THE BITTER CONSTITUENT OF *GESHO* (*RHAMNUS PRINOIDES*) LEAVES

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ABSTRACT

Gesho, Rhamnus prinoides L'Herit (Rhamnaceae) is a shrub or tree which can be up to 6 m tall. It is cultivated in Ethiopia for use as an ingredient in the making of the home brewed drinks-*Telia* and *Tej*.

Phytochemical investigations of the leaves of this plant resulted in the isolation and characterization of the bitter prinicple- a naphthaenic lactone glucoside named geshoidin, in addition to three anthraquinones, a pre-anthraquinone and a flavonol. These compounds are chrysophanol (1), physcion (2), musizin (19), emodin (3), rhamnocitrin (40) and β-sorigenin-8-O-β-D-glucoside (68). Acetylated and methylated derivatives as well as hydrolysis products of the glucoside and the permethylated compound are also characterized. These include β-sorigenin-8-O-β-D-glucoside-peracetate (69), β-sorigenin-8-O-β-D-glucoside-permethylate (70), β-sorigenin (22) and β-sorigenin-1-methylether (71), respectively. Compounds **2** and **3** have been isolated previously from the fruits of *R. prinoides*. **19** and **40** have not been reported from this plant while **68** is a novel natural product.

Based on spectroscopic data, two possible explanations are offered for the upfield chemical shift of the *peri* hydroxyl proton in 68. A sensory panel concluded that compound 68 is a bitter principle in the leaves of *Gesho*. At least 1% of the dry leaves of *Gesho* contain this bitter component.

The species *C. faralaorta* is known for the 3-glucosyl-acetylrhamnosyl galactoside of the two major flavonoids: kaempferol (38) and quercetin (43) (Guinaudeau *et al.* 1981). Z. *vulgaris* and Z. *nummularia* are reported for spinosin (swertisin-2''-O-glucoside) (57) and the 3-hydroxyflavanone glucoside (taxifolin-3-glucoside) (55), (Woo, *et al.* 1979 and Srivastava, *et al.* 1977), respectively.

Most *Zizyphus* species contain cyclopeptide alkaloids. Among these, Z. *sativa* (Shah, *et al.* 1987,1988), Z. *nummularia* (Dwivedi, *et al.* 1987; Devi, *et al.* 1987), Z. *rugosa* (Tripathi, *et al.* 1989), Z. *vulgaris* (Han, *et al.* 1990), Z. *spina-christi* (AbdeLGalil and EL-Jissry, 1991) and Z. *lotus* (Ghedria, *et al.* 1993) have yielded different cyclopeptide alkaloids. A cyclopeptide alkaloid called frangulanine (Schmidt, *et al.* 1991) and three cyclopeptide alkaloids, *viz.* aralionin A (Tschesche, *et al.*1969), aralionin B (Tschesche, *et al.* 1970) and aralionin C (Tschesche, *et al.* 1977) have been isolated from *R. frangula* and *A. vaginata*, respectively.

The occurrence of a biflavonoid in the Rhamnaceae is reported from *Phyllogeiton* only, which contains a compound known as zeyherin (Geiger and Quinn, 1975). No isoflavonoid structure has been reported from this family.

1.2 The genus *Rhamnus*

Taxa belonging to the genus *Rhamnus* are found in all tropical, subtropical and temperate regions. There are about 150 taxa in this genus and only two, *viz. R. prinoides* and *R. staddo* occur in Ethiopia. *R. staddo*, like *R. prinoides*, is used to make the traditional drinks of *Tej* and *Telia*, but it is not recommended for *Telia* although it is sometimes used.

One feature of the genus is its tendency to elaborate anthraquinone and some flavonoid glycosides in addition to a variety of anthrones, anthraquinones and their dimeric derivatives. Flavonoids appear in few of the *Rhamnus* species where kaempferol, quercetin and their derivatives are the main aglycones. These exist mainly as glycosides in which glucose alone or glucose, rhamnose and galactose as a trioside, are the sugars most commonly observed. Flavonol triosides are reported from *R. petiolaris* (Wagner, *et al.* 1974), *R. nakaharia* (Lin, *et al.* 1982, 1994), *R. catharticus*, *R. alaternus*, *R. saxatilis* (Riess-Maurer and Wagner, 1982), *R. formosana* (Lin, *et al.* 1991) and *R. thymifolius* (Satake, *et al.* 1993). The synthesis of rhamnocitrin (40), rhamnazin (46) and rhamnetin (44) glycosides, particularly the 3-Otriosides, and nC NMR spectroscopic investigations of these compounds proved that all naturally occurring flavonol triosides so far isolated from different *Rhamnus* species, contain the sugar-moiety rhamninose (Riess-Maurer and Wagner, 1982).

1.3 *Rhamnus pnnoides*

R. prinoides L'Herit; Amharic name: *Gesho*, family Rhamnaceae, order Rhamnales, is a dicotylendenous angiospermic plant cultivated in Ethiopia. It is a shrub or tree which grows up to 6 meters and is also known to occur in Cameroon, Sudan, throughout East Africa to South Africa and Angola, and also in Arabia (Thulin, 1988). In Ethiopia, the plant is used to add flavour to the fermented drinks *Telia* and *Tej*,

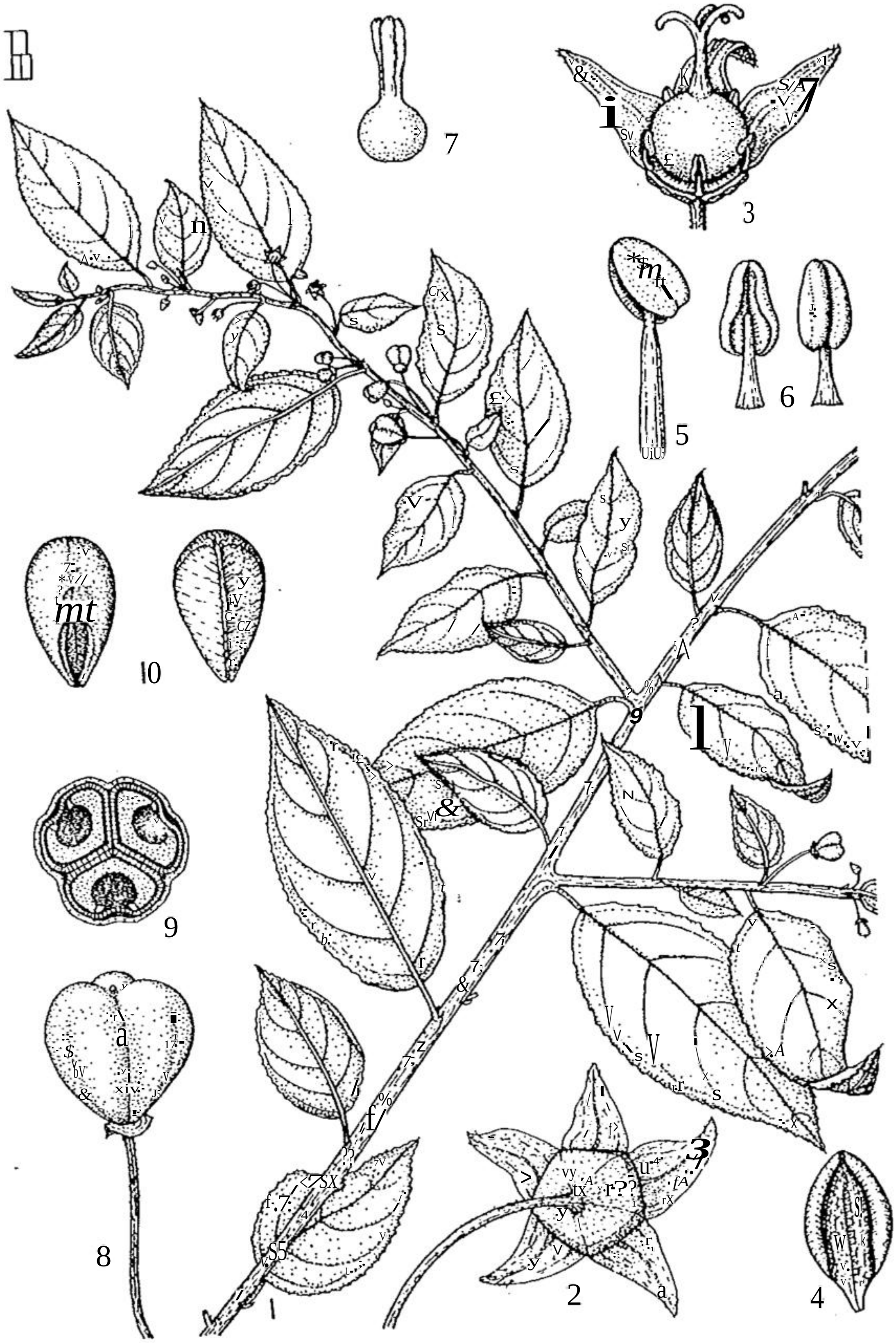
One of the early scientific reports on *R. prinoides* is that of Salgues (1962) and Coady (1965). Salgues described the presence of inorganic cations, organic acids and the flavonoid derivative rhamnetin rhamnoside (51). He also made some toxicological studies on the plant tissues.

The fungistatic effect of the extracts of *R. prinoides* fruits were investigated (Biftu, *et at.* 1979) and the minimum concentration responsible for this effect was reported.

The secondary metabolites of *R. prinoides* fruits were investigated and the presence of the known anthracene derivatives: physcion, emodin, emodin anthrone, emodin bianthrone and a new anthrone rhamnoside derivative, named prinoidin (32), were reported (Abegaz and Dagne 1988).

1.4 Bitter Sources in *Telia* and Other Substances

It is assumed that *Gesho* (*R. prinoides*) maintains acidic pH during *Telia* fermentation so as to modify the nature of the mash and inhibits the growth of undesirable micro-organisms (Kleyn and Hough, 1971). *Telia* (a malt beverage like beer) on an average contains three parts (by volume) of powdered *Gesho* leaves (and/or stem), four



RHAMNUS PRINOIDES: 1 - branch x 2/3; 2 - flower viewed from beneath x 6; 3 - flower with 2 sepals removed x 10; 4 - petal x 20; 5 - long stamen x 20; 6 - short stamen, back and front views x 20; 7 - ovary x 20; 8 - fruit x 4; 9 - transverse section of fruit x 4; 10 - stone, two views x 4. 1-3 from Wright 234; 4 from *Stolz* 451; 5 & 7 from *Glover* et al. 1602; 6, 8-10 from *Tweedie* 60/458. (Reproduced with permission from *Fl. Trop. E. Afr.*, Rhamnaceae: fig. 6, 1972.)

FIGURE 1: SKETCH OF GESHO PLANT PARTS

parts (by volume) of powdered *Bikil* (dried germinated seeds of barley, *Hordium vulgare* or wheat, *Triticwn durum*), an amount of maize (*Zea mays*) or barley or brown teff (*Eragostis tef*), or a mixture of the three, roughly equivalent (by volume) to two to three times that of *Bikil*, all combined and made into cakes; an amount of maize or barley roughly equivalent (by volume of powder) to three to four times that of *Bikil*, roasted, powdered, and granulated with water on a heated pan (Desta, 1977). Each part of the ingredient is added to the starter (water-G&s/?o mixture) at different stages taking up to a total of eight to ten fermenting days, and varying amount of water is added to the final mixture and left sealed for a final fermentation and settling.

The other home brewed fermented beverage in which *Gesho* is used is *Tej*, It is a yellow to olive-green, alcoholic beverage similar to mead. *Tej* uses not the leaves, but *Gesho* stems only. The alcoholic contents (% v/v) of *Telia* and *Tej* are 5.65-6.56 and 13.18-13.73, respectively (Desta, 1977).

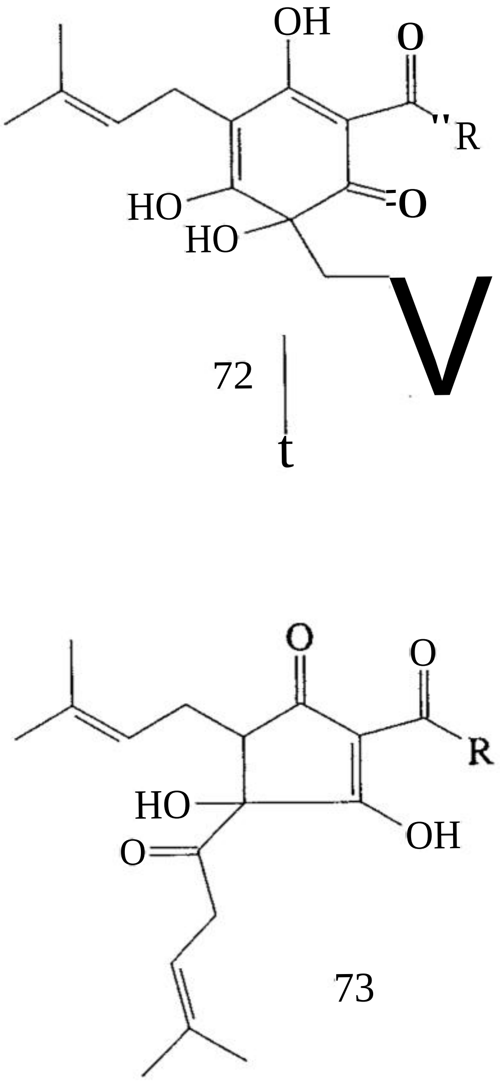
Gesho imparts a bitter taste to *Telia* and *Tej* and the bitterness of *Telia* is directly related to the amount of *Gesho* added during brewing (Sahle and Abegaz, 1991). But apart from such general conclusions, no attempt has been made to isolate and chemically characterize the bitter principle(s) in *Gesho* prior to this work.

The present work had two major thrusts. The first involves isolation and characterization of the compound responsible for the bitterness of *Gesho* and the second part requires conducting a sensory analysis to establish this compound as the real bitter principle of *Gesho*.

A sensory analysis is a multidisciplinary science that uses human panelists and their senses of sight, smell, taste, touch and hearing to measure the sensory characteristics and acceptability of food products, as well as many other materials (Watts, *et al*. 1989). There is no one instrument that can replicate or replace the human response. Thus, the testing instrument for sensory analysis is the panel of human judges who have been recruited and/or trained to carry out specific tasks of sensory evaluation. Hence both the above tasks have to be fulfilled to establish the identity of the bitter principle of *Gesho*.

On the other hand, the industrial beer, which uses hops (*Humulus lupulus*) (Ellis, 1982) for the particular effect has a phioroglucinol skeleton in the bitter component. There are six major bitter iso-a-acids in hops which have isoprenoid side chains attached to the phioroglucinol nucleus or to a cyclopentatrione ring (Scheme 1).

In the brewing of beer, hops are boiled with malt and other cereal extracts (wort) in the brew kettle for a given period. During this period, the three major a-acids (72) humulone, cohumulone and adhumulone undergo heat induced isomerization into iso-a-acids (73) (Raphael, 1991).



