Flavonoids of *Lupinus princei* and *Lupinus albus*

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By
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ABSTRACT

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By

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This thesis covers the phytochemical investigation of two Lupinus species (Leguminosae), namely *L. albus* and *L. princei*.

L. albus L. known locally as "Gebto" (Amharic) is a plant which grows widely in Gojjam province. Chemical investigation of this plant afforded four known isoflavonoids, namely genistein, 2'-hydroxygenistein, 2'-hydroxydaidzein. 2'-Hydroxydaidzein is a new record for the genus *Lupinus*.

L. princei Harms locally known as "Dergie Gerbie" (Oromo) is an indigenous plant which grows in Sidamo province. The phytochemical investigation of this plant was carried out for the first time resulting in the isolation of 4 isoflavonoids and a triterpone. The compounds are genistein, 2'-hydroxygenistein, luteone, 2'-hydroxyisolupslbigenin and B-sitosterol. This is the second report of 2'-hydroisolupalbigenin, which was recently (1909) discovered to occur in the roots of *L. albus*.

The structures of the isolated compounds were principally deduced by spectroscopic techniques.

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1. INTRODUCTION

The genus Lupinus is one of the largest and widely distributed of the 600 genera in the family Leguminosae subfamily Papilionadeae, It has about 200 species and is found predominantly in Mediterranean region, Asia, North and South America, Tropical and Equatorial region of Africa A few of the species notably L, albus L., L* luteus L. and L, angustifolius L. are widely cultivated for use as forage crop, as source of edible oil and as food(2. A number of Lupinus species are also used in folk medicine of many countries!

In Ethiopia six Lupinus species are known to occur(5), four of these have been introduced into the country recently and these include L. luteus L., k* angustifolius L., L. mutabilis sweet and L. tnoxicanus Cerv. ex Lag, "Gebto" or L. albus L. (L. termis forsk) was probably introduced to North Ethiopia by the Egyptians many centuries back and cultivated in Gojam for fodder and for its edible seeds. The ripe seeds have a bitter taste which is removed by boiling. The only truely indigenous member of the genus is L, princei Harms which has so far been found only in Sidamo probince near Mega,

This species is also known to occur in

Kenya and Tanzania.(5) Nearly 30 species of *Luninus* hove so far been investigated for their chomical constituents. The most studied species are *L*, *anqustifolius*, *L*. *luteus*, *L*. *arboreus*, *L*. *albus*, *L*. *polyphyllus*. These phytochemical study have resulted in the isolation and characterization of nearly 45 alkaloids, 75 flavonoids and 10 miscellaneous compounds. The recent study on the roots of *L*, *albus*(6) (source of material - Experimental farm) resulted in the isolation of 17 isoflavonoids 13 of which turned out to be novel.

The aim of this project is to perform chemical investigation on the roots of *L*. *albus* and k* *Princei* of the two, literature reports are available only on *L*, *albus* which was grown in an experimental farm. On the contrary no prior report is avilable in the literature on *L*. *princei*,

2. LITERATURE BACKGROUND

2,1 Flavonoids of Lupinus Species

phytochemical investigation on roots and above ground parts of several species belonging to the genus Lupinus (Leguminosae - Papilionoidsae, tribe Genisteae, subtribe Lupininae) have shown this genus to be a rich source of flavonoids and isoflavonoids,(7)

Hitherto nearly 75 flavonoids have been isolated and structurally elucidated, A list of the flavonoids, so far known from Lupinus species, their structure, distributions and literature references are given in the Appendix.

2,2 The Flavonoid

Flavonoids constitutes one of the largest groups of naturally occuring phenols.(8) In plants flavonoids exist as aglycones (flavonoid without attached sugars), glycosides, sulfates and biflavonoids.(9)

2.2.1 Classification of Flavonoids

Flavonoids contain fifteen carbon atoms in their basic skeleton and these are arranged in a C_6-C_6-C_D manner in which the C_3 units links two aromatic rings which may or may not form a third ring,(9,10) For convenience the rings are labelled as A, B and C. The oxidation state of the C_3 fragment (ring C) and the size of this ring is involved in, form the basis for the classification of flavonoids to the major classes[11,12]

2,2.2 Isoflavonoids

In view of the fact that the genus Lupinus is a rich source of isoflavonoids, A brief review of the chemistry of isoflavonoids will be presented below.

Distribution and Occurrence

Isoflavonoids (aglycone + glycosides) of plant origin are known to be predominantly produced by species belonging to the large (about 430 genera; 12000 species) and taxomomically advanced subfamily Papilionoideae of the Leguminosae/Λ Their chief source of occurrence is in leguminous plants, some of which are very much in use as food materials. Moreover, leguminous plants sometimes exhibit remarkable physiological activities sometimes attributable to their isoflavone content.

2*2B3 Types ©f la®fXsvonoids

Although iaoflavonoids are biogentleally related to the flavonoide, they constitute a distinctly separated class, because they possess a 1,2 diphenyl propane skeleton Fig, 1 formed by the rearrangement of the flavonold skeleton, the Bering being attached to the 3 position of the hetro ring,ΛΛ

i

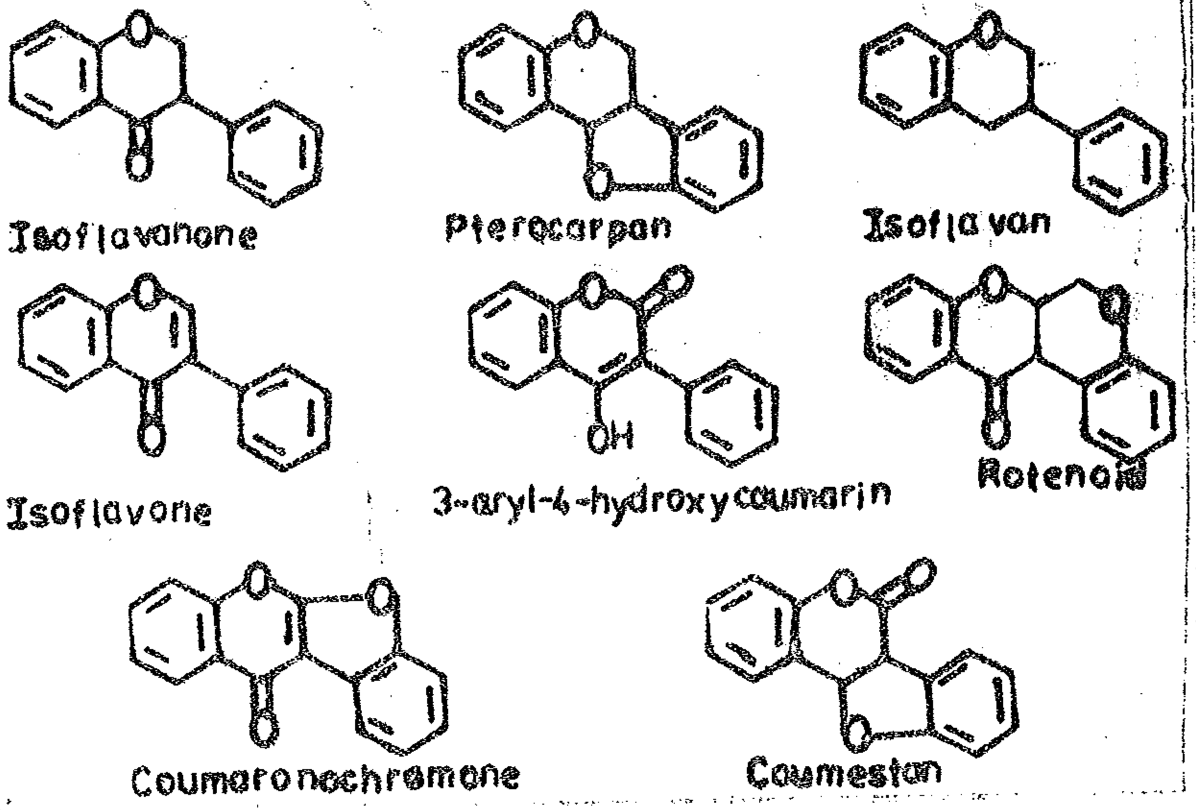
CUTD

if

Fig* i⊙ ffasic structural unit of ieoflavonoid◇

The branched Cg-C∧-Cg skeleton (Fig, %) ie characterised by its chemical flexibility, the various distinct isoflavonoid groups considered in Fige 2 arise via comparatively simple stepwise oxidative and reductive raodification at one or more of several central, or near central, molecular sites. Thus, despite their comparatively restricted distribution within the plant kingdom, the laoflavonoido as a V#hal® display a quite remarkable array of structures* 10)

Fig-2 Structure of various isoflavonoids



2.3 Biological Significance of Flavonoids

The flavonoids are remarkable in that they display an exceedingly diverse range of biological properties, some of which are particularly characteristic of certain compounds. Rotenone and many related rotenoid derivatives are highly effective fish poisons, an attribute not generally associated with other types of isoflavonoids. In fact, rotenoid containing plants belonging to genera such as Derris, Lonchocarpus, Piscidia and Tephrosia have long been used as fish poisons by tribal groups in parts of South America, tropical Africa and Asia.(13)

A considerable number of pterocarpans and isoflavans as well as certain isoflavones and isoflavanones especially those with isoprenoid substituents are extremely toxic to saprophytic and plant pathogenic fungi.(13) A great many species of the Leguminosae, typically produce isoflavonoid phytoalexins (*de novo* antifungal compounds) following infection of their leaves and other tissues by non pathogenic fungi. The first known phytoalexin isoflavonoids are pisatin from pea (*Piaum satium*) and phaseollin from bean (*Phaseolus vulgare*).(14) In contract, phytoalexins were apparently not produced by the fungus infected leaves or other tissues of *Lupinus* species, these species were characterized by the presence of constitutive antifungal isoflavones.(15)

The antifungal activity of the prenylated isoflavones wighteone and luteone, found in a number of *Lupinus* species have been recently demonstrated and may serve to highlight an important role for isoflavonoids in protecting plants against potentially pathogenic fungi. Apart from their action on fungi, it has been shown that a few isoflavonoids are potent feeding deterrent to certain larvae species. The isoflavan vestitol in *Lotus pedunculatus* and the isoflavone angustone-A in Lupinus angustifolius are found to have high insect feeding deterrant activity to feeding insect larvae. The isoflavones luteone, Licoisoflavone-A, licoisoflavone-B of L. angustifolius show the association of high antifungal activity, this suggests a dual defensive role for isoflavonoids in legumes against both insects and fungi.(16)

Certain plants commonly used for pasture grazing contain isoflavonoids that mimic the activity of animal oestrogens, regular ingestion of these substances for periods of 6 months to several years leading to the development of severe infertility problems in the female, which is often referred to as "clover disease" the legume most responsible for "clover disease" is Trifolium subterraneum (Subterranean clover). The cause was located in the isoflavone content of this plant, which includes formonontin, genistein and biochanin-A.

Some additional activity of isoflavonoids include antispasmodic activity (daidzein), antiarthritic activity (pseudobaptigenin), antiulcer activity (formononotin, 3'-methoxydaidzein, genistein), diuretic activity(tectorigenin), hypotensive activity (orbol, iristectorigenin-A.(13)

Rutin a flavone glycoside has been used with benefit in the coagulation of blood. It has been shown to have therapeutic effect for certain persons with thrombocytopenicpurpura.(17)

2.4 Biosynthesis of Flavonoids

All classes of flavonoid are closely related by a common biosynthetic pathway which incorporate precursors from both the shikimate and acetate malonate pathway.(18) The flavonoid initially formed in the biosynthesis is thought to be the chalcone and all other forms are derived from this. It has been established that ring-A is formed by a head to tail condensation of three acetate units and ring-B as well as carbon atoms 2,3 and 4 of the heterocyclic ring-C arise from phenylalanine.(19)

The transformation leading to formation of chalcone, consists of 3 successive condensation steps with acetate units which result in the elongation of aliphatic side chain of p-coumarate by six carbon atoms which then cyclizes to give the aromatic ring-A.(18,20)

The isoflavanoids share a common biosynthetic pathway with the other flavonoids as far as chalcone intermediates, but then a 1,2 aryl migration occurs to give the characteristic rearranged skeleton. The biosynthetic pathways which lead to the formation of isoflavonoids may be divided into three parts.

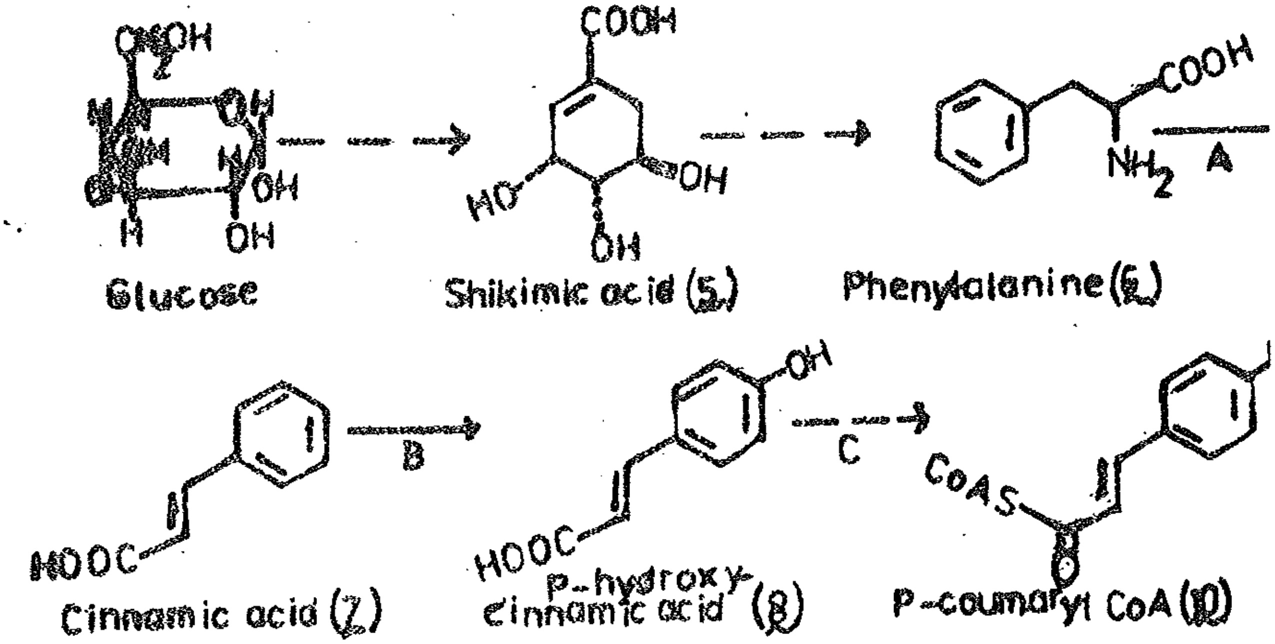
I. Those early pathways shared with other secondary metabolites. The essential C_{15} molecular frame work of both flavonoids and isoflavonoids results from the convergence of the acetate/malonate and shikimic acid pathways. The precursor for the acetate/malonate pathways is acetyl Co-A while P-coumaroyl Co-A (10) derived from phenylalanine (6) as shown in Scheme-1 serves as the precursor of the shikimate route.

II. Those steps common to flavonoid and isoflavonoid biosynthesis. The transformation leading to the formation of chalcones as shown in Scheme-2 consists of three successive condensation steps with acetate units which results in the elongation of the aliphatic side chain of P-coumarate by six carbon atoms which then cyclizes to give the aromatic ring A.

III. Those reactions unique to the isoflavonoids. Feeding experiments by Griseabach and Derr demonstrated that the isoflavone skeleton results from the flavonoid precursors by a 1,2-aryl migration of the B-ring. This shift is believed to take place after the formation of the C_{15} chalcone (14) intermediate, this was established by feeding experiments that showed the chalcone rather than the isomeric flavanone (15) acted as a substrate for aryl migration.(14,21) Thus the proposal that chalcones are the more immediate precursors of isoflavones enjoys general acceptance. Indeed, it is probable that only two chalcones 4,2,4',6'-trihydroxy chalcone and 4,2,4',4'- -tetrahydroxy chalcone (14) normally act as a substrates for aryl migration.(22) As shown in Scheme-3 the presence of a para hydroxyl group on ring-B of the chalcone precursor is essential for the rearrangement to isoflavone (18).

