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INVESTIGATION OF THE EXTRACTIVES  
OF  
NEORAUTANENIA amboensis SCHINZ  
WITH SPECIAL REFERENCE TO  
NUCLEAR MAGNETIC RESONANCE STUDIES  
ON  
HYDROXYBENZOFURANS

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of the requirements  
for the degree

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BLOEMFONTEIN

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BY

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## SUMMARY

Four new compounds were isolated from *Neorautanenia amboensis*, three minor isoflavanoids, the dehydrorotenoids DEHYDRODOLINEONE, NEBOENSINONE and NAMBINONE, and the isoflavone glycoside AMBONIN. In addition four known isoflavanoids, NEODULIN, NEOTENONE, PACHYRRHIZIN and DEHYDRO-NEOTENONE were also isolated from *N. amboensis*. This dissertation is concerned with:

- (i) The elucidation of the structures of the new compounds mentioned above by chemical and physical methods.
- (ii) Nuclear magnetic resonance studies on hydroxybenzofurans and related phenolic derivatives of natural isoflavanoids with special reference to the relation between anion shift and chemical structure.

A summary is given below of the most important results which were obtained and which represent a contribution to our knowledge of the isoflavanoids and the physical methods used in their structural elucidations.

### (i) (a) THE ISOFLAVONE GLYCOSIDE AMBONIN (I)

The glycoside ambonin (I) was obtained as the major constituent from *N. amboensis*. Hydrolysis of ambonin with dilute sulphuric acid afforded the aglycone DAIDZEIN (II) (7, 4'-dihydroxyisoflavone)  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3160 (hydroxyl), 1621 ( $\alpha : \beta$  - unsaturated ketone) and two sugars, glucose and a pentose. Methylation of (II) gave 7, 4'-DIMETHOXYISOFLAVONE (III),  $\nu_{\max}$   $1625 \text{ cm}^{-1}$  ( $\alpha : \beta$  - unsaturated ketone). Alkaline hydrogen peroxide oxidation of (III) gave FORMIC ACID, 4 - METHOXY SALICYLIC ACID (IV) and 4 - METHOXY BENZOIC ACID (V) which established the structure of (II) beyond any doubt.

Catalytic hydrogenation of (III) over 10% Pd/C gave two products, 7, 4'-DIMETHOXYISOFLAVANONE (VIII) and 7, 4'-DIMETHOXYISOFLAVAN (IX). Methylation of ambonin (I) with dimethyl sulphate afforded an oily product which was hydrolysed with hydrochloric acid to yield FORMONONETIN (X) (7-hydroxy-4'-methoxyisoflavone)  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3100 (hydroxyl), 1628 ( $\alpha : \beta$  - unsaturated ketone), which established that the sugar moiety is attached to the aglycone, daidzein (II), as a disaccharide in the 7-position.

The separation of the sugars of the glycoside ambonin (I) was successfully achieved by chromatography of the neutralised, evaporated hydrolysate on a

column of powdered cellulose. The second sugar from the column was found to be  $\alpha$ -D-GLUCOSE. The first sugar from the column, SUGAR X  $\left[ \begin{array}{c} \alpha \\ \text{D} \end{array} \right]^{21} + 8.2^{\circ}$ , was obtained as a syrup and could not be induced to crystallise. Acetylation of the sugar afforded the acetoxy derivative as a colourless oil. The mass spectra of sugar X and its acetoxy derivative as well as the NMR spectrum of the latter strongly indicated that the sugar is most likely a pentose.

(b) THE DEHYDROROTENOID DEHYDRODOLINEONE C<sub>19</sub>H<sub>10</sub>O<sub>6</sub>

The infrared spectrum of DEHYDRODOLINEONE (XVI), clearly shows the presence of a methylenedioxy group,  $\nu_{\text{max.}}$  (cm<sup>-1</sup>): 936, 1032 and 1156, which was further substantiated by a positive Labat test for this group and an  $\alpha$  :  $\beta$  - unsaturated ketone  $\nu_{\text{max.}}$  1634 cm<sup>-1</sup>. The ultraviolet spectrum shows the presence of a benzofuran system,  $\lambda_{\text{max.}}$  237 m $\mu$  (log  $\epsilon$  4.42). Hydrogenation of (XVI) over 10% Pd/C in ethyl acetate afforded DIHYDRODOLINEONE (XXIX). The saturation of the 6a, 12a-double bond of (XVI) increased the carbonyl frequency of (XVI),  $\nu_{\text{max.}}$  1634 cm<sup>-1</sup>, to 1659 cm<sup>-1</sup> for (XXIX) as expected.

The oxidation of (XVI) with n-amyl nitrite in glacial acetic acid gave NEBOENSINONE (XVII) (see under (c) ), identical in every way with the natural product. The mass-spectral fragmentation pattern of (XVI) (M334) is in full agreement with the proposed structure for this compound.

(c) THE DEHYDROROTENOID NEBOENSINONE C<sub>19</sub>H<sub>8</sub>O<sub>7</sub>

The infrared spectrum of NEBOENSINONE (XVII) shows in addition to the absorption band at  $\nu_{\text{max.}}$  1639 cm<sup>-1</sup> (  $\alpha$  :  $\beta$  - unsaturated ketone) the presence of an  $\alpha$  :  $\beta$  - unsaturated lactone group at 1724 cm<sup>-1</sup>. The presence of a methylenedioxy group is shown by the absorption bands at  $\nu_{\text{max.}}$  930, 1028 and 1155 cm<sup>-1</sup>. The ultraviolet spectrum of (XVII)  $\lambda_{\text{max.}}$  m $\mu$  (log  $\epsilon$  ): 252 (4.10) and 262 (4.12) (benzofuran and carbonyl) also has a very high absorption band at 404 (3.85). As this compound is identical in all respects with the oxidation product of (XVI) (see under (b)) there can be little doubt as to the structure of this compound, which is also in full agreement with data obtained from its mass spectrum, (M 348).

(d) THE DEHYDROROTENOID NAMBINONE C<sub>20</sub>H<sub>12</sub>O<sub>7</sub>

An accurate mass-spectral molecular weight determination showed that NAMBINONE (XVIII) has a formula of C<sub>20</sub>H<sub>12</sub>O<sub>7</sub> (M364). As with neboensinone (XVII) the infrared spectrum shows the presence of an  $\alpha$  :  $\beta$  - unsaturated ketone at  $\nu_{\text{max.}}$  1650 cm<sup>-1</sup> and a lactone at  $\nu_{\text{max.}}$  1735 cm<sup>-1</sup>. In contrast

to dehydrodolineone (XVI) and neboensinone (XVII) the infrared spectrum shows no bands for a methylenedioxy group, but absorption bands at  $\nu_{\text{max}}$ . 2920, 2850 and  $1457 \text{ cm}^{-1}$  indicate the presence of methoxyl groups. The ultraviolet spectrum of this compound is virtually identical to that of neboensinone,  $\lambda_{\text{max}}$ .  $m\mu$  ( $\log \epsilon$ ) : 258 (4.04) (benzofuran and ketone) and 406 (3.51), suggesting a similar structure. The mass-spectral fragmentation pattern is also in full agreement with the proposed structure for this compound. Due to the fact that only very small amounts of this compound is available from the natural source, extensive chemical investigation was impossible. Because of the insolubility of these compounds in most organic solvents used in NMR-spectral studies, the latter was also excluded as a possible method for confirming the tentative structure suggested.

(e) MASS-SPECTRAL FRAGMENTATION PATTERNS OF ISOFLAVANOIDS AND DEHYDROROTENOIDS

In order to obtain more information on the mass-spectral fragmentation patterns of isoflavanoids and related compounds, studies on the fragmentation patterns of seven isoflavanoids and three dehydrorotenoids were undertaken. It was found that these compounds are relatively stable to electron impact. The most pronounced cleavage mode of the isoflavanones and derivatives is via a retro-Diels - Alder fragmentation of the molecule. In the case of the furocoumarins the retro-Diels-Alder fragmentation pattern is not an important process due to the stabilization effect of the 3, 4 - double bond. The loss of carbon monoxide is the breakdown pathway mostly favoured for these compounds. The stability of the dehydrorotenoids is due to the 6a, 12a-double bond which causes a high degree of conjugation and very little fragmentation occurs. The major breakdown paths for the dehydrorotenoids are via the loss of a hydrogen atom and the elimination of carbon monoxide.

(ii) (a) THE SYNTHESIS OF HYDROXYBENZOFURANS

Three hydroxybenzofurans were synthesized. The preparation of 6-HYDROXY -, 4, 6 - DIHYDROXY -, and 6, 7 - DIHYDROXY - 2, 3 - DIHYDROBENZOFURAN - 3 - ONE, (XXXIV), (XLI) and (XLVI) according to known methods is described. Acetylation of (XXXIV), (XLI) and (XLVI) with acetic anhydride in pyridine and hydrogenation of the acetoxy derivatives over Pd/C at  $60^{\circ}$ - $70^{\circ}\text{C}$

under 4 atmospheres pressure afforded 6 - ACETOXY - , 4, 6 - DIACETOXY - and 6, 7 - DIACETOXY - 2, 3 - DIHYDROBENZOFURAN, (XXXVI), (XLIII) and (XLVIII) respectively.

By refluxing compounds (XXXVI), (XLIII) and (XLVIII) in dry benzene with DDQ (2, 3 - dichloro-5, 6 - dicyano - 1, 4 - benzoquinone) for different periods of time afforded 6 - ACETOXY - , 4, 6 - DIACETOXY - and 6, 7 - DIACETOXYBENZOFURAN, (XLIX), (L) and (LI) respectively in good yields. This is to the best of our knowledge the first instance where a high potential quinone (DDQ) was used to dehydrogenate dihydrobenzofuran compounds. It was found that the hydroxybenzofurans are very sensitive to oxygen in alkaline medium which excluded the use of alkali for the hydrolysis of the acetoxybenzofurans. The reductive cleavage of the acetyl groups with lithium aluminium hydride in ether under a nitrogen atmosphere yielded the corresponding hydroxybenzofurans, 6 - HYDROXY - , 4, 6 - DIHYDROXY - and 6, 7 - DIHYDROXYBENZOFURAN, (LII), (LIII) and (LIV) respectively.

(b) PHENOLIC DERIVATIVES OF ISOFLAVANOIDS

The preparation of the phenolic compounds PACHYRRHIZINOL (LV); the DEOXYBENZOIN OF DEHYDRONEOTENONE (LVI) and FORMONONETIN (X) from the isoflavanoids pachyrrhizin (XIII), dehydroneotenone (XV) and, in the case of formononetin, from the glycoside ambonin (I), is described.

(c) NUCLEAR MAGNETIC RESONANCE STUDIES OF HYDROXY-BENZOFURANS AND RELATED COMPOUNDS

Nuclear magnetic resonance studies of hydroxybenzofurans and related compounds were undertaken in which extensive use was made of the relatively new anion shift technique in order to establish whether this method can be used as an aid in the structural elucidation of isoflavanoids and related compounds. The nuclear magnetic resonance anion shift data obtained from ten phenolic compounds are given and discussed (Chapter 7). In benzofuran compounds long-range spin-spin coupling between the protons in positions 3 and 7 was observed, a fact which can be very useful in detecting substitution in these positions.

GENERAL PART

CHAPTER 1

INTRODUCTION

## INTRODUCTION

The genus *Neorautanenia* belongs to the tribus Phaseoleae of the sub-family Papilionatae of the Leguminosae. The Leguminosae has proved to be one of the richest sources of isoflavanoids. The genera *Mundulea*, *Milletia*, *Lonchocarpus*, *Tephrosia* and *Derris*, which also belong to the sub-family Papilionatae, are known to be very potent fish-poisonous plants. These plants are also very toxic to insects, the *Derris* genus for example has been commercially exploited as an insecticide. It has been established that the toxic properties of these plants are due to a group of chemically closely related compounds, the rotenoids, of which the most important is rotenone from which the name of the group is derived.

The *Neorautanenia* species occur in several regions of the Republic as well as in Central Africa. The National Herbarium lists eight *Neorautanenia* species:

- (1) *N. amboensis* Schinz.
- (2) *N. brachypus* (Harms) C. A. Sm.
- (3) *N. coriacea* C. A. Sm.
- (4) *N. deserticola* C. A. Sm.
- (5) *N. edulis* C. A. Sm.
- (6) *N. ficifolia* (Benth.) C. A. Sm.
- (7) *N. lugardii* (N. E. Br.) C. A. Sm.
- (8) *N. pseudopachyrrhiza* (Harms) Milne Redhead.

Several taxonomists hold the view that the classification of the *Neorautanenia* species as given above should be revised as they are convinced that *N. edulis* and *N. coriacea* are variations of the same species, that *N. ficifolia* and *N. deserticola* are identical and that *N. brachypus* and *N. lugardii* are variations of *N. amboensis*. Although the four *Neorautanenia* species which have been investigated so far have one or more isoflavanoids in common, the basic isoflavanoid pattern of each species differ considerably (Table A) and this fact can be of considerable taxonomic importance in the re-classification of this species.

Three *Neorautanenia* species have been investigated in this department, *N. amboensis*, *N. ficifolia*<sup>44, 55</sup> and *N. edulis*<sup>30, 31</sup>. *N. amboensis* occurs in

the Kruger National Park near Shingwidzi and Tshokwane and also in the Khomas Highlands in South West Africa. *N. ficifolia* occurs in the Bothavillé (O.F.S.) and adjoining districts as well as in the Pretoria district. The third species, *N. edulis*, which was also the first of the three species to be investigated, occurs in the northern Soutpansberg bushveld and Koedoesrand areas in the Transvaal. It is known that *N. edulis* and *N. ficifolia* are fish-poisonous plants, but the toxic properties of *N. amboensis*, if any, are not known as no tests in this regard have been carried out. *N. pseudopachyrrhiza*, which occurs in Tanzania, is to the best of our knowledge the only *Neorautanenia* species which has been investigated outside the Republic by Crombie et al.<sup>15</sup> This species is also reported to be toxic to insects. The *Neorautanenia* species are characterized by their exceptionally large roots (tubers), which often attain weights of 40 to 60 pounds.

A variety of isoflavanoids and rotenoids have been isolated from the four *Neorautanenia* species which have so far been investigated and the results to date are summarized in Table A.

TABLE A.

Isoflavanoids and rotenoids isolated from the *Neorautanenia* species.

Compound	<i>N. amboensis</i>	<i>N. edulis</i>	<i>N. ficifolia</i>	<i>N. pseudo-pachyrrhiza</i>
Neodulin	+	+	-	-
Neotenone	+	+	+	+
Pachyrrhizin	+	+	-	+
Dehydroneotenone	+	+	-	-
Neofolin	-	-	+	-
Ficinin	-	-	+	-
Neficin	-	-	+	-
Nepseudin	-	-	-	+
Dolineone	-	-	-	+
Dehydrodolineone	+	-	-	-
Neboensinone	+	-	-	-
Nambinone	+	-	-	-
Daidzein (as the 7-O-glycoside ambonin)	+	-	-	-

From Table A it can be seen that the furoisoflavanone neotenone is common to all four *N.* species. *N. amboensis* and *N. edulis* have four isoflavanoids in common and it is worth mentioning that the pterocarpan, neodulin, was previously thought to be characteristic for *N. edulis*. *N. amboensis* has a remarkably rich variety of isoflavanoids and rotenoids, i. e. a pterocarpan (neodulin), a furocoumarin (pachyrrhizin), an isoflavone (dehydroneotenone), an isoflavanone (neotenone) as well as three new dehydrorotenoids, dehydrodolineone, neboensinone and nambinone, the latter two representing a new class of compound which contain isoflavone and coumarin structures. In addition a dihydroxyisoflavone (daidzein) was also isolated from *N. amboensis* as the new 7-O-glycoside ambonin, which, to the best of our knowledge, is reported for the first time from the *Neorautanenia* species. It is remarkable that an isoflavone (daidzein) of such a relatively simple structure should be associated with the more complex furoisoflavanoids and dehydrorotenoids. The biogenetic relationships of the compounds isolated from *N. amboensis* and the other *N.* species raise interesting questions and should prove to be a very promising field of study.

The three dehydrorotenoids from *N. amboensis* mentioned above are the first natural dehydrorotenoids that have been isolated and, in addition, the dehydrorotenoids neboensinone and nambinone represent an entirely new (ketolactone) type of rotenoid. The isolation of an isoflavanone (neotenone) and its corresponding rotenoid (dolineone) from the same plant, *N. pseudopachyrrhiza*,<sup>15</sup> was the first fully authenticated instance where these two compounds were shown to occur together. In this regard it is interesting to note that the corresponding dehydro- compounds i. e. dehydroneotenone and dehydrodolineone also occur together in *N. amboensis*. All the furoisoflavanoids and rotenoids isolated from *N. amboensis* and *N. pseudopachyrrhiza* possess a methylenedioxy group except the isoflavanone nepseudin (from *N. pseudopachyrrhiza*) and the rotenoid nambinone (from *N. amboensis*) which have two methoxyl groups in lieu of a methylenedioxy group. From a biosynthetic point of view the synthesis of rotenoids from isoflavanoids appears very attractive,

*N. edulis* was extensively examined by several workers but no rotenoids could be found. In the case of *N. ficifolia*, which is still being investigated, three minor compounds, compound A m. p. 230°C, compound B m. p. 270°C and compound X m. p. 400°C, were isolated but were not further investigated.<sup>44</sup> Of these three compounds any one or all three might possibly be rotenoids.

It was found that the relative proportions of the compounds isolated from *N. amboensis* varied considerably during the different seasons. Neodulin, for example, was only found in very small quantities from plant material collected during May, whereas the relative proportion of this compound increased considerably from plant material obtained during January. This seasonal variation in the relative abundance of the components are also known to occur in the other three *N.* species. Due to this seasonal fluctuations in the relative abundance of these compounds it is very important to examine batches of the plant material throughout the plant's growth cycle as certain components, especially in the case of the minor constituents, can easily be overlooked.

Four of the isoflavanoids isolated from *N. amboensis* are known compounds which have previously been isolated.<sup>15, 30</sup> Chemical degradation of the new glycoside ambonin showed the aglycone to be the known 7, 4'- dihydroxy-isoflavone (daidzein) which was first isolated by Walz<sup>7</sup> from soya beans (*Soja hispida*). The sugar moiety was found to be a disaccharide (glucose + a pentose) attached to the 7-position of the aglycone.

The structural elucidations of the three new dehydrorotenoids isolated from *N. amboensis* were mainly achieved by spectrophotometric methods as a result of the very small quantities of materials that were available which precluded conventional chemical methods of investigation. In this regard extensive use was made of mass-spectral data obtained from related compounds i. e. isoflavanoids. The final proof for the structures advanced for these dehydrorotenoids will have to await further chemical and spectrophotometric examinations when more material becomes available or total synthesis.

A new route for the synthesis of hydroxybenzofurans was also developed. Nuclear magnetic resonance studies of the hydroxybenzofurans and related compounds using the relatively new anion shift technique, afforded much information as to the possible application of this method as an aid in the structural elucidation of isoflavanoids and related compounds.

## CHAPTER 2.

### STRUCTURE OF THE ISOFLAVONE GLYCOSIDE AMBONIN

- A. GENERAL
- B. HYDROLYSIS OF AMBONIN
- C. METHYLATION OF DAIDZEIN
- D. ALKALINE HYDROGEN PEROXIDE OXIDATION OF DIMETHOXYDAIDZEIN
- E. HYDROGENATION OF DIMETHOXYDAIDZEIN
- F. METHYLATION OF AMBONIN
- G. SEPARATION AND IDENTIFICATION OF THE CONSTITUENT MONOSACCHARIDES.

## STRUCTURE OF THE GLYCOSIDE AMBONIN (I)

### A. GENERAL

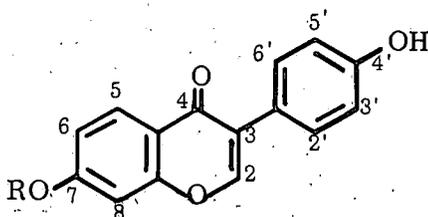
The glycoside ambonin (I) was obtained as the major constituent from *N. amboensis* as a white crystalline compound melting point  $230^{\circ} - 232^{\circ}\text{C}$  (from water). The drying of ambonin at  $110^{\circ}\text{C}$  for 16 hours lowered the melting point to  $225.5^{\circ} - 227.5^{\circ}\text{C}$  with a corresponding loss of weight, indicating the presence of water of crystallisation. Ambonin (I) is the only isoflavone glycoside that has been isolated so far from any of the three *Neorautanenia* species, *N. edulis*, *N. ficifolia* and *N. amboensis*, which have been investigated in this department.

The infrared spectrum of ambonin (plate 1) shows strong absorption bands at  $\nu_{\text{max.}} (\text{cm}^{-1})$ : 3340 (hydroxyl); 1630 ( $\alpha$ :  $\beta$  - unsaturated ketone); 1615, 1563, 1515 (aromatic). The ultraviolet spectrum of ambonin shows absorption bands at  $\lambda_{\text{max.}} (\text{m}\mu)$  ( $\log \epsilon$ ): 232 (4.30) ( $\alpha$ :  $\beta$  - unsaturated ketone); 262 (4.46), and  $302^1$  (3.94) (aromatic).

Genistin and sophoricoside<sup>1,2</sup>, the 7- and 4'-glucosides of genistein (5, 7, 4' - trihydroxyisoflavone) have very similar ultraviolet spectra. Analysis showed that ambonin contains no methoxyl - or C - methyl groups, but phenolic hydroxyl groups. Ambonin has a specific rotation of  $[\alpha]_{\text{D}}^{20} - 73.8^{\circ}$  in a 0.02 M, potassium hydroxide solution and gave a positive Molisch test.

### B. HYDROLYSIS OF AMBONIN (I).<sup>3,4,5</sup>

Hydrolysis of (I) with dilute sulphuric acid afforded the aglycone daidzein (II) (7, 4'-dihydroxyisoflavone) m. p.  $330^{\circ} - 333^{\circ}\text{C}$  (decomp.) as a white amorphous powder.<sup>7,8,9,10</sup> Thin-layer chromatography of the neutralised filtrate showed the presence of two sugars.



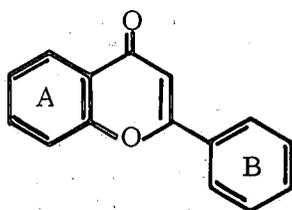
(I) R = Disaccharide (glucose + pentose)

(II) R = H.

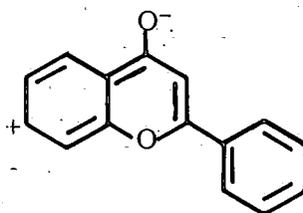
The infrared spectrum of daidzein (II) (plate 1) has strong bands at  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3160 (phenolic hydroxyl); 1621 ( $\alpha : \beta$  - unsaturated ketone); 1598, 1587, 1513 (aromatic); 1235 (hydroxyl). Ultraviolet spectrum  $\lambda_{\max}$  ( $\text{m}\mu$  ( $\log \epsilon$ )): 232<sup>i</sup> (4.22); 249 (4.30) ( $\alpha : \beta$  - unsaturated ketone); 300 (3.87) (aromatic).

Infrared and ultraviolet spectroscopic examination of flavanoids is very informative and can yield much information about the compounds. Isoflavones have fairly characteristic ultraviolet spectra and their infrared spectra usually show strong multiple bands in the 1400 - 1600  $\text{cm}^{-1}$  region.

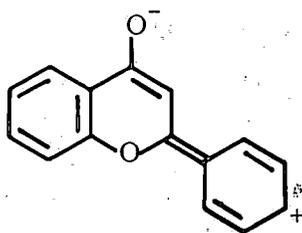
The most distinctive difference between isoflavones and flavones is in their ultraviolet spectra.<sup>2,6</sup> Flavones (a) and flavonols generally exhibit high intensity absorption in the 320-380  $\text{m}\mu$  region (Band II) and a lower intensity in the 240-270  $\text{m}\mu$  region (Band I). The position and intensity of the absorption of each of these bands vary with the relative resonance contributions of the benzoyl (b), cinnamoyl (c) and pyrone ring (d) groupings to the total resonance of the flavone molecule.



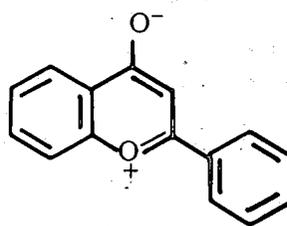
(a)



(b)

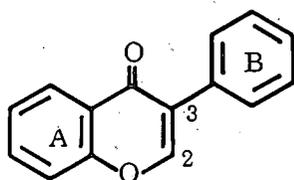


(c)



(d)

Although these groupings interact, Band II is mainly associated with absorption in the cinnamoyl grouping (c) and Band I with absorption in the benzoyl grouping (b).<sup>11</sup>



(e)

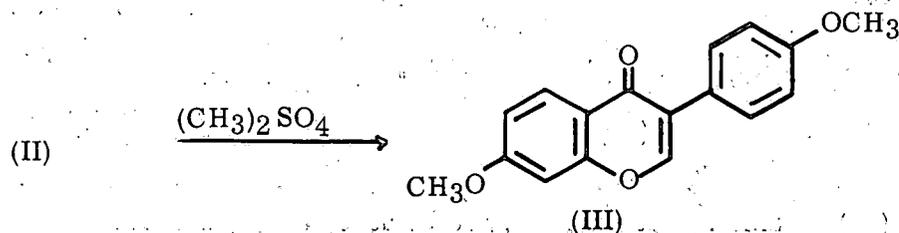
In isoflavones (e) the phenyl ring at position 3 is not conjugated with the pyrone carbonyl group. Consequently, Band II which in flavones is associated with the conjugated lateral B ring (c), is either absent or considerably diminished in intensity in the spectra of isoflavones. Isoflavones, therefore, show one intense absorption maximum at 240-270  $m\mu$  (Band I) and a peak or inflection at 290-330  $m\mu$  (Band II) of much lower intensity.

Band II is thus of diagnostic value since isoflavones show weaker absorption than is shown by their flavone analogues. Isoflavanones show absorption which is not very different from that of isoflavones.<sup>12, 13</sup>

The ultraviolet and infrared spectra of daidzein (II) thus strongly indicated that the compound was an hydroxyisoflavone.

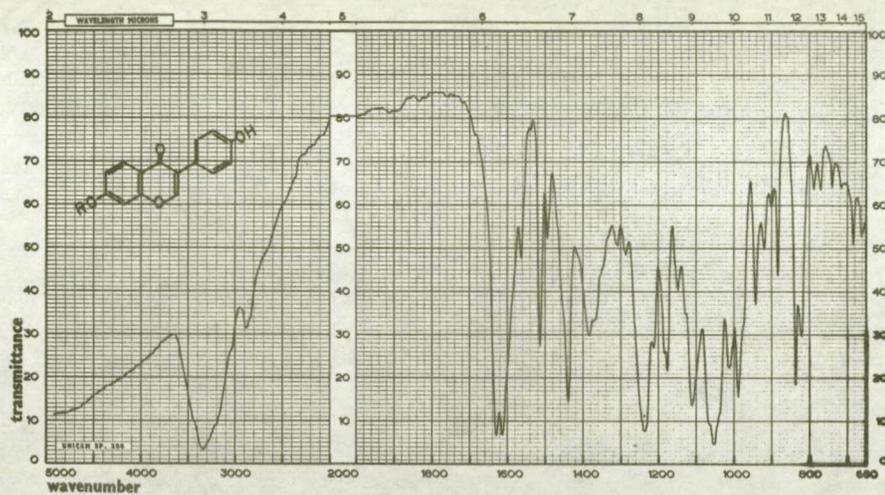
#### C. METHYLATION OF DAIDZEIN (II) <sup>9, 10, 14.</sup>

Methylation of (II) with dimethyl sulphate in dry acetone and the subsequent chromatography of the reaction product on alumina afforded the pure methoxy derivative of daidzein (III). Analysis showed that the compound contains two methoxyl groups and thus established that daidzein is a dihydroxyisoflavone.

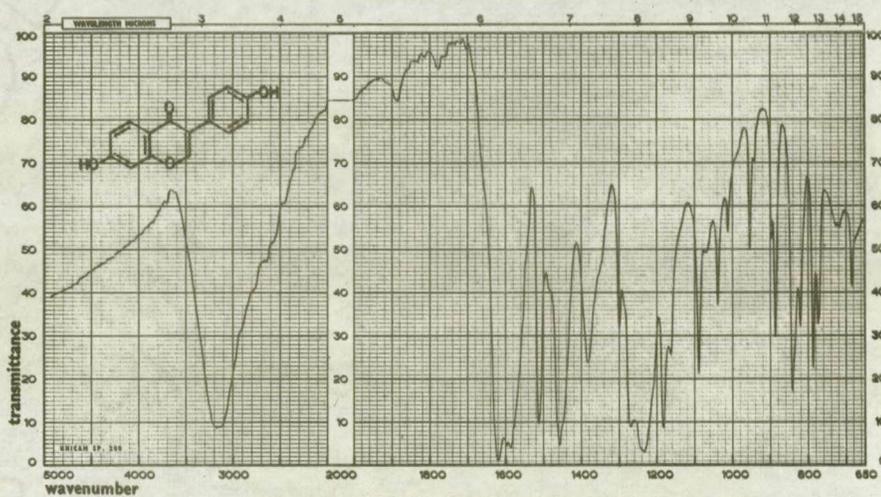


The infrared spectrum of 7, 4' - dimethoxydaidzein (III) (plate 1) showed no hydroxyl groups and has absorption bands at  $\nu_{\max}$ . ( $cm^{-1}$ ): 1625 ( $\alpha$  :  $\beta$  - unsaturated ketone); 1597, 1560 and 1510 (aromatic).

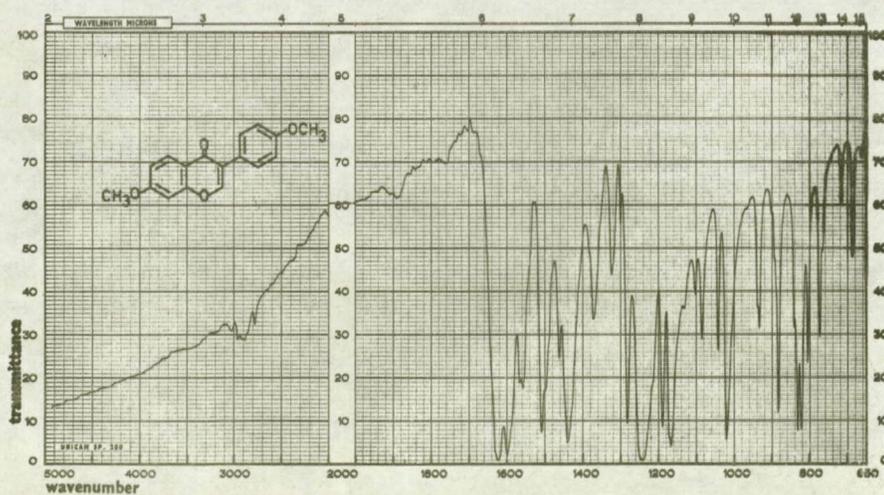
## PLATE 1



(a) AMBONIN (I)



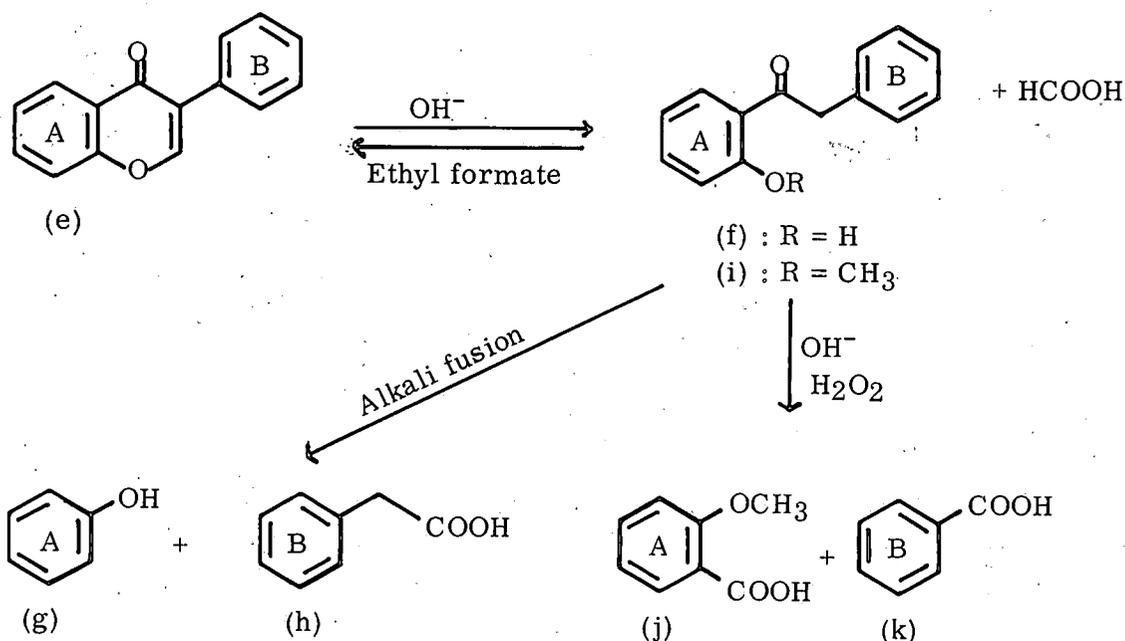
(b) DADZEIN (II)



(c) 7,4' - DIMETHOXYDADZEIN (III)

D. (i) ALKALINE HYDROGEN PEROXIDE OXIDATION OF  
7,4' - DIMETHOXYDAIDZEIN (III)<sup>15</sup>

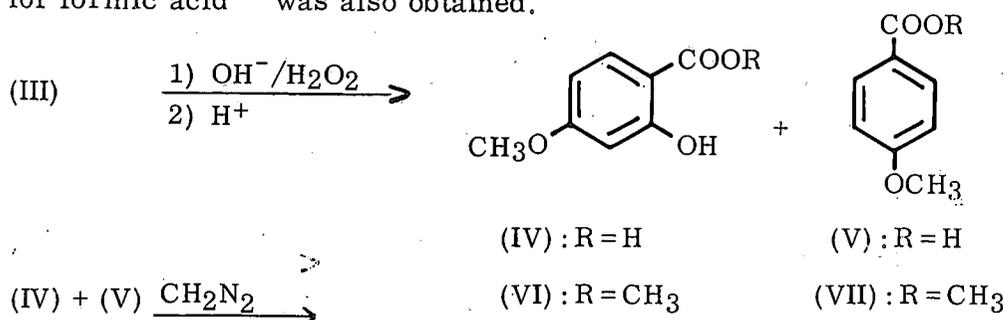
The usual methods of isoflavone degradation are shown below.



Isoflavones (e) are generally stable towards acidic reagents and basic hydrolysis is usually more informative. Alkaline hydrolysis under mild conditions will usually transform an isoflavone into the corresponding deoxybenzoin (f) and formic acid. This reaction is diagnostic for an isoflavone and may be confirmed by resynthesis of the isoflavone from the deoxybenzoin (f) with ethyl formate<sup>16</sup> or ethyl orthoformate<sup>17</sup>. Vigorous alkaline hydrolysis of the deoxybenzoin (f) leads to the formation of the phenol (g) and the phenylacetic acid (h).

Alkaline hydrogen peroxide oxidation of the fully methylated deoxybenzoin (i) leads to the formation of the two acids (j) and (k).

The alkaline hydrogen peroxide oxidation of (III) afforded the two acids 4-methoxy salicylic acid (IV) and 4-methoxy benzoic acid (V). A positive test for formic acid<sup>18</sup> was also obtained.



(ii) METHYLATION OF THE ACIDS (IV) AND (V)

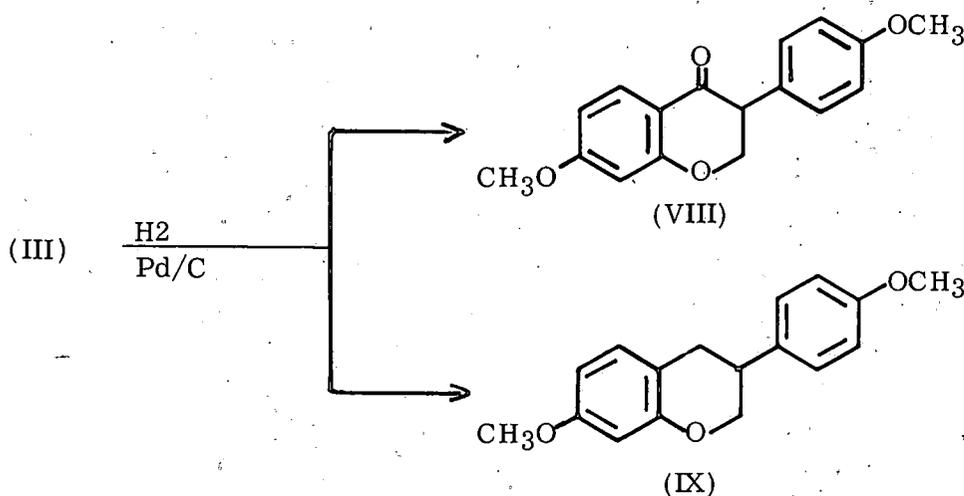
Methylation of the acids (IV) and (V) in anhydrous ether with diazomethane yielded the corresponding methyl esters, 4-methoxy methyl salicylate (VI) and 4-methoxy methyl benzoate (VII).

Evaporation of the ether gave the esters (VI) and (VII) as an oily residue. The residue was taken up in benzene and separated by extracting with cold potassium hydroxide solution. Acidification of the alkaline layer gave (VI) and the ester (VII) was obtained from the benzene layer.

Hydrolysis of the esters (VI) and (VII) with dilute sodium hydroxide solution at room temperature and acidification afforded the acids (IV) and (V), which were positively identified by comparison with authentic samples (infrared, melting points and  $R_f$  values). The structure of the aglycone of ambonin (I) was thus unambiguously proved as 7, 4' - dihydroxyisoflavone (daidzein) (II) 7, 8, 9, 10. Daidzein was first isolated by Walz<sup>7</sup> from soya beans (*Soja hispida*) as the 7-O-glucoside daidzin and subsequently also from the roots of *Peuraria thunbergiana* Benth.<sup>19</sup> as well as from several other members of this species.

E. HYDROGENATION OF DIMETHOXYDAIDZEIN (III)

Catalytic hydrogenation of isoflavones can lead to a variety of products, depending on the catalyst and solvent used<sup>12,20</sup>. Hydrogenation of (III) in ethyl acetate over 10% Pd/C catalyst until the hydrogen absorption ceased, yielded two products, 7, 4' - dimethoxyisoflavanone (VIII)<sup>12</sup> and 7, 4' - dimethoxyisoflavan (IX)<sup>12</sup>.

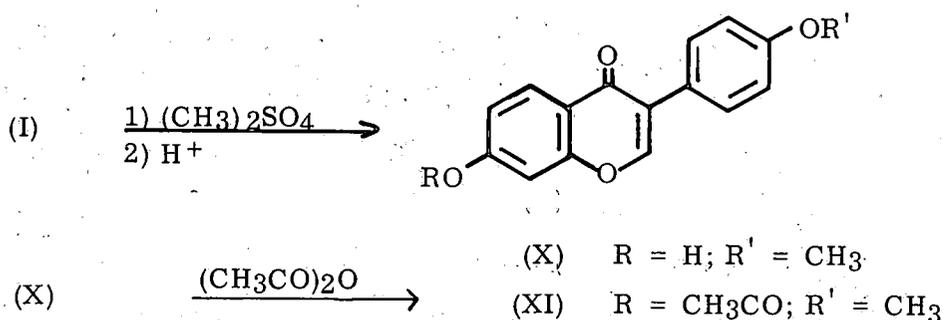


Infrared spectrum of (VIII)  $\nu_{\max}$ . ( $\text{cm}^{-1}$ ): 1664 (carbonyl); 1595, 1563, 1510 (aromatic).

Infrared spectrum of (IX)  $\nu_{\max}$ . ( $\text{cm}^{-1}$ ): 1602, 1572, 1510 and 1500 (aromatic).

#### F. METHYLATION OF AMBONIN (I)<sup>14</sup>

In order to ascertain whether the sugar moiety is attached to the aglycone as a disaccharide or as two monosaccharides ambonin was methylated with dimethyl sulphate in acetone. The fully methylated derivative of ambonin was obtained as an oil which was hydrolysed with hydrochloric acid in methanol. The hydrolysis yielded a crystalline product which was positively identified by its ultraviolet and infrared spectra, melting point and acetoxy derivative (7-acetoxy-4'-methoxyisoflavone) (XI) m. p.  $170^{\circ}$ - $171^{\circ}\text{C}$  as 7-hydroxy-4'-methoxyisoflavone (formononetin) (X). Formononetin (X) is a naturally occurring isoflavone which has been isolated from subterranean clover (*Trifolium subterraneum* L.)<sup>9</sup> and from *Ononis spinosa* L.<sup>8,9</sup> as the glucoside ononin.



It was thus established that the sugar moiety is attached to the aglycone (II) at the 7-position as a disaccharide.

Formononetin (X) infrared spectrum  $\nu_{\max}$ . ( $\text{cm}^{-1}$ ): 3100 (phenolic hydroxyl); 1628 ( $\alpha$  :  $\beta$  - unsaturated ketone); 1612, 1600, 1590, 1562 and 1510 (aromatic).

Ultraviolet spectrum  $\lambda_{\max}$ .  $m_{\mu}$ . ( $\log \epsilon$ ): 249 (4.45); 255 (4.44); 302 (4.03).

7-Acetoxyformononetin (XI) infrared spectrum  $\nu_{\max}$ . ( $\text{cm}^{-1}$ ): 1747 (O-acetyl); 1637 ( $\alpha$  :  $\beta$  unsaturated ketone); 1610, 1565 and 1513 (aromatic).

G. SEPARATION AND IDENTIFICATION OF THE CONSTITUENT MONOSACCHARIDES<sup>21</sup>

The neutralised filtrate from the acid hydrolysis of ambonin (I) was evaporated under diminished pressure at 40°C until a syrup was obtained. The individual monosaccharides were very successfully separated by chromatography of the sugar mixture on a column of powdered cellulose, as described by Hough et al.<sup>21</sup>, using acetone - water and methanol - water mixtures as eluents. The eluate fractions from the column were examined by thin-layer chromatography using kieselguhr plates buffered with sodium acetate.

Combination of the appropriate first fractions of acetone-water eluate from the column, fraction 1, yielded a colourless syrup which consisted of the monosaccharide sugar X. After a small transition zone the second monosaccharide was obtained from the column, fraction 2, using a methanol-water mixture as eluent. Thin-layer chromatography (TLC) of this sugar showed that it had the same  $R_f$  value and colour reaction (with p - anisaldehyde - sulphuric acid) as glucose. The sugar was obtained crystalline from methanol-acetone and positively identified from its melting point, mixed melting point, osazone and specific rotation as  $\alpha$  - D - glucose.

Despite all efforts sugar X (fraction 1 from column) could not be induced to crystallise. Addition of dry acetone to the syrup dissolved in the minimum amount of absolute methanol caused the sugar to precipitate as a fine white amorphous powder which was, however, found to be highly hygroscopic. TLC of the sugar showed that it has approximately the same  $R_f$  value as rhamnose. Sugar X has a characteristic colour reaction with anisaldehyde-sulphuric acid reagent. Upon heating, the spot first becomes red-violet, then turns blue and then reverts back to the original red-violet colour after approximately 12 hours at room temperature. Comparison of sugar X on TLC plates with other available monosaccharides showed that it is not any of the following: fructose, sorbose, galactose, mannose, ribose, arabinose, xylose or rhamnose. The high  $R_f$  value of sugar X indicated that it might possibly be a pentose or deoxy sugar.

The syrup (dried under high vacuum) gave a specific rotation value of + 8.2° in aqueous solution at 21°C. The phenylhydrazone derivative of the sugar was obtained as a brown resinous compound which yielded an amorphous product m.p. 70°-80°C. The p-nitrobenzoate derivative was obtained as a yel-

low crystalline product m. p. 70<sup>o</sup>-75<sup>o</sup>C. Tests for deoxy sugars were all negative. Acetylation of the syrup and purification by preparative TLC<sup>22</sup> afforded the sugar X acetate as a colourless oil.

The NMR spectrum of the acetoxy derivative (in CDCl<sub>3</sub>) integrated for 18 protons, six low field protons with resonances centred at  $\tau = 3.89; 4.54; 5.24; 5.44; 5.65$  and  $5.80$ , and four three-proton signals at  $\tau = 7.82; 7.83; 7.85$  and  $7.97$  which are in the characteristic range for the chemical shifts of carbohydrate acetoxy groups<sup>23</sup>. This data is thus in agreement for a pentose acetoxy derivative.

The mass spectra of sugar X and its acetoxy derivative further indicated that the sugar is a pentose. 24a, 25, 26, 27. The data of the mass spectra are given in Table 1

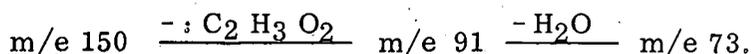
TABLE 1

Sugar X	m/e	132	119	101	91	86	73	60	59	40			
	I(%)	5	4	11	15	23	100	57	28	35			
Acetoxy Derivative	m/e	259	216	170	156	145	139	128	110	103	97	85	43
	I(%)	34	5	9	8	6	10	9	11	5	4	4	100

I = relative abundance.

Very little work has been done on the fragmentation patterns of carbohydrates. As may be expected<sup>26,27</sup> the parent molecular ion (at m/e 150 for a pentose) could not be observed. The peak at m/e 132 is in agreement with the M<sup>+</sup>-18 ion which could be expected by the loss of H<sub>2</sub>O from the M<sup>+</sup> ion (m/e 150) for a pentose.

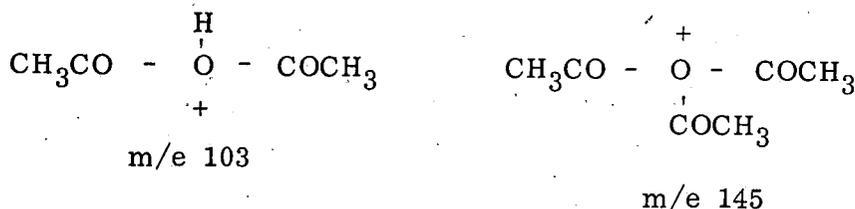
The ion at m/e 119 may be due to the loss of CH<sub>2</sub>OH from m/e 150. A possible fragmentation pattern for the formation of the base peak at m/e 73 is shown below.<sup>26</sup>



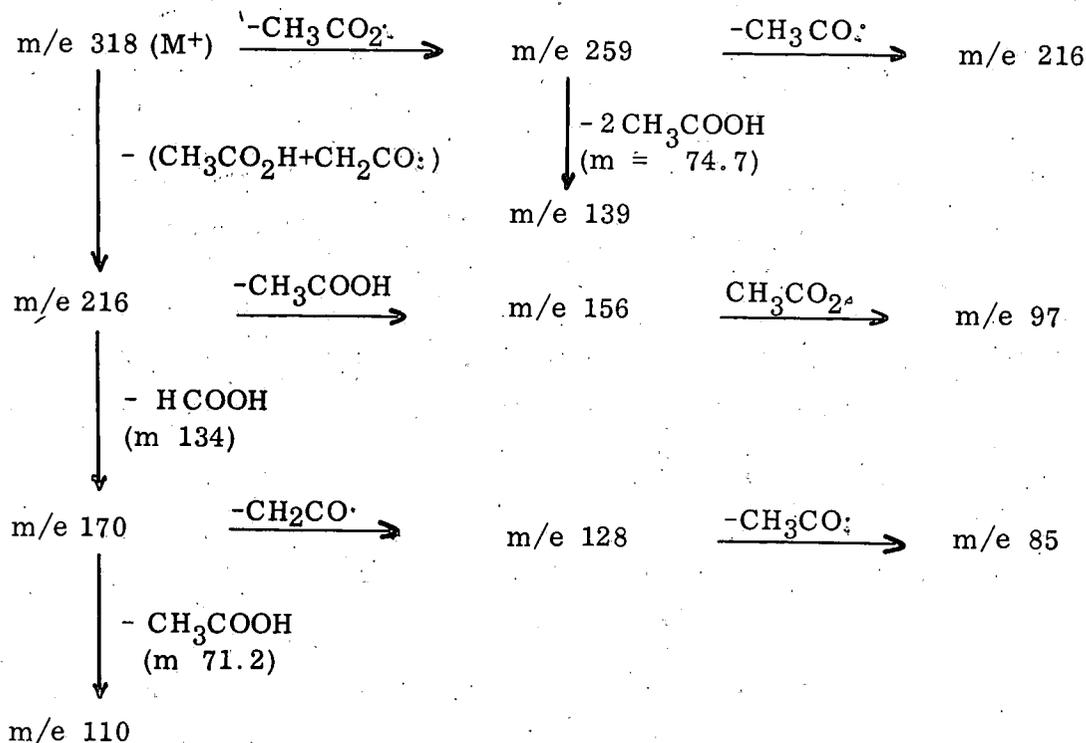
As a result of the low volatility of carbohydrates and their thermal lability most mass spectrometry work on carbohydrates has been done mainly on the more volatile derivatives such as the methyl and phenyl ethers, acetates and

acetonides. The spectra of all these derivatives are subject to certain limitations, the most notable being the absence of a measurable molecular ion.

