5.70, dd, J = 9.0, 10.0Hz), H-4(δ5.22, dd, 9.0, 10.0Hz), H-2(δ5.16, dd, 3.5, 10.5Hz), 1 x H-6'(δ4.28, dd, J = 4.5, 12.5Hz), 1 x H-6'(δ4.11, dd, J = 2.0, 12.5Hz), H-5'(δ4.09, m), $C\underline{H}_3$(δ2.87, s) and 4 x OAc at δ2.08-δ2.06.

Appendix I

PHENOLIC COMPOUNDS FROM *CYCLOPIA SUBTERNATA* (Honeybush Tea)

Penta-*O*-acetyl-(+)-pinitol (53)

^{1}H NMR ($CDCl_3$) (298K) (δ_H): 5.37 (3H, m, H-1,4,6), 5.23 (1H, dd, J = 3.0Hz and 10.0Hz, H-5), 5.21 (1H, dd, J = 3.0Hz and 10.0Hz, H-2), 3.64 (1H, dd, J = 10.0Hz, H-3), 3.49 (3H, s, OMe).

6-Acetoxy-3,3-dimethyl-7-[*O*-α-2",3",4"-tri-*O*-acetylapiofuranosyl-(1"→6')-β-D-2',3',4'-tri-*O*-acetylglucopyranosyloxy] oct-4-enoic acid (54)

^{1}H NMR ($CDCl_3$) (298K) (δ_H): 2.44 (1H, d, J = 17.0Hz, H-2), 2.25 (1H, d, J = 17.0Hz, H2), 5.78 (2H, m, H-5,4), 5.84 (1H, m, H-6), 4.31 (1H, t, J = 6.0Hz, H-7), 4.58 (1H, d, J = 8.0Hz, H-1'), 4.95 (2H, m, H-3',2'), 5.20 (1H, t, J = 9.5Hz, H-4'), 3.60 (1H, m, H-5'), 3.71 (1H, dd, J = 2.0Hz, 11.0Hz, H-6'), 3.54 (1H, dd, J = 6.0Hz, 11.0Hz, H-6'), 5.38 (1H, d, J = 0.5Hz, H-1''), 5.01 (1H, d, J = 0.5Hz, H-2''), 4.15 (1H, d, J = 12.0Hz, H-4"), 4.23 (1H, d, J = 12.0Hz, H-4"), 4.62 (1H, d, J = 12.0Hz, H-5"), 4.72 (1H, d, J = 12.0Hz, H-5"), 1.24 (3H, d, J = 6.5Hz, C-7-CH_3), 1.02 (3H, s, C-3-C$\underline{H}_3$), 1.09 (3H, s, C-3-C$\underline{H}_3$), 1.89 (3H, s, OAc), 2.12 (3H, s, OAc), 2.10 (3H, s, OAc), 2.05 (9H, s, 3 x OAc), 2.01 (3H, s, OAc).

3',3",4',4",5,5',5",7-Octa-*O*-acetyl-epicatechin-3-*O*-gallate (55)

^{1}H NMR ($CDCl_3$) (298K) (δ_H): 7.64 (2H, s, H-2",6"), 7.25 (2H, s, H-2',6'), 6.75 (1H, d, J = 2.0Hz, H-8), 6.63 (1H, d, J = 2.0Hz, H-6), 5.65 (1H, s, H-3), 5.20 (1H, s, H-2), 3.05 (2H, m, H-4), 2.25-2.31 (H24, s, 8 x OAc).

3',4',5,7-Tetra-*O*-acetyl-luteolin (56)

^{1}H NMR ($CDCl_3$) (298K) (δ_H): 6.63 (1H, s, H-3), 7.76 (1H, dd, J = 2.0 and 8.0Hz, H-6'), 7.72 (1H, d, J = 2.0Hz, H-2'), 7.38 (1H, d, J = 8.0Hz, H-5'), 7.38 (1H, d, J = 2.0Hz, H-6), 6.88 (1H, d, J = 2.0Hz, H-8), 2.35, (3H, s, OAc), 2.37 (6H, s, 2 x OAc), 2.46 (3H, s, OAc).

3',4',5,7-Tetra-O-methyl-orobol (57)

^{1}H NMR ($CDCl_3$) (298K) (δ_H): 7.81 (1H, s, H-2), 7.24 (1H, dd, J = 2.0 and 8.0Hz, H-6'), 7.15 (1H, d, J = 2.0Hz, H-2'), 6.92 (1H, d, J = 8.0Hz, H-5'), 6.14 (1H, d, J = 2.0Hz, H-8), 5.99 (1H, d, J = 2.0Hz, H-6), 3.96 (3H, s, OMe), 3.95 (3H, s, OMe), 3.94 (3H, s, OMe), 3.86 (3H, s, OMe).

***O*-Acetyl-4-(*O*-β-D-2',3',4',6'-tetra-*O*-acetylglucopyranosyl) tyrosol (58)**

^{1}H NMR ($CDCl_3$) (298K) (δ_H): 6.95 (2H, d, J = 8.0Hz, H-2,6), 7.16 (2H, d, J = 8.0Hz, H-3,5), 2.95 (2H, t, J = 7.0Hz, α-H), 4.25, t, J = 7.0Hz, β-H), 5.07 (1H, d, J = 8.0Hz, H-1'), 5.30 (2H, m, H-2', 3'), 5.18 (1H, m, H-4'), 3.87 (1H, m, H-5'), 4.31 (1H, dd, J = 5.0Hz and 12.0Hz, H-6'), 4.18 (1H, dd, J = 5.0Hz and 12.0Hz, H-6'), 2.19 (3H, s, OAc), 2.10 (3H, s, OAc), 2.08 (3H, s, OAc), 2.07 (3H, s, OAc), 2.05 (3H, s, OAc).

1-[β-D-2',3',4'-Tri-*O*-acetylglucopyranosyloxy]-2-(3,5-diacetoxyphenyl)ethane(59)

^{1}H NMR ($CDCl_3$) (298K) (δ_H): 2.90 (2H, m, H-α), 3.69 (2H, m, H-β), 7.10 (2H, d, J = 2.0Hz, H-2,6), 7.04 (1H, t, J = 2.0Hz, H-4), 4.28 (1H, dd, J = 4.5Hz and 12.0Hz, H-6'), 4.15 (1H, dd, J = 4.5Hz and 12.0Hz, H-6'), 5.01 (1H, t, J = 10.0Hz, H-2'), 5.20 (1H, t, 9.0Hz, H-3'), 5.10 (1H, t, J = 9.5Hz, H-4'), 4.15 (1H, m, H-5'). 2.32 (6H, s, 2 x OAc), 2.11 (3H, s, OAc), 2.04 (3H, s, OAc), 2.01 (3H, s, OAc), 1.94 (3H, s, OAc).

1-Acetoxy-2-(β-D-2',3',4',6'-tetra-*O*-acetylglucopyranosyloxy)ethene (60)

^{1}H NMR ($CDCl_3$) (298K) (δ_H): 6.70 (2H, m, H-α,β), 5.10 (1H, d, J = 7.0Hz, H-1'), 5.27 (1H, t, J = 8.0Hz, H-2'), 5.10 (2H, m, H-1',3'), 5.18 (1H, t, J = 10Hz, H-4'), 3.78 (1H, m, H-5'), 4.25 (2H, m, H-6'), 2.17 (3H, s, OAc), 2.08 (3H, s, OAc), 2.07 (3H, s, OAc), 2.06 (3H, s, OAc), 2.04 (3H, s, OAc).

4-[*O*-α-2",3",4"-Tri-*O*-acetylapiofuranosyl-(1"→6')-β-D-2',3',4'-tri-*O*-acetylglucopyranosyloxy] benzaldehyde (61)

^{1}H NMR ($CDCl_3$) (298K) (δ_H): 7.88 (2H, d, J = 8.0Hz, H-2,6), 7.12 (2H, d, J = 8.0Hz, H-3,5), 9.50 (1H, s, CHO), 5.05 (2H, m, H-1',4'), 5.28 (2H, m, H-2',3'), 5.36 (1H, s, H-2"), 4.98 (1H, s, H-1"), 4.22 (1H, d, J = 10.0Hz, H-4"), 4.14 (1H, d, J = 10.0Hz, H-4"), 4.80 (1H, d, J = 12.0Hz, H-5"), 4.56 (1H, d, J = 12.0Hz, H-5"), 3.63 (1H, dd, J = 7.0 and 11.0Hz, H-6"), 3.76 (1H, dd, J = 2.0 and 11.0Hz, H-6"), 3.90 (1H, m, H-5"), 2.03 (3H, s, OAc), 2.04 (3H, s, OAc), 2.06 (3H, s, OAc), 2.08 (3H, s, OAc), 2.10 (3H, s, OAc), 2.16 (3H, s, OAc).

3',4',7-Triacetoxy-5-(β-D-2'',3'',4'',6''-tetra-*O*-acetylglucopyranosyloxy)flavan (62)

^{1}H NMR (C_6D_6) (298K) (δ_H): 1.65 (2H, m, H-4), 2.60 (2H, m, H-3), 4.58 (1H, dd, J = 3.0 and 9.0Hz, H-2), 7.01 (1H, dd, J = 2.0 and 8.0Hz, H-6') 7.33 (1H, d, J = 2.0Hz, H-2'), 7.15 (1H, d, J = 8.0Hz, H-5'), 6.79 (1H, d, J = 2.0Hz, H-6), 6.87 (1H, d, J = 2.0Hz, H-8), 4.87 (1H, d, J = 8.0Hz, H-1"), 5.69 (1H, dd, J = 8.0 and 10.0Hz, H-2"), 5.50 (1H, t, J = 9.0Hz, H-3"), 5.35 (1H, t, J = 9.0Hz, H-4"), 3.25 (1H, m, H-5"), 4.28 (1H, dd, J = 6.0 and 12.0Hz, H-6"), 4.11 (1H, dd, J = 3.0 and 12.0Hz, H-6"), 1.89 (3H, s, OAc), 1.87 (9H, s, 3 x OAc), 1.86 (3H, s, OAc), 1.81 (3H, s, OAc), 1.76 (3H, s, OAc).

3', 4', 5-Triacetoxy-7-(β-2'',2''', 3'',3''', 4'', 4'''-hexa-*O*-acetyl-rutinosideoxy) flavone (63)

^{1}H NMR (C_6D_6) (δ_H): 7.75 (1H, d, J = 2.0Hz, H-2'), 7.33 (1H, dd, J = 2.0 and 8.0 Hz, H-6'), 7.21 (1H, d, J = 8.0Hz, H-5'), 6.81 (1H, d, J = 2.0Hz, H-8), 6.77 (1H, d, J = 2.0Hz, H-6), 6.58 (1H, s, H-3), 5.72 (1H, dd, J = 3.0 and 10.0Hz, H-3'''), 5.66 (1H, m, H-2'''), 5.57 (3H, m, H-2'', H-3'',H-4'''), 5.16 (1H, t, J = 10.0Hz, H-4''), 5.05 (1H, d, J = 8.0 Hz, H-1''), 4.88 (1H, d, J = 2.0 Hz, H-1'''), 4.02 (1H, m, H-5''') 3.63 (2H, m, H-6"), 3.36 (1H, m, H-5''), 1.99 (3H, s, OAc), 1.90 (3H, s, OAc), 1.83 (3H, s, OAc), 1.86 (6H, s, 2 x OAc), 1.80 (3H, s, OAc), 1.72 (3H, s, OAc), 1.69 (3H, s, OAc), 1.66 (3H, s, OAc), 1.28 (3H, d, J = 6.0Hz, Me)

3', 5, -Diacetoxy-7-(β-2'',2''', 3'',3''', 4'',4'''-hexa-*O*-acetyl-rutinosideoxy)-4'-metoxyflavanone (64)

^{1}H NMR ($CDCl_3$) (298K) (δ_H): 7.28 (1H, dd, J = 2.0Hz and 8.0Hz, H-6'), 7.17 (1H, d, J = 2.0Hz, H-2'), 7.03 (1H, d, J = 8.0Hz, H-5'), 6.49 (1H, d, J = 2.0Hz, H-6), 6.33 (1H, d, J = 2.0Hz, H-8), 5.46 (1H, dd, J = 3.0, 12.5Hz, H-2), 3.01 (1H, dd, J = 12.5, 16.5Hz, H-3α), 2.78 (1H, dd, J = 3.0, 16.5Hz, H-3β), 5.18 (1H, d, J = 7.5Hz, H-1''), 5.27 (1H, t, J = 6.0Hz, H-2''), 5.80 (1H, t, J = 9.0Hz, H-4'''), 5.23 (4H, m, H-2''',3'',3''',4''), 5.03 (1H, t, J = 9.5Hz, H-2'''), 4.70 (1H, d, J = 1.0Hz, H-1'''), 3.87 (2H, m, H-5'',5'''), 3.82 (1H, dd, J = 2.5 and 11.5Hz, 1 x H-6''), 3.63 (1H, dd, J = 5.5 and 11.5Hz, 1 x H-6''), 1.16 (3H, d, 6.0Hz, 5'''-CH_3), 2.11 (3H, s, OAc), 2.09 (3H, s, OAc), 2.06 (3H, s, OAc), 2.05 (6H, s, 2 x OAc), 1.96 (3H, s, OAc), 2.40 (3H, s, OAc), 2.35 (3H, s, OAc).

4',5-Diacetoxy-7-(β-2'',2''',3'',3''',4'',4'''-hexa-O-acetyl-rutinosideoxy) flavanone (65)

^{1}H NMR ($CDCl_3$) (298K) (δ_H): 7.49 (2H, d, J = 8.0Hz, H-2',6'), 7.17 (2H, d, J = 8.0Hz, H-3',5'), 6.50 (1H, d, J = 2.0Hz, H-6), 6.34 (1H, d, J = 2.0Hz, H-8), 5.46 (1H, dd, J = 2.0, 12.0Hz, H-2), 3.02 (1H, dd, J = 12.5, 16.5Hz, H-3α), 2.78 (1H, dd, J = 3.0, 16.5Hz, H-3β), 5.23 (7H, m, H-1'',2'',3'',4'',2''',3''',4'''), 3.85 (3H, m, H-5'',5''', 1 x H6''), 3.84 (1H, dd, J = 5.0 and 11.5Hz, H-6''), 4.70 (1H, d, J = 1.5Hz, H-1'''), 5.03 (1H, t, 9.5Hz, H-2'''), 1.16 (3H, d, 6.0Hz, 5'''-CH_3), 2.11 (3H, s, OAc), 2.09 (3H, s, OAc), 2.06 (3H, s, OAc), 2.05 (6H, s, 2 x OAc), 1.96 (3H, s, OAc), 2.40 (3H, s, OAc), 2.35 (3H, s, OAc).

3', 4', 5, -Triacetoxy-7-(β-2'',2''', 3'',3''', 4'', 4'''- hexa-*O*-acetyl-rutinosideoxy) flavanone (66).

^{1}H NMR (CD_6CO) (298K) (δ_H): 7.53 (2H, m, H-2',6'), 7.35 (1H, d, J = 8.0Hz, H5'), 6.45 (1H, d, J = 2.0Hz, H-6), 6.69 (1H, d, J = 2.0Hz, H-8), 5.64 (1H, m, H-2), 3.51 (1H, dd, J = 12.5, 16.5Hz, H-3α), 2.75 (1H, dd, J = 3.0, 16.5Hz, H-3β), 5.70 (1H, d, J = 8.0Hz, H-1''), 5.20 (4H, m, H-2'', 2''', 3''', 4''), 4.33 (1H, m, H-5''), 3.73 (1H, m, 1 x H-6''), 3.90 (2H, m, H-5''', 6''), 5.42 (1H, t, J = 10.0Hz, H-3''), 4.96 (1H, t, J = 10.0Hz, H-4'''), 3.91 (1H, m, H-5'''),4.77 (1H, d, J = 2.0Hz, H-1'''), 1.08 (3H, t, 6.0Hz, 5'''-CH_3), 2.03-2.08 (21H, s, 9 x OAc).

1,3,6,7-Tetraacetoxy-4-(β-D-2'', 3'', 4'', 6''-tetra-*O*-acetyl-glucopyranosyl) xanthone (67)

^{1}H NMR ($CDCl_3$) (343K) (δ_H): 8.10 (1H, s, H-8), 7.37 (1H, s, H-5), 7.25 (1H, s, H-2), 5.71 (1H, t, J = 9.5Hz, H-2'), 5.30 (1H, t, J = 9.5Hz, H-3'), 5.70 (1H, t, J = 9.5Hz, H-4'), 4.87 (1H, d, J = 10.0Hz, H-1'), 4.37 (1H, dd, J = 4.5Hz and 12.5Hz, 1 x H-6'), 4.04 (1H, dd, J = 2.0Hz and 12.5Hz, 1 x H-6'), 3.80 (1H, m, H-5'), 2.51 (3H, s, OAc), 2.43 (3H, s, OAc), 2.29 (3H, s, OAc), 2.28 (3H, s, OAc), 2.05 (3H, s, OAc), 2.04 (3H, s, OAc), 2.01 (3H, s, OAc), 1.78 (3H, s, OAc).

3,4',5,7-Tetraacetoxy-6-(β-D-2'', 3'', 4'', 6''-tetra-*O*-acetyl-glucopyranosyl) flavonol (68)

^{1}H NMR ($CDCl_3$) (298K) (δ_H): 7.76 (2H, d, J = 8.5Hz, H-2',6'), 7.16 (2H, d, J = 8.5Hz, H-3',5'), 6.72 (1H, s, H-8), 4.94 (1H, d, J = 10.0Hz, H-1"), 5.42 (1H, dd, J = 9.0 and 10.0Hz, H-2"), 5.35 (1H, t, J = 9.0Hz, H-3"), 5.29 (1H, t, J = 10.0Hz, H-4"), 3.92 (1H, m, H-5"), 4.36 (1H, dd, J = 3.5 and 12.0Hz, 1 x H-6"), 4.18 (1H, dd, J = 2.5 and 12.0Hz, 1 x H-6"), 2.34 (3H, s, OAc), 2.19 (3H, s, OAc), 2.16 (3H, s, OAc), 2.09 (6H, s, 2 x OAc), 2.03 (3H, s, OAc), 1.88 (3H, s, OAc), 1.74 (3H, s, OAc).

3,4',6,7-Tetraacetoxy-5-(β-D-2'', 3'', 4'', 6''-tetra-*O*-acetyl-glucopyranosyl) flavonol (69)

^{1}H NMR ($CDCl_3$) (333K) (δ_H): 7.19 (1H, d, J = 9.0Hz, H-3',5'), 7.82 (1H, d, J = 9.0Hz, H-2',6'), 7.07 (1H, s, H-8), 4.77 [1H, d, 10.0Hz, H1" (β-coupled)], 5.65 (1H, t, J = 9.0Hz, H-2"), 5.29 (1H, t, J = 9.0Hz, H-3"), 5.15 (1H, t, J = 9.5Hz, H-4"), 3.78 (1H, m, H-5"), 4.39 (1H, dd, J = 4.5 and 12.5Hz, 1 x H-6"), 4.03 (1H, dd, 2.0 and 12.5Hz, 1 x H-6"), 2.32 (3H, s, OAc), 2.17 (6H, s, 2 x OAc), 2.02 (3H, s, OAc), 2.04 (3H, s, OAc), 2.06 (6H, s, 2 x OAc), 2.41 (3H, s, OAc).

3',4',5,5',7-Pentaacetoxy 8-(β-D-2'', 3'', 4'', 6''tetra-*O*-acetyl-glucopyranosyl) flavanone (70)

^{1}H NMR ($CDCl_3$) (343K) (δ_H): 5.24 (1H, t, J = 9.0Hz, H3"), 5.13 (1H, t, J = 9.5Hz, H4"), 4.03 (1H, dd, J = 2.0 and 12.5Hz, 1 x H-6"), 3.68(1H, m, H-5"), 6.97 (1H, d, J = 2.0, H-2'), 7.11 (1H, d, J = 2.0Hz, H-6'), 7.13 (1H, s, H-6), 5.68 (1H, t, J = 9.5Hz, H-2"), 4.77 [(1H, d, J = 7.5Hz, H-1") β-coupled], 4.33 (1H, dd, J = 5.0 and 12.5Hz, 1 x H-6"), 2.28

(3H, s, OAc), 2.27 (3H, s, OAc), 2.15 (3H, s, OAc), 2.16 (3H, s, OAc), 2.06 (3H, s, OAc), 2.04 (6H, s, 2 x OAc), 2.01 (6H, s, 2 x OAc).

Opsomming

Cyclopia subternata (Fabaceae), algemeen bekend as Heuningbostee, is een van nagenoeg 24 *Cyclopia* spesies endemies tot die Kaapse fynbos gebied van Suid Afrika. Met die rugsteuning van ons aanvanklike ondersoek op *C intermedia* wat die teenwoordigheid van n verskeidenheid fenoliese vebindings, insluitend kumestane, isoflavone, flavanone, xanthone, flavone, pinitol, *p*-kumaarsuur en flavonoïed glukosiede, 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 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 *subternata* spesie.

Die asetoon en methanol ekstrakte van die ongefermenteerde lote van *C. subternata* het na chromatografiese skeidings (kolom met Sephadex LH 20 en preperatiewe dunlaag) n nuwe flavaan, flavonole, flavanone, flavone, isoflavone en $C_6.C_1$- en $C_6.C_2$-tipe verbindings gelewer. Die structure van die verbindings, as die volledige asetaat of gemetieleerde derivate, is met behulp van hoë-resolusie (300 MHz) ^{1}H KMR spektrometrie, (insluitend COSY, NOESY, HMQC, HMBC en DEPT 135° eksperimente) ^{13}C KMR spektroskopie, Molekulêre Modellering en Sirkulêre dichroïsme bepaal.

(+)-Pinnitol is saam met n nuwe apiofuranosiel(1"→6")-glukopiranosiel karboksiel suur as nie-flavonoïed verbindings geïsoleer. Die aglikone behels epikatesjien-3-*O*-gallaat (flavanol), luteolien (flavoon), en orobol (isoflavoon). Die nie-flavonoïed glikosiede word verteenwoordig deur 'n 4-*O*-glikopiranosiel tyrosol, apiofuranosiel(1"→6")-glikopiranosiel-bensaldehied en die asetaat derivate van twee nuwe O-glikosiede naamlik, 1-[β-D-2',3',4'-tri-*O*-asetielglukopiranosieloksi]-2-(3,5-diasetoksifeniel)etaan en 1-asetoksi-2-(β-D-2',3',4',6'-tetra-*O*-asetielglukopiranosieloksi)etaan. Die flavonïed-O-glukosiede word verteenwoordig deur n nuwe 3',4',7-trihidroksi-5-(glukopiranosieloksi) flavan, die flavoon, scolymosied en die flavanone, hesperedien, narirutien en eriocitrien. Die *C*-glukosiede bestaan uit die xantoon, mangiferin, n *C*-6 kaemferolglukosied en twee nuwe C-glukosiede naamlik, 3,4',6,7-tetrahidroksi-5-(β-D-

glukopiranosiel) flavonol en 3',4',5,5',7-pentahidroksi-8-(β-D-glukopiranosiel) flavanoon.

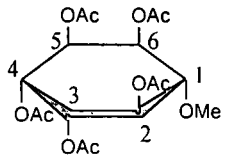
Die sintese van die flavaanglukosied wat geïsoleer is, is gemotiveer deur die feit dat die flavaan nuut is, die bweerde gesondheidseienskappe van flavane in die algemeen en die relatief onverkende roetes na flavonoïedglukosiede. Die effek van flavonoïedglikosilering op die oplosbaarheid en gedrag van die aglikoon, die verbeterde opname in die weefsel van die mens asook die onsekerheid oor die posisie waar die suiker gekoppel is aan die aglikoon, gewoonlik wanneer min material beskikbaar is, het verder die beoogde sintese van die flavaan glikosied ondersteun. In n poging om die 5-*O*-flavaanglukosied te sintetiseer is die gekose glukosilerings prosedure op 'n analoë 5,7-dihidroksi-3',4'-dimetoksiflavanoon toegepas, maar was onsuksesvol as gevolg van die suurheid van die 3-protone wat tot ringopening lei onder basiese toestande. Dieselfde glikosileringsprosedure is gepoog op die analoë chalkoon met 'n onbeskermde A-ring. Glukosilerings is suksesvol uitgevoer op resasetofenoon, floroasetofenoon en 4-metoksifloroasetofenoon as substrate vir die vorming van die chalkoonglikosied. Die kondensasie van die asetofenoonglikosiede met die beskermde bensaldehied om die chalkoonglikosied as voorloper vir die flavaan glikosied te verkry, was egter ook onsuksesvol.

Die voorgestelde fisiologiese en terapeutiese eienskappe van fenoliese metaboliete kan vereenselwig word met verbindings wat uit die tee geïsoleer is. Luteolien en eriodiktol vorm deel van die groep metaboliete met 'n 3',4'-diol funktionaliteit wat gekoppel word aan antioksidant aktiwiteit. Die antimikrobiese aktiwiteit van flavonoïede in plante is ook deeglik gedokumenteer en so ook die antivirale eienskappe en flavonoïede se vermoë om sleutel ensieme in die mitokondria respirasie te inhibeer. Daar is ook gevind dat n C-2, C-3-dubbelbinding, n 4-keto groep en die 3',4',5'-trihidroksilering van die B-ring sleutel vereistes is vir die inhibisie van NADH-oksidase. Verder word beweer dat hartaanvalle verminder word onder mense met 'n hoë flavonoïedinname. Die anti-inflamatoriese en anti-kanker aktiviteit saam met die toenemende belangstelling in plant flavonoïede om siektes te behandel en ook om die HI virus onder beheer te hou het 'n belang by hierdie tee aangewakker. 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 *C. subternata.*

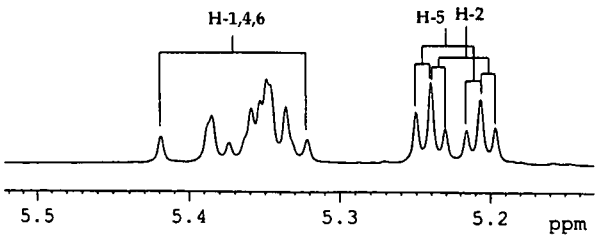
APPENDIX II

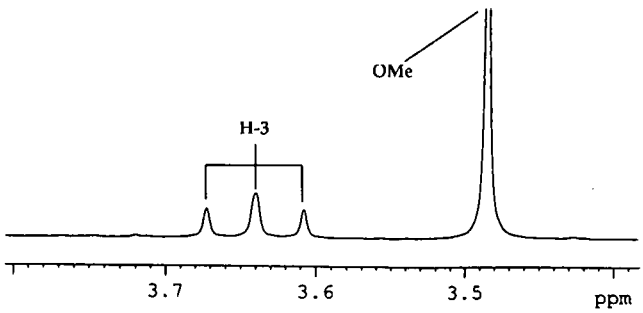
NMR DATA

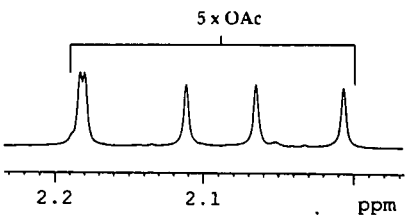
Plate 1 (CDCl3 298K)
(^{1}H NMR)



(53)







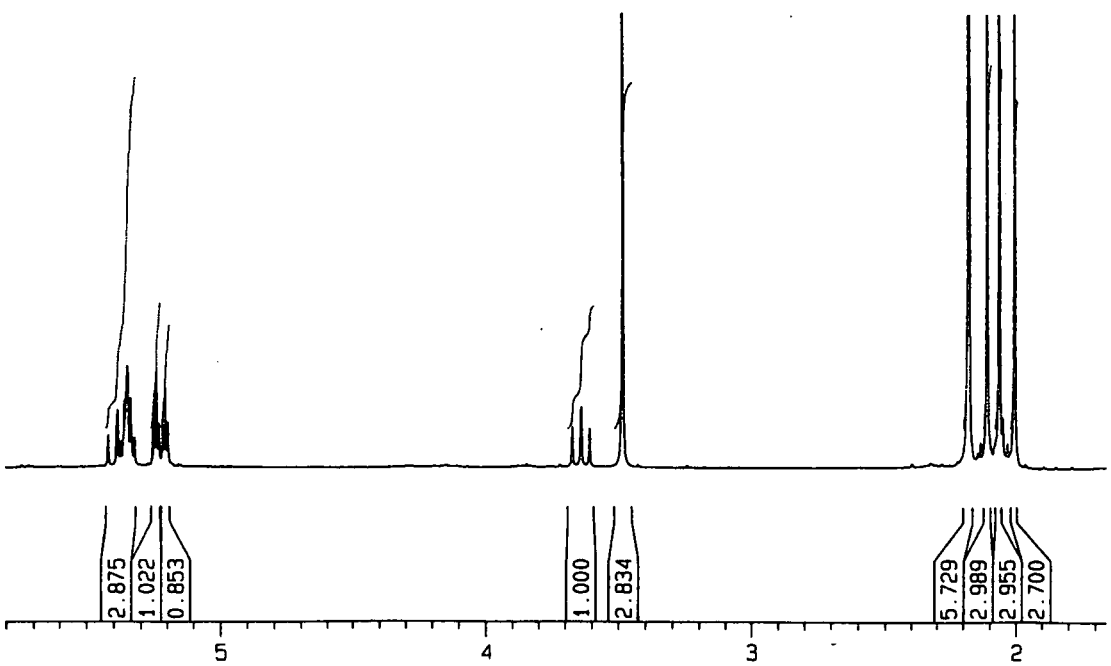
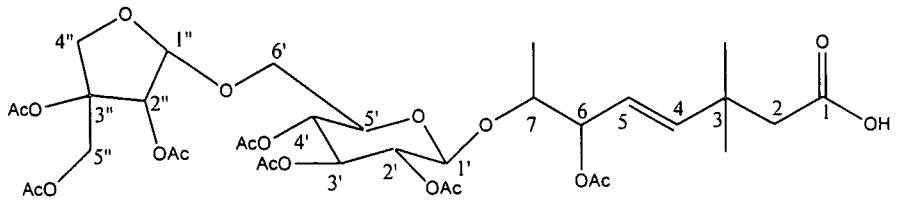


Plate 2 (CDCl3 298K)
(^{1}H NMR)



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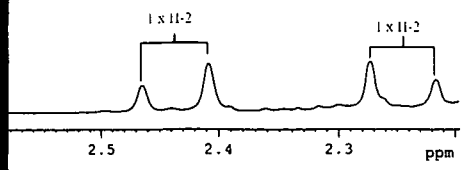
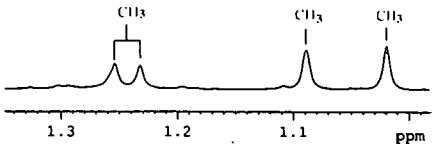
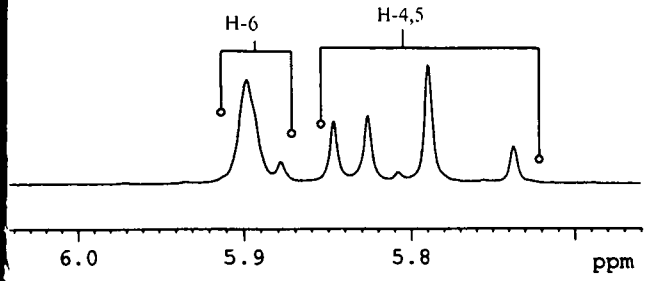
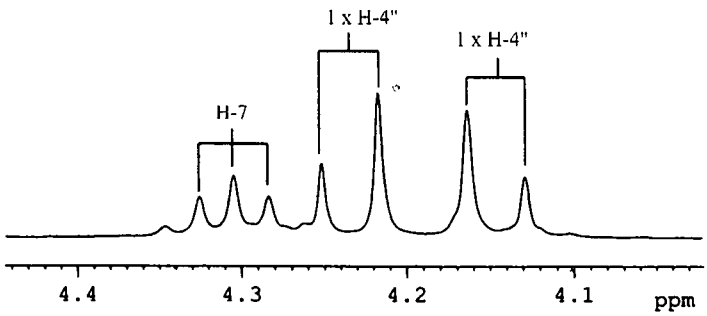
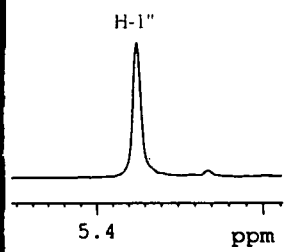
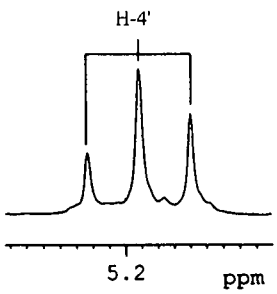


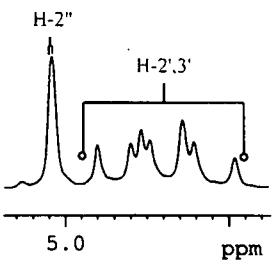


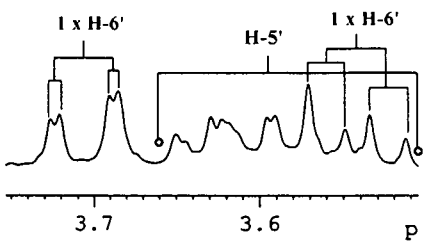


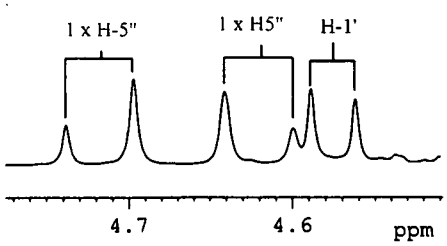


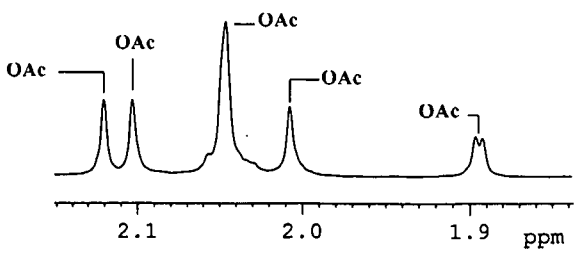


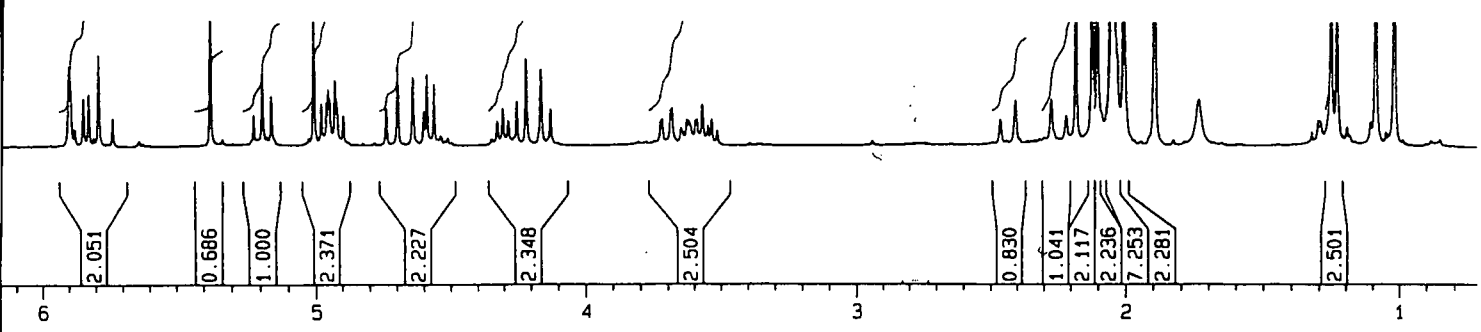




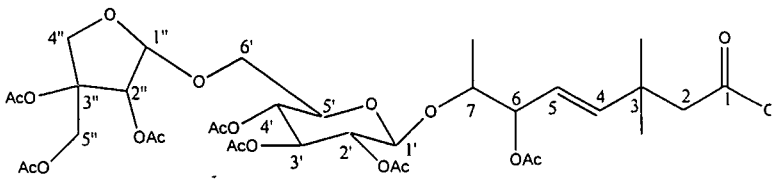








late 2 (CDCl3- 298K)
(COSY 2a-1)



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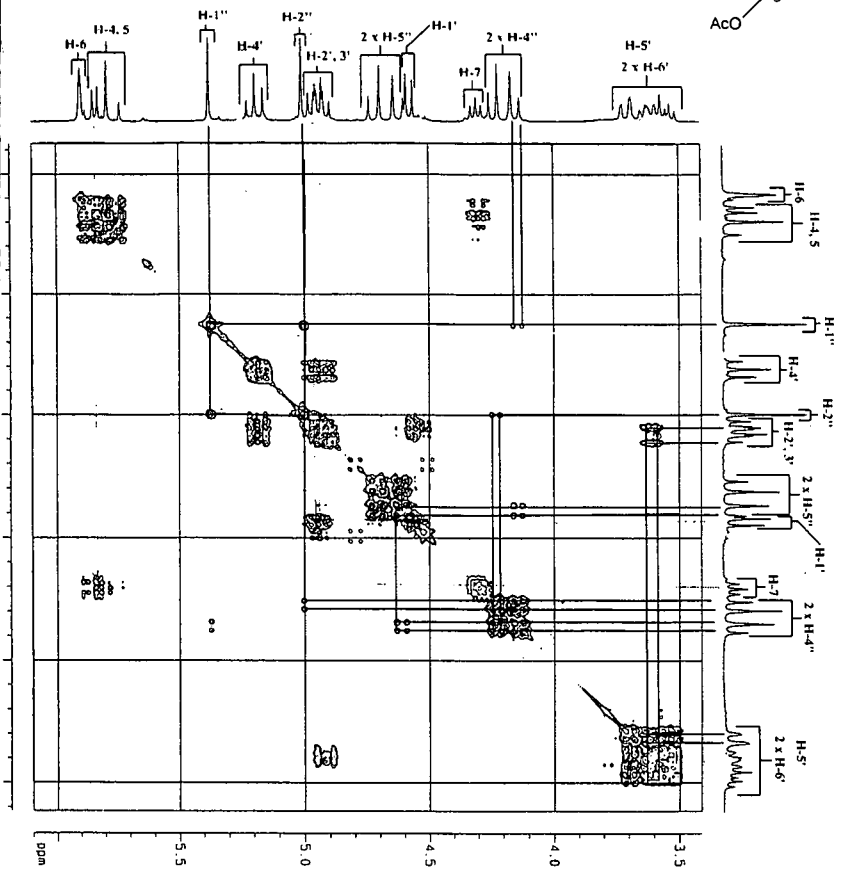


Plate 2 (CDCl3- 298K)
(COSY 2a-2)