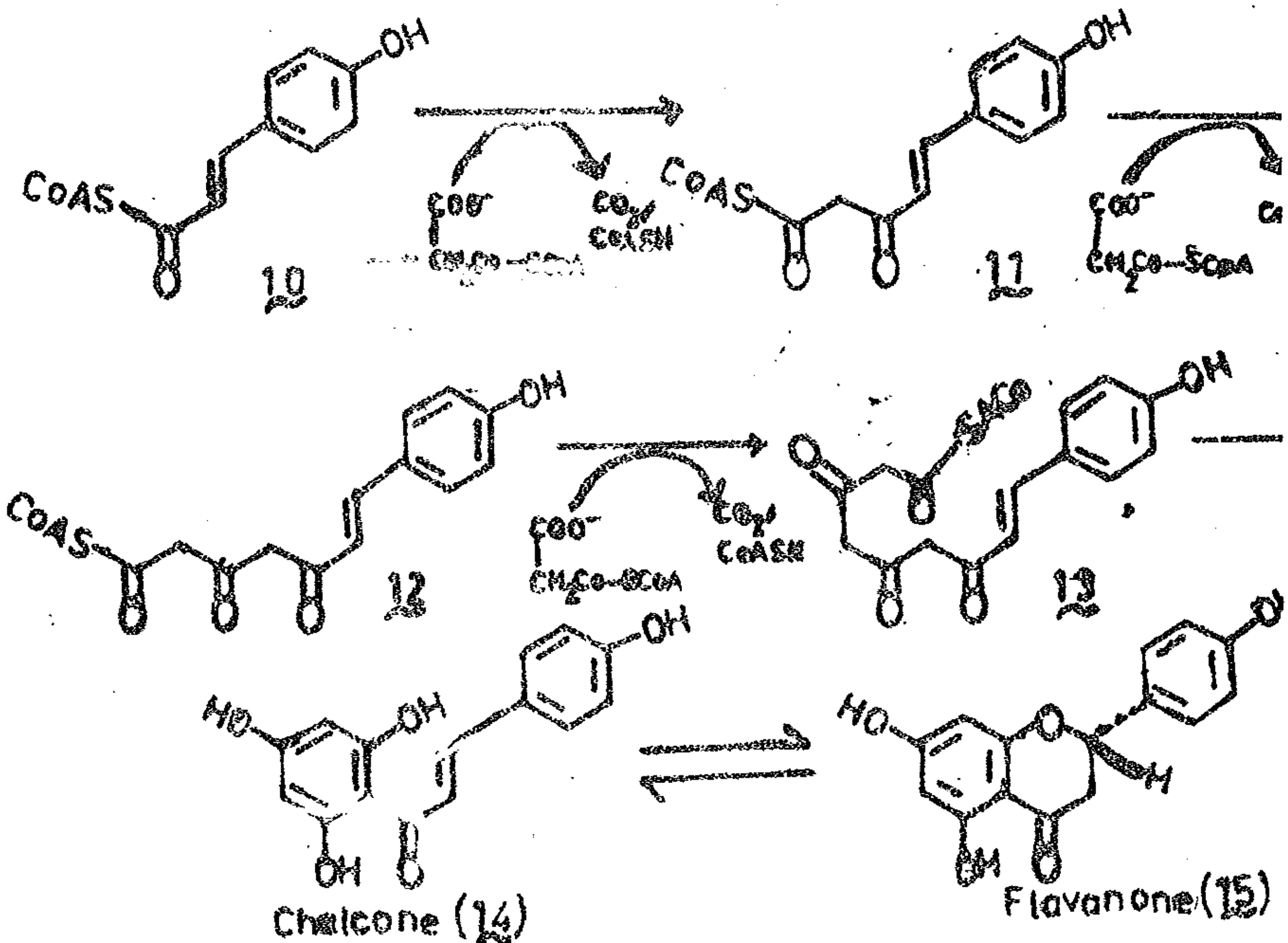
Isoflavones may be further oxidized either at C-2 leading to 3-aryl-4-hydroxy coumarins or at C-2' leading to 2'-hydroxy isoflavones. The presence of 2'-oxygenation is explicit in the majority of isoflavanones, isoflavans and complex isoflavans and implicit in the ring system of pterocarpans, coumestans and rotenoids.(18)

Scheme-1 Formation of P-coumaroyl CoA

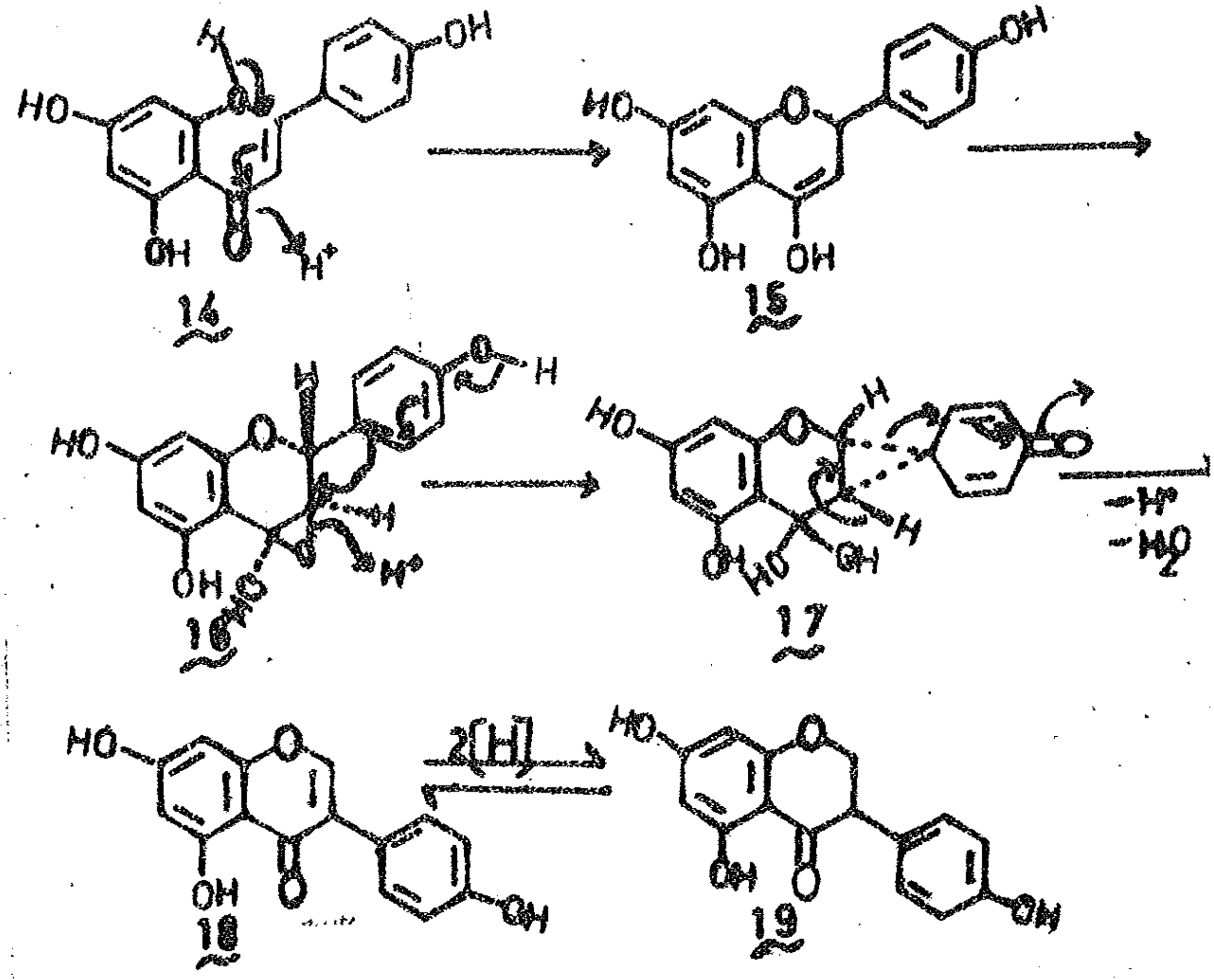


A - phenyl alanine lyase
B - Cinnamate-4-hydroxylase
C- 4-coumarate CoA ligase

Scheme-2 Biosynthesis of chalcones



Scheme-3 Proposed pathway for 1,2-aryl migration

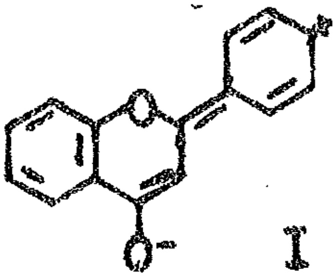


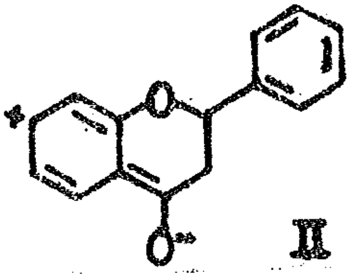
2.5 Spectral Properties of Flavonoids

The main steps generally followed in the structural elucidation of flavonoids are recognition of the class to which the compound belongs and determination of the nature and orientation of substituent groups in the aromatic rings. These steps are well served by the application of UV, IR, NMR and MS spectroscopic techniques.

2.5.1 Ultra-Violet-Visible Absorption Spectroscopy

UV absorption spectroscopy is one of the most useful techniques for flavonoid structural analysis. The spectrum exhibits two absorption maxima in the region 300-550 nm (Band-I) and 240-285 nm (Band-II). The position and intensities of these maxima give valuable information about the nature of the flavonoid and its oxygenation pattern. Changes in the substitution of the A-ring result in changes of Band-II while alteration of the B and C rings affect Band-I absorption.(9,12) Band-I is considered to be due to absorption of the B-ring cinnamoyl system (I) and Band-II with absorption involving the A-ring benzoyl system (II).


I


II

The introduction of electron donating groups such as hydroxyl in the B ring increases the relative resonnance contribution of (I) and consequently produce considerable bathochromic shift of Band-I. In isoflavones the phenyl ring at position 3 is not conjugated with the carbonyl group consequently, Band-I, which in flavones is associated with conjugated "B" ring, is either absent or considerably diminished in intensity. Isoflavones therefore, show one intensity maxima at 250-270 nm (Band-II) and a peak or inflection of very low intensities for Band-I.(12) Increased hydroxylation of isoflavones in the B-ring does not usually have a significant effect in the absorption of Band-II, in 5,7 dihydroxy isoflavone (259 nm), 5,7,4'-trihydroxy isoflavone (261 nm) and 5,7,3',4'-tetrahydroxy isoflavone (262 nm). However, Band-II is shifted bathochromically by increased oxygenation in the A-ring.(23) Additional information on the substitution pattern of the hydroxyl group can be made using shift reagents.

Shift reagents in UV-visible study of flavonoids

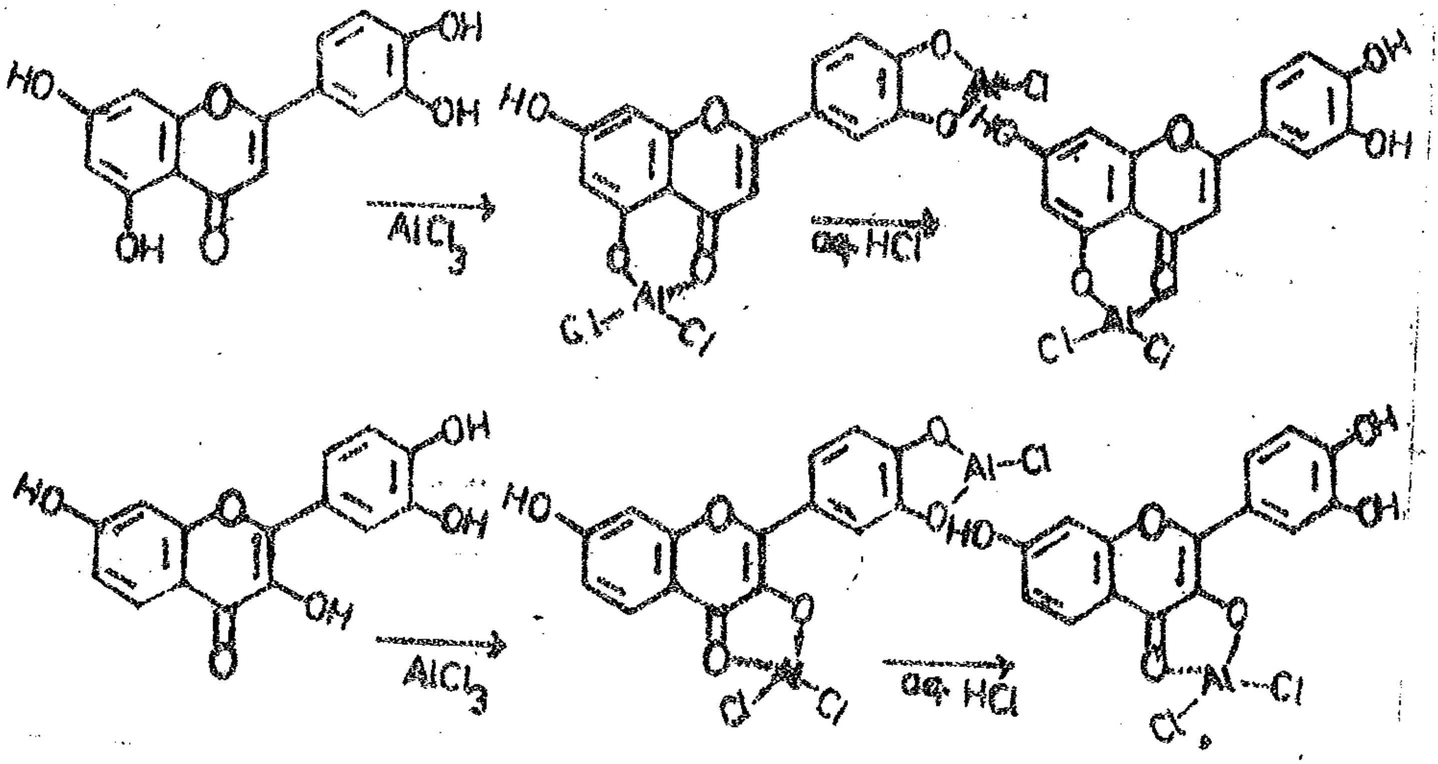
The oxygenation pattern of a flavonoid nucleus may be established by adding "shift reagents" to the sample solution and observing the resultant shift absorption peaks.(9,12,23)

Some of the shift reagents used are:

1) $AlCl_3/HCl$ (2) NaOAc (3) $H_3BO_3/NaOAc$

4) NaOMe

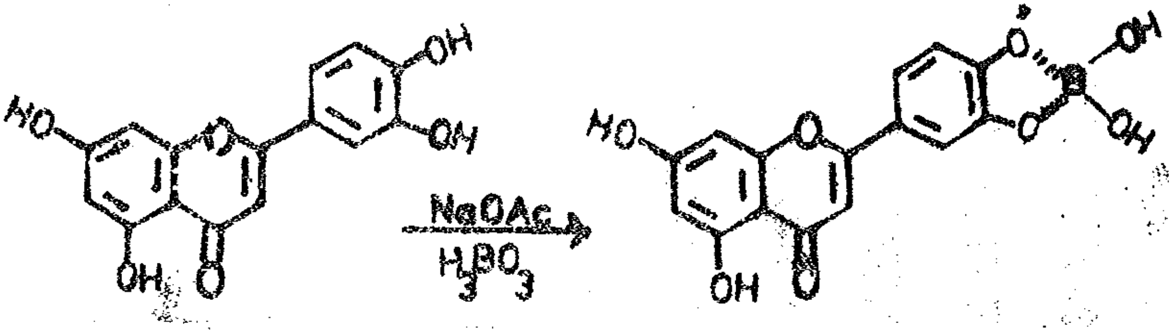
$AlCl_3$ has the ability of forming acid stable complexes between hydroxyls and neighbouring keto group and acid labile complexes with ortho dihydroxy groups as shown below.



The reagent can therefore be used to detect the presence of both groupings on the flavonoid nucleus. The $AlCl_3$ spectrum represents a bathochromic shift due to all of the complex formed. In $AlCl_3/HCl$ spectrum, however only the hydroxy keto complexes, will be observed because the acid labile complexes (complexes formed by ortho dihydroxyl groups) are destroyed by the acid. The $AlCl_3/HCl$ spectrum therefore indicates the presence of 3 or 5 hydroxy groups in the flavonoids nucleus and the presence of ortho dihydroxy group in the B-ring of flavonoids can be detected by a comparison of the spectrum of the flavonoid in the presence of $AlCl_3$ with that obtained in $AlCl_3/HCl$.(9,23)

NaOAc is a weaker base than NaOMe and ionizes the most acidic of the flavanoid hydroxyl groups that is, hydroxyl groups at position 3,7 and 4' of flavanoid nucleus. Ionization of 7-OH group mainly affect Band-II. NaOAc is useful diagnostic reagent for the specific detection of hydroxyl group at position 7.(9,23)

$NaOAc/H_3BO_3$ chelates with ortho dihydroxy group on the flavonoid nucleus as shown below.



Due to this chelate formation a bathochromic shift on the spectrum will be observed therefore, the reagent is used to detect the presence of ortho dihydroxyl groups on the flavonoid nucleaus.(9,23)

NaOMe is a strong base and ionizes to some extent all hydroxyl groups on the flavonoid nucleaus. Addition of NaOMe to flavonoids usually produce bathochromic shift in all absorption band, a large bathochromic shift of Band-I without a decrease in intensity confirm the presence of free 4'-hydroxyl group.(23)

2.5.2 Infra-red Spectroscopy

Infra-red spectroscopy is the primary probe for the detection of functional groups and electronic conjugation in a molecule.

The IR spectra of all the flavonoids and isoflavonoids show absorption bands in the region 1500-1600 cm^{-1} due to aromatic rings, along with a carbonyl band at 1620-1670 cm^{-1}. The presence of hydroxyl group in hydroxy flavonoids is evidenced by absorption in the region 3300-3450 cm^{-1}. An absorption at *ca.* 925 cm^{-1} is indicative of a methylenedioxy group and the presence of a *gem*-dimethyl group is indicated by the appearance of a band of *ca.* 1400 cm^{-1}. The glycosidic nature of a flavonoid is reflected by broad bands at *ca.* 3250 and 1060 cm^{-1}(24)

2.5.3 ^{1}H NMR Spectroscopy

The structural elucidation of flavonoids also rely on NMR techniques to a considerable extent. Chemical shifts as well as coupling patterns and coupling constant values provide information about the substitution pattern of flavonoids.

The proton of ring-A located at C-6 and C-8 of flavones, flavonols and isoflavones, which contain the common 5,7-dihydroxy substitution pattern give rise to two doublete (J = 2.5 Hz) in the range of 6.0-6.5 ppm due to *meta* coupling. As a result of the *ortho* shielding effect of the two oxygenated substituent the signal for H-6 is usually observed at higher field (6.0-6.2 ppm) than H-8(6.3-6.5 ppm). Attachment of sugar residue at C-7 causes a down field shift for H-6 and H-8 signals.(23) If a flavonoid is oxygenated only at the 7 position of the A-ring, the C-5 proton appear as a doublet due to *ortho* coupling (J = 9 Hz) with the C-6 proton and occurs at lower field ([illegible]) than the most aromatic protons are observed, this is due to the *ortho* deshielding effect of the C-4 keto group. In a fully substituted A-ring except at C-5, where the 6 position is oxygenated the C-5 proton appears as a singlet in the range 7.5-7.8 ppm. In this case, the <u>ortho</u> deshielding effect is minimized to certain extent by the <u>ortho</u> shielding effect of the C-6 oxygenated substituent.

The protons of ring-B usually appear in the range of 6.7-7.9 ppm which is down field from the region where the A ring proton absorb. The signal pattern observed for the B-ring protons is characteristic for the substitution of the ring. If the B-ring is in conjugation with a double bond of ring-C as in the case of isoflavone, flavone and chalcone, H-2' and H-6' appear together and likewise H-3' and H-4' appear with H-5'. If ring B is oxygenated at C-4' a typical four peak pattern of two doublets (J = 8.5 Hz) is observed. The doublete for H-3' and H-5' always appear up field (6.65-7.1 ppm) from the H-2' and H-6' protons (7.2-7.5 ppm) because they will be shielded by the C-4' oxygen substitution. For a C-3', 4'-oxygenated B-ring the NMR spectra will be more complex than the C-4' oxygenated isoflavones. In such a case multiplet of ABX pattern at the region (6.7-7.1 ppm) is observed.

Considerable variation is found in the chemical shifts of the C-ring protons among the different flavonoid class depending upon the oxygenation level of the C-ring. The C-3 proton is flavones gives a sharp singlet near 6.3 ppm while the C-2 proton in isoflavone which is in the <u>beta</u> position to the C-4 keto function, occurs in the range 7.6-7.8 ppm down field from where most aromatic proton signal appear. Thus ring-C proton chemical shift values aid in differentiating flavone from isoflavone.