In the spectra of carbohydrate acetate derivatives there are three ions (at  $m/e$  43, 103 and 145) which are derived from the acetate groups and which are characteristic among polyacetate spectra.<sup>24a</sup> The peak at  $m/e$  43 is due to the acetylum ion  $\text{CH}_3\text{CO}^+$  and it forms the base peak in all of the published spectra. The other two peaks at  $m/e$  103 and  $m/e$  145 are the di- and triacetyloxonium ions.



In the spectrum of the acetoxy derivative of sugar X (Table 1) all three these peaks are present, the peak at  $m/e$  43 being the base peak. The molecular ion peak at  $m/e$  318 (for a pentose tetra-acetate) is not visible. The highest mass peak is at  $m/e$  259 ( $M^+ - 59$ ). The fragmentation processes for this compound may possibly be interpreted as shown below.



Where  $m$  is the corresponding metastable peak.

Due to limited time no further work was done on sugar X, but it is envisaged that further work will be done in this department to determine the structure of sugar X. The complete structure of the glycoside ambonin (I) thus remains to be established.

In this regard it may be mentioned that Malhotra et al<sup>28</sup> recently isolated a new glycoside, lanceolarin, the 7-apioglucoside of the isoflavone biochanin - A. The branched chain sugar apiose (a pentose) has previously been found in only 3 flavone glycosides, lanceolarin being the first isoflavone glycoside which contains apiose. It is just possible that sugar X may also be a branched chain sugar similar to apiose.

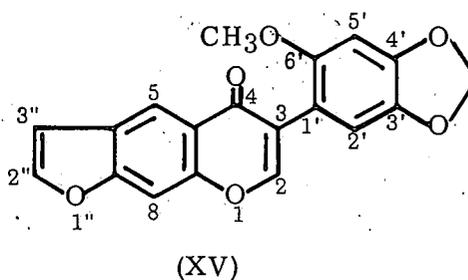
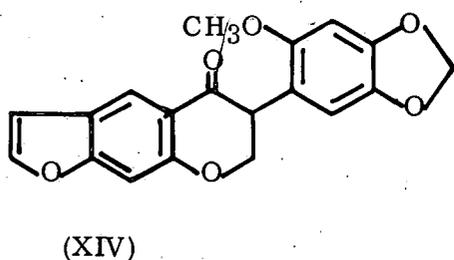
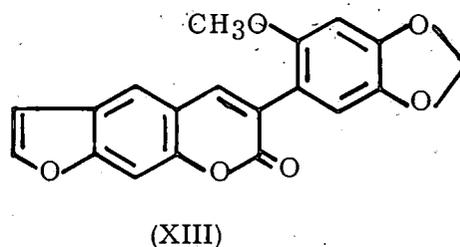
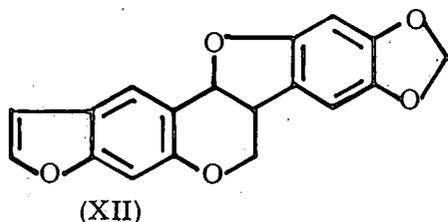
## CHAPTER 3.

- I. KNOWN ISOFLAVANOIDS FROM N. AMBOENSIS.
- II. THE STRUCTURAL ELUCIDATION OF THREE NEW DEHYDROROTENOIDS.
  - A. INTRODUCTION
  - B. DEHYDRODOLINEONE
  - C. NEBOENSINONE
  - D. NAMBINONE

## I. KNOWN ISOFLAVANOIDS FROM N. AMBOENSIS.

In this investigation four known isoflavanoids were isolated from *N. amboensis*, the pterocarpan neodulin (XII), the furocoumarin pachyrrhizin (XIII), the furoisoflavanone neotenone (XIV) and the furoisoflavone dehydroneotenone (XV).

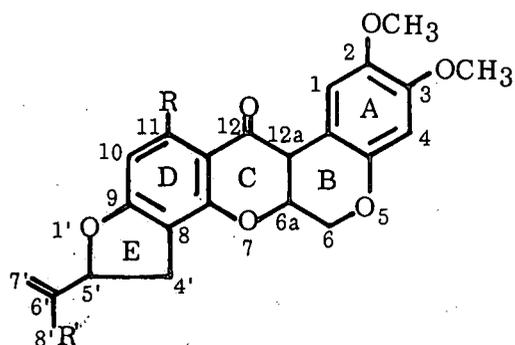
Neodulin (XII) has previously been found to occur only in *N. edulis*,<sup>29, 30</sup> from which the other three, (XIII), (XIV) and (XV) were also isolated.<sup>30, 31, 32.</sup> In addition pachyrrhizin (XIII), neotenone (XIV) and dehydroneotenone (XV) have also been isolated from *Pachyrrhizus erosus*<sup>15, 33, 34</sup> and *Neorautanenia pseudopachyrrhiza*.<sup>15</sup>



## II. THE STRUCTURAL ELUCIDATION OF THREE NEW DEHYDRO-ROTENONDS: DEHYDRODOLINEONE (XVI), NEBOENSINONE (XVII) AND NAMBINONE (XVIII).

### A. INTRODUCTION

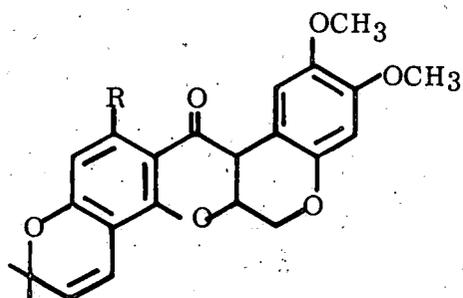
Prior to this investigation only ten natural rotenoids were known,<sup>15, 35, 36, 37.</sup> They all have methoxyl groups at positions 2 and 3 with the exception of pachyrrhizone (XXVII)<sup>35</sup> and dolineone (XXVIII)<sup>15</sup> which have a methylene-dioxy group in these positions. The structures of the known rotenoids are given below.



Rotenone (XIX) :  $R = H$ ;  $R' = CH_3$

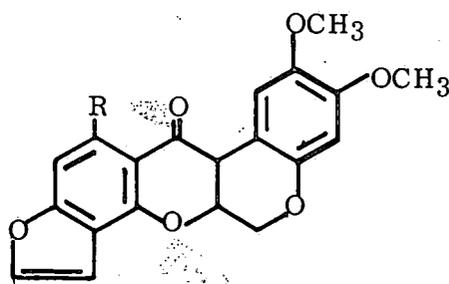
Sumatrol (XX) :  $R = OH$ ;  $R' = CH_3$

Amorphigenin (XXI) :  $R = H$ ;  $R' = CH_2OH$



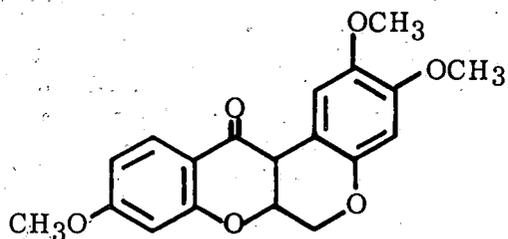
Deguelin (XXII) :  $R = H$

$\alpha$ -Toxicarol (XXIII) :  $R = OH$

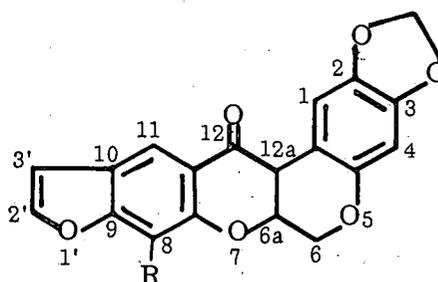


Elliptone (XXIV) :  $R = H$

Malaccol (XXV) :  $R = OH$



Munduserone (XXVI)

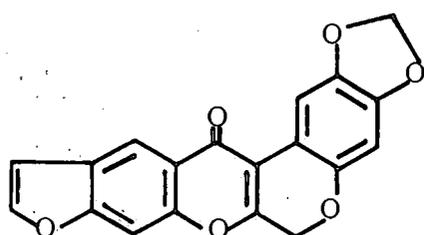


Pachyrrhizone (XXVII) :  $R = OCH_3$

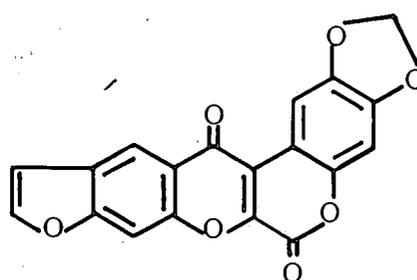
Dolineone (XXVIII) :  $R = H$

Amorphigenin (XXI)  $C_{23}H_{22}O_7$ , the aglycone of the first rotenoid glycoside amorphin, was the latest of the rotenoids thus far isolated. <sup>36</sup>

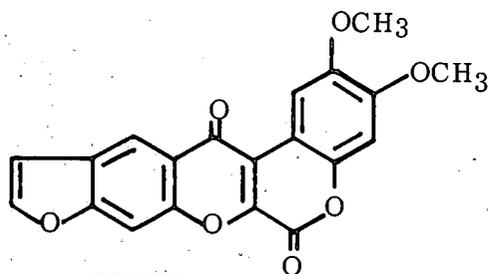
The three new rotenoids isolated in this investigation, dehydrodolineone (XVI), neboensinone (XVII) and nambinone (XVIII) are the first natural 6a, 12a - dehydrorotenoids that have been isolated and in addition neboensinone (XVII) and nambinone (XVIII) represent to the best of our knowledge also the first keto-lactone type of rotenoid that has been isolated.



(XVI)



(XVII)



(XVIII)

These rotenoids occur in extremely small quantities in *N. amboensis*, from approximately 70 kg dried raw material 80 mg (XVI), 110 mg (XVII) and 0.3 mg (XVIII) were obtained. The isolation of these compounds also presented major problems. The most successful method was found to be by slow fractional crystallisation from the resinous acetone extract. Due to the small amounts of material available no extensive chemical investigations were possible on these compounds and the structural elucidations of these rotenoids were mainly achieved by means of infrared-, ultraviolet- and mass spectrometry.

The solubility of these dehydrorotenoids was found to be extremely low in all the common organic solvents. Even in solvents such as pyridine and dimethyl sulphoxide the solubility of these compounds is in the order of 1 mg per 0.5 ml at a temperature of 100°C. As a result the NMR spectra of these rotenoids could unfortunately not be obtained and the determination of their specific

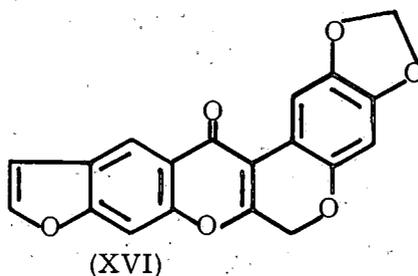
rotations was also not possible. The Rogers and Calamari<sup>38</sup> and Durham<sup>39</sup> tests for rotenoids were negative for these compounds, a fact which is attributed to the low solubility of these compounds. It is also not certain whether these tests apply to dehydrorotenoids.

B. DEHYDRODOLINEONE (XVI) C<sub>19</sub>H<sub>10</sub>O<sub>6</sub>.

Dehydrodolineone (XVI) was obtained as pale yellow needles from chloroform m. p. 280°C (decomp.). In solution dehydrodolineone has a light-green fluorescence under ultraviolet light. Infrared spectrum (plate 2),  $\nu_{\max}$  (cm<sup>-1</sup>): 1634 (  $\alpha$  :  $\beta$  - unsaturated carbonyl); 1605, 1542 and 1500 (aromatic); 1156, 1032 and 936 (methylenedioxy group).

Ultraviolet spectrum (plate 3)  $\lambda_{\max}$  (m $\mu$ .) (log  $\epsilon$  ) in CH<sub>2</sub>Cl<sub>2</sub>: 237 (4.42) (  $\alpha$  :  $\beta$  - unsaturated carbonyl and benzofuran); 274 (4.22) and 310 (4.18) aromatic) Mass 334.

Analysis showed that the compound contained no methoxyl or C-methyl groups, which facilitated the structural elucidation. The molecular formula C<sub>19</sub>H<sub>10</sub>O<sub>6</sub> is in exact agreement with its molecular weight (334), obtained by mass spectrometry.



The presence of a methylenedioxy group was shown by the positive Labat<sup>40</sup> chromotropic acid<sup>41</sup> and phloroglucinol<sup>42</sup> tests and by the characteristic absorption bands for a methylenedioxy group in the infrared spectrum<sup>43, 44</sup> (plate 2) Table 2.

TABLE 2.

Infrared absorption maxima of the methylenedioxy group.

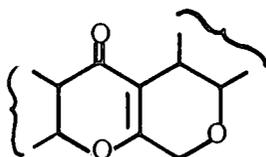
Compound	$\nu_{\text{max.}}$ (cm <sup>-1</sup> )			Reference
Dehydrodolineone (XVI)	936,	1032,	1156	
Neboensinone (XVII)	930,	1028,	1155	
Nambinone (XVIII)	-	-	-	
Neofolin (XXX)	941,	1033,	1199	44
Ficinin ("ficifolin")	930,	1030,	1196	44
Neficin	930,	1030,	1196	44
Pachyrrhizin (XIII)	936,	1039,	1191	29, 30, 32, 34
Neotenone (XIV)	930,	1033,	1182	30
Dehydroneotenone (XV)	942,	1042,	1190	30
Neodulin (XII)	915,	1040,	1166	29, 30
Tlatlancuayin	-,	1051,	1162	98
Pterocarpin	-,	1037,	1165	99
Jamaicin	933,	1034,	-	100
Pisatin	945,	1047,	1163	101
Sophorol	939,	1044,	-	102

An  $\alpha : \beta$  - unsaturated ketone is clearly shown by the characteristic band at 1634 cm<sup>-1</sup> in the infrared spectrum (plate 2).<sup>15, 36, 45, 46.</sup> The intense absorption band at 237 m $\mu$  ( $\log \epsilon = 4.42$ ) in the ultraviolet spectrum of dehydrodolineone (XVI) (plate 3) is ascribed to both the  $\alpha : \beta$  - unsaturated keto-carbonyl group and the benzofuran system as it is well known that these systems absorb in the 230-260 m $\mu$  region.<sup>15, 30, 44, 47-54.</sup> The ultraviolet spectrum of this compound (plate 3) closely resembles that of 6a, 12a - dehydropachyrrhizone<sup>35</sup> (XXVIIb) which suggests a similar structure. Ultraviolet data are presented in Table 3 for comparison purposes.

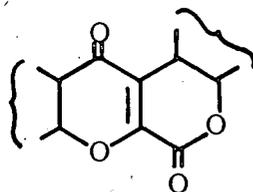
TABLE 3.

Ultraviolet absorption maxima of rotenoids and rotenoid derivatives

Compound	$\lambda_{\text{max.}}$ m $\mu$ (log $\epsilon_{\text{cm}^2}$ )	Reference
Dehydrodolineone (XVI)	237(4.42) ; - ; 274(4.22) ; 310(4.18) ; -	
Neboensinone (XVII)	252(4.10) ; 262(4.12) ; 274(4.17) ; 302(4.19) ; 404(3.85)	
Nambinone (XVIII)	258(4.04) ; - ; 274(4.04) ; 296(3.98) ; 406(3.51)	
Rotenone (XIX)	236(4.18) ; 244(4.11) ; - ; 295(4.23) ; -	45
Rotenone (XIX) (c)	; 265(4.40) ; - ; 298(4.33) ; 345(3.95)	46
Sumatrol (XX)	235(4.22) ; - ; - ; 298(4.36) ; -	103
Amorphigenin (XXI)	236(4.14) ; 242(4.09) ; - ; 293(4.22) ; -	36
Dehydroarmorphigenin (XXI (b))	238(4.45) ; - ; 279(4.37) ; 309(4.25) ; -	36
Amorphigenin keto-lactone (XXI(c))	; 261(4.37) ; 268(4.36) ; 298(4.29) ; 340(3.91)	36
Deguelin (XXII)	- ; - ; 269(4.47) ; 315(3.97) ; -	103
$\alpha$ -Toxicarol (XXIII)	- ; - ; 272(4.54) ; 297(4.05) ; -	103
Dehydro- $\alpha$ -Toxicarol(XXIII)(b))	237(4.43) ; - ; 270(4.61) ; 280(4.61) ; 332(4.17)	45
Dehydro- $\alpha$ -malaccol (XXV) (b))	233(4.45) ; 257(4.43) ; - ; 315(4.16) ; -	104
Pachyrrhizone (XXVII)	242(4.65) ; - ; 284(3.93) ; - ; 334(3.50)	35
Dehydropachyrrhizone(XXVII)(b))	248(4.40) ; - ; 275(4.34) ; 314(4.18) ; -	35
Pachyrrhizonone (XXVII) (c)	225(4.62) ; 263(4.25) ; 278(4.32) ; 301(4.38) ; 412(3.90)	35
Dolineone (XXVIII)	237(4.56) ; - ; 275(3.84) ; 305(3.71) ; 335(3.50)	15



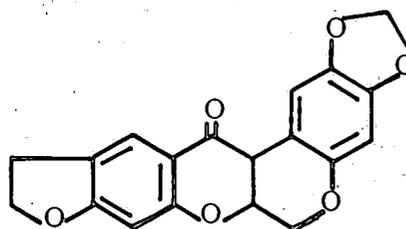
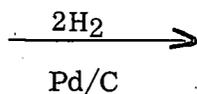
(XXI (b) )  
 (XXIII (b) )  
 (XXV (b) )  
 (XXVII (b) )



(XIX (c) )  
 (XXI (c) )  
 (XXVII (c) )

It has been found that in the catalytic hydrogenation of furoisoflavanoids the furan double bond is easily hydrogenated, whereas the double bond of the pyrone ring often necessitates the use of more drastic conditions such as higher temperature and pressure.<sup>29, 30-32, 34, 44, 55.</sup> Hydrogenation of dehydrodolineone (XVI) over 10% Pd/C in ethyl acetate until the absorption of hydrogen ceased, afforded a small amount of colourless crystals m. p. 149°C, probably dihydrodolineone (XXIX) C<sub>19</sub>H<sub>14</sub>O<sub>6</sub>, infrared spectrum  $\nu_{\text{max.}}$  (cm<sup>-1</sup>): 2850; 1659 (keto-carbonyl); 1610, 1575, 1503 (aromatic); 1160, 1030, 933 (methylene-dioxy group). As expected, the saturation of the 6a, 12a - double bond increased the carbonyl frequency of dehydrodolineone (XVI) (1634 cm<sup>-1</sup>) to 1659 cm<sup>-1</sup>.<sup>15, 30, 56.</sup>

(XVI)



(XXIX)

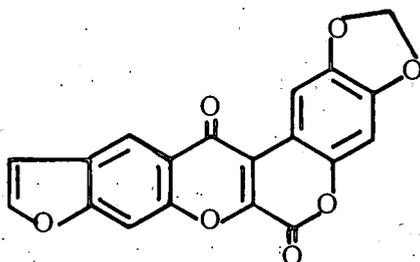
Oxidation of dehydrodolineone (XVI) with *n*-amyl nitrite presented further evidence in favour of structure (XVI) for this compound, (see under neboensinone (XVII), C.)

Crombie and Whiting<sup>15</sup> synthesized dehydrodolineone (XVI) by dehydrogenating dolineone (XXVIII), isolated by them from *N. pseudopachyrrhiza*, with active MnO<sub>2</sub> and the data of the synthetic 6a, 12a - dehydrodolineone m. p. 270°C (decomp.)  $\nu_{\text{max.}}$  (cm<sup>-1</sup>): 1639 (  $\alpha$  :  $\beta$  - unsaturated ketone): 1613, 1587,

1538 and 1499 (aromatic) are in agreement with that of natural dehydrolineone isolated in this investigation, m. p. 280°C (decomp.)  $\nu_{\max}$ . ( $\text{cm}^{-1}$ ): 1634 ( $\alpha : \beta$  - unsaturated ketone); 1605, 1578, 1542 and 1500 (aromatic).

C. NEBOENSINONE (XVII)  $\text{C}_{19}\text{H}_8\text{O}_7$ .

Neboensinone (XVII) was obtained as a yellow crystalline compound (from chloroform) m. p. 350°C (decomp.). It has an intense green fluorescence in solution under ultraviolet light. Infrared spectrum (plate 2)  $\nu_{\max}$ . ( $\text{cm}^{-1}$ ): 1724 (lactone); 1639 ( $\alpha : \beta$  - unsaturated ketone); 1618, 1540, 1504 (aromatic); 1155, 1028, 930 (methylenedioxy group). Ultraviolet spectrum (plate 3), (in  $\text{CH}_2\text{Cl}_2$ ),  $\lambda_{\max}$ .  $m\mu$ . ( $\log \epsilon$ ): 252 (4.10) ( $\alpha : \beta$  - unsaturated carbonyl + benzofuran); 262 (4.12), 274 (4.17) and 302 (4.19) (aromatic); 404 (3.85) (lactone). Mass-spectral molecular weight 348.

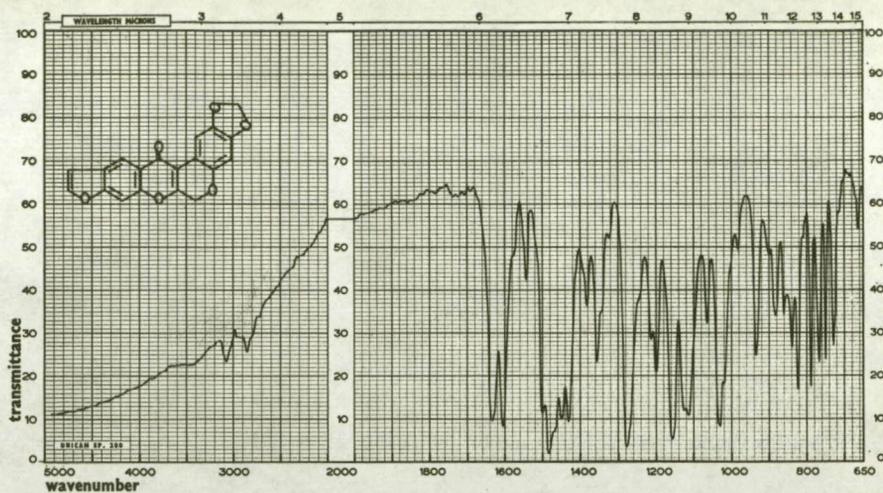


(XVII)

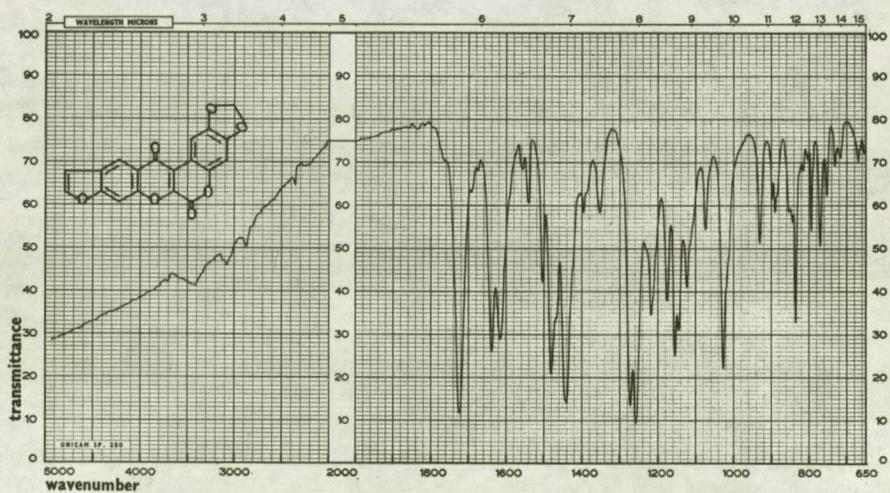
As in the case of dehydrolineone (XVI), neboensinone (XVII) also gave positive Labat<sup>40</sup>, chromotropic acid<sup>41</sup> and phloroglucinol<sup>42</sup> tests for a methylenedioxy group, which were further substantiated by the characteristic absorption bands for a methylenedioxy group<sup>43,44</sup> in the infrared spectrum (plate 2), Table 2. Analysis also showed that this compound contains no methoxyl- or C-methyl groups. In addition to the absorption band of an  $\alpha : \beta$  - unsaturated carbonyl group at  $1639\text{cm}^{-1}$ <sup>15,36,45,46</sup> the infrared spectrum (plate 2) also shows the presence of a lactone group at  $1724\text{cm}^{-1}$ ,<sup>15,36,44-46</sup> (cf. rotenone  $1730\text{cm}^{-1}$  (lactone); pachyrrhizonone  $1745\text{cm}^{-1}$  (lactone) ) The compound was found to be soluble in a 10% methanolic potassium hydroxide solution and upon the evaporation of the methanol and acidification the unchanged compound was again obtained, a reaction which is characteristic for  $\alpha : \beta$  - unsaturated lactones.<sup>30,44,57</sup>

The ultraviolet spectrum of neboensinone (XVII) (plate 3) is strikingly

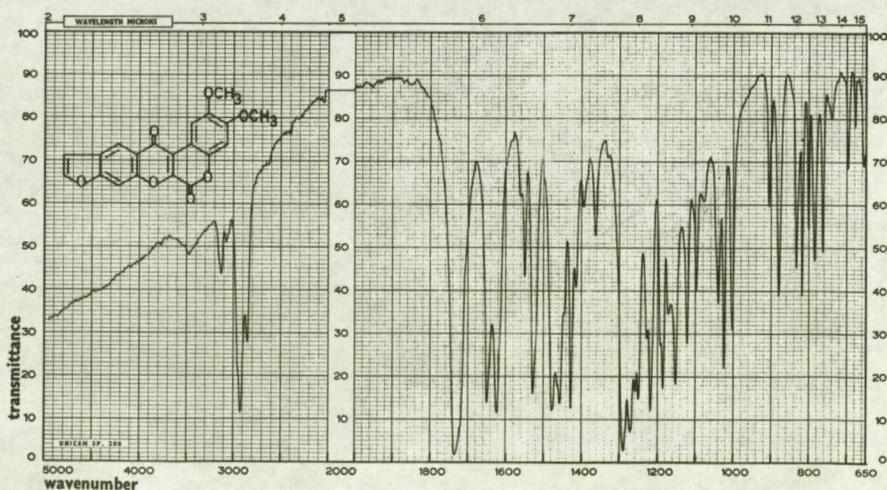
## PLATE 2



(a) DEHYDRODOLINEONE (XVI)



(b) NEBOENSINONE (XVII)

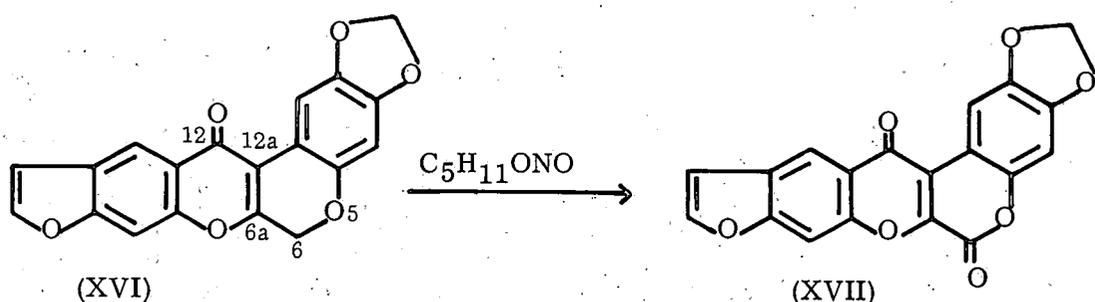


(c) NAMBINONE (XVIII)

similar to that of the keto-lactone derivative of pachyrrhizone (XXVII), pachyrrhizonone (XXVII (c)),<sup>35</sup> (Table 3), which suggested a similar structure. Both neboensinone (XVII) and pachyrrhizonone (XXVII (c)) have an exceptionally strong absorption maximum in the 410 m $\mu$  region.

The proposed molecular formula of neboensinone, C<sub>19</sub>H<sub>8</sub>O<sub>7</sub>, is in exact agreement with the mass-spectral molecular weight of 348 which indicated the replacement of two hydrogen atoms by an oxygen atom in the formula of dehydrodolineone (XVI) C<sub>19</sub>H<sub>10</sub>O<sub>6</sub> (M334), a proposal which was further substantiated by the presence of the additional lactone absorption band in the infrared spectrum of neboensinone (plate 2) compared to that of dehydrodolineone (plate 2).

In order to establish the validity of this proposal, dehydrodolineone was oxidised<sup>15, 35, 36, 46</sup> with *n*-amyl nitrite in glacial acetic acid. The product from this reaction was found to be identical to natural neboensinone (XVII) (m. p., infrared-, ultraviolet and mass spectra, and R<sub>f</sub> values).

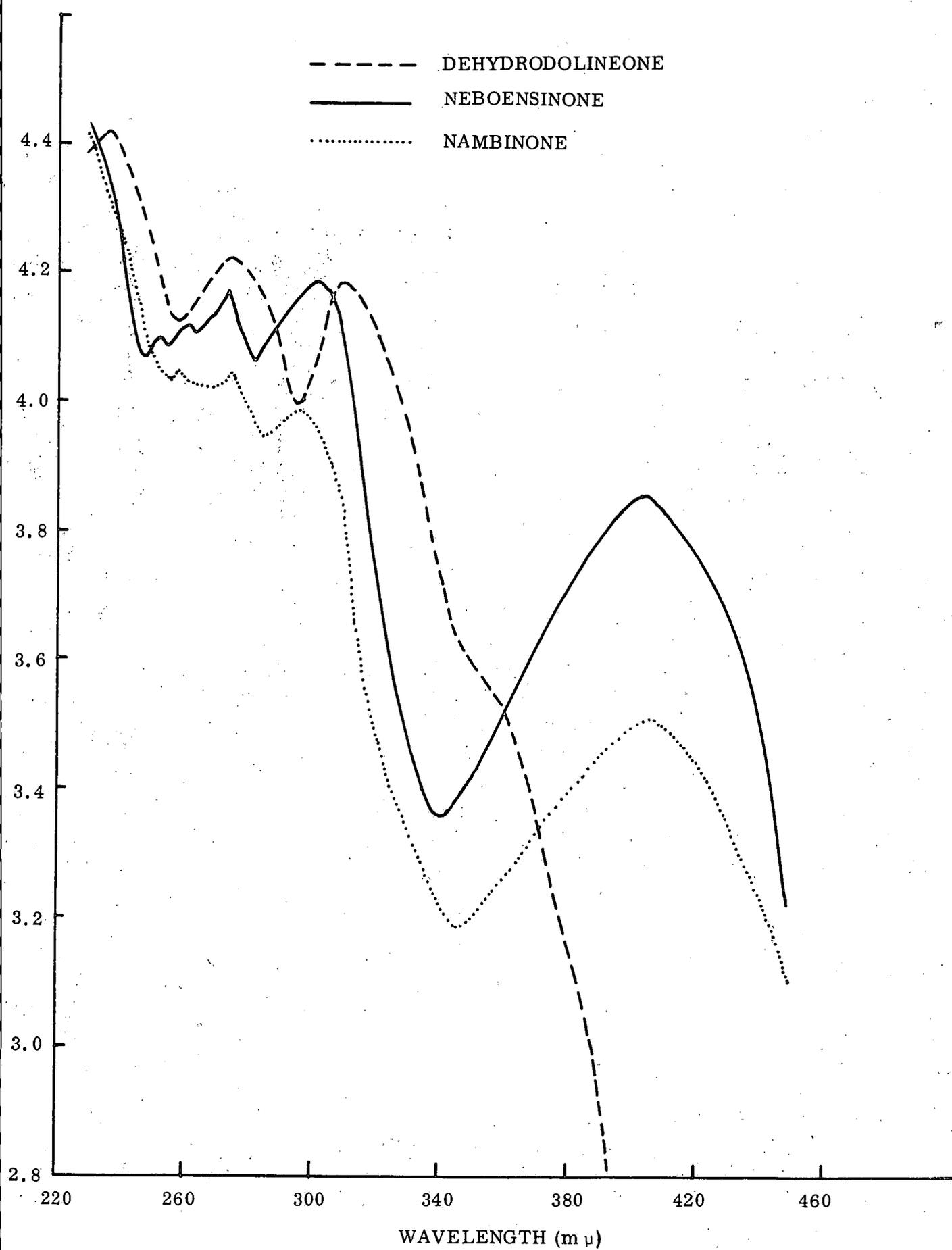


The oxidation of the active methylene group at position 6 in (XVI) to the  $\alpha$  :  $\beta$  - unsaturated lactone in (XVII) further established the 5, 6, 6a, 12a and 12 sequence of oxygen and carbon atoms in dehydrodolineone (XVI) and also unambiguously proved the structure of neboensinone (XVII).

#### D. NAMBINONE (XVIII) C<sub>20</sub>H<sub>12</sub>O<sub>7</sub>

Nambinone (XVIII) was obtained as a deep yellow crystalline compound m. p. 400°C (decomp.) and it has a pale yellow fluorescence in solution under ultraviolet light. Infrared spectrum (plate 2)  $\nu_{\text{max}}$  (cm<sup>-1</sup>): 2920, 2850 (methoxyl); 1735 (lactone); 1650 ( $\alpha$  :  $\beta$  - unsaturated carbonyl); 1622, 1550 and 1530 (aromatic); 1457 (methoxyl group).

## PLATE 3

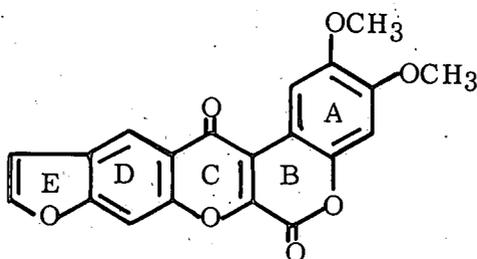


Ultraviolet spectrum (plate 3) (in  $\text{CH}_2\text{Cl}_2$ ),  $\lambda_{\text{max}}$ .  $m\mu$  ( $\log \epsilon$ ): 258 (4.04) benzofuran and carbonyl; 274 (4.04) and 296 (3.98) (aromatic); 406(3.51) (lactone). Mass-spectral molecular weight 364.

As a result of the very small amount of nambinone which was available ( $\pm 3$  mg) no analysis or chemical reactions were possible and consequently the structural elucidation of this compound was attempted purely by making use of physical methods. An accurate mass-spectral molecular weight determination showed that the compound has a molecular formula of  $\text{C}_{20}\text{H}_{12}\text{O}_7$  (required 364.058293, found 364.058510).

The infrared spectrum of nambinone (XVIII) has the same general absorption pattern as dehydrodolineone and nevoensinone (plate 2) and also clearly shows the presence of an  $\alpha$  :  $\beta$  - unsaturated carbonyl at  $1650\text{ cm}^{-1}$ , 15, 36, 45, 46 and an  $\alpha$  :  $\beta$  - unsaturated lactone at  $1735\text{ cm}^{-1}$ , 15, 36, 44-46 as in the case of nevoensinone (XVII) (plate 2). In contrast to dehydrodolineone and nevoensinone the characteristic methylenedioxy group absorption bands are absent in the infrared spectrum of nambinone, but strong absorption bands at 2920, 2850 and  $1457\text{ cm}^{-1}$  indicate the presence of methoxyl groups<sup>43</sup> which is further substantiated by the mass spectrum of this compound (see Chapter 4). The molecular weight of nambinone (XVIII) 364, is 16 mass units higher than the molecular weight of nevoensinone (348) which the accurate molecular weight determination showed to be due to an additional C and 4H atoms and not to an oxygen atom. This is in exact agreement with the replacement of the methylenedioxy group of nevoensinone (XVII) with two methoxyl groups.

In addition the ultraviolet spectrum of nambinone (XVIII) (plate 3) is virtually identical to that of nevoensinone (plate 3) which further indicates the close structural relationship between these two compounds.



(XVIII)

The proposed structure of nambinone (XVIII) is thus in complete agreement with the infrared- and ultraviolet spectral data. The mass-spectral fragmentation pattern of nambinone is also completely reconcilable with the suggested structure (see Chapter 4.)

The methoxyl groups of nambinone were assigned to positions 2 and 3 from biogenetic considerations, as all the known rotenoids (with methoxyl groups) have these groups attached to these positions. Due to the close biogenetic relationship of nambinone with dehydrodolineone and neboensinone it is also suggested that nambinone has a linear D/E ring fusion and not angular as in the case of elliptone (XXIV) and malaccol (XXV).

Confirmation for the proposed structure of nambinone, when more material becomes available, can be obtained from NMR data if a more soluble derivative of nambinone can be synthesized.

It is interesting to note that the dehydrorotenoids and the keto-lactone rotenoids have very high melting point ranges, 220°-280°C and 300°-400°C respectively. It has also been found that as the case with dehydrodolineone, neboensinone and nambinone, the solubility of the keto-lactone derivatives of pachyrrhizone (XXXVII (c))<sup>35</sup> and amorphigenin (XXI (c))<sup>36</sup> is very low in most known solvents.

Further evidence as to the structures of compounds (XVI), (XVII) and (XVIII) was obtained from the mass-spectral fragmentation patterns of these compounds, which are discussed in the following chapter.

CHAPTER 4.

THE INVESTIGATION OF THE MASS-SPECTRAL FRAGMENTATION PATTERNS OF ISOFLAVANOIDS AND ROTENOIDS

- I. THE MASS SPECTRA OF ISOFLAVANOIDS.
  - A. INTRODUCTION
  - B. SPECTRAL DATA OF ISOFLAVANOIDS
  - C. NEOTENONE
  - D. DEHYDRONEOTENONE
  - E. PACHYRRHIZIN
  - F. NEOFOLIN
  - G. NEOTENANE
  - H. TETRAHYDRONEOFOLIN
  - I. NEODULIN
  
- II. THE MASS SPECTRA OF THE DEHYDROROTENOIDS, DEHYDRODOLINEONE, NEBOENSINONE AND NAMBINONE.
  - A. DEHYDRODOLINEONE
  - B. NEBOENSINONE
  - C. NAMBINONE
  - D. CONCLUSIONS.

THE INVESTIGATION OF THE MASS-SPECTRAL FRAGMENTATION  
PATTERNS OF ISOFLAVANOIDS AND ROTENOIDS

I. ISOFLAVANOIDS

A. INTRODUCTION

Although a fairly recent development, the application of mass spectrometry in organic chemistry has proved to be a very powerful tool in the structural elucidations of natural products such as the steroids, triterpenes, aliphatic compounds, amino acids, alkaloids and flavanoids. In many cases the accurate molecular weight of a compound, and hence the molecular formula, can be obtained from the spectrum. A further advantage of this technique is the very small samples needed for the determination of a spectrum, which is often of considerable importance especially if only a small amount of material is available.

In order to facilitate the interpretation of the mass spectra of the dehydro-rotenoids dehydrodolineone (XVI), neboensinone (XVII) and nambinone (XVIII), a study of the fragmentation patterns of the isoflavanoids isolated from *N. amboensis* and *N. ficifolia* <sup>44, 45</sup> was undertaken. Isoflavanoids and flavanoids do not possess sites of facile bond fission and the relative high stability of these compounds to electron impact is reflected in the high abundance of their molecular ions. <sup>24b, 58, 59, 60</sup>

B. SPECTRAL DATA OF ISOFLAVANOIDS

The relevant data are given in Tables 4 and 5. Metastable ions are indicated by m.

TABLE 4

Neotenone (XIV) (Isoflavanone)		Dehydroneotenone (XV) (Isoflavone)		Pachyrrhizin (XIII) (furocoumarin)		Neofolin (XXX) (furocoumarin)	
m/e	I	m/e	I	m/e	I	m/e	I
339	4.8	337	21	337	20.0	367	24.0
338(M <sup>+</sup> )	23.0	336(M <sup>+</sup> )	100	336(M <sup>+</sup> )	100.0	366(M <sup>+</sup> )	100.0
179	11.4	335	5.0	321	10.0	351	6.2
178	100.0	321	3.5	319	5.0	338	7.0
165	7.0	307	10.0	305	5.5	323	21.0
163	6.4	306	15.0	294	9.0	307	7.5
160	3.1	305	74.0	293	42.0	280	8.2
149.3(m)	-	293	6.0	291	7.5	229	8.5
133	13.2	291	10.0	265	15.0	183	10.4
108.5(m)	-	265	4.0	239.3(m)	-	169	15.5
105	2.8	263	9.0	207	5.0	161.5	11.0
93.7(m)	-	235	5.0	199	17.5	149	4.6
77	4.4	211	5.0	179	7.0	44	56.3
44	5.4	176	12.0	168	10.5	28	91.6
				160	5.0		
28	16.8	175	15.0	154	17.0		
		161	24.0	150	5.5		
		160	6.0	146.5	8.5		
		152.5	17.0	103.5	5.5		
		152	9.0	75	6.5		
		132	5.5	53	10.0		
		53	7.0	28	21.0		
		28	27.0				

Where M<sup>+</sup> = molecular parent ion

I = relative intensities

TABLE 5

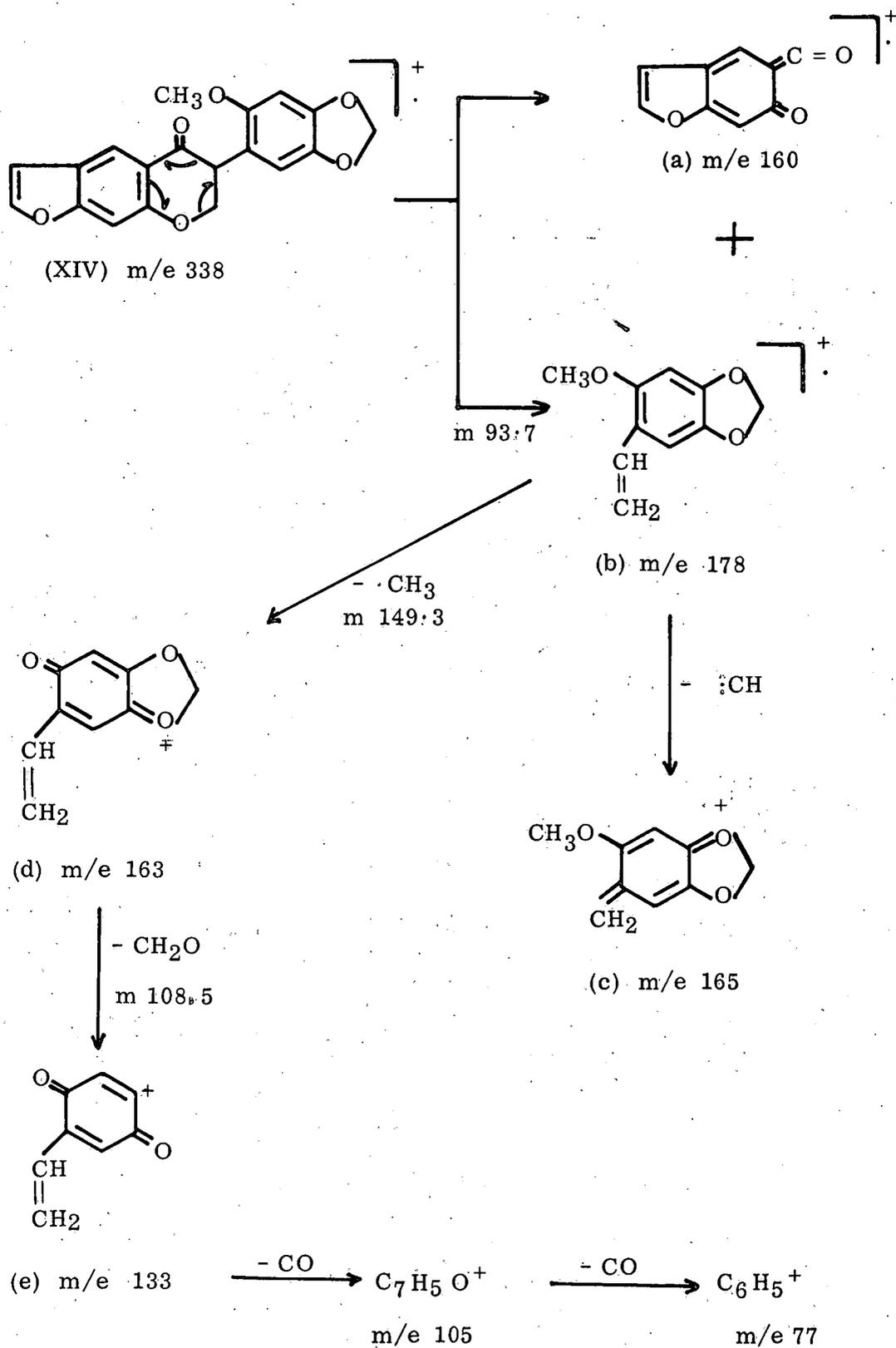
Neotenane (XXXI) (Isoflavan)		Tetrahydroneofolin (XXXII) (Dihydrocoumarin)		Neodulin (XII) (Pterocarpan)	
m/e	I	m/e	I	m/e	I
327	20.3	371	18.8	309	10
326(M <sup>+</sup> )	100.0	370(M <sup>+</sup> )	82.7	308(M <sup>+</sup> )	100
178	33.7	343	17.0	307	14.5
165	4.8	342	74.2	291	8.0
163	3.2	327	6.0	275(m)	-
149.5(m)	-	316(m)	-	265	4.0
133	5.2	312.8(m)	-	171	6.0
69	4.8	299	19.0	162	14.5
28	21.4	223	5.8	158	10.4
		193	9.5	28	3.3
		192	76.3		
		180	12.7		
		179	100.0		
		177	27.0		
		149	9.2		
		78	12.0		
		44	30.4		
		28	52.6		

Where M<sup>+</sup> = molecular parent ion

I = relative intensities

### C. NEOTENONE(XIV)

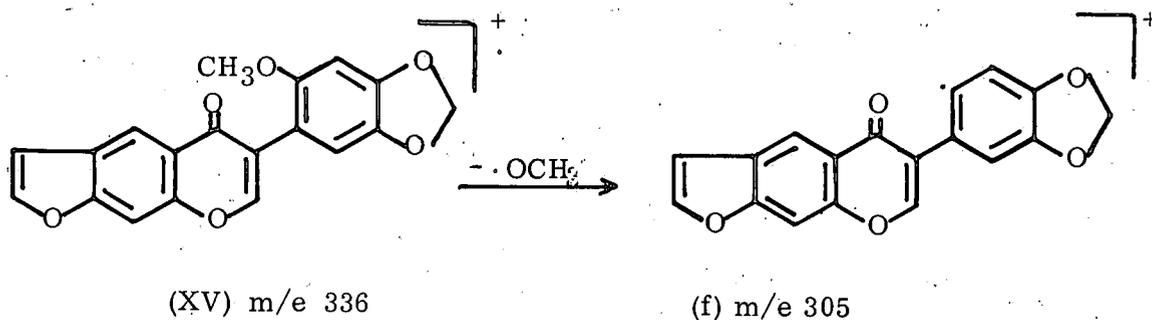
The major fragmentation route of neotenone (XIV) is via a retro-Diels-Alder breakdown to give the ion (b) at m/e 178 (base peak) substantiated by the meta-stable ion at m/e 93.7 and the ion (a) at m/e 160.



The loss of a methyl group from (b)  $m/e$  178 gives the ion (d) at  $m/e$  163 which in turn forms the ion (e) at  $m/e$  133 due to the loss of formaldehyde, breakdown pathways which are confirmed by the appropriate metastable peaks at  $m/e$  149.3 and  $m/e$  108.5 respectively. It has been found<sup>61</sup> that the methylenedioxy group is relatively stable to electron impact and ions associated with its cleavage are usually small.