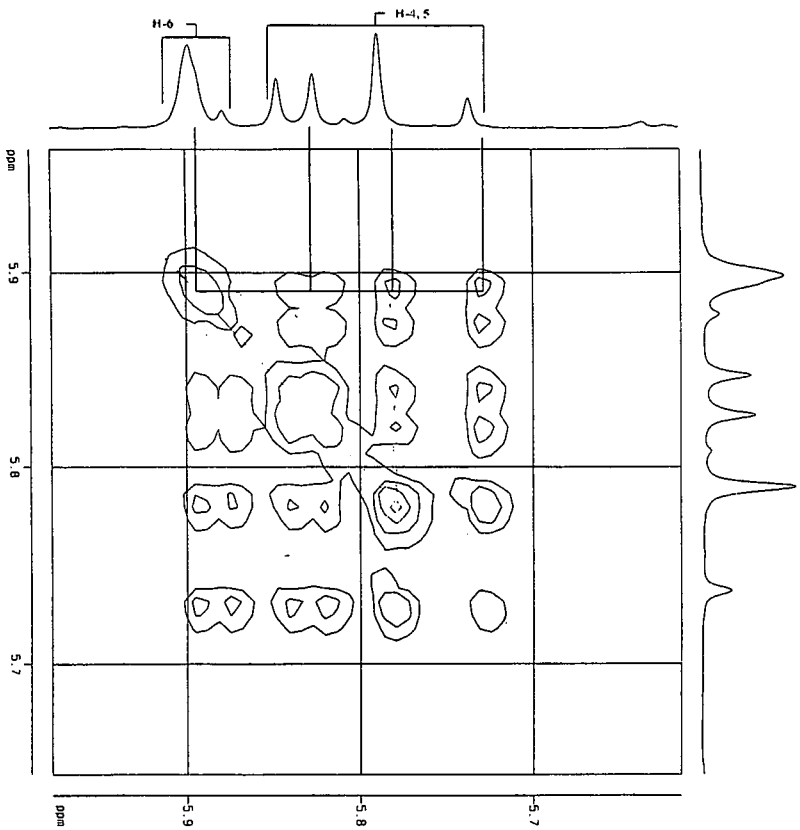
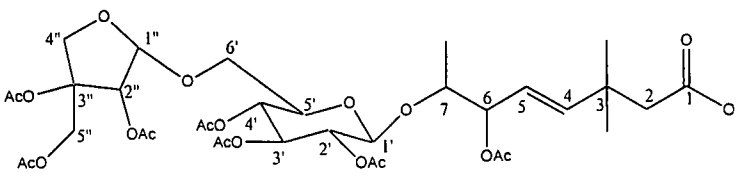


Plate 2 (CDCl3- 298K)
(COSY 2a-3)



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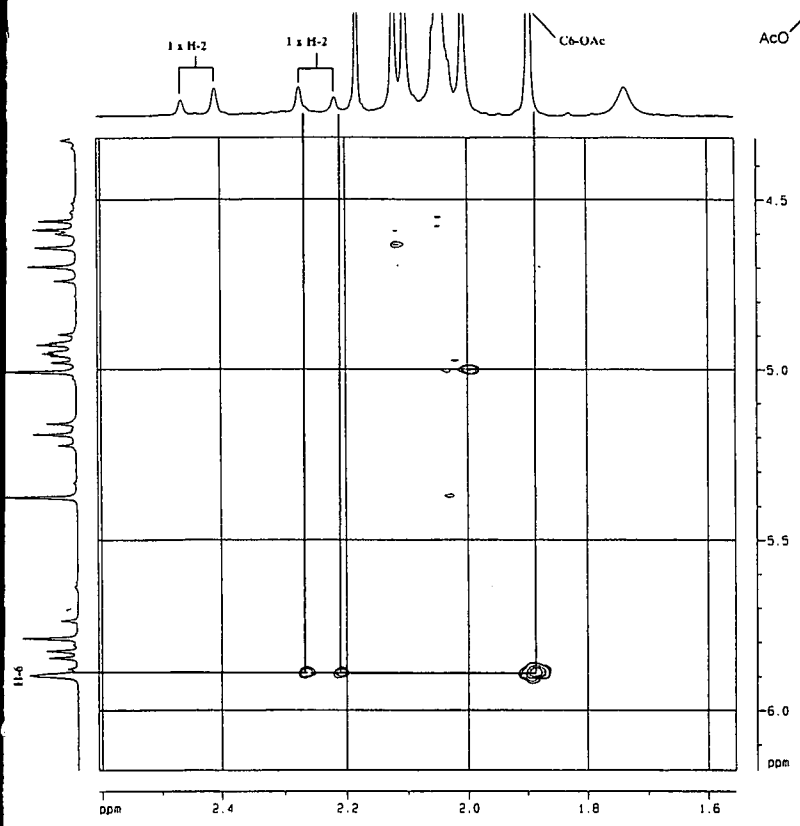

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(COSY 2a-4)

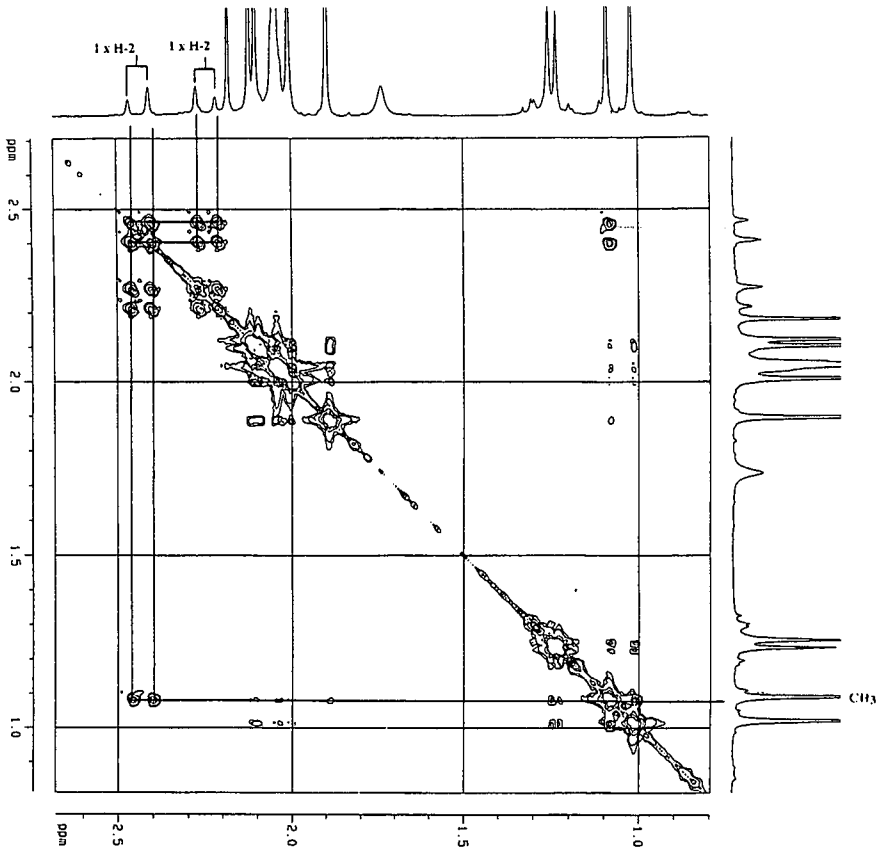
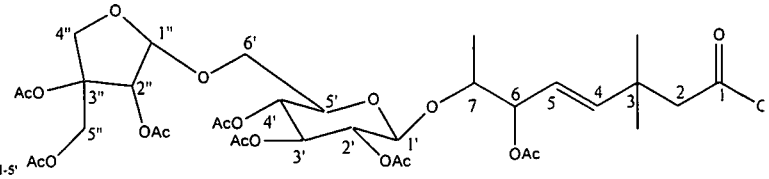


Plate 2 (CDCl3- 298K)
(NOESY 2b-1)



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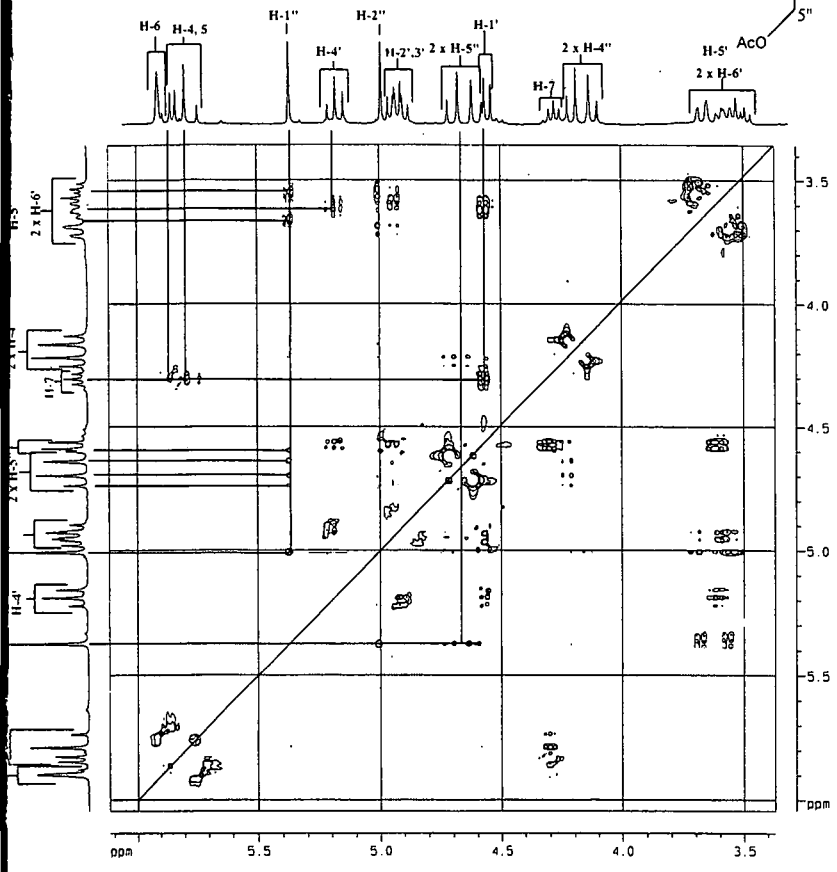


Plate 2 (CDCl3- 298K)
(NOESY 2b-2)

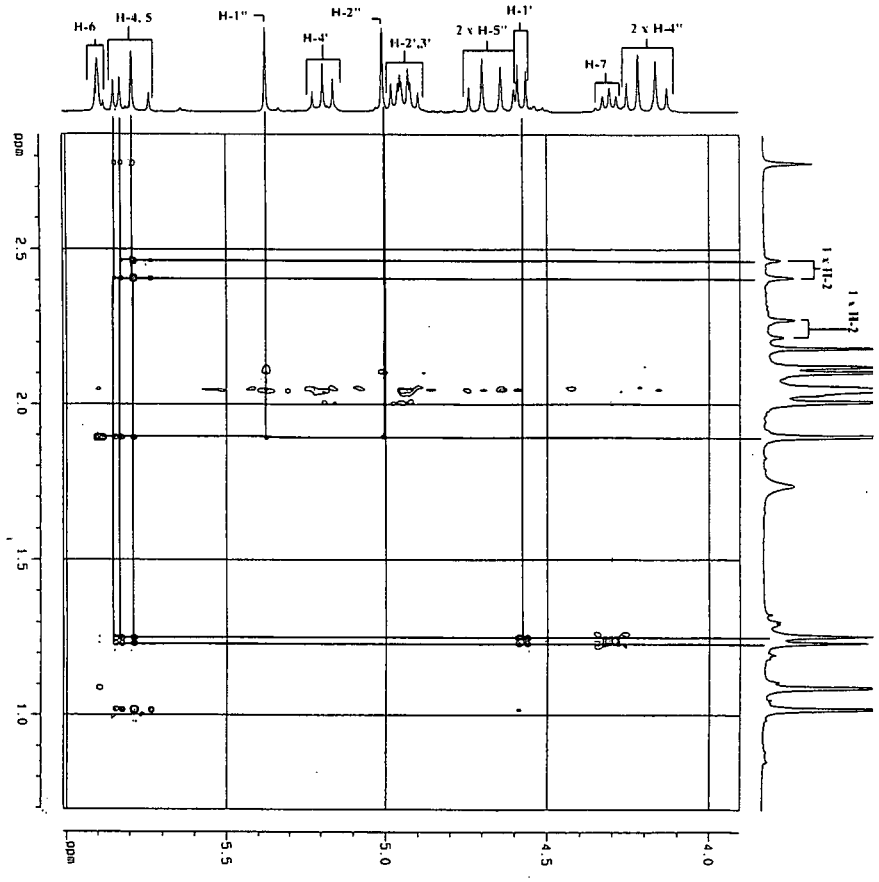
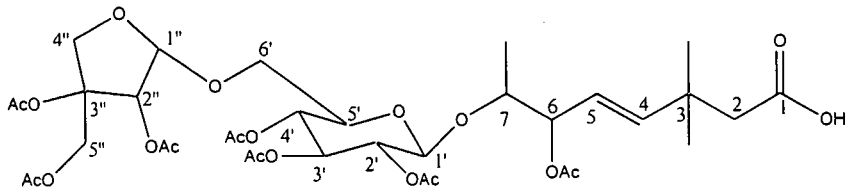


Plate 2 (CDCl3-298K)
(^{13}C NMR 2c-1)



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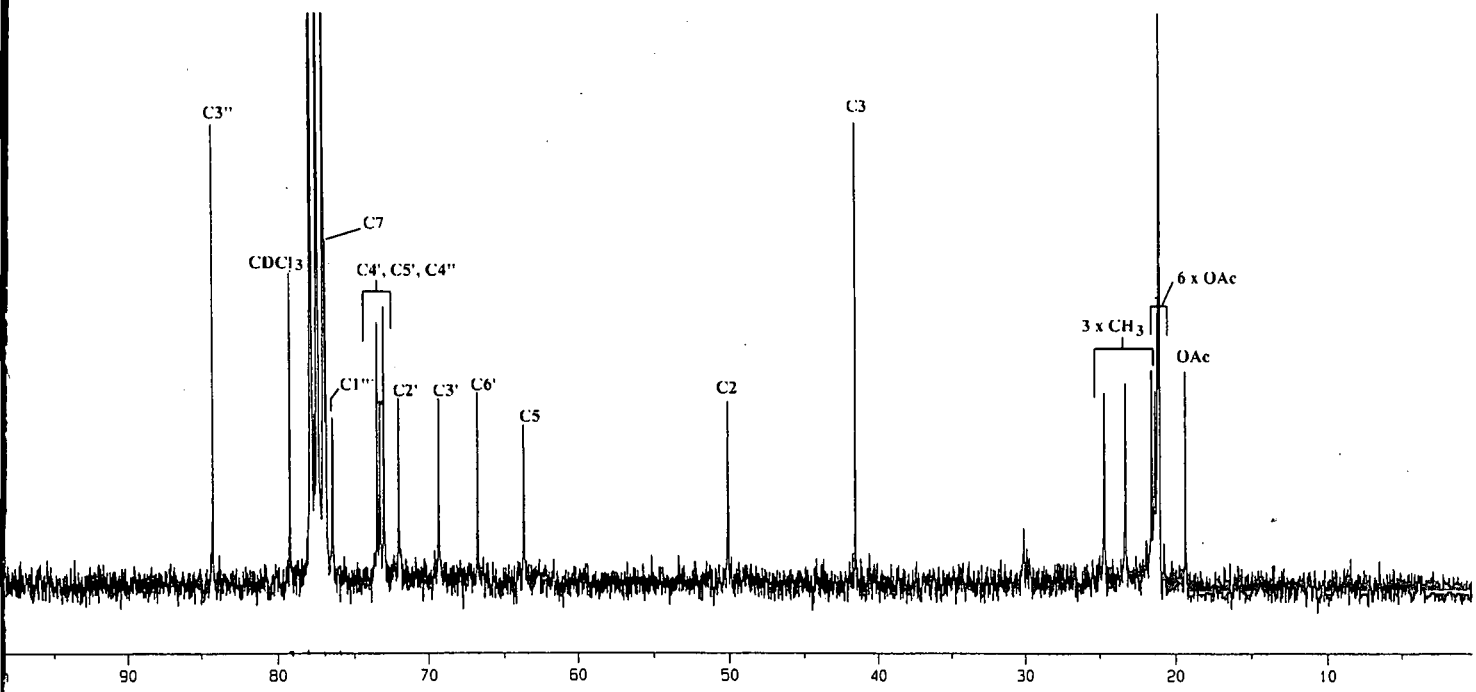


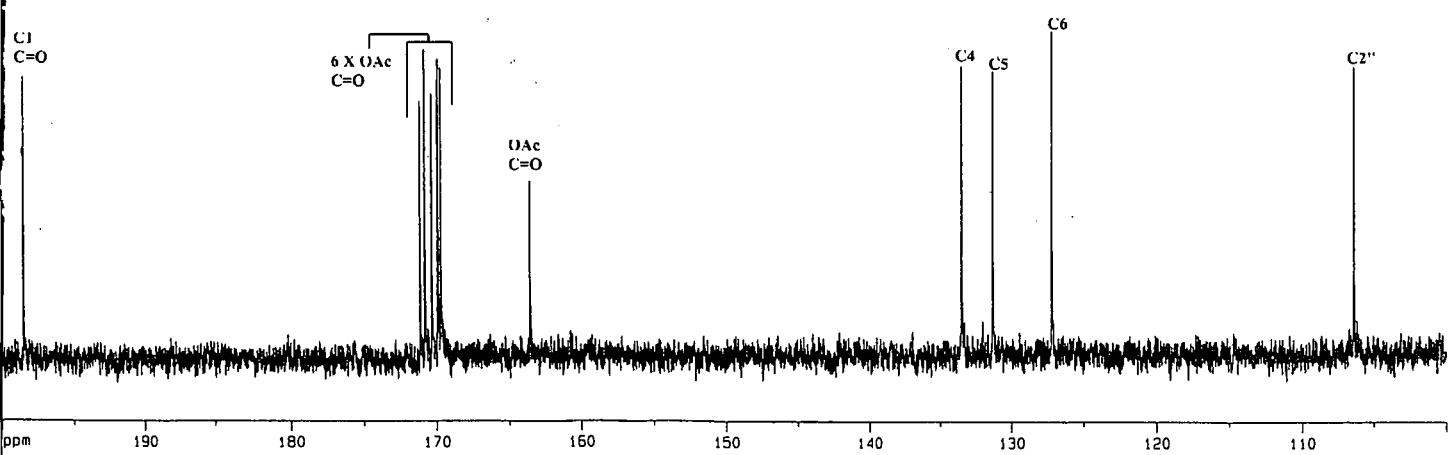
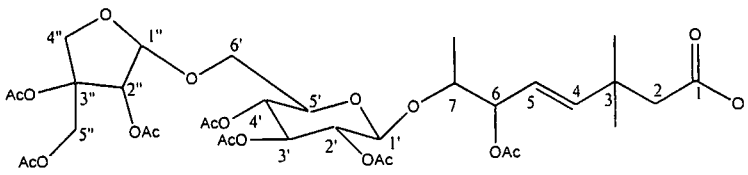


Plate 2 (CDCl3- 298K)
(HMQC 2d-1)



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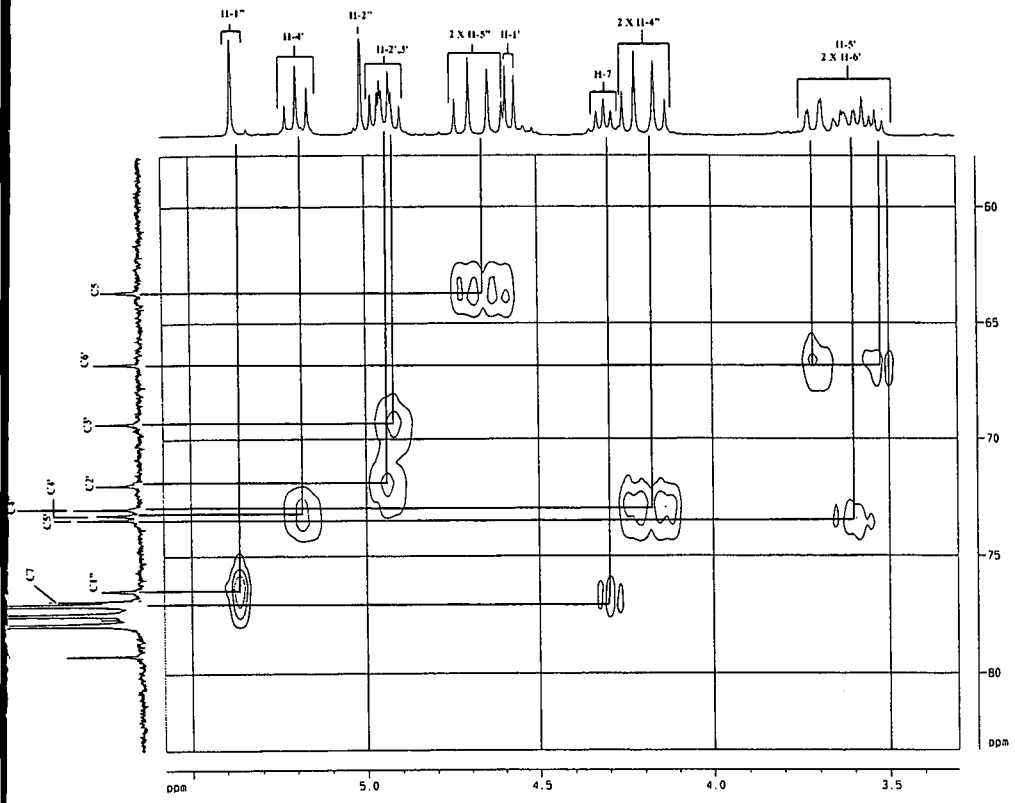


Plate 2 (CDCl3- 298K)
(HMQC 2d-2)

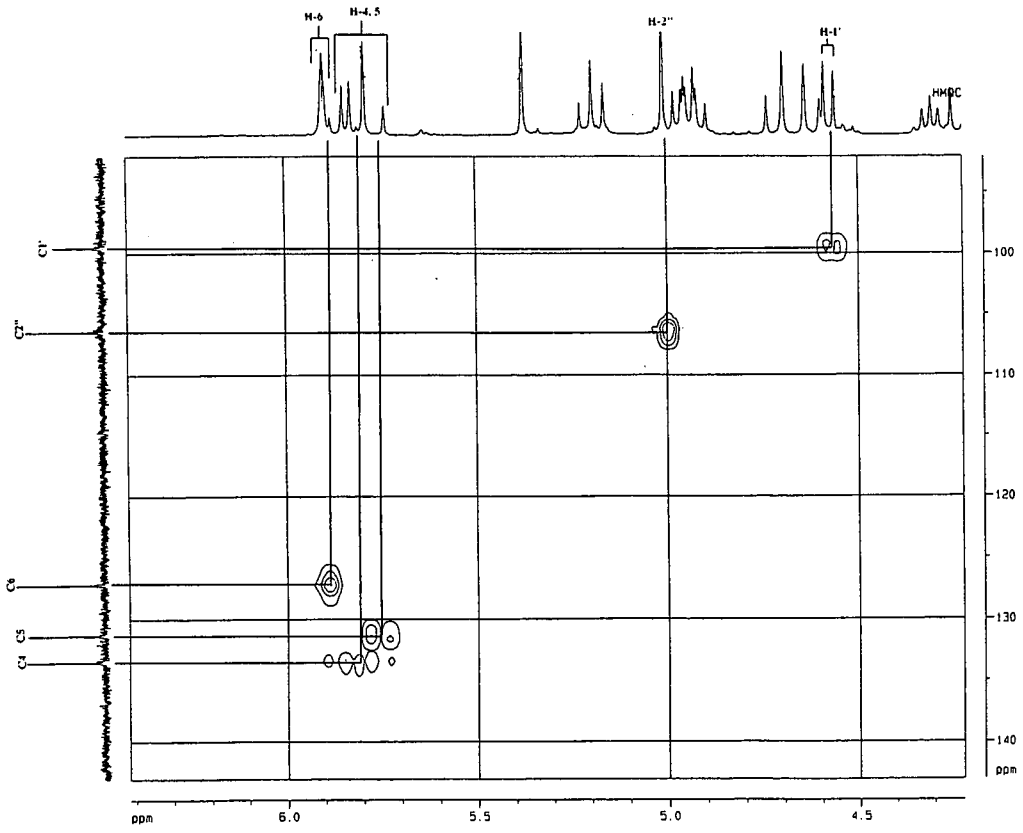
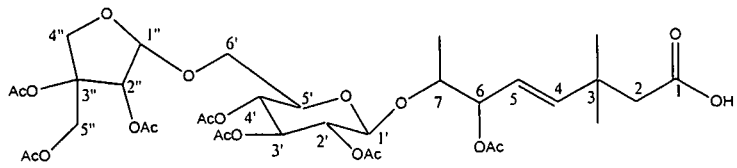


Plate 2 (CDCl3- 298K)
(HMBC 2e-1)



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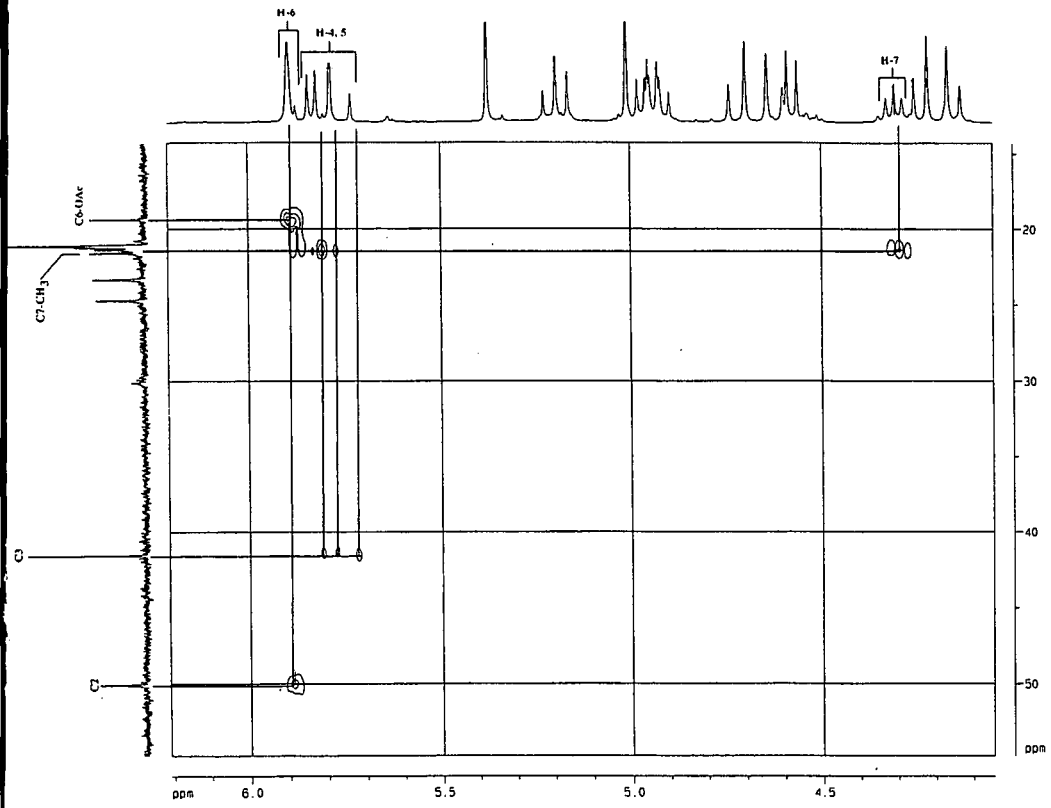


Plate 2 (CDCl3- 298K)
(HMBC 2e-2)

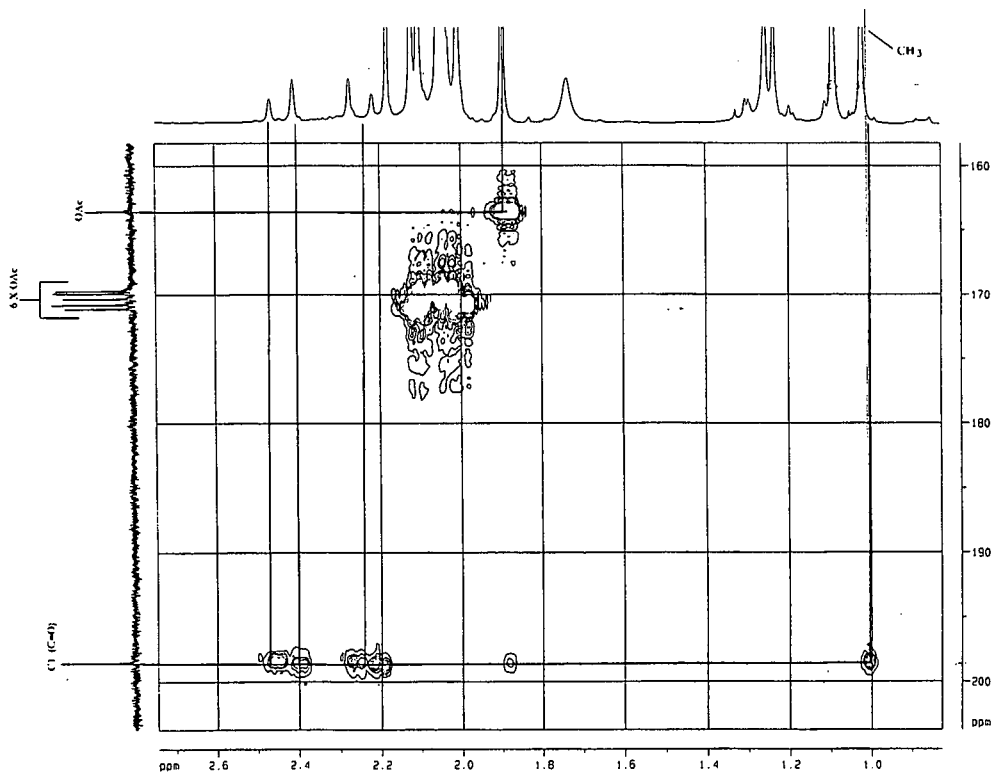
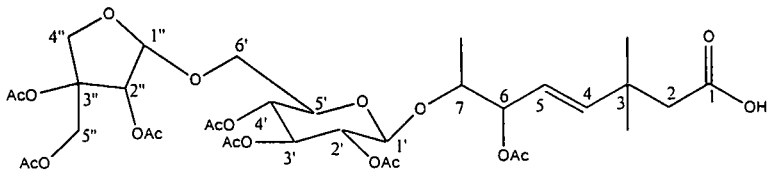


Plate 2 (CDCl3- 298K)
(DEPT 135° 2f-1)



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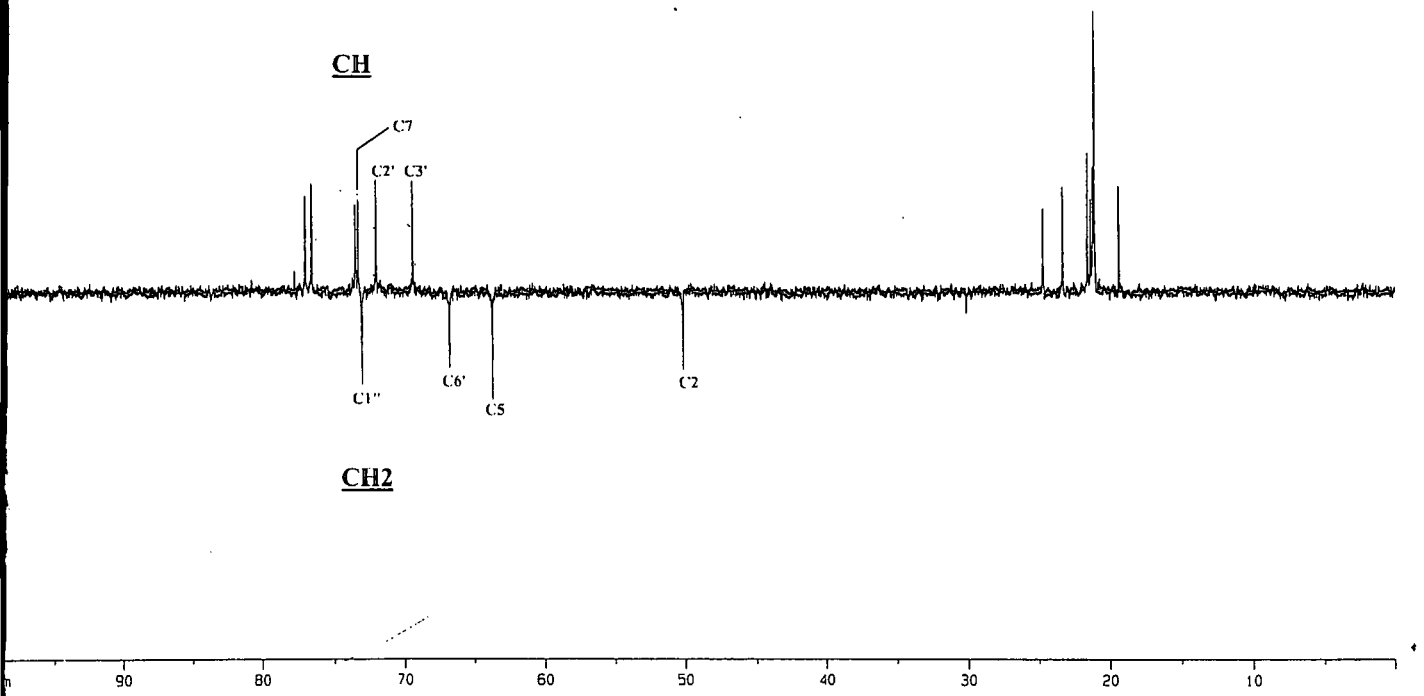


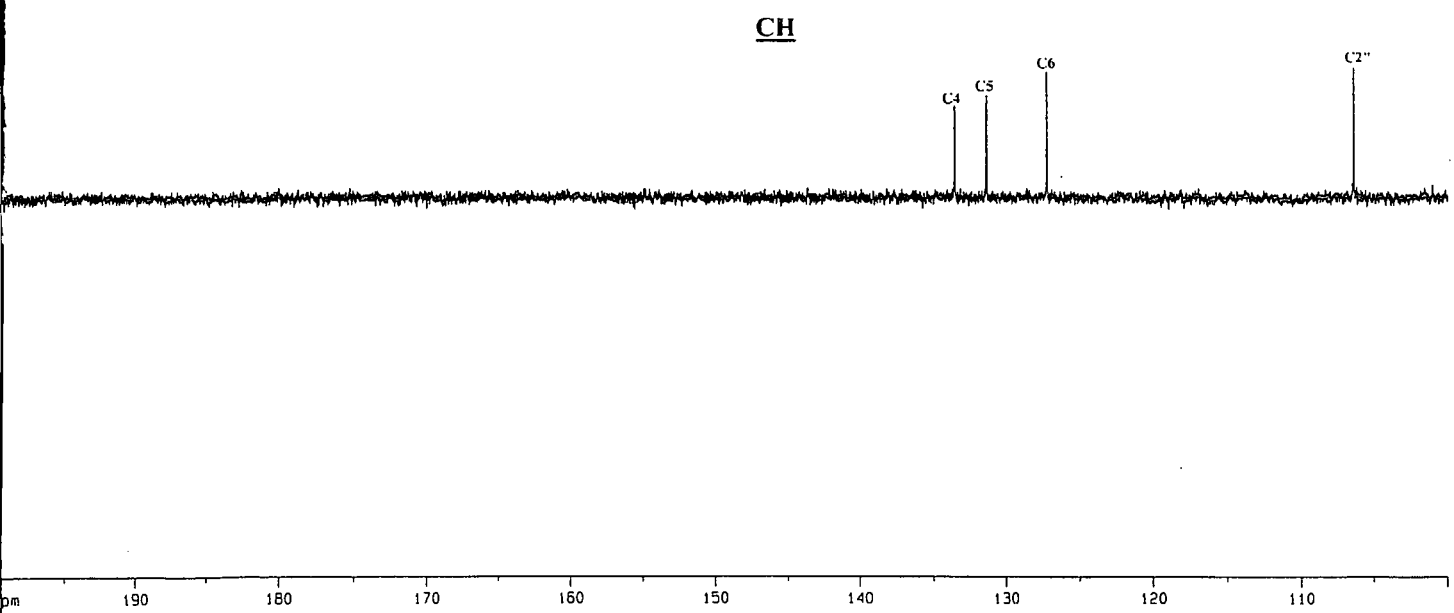
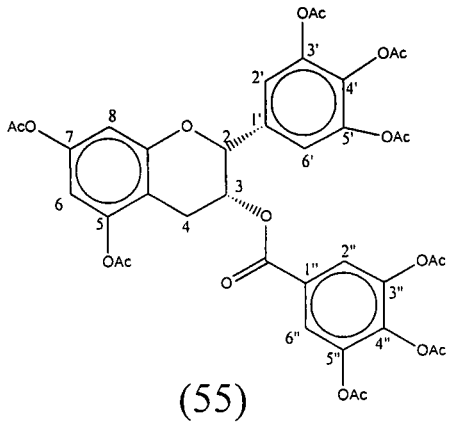


Plate 3 (CDCl3 298K)
(^{1}H NMR)



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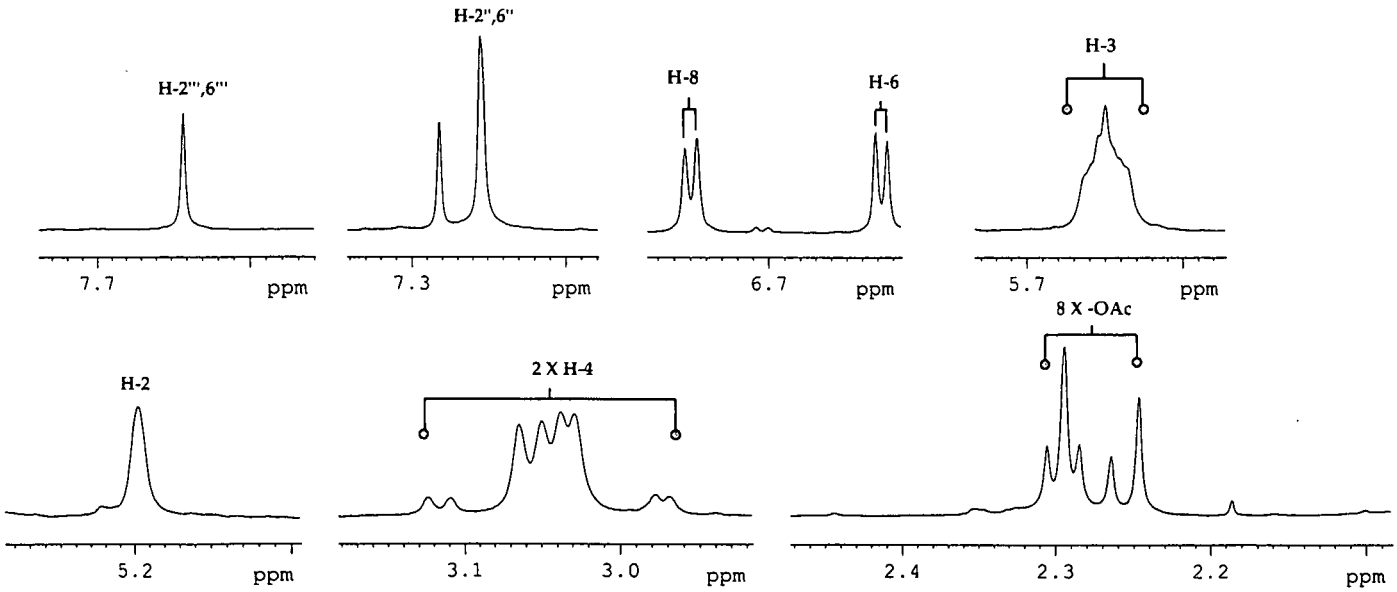


