A SYNTHESIS OF MUNINGIN AND COMPUTERISED OTHER ISOFLAVONES

A thesis

submitted by

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- C O N T E N T S -

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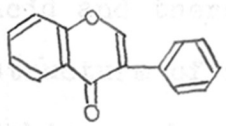
Part I

GENERAL INTRODUCTION

Isoflavones

Isoflavones (I), as the term implies, are the isomers of flavones and possess a 3-phenyl substituent in the benzo-γ-pyrone ring system. Until recently isoflavones were of academic interest because of their occurrence in nature and in connection with methods for their synthesis. It was found that ~~o~~gajin and pomiferin, as well as several synthetic isoflavones related to rotenone, had no value as insecticides.[1,2,3] They assumed importance from the point of view of biological activity when Bradbury and White[4] showed by isolating formononetin (II) and genistein (IV) from Australian clover, that these isoflavones were the principal oestrogens responsible for ~~x~~fertility in sheep. Since the time of this discovery several other workers have studied the oestrogenic activity of isoflavones and



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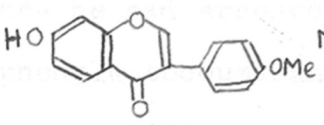

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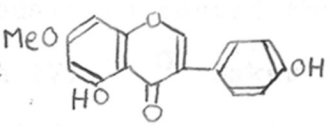


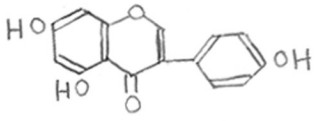
(III)

related compounds, such as isoflavans and isoflav-3-enes.[5,6]

Naturally occurring isoflavones

Isoflavones have been isolated from various parts of plants, including roots and barks, but they are much less widely distributed in nature than flavones. The first compound to be regarded as an isoflavone was prunetin (III). It was suggested by Finnemore[7] that prunetin, which was isolated from an unidentified species of Prunus as the glucoside prunetrin, was a monomethyl ether of 5:7:4'-trihydroxyisoflavone. He based his conclusion on the degradation products, molecular weight and the methoxyl content. He was not able to fix the position of the methoxyl group as demethylation took place during alkali fusion. A. G. Perkin[8] realized that prunetol (IV), the demethylation product of prunetin (III), was possibly identical with genistein, but in examining the products of alkaline hydrolysis he missed the presence of formic acid and therefore he had erroneously suggested the structure of a phenylketocoumaran (V).[9,10] Baker and Robinson, however, in 1928 succeeded in synthesising 5-hydroxy-7:4'-dimethoxy-C-methylisoflavone and showed it to be identical with the product obtained from genistein by methylation.[8] This was the first conclusive

proof that the isoflavones occur in nature.



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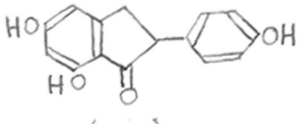

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Eighteen isoflavones (excluding different glycosides of the same aglycone) have been mentioned as occurring in nature, but ~~the~~ recent researches[11,12,13,14] have reduced this number to thirteen. The observations of Okano and Beppu[15] describing isogenistein, methyl isogenistein, methyl genistein and tatoin as new isoflavones occurring in the soya bean have been shown to be incorrect, since the properties mentioned by them did not agree with those of synthetic specimens possessing the proposed isoflavone structures. Baker[13] had predicted that what Okano and Beppu thought to be isogenistein and other unknown isoflavones must be a mixture of genistein and daidzein in various proportions. Seshadri[16] is in agreement with this view, since he was able to isolate only genistein and daidzein, and paper chromatography did not give evidence of the presence of other isoflavones. However, in view of the work

carried out in this laboratory on the colouring matters of Ponderosa pine bark[17] which led to the isolation of pinoquercetin (6-methylquercetin) and pinomyricetin (6-methylmyricetin), a re-examination of the constituents of the soyabean, using more exhaustive chromatographic methods, is desirable. Pratol, a phenolic substance, which was isolated by Power and Salway[18] from the blossoms of red clover, has long been considered to be 7-hydroxy-4'-methoxyflavone;[19] the suggestion was based on the close similarity in properties between pratol and synthetic 7-hydroxy-4'-methoxyflavone, but a direct comparison was not made since the natural substance was not available. Pratol has now been shown by Bate-Smith and Swain[20] to be an isoflavone (formonenetin) on the basis of paper chromatography and absorption spectra.

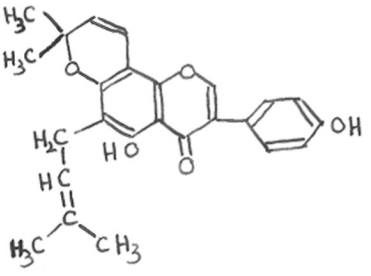
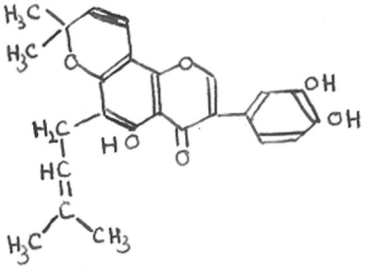
A list of isoflavones occurring in nature is given in Table I.

Table I/

Table I

No.	Compound	Isoflavone	References
1.	Daidzein	7:4'-Dihydroxy	21, 22
2.	Formononetin	7-Hydroxy-4'-methoxy	4, 21, 23
3.	Pseudobaptigenin	7-Hydroxy-3':4'-methoxylene dioxy	21, 24
4.	Genistein	5:7:4'-Trihydroxy	4, 9, 22, 25, 26
5.	Prunetin	5:4'-Dihydroxy-7-methoxy	7, 27
6.	Biochanin (A)	5:7-Dihydroxy-4'methoxy	24
7.	Orobol	5:7:3':4'-Tetrahydroxy	28
8.	Santal	5:3':4'-Trihydroxy-7-methoxy	28, 29
9.	Muningin	6:4'-Dihydroxy-5:7-dimethoxy	30, 31
10.	Tectorigenin	5:7:4'-Trihydroxy-6-methoxy	32, 33, 34

Table I contd.

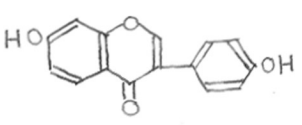
No.	Compound	Isoflavone	References
11.	Irigenin	5:7:3'-Trihydroxy-6:4':5'-trimethoxy	35
12.	Osajin		1, 54
13.	Pomiferin		1, 54

Isolation and reactions of isoflavones

Isoflavones are generally isolated by extracting the powdered plant material with ethanol or water according as they are present as aglycones or glycosides. They are separated by making use of their functional groups or by chromatography.[4] On chromatograms they are identified by their fluorescence in ultraviolet light or by spraying a suitable reagent for the development of coloured spots.

Isoflavones are much more susceptible to alkaline hydrolysis than flavones. Relatively mild treatment with alkali results in the formation of deoxybenzoins, which can be recyclized to the isoflavones, together with formic acid. Daidzein (VI) for instance breaks down readily to the deoxybenzoin (VII; 2:4-dihydroxyphenyl 4-hydroxybenzyl ketone) and formic acid. More vigorous hydrolysis naturally results in further degradation of the deoxybenzoin to a phenol and a phenylacetic acid. The isolation of formic acid is of the utmost importance, as it constitutes the main evidence of an isoflavone structure. Nitric acid oxidation is of value occasionally; this (VIII) gave picric acid, showing thereby the presence of a hydroxyl

group in the 3-phenyl nucleus. Isoflavones exhibit characteristic absorption spectra: a strong absorption band in the region 262 to 270 mμ and sometimes a very weak band in the carbonyl region at 320-360 mμ.



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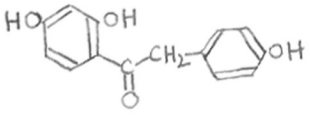

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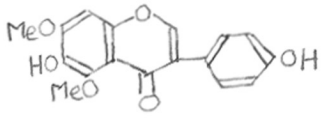

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Seshadri has exploited the Elbs persulphate oxidation of phenols to introduce an additional hydroxyl para to a free hydroxyl in both the flavone and isoflavone series. 5:7-Dihydroxyisoflavone was oxidized in this manner to 5:7:8-trihydroxyisoflavone in about 25% yield, and 8-hydroxy-7-methoxyisoflavone to 5:8-dihydroxy-7-methoxyisoflavone.[36,37]

The 5-hydroxyl group in an isoflavone, as in other chromones and in o-hydroxycarbonyl compounds, is difficult to methylate because of chelation between the hydroxyl and carbonyl groups. It can be methylated, however, by prolonged boiling with acetone, potassium carbonate and dimethyl sulphate. Methyl iodide can also be used as a methylating agent, but it is usually avoided in

view of its tendency to effect nuclear methylation.

Isoflavones containing methoxyl groups are generally demethylated by hydriodic acid, but this is sometimes accompanied by rearrangement, as frequently observed in the flavone series. Thus 5:8-dimethoxy-isoflavone leads to the 5:6-isomer.[38] This is explained on the basis that during demethylation hydrolytic opening of the pyrone ring takes place and that recyclization takes place in the opposite direction. According to Seshadri aluminium chloride in benzene does not bring about any rearrangement during demethylation,[39] but very recently Whalley[40] has shown that 5:7:2'-trimethoxy-8-methylisoflavone gives, during demethylation with aluminium chloride in benzene, both the corresponding trihydroxy compound and its 6-methyl isomer. This observation could not be explained on the basis of the hydrolytic opening of the pyrone ring, and Wheeler[41] has suggested that it may be due to simple migration of the methyl group from position 8 to position 6, since aluminium chloride is well known to produce such migration of alkyl groups.

It was first shown by Bharadwaj and Venkataraman[42] that the 5-methoxyl group in a polymethoxyflavone can

be preferentially demethylated by treatment with aluminium chloride in nitrobenzene solution under controlled conditions; boiling ether and benzene have subsequently been used as suitable solvents.[43] The first application of the partial demethylation technique to isoflavones was made by Iyer, Shah and Venkataraman in connection with the synthesis of prunetin (III).[27]

Seshadri[44] has shown that a 7-methoxyl group in the isoflavone nucleus is more resistant towards demethylation than methoxyl groups in the 5- and 4'-positions. This observation has been utilized in Part II of this thesis for the synthesis of muningin. It has also been shown in the present work that a 7-methoxyl is more resistant towards demethylation than a 6-methoxyl group, and that a methoxyl in the 4'-position in the isoflavone nucleus is more resistant to demethylation than a 6-methoxyl group.

Synthesis of deoxybenzoins

Both as products of hydrolysis of isoflavones and as the essential intermediates for the synthesis of isoflavones, o-hydroxydeoxybenzoins (o-hydroxyphenyl benzyl ketones) are of great importance in

in isoflavone chemistry. These deoxybenzoins are synthesised by making use of normal methods for the preparation of phenolic ketones.

(1) The Hoesch reaction: Resorcinol and similar polyhydric phenols are condensed with an arylaceto-nitrile in ether in presence of hydrochloric acid and zinc chloride.

(2) The Friedel-Crafts reaction: A phenolic ether is treated with phenylacetyl chloride or a substituted phenylacetyl chloride in presence of aluminium chloride. A solvent such as nitrobenzene, carbon disulphide or ether may be used.

(3) Fries rearrangement: The O-phenylacetyl derivatives of phenols do not undergo the Fries migration smoothly, and bad yields are usually obtained.

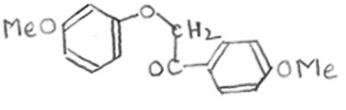
(4) The Nencki reaction between resorcinol (or pyrogallol) and an arylacetic acid, using zinc chloride as condensing agent, can be used for the preparation of deoxybenzoins. Boron trifluoride, which offers several advantages as condensing agent in the Nencki reaction, has received little attention. It has been used solely or mainly for condensations involving acetic acid.[45]

In Parts II and III of this thesis the preparation of ketones from phenylacetic acids and various phenols (resorcinol, pyrogallol, antiarol, 2:6-dimethoxyhydroquinone) in presence of boron trifluoride is described. The procedure proved to be invaluable for the deoxybenzoins from 2:6-dimethoxyhydroquinone, which were inaccessible by other methods. The preparation of various phenolic ketones and chalkones by means of boron trifluoride has also been studied in this laboratory by Mani,[46] and more recently Buu-Hoi and Seailles[47] have used the same condensing agent for the preparation of several phenolic ketones.

Synthesis of isoflavones

The first isoflavone syntheses were due to Baker and Robinson, but these are only of historical interest at the present time because simpler and more practicable methods are now available. Baker and Robinson synthesized 7-methoxyisoflavone by the oxidation of 7-methoxy-2-styryl-isoflavone to the 2-carboxylic acid, followed by decarboxylation.[48] Genistein and daidzein were later synthesised similarly, but in the case of irigenol (5:6:7:3':4':5'-hexahydroxyisoflavone) the

oxidation of the 2-styryl derivative of the hexamethyl ether proved impracticable.[49] Baker, Pollard and Robinson[50] developed a second synthesis of isoflavones involving a Hoesch reaction on the cyanhydrin of an ω-phenoxyacetophenone (IX), which was applied by Späth and Lederer[51] to ψ-baptigenin, but was inadequate for other syntheses in the field.[52] The third synthesis, due to Späth and Lederer, consisted in heating derivatives of 2-hydroxyphenyl benzyl ketone (X) with ethyl formate and sodium in a sealed tube, followed by treatment of the reaction mixture with boiling alcohol and fuming hydrochloric acid in order to effect cyclisation of the oxymethylene compound (XI), assumed to be an intermediate product, to the isoflavone (XII).



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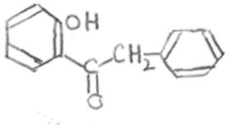

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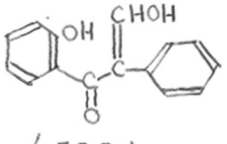

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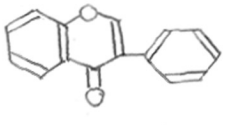

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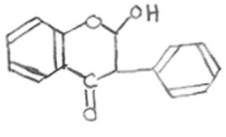
By sublimation in high vacuum, poor yields of the isoflavones (ψ-baptigenin, daidzein and formononetin) were obtained.[23; 51] The action of sodium on an ice-cooled solution of o-hydroxyphenyl benzyl ketones in ethyl formate proceeds, however, in one stage to the isoflavone;[53] a series of isoflavones (isoflavone, the parent member, 7-hydroxyisoflavone, α- and ββ-naphtha-isoflavone, formononetin, daidzein, and ψ-baptigenin, santal, 5:7:2'-trihydroxyisoflavone, 5:7:2'-tri-hydroxy-8-methylisoflavone) have thus been synthesised with simplicity and in excellent yields.

In connection with their work on the osage orange pigments (osajin and pomiferin) Wolfrom et al.[54] in 1941 isolated four compounds of the 2-hydroxyisoflavanone type (XIII) by the condensation of the appropriate deoxybenzoin with ethyl formate and sodium under Joshi and Venkataraman's conditions.[53] They found that these compounds on treatment with glacial acetic acid yielded the corresponding isoflavones. Seshadri[12] claims to have isolated a 2-hydroxyisoflavanone by the ethyl formate reaction of 2-hydroxy-4:6-dimethoxyphenyl 2-methoxybenzyl

ketone, but the present author[11] directly obtained the isoflavone in the same condensation. Under the conditions employed by Venkataraman and his collaborators or by Robertson, Suckling and Whalley[28] there is no evidence that 2-hydroxyisoflavanones (XIII) are isolable as intermediates in the synthesis of isoflavones by means of ethyl formate. From all the available data it appears probable that the reaction can proceed along alternative routes, and further work on the reaction mechanism is necessary.