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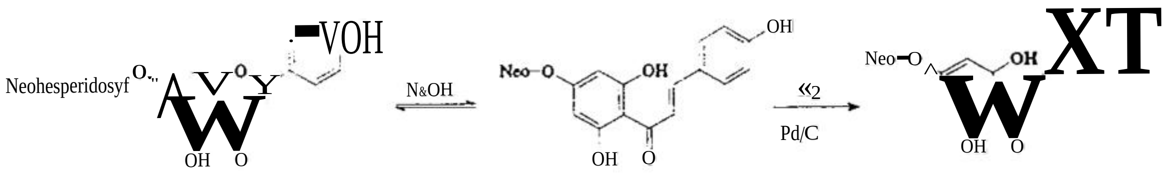
R = $CH_2CH(CH)_3$: humulone
R = $CH(CH_3)_2$: cohumulone
R = $CH(CH_3)CH_2CH_3$: adhumulone

Scheme 1: The bitter iso-a-acids in beer

Bitter principles in some fruits are also known. For example, the bitter compound neohesperidin (63) occurs in the bitter orange, *Citrus aurantiurn*. The isomer hesperidin (62), is a non-bitter compound which is the predominant flavonoid in lemon and the ordinary sweet orange, *Citrus sinesis*.

Grapefruit acquires bitterness from naringin (74). It can be converted to the non-bitter naringenin (64) by adding a hydrolytic enzyme (naringinase) to grapefruit juice. On the other hand, if the bitter naringin is treated with NaOH and then reduced catalytically (Scheme 2), naringin dihydrochalcone (75), which is about 1000 times sweeter than sucrose (Raphael, 1991), can be obtained.



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75

Scheme 2: Conversion of the bitter naringin into a sweetening agent

1.5 Anthraquinones and Related Compounds of the Rhamnaceae

A. Occurrence and Distribution

Anthraquinones are the largest group of natural quinones. They are found in higher plants and also occur in animals, particularly in insects (*Coccidae*) and feather stars (*Crinoidea*). In higher plants, anthraquinones are located chiefly in heart wood, bark and roots (often as glycosides), occasionally in stems, seeds and fruit. The anthracene derivatives, chrysophanol, physcion and emodin, are very common and appear in almost all anthraquinone containing plants of Rhamnaceae.

Of the two species of *Araliorhamnus*, only *A. vaginata* has been investigated and this contributed 24 new anthraquinones and related compounds to the family. Anthrones in the Rhamnaceae are distributed in *R. purshianus*, *R. frangula* and *R. prinoides*. The only reported anthrones are chrysophanol, physcion (34), aloe-emodin (28), and emodin anthrones (33).

Bianthrones, on the other hand, are reported from five species of the family, *viz*. *R. purshianus*, *R. frangula*, *R. prinoides*, *K. humboUltinna* and *A. vaginata*. Physcion

10,10'-bianthrone and karwinskia toxins 1 (58), 2, 3 and 4 are the only reported bianthrones from *A. vaginara* (Mammo, 1989) and *K. humbokitiana* (Dreyer, *et al.* 1975), respectively.

Of the 37 naphthoquinones (benzoisochroman quinones included) isolated and characterized from the Rhamnaceae, 29 occur in the four *Venrilago* species: *V. maderaspatana*, *V. calyculata*, *V. goughii*, and *V. viminalis*, while 7-methoxyeleutherin and its 6-hydroxy derivative appear in *K. humboldtiana* only. 2-Methoxystypandrone (53) has been isolated from *A. vaginara*, *V. calyculata* and *R. fallax*. The remaining five naphthoquinone monomers and dimers are reported from *A. vaginata*.

There are a total of 19 naphthalene derivatives among which 6 are glycosides. Musizin has been isolated from *M. eminii*, *R. procumbens* and *R. wightii* whereas nakahalene (20) and nakahalene-8-O-primeveroside occur in *R. nakaharia*.

The naphthalene lactones α- and β-sorigenin (21, 22) (Haber, *et al.* 1956) together with the primeverosides α- and β-sorinin (23, 24) (Hegnauer, 1973) have been isolated from *R. japonicus*. α-Sorigenin-8-O-β-D-glucoside (26) has been reported (Rauwald and Just, 1983) and a recent paper by Pepaila, *et al.* (1991) revealed that β-sorigenin-1-O-β-D-glucoside (25) is present in *R. wightii*. The occurrence of 1- and 8-O-β-D-glucosides of α- and β-sorigenin as natural products, respectively, have not been reported prior to this work.

There are three monoaromatic compounds isolated from Rhamnaceae. These are 2,6-dihydroxy-4-methoxyacetophenone 2-O-β-rutinoside (66) which was isolated together with a new naphthalide glycoside: 8,9-dihydroxy-6-methoxynaphthalide 8-O-β-rutinoside (α-sorigenin 8-O-rutinoside) (Satake, *et al.* 1991) and 7-hydroxy-5-methoxyphthalide 7-O-β-D-glucoside (67) (Jan, *et al.* 1973).

To date, 144 anthraquinones and related compounds have been reported from the Rhamnaceae. Their distributions according to structural type is given in Table 1.

Table 1. Anthraquinones and related compounds from Rhamnaceae

Structural type	Up to 1988	1989	1990-94	Total
Athraquinones	26	16	-	42
Anthrones	4	-	-	4
Bianthrones	14	1	-	15
Naphthoquinones	27	5	5	37
Naphthalenes	11 (2)*	2	6 (4)*	19(6)*
Anthraquinone gly.	20	-	4	24
Monoaromatics	-	-	3	3
Total	102 (2)*	24	18 (4)*	144 (6)*

* Number indicates glycosides.

B. Identification of Anthraquinones

1. Color Reactions

Anthraquinones are detected on chromatographic plates by their visible and UV colors. By spraying plates with 10% methanolic KOH, the original yellow and yellow-brown colors change to red, violet, green or purple. The reaction can be done on crude extracts, purified materials or on chromatograms.

2. Ultraviolet and Visible Spectroscopy

Spectral measurements are essential for identification of quinone structures. The UV and visible spectra indicate the class of quinones present, since the number and position of bands increase with the complexity of the structure.

The spectra of benzoquinones characteristically have one strong band between 260 and 290 nm and one band of less intensity between 375 and 410 nm. All naphthoquinones have three or four spectral maxima: one or two below 300 nm, one at about 330 to 340 nm and one above 400 nm. Anthraquinones can be distinguished from other classes of quinones by the fact that they have four or five absorption bands in the UV and visible regions. At least three of these lie between 215 and 300 nm and another one above 430 nm. The pattern in the UV region is not strongly affected by substitution. On the other hand, absorption in the visible region is influenced by the number of *alpha* hydroxyl groups. The influence of *beta* hydroxyl is much weaker except when adjacent to an *alpha* hydroxyl.

The UV-Visible spectra of 1, 8-dihydroxyanthraquinones show a peak at 430-450 nm and those of 1, 4-dihydroxyanthraquinones exhibit absorption at 470-500 nm. This is also reflected in the color of anthraquinones, where 1, 8-dihydroxyanthraquinones are yellow or orange and 1, 4-dihydroxyanthraquinones are red. Additional *alpha* hydroxylation results in a bathochromic shift of the longer wave absorption.

3. IR spectra

The carbonyl stretching vibrational frequencies are useful aids in structural determination of anthraquinones. The stretching vibration of anthraquinones having positions 9 and 10 in keto form shows a second carbonyl band at a lower frequency if they contain a hydroxyl group in the *alpha* position due to conjugation and chelation. The correlations between the carbonyl frequency range and the number of *alpha* hydroxyl groups are summarized below.

Table 2. Carbonyl frequencies of hydroxyanthraquinones (Bloom, *et. al* 1959)

Number of o-OH groups	CO (Nujol) cm^{-1}
None	1678-1653
1	1675-1647, 1637-1621
2 (1, 4- and 1, 5-)	1645-1608
2 (1, 8-)	1673-1661, 1626-1616
3	1616-1592
4	1592-1572

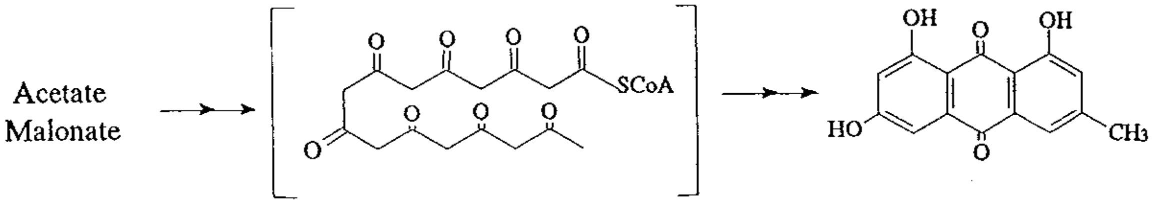
4. NMR Spectra

Chelated *alpha* hydroxyl groups (1-, 4-, 5-, 8-OH) are easily recognized as they resonate at very low field (δ 12-14 ppm). Splitting patterns and coupling constants are also useful aids for the determination of orientation of aromatic substituents by nuclear magnetic resonance. Since coupling with a *para* proton is very small, only *ortho* and *meta* couplings are considered. Anthraquinones of the chrysophanol type show *ortho-meta* (doublet of doublets) and di-*ortho* (broad triplet) splitting patterns for the protons at positions 5 or 7 and 6, respectively. They also show the *meta* multiplicity (narrow doublet) for the protons at positions 2 and 4, where the signals are broadened by the allylic coupling with the methyl protons at carbon 3. Emodin or physcion type anthraquinones show only the *meta* coupling patterns (narrow doublets).

C. The Biosynthesis of Anthraquinones in Rhamnaceae

Generally, the biosynthesis of anthraquinones is believed to follow two pathways, the acetate-malonate and the shikimate-mevalonate pathways (Manitto, 1981).

1. Acetate-malonate pathway: The aromatic polyketides are built up from a starter unit (usually acetate) and chain of malonate units formed by carboxylation of acetyl Co-enzyme A. The overwhelming majority of the anthraquinones isolated from the Rhamnaceae are of the emodin type (3) and they appear to arise by suitable folding and condensation of a polyketide chain derived from eight acetate units as shown in Scheme 3. Structural variations of this basic structure may arise from O-methylation, side chain oxidation, dimerization and the introduction or elimination of hydroxyl groups.



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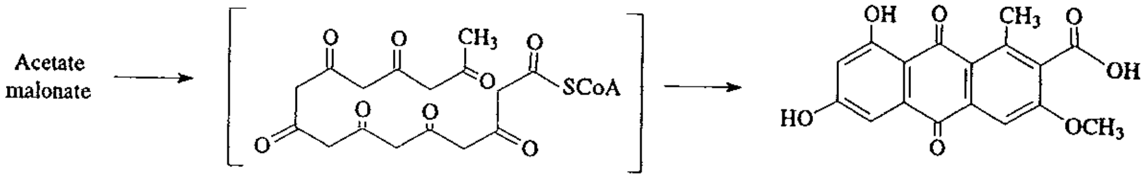
Scheme 3: The biosynthesis of emodin-type anthraquinons

2. Shikimate-mevalonate pathway: The biosynthesis of alizarin (17) is a direct consequence of this pathway. In such types, ring A is derived from shikimic acid and ring C from mevalonic acid.

Thus, based on their structure and biosynthesis, anthraquinones are classified into either the emodin or the alizarin-type. Those anthraquinones with substituents on both benzenoid rings are derived from the acetate-malonate pathway (emodin-type) while those devoid of substituents on one benzenoid ring come through the shikimate-mevalonate pathway (alizarin-type). All reported anthraquinones isolated from Rhamnaceae have substituents on both rings (A and C) and thus, the acetate-malonate pathway seems to be the most plausible biosynthetic pathway for the anthraquinones of this family.

Even though such anthraquinones have a polyketide chain derived from eight acetate units as a precursor, the mode of cyclization to form a three ring skeleton is not always the same as depicted in Schemes 3 and 4. The isolation of rhamnalpinoside and its aglycone (74) from *R. fallax* (Rauwald and Miething, 1985) and different α-methylanthraquinones from *A. vaginata* (Mammo, 1989) demonstrate a second mode of cyclization of an octaketide in the Rhamnaceae.



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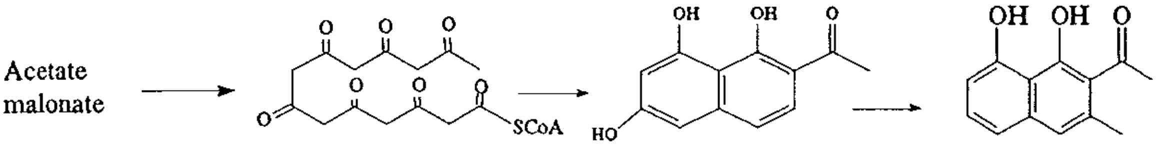
Scheme 4: The mode of cyclization in rhamnalpinogenin

Since both emodin (3) and rhamnalpinogenin (74) appear in *R. fallax*, it can be speculated that the same plant can operate with both folding modes of an octaketide chain.

On the other hand, the occurrence of two structurally related compounds in the same plant does not necessarily imply that both have started from the same polyketide skeleton. This can be observed from the two naphthalene derivatives, musizin (19) and glucoside of *β*-sorigenin. These structurally similar natural products are isolated from *R. wightii* (Pepalla, *et al.* 1991) and *R. prinoides* (see Results and Discussion). But the biosynthetic origin of musizin requires a heptaketide chain (Scheme 5) while that of *β*-sorigenin (22) is believed to arise from a hexaketide chain (Scheme 6). In the latter case, oxidation of the methyl group will enable it to lactonize with the carboxylic side chain. Such type of ring formation (lactonization) could also be considered a plausible mechanism for the co-isolated compound: 7-hydroxy-5-methoxyphthalide (67), from *R. libanoticus* (Satake, *et al.* 1991). But, this will not exclude the possibility of one compound to be the very precursor of the other, as it can be speculated from musizin and β-sorigenin.