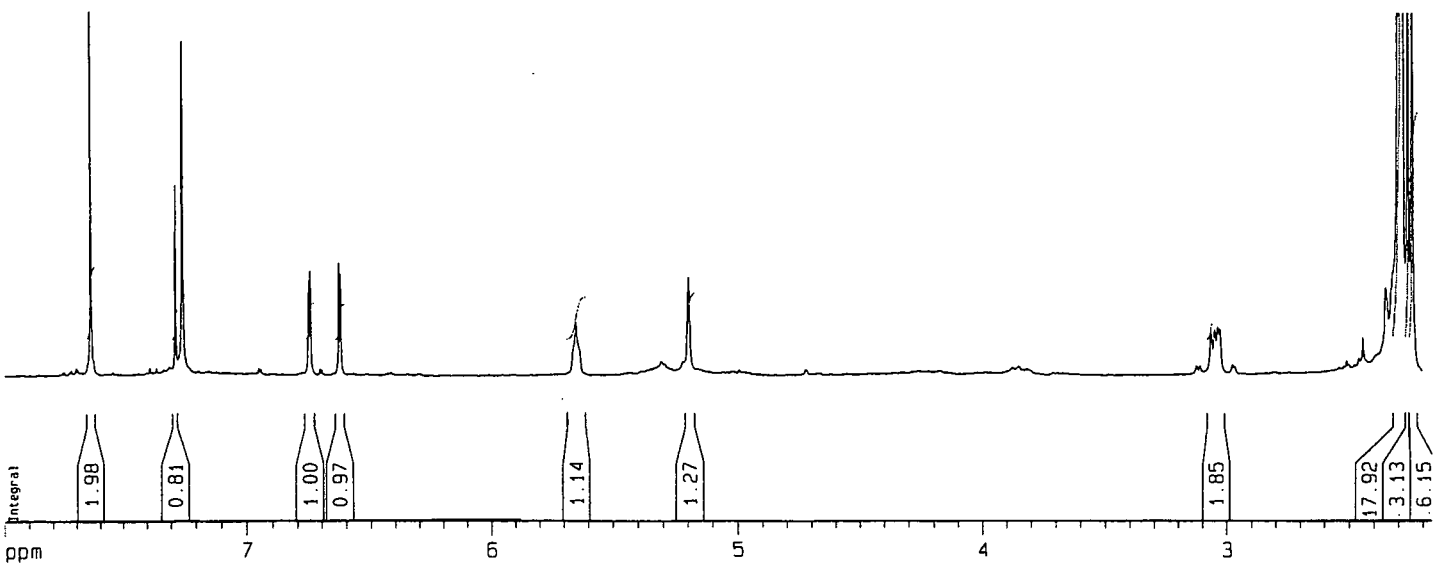
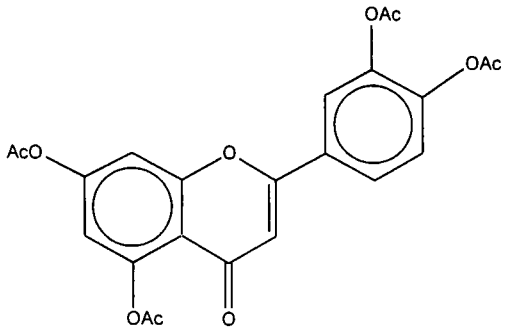


Plate 4 ($CDCl_3$ 298K)
(^1H NMR)



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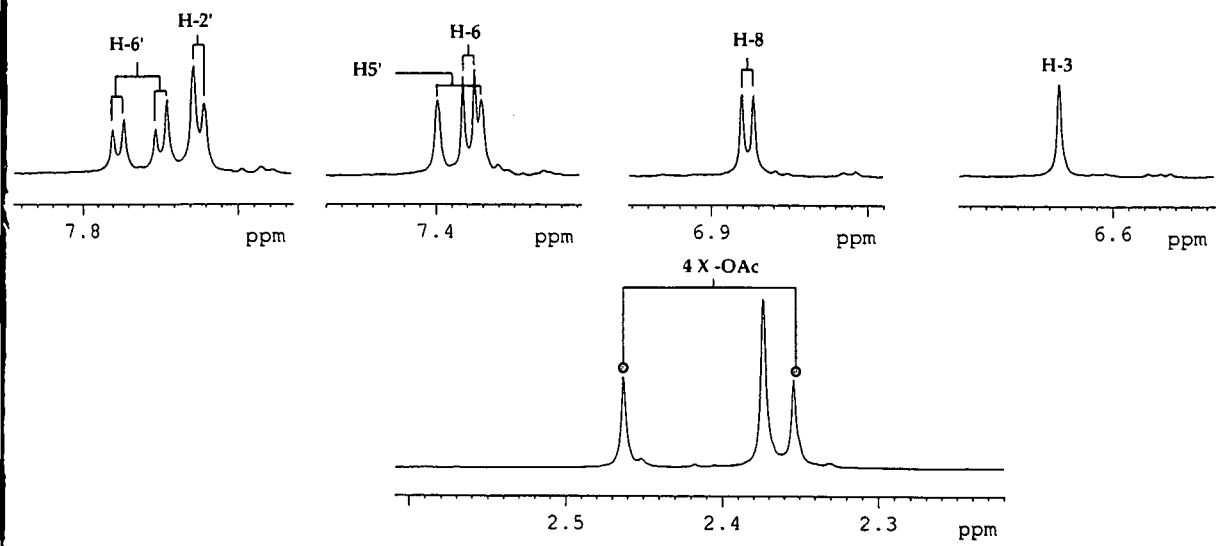


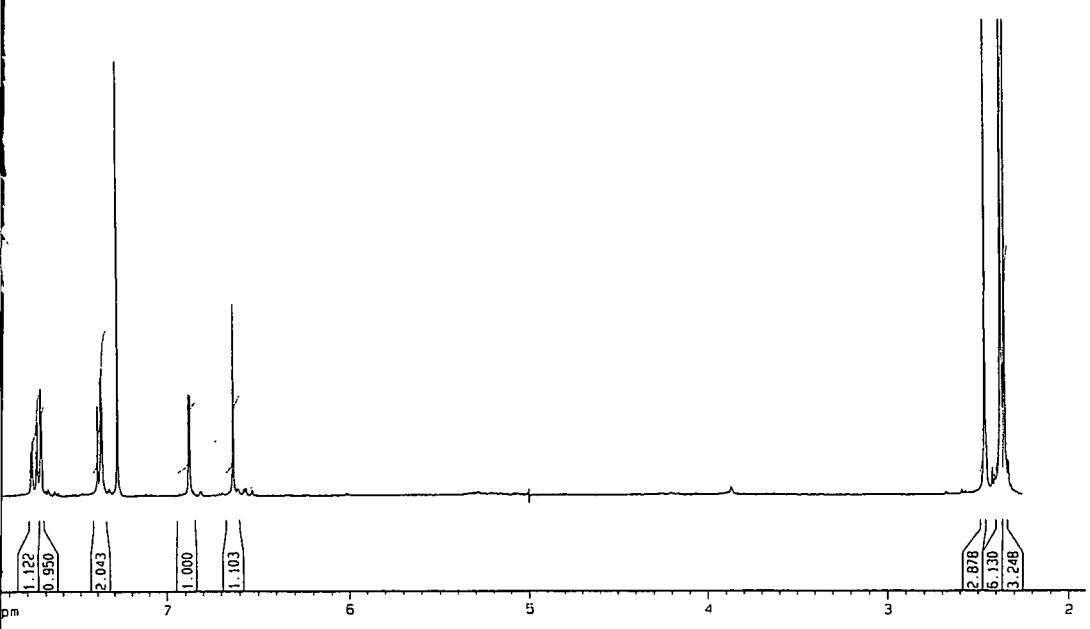
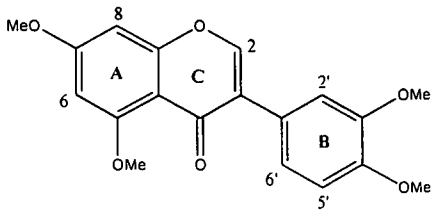


Plate 5 (CDCl3 298K)
(^{1}H NMR)



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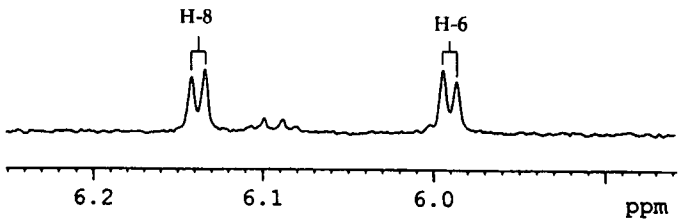
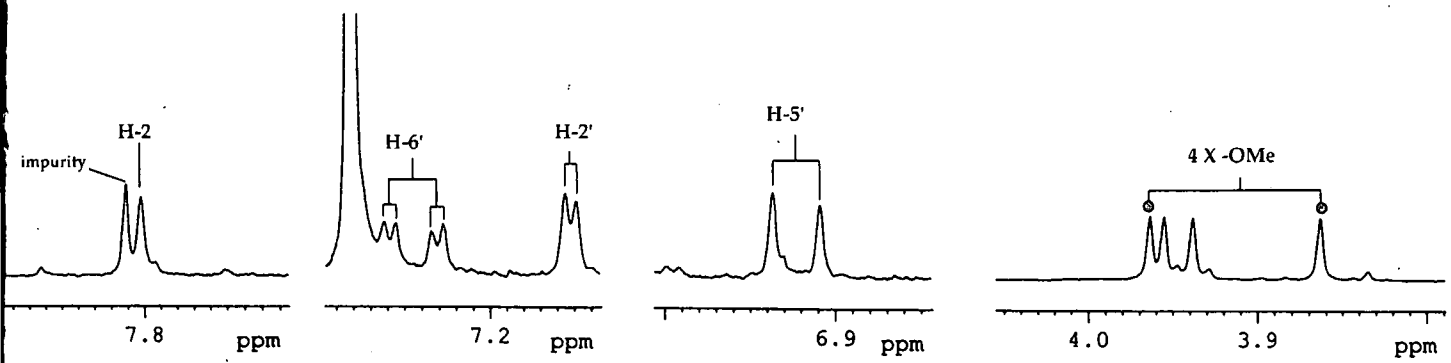




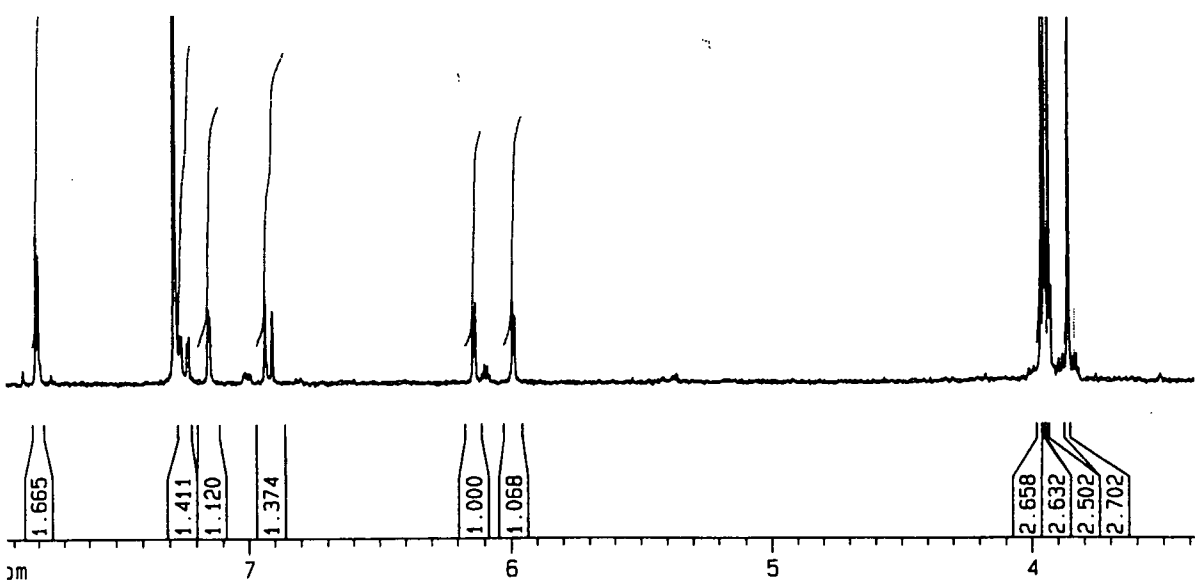
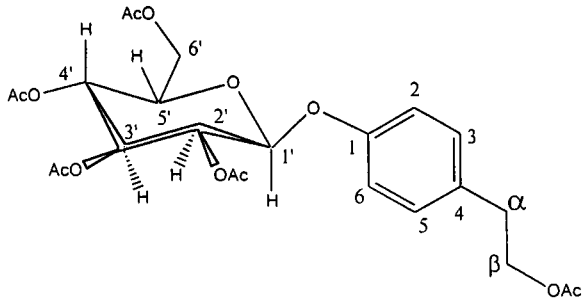


Plate 6 (CDCl3 298K)
(^{1}H NMR)



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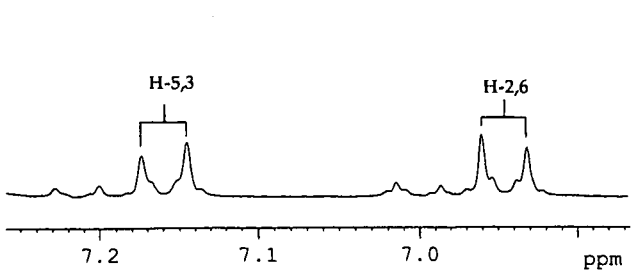
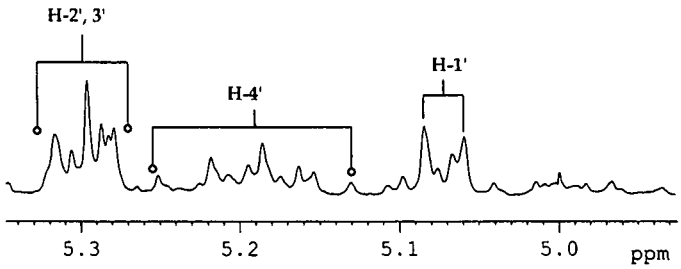
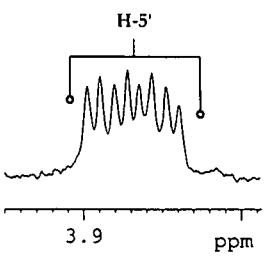
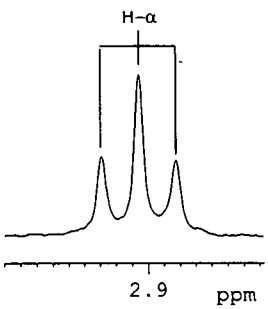
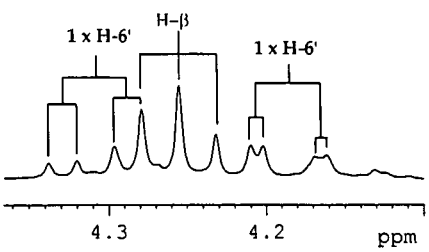
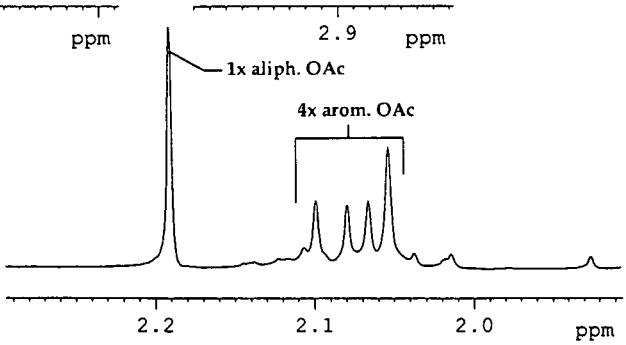


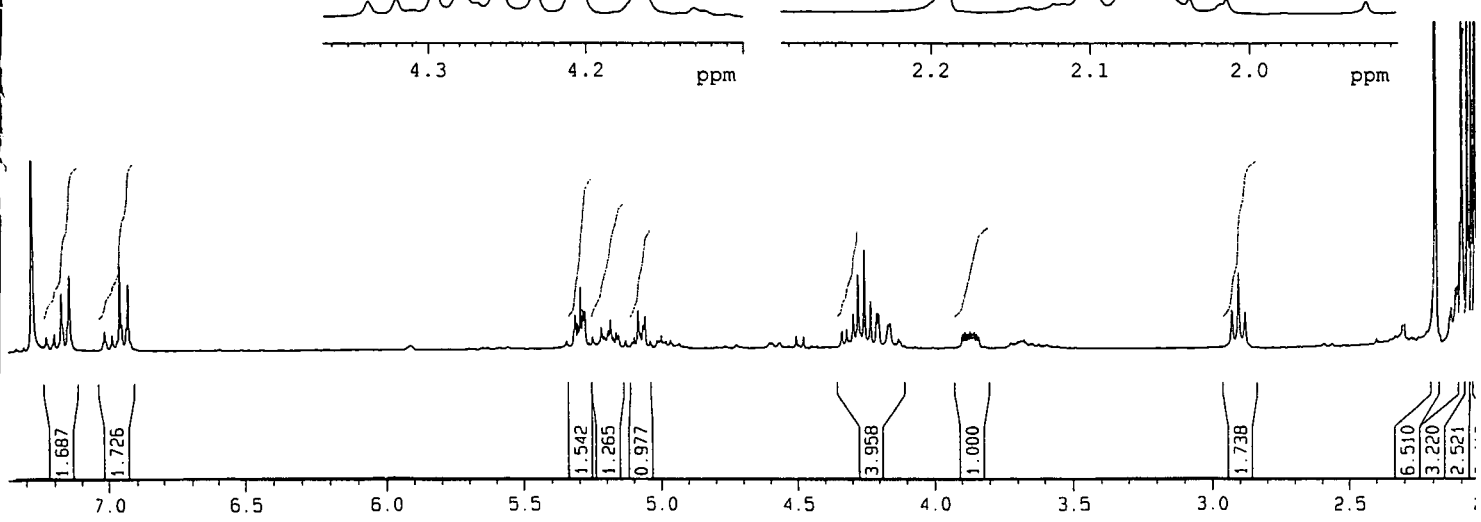




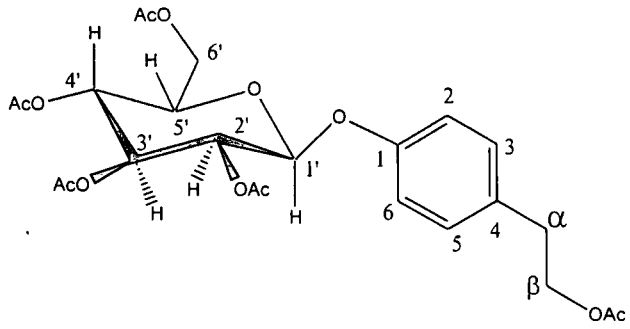








late 6 (CDCl3- 298K)
(COSY 6a-1)



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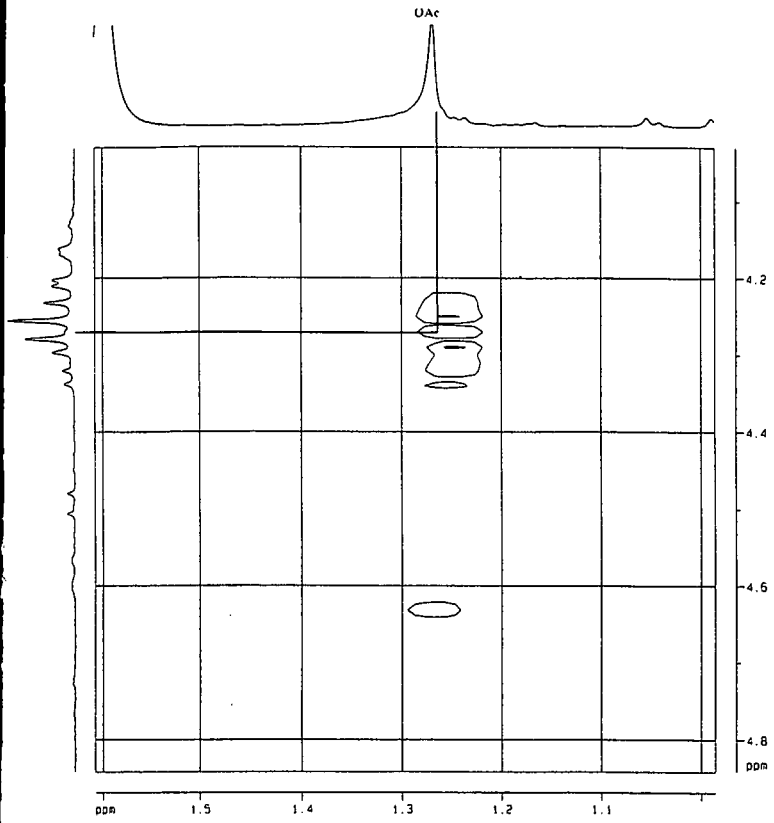
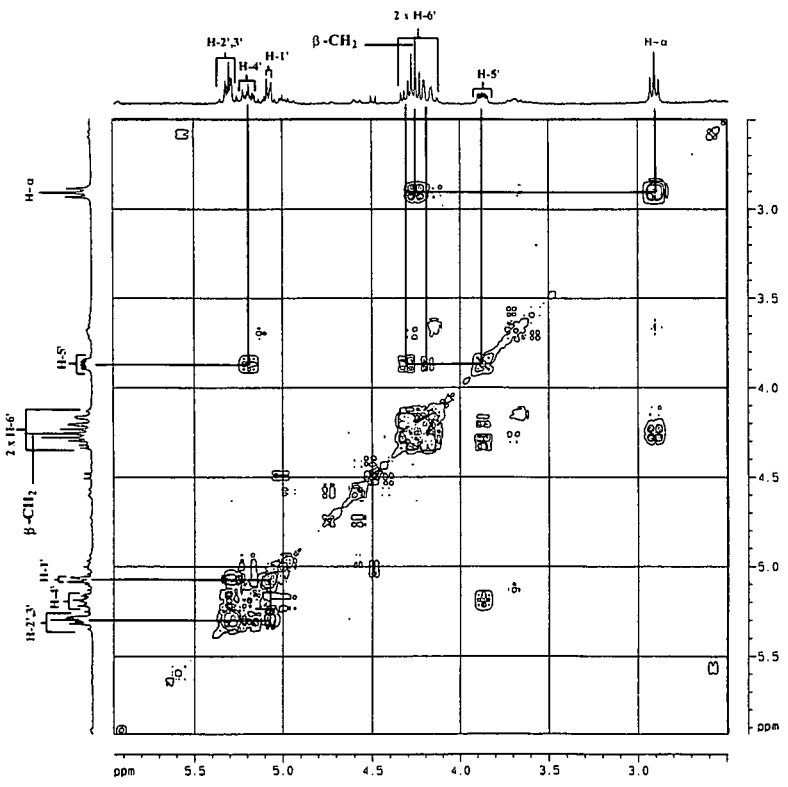
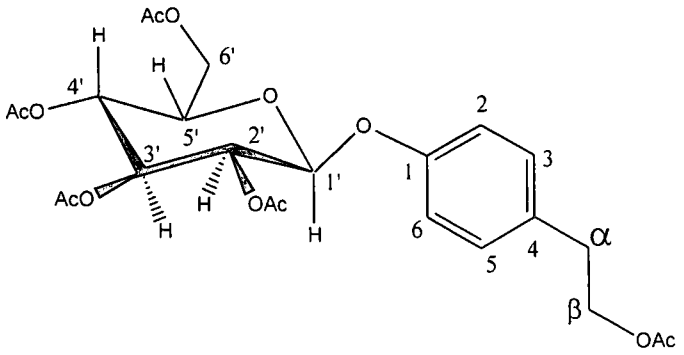


Plate 6 (CDCl3- 298K)
(COSY 6a-2)



te 6 (CDCl3- 298K)
(NOESY 6b-1)



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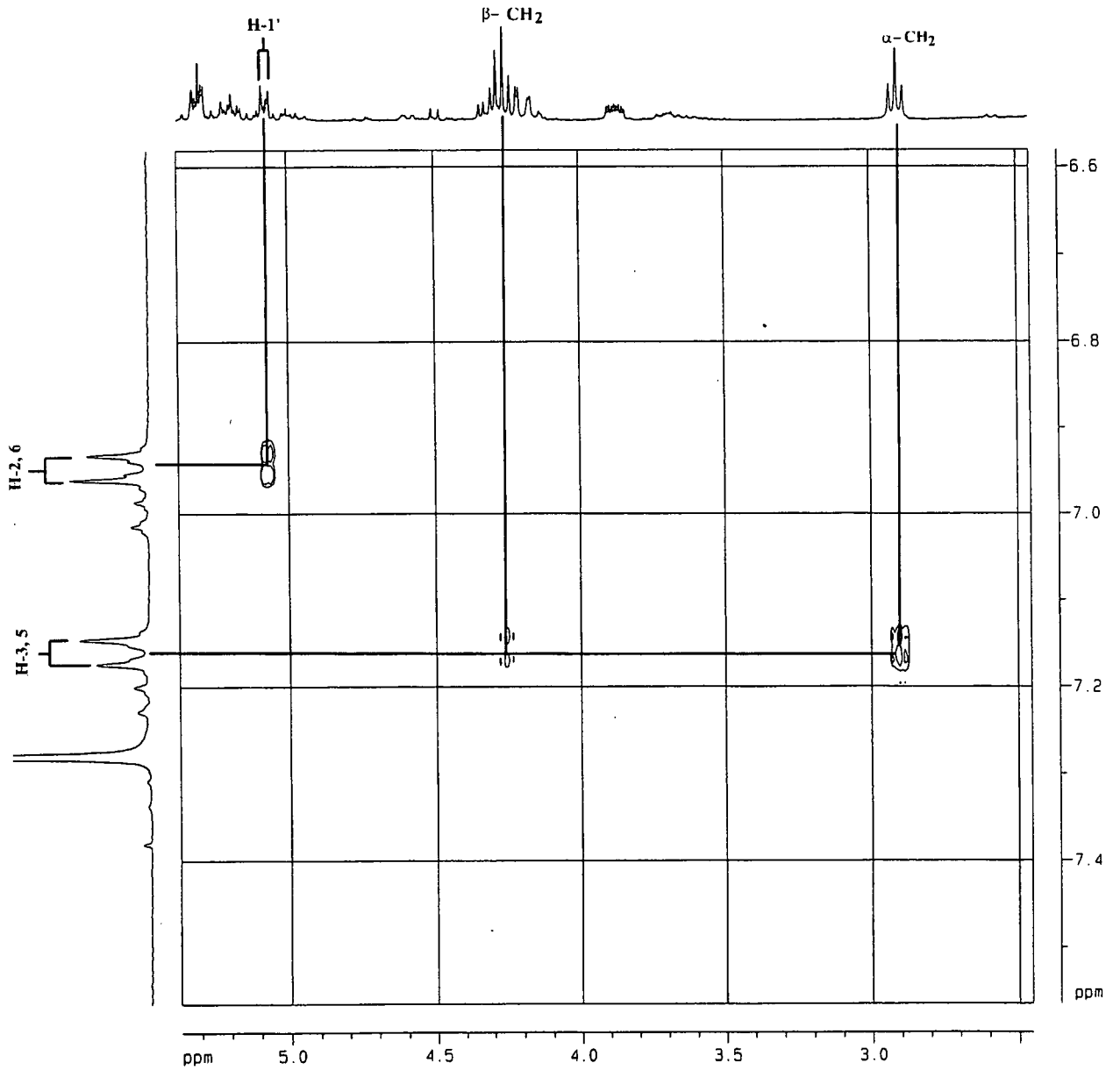
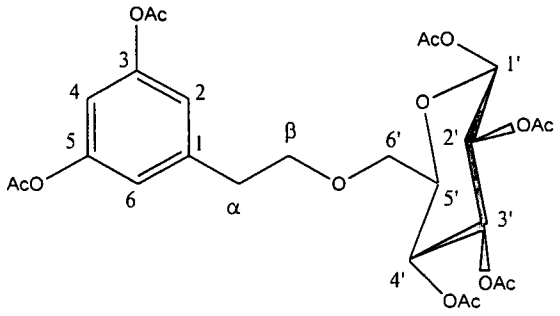


Plate 7 (CDCl3 298K)
(^{1}H NMR)



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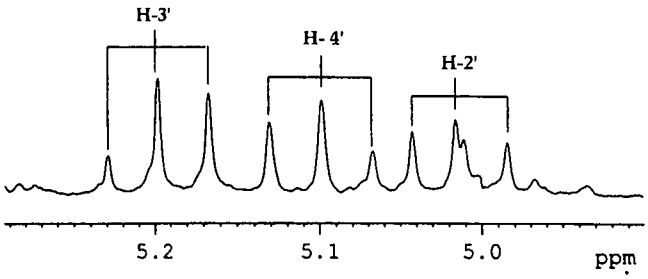
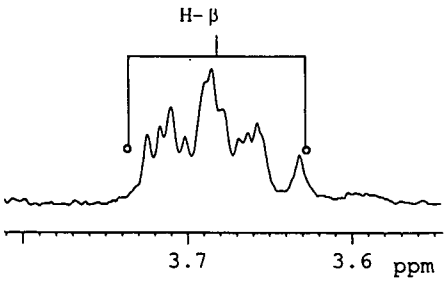
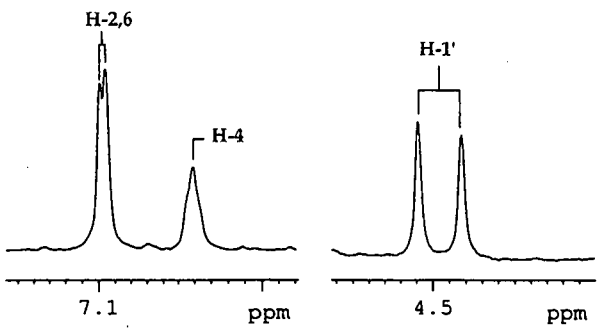
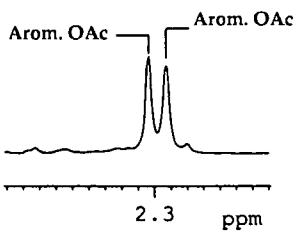
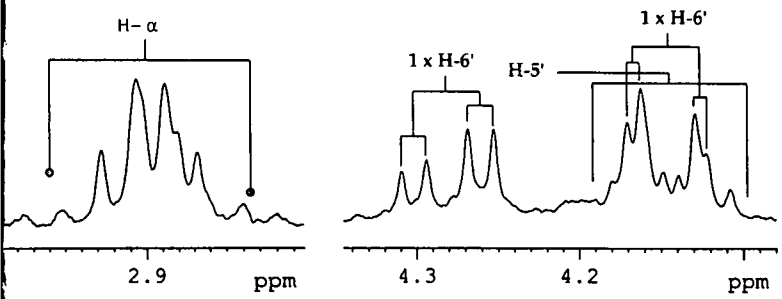
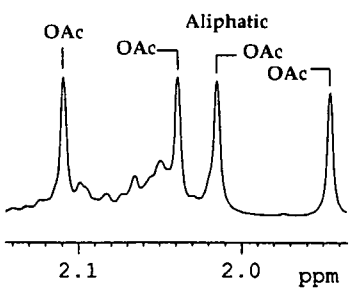


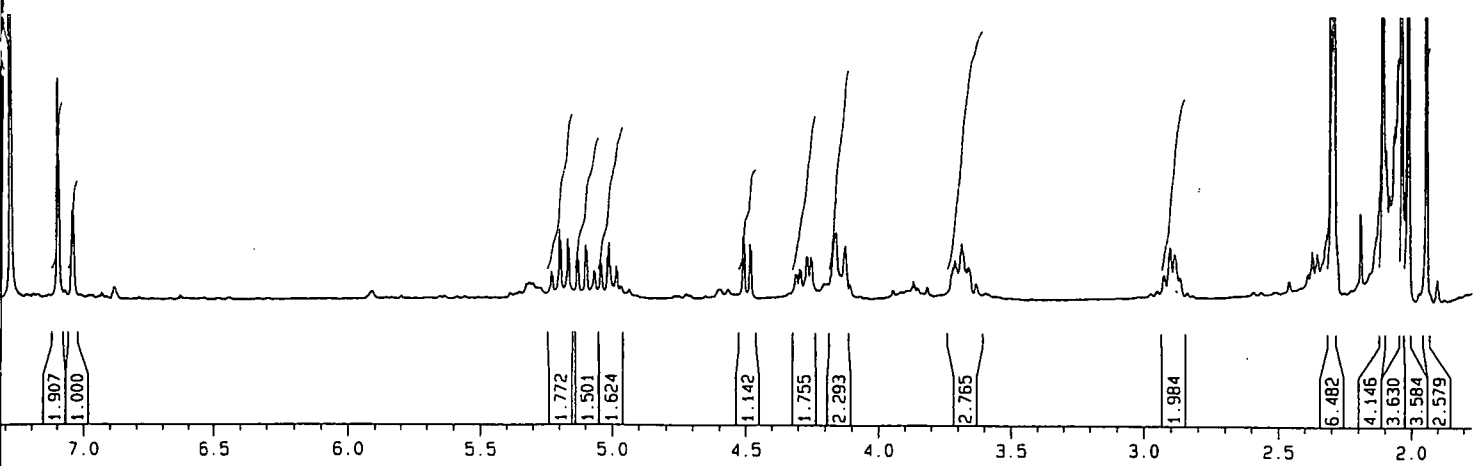




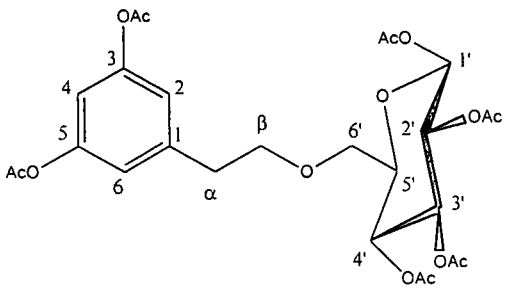








ate 7 (CDCl3- 298K)
(COSY 7a-1)



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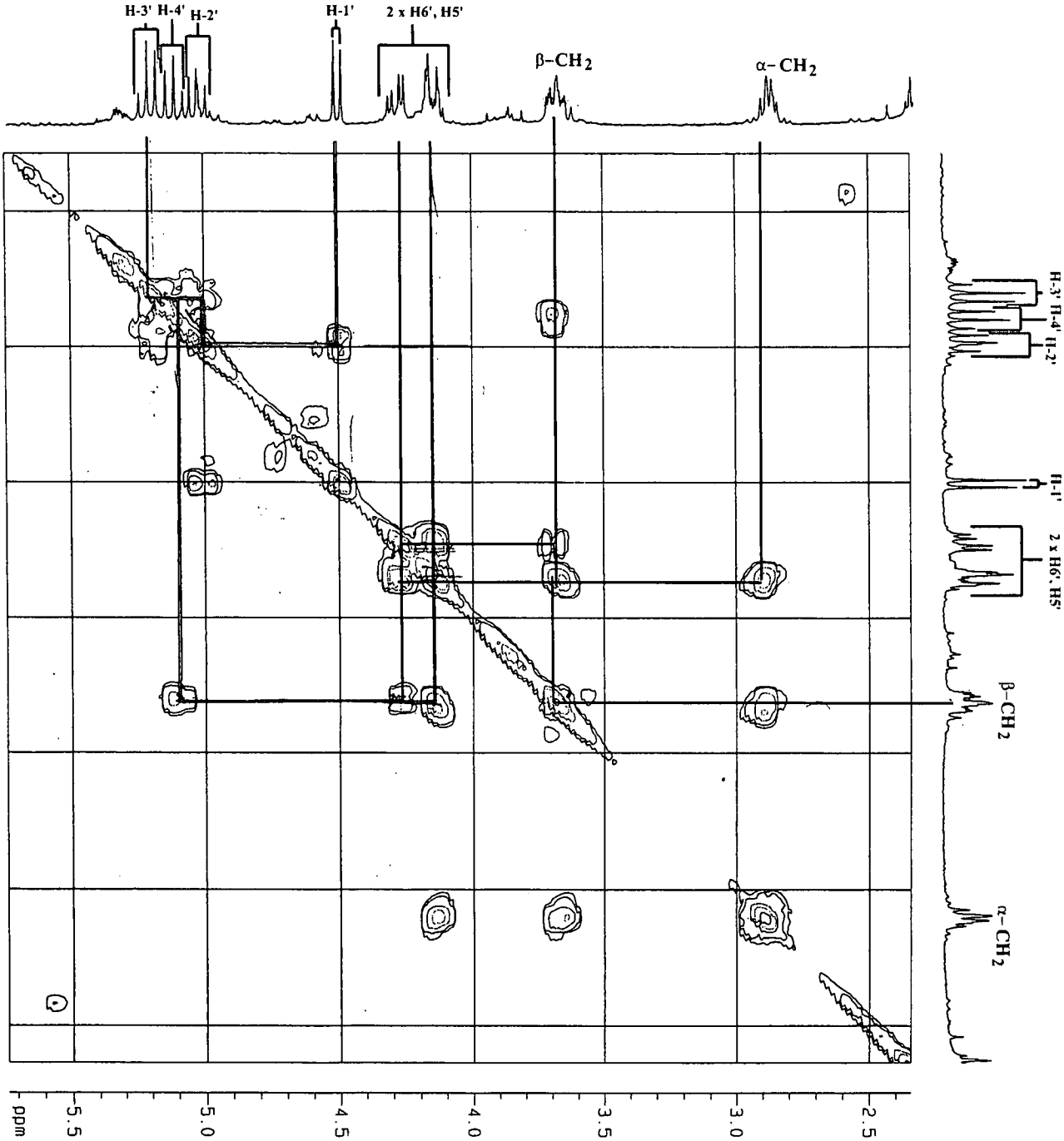
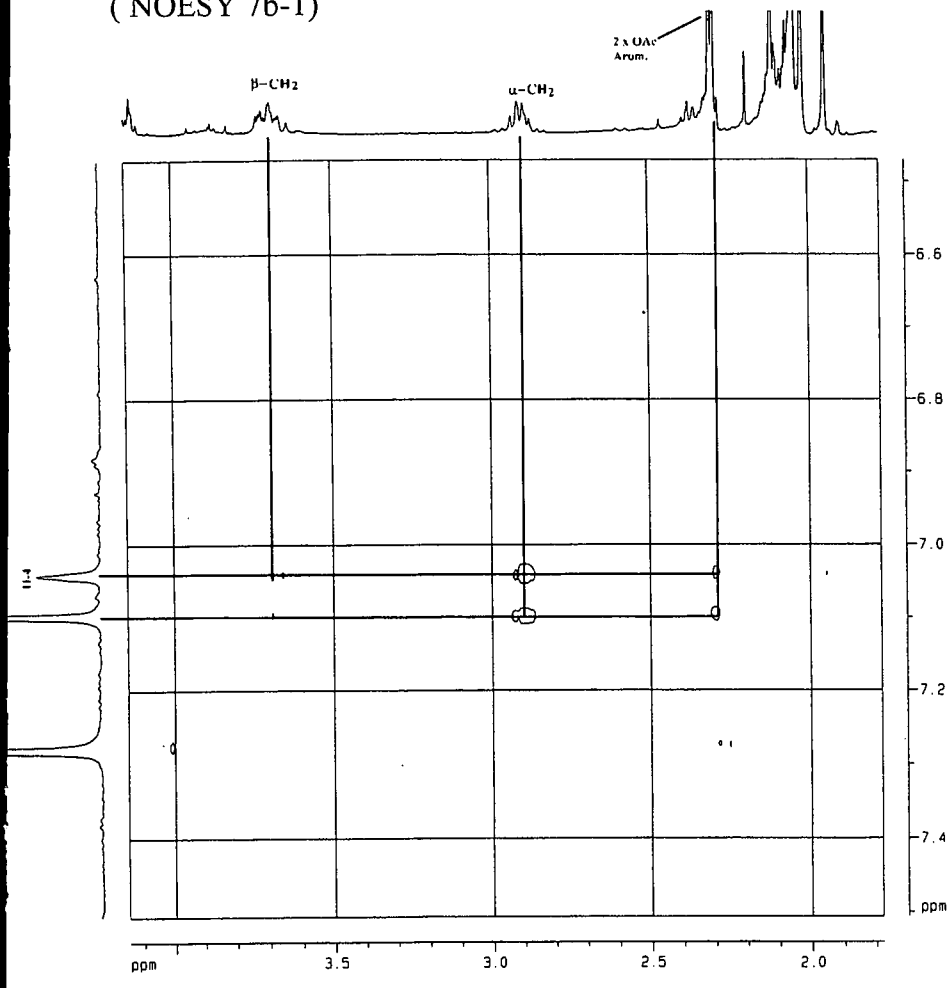


late 7 (CDCl3- 298K)
(NOESY 7b-1)