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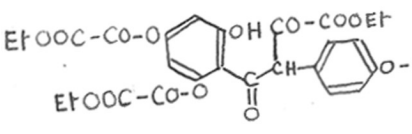
In 1949 Sathe and Venkataraman[55] described a new and very facile synthesis of isoflavones by the action of ethyl orthoformate on o-hydroxyphenyl benzyl ketones in presence of pyridine and piperidine. They prepared by this method 7-hydroxy- and other isoflavones. Iyer et al.[27] used it for the synthesis of prunetin, and it has been employed in this thesis for the synthesis of numerous isoflavones. Bose and

Dutta[56] have reported its use for synthesising 5:7-dihydroxy-4'-nitroisoflavone, obtained in 20% yield. The ethyl orthoformate reaction proceeds smoothly with deoxybenzoins derived from phenol, hydroquinone, resorcinol, pyrogallol, 2:6-dimethoxyhydroquinone and antiarol, but not with phloroglucinol derivatives because of a tendency for the formation of polymeric compounds by the intervention of methine groups.

Baker et al.[57] in 1952 reported the use of ethoxalyl chloride for supplying the 2-carbon atom



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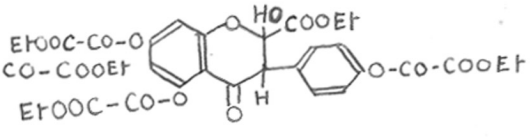

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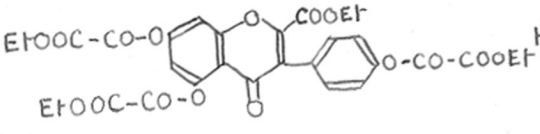

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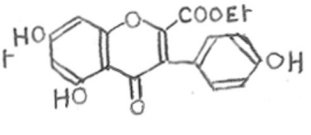


(XVII)

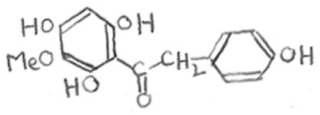
which is necessary for the formation of isoflavones from deoxybenzoins. By this method they obtained an

isoflavone 2-carboxylic esters (XVI) which was then hydrolysed and decarboxylated to give the isoflavone.

They have also investigated the mechanism of this reaction; ethoxylation of all the free hydroxyl groups except one hydroxyl ortho to the carbonyl first takes place; the reactive methylene group is then attacked to give the keto ester (XIV) which cyclizes to a 2-hydroxyisoflavone 2-carboxylic ester (XV). This ester loses a molecule of water to form the isoflavone 2-carboxylic ester (XVI). The ethoxalyl groups are then removed by the action of acid to give the corresponding polyhydroxy-isoflavone 2-carboxylic ester (XVII).

Baker's ethoxyalyl chloride reaction is a useful supplement to the ethyl formate and orthoformate methods and has the advantage that protection of hydroxyl groups is not necessary, but the latter may be exaggerated. There is no real difficulty in protecting hydroxyl groups, a variety of procedures employing dimethyl sulphate, arylsulphonyl chlorides, dihydropyran, etc., being available. With the possible exception of dehydro-homoferreirin, which needs to be verified, no example has yet been cited by Baker, Whalley or others in which an isoflavone

cannot be synthesized by the ethyl formate or ethyl orthoformate methods, provided the appropriate deoxybenzoin can be prepared. It is of interest to refer in this connection to Baker's attempted synthesis of tectorigenin by the action of ethoxalyl chloride on the deoxybenzoin (XVIII). Baker found that cyclization took place, but not in the desired direction, and the product was (XIX). In a private communication to Professor Venkataraman, Baker and Ollis state that the action of ethyl orthoformate on the dibenzyl ether of (XVIII) results in cyclization in the desired direction, leading to a synthesis of tectorigenin.



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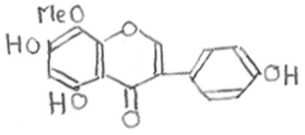

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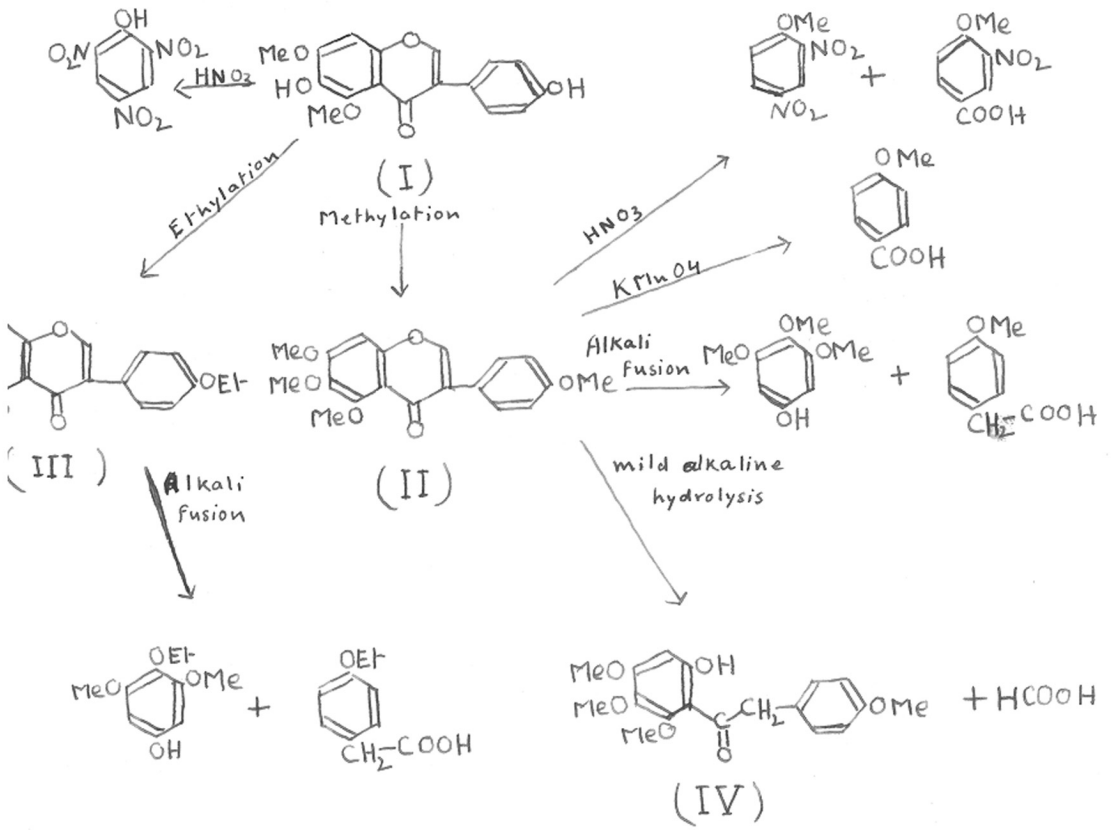
Part II

A SYNTHESIS OF MUNINGIN AND ITS DERIVATIVES

King, King and Warwick[1] isolated from the heartwood of Pterocarpus angolensis a colourless compound, which was very sparingly soluble in ether and chloroform, and slightly soluble in dioxane, ethanol, acetone and acetic acid. Crystallization from dioxane gave colourless minute hexagonal plates, m.p. 285° (decomp.). Reduction with magnesium and hydrochloric acid did not give any colour, but a pink colour developed on acidifying an alcoholic solution of muningin previously reduced by sodium amalgam. With hydrobromic acid an easily hydrolysable salt was obtained. Molecular weight determination, X-ray crystallographic measurements and the elementary analysis corresponded to the formula $C_{17}H_{14}O_6$. The formation of a dimethyl ether, m.p. 176°, a diethyl ether, m.p. 156°, a diacetate, m.p.232-33°, and a dibenzoate, m.p. 180°, indicated the presence of two hydroxyl groups. The isolation of picric acid from the vigorous reaction of concentrated nitric acid with muningin, and of a mixture of dinitroanisole and 4-methoxy-3-nitrobenzoic acid

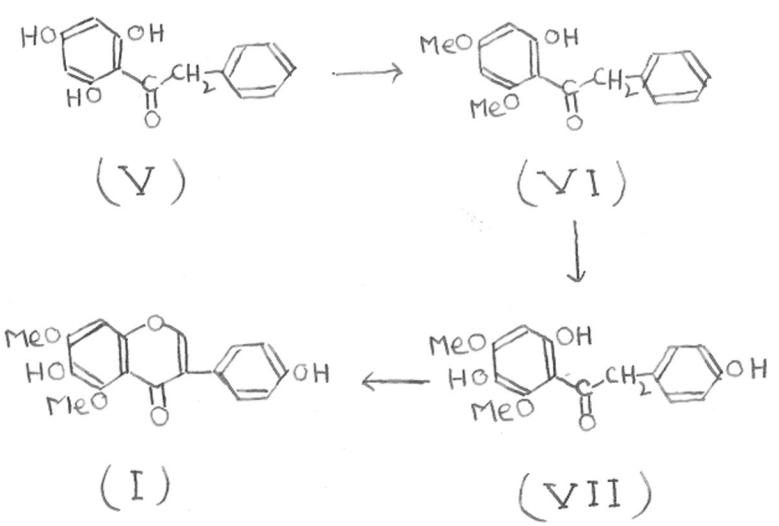
from the action of this reagent on the dimethyl ether of muningin, indicated the presence of a p-hydroxyphenyl group. Permanganate oxidation of the dimethyl ether of muningin gave anisic acid. On mild alkaline hydrolysis of the methyl ether formic acid was liberated, and the resulting crystalline product was a ketone which readily formed a semicarbazone. More vigorous alkali fusion of muningin dimethyl ether (II) led to p-methoxyphenylacetic acid and antiarol. These results and the recyclization of the ketonic product (IV) obtained by mild alkaline hydrolysis with sodium and ethyl formate clearly indicated the presence of an isoflavone nucleus, having hydroxyl and methoxyl groups in the 5:6:7 and 4'-positions. Since the oxidation experiments showed the presence of a p-hydroxyphenyl group, one hydroxyl group was in the 4'-position. Muningin did not give any colouration with ferric chloride, and a hydroxyl group in the 5-position was therefore excluded. Hence the second hydroxyl group was in the 6- or 7-position. The former alternative was shown to be correct, when alkali fusion

of the diethyl ether of muningin (III) gave 4-ethoxy-3:5-dimethoxyphenol. The structure of muningin was thus proved to be 5:7-dimethoxy-6:4'-dihydroxyisoflavone (I).



Before King's discovery of muningin no exception to Bose's generalization,[2] that in partially methylated polyhydroxyflavones occurring in nature a methoxyl group is never found in the 5-position, was noticed in the flavone or isoflavone series.

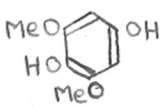
Prunusetin, which was regarded by Chakravarti[3] as 7:4-dihydroxy-5-methoxyisoflavone, has been shown by Narasimhachari and Seshadri[4] to be identical with prunetin, 5:4'-dihydroxy-7-methoxy-isoflavone. Although isopedicin (6-hydroxy-5:7:8-trimethoxyisoflavanone), an example of such a type, has been encountered among flavanones,[5] it seemed desirable to confirm by an unambiguous synthesis the structure assigned by King to muningin. There is no difficulty in visualising a biogenetic scheme, such as the following, for the conversion of a phloroglucinol derivative into muningin.



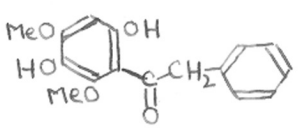
(V) (VI) (VII) (I)

As a preliminary to the synthesis of muningin, the synthesis of the unknown 6-hydroxy-5:7-dimethoxyisoflavone was first undertaken. As stated by Chapman, Perkin and Robinson,[6] 2:6-dimethoxyhydroquinone (VIII) was found to be unreactive in the Friedel-Crafts and Hoesch reactions. Mauthner[7] prepared 2:5-dihydroxy-4:6-dimethoxy-acetophenone by the Fries migration of the diacetate of (VIII), but a similar method has not proved to be feasible for the preparation of 2:5-dihydroxy-4:6-dimethoxyphenyl benzyl ketone (IX) and other derivatives of phenylacetic acids. The bis-phenylacetyl and bis-p-nitrophenylacetyl esters of 2:6-dimethoxyhydroquinone, required for studying the applicability of the Fries migration, were prepared by treating the phenol (VIII) with the appropriate acid chloride in boron trifluoride-ether complex. The ketone (IX), m.p. 108°, was ultimately obtained by the interaction of 2:6-dimethoxyhydroquinone (VIII) and the boron trifluoride complex of phenylacetic acid.[8] It was isolated as an oil in the beginning and was difficult to crystallize. The difficulty was traced to the presence of the monophenylacetyl

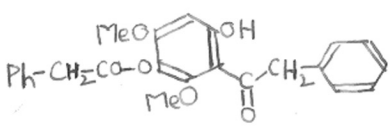
ester (X) of the ketone (IX), which was simultaneously formed during the boron trifluoride condensation. The ester (X), m.p. 105°, and the ketone, m.p. 108°, were separated by fractional crystallization. The ester (X) could be readily hydrolysed by aqueous sodium hydroxide in the cold to give the ketone (IX). This ketone has also been prepared by the persulphate oxidation of 2-hydroxy-4:6-dimethoxyphenyl benzyl ketone, but in extremely poor yield. The ketone (IX), on treatment with ethyl orthoformate, gave 5:7-dimethoxy-6-hydroxyisoflavone (XI), m.p. 186-87°, which on demethylation with hydriodic acid gave 5:6:7-trihydroxyisoflavone (XII), m.p. 189-90°.



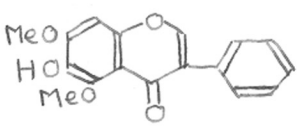
(VIII)



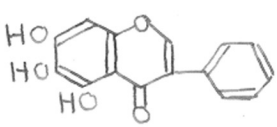
(IX)



(X)



(XI)



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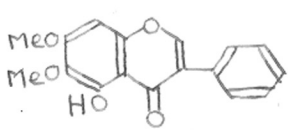
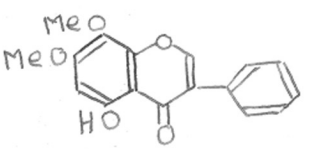
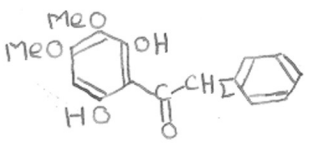


(XIII)

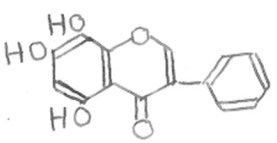
The possibility of rearrangement during demethylation of (XI) was eliminated by remethylating the compound (XII) with dimethyl sulphate, when 5:6:7-trimethoxyisoflavone, m.p. 149°, identical with the compound prepared by a different method described elsewhere in this thesis, was obtained. The compound (XII) has also been described, after the publication of the present author's work, by Mahesh and Seshadri.[9] The m.p. quoted by them is 282-83° (decomp.). They obtained it by the hydriodic acid demethylation of 5-hydroxy-6:7-dimethoxyisoflavone (XIII), which was prepared from 5-hydroxy-7:8-dimethoxyisoflavone (XIV) by alkaline hydrolysis to the ketone (XV) and cyclization with ethyl formate. It was assumed by Mahesh and Seshadri that cyclization took place in the direction leading to (XIII) because the product



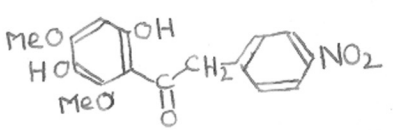
(XIV)



(XV)



(XVI)

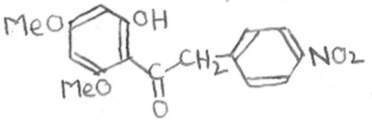


(XVII)

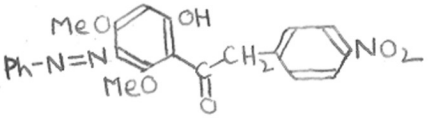
was different from (XIV). However, it may be recorded that the m.p. of 5:6:7-trihydroxyisoflavone found by Mahesh and Seshadri is very close to that of 5:7:8-trihydroxyisoflavone (XVI).