#### D. DEHYDRONEOTENONE (XV)

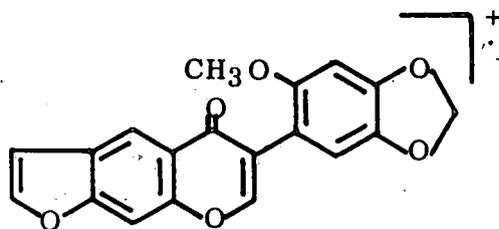
The parent molecular ion at  $m/e$  336 forms the base peak which shows the high stability of this compound. The next most abundant peak is due to the loss of a methoxyl group from the parent molecular ion to form the ion (f) at  $m/e$  305.



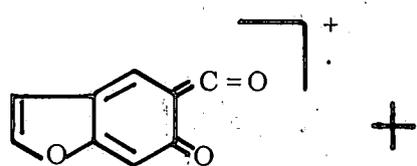
A retro-Diels-Alder fragmentation of the parent ion results in the formation of the ions (g) and (h) at  $m/e$  160 and  $m/e$  176 respectively, but this breakdown process is not so prominent as in the case of neotenone (XIV). Possible routes for the formation of the peaks at  $m/e$  132 and  $m/e$  161 from the ions (g) and (h) are shown below.

The transfer of the acetylenic side chain hydrogen atom of the ion (h)  $m/e$  176 and the subsequent elimination of the two carbon atoms may account for the ion at  $m/e$  152.

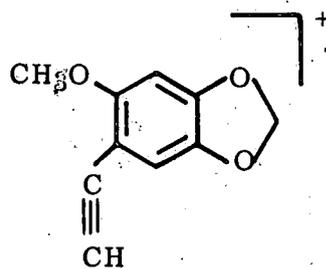
The elimination of a methyl group from the molecular ion at  $m/e$  336 gives rise to the ion (k) at  $m/e$  321, which may lose CO to form (l)  $m/e$  293. The loss of formaldehyde from (k) may be responsible for the peak (m) at  $m/e$  291.



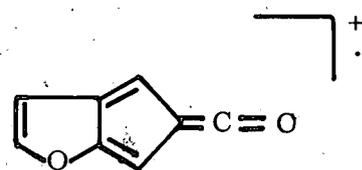
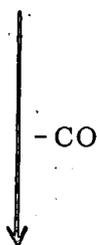
(XV) m/e 336



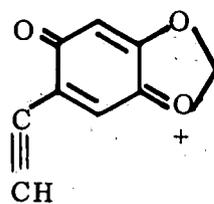
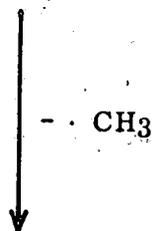
(g) m/e 160



(h) m/e 176

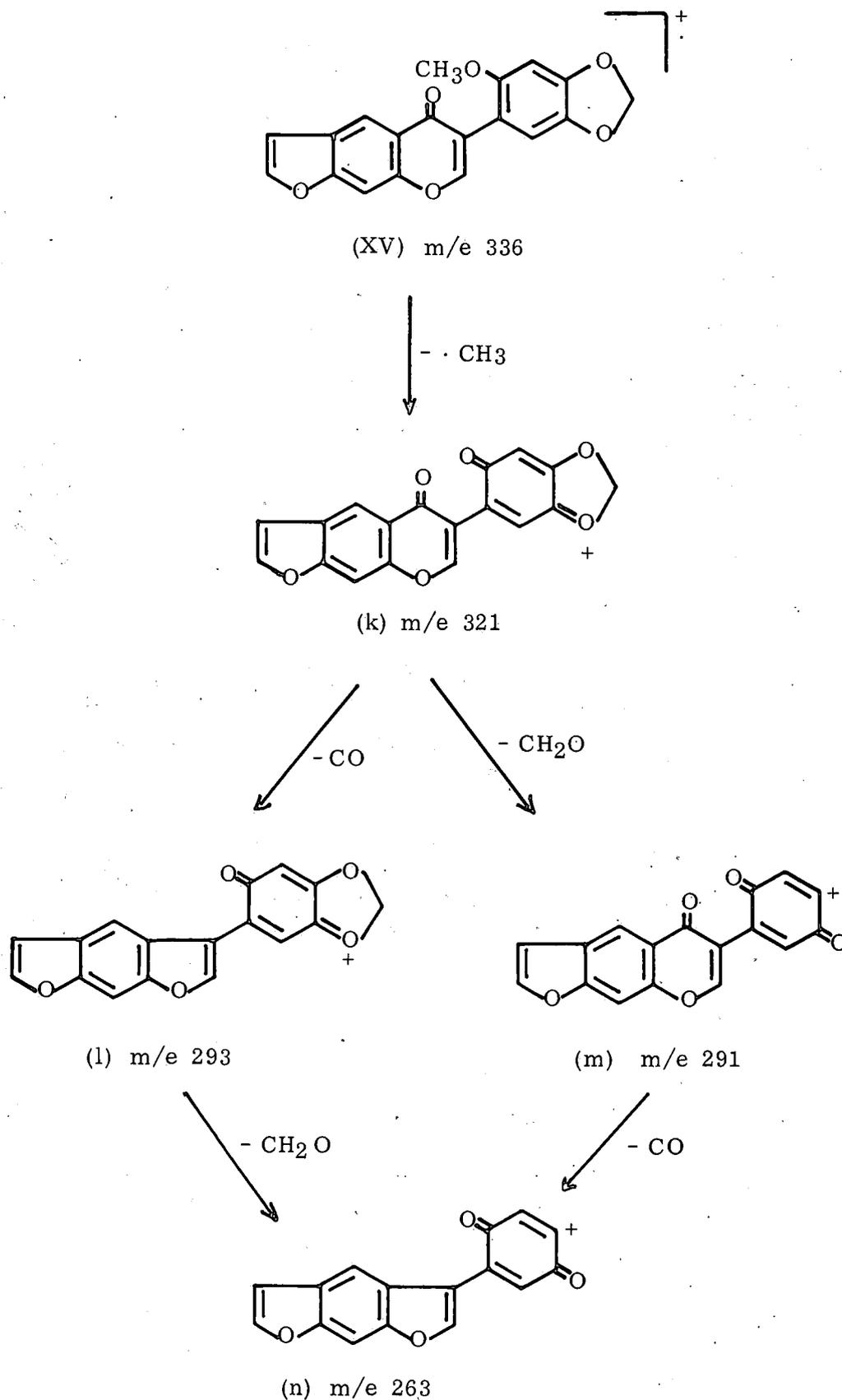


(i) m/e 132



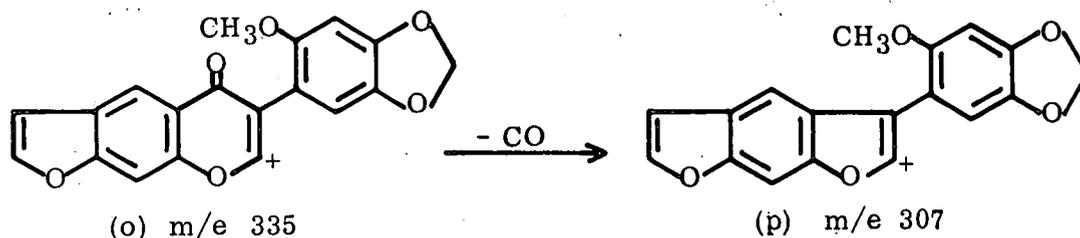
(j) m/e 161

The further elimination of  $\text{CH}_2\text{O}$  from (l) and  $\text{CO}$  from (m) gives the ion (n) at  $m/e$  263. Alternative positions for the loss of  $\text{CO}$  are available as illustrated in the scheme shown below.



The ion at  $m/e$  265 is possibly formed by the elimination of CO from (l)  $m/e$  293. The loss of  $\text{CH}_2\text{O}$  from  $m/e$  265 will afford the ion  $m/e$  235. The ion at  $m/e$  235 can also be formed by the loss of CO from (n)  $m/e$  263.

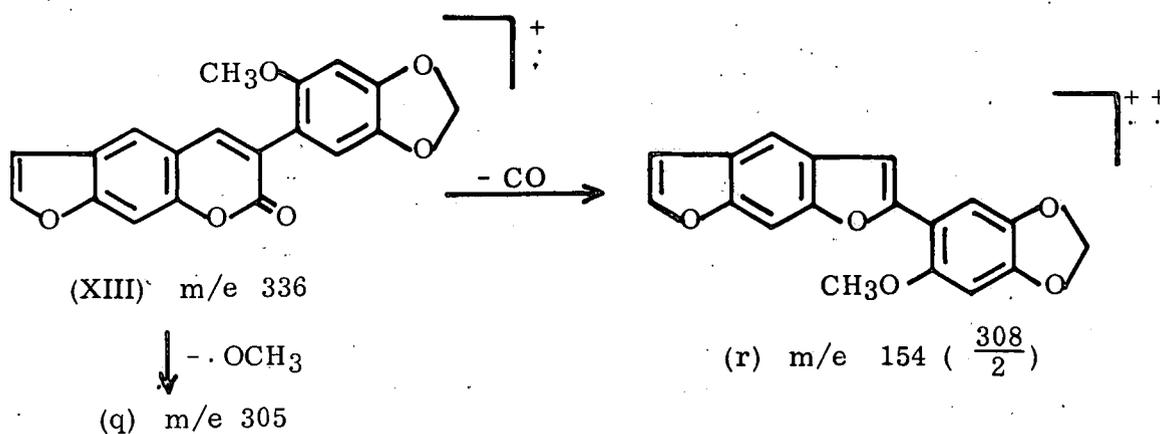
The species at  $m/e$  307 (p) may be due to the loss of CO from the (M-1) ion (o)  $m/e$  335.



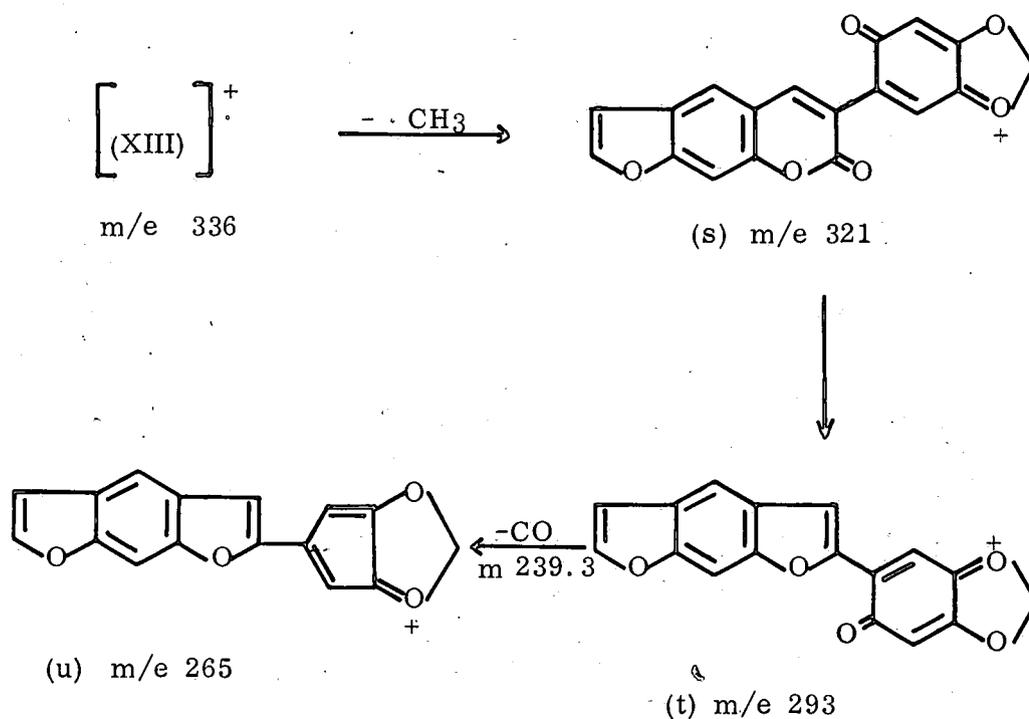
A retro-Diels-Alder fragmentation of (o)  $m/e$  335 can yield the ion (g)  $m/e$  160 and the ion at  $m/e$  175. It is suggested that the peaks at  $m/e$  161 and  $m/e$  175 may be formed as the result of a retro-Diels-Alder fragmentation of the parent molecular ion  $m/e$  336 with a concomitant hydrogen atom shift. The peak at  $m/e$  152.5 is a doubly charged ion ( $m/e$  305).

#### E. PACHYRRHIZIN (XIII)

This compound is also very stable to electron impact, the base peak being the parent molecular ion ( $m/e$  336). As with dehydroneotenone (XV) the loss of the methoxyl group from the molecular ion affords the species (q) at  $m/e$  305. The elimination of CO from the parent molecular ion ( $m/e$  336) affords the ion (r) at  $m/e$  308, which appears in the spectrum as a doubly charged ion at  $m/e$  154.

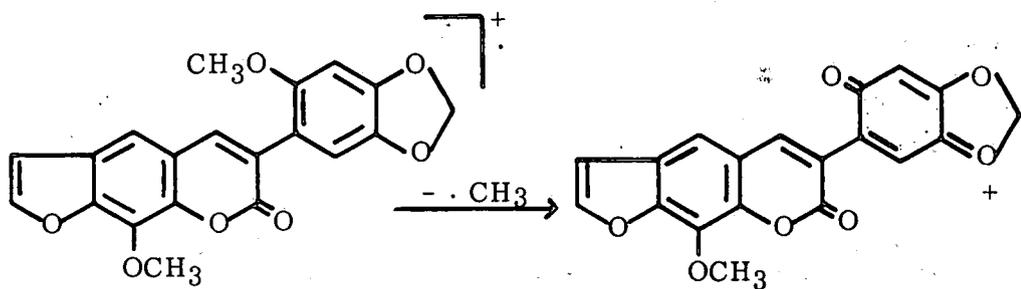


The breakdown of the parent ion  $m/e$  336 via the loss of a methyl group yields the ion (s)  $m/e$  321 which by the elimination of CO gives the peak (t) at  $m/e$  293. The loss of CO from (t) affords the ion (u)  $m/e$  265, a transition which is substantiated by the metastable peak at  $m/e$  239.3. The formation of the species at  $m/e$  207 can be explained by the expulsion of CO and  $\text{CH}_2\text{O}$  from (u). The peaks at  $m/e$  168, 146.5 and 103.5 are doubly charged ions



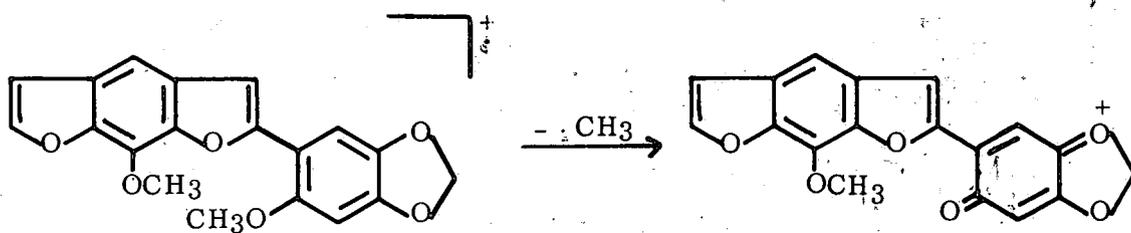
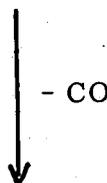
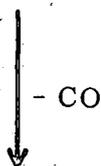
#### F. NEOFOLIN (XXX) 44, 55

The fragmentation of neofolin (XXX) is analogous to that of pachyrrhizin. As there are alternative positions for the elimination of methoxyl-, methyl and CO groups the fragmentation patterns shown below is merely an illustration of one such pathway. The peaks at  $m/e$  183, 169 and 161.5 are doubly charged ions. The base peak is formed by the parent molecular ion  $m/e$  366.



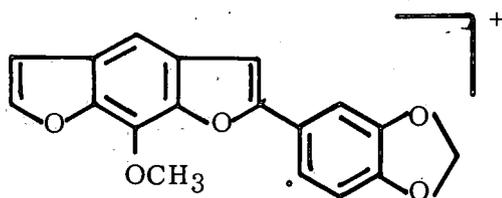
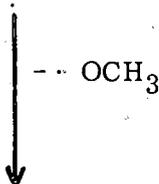
(XXX) m/e 366

(v) m/e 351



(w) m/e 338

(x) m/e 323

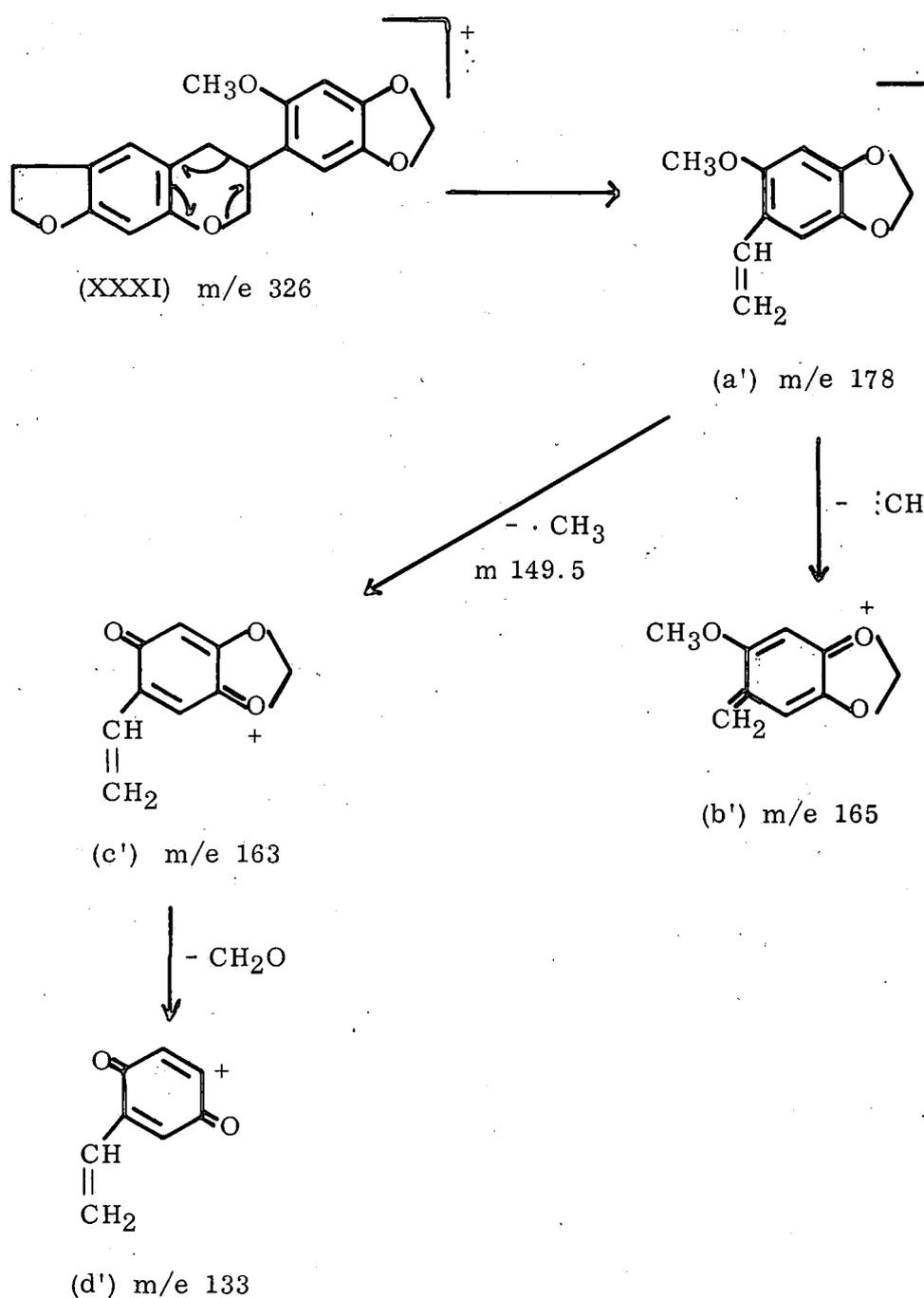


(y) m/e 307

A possible route for the formation of the species at m/e 280 is via the loss of a methyl- and a CO group from (X) m/e 323.

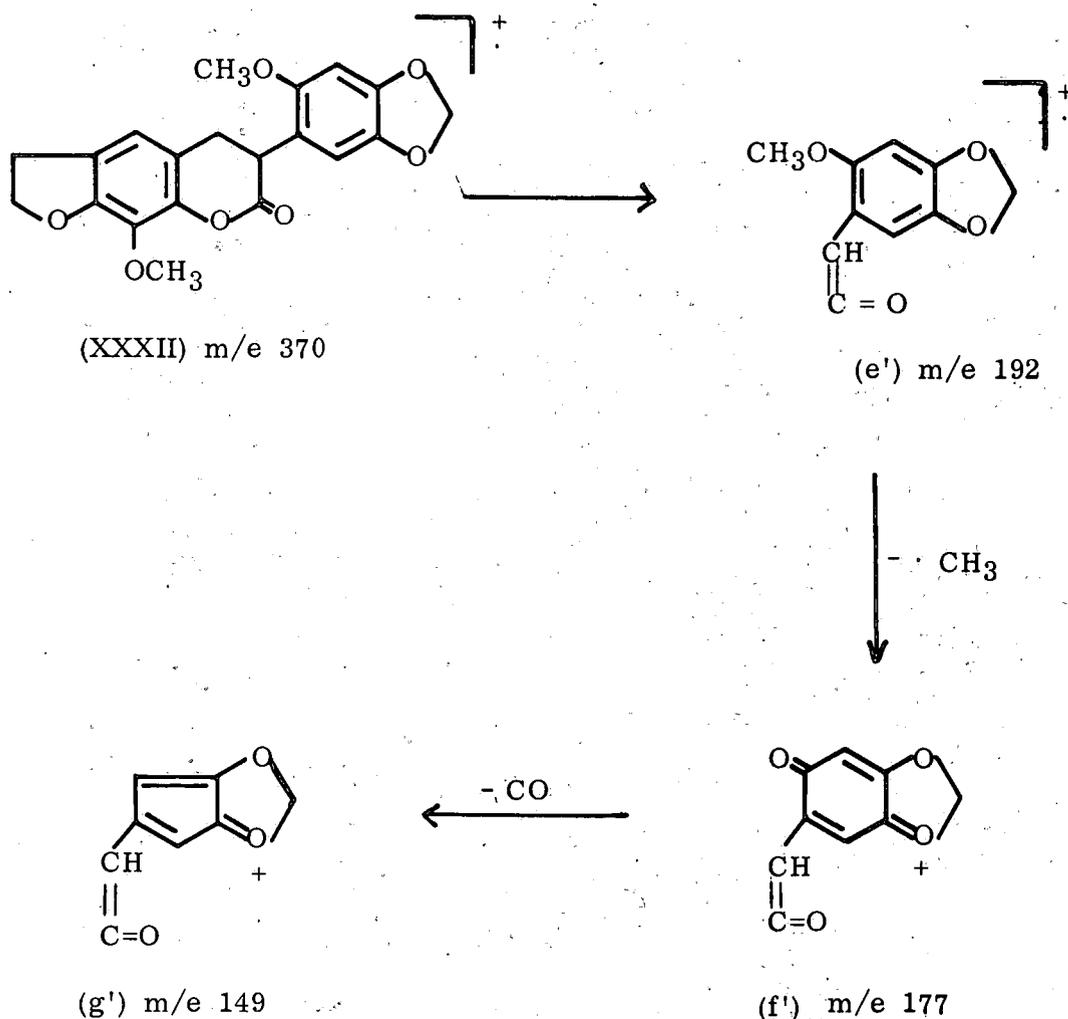
G. NEOTENANE (XXXI) 31

As with neotenone the major fragmentation pattern of this compound is via a retro-Diels-Alder fragmentation with the formation of the species (a') at  $m/e$  178. The parent molecular ion forms the base peak at  $m/e$  326. The elimination of 13 mass units from (a') affords the ion (b') at  $m/e$  165. The loss of a methyl group from (a') to yield the peak (c') at  $m/e$  163 is confirmed by the appropriate metastable peak at  $m/e$  149.5. Elimination of formaldehyde from (c') will account for the ion (d') at  $m/e$  133.



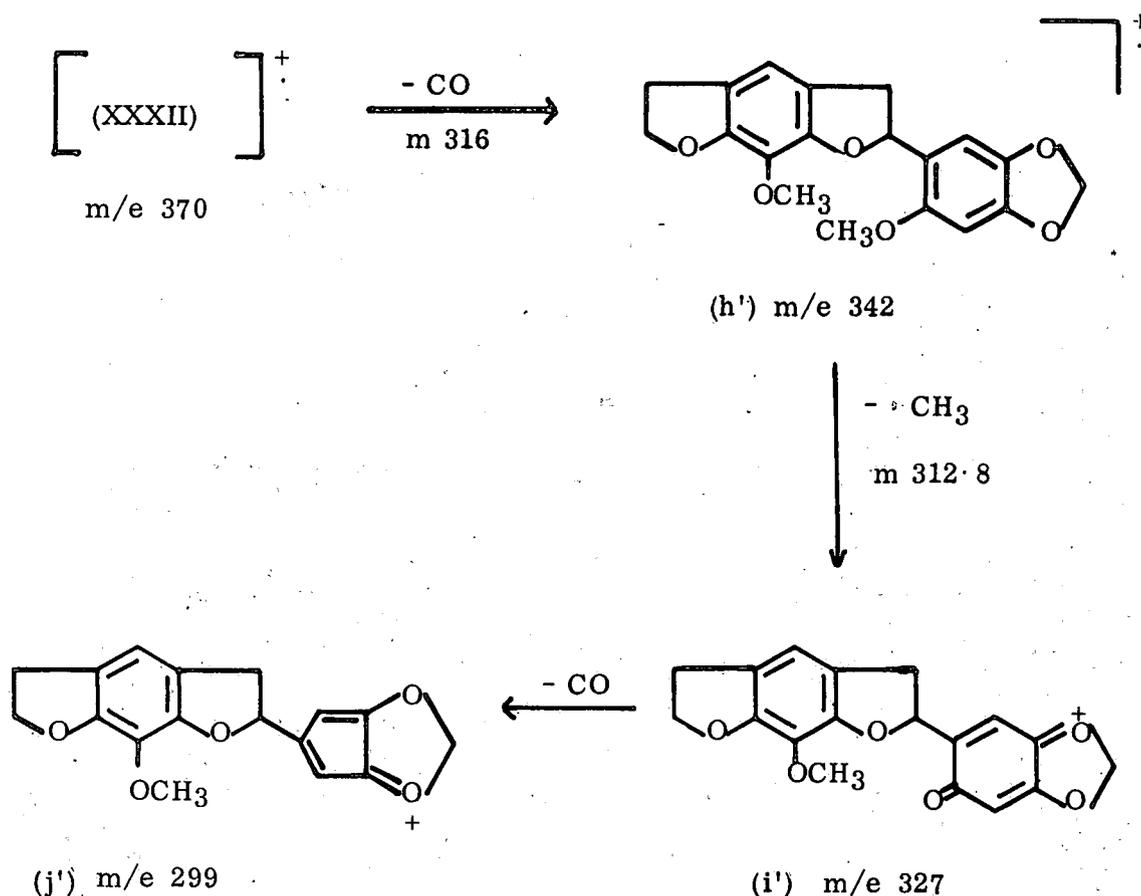
H. TETRAHYDRONEOFOLIN (XXXII) 55

Tetrahydroneofolin is subject to a pronounced retro-Diels-Alder fragmentation to give the species (e') at m/e 192 which in turn loses 15 and 28 mass units to yield the peaks (f') m/e 177 and (g') m/e 149 respectively.

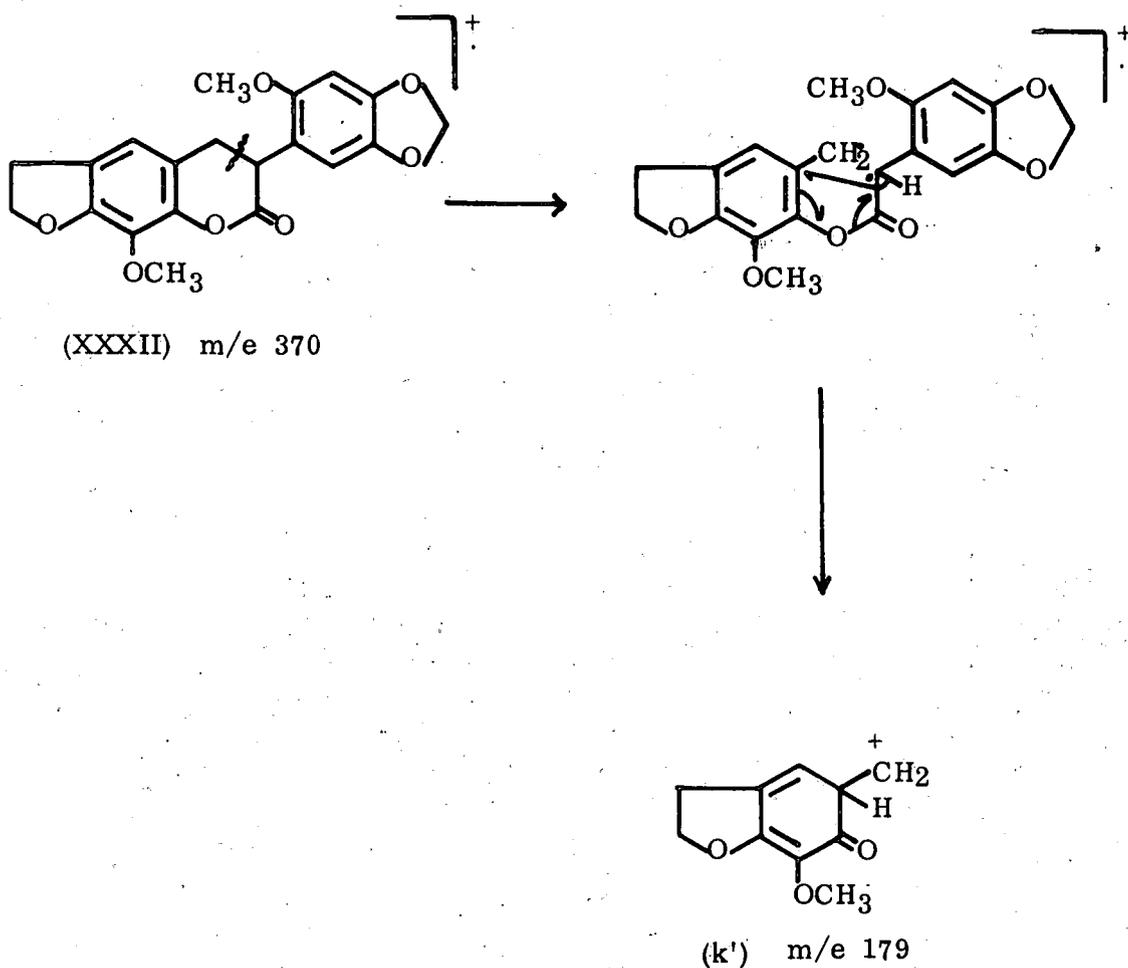


A second major breakdown pattern of (XXXII) is a pronounced loss of carbon monoxide to yield the ion (h') m/e 342 (confirmed by the metastable peak at m/e 316) which further breaks down by the successive loss of a methyl group (substantiated by the metastable peak at m/e 312.8) and CO to form the ions (i') and (j') at m/e 327 and 299 respectively.

As with neofolin there are alternative groups from which eliminations can occur, and the scheme below illustrates only one possible breakdown route.

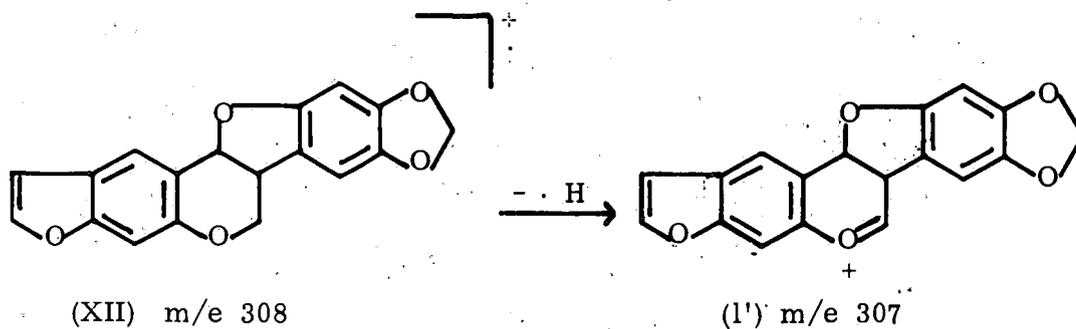


Studies by Pelter et al.<sup>59,60</sup> on flavanoids, isoflavonols and isoflavans showed that in addition to the retro-Diels-Alder fragmentation pattern a second major breakdown mode is operative in many of these compounds. This fragmentation pattern involves the homolytic fission of the  $C_3 - C_4$  bond which gives rise to a diradical in which both electrons are stabilised by mesomerism with the adjacent benzene rings. A hydrogen atom transfer then occurs, as shown below, to give the corresponding fragments of which either are capable of carrying the positive charge. In tetrahyroneofolin this breakdown path yields the ion (k')  $m/e \text{ 179}$  which forms the base peak.

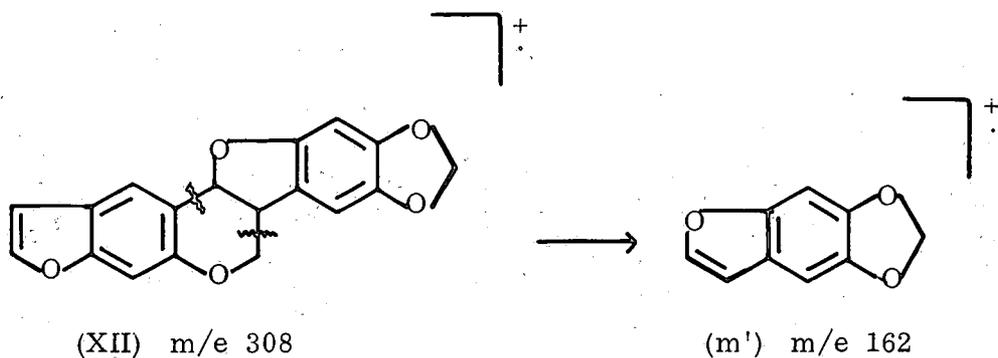


### I. NEODULIN (XII) 31

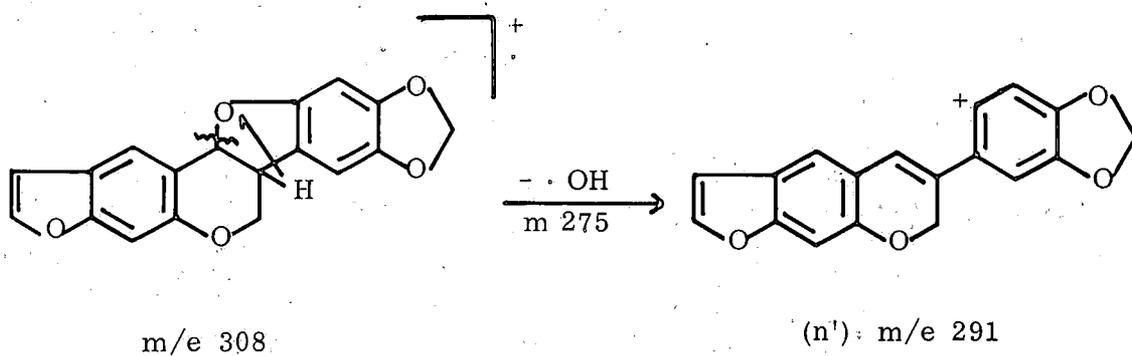
The high stability of the pterocarpans (coumarano-chromans) are shown by the resistance to fragmentation of these compounds under electron impact. The loss of a hydrogen atom is favoured to form the M-1 ion (1') at  $m/e$  307. The parent molecular ion at  $m/e$  308 forms the base peak.



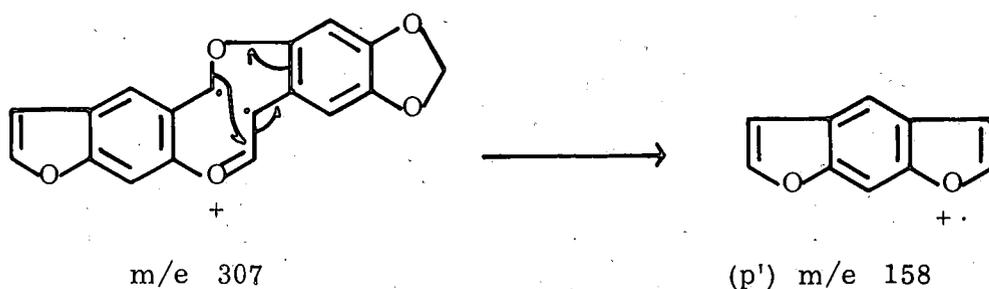
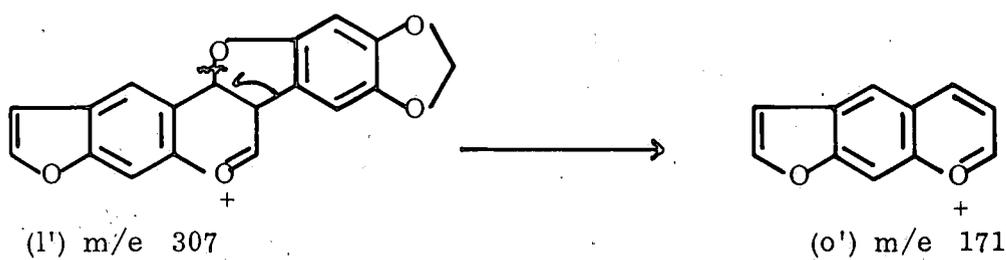
With reference to work done by Pelter et al.<sup>60</sup> on pterocarpin the following fragmentation processes are proposed for neodulin (XII).



The loss of 17 mass units from the parent ion m/e 308 to form the ion (n') m/e 291 is confirmed by the metastable peak at m/e 275.



The formation of the ions (o') and (p') at m/e 171 and 158 respectively may possibly be by the fragmentation of the M-1 ion (l') and may be rationalised as shown.<sup>60</sup>



## II. THE MASS SPECTRA OF THE DEHYDROROTENOIDS, DEHYDRODOLINEONE, NEBOENSINONE AND NAMBINONE

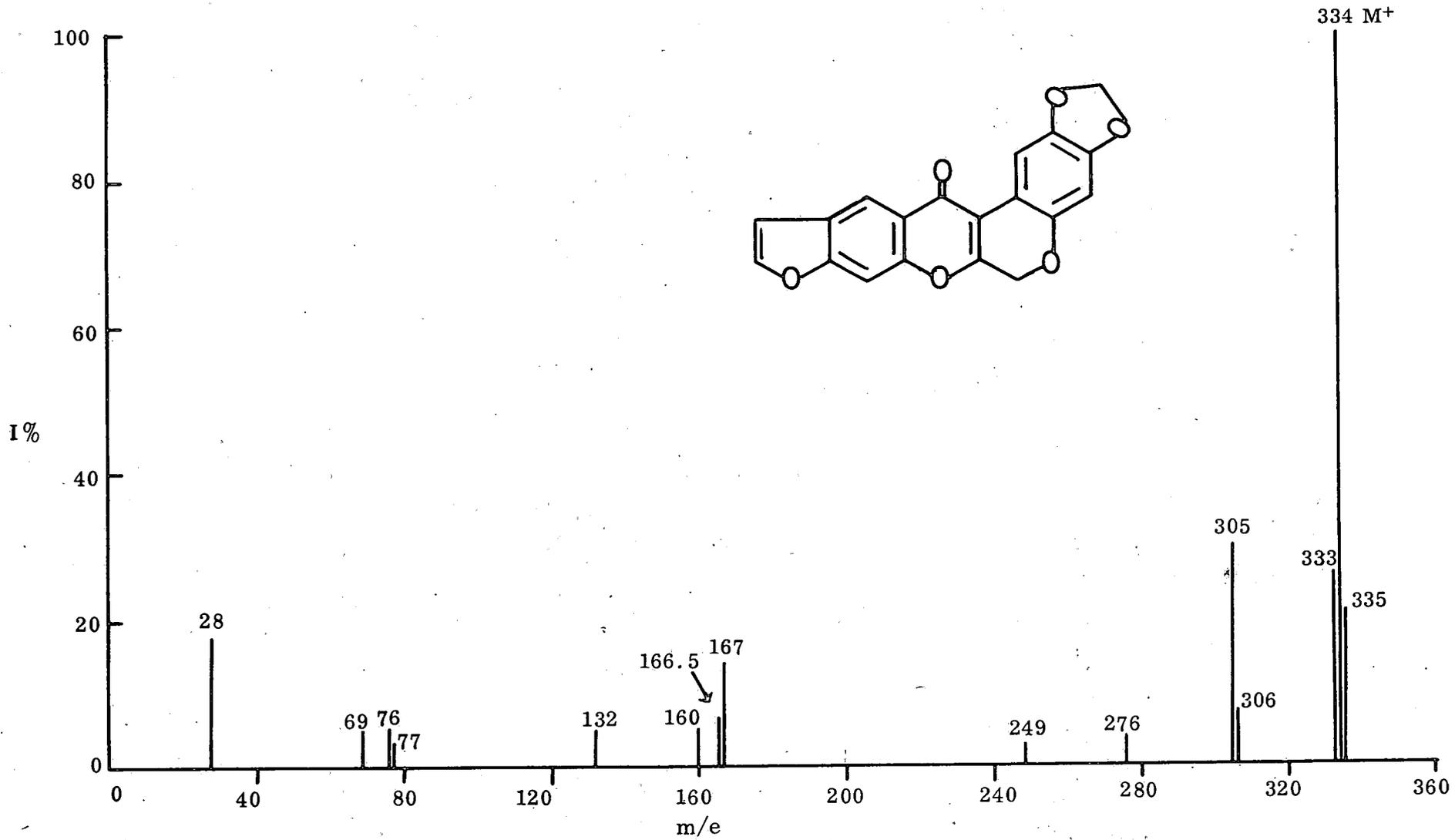
The mass-spectral data for dehydrodolineone (XVI), neboensinone (XVII) and nambinone (XVIII) are given in Table 6, (plate 4). The data for the rotenoid pachyrrhizone (XXVII) were obtained from a publication by Reed and Wilson.<sup>62</sup> The dehydrorotenoids also have alternative positions from which groups such as CO and  $\cdot\text{CH}_3$  can be eliminated, but for the sake of convenience only one route is given in each case.

TABLE 6

Dehydrodolineone (XVI)		Neboensinone (XVII)		Nambinone (XVIII)		Pachyrrhizone (XXVII)	
m/e	I	m/e	I	m/e	I	m/e	I
335	21.1	349	20.0	365	27.0	367	10.5
334(M <sup>+</sup> )	100.0	348(M <sup>+</sup> )	100.0	364(M <sup>+</sup> )	100.0	366(M <sup>+</sup> )	36.5
333	26	347	8.1	350	7.1	191	6.5
332(m)	-	320	5.3	349	7.0	190	9.0
306	7.2	319	3.0	334.4(m)	-	177	19.3
305	30.0	294.2(m)	-	321	18.0	176	100.0
276	3.1	234	5.4	295.4(m)	-	175	59.8
249	2.5	174	12.0	293	4.6	163	9.0
167	14.0	173.5	7.5	278	7.8	162	6.2
166.5	7.1	160	9.3	267.3(m)	-	161	4.4
160	5.3	159.5	6.8	250	4.8	147	7.9
132	5.0	132	3.8	182	13.3	133	3.7
77	2.8	116	4.0	160	4.6		
76	5.1	85	5.2	85	6.6		
69	4.3	71	7.0	71	7.1		
28	17.3	69	4.5	69	5.2		
		57	5.1	57	14.8		
		43	3.5	55	5.1		
		28	17.2	43	5.0		
				28	6.3		

Where M<sup>+</sup> = molecular parent ion

I = relative intensities.