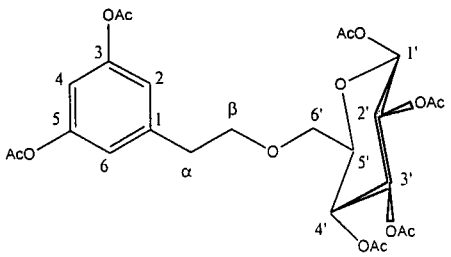


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Plate 7 (CDCl3- 298K)
(NOESY 7b-2)

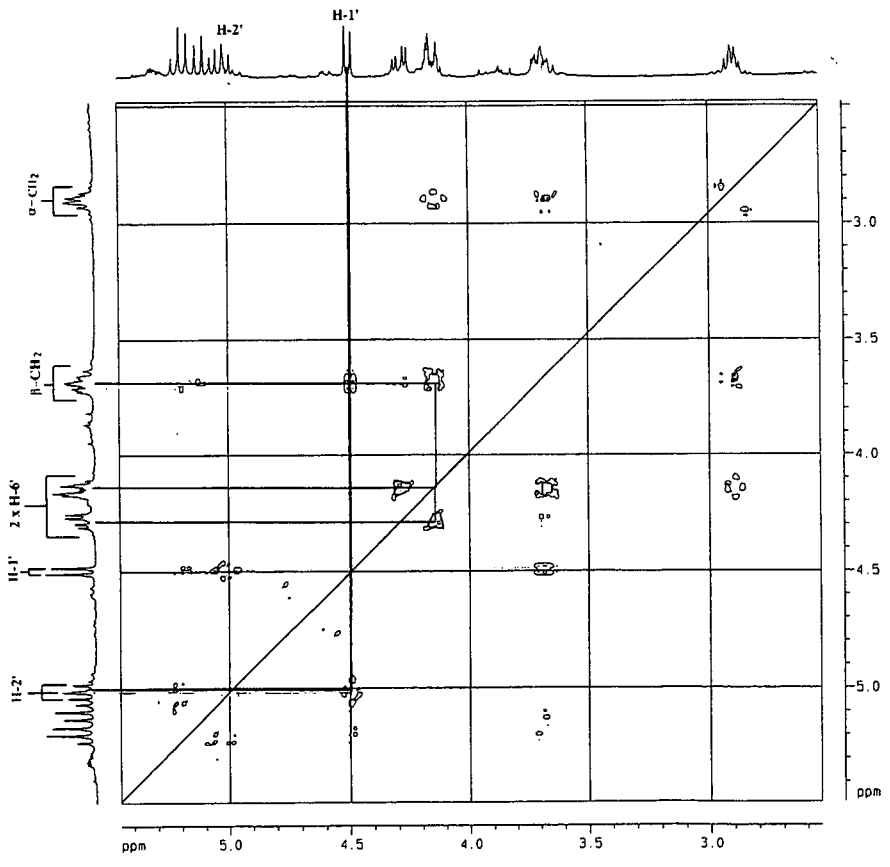
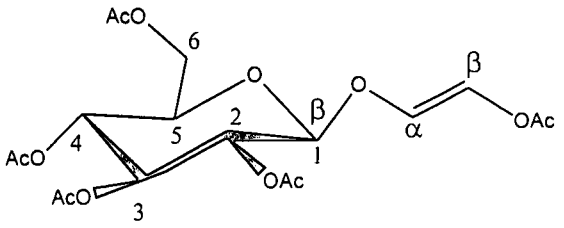


Plate 8 (CDCl3 333K)
(^{1}H NMR)



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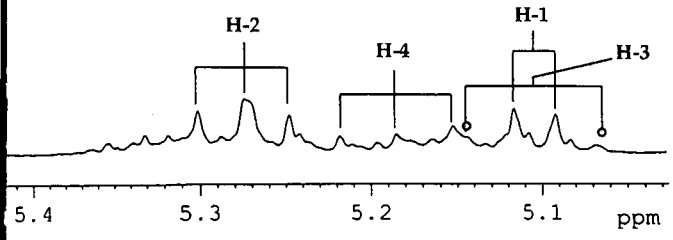
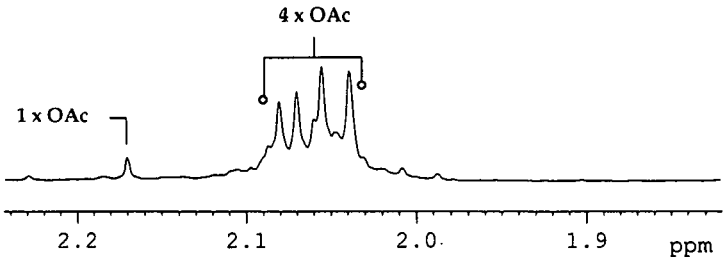
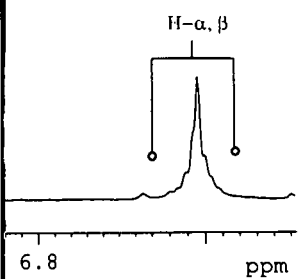
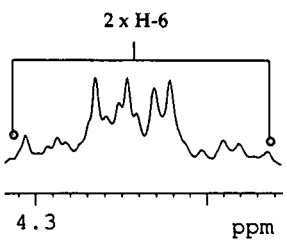
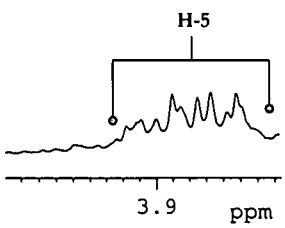










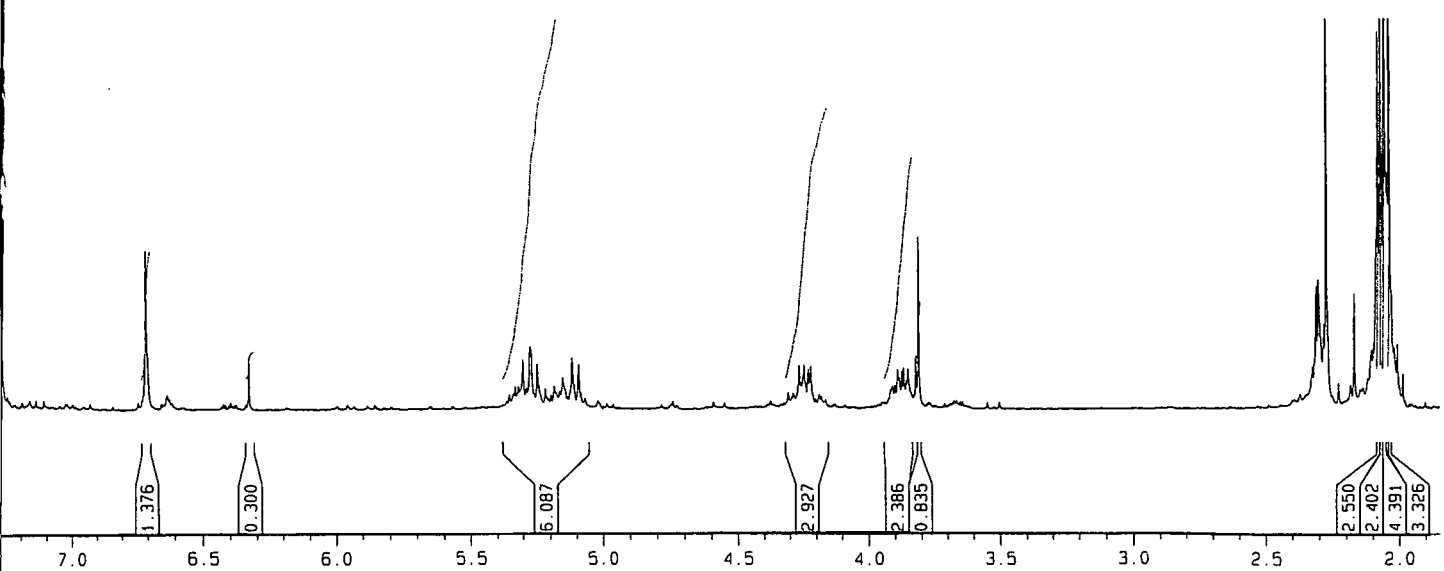
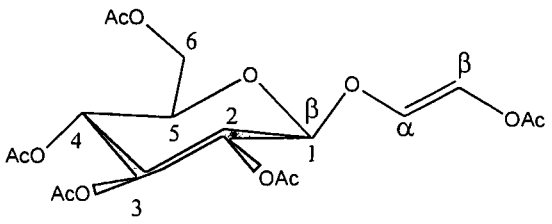


Plate 8 (CDCl3- 333K)
(COSY 8a-1)



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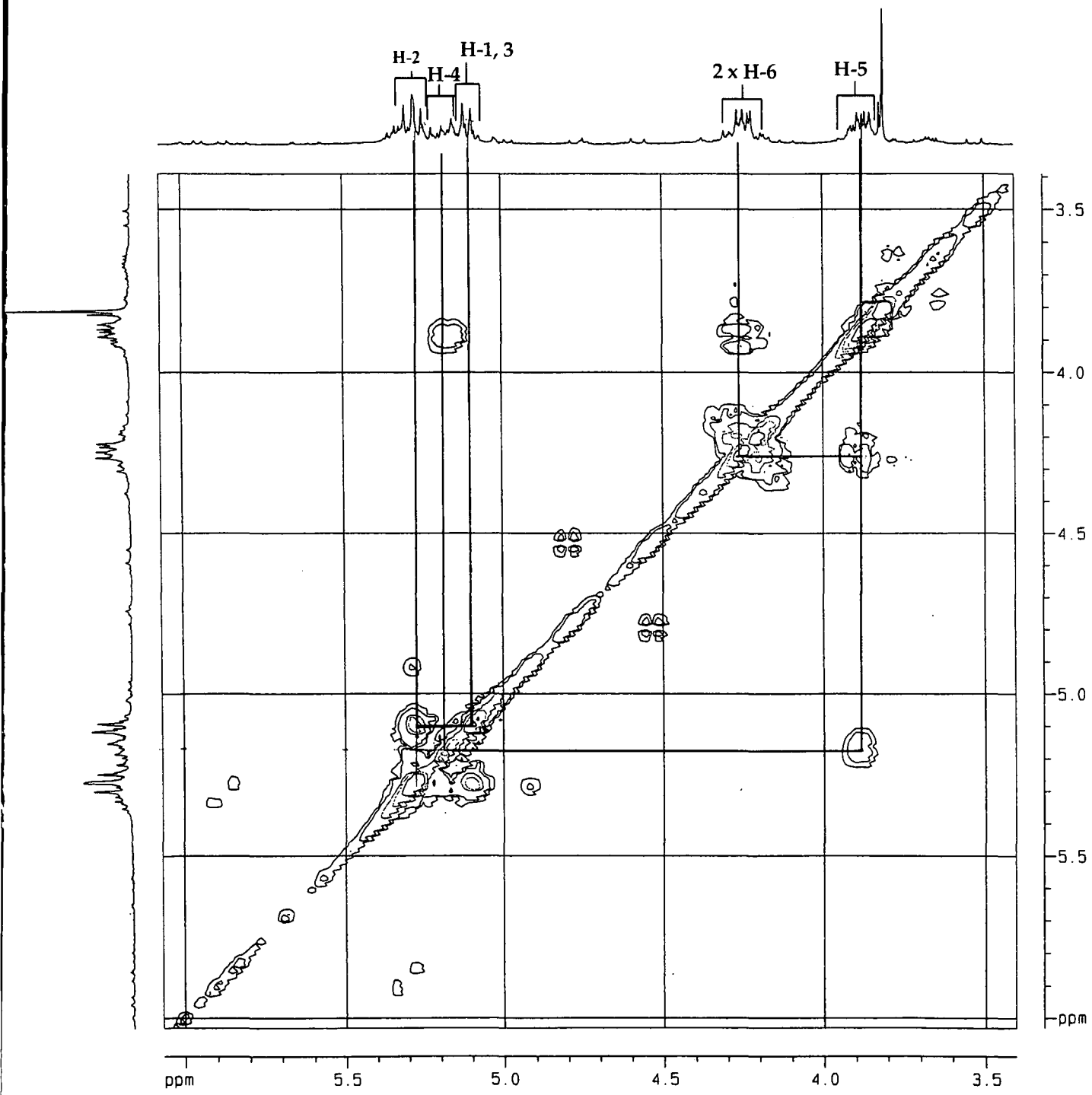
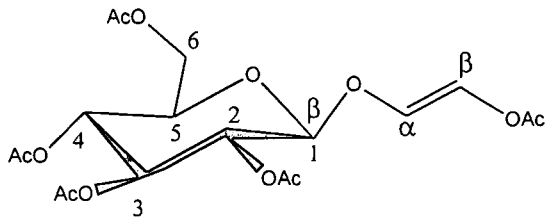


Plate 8 (CDCl3- 333K)
(NOESY 8b-1)



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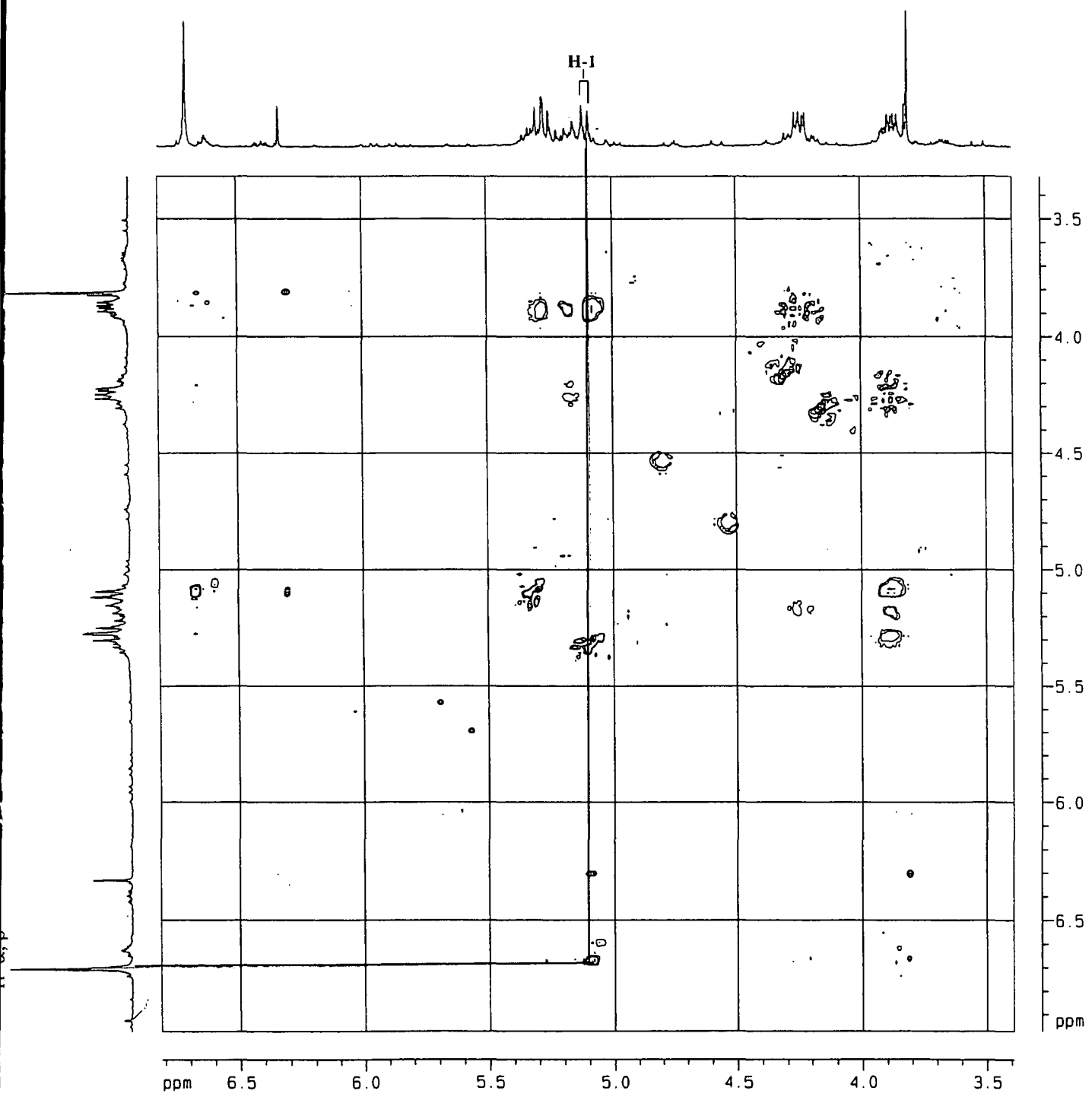
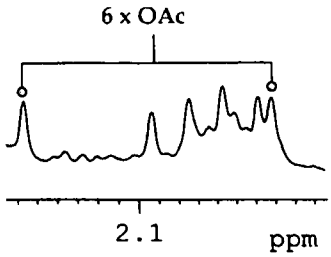
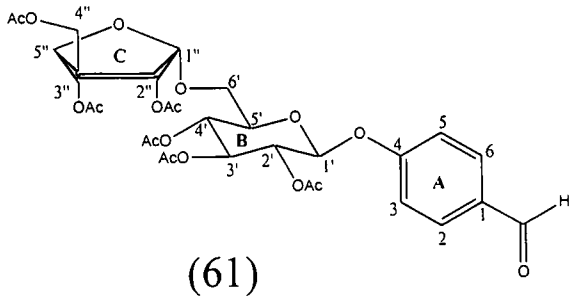


Plate 9 (CDCl3 298K)
(^{1}H NMR)





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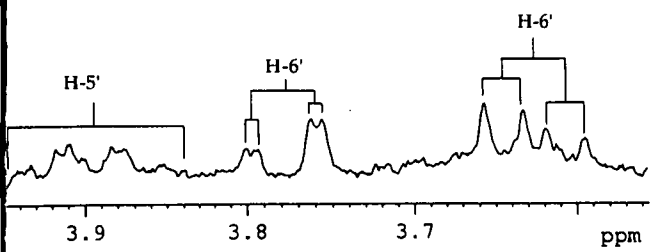
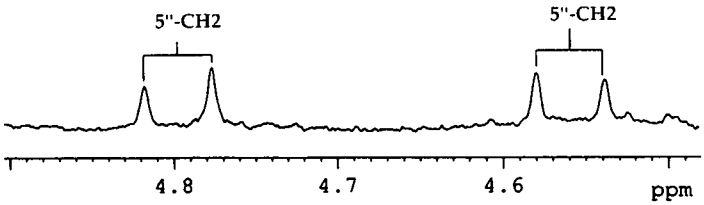
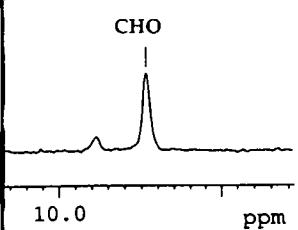
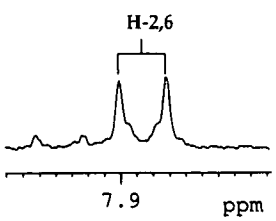
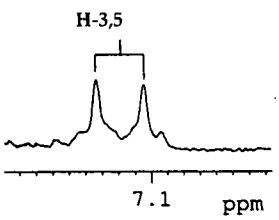
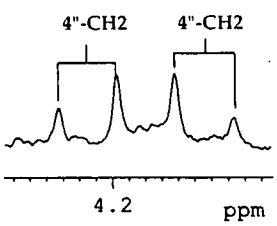


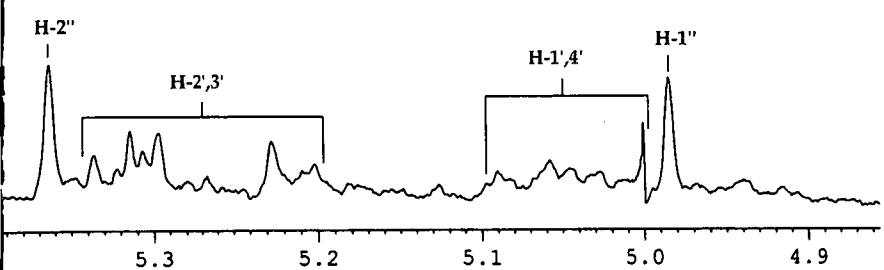












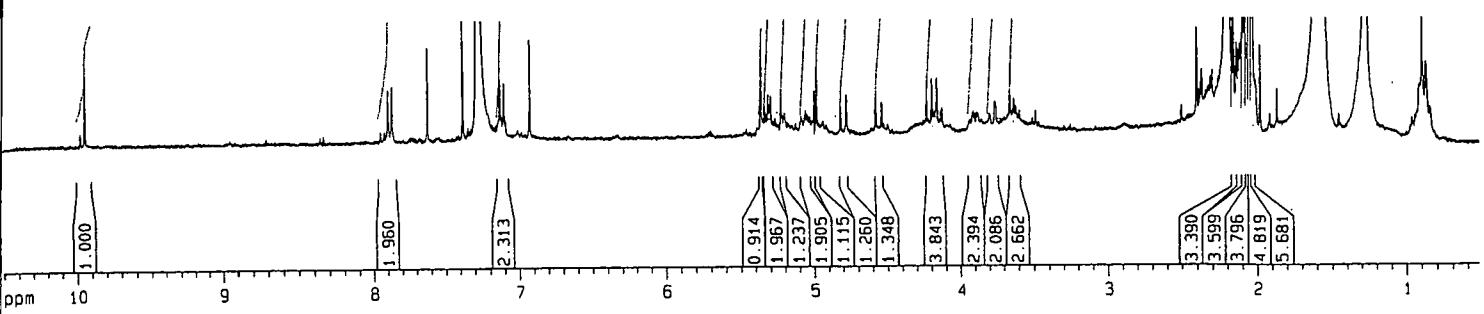
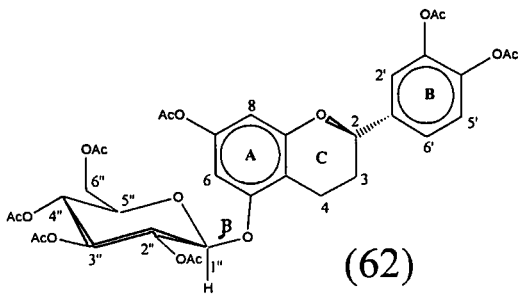


Plate 10 (C6D6 298K)
(^{1}H NMR)



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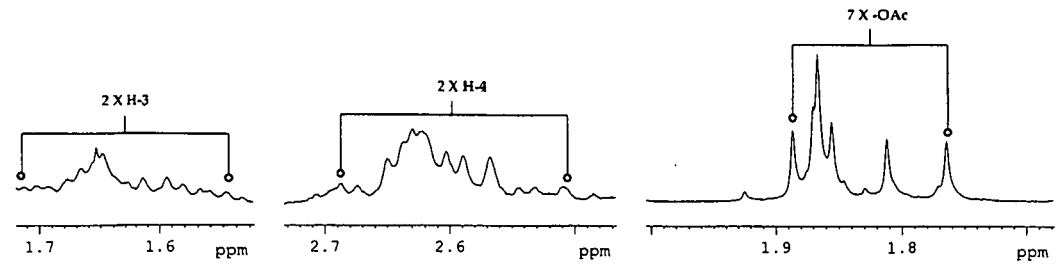
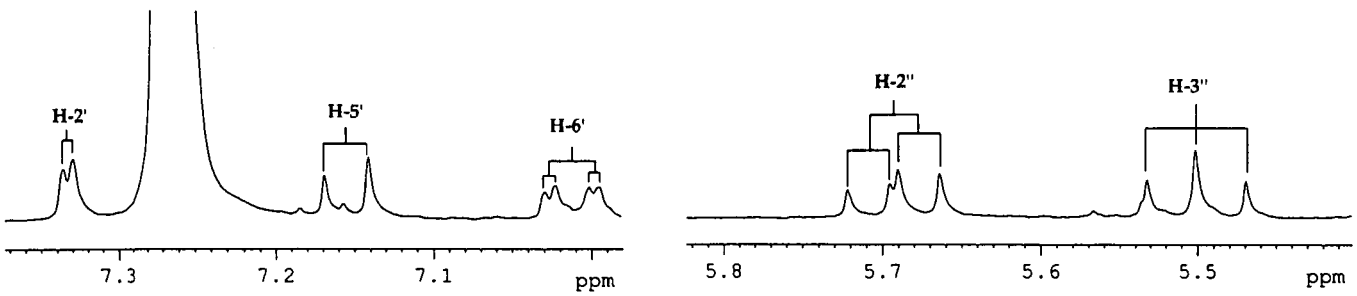
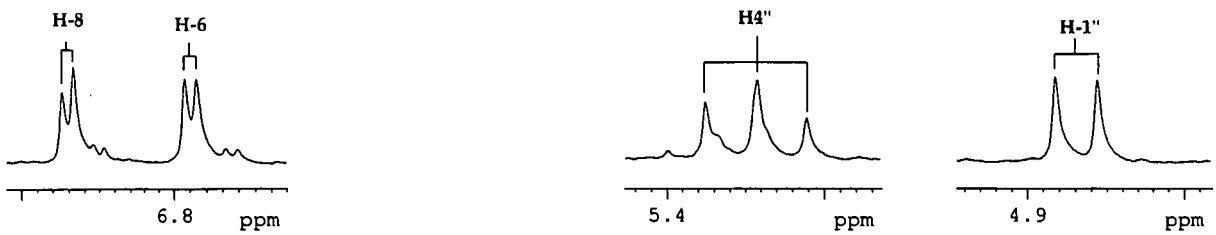
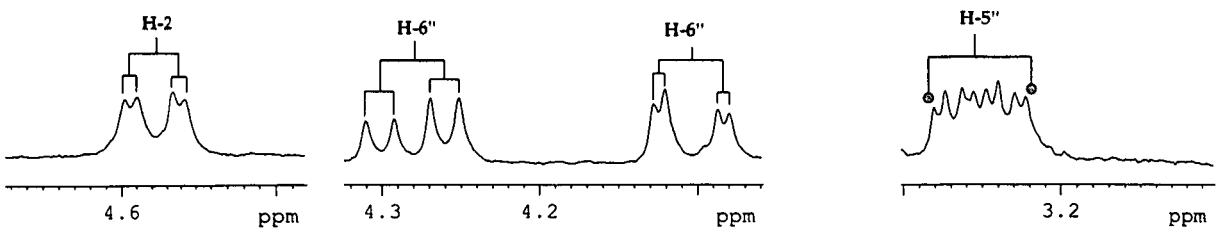








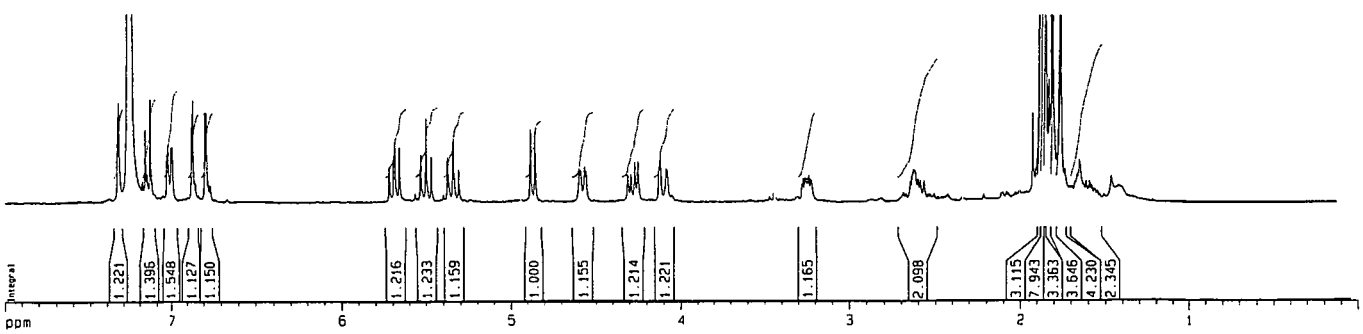
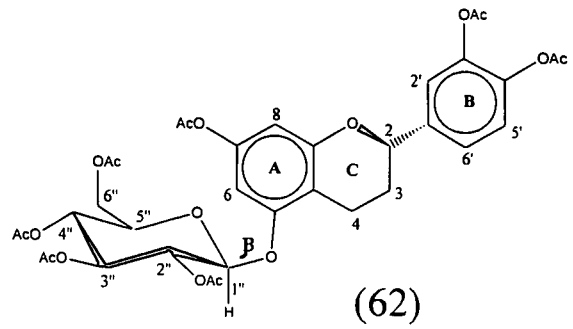


Plate 10 (**C6D6**- 298K)
(COSY 10a-1)



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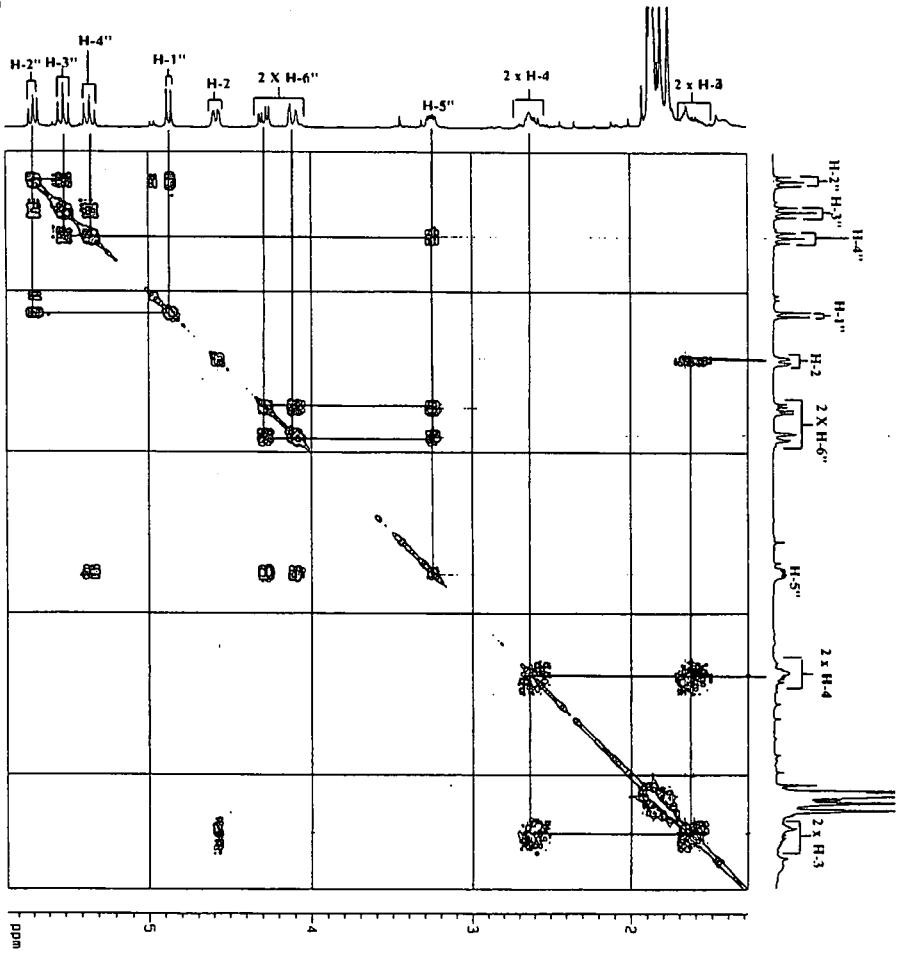


Plate 10 (**C6D6**- 298K)
(COSY 10a-2)

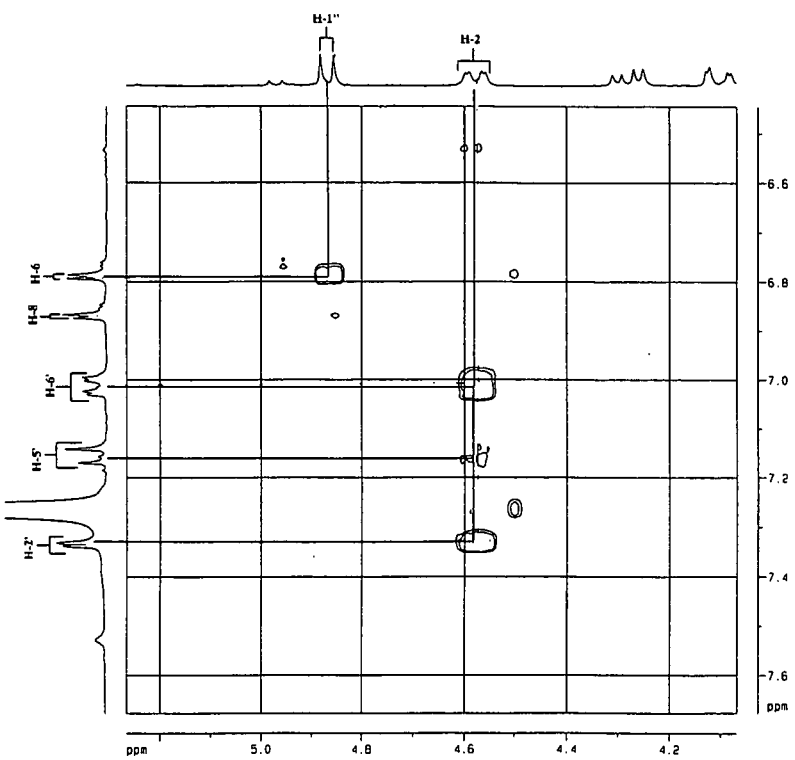
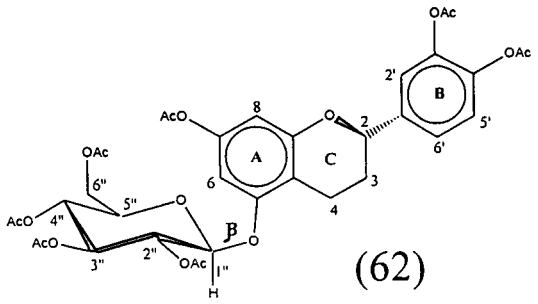


Plate 10 (C_6D_6- 298K)
(COSY 10a-3)



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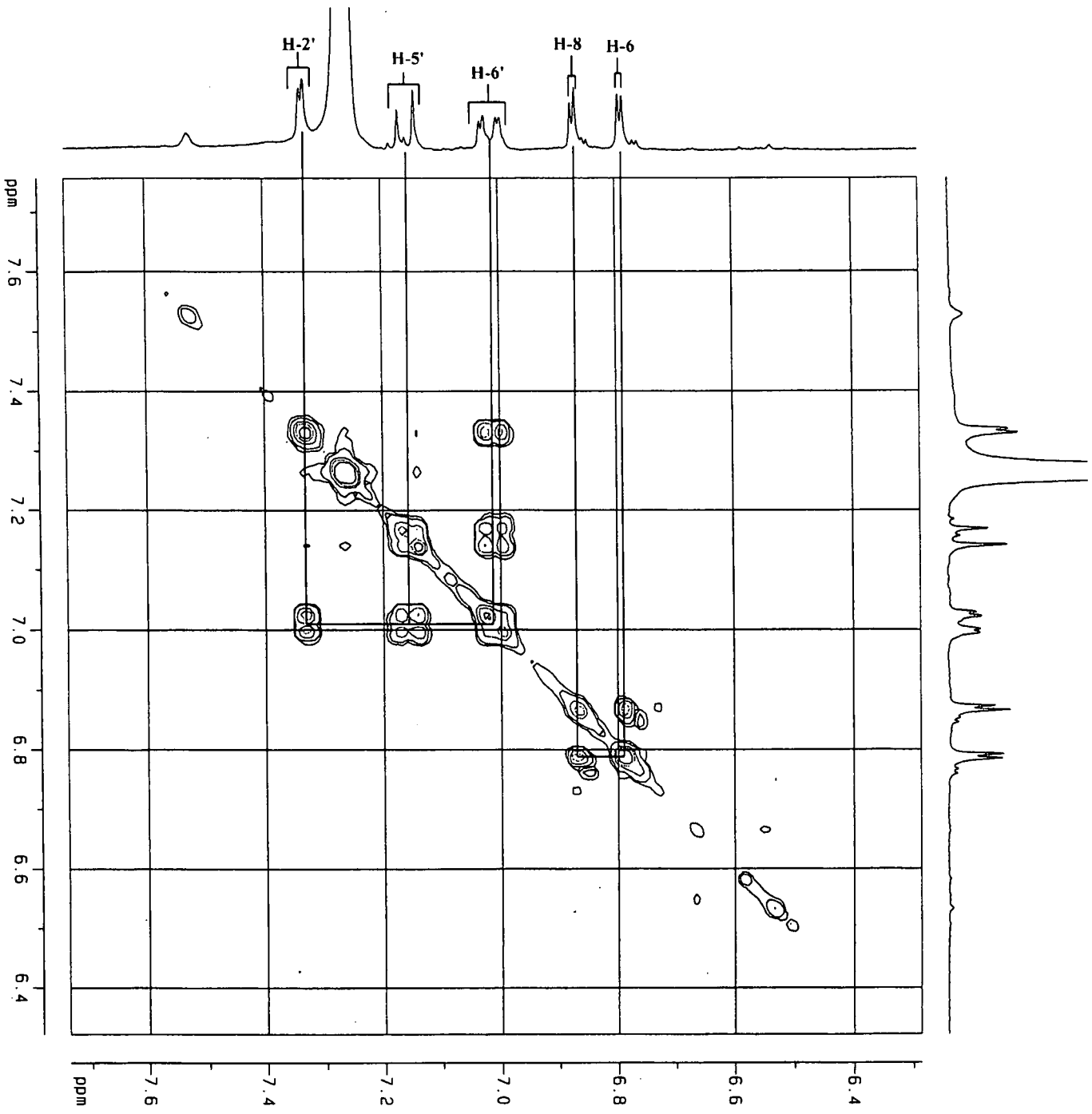
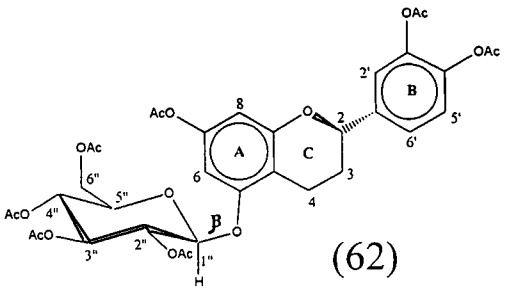