The presence of a methoxyl and methylene dioxy groups is readily recognized by the appearance of strong signal at 3.5-4.1 ppm and at about 6.0 ppm respectively.(23)

2.5.4 ^{13}C NMR Spectroscopy

^{13}C NMR spectroscopy aids for elucidation of the structure of flavonoids and related compounds. All fifteen signals due to the flavonoid nucleus resonate in a region 90-200 ppm in the case of 2,3-unsaturated flavonoids and isoflavonoids whereas 2,3-saturated flavonoids and isoflavonoids possess only thirteen signals in this region, the two additional signals resonating at higher field. The flavanoids, pterocarpanoids and chalcanes possess three aliphatic resonances and twelve aromatic resonances in above mentioned chemical shift range. The presence of many signals in the 60-80 ppm region is generally indicative of glycosidic carbons.(24)

The carbonyl signals of both flavone and isoflavone resonate in the region 174-178 ppm but C-2 and C-3 are sufficiently different in the two series to permit an immediate distinction. In the flavone the C-2 signal appear at 160-163 ppm and that of C-3 104-118 ppm, whereas in the isoflavones the C-2 resonance is seen at 149-155 ppm and that of C-3 122-123 ppm. A carbonyl resonance of 181 ± 1 ppm clearly indicates the presence of a hydroxyl moiety at C-5.(25)

A 4'-substituted isoflavones (OH, OCH_3) is well characterized by signal sequences 123+2 ppm, 130+1 ppm (strong) 114+1 ppm (strong) and 152+2 ppm. 55 ppm resonance is very likely due to a 4'-methoxy group while all other methoxy groups of isoflavones resonates near 56 ppm.(26)

2.5.5 Mass Spectrometry

Mass spectrometry is one of the most useful spectroscopic techniques used for flavonoid structural elucidation. The main application of MS in structural analysis of flavonoids are:

1) Determination of molecular weight of the compound.

2) Establishment of the distribution of substituents between the A and B rings.

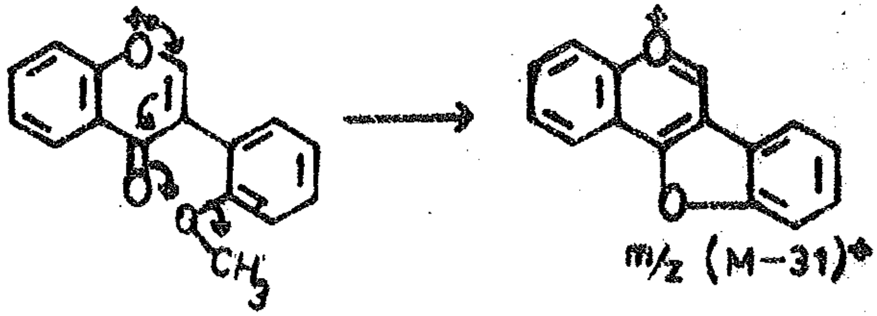
3) Determination of the nature and site of attachment of the sugar in flavonoid C and O-glycosides. The first objective in interpreting a flavonoid MS is to identify the unfragmented molecular ion (M^+), and then to relate other major fragments to it by rationalizing the loss in molecular weight using recognized fragmentation pathways.(9) The commonly encountered fragements in the MS of flavonoids are:

A) The molecular ion (M^+) appears as a major peak in the MS of aglycones and the exact mass measurment of this permits calculation of the elemental composition of the aglycone.

B) M^+-1 loss of hydrogen is common in most flavonoid containing 3-OH or 6-OH group and flavone methyl ethers which have an OMe group at either the 3 or 5 position, because these give rise to a stable quinonoid ions.

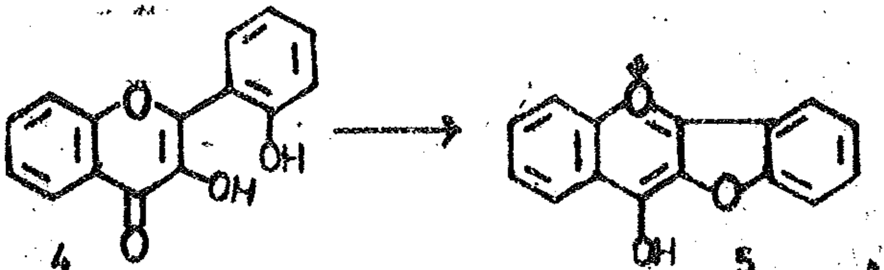
C) M^+-15 For flavonoids containing a methoxyl at C-6 or C-8, the loss of CH_3 produces an intense ion which may be more intense than M^+, lower intensity M-15 ions can originate from flavonoids O-methylated at other sites.

D) M^+-17 loss of OH is indicated by the presence of M-17 ions, this usually involves internal ring formation and is commonly associated with 2'-hydroxylation in flavones, flavonols, isoflavones etc. A loss of 31 mass unit may indicate loss of $^{\bullet}OCH_3$ from 2'-methoxylated isoflavones that are unique for the 2'-substitution pattern,



Scheme -6

In similar way 2',3-dihydroxy flavone (4) was shown to lose 17 mass units to give as its base peak an ion of postulated structure (5), suggesting that this fragmentation might be diagnostic for 2',3-dihydroxy flavones.(9,27)



4 5

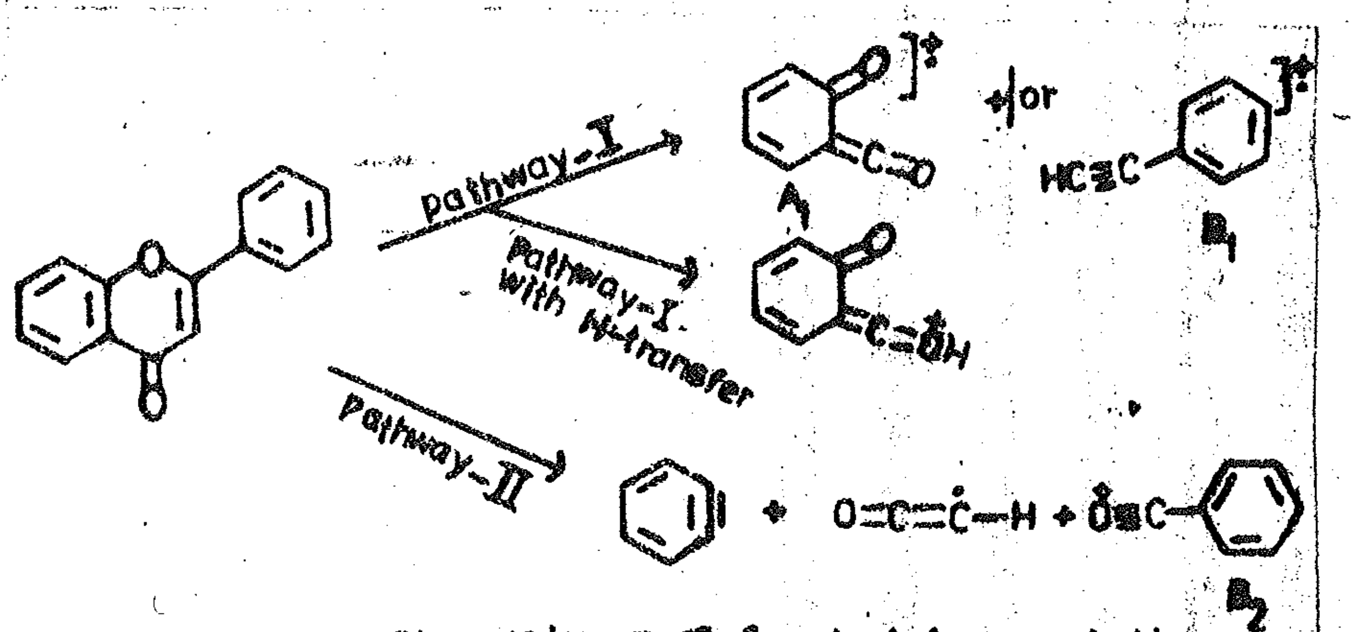
E) M^+-18 loss of H_2O is common in a flavens, flavan 3,4-diols and c-glycosides.

F) M^+-28(29) indicates loss of CO(CHO) from the 4 keto function to form a 5 membered ring (especially with 3-hydroxy flavones and dihydroflavonols).

G) M^+-43 The loss of CH_3 in concert with CO (ie. CH_3CO) may also occur in methoxylated flavonoids leading to a loss of 43 mass units, it is most intense in the spectra of 6-methoxy flavones and isoflavones.

H) M^+-55 (56) These ions may indicate the presence of a prenyl ($-CH_2-CH=C(CH_3)_2$) substituent and relate to the loss of a portion of the substituent.

I) A and B ring fragments - Fission of the M^+ ion into A and B ring containing fragments of ten aid in the identification of the substitution pattern, these fragmentation usually involve one of the two competing pathways, I (retro-Diels Alder) and II (See Scheme-5). The dominant pathway is determined by the aglycone type, although on occasions neither pathways produces significant fragments.(24)



Scheme-5 Diagnostic mass Spectral fragmentation Pathway for flavones

Flavones and isoflavones tend to produce $A_1^+(A_1+H^+)$ plus B_1^+ fragments; flavonols $(A_1+H)^+$ plus B_2^+ fragments; flavanones, (A_1^+, A_1+H^+) plus (B_1+2H^+) fragments.

Chalcones tend to produce fragments derived from cleavage either side of the carbonyl, although 2'-hydroxychalcones may isomerize to flavones and produce typical flavone fragments instead.(9)

The useful diagnoistic RDA fragmentation is almost entirely absent in the spectra of flavones with four oxygen substituents (OH or OMe groups), and the spectra are dominated by such ions as the molecular ion, the (M-15) ion, the (M-28) ion and the (M-43) ion.(27)

3. RESULTS AND DISCUSSION

The genus *Lupinus* with nearly 200 species is well known for elaborating interesting secondary metabolites such as isoflavonoids. To our knowledge so far 31 species have been subjected to phytochemical study and an attempt has been made here to list all the flavonoids so far isolated in the Appendix.

TLC examination of the crude ethanolic extract of the roots *L. albus* and *L. princei* using solvent system-2B showed 9 spots for the former and 10 spots for the latter (Fig. 3) combined column chromatography and prep. TLC on the crude extract of *L. albus* resulted in the isolation of 4 isoflavonoids, on the other hand *L. princei* aforded 4 isoflavonoids and one triterpene.

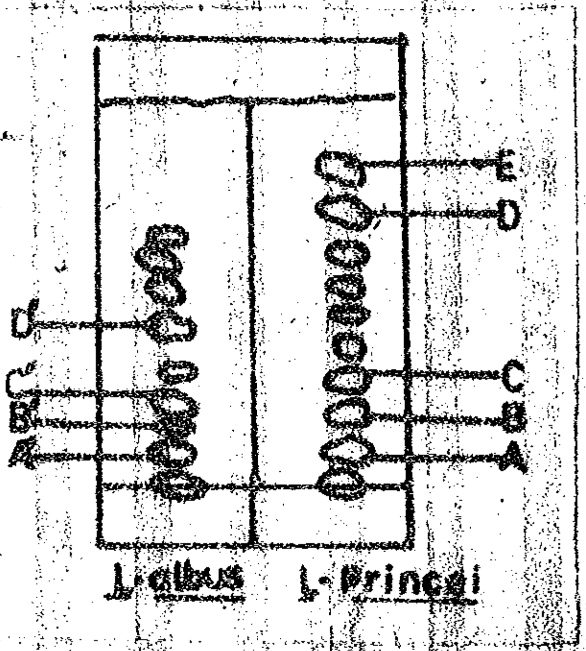


Fig. 3 Comparison of the TLC Chromatograms of *L. albus* and *L. princei*

As shown in Fig. 3 the compounds isolated from *L. albus* and *L. princei* were named as A', B', C', D' and A, B, C, D, E respectively, based on increasing TLC R_f values. Compound A' and C' that correspond to 2'-hydroxygenistein (13) and genistein (9) isolated from *L. albus* were reisolated from *L. princei* as compound A and B.

The characterization of these compounds have been done mainly by spectroscopic methods. The spectral and physical data of the known compounds isolated have been compared with those reported in the literature. In some cases characterization was augmented by direct comparison with authentic samples.

3.1 Compounds Isolated from *L. princei*

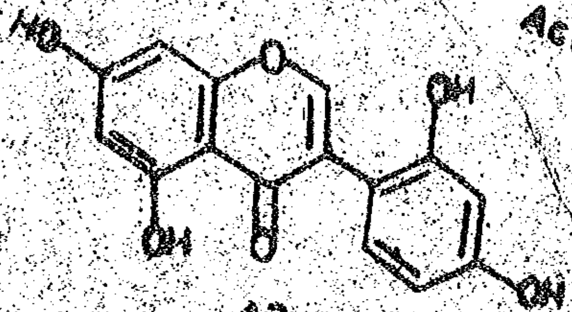
3.1.1 Characterization of Compound-A as 2'-hydroxygenistein (13)

This yellow amorphous solid, MP 223 - 225°(dec) showed a strong molecular ion peak at m/z 286 in its low resolution MS.

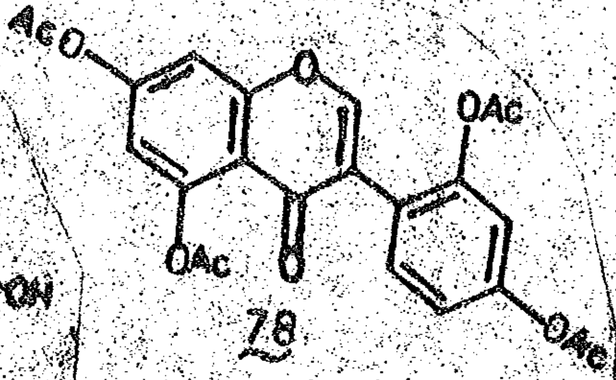
The IR spectrum showed the presence of an OH (3250 cm^{-1}) and a chelated carbonyl

(1645 cm^{-1}). The UV spectrum displayed an absorption maximum at 262 nm (MeOH). A bathochromic shift with NaOAc (+8 nm), $AlCl_3$(+7 nm) and NaOMe (+7 nm) revealed a 5,7-dihydroxy isoflavone (28). The ^1H NMR showed an isoflavone H-2 singlet (δ7.9). The <u>meta</u> coupled doublets (J = 2 Hz) at 6.30 and 6.50 suggest the presence of a hydroxyl group at 5 and 7 position of ring A. The presence of a peri OH group was further established by the DMSO-d_6 NMR spectrum which displayed a singlet at δ 12.71. The 2',4'-dihydroxylation nature of ring B was evident from the proton resonances at δ 6.45 (incomplete doublet, H-3'), 6.40 (dd,J = 7.8 and 2.2 Hz, H-5'), 7.10 (d, J = 7.8 Hz, H-6') in the ^1H NMR which is comparable with the reported data (30). Furthermore, the characteristic fragment ion peaks caused by usual retro-Diels Ader cleavage with H transfer were apparent at m/z 153 (100%) (A ring derived fragment with dihydroxylation). Support the <u>meta</u> disposition of the two hydroxyls in ring B is obtained from ^{13}C NMR spectrum (Table 3) which shows diagnostic signals at 159.4 and 159.9 ppm due to <u>meta</u> carbons bearing hydroxyl substituent. The presence of four hydroxyl groups was provided by the formation of a tetracetyl derivative (78) which displayed four methyl signals (2.2, 2.3, 2.41, 2.42),6.5-6.6 (unresolved dd, H-6 and H-8), 6.8-6.9 (unresolved d, H-5' and H-6') and 7.8 (5, H-2) in its ^1H NMR spectrum.

All the above spectroscopic data allow for the assignment of structure (13) for this compound. 2'-hydroxygenistein (13) is a known natural product which was earlier isolated from the roots of *L. albus* by Tahara and Mizutani (30).



13



78

Table - 1

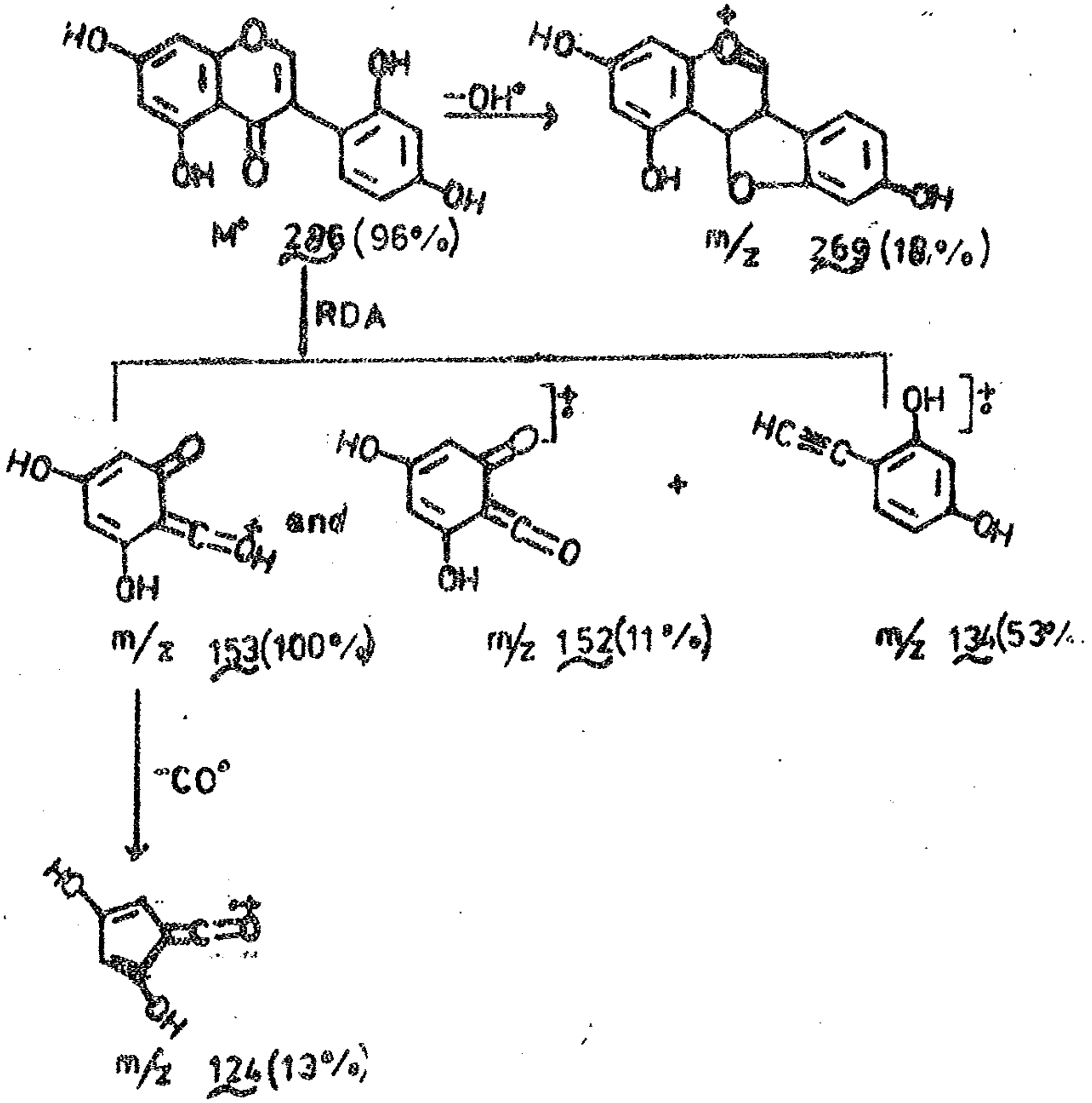
<u>^{13}C Spectral data of Compound A (2'-hydroxygenistein)</u>