(a) DEHYDRODOLIONEONE (XVI)

PLATE 4(a)

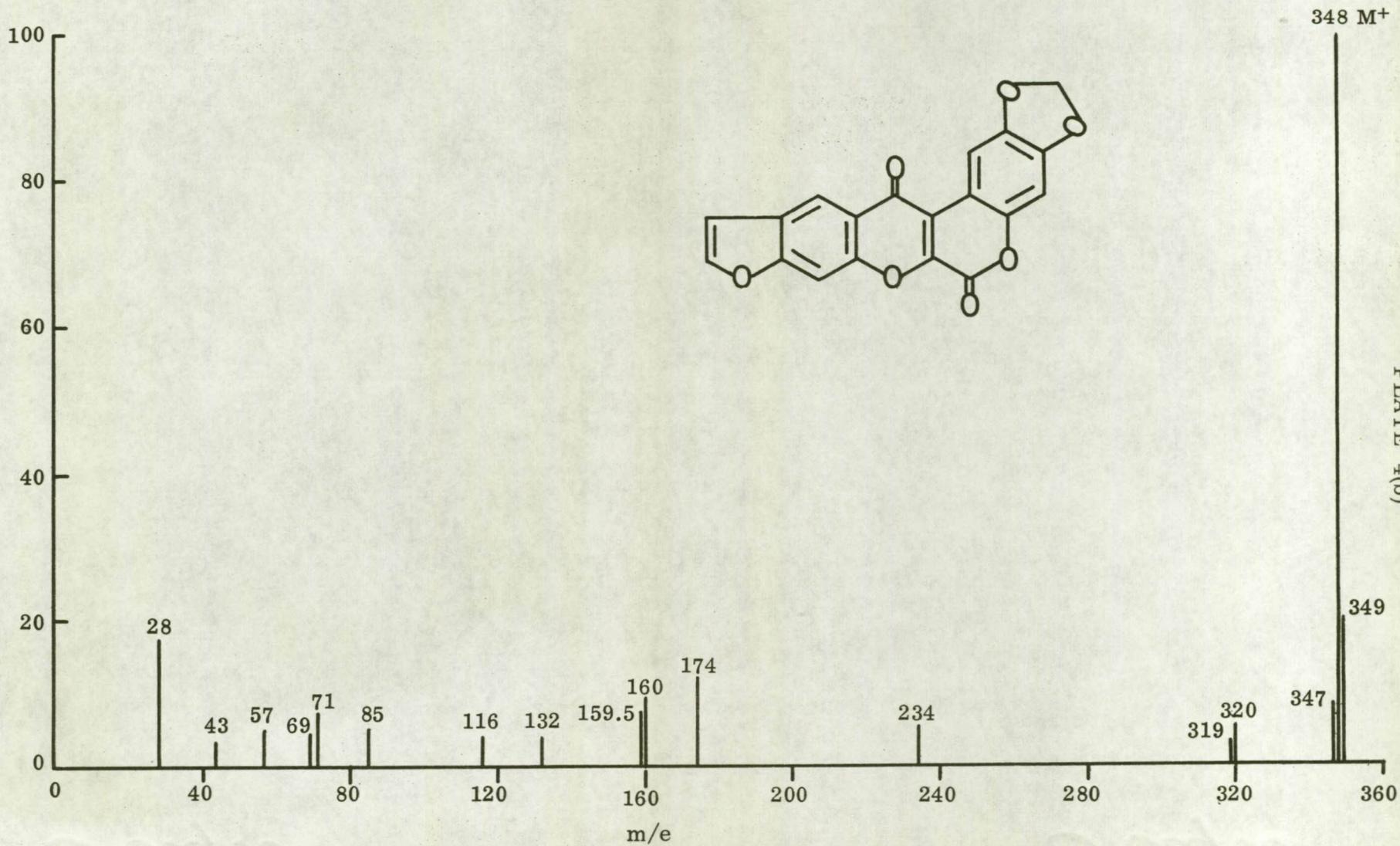
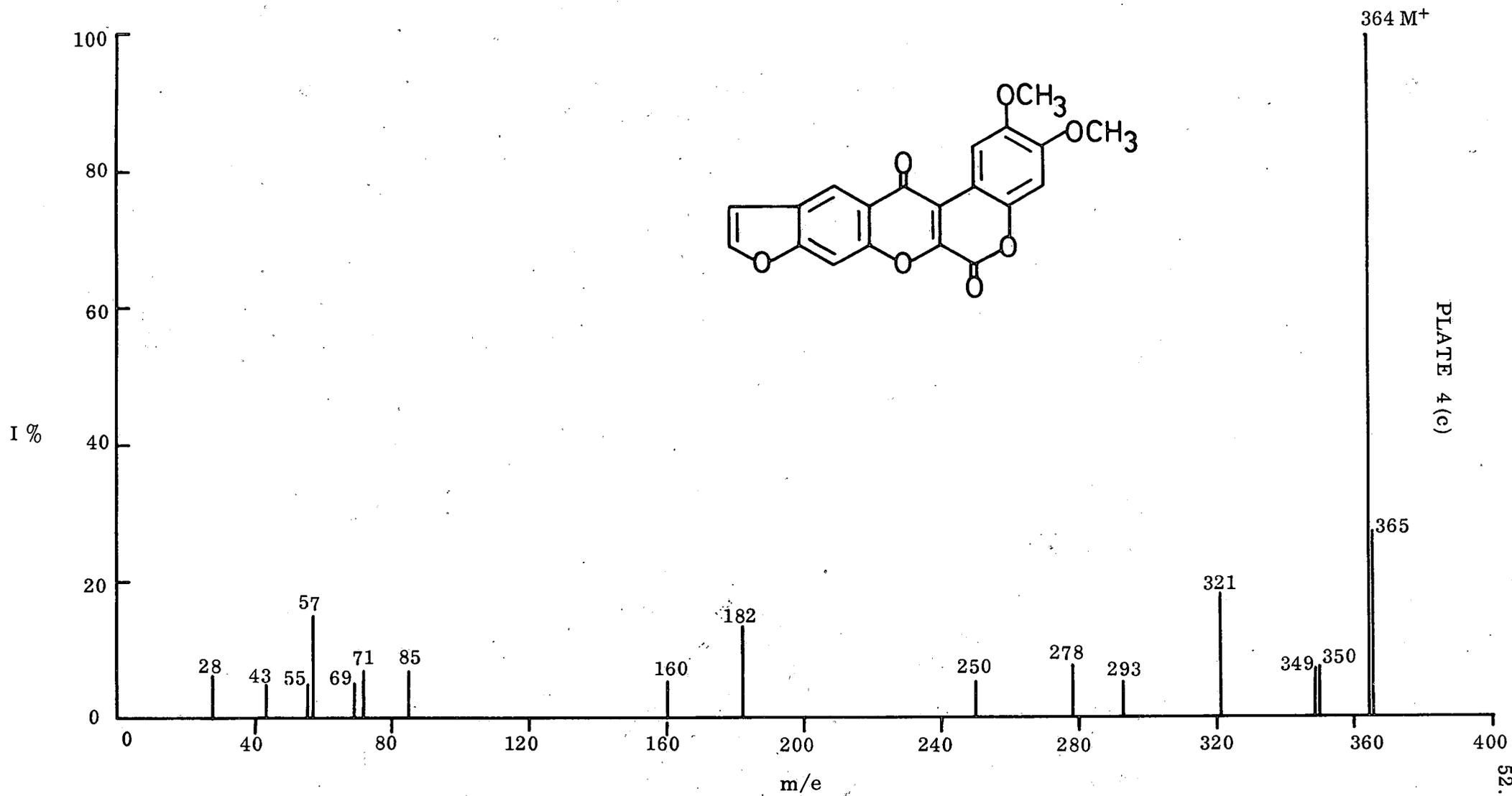


PLATE 4(b)

(b) NEBOENSINONE (XVII)



95464

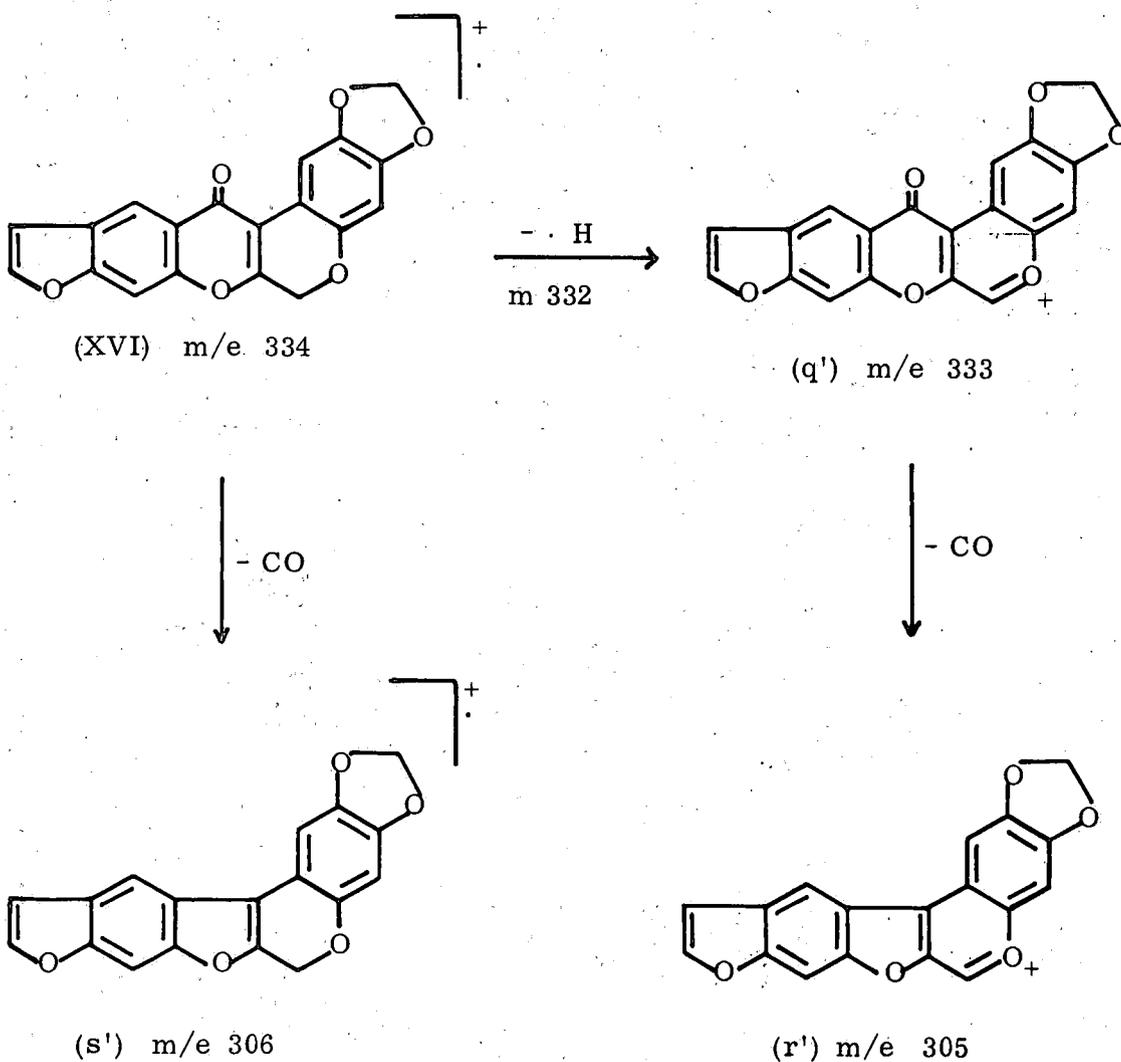


(c) NAMBINONE (XVIII)

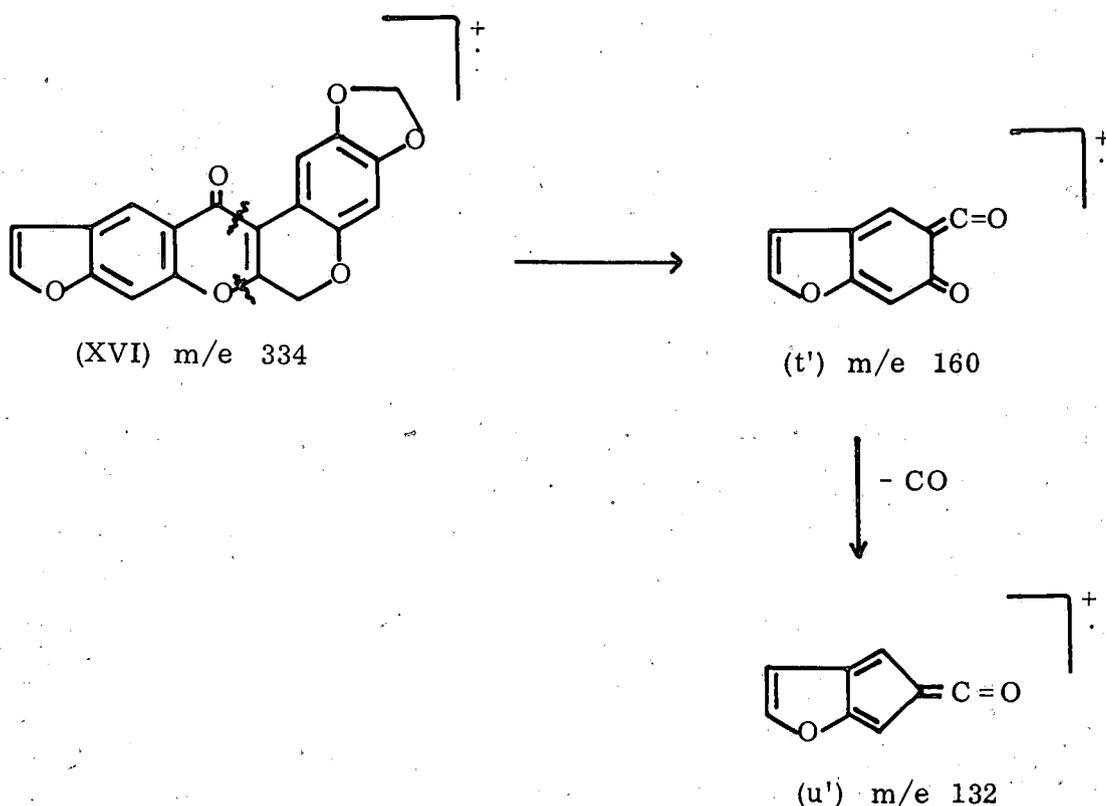
PLATE 4(c)

A. DEHYDRODOLINEONE (XVI)

In dehydrodolineone (XVI) very little fragmentation occurs as the retro-Diels-Adler breakdown pattern is not operative. Due to the stability of this compound a loss of a hydrogen atom from the parent molecular ion,  $m/e$  334 (base peak), is favoured to form the M-1 peak (q') at  $m/e$  333, (confirmed by the metastable peak at  $m/e$  332). The loss of CO from the ion (q') and from the parent ion  $m/e$  334 affords the ions (r')  $m/e$  305, which is the major breakdown route of this compound, and the ion (s') at  $m/e$  306. The peak at  $m/e$  276 is possibly formed by the expulsion of  $\text{CH}_2\text{O}$  from the methylenedioxy group of the ion (s')  $m/e$  306.



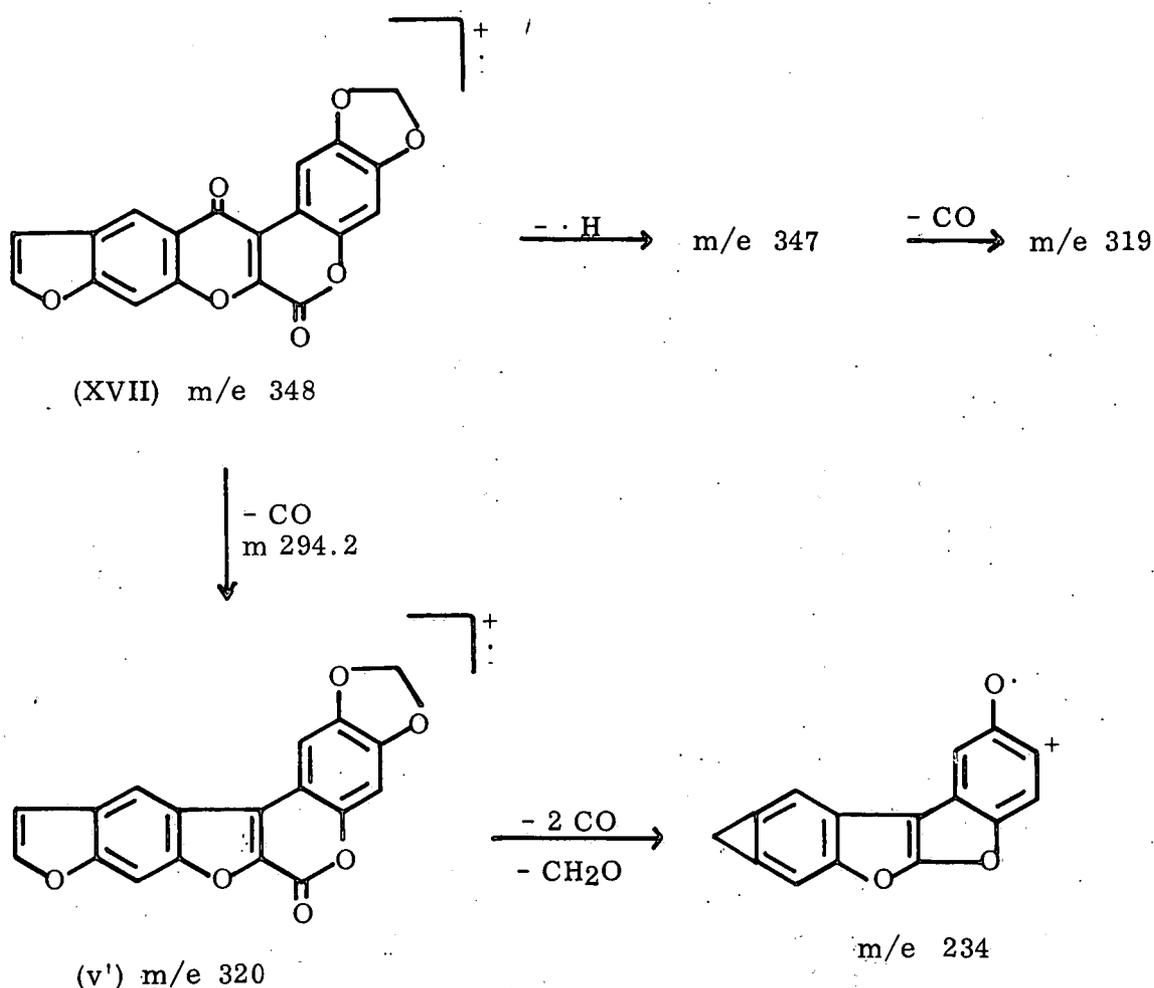
The cleavage of the  $\gamma$ -pyrone ring affords the species (t') at m/e 160 which undergoes further fragmentation by the loss of CO to form the peak (u') m/e 132.



The peaks at m/e 167 and 166.5 are doubly charged ions.

#### B. NEBOENSINONE (XVII)

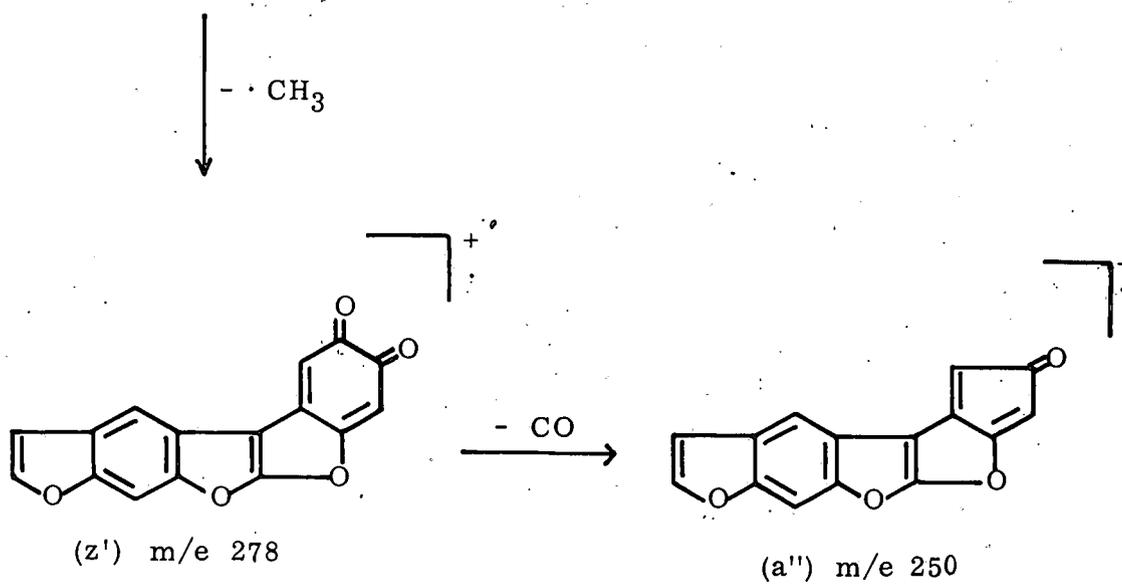
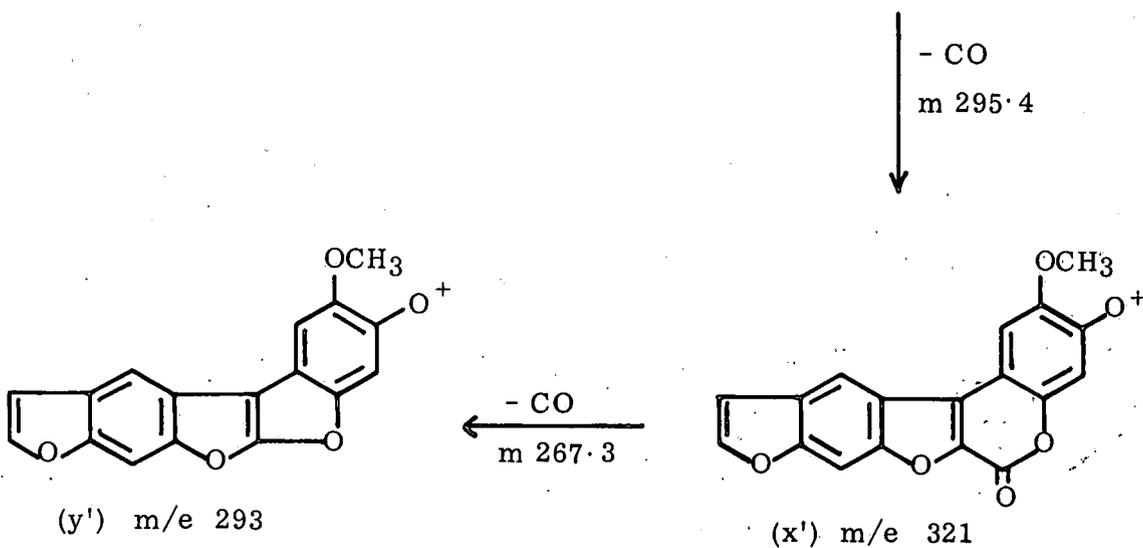
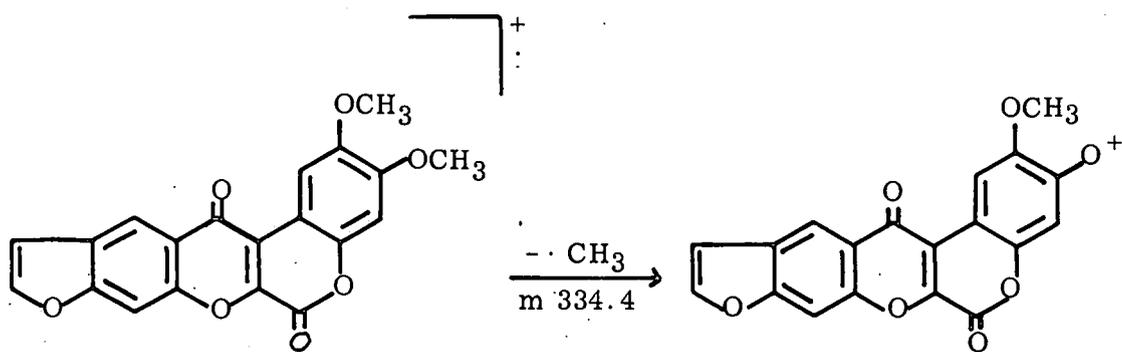
The fragmentation pattern of neboensinone, parent molecular ion m/e 348 (base peak, closely resembles that of dehydroloneone. The loss of a hydrogen atom from m/e 348 affords the ion at m/e 347 which in turn gives the ion at m/e 319 by the loss of CO. The elimination of CO from the parent molecular ion, confirmed by the metastable peak at m/e 294.2, gives rise to the ion (v') at m/e 320. The peak at m/e 234 is probably formed by the successive loss of two CO groups and formaldehyde (from the methylenedioxy group) from the ion (v') m/e 320.



As with dehydrodolioneone (XVI) the fragmentation of the  $\gamma$  - pyrone ring leads to the formation of the ion (t') m/e 160, which breaks down further by the loss of CO to form the ion (u') m/e 132 (see under dehydrodolioneone). Doubly charged ions are found at m/e 174, 173.5 and 159.5.

### C. NAMBINONE (XVIII)

The base peak in the spectrum of nambinone is also formed by the parent molecular ion at m/e 364. The transition from m/e 364 to the species (w') at m/e 349 corresponds to the loss of a methyl radical, a transition which is substantiated by a metastable peak at m/e 334.4. The elimination of CO from (w') affords the ion (x') at m/e 321 which in turn breaks down by the expulsion of CO to yield the peak (y') at m/e 293, both these transitions are confirmed by the appropriate metastable peaks at m/e 295.4 and 267.3 respectively. The subsequent decomposition of (y') by the loss of a methyl radical gives the ion (z') at m/e 278 which breaks down by the elimination of 28 mass units to yield the ion (a'') at m/e 250. The possible routes of fragmentation are given below.



Fragmentation of the  $\gamma$  - pyrone ring of the parent molecular ion also yields the peak (t') m/e 160 (see under dehydrodolineone A.) The peak at m/e 350 is possibly due to the (M+1) - 15 ion and the peak at m/e 182 is the doubly charged parent molecular ion (m/e 364). Both nambinone and neboensinone have peaks at m/e 160, 85, 71, 69, 57 and 43 which indicate the similarity in structure of these compounds. The possible fragmentation pattern of nambinone as suggested above is thus in complete agreement with the proposed structure for this compound (Chapter 3 II, D).

#### D. CONCLUSIONS

The mass spectra of the isoflavanoids investigated in this work show that their general modes of fragmentation are in agreement with similar types of compounds.<sup>58-62</sup> Neotenone, neotenane and tetrahedroneofolin undergo a pronounced retro-Diels-Alder fragmentation of the heterocyclic ring, a cleavage which is less prominent in dehydronotenone as a result of the stabilisation effect of the 2, 3 - double bond of the pyrone ring.<sup>24b, 62</sup> It has been found<sup>58-62</sup> that the elimination of methoxyl groups from oxygen heterocycles by the fission of the phenyl-oxygen bond does not readily occur. This was also found to be true for the compounds investigated in this work, with the notable exception of dehydronotenone where the loss of the methoxyl group from the parent molecular ion is the major breakdown path, in fact the only one possible.

The retro-Diels-Alder breakdown mode is not an important process in the case of the furocoumarins pachyrrhizin and neofolin since the rupture of the  $\alpha$  - pyrone ring is prevented by the 3, 4 - double bond.<sup>24b</sup> The major fragmentation mode of the furocoumarins is the pronounced elimination of carbon monoxide. The relatively insignificant breakdown of the isoflavanoids and the fact that most of these compounds have the parent molecular ion as the base peak clearly shows the stability of these compounds. (Tables 4 and 5).

Studies on the fragmentation patterns of rotenoids by Reed and Wilson<sup>62</sup> showed that the major breakdown pattern of these compounds is via a retro-Diels-Alder fragmentation of the  $\gamma$  - pyrone ring to yield the corresponding fragments. All the rotenoids which have methoxyl groups in the 2 - and 3 positions give the ion m/e 192 as their base peak<sup>36, 62</sup> which corresponds to the dimethoxychromene group common to all of these (see Chapter 3). In the case of pachyrrhizone, (XXVII) (Table 6) which has a methylenedioxy group attached

to positions 2 and 3 ( <sup>cf.</sup> ~~of~~ dolineone (XXVIII), dehydrodolineone (XVI) and neboensinone (XVII) , the base peak is formed by the ion at  $m/e$  176 which corresponds to the methylenedioxychromene group.

In contrast to the rotenoids the retro-Diels-Alder fragmentation is not an important process in the breakdown of the dehydrorotenoids investigated in this work as a result of the 6a, 12a - double bond which tends to prevent the collapse of the  $\gamma$  - pyrone ring. In this regard the dehydrorotenoids can be compared to the isoflavones ( cf. dehydroneoteneone)<sup>58-62</sup> and the furocoumarins pachyrrhizin and neofolin. As with the furocoumarins the major breakdown path of the dehydrorotenoids is the pronounced loss of carbon monoxide. The ion (a) = (g) = (t') at  $m/e$  160 is present in the spectra of all the compounds examined which contains a furo -  $\gamma$  - chromanone ring system (neoteneone, dehydroneoteneone, dehydrodolineone, neboensinone and nambinone, Tables 4, 6). This peak ( $m/e$  160) can thus possibly serve as a very useful indication as to the presence of such a ring system in isoflavanoids, rotenoids and related compounds. The corresponding ion in the spectrum of pachyrrhizone (XXVII) (Table 6) is at  $m/e$  190 due to the additional methoxyl group in position 8.

The possible routes for the formation of the most prominent peaks in the mass spectra of the dehydrorotenoids (XVI), (XVII) and (XVIII) are completely reconcilable with the proposed structures for these compounds (Chapter 3). Despite their high stability to electron impact, the mass spectra of isoflavanoids, rotenoids and related compounds can yield very valuable information which could facilitate the structure elucidation of these types of compounds considerably.

## C H A P T E R 5

### I. THE SYNTHESIS OF HYDROXYBENZOFURANS

#### A. PREPARATION OF ACETOXY - 2, 3 - DIHYDROBENZOFURANS.

- (i) 6 - ACETOXY - 2, 3 - DIHYDROBENZOFURAN
- (ii) 4, 6 - DIACETOXY - 2, 3 - DIHYDROBENZOFURAN
- (iii) 6, 7 - DIACETOXY - 2, 3 - DIHYDROBENZOFURAN

#### B. QUINONE DEHYDROGENATION OF 2, 3 - DIHYDROBENZOFURANS

- (i) INTRODUCTION
- (ii) PREPARATION OF ACETOXYBENZOFURANS BY QUINONE DEHYDROGENATION
  - (a) 6 - ACETOXYBENZOFURAN
  - (b) 4, 6 - DIACETOXYBENZOFURAN
  - (c) 6, 7 - DIACETOXYBENZOFURAN

#### C. PREPARATION OF HYDROXYBENZOFURANS

- (i) 6 - HYDROXYBENZOFURAN
- (ii) 4, 6 - DIHYDROXYBENZOFURAN
- (iii) 6, 7 - DIHYDROXYBENZOFURAN

### II. SYNTHESIS OF PHENOLIC DERIVATIVES OF NATURAL ISOFLAVANOIDS

#### A. PREPARATION OF PACHYRRHIZINOL

#### B. PREPARATION OF THE DEOXYBENZOIN OF DEHYDRONEOTENONE

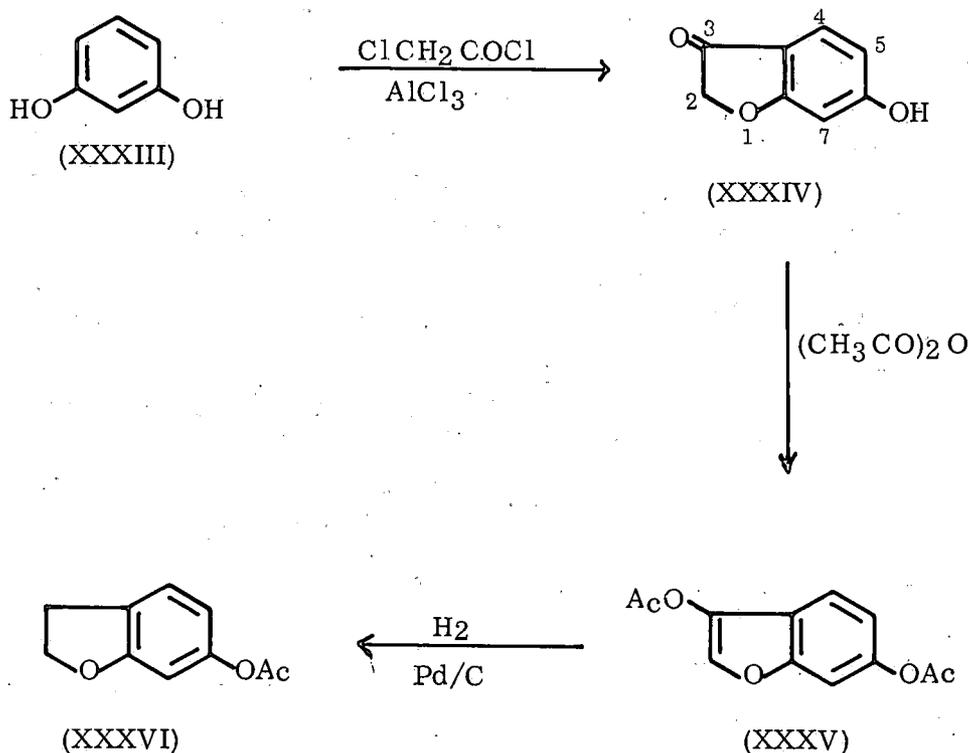
#### C. PREPARATION OF FORMONONETIN.

I. THE SYNTHESIS OF HYDROXYBENZOFURANS

A. PREPARATION OF ACETOXY - 2, 3 - DIHYDROBENZOFURANS.

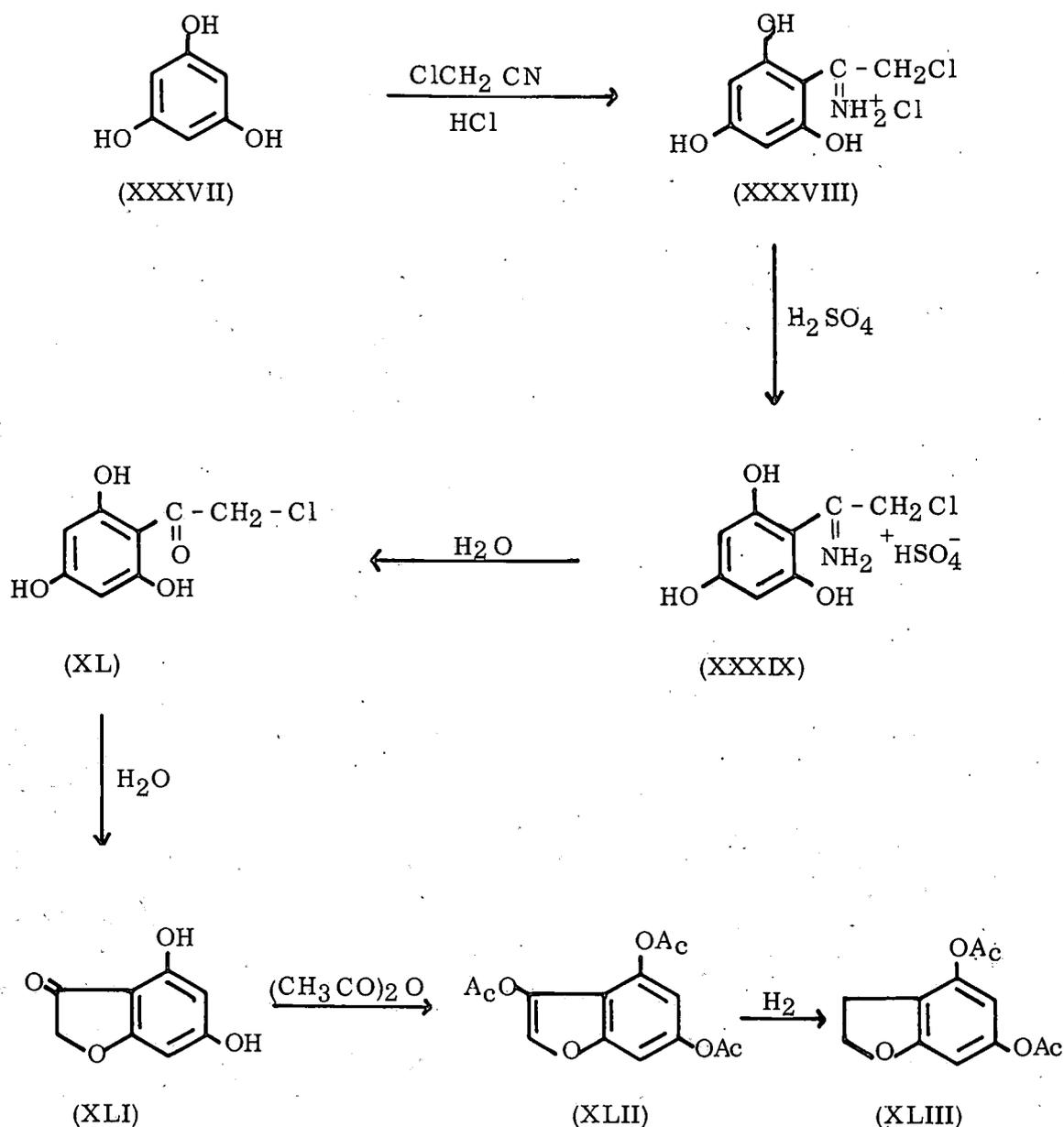
(i) 6 - ACETOXY - 2, 3 - DIHYDROBENZOFURAN (XXXVI)

6 - Hydroxy - 2, 3 - dihydrobenzofuran - 3 - one (XXXIV) was prepared by a Friedel-Crafts reaction with resorcinol (XXXIII) and chloroacetyl chloride in nitrobenzene.<sup>63, 64, 65, 66</sup> Acetylation of (XXXIV) with acetic anhydride in pyridine medium gave 3, 6 - diacetoxybenzofuran (XXXV). Hydrogenation of (XXXV) in glacial acetic acid over 30% Pd/C catalyst<sup>67</sup> at 70°C and 4 atmospheres pressure afforded 6 - acetoxy - 2, 3 - dihydrobenzofuran (XXXVI)<sup>65</sup>.



(ii) 4, 6 - DIACETOXY - 2, 3 - DIHYDROBENZOFURAN (XLIII).

A Hoesch reaction between phloroglucinol (XXXVII) and chloroacetonitrile produced 2, 4, 6 - trihydroxyphenyl chloromethylketimine hydrochloride (XXXVIII) which was converted to the corresponding ketimine sulphate (XXXIX). Hydrolysis of (XXXIX) yielded 2, 4, 6 - trihydroxy -  $\alpha$  - chloroacetophenone (XL) which on heating under reflux in water gave 4, 6 - dihydroxy - 2, 3 - dihydrobenzofuran - 3 - one (XLI)<sup>66, 68, 69</sup>



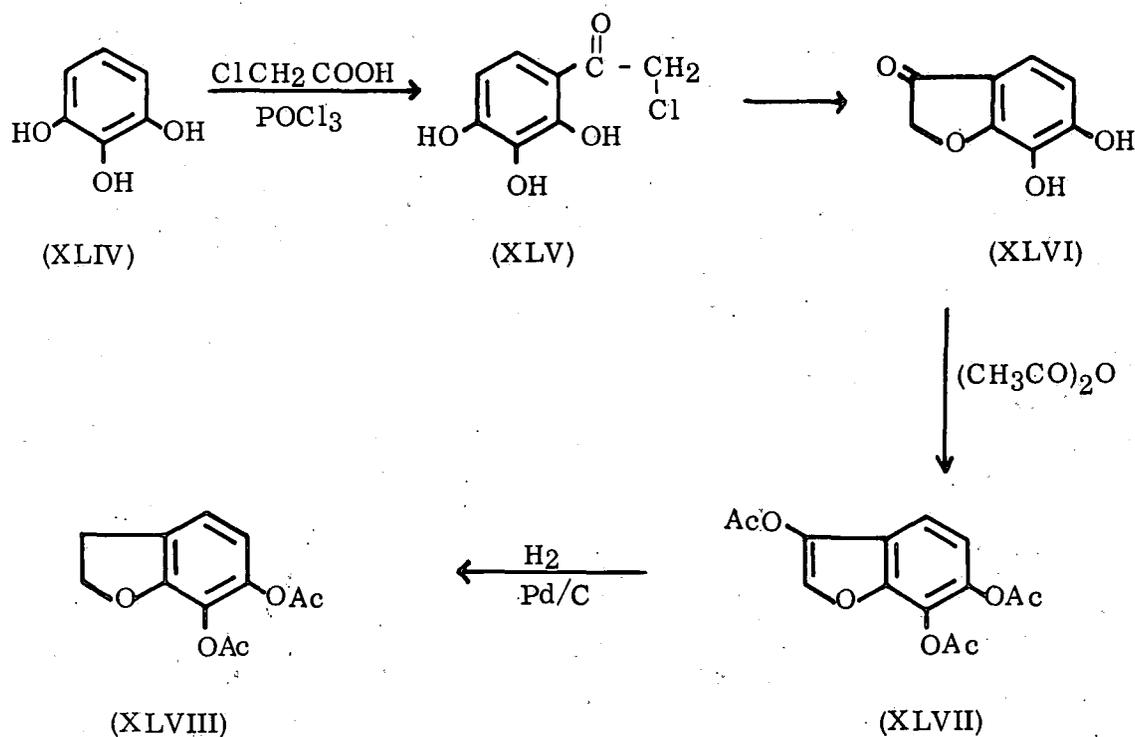
Acetylation of (XLI) with acetic anhydride in pyridine gave 3,4,6-triacetoxybenzofuran (XLII)<sup>69</sup> which on hydrogenation in glacial acetic acid over 30% palladium-charcoal catalyst at 60° - 70°C under 4 atmospheres pressure afforded 4,6, - diacetoxy - 2,3 - dihydrobenzofuran (XLIII)<sup>69</sup>.

(iii) 6,7-DIACETOXY-2,3-DIHYDROBENZOFURAN (XLVIII)

On heating pyrogallol (XLIV) with monochloroacetic acid and phosphorus oxychloride at 60° - 70°C  $\omega$ -chlorogallacetophenone (XLV) was obtained which was cyclised by heating with sodium acetate in ethanol to yield

6,7-dihydroxy-2,3-dihydrobenzofuran-3-one (XLVI)<sup>35, 68, 70, 71, 72.</sup>

Acetylation of (XLVI) with acetic anhydride in pyridine at room temperature gave 3,6,7-triacetoxybenzofuran (XLVII)<sup>70.</sup> Hydrogenation over 30% Pd/C as under (i) and (ii) afforded 6,7-diacetoxy-2,3-dihydrobenzofuran (XLVIII)<sup>70, 72.</sup>



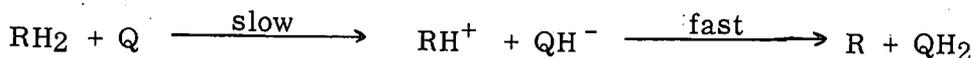
## B. QUINONE DEHYDROGENATION OF 2,3-DIHYDROBENZOFURANS<sup>73</sup>

### (i) INTRODUCTION

Quinones are amongst the most powerful organic hydrogen acceptors known at present. Although the use of a quinone for dehydrogenation was first reported as early as 1930,<sup>74</sup> this method for the dehydrogenation of organic compounds has received limited attention in the past. In the last decade the use of this method has steadily increased, especially since a variety of high potential quinones became available which were found to be very useful in the dehydrogenation of hydroaromatic compounds.

Braude, Jackman and Linstead<sup>75, 76</sup> studied the mechanism of this reaction and established that the dehydrogenation of hydroaromatic compounds by

quinones proceeds by way of a two stage ionic process.

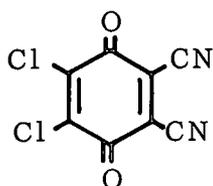


In the first and rate-determining step abstraction of hydrogen occurs as a hydride ion and is followed by the rapid transfer of a proton from the resulting conjugate acid of the aromatic hydrocarbon to the quinol anion. It has been found<sup>75</sup> that electron withdrawing substituents enhance the dehydrogenating reactivity of quinones. The substituents of a quinone affect the oxidation-reduction potential of the quinone which has been correlated with the dehydrogenating power of the quinone. It has been established that quinones with high redox potentials are the most powerful acceptors of hydrogen in dehydrogenation reactions.

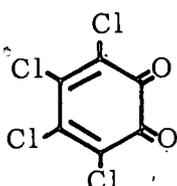
Quinones are generally to a higher or lesser degree susceptible to various side reactions which may compete with the dehydrogenation process and in some cases may even completely overshadow the dehydrogenation process in some systems. The most common side reactions are a Diels-Alder reaction (diene addition)<sup>77, 78</sup>, nucleophilic substitution (with halogenated quinones)<sup>79</sup> and nucleophilic addition (in unsubstituted quinones). Despite these side reactions it is possible to dehydrogenate a wide variety of compounds by the judicious choice of a suitable quinone.

Chloranil (tetrachloro-1,4-benzoquinone) has been employed most frequently as an acceptor in dehydrogenation reactions<sup>80-82</sup> but it is not as reactive as the high potential quinones which are available at present. The oxidation potential of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone is c.a. 1.0V compared to the 0.71 V for chloranil. In the dehydrogenation of tetralin, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone reacts 5,500 times faster than chloranil.

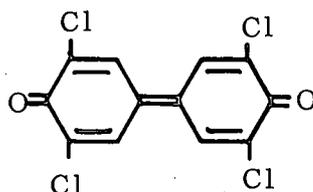
The three most useful high potential quinones known today are 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (a), tetrachloro-1,2-benzoquinone (b) and 3,3',5,5'-tetrachloro-4,4'-diphenoquinone (c).



(a)



(b)



(c)

DDQ (a) is regarded as one of the most powerful dienophiles known today and has found wide applications as an acceptor in dehydrogenations reactions<sup>83-88</sup>.

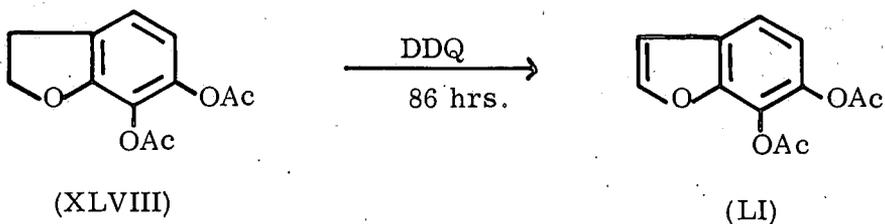
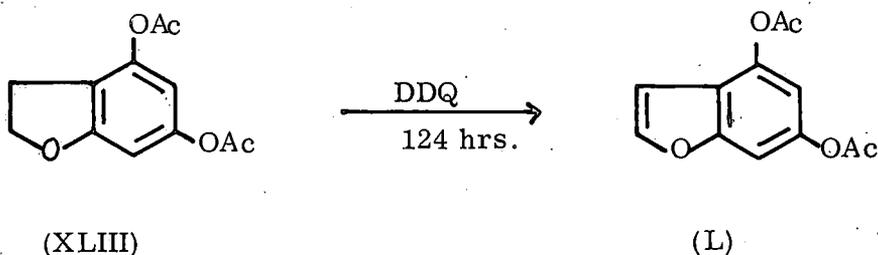
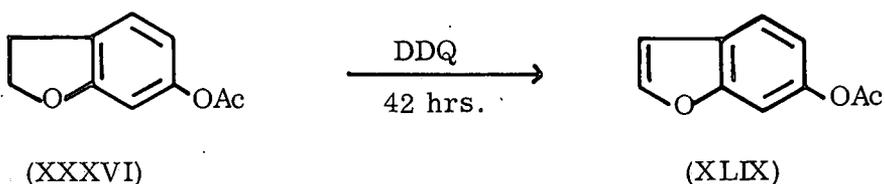
The use of quinones in the preparation of furans has received very little attention and only one reference in this regard was found in the literature where chloranil was used in the dehydrogenation of 3-phenyl-2,5-dihydrofuran which gave only a 10% yield of the corresponding furan<sup>89</sup>. Dehydrogenation of the same compound with sulphur in dimethylformamide gave a much better yield. The dehydrogenation of dihydrofuran compounds to the corresponding furans was also achieved by Baxter<sup>90</sup> and Davies et al.<sup>65,69,70</sup> by using a column packed with palladium-charcoal catalyst through which they sublimed their respective dihydrofuran compounds.

The work carried out in this investigation is, to the best of our knowledge, the first where dihydrofuran compounds were successfully dehydrogenated by means of a high potential quinone.

## (II) PREPARATION OF ACETOXYBENZOFURANS BY QUINONE DEHYDROGENATION

The dehydrogenation of 6-acetoxy-2,3-dihydrobenzofuran (XXXVI) with chloranil in benzene was not successful as only approximately 1% 6-acetoxybenzofuran (XLIX) was obtained after 112 hours.

Dehydrogenation of 6-acetoxy-2,3-dihydrobenzofuran (XXXVI), 4,6-diacetoxy-2,3-dihydrobenzofuran (XLIII) and 6,7-diacetoxy-2,3-dihydrobenzofuran (XLVIII) with DDQ in benzene to the corresponding acetoxybenzofurans (XLIX), (L) and (LI) proved to be very successful and good yields were obtained.



The reactions were carried out under strictly anhydrous conditions by refluxing the compounds in sodium-dried benzene (10-60 ml) with DDQ, as it is known<sup>91</sup> that DDQ is very sensitive to hydrolysis by moisture, resulting in the liberation of hydrocyanic acid.

In order to establish the optimum reaction conditions for each compound different mole ratios of the compounds to DDQ were used. It was found that with a mole ratio of 1:1 the reactions did not go to completion, probably as the result of side reactions. Some of the side reaction products were isolated, but they were not investigated in any great detail as these types of complex compounds represent a field of study in their own right. As expected the reaction of DDQ with the respective hydroxy-2,3-dihydrobenzofurans immediately led to the formation of complex compounds. The relevant data obtained from the different dehydrogenation reactions are given in Table 7.

TABLE 7

Compound	Compd. :DDQ mole ratio	Time (hrs)	Yield %*	Total Yield %**
(XXXVI)	1 : 1	24	71	92
"	1 : 1	36	77	88
"	1 : 1.5	36	81	84
"	1 : 1.5	42	52.6	52.6
(XLIII)	1 : 1	26	63	90
"	1 : 1.3	26	74	89
"	1 : 1.5	124	87	87
(XLVIII)	1 : 1	38	76	94
"	1 : 1.25	38	81	89
"	1 : 1.3	46	84	87
"	1 : 1.5	86	62	62

Yield %\* = % dehydrogenated compound

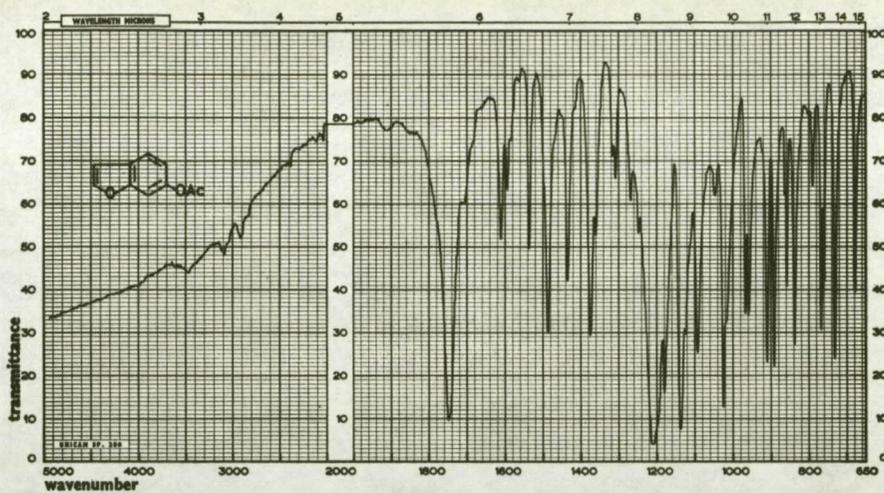
Total Yield %\*\* = Total material recovered.

From the table it can be seen that the dehydrogenation reactions proceed rapidly at first but then slows down considerably and relatively long times are required for the reactions to go to completion. The yields from the reactions, with the notable exception of (XLIII), are adversely influenced by the time factor and the excess DDQ required for the completion of the reactions as these factors favour the side reactions.