Plate 10 (**C6D6**- 298K)
(NOESY 10b-1)



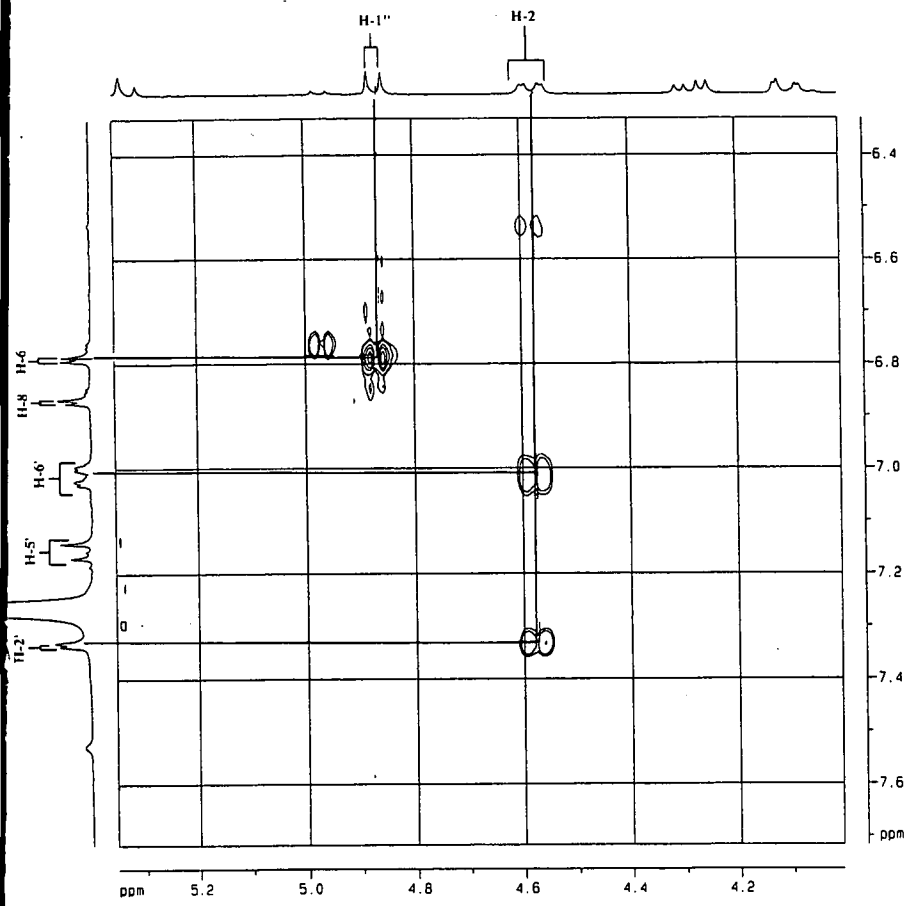


Plate 10 (**C6D6**- 298K)
(NOESY 10b-2)

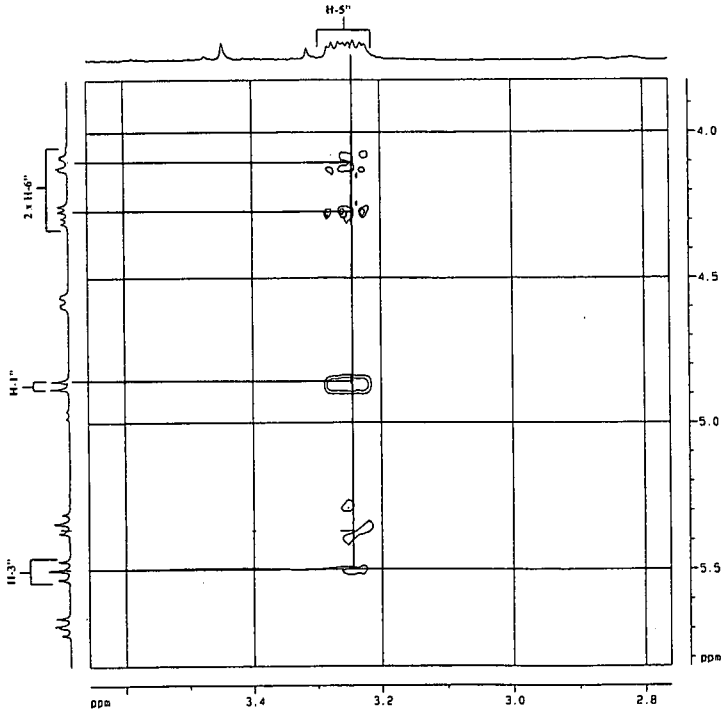
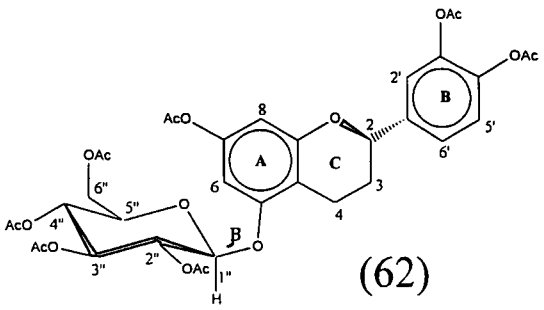


Plate 10 (C_6D_6- 298K)
(NOESY 10b-3)



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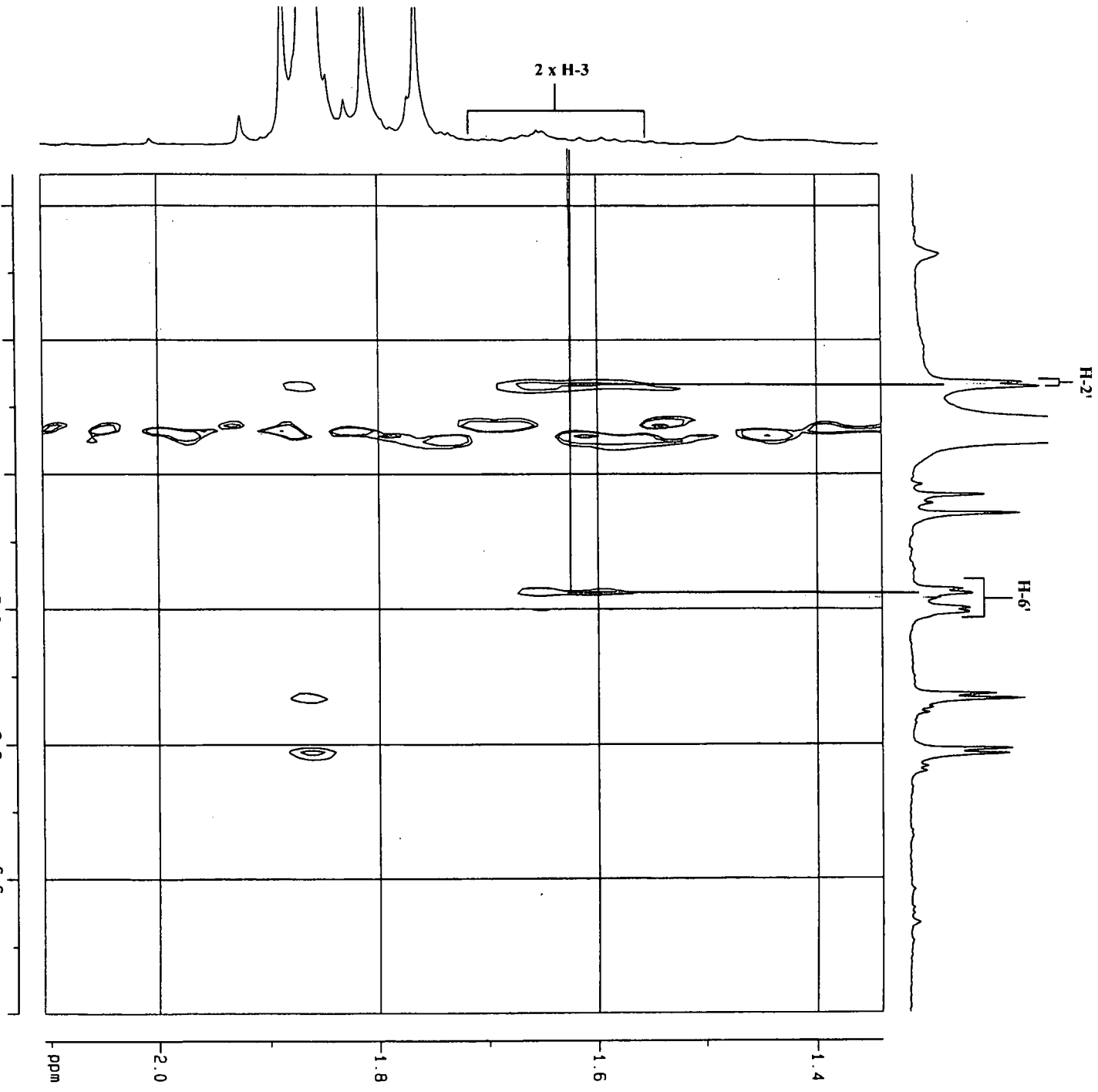
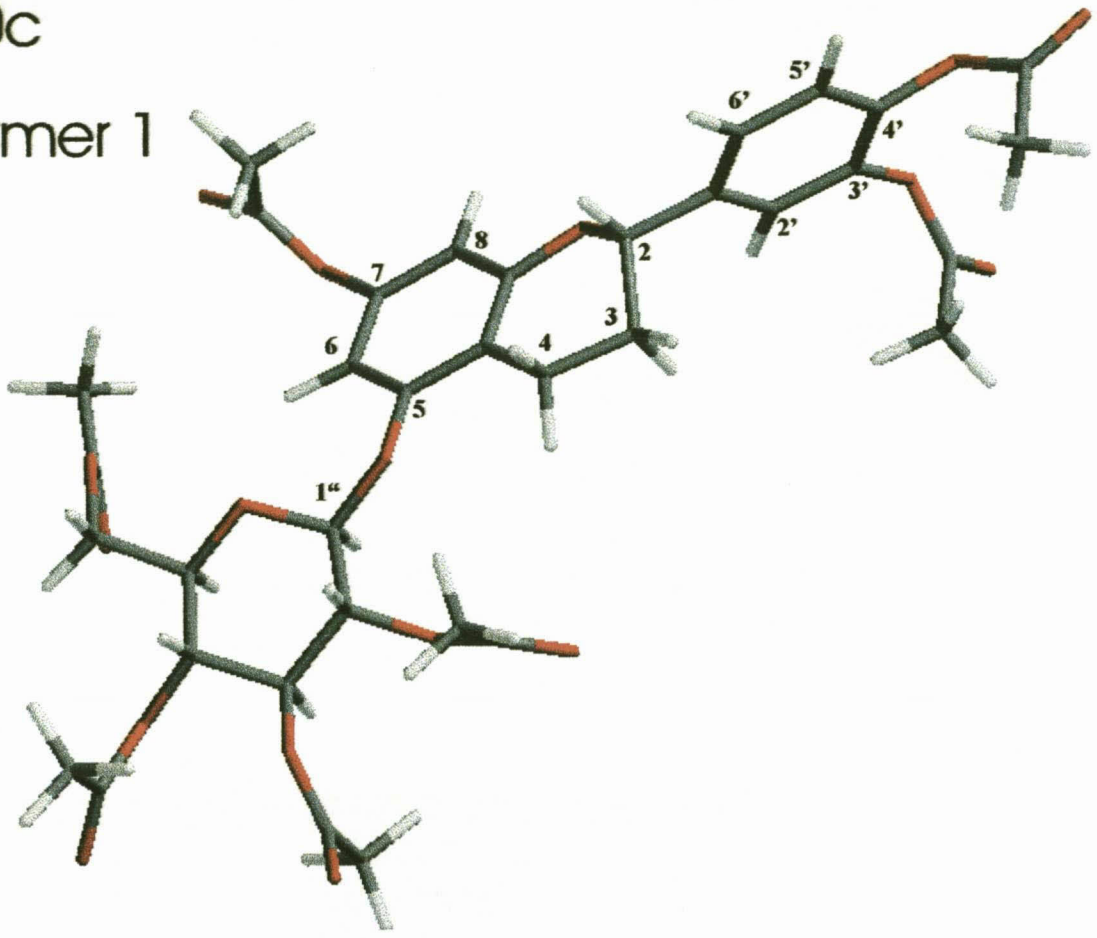


Plate 10c

Conformer 1



Conformer 2

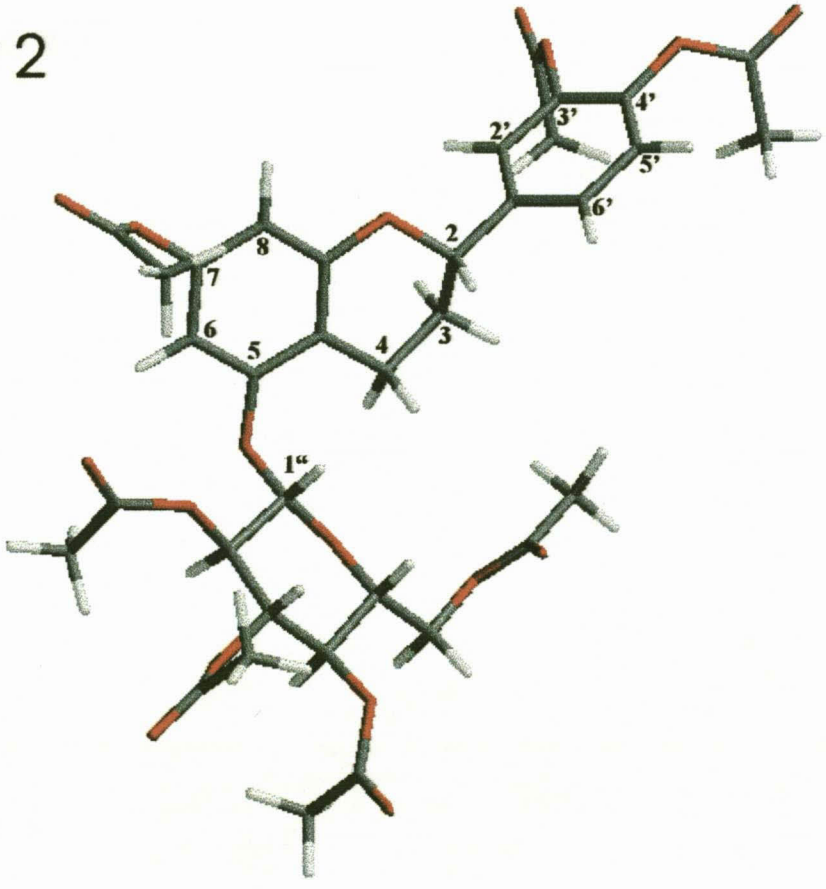
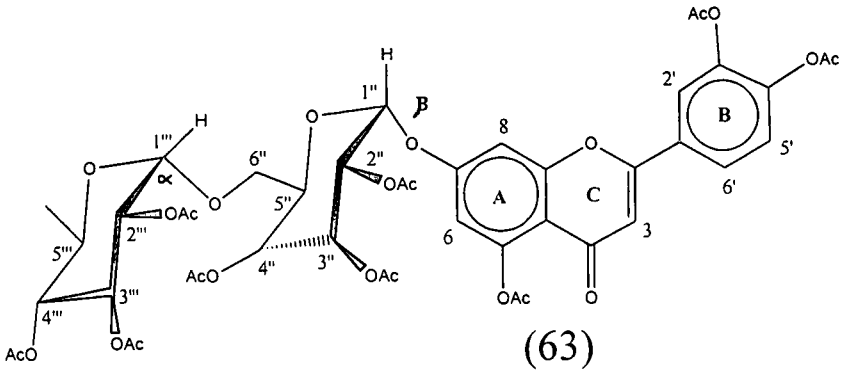


Plate 11 (C6D6 298K)
(^{1}H NMR)



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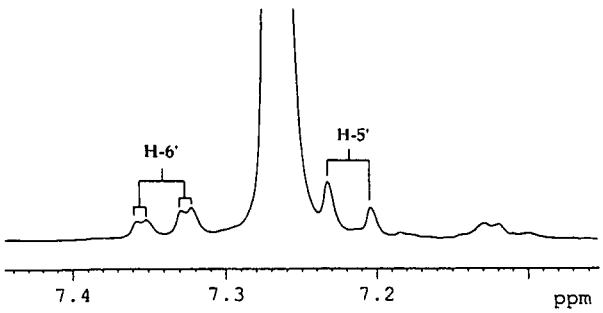
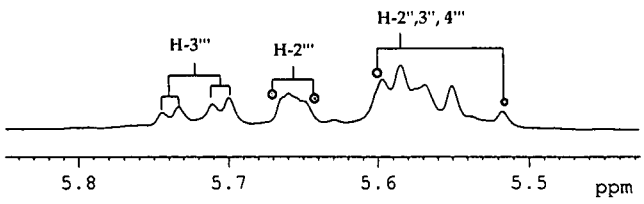
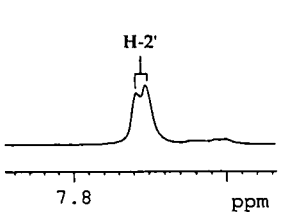
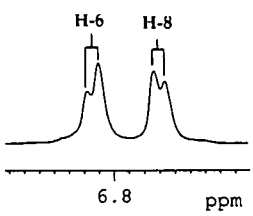
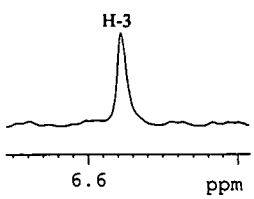
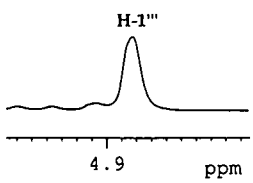


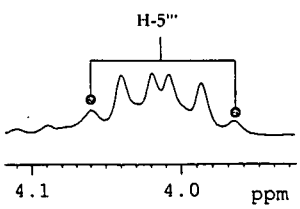


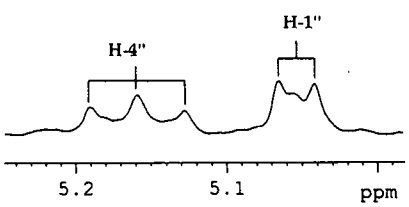


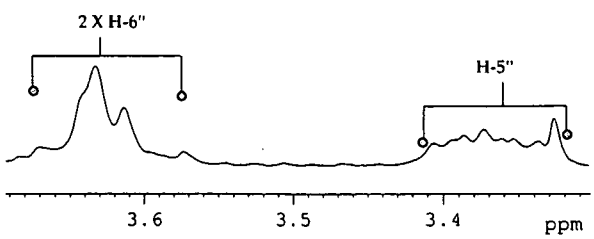












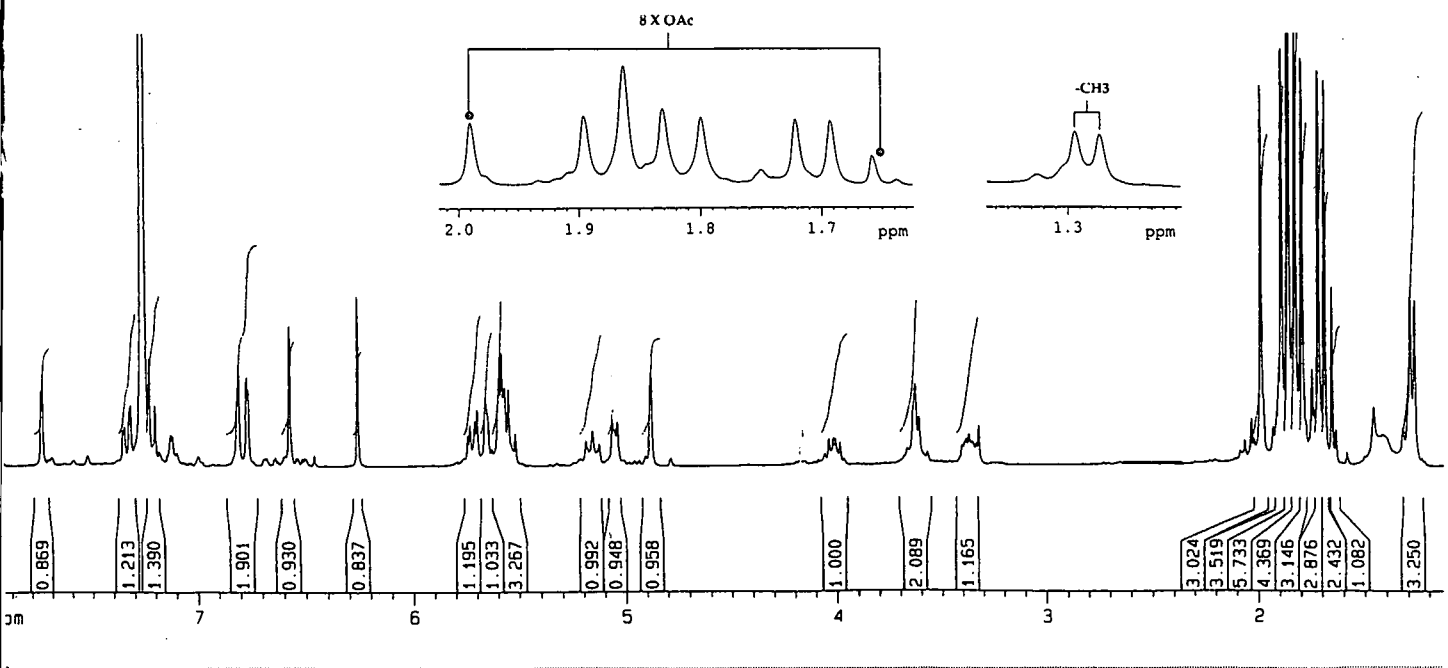
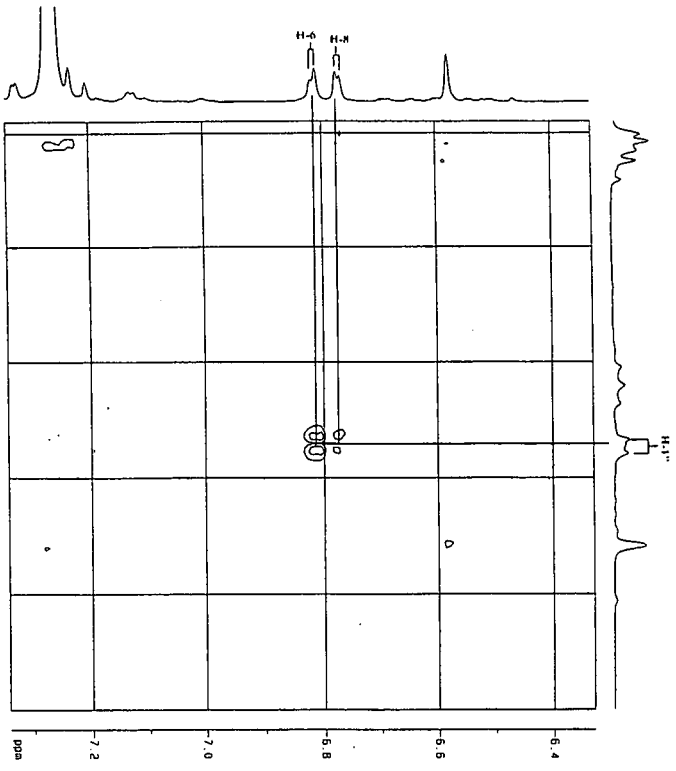
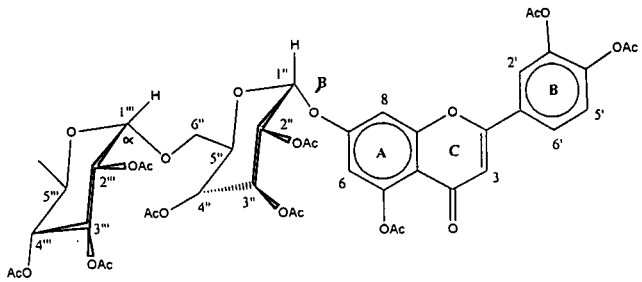


Plate 11 (C6D6 -298K)
(COSY11a-1)





(63)

Plate 11 (C6D6 -298K)
(COSY11a-2)

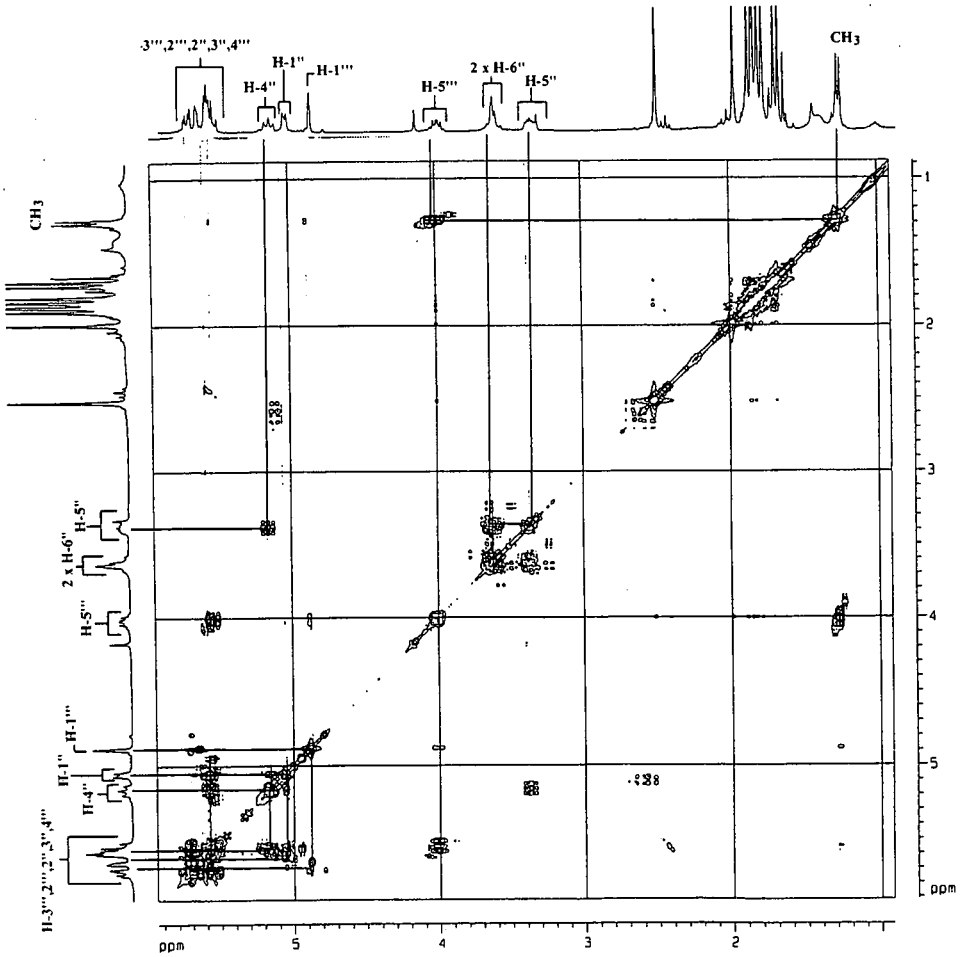
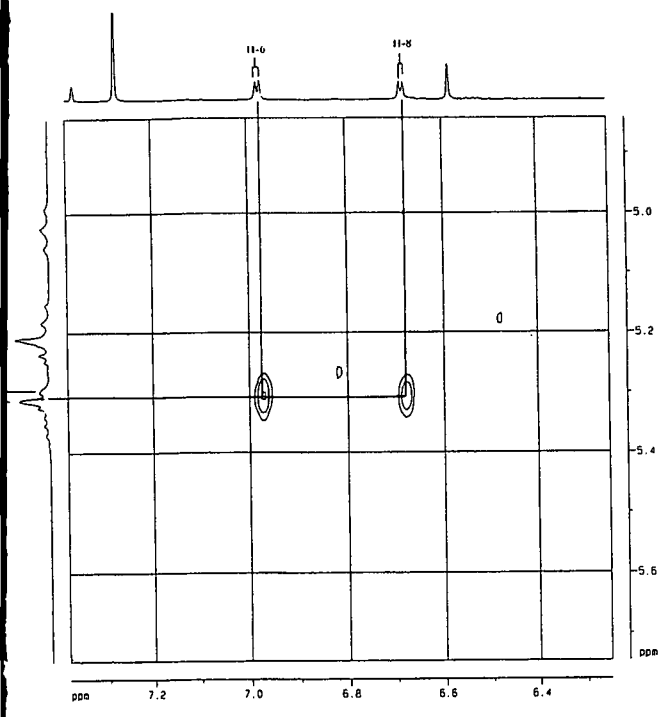


Plate 11 (CDCl3 298K)
(NOESY11b-1)



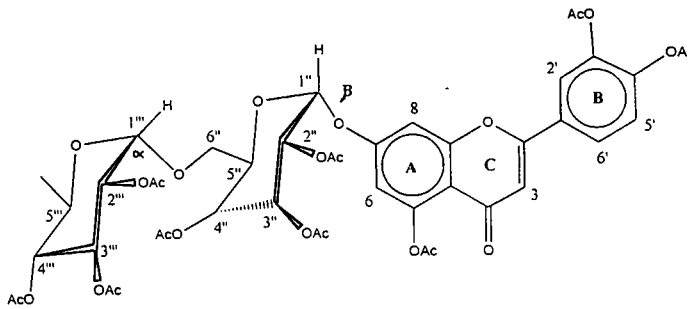


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Plate 11 (CDCl3 298K)
(NOESY11a-2)

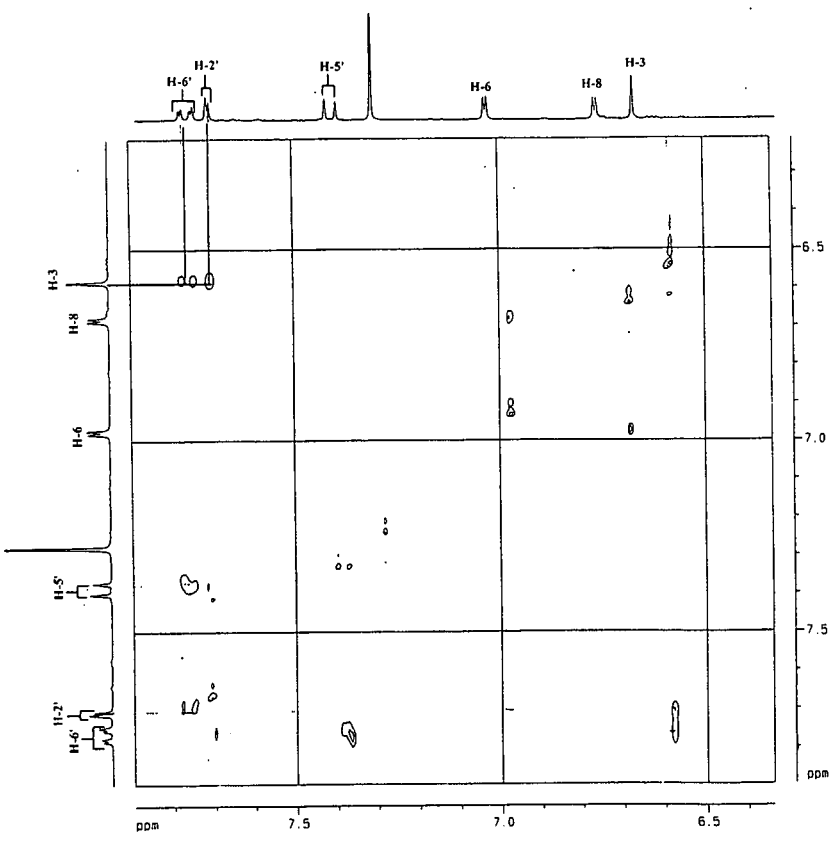
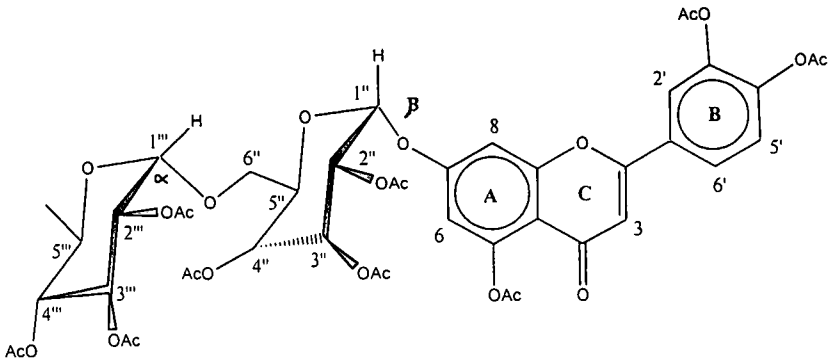


Plate 11 (CDCl3 298K)
(NOESY11b-3)



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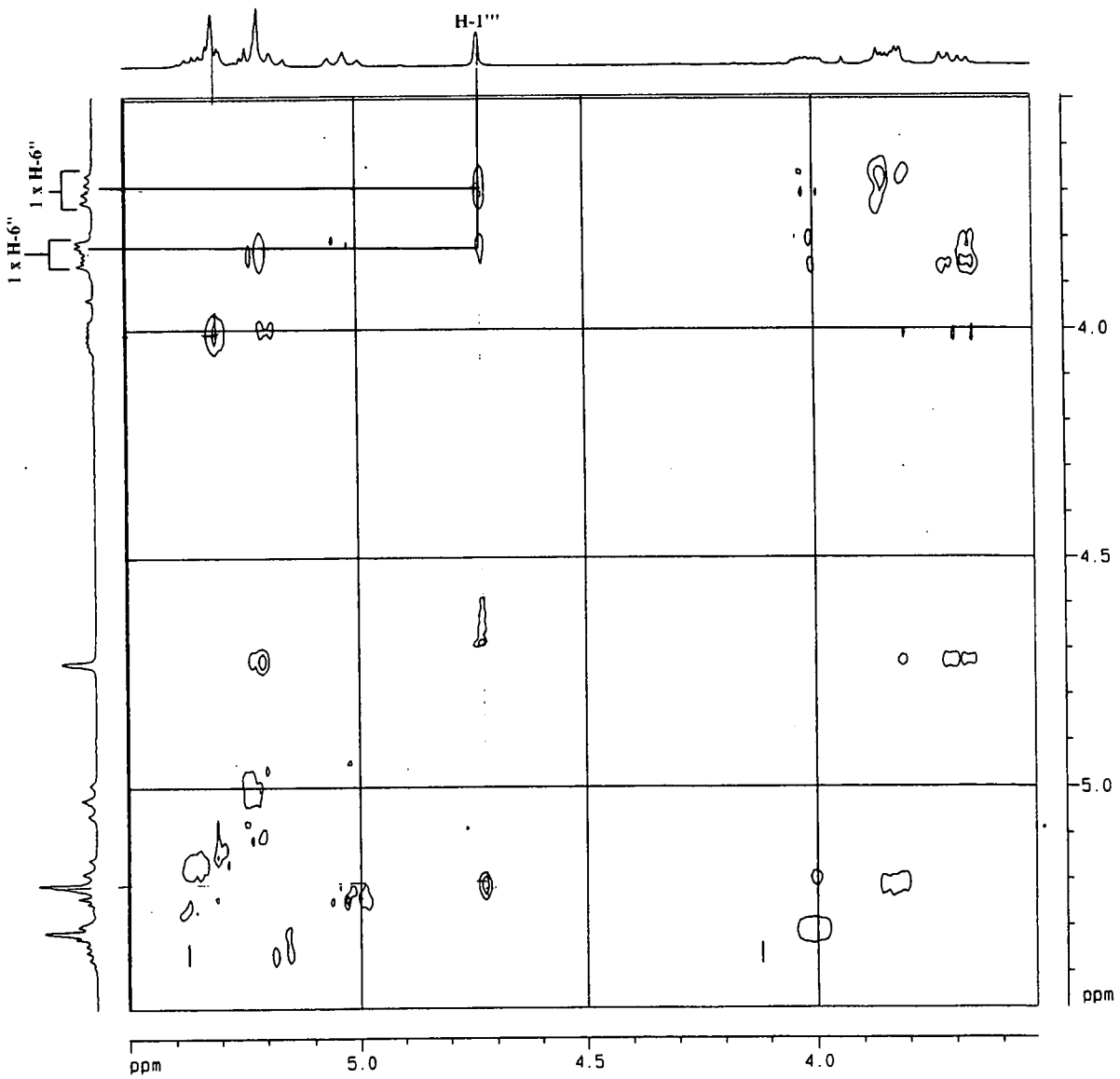
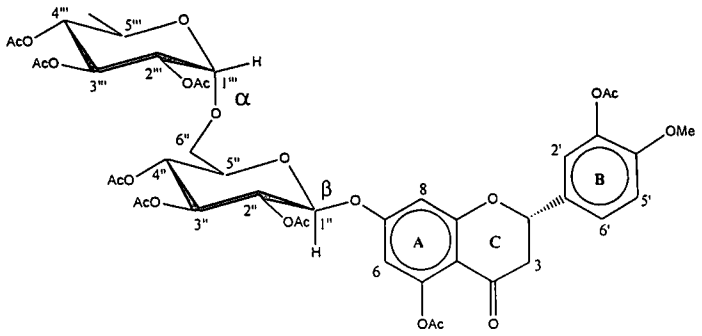


Plate 12 (CDCl3 298K)
(^{1}H NMR)



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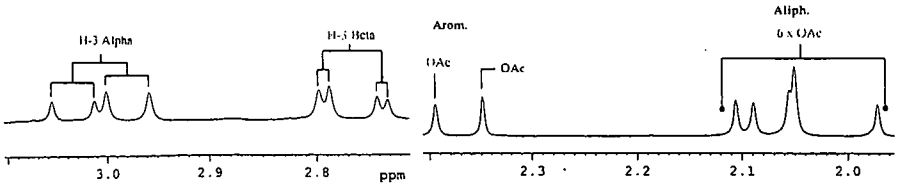
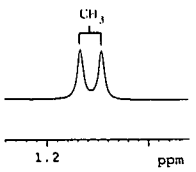
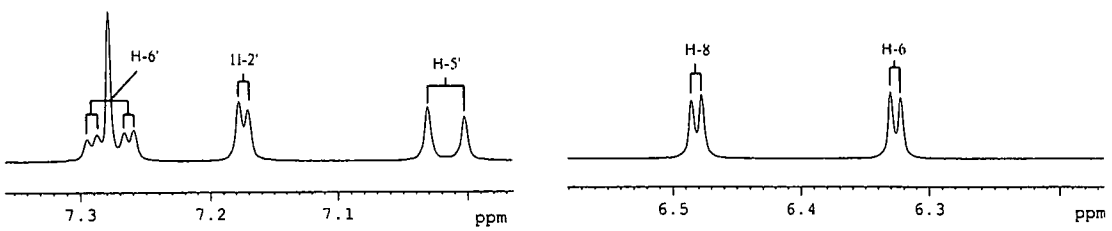
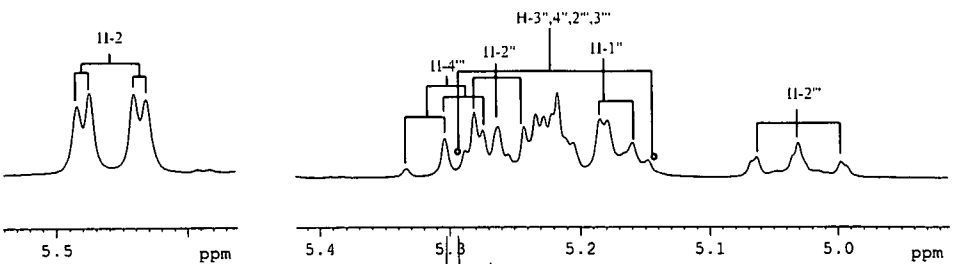
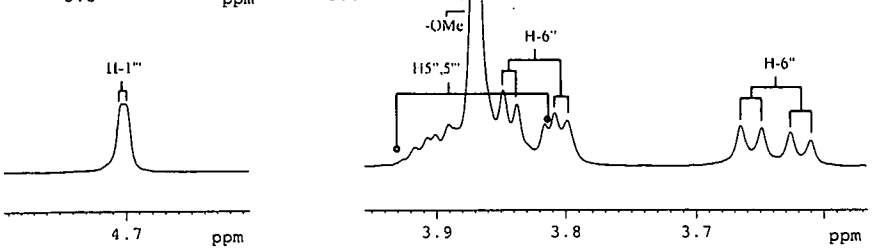