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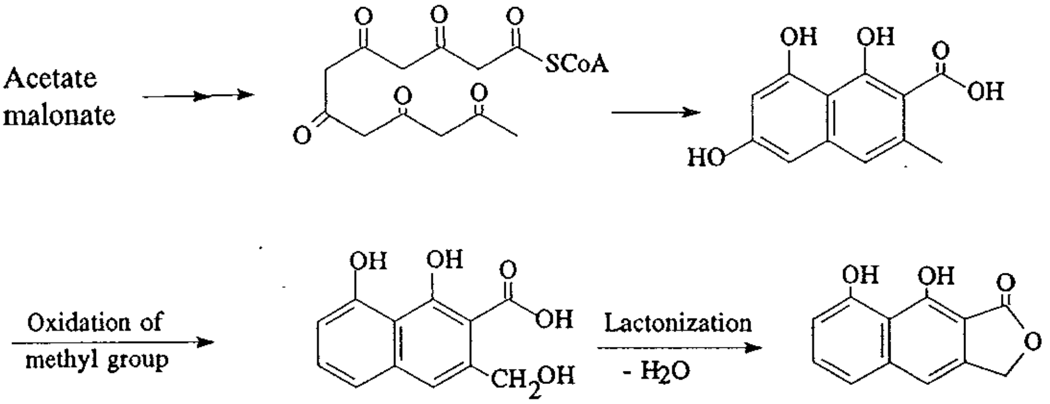
Scheme 5: Biosynthesis of musizin



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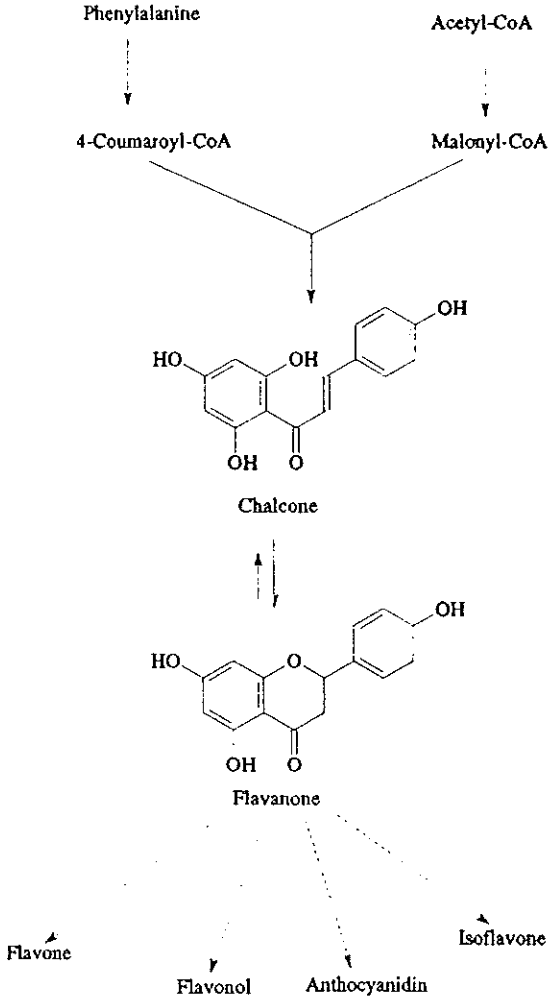
Scheme 6: Proposed biosynthetic pathway for β-sorigenin

1.6 Flavonoids of the Rhamnaceae

A. Occurrence and Distribution

Flavonoids are structurally derived from flavones and share a number of common properties. These phenolic compounds are generally present in all plants bound to sugar as glycosides and any one flavonoid aglycone may occur in a single plant in several glycosidic combinations (Harborne, 1984). Flavonoids appear in all vascular plants but some classes are more widely distributed than others; while flavones and flavonols are universal, isoflavones and biflavonyls are found in only a few plant families. The classes of flavonoids encountered in the Rhamnaceae include flavonols, dihydroflavonols (e.g. **54**), C-glycoflavones (e.g. **56**), a biflavonoid and a chalcone or aurone derivative (e.g. **65**). The



Scheme 7: Biosynthesis of all classes of flavonoids

1.7 Biological Significance of Rhamnaceae Plants

A. vaginata is used in the southern part of Madagascar for the treatment of stomach problems. The purgative extracts of *R. frangula*, *R. purhsianus* and *R. catharticus* contain anthracene derivatives as their active constituents. Emodin is a strong inhibitor of a protein tyrosine kinase, which is a potential target for modulating cancer cell growth. It also possesses intestinal and cardiac stimulant, analgesic and anti-inflammatory properties (Dwivedi, *et al*. 1988). Emodin anthrone, the precursor of the antiviral hypericin, has been isolated from *R. frangula*, *R. purshianus* (Labadie, 1970) and *R. prinoides* (Abegaz and Dagne, 1988).

The fruits of *R. prinoides* are traditionally used to treat ring-worm infections in Ethiopia and the methanol fraction from Soxhlet extraction of this fruit was found to inhibit the growth of the dermatophytes *Microsporum*, *Gypseum*, *Ferrugenium* and

2. RESULTS AND DISCUSSION

Six compounds isolated from the leaves of *R. prinoides* (*Gesho*) were fully characterized during the course of this chemical study. They are:

Chrysophanol (1)
Physcion (2)
Musizin (19)
Emodin (3)
Rhamnocitrin (40)
β-Sorigenin-8-O-β-D-glucoside (68)

Derivatization of geshoidin (68) was necessarry for complete structural elucidation and identification. Thus, β-sorigenin-8-O-β-D-glucoside-peracetate (69), permethylated β-sorigenin-8-O-β-D-glucoside (70), β-sorigenin-1-methylether (71) and β-sorigenin (22) were made from **68** by acetylation, methylation and hydrolysis procedures.

Compounds **2** and **3** have been obtained from the fruits of *R. prinoides* (Abegaz and Dagne, 1988). The occurrence of the known compounds **19** and **40** have not been reported before from this taxon while geshoidin (68) is a novel natural product. Spectroscopic data for an acetylated, permethylated and 1-methyl ether derivatives of a naphthalene glucoside lactone are also reported here for the first time. The upfield chemical shift of the *peri* hydroxyl proton in 68, relative to that observed for the non-lactonic compound 19 is explained in terms of electronic and bond length differences. All compounds were characterized by spectroscopic methods and other physical data, and wherever possible by direct comparison with authentic materials. ^{1}H NMR or ^{1}H NMR and UV-Visible spectroscopy were used for structural elucidation of all the ten compounds, and ^{13}C NMR and IR spectroscopic methods were utilized additionally for compounds **68**, **69** and **70**.

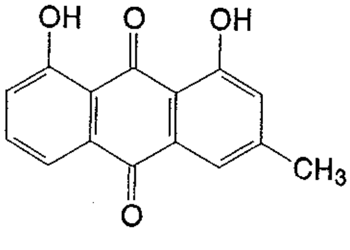
The bitter attributes of β-sorigenin-8-O-β-D-glucoside have been established for the first time by a sensory panel. The name geshoidin is proposed for this bitter natural product.

2.1 Characterization of Compounds 1, 2 and 3

All the three compounds showed four or more absorption bands in the UV-Visible regions, one of which is above 430 cm^{-1} . This, and the color detected during TLC with KOH indicated an anthraquinone skeleton. The characterization of each compound was made possible by co-chromatography and comparison of their observed UV and NMR spectroscopic data with authentic samples.

Compound **1**

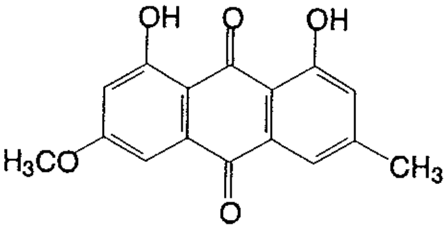
^{1}H NMR spectroscopic data revealed two broad singlet aromatic protons at δ 7.1 and 7.65 which can be assigned to H-2 and H-4 of a chrysophanol structure (1). The peaks appear broad because of allylic coupling with the methyl protons at C-3. The other peaks at δ 7.32, 7.58 and 7.75 are for the protons H-7, H-6 and H-5, respectively. Methyl protons resonated at δ 2.46 while the two chelated hydroxyl protons appeared at δ 12.02 and 12.15. This 1, 8-dihydroxyanthraquinone skeleton is further supported by the UV-Visible spectrum at λ_{max} =431 nm.



1

Compound **2**

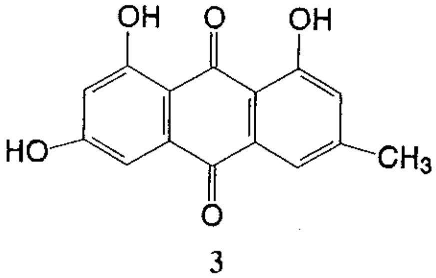
Only *meta* coupling patterns (narrow doublets and broad singlets) are observed here which goes well with the structure of physcion (2). H-2 and H-4 resonated at δ 7.04 and 7.62, respectively, both broadened by the methyl protons at C-3. The narrow doublets at δ 6.65 and 7.35 can be assigned to H-7 and H-5, respectively, whose coupling constant is 2.5 Hz. Methyl protons appeared as a singlet at δ 2.45 whereas the methoxy protons resonated at δ 3.92. 1, 8-dihydroxy protons are observed at δ 12.30 and 12.10, respectively. The UV-Visible spectrum displayed an absorption maximum at λ_{max} =435 nm. All the above data and co-chromatography with an authentic sample confirmed the physcion structure (2).



2

Compound **3**

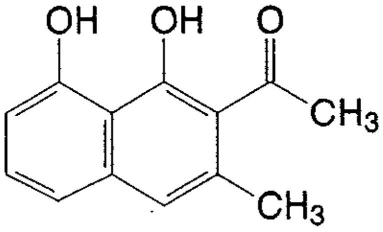
Similar splitting patterns as that of **2** were observed here. There are two broad singlets at δ 7.1 and 7.5, assignable to H-2 and H-4 of emodin (3), respectively. The *meta* coupled protons of C-7 and C-5 appeared at δ 6.6 and 7.2, respectively. Protons on C-3 methyl group resonated at δ 2.4 and the two 1, 8-OH protons showed peaks at δ 12.15 and 12.05. Co-chromatography with authentic sample together with the above data suggested compound **3** to be emodin (3).



3

2.2 Characterization of Compound 19

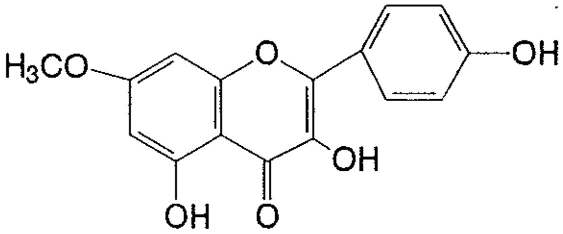
The ^1H NMR signals at δ 17.3 (s) and 10.24 (s) indicated the presence of doubly chelated and phenolic hydroxyl protons, respectively. The same spectrum also showed one methyl, one acetyl and four aromatic protons. Three of the aromatic protons, δ 6.8, 7.0 and 7.15, formed an ABC pattern and a fourth proton appeared at δ 7.48 (s). The UV absorption patterns are similar to that of naphthalene but are shifted to longer wavelengths due to the substituents in **19**. Co-chromatography with an authentic sample, UV and proton NMR spectroscopic data established compound **19** to be musizin (19).



19

2.3 Characterization of Compound 40

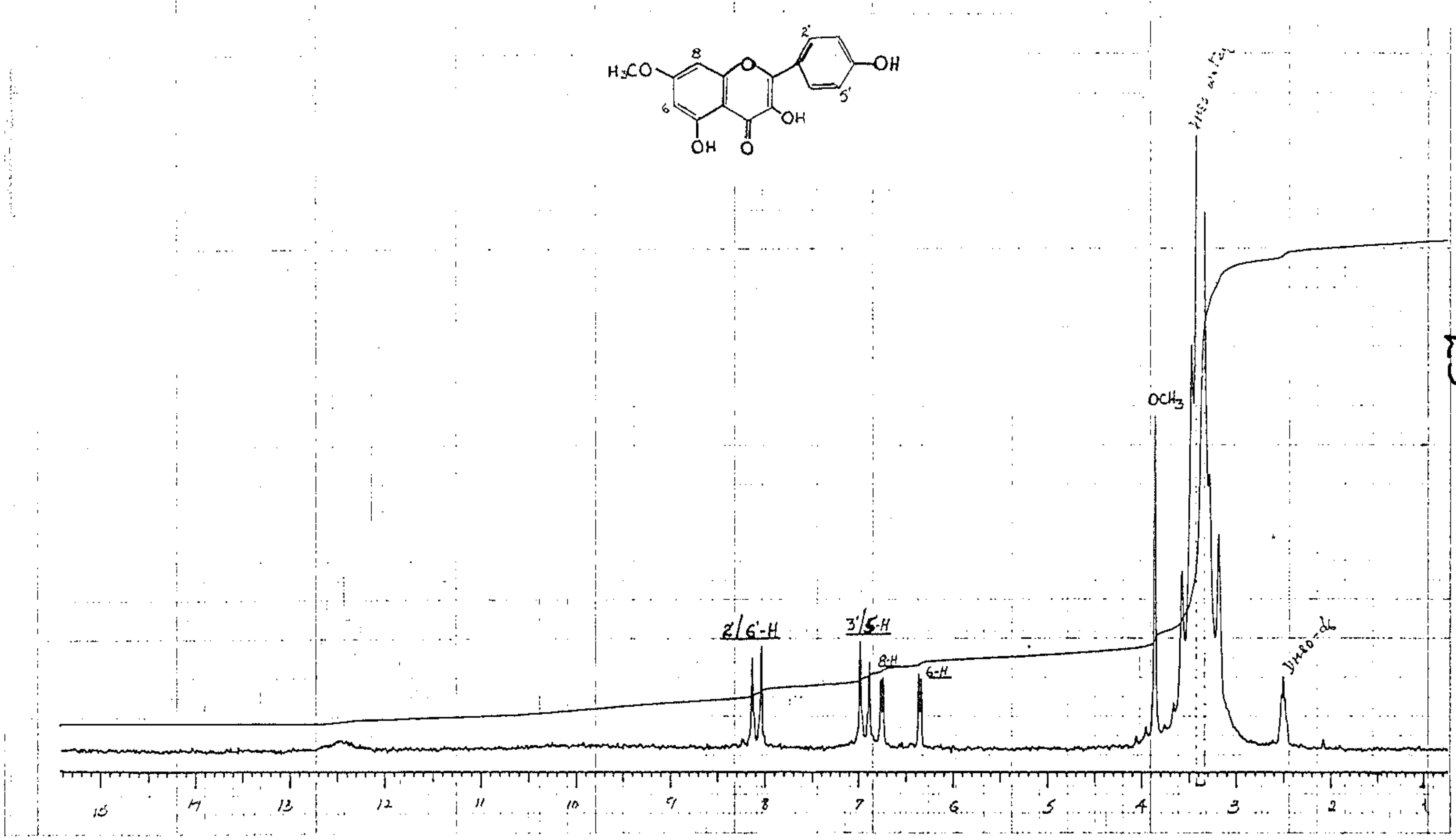
All UV-Visible absorption bands lie below 400 nm and TLC color with KOH appeared yellow, suggesting a non-anthraquinone skeleton. There was no significant bathochromic shift in the UV spectrum on addition of powdered NaOAc. Proton NMR spectroscopic data (Fig. 2) showed one methoxy (δ 3.91, 3H), *meta* coupled aromatic protons at δ 6.32 (1H, d, J=2.5 Hz) and 6.7 (1H, d, J=2.5 Hz), an A_2B_2 pattern at δ 7.0 (2H, d, J=9 Hz) and 8.18 (2H, d, J=9 Hz). The above data are consistent with a flavonol of 5, 7, 4'-trisubstituted structure. The methoxy group to be at C-7 is strongly suggested from the absence of a shift in the UV spectrum upon addition of powdered NaOAc. The above explanations are consistent with the structure of kaempferol 7-methylether (Rhamnocitrin) (40).



40

FX 90
RPL-5

FIGURE 2 : ^{1}H NMR SPECTRUM [redacted]
OF COMPOUND 40



23

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REFERENCE DMSO-d₆ 2.5 ppm
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MULTI
DATA POINTS (FID)
WINDOW EX- 2
NO. OF PULSES
SPECTRAL WIDTH 1200
AMPLITUDE 14 x 2ds
DECOUPLING
NOISE KHz POWER
DATE 28th Oct 1983
OPERATOR EWR
REMARKS ADRES

BA8
SD5

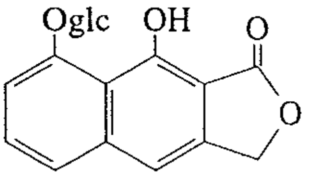
JEOL LT

2.4 Characterization of 68

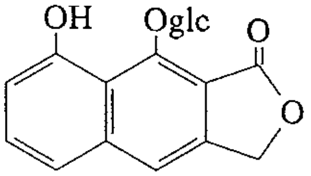
Compound **68** is the major costituent isolated from the leaves of *R. prinoides*. 1 g of the precipitate which contained **68** gave 378 mg of the compound upon purification. This indicated that at least 1.0% of *Gesho* leaves contain **68**.