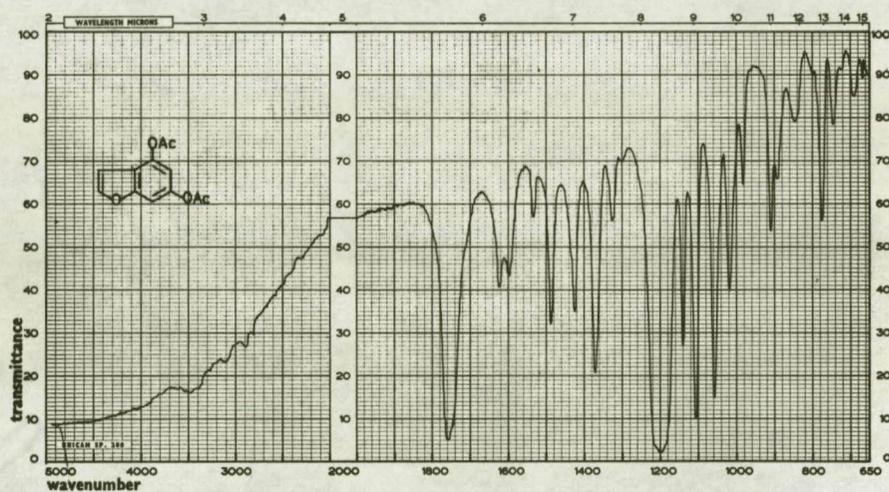
(a) 6-ACETOXYBENZOFURAN (XLIX)

6-Acetoxybenzofuran was obtained as long colourless needles m. p. 44° - 45°C in a 52.6% yield. Infrared spectrum (plate 5),  $\nu_{\max}$  (cm<sup>-1</sup>): 1751 (acetyl); 1610, 1594 and 1537 (aromatic). Ultraviolet spectrum (plate 6),  $\lambda_{\max}$  m  $\mu$  (log  $\epsilon$ ): 244 (4.05) (benzofuran); 250<sup>1</sup> (4.01) (benzofuran); 278 (3.47) and 284 (3.45) (aromatic).

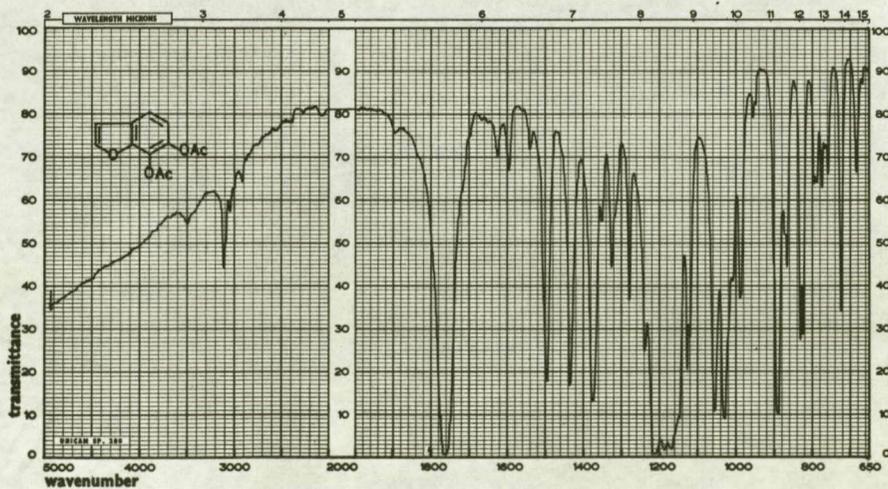
## PLATE 5



(a) 6 - ACETOXYBENZOFURAN (XLIX)

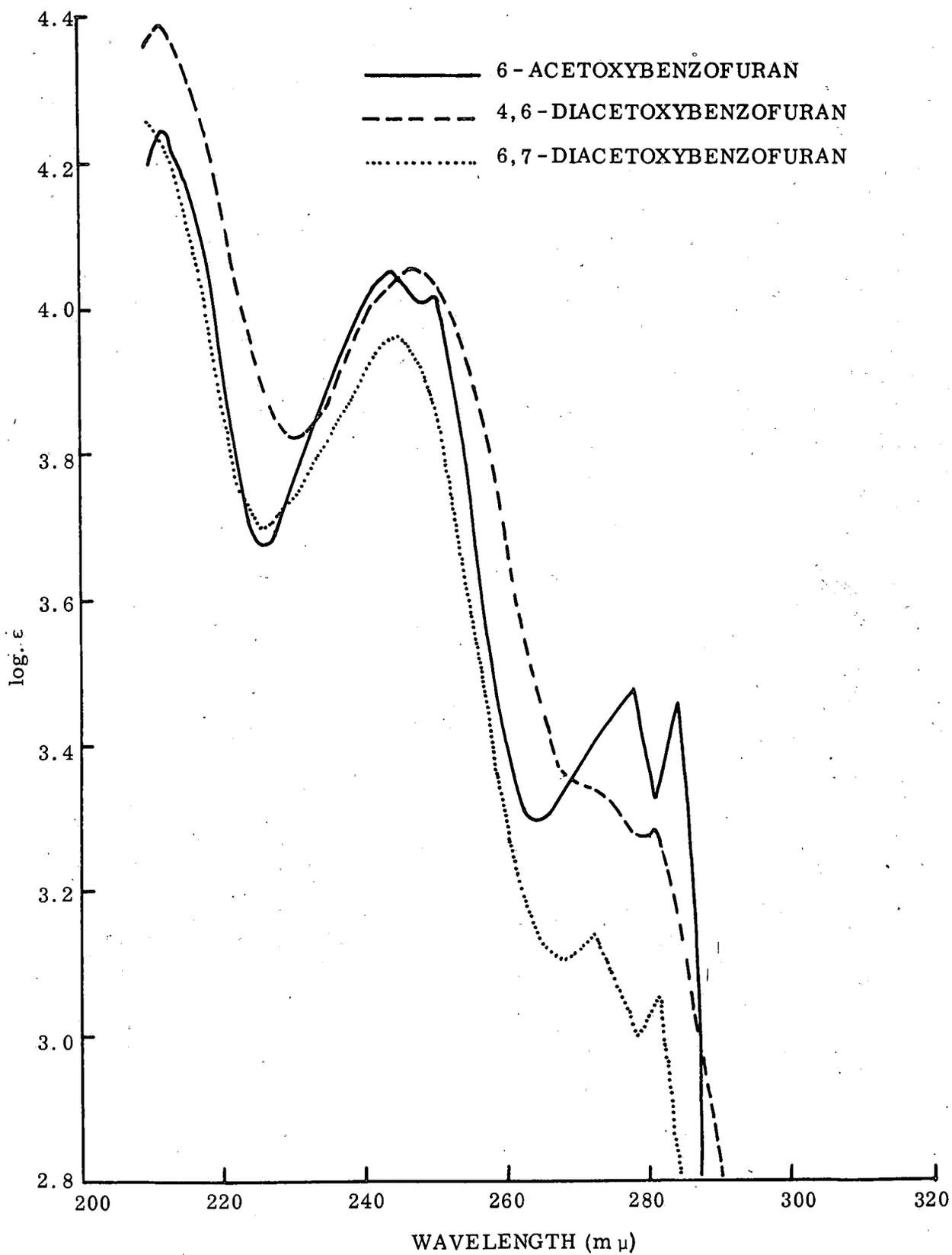


(b) 4,6 - DIACETOXYBENZOFURAN (L)



(c) 6,7 - DIACETOXYBENZOFURAN (LI)

## PLATE 6



(b) 4,6-DIACETOXYBENZOFURAN (L)

This compound was obtained as a light yellow oil in a 87% yield. Infrared spectrum (plate 5)  $\nu$  max. ( $\text{cm}^{-1}$ ): 1760 (acetyl); 1623, 1598 and 1537 (aromatic). Ultraviolet spectrum (plate 6),  $\lambda$  max.  $m\mu$  ( $\log \epsilon$ ): 247 (4.05) (benzofuran); 272<sup>1</sup> (3.34) and 281 (3.28) (aromatic).

(c) 6,7-DIACETOXYBENZOFURAN (LI)<sup>35</sup>

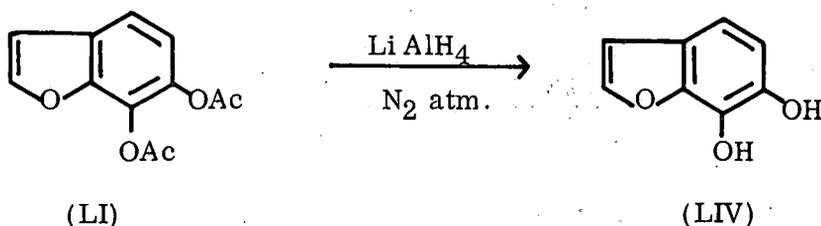
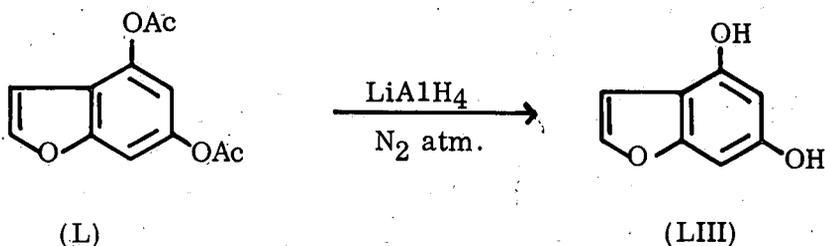
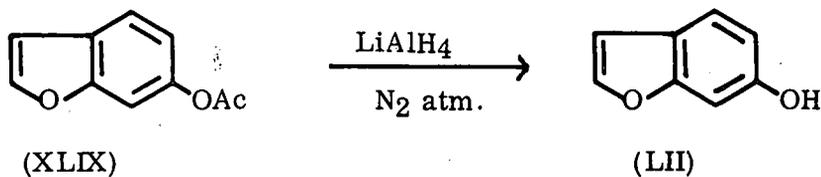
Dehydrogenation of (XLVIII) afforded (LI) as colourless needles m. p. 97°C-98°C in a 62% yield. Infrared spectrum (plate 5);  $\nu$  max. ( $\text{cm}^{-1}$ ); 1762 (acetyl); 1625, 1598 and 1540 (aromatic). Ultraviolet spectrum (plate 6)  $\lambda$  max.  $m\mu$  ( $\log \epsilon$ ): 244 (3.96) (benzofuran); 272 (3.13) and 281 (3.04) (aromatic).

The ultraviolet spectra of the acetoxy - 2,3-dihydrobenzofurans (XXXVI), (XLIII) and (XLVIII) showed no absorption in the 240-250  $m\mu$  region in contrast to the acetoxybenzofurans (XLIX), (L) and (LI) (plate 6) which show strong absorption in this region. This is in full agreement with the assignment of these bands to a benzofuran structure. 30, 44, 47-53.

C. PREPARATION OF HYDROXYBENZOFURANS.

The hydrolysis of the acetoxybenzofurans with alkali proved to be unsuitable as only small yields were obtained. In the case of 6,7-diacetoxybenzofuran (LI) it was found that the hydrolysis product 6,7-dihydroxybenzofuran (LIV) is extremely unstable in alkaline medium in the presence of oxygen. It immediately oxidised and resinified with the result that no product was obtained.

The hydrolysis of the acetoxybenzofurans (XLIX), (L) and (LI) were successfully achieved by the reductive cleavage of the acetyl groups with lithium aluminium hydride<sup>92</sup>.  $\text{LiAlH}_4$  was added to the compounds dissolved in ether and kept under nitrogen at 10°C. After three minutes the excess  $\text{LiAlH}_4$  was destroyed by aqueous ether, water and hydrochloric acid while still working in a nitrogen atmosphere. In acidic medium the hydroxybenzofurans were found to be stable and the reaction products were worked up in the usual manner.



(i) 6-HYDROXYBENZOFURAN (LII)<sup>93</sup>

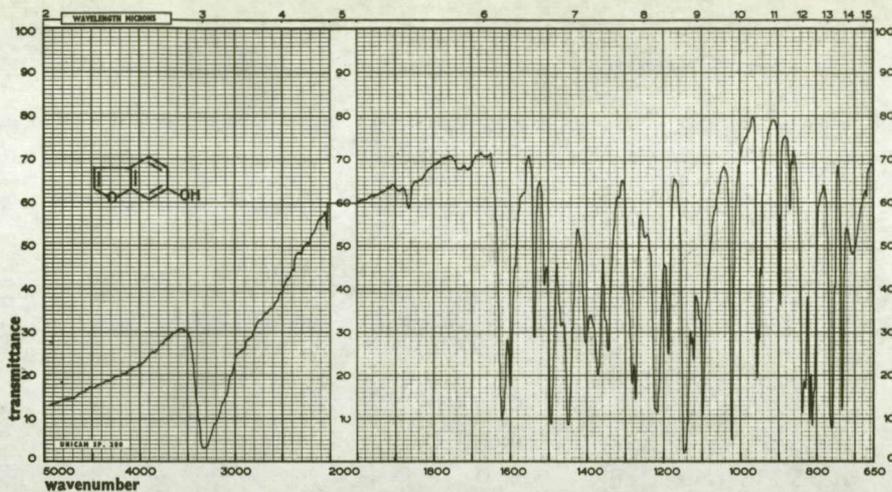
This compound was obtained as colourless prisms m. p. 57°-58°C (58% yield). Infrared spectrum (plate 7),  $\nu_{\text{max.}}$  ( $\text{cm}^{-1}$ ): 3325 (hydroxyl); 1620, 1600, 1538 and 1510 (aromatic). Ultraviolet spectrum (plate 8),  $\lambda_{\text{max.}}$   $m\mu$  ( $\log \epsilon$ ): 244 (4.10) and 251 (4.09) (benzofuran); 288 (3.72) (aromatic).

(ii) 4,6-DIHYDROXYBENZOFURAN (LIII)

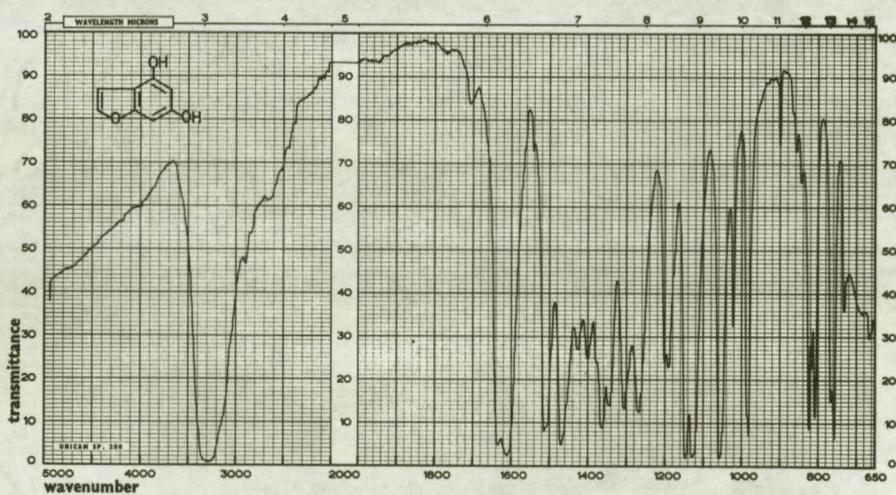
A yield of 64% was obtained for (LIII) m. p. 121°-122°C. Infrared spectrum (plate 7),  $\nu_{\text{max.}}$  ( $\text{cm}^{-1}$ ): 3285 (hydroxyl); 1635, 1610 and 1517 (aromatic). Ultraviolet spectrum (plate 8),  $\lambda_{\text{max.}}$   $m\mu$  ( $\log \epsilon$ ): 226 (4.40); 249<sup>i</sup> (4.04) and 254 (4.07) (benzofuran); 287<sup>i</sup> (2.80) (aromatic).

(iii) 6,7-DIHYDROXYBENZOFURAN (LIV)<sup>35</sup>

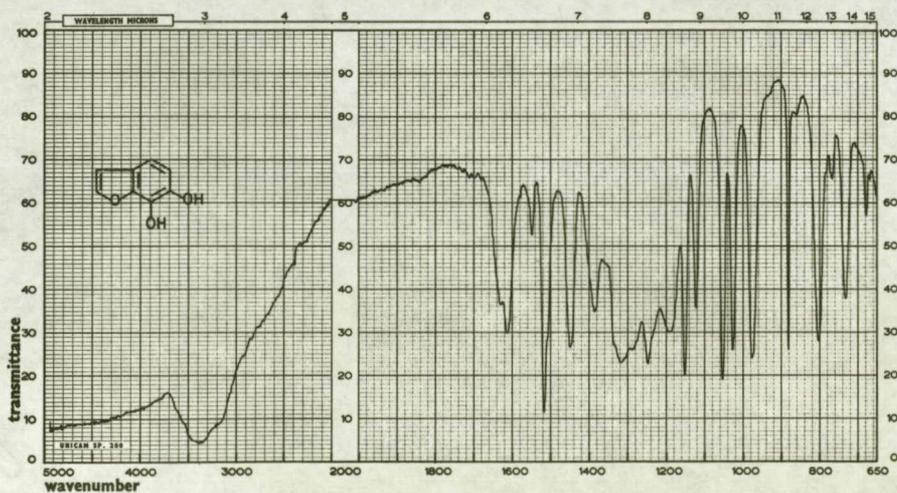
The  $\text{LiAlH}_4$  reduction of (LI) afforded 6,7-dihydroxybenzofuran as clusters of needles from benzene, m. p. 73°-74°C - (Yield 62%). Infrared spectrum (plate 7),  $\nu_{\text{max.}}$  ( $\text{cm}^{-1}$ ): 3380 (hydroxyl); 1630, 1613, 1550 and 1518 (aromatic).



(a) 6 - HYDROXYBENZOFURAN (LII)

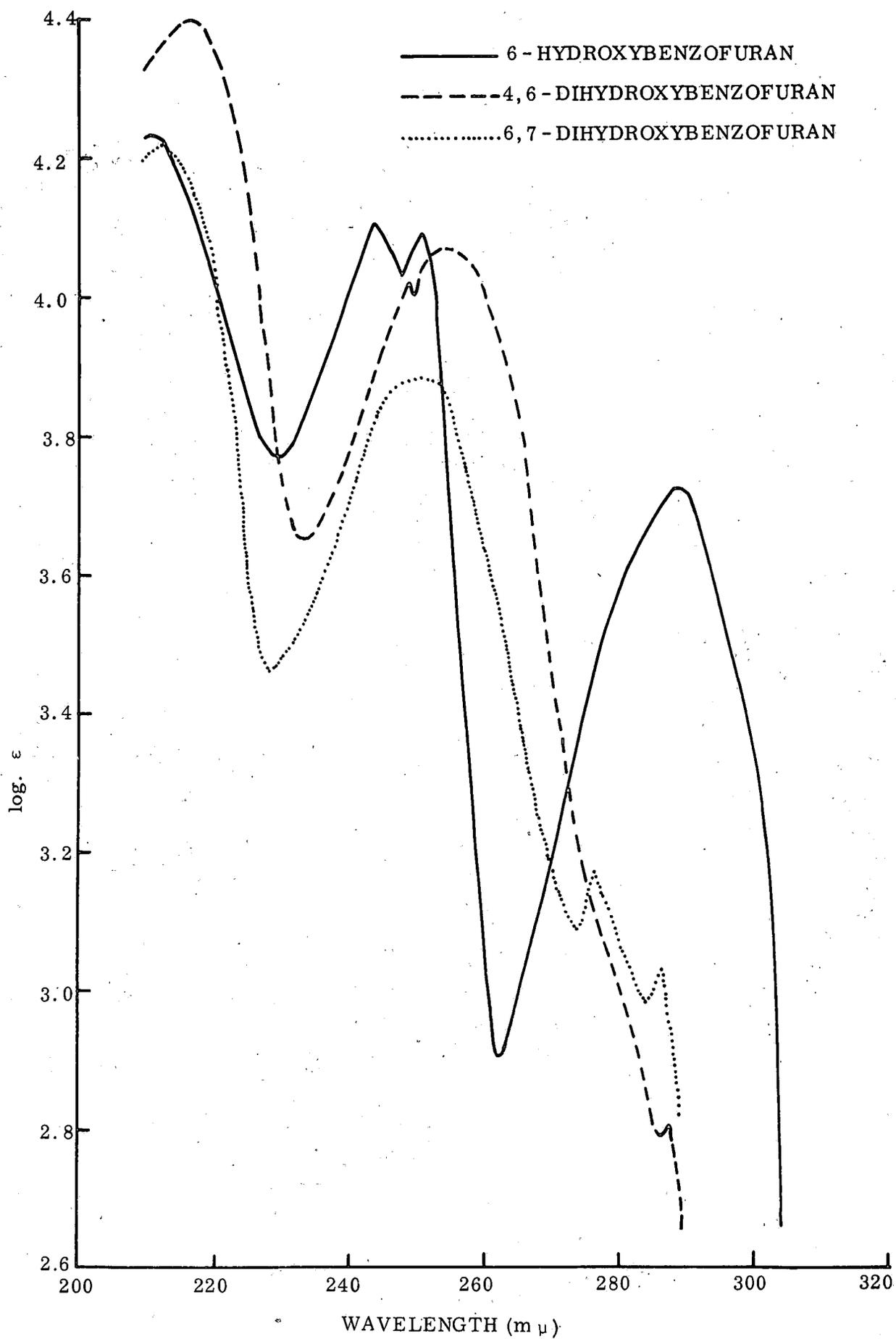


(b) 4,6 - DIHYDROXYBENZOFURAN (LIII)



(c) 6,7 - DIHYDROXYBENZOFURAN (LIV)

## PLATE 8



The ultraviolet spectrum of (LIV) was recorded in iso-octane as it was found that this compound rapidly decomposed in ethanol, probably as a result of oxidation.

Ultraviolet spectrum (plate 8);  $\lambda_{\text{max. m}\mu}$  ( $\log \epsilon$ ): 251 (3.88) (benzofuran); 276 (3.16) and 286 (3.03) (aromatic).

6,7-Dihydroxybenzofuran (LIV) was found to be unstable when in contact with oxygen and that it slowly decomposed over a period of 1-2 months. This compound was consequently stored under nitrogen.

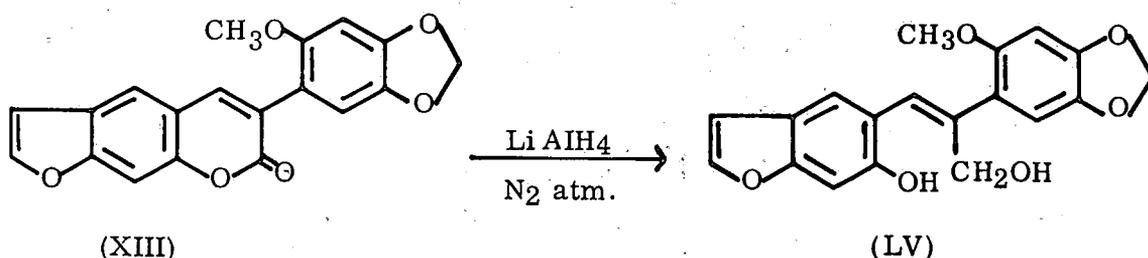
A qualitative test for benzofurans was developed which seems quite promising (see experimental section).

## II. SYNTHESIS OF PHENOLIC DERIVATIVES OF NATURAL ISOFLAVANOIDS

### A. PREPARATION OF PACHYRRHIZINOL (LV)

The reduction of  $\alpha : \beta$  - unsaturated lactones by lithium aluminium hydride 30,44 94-96 results in the cleavage of the  $\alpha$  - pyrone ring to yield either the  $\alpha : \beta$  - unsaturated - or  $\alpha : \beta$  - saturated diol<sup>97</sup> or both. The reaction conditions for the reduction of pachyrrhizin (XIII)<sup>30,32</sup> were found to be critical and in a series of experiments the best results were obtained by using the reverse procedure. Lithium aluminium hydride was added to pachyrrhizin dissolved in tetrahydrofuran in a nitrogen atmosphere at a temperature of 50°C. The  $\alpha : \beta$  - unsaturated diol, pachyrrhizinol (LV)

$\beta$  - (6-hydroxybenzofuran-5) -  $\alpha$  - (2'-methoxy-4', 5' methylenedioxyphenyl) allyl alcohol was obtained in a 22% yield m. p. 203°-204°C. The reduction of 2''3'' - dihydropachyrrhizin was unsuccessful and no products were isolated. In the case of the furocoumarin neofolin<sup>44,55</sup> (8-methoxy pachyrrhizin) and dihydroneofolin similar reductions with  $\text{LiAlH}_4$  proceeded smoothly and the respective diols were obtained in good yields.

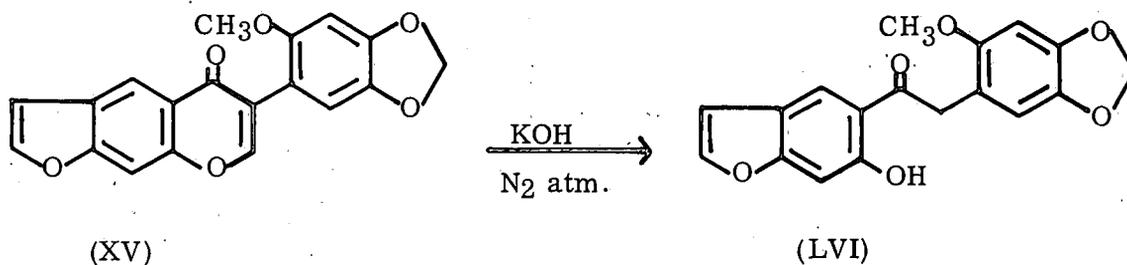


Pachyrrhizinol (LV): Infrared spectrum,  $\nu_{\text{max.}}$  ( $\text{cm}^{-1}$ ): 3350, 3060 (hydroxyl); 1618, 1503 (aromatic); 1165, 1037 and 938 (methylenedioxy group).

B. PREPARATION OF THE DEOXYBENZOIN OF DEHYDRONEOTENONE (LVI) 15

The deoxybenzoin of dehydroneotenone (LVI)

6 - hydroxy - 5 - (2' - methoxy - 4'5' methylenedioxyphenylacetyl) benzofuran m. p.  $161.5^{\circ}\text{C} - 162.5^{\circ}\text{C}$  was obtained by refluxing dehydroneotenone (XV) in an ethanolic potassium hydroxide solution for 3 hours under a nitrogen atmosphere.



Infrared spectrum of (LVI):  $\nu_{\text{max.}}$  ( $\text{cm}^{-1}$ ): 1641 (chelated carbonyl); 1598, 1545, and 1518 (aromatic); 1163, 1038 and 933 (methylenedioxy group).

C. PREPARATION OF FORMONONETIN (X)

The preparation of this compound is discussed in Chapter 2 F.

CHAPTER 6

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

## NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

### INTRODUCTION

Since the first measurements in nuclear magnetic resonance (NMR) spectroscopy were made in 1945, this technique has found wide applications in the different fields of physics and chemistry. NMR spectroscopy is now recognized as one of the most important methods for the structural elucidation and determination of the stereochemistry of organic compounds.

### PRINCIPLES OF NMR SPECTROSCOPY

#### A. MAGNETIC PROPERTIES OF ATOMIC NUCLEI

The atomic nuclei of approximately half of the known isotopes possess an intrinsic mechanical spin or angular momentum. The total angular momentum depends on the nuclear spin or spin number  $I$ , which may have values of 0,  $\frac{1}{2}$ , or larger integral multiples of  $\frac{1}{2}$  depending on the particular nucleus. Since an electrical charge is associated with atomic nuclei, the spin of an atomic nucleus ( $I > 0$ ) produces a magnetic field whose axis coincides with the axis of spin so that a nuclear magnetic moment  $\mu$  results. A spinning nucleus may thus be considered as a tiny bar magnet. Nuclei with  $I = \frac{1}{2}$  can be described as spinning spherical bodies possessing uniform charge distributions and include  $H^1$ ,  $C^{13}$ ,  $N^{15}$ ,  $F^{19}$  and  $P^{31}$  which are particularly useful in organic chemistry. The most common element isotopes in organic chemistry are  $H^1$ ,  $C^{12}$  and  $O^{16}$  of which only  $H^1$  nuclei (protons) can give NMR signals as  $C^{12}$  and  $O^{16}$  are non-magnetic ( $I = 0$ ). NMR studies are thus, with a few exceptions, only concerned with protons.

In a static uniform magnetic field  $H_0$  a magnetic nucleus ( $I$  and  $\mu \neq 0$ ) may take up any one of  $(2I+1)$  orientations with respect to the direction of the applied magnetic field. A proton ( $I = \frac{1}{2}$ ) is thus restricted to only two possible orientations. As a result of the spin angular momentum that a nucleus possesses the axis of the nuclear magnet is not aligned exactly parallel or anti-parallel with respect to the applied magnetic field and the force exerted by the applied field will tend to reorientate it. This causes the axis of spin of the nuclear magnet to precess around the direction of the applied field. The frequency of this precessional motion is directly proportional to the magnetic moment  $\mu$  of the nucleus and to the strength of the applied field.

## B. THEORY OF NMR SPECTROSCOPY

The two possible orientations which a proton can assume in a uniform magnetic field can be described as a low energy orientation in which the nuclear magnetic moment is aligned parallel to the field, and a high energy orientation in which the nuclear magnetic moment is aligned anti-parallel to the field.

When a proton is placed in a strong uniform magnetic field  $H_0$  and subjected to a second fluctuating magnetic field  $H$ , which is applied at right angles to  $H_0$  and which is of the same frequency as the precessing nucleus, absorption or emission of energy by the nucleus can occur. Energy is absorbed if the angle of precessing nucleus (relative to  $H_0$ ) is caused to change from the parallel orientation (low energy level) to the anti-parallel orientation (high energy level.) The frequency of the electromagnetic radiation which will effect such transitions is given by the equation.

$$\nu = \frac{\gamma H_0}{2\pi}$$

where  $\gamma$  is the gyromagnetic ratio, a fundamental nuclear constant.

Electromagnetic radiation theory shows that the probability of a transition from the lower energy state to the higher state by absorption of energy is equal to the probability of transition downwards by radiation-induced emission. If, in a collection of nuclei of the same isotope, the numbers of nuclei in each energy state were equal, the rate of transitions up and down would be equal and there would be no net absorption or emission. In a static uniform magnetic field, however, a collection of nuclei are not equally distributed between the possible energy states, they tend to assume a Boltzmann distribution with a very small but finite excess in favour of the lower energy state, which permits a net observable absorption of energy from the radiofrequency field, since the number of upward transitions (absorption) is now slightly greater than downward transitions.

If a collection of protons is irradiated, the rate of absorption is initially greater than the rate of emission as a result of the small excess of protons in the lower energy state. The original excess of protons in the lower energy state may steadily diminish until the two energy states are equally populated. The intensity of the absorption signal will accordingly dwindle and vanish completely. This type of behaviour, known as saturation, is sometimes observed.

There exist, however, various types of radiationless transitions by which a nucleus in a higher energy state returns to a lower state. These transitions are called relaxation processes which maintain an excess of nuclei in the lower energy state, a condition which is necessary for the observation of NMR signals.

When a collection of nuclei <sup>is</sup> placed in a strong uniform magnetic field and subjected to the radiofrequency field of the oscillator, the absorption of the radiofrequency energy, which occurs at particular combinations of the oscillator frequency and the magnetic field strength, is measured by a suitable detector. The absorption signal so obtained is usually measured at a constant radiofrequency as a function of the strength of the magnetic field, i. e. the oscillator frequency remains constant while the strength of the applied magnetic field is varied.

### C. APPLICATIONS OF NMR IN ORGANIC CHEMISTRY.

#### (i) SHIELDING MECHANISMS

With a few exceptions, the major application of NMR in organic chemistry involves the study of protons. The magnetic field strength at which a nucleus absorbs energy of a particular radiofrequency is primarily determined by the nucleus. To a very small extent, however, the resonance frequency of a nucleus is dependent on its molecular (i. e. electronic) environment. Protons in different positions in a molecule absorb at slightly different magnetic field strengths at a particular frequency. This is because the extranuclear electrons magnetically screen (shield) the nucleus. The total shielding effect is directly proportional to the magnitude of the applied field, as expressed in the equation.

$$H_i = H_0 (1 - \sigma_i)$$

where  $H_i$  is the field at nucleus  $i$ ,  $H_0$  the applied magnetic field and  $\sigma_i$  the nondimensional shielding or screening constant of nucleus  $i$ .

Electronic shielding of protons arises from the circulations of electrons about the nuclei and bonds which are induced by the applied magnetic field. The three types of electronic shielding are due to local diamagnetic currents, diamagnetic currents in neighbouring anisotropic groupings and interatomic diamagnetic currents.

In local diamagnetic shielding the induced circulation of electrons about a nucleus is in such a direction as to produce a secondary magnetic field, the centre (i. e. in the region of the nucleus) of which is opposed to the applied magnetic field. The resultant field experienced by the nucleus is thus slightly less than the applied magnetic field. The degree of local diamagnetic shielding is dependent on the electron density around the nucleus concerned, the higher the electron density the larger the shielding effect and consequently the higher the external field ( $\tau$  - value) at which the nucleus absorbs. The inductive effect of electronegative substituent groups in saturated molecules will decrease the electron density around a neighbouring nucleus (i. e. decreasing the shielding effect) and cause a downfield shift of the signal.

The second type of electronic shielding may arise from the circulations of electrons about neighbouring atoms when they are specifically orientated with respect to the applied field. The induced secondary field may lead to the shielding or deshielding of nearby protons. Electrons associated with nuclei such as carbon, nitrogen and oxygen often play an important role in determining the shielding of neighbouring protons. Examples are the high diamagnetic shielding of acetylenic protons and the greatly reduced shielding of aldehydic protons.

The interatomic diamagnetic shielding effect is concerned with certain structures which allow the circulation of electrons over a number of atoms. Aromatic structures contain large closed circuits of  $\pi$  electrons in which strong diamagnetic currents are induced by the applied magnetic field, figure 1.

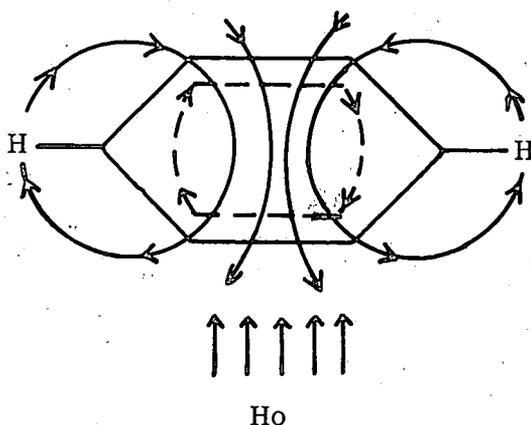


Fig. 1.

The induced secondary magnetic field at the centre of a benzene molecule is opposed to the applied magnetic field but parallel to the field at the positions of the aromatic protons. The ring-current effect thus deshields the aromatic protons or any such groups orientated in the plane of the benzene ring. Protons or groups

occupying positions above or below the centre of the aromatic ring may however be highly shielded.

All three shielding mechanisms are the result of induced secondary magnetic fields the magnitudes of which are directly proportional to the strength of the applied magnetic field.

(ii) THE CHEMICAL SHIFT

Protons in the usual types of organic environments have relative resonance frequencies spread over about 600 cycles per second (cps) at a magnetic field strength of about 14,000 gauss. At this field strength protons absorb at a frequency of about  $60 \times 10^6$  cps. This spread is thus equivalent to approximately 10 parts per million (ppm). The resonance frequency of a given proton or group of protons cannot be obtained directly from the instrument because the absolute strength of the applied magnetic field cannot be determined to the required degree of accuracy (one part in  $10^8$ ). The relative values for proton signals can however be readily determined with an accuracy of about 1 cps by using a suitable reference compound as a standard.

The most suitable internal reference compound at present is tetramethylsilane  $(\text{CH}_3)_4\text{Si}$ , (TMS). All the protons in TMS absorb at the same magnetic field strength and TMS has the added advantage that its absorption line occurs at a higher field strength (taken as 0 cps) than all the common types of organic protons. As TMS is insoluble in  $\text{D}_2\text{O}$  it cannot be used with this solvent. Sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS),  $(\text{CH}_3)_3\text{SiCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3\text{Na}$ , is a suitable aqueous and dimethyl sulphoxide soluble reference compound.

The separation of the resonance frequencies of nuclei in different structural environments from the absorption signal of the reference compound is termed the chemical shift. The chemical shift values can be expressed in different units. The symbol commonly used for the cps scale is  $\nu$  and the difference between two signal positions (in cps) is designated as  $\nu\Delta\nu$ . There are also two other ways of expressing the magnetic field strength scale other than in cps, the delta ( $\delta$ ) and tau ( $\tau$ ) scales. The chemical shift parameter is independent of the magnetic field strength and oscillator frequency and is defined by equation (1).

$$\delta \equiv \frac{(\nu_i - \nu_r) \text{ cps} \times 10^6}{\text{Oscillator frequency (cps)}} \quad (1)$$

where  $\nu_i$  = resonance frequency of nucleus  $i$

$\nu_r$  = resonance frequency of the reference compound

The  $\delta$  scale, which is dimensionless, is expressed in parts per million (ppm) to give convenient numbers. The  $\tau$  chemical shift parameter is given by equation (2)

$$\tau = 10 - \delta \quad (2)$$

As it is now conventional to present spectra with the magnetic field strength increasing from left to right the  $\tau$  scale, whose numbers also increase from left to right and mostly gives positive values, is often preferred to the  $\nu$  and  $\delta$  scales whose values increase from right to left. The larger the numerical value of  $\tau$ , the greater the magnetic shielding of the nucleus to which it refers.

The NMR spectrum of a given organic compound thus reveals the number of different molecular (electronic) environments in which protons or groups of protons are located from the number of absorption bands. The intensity of the absorption of a signal is proportional to the number of nuclei causing that particular signal. The relative number of nuclei in each different environment can thus be obtained from the relative areas under the signals, but it must be noted that these areas reveal only the ratio of the number of nuclei causing each signal.

### (iii) FACTORS WHICH INFLUENCE THE CHEMICAL SHIFT

In general the chemical shift parameter  $\tau$  will be a function of the shielding effects for any given nucleus or group of nuclei as described under C(i). There are however other factors which can influence the chemical shift parameter.

#### (a) SOLVENT EFFECTS.

In solution the positions of the resonance signals of a particular molecule are affected by the surrounding molecules. The proton chemical shifts of aromatic compounds dissolved in non-aromatic solvents and vice versa have been found to be strongly concentration dependent. By extrapolating to infinite dilution the effect of the neighbouring molecules can be ascertained. In general two different effects can be distinguished.

- (1) Shifts due to a difference in the bulk diamagnetic susceptibility of the solute and the solvent.
- (2) Shifts arising from intermolecular interactions between solute molecules or between solute and solvent molecules. An example of this kind is that of hydrogen bonding which gives rise to pronounced proton chemical shifts. Protons attached to oxygen, nitrogen and sulphur as in alcohols, phenols, carboxylic acids, ~~enols~~ amines, amides and sulphhydryl compounds are commonly considered to be active hydrogens. The positions of the resonances of such protons depend on the extent of intermolecular hydrogen bonding, concentration, temperature and the nature of the solvent.

(b) CHEMICAL EXCHANGE

An example of chemical exchange is when a hydroxyl proton exchanges with similar protons so that in a certain period of time this proton is attached to a number of different molecules. For example in the absence of acidic or basic catalysts the NMR spectrum of a mixture of ethanol and water possesses absorption signals which are characteristic for the protons of water and the hydroxylic protons of ethanol. If a trace of acid or base is added to the mixture these two signals coalesce to a single sharp line as a result of the rapid proton exchange between water and ethanol which averages the shielding of the protons in each environment. At intermediate rates of exchange the mutual absorption signal may occur as a broad peak. The rate of chemical exchange and the position of the mutual absorption signal is also dependent on the concentration, temperature and the nature of the solvent. Protons attached to nitrogen and sulphur are also subject to chemical exchange and influenced by the same factors as described above for hydroxylic protons.

D. ELECTRON COUPLED SPIN-SPIN INTERACTIONS  
(SPIN-SPIN COUPLING)

As previously explained differently situated magnetic nuclei within a molecule give rise to resonance signals chemically shifted from one another as a result of differences in electronic shielding. The intensities of the absorption bands, as given by the areas which they enclose, is the ratio of the number of protons in each group. The low resolution spectrum of ethanol shows three absorption peaks in an area ratio of 1:2:3 arising from the hydroxyl, methylene and methyl protons respectively.

Under higher resolution however, the signals of ethanol attributed to the methylene and methyl protons appear as multiplets whose relative areas are still 2:3 respectively. The spacings of the three components of the methyl group triplet are exactly equal to the spacings of the four components of the methylene group quartet. The areas of the components of each multiplet approximate to simple integral ratios of 1:2:1 for the triplet and 1:3:3:1 for the quartet. The observation of these splitting patterns can be explained as follows.

The two methylene protons can assume four possible spin arrangements of which two arrangements are equivalent, thus giving a total of three different spin arrangements, figure 2(a)

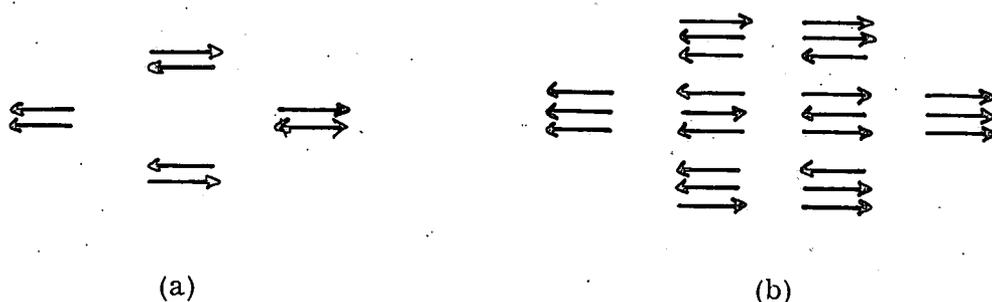


Fig. 2 Possible spin arrangements of (a) methylene protons and (b) methyl protons.

The magnetic effects of these three arrangements are transmitted to the methyl group protons primarily by means of the bonding electrons so that the methyl protons will in effect be subjected to three different effective magnetic fields. There will thus be three equally spaced transition energies (frequencies) for the methyl protons, and since two spin arrangements are equivalent it follows that the intensities of the three transitions will be 1:2:1 in the methyl triplet.

In the same manner we find that the protons of the methyl group have eight possible spin arrangements of which there are two sets of three equivalent arrangements (fig 2b), a total of four different arrangements, thus accounting for the methylene quartet having relative areas of 1:3:3:1.

Each pair of peaks of the multiplets is separated by exactly the same distance and this separation is known as the coupling constant  $J$ . The magnitude of  $J$  is independent of the strength of the applied magnetic field. Although the absorption position of a group (in cps) is changed with different field strengths, the value of  $J$  remains constant. Values of  $J$  are always expressed in cps. The

numerical value of  $J$  is dependent on the gyromagnetic ratio of the nuclei and on the structural relationship of the groups involved. The magnitude of  $J$  decreases sharply as the number of intervening bonds between the interacting nuclei increases. Spin-spin coupling usually occurs across one, two or three bonds although long-range coupling across four or even five bonds are known to occur in certain systems.

The number of absorption signals expected in each multiplet when two groups of protons interact can be predicted if the difference in chemical shifts ( $\Delta\nu$ ) between these groups is at least six times the value of  $J$  and if each proton in one group is coupled equally to each and every proton in the second group. When these conditions are met, the resulting patterns are said to be "first order" and the simple multiplicity rules, given below, apply.

- (1) The nuclei of an equivalent group (e.g. the three protons of the methyl group in ethanol) do not interact with each other to cause observable multiplicity.
- (2) The multiplicity of peaks in the band arising from a group of equivalent protons is determined by the neighbouring groups of equivalent protons and is given by the formula  $(2nI+1)$  where  $n$  is the number of equivalent protons ( $I = \frac{1}{2}$ ) in the neighbouring groups.
- (3) The relative intensities of a multiplet are symmetric about the midpoint of the band (chemical shift value) and are separated from each other by the coupling constant.

#### E. COMPLEX SPIN-SPIN INTERACTIONS

Interacting protons for which the difference in chemical shifts is of approximately the same magnitude as the coupling constant give complicated spectra, that are usually unrecognizable in terms of the simple splitting rules given under D. In many cases a mathematical analysis of the spectral data is required in order to obtain accurate values for the chemical shifts of the protons and the coupling constants. In the analysis of complex spectra interacting groups of nuclei are by convention labeled  $A_n B_m \dots$  (in order of increasing  $\tau$  value) if they have approximately the same chemical shift values and  $X_n Y_m \dots$  if the chemical shift values are very much different from  $A, B \dots$ , the subscripts refer to the number of nuclei in the group. Equivalent nuclei are assigned the same letter. Thus ethyl bromide is an  $A_2 B_3$  system, vinyl bromide an ABC system and 1,3,5-trifluorobenzene an  $A_3 X_3$  system.

## CHAPTER 7.

- I. ANION SHIFTS OF HYDROXYBENZOFURANS AND RELATED COMPOUNDS.
  - A. INTRODUCTION
  - B. ASSIGNMENTS OF THE PROTON RESONANCES
  - C. LONG-RANGE SPIN-SPIN COUPLING
  - D. ANION SHIFTS OF HYDROXYBENZOFURANS
  - E. POTENTIOMETRIC TITRATIONS OF HYDROXYBENZOFURANS
  - F. DISCUSSION
- II. ANION SHIFTS OF PHENOLIC DERIVATIVES OF NATURAL ISOFLAVANOLS
  - A. ASSIGNMENTS OF THE PROTON RESONANCES
  - B. ANION SHIFTS OF COMPOUNDS (LV), (LVI) AND (X)
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# I. ANION SHIFTS OF HYDROXYBENZOFURANS AND RELATED COMPOUNDS

## A. INTRODUCTION

It has been proved that the  $\pi$ -electron density (charge density) on the carbon atoms of aromatic rings has an important effect on the chemical shifts of the protons which are attached to these carbon atoms.<sup>107-111</sup> Fraenkel et al.<sup>112</sup> presented evidence that the changes in chemical shifts of protons are approximately proportional to the excess  $\pi$ -electron density ( $\Delta q$ ) on the carbon atoms of conjugated molecules to which the protons are attached. This relationship is given by the expression

$$\Delta\tau = k(\Delta q) \quad (1)$$

where  $\Delta\tau$  is the difference in chemical shift from that of benzene,  $\Delta q$  the excess in charge density from unity (unity being the charge density on benzene carbon atoms) and  $k$  is a constant estimated at 10.7 ppm/electron. As a general statement it can thus be said that an increase in the electron density on a carbon atom will increase the shielding of a proton attached to it resulting in a high field chemical shift of the proton.

Schaefer and Schneider<sup>113</sup> found that the abovementioned relationship was applicable to various aromatic compounds by correlating the calculated charge densities with the observed chemical shifts. This relationship was however not always applicable to heterocyclic and polycyclic aromatic compounds. In applying equation (1) to the evaluation of charge densities, discrepancies are often encountered which are attributed to significant additional effects.<sup>113, 114</sup> These additional contributions are due to:

- (a) The magnetic anisotropy of substituents or of hetero atoms in the aromatic ring.
- (b) Ring-current effects of neighbouring rings in polycyclic aromatic compounds.
- (c) Inductive effects.
- (d) Ion association effects of aromatic rings.
- (e) Solvent effects.

Large contributions arising from any one or combination of these effects seriously limit the reliability of the charge density evaluations.

Studies on substituted benzenes<sup>107, 108, 114, 115</sup> indicated differences in the electron charge distributions at the ortho, meta and para positions which appeared to be closely associated with the substituents. The differences in the electron densities at these positions are ascribed to inductive and mesomeric effects caused by the substituents.<sup>108</sup> The mesomeric effects caused by electron-donating substituents produce high field shifts for protons in the ortho and para positions whereas the mesomeric contributions of electron-withdrawing substituents cause low field shifts for protons in these positions. Similar but smaller changes in shifts are obtained for protons in the meta position.