Attempts were then made to condense (VIII) with p-nitrophenylacetic acid under a variety of conditions, using boron trifluoride, as well as other condensing agents, but the corresponding deoxybenzoin (XVII) was not obtained; the only products were 2:6-dimethoxybenzoquinone and the bis-p-nitrophenylacetate of (VIII), apart from intractable tarry material. Unsuccessful attempts were also made to prepare the analogues of (XVII) from p-benzyloxy-, p-tosyloxy-, and p-phenylazophenylacetic acid. These deoxybenzoins would have led to an unambiguous synthesis of muningin, but they proved to be inaccessible.

An attempt to couple the ketone (XVIII) under various conditions with diazonium salts failed; the object was to prepare (XIX) and convert it by reduction, diazotization and hydrolysis to the ketone (XX).

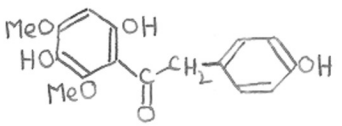


(XVIII)

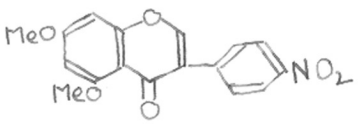


(XIX)

Persulphate oxidation of the ketone (XVIII) could not be carried out, since it was sensitive to alkali.

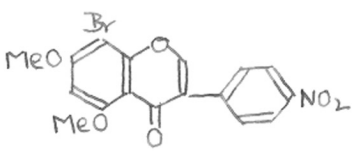


(XX)

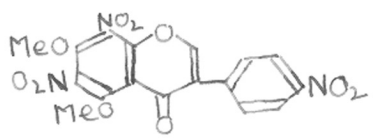


(XXI)

In an attempt to get the nitro group in the 6- and 4'-positions of the isoflavone nucleus, 5:7-dimethoxy-4'-nitroisoflavone[10] (XXI) was monobrominated and the 8-bromo derivative (XXII), m.p. 275°, was nitrated. The product, m.p. 225-26°, gave a



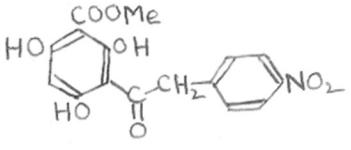
(XXII)



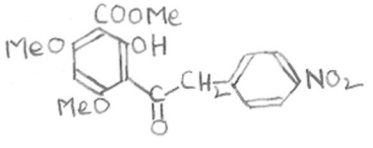
(XXII A)

negative test for bromine, and analysed for the trinitroisoflavone (XXIIA). Instead of the 8-bromo derivative, the 8-carbomethoxy derivative was then prepared in the following manner. Methyl

phloroglucinol carboxylate was condensed with p-nitrobenzyl cyanide by the Hoesch method to give the ketone (XXIII), m.p. 185-86°. This ketone on dimethylation gave (XXIV), m.p. 173-74°, which on treatment with ethyl orthoformate, pyridine and ~~x~~ piperidine cyclized to the isoflavone (XXV). Nitration of (XXV) gave a poor yield of a product which could not be purified.



(XXIII)



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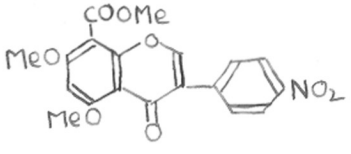
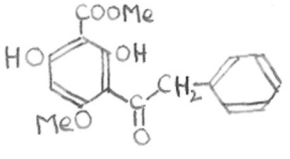
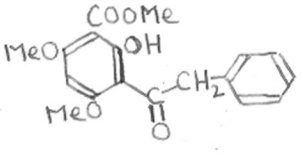


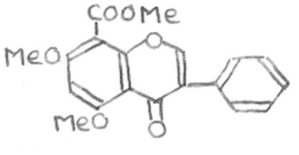
(XXV)



(XXVI)



(XXVII)



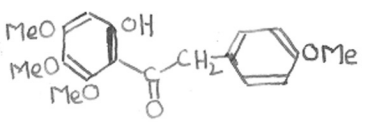
(XXIII)

Methyl 4-O-methylphloroglucinol carboxylate did not condense with p-nitrobenzyl cyanide or the corresponding acid, but it condensed with phenylacetic acid and boron trifluoride complex to give the ketone (XXVI), m.p. 149°, which on selective methylation gave (XXVII), m.p. 133°. The ethyl formate cyclization of (XXVII) gave the isoflavone (XXVIII), m.p. 166-68°. Nitration of (XXVIII) did not yield a pure, homogeneous product.

After having failed in all the above methods, a route involving partial demethylation, selective benzoylation or tosylation, methylation, and debenzoylation or detosylation was attempted. For this purpose 2:6-dimethoxyhydroquinone (VIII) was converted to antiarol (XXIX), which was condensed by the boron trifluoride method with p-methoxyphenylacetic acid to give the ketone (IV), m.p.69°. p-Methoxyphenylacetic acid was obtained by the direct oxidation of p-methoxyphenylpyruvic acid.[11] King et al.[1] and Seshadri et al.[12] have quoted the melting points of the ketone (IV) as 73° and 93° respectively. Seshadri has prepared this ketone from antiarol and p-methoxyphenylacetyl chloride by the Friedel-Crafts reaction using

anhydrous aluminium chloride in dry ether. On treating the ketone (IV) with ethyl orthoformate, pyridine and piperidine, 5:6:7:4'-tetramethoxy-isoflavone (II), m.p. 176°, was obtained. This m.p. agrees with the m.p. quoted by Seshadri,[12] and with the m.p. quoted by King[1] for the dimethyl ether of muningin.

The compound (II), on treatment with hydrobromic acid in acetic acid at 100°, gave 5-hydroxy-6:7:4'-trimethoxyisoflavone (XXX), m.p. 186° (King *et al.*[1] 186°). Further treatment with excess of hydrobromic



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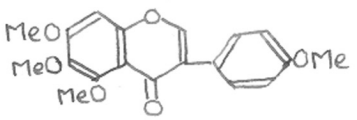
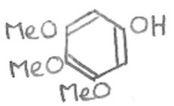
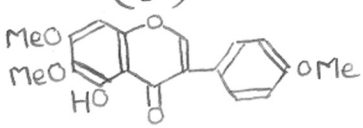


(II)



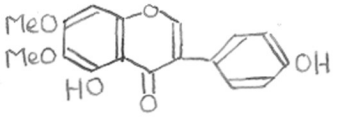
(XXIX)



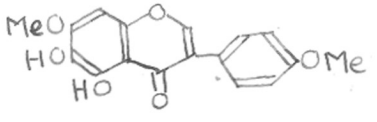
(XXX)

acid and acetic acid on a boiling water-bath resulted in a new compound, m.p. 218-20°. Its analysis showed that it was a dihydroxydimethoxy-isoflavone. As the 7-methoxyl group has been shown by Seshadri[13] to be stable under the above conditions of demethylation, the isoflavone, m.p. 218-20°, can be either 5:4'-dihydroxy 6:7-

dimethoxyisoflavone (XXXI) or 5:6-dihydroxy-7:4'-dimethoxyisoflavone (XXXII). It was proved to



(XXXI)



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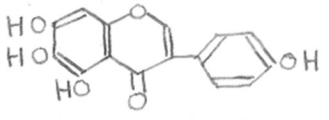


(XXXIII)

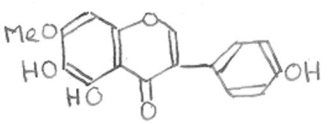
to be (XXXII) as its properties agreed with those of 5:6-dihydroxy-7:4'-dimethoxyisoflavone, prepared by the partial demethylation of 6-hydroxy-5:7:4'-trimethoxyisoflavone (XLII).

Demethylation of (II) with hydriodic acid in acetic acid and acetic anhydride during 4 hours gave 5:6:7:4'-tetrahydroxyisoflavone (XXXIII), m.p. 275° (decomp.) for the substance crystallizing with 1 H_2O. After sublimation at 0.2 mm. this isoflavone (XXXIII), which resembled baicalein and 5:6:7-trihydroxyisoflavone[8] in giving a flocculent bluish green precipitate by the addition of a drop of aqueous caustic soda to an alcoholic solution, melted at 280° (decomp.) and analysed for $C_{15}H_{10}O_6$, $2H_2O$.

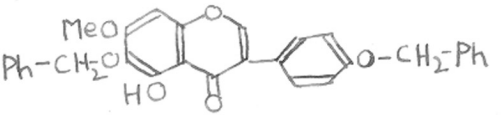
Muningin does not appear to have been demethylated to (XXXIII). The acetyl derivative, analysing correctly for a tetra-acetoxyisoflavone, melted at 205° (after shrinking at 197°) or 227° according as it was prepared from the crystallized or sublimed sample of (XXXIII). Methylation of (XXXIII), before or after sublimation, with potassium carbonate and dimethyl sulphate yielded the tetramethyl ether (II), m.p. 176°, from which (XXXIII) had been prepared by treatment with hydriodic acid.

When the tetramethoxyisoflavone (II), m.p. 176°, was heated with hydriodic acid and acetic anhydride at 120° for half an hour, a new compound, m.p.263-64° (decomp.) , was obtained. Its analysis showed that it was a trihydroxymonomethoxyisoflavone. The structure of this isoflavone can only be (XXXIV) in view of the relative stability of the 7-methoxyl group.[13] With alkali it gave the Bargellini reaction, which is therefore not restricted to chromones containing hydroxyls in the 5:6:7-positions. Seshadri has recorded that herbacetin (3:5:7:8:4'-pentahydroxyflavone) responds to the Bargellini test.[13a] The preparation of this isoflavone

by the partial demethylation of muningin has been



(XXXIV)



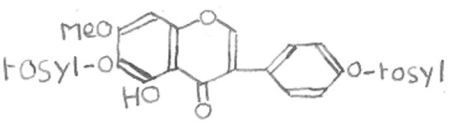
(XXXV)

reported by King & *et al.*[1] but the m.p. quoted by them is 257° (*decomp.*)

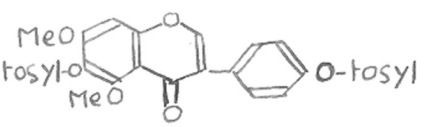
The isoflavone (XXXIV) could not be selectively dibenzylated to (XXXV) with benzyl chloride, potassium carbonate and acetone. When exactly two moles of benzyl chloride were used, a compound, m.p. 155°, was obtained, which analysed nearly for the monobenzyl derivative. When excess of benzyl chloride was used, the product, m.p. 161°, was insoluble in caustic soda, had no ferric chloride colouration, and analysed for the tribenzyl derivative.

On treating (XXXIV) with two moles of *p*-toluenesulphonyl chloride a ditosyl derivative (XXXVI), m.p. 185°, was obtained in poor yield. This compound on methylation gave the ditosyl derivative (XXXVII), m.p.205°, of muningin. Its mixed m.p. with the ditosyl derivative prepared

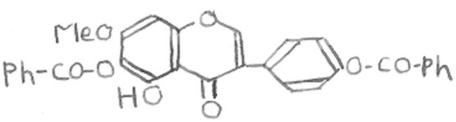
from natural muningin showed no depression. Attempts to detosylate the ditosyl derivative under various conditions failed. It may be mentioned, however, that 7:8-ditosyloxyisoflavone could be detosylated to 7:8-dihydroxyisoflavone with concentrated sulphuric acid.



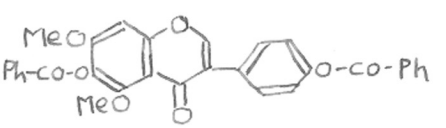
(XXXVI)



(XXXVII)



(XXXVIII)



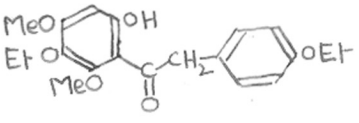
(XXXIX)

Compound (XXXIV)on treatment with two moles of benzoyl chloride in pyridine gave the dibenzoyl derivative (XXXVIII), m.p.187°, in extremely poor yield. Considerable improvement in the yield was noticed when the reaction was carried out in acetone solution in presence of potassium carbonate. Methylation of (XXXVIII) by means of dimethyl sulphate

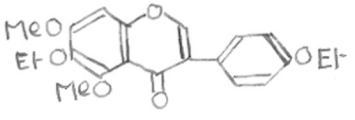
and potassium carbonate in acetone gave 6:4'-dibenzoyloxy-5:7-dimethoxyisoflavone (XXXIX), m.p. 195°. The m.p. recorded by King[1] for the O-dibenzoyl derivative of natural muningin is 180°; but when natural muningin, kindly supplied by Professor F. E. King, was benzoylated by the acetone-potassium carbonate method, the product melted at 195°, not depressed by mixing with (XXXIX). The ester (XXXIX) was finally hydrolysed to muningin (I), m.p. 285° (decomp.), by shaking with 2% methanolic caustic potash (4 mol.) for about an hour at room temperature (25-28°). The mixed m.p. with the natural sample showed no depression, and all the other properties of the natural and synthetic substances were identical.

The diethyl derivative of muningin (III) has also been synthesized independently. 3:5-Dimethoxy-4-ethoxyphenol was condensed with p-ethoxyphenylacetic acid, using boron trifluoride as a catalyst, to give an oily ketone (XL), which could be crystallized (m.p.105°) with very great difficulty from ethyl acetate. Krishnamurti and Seshadri[14], m.p. 105°) The ketone (XL) on treatment with ethyl orthoformate, pyridine and a few drops of piperidine gave diethyl ether of muningin (III), m.p. 160°

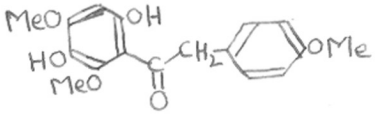
(King[1], m.p. 156°). The mixed m.p. was found to be 151-53°. After the present work was completed Seshadri[14] described the preparation of the ketone (XL) by the Friedel-Crafts reaction and the isoflavone (III) by ethyl formate cyclization; for (III) he quotes the m.p. 156-58°.



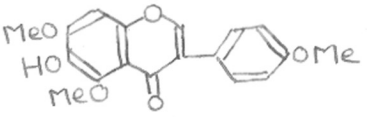
(XL)



(III)



(XLI)



(XLII)

The ketone (XLI) was prepared by condensing 2:6-dimethoxyhydroquinone (VIII) with p-methoxyphenylacetic acid-boron trifluoride complex. It crystallized from hexane in yellow needles, m.p. 110°. On treating (XLI) with ethyl orthoformate muningin 4'-methyl ether (XLII), m.p. 177°, was obtained. The O-acetyl derivative melted at 213°. The isoflavone

(XLII) on treatment with hydrobromic acid in acetic acid gave (XXXII), m.p.218-20°, identical with the compound obtained by treating compound (XXX) with excess of hydrobromic acid in acetic acid on a boiling water-bath.