The IR and UV spectral data of **68** appear to be comparable with that of β-sorigenin-1-O-β-D-glucoside (25) (Pepalla, *et al.* 1991) but significant differences are observed between the ^{13}C NMR spectroscopic data of the two compounds. In the IR spectrum of **68** (Fig. 5), α, β-unsaturated γ-lactone ring is indicated at $\nu = 1741$ cm^{-1}. The presence of a chelated carbonyl absorption at 1643 cm^{-1} and a bathochromic shift with $AlCl_3$ (Fig. 4) in the IR and UV spectra, respectively, suggested that it possessed an aromatic *peri* hydroxyl carbonyl moiety and the sugar was located at C-8 of the ring. Hydrogen bonded hydroxyl signal is also observed at $\nu = 3362.8$ cm^{-1}. ^{1}H NMR spectrum of **68** (Fig. 3) showed four aromatic, one anomeric and six sugar protons together with a lactonic group. On acid hydrolysis, **68** gave glucose, detected by paper chromatography. The β-linkage of the sugar was established from the ^{1}H NMR coupling constant (J = 8 Hz) of the H-1' in the glucoside.

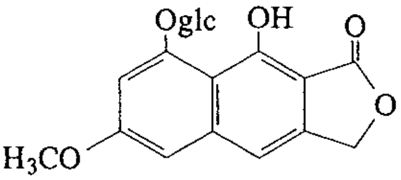
The above findings together with ^{13}C NMR spectrscopic data suggested **68** to represent β-sorigenin-8-O-β-D-glucoside (68). The ^{13}C NMR spectroscopic data of **68** was assigned from the DEPT spectrum and comparison with the chemical shifts of β-sorigenin 1-O-β-D-glucoside (25) and α-sorigenin 8-O-β-D-glucoside (26). ^{13}C NMR spectroscopic data of **68**, its acetylated derivative (i.e. **69**), (25) and (26) are given in Table 4.



68



25



26

Table 4. ^{13}C NMR spectral data of **68**, **25**, **26** and **69**.

Carbon	**68**[a]	**25**[a]	**26**[b]	**69**[c]
1	155.3[d]	155.907	157.1	147.3
2	114.3	113.759	111.3	120.3
3	139.5	142.709	141.9	140.3
4	110.97	119.354	110.2	118.4
4a	142.9	139.797	144.3	141.3
5	122.98	110.397	105.0	123.5
6	129.5	130.134	160.7	129.2
7	110.97	104.373	104.6	111.3
8	155.84[d]	156.911	158.4	155.2
8a	105.83	108.789	105.5	115.3
Lactone-CH_2	67.93	68.158	68.3	68.20
Lactone CO	168.8	168.377	169.3	170.3[e]
1'	102.85	102.893	102.6	99.85
2'	73.4	73.286	74.7	72.05
3'	77.8	77.820	78.4	72.50
4'	69.9	69.737	71.2	68.20
5'	72.26	76.237	79.7	73.30
6'	60.78	60.698	62.3	62.00
$OCOCH_3$ (sugar methyls)				20.50
$OCOCH_3$ (Aryl methyl)				20.89
OC=O (Acetate)				170.1[e], 169.48, 169.4, 169.2, 167.6

---

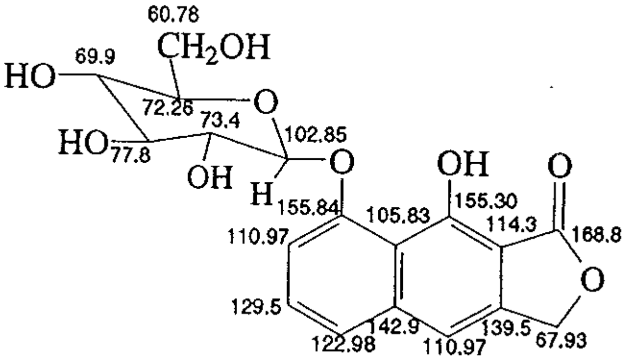
[a] Measured in DMSO-d_6.

[b] Measured in pyridine-d_5 (Lin and Wei, 1994).

[c] Measured in $CDCl_3$.

[d, e] Interchangeable.

Comparison of ^{13}C NMR spectral data of **68** and **25** show significant differences at C-4, C-5 and C-7 which arise from the substitution effect of the glucose moiety. On the other hand, C-4 and C-8a of **68** and **26** showed about equal chemical shifts supporting similar substituents at C-1 and C-8.



68

Interestingly, the signals of the *peri* hydroxyl proton in **68** appeared at higher field compared with non-lactonic compounds like musizin (δ 17.3). The chemical shift of this hydroxyl proton in β-sorigenin 8-methylether (27) has been reported to resonate at δ 9.76 (Pepalla, *et al.* 1991), whereas in **68** and its aglycone **22**, the signals were observed at δ 10.45.

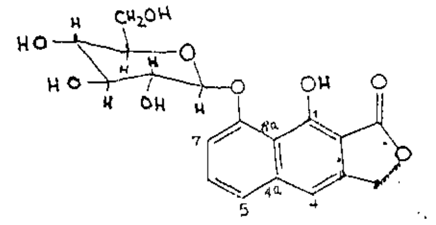
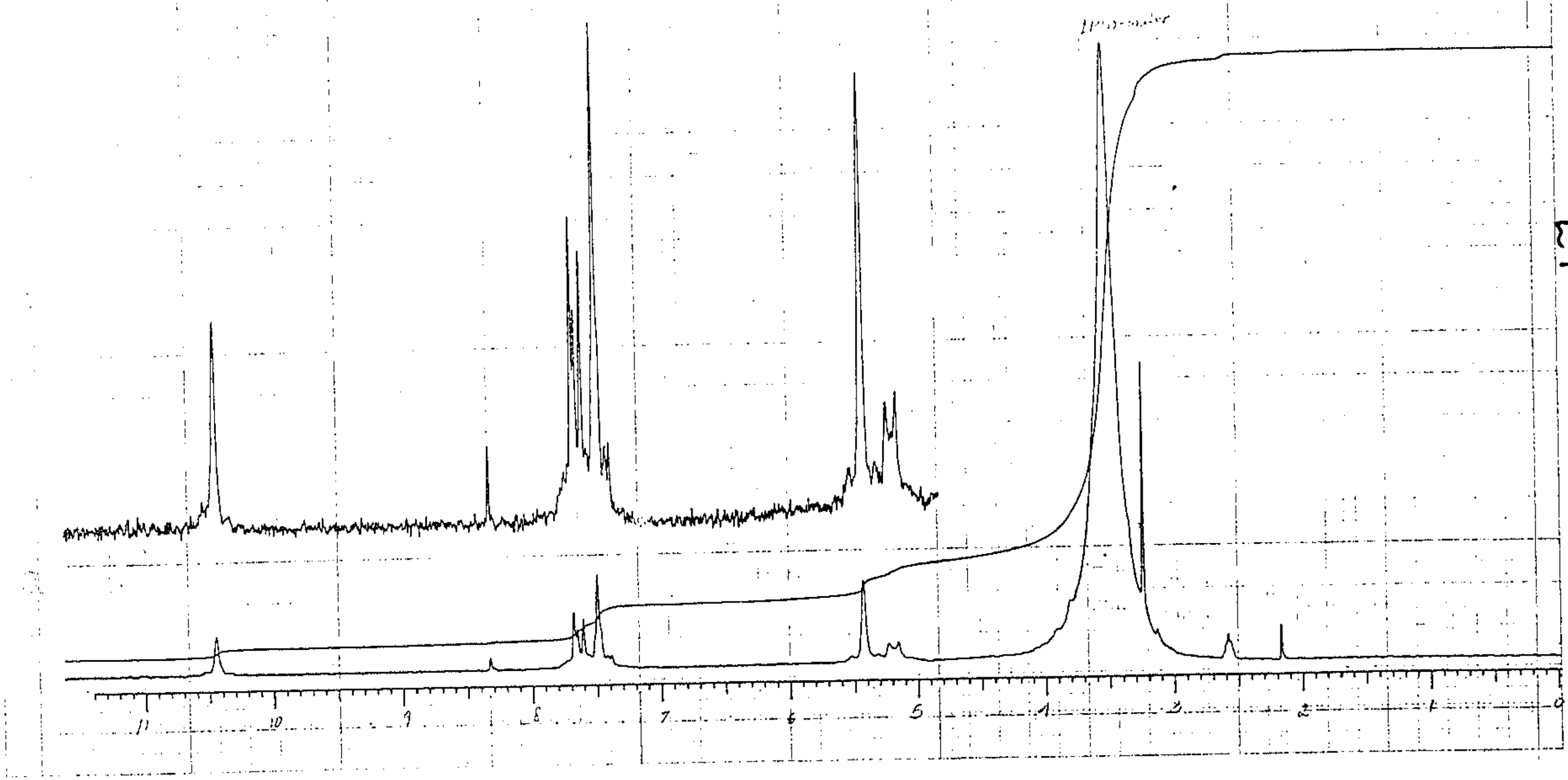


FIGURE 3 : ¹H NMR SPECTRUM OF COMPOUND 68

[redacted]



FX / 90
Technical
RPL-6

SOLVENT DMSO/TUBE 5
CONCENTRATION 3.2 mg
REFERENCE DMSO-d6 2.5 ppm
TEMPERATURE 27°C
/ SINGLE
MULTI
WINDOW EX- 2
NO. OF PULSES
SPECTRAL WIDTH 1800
AMPLITUDE 14 x 3de
DECOUPLING
MHz POWER
DATE 22nd Feb 1992
OPERATOR EWR
REMARKS: ADRES

BA-10
TK1

JEOL LT

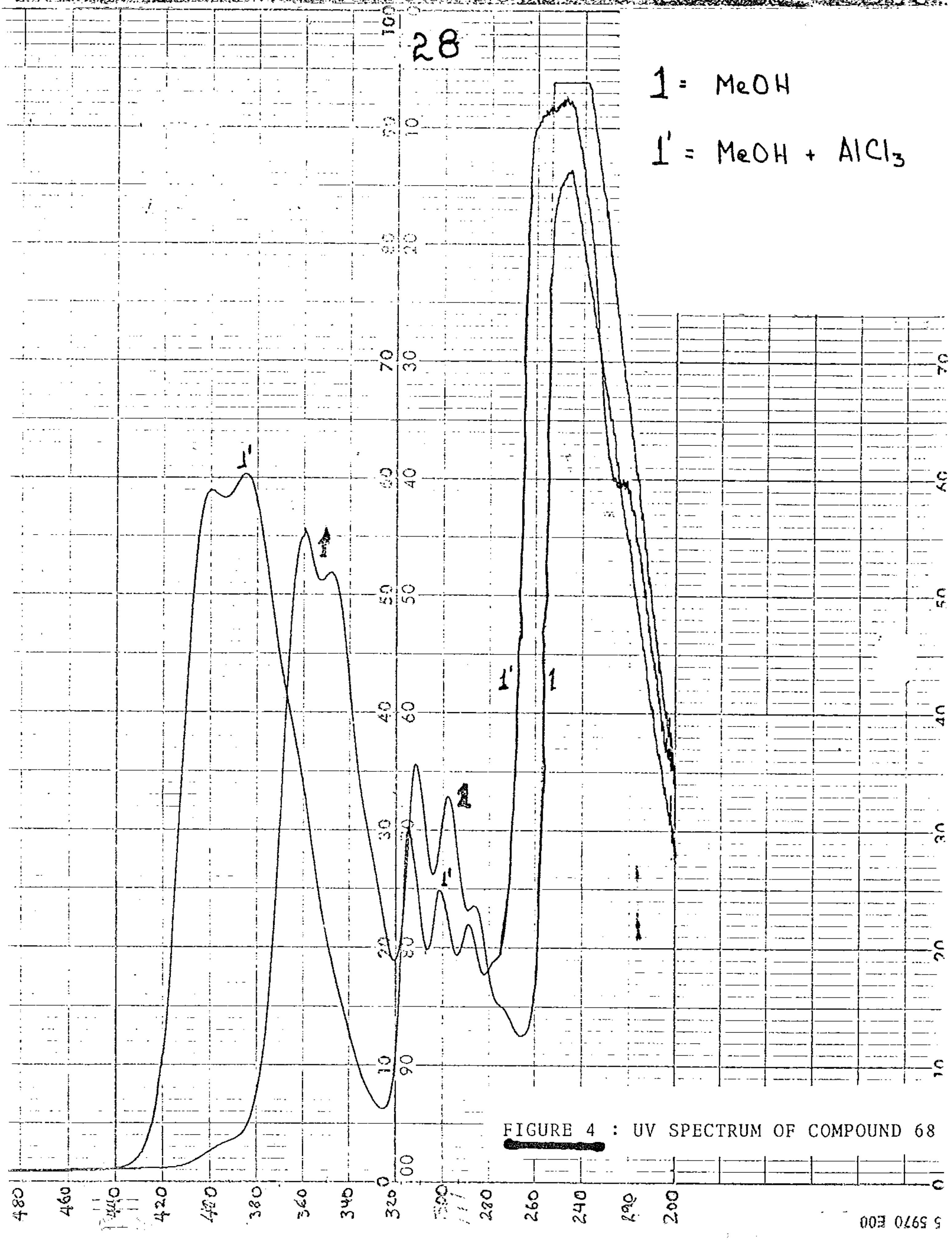


FIGURE 4 : UV SPECTRUM OF COMPOUND 68

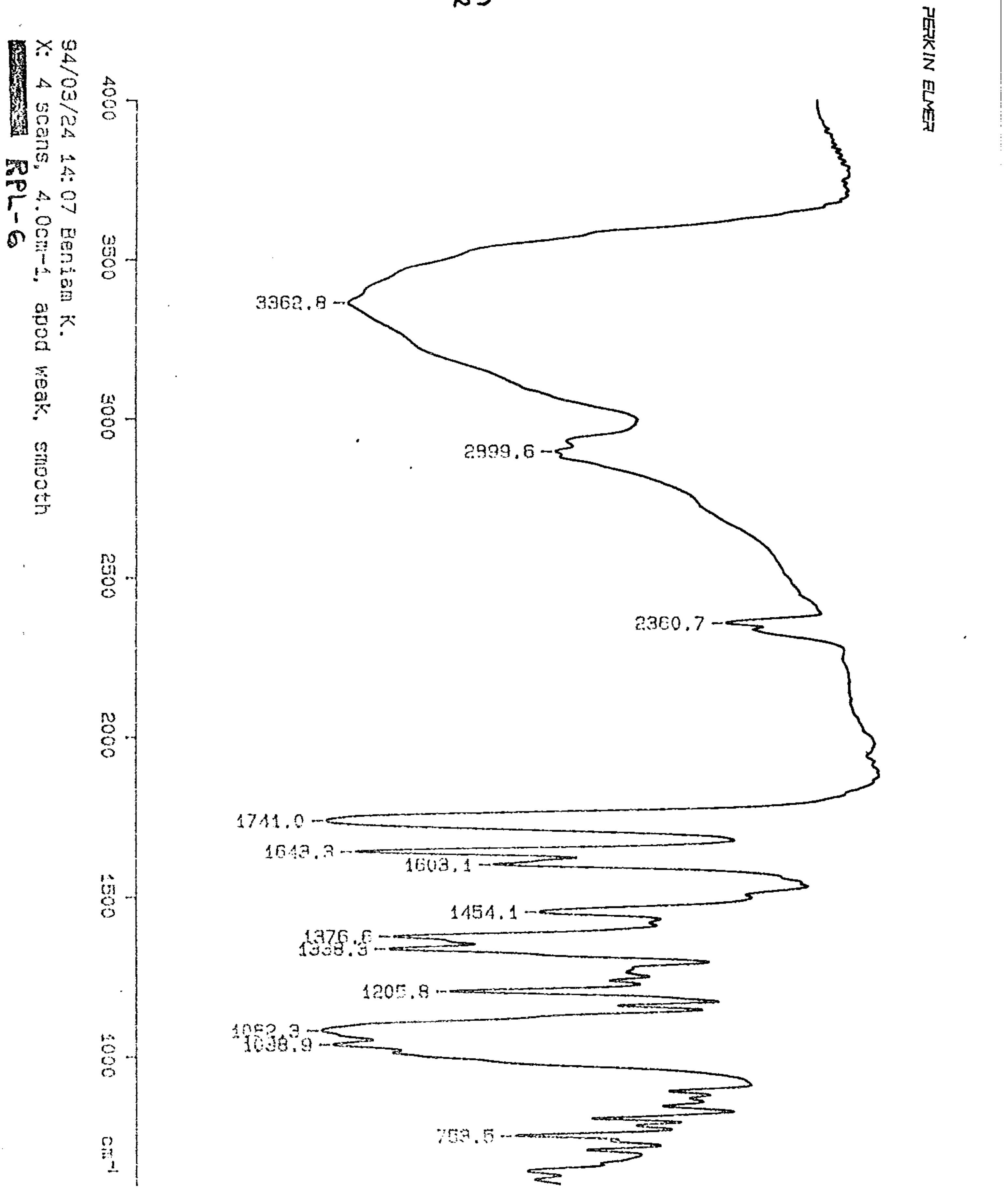


FIGURE 5 : IR SPECTRUM OF COMPOUND 68 .