Ballantine and Pillinger<sup>116</sup> investigated a large number of substituted phenolic compounds in order to ascertain the shielding values of different substituents. They analysed the chemical shift values of the aromatic protons of the compounds in terms of additive substituent shielding values. They found that the substituent shielding constants so obtained for the different substituents can be used with considerable success in predicting the chemical shift values of aromatic protons. They also found that these shielding constants cannot be used with equal success for certain structural types such as flavones, iso-flavones and heterocyclic compounds containing a benzofuran system and they ascribed this fact to the influence of long-range magnetic anisotropic effects.

In recently published papers by Brown<sup>117</sup> and by Highet and Highet<sup>118</sup> the anion shift technique was described in which they showed that the chemical shifts of the aromatic protons of phenols experience a high field shift when the phenols dissociate to form the corresponding phenoxide ions. This high field shift (anion shift) of the aromatic protons was found to be characteristic of the positions of the protons relative to the phenolic hydroxyl group. As it has generally been found that the anion shifts of protons are affected the most in the ortho and para positions, it has been suggested<sup>117, 118</sup> that these shifts are due to the increased electron charge densities at these positions which are contributed by the mesomeric structures of the phenolic compound. It was also found<sup>118</sup> that the substitution pattern of the compound concerned considerably influenced the values of the anion shifts.

Dimethyl sulphoxide was found to be the most suitable solvent for determining anion shifts as considerably higher shift values are obtained in this solvent compared to methanol or deuterated chloroform. The ranges for the maximum

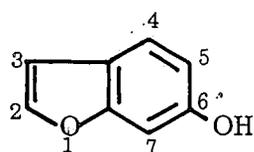
anion shifts for alkyl and alkoxy substituted phenols in dimethyl sulphoxide solution are given <sup>118</sup> as ortho: 0.42 - 0.59 ppm; meta : 0.19 - 0.38 ppm; para: 0.71 - 0.79 ppm. Phenols which have a carbonyl group or vinylogous carbonyl system para to the phenolic hydroxyl group showed anion shifts in the ranges ortho : 0.60 - 0.84 ppm and meta: 0.22 - 0.47 ppm.

By using the anion shift technique Highet and Highet <sup>118</sup> were able to determine the exact position of the phenolic hydroxyl group in the alkaloid amaryllisine. Pachler et al. <sup>119</sup> showed that the previously proposed structure of the phenolic aporphine alkaloid, rogersine, was incorrect. As the NMR spectra of certain closely related phenolic aporphine alkaloids are virtually identical, the small differences did not permit the assignment of the position of the phenolic hydroxyl group in rogersine. The anion shift data obtained from closely related compounds enabled them to locate the exact position of the hydroxyl group, proving rogersine to be N-methyl-laurotetanine. Subsequent anion shift studies by Baarschers and Pachler <sup>120</sup> in the aporphine alkaloid field showed the value of this method in the structural elucidations of these compounds.

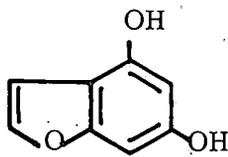
This technique may prove to be a very valuable aid in the structural elucidations of all types of phenolic compounds, especially if anion shift data of closely related compounds are available. This study of the anion shifts of hydroxybenzofurans and related compounds was undertaken mainly in order to ascertain whether this method can be used to facilitate the structural elucidations of natural isoflavanoids and related compounds.

## B. ASSIGNMENTS OF THE PROTON RESONANCES

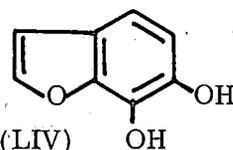
The structures of the hydroxybenzofurans and related compounds which were investigated are given below. (See Chapter 5).



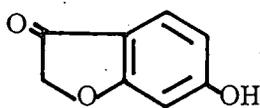
(LH)



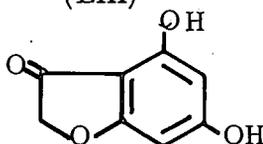
(LIII)



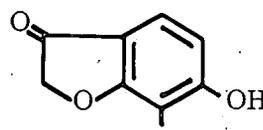
(LIV)



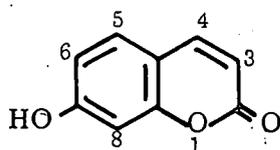
(XXXIV)



(XLI)



(XLVI)



(LVII)

The NMR data obtained from dimethyl sulphoxide solutions for these compounds are summarized in Table 8.

TABLE 8.

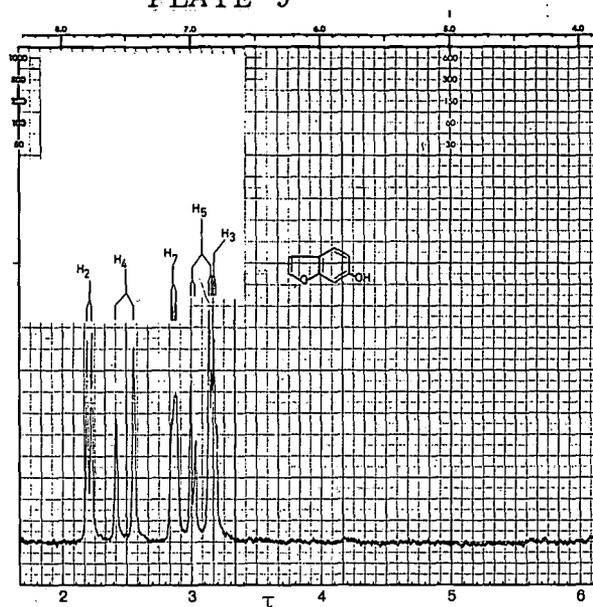
$\tau$  - VALUES FOR (CH<sub>3</sub>)<sub>2</sub> SO SOLUTIONS

Compound No.	$\tau$ - Values of groups at positions					Coupling constants (cps)
	2	3	4	5	7	
(LII)	2.20	3.18	2.50	3.08	2.86	$J_{23} = 2.0$ ; $J_{37} = 1.0$ $J_{45} = 8.4$ ; $J_{57} = 2.1$
(LIII)	2.37	3.11	-	3.69	3.47	$J_{23} = 2.2$ ; $J_{37} = 0.9$ $J_{57} = 2.0$
(LIV)	2.19	3.20	3.11	3.04	-	$J_{23} = 2.0$ ; $J_{45} = 8.2$
(XXXIV)	5.28	-	2.49	3.33	3.42	$J_{45} = 8.2$ ; $J_{47} = 1.0$ ; $J_{57} = 2.0$
(XLI)	5.46	-	-	4.10	4.10	
(XLVI)	5.23	-	2.94	3.30	-	$J_{45} = 8.1$
	3	4	5	6	8	
(LVII)	3.74	2.03	2.42	3.11	3.20	$J_{34} = 9.5$ ; $J_{56} = 8.0$ $S_{58} = 1.2$ ; $S_{68} = 1.9$

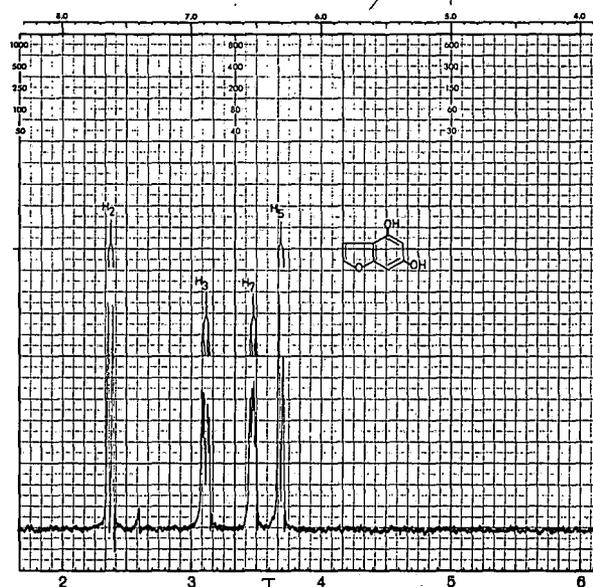
(i) 6-HYDROXYBENZOFURAN (LII)

In the NMR spectrum of this compound (plate 9) the furan ring protons ( $\tau = 2.20$  and  $\tau = 3.18$ ) are identified by their spin-spin coupling ( $J_{23} = 2.0$  cps). The signal at lower field is assigned to the proton in position 2, next to the electronegative oxygen atom.<sup>121a, 122</sup> The three aromatic protons between  $\tau = 2.50$  and  $\tau = 3.08$  are easily assigned from their splitting patterns. The protons in the 4 and 5 positions ( $\tau = 2.50$  and  $\tau = 3.08$  respectively) are ortho coupled ( $J_{45} = 8.4$  cps) and appear basically as an AB system where one of them (5, at higher field) exhibits a further small meta splitting

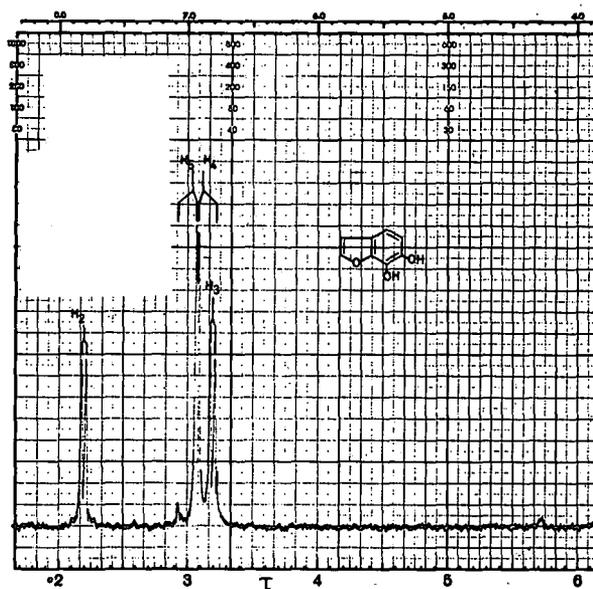
## PLATE 9



(a) 6 - HYDROXYBENZOFURAN (LII)



(b) 4, 6 - DIHYDROXYBENZOFURAN (LIII)



(c) 6, 7 - DIHYDROXYBENZOFURAN (LIV)

( $J_{57} = 2.1$  cps), the para coupling ( $J_{47}$ ) not being resolved. The  $\tau$ -values for the protons in positions 4 and 5 are determined from an AB-analysis after removing the small meta coupling. The relatively high  $\tau$ -values for the protons in positions 5 and 7 are as expected as it is known that hydroxyl substituents  $107, 110, 111, 114, 116, 123$  shift the resonances of ortho and para aromatic protons to higher field by approximately 0.5 ppm. The hydroxyl proton signal is at  $\tau = 0.4$ . The resonances of the protons in positions 3 ( $\tau = 3.18$ ) and 7 ( $\tau = 2.86$ ) show long-range coupling ( $J_{37} = 1.0$  cps), see under C).

(ii) 4,6 - DIHYDROXYBENZOFURAN (LIII)

The resonances at  $\tau = 2.37$  and  $\tau = 3.11$  (plate 9) are assigned to the furan ring protons in positions 2 and 3 respectively ( $J_{23} = 2.2$  cps) as described under 6-hydroxybenzofuran. The aromatic protons in positions 5 and 7 are meta coupled ( $J_{57} = 2.0$  cps) and as expected their resonances appear at a relatively high field ( $\tau = 3.69$  and  $\tau = 3.47$ ) as they are subject to the additive substituent effects of the two hydroxyl groups (see under (i)). The signal at lower field exhibits long-range coupling ( $J_{37} = 0.9$  cps) and is assigned to the proton in position 7 (see under C). The hydroxyl protons appear as two broadened peaks at  $\tau = 0.19$  and  $\tau = 0.65$ , (not shown in the spectrum).

(iii) 6,7 - DIHYDROXYBENZOFURAN (LIV)

The aromatic protons of this compound in positions 4 and 5 (plate 9) constitute an AB system with  $\tau$ -values at  $\tau = 3.04$  and  $\tau = 3.11$  ( $J_{45} = 8.2$  cps). The A doublet (at lower field) is assigned to the proton in position 5 from anion shift data (see under D(iii)). The doublets at  $\tau = 2.19$  and  $\tau = 3.20$  ( $J_{23} = 2.0$  cps) are assigned to the furan protons in positions 2 and 3 respectively as described under (i). The two hydroxyl protons appear as a single line at  $\tau = 0.94$ .

(iv) HYDROXY - 2,3 - DIHYDROBENZOFURAN - 3 - ONES (XXXIV), (XLI) AND (XLVI)

The aromatic protons of these compounds are assigned in a similar manner from their chemical shifts and coupling constants as described above for the hydroxybenzofurans. The degenerate spectrum of 4,6 - dihydroxy - 2,3 - dihydrobenzofuran - 3 - one (XLI) is deceptively simple and the aromatic protons (in positions 5 and 7) accidentally have the same  $\tau$ -values ( $\tau = 4.10$ ). The two-proton methylene signals have  $\tau$ -values of 5.28, 5.46 and 5.27 for

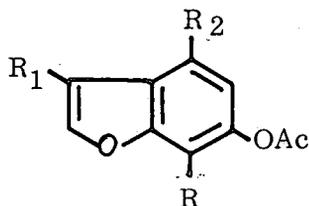
compounds (XXXIV), (XLI) and (XLVI) respectively. The NMR data for these compounds are summarized in Table 8.

(v) 7 - HYDROXYCOUMARIN (LVII)<sup>124</sup>

In the NMR spectrum of this compound the  $\alpha$  - pyrone ring protons in positions 3 and 4 constitute an AB system with  $\tau$  - values at  $\tau = 2.03$  and  $\tau = 3.74$  of which the one at higher field is assigned to the proton in position 3.<sup>121b, 125</sup> The three aromatic protons in positions 5, 6 and 8 ( $\tau = 2.42$ ,  $\tau = 3.11$  and  $\tau = 3.20$  respectively) constitute a three-spin system and are easily assigned from their splitting patterns. The protons in the 5 and 6 positions are ortho coupled ( $J_{56} = 8.0$  cps) and appear basically as an AB system of which the doublet at higher field, (6, ortho to the hydroxyl group, see under (i) ), exhibits a further small meta splitting ( $S_{68} = 1.9$  cps) and the one at lower field shows a small para splitting ( $S_{58} = 1.2$  cps). The  $\tau$  - values for the protons in positions 5 and 6 are determined from an AB - analysis after removing the small meta and para spin-spin splittings.

(vi) ACETOXY DERIVATIVES

The NMR data of the acetoxy derivatives of compounds (LII), (LIII), (LIV), (XXXIV), (XLI) and (XLVI) are summarized in Table 9. The spectra of compounds (XLIX) - (LI) are presented in plate 10. The assignments of the protons of the acetoxy derivatives are made in a similar manner as for the hydroxybenzofurans from (a) long-range coupling, (b) substituent effects and (c) splitting patterns and are summarized as follows. Compound (XLIX) (a) and (c); - (L) (a) and (c); - (LI) (b) and (c); - (XXXV) (c); (XLII) (b); - (XLVII) (b). The structures of these compounds are given below.



(XLIX) :  $R = R_1 = R_2 = H$

(L) :  $R = R_1 = H; R_2 = OAc$

(LI) :  $R_1 = R_2 = H; R = OAc$

(XXXV) :  $R = R_2 = H; R_1 = OAc$

(XLII) :  $R = H; R_1 = R_2 = OAc$

(XLVII) :  $R_2 = H; R = R_1 = OAc$

TABLE 9

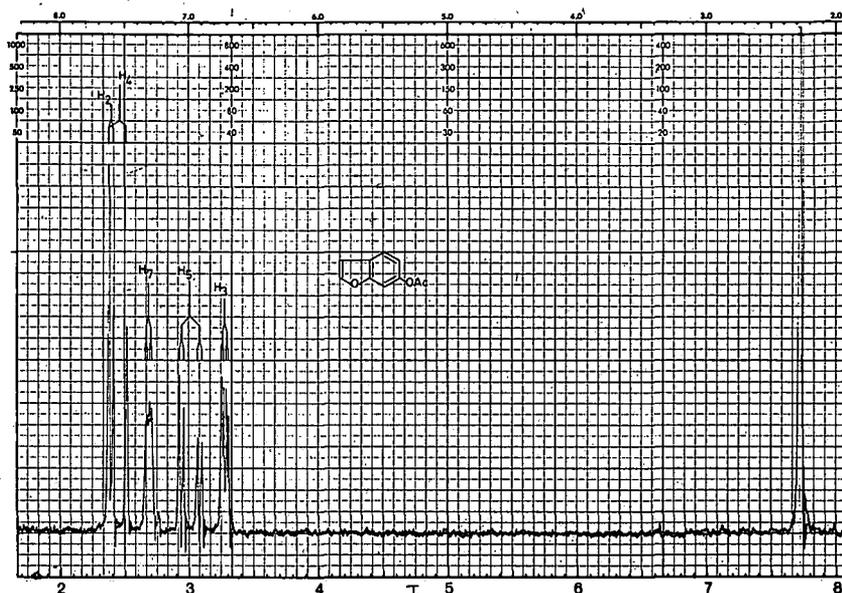
 $\tau$  - VALUES FOR CDCl<sub>3</sub> SOLUTIONS

Compound No.	$\tau$ - Values of groups at positions						Coupling constants (cps)
	2	3	4	5	6	7	
(XLIX)	2.39	3.28	2.46	3.01	7.72	2.68	J <sub>23</sub> = 2.0 ; J <sub>37</sub> = 1.0 J <sub>45</sub> = 8.5 ; J <sub>57</sub> = 2.0
(L)	2.45	3.38	7.80	3.10	7.75	2.76	J <sub>23</sub> = 2.2 ; J <sub>37</sub> = 1.0 J <sub>57</sub> = 1.8
(LI)	2.40	3.26	2.58	2.94	7.71	7.63	J <sub>23</sub> = 2.0 ; J <sub>45</sub> = 8.5
(XXXV)	1.97	7.74	2.49	2.99	7.71	2.75	J <sub>45</sub> = 8.3 ; J <sub>57</sub> = 1.9
(XLII)	2.0	7.76	7.76	3.16	7.71	2.82	J <sub>57</sub> = 1.8
(XLVII)	1.94	7.72	2.61	2.92	7.72	7.66	J <sub>45</sub> = 8.5

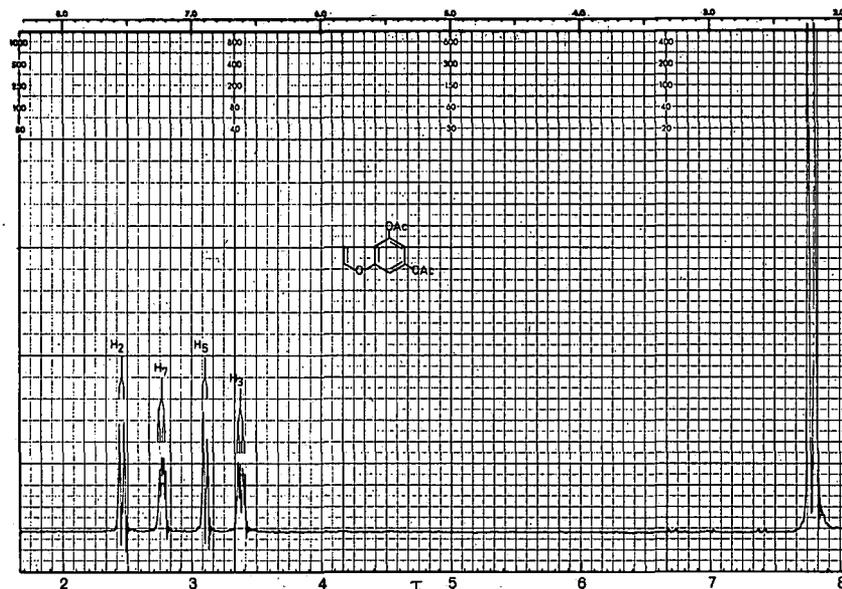
C. LONG-RANGE SPIN-SPIN COUPLING

The signals of the protons in positions 3 and 7 of compounds (LII), (LIII), (XLIX) and (L) (plates 9 and 10) exhibit additional splitting whereas this splitting pattern is not observed in compounds (LI), (LIV), (plates 9 and 10) and in the spectra of the hydroxy -2,3-dihydrobenzofuran -3-ones and their acetoxy derivatives where only one of the protons in positions 3 and 7 is present. This additional splitting can thus only be due to long-range coupling between the protons in positions 3 and 7 (J<sub>37</sub> = 0.9 - 1.0 cps). Similar long-range couplings were also observed in indene and benzofuran<sup>126</sup>, quinolines<sup>127</sup>, methylnitronaphthalenes<sup>128</sup>, indole and carbazole<sup>129</sup> which is in complete agreement with our findings. In all these compounds the long-range coupling path is over bonds which have a planar zig-zag arrangement.<sup>130, 131</sup>

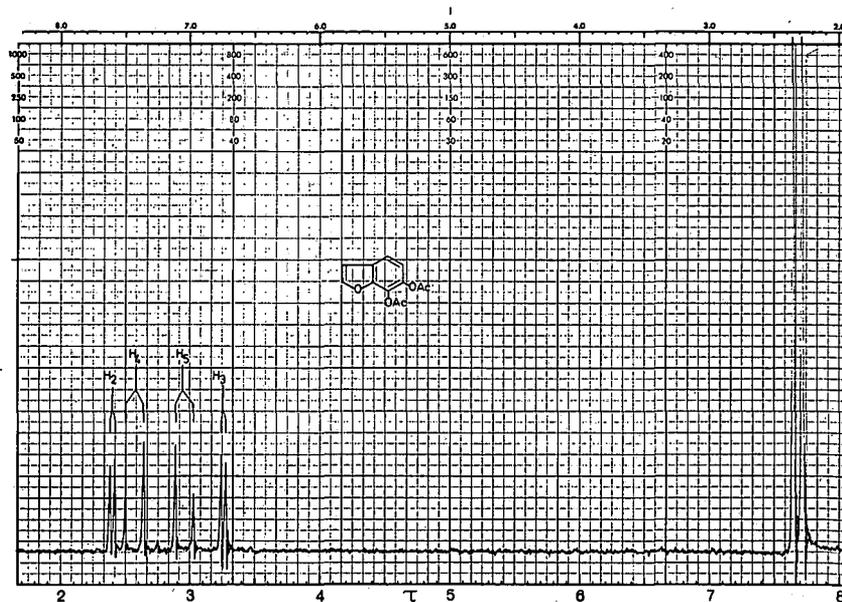
The observation of long-range coupling between the protons in positions 3 and 7 considerably facilitated the assignments of the protons in the relevant compounds mentioned above, and should also prove to be very useful in detecting substitution in position 8 (which is equivalent to position 7 in benzofurans) in furoisoflavanoids or in compounds possessing a benzofuran system.



(a) 6 - ACETOXYBENZOFURAN (XLIX)



(b) 4, 6 - DIACETOXYBENZOFURAN (L)



(c) 6, 7 - DIACETOXYBENZOFURAN (LI)

#### D. ANION SHIFTS OF HYDROXYBENZOFURANS

It was found by Pachler et al.<sup>119</sup> that a good method of studying anion shifts is by successively adding small amounts of concentrated alkali (NaOD) solution to the compound dissolved in dimethyl sulphoxide and recording the spectrum after each addition of alkali. By plotting the chemical shifts so obtained versus the relative amount of alkali added, expressed as  $\tau$  - values versus mole alkali per mole compound, considerably facilitates the interpretation of the data obtained. This method can be most valuable in the assignments of the proton resonances and can also yield much additional information as regards the dissociation sequence of the phenolic hydroxyl groups of compounds possessing two or more such groups. These graphs are given in figures 1-7 (plates 11 - 14).

The maximum anion shift data obtained for the compounds mentioned under B(i) - (v) are given in Table 10.

TABLE 10.

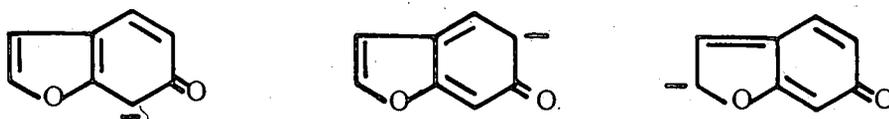
#### MAXIMUM VALUES OF ANION SHIFTS

Compound No.	Maximum shift (ppm) for protons at positions						
	2	3	4	5	6	7	8
(LII)	0.38n	0.21n	0.35m	0.37o	-	0.53o	-
(LIII)	0.49n	0.22n	-	0.69o,o	-	0.75o,p	-
(LIV)	0.45n	0.33n	0.40m,p	0.68o,m	-	-	-
(XXXIV)	-	-	0.27m	0.48o	-	0.67o	-
(XLI)	-	-	-	0.80o,o	-	0.80o,o	-
(XLVI)	-	-	0.41m,p	0.42o,m	-	-	-
(LVII)	-	0.51n	0.25n	0.39m	0.45o	-	0.55o

The designations o, m, p indicate that there is a hydroxyl group respectively in the ortho, meta and para position relative to the proton concerned while n indicates a hydroxyl group (or groups) on the neighbouring aromatic ring.

(i) 6 - HYDROXYBENZOFURAN (LII)

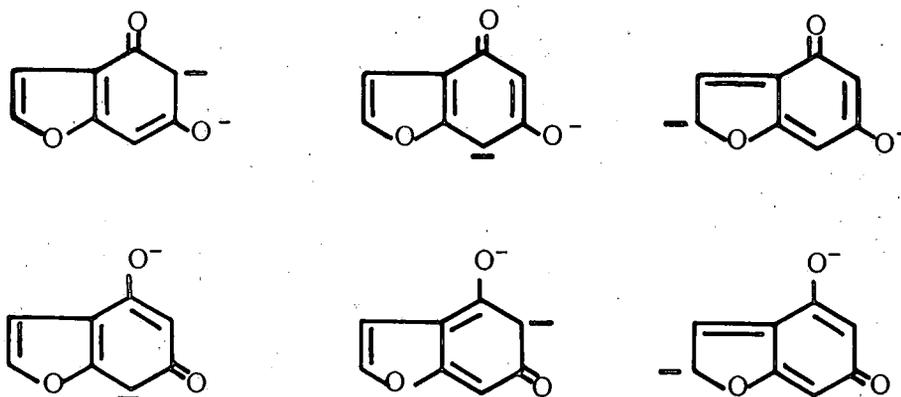
The mesomeric structures of the phenoxide ion of 6-hydroxybenzofuran which affect the chemical shifts of the protons in positions 7, 5 and 2 are given below



In figure 1 (plate 11) it can be seen that the proton in position 7 shows the largest high field shift (0.53 ppm) whereas the protons in positions 2, 4 and 5 have approximately the same maximum anion shift values, 0.38, 0.35 and 0.37 ppm respectively.

(ii) 4, 6 - DIHYDROXYBENZOFURAN (LIII)

The mesomeric structures which influence the chemical shifts of the protons in positions 7, 5 and 2 are shown below.



The large high field shifts of the protons in positions 5 (0.69 ppm) and 7 (0.75 ppm) (figure 2 plate 11) are as expected as the dissociation of both the hydroxyl groups affect these protons (see mesomeric structures above). The dissociation sequence of the hydroxyl groups cannot be determined from the plots of the anion shift values.

## PLATE 11

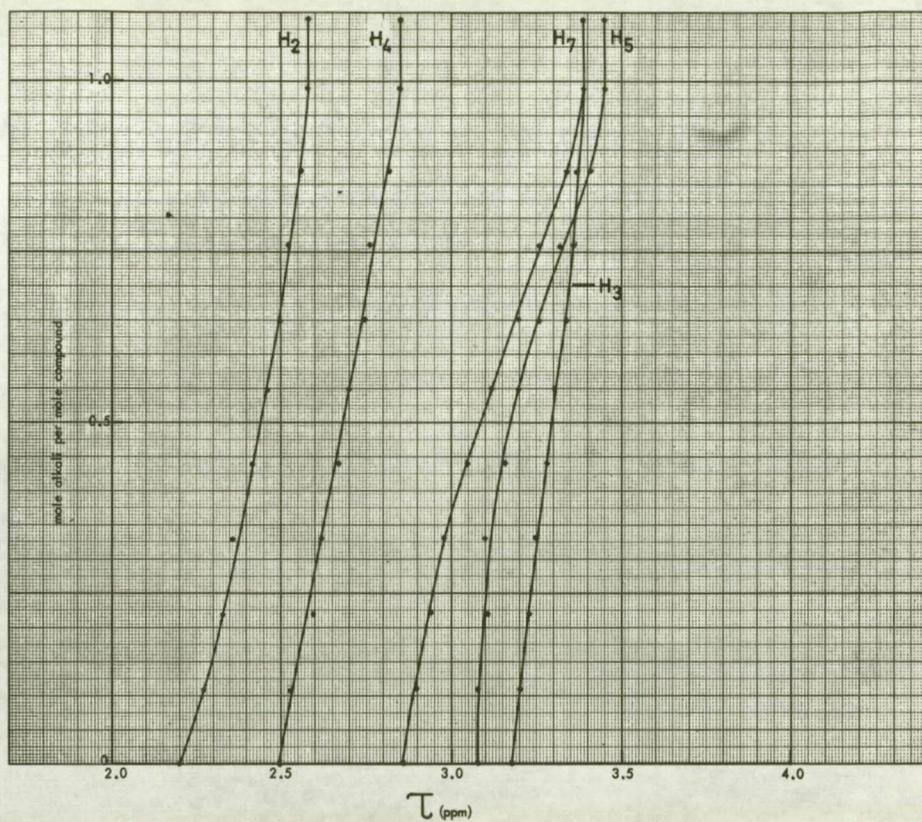


FIG. 1. 6-HYDROXYBENZOFURAN (LII)

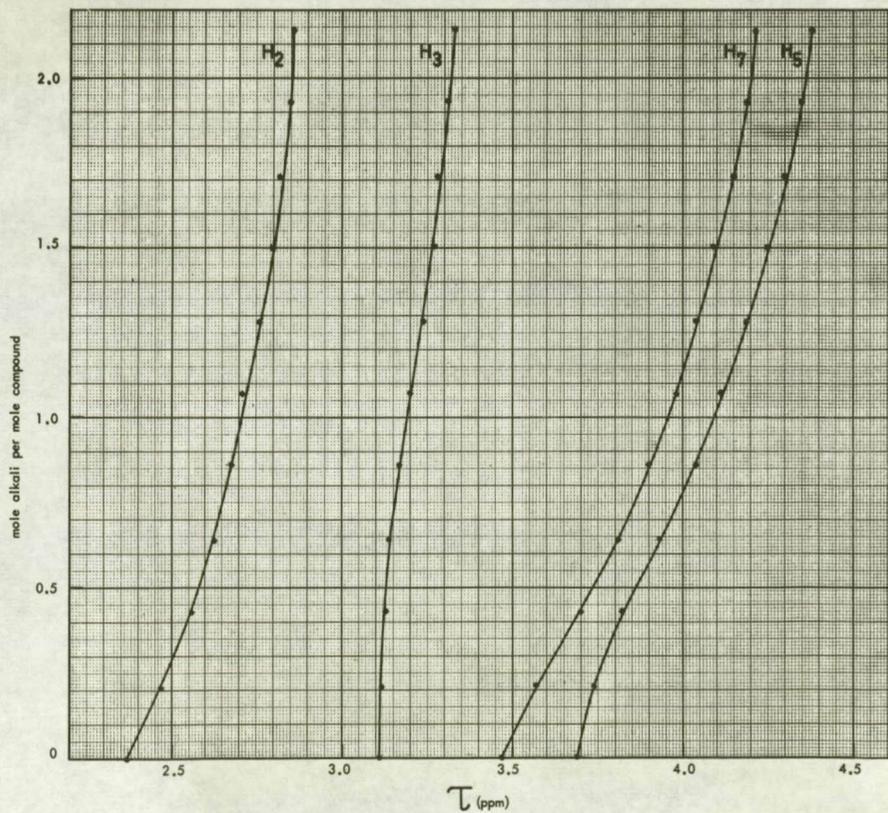
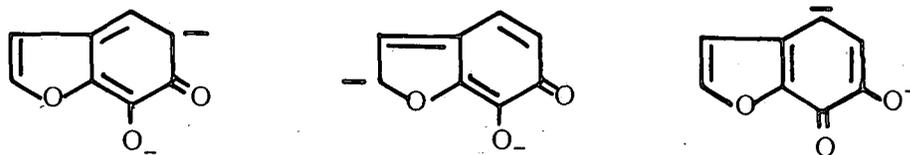


FIG. 2. 4,6-DIHYDROXYBENZOFURAN (LIII)

It was found that the aromatic hydrogen atoms in positions 5 and 7 are readily exchanged by deuterium atoms if an NaOD solution is used, and consequently an NaOH solution was used in the determination of the anion shift values for this compound. This deuterium - hydrogen exchange was not observed in any of the 6 - and 6,7 -hydroxy compounds investigated in this work, which is in complete agreement with the work done by Hand and Horowitz<sup>132</sup> and Massicot et al.<sup>133, 134</sup> on the hydrogen-deuterium exchange in resorcinols and related compounds. They<sup>132</sup> found that the combined activating effects of two or more meta orientated hydroxyl groups sufficed for ready exchange of protons ortho and para to them, while protons of aromatic rings containing only one hydroxyl group or two adjacent hydroxyl groups did not exchange.

(iii) 6,7-DIHYDROXYBENZOFURAN (LIV)

The relevant phenoxide mesomeric structures for the di-anion of this compound are given below.



From figure 3 (plate 12) it can be seen that after the addition of approximately 1 mole equivalent alkali one of the two aromatic protons (5) shows a large high field shift to very nearly its maximum shift value (0.68 ppm). On further addition of alkali there is a very small increase in the shift value for this proton whereas the other protons (in positions 2, 3 and 4) continue to shift to high field. As both the aromatic protons (in positions 4 and 5) are theoretically subject to mesomeric effects, as shown in the structures above, it is reasonable to expect that their maximum anion shift values should be approximately the same. One of the aromatic protons (4) however has a comparatively low shift value (0.40 ppm) and the shape of its curve (compared to that of proton 5) indicates that it is not subject to mesomeric effects. This indicates that only one of the hydroxyl groups dissociates. In order to test this proposal potentiometric titrations were carried out on several phenolic compounds which established that only one of the hydroxyl groups of 6,7 - dihydroxybenzofuran does dissociate, (see under E).

## PLATE 12

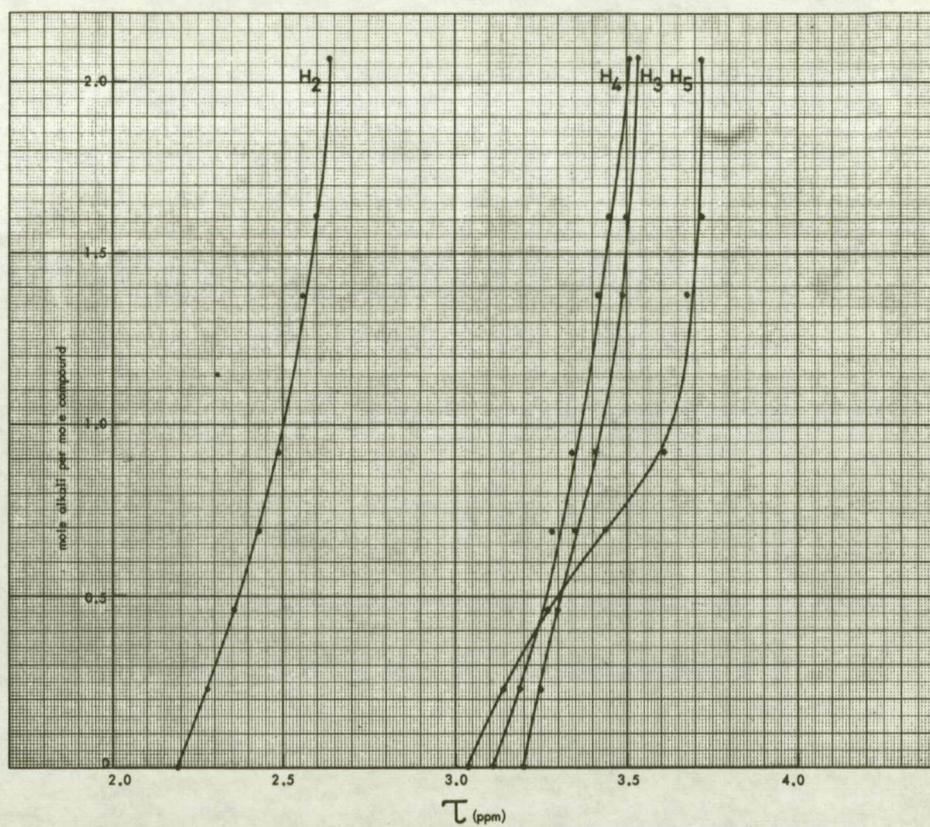


FIG.3. 6,7-DIHYDROXYBENZOFURAN (LIV)

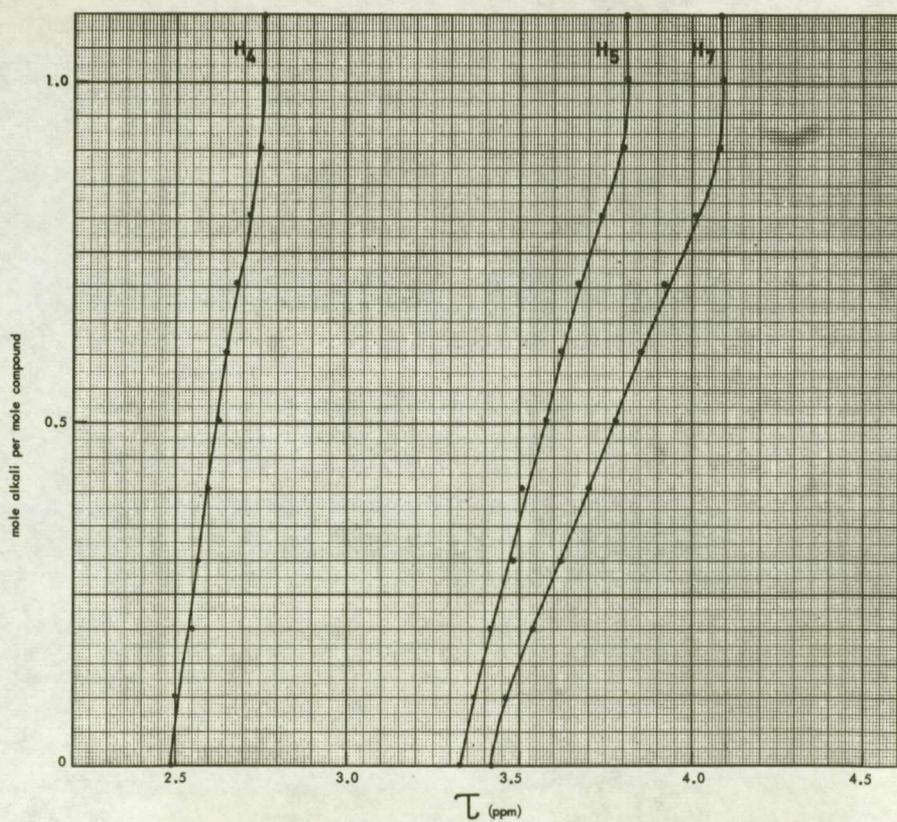


FIG.4. 6-HYDROXY-2,3-DIHYDROBENZOFURAN-3-ONE (XXXIV)

HYDROXY - 2,3 - DIHYDROBENZOFURAN - 3 - ONES

The relevant mesomeric structures for these compounds, (XXXIV), (XLI) and (XLVI) can be written in a similar manner as shown for the hydroxybenzofurans. The maximum anion shift values of the methylene protons for these compounds could not be determined as the water resonance obscured the final shift values of the methylene group. The approximate maximum shift values obtained for the methylene groups of compounds (XLI) and (XLVI) are 0.24 ppm and 0.32 ppm respectively.

(iv) 6 - HYDROXY - 2,3 - DIHYDROBENZOFURAN - 3 - ONE (XXXIV)

The aromatic protons of this compound show a constant rate of increase in their shift values, figure 4 (plate 12). As expected the protons in positions 5 and 7 have the highest anion shift values (0.48 ppm and 0.67 ppm respectively) as they are ortho to the hydroxyl group.

(v) 4,6 - DIHYDROXY - 2,3 - DIHYDROBENZOFURAN - 3 - ONE (XLI)

The large maximum anion shift values obtained for the aromatic protons in positions 5 and 7 (0.80 ppm) of this compound, figure 5 (plate 13), is ascribed to the combined effects of the hydroxyl groups. The hydrogen atoms in positions 5 and 7 are also exchanged by deuterium atoms, but not to such a marked extent as was found for 4,6 - dihydroxybenzofuran.

(vi) 6,7 - DIHYDROXY - 2,3 - DIHYDROBENZOFURAN - 3 - ONE (XLVI)

The dissociation of the hydroxyl groups in positions 6 and 7 of this compound can readily be seen from the anion shift values of the aromatic protons in positions 4 and 5, figure 6 (plate 13). The hydroxyl group in position 6 dissociates first as the resonance of the proton in position 5 shifts to very nearly its maximum anion shift value (ortho anion effect) whereas the proton in position 4 has a very small shift value after the addition of approximately 1 mole equivalent alkali. On further addition of alkali the dissociation of the second hydroxyl group (in position 7) then begins as the proton in position 4 shows a sharp increase in its shift values (para anion effect) while the proton in position 5 hardly shows any further increase in its shift value. The maximum shift values for protons 4 and 5 are 0.41 ppm and 0.42 ppm respectively.

## PLATE 13.

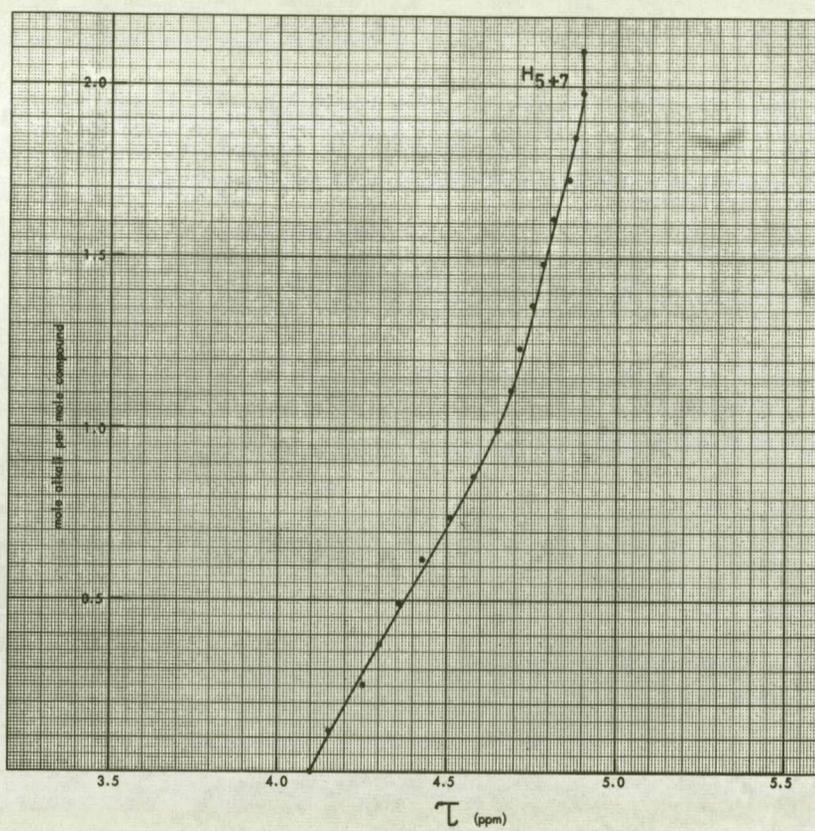


FIG. 5. 4,6-DIHYDROXY-2,3-DIHYDROBENZOFURAN-3-ONE (XLI)

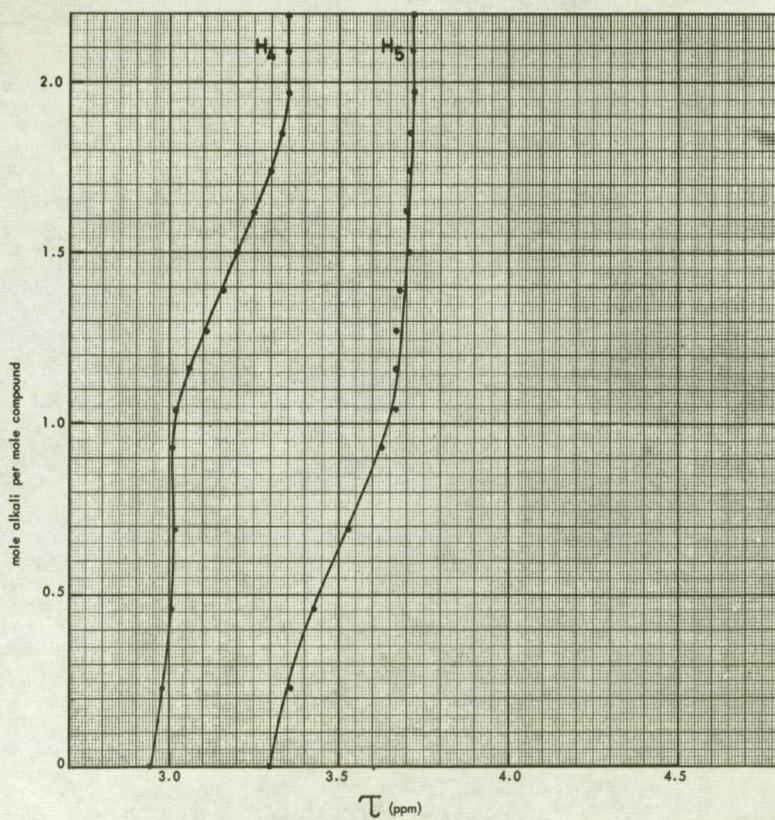
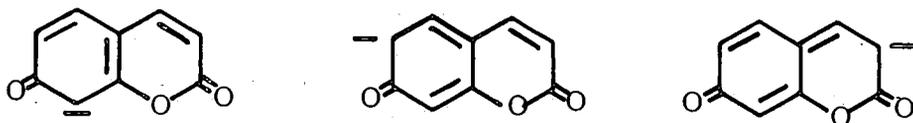


FIG. 6. 6,7-DIHYDROXY-2,3-DIHYDROBENZOFURAN-3-ONE (XLVI)

(vii) 7 - HYDROXYCOUMARIN (LVII)

The protons in positions 3, 6 and 8 in this compound have the highest anion shift values, 0.51 ppm, 0.45 ppm and 0.55 ppm respectively. This is in accordance with the mesomeric contributions as shown in the structures below.



The anion shift value for the proton in position 5 (0.39 ppm) is in agreement with the values reported by Highet and Highet<sup>118</sup> for meta shifts in compounds which have a vinylogous acyl group para to the hydroxyl group. The anion shift values of the protons of this compound is graphically presented in figure 7 (plate 14).

E. POTENTIOMETRIC TITRATIONS OF HYDROXYBENZOFURANS

Potentiometric titrations were carried out on several phenolic compounds including 4,6-dihydroxy-2,3-dihydrobenzofuran-3-one (XLI), 4,6-dihydroxybenzofuran (LIII), 6,7-dihydroxy-2,3-dihydrobenzofuran-3-one (XLVI) and 6,7-dihydroxybenzofuran (LIV). These compounds, dissolved in dimethyl sulphoxide, were titrated with a NaOH solution and the results were plotted as mV versus mole alkali per mole compound. The graphs are given in figures 8 and 9 (plate 15). From blank titrations it was established that any decrease in the slope of a curve is due to a dilution effect.