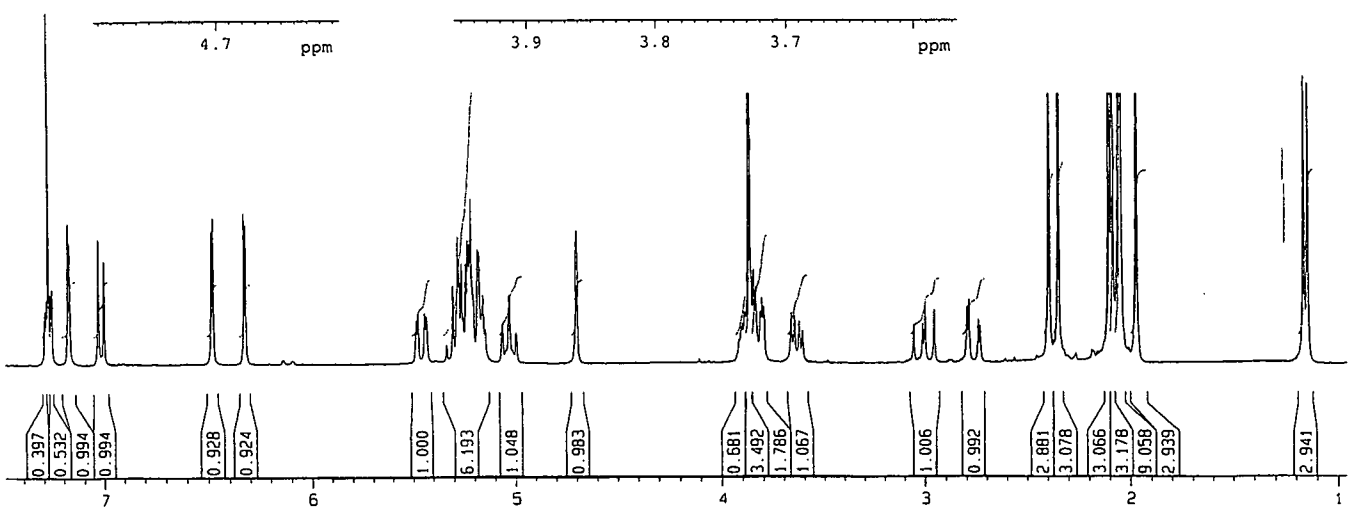
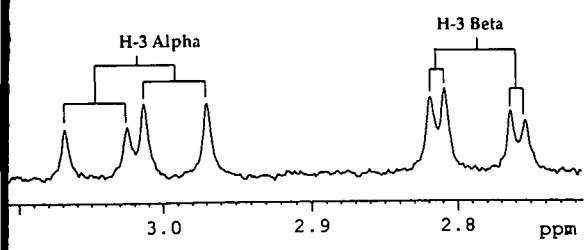
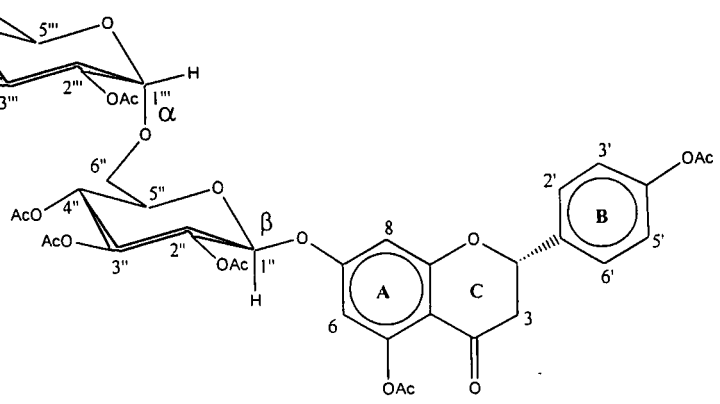


Plate 13 (CDCl3 298K)
(^{1}H NMR)





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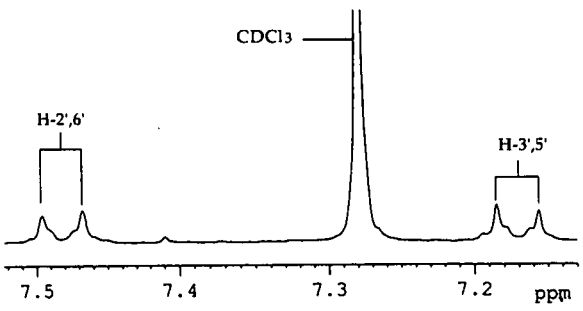
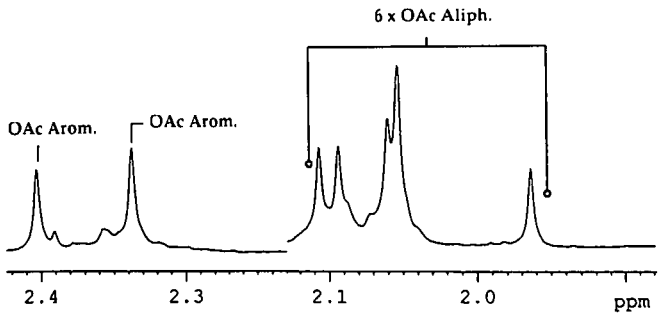
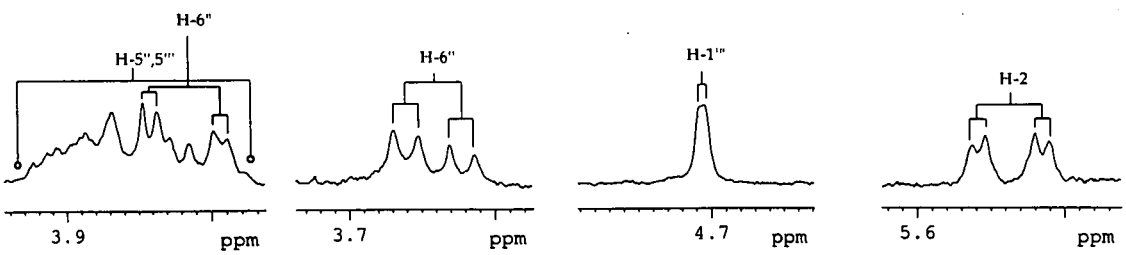
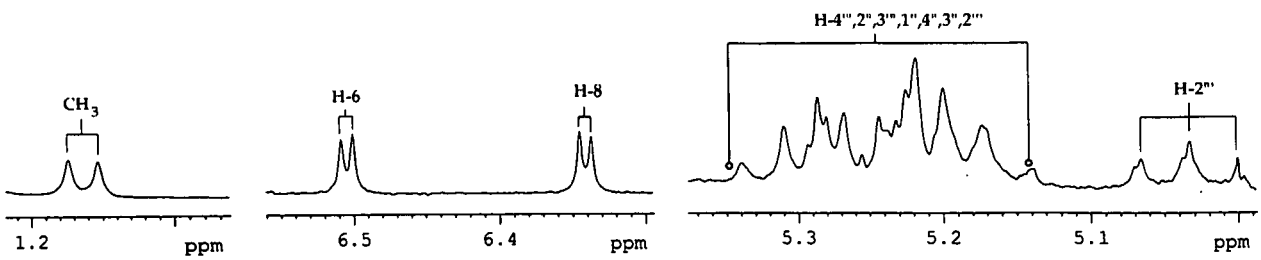








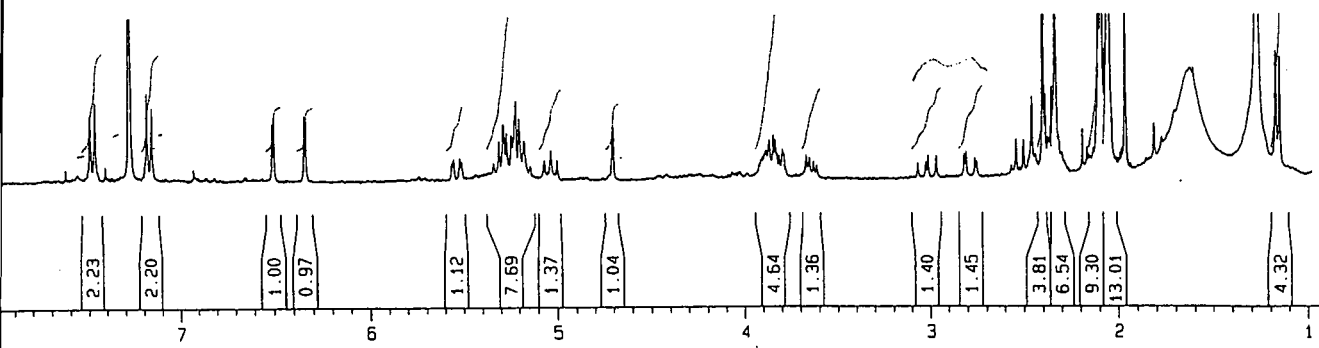
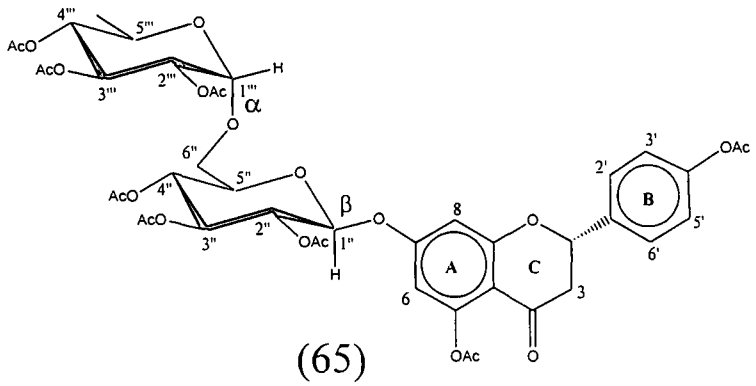


Plate 13 (CDCl3- 298K)
(NOESY 13b-1)



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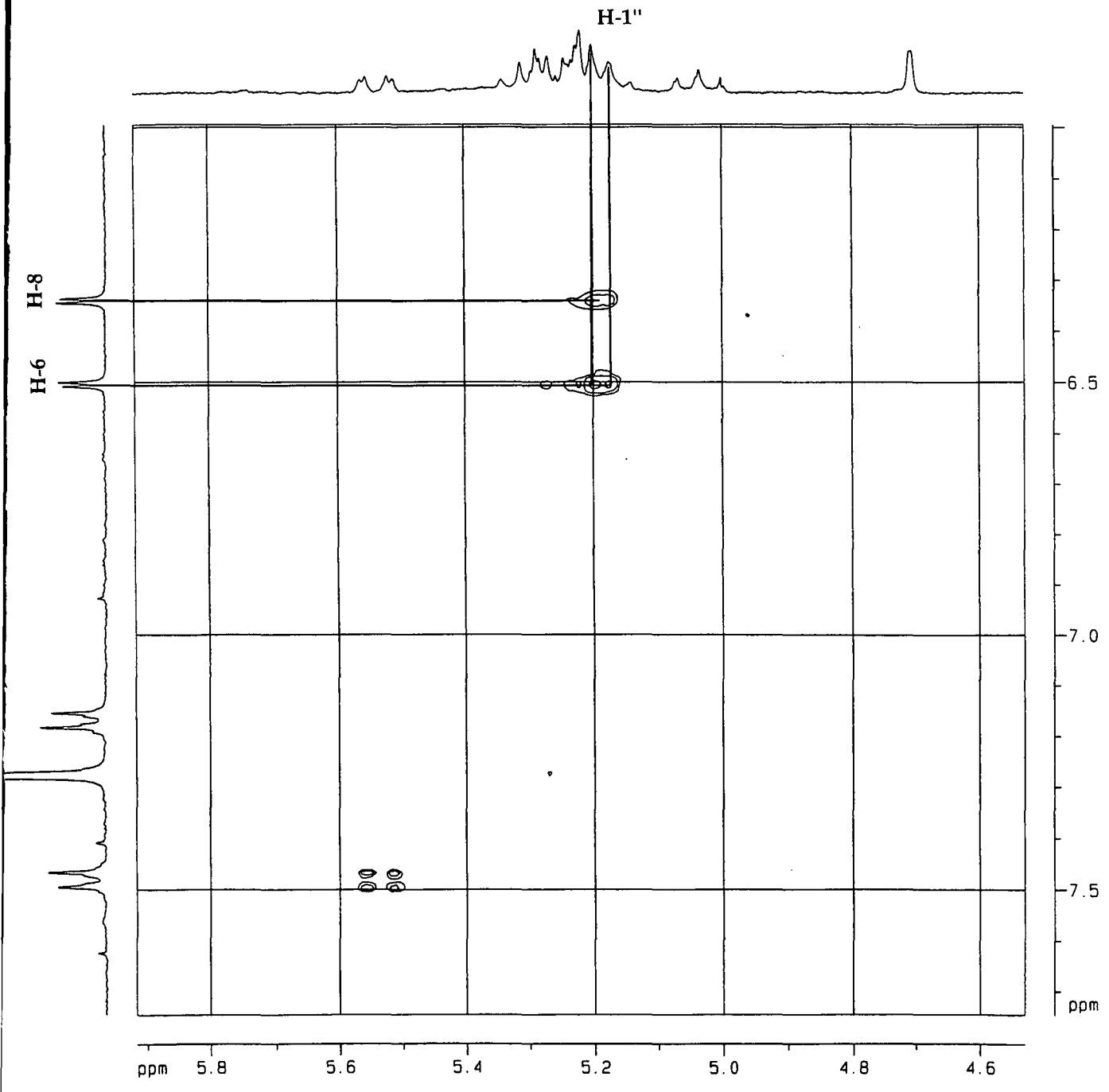
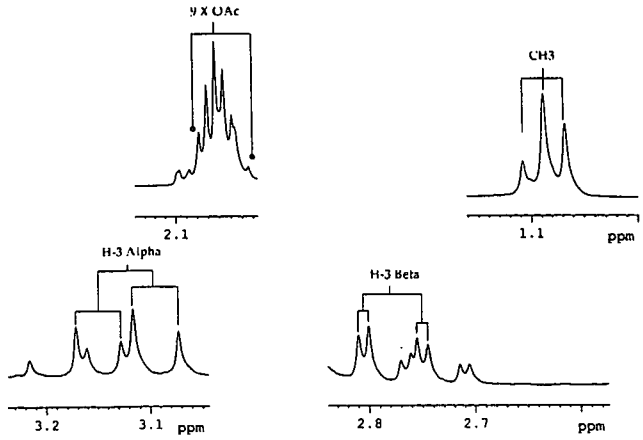
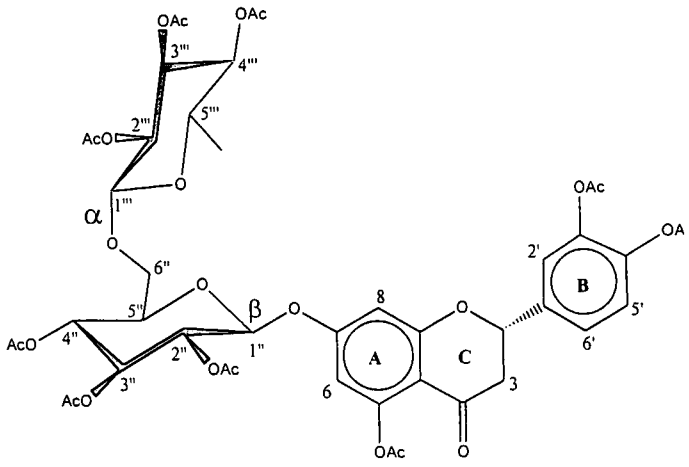


Plate 14 (C2D6CO 298K)
('H NMR)





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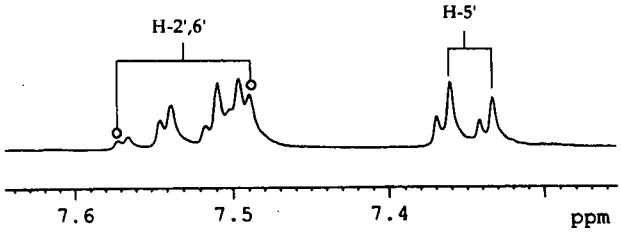
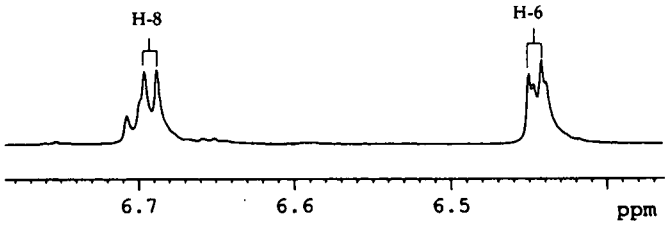
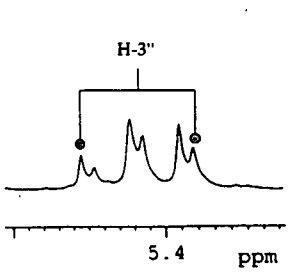
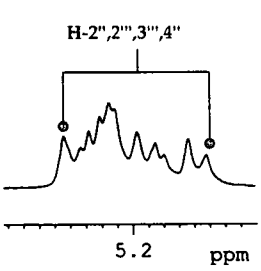
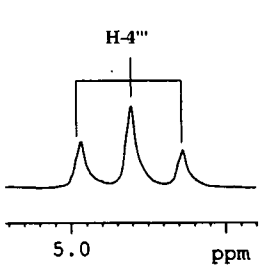
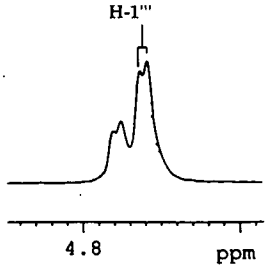


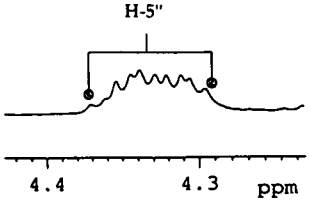


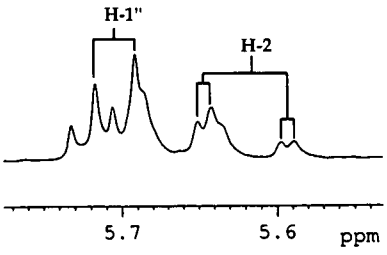


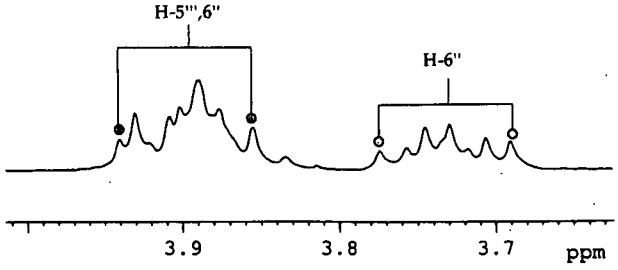












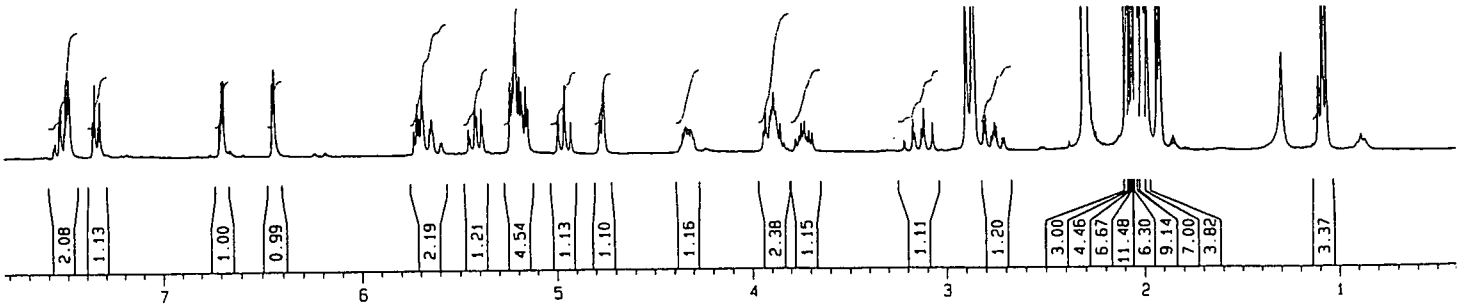
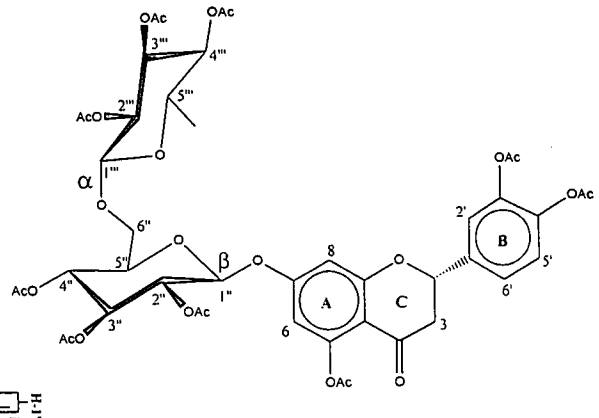


Plate 14 (C2D6CO 298K)
(COSY 14a-1)



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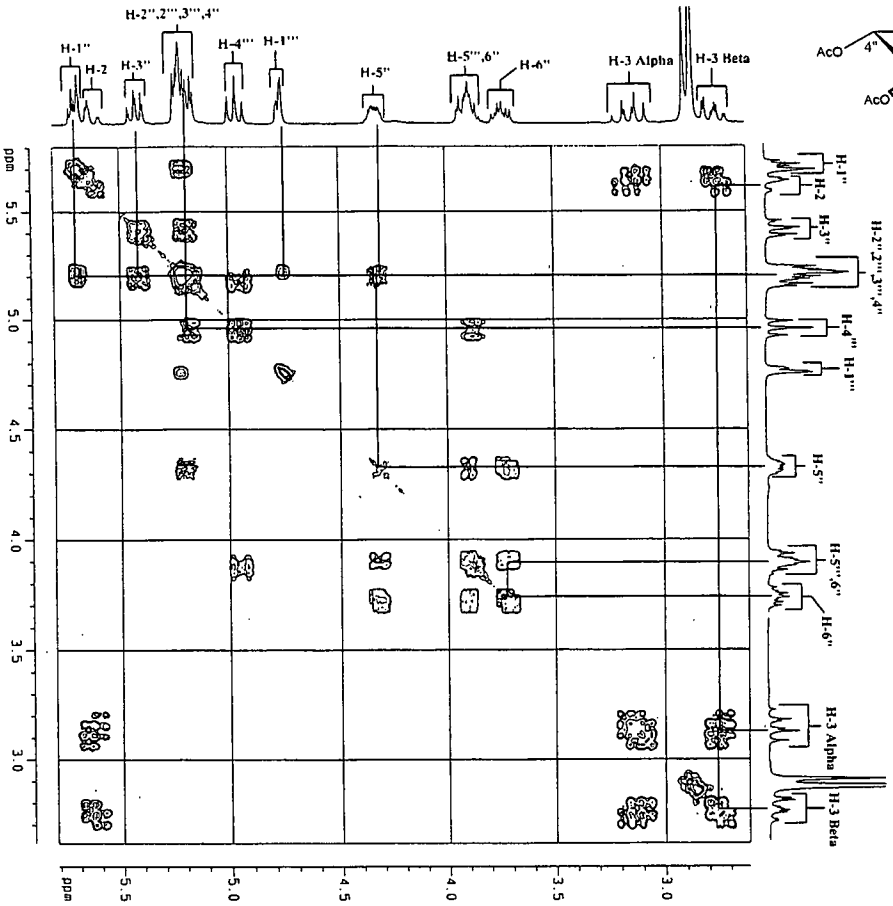


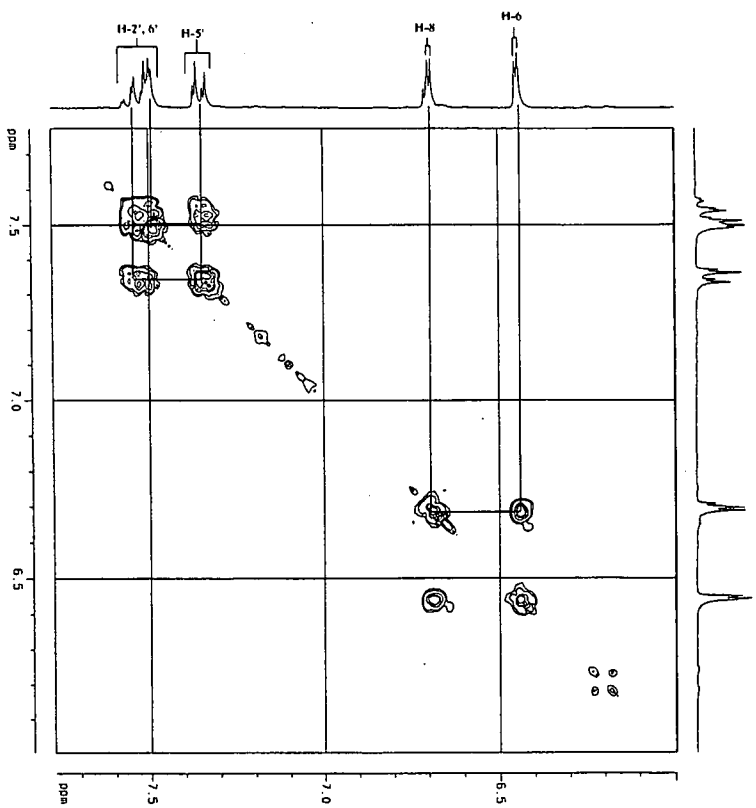
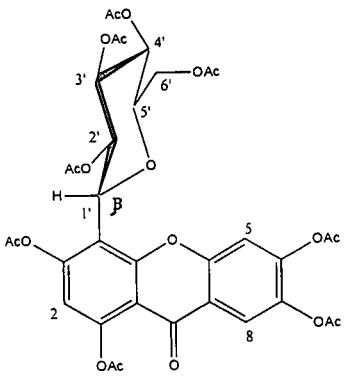


Plate 14 (C2D6CO 298K)
(COSY 14a-2)

Plate 15 (CDCl3 343K)
(^{1}H NMR)



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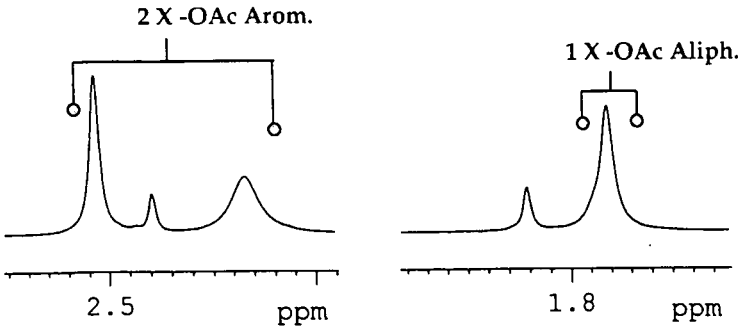
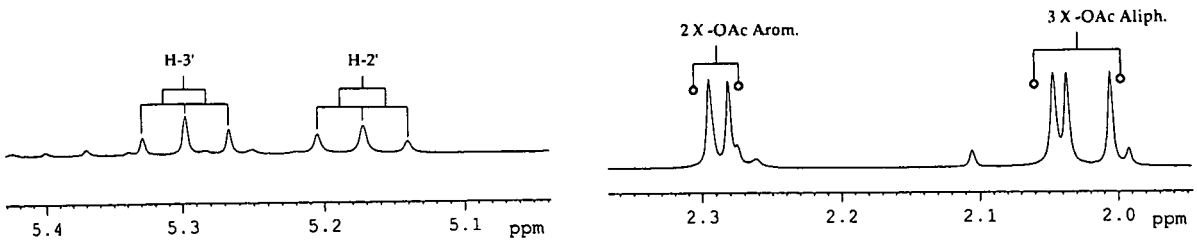
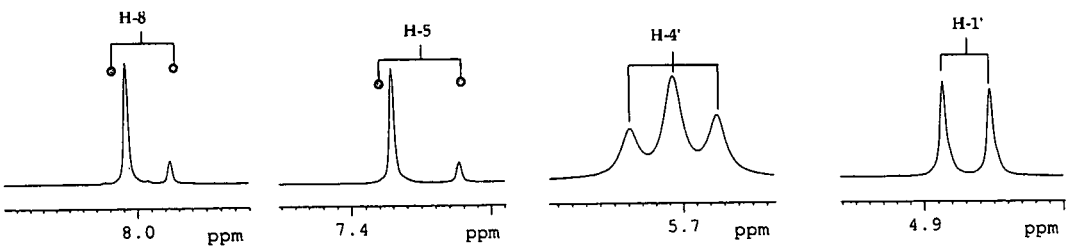






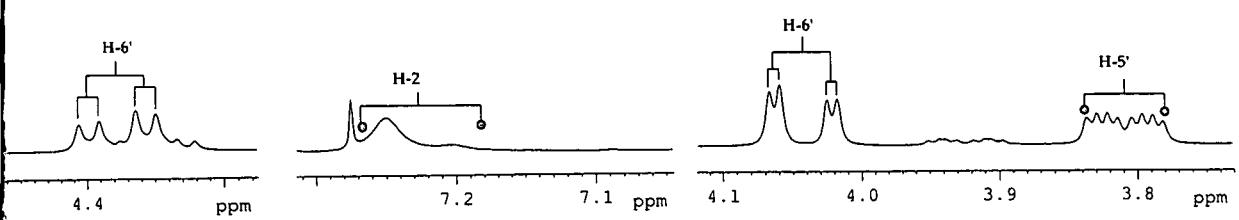
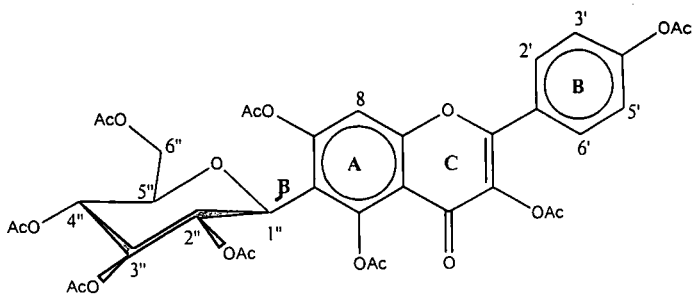


Plate 16 (CDCl3 298K)
(^{1}H NMR)



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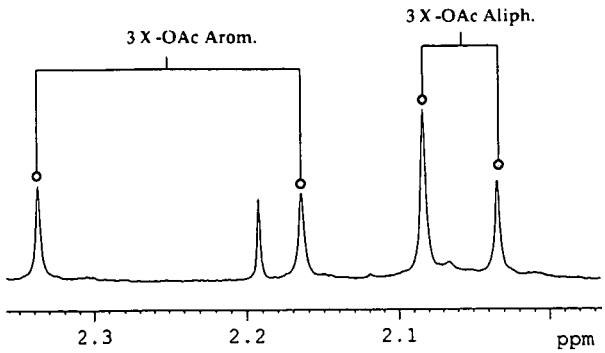
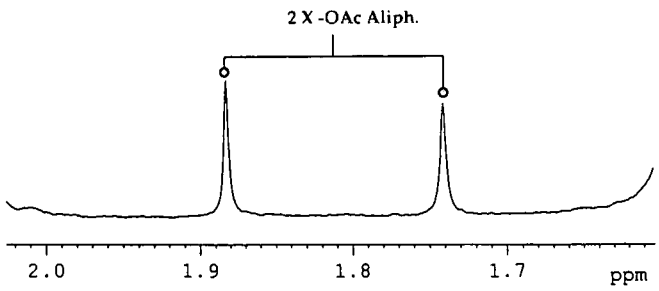
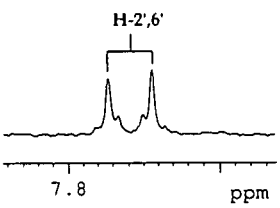
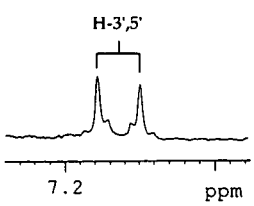
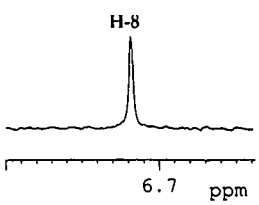
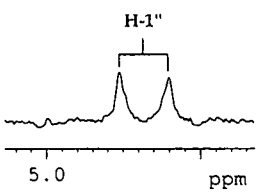


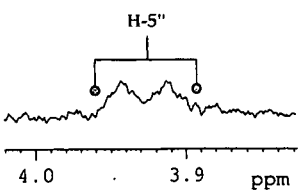


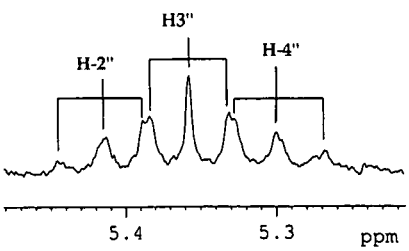


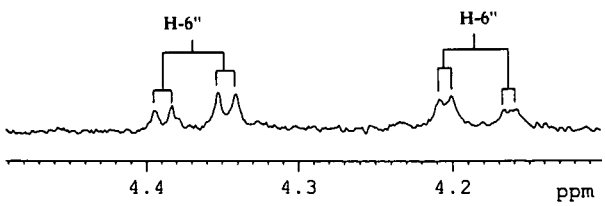












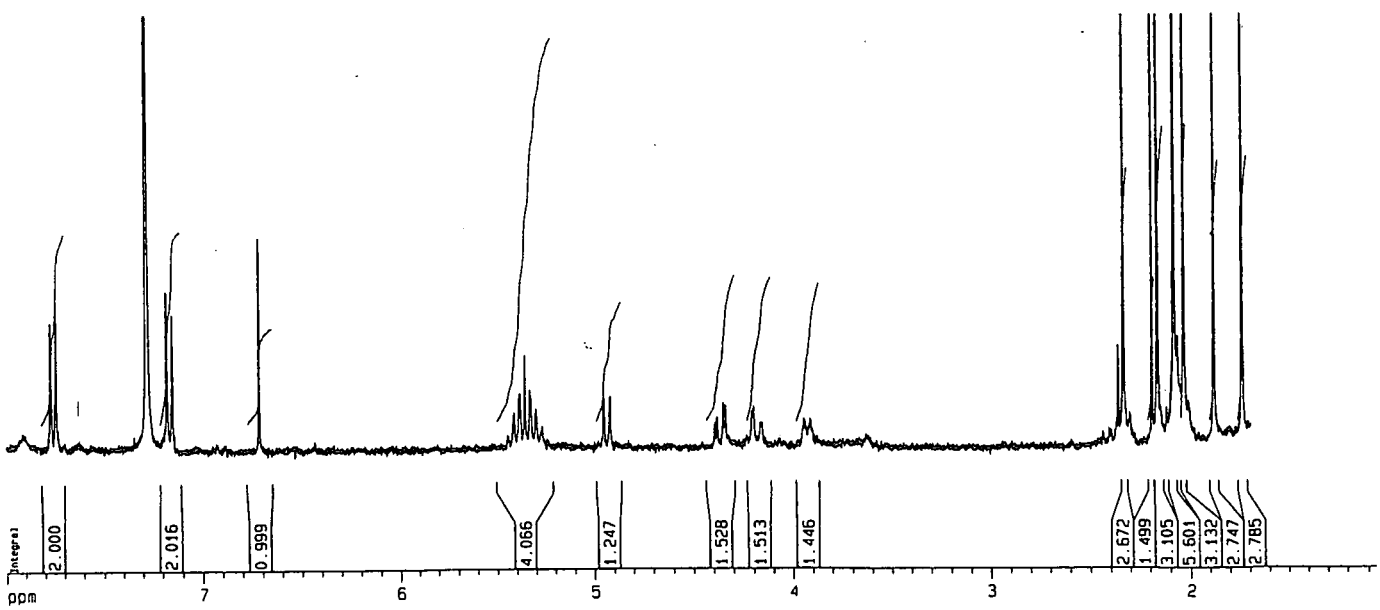
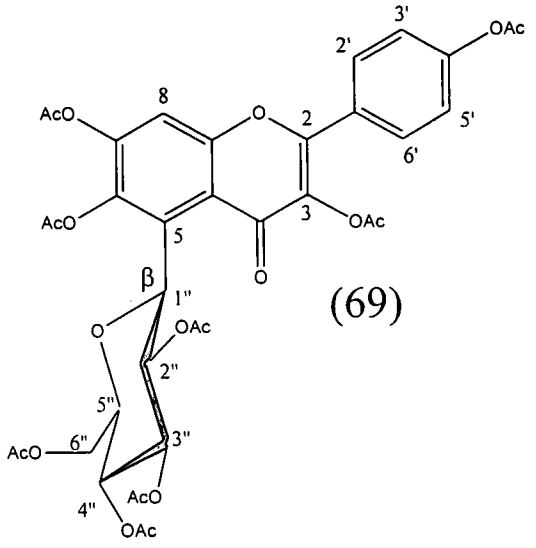


Plate 17 (CDCl3 333K)
(^{1}H NMR)



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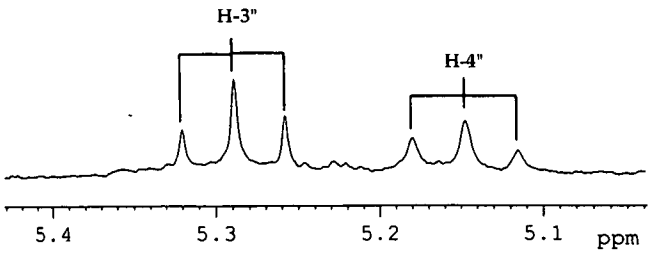
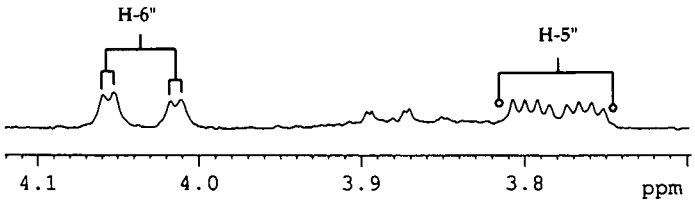
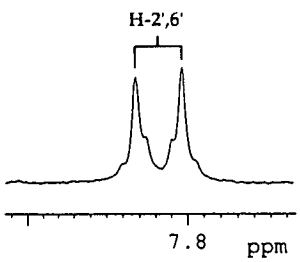
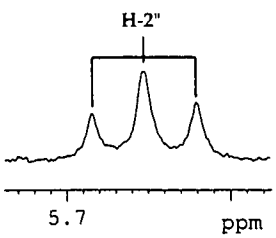
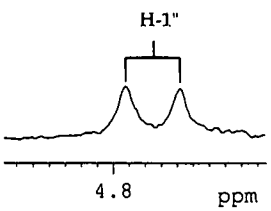
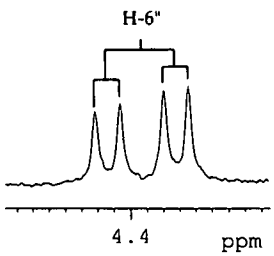