906.
Teshome

FIGURE 6 : [redacted] 13C NMR SPECTRUM OF COMPOUND 8

```
TOTAL        23
FSAMPL 6??00 -4 HZ
CNFRQ  22.5??????
RES   1015.0633 HZ
NGRID        11
```

NO	FREQ(HZ)	PPM	INT%
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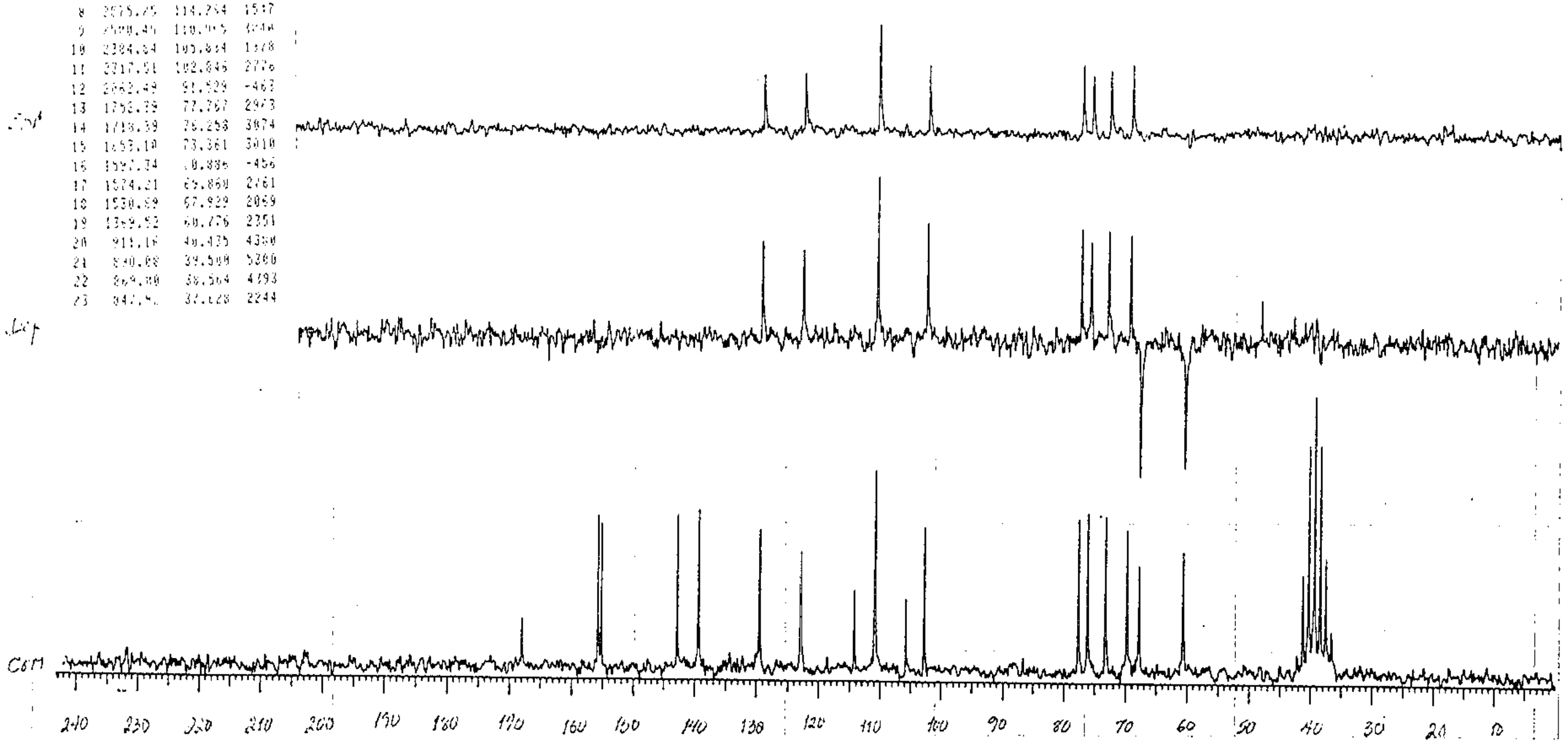
2	3511.59	155.542	2951
3	3499.45	155.003	2085
4	3219.14	142.595	2575
5	3148.12	139.424	7073
6	2910.60	129.525	2710
7	2771.11	122.976	2303
8	2575.75	114.254	1547
9	2500.45	110.765	4044
10	2384.64	105.634	1378
11	2317.51	102.846	2776
12	2062.49	91.529	-463
13	1755.79	77.767	2973
14	1718.39	76.258	3074
15	1653.10	73.361	3010
16	1597.74	70.886	-456
17	1574.21	69.860	2761
18	1530.59	67.929	2069
19	1369.52	60.776	2351
20	911.16	40.435	4380
21	890.08	39.500	5300
22	869.00	38.564	4793
23	847.9	37.628	2244



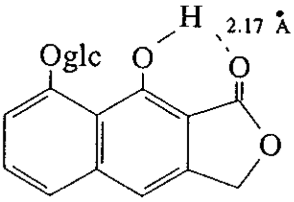
DMSOd6 - δ
54.5
DMSO-d6 39.5
27°C
13C NMR

30

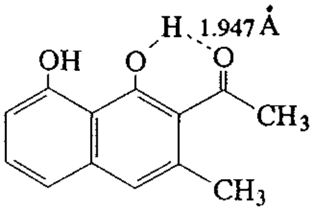
11
1425
5370
19X 30'2

9th March 15
2002
BA 6
TK 12 c
TK 12 J
TK 14 :
JEOL

Two possible explanations are now offered for the upfield chemical shift of this proton. One is the delocalization of the lone-pair electrons in lactonic oxygen which enters into conjugation with the carbonyl double bond. This results in a net negative charge or accumulation of electron density around carbonyl oxygen, thus increasing the shielding effect on the *peri* hydroxyl proton. The second is the differences in bond lengths between chelated hydrogen and carbonyl oxygen in the lactonic and non-lactonic systems. From molecular mechanics calculations, these distances were 2.171 Å for **68** and 1.947 Å for **19**. In the Dreiding models of **68** and **19**, the measured intramolecular hydrogen bond distances were found to be 2.3Å and 2.1Å, respectively. Thus, the longer bond length in **68** makes chelation of the hydroxyl proton weaker; as a result its chemical shift appeared upfield.

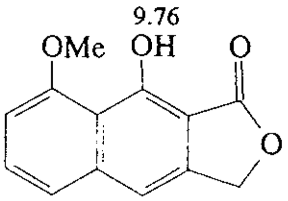


68

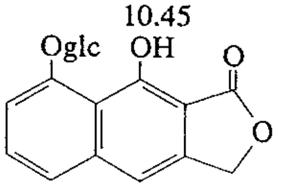


19

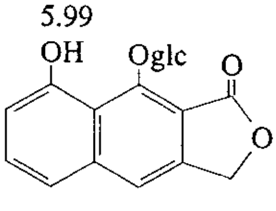
Chemical shifts of hydroxyl protons in the different structures are given below.



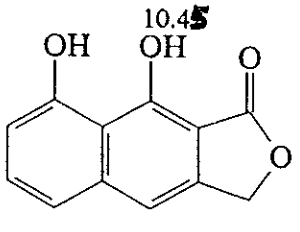
27



68



25



22

2.5 Characterization of Derivatives and Hydrolysis Products from 68

Acetylation of **68** with pyridine-acetic anhydride gave white leaflet crystals (**69**), which showed a blue-green spot when sprayed with vanillin-H_2SO_4 on TLC. The ^1H NMR spectrum revealed four aliphatic acetate signals (12H) in the region δ 2.01-2.10, one aromatic acetate peak at δ 2.5 (3H) and four aromatic protons. Three of these protons form an ABC pattern while the fourth proton is observed as a singlet.

In the IR spectrum α, β-unsaturated γ-lactone ring is indicated at $\nu = 1765$ cm^{-1}. which is supported by the methylene protons peak at δ 5.37 in the ^1H NMR spectrum. IR data also showed no hydroxyl signals suggesting a peracetylated compound.

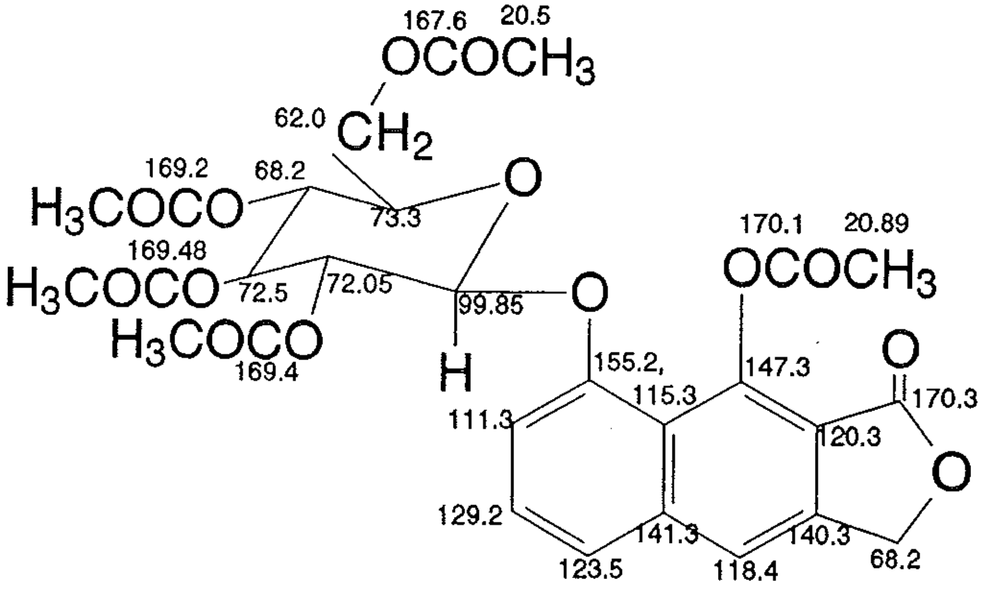
In the DEPT spectrum of **69** (Fig. 7), twelve peaks corresponding to 12 quaternary carbons, nine CH signals, five of them appearing below δ 100 and four between δ 111 and 124, accounting for 9 carbons were observed. In addition to these, two CH_2 groups and acetate methyl peaks representing five carbons were indicated.

The above data are consistent with a molecular formula of $C_{28}H_{28}O_{14}$, which corresponds to six carbonyls, four rings and five carbon-carbon double bonds [Ring + unsaturation = 28-1/2 (28)+1 = 15].

6 C=O	170.3,	170.1,	169.48,	169.4,	169.2,	167.6.
6 C quater.	155.5,	147.3,	141.30,	140.3,	120.3,	115.3.
5 CH (Glc)	99.85,	73.3,	72.50,	72.05,	68.2.	
4 CH (Ar)	129.2,	123.5,	118.40,	111.3.		
2 CH_2	68.2,	62.0.				
4 $OCOCH_3$ (methyl)	20.5.					
1 $OCOCH_3$ (methyl)	20.89.					

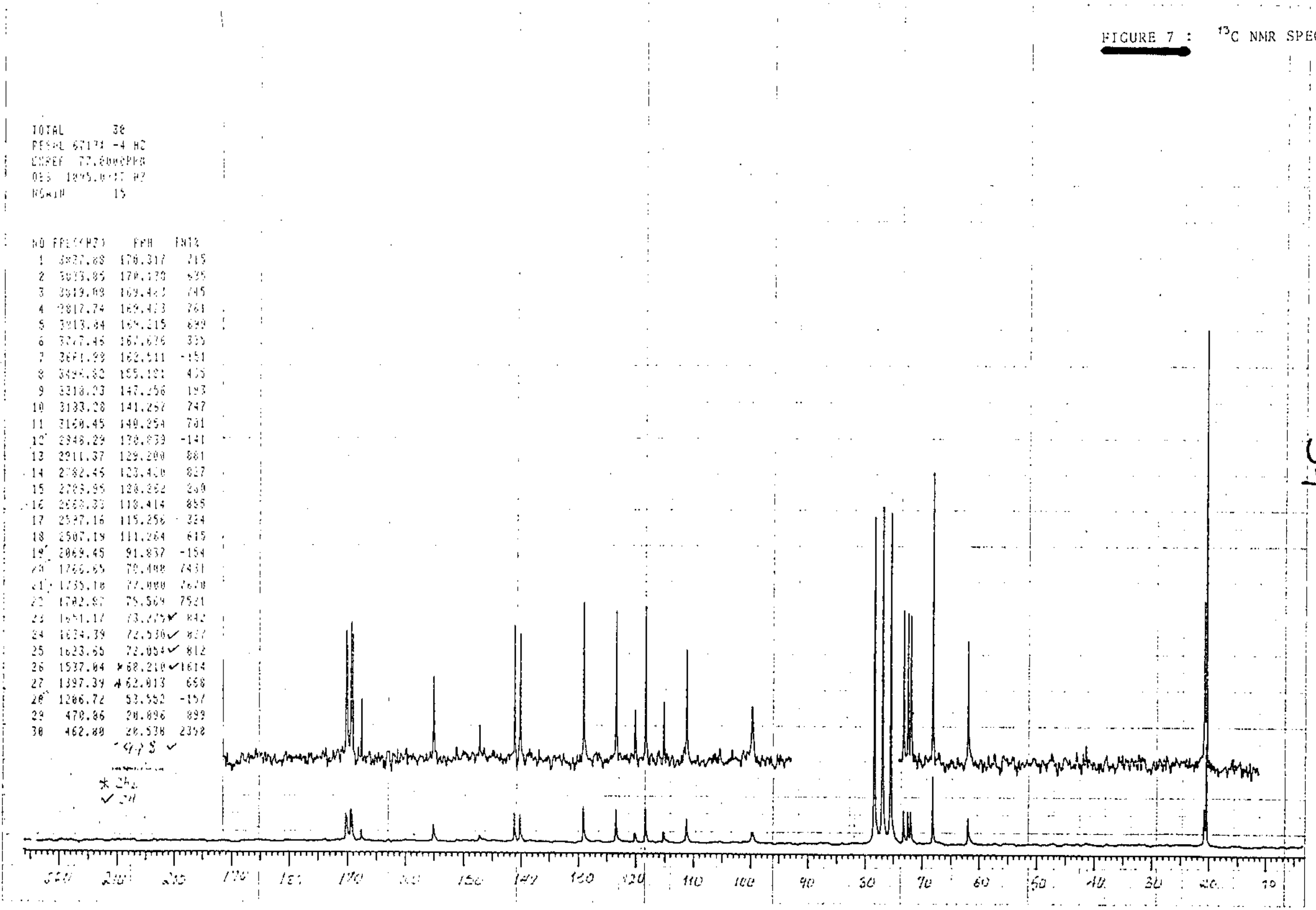
The two CH_2 peaks of different chemical shifts can be assigned to C-6' of sugar (δ 62.0) and lactonized CH_2 group (δ 68.2).