From figure 8 it can clearly be seen that both the hydroxyl groups of compounds (XLI) and (LIII) dissociate. The curve of 6,7-dihydroxy-2,3-dihydrobenzofuran-3-one (XLVI) (figure 9, plate 15) shows that after the dissociation of the first hydroxyl group (sharp increase in the slope of the curve after the addition of 1 mole equivalent alkali), the second hydroxyl group slowly dissociates (gradual increase in the slope of the curve on further addition of alkali). The fact that both the hydroxyl groups of this compound dissociate is in full agreement with the anion shift data obtained for this compound, (see under D(vi).) In the case of 6,7-dihydroxybenzofuran (LIV), (figure 9, plate 15) the

## PLATE 14

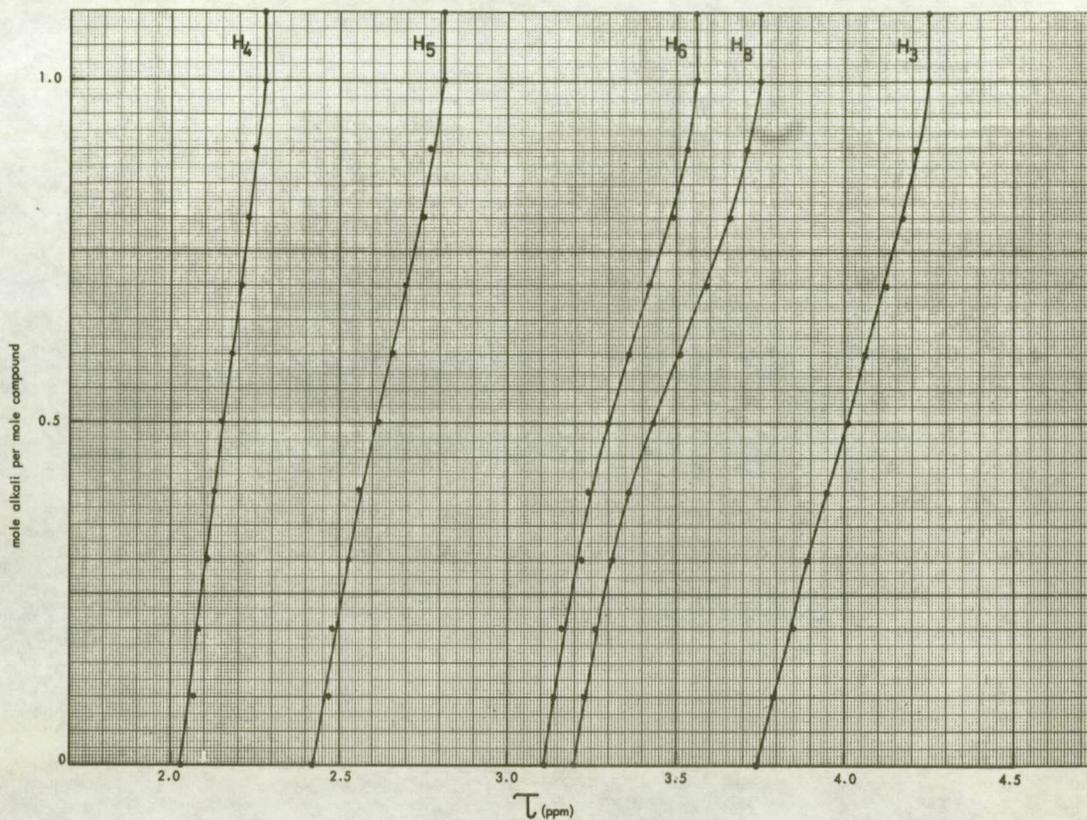


FIG. 7. 7-HYDROXYCOUMARIN (LVII)

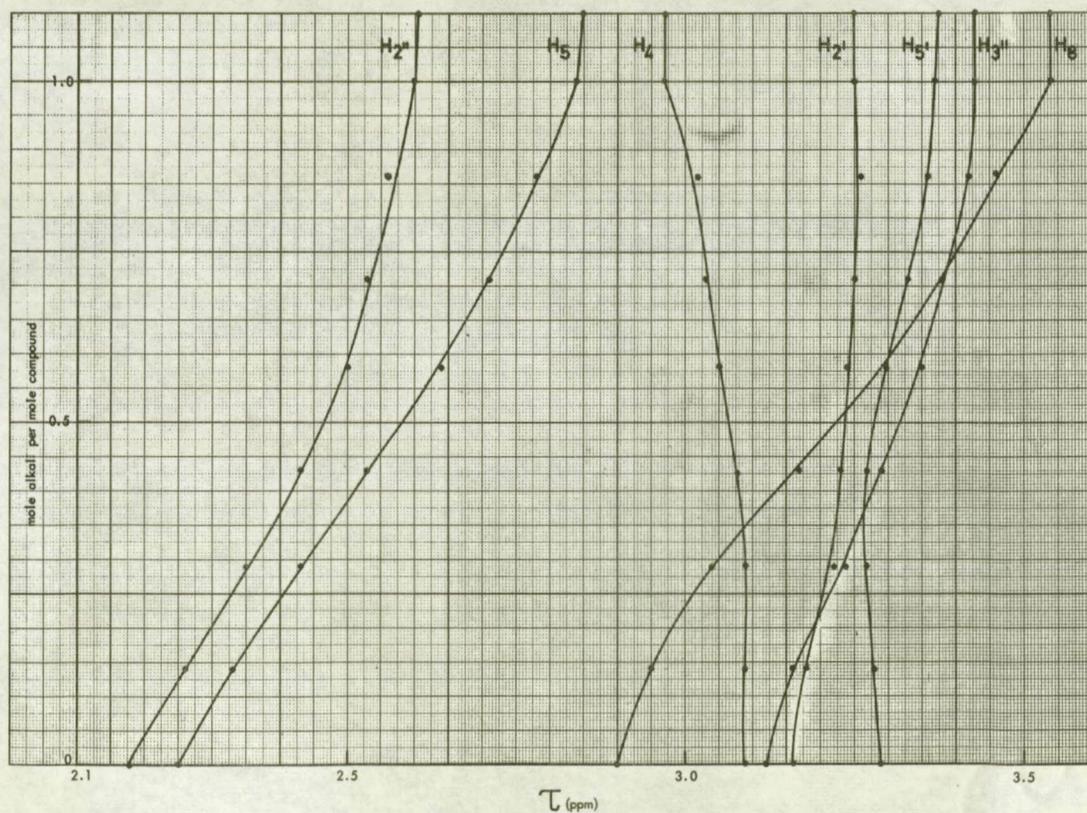
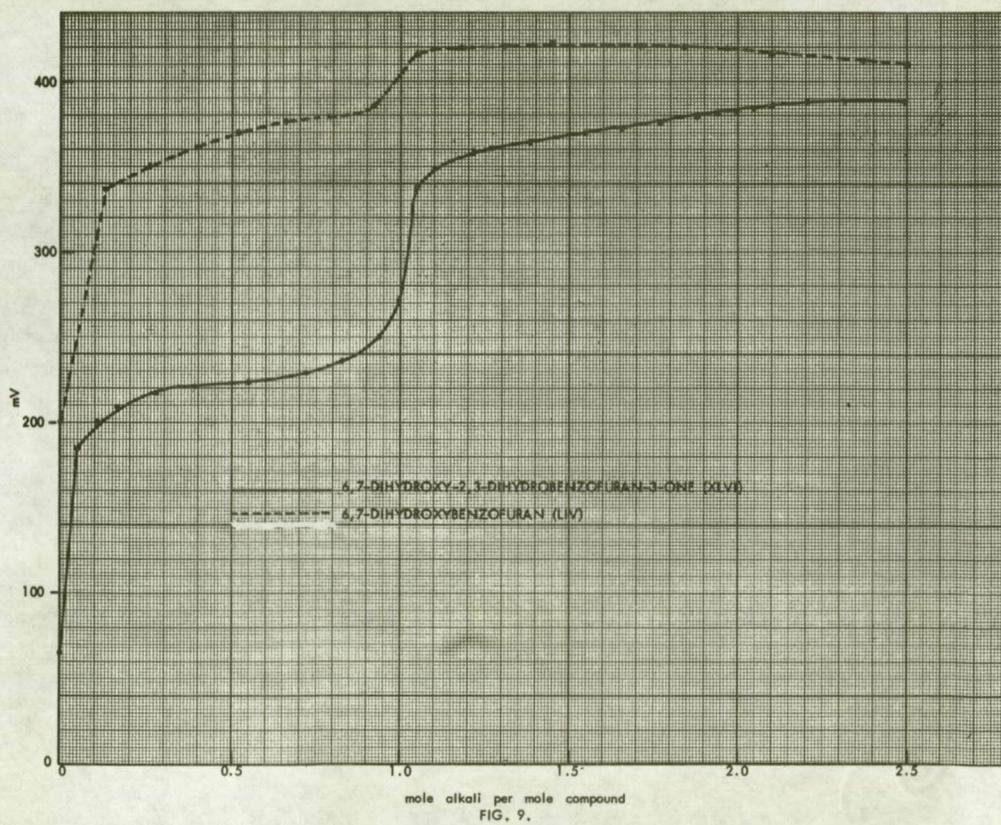
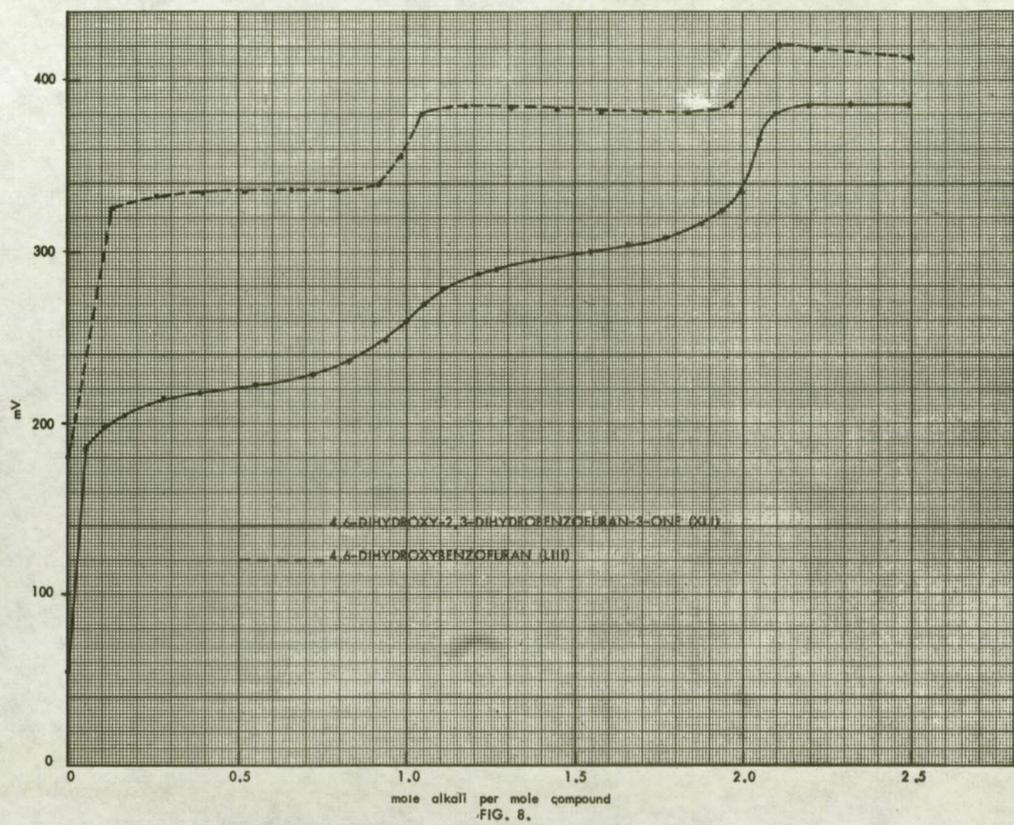


FIG. 10. PACHYRRHIZINOL (LV)

## PLATE 15



curve clearly shows that only one hydroxyl group dissociates. After the addition of approximately 1 mole equivalent alkali a single sharp increase in potential is obtained after which, on further addition of alkali, the slope of the curve has a steady downwards trend (dilution effect).

#### F. DISCUSSION

The anion shifts observed for the furan ring protons of compounds (LII), (LIII) and (LIV) are as expected. The proton in position 2, which is affected by mesomerism, always shows a greater effect than the proton in position 3. The two compounds with one 6-hydroxyl substituent, (LII) and (XXXIV), show anion shifts for the protons in positions 4 and 7 which are within the ranges given by Highet and Highet.<sup>118</sup> Of these two compounds compound (XXXIV), which has a carbonyl group para to hydroxyl group, shows larger anion shift values for the ortho protons in positions 5 and 7. The shift values found for the proton in position 5 of these compounds are however unusually small for an ortho effect. The 4,6-dihydroxy compounds, (LIII) and (XLI), exhibit effects within the expected ranges. The anion shifts of the protons in positions 5 and 7 are subject to the cumulative effects of the two hydroxyl groups.

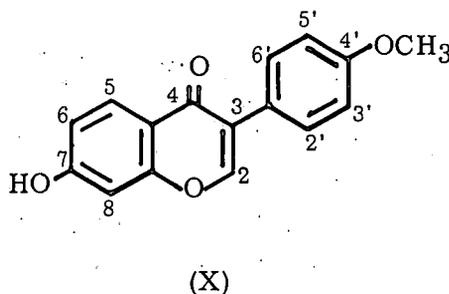
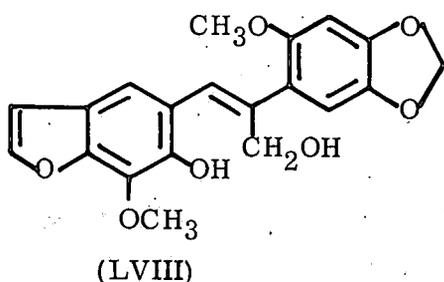
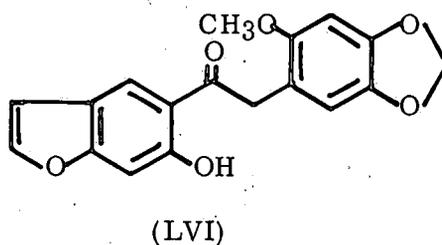
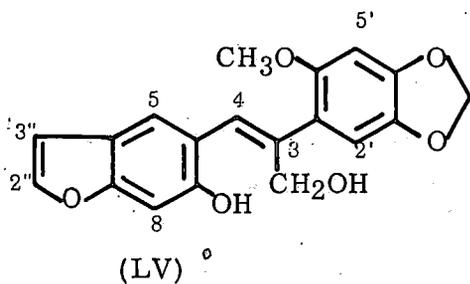
The interpretation of the results obtained for the 6,7-dihydroxy compounds, (LIV) and (XLVI) appears to be difficult. Both the hydroxyl groups of 6,7-dihydroxy-2,3-dihydrobenzofuran-3-one (XLVI) dissociate as evidenced by the anion shift data (fig. 6, plate 13) and the potentiometric titration (fig. 9, plate 15). The second step in the potentiometric curve of this compound appears rather diffuse in contrast to the anion shift results which are quite normal. This may be due to the differences in experimental conditions (concentrations). From the anion shift data of this compound it follows that the hydroxyl group in position 6 dissociates first. The total shifts observed are however lower than expected. In contrast to the compound mentioned above, (XLVI), only one of the hydroxyl groups of 6,7-dihydroxybenzofuran (LIV) dissociates, a fact which is proved by the anion shift data (fig. 3 plate 12) and the potentiometric titration results (fig. 9, plate 15). From the facts that the hydroxyl group in position 6 of compound (XLVI) dissociates first and that the anion shift value of the proton in position 2 of 6,7-dihydroxybenzofuran (LIV) agrees well with the shift value observed for the corresponding proton in 6-hydroxybenzofuran (LII) we conclude that it is the hydroxyl group in position 6 of 6,7-dihydroxybenzofuran (LIV) which dissociates as the proton in position 2

of this compound will not be subject to mesomeric contributions on the dissociation of the hydroxyl group in position 7. The magnitudes of the anion shifts observed for the 6,7-dihydroxy compounds do not fit into any simple scheme.

Although some general features can be observed for the anion shifts of the hydroxybenzofurans and hydroxy-dihydrobenzofuran-3-ones which are in good agreement with the values given by Highet and Highet<sup>118</sup> no scheme could be designed which could accommodate all the data obtained. It is particularly the 6,7-dihydroxy compounds (LIV) and (XLVI) which show exceptional behaviour due to the ortho relationship of the two hydroxyl groups.

## II. ANION SHIFTS OF PHENOLIC DERIVATIVES OF NATURAL ISOFLAVANOIDS

The structures of the phenolic isoflavanoid derivatives pachyrrhizinol (LV), the deoxybenzoin of dehydroneotene (LVI), formononetin (X) and neofolinol (LVIII) are given below. Purely for the sake of convenience the same nomenclature used for the furoisoflavanoids (Chapter 3) is also used for their phenolic derivatives



The NMR data of these compounds, obtained from dimethyl sulphoxide solutions are summarized in Table 11. The data of neofolinol (LVIII) are taken from a publication by Brink et al.<sup>55</sup>

## A. ASSIGNMENTS OF THE PROTON RESONANCES

### (i) PACHYRRHIZINOL (LV)

The low field part of the spectrum (plate 16) contains the signals of nine protons. The furan ring protons ( $\tau = 2.18$  and  $\tau = 3.12$ ) are identified by their spin-spin coupling ( $J_{2''3''} = 2.1$  cps). The lower signal is assigned to the proton in position 2'' next to the electronegative oxygen atom<sup>121a, 122</sup>. The resonances at relatively high field ( $\tau = 3.16$  and  $\tau = 3.29$ ) are assigned to the protons in positions 2' and 5' respectively as it is known that methylenedioxy<sup>135</sup> methoxyl and hydroxyl<sup>107, 110, 114, 116, 123</sup> substituents shift the resonances of ortho and para aromatic protons to higher field. The signal at  $\tau = 2.90$  is assigned to the proton in position 8 as the resonance shows long-rang spin-spin coupling ( $J_{3''8} = 1.0$  cps) (see under IC). The low field signal at  $\tau = 2.25$  is assigned to the aromatic proton in position 5 (from anion shift data). The two-proton signal at  $\tau = 3.98$  is characteristic for a methylenedioxy group.<sup>135-137</sup> The remaining low field signal at  $\tau = 3.09$  must thus be due to the olefinic proton in position 4. The two proton signal at  $\tau = 5.52$  and the three-proton signal at  $\tau = 6.21$  are assigned to the methylene - and methoxyl groups respectively. The phenolic hydroxyl proton resonance is at  $\tau = 0.3$ .

### (ii) DEOXYBENZOIN OF DEHYDRONEOTENONE (LVI)

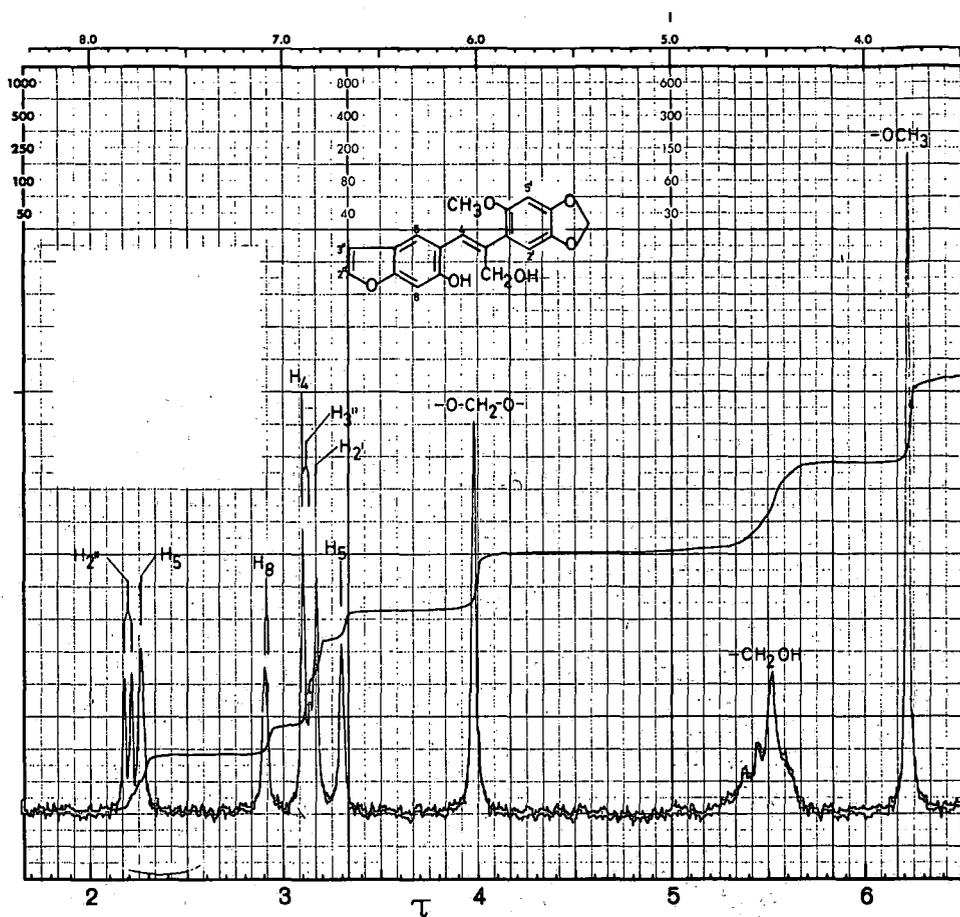
The assignments of the protons of this compound~~s~~ (plate 16) are made in a similar manner as described above. The signals at  $\tau = 2.27$  and  $\tau = 3.21$  are assigned to the furan ring protons in positions 2'' and 3'' respectively. The proton in position 3'' clearly shows long-range coupling with the proton in position 8 ( $\tau = 3.06$ ) ( $J_{3''8} = 0.9$  cps). The signals at higher field ( $\tau = 3.35$  and  $\tau = 3.39$ ) are assigned to the protons in positions 2' and 5' respectively as described under (i). The remaining low field signal at  $\tau = 1.80$  must thus be due to the proton in position 5. The low  $\tau$  - value of this proton is ascribed to deshielding by the anisotropic carbonyl group<sup>138</sup> ortho to this proton. The two-proton resonance at  $\tau = 4.18$  is characteristic for the methylenedioxy group<sup>135-137</sup> and the three-proton signal (methoxyl group) is located at  $\tau = 6.41$ . The remaining two-proton signal at  $\tau = 5.75$  is thus assigned to the methylene protons.

TABLE 11

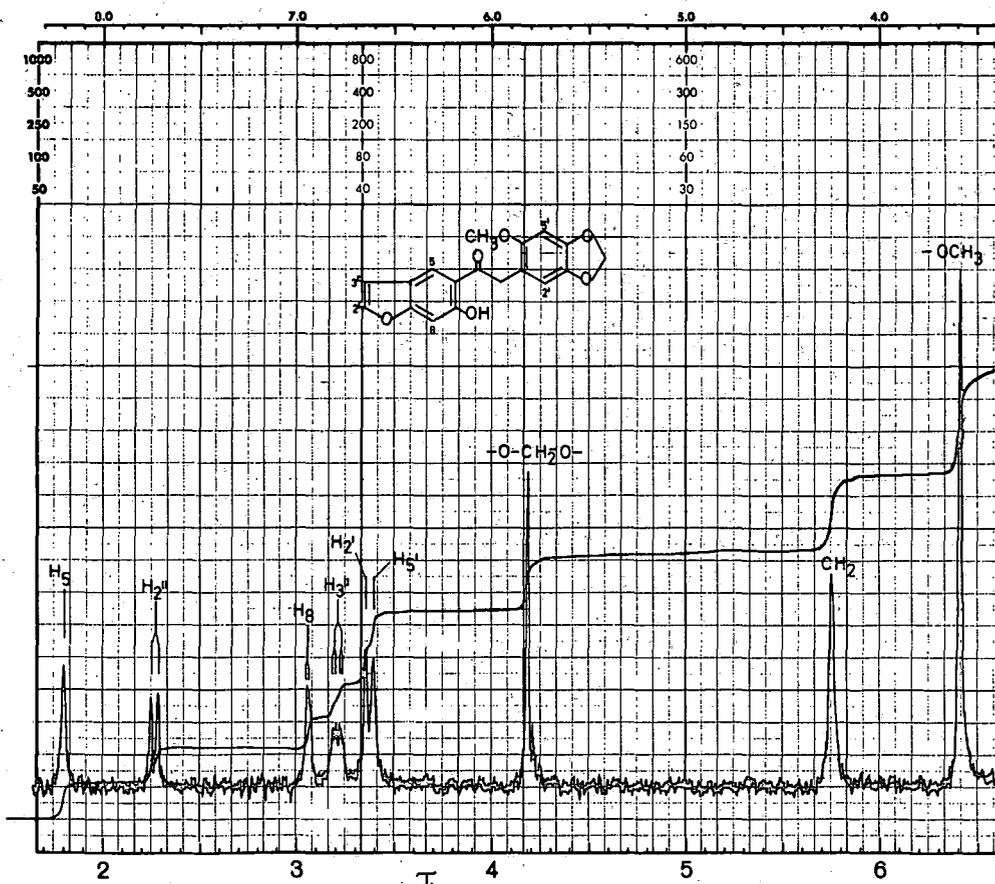
$\tau$  - VALUES FOR (CH<sub>3</sub>)<sub>2</sub> SO SOLUTIONS

Compound No.	$\tau$ - VALUES OF GROUPS AT POSITIONS										
	2''	3''	4	5	8	2'	5'	CH <sub>2</sub>	OCH <sub>3</sub>	-OCH <sub>2</sub> -O-	J (cps)
(LV)	2.18	3.12	3.09	2.25	2.90	3.16	3.29	5.52	6.21	3.98	J <sub>2''3''</sub> =2.1; J <sub>3''8</sub> =1.0
(LVI)	2.27	3.21	-	1.80	3.06	3.35	3.39	5.75	6.41	4.18	J <sub>2''3''</sub> =2.1; J <sub>3''8</sub> = 0.9
(LVIII)	2.24	3.18	3.14	2.59	-	3.27	3.33	-	-	-	
	2	5	6	8	2'+6'	3'+5'					
(X)	1.63	1.96	3.00	3.05	2.43	2.96	-	-	6.18	-	J <sub>56</sub> = 8.8; J <sub>68</sub> = 2.0

## PLATE 16



(a) PACHYRRHIZINOL (LV)



(b) DEOXYBENZOIN OF DEHYDRONEOTENONE (LVI)

(iii) FORMONONETIN (X) <sup>139</sup>

The aromatic protons in positions 5, 6 and 8 ( $\tau = 1.96$ ,  $\tau = 3.00$  and  $\tau = 3.05$  respectively) constitute a three-spin system which appears basically as an AB system. The assignments of these protons are easily made from their splitting patterns. The protons in positions 5 and 6 are ortho coupled ( $J_{56}=8.8$  cps) and one of them (6, at higher field) exhibits a further small meta splitting ( $J_{68} = 2.0$  cps), the para coupling ( $J_{58}$ ) not being resolved. The  $\tau$  - values for the proton in positions 5 and 6 were determined from an AB-analysis after removing the small meta coupling. The relatively low  $\tau$  - value for the proton in position 5 is ascribed to deshielding by the anisotropic carbonyl group in position 4. The one-proton signal at lowest field,  $\tau = 1.63$  is assigned to the proton in position 2. The low  $\tau$  - value of this proton is caused by the inductive effect of the neighbouring electronegative ring oxygen atom and a mesomeric effect originating in the 4- carbonyl group as shown below.



This value is in good agreement with these found for similar compounds <sup>139</sup> (genistein and biochanin A). The resonances of corresponding protons in similar compounds which do not have a 4 - carbonyl group appear at considerably higher  $\tau$  - values ( $\tau = 3.7 - 3.8$ ) <sup>121c, 142</sup>.

The aromatic protons in positions 2'6' and 3'5' constitute an AA'BB' system which approximates a simple AB system the doublets of which are located at  $\tau = 2.43$  and  $\tau = 2.96$ . The higher field doublet is assigned to the protons in positions 3' and 5' as they are ortho to the methoxyl group. <sup>107, 110, 114, 116, 123</sup>

B. ANION SHIFTS OF COMPOUNDS (LV), (LVI) AND (X)

The maximum anion shift data obtained for compounds (LV), (LVI), (X) and neofillinol (LVIII) <sup>55</sup> are given in Table 12.

TABLE 12

MAXIMUM VALUES OF ANION SHIFTS

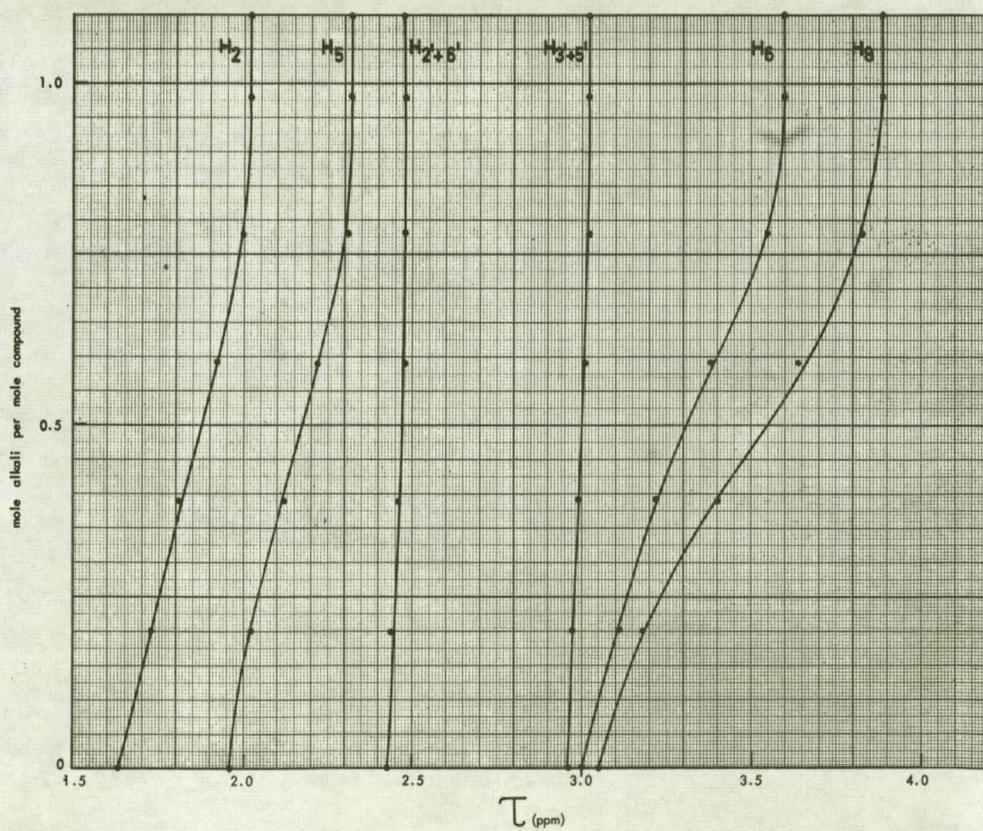
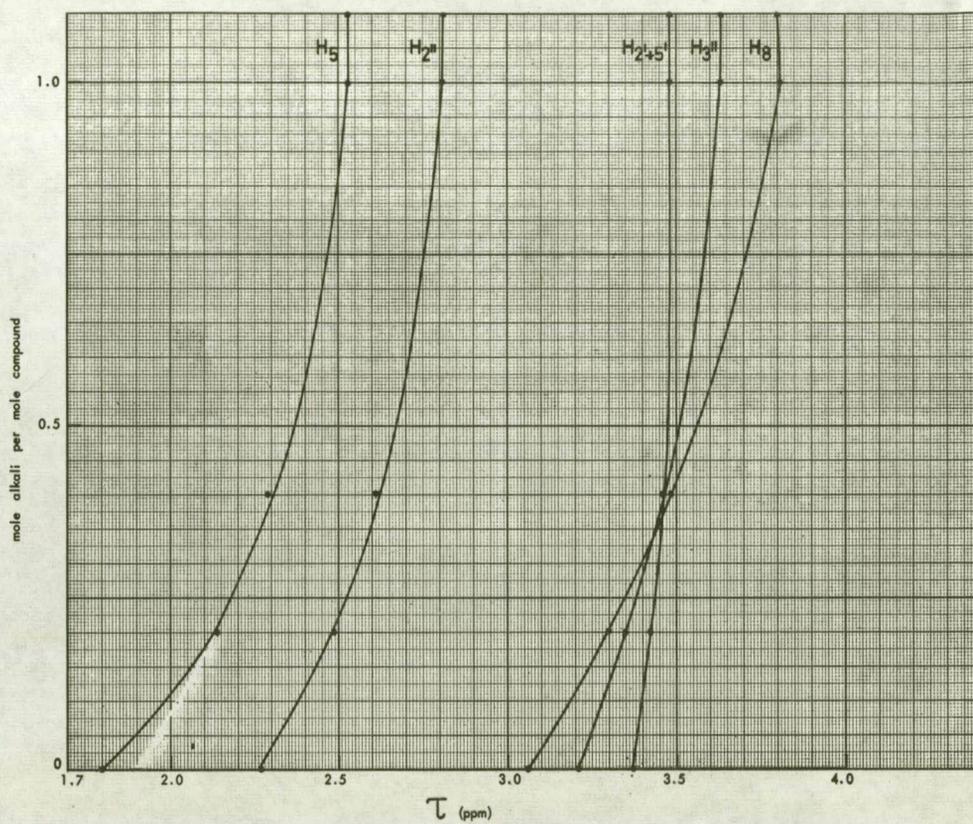
Proton	Maximum shifts (ppm) for Compound No.			
	(LV)	(LVI)	(LVIII)	(X)
2''	0.43 n	0.54 n	0.38 n	-
3''	0.31 n	0.42 n	0.26 n	-
2	-	-	-	0.39 n
4	- 0.13	-	- 0.19	-
5	0.61 m	0.73 m	0.50 m	0.37 m
6	-	-	-	0.60 o
8	0.64 o	0.75 o	-	0.84 o
2'	0.11	0.11	0.07	0.05
3'	-	-	-	0.06
5'	0.09	0.11	- 0.01	0.06
6'	-	-	-	0.05

The designations o, m, p and n have the same meaning as in Table 10.

(i) PACHYRRHIZINOL (LV)

In figure 10 (plate 14) it can be seen that the protons in positions 8, 5 and 2'' have the largest high field shifts with maximum anion shift values of 0.64, 0.61 and 0.43 ppm respectively. The relevant mesomeric structures for the benzofuran part of the molecule can be written in a similar manner as for 6-hydroxybenzofuran. It is important to note that the meta anion shift value 0.61 ppm (proton 5) is approximately the same as the ortho anion shift value, 0.64 ppm (proton 8), a fact which cannot be accounted for at present. As expected the dissociation of the phenolic hydroxyl group has hardly any effect on the protons in positions 4, 2' and 5' (Table 12). The proton in position 4 shows a small negative (low field) shift. 55, 119, 140 The unambiguous assignments of the protons in positions 4 and 5 are made possible by the anion shift data as a result of the fact that of these two protons only the proton in position 5 is expected to have a significant anion shift value. The anion shift data of similar compounds, (LVI) and (LVIII) clearly show that only the protons

## PLATE 17



attached to the benzofuran part of the molecule are significantly influenced by the dissociation of the phenolic hydroxyl group.

(ii) DEOXYBENZOIN OF DEHYDRONEOTENONE (LVI)

The anion shift values of this compound figure 11 (plate 17), are very similar to the values obtained for pachyrrhizinol (LV). The protons in positions 8, 5 and 2'' have the highest anion shift values, 0.75, 0.73 and 0.54 ppm respectively. As in the case of pachyrrhizinol the meta and ortho anion shifts (protons 5 and 8) are of the same magnitude while the protons in positions 2' and 5' are hardly affected.

(iii) FORMONONETIN (X)

As expected the aromatic protons ortho to the hydroxyl group, in positions 6 and 8, have the highest maximum anion shift values, 0.60 and 0.84 ppm respectively, figure 12 (plate 17). The protons in positions 2 and 5 have approximately the same shift values, 0.39 and 0.37 ppm. Very small shifts are obtained for the aromatic protons in positions 2', 3', 5' and 6' (no mesomeric contributions). The slight backward shift which is observed in some of the curves after passing the equivalence point can possibly be ascribed to a dilution effect<sup>117, 119</sup>.

(III) GENERAL CONCLUSIONS

The maximum anion shift values which were obtained for the compounds investigated in this work are in good agreement with the anion shift values found by other authors.<sup>117-120</sup> In compounds (X), (XXXIV), (LII) and (LVII), which have two ortho protons with respect to the hydroxyl group, the proton in position 7 (position 8 for compounds (X) and (LVII)) has the higher anion shift value of the two. The relatively high meta anion shift values of the hydroxybenzofurans and the phenolic derivatives of the isoflavanoids are also in agreement with the high meta anion shift values found by Hight and Hight<sup>118</sup> for phenols which have vinylogous <sup>acyl</sup> and groups para to the hydroxyl groups. It appears that the furan ring in phenolic benzofuran compounds has a similar effect on the magnitude of the meta anion shift. As only a few compounds have been studied it is not possible to draw any definite conclusions in this regard.

In compounds (LV) and (LVI) the anion shift values of the protons in the ortho and meta positions (positions 8 and 5) are approximately the same. It

therefore appears that the anion shift technique is not a suitable method to distinguish between these protons in compounds of this type. In furoisoflavanoids the proton in position 8 can easily be identified as a result of the long-range coupling between this proton and the proton in position 3".

It has been found <sup>117, 118</sup> that the anion shift values are dependent on the entire substitution pattern of the compound concerned and that the substituent shielding values are in turn influenced by contributions from inductive, resonance, hetero atoms, steric and magnetic effects. <sup>113, 114, 116</sup> Substituents ortho to the hydroxyl group as well as solvent effects may also significantly influence the anion shift values.

As a result of the limited amount of information which is at present available on the effects which substituents and the other factors mentioned above have on the anion shifts of protons, it must be strongly emphasised that considerable care should be taken in using this method for structural elucidations unless anion shift data of closely related compounds are available. This may prove to be especially true in the case of heterocyclic compounds where additional effects may be expected.

#### (IV) EXPERIMENTAL

The NMR spectra were recorded on a pre-calibrated Varian A-60 spectrometer (probe temperature 35°C). Compounds (XXXV), (XLII), (XLVII), (XLIX), (L) and (LI) were determined in deuteriochloroform (dilute solutions) with tetramethylsilane (TMS) as internal reference. Dimethyl sulphoxide was used as solvent in the anion shift studies with sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS) as internal reference. The chemical shifts are expressed in ppm on the  $\tau$  scale (with  $\tau$  TMS or  $\tau$  DSS = 10.00) and are estimated to be accurate to + 0.02 ppm.

The anion shifts were studied by successively adding small portions (approximately 10% of the equivalent amount) of alkali (+ 5 N Na OD solution) to the compound and recording the low field part of the spectrum after each addition until no further changes were observed. The NaOD solution was prepared by adding sodium to D<sub>2</sub>O.

Special precautions, however, had to be taken in the anion shift determinations of 6,7-dihydroxybenzofuran (LII). It was found that this compound is extremely sensitive to oxygen in alkaline medium, it immediately oxidises and resinifies in the presence of oxygen. The experiment was accordingly carried out under a nitrogen atmosphere, using oxygen-free dimethyl sulphoxide and NaOD solutions. As a result of the hydrogen-deuterium exchange of the aromatic protons of 4,6-dihydroxybenzofuran (LIII), a NaOH solution was used.

AB systems were analysed according to the usual procedure<sup>108,141</sup>. Three-spin systems of aromatic protons were reduced to AB patterns, as described, and analysed as such.

The potentiometric titrations were carried out with a "Radiometer type TTT1c" automatic titrator. The compounds were dissolved in dimethyl sulphoxide and titrated with a 1 N NaOH solution under a nitrogen atmosphere.

EXPERIMENTAL PART

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EXPERIMENTAL PART

CHAPTER 8

- A. GENERAL.
- B. PREPARATION AND EXTRACTION OF PLANT MATERIAL.
- C. ISOLATION AND PURIFICATION OF THE GLYCOSIDE AMBONIN.
- D. SEPARATION OF THE KNOWN ISOFLAVANOIDS BY COLUMN CHROMATOGRAPHY.
- E. SEPARATION OF THE DEHYDROROTENOIDS.

## A. GENERAL

Unless specified to the contrary, melting points are corrected. Infrared spectra refer to KBr discs and were recorded on a Unicam SP 200 spectrophotometer. Ultraviolet spectra were determined in spectroscopically pure ethanol, except where otherwise stated, and were recorded on a Zeiss PMQ II spectrophotometer. Optical rotations were determined with a Hilger-Watts M412 polarimeter. Activated alumina refers to Riedel-de-Haen "Alumina for Chromatography" treated with 2N hydrochloric acid, washed with water until neutral, dried and activated at 220°C for 24 hours. Thin-layer chromatography refers to Silica Gel G (Merck) and Kieselguhr G (Merck) buffered with sodium acetate (0.02 M) (layer thickness 0.3 mm) activated at 110°C for 35 minutes. Where the multiple development technique was used the factor is given in parentheses. Vanillin - sulphuric acid spray reagent refers to vanillin (1g) and sulphuric acid (1.5 ml) dissolved in 35 ml 96% ethanol. Anisaldehyde - sulphuric acid spray reagent refers to p-anisaldehyde (1.5 ml) and sulphuric acid (1.5 ml) dissolved in 27 ml 96% ethanol. The thin-layer plates were developed by heating for 10 - 20 minutes at 100°C. The mass spectra were recorded on a A. E. I. MS 9 mass spectrometer. The micro-analysis were done by Janssen Pharmaceutica, Beerse, Belgium and by the C. S. I. R., Pretoria.

## B. PREPARATION AND EXTRACTION OF PLANT MATERIAL

Roots of *N. amboensis* were obtained from the Khomas highlands in South West Africa during April and May and from the Kruger National Park, near Shingwidzi and Tshokwane, during January and May. After removal of the bark, the roots were sliced, sun-dried and ground to pass 100 mesh. Approximately 70 kg of the ground material was extracted in 5 kg portions with hot acetone for 24 hours. The acetone extracts were concentrated under diminished pressure to a third of its original volume. The extracts were left to evaporate slowly at room temperature and after 10-14 days the resinous brown syrup slowly started to crystallise. Treatment of the syrup with the minimum amount of cold methanol and filtration afforded a small amount of crystalline material, Product A. The methanol filtrate yielded a further small amount of crystalline material after 5-6 days which was filtered and added to Product A.

C. ISOLATION AND PURIFICATION OF THE GLYCOSIDE AMBONIN (I)

After several attempts a very successful method was found for the isolation of the glycoside ambonin. The methanol filtrate from which Product A was obtained, was evaporated until a resinous syrup was obtained. This syrup was repeatedly extracted with boiling benzene until all the benzene soluble compounds were removed. The excess benzene was evaporated from the benzene insoluble residue. After cooling cold methanol was added to the benzene insoluble residue to form a layer a quarter of an inch thick on top of the resinous product. The resinous syrup slowly started to crystallise and with the addition of further small quantities of cold methanol the entire resinous product was obtained crystalline after 10 - 12 days. The crystalline product was filtered and washed with small amounts of cold methanol. A white crystalline compound was obtained which consisted mainly of the glycoside ambonin. Evaporation of the methanol from the filtrate and treating the residue with cold methanol as described above afforded a further crop of glycoside. The yield of impure glycoside from 5.8 kg plant material was 11.0 g. The benzene soluble fraction as well as the benzene insoluble fraction, from which the glycoside was obtained, were combined, concentrated and kept for further processing (Residue A).

After two recrystallisations from methanol and seven crystallisations from distilled water the glycoside AMBONIN (I) was obtained pure as white needles m. p. 230° - 232°C. On drying ambonin at 110°C for 16 hours the melting point was lowered to 225.5° - 227.5°C with a corresponding loss of weight which showed the presence of water of crystallisation, Ambonin  $\left[ \begin{array}{c} \alpha \\ \text{D} \end{array} \right]^{21} - 73.8^\circ$  (in 0.02 MKOH solution).

$C_{26}H_{28}O_{13} \cdot \frac{1}{2}H_2O$ (557.5)	Calc :	C, 55.97; H, 5.24
	Found:	C, 55.87; H, 4.97
		C, 56.21; H, 5.04

Thin-layer chromatography (Silica Gel G) of ambonin (solvent system ethyl acetate - methyl ethyl ketone - methanol (5:3:2)) showed only one spot,  $R_f$  0.39, which had a light-green fluorescence under ultraviolet light. With vanillin-sulphuric acid a yellow spot was obtained.

D. SEPARATION OF THE KNOWN ISOFLAVANOIDS  
BY COLUMN CHROMATOGRAPHY

The resinous syrup from which the glycoside was obtained, Residue A (see under C) was mixed with activated alumina (100g alumina to the extract obtained from each kilogram plant material) to give a dry porous mixture which was successively extracted in a soxhlet with petroleum ether (8 hours) and benzene (8 hours). The petroleum ether extract deposited a yellow waxy precipitate which crystallised on trituration with ether, Product B. Evaporation of the benzene extract and trituration with ether afforded a brown-yellow crystalline product, Product C. The total yield of crude crystalline material obtained (excluding the glycoside ambonin) was approximately 0.18% calculated on the dry plant material.

The crude crystalline mixture (3g), Product B, was chromatographed on activated alumina (300g) with benzene as eluent to separate neodulin, neotenone, pachyrrhizin and dehydroneotenone.

FRACTION 1

A colourless band on the column which showed an intense purple fluorescence on the column and in solution under ultraviolet light. A colourless crystalline product was obtained which on rechromatography and crystallisation from benzene afforded NEODULIN (XII) m. p.  $225.5^{\circ}\text{C}$  as colourless needles. (110 mg).

FRACTION 2

A colourless band on the column which had a cream colour under ultraviolet light. Rechromatography of the compound obtained from this fraction and recrystallisation from benzene yielded NEOTENONE (XIV) as white needles m. p.  $179.5^{\circ} - 180.5^{\circ}\text{C}$  (950 mg).

FRACTION 3

A light yellow band on the column which showed an intense green fluorescence under ultraviolet light on the column and in solution. The product of this fraction was rechromatographed several times and recrystallised from benzene. PACHYRRHIZIN (XIII) melting point  $206.5^{\circ} - 207.5^{\circ}\text{C}$  was obtained as yellow-green needles (1.25g).

FRACTION 4

A cream-coloured band on the column under ultraviolet light which eluted slowly from the column. Rechromatography of this compound and recrystallisation from benzene afforded Dehydroneotenone (XV) melting point  $239.5^{\circ} - 240.5^{\circ} \text{C}$  as white needles (45 mg).

Thin-layer chromatography gave the following  $R_f$  values on Silica Gel G plates with benzene-chloroform (1:1) as the solvent system:

Neodulin  $R_f$  0.57; neotenone  $R_f$  0.32; pachyrrhizin  $R_f$  0.20; dehydroneotenone  $R_f$  0.10. The colour reactions with vanillin-sulphuric acid spray reagent gave the following: neodulin, blue; neotenone, khaki-grey; pachyrrhizin, grey-yellow; dehydroneotenone, grey-yellow.

Product C (benzene extract from soxhlets) was dissolved in the minimum amount of ether and left for 24 hours, filtered and the crystalline material so obtained was combined with Product A (see under B). Chromatography on activated alumina of the filtrate gave the same compounds as Product B as described above.

E. SEPARATION OF THE DEHYDROROTENONDS

When Product A (1.5g) crystalline product obtained from the fractional crystallisation of the acetone extract, see under B) was dissolved in benzene a viscous solution was obtained. The viscous solution was filtered and the filtrate was chromatographed on activated alumina (300g) with benzene as eluent.

FRACTION 1

A colourless band on the column which had a cream colour under ultraviolet light. In solution this fraction had an intense light-green fluorescence under ultraviolet light. Rechromatography of this fraction and recrystallisation from benzene afforded DEHYDRODOLINEONE (XVI) melting point  $280^{\circ} \text{C}$  (decomp.) as white-yellow needles (80 mg).

$\text{C}_{19}\text{H}_{10}\text{O}_6$  (334.3) Calc. : C, 68.26; H, 3.01; -  $\text{OCH}_3$ , 0.0

Found : C, 68.01; H, 3.07; -  $\text{OCH}_3$ , 0.0

Molecular weight 334.

FRACTION 2

Consisted mainly of neotenone.

FRACTION 3

Consisted mainly of pachyrrhizin.

FRACTION 4

A light yellow band on the column which showed an intense yellow-green fluorescence on the column and an intense green fluorescence in solution under ultraviolet light. Evaporation of the benzene afforded a yellow crystalline compound which was rechromatographed on alumina. The product so obtained was found to be impure and the compound was consequently purified by preparative thin-layer chromatography on Silica Gel G plates with benzene as eluent.

NEBOENSINONE (XVII) was obtained as fine yellow needles melting point  $350^{\circ}\text{C}$  (decomp.) 110 mg).

$\text{C}_{19}\text{H}_8\text{O}_7$  (348.2) Calc. : C, 65.52; H, 2.31; -  $\text{OCH}_3$ , 0.0.

Found : C, 64.37; H, 2.51; -  $\text{OCH}_3$ , 0.0.

Molecular weight 348.

(The low carbon value may possibly be due to incomplete combustion as a result of the high melting point of nevoensinone).