(22.5 MH_z, $CDCl_3$ - CD_3OD (1:1)

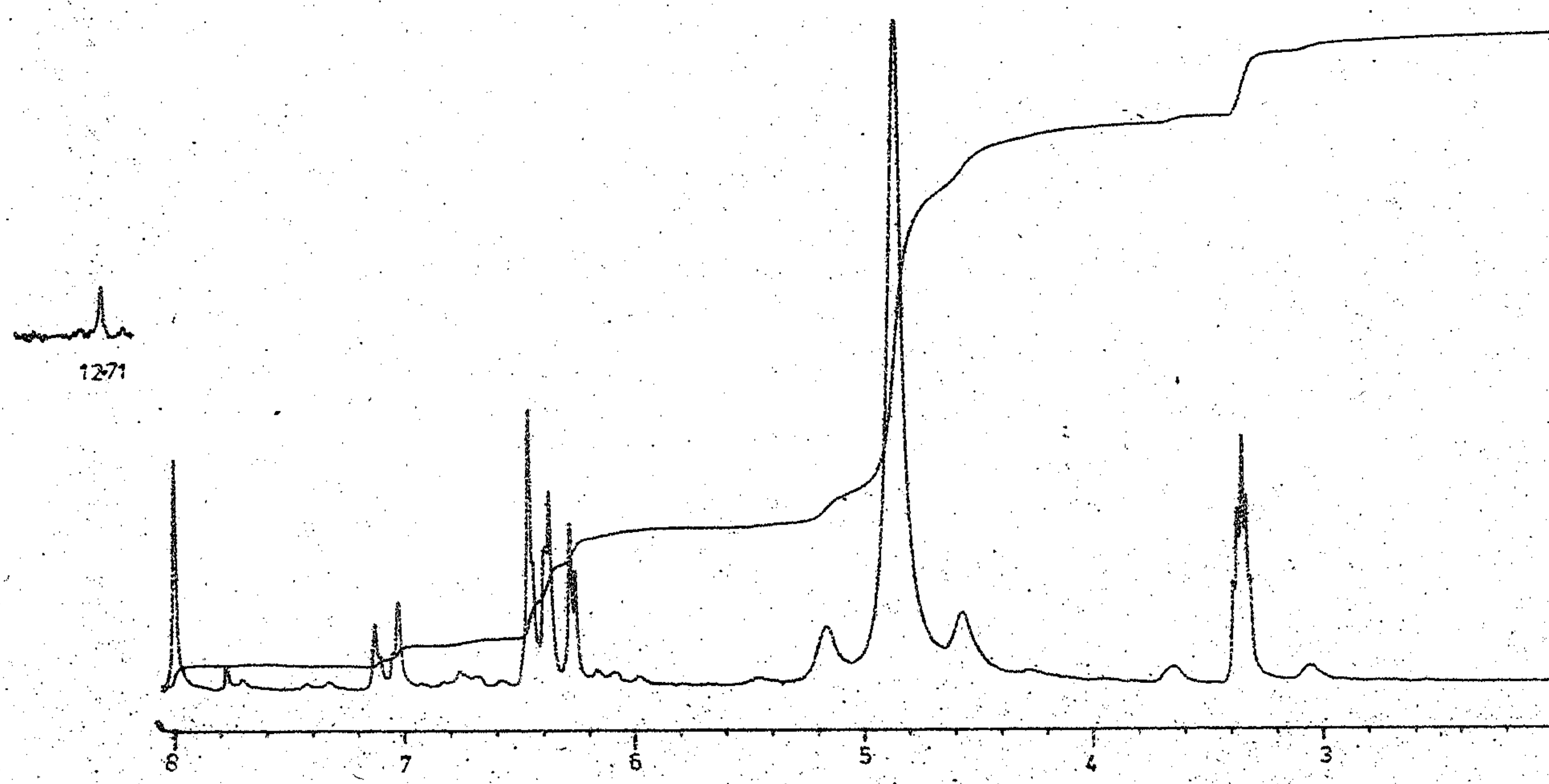
Carbon	ppm
2	156.2
3	129.1
4	182.7
5	163.1[a]
6	100.6
7	165.7[a]
8	95.1
9	157.4
10	111.4
1'	122.8
2'	159.4[b]
3'	105.4
4'	159.9[b]
5'	108.9
6'	132.6

Note: a,b assignment may be interchanged.

Scheme-8 MS spectral fragmentation of 2'-hydroxygenistein



Fig— 5 ^1H NMR spectrum of compound-A or 2'-hydroxygenistein (13)



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EXREF  49.5000PPM
OBS  1228.2714.52
NGAIN        5


NO FREQ(HZ)    PPM    INTZ
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 2  3732.91  165.659  2786
 3  3603.82  159.930  2947
 4  3591.19  159.378  2426
 5  3547.79  157.444  3247
 6  3520.33  156.225  3781
 7  2988.59  132.627  4414
 8  2812.81  124.826 -1022
 9  2456.38  109.005  4174
10  2375.55  105.422  4074
11  2268.43  100.668  4074
12  2144.29   95.159  3622
13  1801.51   79.947  2882
14  1736.69   77.071  3248
15  1159.36   51.459  4079
16  1137.94   50.499  8196
17  1115.97   49.524  9951
18  1094.54   48.573  8297
19  1073.12   47.622  4392
20   812.74   36.068 -1963
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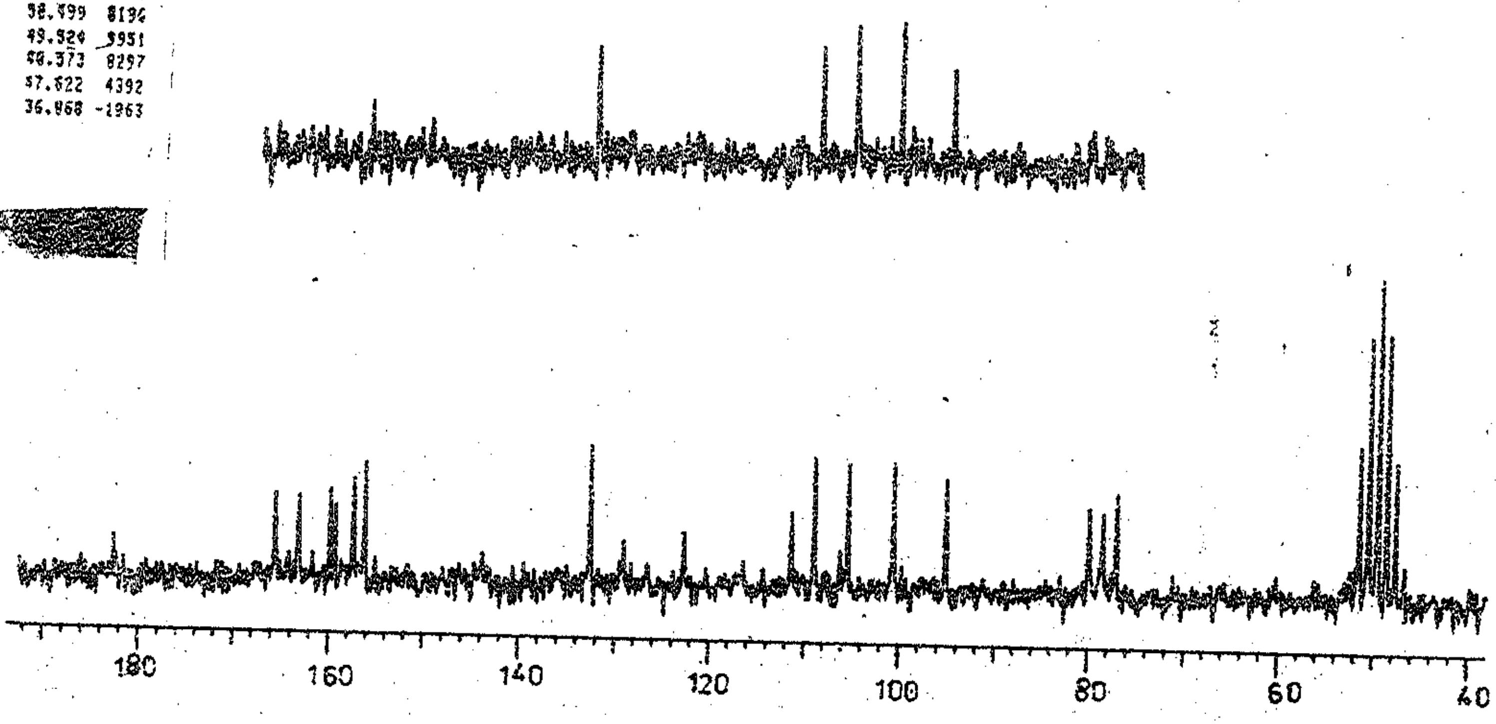
CDCl$_3$— CD$_3$OD

90 MHz

Fig-6 ^{13}C NMR spectrum of compound-A or 2'-hydroxygenistein (13)



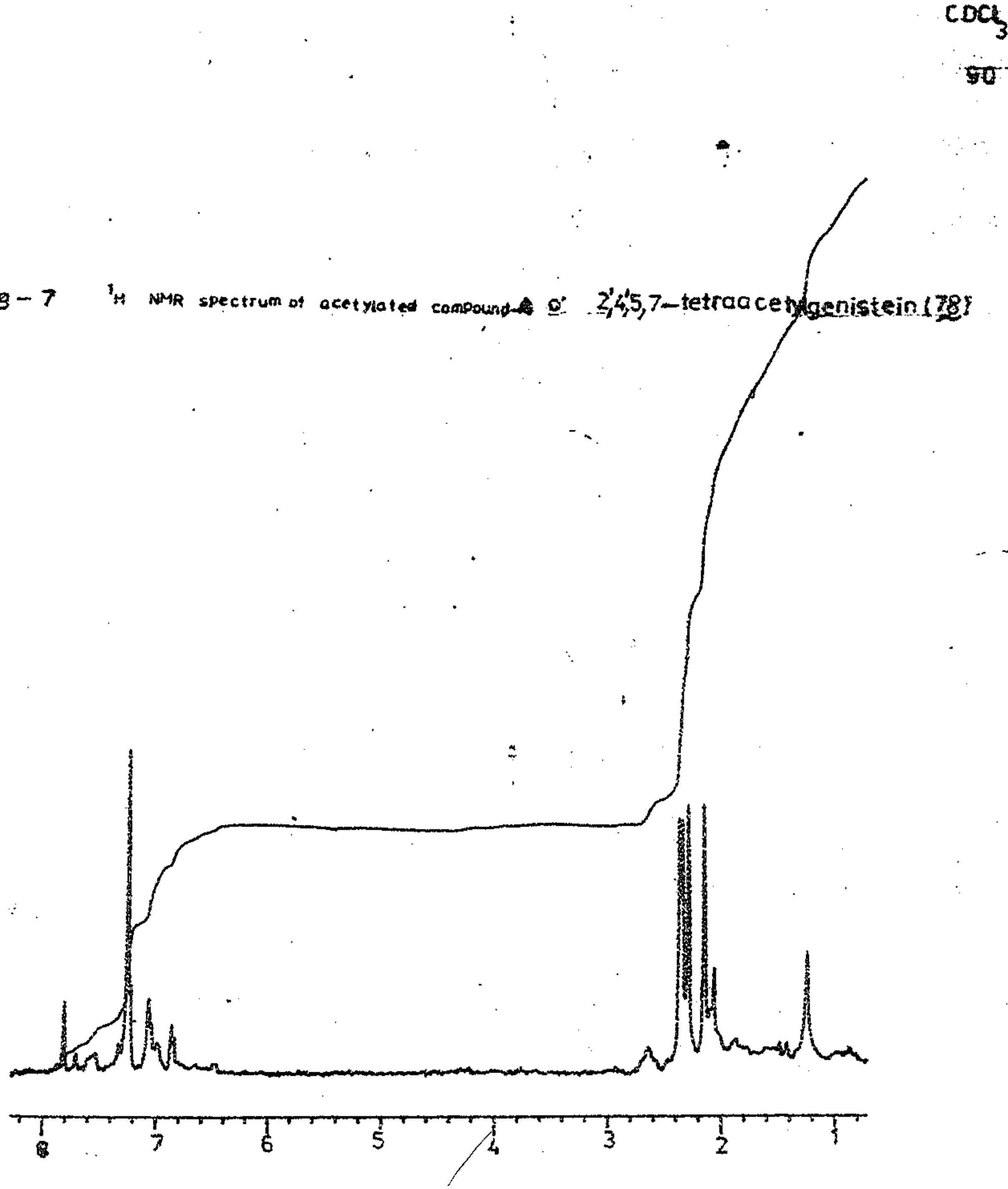


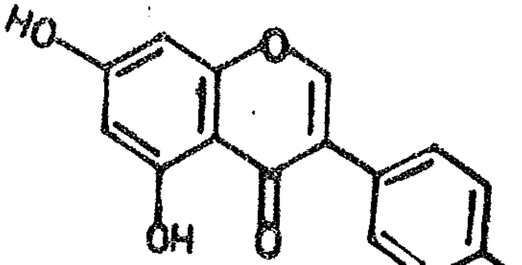
Fig – 7 ^1H NMR spectrum of acetylated compound-A or 2',4',5,7-tetraacetylgenistein (78)

3.1.2 Characterization of Compound-B as Genistein (9)

Compound-B is a pale yellow amorphous solid, MP 220-223°(dec), a homogenous on TLC (R_f 0.54 solvent system 1A) that turned brown when sprayed with 0.5% aq. fast blue B followed by 0.1 N NaOH. The IR spectrum displayed absorption bands at 3350 cm^{-1} (OH) and 1620 cm^{-1} (chelated carbonyl) (24). The UV spectrum showed absorption maxima at 261 nm (MeOH). A bathochromic shift with NaOAc (+9 nm), $AlCl_3$ (+6 nm) and NaOMe (+10 nm) revealed that this compound is a 5,7-dihydroxy isoflavone.(28) The presence of two meta coupled doublets (J = 2 H_z) at 6.30 and 6.40 in the ^1H NMR spectrum further supported the UV finding discussed above. Apart from the isoflavone H-2 singlet (δ 7.9) the ^1H NMR spectrum consists of two pairs of ortho coupled proton at δ 7.25 (H-2' and 6') δ 6.92 (H-3' and 5') this fact recquired the placement of an OH at 4'-position. This was further supported by ^{13}C NMR spectrum (Table - 4). The chemical shifts of C-2' and 6' (δ 129.8) and C-3' and 5' (δ 115.2) are comparable with the chemical shifts reported for carbon atoms (δ 130.0 and 115.2) in similar compound.(29) The location of two hydroxyls on ring A and the mono hydroxy substituent on ring B, was apparent from the MS. RDA fragments were observed at m/z 152 (64%), 153 (RDA with H transfer 42%) and

118 (32%) and a rationalization of their formation is given in Scheme 7.

Based on these information (^1H NMR, ^{13}C NMR, IR, UV EIMS) structure-9 could be assigned to compound B. This structure corresponds to a natural product genistein that has been isolated from some plants belonging to the genus Lupinus.



9

Scheme - 7 MS spectral fragmentation of genistein

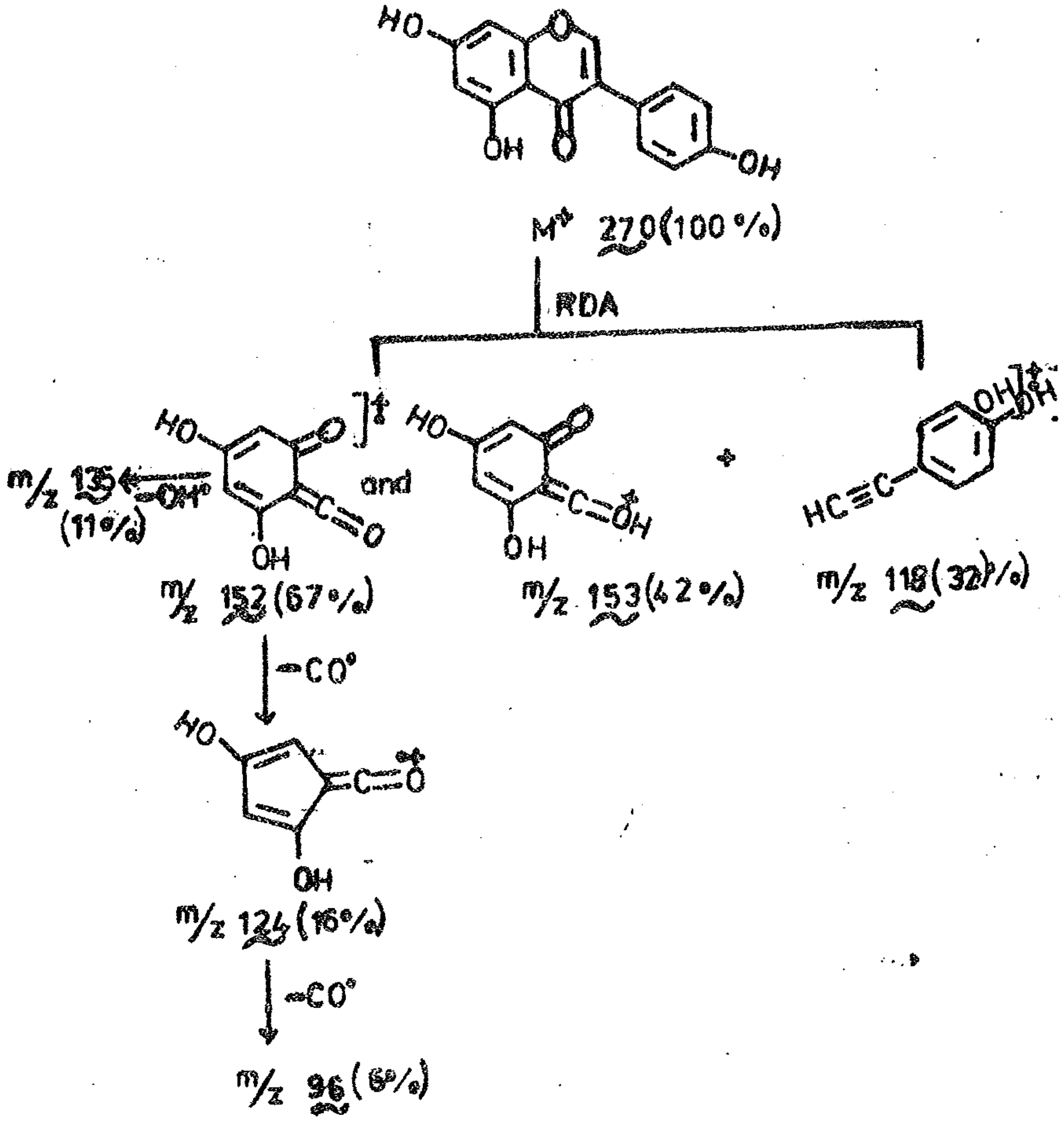


Table 2

^{13}C NMR Spectral Data of Compound-B (genistein)

(22.5 MH_z, CD_3OD = $CDCl_3$ (1:1)

Carbon	C-Shift in ppm
2	152.5
3	121.2
4	
5	162.0^{xx}
6	93.7
7	163.9^{xx}
8	99.1
9	157.0^{x}
10	105.2
1'	122.2
2'	129.8
3'	115.2
4'	156.8^{x}
5'	115.2
6'	129.8

Note: ^{xx,x}interchangeable pairs

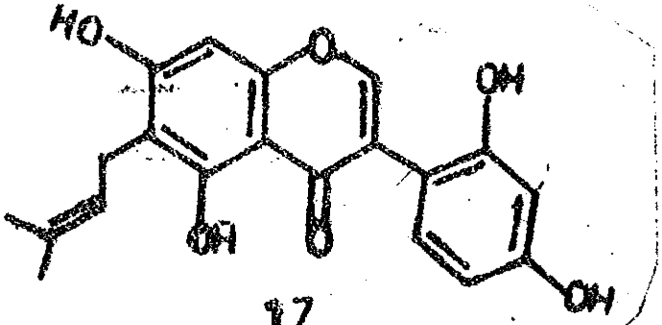
Carbonyl carbon (C-4) signal was not observed.