EXPERIMENTAL

2-Hydroxy-4:6-dimethoxy-5-phenylacetoxyphenyl benzyl ketone (X):

Phenylacetic acid (5 g.) was dissolved in dry chloroform (8 c.c.), and a stream of boron trifluoride was passed into the solution cooled to 10-15°. After the separation of the phenylacetic acid-boron trifluoride complex, 2:6-dimethoxyhydroxyquinone (VIII) (1 g.) was added. The colour of the solution immediately turned yellow. Boron trifluoride was again passed for 15 minutes, and the reaction mixture was left overnight at room temperature (25-28°). The clear yellow solution was added to crushed ice and left for two hours. It was then extracted with ether, and the ether-chloroform washed with sodium bicarbonate solution and then with water. The ether-chloroform extract was dried over anhydrous sodium sulphate and the solvents removed by distillation, when a yellow oil (1.75 g.) was obtained. This was distilled at 1 mm. and the fraction coming over at 230-50° (air bath temperature) was dissolved in the minimum amount of hot absolute alcohol and was left overnight at room temperature. After storage in a

refrigerator for a few weeks, colourless needles separated. Recrystallization from dilute methyl or ethyl alcohol yielded colourless needles, m.p. 105° (Found: C, 71.0; H, 5.7. $C_{24}H_{22}O_6$ requires C, 70.9; H, 5.5%). The substance gives a reddish brown colouration with alcoholic ferric chloride.

2:5-Dihydroxy-4:6-dimethoxyphenyl benzyl ketone (IX):

Method (a): The above substance, m.p. 105°, was heated with 10% alcoholic potassium hydroxide for 2 hours on a water-bath. After dilution with water, removal of alcohol and acidification with conc. hydrochloric acid, the solution was extracted with ether. Bicarbonate washings of the ether layer of acidification gave phenylacetic acid. The ether layer yielded an oil which crystallized from dilute alcohol in yellow prismatic needles, m.p. 108° (Found: C, 66.9; H, 5.8. $C_{16}H_{16}O_5$ requires C, 66.7; H, 5.6%). The mixed m.p. with the monophenylacetate described above was considerably depressed.

Method (b): The condensation of phenylacetic acid (20 g.) and 2:6-dimethoxyhydroquinone (5 g.) was carried out as described above. The chloroform-ether layer was washed with sodium bicarbonate solution and

then with aqueous caustic soda (500 ml.). The caustic soda extract was acidified with conc. hydrochloric acid and then thoroughly extracted with ether. The oily residue from the ether layer solidified on keeping (7 g.) and crystallized from dilute alcohol in stout prismatic needles, m.p. 108°. No depression in the m.p. was observed when mixed with the substance obtained by method (a). The substance gives a green colour with ferric chloride, which immediately changes to yellows and finally to brownish red.

Method (c): 2-Hydroxy-4:6-dimethoxyphenyl benzyl ketone (1.36 g.) was dissolved in pyridine (5 ml.), and caustic potash (1.4 g. in 14 ml. of water) was added. The solution was cooled to 10°, and treated with 5% aqueous potassium persulphate (25 ml.) during 3 hours with stirring. The reaction mixture was left overnight at room temperature, made acidic to Congo Red, the precipitate filtered and crystallized from dilute alcohol. The m.p. and mixed m.p. showed that this product was the starting material (0.3 g.). After extracting the filtrate with ether it was made strongly acidic with conc. hydrochloric acid (20 ml.), heated on a boiling water-bath for 1 hour with the addition

of a little sodium sulphite, cooled, and extracted with ether. The ether extract yielded an oil, which distilled at 220-40°/1 mm. (0.12 g.). When it was kept in contact with petroleum ether in a refrigerator for a long time, it crystallized in yellow needles, m.p. 108° (Found: C, 66.6; H, 5.6. $C_{16}H_{16}O_5$ requires C, 66.7; H, 5.6%). The mixed m.p. with the ketone obtained by the boron trifluoride method showed no depression.

6-Hydroxy-5:7-dimethoxyisoflavone (XI):

The ketone (2.5 g.) was dissolved in ethyl orthoformate (3.6 g., 3 mols.) and dry pyridine (20 c.c.). The colour of the solution was yellow. After the addition of piperidine (6 drops) the colour changed to faint brownish yellow. On boiling for 1 hour the colour changed to faint yellow and finally to brownish red. After boiling for 5 hours, the solution was cooled, poured over ice and hydrochloric acid and left overnight. The precipitate was collected (2.5 g.), washed and crystallized from alcohol. The colourless needles had m.p. 186-87° (Found: C, 68.4; H, 4.6. $C_{17}H_{14}O_5$ requires C, 68.4; H, 4.7%). The substance gives a bright yellowish green colour with

ferric chloride. It dissolves in conc. sulphuric acid with a very pale yellow colour and in aqueous sodium hydroxide to form a yellow solution. Acetylation in the usual manner by refluxing with acetic anhydride and a drop of pyridine gave the acetyl derivative which crystallized from dilute alcohol in colourless needles, m.p. 203-4° (Found: C, 66.8; H, 5.1. $C_{19}H_{16}O_6$ requires C, 67.1; H, 4.7%).

5:6:7-Trihydroxyisoflavone (XII):

The dimethyl ether (XI) (0.5 g.) was heated with acetic acid (1 c.c.), acetic anhydride (1 c.c.) and hydriodic acid (d. 1.7; 1 c.c.) at the boil for 4 hours, cooled, diluted with water and treated with sodium bisulphite to destroy free iodine. The precipitate (0.4 g.) was collected, and it crystallized from dilute alcohol in clusters of colourless needles, m.p. 189-90° (Found: C, 66.6; H, 3.6. $C_{15}H_{10}O_5$ requires C, 66.7; H, 3.7%). The substance gives an intense olive green colouration with ferric chloride. It dissolves in concentrated sulphuric acid with a yellow colour. With aqueous sodium hydroxide it forms a greenish yellow solution, and on shaking a flocculent dark bluish green precipitate separates immediately. A dark bluish green

flocculent precipitate separates immediately when a drop of sodium hydroxide solution is added to an alcoholic solution of the isoflavone.

The triacetyl derivative crystallized from alcohol in colourless needles, m.p. 178° after sintering at 170° (Found: C, 63.4; H, 4.5. $C_{21}H_{16}O_8$ requires C, 63.6; H, 4.4%).

5:6:7-Trimethoxyisoflavone:

A solution of the above trihydroxyisoflavone (0.1 g.) in acetone (15 c.c.) was treated with dimethyl sulphate (0.2 c.c.) and potassium carbonate (0.4 g.). The mixture was refluxed for 8 hours. Acetone was evaporated and potassium carbonate was dissolved in water (20 c.c.). The solid which separated was filtered and crystallized from dilute alcohol in colourless needles, m.p. 149°. Its mixed m.p., with the compound obtained by cyclizing 2-hydroxy-4:5:6-trimethoxyphenyl benzyl ketone with ethyl orthoformate, described in the next part, showed no depression.

2-Hydroxy-4:5:6-trimethoxyphenyl 4'-methoxybenzyl ketone (IV):

p-Methoxyphenylacetic acid (1.2 g.) was dissolved in dry alcohol-free chloroform (20 ml.) and the ice-

cooled solution was saturated with boron trifluoride. To the red oily complex antiarol (0.6 g.) was added. The reaction mixture was left overnight at room temperature. Crushed ice was then added and the mixture extracted with ether. The ether-chloroform layer was washed with sodium bicarbonate solution and then with water, dried over anhydrous sodium sulphate, and the solvents distilled off. The residue was extracted with hexane and the insoluble portion proved to be antiarol. The hexane solution led to a pale yellow oil, which distilled at 210-20°/2 mm. (0.4 g.). When the oil was kept in contact with petroleum ether at -5° for two days, it solidified, and then crystallized from the same solvent in colourless plates, m.p. 69° (King[1] and Seshadri[12] quote m.p.s 73° and 96°) (Found: C, 65.2; H, 6.3. $C_{18}H_{20}O_6$ requires C, 65.1; H, 6.1%). The alcoholic solution gives a greenish brown colour with ferric chloride.

5:6:7:4'-Tetramethoxyisoflavone (II):

On the addition of ethyl orthoformate (5 ml.) and piperidine (10 drops) to a solution of the ketone (IV) (2 g.) in pyridine (20 ml.), a pink colour developed, which turned to green, greenish yellow and finally

yellowish brown when the solution was refluxed for 5 hours. The product obtained by treatment with ice and hydrochloric acid crystallized from methanol in colourless needles (1.4 g.), m.p. 176° (Found: C, 67.0; H, 5.6. $C_{19}H_{18}O_6$ requires C, 66.7; H, 5.3%). (King[1] and Seshadri[12] quote the same m.p.).

5-Hydroxy-6:7:4'-trimethoxyisoflavone (XXX):

Method (a): The above tetramethyl ether (0.5 g.) was heated with glacial acetic acid (2.5 ml.) and hydrobormic acid (b.p. 126°; 7.5 c.c.) at 100°. As soon as crystals started separating from the clear solution, heating was discontinued, and the product was collected after cooling (0.42 g.). It crystallized from dilute acetic acid in colourless needles, m.p. 186° (King[1] m.p. 186°) (Found: C, 66.2; H, 5.1. $C_{18}H_{16}O_6$ requires C, 65.9; H, 4.9%). The ferric coloration is green.

Method (b): Methylation of (XXXIII) (0.1 g.) in acetone, with anhydrous potassium carbonate (0.4 g.) dimethyl sulphate (1 mol., 0.033 c.c. or 3 mols.) for 1/2 hour resulted in a compound, m.p. 186°. Mixed m.p. with the substance obtained in method (a) showed no depression.

5:6-Dihydroxy-7:4'-dimethoxyisoflavone (XXXII):

The above isoflavone (XXX) (0.2 g.) was heated on a boiling water bath with a mixture of constant boiling hydrobromic acid (b.p. 126°, 4 c.c.) and acetic acid (4 c.c.) for 3 hours. Slowly the isoflavone went in solution. The solution was cooled and poured into water. The precipitate was filtered and crystallized from alcohol or dilute acetic acid at room temperature in yellow prisms, m.p. 218-20° (Found: C, 65.0; H, 4.8. $C_{17}H_{14}O_6$ requires C, 65.0; H, 4.5%). Its mixed m.p. with the compound obtained by the partial demethylation of (XLII) showed no depression.

5:6:4'-Trihydroxy-7-methoxyisoflavone (XXXIV):

5:6:7:4'-Tetramethoxyisoflavone (0.5 g.) was heated with acetic anhydride (5 ml.) and freshly distilled hydriodic acid (5 ml.) at 120° for half an hour. Worked up as usual, the product (0.4 g.) was boiled with water (200 ml.); the insoluble portion crystallized from glacial acetic acid in yellow prisms, m.p. 263-64° (decomp.) after shrinking at 258° (Found: C, 64.2; H, 4.1; OMe, 9.9. $C_{16}H_{12}O_6$ requires C, 64.0; H, 4.0; OMe, 10.3%). The m.p. quoted by King[1] is 257° (decomp.). The ferric reaction is green.

6:4'-Dibenzoyloxy-5-hydroxy-7-methoxyisoflavone (XXXVIII):

5:6:4'-Trihydroxy-7-methoxyisoflavone (XXXIV) (0.6 g.) in boiling acetone (300 c.c.) was treated with benzoyl chloride (2 mols., 0.46 c.c.) and freshly ignited potassium carbonate (2 g.) during 5 hours. Potassium carbonate was filtered off and washed with hot acetone. Distillation of the acetone solution led to a greenish residue, which was refluxed with alcohol (Norit) and filtered. The filtrate concentrated to a small volume and left at room temperature overnight. The colourless needles which separated (0.4 g.) melted at 185-86° (Found: C, 71.2; H, 4.2. $C_{30}H_{20}O_8$ requires C, 70.9; H, 3.9%). An alcoholic solution of the substance gives a brown colour with ferric chloride.

6:4'-Dibenzoyloxy-5:7-dimethoxyisoflavone (XXXIX):

The above isoflavone (XXXVIII) (0.4 g.) in boiling acetone (200 c.c.) was treated with potassium carbonate (2 g.) and dimethyl sulphate (0.5 c.c.) for 12 hours. Acetone was recovered and water added to the residue. The precipitate was filtered and washed with water. It crystallized from alcohol in flat colourless needles, m.p. 185° (Found in material dried at 110°/1 mm.: C, 71.5; H, 4.5. $C_{31}H_{22}O_8$ requires C, 71.2;

H, 4.2%). When a sample of natural muningin was methylated similarly by the acetone-potassium carbonate method, the product crystallized from alcohol in flat colourless needles, m.p. 195°, not depressed by mixing with (XXXIX). King, et al.[1], quote m.p. 180° for the O-dibenzoyl derivative of muningin prepared by means of benzoyl chloride and aqueous caustic soda.

Muningin (I):

A suspension of 6:4'-dibenzoyloxy-5:7-dimethoxy-isoflavone (XXXIX) (0.2 g.) in 2% methanolic caustic potash (5 c.c.) was mechanically shaken at room temperature (30°) for 1 hour, when a clear yellow solution resulted. After dilution with water (10 c.c.) and acidification with conc. hydrochloric acid, the colourless needles which separated were collected. washed with sodium bicarbonate and filtered. As described by King[1] for natural muningin, synthetic muningin crystallized from dioxane in colourless minute hexagonal plates; the m.p. was 285° (decomp.). The m.p. of natural muningin being 285° (decomp.). The mixed m.p. was 285-86° (Found: in material dried at 120°/1 mm.: C, 64.7; H, 4.8; $C_{17}H_{14}O_6$ requires C, 65.0; H, 4.5%). The substance gives no colouration with ferric chloride. It dissolves in sulphuric acid

with a yellow colour, which turns deep red on the addition of a drop of conc. nitric acid. It dissolves in aqueous sodium hydroxide with a yellow colour.

5-Hydroxy-7-methoxy-6:4'-ditosyloxyisoflavone (XXXVI):

The monomethyl ether (XXXIV) (0.35 g.) in boiling acetone (150 c.c.) was treated with p-toluenesulphonyl chloride (0.39 g.; 2 mols.) and anhydrous potassium carbonate (1 g.) during 3 hours. On working up the product as described for the benzoyl analogue, and crystallizing thrice from alcohol, pale brown prisms, m.p. 185° were obtained (Found: C, 59.6; H, 4.2. $C_{30}H_{24}O_{10}S_2$ requires C, 59.2; H, 4.0%). An alcoholic solution of the substance gives a reddish brown colour with ferric chloride.

5:7-Dimethoxy-6:4'-ditosyloxyisoflavone (XXXVII):

An acetone solution of the above isoflavone (0.1 g.) was refluxed for 18 hours with potassium carbonate (1 g.) and dimethyl sulphate (0.6 c.c.). Acetone was removed by distillation, and water was added. The oily product solidified partly on leaving in a refrigerator, and crystallization from dilute acetic acid gave colourless prismatic needles, m.p. 204° (Found: C, 60.1;

H, 4.2. $C_{31}H_{26}O_{11}S_2$ requires C, 59.8; H, 4.2%). The ditosylate prepared similarly from natural muningin had the same m.p. and mixed m.p.