FRACTION 5

A yellow band on the column which did not show any fluorescence under ultraviolet light and which eluted very slowly with benzene. The band was consequently eluted with chloroform. In solution the compound showed a faint yellow fluorescence under ultraviolet light. The compound was purified by preparative thin-layer chromatography on Silica Gel G plates with benzene-chloroform (1:1) as the solvent system. NAMBINONE (XVIII) was obtained as a fine deep yellow crystalline compound melting point  $400^{\circ}\text{C}$  (decomp.) (4 mg), molecular weight 364.

Thin-layer chromatography of the dehydrorotenoids on Silica Gel G (solvent system benzene-chloroform (1:1) gave the following  $R_f$  values: dehydrodolineone  $R_f$  0.27; nevoensinone  $R_f$  0.25; nambinone  $R_f$  0.09. The colour reactions with vanillin-sulphuric acid spray reagent are: dehydrodolineone, grey-blue; nevoensinone, yellow; nambinone, grey.

## CHAPTER 9

### STRUCTURE ELUCIDATION OF AMBONIN

- A. ACETYLATION OF AMBONIN
- B. HYDROLYSIS OF AMBONIN
- C. METHYLATION OF DAIDZEIN
- D. ACETYLATION OF DAIDZEIN
- E. (i) ALKALINE HYDROGEN PEROXIDE OXIDATION OF DIMETHOXYDAIDZEIN
  - (ii) METHYLATION OF THE CARBOXYLIC ACIDS WITH DIAZOMETHANE
  - (iii) HYDROLYSIS OF THE METHYL ESTERS.
- F. HYDROGENATION OF DIMETHOXYDAIDZEIN
- G. (i) METHYLATION OF AMBONIN
  - (ii) HYDROLYSIS OF METHYLATED AMBONIN
- H. ACETYLATION OF FORMONONETIN
- I. SEPARATION AND IDENTIFICATION OF THE CONSTITUENT MONOSACCHARIDES.

STRUCTURAL ELUCIDATION OF AMBONIN (I)

A. ACETYLATION OF AMBONIN (I)

Ambonin (400 mg) was dissolved in dry pyridine (5 ml), acetic anhydride (9 ml) was added and the reaction mixture was heated on a water bath for 1 hour.

The mixture was cooled, ice water (200 ml) was added and after 2 hours the precipitate was filtered off, washed with dilute cold hydrochloric acid, water until neutral and dried. The product was chromatographed on activated alumina with methanol as eluent. Evaporation of the eluate afforded a colourless oil which solidified after 2 weeks in a vacuum desiccator as a glassy solid m. p.  $95^{\circ} - 97^{\circ} \text{C}$ .

B. HYDROLYSIS OF AMBONIN <sup>3, 4, 5</sup>

Ambonin (4g) was dissolved in distilled water (120 ml) <sup>by refluxing on a water bath.</sup> ~~was~~ <sup>Sulphuric acid 28ml (25%)</sup> slowly added (dropwise) to the solution and refluxed on a water bath for 4.5 hours. The white precipitate which formed (aglycone) was filtered and washed with water. On further heating the filtrate for a further 3 hours a small amount of precipitate was formed, which was filtered and added to the first batch. Crystallisation from methanol gave DAIDZEIN (II) m. p.  $330^{\circ} - 333^{\circ} \text{C}$  (lit. <sup>10</sup>  $320^{\circ} - 325^{\circ} \text{C}$ ) as white needles. Yield 1.78g.

Thin-layer chromatography of daidzein showed only one spot  $R_f$  0.75 which had a light-blue fluorescence under ultraviolet light. With vanillin-sulphuric acid a faint yellow spot was obtained. (Silica Gel G, solvent system: ethyl acetate - methyl ethyl ketone - methanol (5:3:2).

C. METHYLATION OF DAIDZEIN (II) <sup>14</sup>

Daidzein (1.5 g) was dissolved in anhydrous acetone (550 ml, daidzein is not very soluble in acetone) by heating under reflux on a water bath under anhydrous conditions. Anhydrous potassium carbonate (prepared by heating for 12 hours at  $140^{\circ} - 150^{\circ} \text{C}$ ) (15.0 g) was added to the acetone mixture. Dimethyl sulphate (18.0 ml) was added dropwise to the reaction mixture over a period of 60 minutes at a temperature of  $50^{\circ} \text{C}$  with stirring. The reaction mixture was heated under reflux for a further 9 hours, cooled and filtered. Evaporation of the acetone filtrate afforded an oily residue to which a solution of 5% potassium hydroxide (50 ml) was added to decompose the excess dimethyl sulphate. After 5 hours at room temperature a crystalline product was obtained which was filtered, washed with distilled water and dried.

The crystalline product was chromatographed on activated alumina (200g) with benzene as eluent. The first fraction yielded an oily by-product. Fraction 2 showed a blue-green fluorescence on the column under ultraviolet light. Evaporation of the eluate (fraction 2) afforded a white crystalline compound which was obtained pure after recrystallisations from benzene (2x) as white needles, DIMETHOXYDAIDZEIN (III) (7, 4' - dimethoxyisoflavone) m. p. 163.5 - 164.5°C (lit. <sup>9</sup> 162° - 163° C). Yield 1.2 g.

$C_{17}H_{14}O_4$  (282.2) Calc. : C, 72.36; H, 5.00; -OCH<sub>3</sub>, 21.98

Found : C, 72.25; H, 4.97; -OCH<sub>3</sub> 22.10.

#### D. ACETYLATION OF DAIDZEIN (II)

Daidzein (150 mg) was dissolved in dry pyridine (5 ml), acetic anhydride (5 ml) was added and the reaction mixture was heated on a water bath for 45 minutes, cooled and poured into ice water (200 ml). The precipitate was filtered, washed with a cold dilute hydrochloric acid, water and dried. After recrystallisation from benzene-methanol (1:1) 7, 4' - DIACETOXYDAIDZEIN was obtained as white needles m. p. 187° - 188° C (lit. <sup>10</sup> 187°C).

#### E. (i) ALKALINE HYDROGEN PEROXIDE OXIDATION OF DIMETHOXYDAIDZEIN (II) <sup>15</sup>

Dimethoxydaidzein (1.12 g) was added to a mixture of ethanol (98 ml), water (24 ml), potassium hydroxide (6.5 g) and a solution of 30% hydrogen peroxide ("Perhydrol") (14 ml) with stirring at room temperature until all the dimethoxydaidzein was dissolved, (60 minutes). The mixture was slowly heated up to 40°C and stirred for a further 60 minutes at this temperature after which three further amounts of 30% hydrogen peroxide solution (7 ml) <sup>were</sup> ~~was~~ added with 10 minute intervals. The temperature of the reaction mixture was raised to 40° - 45°C and stirred for another 45 minutes, after which the temperature of the reaction mixture was finally increased to 70°C and stirred for 15 minutes, cooled and acidified with concentrated hydrochloric acid. Evaporation of the ethanol under diminished pressure afforded a precipitate (545 mg) which was filtered and washed with cold water. Recrystallisation of the crystalline precipitate from benzene - petroleum ether (1:2) gave colourless needles. The ethanol distillate from the reaction mixture gave a positive test for formic acid. <sup>18</sup>

Thin-layer chromatography of the crystalline product showed the presence of two carboxylic acids. Silica Gel G, 110°C /45 minutes, solvent system benzene-methanol (3:1) (3x) : R<sub>f</sub> 0.11 (purple-brown spot) and R<sub>f</sub> 0.17 (yellow spot). Spray reagent hydrogen peroxide - ferric chloride (Method: spray with 0.5% aqueous 30% hydrogen peroxide solution, dry at 105°C and spray with a 2% aqueous ferric chloride solution and heat at 110°C for 20-30 minutes.)

The acids could not be separated by fractional crystallisation.

(ii) METHYLATION OF THE CARBOXYLIC ACIDS WITH DIAZOMETHANE

The mixture of the two acids, obtained under (i) above, (540 mg) was dissolved in ether (65 ml) and cooled in ice. Diazald (p - toluenesulphonyl - N - methyl - N - nitrosamide) (10.7g) was dissolved in ether (160 ml) and cooled in a freezing mixture of ice and salt. To this cooled mixture a solution of potassium hydroxide (2g) dissolved in 96% ethanol (50 ml) was added. After 10 minutes the diazomethane-ether solution was distilled (dropwise) into the flask containing the carboxylic acid solution until gas evolution ceased. The ether was evaporated and the residue was taken up in benzene (200 ml) and extracted with a 5% sodium bicarbonate solution (3x100 ml) and washed with water (5x100 ml). (Acidification of the sodium bicarbonate layer gave no precipitate). The benzene solution was subsequently extracted with a cold 6% solution of potassium hydroxide (4x50 ml). The alkaline layer was separated, acidified with concentrated hydrochloric acid and extracted with ether (3x100 ml). The ether layer was washed with water, dried over anhydrous sodium sulphate and evaporated. A colourless oil (4 - methoxy methyl salicylate (VI) ) was obtained which did not crystallise.

The benzene layer(after extraction with alkali) was washed with water, dried over anhydrous sodium sulphate and evaporated. A colourless oil (4 - methoxy methyl benzoate (VII) ) was obtained which also could not be induced to crystallise.

(iii) HYDROLYSIS OF THE METHYL ESTERS

The oily product obtained from the alkaline fraction (E (ii) ), 4 - methoxy methyl salicylate (VI), was hydrolysed with a 10% solution of sodium hydroxide for 4 days at room temperature. The alkaline mixture was acidified

with concentrated hydrochloric acid and extracted with ether (3x200 ml). The ether layer was washed with water, dried over anhydrous sodium sulphate and evaporated. A crystalline product was obtained which was recrystallised from benzene-methanol (9:1) to give 4-METHOXY SALICYLIC ACID (IV) as colourless needles m. p.  $157^{\circ} - 158^{\circ}\text{C}$  (lit. <sup>105</sup>  $157^{\circ}\text{C}$ )  $R_f$  0.11 (purple-brown spot, see under E(i)).

The oily product (4-methoxy methyl benzoate (VII)) obtained from the benzene layer (E(ii)) was hydrolysed as described above with a 10% sodium hydroxide solution. The product was worked up as described above. Recrystallisation from benzene afforded 4-METHOXY BENZOIC ACID (V) as colourless needles m. p.  $179^{\circ} - 180^{\circ}\text{C}$  (lit. <sup>106</sup>  $184^{\circ}\text{C}$ ),  $R_f$  0.17 (yellow spot, see under E(i)).

#### F. HYDROGENATION OF DIMETHOXYDAIDZEIN (III) <sup>12</sup>

Dimethoxydaidzein (400 mg) was dissolved in ethyl acetate (50 ml) and hydrogenated over 10% Pd/C (600 mg) (previously saturated with hydrogen) until absorption of hydrogen ceased. The catalyst was removed by filtering through a column of celite and the filtrate was evaporated. An oily product was obtained which was chromatographed on activated alumina (200 g) with benzene as eluent.

##### FRACTION 1

A colourless band on the column which showed a light purple fluorescence under ultraviolet light. Evaporation of the eluate and recrystallisation from benzene afforded 7,4'-DIMETHOXYISOFLAVAN (IX) m. p.  $111^{\circ} - 112^{\circ}\text{C}$  (lit. <sup>12</sup>  $112^{\circ} - 113^{\circ}\text{C}$ ) as white needles.

##### FRACTION 2

A colourless band on the column which had a light purple fluorescence under ultraviolet light. Evaporation of the eluate and recrystallisation from benzene gave 7,4'-DIMETHOXYISOFLAVANONE (VIII) m. p.  $125^{\circ} - 126^{\circ}\text{C}$  (lit. <sup>12</sup>  $125^{\circ} - 126^{\circ}\text{C}$ ) as white needles.

##### FRACTION 3

A colourless band on the column which showed a blue-green fluorescence under ultraviolet light. This fraction consisted of a small amount of unchanged 7,4'-dimethoxydaidzein.

Thin-layer chromatography (Silica Gel G, eluent benzene (2x) vanillin-sulphuric acid spray reagent.

7,4'-dimethoxyisoflavanone	R <sub>f</sub> 0.24 (grey spot)
7,4'-dimethoxyisoflavan	R <sub>f</sub> 0.75 (red spot)

G. (i) METHYLATION OF AMBONIN (1)<sup>14</sup>

Ambonin (2g) was added to dry acetone (200 ml) (ambonin is very slightly soluble in acetone) and refluxed on a water bath (anhydrous conditions). Anhydrous potassium carbonate (20g) (heated at 150°C for 12 hours) was added to the mixture. Dimethyl sulphate (30 ml) was added over a period of 60 minutes to the mixture with stirring at 50°C. The reaction mixture was heated for 9 hours under reflux (with stirring) cooled and left overnight. The mixture was filtered and the acetone filtrate was evaporated. A solution of 5% potassium hydroxide (150 ml) was added to the oily residue obtained after evaporation and left for 7 hours at room temperature to decompose the excess dimethyl sulphate. The reaction mixture was extracted with benzene, the benzene layer was separated, washed with water, dried over anhydrous sodium sulphate and evaporated. A light yellow oil was obtained which did not crystallise.

(ii) HYDROLYSIS OF METHYLATED AMBONIN

The oily product which was obtained under (i) was dissolved in methanol, 3 N hydrochloric acid (20 ml) was added and the reaction mixture was heated on a water bath for 3 hours. The methanol was evaporated under reduced pressure, distilled water was added to the residue and the precipitate that formed was filtered off and washed with water.

Recrystallisation from methanol (3x) afforded FORMONONETIN (X) (7-hydroxy-4'-methoxyisoflavanone) m. p. 256° - 256°C (lit.<sup>9</sup> 258°C) as white needles.

H. ACETYLATION OF FORMONONETIN (X)<sup>9</sup>

Formononetin (180 mg) was dissolved in dry pyridine (3 ml), acetic anhydride (5 ml) was added to the mixture and heated on a water bath for 1 hour. After cooling ice water (100 ml) was added to the reaction mixture and left for 2 hours. The precipitate was filtered, washed with distilled water and dried. Recrystallisation from methanol (4x) afforded Acetylformononetin (XI) (7-acetoxy-4'-methoxyisoflavone) m. p. 170° - 171°C (lit.<sup>9</sup> 170°-171°C) as white needles.

I. SEPARATION AND IDENTIFICATION OF THE  
CONSTITUENT MONOSACCHARIDES

The filtrate from the acid hydrolysis of ambonin was neutralised with finely powdered barium carbonate and filtered. The neutral aqueous solution was heated at 60°C with decolourising charcoal ("Norit NK"), filtered and evaporated at 40°C under reduced pressure. Thin-layer chromatography of the colourless syrup so obtained revealed the presence of two sugars,  $R_f$  0.32 (blue spot) and  $R_f$  0.75 (red-violet spot). (Kieselguhr G buffered with sodium acetate (0.02 m), solvent system ethyl acetate - isopropanol - water (65:23:12) and spray reagent p - anisaldehyde - sulphuric acid, see Chapter 8 under general). The colour reaction of the sugar  $R_f$  0.75 is characteristic as it first turned violet-red, then blue and finally it reverted back to its original red-violet colour on standing at room temperature for approximately 12 hours.

A column packed with powdered cellulose was used for the separation of the sugars.<sup>21</sup> Cellulose, washed with acetone, ether and methanol, dried and passed through a 100 mesh sieve, was tightly and uniformly packed in a column (3.3 cm diameter) to a height of 40 cm in a solvent mixture of acetone-water (95:5 v/v). (The uniformity of the packing can be tested by placing bromocresol-green indicator on the column and eluting with acetone-water (95:5 v/v). The column is regarded as satisfactory for use if the dye elutes in the form of a regular horizontal band).

The sugar mixture (3g syrup) was introduced on the column as a thin aqueous syrup and eluted with an acetone-water (95:5 v/v) solvent mixture. The eluate was fractionated in 50 ml portions and each portion was examined by their <sup>thin-</sup>layer chromatography. The appropriate first fractionated portions were combined (1.9 liter), fraction 1, and evaporated at 40°C under diminished pressure. A colourless syrup was obtained (0.96 g) which consisted of the monosaccharide, sugar X ( $R_f$  0.75, red-violet spot). After a transition zone (600 ml) which consisted of both monosaccharides, the second sugar was obtained ( $R_f$  0.32, blue spot) by eluting the column with a methanol-water (95:5 v/v) solvent mixture, (550 ml), fraction 2. This sugar had the same  $R_f$  value (0.32) and gave the same colour reaction (blue spot) as glucose. The syrup (fraction 2) was obtained crystalline from methanol-acetone (9:1). The crystalline product was recrystallised from methanol, m. p. 147°C. The osazone derivative

of this sugar m. p.  $204^{\circ}\text{C}$  and optical rotation  $\left[ \alpha \right]_{\text{D}}^{20} + 112^{\circ}$  to  $+ 52^{\circ}$  further positively identified the sugar as  $\alpha - \text{D} - \text{GLUCOSE}$ .

Despite all efforts the sugar X (fraction 1) could not be induced to crystallise. Addition of dry acetone to the syrup dissolved in the minimum amount of absolute methanol caused the sugar to precipitate as a fine amorphous white powder which could not be isolated as it was found to be highly hygroscopic. The syrup (dried under high vacuum) gave a optical rotation value of  $\left[ \alpha \right]_{\text{D}}^{21} + 8.2^{\circ}$  (in water).

Sugar X (260 mg) was heated on a waterbath for one hour with acetic anhydride (2 ml) and dry pyridine (1.5 ml). The reaction mixture was poured into a mixture of ice water and dilute hydrochloric acid. The oily product which separated was extracted with ether, the ether layer washed successively with a 5% solution of sodium bicarbonate, water and finally dried over anhydrous sodium sulphate. Evaporation of the ether afforded the acetoxy derivative of sugar X as a light-yellow oil. The sugar acetate was purified by preparative thin-layer chromatography<sup>22</sup> (Silica Gel G, eluent benzene - methanol (93 :7)). The pure acetoxy derivative of sugar X was obtained as a colourless oil which was dried in vacuo.

The mass-spectral - and NMR data of the acetate indicated the sugar to be a pentose (Chapter 2 G).

CHAPTER 10

STRUCTURE ELUCIDATION OF DEHYDRODOLINEONE  
AND NEBOENSINONE

- A. HYDROGENATION OF DEHYDRODOLINEONE
- B. OXIDATION OF DEHYDRODOLINEONE WITH  
N-AMYL NITRITE

STRUCTURAL ELUCIDATION OF DEHYDRODOLINEONE  
AND NEBOENSINONE

A. HYDROGENATION OF DEHYDRODOLINEONE (XVI)

Dehydrodolineone (25 mg) was hydrogenated in ethyl acetate (15 ml) over 10% Pd/C catalyst, previously saturated with hydrogen, until absorption of hydrogen ceased. The catalyst was removed by filtering through a short column packed with celite and the eluate was evaporated. A waxy residue was obtained which was taken up in benzene and chromatographed on activated alumina (50 g) with benzene as eluent. Evaporation of the eluate afforded DIHYDRODOLINEONE (XXIX) m. p. 149<sup>o</sup> C as white needles (1.5 mg).

B. OXIDATION OF DEHYDRODOLINEONE (XVI) WITH  
N-AMYL NITRITE<sup>29</sup>

Dehydrodolineone (15 mg) was dissolved in warm glacial acetic acid (5 ml) and cooled in a freezing mixture to - 5<sup>o</sup> C. Freshly prepared n-amyl nitrite (0.5 ml) was added before the compound crystallised. The reaction mixture was kept at 0<sup>o</sup> C for one hour and then allowed to warm to room temperature. A small amount of a yellow crystalline material precipitated and was filtered. The reaction mixture was extracted with benzene, washed with water and dried over anhydrous sodium sulphate. Evaporation of the benzene afforded a yellow crystalline compound which was combined with the yellow precipitate which was filtered off, dissolved in benzene and chromatographed on activated alumina (50 g) with benzene as eluent. Evaporation of the eluate gave a yellow crystalline compound (4 mg) which was purified by preparative thin-layer chromatography (Silica Gel G). NEBOENSINONE (XVII) m. p. 350<sup>o</sup> C (decomp.) was obtained a fine yellow needles (1.5 mg), identical in every way with the natural product.

## C H A P T E R 11

### I. SYNTHESIS OF HYDROXYBENZOFURANS

#### A. SYNTHESIS OF 6 - HYDROXYBENZOFURAN

- (i) PREPARATION OF 6 - HYDROXY - 2, 3 - DIHYDROBENZOFURAN - 3 - ONE.
- (ii) PREPARATION OF 3, 6 - DIACETOXYBENZOFURAN
- (iii) PREPARATION OF 6 - ACETOXY - 2, 3 - DIHYDROBENZOFURAN
- (iv) PREPARATION OF 6 - ACETOXYBENZOFURAN BY QUINONE DEHYDROGENATION
- (v) PREPARATION OF 6 - HYDROXYBENZOFURAN

#### B. SYNTHESIS OF 4, 6 - DIHYDROXYBENZOFURAN

- (i) PREPARATION OF 4, 6 - DIHYDROXY - 2, 3 - DIHYDROBENZOFURAN - 3 - ONE
- (ii) PREPARATION OF 3, 4, 6 - TRIACETOXYBENZOFURAN
- (iii) PREPARATION OF 4, 6 - DIACETOXY - 2, 3 - DIHYDROBENZOFURAN
- (iv) PREPARATION OF 4, 6 - DIACETOXYBENZOFURAN BY QUINONE DEHYDROGENATION
- (v) PREPARATION OF 4, 6 - DIHYDROXYBENZOFURAN

#### C. SYNTHESIS OF 6, 7 - DIHYDROXYBENZOFURAN

- (i) PREPARATION OF 6, 7 - DIHYDROXY - 2, 3 - DIHYDROBENZOFURAN - 3 - ONE<sub>s</sub>
- (ii) PREPARATION OF 3, 6, 7 - TRIACETOXYBENZOFURAN
- (iii) PREPARATION OF 6, 7 - DIACETOXY - 2, 3 - DIHYDROBENZOFURAN
- (iv) PREPARATION OF 6, 7 - DIACETOXYBENZOFURAN BY QUINONE DEHYDROGENATION
- (V) PREPARATION OF 6, 7 - DIHYDROXYBENZOFURAN.

### II. QUALITATIVE TEST FOR SUBSTITUTED BENZOFURANS

### III. SYNTHESIS OF PHENOLIC DERIVATIVES OF NATURAL ISOFLAVANOIDS

#### A. PREPARATION OF PACHYRRHIZINOL

#### B. PREPARATION OF THE DEOXYBENZOIN OF DEHYDRONEOTENONE

#### C. PREPARATION OF FORMONONETIN.

I. SYNTHESIS OF HYDROXYBENZOFURANS

A. SYNTHESIS OF 6 - HYDROXYBENZOFURAN (LII)

(i) PREPARATION OF 6 - HYDROXY -  
2,3 - DIHYDROBENZOFURAN - 3 - ONE (XXXIV) 63-66

Chloroacetyl chloride (28.25 g) was added dropwise during 90 minutes to a stirred mixture of resorcinol (250 ml) at a temperature of  $50^{\circ} - 55^{\circ}\text{C}$ . The mixture was stirred for a further 15 minutes at this temperature, cooled and poured into an excess of ice and dilute hydrochloric acid and set aside overnight. The organic layer was separated and extracted with 1N aqueous sodium hydroxide (300 ml). The alkaline extract was acidified with concentrated hydrochloric acid and the precipitate was filtered and washed with cold water. The crystalline product was dissolved in ethanol and refluxed with decolourising charcoal (2 g) for 30 minutes on a water bath. The charcoal was filtered through a short column of Celite and the ethanol was evaporated under reduced pressure. Recrystallisation (twice) from methanol afforded 6 - HYDROXY - 2,3 - DIHYDRO - BENZOFURAN - 3 - ONE (XXXIV) (8 g) as golden yellow plates m. p.  $243^{\circ} - 244^{\circ}\text{C}$  (lit. <sup>65</sup> $245^{\circ}\text{C}$ ).

(ii) PREPARATION OF 3,6 - DIACETOXYBENZOFURAN (XXXV)

6 - Hydroxy - 2,3 - dihydrobenzofuran - 3 - one (8 g) was dissolved in dry redistilled pyridine (30 ml) and acetic anhydride (40 ml) was added under stirring and cooling. After 24 hours at room temperature the mixture was stirred into ice water (350 ml) and left for 2 hours. The yellow precipitate formed was filtered off and washed with cold dilute hydrochloric acid, distilled water until neutral and then dried. The crystalline product was taken up in methanol and chromatographed on a short column of unactivated alumina. Evaporation of the methanol and recrystallisation from methanol-water (2:1) gave 3,6 - DIACETOXYBENZOFURAN (XXXV) (7 g) as white needles m. p.  $80^{\circ} - 81^{\circ}\text{C}$  (lit. <sup>65</sup> $81^{\circ}\text{C}$ ).

(iii) PREPARATION OF 6 - ACETOXY - 2,3 - DIHYDROBENZOFURAN (XXXVI)

3,6 - Diacetoxybenzofuran (7 g) was hydrogenated in glacial acetic acid (30 ml) over 30% palladium - charcoal catalyst (0.5 g) at  $60^{\circ} - 70^{\circ}\text{C}$  under 4 atmospheres pressure for 3 hours. (The Pd/C catalyst was prepared according to the method of Linstead and Thomas<sup>67</sup>, catalyst d, which was dried at  $110^{\circ}\text{C}$  for 20 hours).

The catalyst was removed by filtering through a short column of Celite, methanol (4 x 200 ml) was added to the filtrate and evaporated under reduced pressure. A yellow-green oil was obtained to which water (30 ml) was added. After 36 hours a crystalline product was obtained which was filtered and washed with cold water. Recrystallisation from methanol - water (3:1) (2x) afforded 6 - ACETOXY - 2, 3 - DIHYDROBENZOFURAN (XXXVI) (2.3 g) as white needles m. p. 73.5° - 74.5° C (lit.<sup>65</sup> 73° - 74° C).

(iv) PREPARATION OF 6 - ACETOXYBENZOFURAN (XLIX) BY QUINONE DEHYDROGENATION

(a) WITH CHLORANIL

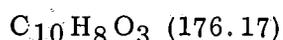
6 - Acetoxy - 2, 3 - dihydrobenzofuran (250 mg) was added to chloranil (tetrachloro - 1, 4 - benzoquinone) (345 mg), 1:1 mole, in a flask which was equipped with a condenser with a calcium chloride guard tube and a nitrogen inlet tube. Pure sodium-dried benzene was added to the mixture until a homogenous solution was obtained (20 ml). The reaction mixture was heated under reflux under a nitrogen atmosphere with stirring. The progress of the reaction was periodically tested by taking a drop from the red-brown reaction mixture and heating it with 5 N sodium hydroxide solution (1 ml) on a waterbath. A pink-red colour showed the presence of unreacted chloranil.<sup>80</sup> After 52 hours under reflux and 60 hours at room temperature the test for chloranil was still positive. The reaction mixture was chromatographed on a short column of unactivated alumina. The benzene eluate was washed with a saturated solution of sodium bisulphite to remove any unreacted chloranil, washed with water, and dried over anhydrous sodium sulphate. Evaporation of the benzene gave a crystalline product m. p. 64° - 68° C. Thin-layer chromatography (Silica Gel G) showed that the product consisted mainly of the starting material, 6 - acetoxy - 2, 3 - dihydrobenzofuran and only a small amount ( $\pm$  1%) of 6 - acetoxybenzofuran.

(b) WITH DDQ (2, 3 - DICHLORO - 5, 6 - DICYANO - 1, 4 - BENZOQUINONE)

6 - Acetoxy - 2, 3 - dihydrobenzofuran (2.3 g) was added to DDQ (4.39 g) (1:1.5 mole) in a flask equipped with a condenser and a calcium chloride tube. Pure, sodium-dried benzene (30 ml) was added to the mixture and heated under reflux with stirring. (It is important to work under strictly anhydrous conditions as DDQ is hydrolysed by water with the liberation of hydrocyanic acid).<sup>91</sup>

Initially the reaction mixture had a brown-green colour and after approximately 60 minutes a brown precipitate began to form which consisted mainly of the quinol, (2,3-dichloro-5,6-dicyano-1,4-benzoquinol, DDK) which is insoluble in benzene. As the reaction progressed the colour of the reaction mixture changed from brown-green to brown-red. The mixture was refluxed for 42 hours, cooled and the precipitate filtered off, washed with hot benzene, dried and weighed. The weight of the precipitate (3.38 g) showed that, besides the expected quinol DDK (theoretical amount 2.972 g) benzene insoluble by-products were also formed.

The brown-red filtrate was chromatographed on a short column unactivated alumina with benzene as eluent to remove the excess DDQ. Evaporation of the benzene afforded a yellow-green oil which rapidly solidified. The crystalline product was triturated with cold petroleum ether and filtered. Recrystallisation of the petroleum ether insoluble product from benzene-petroleum ether (1:9) gave a crystalline compound (25 mg) m. p.  $213^{\circ}$  -  $216^{\circ}$ C as yellow needles, probably a complex by-product which was not investigated further. The petroleum ether filtrate was chromatographed on unactivated alumina with petroleum ether as eluent. Evaporation of the solvent and crystallisation of the product from petroleum ether gave 6-ACETOXYBENZOFURAN (XLIX) m. p.  $44^{\circ}$  -  $45^{\circ}$ C as long colourless needles. Yield 1.21 g (52.6% theor.)



Calc.: C, 68.17 ; H, 4.58

Found : C, 68.01 ; H 4.69

Thin-layer chromatography (Silica Gel G) with benzene (2x) as eluent and vanillin-sulphuric acid spray reagent gave the following colour reactions and  $R_f$  values:

6-Acetoxybenzofuran  $R_f$  0.19 (blue spot)

6-acetoxy-2,3-dihydrobenzofuran  $R_f$  0.09 (red spot)

by-product

$R_f$  0.02 (green-brown spot)

#### (V) PREPARATION OF 6-HYDROXYBENZOFURAN (LII)

6-Acetoxybenzofuran (1.1 g) dissolved in dry ether (35 ml) was added to a suspension of lithium aluminium hydride (500 mg) in dry ether (40 ml) by means of a dropping funnel with stirring over a period of 1 minute at  $10^{\circ}$ C while a continuous stream of nitrogen was passed through the reaction mixture and

apparatus. After 3 minutes the excess lithium aluminium hydride was decomposed by the addition of moist ether (60 ml) and water. The aluminium complexes were decomposed by the subsequent addition of 2N hydrochloric acid (100 ml) while still working under a nitrogen atmosphere. The ether layer was separated and the aqueous layer was extracted with ether (3x100 ml). The ether fractions were combined and washed neutral with water (7x100 ml), dried over anhydrous sodium sulphate and evaporated. A yellow oil was obtained (760 mg) which was kept at 0°C for 24 hours, triturated with cold benzene and filtered. Evaporation of the benzene gave an oil which slowly crystallised (3 days). The crystalline product was dissolved in petroleum ether (250 ml), filtered and the petroleum ether filtrate was concentrated to 50 ml. After 6 hours a crystalline compound was obtained which was filtered off. Recrystallisation of the crystalline compound from petroleum ether afforded 6-HYDROXYBENZOFURAN (LII) m. p. 57°-58°C (lit. <sup>93</sup> 56°C) as long flat colourless needles. A green colour reaction was obtained with methanolic ferric chloride. Yield 484 mg (58% theor.)

#### B. SYNTHESIS OF 4,6-DIHYDROXYBENZOFURAN (LIII)

##### (i) PREPARATION OF 4,6-DIHYDROXY-2,3-DIHYDROBENZOFURAN-3-ONE (XLI) <sup>66, 68, 69</sup>

Chloroacetonitrile (17 g) was added to phloroglucinol (25 g) dissolved in dry ether (130 ml) with stirring and dry hydrogen chloride gas was passed through the mixture for 6.5 hours.

The ether layer was decanted from the yellow precipitate (2,4,6-trihydroxyphenyl chloromethylketimine hydrochloride (XXXVIII)) and the ketimine hydrochloride was dissolved in distilled water (200 ml) and the solution extracted with ether (2x100 ml). The aqueous layer was separated and acidified with 50% sulphuric acid (100 ml). After approximately 15 minutes the ketimine sulphate (XXXIX) started to precipitate as a yellow crystalline compound. The mixture was kept overnight, the solid was filtered and washed with cold water (400 ml). The ketimine sulphate was taken up in water (400 ml) and refluxed for a further 3 hours. A red precipitate was obtained which was filtered off and recrystallised twice from water-ethanol (5:1).

4,6-DIHYDROXY-2,3-DIHYDROBENZOFURAN-3-ONE (XLI)  
m. p. 248°-253°C (lit. <sup>66</sup> 250°C) was obtained as light pink needles. Yield 15 g.

(ii) PREPARATION OF 3, 4, 6 - TRIACETOXYBENZOFURAN (XLII)<sup>69</sup>

4, 6 - Dihydroxy - 2, 3 - dihydrobenzofuran - 3 - one (10 g) was dissolved in dry pyridine (35 ml), acetic anhydride (100 ml) was added with stirring and the mixture was kept for a further 16 hours at room temperature. The reaction mixture was poured into ice-water and after 2 hours the precipitate was filtered off, washed with cold dilute hydrochloric acid, water and dried. Crystallisation of the product from ethanol yielded 3, 4, 6 - TRIACETOXYBENZOFURAN (XLII) m. p. 103° - 104° C (lit.<sup>69</sup> 102° C) as white needles, yield 13.75 g.

(iii) PREPARATION OF 4, 6 - DIACETOXY - 2, 3 - DIHYDROBENZOFURAN (XLIII)<sup>69</sup>

3, 4, 6 - Triacetoxybenzofuran (13.75 g) was hydrogenated in glacial acetic acid over 30% Pd/C (1 g) (catalyst d)<sup>67</sup> (see under A (iii) ) at 60° - 70° C under 4 atmospheres pressure for 8 hours. The catalyst was removed by filtration through a Celite column, methanol (4 x 200 ml) was added to the filtrate and evaporated under diminished pressure. A light-yellow oil was obtained which was taken up in ether, washed with 5% sodium bicarbonate solution (3 x 50 ml) and dried over anhydrous sodium sulphate. Evaporation of the ether and crystallisation from benzene-ether (1:5) gave 4, 6 - DIACETOXY - 2, 3 - DIHYDROBENZOFURAN (XLIII) m. p. 68° - 69° C (lit.<sup>69</sup> 69° C) as white needles, yield 8.8 g.

(iv) PREPARATION OF 4, 6 - DIACETOXYBENZOFURAN (L) BY QUINONE DEHYDROGENATION

A mixture of 4, 6 - diacetoxy - 2, 3 - dihydrobenzofuran (3.0 g) and DDQ (4.32 g) (1 : 1.5 mole) was dissolved in sodium-dried benzene (60 ml) and heated under reflux with stirring, (anhydrous conditions, see under A(iv) b). The reaction mixture had a brown-green colour, and after 5 minutes a fine brown precipitate began to form which consisted mainly of the benzene-insoluble quinol, DDK. The colour of the reaction mixture gradually changed from brown-green to brown-red as the reaction time increased. The progress of the reaction was followed by thin-layer chromatography (Silica Gel G, solvent system benzene-chloroform (1:1), vanillin-sulphuric acid reagent).

The reaction mixture was heated under reflux for 124 hours, cooled and filtered. The filtered precipitate was washed with hot benzene, dried and weighed (3.03 g, theoretical 2.90 g). The difference in weight from the theoretical

amount showed the presence of benzene-insoluble complex by-products. The brown-red filtrate was chromatographed on unactivated alumina with benzene as eluent to remove the excess DDQ. Evaporation of the solvent under reduced pressure afforded 4,6-DIACETOXYBENZOFURAN (L) as a light-yellow oil. Yield 2.61 g (87% theor.).

$C_{12}H_{10}O_5$  (234.2) Calc.: C, 61.53; H, 4.30

Found : C, 61.41; H, 4.36

Thin-layer chromatography (Silica Gel G, benzene-chloroform (1:1), vanillin-sulphuric acid spray reagent): 4,6-diacetoxybenzofuran:  $R_f$  0.19 (blue spot)  
4,6-diacetoxy-2,3-dihydrobenzofuran:  $R_f$  0.14 (red spot).

(v) PREPARATION OF 4,6-DIHYDROXYBENZOFURAN (LIII)

4,6-Diacetoxybenzofuran (1.45 g) dissolved in dry ether (40 ml) was added to a suspension of lithium aluminium hydride (900 mg) in dry ether (40 ml) with stirring over a period of 1 minute at  $10^{\circ}C$  while nitrogen was passed through the reaction mixture and apparatus. After 5 minutes the excess lithium aluminium hydride was decomposed by the addition of moist ether (100 ml) and water, (10 ml). The aluminium complexes were decomposed by the addition of 2N hydrochloric acid (150 ml) whilst still working under a nitrogen atmosphere. (4,6-Dihydroxybenzofuran is sensitive to oxygen in alkaline medium and oxidizes rapidly to form a red-brown oxidation product). The ether layer was separated and the aqueous layer was extracted with ether (3 x 100 ml). The ether fractions were combined and washed with water (7 x 100 ml), dried over anhydrous sodium sulphate and evaporated. A light pink-brown oil was obtained (905 mg theor. 935 mg) which crystallised on standing after 24 hours. After three recrystallisations from benzene (the compound is not very soluble in benzene) -  $R_D$  100.00  
4,6-DIHYDROXYBENZOFURAN (LIII) m. p.  $121^{\circ} - 122^{\circ}C$  was obtained as light pink prisms. Yield 643 mg (64% theor.)

$C_6H_8O_3$  (150.13) Calc. : C, 64.00; H, 4.03

Found : C, 64.02; H 4.06

Thin-layer chromatography (Silica Gel G<sup>+</sup> with benzene-methanol-glacial acetic acid (90:7:3) as eluent and a 0.1% methanolic "Fast blue salt BB" solution as spray reagent.

4, 6 - dihydroxybenzofuran  $R_f$  0.18 (red-violet spot) (The plate was heated for 8 minutes at 100°C after spraying).

A red-brown colour reaction was obtained with a methanolic ferric chloride solution.

C. SYNTHESIS OF 6, 7 - DIHYDROXYBENZOFURAN (LIV)

(i) PREPARATION OF 6, 7 - DIHYDROXY - 2, 3 - DIHYDROBENZOFURAN - 3 - ONE (XLVI)<sup>35, 68, 70, 71</sup>

A stirred mixture of anhydrous pyrogallol (50 g), monochloroacetic acid (40g) was heated at 60° - 70°C for 5.5 hours. The reaction mixture was cooled and ice water (300 ml) was added and left for 15 minutes. The mixture was heated until the dark brown solid dissolved, and after clarification (charcoal (10g)) and filtration of the charcoal, the mixture was left for 24 hours at 0°C. The crystalline material which separated was filtered off, washed with a small amount of cold water and dried. Pure  $\omega$ -CHLOROGALLACETOPHENONE (XLV) was obtained as light-brown needles m. p. 168° - 169°C (lit.<sup>68, 70</sup> 169°C). Yield 20.5 g.

$\omega$ -Chlorogallacetophenone (20.5 g) was dissolved in ethanol (250 ml), sodium acetate (25 g) was added and the mixture was heated under reflux for 5.5 hours. The ethanol was evaporated under reduced pressure and distilled water (120 ml) was added to the solid residue. After approximately 16 hours the crystalline product was filtered and washed with water. The product was taken up in ethanol (400 ml) and chromatographed on unactivated neutral alumina. The eluate was concentrated and left overnight. Filtration afforded pure 6, 7 - DIHYDROXY - 2, 3 - DIHYDROBENZOFURAN - 3 - ONE (XLVI) m. p. 229°-230°C (lit.<sup>70</sup> 229°C) as yellow plates. Yield 12 g.

(ii) PREPARATION OF 3, 6, 7 - TRIACETOXYBENZOFURAN (XLVII)<sup>70</sup>

6, 7 - Dihydroxy - 2, 3 - dihydrobenzofuran - 3 - one (8 g) was dissolved in dry pyridine (30 ml) and acetic anhydride (80 ml) was added while stirring. After 11 hours at room temperature the red solution was poured into ice water (400 ml) and left overnight. The yellow precipitate was filtered, washed with dilute cold hydrochloric acid, then water until neutral and finally dried. Recrystallisation from ethanol-water (1:1) gave 3, 6, 7 - TRIACETOXYBENZOFURAN (XLVII) m. p. 105° - 106° C (lit.<sup>70</sup> 105.5°C) as white needles. Yield 10.0 g.

(iii) PREPARATION OF 6,7-DIACETOXY-2,3-DIHYDROBENZOFURAN (XLVIII)<sup>70,72</sup>

3,6,7-Triacetoxybenzofuran (10 g) was hydrogenated in glacial acetic acid (30 ml) over 30% Pd/C<sup>67</sup> (see under A (iii)) at a temperature of 60° - 70°C under 4 atmospheres pressure for 6 hours. The catalyst was filtered through a short column of Celite, methanol (4x200 ml) was added to the filtrate and evaporated under reduced pressure. A light yellow oil was obtained which solidified after 2 hours. The crystalline product was dissolved in chloroform (250ml) and washed with a 5% sodium bicarbonate solution (3x100 ml), water and dried over anhydrous sodium sulphate. Evaporation of the chloroform afforded a light yellow oil which slowly solidified. Recrystallisation (twice) from methanol gave 6,7-DIACETOXY-2,3-DIHYDROBENZOFURAN (XLVIII) m. p. 114° - 115°C (lit.<sup>70</sup> 116°C) as white needles. Yield 7.0 g.

(iv) PREPARATION OF 6,7-DIACETOXYBENZOFURAN (LI) BY QUINONE DEHYDROGENATION

A mixture of 6,7-diacetoxy-2,3-dihydrobenzofuran (4.0g) and DDQ (5.76 g) (1:1.5 mole) was dissolved in sodium-dried benzene (25 ml) and heated under reflux with stirring (anhydrous conditions, see under A (iv) (b).) Initially the reaction mixture had a dark brown-green colour and after 2 hours a precipitate began to form which consisted mainly of the benzene-insoluble quinol DDK. As the reaction time increased the colour of the mixture changed to brown-red. The reaction mixture was heated for 86 hours with stirring. On cooling a red-brown viscous oily product was obtained. Benzene (50 ml) was added to the reaction mixture, heated under reflux, cooled and filtered. The red-brown filtrate was chromatographed on unactivated neutral alumina with benzene as eluent to remove the excess DDQ. Evaporation of the eluate afforded a light-yellow oil which rapidly solidified (2.48 g).

The benzene-insoluble product was dissolved in benzene-chloroform (1:1) (50 ml) and chromatographed as described above. A further amount 248 mg) of the light-yellow oil was obtained which was added to the first crop (2.48 g). Recrystallisation (2x) of the crystalline product from benzene-petroleum ether (1:4) gave pure 6,7-DIACETOXYBENZOFURAN (LI) m. p. 97.5° - 98.5°C (lit.<sup>35</sup> 96°-97°C) as colourless needles. Yield 2.5 g (62 % theor.)

$C_{12}H_{10}O_5$  (234.2) Calc. : C, 61.53; H, 4.30

Found : C, 61.50; H, 4.21

Thin-layer chromatography (Silica Gel G, solvent system benzene - chloroform (3:2), spray reagent vanillin - sulphuric acid)

6,7 - diacetoxybenzofuran :  $R_f$  0.16 (blue spot)

6,7 - diacetoxy - 2,3 - dihydrobenzofuran :  $R_f$  0.08 (red spot).

(v) PREPARATION OF 6,7 - DIHYDROXYBENZOFURAN (LIV)

6,7 - Diacetoxybenzofuran (1.0 g) dissolved in dry ether (35 ml) was added to a suspension of lithium aluminium hydride (750 mg) in dry ether (40 ml) with stirring over a period of 1 minute at  $10^\circ\text{C}$  while nitrogen was passed through the reaction mixture and apparatus. After 3 minutes the excess lithium aluminium hydride was decomposed by the addition of moist ether (100 ml) and water (15 ml). The aluminium complexes were decomposed by the addition of 2 N hydrochloric acid (150 ml) whilst still working under a nitrogen atmosphere.

(6,7 - Dihydrobenzofuran is extremely sensitive to oxygen in alkaline medium and upon contact immediately oxidizes and resinifies). The ether layer was separated and the aqueous layer was extracted with ether. The ether fractions were combined and washed with water (7 x 100 ml), dried over anhydrous sodium sulphate and evaporated. A light-yellow oil was obtained which slowly solidified. After recrystallisation from anhydrous benzene (3x) 6,7 - DIHYDROXY-BENZOFURAN (LIV) m.p.  $73^\circ - 74^\circ\text{C}$  (lit. <sup>35</sup>  $72.5^\circ - 74^\circ\text{C}$ ) was obtained as thick colourless needles which crystallised as rosettes. Yield 402 mg (62% theor.)

$C_6H_8O_3$  (150.13) Calc. : C, 64.00; H, 4.03

Found : C, 64.17; H 4.03

Thin-layer chromatography (Silica Gel G, solvent system benzene - methanol - glacial acetic acid (90:7:3), spray reagent 0.1% methanolic

"Fast blue salt BB" solution heated at  $100^\circ\text{C}$  for 8 minutes):

6,7 - dihydroxybenzofuran  $R_f$  0.28 (grey-blue spot)

A Dark-brown precipitate was obtained with methanolic ferric chloride solution.

## II. QUALITATIVE TEST FOR SUBSTITUTED BENZOFURANS

A few crystals of the compound are dissolved in 0.3 ml 96% ethanol to which 0.4 ml 40% sulphuric acid is added. Concentrated sulphuric acid (2 ml) is then carefully added (down the side of the test tube) so as to form 2 layers. The formation of a red or purple-red ring between the layers indicate the presence of a benzofuran compound. On mixing the layers by shaking, a uniform red to red-violet colour is obtained. Dihydrobenzofurans give a light-green or light-yellow colour reaction (negative result). Benzofurans with substituents attached to the furan ring (positions 2 and 3) also give negative tests.

## III. SYNTHESIS OF PHENOLIC DERIVATIVES OF NATURAL ISOFLAVANOIDS.

### A. PREPARATION OF PACHYRRHIZINOL (LV)<sup>30, 44, 94-96</sup>

Pachyrrhizin (XIII) (250 mg) was dissolved in dry tetrahydrofuran (60 ml) in a flask equipped with a condenser and a nitrogen inlet tube. A suspension of lithium aluminium hydride (250 mg) in dry tetrahydrofuran (50 ml) was added to the pachyrrhizin solution over a period of 5 minutes with stirring at a temperature of 50° - 55°C while nitrogen was passed through the reaction mixture. After a further 5 minutes the excess lithium aluminium hydride was decomposed by the addition of ethyl acetate (100 ml) and water (10 ml). The solvent mixture was evaporated under reduced pressure and 10% sulphuric acid (40 ml) was added to the residue to decompose the aluminium complexes. The solution was extracted with ether (4 x 100 ml), the ether layer was washed with a 5% solution of sodium bicarbonate, water and dried over anhydrous sodium sulphate. Evaporation of the ether and recrystallisation of the residue from benzene - acetone (6:4) afforded PACHYRRHIZINOL (LV) m. p. 203° - 204°C (lit.<sup>30, 32</sup> 201°C) as colourless needles. Yield 55 mg (22% theor.)

### (ii) PREPARATION OF THE DEOXYBENZOIN OF DEHYDRONEOTENONE (LVI)<sup>15</sup>

Dehydroneotene (XV) (600 mg) was added to a solution of 5% methanolic potassium hydroxide (60 ml) and refluxed for 3 hours at under a nitrogen atmosphere. After cooling the reaction mixture was acidified with concentrated hydrochloric acid and the methanol was evaporated under reduced pressure. Distilled water (100 ml) was added to the residue and the solution was extracted with ether (4x100 ml),

the ethereal layer was washed with water, dried over anhydrous sodium sulphate and evaporated. The crystalline residue was taken up in benzene and chromatographed on unactivated alumina with benzene as eluent. Evaporation of the eluate and recrystallisation of the residue from benzene (3x) afforded the DEOXYBENZOIN (LVI) m. p.  $161.5^{\circ} - 162.5^{\circ}\text{C}$  (lit.<sup>15</sup>  $162^{\circ} - 163^{\circ}\text{C}$ ) as yellow needles. Yield 322 mg.

(iii) PREPARATION OF FORMONONETIN (X)

The preparation of formononetin is discussed in Chapter 9 G.

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