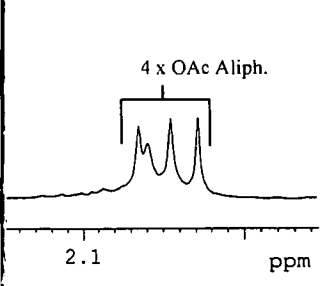


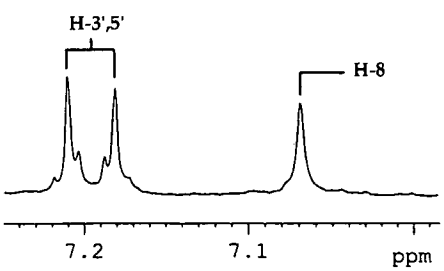


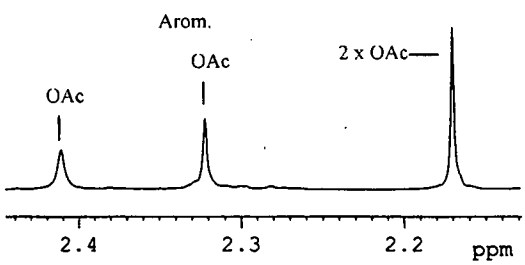












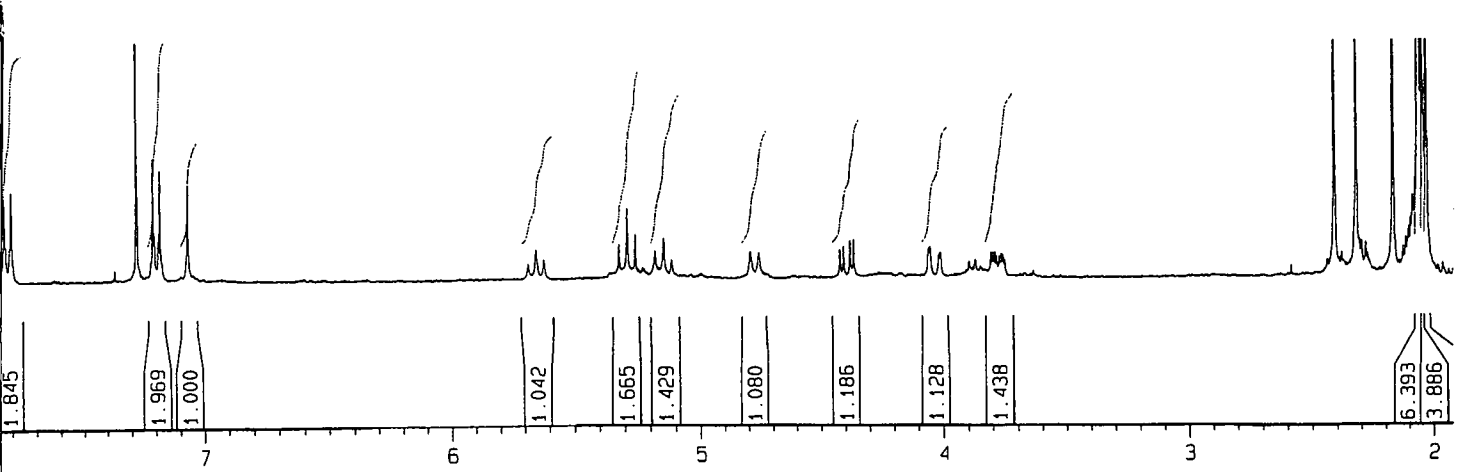
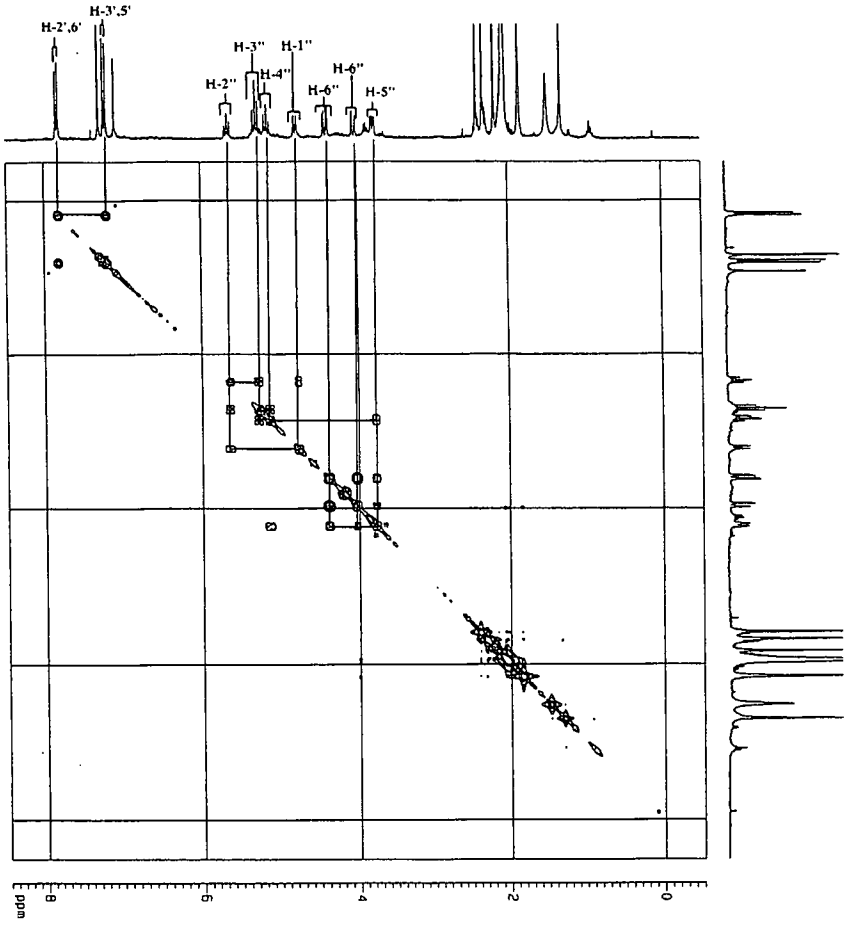


Plate 17 (CDCl3 333K)
(COSY17a-1)



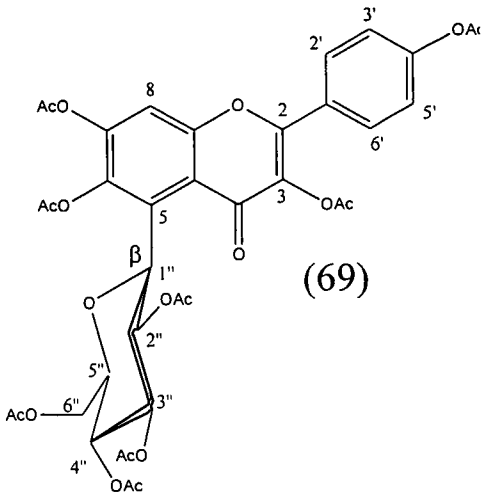


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Plate 17 (CDCl3 333K)
(COSY17a-2)

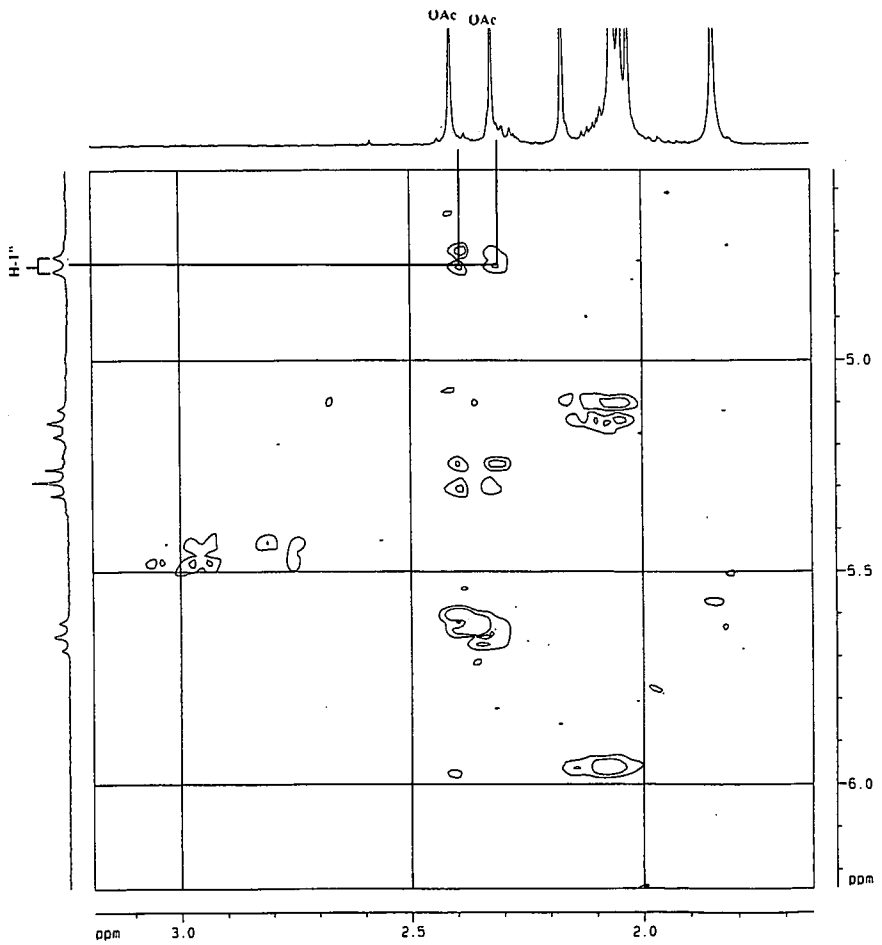
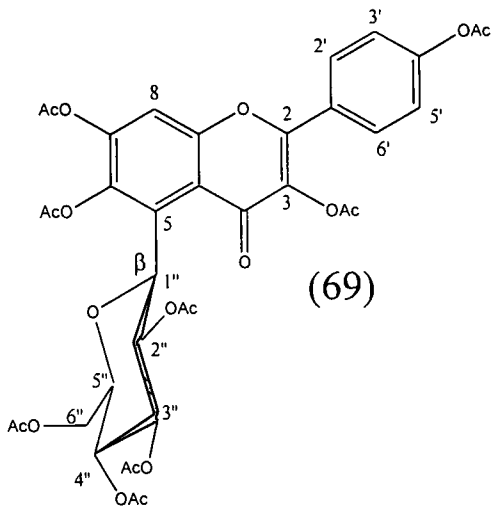


Plate 17 (DMSO d6 333K)
(HMQC 17d -1)



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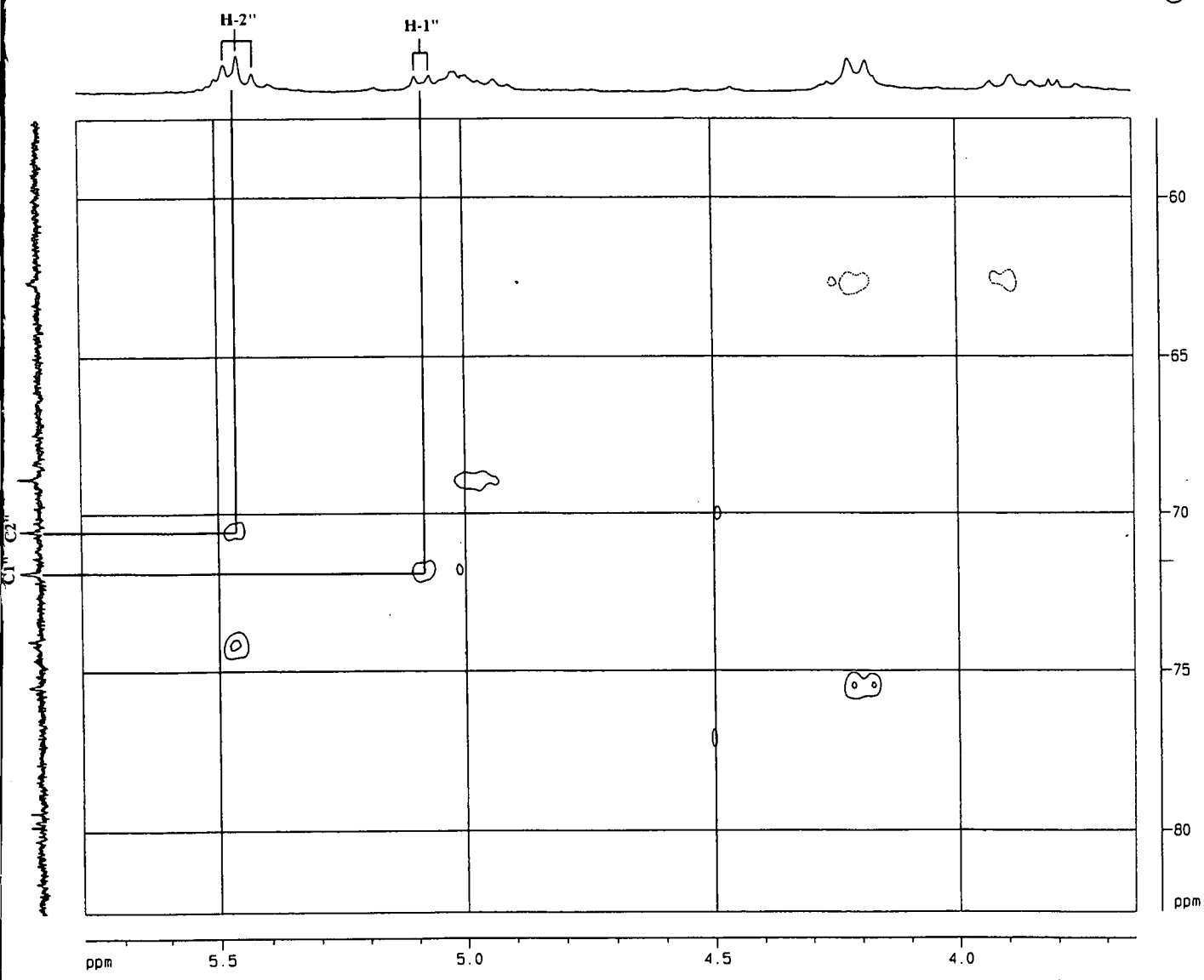
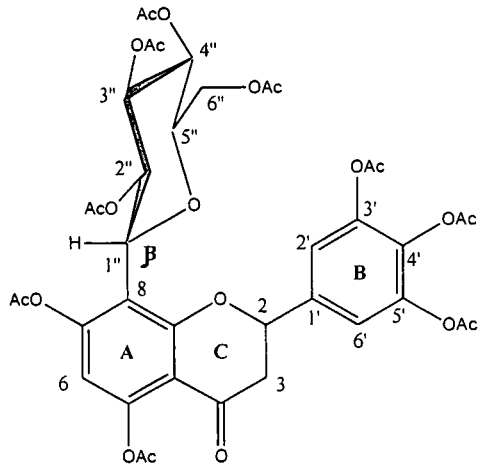


Plate 18 (CDCl3 - 343K)
(^{1}H NMR)



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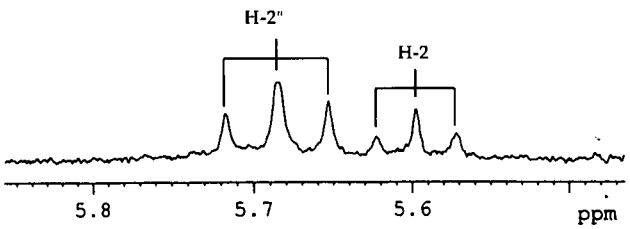
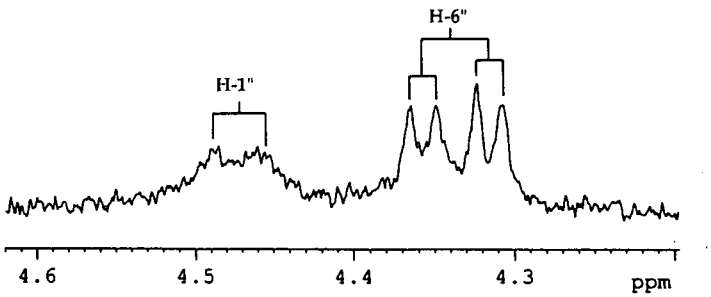
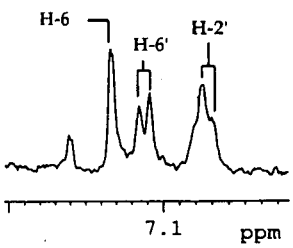
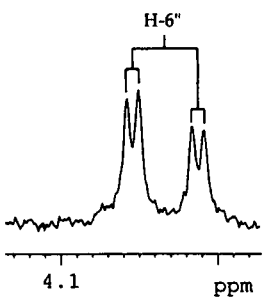
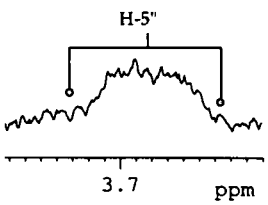
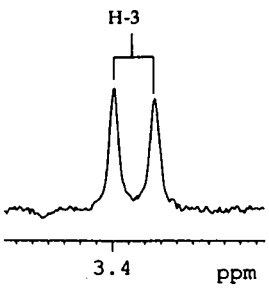


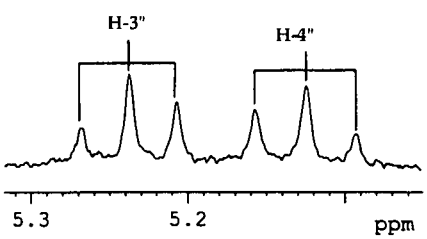


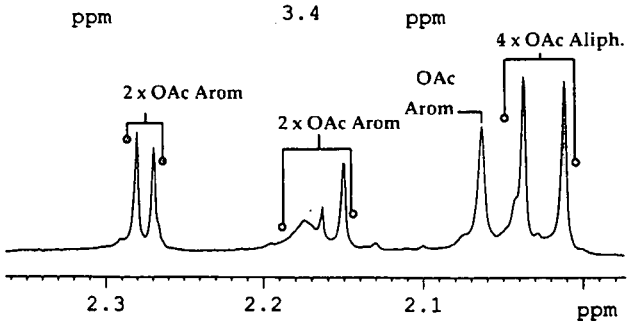












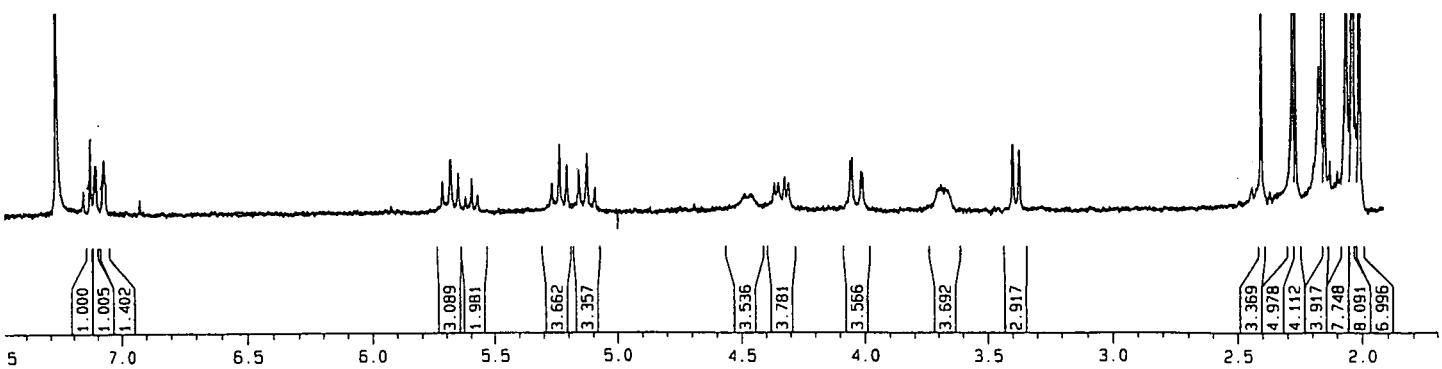
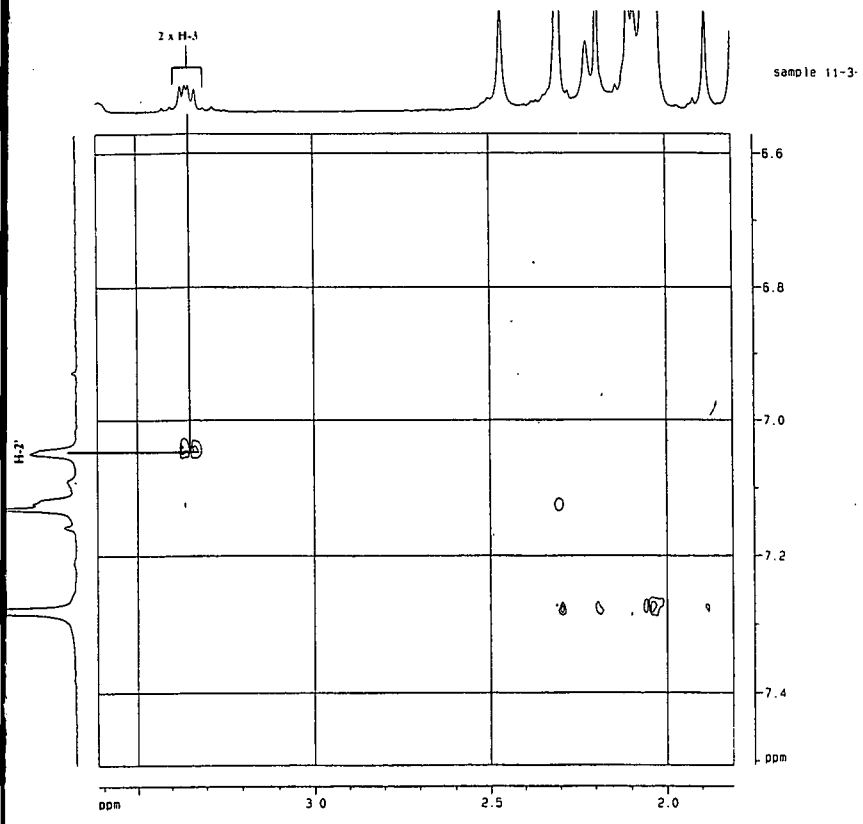


Plate 18 (CDCl3 - 298K)
(COSY 18a-1)



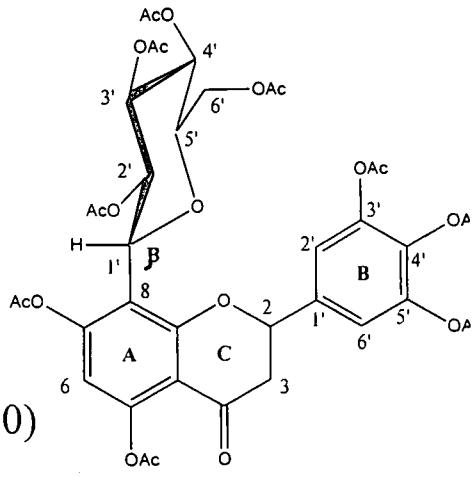


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Plate 18 (CDCl3 - 298K)
(COSY18a-2)

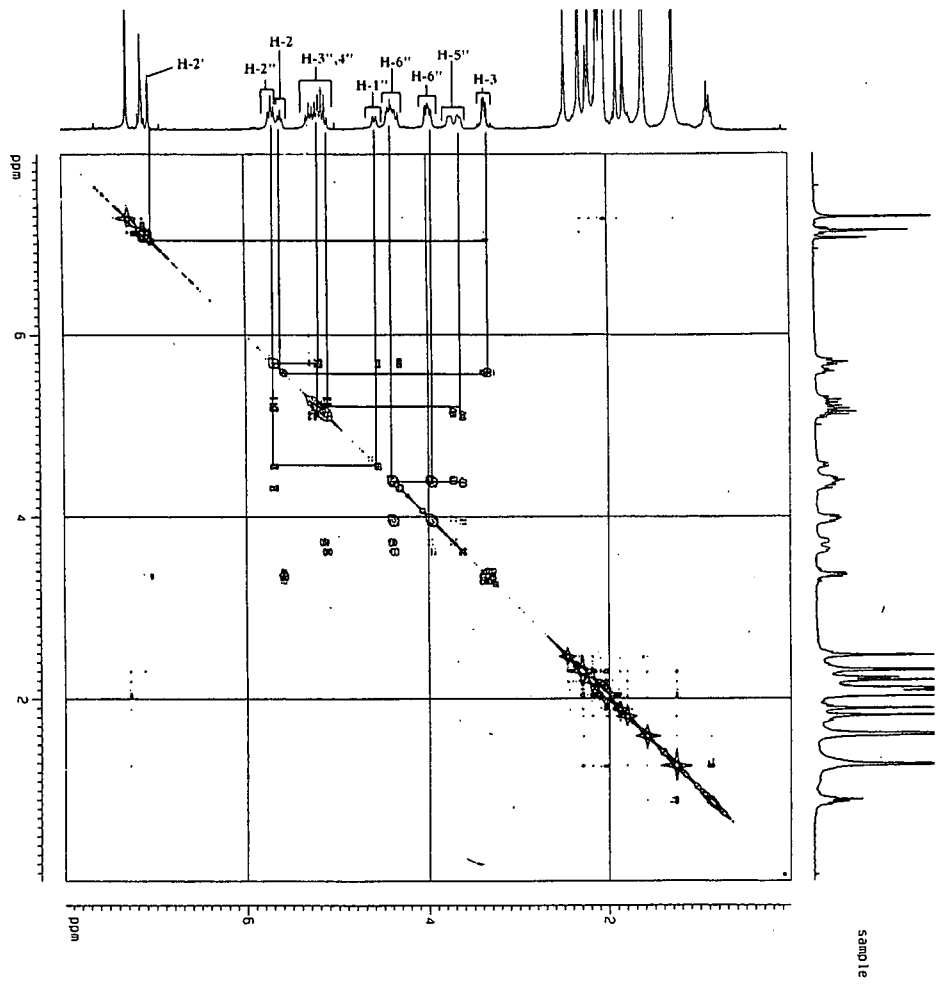
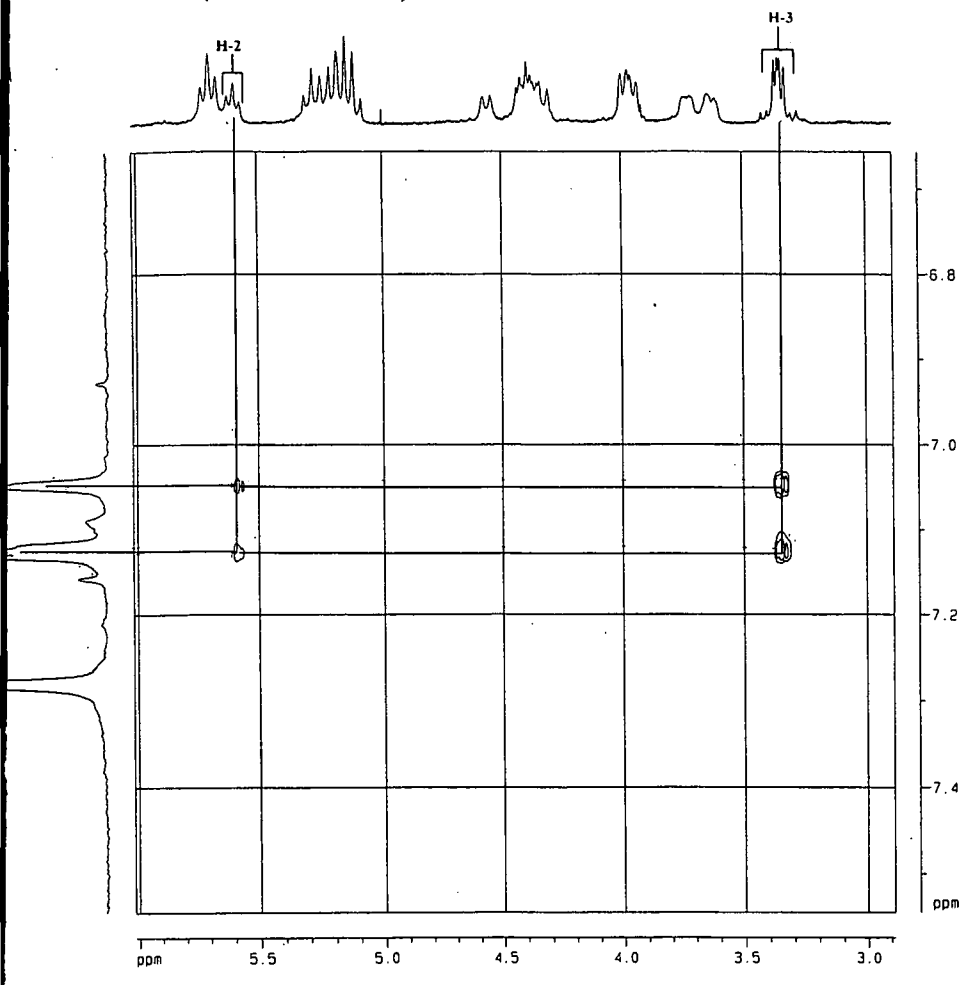
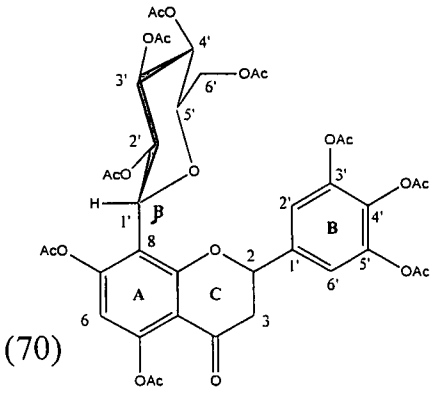


Plate 18 (CDCl3 - 298K)
(NOESY 18b-1)





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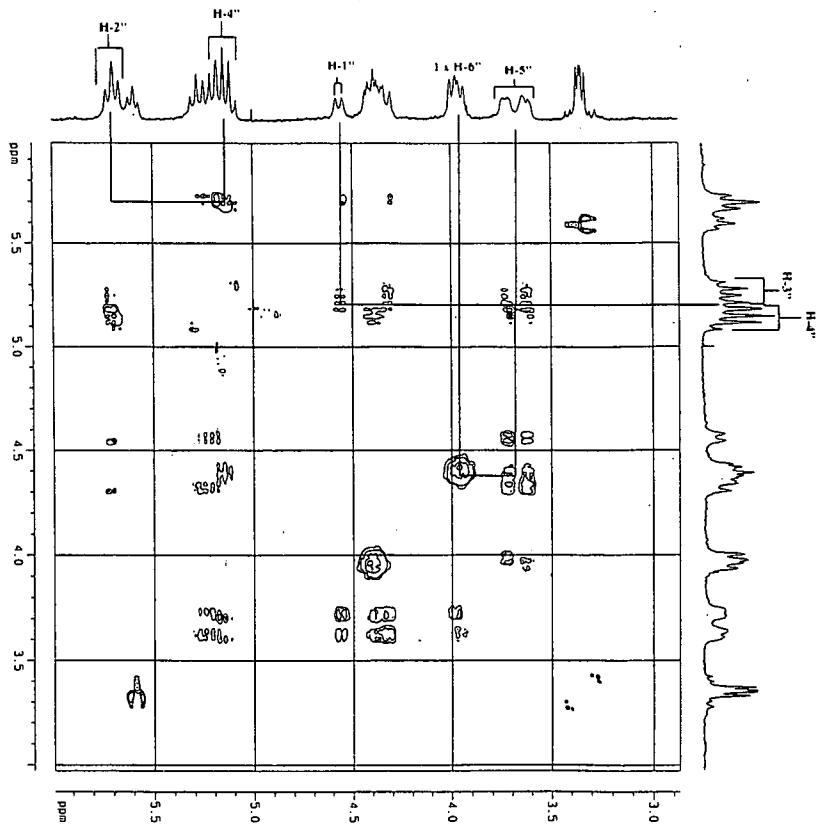
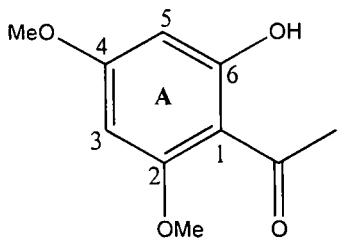
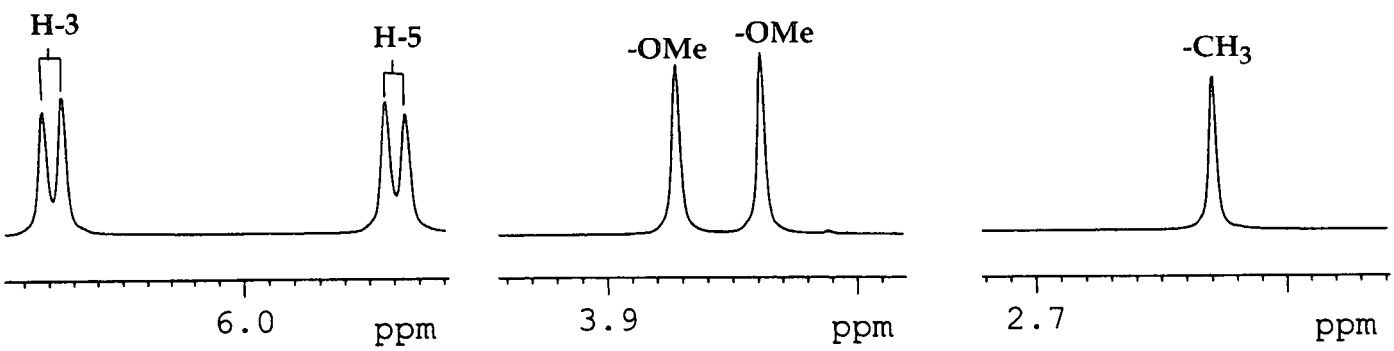
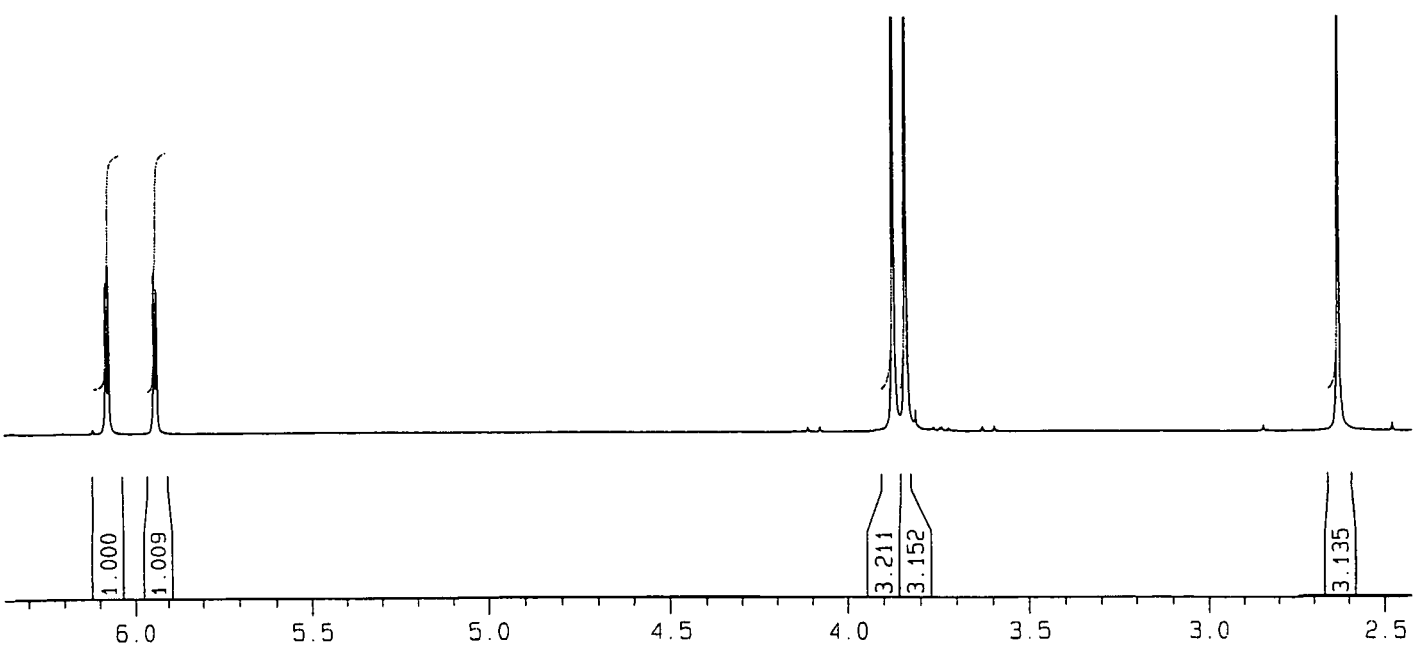


Plate 18 (CDCl3 - 298K)
(NOESY 18b-2)

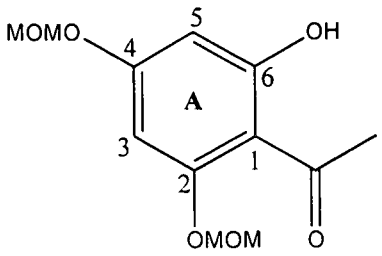
Plate 19 (CDCl3 298K)
(^{1}H NMR)

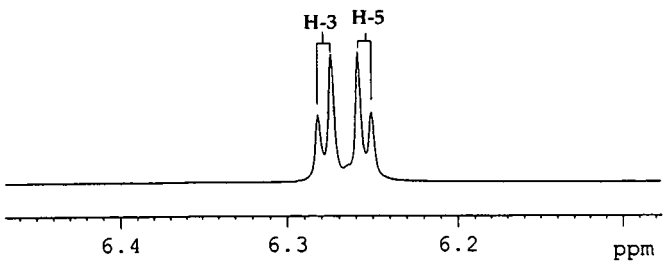


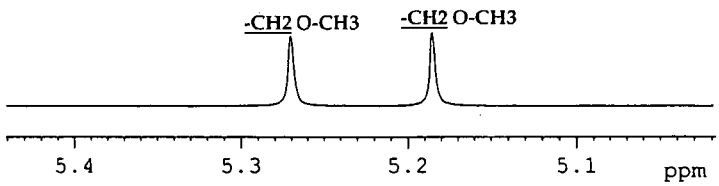


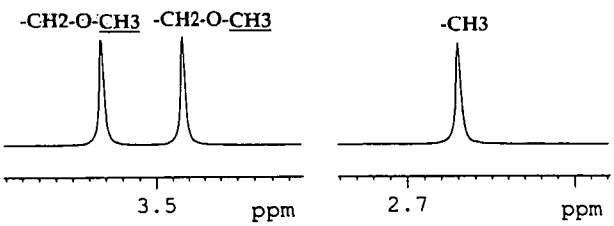


te 20 (CDCl3 298K)
(^{1}H NMR)