5:6:7:4'-Tetrahydroxyisoflavone (XXXIII):

5:6:7:4'-Tetramethoxyisoflavone (0.1 g.) was refluxed with acetic anhydride (1 c.c.), acetic acid (1 c.c.) and hydriodic acid (d. 1.7; 1 ml.) for 4 hours. After dilution with aqueous sodium bisulphite and cooling, the product (70 mg.) was crystallized from dilute alcohol. The pale yellow needles had m.p. 275° (decomp.) (Found: in material dried at 130°/2 mm. for 2 hours: C, 59.4; H, 4.1. $C_{15}H_{10}O_6$, H_2O requires C, 59.2; H, 4.0%). The substance gives a deep green colour changing to greenish brown with alcoholic ferric chloride. A green colouration, followed by a bluish green precipitate, appears when a drop of aqueous caustic soda is added to an alcoholic solution of the substance. The acetyl derivative, prepared in the usual manner, crystallized from dilute acetic acid in colourless prisms, m.p. 205°, with shrinking at 197° (Found: C, 61.0; H, 4.0. $C_{23}H_{18}O_{10}$ requires C, 60.7; H, 3.9%).

After sublimation at 0.2 mm. the m.p. of the tetrahydroxyisoflavone rose to 280° (decomp.) (Found: C, 56.3; H, 4.1. $C_{15}H_{10}O_6$, $2H_2O$ requires C, 55.9; H, 4.3%). The acetyl derivative crystallized from dilute acetic acid in colourless prisms, m.p. 227° (Found: C, 61.1; H, 4.1. $C_{23}H_{18}O_{10}$ requires C, 60.7; H, 3.9%). The mixed m.p. with the acetyl derivative, m.p. 205°, was 205° after shrinking at 197°. After prolonged storage the acetyl derivative, m.p. 227°, melted at 205° after shrinking at 197°.

On methylation of the tetrahydroxyisoflavone before or after sublimation with dimethyl sulphate and potassium carbonate in acetone, the parent tetramethoxyisoflavone, m.p. 176°, was obtained. The tetrabenzyl derivative, prepared by the acetone potassium carbonate method, crystallized from acetone in colourless needles, m.p. 153° (Found: C, 80.3; H, 5.4. $C_{43}H_{34}O_6$ requires C, 79.9; H, 5.3%).

2-Hydroxy-5-ethoxy-4:6-dimethoxyphenyl 4-ethoxybenzyl ketone (XL):

This ketone was prepared from p-ethoxyphenylacetic acid (1.2 g.) and 4-ethoxy-3:5-dimethoxyphenol (0.6 g.) by the procedure described for the ketone (IX). The

yellow oil distilled at 220-30°/0.5 mm. (0.34 g.), and crystallized from ethyl acetate with great difficulty, in colourless plates, m.p. 104° (Krishnamurti and Seshadri,[14] m.p. 104°) (Found: C, 66.3; H, 7.1. $C_{20}H_{24}O_6$ requires C, 66.7; H, 6.7%). The ferric colouration is red-brown.

6:4'-Diethoxy-5:7-dimethoxyisoflavone (III):

The orange solution of the above ketone (0.15 g.) in pyridine (2 ml.), ethyl orthoformate (1 ml.) and piperidine (2 drops) changed to brown on heating. After refluxing for 5 hours and adding to ice and hydrochloric acid, the product (0.11 g.) was collected and crystallized from alcohol. The colourless needles had m.p. 160°. The mixed m.p. with a sample of O-diethyl muningin which melted at 153-56°, was 151-53° (Krishnamurti and Seshadri,[13] m.p. 156-58°) (Found: C, 68.2; H, 6.2. $C_{21}H_{22}O_6$ requires C, 68.1; H, 6.0%).

2:5-Dihydroxy-4:6-dimethoxyphenyl 4-methoxybenzyl ketone (XLI):

Using p-methoxyphenylacetic acid (10 g.) and 2:6-dimethoxyhydroquinone (4 g.) and following the same

procedure as for the ketone (II), the ether-chloroform layer was washed with sodium bicarbonate solution, from which p-methoxyphenylacetic acid (8 g.) was recovered. Aqueous sodium carbonate extraction led to an unworkable sticky brownish product. Final extraction with 10% caustic soda solution, acidification and ether extraction yielded a reddish viscous oil (2.7 g.). Extraction of this oil with hexane gave a crystalline substance (0.7 g.), part of which was soluble in aqueous sodium bicarbonate. Acidification of the solution gave p-methoxyphenylacetic acid, resulting apparently from the hydrolysis of the mono-p-methoxyphenylacetate of the desired ketone (XLI), by the action of aqueous caustic soda. This ester could not be isolated in crystalline form. The bicarbonate-insoluble substance crystallized from hexane in yellow prismatic needles (0.1 g.), m.p. 110° (Found: C, 64.5; H, 5.7. $C_{17}H_{18}O_6$ requires C, 64.1; H, 5.7%). On the addition of alcoholic ferric chloride an alcoholic solution of the substance turns green, then yellow and red.

6-Hydroxy-5:7:4'-trimethoxyisoflavone (XLII):

The ketone (XLI) (50 mg.) was refluxed with

pyridine (1 ml.), ethyl orthoformate (0.5 ml.) and piperidine (2 drops) for 5 hours. The isoflavone was isolated by adding ice and hydrochloric acid and extracting with ether, and it crystallized from dilute alcohol in colourless needles (20 mg.), m.p. 177° (Found: C, 65.6; H, 5.2. $C_{18}H_{16}O_6$ requires C, 65.9; H, 4.9%). The substance has no ferric reaction. The acetyl derivative crystallized from dilute alcohol in colourless needles, m.p. 213° (Found: C, 64.8; H, 4.9. $C_{20}H_{18}O_7$ requires C, 64.9; H, 4.8%).

5:6-Dihydroxy-7:4'-dimethoxyisoflavone (XXXII):

The isoflavone (XLII) (0.1 g.) was heated with glacial acetic acid (2.5 c.c.) and hydrobromic acid (b.p. 126°; 2.5 c.c.) at 100° for about 7 minutes. Everything went in solution. On cooling yellow crystals separated. Crystals were filtered and crystallized from dilute acetic acid in yellow rectangular rods, m.p. 218-20° (Found: C, 65.3; H, 4.9; OMe, 20.0. $C_{17}H_{14}O_6$ requires C, 65.0; H, 4.5; OMe, 19.7%). It gives a green colour with ferric chloride. Its mixed m.p. with the compound obtained by the partial demethylation of (XXX) showed no depression.

Bisphenylacetate of 2:6-dimethoxyhydroquinone:

2:6-Dimethoxyhydroquinone (0.5 g.; 1 mol.) was mixed with phenylacetyl chloride (1.17 g., 2 mols.) and boron trifluoride-ether complex (5 c.c.) was added. Phenylacetyl chloride immediately went in solution. The solution was left overnight at room temperature. On pouring into ice, the sticky product was extracted with ether, and the ether extract washed with aqueous sodium bicarbonate and then with water. The ether solution was dried over anhydrous sodium sulphate and distilled. The sticky residue crystallized from alcohol in colourless needles, m.p. 104° (Found: C, 70.7; H, 5.6. $C_{24}H_{22}O_6$ requires C, 70.9; H, 5.4%).

Bis-p-nitrophenylacetate of 2:6-dimethoxyhydroquinone:

The reaction was carried out in exactly the same way as described earlier with 2:6-dimethoxyhydroquinone and p-nitrophenylacetyl chloride. The reaction mixture was poured over ice and after some time the dark brown solid was filtered. The solid was treated with sodium bicarbonate and was crystallized from acetone in colourless flat needles, m.p. 178° (Found: C, 58.0; H, 4.0; N, 5.9. $C_{24}H_{20}O_{10}N_2$ requires C, 58.1;

H, 4.0; N, 5.6%). It is very sparingly soluble in ether.

p-Methoxyphenylacetic acid:

A solution of p-methoxyphenylpyruvic acid[15] (5 g.) in 10% aqueous caustic soda (30 ml.), cooled to 0°, was treated with 3% hydrogen peroxide (30 ml.) gradually (cf. Robinson and Young[16]). Acidification after 12 hours gave p-methoxyphenylacetic acid, which crystallized from water in colourless plates (3.8 g.), m.p. 84-85°.

p-Ethoxyphenylacetic acid:

This was also prepared by the oxidation of p-ethoxyphenylpyruvic acid[17] with 3% hydrogen peroxide.[16]

Monobenzyl ether of (XXXIV):

The monomethyl ether (XXXIV) (0.3 g.) was refluxed in dry acetone (40 c.c.) with anhydrous potassium carbonate (0.6 g.) and benzyl chloride (2 mols., 0.23 c.c.) for 20 hours. Colour of the solution became yellow. Acetone was distilled off and water was added. The alkaline solution was ether extracted. Ether on distillation gave an oily product, which

crystallized from dilute alcohol, m.p. 154.55° (Found: C, 69.2; H, 4.7. $C_{23}H_{18}O_6$ $1/2H_2O$ requires C, 69.3; H, 4.7%). It gave a greenish brown colour with alcoholic ferric chloride.

The alkaline solution was acidified with hydrochloric acid and was extracted with ether. Ether on distillation gave some oil which could not be crystallized. It gave a green colouration with ferric chloride.

Tribenzyl ether of (XXXIV):

Same experiment as described above was carried out with the monomethyl ether (XXXIV) (0.3 g.) in dry acetone (150 c.c.), anhydrous potassium carbonate (4 g.), benzyl chloride (2 mols., 0.23 c.c.) and a pinch of dry potassium iodide. Refluxed for 45 hours. Benzyl chloride (6 mols., 0.69 c.c.) was again added and the refluxing was continued for further 25 hours. Acetone was recovered and water was added. Oily product which separated was ether extracted. Very little substance went into the ether layer. Ether insoluble portion was filtered and crystallized from acetone in colourless plates, m.p. 160-61° (Found: C, 78.1; H, 5.2. $C_{37}H_{30}O_6$ requires C, 77.9; H, 5.3%). Its alcoholic solution gave

gave no colouration with ferric chloride.

Potassium carbonate solution on acidification gave no precipitate. Ether soluble portion was found to be the same as the insoluble one. It appears that the compound, m.p. 160-61° has got a very little solubility in ether.

8-Bromo-5:7-dimethoxy-4'-nitroisoflavone (XXII):

5:7-Dimethoxy-4'-nitroisoflavone (0.1 g.) was dissolved in warm glacial acetic acid (3 c.c.). To this solution was then added one mole of bromine in acetic acid. Instantaneous separation of a solid occurred. It was then cooled and filtered. It crystallized from glacial acetic acid in colourless needles, m.p. 275° (Found: C, 50.6; H, 3.4. $C_{17}H_{12}O_6NBr$ requires C, 50.2; H, 3.0%). It dissolves in conc. sulphuric acid with yellow colour.

5:7-Dimethoxy-6:8:4'-trinitroisoflavone (XXIIA):

Nitration mixture was prepared by adding conc. nitric acid (1 c.c.) to con. sulphuric acid (1 c.c.) with cooling. The mixture was cooled to 0° and powdered 8-bromo-5:7-dimethoxy-4'-nitroisoflavone (0.1 g.) was added, very slowly, within half an hour with

intermittent shaking. It was then left at room temperature for 6 hours. A red oily layer had separated at the top. It was then poured over ice and filtered. The pale yellow solid was then crystallized from dilute acetic acid in pale yellow needles, m.p. 225-26° (Found: N, 10.4. $C_{17}H_{11}O_{10}N_3$ requires N, 10.0%). The compound did not give any test for bromine.

8-Bromo-6-hydroxy-5:7-dimethoxyisoflavone:

6-Hydroxy-5:7-dimethoxyisoflavone (XI) (0.1 g.) was dissolved in glacial acetic acid (3 c.c.) and at room temperature one mole of bromine in acetic acid was added. It was then left at room temperature overnight and was poured over water. The solid was filtered and crystallized from dilute acetic acid in yellow needles, m.p. 166° (Found: C, 53.7; H, 3.0. $C_{17}H_{13}O_5Br$ requires C, 54.1; H, 3.4%).

3-Carbomethoxy-2:4-dihydroxy-6-methoxyphenyl-benzyl ketone (XXVI):

A solution of phenylacetic acid(5 g.) in dry chloroform (50 c.c.) was saturated with boron trifluoride gas at 0-10°. Phloroglucinol carboxylic acid 4-methyl ether ester (1.2 g.) was then added to the

phenylacetic acid boron trifluoride complex, which had separated and was resaturated with boron trifluoride gas. Next day it was poured over crushed ice and after leaving it at room temperature, the solid which remained insoluble in ether-chloroform mixture was then filtered. It was dissolved in sodium hydroxide (5%), filtered and acidified. The compound which separated crystallized from alcohol in colourless needles (1.2 g.), m.p. 149° (Found: C, 64.5; H, 5.3. $C_{17}H_{16}O_6$ requires C, 64.5; H, 5.0%). It gives a cherry red colouration with alcoholic ferric chloride.

The ether-chloroform layer contained only a very small quantity of the ketone.

3-Carbomethoxy-2-hydroxy-4:6-dimethoxy-phenyl-benzyl ketone (XXVII):

The above ketone (XXVI) (1.35 g.) was dissolved in dry acetone (50 c.c.), and potassium carbonate (3.0 g.) and dimethyl sulphate (1 mole, 0.4 c.c.) were added. It was refluxed for 24 hours. The colour of the solution became deep violet. Acetone was distilled and water was added. The precipitate which separated was filtered and crystallized from dilute alcohol in colourless needles, m.p. 131-32° (Found: C, 65.8;

H, 5.7. $C_{18}H_{18}O_6$ requires C, 65.4; H, 5.5%). It gives a cherry red colour with a drop of ferric chloride.

8-Carbomethoxy-5:7-dimethoxyisoflavone (XXVIII):

The ketone, m.p. 131-32° (0.1 g.) was dissolved in dry ethyl formate (5 c.c.), cooled to 0° and was added to powdered sodium (0.1 g.), with external cooling. Left over night in the refrigerator and was poured over ice. The ice cooled solution was made slightly acidic to Congo Red by the addition of dilute hydrochloric acid. Ethyl formate was allowed to evaporate at room temperature and the solid which separated was filtered. It crystallized from dilute alcohol in clusters of very minute colourless prismatic needles, m.p. 166-68° (Found: C, 66.6; H, 4.8. $C_{19}H_{16}O_6$ requires C, 67.0; H, 4.7%). The compound gave no colouration with ferric chloride.

3-Carbomethoxy-2:4:6-trihydroxyphenyl-4-nitrobenzyl ketone (XXIII):

A suspension of methyl phloroglucinol carboxylate (0.4 g.), p-nitrobenzyl cyanide (0.4 g.) and fused zinc chloride (0.5 g.) in dry ether (50 c.c.) was

saturated with dry hydrochloric acid gas at 0°. During saturation a faint red solution was obtained. It was left for 24 hours at 10° and the ether layer was separated by decantation from the red oil which had separated. Ice water was then added to the red oil. Excess of hydrochloric acid was neutralized by adding solid sodium bicarbonate and the solution was made slightly acidic with dilute hydrochloric acid. It was then heated at 60° for 2 hours and the solid was filtered. The solid substance was then dissolved in excess of alcohol, norited and filtered. Filtrate was concentrated and was left overnight at room temperature. Next day the cream coloured needles were filtered (0.5 g.), m.p. 185-86° (Found: C, 55.2; H, 3.8; N, 4.4. $C_{16}H_{13}O_8N$ requires C, 55.3; H, 3.7; H, 4.0%). It gives a brown colouration with alcoholic ferric chloride.