Therefore, compound **69** is the acetylated derivative of **68**, namely, β-sorigenin 8-O-β-D-glucoside-peracetate.


167.6
20.5
OCOCH3
62.0
CH2
169.2
68.2
H3COCO
73.3
O
169.48
H3COCO
72.5
72.05
H3COCO
169.4
99.85
H
O
170.1
20.89
OCOCH3
155.2,
115.3
147.3
O
170.3
111.3
120.3
O
129.2
141.3
140.3
123.5
118.4
68.2

69

FIGURE 7 : ^{13}C NMR SPECTRUM OF COMPOUND 61



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OBS  1995.0917 HZ
NGAIN        15

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 2  3823.85  170.170   635
 3  3819.08  169.945   745
 4  3812.74  169.643   761
 5  3803.04  169.215   699
 6  3767.46  167.626   335
 7  3651.99  162.511  -151
 8  3486.82  155.141   435
 9  3318.23  147.256   193
10  3183.28  141.257   747
11  3160.45  140.254   701
12  2948.29  130.839  -141
13  2911.37  129.200   881
14  2782.45  123.420   827
15  2709.95  120.262   269
16  2668.33  118.414   855
17  2597.16  115.256   334
18  2507.19  111.264   615
19  2069.45   91.837  -154
20  1766.65   78.408  7431
21  1735.18   77.000  7670
22  1702.87   75.569  7521
23  1651.17   73.275✓  842
24  1634.39   72.530✓  827
25  1623.65   72.054✓  812
26  1537.04 *68.210✓1614
27  1397.39 *62.013   658
28  1206.72   53.552  -157
29   470.86   20.896   899
30   462.80   20.538  2358
```

-4.75 ✓

* CH_2
✓ CH

FX 100
Tcl

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CONCENTRATION 25 mg
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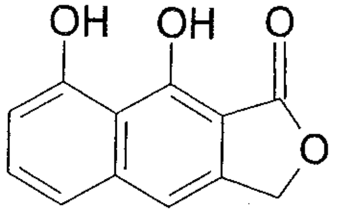
TEMPERATURE 27°C
DATA POINTS (FID)
WINDOW EX-12
NO OF PULSES
SPECTRAL WIDTH 5500
15x3dB
NOISE KHz POWER
DATE 3rd Feb 1984
OPERATOR
REMARKS ADRES

32000 scans

BAE

TK 22

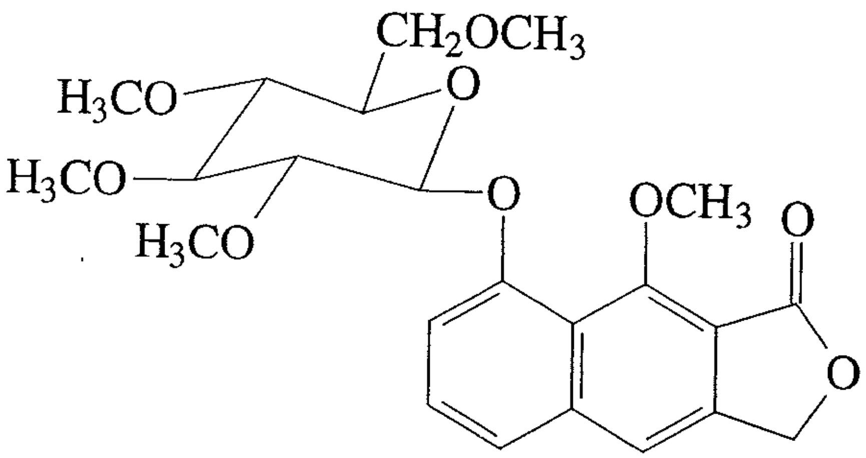
Hydrolysis of **68** with MeOH-H_2SO_4 (3N) gave faint-yellow needles of **22**. The ^{1}H NMR spectrum of this compound (Fig. 8) showed four aromatic proton signals at δ 7.31, 7.4 (2H), and 6.84 and lactone CH_2 protons peak at δ 5.34 (2H). The ^{1}H NMR, UV and melting point data of this compound are the same as the reported values of β-sorigenin (22). This compound was not detected in the crude extract of *R. prinoides* leaves on TLC.



22

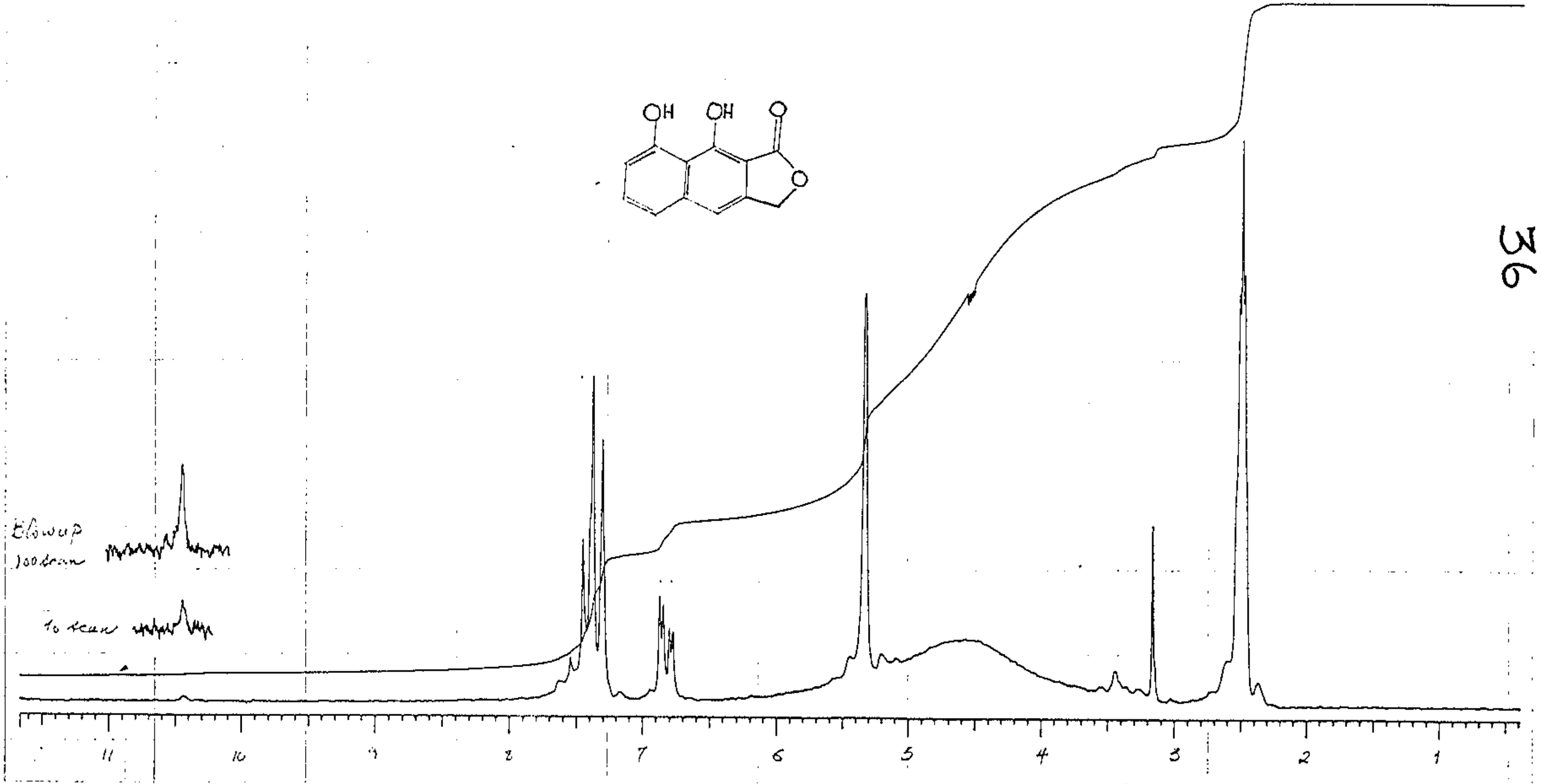
β-Sorigenin 8-O-β-D-glucoside (68) was methylated by Kuhn method (Kuhn, 1955) using CH_3I as methylating reagent (see Experimental). The ^{1}H NMR spectrum of the methylated glucoside (Fig. 9) showed four aromatic proton peaks at δ 7.05, 7.48 and 7.5 (2H), an anomeric sugar proton signal at δ 5.1 (d, J=8 Hz), a lactone CH_2 proton signal at δ 5.32 and five methoxy signals at δ 4.12, 3.72, 3.68, 3.58 and 3.35. The five methoxy signals indicated that all sugar and phenolic hydroxyl groups have been fully methylated. The aromatic methoxy substituent in the ^{13}C NMR spectrum appeared low field (δ 64) indicating that it is flanked between the carbonyl oxygen and the methylated sugar substituent, thus putting the glucoside at C-8.

The above spectral information is consistent with the structure of permethylated β-sorigenin 8-O-β-D-glucoside (70).



70

FIGURE 8 : ^{1}H NMR SPECTRUM OF COMPOUND 22



Addis Ababa University
Department of Chemistry

NMR Laboratory
Jeol FX-90Q Spectrometer

Spectrum No. [illegible]

Sample Code RPL-8
Sample Source

Lock 2D/INT
Nucleus
^{1}H (89.6 MHz)
^{13}C (22.5 MHz)

Solvent DMSO d_6
Amount in mg 10
Reference [illegible]
No. of Pulses 16
Spectral Width 1800 Hz
Amplitude [illegible]

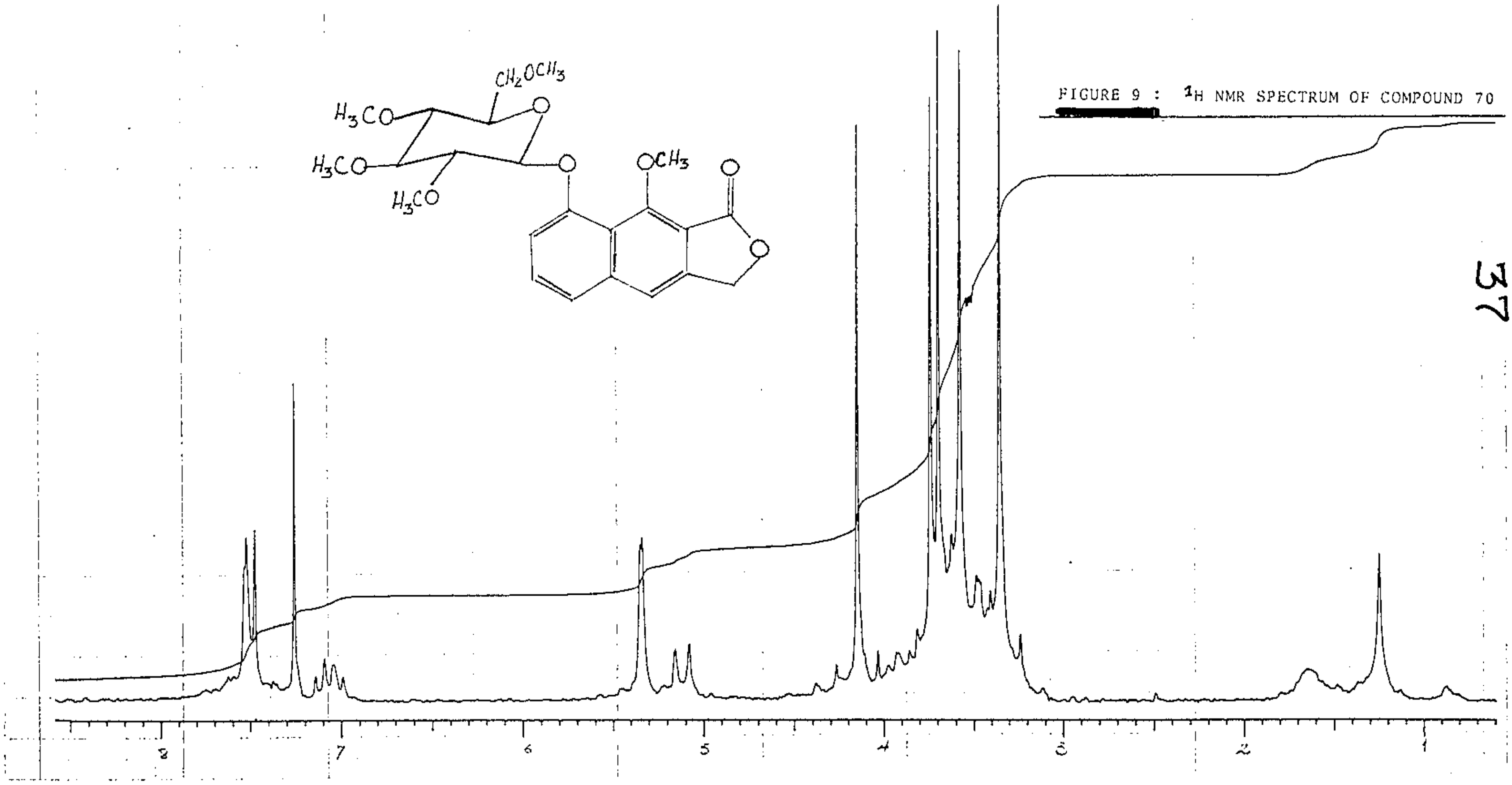
Saved on EAC File Name
Operator Zack

Day 19th Month May 199

Suggested structure/Remarks :

FIGURE 9 : ^{1}H NMR SPECTRUM OF COMPOUND 70

[redacted]



37

Addis Ababa University
Department of Chemistry

NMR Laboratory
Jeol FX-90Q Spectrometer

Spectrum No. Tachome L

Sample Code RPL-9
Sample Source

Lock 2D/INT
Nucleus
^{1}H (89.6 MHz) ✓
^{13}C (22.5 MHz)

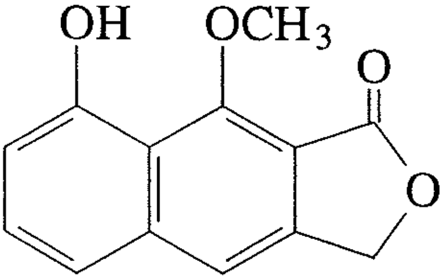
Solvent CDCl3
Amount in mg 16
Reference 7.26 ppm
No. of Pulses 16
Spectral Width 1010 H
Amplitude [illegible]

Saved on RA4 File Name
Operator [illegible]

Day 22nd Month May 19

Suggested structure/Remarks:

Hydrolysis of the permethylated compound (**70**) with 6% HCl gave brown leaflets of compound **71**. The ^{1}H NMR of this compound (Fig. 10) showed signals at δ 7.5 (1H, s, H-4), 7.4 (2H, t, H-5 and H-6), 6.95 (1H, d, H-7), 5.38 (2H, s) and 4.42 (3H, s). The signal of H-7 in **71** has moved upfield compared with that of compounds **68** and **70** suggesting the methylated glucoside substituent to be at C-8. Thus compound **71** represents β-sorigenin-1-methylether.



71

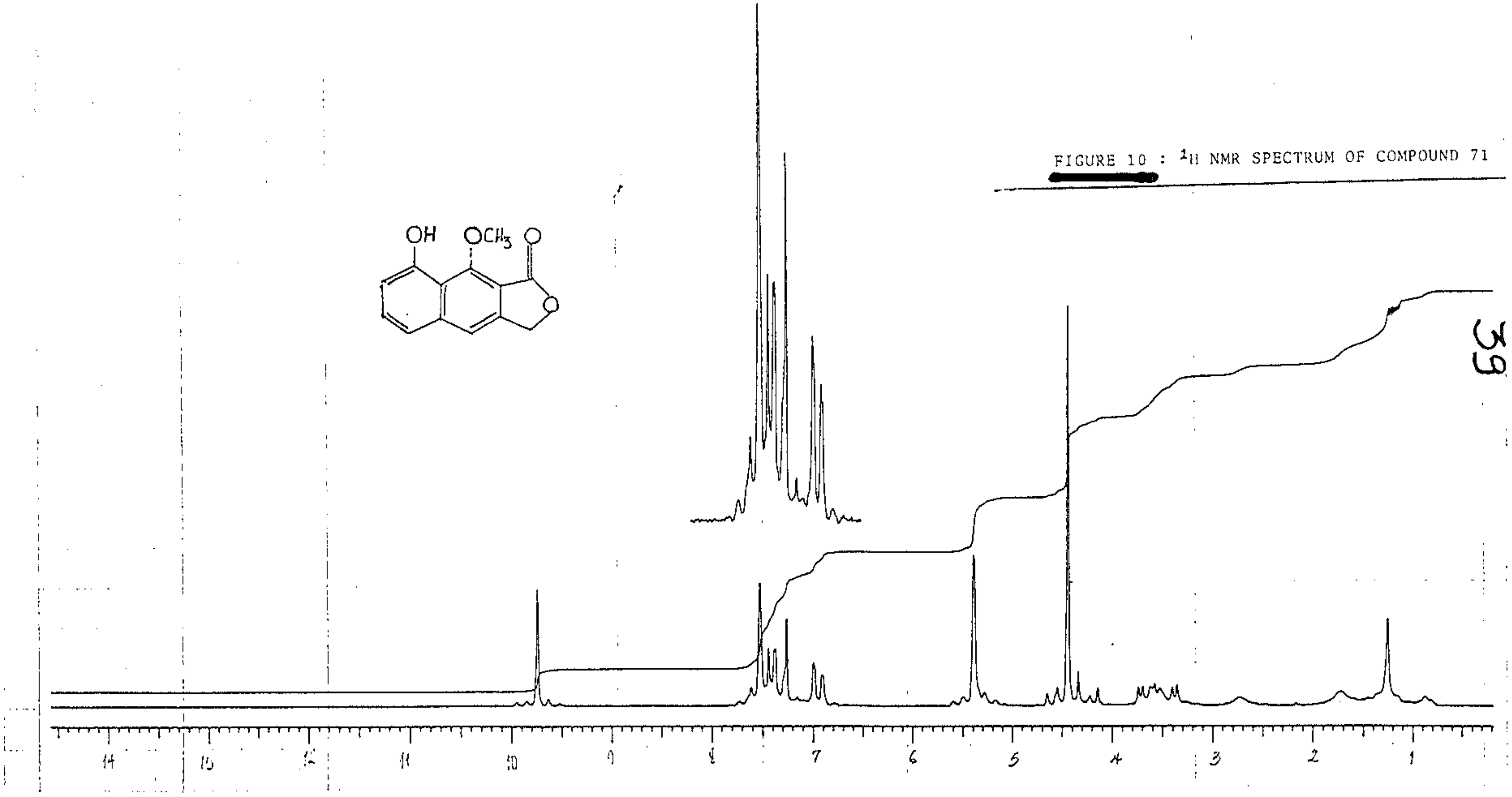


FIGURE 10 : ^{1}H NMR SPECTRUM OF COMPOUND 71

Addis Ababa University
Department of Chemistry

NMR Laboratory
Jeol FX-90Q Spectrometer

Spectrum No.______

Sample Code PPL-10
Sample Source______

Lock 2D/INT
Nucleus
^{1}H (89.6 MHz) ✓
^{13}C (22.5 MHz) ___

Solvent CDCl3
Amount in mg 5
Reference 7.26 ppm
No. of Pulses 16
Spectral Width 1800 Hz
Amplitude 15×2 dk

Saved on KA4 File Name 7.2
Operator [illegible]

Day 30th Month May 199_

Suggested structure/Remarks

B. Hydrolysis of Compound 70

50 mg of the permethylated glucoside (70) was dissolved in 4 ml methanol and 2 ml of 6% HCl added to the solution. This was refluxed for 8 hr and extracted with $CHCl_3$. After removing the solvents, brown leaflets of **71** were collected.

3.5 Organoleptic Evaluation of 68

Four male volunteers were recruited as panelists to perform a sensory analysis. Three were graduate students and the fourth was a lecturer in the Department of Biology. Samples were made and labelled as follows:

Code 347: *Tella* of unknown concentration, commercial sample

Code 472: 180 mg crude precipitate from the EtOAc-MeOH solvent system dissolved in 60 ml tap-water.

Code 565: 60 mg of **68** dissolved in 60 ml tap-water.

15 ml from each sample was transferred to the corresponding labelled test tubes and arranged in four different orders as observed from left to right, i.e. rack 1: 472, 347, 565; rack 2: 347, 565, 472; rack 3; 565, 347, 472; rack 4: 347, 472, 565.

Orientation meeting was arranged for the panelists prior to the sensory analysis. Each panelist was told to taste about equal portions from each sample, starting from left to the right. A questionnaire was given (see Appendix III) to each panelist and responses registered. These responses were evaluated subsequently.

4. SPECTROSCOPIC DATA OF COMPOUNDS