3.1.3 Characterization of Compound-C as Luteone (17)

This pale amorphous solid, MP 190-192^{0} showed in its IR spectrum on OH absorption (3400 cm^{-1}) and a chelated carbonyl (1630 cm^{-1}) band. The presence of a dimethyl allyl substituent was evident from the proton resonances at δ 1.7 (3H, s), 1.80 (3H, s), 3.41 (2H, brd, J = 6.8 H$_{z}$) and 5.3 (1H, brt, J = 6.8 H$_{z}$) in the ^{1}H NMR spectrum (Table 6). In addition, the singlet at δ 6.58 and 7.9 ppm are assignable to H-2 and H-8. The placement of the prenyl group at C-6 was supported by the fast and intense blue colour formation of Gibb's test.$^{(31)}$ In the EIMS, the fragment ion peaks at m/z 299 (100%) and 311 (78%) must originate from fragmentation with the loss of the prenyl side chain from the molecular ion. The ion at m/z 220 (7%) presumably originates from a RDA cleavage of ring A and therefore suggests the placement of the prenyl group together with two OH group on ring A. A rationalization of the mass spectral fragmentation is given in Scheme 8. The UV spectrum displayed absorption maxima at 261 nm (MeOH), a bathochromic shift with NaOAc (+11 nm) and AlCl$_{3}$ (+6 nm) revealed a 5,7-dihydroxy isoflavone.

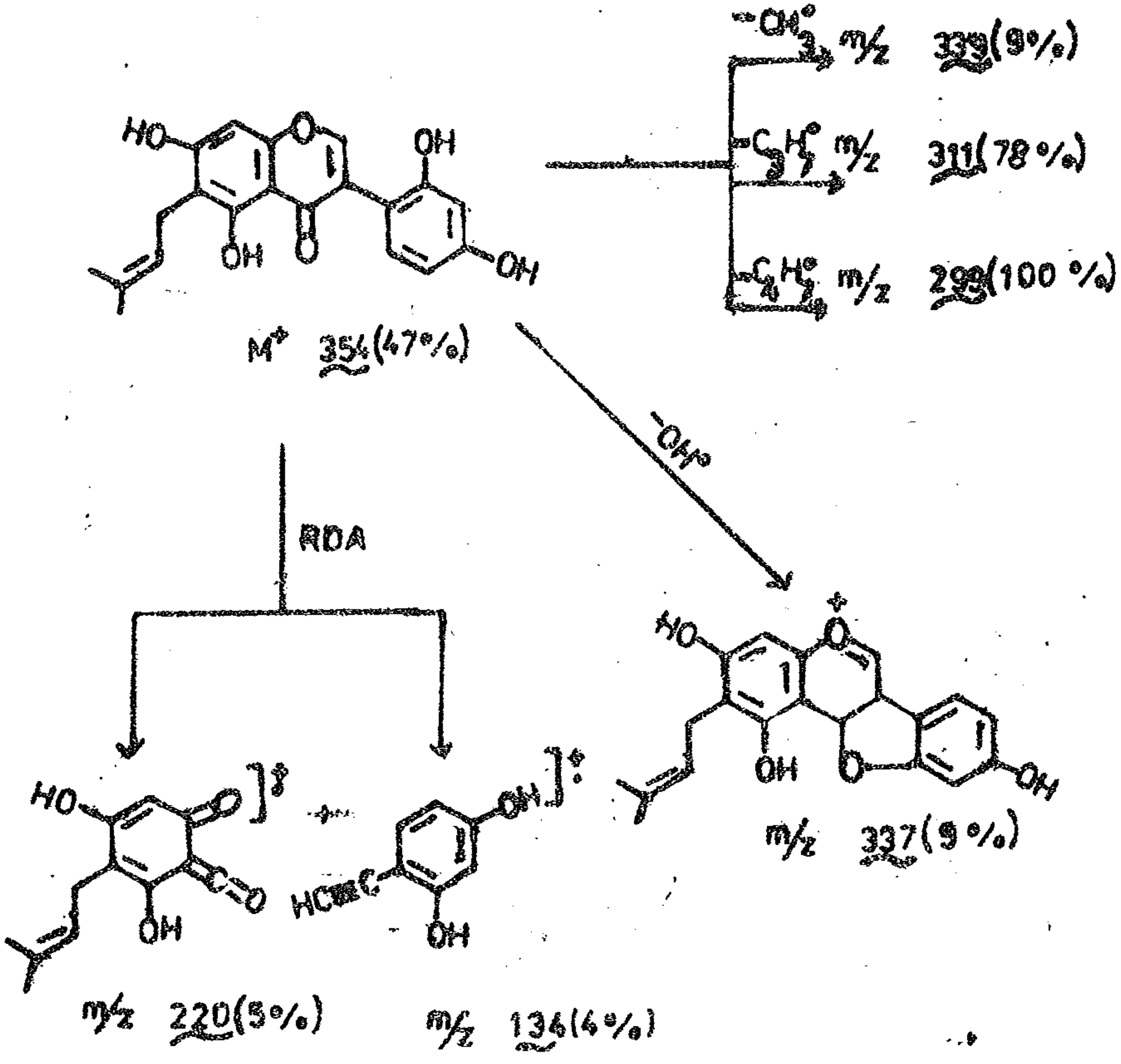
The 2',4'-dihydroxylation nature of ring B was evident from the ^{1}H NMR spectrum which displayed signals at 6.41 (d,J = 1.8 H_z, H-3'); 6.5 (dd,J = 8 and 1.8 H_z, H-5') and 7.10 (d,J = 8 H_z, H-6'). Moreover, a minor MS fragment at m/z 134 (5%) which presumably originates from a RDA cleavage of ring B through loss of ring A further supports the 2',4'-dihydroxylation of ring B. The ion at m/z 337 which originates from the molecular ion by loss of $^{\bullet}$OH is also characteristic of 2'-hydroxylated isoflavonoids.

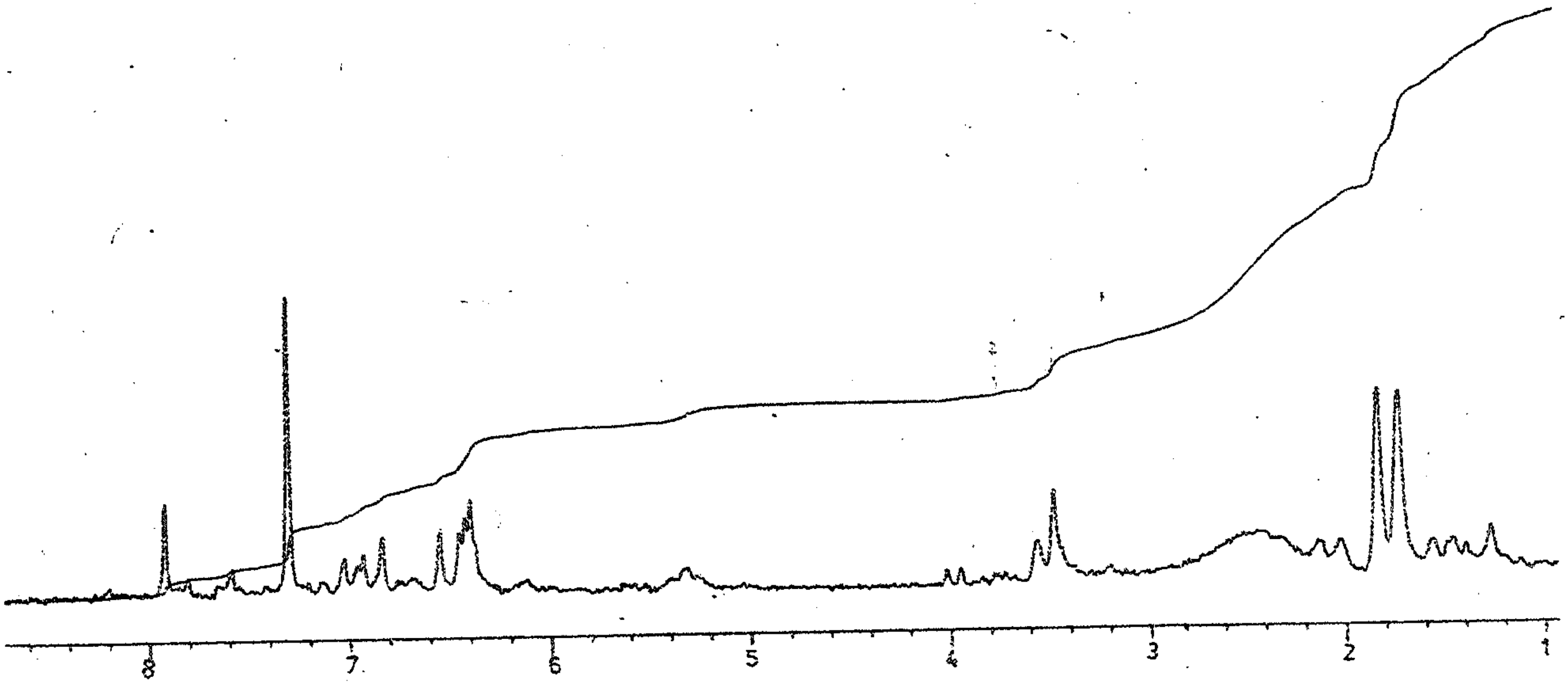
The above presented evidence is consistent with the structure assigned for luteone (17). Luteone (17) has previously been reported from some plants belonging to the genus *Lupinus*.(7,40)



17

Scheme—8 MS spectral fragmentation of luteone





CDCl$_3$ & drops of CD$_3$OD

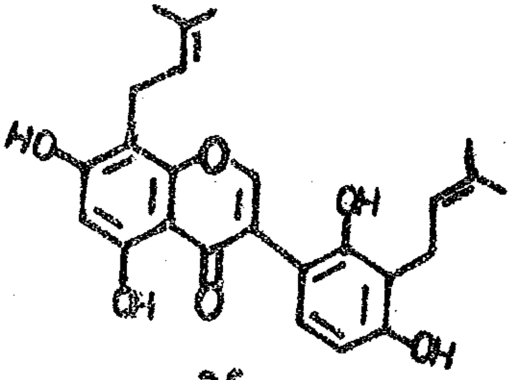
90 MH$_z$

Fig— 8 ^{1}H NMR spectrum of compound-C of luteone (17)

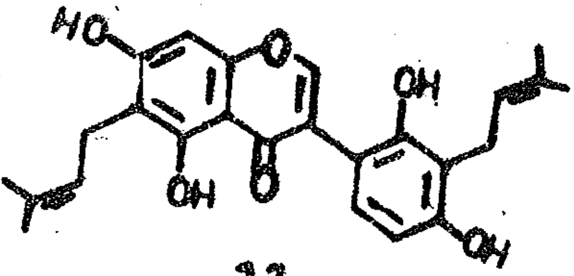
3.1.4 Characterization of Compound-D as 2'-hydroxyisolupalbigenin (26)

Compound-D was isolated as partly solidified gum, homogeneous on TLC (R_f 0.45, solvent system 2A) and turned to light brown when sprayed with 0.5% aq. fast blue B followed by 0.1 N NaOH. The IR spectrum revealed on OH absorption band ($3220 cm^{-1}$) and a chelated carbonyl band ($1640\ cm^{-1}$). The UV spectrum showed absorption maxima at 266 nm (MeOH). A bathochromic shift with NaOAc (+6 nm), $AlCl_3$ (+6 nm), could be due to the presence of hydroxyl groups at position 5 and 7. The ^1H NMR spectrum (Table 6) revealed two hydroxyl singlet at δ 12.85 and 8.18, an isoflavone H-2 singlet (δ 7.91) and the characteristic signals for two methylene, two olefinic methine and four non equivalent terminal methyl protons of two 3,3-dimethyl allyl side chains.

The presence of two set of aromatic protons was evident from the proton resonances at 6.9 (d,J = 8.1 H_z), 6.45 (d,J = 8.1 H_z) and 6.40 (s). This allows the placement of the prenyl groups at ring A and ring B. From the above data two alternative structures 26 and 27 could be assigned to compound-D.



26



27

Structure 26 could be suggested to compound-D based on comparison with the ^{1}H NMR data of Angustone-A (27) (2'-hydroxylupalbigenin) and 2'-hydroxyisolupalbigenin (26 (Table 5). The signal at δ 6.40 (s) in the ^{1}H NMR spectrum of compound-D assignable to H-6 more closely resembled the corresponding signal in 5,7-dihydroxy-8-prenyl isoflavone than its 6 prenyl isomer luteone (17) (8H at δ 6.53, as an isolated signal). This is further supported by the slow and less intense blue colour formation of compound-D on Gibb's test(16) which is in agreement with the placement of the prenyl side chain of ring A at C-8.

Based on the above spectroscopic observations, structure 26 was assigned to compound-D. This structure corresponds to 2'-hydroxyisolupalbigenin which was earlier isolated from the roots of <u>L. albus</u>.(6)

Table 3

^{1}H NMR data of Angustone-A (2'-hydroxylupalbigenin), 2'-hydroxyisolupalbigenin and Compound-D.(29,33)

Proton	2'-hydroxyisolupalbigenin (structure-26) 100 MH$_z$, Acetone-d$_6$	Compound-D (Structure-26) 90 MH$_z$, CDCl$_3$	2'-hydroxylupalbigenin (Structure-27) 80 MH$_z$, DMSO-d$_6$
2-H	8,29 (s)	7.99 (s)	8.09 (s)
5-OH	12.46 (s)	12.85 (s)	13.09 (s)
6-H	6.43 (1H,s)	6.40 (1H,s)	-
8-H	-	-	6.47 (1H,s)
5'-H	6.51 (1H,d,8.3)	6.45 (1H,d,8.1)	6.38 (1H,dJ, 8 H$_z$)
6'-H	6.96 (1H,d,8.3)	6.91 (1H,d,8.1)	6.77 (1H,d,J,8 H$_z$)
A or B ring Attachement			
1" Ha	3.44 brd (2H)	3.37 brd (2H)	3.30 (4H, m)
Hb	3.46 brd (2H)	3.41 brd (2H)	
2"-H	5.32 brt (2H)	5.23 brt (2H)	5.19 (2H, m)
4"-H	1.78 (s,6H)	1.81 (s,6H)	1.74 (6H,brs)
5"-H	1.65 (s,6H)	1.71 (s,6H)	1.65 (6H,brs)

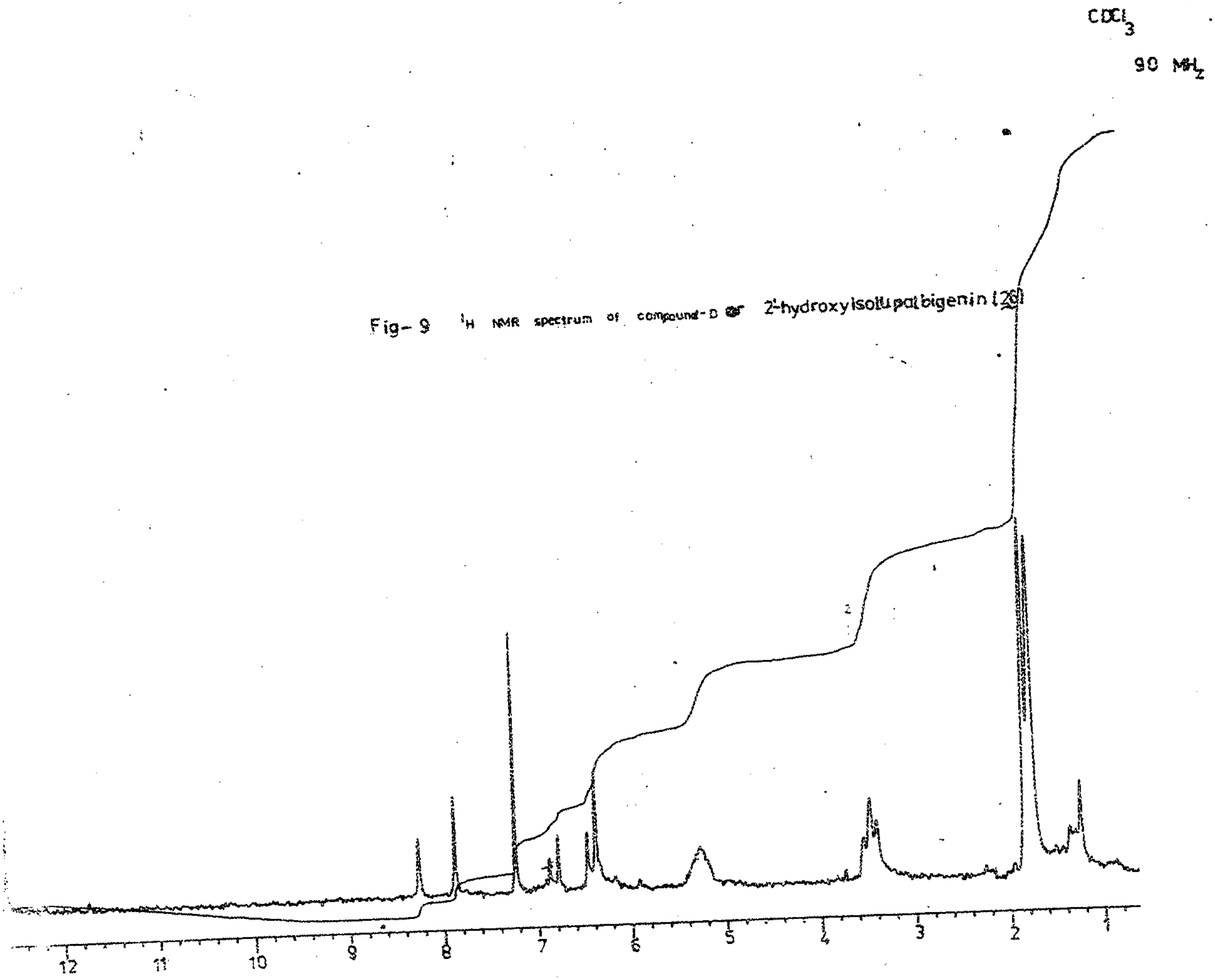
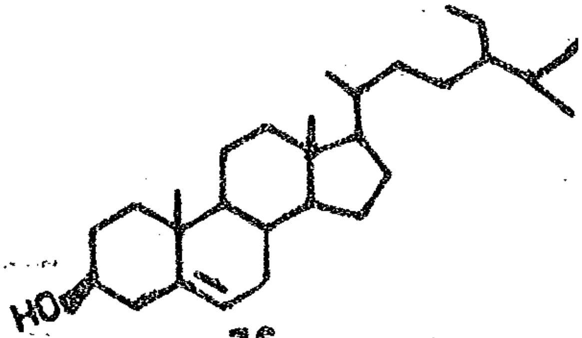


Fig- 9 ^1H NMR spectrum of compound-D or 2'-hydroxyisolupalbigenin (26)

3.1.5 Identification of Compound-E as β-sitosterol (76)

Compound-E is a colourless crystalline, MP 128-130°C and homogeneous on TLC (R_f 0.65 solvent system-1A), that turned purple when sprayed with vanillin-sulfuric acid and heated.(32)

It gave a rather complex ^{1}H NMR spectrum which however, suggested to be a sterol. Identification of this compound as β-sitostrol (76) was based on direct comparison (CO-TLC, mixed melting point, IR, ^{1}H NMR) with authentic sample.