3-Carbomethoxy-2-hydroxy-4:6-dimethoxyphenyl-4-nitrobenzyl ketone (XXIV):

The ketone (XXIII) (1.2 g.) was dissolved in dry acetone (60 c.c.) and treated with dimethyl sulphate (2 mols., 0.66 c.c.) and freshly ignited potassium

carbonate (3.5 g.). The colour changed to very deep violet. After refluxing for 22 hours acetone was distilled off and water was added (200 c.c.). The brown solid which separated was filtered (0.96 g.) and crystallized from dilute acetic acid in colourless prisms, m.p. 173-74° (Found:C, 57.7; H, 4.6; N, 4.0. $C_{18}H_{17}O_8N$ requires C, 57.6; H, 4.6; N, 3.7%). Its alcoholic solution gives a cherry red colour with a drop of ferric chloride.

8-Carbomethoxy-5:7-dimethoxy-4'-nitroisoflavone (XXV):

A solution of the ketone (XXIV) (0.1 g.) in freshly distilled pyridine (1 c.c.), ethyl orthoformate (0.5 c.c.) and piperidine (1 drop) was refluxed for 1 hour. The colour of the solution, before the addition of piperidine was brown. After the addition of piperidine it turned to deep violet and after refluxing it changed to dark brown. The solution was cooled and was poured over ice. It was made acidic to Congo Red by the addition of dilute hydrochloric acid very cautiously. The solid which separated (0.07 g.) was immediately filtered and crystallized from alcohol in cream coloured needles, m.p. 179-80° (Found: C, 59.5;

H, 4.2; N, 4.0. $C_{19}H_{15}O_8N$ requires C, 59.2; H, 3.9; N, 3.6%). Cyclization of the ketone (XXIV), to the isoflavone (XXV) was indicated by the absence of ferric chloride colouration.

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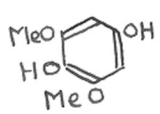
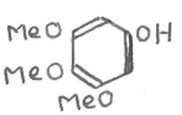
Part III

THE SCOPE AND LIMITATIONS OF THE ETHYL ORTHOFORMATE METHOD FOR THE SYNTHESIS OF ISOFLAVONES

All the practicable methods for the synthesis of isoflavones involve the formylation of benzyl o-hydroxyphenyl ketones. These ketones have been usually prepared by the Hoesch or Friedel-Crafts reactions. However, certain phenols have been found to be unreactive in these reactions, e.g. 2:6-dimethoxyhydroquinone (I), as stated by Chapman, Perkin and Robinson.[1] Antiarol (3:4:5-trimethoxyphenol; II) does not undergo the Hoesch reaction.[1] Both these phenols (I and II) have been shown in the present work to react with boron trifluoride complexes of acids to form the corresponding ketones. The ethyl esters of the acids are also formed if the chloroform, which is usually used as solvent, contains ethyl alcohol. Esters of the phenols have also been isolated.[2]

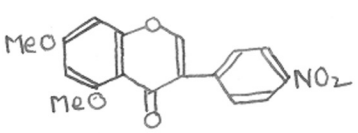


(I)

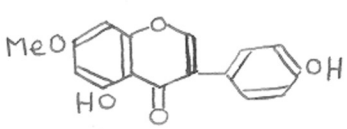


(II)

Sathe and Venkataraman[3] in 1949 showed that 7-hydroxyisoflavone can be obtained very readily by treatment of 2:4-dihydroxyphenyl benzyl ketone with ethyl orthoformate in boiling pyridine containing a little piperidine. This new reaction for isoflavone synthesis is of interest because of the simplicity of the technique, and therefore a brief study of its scope and limitations has now been made. Iyer, Shah and Venkataraman[4] ~~xm~~ utilized the new method for the preparation of 5:7-dimethoxy-4'-nitroisoflavone (III), which was converted to prunetin (IV) by replacement of the nitro group by hydroxyl via the amine and the diazonium salt, followed by selective demethylation of the 5-methoxyl group.



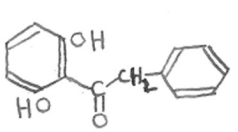
(III)



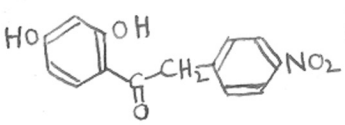
(IV)

2:4-dihydroxyphenyl 4-nitrobenzyl ketone (VI) and 2:6-Dihydroxyphenyl benzyl ketone (V), 2:4-dihydroxyphenyl 4-methoxybenzyl ketone (VII) have been cyclized to the corresponding isoflavones. The

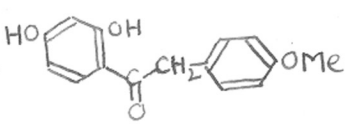
yields obtained, stated in Table I, indicate that the nitro group in the <u>para</u> position of the benzyl group, favours isoflavone formation, obviously on account of the resonance and inductive effects of the nitro group on the reactivity of the methylene group which thus becomes more acid in character; the displacement of a proton from the methylene group and condensation with an ester in the presence of a base (pyridine, piperidine) are thus facilitated.



(V)



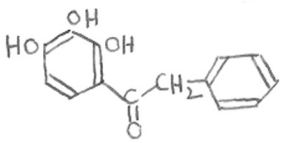
(VI)



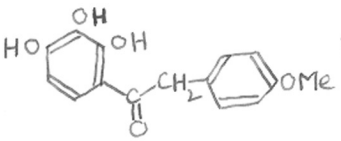
(VII)

Deoxybenzoins derived from pyragallol undergo cyclization to isoflavones by ethyl orthoformate, protection of the hydroxyl groups not being necessary. 2:3:4-Trihydroxyphenyl benzyl ketone (VIII), 2:3:4-trihydroxyphenyl 4-methoxybenzyl ketone (IX) and 2:3:4-trihydroxyphenyl 4-nitrobenzyl ketone (X), have been cyclized without difficulty to the corresponding isoflavones. Here also it has

been observed that the nitro group in the 4-position of the benzyl group favours the condensation.



(VIII)



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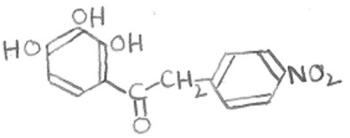
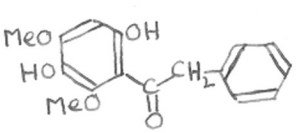


(X)

2:5-Dihydroxy-4:6-dimethoxyphenyl benzyl ketone (XI) can be readily cyclized to 5:7-dimethoxy-6-hydroxyisoflavone (XII) in excellent yield; but if a methoxyl group is present in the benzyl nucleus in the 4-position (XIII), the isoflavone (XIV) is obtained in low yield. At the same time it should be noted that a 4-methoxyl or 4-ethoxyl in the benzyl group does not hinder the cyclization and the corresponding isoflavones (e.g. XV and XVI) are formed without any difficulty, if 2:6-dimethoxyhydroquinone is replaced by antiarol or 4-ethoxy-3:5-dimethoxyphenol.



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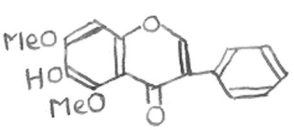

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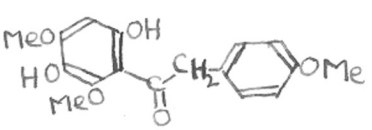

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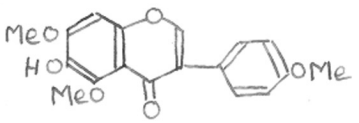

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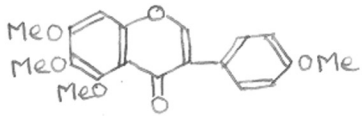

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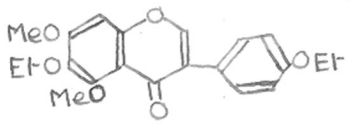
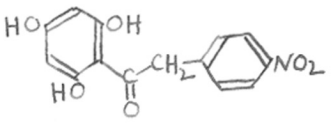


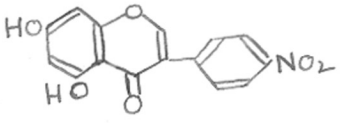
(XVI)

Deoxybenzoins derived from phloroglucinol largely yield high melting, red products in the ethyl orthoformate reaction, if all the hydroxyl groups are unprotected. Polymerization takes place by the intervention of methine groups and dyes of the cyanine type are formed. Bose and Dutta[5] have reported that 5:7-dihydroxy-4'-nitroisoflavone (XVIII) is obtained in about 20% yield from the corresponding ketone (XVII). If the 4- and 6-hydroxyls are protected by methylation in the above ketone (XVII) the cyclization with ethyl orthoformate proceeds in much better yield.[4] 2-Hydroxy-4:6-dimethoxyphenyl benzyl ketone also undergoes cyclization with ethyl orthoformate, but not in such good yield as the nitro derivative.

If the phloroglucinol nucleus is C-substituted by a methyl or carbomethoxyl group, and if the 6-hydroxyl or 4- and 6-hydroxyls are methylated as

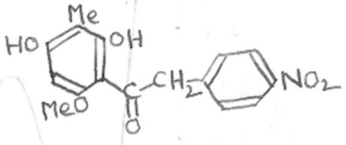


(XVII)

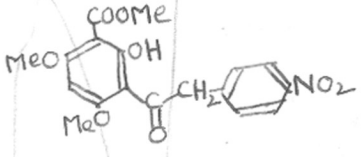


(XVIII)

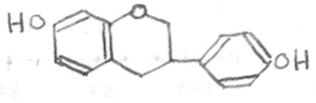
in the ketones (XIX), and (XX), the ethyl orthoformate reaction proceeds smoothly.



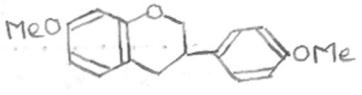
(XIX)



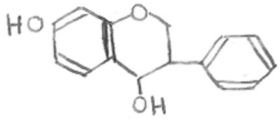
(XX)



(XXI)



(XXII)



(XXIII)

Equol (XXI) was originally isolated by Marrian and Haslewood[6] from the urine of pregnant mares. Later Marrian and Beall[7] isolated it from the urine of non-pregnant mares also.

Wessely et al.[8] suggested the correct constitution for equol as 7:4'-dihydroxyisoflavan. Wessely and Prillinger[9] have synthesized it by the catalytic hydrogenation of daidzein using palladized charcoal. Because the substance obtained was difficult to purify, they prepared the dimethyl ether, but found that the dimethyl ether (XXII) was difficult to demethylate by the usual demethylating agents (hydriodic acid, hydrobromic acid, aluminium chloride or aluminium bromide). They finally demethylated it by a Grignard reagent (ethyl magnesium iodide), and isolated a small quantity of of synthetic equol. They also showed that the spectra of the ~~xxxxxx~~ synthetic dimethyl ether and the diacetyl derivative were identical with the dimethyl ether and diacetate of natural equol. The colour of equol, as visually observed, is yellow, although no colour will be anticipated from the structure.

It appeared therefore that a direct synthesis of equol, which does not involve demethylation and where the reaction product is easier to work up, is needed. As a preliminary to such a synthesis the Raney nickel reduction of 7-hydroxyisoflavone under Mozingo's conditions[10] was carried out. The product which was obtained melted at 136°, and was 4:7-dihydroxyisoflavan (XXIII), as shown by the analysis. Further work to convert this compound to 7-hydroxyisoflavan and the preparation of the corresponding products from daidzein are in progress.

Table I

Yields of isoflavones obtained by the ethylorthoformate reaction.

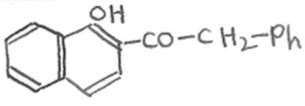
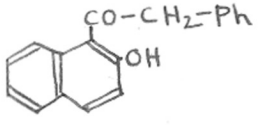
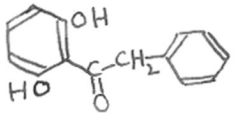
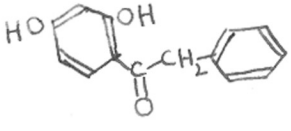
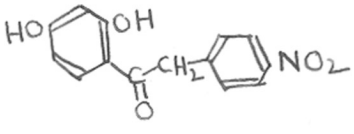
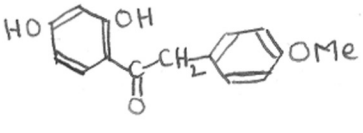
Ketone		Percentage yield	Ethyl-ortho-formate mols.	Time in hrs.
* 1)		45	10	4
* 2)		25	10	4
3)		96	3	8
4)		82	3	solid separates after 1/2 hr.
5)		96	3	solid separates in about 45 mins.
6)		70	9	2

Table I (contd.)

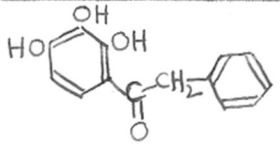
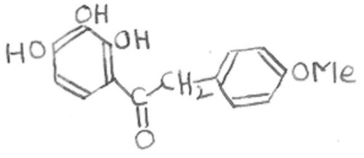
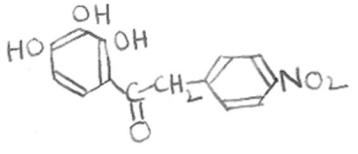
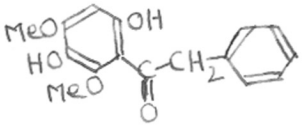
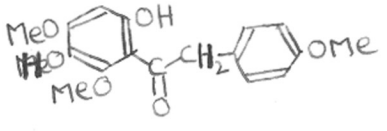
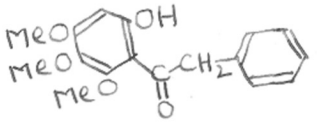
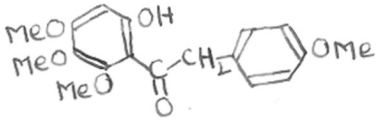
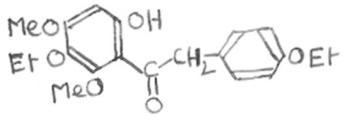
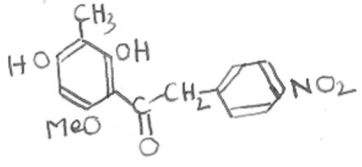
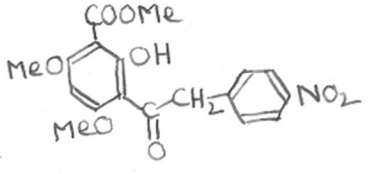
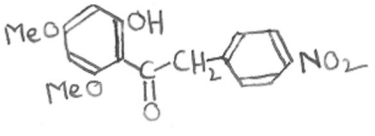
Ketone		Percentage yield	Ethyl orthoformate (mols.)	Time in hrs.
7)		67	3	1
8)		68	1	5
9)		68	10	1/2
10)		90	3	5
11)		40	3	5
12)		87	5	7
13)		68	6	5

Table I (contd.)

Ketone	Percentage yield	Ethyl orthoformate (mols.)	Time in hrs.
14) 	72	15	5
15) 	72	10	solid separates after one hour
16) 	70	13	1
17) 	82	10	8

* Sathe, private communication.