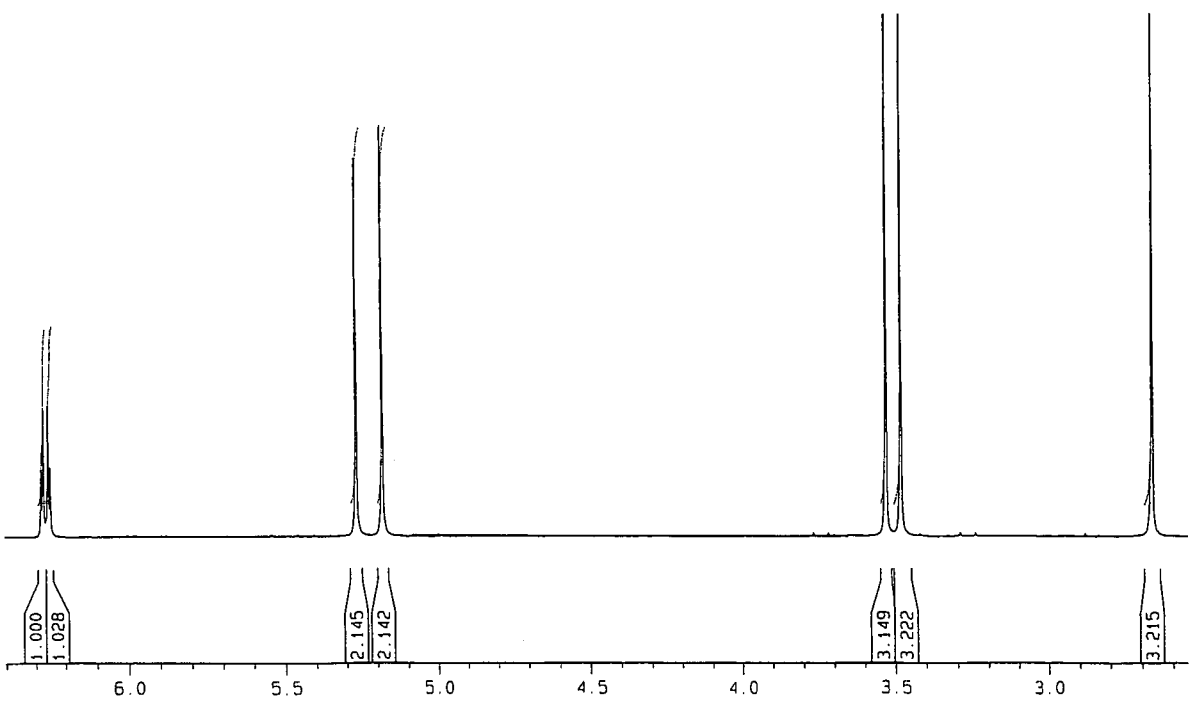
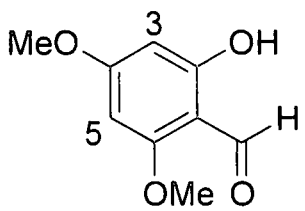


Plate 21 (CDCl3 298K)
(^{1}H NMR)



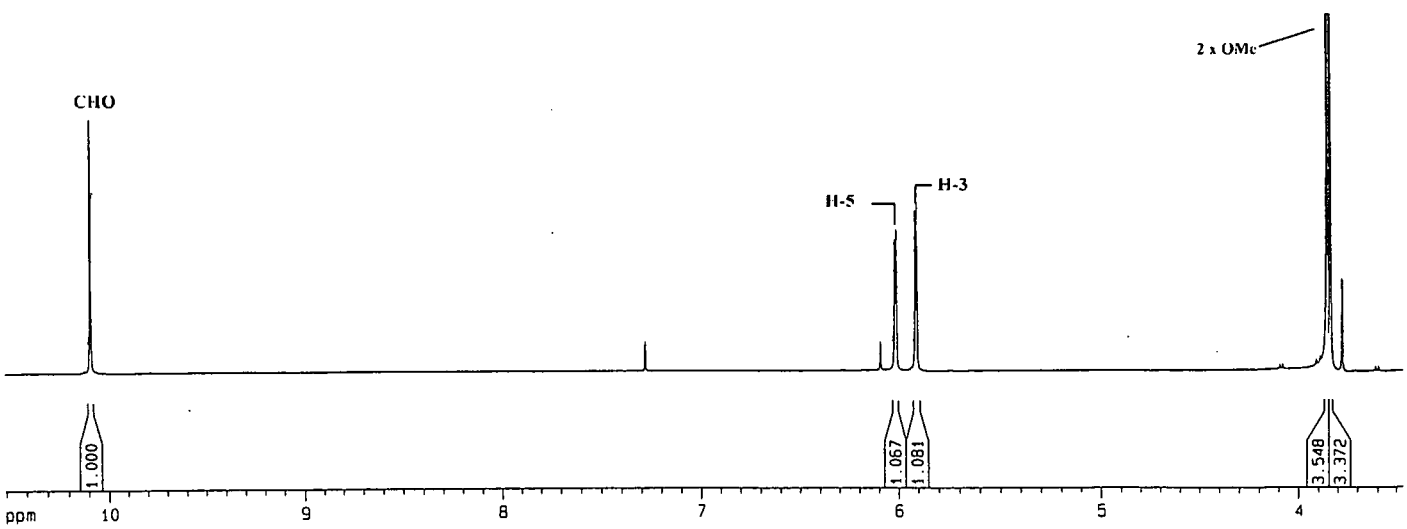
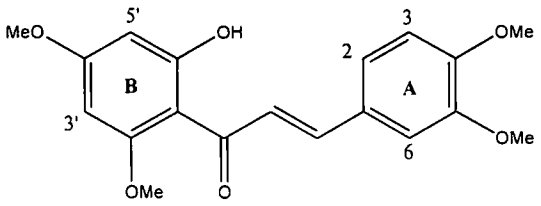
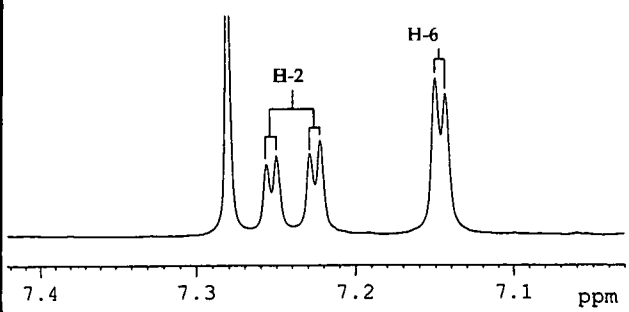
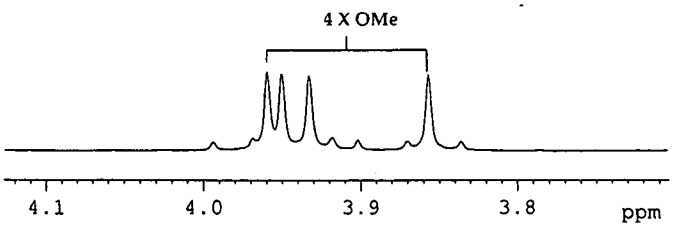
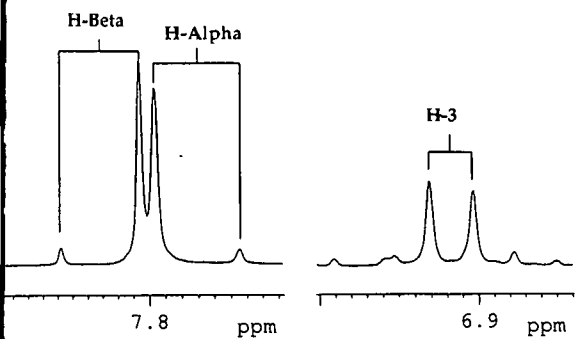


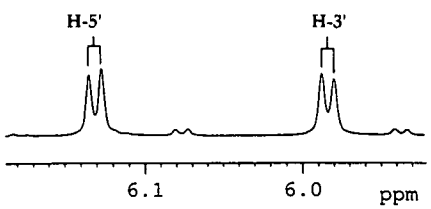
Plate 22 (CDCl3 298K)
(^{1}H NMR)











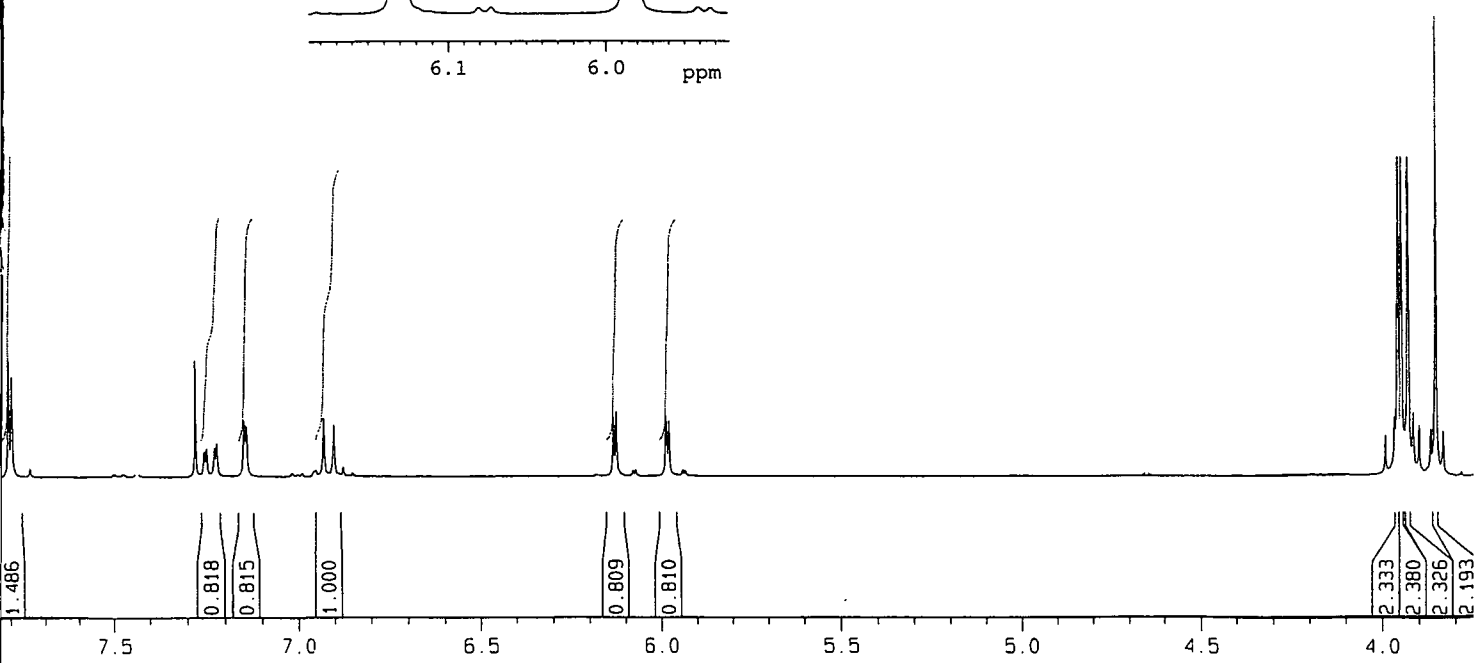
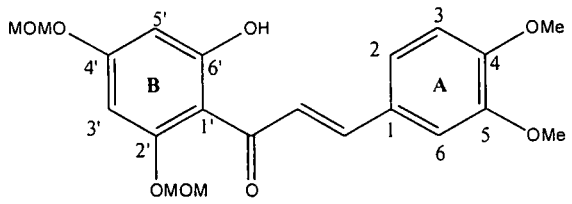
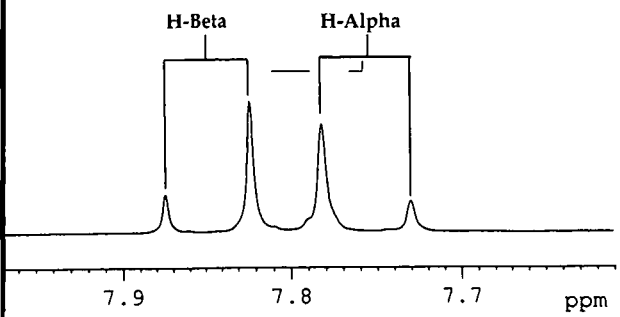
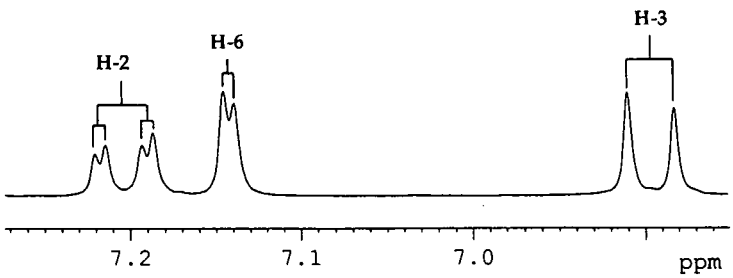
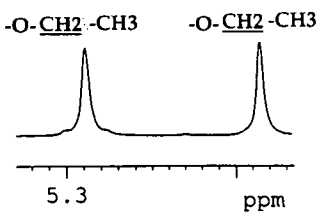


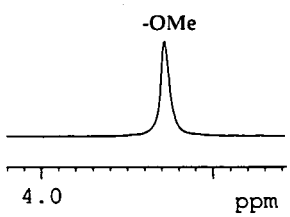
Plate 23 (CDCl3 298K)
(^{1}H NMR)

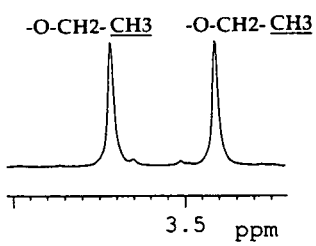


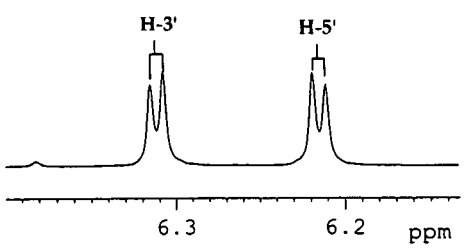












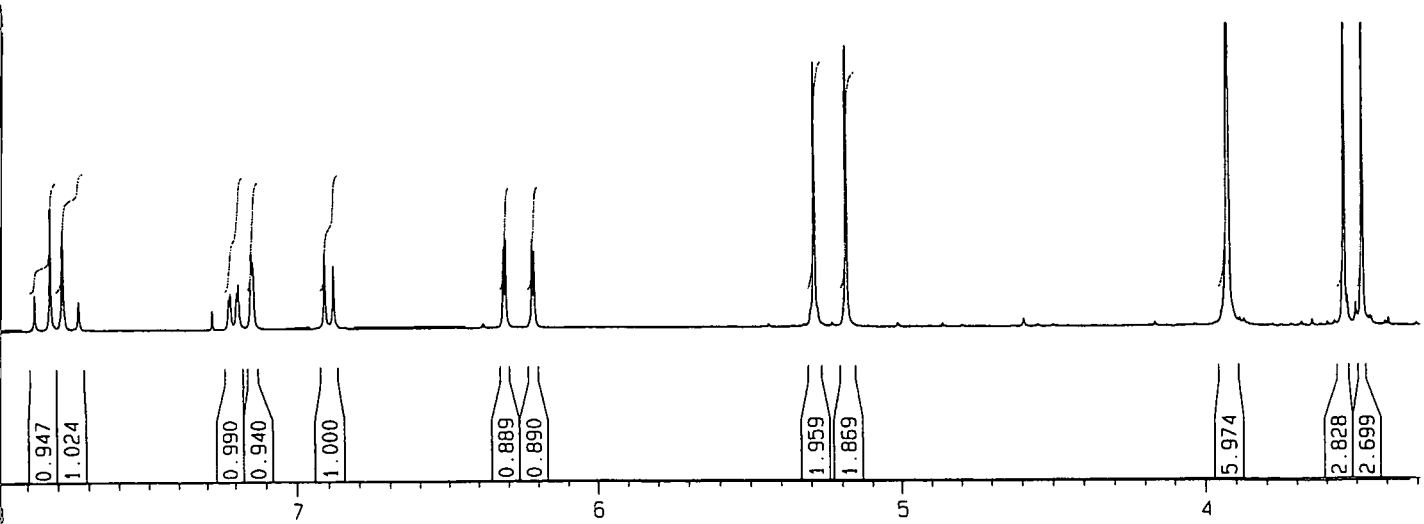
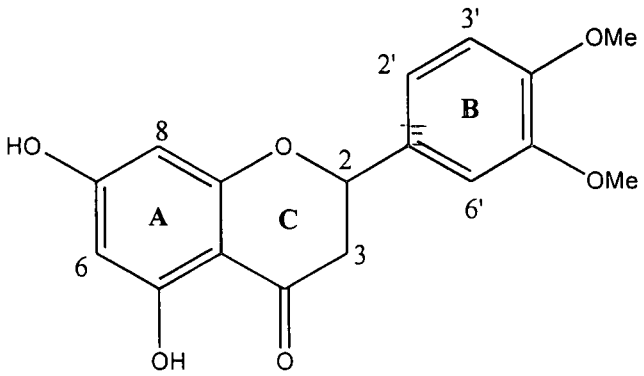
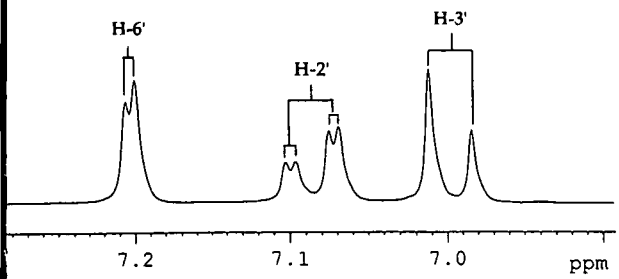
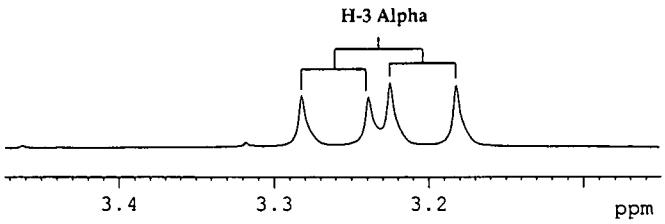
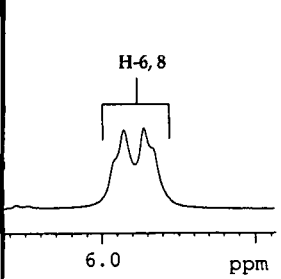


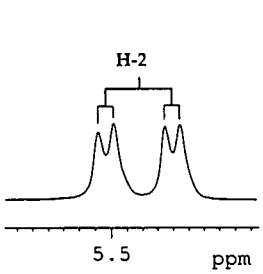
Plate 24 (CDCl3 298K)
(^{1}H NMR)

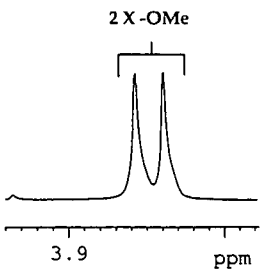


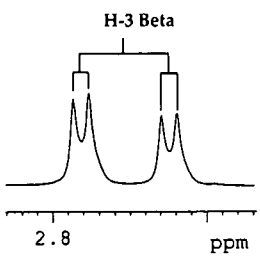


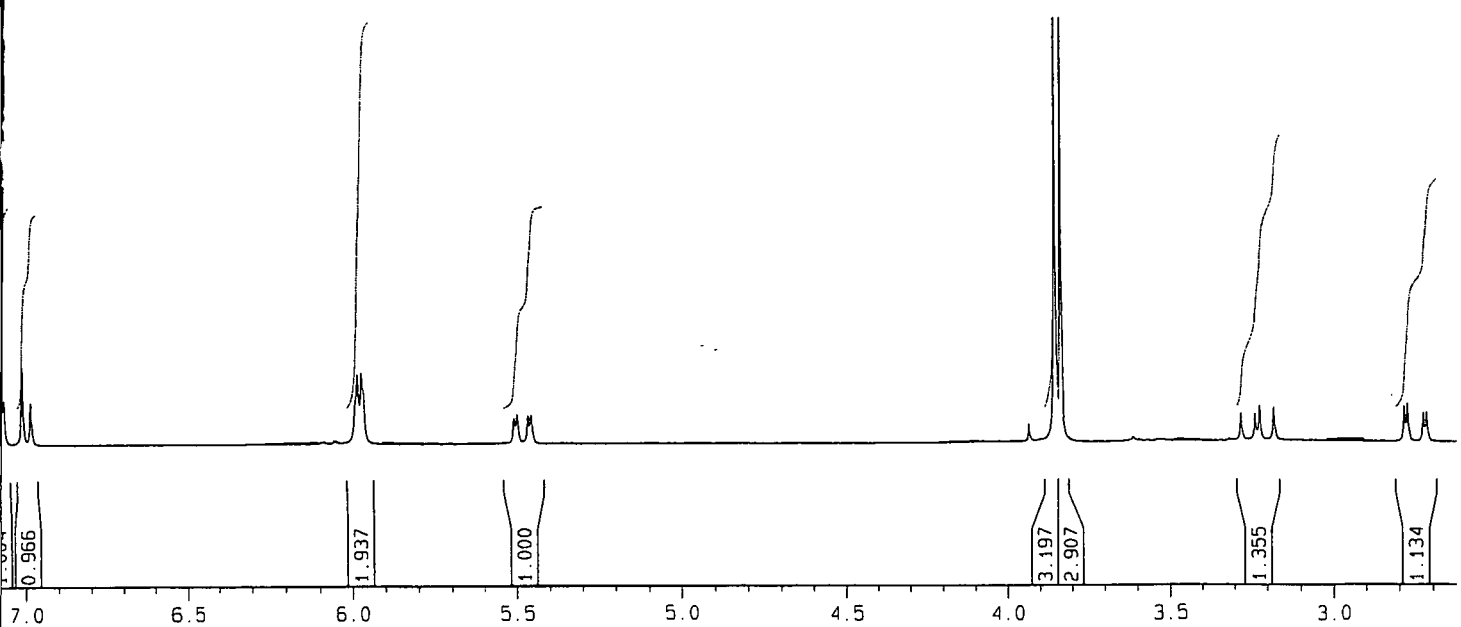




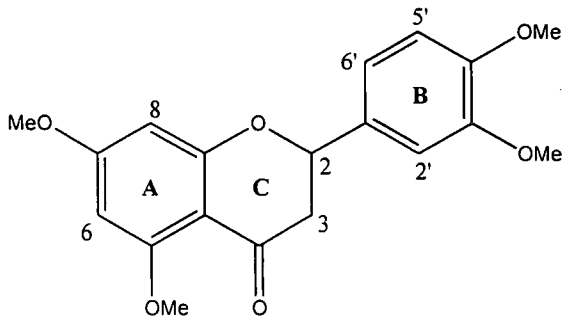


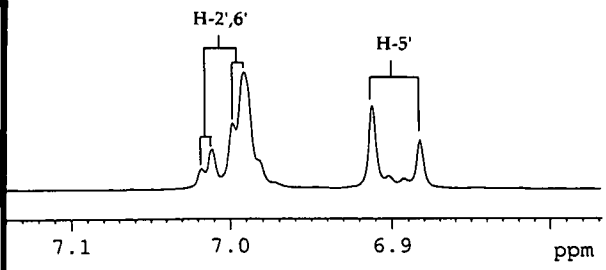


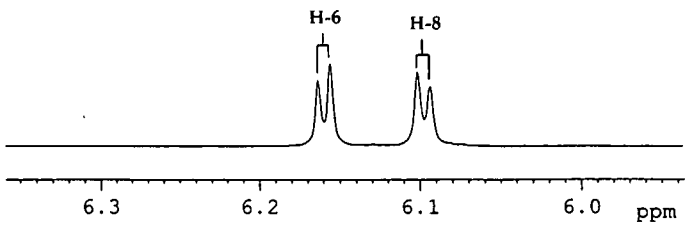


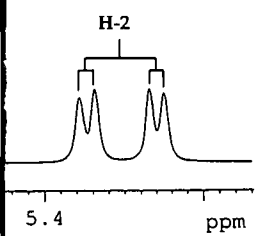


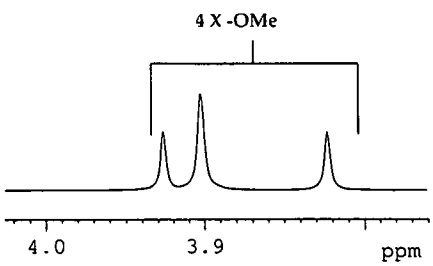
ate 25 (CDCl3 298K)
(^{1}H NMR)

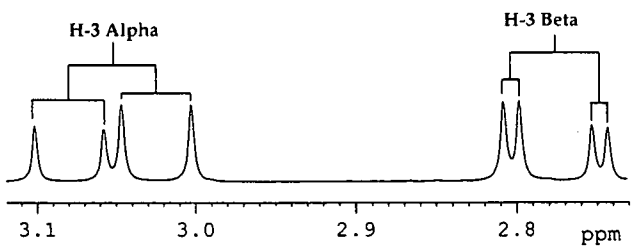


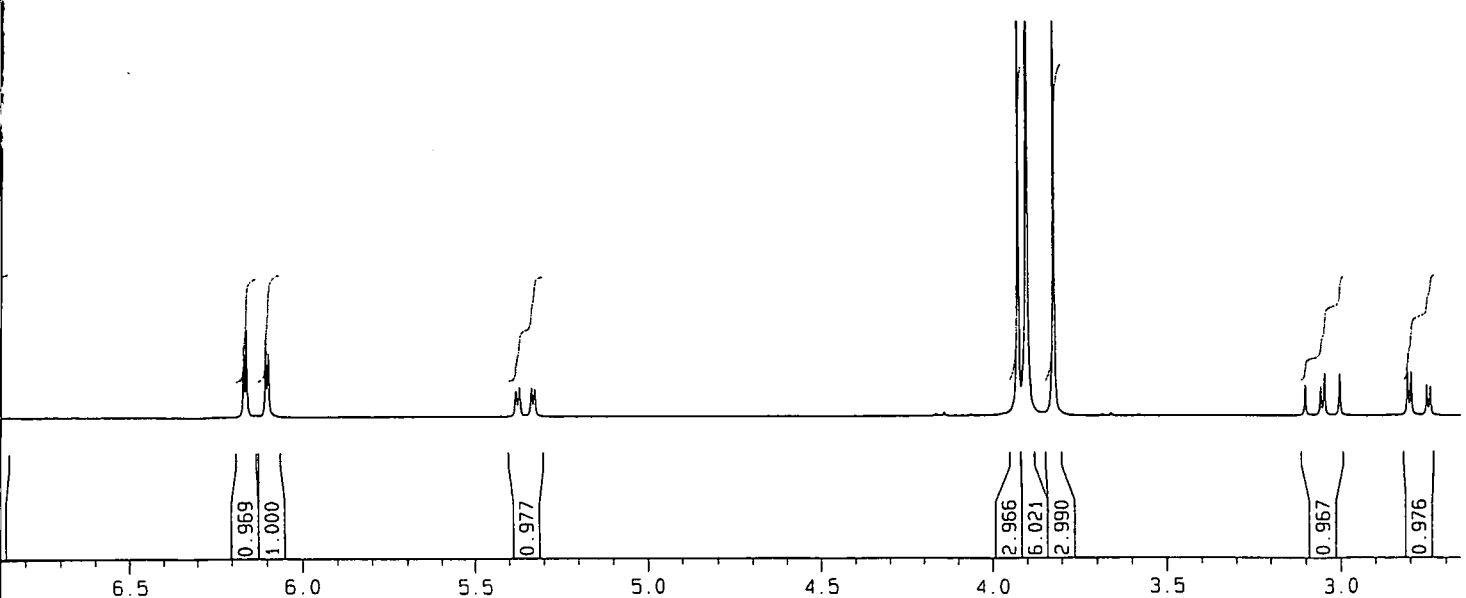




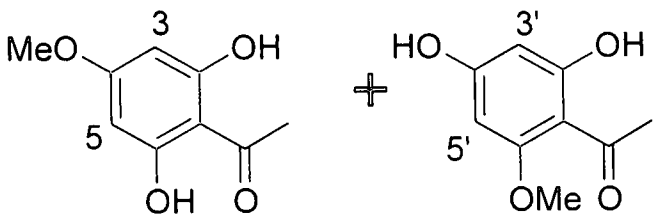








e 26 (CD6CO - 298K)
(^{1}H NMR)



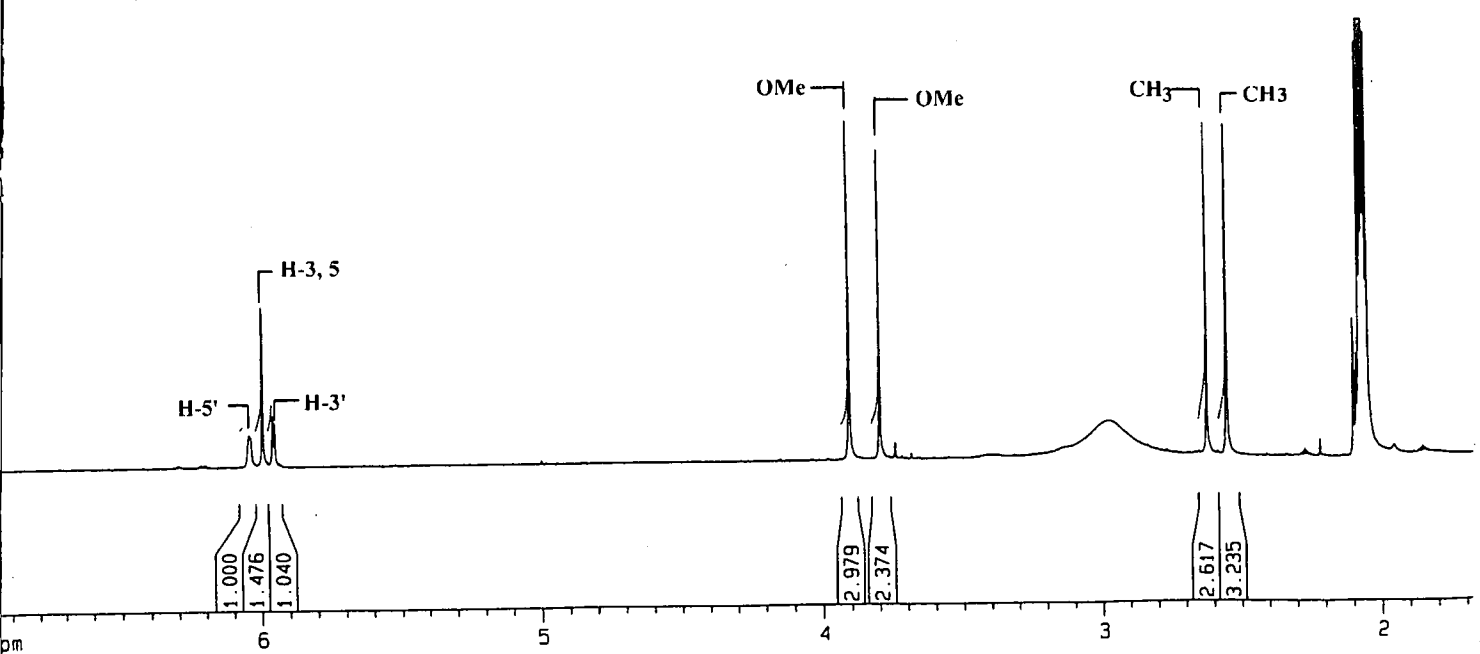
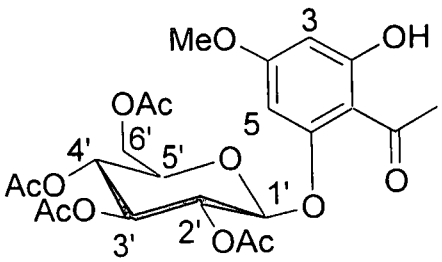
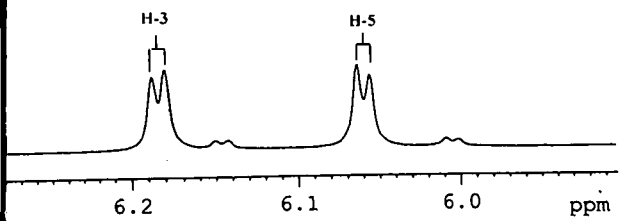
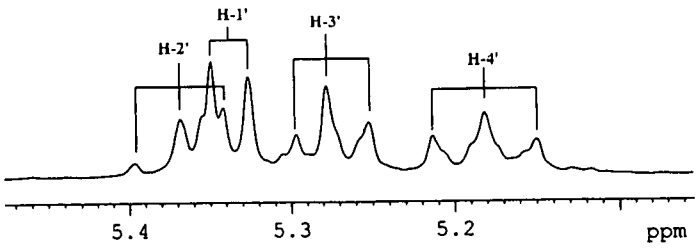
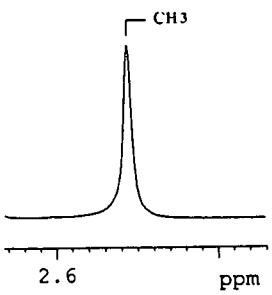


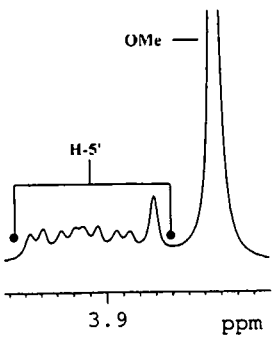
Plate 27 (CDCl3 - 298K)
(^{1}H NMR)

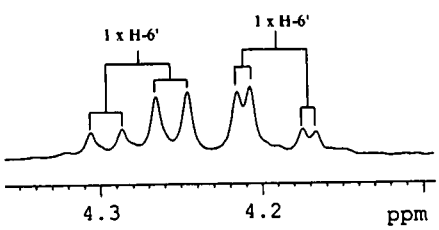


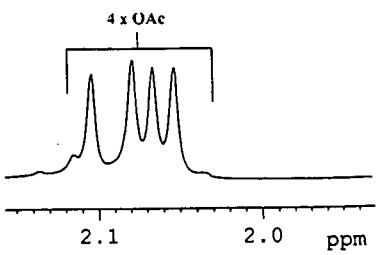


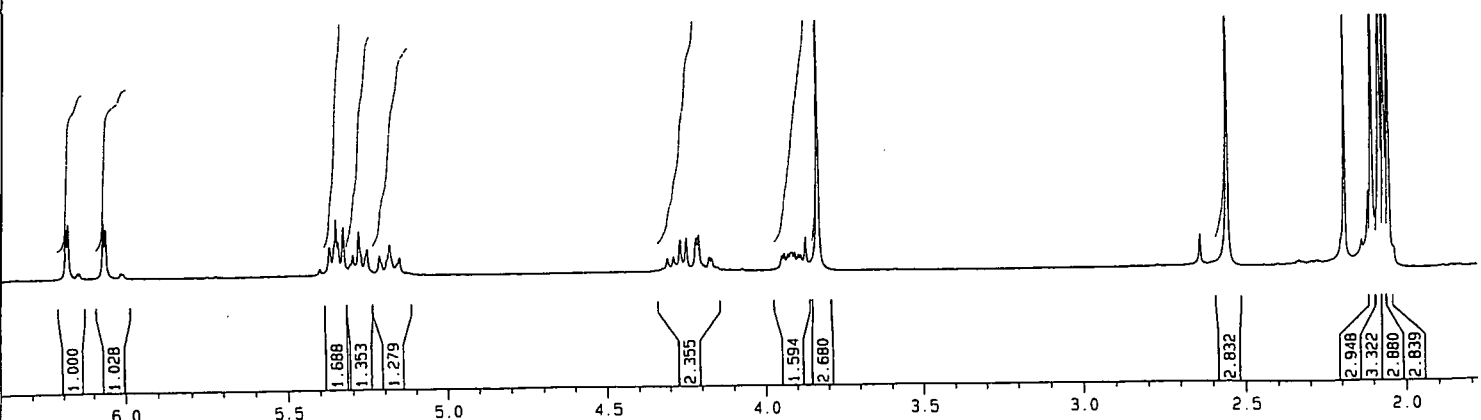




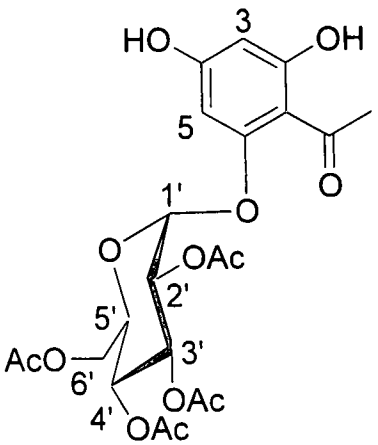








late 28 (CDCl3 - 298K)
(^{1}H NMR)



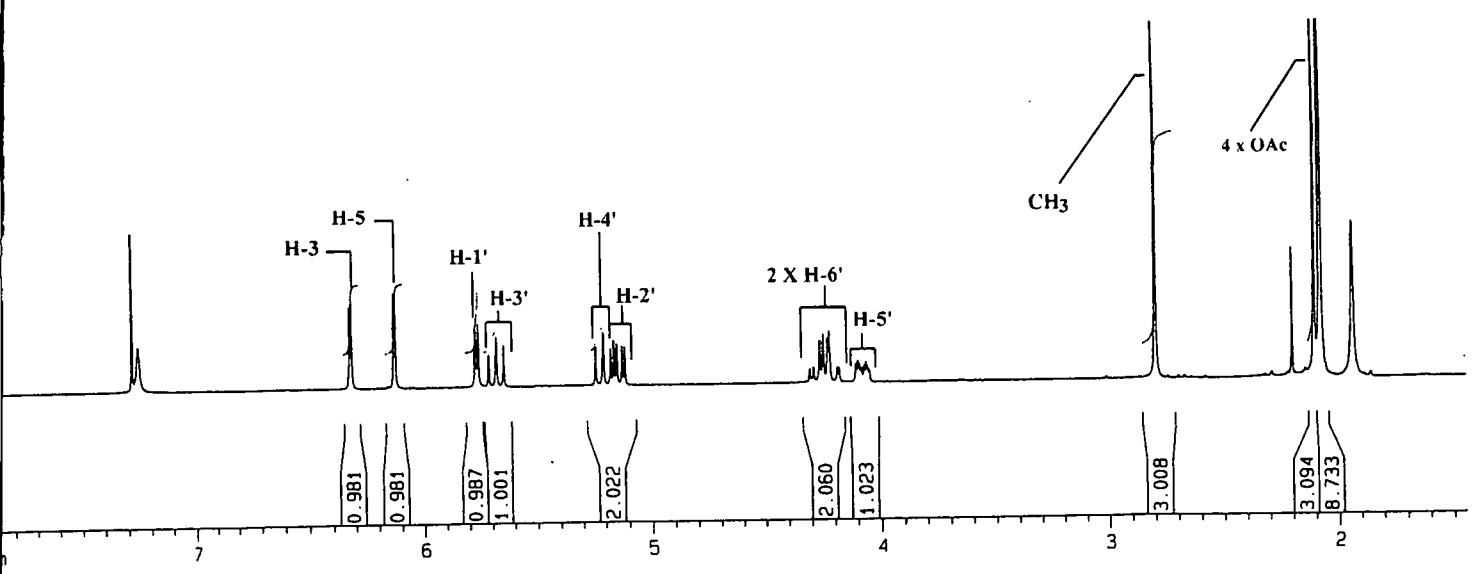


Plate 29 (CDCl3 - 298K)
(^{1}H NMR)