76

$CDCl_3$

90 MH_z

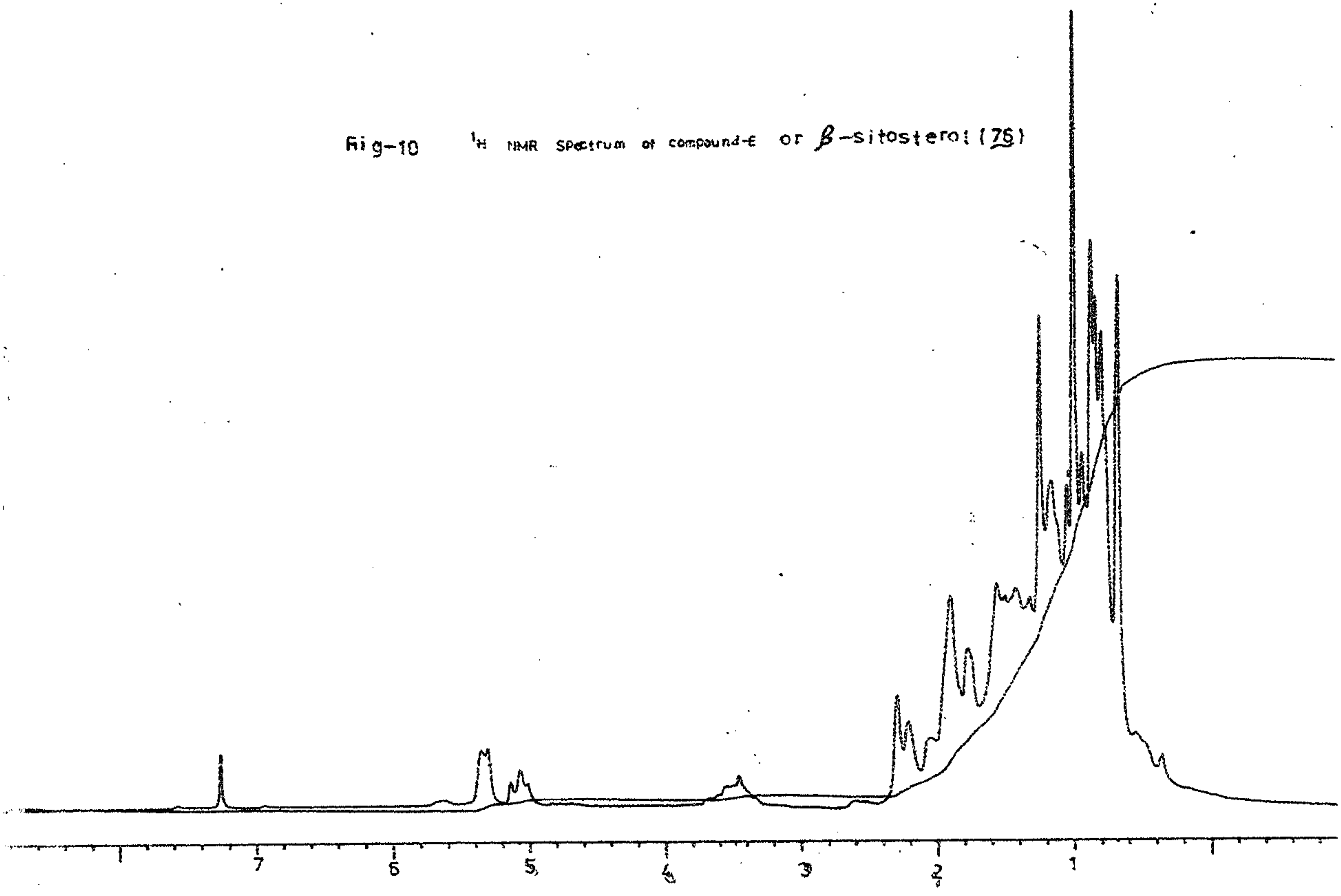


Fig-10 ^1H NMR Spectrum of compound-E or β-sitosterol (76)

3.2 Compounds Isolated from *L. albus*

3.2.1 Identification of Compound-A' as genistein (9)

It is pale yellow amorphous solid, MP 217-219° (dec) homogenous on TLC (R_f 0.51, solvent system-1A), that turned brown when sprayed with 0.5% aq. fast blue B followed by 0.1 M NaOH. Identification of this compound as genistein (9) was based on direct comparison (^{1}H NMR, CO-TLC) with genistein isolated from *L. princei*.

3.2.2 Identification of Compound-C' as 2'-hydroxygenistein (13)

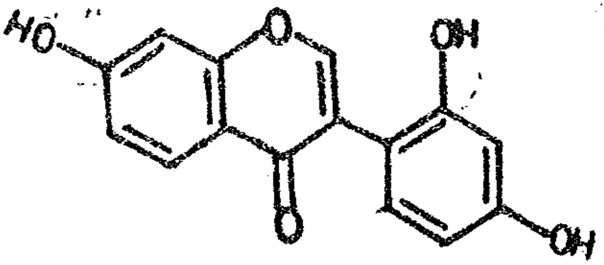
This yellow amorphous solid, MP 218-222° (dec) has similar CO-TLC, ^{1}H NMR, IR, UV data with 2'-hydroxygenistein isolated from *L. princei*.

3.2.3 Characterization of Compound-B' as 2'-hydroxydaidzein (77)

Compound-B' was isolated as grey amorphous solid, MP 180-184°. (dec). The IR spectrum revealed an OH absorption band (3450 cm^{-1}) and a non-chelated band (1665 cm^{-1}). The ^{1}H NMR spectrum revealed an isoflavone H-2 singlet at δ 7.82. The six aromatic proton resonances appeared as two ABX systems with signals at δ 6.96

(d, J = 7.4 H_z, H-6'), 6.46 (d, J = 1.8 H_z, H-3') and 6.24 (dd, J = 7.24 H_z and 1.8 H_z, H-5') assignable to ring-B protons by comparison with the corresponding B-ring proton resonances of 2'-hydroxygenistein. The typical down field doublet at δ 7.7 (J = 7.4 H_z) could be assigned for H-5 and the double doublet at δ 6.7 (J = 7.4 and 1.8 H_z), the doublet at δ 6.3 (J = 1.8 H_z) are attributable to H-6 and H-8 respectively. The UV spectrum showed an absorption maximum at [illegible] nm (MeOH). A bathochromic shift with NaOAc (+11 nm could be due to the presence of OH at position 7.

Based on the above grounds structure 77 was established for compound-B'. This structure corresponds to a 5-deoxy isoflavone known as 2'-hydroxydaidzein which was earlier isolated from Phaseolus vulgaris.(34) This therefore, is the first report on the presence of 2'-hydroxydaidzein in the genus Lupinus.

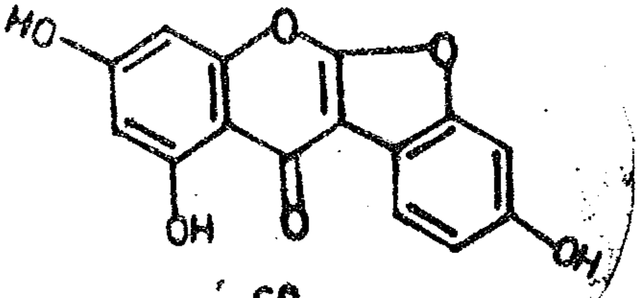


77

3.2.4 Characterization of Compound-D' as lupinalin-A (69)

This pale yellow amorphous solid, MP 219-223 (dec), showed in its IR spectrum an OH absorption (3220 cm^{-1}) and a chelated carbonyl (1620 cm^{-1}) band. The UV spectrum displayed absorption maximum at 256 nm (MeOH). Bathochromic shifts with NaOAc (+6 nm) and $AlCl_3$ (+6 nm) are indicative of 5,7-dihydroxylation.(28) The aromatic in the ^1H NMR spectrum were evident as two *meta* coupled doublets (δ 6.50, 1H, J = 1.8 H_z and δ 6.45, 1H, J = 1.8 H_z) which were assigned to the H-6 and H-8 A ring protons of 5,7-dioxygenated isoflavone. An ABX system (δ 6.94 dd, 1H, J = 7.4 and 1.8 H_z; δ 7.0 d, 1H, J = 1.8 H_z and δ 7.8 d, J = 7.4 H_z) is attributable to the B ring protons of an isoflavone with 2',4'-dioxygenation. However, the spectrum lacks a characteristic H-2 singlet and hence this compound must possess a modified isoflavone type skeleton.

The above spectroscopic data allow for the assignment of structure 69 for this compound. Lupinabin-A (69) is a coumarnochromone which was earlier isolated from the root of *L. albus*.(30)



69

Table 5 UV spectral data of compound A-D of <u>L</u>. <u>princei</u> and compound B' and D' of <u>L</u>. <u>albus</u>

Compound	UV max(nm)					
	MeOH	$AlCl_3$	$AlCl_3/HCl$	NaOAc	NaOMe	$NaOAc/H_3BO_3$
A	262,329(sh) 328(sh)	269,311(sh) 367(sh)	269,311(sh) 374	270,322	269,318	262,286(sh)
B	261,329(sh	267,378	268,374	270,374	271,308	260,329(sh)
C	261,329(sh)	267,364	270,374	272,336	276,324	262,330(sh)
D	266,324(sh)	272,326(sh)	271,324(sh)	272,337		
B'	256.9,332	257.7,333	257.6,334	268,342		
D'	256,284, 335(sh)	262,284 377	263,284 379	262,292 246		

4. EXPERIMENTAL

4.1 Plant Material

The roots of *L. albus* in this study were collected near Debre Markos, Gojam province in August 1989 at an altitude of 2400 m. The roots of *L. princei* were collected near Mega, Sidamo provinće in October 1988 at an altitude of 1950 m. Both species were identified by Dr. Sebsibe Demissew of the National Herbarium. Voucher specimen representing *L. albus* (voucher No 012423) and *L. princei* (voucher No 045733) were deposited at National Herbarium, Addis Ababa University.

4.2 Material and Apparatus

Melting point were determined using Thomas Hoover capillary melting point apparatus. UV spectral were recorded on Beckman DU-65 spectrometer in methanol. IR spectra were run on Perkin-Elmer model 727 infrared spectrometer on KBr disc. EIMS was run at 70 ev. ^1H NMR spectra were measured on Joel FX 90 at 90 MH$_z$ and chemical shifts are given in δ values with TMS as internal standard. ^{13}C NMR spectra were recorded on Joel FX 90 at 22.5 MH$_z$ in $CDCl_3$-CD_3OD (1:1). Analytical thin layer chromatography (TLC) were run on 0.2 mm silica gel plates and the spots were detected by their UV fluorescence and by spraying with 0.5% aq. fast blue B solution followed by 0.1 N NaOH.(32)

Circular preparative thin layer chromatography (CPTLC) was run on 1 mm thick silica gel and bands were detected by their UV fluorescence. Vaccum liquid chromatography (VLC) was carried out on PTLC grade silica gel. Column chromatography was done on silica gel (70-230 mesh) and Sephadex LH-20 ($CHCl_3$-MeOH, 1:1). All solvents were distilled prior to chromatography. The petrol used through out the study has B.P (60-80°.).

Table 6. Solvent Systems - The following solvent systems were used for thin layer, preparative layer and column chromatography.

No.	Solvent System	Ratio
1-A	Benzene - EtOAc	7:3
B	Benzene - EtOAc	1:1
2-A	Chloroform - Methanol	9.5:0.5
B	Chloroform - Methanol	9:1
C	Chloroform - Methanol	1:1
3-A	Petrol - EtOAc	95:5
B	Petrol - EtOAc	90:10
C	Petrol - EtOAc	80:20
D	Petrol - EtOAc	70:30
E	Petrol - EtOAc	60:40
F	Petrol - EtOAc	50:50

4.3 Extraction and Fractionation of *L. princei* Root

The air dried and ground roots (120 g) were first extracted with chloroform and the marc was then further extracted with Ethanol.

The oily residue (3 g) obtained from the chloroform extract after removal of solvent was applied on a silicagel column (60 g) and eluted with petrol-EtOAc mixtures of increasing polarities fractions were monitored by TLC and a total of 23 fractions each of 150 ml were collected.

Fraction 6 eluted with solvent system 3B afforded compound-E (18 mg) which was crystalized from MeOH as colourless needle.

Fractions 11-15 eluted with solvent system 3D and E gave compound-D (6 mg), which was further purified by CPTLC using solvent system 2A.

The EtOH extract was concentrated and the residue (10 g) was applied on silica gel column and eluted with petrol-EtOAc mixtures of increasing polarities fractions were monitored by TLC and a total of 30 fractions each of 150 ml were collected.

Fractions 12-18 eluted with solvent system 3E gave a mixture of compound A,B and C. The mixture was separated by repeatitive application on CPTLC using solvent system 2A and 2B to give compound-C (upper zone, 20 mg); compound-A (lower zone, 240 mg, compound-B (middle zone, 50 mg).

4.4 Extraction and Fractionation of *L. albus* Root

The air dried and ground roots (623 g) were percolated with EtOH at room temperature for 6 days. The extract was then filtered and concentrated under reduced pressure to give a 22 g syrupy residue which was fractionated into petrol soluble (2 g) and chloroform soluble (20 g) portions. The chloroform soluble portion (20 g) was subjected to vaccum liquid chromatography (VLC) and eluted with petrol-EtOAC mixtures of increasing polarities. Twelve fractions each of 250 ml were collected and the pure compounds were obtained from the combined fractions after further purification by CC followed by prep. TLC and chromatography on Sephadex LH-20 ($CHCl_3$-MeOH, 1:1)

Fractions 5-7 eluted with solvent system 3D and E gave a mixture of compounds A' and D'. The mixture was separated by multiple development on prep. TLC in solvent system-1A to give compound-D' (upper zone, 12 mg) and compound A' (lower zone, 14 mg).

Fractions 8-11 eluted with solvent system

3E and F gave a mixture of compounds B' and C'. The mixture was chromatographed on Sephadex LH-20 ($CHCl_3$-MeOH, 1:1) to give compound-B' (50 mg) and compound-C (13 mg) which was further crystalized from dichloromethane.

4.5 Acetylation of Compound-A

A mixture of 5 mg compound-B, 1 ml of acetic-anhydride and 2 drops of pyridine was heated with stirring at 60-70° for 4 hrs, 5 ml of ice water was added and the mixture stirred for 30 minutes. The solution was neutralized with 20 ml of saturated sodium bicarbonate solution and extracted with 80 ml of chloroform. The organic layer was washed with 0.1N HCl and dried with anhydrous sodium sulpate. The solvent was evaporated to give 4 mg of the acetylated product. This product was homogeneous on TLC R_f 0.6, solvent system-2A and did not give colour when sprayed with 0.5% aq fast blue B followed by 0.1N NaOH.

4.6 Physicochemical Data

4.6.1 Compounds of *L. princei*

Genistein (9). R_f 0.54 (solvent system 1A), pale yellow amorphous solid, MP 220-223°(dec). UV (254) fluorescence, purple. Gibbs test; (+), slow, blue. Fast blue B: (+), brown. UV λ_{max} MeOH nm (see Table 7), IR ν_{max} KBr cm^{-1}: 3350, 1620, 1560, 1440, 1300, 1080, 940. ^{1}H NMR (see Table 6), ^{13}C NMR (see Table 3). EIMS m/z (rel. int.): 270(M^{+} 100), 153 (42), 152(67), 135(11), 124(16), 118(32), 96(6), 69(16).

2'-hydroxygenistein (13). R$_{f}$ 0.38 (solvent system 1A), yellow amorphous solid, MP 223-225^{o} (dec). UV(254) fluorescence, deep purple. Gibbs test: (+), fast, dark purple, fast blue B(+), dark brown. UV λ_{max} MeOH (see Table 7). IR ν_{Max} KBr cm^{-1}: 3250, 1680, 1645, 1520, 1330, 1200. ^{1}H NMR (see Table 6), ^{13}C NMR (see table 4). EIMS m/z (rel. int.), 286 (M^{+} 96), 274(24), 269(18), 153(100), 152(11) 124(13), 134(53), 69(33), 50(19).