EXPERIMENTAL

Benzyl 2:3:4-trihydroxyphenyl ketone (VIII):

Phenylacetic acid (12 g.) was dissolved in dry alcohol free chloroform (40 cc.) and the ice cooled solution was saturated with boron trifluoride gas. Then to the complex, which separated, pyragallol (6 g.) was added.The reaction mixture was left overnight, at room temperature, after resaturating the mixture with boron trifluoride gas. Next day crushed ice was added and it was extracted with ether. Ether-chloroform layer was then washed with sodium bicarbonate solution and then with water. Ether chloroform layer was then distilled after drying over anhydrous sodium sulphate. The solid which remained behind crystallized from alcohol in colourless needles, m.p. 140-41° (Found: C, 69.0; H, 5.1. $C_{14}H_{12}O_4$ requires C, 68.8; H, 4.9%). Yield (11.1 g.). Its alcoholic solution gives a green ferric reaction (Desai *et al.*[11] quote the m.p. as 144-45°).

7:8-Dihydroxyisoflavone:

Benzyl 2:3:4-trihydroxyphenyl ketone (1 g.) was dissolved in dry pyridine (4 cc.). Piperidine (0.3 cc.)

and ethyl orthoformate (1.8 cc.) were then added. The solution was then refluxed for one hour. It was then poured over ice and hydrochloric acid, after cooling. Next day the precipitate was collected and crystallized from alcohol, with norit, in colourless needles (0.7 g.), m.p.216°, after shrinking at 210°. (Found: C, 68.6; H, 4.2. $C_{15}H_{10}O_4$, 1/2 H_2O requires C, 68.4; H, 4.2%). On drying in vacuo at 150° for 3 hours over phosphorous pentoxide the melting point rose to 219° (Found: C, 71.2; H, 4.1. $C_{15}H_{10}O_4$ requires C, 70.8; H, 3.9%). Its alcoholic solution gives a green ferric reaction, ~~xxxxxxxxx~~ (Ballio and Pocchiari,[12] m.p.221°. Seshadri et al.[13] quote m.p.218-20°).

Its acetyl derivative, prepared in the usual manner, crystallized from dilute alcohol in colourless needles, m.p. 137-39° (Found: C, 67.3; H, 4.1. $C_{19}H_{14}O_6$ requires C, 67.4; H, 4.1%) (Ballio and Pocchiari[12], m.p. 143-44°; Seshadri et al.[13] m.p. 139-40°).

2:3:4-Trihydroxyphenyl 4-nitrobenzyl ketone (X):

p-Nitrophenylacetic acid (1 g.) was suspended in chloroform (50 cc.), and the suspension was saturated with boron trifluoride gas at the temp.

of ice water. Pyrogallol (0.5 g.) was added and the mixture was again saturated with boron trifluoride. On leaving it overnight and working it up as usual, the product crystallized from dilute alcohol in yellow prisms, m.p.227-28° (Found: C, 57.9; H, 3.8; N, 5.0. $C_{14}H_{11}O_6N$ requires C, 58.1; H, 3.8; N, 4.8%). The alcoholic solution gives a greenish brown ferric reaction.

7:8-Dihydroxy-4'-nitroisoflavone:

2:3:4-Trihydroxyphenyl 4'-nitrobenzyl ketone (0.1 g.) was dissolved in dry pyridine (2 cc.) and ethyl orthoformate (0.5 cc.) was added.The colour of the solution changed to red on the addition of piperidine (2 drops). The solution was refluxed for 30 minutes, cooled and poured over ice and hydrochloric acid. The product (70 mg.) crystallized from glacial acetic acid in yellow needles, m.p.325° (decomp.) (Found: C, 60.4; H, 3.4; N, 4.3. $C_{15}H_9O_6N$ requires C, 60.2; H, 3.0; N, 4.7%). The substance gives a green ferric reaction.

2:3:4-Trihydroxyphenyl 4-methoxybenzyl ketone (IX):

This ketone was prepared from p-methoxyphenylacetic acid (6 g.), and pyragallol (3 g.) in exactly the same manner as described for the preparation of 2:3:4-trihydroxyphenyl benzyl ketone, yield (5.0 g.). Crystallized from dilute alcohol in prismatic needles, m.p. 145-46° (Found: C, 65.5; H, 5.0. $C_{15}H_{14}O_5$ requires C, 65.7; H, 5.1%). Its alcoholic solution gives a deep violet colour with ferric chloride.

7:8-Dihydroxy-4'-methoxyisoflavone:

The above ketone (1 g.) was refluxed for five hours with pyridine (2 cc.), ethyl orthoformate (0.5 cc.) and piperidine (2 drops). The colour changed from yellowish brown to dark brown. It was then poured over ice and hydrochloric acid after cooling. Next day the solid was collected (0.7 g.) and crystallized from dilute alcohol in pale yellowish brown prisms, m.p.249° (Found: C, 67.5; H, 4.5; $C_{16}H_{12}O_5$ requires C, 67.6; H, 4.3%). It gives a green colour with ferric chloride.

7:7:8:4'-Trihydroxyisoflavone:

7:8-Dihydroxy-4'-methoxyisoflavone (0.3 g.) was suspended in a mixture of acetic acid (1 cc.) and acetic anhydride (1 cc.). To the cooled suspension was then added hydriodic acid (d. 1.7; 1 cc.). On refluxing, it formed a clear solution. After about ten minutes some solid separated. Refluxing was continued for one hour. The cooled solution was then diluted with water and the excess of iodine was destroyed by the addition of sodium bisulphite. The solid was filtered and crystallized from dilute alcohol

in clusters of prismatic needles, m.p. 210° after darkening at 280° (Found: C, 66.9; H, 4.0. $C_{15}H_{10}O_5$ requires C, 66.7; H, 3.7%). This substance gave a green colour with alcoholic ferric chloride.

The triacetyl derivative crystallized from dilute alcohol in colourless cubes, m.p. 192° (Found: C, 63.8; H, 4.5. $C_{21}H_{16}O_8$ requires C, 63.6; H, 4.1%).

Benzyl 2-hydroxy-4:5:6-trimethoxyphenyl ketone:

It was prepared from antiarol (1 g.) and phenylacetic acid-boron trifluoride complex, prepared from phenylacetic acid (5 g.) in the same manner as described in the first experiment, yield (1.3 g.). First it was obtained as a yellow oil which solidified in contact with methyl alcohol. The oil could be distilled at 180-85°/0.3 mm. It also crystallized from dilute alcohol in colourless needles, m.p. 60° (Found: C, 67.4; H, 5.8. $C_{17}H_{18}O_5$ requires C, 67.5; H, 6.0%). Its alcoholic solution gives a brown colour with ferric chloride (Krishnamurti and Seshadri[14], m.p. 64°).

5:6:7-Trimethoxyisoflavone:

The above ketone (0.1 g.) was refluxed with pyridine (1 cc.), ethyl orthoformate (0.2 cc.) and piperidine (1 drop) for seven hours. It was then worked up as usual, yield 90 mg. It crystallized from dilute alcohol in colourless needles, m.p.149°. (Found: C, 69.5; H, 5.3. $C_{18}H_{16}O_5$ requires C, 69.2; H, 5.2%). It dissolves in conc. sulphuric acid with yellow colour. On demethylation with hydriodic acid it gives the corresponding trihydroxyisoflavone m.p. 188-89°. Its mixed melting point with the isoflavone, mentioned in the earlier part, showed no depression. The possibility of rearrangement during demethylation was eliminated by remethylating the trihydroxyisoflavone, by the usual method. Its mixed melting point with the trimethoxyisoflavone remained undepressed (Mahesh and Seshadri[15] quote the m.p. as 165-66°).

Benzyl-2:4-dihydroxyphenyl ketone:

This ketone was prepared from phenylacetic acid (5 g.) and resorcinol (2.5 g.) by the boron trifluoride method for the synthesis of ketones, as

described earlier. Yield (4.5 g.). It crystallized from alcohol in colourless needles, m.p. 115°. The ketone gives a reddish brown ferric reaction (Chapman and Stephen[16], m.p.115°).

7-Hydroxyisoflavone:

(This isoflavone has been synthesized earlier by Sathe and Venkataraman[3] by using ethyl orthoformate as a cyclizing agent, but the details are not described).

Benzyl 2:4-dihydroxyphenyl ketone (1 g.) was refluxed with pyridine (2 cc.), ethyl orthoformate (1.9 cc.) and piperidine (2 drops). After about an hour copious separation of a solid was observed. It was cooled and poured over ice and hydrochloric acid. Next day it was filtered and the product (0.85 g.) crystallized from alcohol in colourless prismatic needles, m.p. 210°. This isoflavone gives no colouration with ferric chloride (m.p.s varying from 205 to 215° have been quoted in literature[4,17,18].

4:7-Dihydroxyisoflavan (XXIII):

7-Hydroxyisoflavone (0.5 g.) was dissolved in alcohol (30 cc.) and after the addition of Raney nickel (5 g.) the mixture was refluxed with stirring, on a boiling water bath for 3 hours. It was then filtered and washed with hot alcohol (20 cc.). After the evaporation of the filtrate the semi-solid mass which separated was dissolved in methyl alcohol and filtered. On dilution of the filtrate with water the solid which separated, after about a day, crystallized from water in colourless needles, m.p. 136° (Found: C, 74.5; H, 5.6. $C_{15}H_{14}O_3$ requires C, 74.4; H, 5.8%). It is soluble in most of the usual organic solvents.

2:4-Dihydroxyphenyl 4-nitrobenzyl ketone (VI):

This ketone was prepared in the same manner as described earlier by the boron trifluoride method using p-nitrophenylacetic acid (1 g.) and resorcinol (0.5 g.), in chloroform. After the distillation of the residue was extracted with sodium carbonate, chloroform, which on anidification with conc. hydrochloric acid gave the said ketone. It crystallized from dil. alcohol in colourless needles (0.15 g.)

m.p. 210°. Its mixed melting point with the ketone prepared from p-nitrobenzyl cyanide and resorcinol, according to Joshi and Venkataraman[19] showed no depression.

7-Hydroxy-4'-nitroisoflavone:

The above ketone (0.2 g.) was treated with pyridine (1.6 cc.), when it became faint red. On the addition of ethyl orthoformate (0.25 cc.) and piperidine (2 drops) the solution became violet. The solution was refluxed for 45 minutes when a copious separation of a solid occurred. The precipitate (0.19 g.) crystallized from alcohol or glacial acetic acid, m.p. 290° (Found: C, 63.6; H, 3.5; N, 5.3. $C_{15}H_9O_5N$ requires C, 63.6; H, 3.2; N, 4.9%). Its alcoholic solution gives no colouration with ferric chloride (Bose and Dutta[5] quote the same m.p.).

7-Hydroxy-4'-aminoisoflavone:

7-Hydroxy-4'-nitroisoflavone (0.2 g.) was added to the suspension of zinc dust (0.3 g.) and ethyl alcohol (20 cc.). To the refluxing solution was then added glacial acetic acid (4 cc.), gradually in two hours, and the heating continued for an hour. The

mixture was filtered hot, the filtrate concentrated to about 5 cc. and diluted with water. The precipitate (0.13 g.) crystallized from dilute alcohol in colourless needles, m.p.265-66° (Found: N, 5.5. $C_{15}H_{11}O_3N$ requires N, 5.6%). The alcoholic solution gives no colouration with ferric chloride.

7:4'-Dihydroxyisoflavone (Daidzein):

The amine (0.2 g.) was dissolved in a mixture of water (1.5 cc.) and conc. sulphuric acid (2 cc.) The solution was cooled in ice and treated with a solution of sodium nitrite (0.1 g.). After allowing the mixture to stand at 0° for 30 minutes, the excess of nitrous acid was destroyed by the addition of urea. The solution was then poured into a boiling mixture of water (15 cc.) and concentrated sulphuric acid (5 cc.). The boiling was continued till the solution did not give any colour with alkaline β-naphthol (about 5 minutes). The solution was cooled and the precipitate collected (0.14 g.). It crystallized from alcohol in prismatic needles. It melted at 320° after its purification through acetylation (Mahal et al.[20] m.p.322° after darkening

at 310°. Baker et al.[18] m.p.220-28°).

The diethyl ether of daidzein was prepared in the usual manner by refluxing it with acetone, potassium carbonate and diethyl sulphate for 12 hours. It crystallized from dilute alcohol in yellow needles, m.p.134° (Found: C, 73.8; H, 6.1; $C_{19}H_{18}O_4$ requires C, 73.6; H, 5.8%).

7-Methoxy 4'-nitroisoflavone:

7-Hydroxy-4'-nitroisoflavone was methylated in the usual manner by using potassium carbonate, acetone and dimethyl sulphate. It crystallized from alcohol or dilute acetic acid in colourless plates, m.p.245° (Found: C, 64.7; H, 3.8; N, 4.7. $C_{16}H_{11}O_5N$ requires C, 64.6; H, 3.7; N, 4.7%). (Dutta and Bose[5] m.p.245°).

7-Methoxy-4'-aminoisoflavone:

7-Methoxy-4'-nitroisoflavone (0.2 g.) was suspended in alcohol (50 cc.), together with zinc dust (0.5 g.). Refluxed for 15 minutes on a water bath, and glacial acetic acid (4 cc.) was added during two hours. Heating was continued for half an hour and the product was

filtered hot. Filtrate was concentrated to a very small volune and diluted with water. After cooling the solid was filtered (0.14 g.). It crystallized from alcohol in pale yellow needles, m.p.206° (Found: C, 71.5; H, 5.2%. $C_{16}H_{12}O_3N$ requires C, 71.9; H, 4.9%).

7-Methoxy-4'-hydroxyisoflavone (Isoformononetin):

This compound was obtained from 7-methoxy-4'-aminoisoflavone (0.15 g.) in the usual manner by diazotization and hydrolysis.

The precipitate obtained was treated with sodium hydroxide and filtered. The filtrate on acidification gave a solid (70 mg.). It crystallized from dilute alcohol in pale yellow needles, m.p. 216-18° on remelting (Found: C, 71.6; H, 4.5. $C_{16}H_{12}O_4$ requires C, 71.6; H, 4.5%). The alkali insoluble portion was the unreacted aminoisoflavone (Seshadri et al.[21] m.p. 218-20°).

2:4-Dihydroxyphenyl-4'-methoxybenzyl ketone:

This compound was prepared from p-methoxy-phenylacetic acid (1 g.) and resorcinol (0.5 g.) in alcohol free chloroform (15 cc.) by the usual boron trifluoride procedure

described earlier, yield 0.6 g. It crystallized from dilute alcohol in colourless needles, m.p. 159° (Baker and Eastwood[22] m.p. 159°).

7-Hydroxy-4'-methoxyisoflavone (Formononetin):

2:4-Dihydroxyphenyl-4'-methoxybenzyl ketone (0.1 g.), was cyclized to the corresponding isoflavone by refluxing it with pyridine (2 cc.), ethyl orthoformate (0.5 cc.) and piperidine (2 drops), for two hours. It was then worked up in the usual manner. Yield 72 mg. It crystallized from alcohol in colourless prismatic needles, m.p. 257°. It had no ferric reaction. Its mixed m.p. with the sample, prepared gy Mahal showed no depression (Mahal et al.[20] m.p.257°. Baker et al.[18], m.p.257°).