Chrysophanol: $C_{15}H_{10}O_4$, yellow leaflets, pink red with KOH, R_f^* 64.8 in (I); m.p. 194-196°, UV λ_{max} (EtOH) nm 226, 254, 277 (sh), 287 (sh), 431; ^{1}H NMR ($CDCl_3$) δ7.1 and 7.65 (br.s, H-2 and H-4, respectively) 7.32 (t, H-7), 7.58 (m, H-6) and 7.75 (t, H-5), 2.46 (3H, s, Me), 12.02 and 12.15 (s, 1, 8-di-OH).

Physcion: $C_{16}H_{12}O_5$, orange-yellow leaflets, pink-red with KOH, R_f 56.5 in (I), m.p. 206-208°, UV λ_{max} (EtOH) nm 225, 236, 256 (sh), 265, 288 (sh), 435; ^{1}H NMR ($CDCl_3$) δ 2.45 (3H, s, Me), 3.92 (3H, s, MeO), 6.65 and 7.35 (d, J=2.6 Hz, H-7 and H-5, respectively), 7.04 and 7.62 (br.s, H-2 and H-4, respectively), 12.3 and 12.1 (s, 1,8-di-OH).

Musizin: $C_{13}H_{12}O_3$, yellow needles, violet with KOH, R_f 49 in (I), m.p. 162-165°, UV λ_{max} (EtOH) nm 224, 240, 303, 317, 338, 340; ^{1}H NMR ($CDCl_3$) δ 2.65 (3H, s, Me), 2.72 (3H, s, Ac), 6.8 (s, H-7), 7.0 (t, H-6), 7.15 (t, H-5), 7.48 (s, H-4), 17.3 (s, 1-OH), 10.24 (s, 8-OH).

Emodin: $C_{15}H_{10}O_5$, orange-red leaflets, red with KOH, R_f 20.3 in (I), m.p. 253-254°, UV λ_{max} (EtOH) nm 223, 234, 252, 280, 289, 441; ^{1}H NMR (Me_2CO-d_6) δ 2.4 (s, Me), 6.6 (d, J=2.4, H-7), 7.2 (d, J=2.4, H-5), 7.1 and 7.5 (br.s, H-2 and H-4, respectively), 12.15 and 12.05 (s, 1, 8-di-OH).

Rhamnocitrin: $C_{16}H_{12}O_6$, pale-yellow needles, yellow with KOH, R_f 60 in (II), m.p. 223-225°, UV λ_{max} (EtOH) nm 267, 327 (sh), 368; UV λ_{max} (EtOH + NaOAc) 265, 327, 372; ^{1}H NMR (DMSO-d_6) δ 3.91 (3H, s, OMe), 6.32 (1H, d, J=2.5 Hz, H-6), 6.7 (1H, d, J=2.5 Hz, H-8), 7.0 (2H, d, J=8.9 Hz, H-3' and H-5'), 8.18 (2H, d, J=8.9 Hz, H-2' and H-6').

β-Sorigenin-8-O-β-D-glucoside: $[\alpha]_D^{22}$ -114.0° (MeOH, c=0.005), $C_{18}H_{18}O_9$, dull-yellow leaflets, blue-green with vanillin-H_2SO_4, R_f 19 in (III), m.p. 160-162°, UV λ_{max} (MeOH) nm (log ϵ): 250.0 (4.63), 286.6 (4.02), 298.2 (4.17), 311.7 (4.2), 348.2 (4.37), 360.1 (4.4), UV λ_{max} (MeOH + $AlCl_3$): 248.4, 289.9, 302.4, 315.5, 386.1, 400.9; IR ν_{max}

(KBr) cm^{-1}: 3363, 1741, 1643; ^{1}H NMR (DMSO-d_6): δ 3.30-3.75 (6H, Glc), 5.2 (1H, d, J=8 Hz, H-1'Glc), 5.42 (2H, lactone CH$_2$), 7.5 (1H, s, H-4), 7.65 (2H, t, H-5 and H-7), 7.4 (1H, t, H-6), 10.45 (s, 1-OH); ^{13}C NMR (DMSO-d_6): see Table 5.

β-Sorigenin-8-O-β-D-glucoside-peracetate: $[\alpha]_D^{22}$ -80.0° (CDCl$_3$, c=0.005), $C_{28}H_{28}O_{14}$, white leaflets, blue-green with vanillin-H$_2$SO$_4$, R$_f$ 87.0 in (III), m.p. 282°, UV λ_{max} (CHCl$_3$) nm: 215, 245, 299, 311, 349; IR ν_{max} (KBr) cm^{-1}: 1765, 1643; ^{1}H NMR (CDCl$_3$): δ 2.01-2.10 (12H, 4xAc), 2.50 (3H, Ac), 4.25 (2H, t, CH$_2$-Glc), 5.32 (1H, H-1'Glc), 5.36 (2H, lactone-CH$_2$), 5.2-5.3 (4H, Glc), 7.71 (1H, s, H-4), 7.1 (1H, dd, H-7), 7.6 (2H, t, H-5 and H-6); ^{13}C NMR (CDCl$_3$): see Table 5.

β-Sorigenin: $C_{12}H_8O_4$, faint-yellow needles, green with vanillin-H$_2$SO$_4$, R$_f$ 40.4 in (III), m.p. 240-243°, UV λ_{max} (MeOH) nm: 250, 314.5, 364.2; ^{1}H NMR (DMSO-d_6): δ 5.34 (2H, s, CH$_2$), 7.31 (1H, s, H-4), 6.82 (1H, dd, J=8 and 2.6 Hz, H-7) 7.4 (2H, t, J=8 Hz, H-5 and H-6), 10.45 (s, exch. with D$_2$O; *peri*-OH).

Permethylated β-sorigenin-8-O-β-D-glucoside: $C_{23}H_{28}O_9$, yellow-brown crystals, light-green with vanillin-H$_2$SO$_4$, R$_f$ 20 in (IV), ^{1}H NMR (CDCl$_3$): δ 7.05 (1H, t, H-7), 7.52 (2H, br.s, H-5 and H-6), 7.48 (1H, s, H-4), 5.32 (2H, br.s, lactone CH$_2$), 5.1 (1H, d, J=8 Hz, H-1'glc), methoxy proton signals at δ 4.12, 3.72, 3.68, 3.58, and 3.35; ^{13}C NMR (CDCl$_3$): δ 157.97* (C-1), 116.8 (C-2), 141.2** (C-3), 118.92 (C-4), 139.86** (C-4a), 116.3 (C-5), 131.1 (C-6), 111.47 (C-7), 156.55* (C-8), 110.4 (C-8a), 167.96 (C=O), 68.7 (lactone CH$_2$), 90.7 (C-1'), 82.1 (C-2'), 83.2 (C-3'), 79.595 (C-4'), 71.45 (C-5'), 70.03 (C-6'), 58.7, 59.1, 60.2, 60.6 (OCH$_3$-2', 3', 4', 6', respectively), 64.99 (1-OCH$_3$).

*, ** Interchangeable

β-Sorigenin-1-methylether : $C_{13}H_{10}O_4$, brown leaflets, green with vanillin-H$_2$SO$_4$, R$_f$ 61 in (IV), ^{1}H NMR (CDCl$_3$) δ 7.5 (1H, s, H-4), 7.4 (2H, t, H-5 and H-6), 6.95 (1H, d, H-7), 5.38 (2H, s, CH$_2$), 4.42 (3H, s, OMe).

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*: R$_f$x100

I: Petrol-EtOAc (4:1)

II: CHCl$_3$-MeOH (9:1)

III:CHCl$_3$-MeOH (3:1)

IV: Petrol-EtOAc (2:1)

APPENDIX I

Anthraquinones and Related Compounds from Rhamnaceae

No.	Source	Compound name	Reference
1.	*A. vaginata*	Aloesaponarin I, Aloesaporanin 1-3-methylether, 3, 4, 8-Trihydroxy-: 4, 8-Dihydroxy-3-methoxy-:, 3, 8-Dihydroxy-4-methoxy-:, 3, 4, 8-Trihydroxy-7-methoxy-:, 3, 7, 8-Trihydroxy-4-methoxy-:, 3, 4, 7-Trihydroxy-8-methoxy-: 3, 7, 8-Trihydroxy-:, 7, 8-Dihydroxy-3-methoxy-:, 3-Hydroxy-7, 8-dimethoxy-:, 3, 8-Dihydroxy-6-methoxy-:, -1-methylanthra-9, 10-quinone-2-carboxylic acid methylester, physcion-10, 10'-bianthrone, Torosachrysone, *Cis*-3, 4-Dihydro-9-hydroxy-7-methoxy-1, 3-dimethyl-1H-naphthol [2,3-C]-pyran-5, 10-dione.	Mammo, 1989
2.	*C. faralaorta*	Kaempferol-and Quercetin-3-[Glucosyl-(1->3)-4'''-acetyl-rhamnosyl-(1->6) galactoside].	Guinaudeau *et. al* 1981
3.	*K. humboldtiana*	7-Methoxyelutherin; 6-Hydroxy-7-methoxyelutherin. Karwinskia toxins 1, 2, 3 & 4; Tarachrysonemonomethylether, Karwinaphthol A & B.	Thomson, 1971 Dreyer, *et. al* 1975

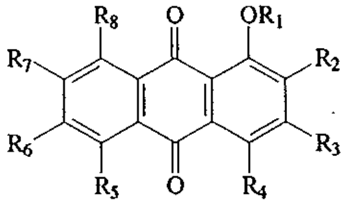
4.	*M. eminii*	Xanthorin, Islandicin, Cyanodontin	Cumming & Thomson, 1970
		Musizin	Covell, *et. al* 1961
		Maesopsin	Janes, *et. al* 1963
5.	*Phyllogeiton*	Zeyherin	Geiger & Quinn, 1975
6.	*R. alaternus*	Alaternin	Briggs, *et. al* 1953
		3-O-β-rhamninosides of Kaempferol Rhamnocitrin, Rhamnazin & Rhamnetin	Riess-Maurer and Wagner, 1982
7.	*R. carniolicus*	Free, methylether and glycosidic derivatives of kaempferol and quercetin.	Tumann, 1915
8.	*R. catharticus*	Alaternin	Rauwald and Just, 1981
		Emodin-8-O-β-D-glucoside, -8-O-gentibioside and -8-O-β-primeveroside	Rauwald, 1983
		3-O-β-rhamninosides of kaempferol, rhamnocitrin, rhamnetin & quercetin	Riess-Maurer & Wagner, 1982
9.	*R. fallax*	2-Methoxystypandrone, Alaternin Rhamnalpinogenin, rhamnalpinoside	Rauwald & Miething, 1983, 1985
		Frangulin A & B, Glucofrangulin A & B	Kinget, 1967

10.	*R. formosana*	Physcion-8-O-rhamnosyl(1->2)glucoside	Lin, *et.al* 1990
		Emodin-1-O-rhamnosyl(1->2)glucoside, rhamnazin-and rhamnocitrin-3-O-iso-rhamninosides.	Lin, *et. al* 1991
		Rhamnetin, rhamnocitrin & kaempferol	Chung, 1983