Luteone (17). R$_{f}$ 0.64 (solvent system 1A), pale amorphous solid, MP 190-192^{o}; UV (254) fluorescence, dark purple. Gibbs test: (+), fast, blue. Fast blue B: (+), brown. VU λ_{max} MeOH (see Table 7). IR ν_{max} KBr cm^{-1}: 3400, 1630, 1560, 1400, 1300, 1200, 1120, 960. ^{1}H NMR (see Table 6). EIMS m/z (rel. int.) 354(M^{+} 47), 339(9), 337(9), 311(78), 299(100), 291(48), 220(7), 134(4), 124(7), 115(7), 91(7), 69(11).

2'-hydroxyisolupalbigenin (26). R$_{f}$ 0.45 (solvent system 2A), partly solidified gum, UV (254, 366) fluorescence, dark purple. Gibbs test: (+), slow, blue. Fast blue:(+), light brown. UV λ_{max} MeOH (see Table 15). IR ν_{max} KBr cm^{-1}: 3220, 2950, 1640, 1560, 1500, 1100, 920. ^{1}H NMR (see table 14).

β- sitosterol (76). R_f 0.42 (solvent system 1A), colourless crystalline, MP 128-130° . UV: non fluoresing, Vanillin-sulfuric acid: (+), purple. IR ν_{max} KBr cm^{-1}: 3460, 3000, 2960, 1400, 1300, 960. ^{1}H NMR ($CDCl_3$): 0.65(3H,s, Me-18), 0.7(6H,d J = 8H_z, Me-26, 27), 0.9(3H, brd, Me-29), 1.0(3H, S, Me-21), 1.25(3H, S, Me-19), 0.95(m), 1.2(m), 1.4-2.4(m).

2',4'5,7-tetraacetylgenistein (78). R_f 0.51 (solvent system-1A), UV (254) fluorescence, deep purple, fast blue B:(-). ^{1}H NMR ($CDCl_3$, 90 MH_z) δ 2.2, 2.3, 2.4, 2.42 (s , 4XCOOCH$_3$), 7.8(s,H-2), 6.8-6.9 (unresolved doublet, H-5' and 6'), 6.5-6.6 (unresolved dd, H-6 and 8).

4.6.2 Compounds of *L. albus*

Genistein (9). R_f 0.54 (solvent system 1A), pale yellow amorphous solid, MP 217-219° (dec). UV (254) fluorescence, purple. Fast blue B: (+), brown. ^{1}H NMR ($CDCl_3$-CD_3OD (1:1): δ 7.85 (1H, s, H-2); 6.30(d, J = 2.1 H_z) H-6); 6.42 (d, J = 2.1 H_z, H-8); 7.23 (d, J = 8.4 H_z, H-2' and 6'); 6.91(d, J = 8.4 H_z, H-3' and 5').

2'-hydroxygenistein (13). R_f 0.39 (solvent system 1A), yellow amorphous solid, MP 218-222° (dec). UV (254) fluorescence, purple. Gibb's test: (+), fast, dark purple, fast blue B: (+), dark brown. UV λ_{max} MeOH 261, 286, 323 (sh); + NaOAc, 272, 372; + $AlCl_3$, 268, 304 (sh), 368; + $AlCl_3$/HCl, 270, 371; + NaOMe, 273, 321 (sh). IR ν_{max} KBr cm^{-1}: 3250, 1620, 1560, 1420, 1240, 940. ^1H NMR ($CDCl_3$-CD_3OD, 1:1) 7.91(s, H-2) 6.30(d, J = 2.2 H_z, H-6); 6.5(d, J = 2.2 H_z, H -8); 6.41(d, J = 2.2 H_z, H-3'); 6.38(d, J = 8.0 and 2.2 H_z, H-5'); 7.00(d, J = 8 H_z, H-6').

2'-hydroxydaidzein (76). R_f 0.43 (solvent system 1A), pale amorphous solid, MP 180-184° (dec). UV (254) fluorescence, purple. Fast blue B: (+), light brown. UV λ_{max} MeOH (see Table 7). IR ν_{max} KBr cm^{-1}: 3450, 1665, 1620, 1520, 1360, 1260, 1080, 940. ^1H NMR (see Table 6).

Lupinalbin-A (69). R_f 0.40 (solvent system 1A), pale amorphous solid, MP 219-223° (dec), UV (254) fluorescence, purple. Fast blue B: (+), brown. UV λ_{max} MeOH (see Table 7). IR ν_{max} KBr cm^{-1} 3220, 1620, 1560, 1540, 1380, 1320, 1300, 1060, 940. ^1H NMR (see Table 6).

Appendix 1. LISTS OF FLAVONES AND FLAVONOLS ISOLATED FROM LUPINUS SPECIES

No.	Compound	MF	MW	Str. No.	Plant-Sourse	References
1	Apigenin	$C_{15}H_{10}O_5$	270	1	L. polyphyllus	35
					L. luteus	35
					L. albus	35
					L. angustifolius	35
					L. hirsutus	36
2	Acacetin	$C_{16}H_{12}O_5$	284	2	L. luteus	35
					L. polyphyllus	35
					L. albus	35
					L. angustifolius	35
3	Luteolin	$C_{15}H_{10}O_6$	286	3	L. luteus	37
					L. angustifolius	35
					L. arboreus	38
					L. albus	35

No.	Compound	MF	MW	Str. No.	Plant-Source	References
4	Kaempferol	$C_{15}H_{10}O_6$	286	4	*L. luteus*	35,39
					L. albus	35
					L. angustifolius	35
					L. polyphyllus	35
					L. hirustus	36
5	Chrysoeriol	$C_{16}H_{12}O_6$	300	5	*L. luteus*	35,39
					L. albus	35
					L. angustifolius	35
					L. polyphyllus	35
6	Diosmentin	$C_{16}H_{12}O_6$	300	6	*L. polyphyllus*	35
					L. albus	35
					L. angustifolius	35
					L. luteus	35

No	Compound	MF	MW	Str. No.	Plant-Source	References
7	Quercetin	$C_{15}H_{10}O_7$	302	7	*L. albus*	35
					L. luteus	35,39
					L. polyphyllus	35
					L. angustifolius	35
8	Isorhamentin	$C_{16}H_{12}O_7$	316	8	*L. albus*	
					L. luteus	35,37,39
					L. polyphyllus	35
					L. angustifolius	37

Appendix 2. SIMPLE ISOFLAVONES

No.	Compound	MF	MW	Str. No.	Plant-Source	Reference
9	Genistein	$C_{15}H_{10}O_5$	270	9	*L. luteus*	35,40
					L. polyphyllus	35
					L. hirustus	36
					L. angustifolius	35
					L. albus	35
10	5,7,3'-trihydroxyisoflavone	$C_{15}H_{10}O_5$	270	10	*L. hirustus*	36
11	5-methoxy genistein	$C_{16}H_{12}O_5$	284	11	*L. luteus*	35
					L. albus	35
					L. polyphyllus	35
					L. angustifolius	35

No.	Compound	MF	MW	Str. No.	Plant-Source	References
12	Orbol	$C_{15}H_{10}O_6$	286	12	*L. polyphyllus*	35
					L. albus	35
					L. luteus	35
					L. angustifolius	35
	2'-hydroxy-genistein	$C_{15}H_{10}O_6$	286	13	*L. albus*	7,30
					L. mutabilis	7
					L. angustifolius	7,29
					L. luteus	40
14	3',4'-methylenedioxy orbol	$C_{16}H_{10}O_6$	298	14	*L. polyphyllus*	35
					L. luteus	35
					L. albus	35
					L. angustifolius	35

Appendix 3. PRENYLATED FLAVONE AND ISOFLAVONES

No.	Compound	MF	MW	Str. No.	Plant-Source	References
15	Wighteone	$C_{20}H_{18}O_5$	338	15	*L. angustifolius*	7
					L. albus	31
					L. mutabilis	7
					L. luteus	40
16	Lupiwighteone	$C_{20}H_{18}O_5$	338	16	*L. luteus*	40
17	Luteone	$C_{20}H_{18}O_6$	334	17	*L. luteus*	7,40
					L. angustifolius	7
					L. albus	7,30,31
					L. mutabilis	7
18	Licoisoflavone-A	$C_{20}H_{18}O_6$	354	18	*L. albus*	31

No	Compound	MF	MW	Str. No.	Plant-Source	References
19	Lupiweighteone-hydrate	$C_{20}H_{20}O_6$	356	19	*L. luteus*	40
20	Lupisoflavone	$C_{21}H_{20}O_6$	368	20	*L. albus*	7
					L. luteus	7
21	Topazin	$C_{21}H_{20}O_6$	368	21	*L. luteus*	33
22	Hydroxyweighteone	$C_{21}H_{21}O_6$	369	22	*L. luteus*	40
23	2,3-dehydrokievetone hydrate	$C_{20}H_{20}O_7$	372	23	*L. luteus*	40
24	Topazine-hydrate	$C_{21}H_{22}O_7$	386	24	*L. luteus*	33
25	Lupalbigenin	$C_{25}H_{26}O_5$	396	25	*L. albus*	7

No.	Compound	MF	MW	Str. No.	Plant-Source	References
26	2'-hydroxyiso-lupalbigenin	$C_{25}H_{26}O_6$	422	26	L. albus	6
27	2'-hydroxyl-lupalbigenin (Angustone-A)	$C_{25}H_{26}O_6$	422	27	L. angustifolius L. albus	29 6,31
28	Lupinisol-A	$C_{25}H_{26}O_6$	422	28	L. albus	6
29	Lupinisol-B	$C_{25}H_{26}O_7$	438	29	L. albus	6
30	Lupinisol-C	$C_{25}H_{26}O_7$	438	30	L. albus	6

Appendix 4. FLAVONE AND ISOFLAVONE GLYCOSIDES

No.	Compound	NF	MW	Str. No.	Plant-Source	References
31	Apigenin-7-O-β D-glucoside	$C_{21}H_{20}O_{10}$	432	31	*L. polyphyllus*	41,42
32	Vitexin	$C_{21}H_{20}O_{10}$	432	32	*L. arboreus*	38
33	Genistein-7-O-β glucoside	$C_{21}H_{20}O_{10}$	432	33	*L. polyphyllus*	41,42
34	Genistein-4'-β O-glucoside	$C_{21}H_{20}O_{10}$	432	34	*L. luteus*	43
35	Genistein-8-C-β -D-glucoside	$C_{21}H_{20}O_{10}$	432	35	*L. luteus*	44
36	Isoquercitrin	$C_{21}H_{21}O_{10}$	433	36	*L. luteus*	37
37	Cystiosoide	$C_{22}H_{22}O_{10}$	446	37	*L. arboreus*	38
38	Orientin	$C_{21}H_{20}O_{11}$	448	38	*L. arboreus*	38

No.	Compound	MF	MW	Str. No.	Plant-Source	References
39	Luteolin-4'-O-glucoside	$C_{21}H_{20}O_{10}$	448	39	L. polyphyllus	41,42
40	Orbol-7-O-β glucoside	$C_{21}H_{20}O_{11}$	448	40	L. luteus	43
41	3',4'-methylenedioxyo-rbol-7-O- D-glucoside	$C_{22}H_{21}O_{11}$	461	41	L. polyphyllus L. luteus	42 37,42,43
42	4-O-methyl-orientin	$C_{22}H_{22}O_{11}$	462	42	L. arboreus	38
43	Acacetin-7-O-β-D-glucoside	$C_{22}H_{22}O_{11}$	462	43	L. polyphyllus	42

No.	Compound	MF	MW	Str. No.	Plant-Source	References
44	Quercetin-3-O-glucoside	$C_{21}H_{21}O_{11}$	465	44	*L. arboreus*	42
45	Rutin	$C_{27}H_{32}O_{16}$	612	45	*L. luteus*	37

Appendix 5. PYRANO SUBSTITUTED ISOFLAVONES

NO.	Compound	MF	MW	Str. No.	Plant-Source	References
46	Isoderrone	$C_{20}H_{16}O_5$	336	46	*L. albus*	6
47	Alpinum isoflavone	$C_{20}H_{16}O_5$	336	47	*L. albus*	7
					L. luteus	7
48	Parvisoflavone B	$C_{20}H_{16}O_6$	352	48	*L. albus*	31
					L. luteus	40
49	Licoisoflavone B	$C_{20}H_{16}O_6$	352	49	*L. angustifolius*	7
					L. albus	7
					L. luteus	7
50	Hydroxyparavisoflavone	$C_{20}H_{16}O_7$	368	50	*L. luteus*	40
51	Isochandalone	$C_{25}H_{24}O_5$	404	51	*L. albus*	6
52	Chandalone	$C_{25}H_{24}O_5$	404	52	*L. albus*	6
53	Angustone-B	$C_{25}H_{24}O_5$	404	53	*L. angustifolius*	29

No.	Compound	MF	MW	Str. No.	Plant-Source	References
54	Angustone-C	$C_{25}H_{24}O_6$	420	54	L. angustifolius L. albus	29 6
55	Lupinisolone-A	$C_{25}H_{26}O_6$	422	55	L. albus	6
56	Lupinisolone-B	$C_{25}H_{26}O_6$	422	56	L. albus	6
57	Lupinisolone-C	$C_{25}H_{26}O_7$	438	57	L. albus	6

Appendix 6. DIHYDROFURANO SUBSTITUTED ISOFLAVONES

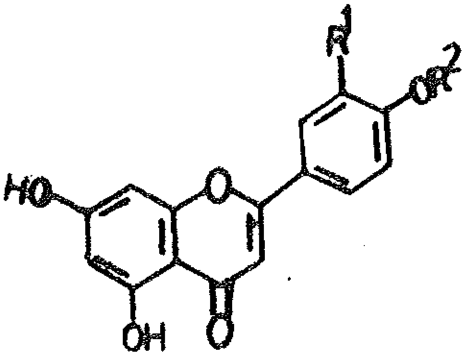
No.	Compound	MF	MW	Str. No.	Plant-Source	References
58	Lupinisoflavone-A	$C_{20}H_{16}O_6$	352	58	*L. albus*	31
					L. luteus	40
59	Lupinisoflavone-C	$C_{20}H_{18}O_6$	354	59	*L. albus*	31
60	Erythrinin-C	$C_{20}H_{18}O_6$	354	60	*L. albus*	30
61	Lupinisoflavone-B	$C_{20}H_{18}O_7$	370	61	*L. albus*	31
62	Lupinisoflavone-D	$C_{20}H_{18}O_7$	370	62	*L. albus*	31
63	Lupinisoflavone-G	$C_{25}H_{26}O_6$	422	63	*L. albus*	6
64	Lupinisoflavone-J	$C_{25}H_{26}O_7$	438	64	*L. albus*	31
65	Lupinisoflavone-E	$C_{25}H_{26}O_7$	438	65	*L. albus*	6
66	Lupinisoflavone-H	$C_{25}H_{26}O_7$	438	66	*L. albus*	6
67	Lupinisoflavone-I	$C_{25}H_{26}O_7$	438	67	*L. albus*	31
68	Lupinisoflavone-F	$C_{25}H_{26}O_8$	454	68	*L. albus*	6

Appendix 7. COUMARNOCHROMONES

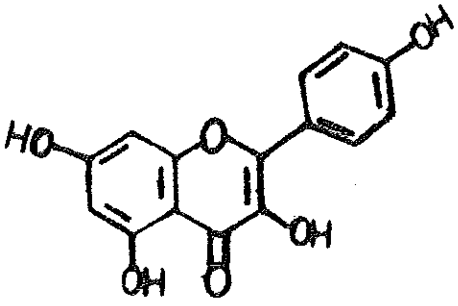
No.	Compound	MF	MW	Str. No.	Plant-Source	Reference
69	Lupinalbin-A	$C_{15}H_{8}O_{6}$	284	69	<u>L</u>. <u>albus</u>	30
70	Lupinalbin-B	$C_{20}H_{16}O_{6}$	352	70	<u>L</u>. <u>albus</u>	30
71	Lupinalbin-D	$C_{20}H_{16}O_{6}$	352	71	<u>L</u>. <u>albus</u>	30
72	Lupinalbin-C	$C_{20}H_{16}O_{7}$	368	72	<u>L</u>. <u>albus</u>	30
73	Lupinalbin-E	$C_{20}H_{16}O_{7}$	368	73	<u>L</u>. <u>albus</u>	30
74	Lupilutin	$C_{20}H_{18}O_{7}$	369	74	<u>L</u>. <u>luteus</u>	40
75	Lupinalbin-F	$C_{25}H_{24}O_{6}$	420	75	<u>L</u>. <u>albus</u>	6

Appendix — 8

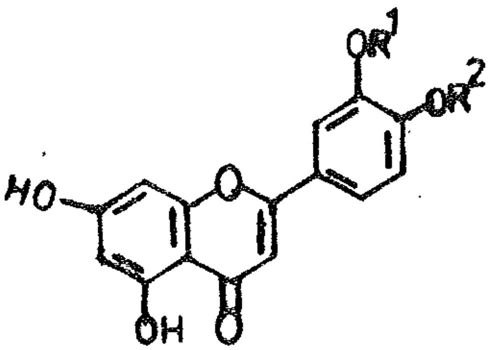
Structures of flavonoids of *Lupinus* species



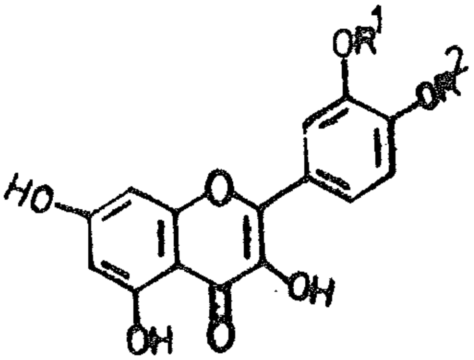
1 $R^1 = R^2 = H$
2 $R^1 = H$, $R^2 = CH_3$
3 $R^1 = OH$, $R^2 = H$



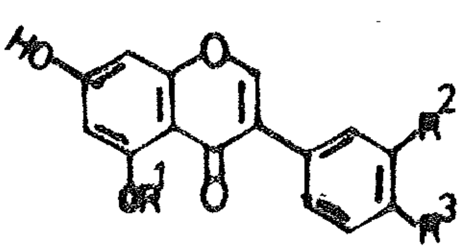
4



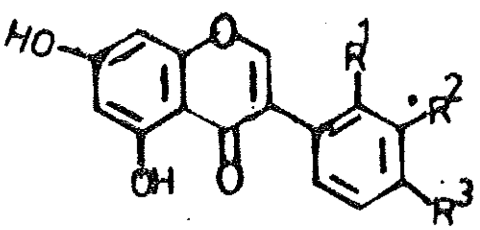
5 $R^1 = CH_3$, $R^2 = H$
6 $R^1 = H$, $R^2 = CH_3$



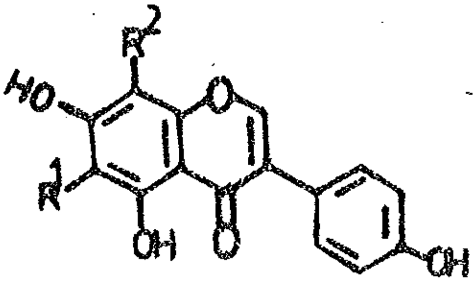
7 $R^1 = R^2 = H$
8 $R^1 = CH_3$, $R^2 = H$



9 $R^1 = R^2 = H$, $R^3 = OH$
10 $R^1 = R^3 = H$, $R^2 = OH$
11 $R^1 = CH_3$, $R^2 = H$ $R^3 = OH$



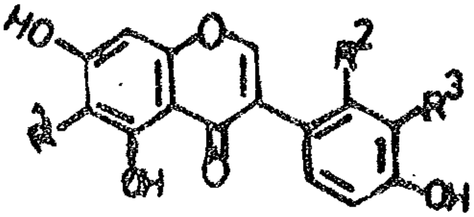
12 $R^1 = H$, $R^2 = R^3 = OH$
13 $R^1 = R^3 = OH$, $R^2 = H$
14 $R^1 = H$, $R^2 + R^3 = -OCH_2O-$



15 $R^1=CH_2CH=C(CH_3)_2$, $R^2=H$

16 $R^1=H$, $R^2=CH_2CH=C(CH_3)_2$

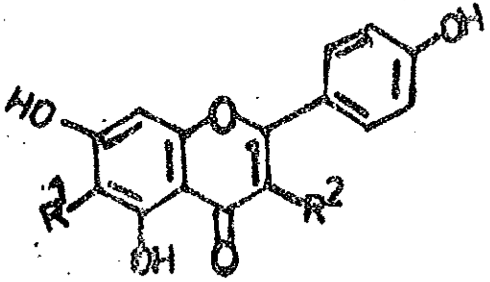
19 $R^1=H$, $R^2=CH_2CH_2C(CH_3)_2OH$



17 $R^1=CH_2CH=C(CH_3)_2$, $R^2=OH$, $R^3=H$

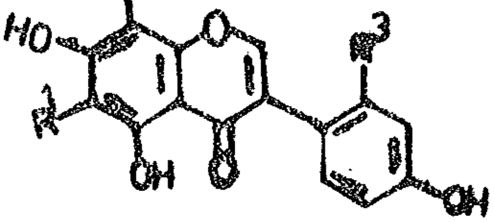
18 $R^1=H$, $R^2=OH$, $R^3=CH_2CH=C(CH_3)_2$

20 $R^1=CH_2CH=C(CH_3)_2$, $R^2=H$, $R^3=OCH_3$



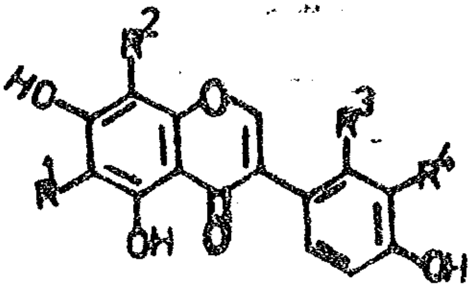
21 $R^1=CH_2CH=C(CH_3)_2$, $R^2=OCH_3$

24 $R^1=CH_2CH_2C(CH_3)_2OH$, $R^2=H$



22 $R^1=CH_2CH=\underset{CH_2OH}{C}CH_3$, $R^2=R^3=H$

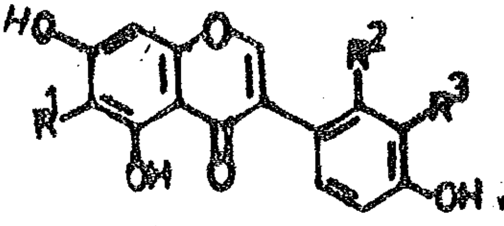
23 $R^1=H$, $R^2=CH_2CH_2C(CH_3)_2OH$, $R^3=OH$



25 $R^1=R^4=CH_2CH=C(CH_3)_2$, $R^2=R^3=H$

26 $R^1=H$ $R^2=R^4=CH_2CH=C(CH_3)_2$, $R^3=OH$

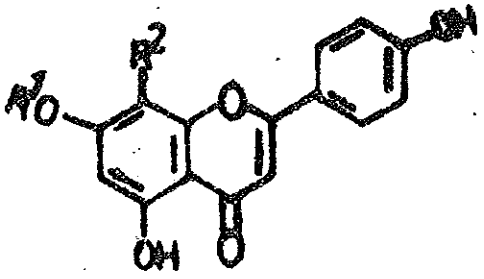
27 $R^1=R^4=CH_2CH=C(CH_3)_2$, $R^2=H$, $R^3=OH$



28 $R^1=CH_2CHOH\underset{CH_3}{C}=CH_2$, $R^2=H$, $R^3=CH_2CH=C(CH_3)_2$

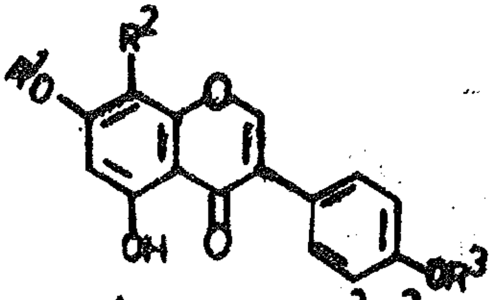
29 $R^1=CH_2CHOH\underset{CH_3}{C}=CH_2$, $R^2=OH$, $R^3=CH_2CH=C(CH_3)_2$

30 $R^1=CH_2CH=C(CH_3)_2$, $R^2=OH$ $R^3=CH_2CHOH\underset{CH_3}{C}=CH_2$



31 R^1 = Glucose , R^2 = H

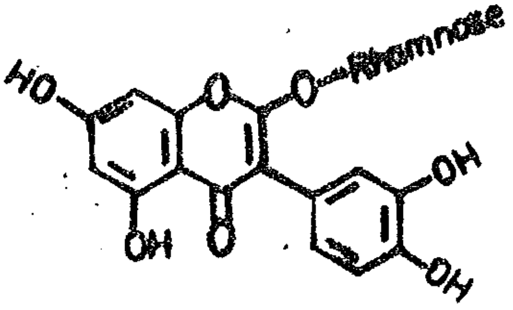
32 R^1 = H , R^2 = Glucose



33 R^1=Glucose , R^2=R^3=H

34 R^1=R^2=H , R^3=Glucose

35 R^1=R^3=H , R^2=Glucose



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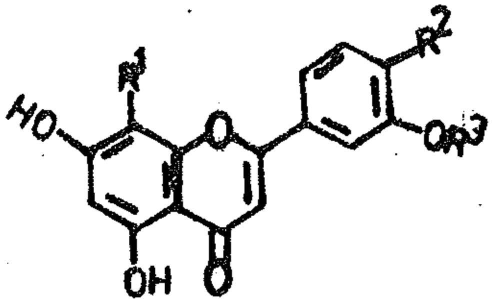
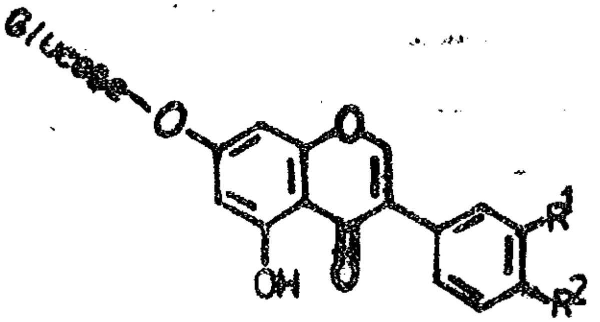


37 R^1=Glucose , R^2=H , R^3=CH_3

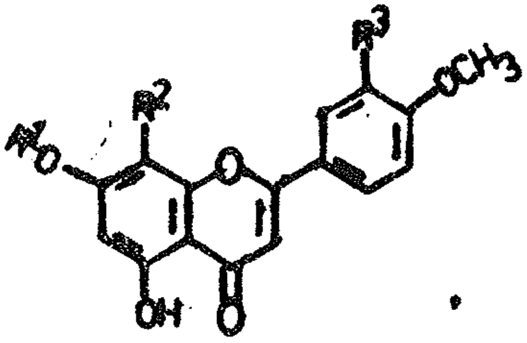
38 R^1=Glucose , R^2=OH , R^3=H

39 R^1=H , R^2=OH , R^3=Glucose



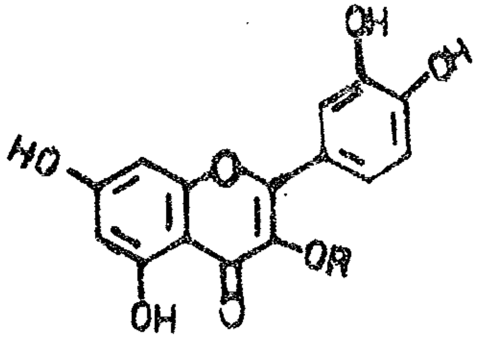
40 R^1 = R^2=OH

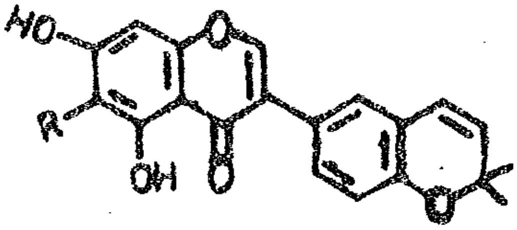
41 R^1 + R^2 = $-OCH_2O-$



42 R^1 = H , R^2=Glucose , R^3=OH

43 R^1=Glucose , R^2 = R^3 = H



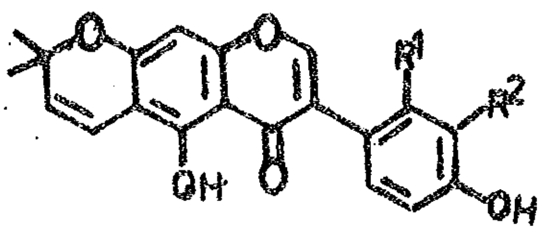


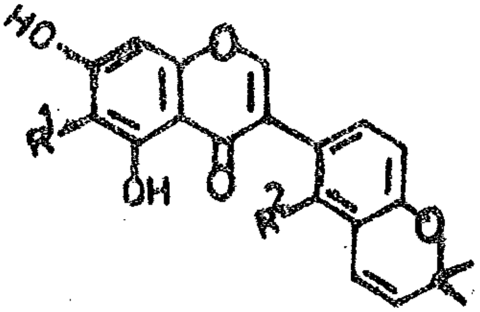
44 R = Glucose

45 R = Rhamnoglucose

46 R = H

51 R = $CH_2CH=C(CH_3)_2$





47 $R^1 = R^2 = H$

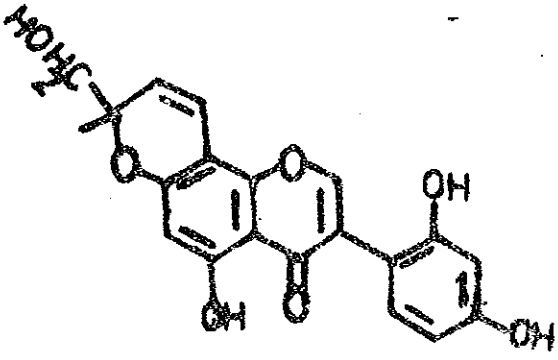
48 $R^1 = OH$, $R^2 = H$

52 $R^1 = H$, $R^2 = CH_2CH=C(CH_3)_2$

54 $R^1 = OH$, $R^2 = CH_2CH=C(CH_3)_2$

49 $R^1 = H$, $R^2 = OH$

53 $R^1 = CH_2CH=C(CH_3)_2$, $R^2 = H$



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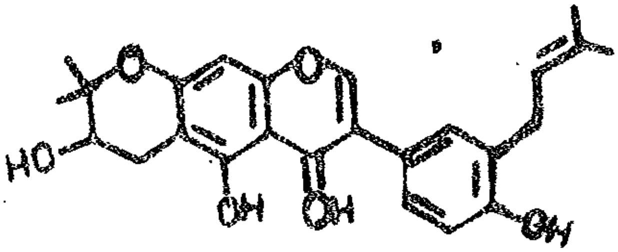

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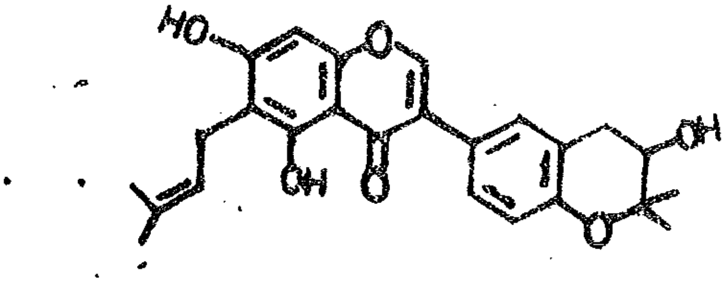

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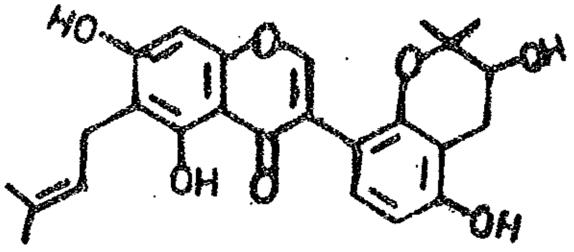

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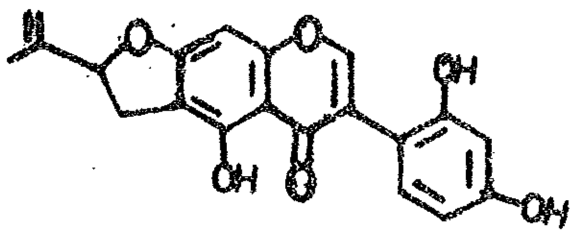

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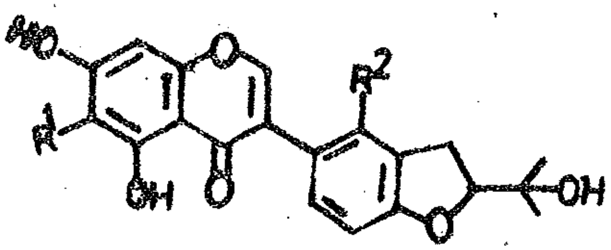
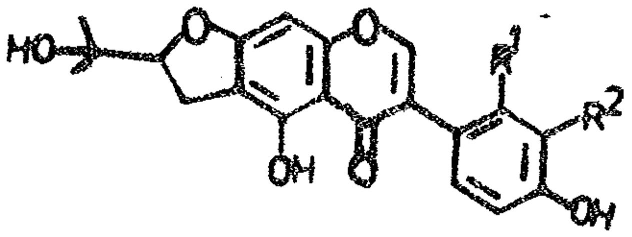


59 $R^1 = R^2 = H$

62 $R^1 = H$, $R^2 = OH$

67 $R^1 = CH_2CH=C(CH_3)_2$, $R^2 = OH$

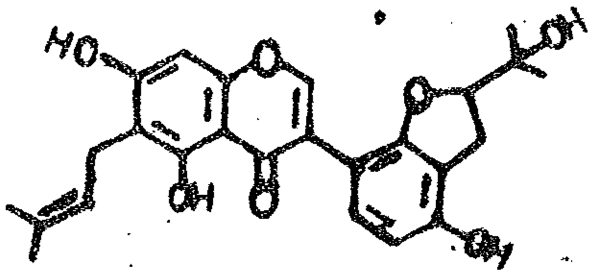


60 $R^1 = R^2 = H$

61 $R^1 = OH$, $R^2 = H$

63 $R^1 = H$, $R^2 = CH_2CH=C(CH_3)_2$

66 $R^1 = OH$, $R^2 = CH_2CH=C(CH_3)_2$



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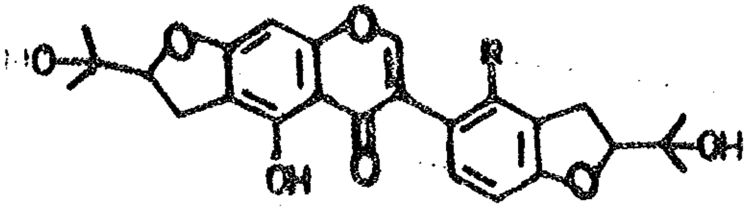
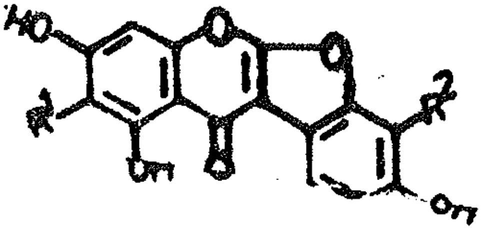


65 R = H

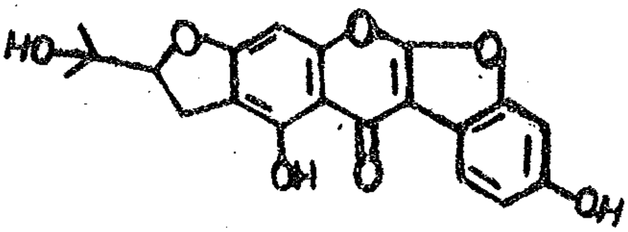
68 R = OH



69 $R^1 = R^2 = H$

70 $R^1 = CH_2CH=C(CH_3)_2$, $R^2 = H$

71 $R^1 = H$, $R^2 = CH_2CH=C(CH_3)_2$



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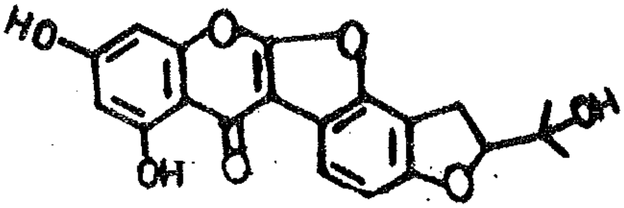

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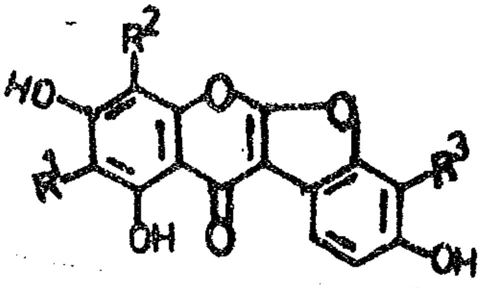

74 $R^1 = R^3 = H$, $R^2 = CH_2CH_2C(CH_3)_2OH$

75 $R^1 = R^3 = CH_2CH=C(CH_3)_2$, $R^2 = H$

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DECLARATION

I the undersigned, declare that this thesis is my work and that all sources of material used for the thesis have been dully acknowledgeed.

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