11.	*R. frangula*	Frangulin A, B and Glucofrangulin A &B	Wagner & Horhammer, 1966, 1969 Horhammer, *et. al* 1964 Muhlemann & Wernli, 1965
		Aloe-emodin-10, 10'-bianthrone, Palmidin B & C, Emodin bianthrone,	Lemi, 1965
		Chrysophanol-, Physcion- and Emodin-9-anthrones.	Labadie, 1970
		1, 8-Dihydroxy-2-acetylnaphthalin	Hegnauer, 1973
		Sennidine C, Rheidien A, B and C	Thomson, 1971
		Emodin-8-genitobioside	Demuth, *et. al* 1978
12.	*R. japonicus*	α- and β-Sorinin; α- and β-Sorigenin	Rauwald and Just, 1983, Haber, *et. al* 1956

29.	*V. maderaspatana*	Xanthorin, islandicin, xanthorin-5-methyl-ether, ventinone A and B.	Perkin & Hummel, 1984; Rao, *et. al* 1983
		Ventiloquinones A-H; cordeauxione, isocordeauxione, maderone, ventilaginone, ventilagol, ventilones A-E.	Hanumaiah, *et. al* 1985
30.	*V. viminalis*	Helminthosporin, islandicin, ventimalin, viminalin, citreosein, ventilagone.	Cooke & Johnson, 1963
31.	*Z. jujuba*	Swertisin (5,4'-di-OH 7-OMe 6-Glc.)	Tanimura *et. al* 1975
32.	*Z. nummularia*	Taxifolin-3-glucoside.	Srivasteva & Chauhan, 1977
33.	*Z. spina-christi*	Quercetin 3-O-xylosyl(1->2)rhamnoside-4'-rhamnoside	Nawwar *et. al* 1984
34.	*Z. vulgaris*	Swertisin-2''-O-glucoside (Spinosin).	Woo *et. al* 1979

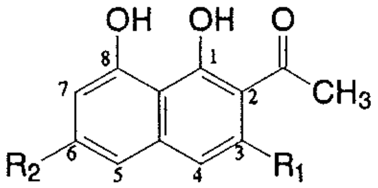
APPENDIX II

Representative Chemical Structures



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1. *Chrysophanol*	H	H	CH_3	H	H	H	H
2. *Physcion*	H	H	CH_3	H	H	OCH_3	H
3. *Emodin*	H	H	CH_3	H	H	OH	H
4. *Aloe-emodin*	H	H	CH_2OH	H	H	H	H
5. *Helminthosporin*	H	H	CH_3	H	OH	H	H
6. *Islandicin*	H	H	CH_3	OH	H	H	H
7. *Cynodontin*	H	H	CH_3	OH	OH	H	H
8. *Ventimalin*	H	OH	CH_3	OH	H	H	OH
9. *Viminalin*	CH_3	OH	CH_3	OH	H	H	OH
10. *Alaternin*	H	OH	CH_3	H	H	OH	H
11. *Xanthorin*	H	H	CH_3	H	OH	OCH_3	H
12. *Citreorosein*	H	H	CH_2OH	H	H	OCH_3	H
13. *4,5-Dihydroxynordigitolutein*	H	CH_3	OH	OH	H	H	H
14. *Calyculatone*	H	OH	CH_3	OH	H	OCH_3	H
15. *Ventinone A*	H	CH_3	CH_3	OH	H	OCH_3	H
16. *Rein*	H	H	CO_2H	H	H	H	H
17. *Alizarin*	H	OH	H	H	H	H	H

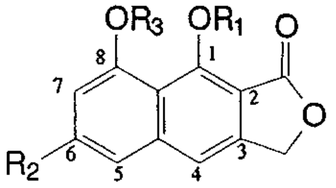
Viminalin $R_8 = OCH_3$ Alizarin $R_8 = H$ Others $R_8 = OH$



18. $R_1 = R_2 = H$: 1, 8-Dihydroxy-2-acetylnaphthalin

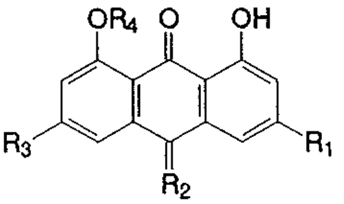
19. $R_1 = CH_3$; $R_2 = H$: *Musizin (= Nepodin = Dianellidin)*

20. $R_1 = CH_3$; $R_2 = OCH_3$: *Nakahalene*

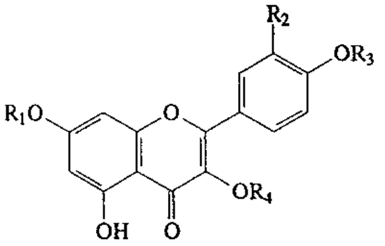


		R_1	R_2	R_3
21.	*α-Sorigenin*	H	OCH_3	H
22.	*β-Sorigenin*	H	H	H
23.	*α-Sorinin*	H	OCH_3	Primeverosyl*
24.	*β-Sorinin*	H	H	Primeverosyl
25.	*β-Sorigenin-1-O-Glucoside*	Glc	H	H
26.	*α-Sorigenin-8-O-Glucoside*	H	OCH_3	Glc
27.	*β-Sorigenin-8-methyl ether*	H	H	CH_3

Primeverosyl = D-Glucose + D-Xylose



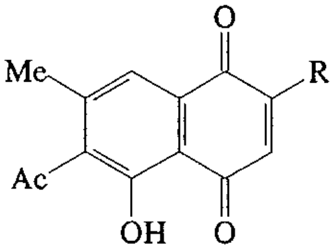
	R_1	R_2	R_3	R_4
28. *Aloe-emodin anthrone*	CH_2OH	H,H	OH	H
29. *Chrysaloin*	CH_3	H,C-Glc	H	H
30. *Aloin*	CH_2OH	H,C-Glc	H	H
31. *Cascaroside*	CH_2OH	H, C-Glc	H	ß-D-Glc
32. *Prinoidin*	CH_3	H,H	-O-L-[2,3-deacetyl rhamnopyranosyl]	H
33. *Emodin anthro.*	CH_3	H,H	OH	H
34. *Physcion anthro.*	CH_3	H,H	OCH_3	H
35. *Frangulin A*	CH_3	O	O-α-rhamnoside	H
36. *Frangulin B*	CH_3	O	O-ß-rhamnoside	H
37. *Glucofrangulin A*	CH_3	O	O-α-rhamnoside	ß-glucoside



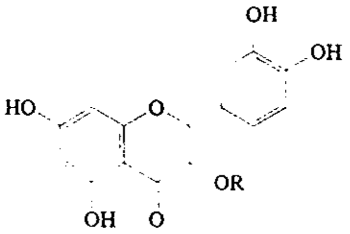
	R_1	R_2	R_3	R_4	
38. *Kaempferol*	H	H	H	H	
39. *Kaempferide*	H	H	CH_3	H	
40. *Rhamnocitrin*	CH_3	H	H	H	
41. *Catharticoside*	CH_3	H	H	Rhamninosyl*	
42. *Rhamnocitrin-3-isorhamninoside*	CH_3	H	H	Glc(6->1)Rha(4->1)Rha	
43. *Quercetin*		H	OH	H	H
44. *Rhamnetin*	CH_3	OH	H	H	
45. *Isorhamnetin*	H	OCH_3	H	H	
46. *Rhamnazin*	CH_3	OCH_3	H	H	
47. *Quercetin 3-rhamninoside*	H	OH	H	Rhamninosyl	

48. *Xanthorhamnin*	CH_3	OH	H	Rhamninosyl
49. *Rhamnazin 3-rhamninoside*	CH_3	OCH_3	H	Rhamninosyl
50. *Rhamnazin 3-isorhamninoside*	CH_3	OCH_3	H	Glc (6->1) Rha (4->1) Rha
51. *Rhamnetin rhamnoside*	CH_3	OH	H	Rhamnosyl

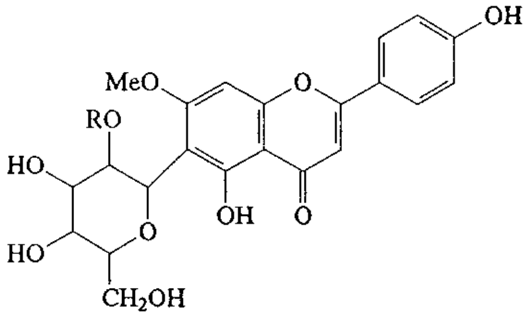
Rhamninose= Trisaccharide (Rhamnose + Rhamnose + Galactose



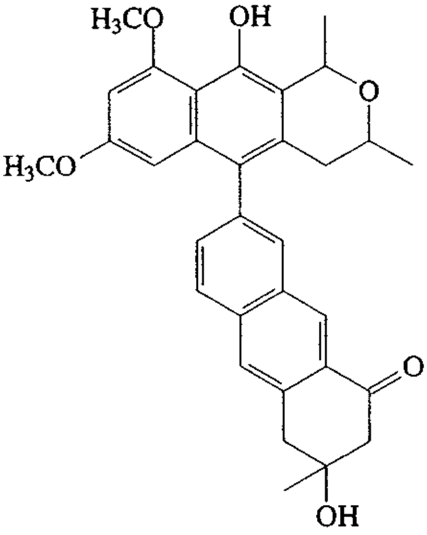
52. R = H : *Stypandrone*
53. R = OMe : *2-Methoxystypandrone*



54. R = H : *Taxifolin*
55. R = Glc : *Taxifolin-3-O-Glucoside*

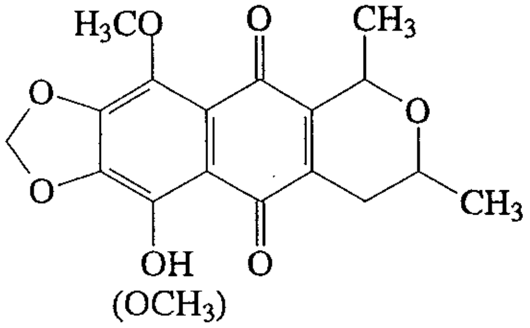


56. R = H: *Sweritisin*
57. R = Glu: *Spinosin*



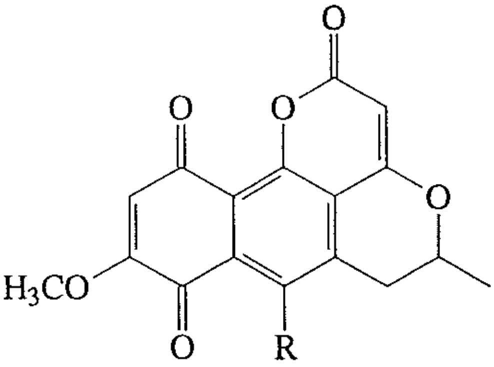
58.

Karwinskia toxin 1



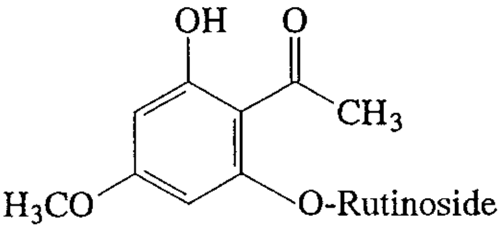
Ventiloquinone A

59.

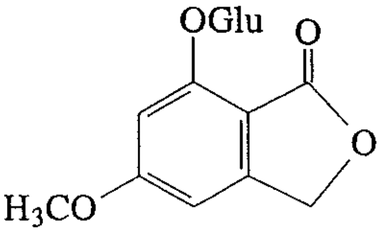


60. R = H: *Ventilatone A*

61. R= OH: *Ventilatone B*



66. 2,6-*Dihydroxy-4-methoxyacetophenone 2-O-ß-rutinoside*



67. *7-Hydroxy-5-methoxynaphthalide 7-O-ß-glucoside*

APPENDIX III

ORGANOLEPTIC EVALUATION FORM

I. Please read this paper before you start any activity. When you are through, take your own time intervals(total time = 10 minutes) and proceed accordingly. In answering each question, please be aware that any slight false response will turn the whole result unreliable. Also, know that you are indirectly evaluating your own sense organs.

The following three questions (1-3) are to be answered prior to testing any of the samples. Rinse your mouth with water to answer them.

1. Which alcoholic beverages, locally or industrially made, are you accustomed to?

2. Which taste group do you like most: SOUR, BITTER, SWEET, SALTY?

3. Which of the above taste groups did you feel when you just rinse your mouth with water? / Please write what you felt, not what you should have felt!/

II. Follow the procedure and answer each question accordingly. If you feel so, add an alternative choice which defines your feeling.
Note. Each sample is safe, so make sure that the sample has touched all your tongue's surface area (top and bottom).

Rinse your mouth with tap water before and after each taste. Start tasting from the left and take equal portion of the sample in all cases.

4. Label each sample by any of the four taste groups, as checked by your senses: SOUR, BITTER, SWEET, SALTY.

5. Which taste feeling do you think is common to any group of samples?

6. Comparatively, which sample showed any degree of difference from the others? Why did you choose it?

7. What beverage(s), locally or industrially made, have you come across (if any) showing

similar taste as each of the samples you are given?

8. What ingredient or substance can you distinguish in any of the three samples? Write the code number of the sample which made you feel so!

NAME:
AGE:
DATE:
SIGNATURE:

Thank you.

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