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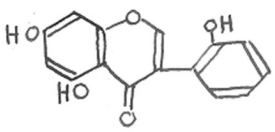
Part IV

SYNTHESIS OF ISOGENISTEIN AND OTHER ISOFLAVONES IN CONNECTION WITH THE CONSTITUENTS OF SOYA BEAN

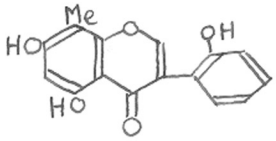
Nineteen natural colouring matters have been assigned isoflavone structures, and six of these have been isolated from soya bean. Walz[1] showed that two constituents of soya bean meal were glucosides of genistein (5:7:4'-trihydroxyisoflavone) and daidzein (7:4'-dihydroxyisoflavone). Okano and Beppu have described four isoflavones (isogenistein, methylisogenistin, methylgenistein and tatoin), three flavones and a "red colouring matter like resene" as constituents of soya bean.[2] Isogenistin and methylisogenistin were glucosides yielding the aglucones, isogenistein and methylisogenistein.

The structures assigned by Okano and Beppu to isogenistein as 5:7:2'-trihydroxyisoflavone (I), methylisogenistein as 5:7:2'-trihydroxy-8-methylisoflavone (II), methylgenistein as 5:7:4'-trihydroxy-8-methylisoflavone (III), and tatoin as 5:4'-dihydroxy-8-methylisoflavone (IV) were based on the results of alkaline fission; (I) gave phloroglucinol

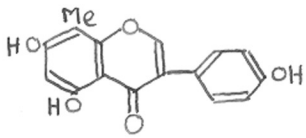
formic acid and o-hydroxyphenylacetic acid, (II) gave



(I)



(II)



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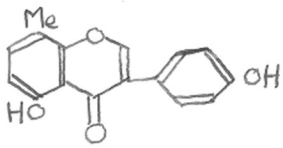


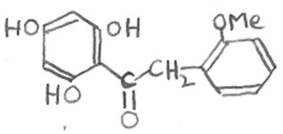
(IV)

C-methylphloroglucinol and the same two acids; (III) gave C-methylphloroglucinol, formic acid and p-hydroxyphenylacetic acid; and (IV) gave 4-methylresorcinol, formic acid and p-hydroxyphenylacetic acid. The products obtained from (IV) can also result from 7:4'- and 5:4'-dihydroxy-6-methylisoflavone; Bhandari, Bose and Siddiqui[3] synthesized the former by the condensation of ethyl formate with 4:6-dihydroxy-m-tolyl p-methoxybenzyl ketone, followed by demethylation, and they found that the properties differed from those of tatoin. They also isolated from the fresh germs of soya bean a

colouring matter which was apparently identical with tatoin. The isoflavones (I), (II) and (III) have now been synthesized, and their properties do not correspond with those described by Okano and Beppu.[2] It may be mentioned that the structure 5:7:4'-trihydroxy-8-methylisoflavone (III) for "methylgenistein" was supported by Shriner and Hull,[4] who found that the product of condensation of ethyl formate with 2:6-dihydroxy-4-methoxy-m-tolyl 4-methoxybenzyl ketone in presence of sodium, followed by demethylation with hydriodic acid, resembled the methylgenistein of Okano and Beppu. However, no direct comparison of the synthetic and natural substances was made. As stated in Parts I and II of this thesis, the ethyl formate or orthoformate reaction on a ketone, containing a phloroglucinol nucleus in which all the hydroxyl groups are unprotected, is not likely to lead to satisfactory results.

In the present work the ketone (V), m.p. 170°, prepared by a Hoesch reaction between phloroglucinol and o-methoxybenzyl cyanide, was dimethylated to (VI)

m.p. 116°, by means of dimethyl sulphate and potassium carbonate in acetone. Condensation of (VI) with ethyl formate and sodium, following the procedure of Joshi and Venkataraman,[5,6] gave 5:7:2'-trimethoxyisoflavone (VII), m.p. 138°,



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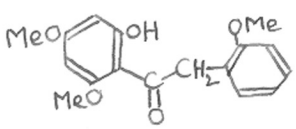

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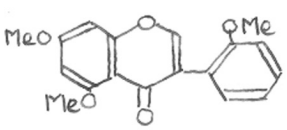

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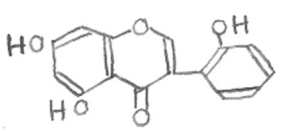
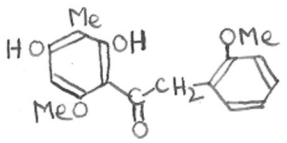


(I)

which was finally demethylated to 5:7:2'-trihydroxyisoflavone (I), m.p. 187°, by the action of aluminium bromide in boiling benzene. The triacetyl derivative melted at 151°. The melting points cited by Okano and Beppu for isogenistein and its triacetate are 302° and 189° respectively.

5:7:2'-Trihydroxy-8-methylisoflavone (II) has

been synthesized by the following route:-



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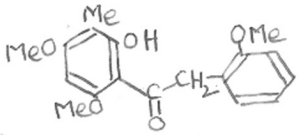

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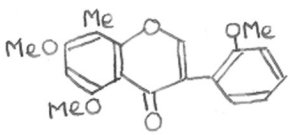

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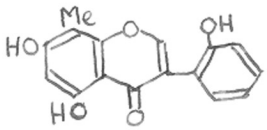


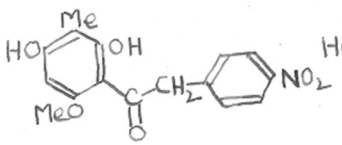
(II)

2:4-Dihydroxy-6-methoxy-m-tolyl 2-methoxybenzyl ketone (VIII), m.p. 194°, was prepared from 2:6-dihydroxy-4-methoxytoluene[4] and o-methoxybenzyl cyanide by the Hoesch reaction. The ketone (VIII) could not be converted to the isoflavone by the ethyl formate-sodium[5,6] or ethyl orthoformate-pyridine reactions;[7] but the trimethyl ether (IX), m.p. 150°, in which the only unprotected hydroxyl was ortho to the carbonyl group, cyclized readily to the isoflavone (X), m.p. 183-84°, on treatment with ethyl formate and sodium; Okano and Beppu obtained from "methylisogenistein" a trimethyl ether melting at 152-53°. Aluminium bromide demethylation of (X) yielded (II), m.p. 234°.——————————→

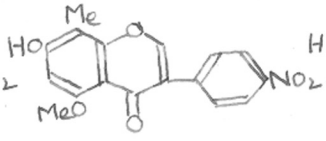
which gave a green colouration with alcoholic ferric chloride; Okano and Beppu's "methylisogenistein" melted at 301-2°, and the ferric colouration was violet red. The triacetate of (II) melted at 105° (139° after dehydration), while the corresponding derivative prepared by Okano and Beppu melted at 188°.

2:6-Dihydroxy-4-methoxytoluene and p-nitrobenzyl cyanide were condensed by the Hoesch reaction to give 2:6-dihydroxy-4'-methoxy-m-tolyl 4-nitrobenzyl ketone (XI), m.p. 201°. The ketone (XI) on cyclization with ethyl orthoformate gave 7-hydroxy-5-methoxy-8-methyl-4-nitroisoflavone (XII), which darkened at 310° and decomposed at 330°. Reduction of the nitro compound to the amine (VIII) with zinc and acetic acid and replacement of the amino group by hydroxyl by the usual diazotization and hydrolysis method resulted in 7:4'-dihydroxy-5-methoxy-8-methylisoflavone (XIV), m.p. 300-302° (Whalley,[8] m.p. 304°). Hydriodic acid demethylation of (XIV) yielded (III), m.p. 231-32°. The melting point recorded by Okano and Beppu for this compound is 298°.

It is well-known in the flavone series that 5:8-dimethoxyisoflavones undergo hydrolytic opening



(XI)



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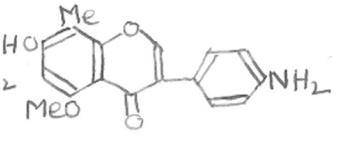

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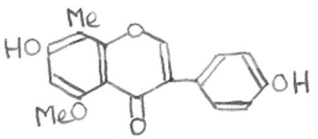

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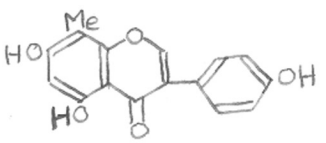


(III)

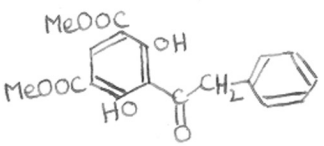
during demethylation with hydrobromic ~~xix~~ acid or hydriodic acid and recyclize in the alternative direction, forming the 5:6-isomer~~s~~. A similar observation in the isoflavone series has been made by Baker[9] and by Whalley.[10] Baker has recorded the isomerization of 5:7:8-trihydroxy-2-methylisoflavone to the 5:6:7-isomer, and of 5:7-dihydroxy-8:3':4':5'-tetramethoxyisoflavone to 5:6:7':3':4':5'-hexahydroxyisoflavone, when boiled with hydrobromic acid and acetic acid for 8 hours. However, he has pointed out that the rearrangement depends upon the conditions of demethylation,

because 5:7:8-trimethoxyisoflavone and its 2-methyl derivative were demethylated by hydriodic acid to the corresponding trihydroxy compound, no rearrangement taking place.[11,12]

Whalley[10] observed that, if 5:7:4'-trimethoxy-8-methylisoflavone was boiled with hydriodic acid for 15 hours, a very small quantity of the 6-methylisomer was formed; but there was no rearrangement during the hydriodic acid demethylation of 7:4'-dihydroxy-5-methoxy-8-methylisoflavone, since remethylation of the final product gave 5:7:4'-trimethoxy-8-methylisoflavone in quantitative yield.[8] Aluminium chloride in benzene, which Seshadri[14a] has repeatedly claimed to be a safer reagent for demethylations because it did not cause rearrangement, was used by Whalley[10] to demethylate 5:7:2'-trimethoxy-8-methylisoflavone, and according to him, two products were isolable: 5:7:2'-trihydroxy-8-methylisoflavone and its 6-methylisomer. Wheeler[13] has suggested that such isomerisation by aluminium chloride may be due to migration of the alkyl group.

As a preliminary to the synthesis of 5:4'-dihydroxy-8-methylisoflavone, 5-hydroxyisoflavone

(IV) has been synthesized. For the synthesis of (IV) the γ-substituted resorcinol derivative (XVI) was needed, and it has been obtained by a new method. Dimethyl resorcinol-4:6-dicarboxylate was condensed with phenylacetyl chloride in presence of anhydrous aluminium chloride to give 2:6-dihydroxy-3:5-dicarbomethoxyphenyl benzyl ketone (XV), m.p. 131°, which on hydrolysis and decarboxylation gave 2:6-dihydroxyphenyl benzyl ketone (XVI), m.p. 171°. The ketone (XVI) on cyclization with ethyl orthoformate gave 5-hydroxyisoflavone (IV), m.p. 102°. The acetyl



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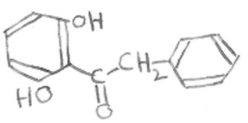
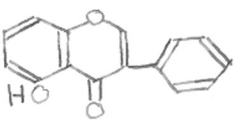


(XVI)



(IV)

derivative of (IV) melted at 144°.

After the completion and publication of the above work,[14] excepting the synthesis of (III), three independent groups of workers,[8,15,16] have described the synthesis of (I), (II) and (III) and they have also come to the same conclusion that (I), (II) and (III) are different from the soya bean isoflavones of Okano and Beppu.

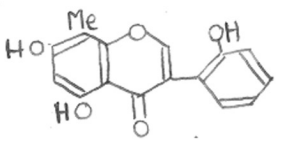
In the synthesis of isogenistein Seshadri *et al.*[16,17] have reported the isolation of the 2-hydroxyisoflavanone, m.p. 180-81°, as an intermediate during the process of cyclization of (VI) with sodium and ethyl formate; treatment with sodium acetate and acetic anhydride then gave 5:7:2'-trimethoxyisoflavone, m.p. 148°. Whalley[8] cyclized the ketone (VI) with sodium and methyl formate, and obtained a compound, m.p. 196°, which was converted by hot acetic acid into 5:7:2'-trimethoxyisoflavone, m.p. 140°. It has been observed in this laboratory that sodium and ethyl formate cyclization of (VI) gives invariably the compound (VII), m.p. 138°. Baker *et al.*[15] have quoted the same m.p. for the trimethyl ether (VII). Baker *et al.*[18] had reported that the m.p. of 5:7:2'-trihydroxyisoflavone was 187°, but later they found[15] that it existed in dimorphic forms, m.p. 187°, and 222-23°. The higher melting compound was more stable. Seshadri[16] has also reported the higher melting compound, but he has also stated[17] that the lower melting compound on crystallization from alcohol was converted to the higher melting form. However, it has been observed in the present work that the compound,

m.p. 187°, is quite stable towards alcohol crystallizations, and it goes to the higher melting form only if it is heated above its melting point. In connection with this synthesis another difference in the findings of different workers may be mentioned. Samples of 2:4:6-trihydroxyphenyl 2-methoxybenzyl ketone (V) obtained by Seshadri and Varadarajan[16] and by Whalley,[8] were monohydrates, whereas Baker *et al.*[15] and the present author[14] obtained it in anhydrous form.

In the synthesis of methylisogenistein, Seshadri *et al.*[16] and Whalley[8] found that the cyclization of the ketone (IX) with sodium and ethyl formate or with sodium and methyl formate yielded the 2-hydroxyisoflavone, which dehydrated to (X) on boiling with sodium acetate and acetic anhydride; but in the author's hands in this laboratory the isoflavone (X) was obtained directly from the ketone (IX) by sodium and ethyl formate cyclization.

Whalley[8] reported earlier that he encountered difficulties in demethylating (VII) and (X) with hydriodic acid, but later[10] he succeeded in demethylating them with aluminium chloride in benzene.

During demethylation of (X) with aluminium chloride he claims to have isolated a mixture of (II) and (XVIII) ; according to Seshadri aluminium chloride



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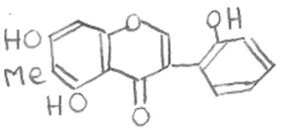

(XVII)

has never caused such an isomeric change.

There are certain differences in the observations of different workers in the synthesis of methylgenistein also. Methylgenistein synthesized by Whalley[8] melted at 252°, while Seshadri[16] reports the m.p. 231-32°. Our melting point agrees with the latter.

In view of the fact that the synthetic isoflavones mentioned above do not agree in their properties with the soya bean isoflavones isolated by Okano and Beppu, Baker[15] compared the properties of genistein, daidzein and their derivatives with the properties of isoflavones isolated by Okano and Beppu and suggested that the latter isoflavones must have been mixtures of genistein and daidzein in various proportions. Seshadri *et al.*[19] have

supported this view by paper chromatography of a soya bean extract. The present author feels, however, that Seshadri's work undoubtedly confirms the presence of daidzein and genistein, but does not really exclude the presence of other isoflavones. Further work on the subject has therefore been undertaken.

EXPERIMENTAL

2:4:6-Trihydroxyphenyl-2-methoxybenzyl ketone (V):

A slow stream of dry hydrogen chloride was passed for 5 hours through an ice-cooled solution of dry phloroglucinol (5 g.), o-methoxybenzyl cyanide (5 g.), fused zinc chloride (6 g.) and anhydrous ether (100 cc.). The reaction mixture was kept in a refrigerator for 48 hours. The ether was decanted off and the solid ketimine hydrochloride was washed with ether, and treated with water (500 cc.) under reflux for 2 hours, crystallized from water in pale yellow needles, m.p. 170° (Found: C, 65.4; H, 5.1. $C_{15}H_{14}O_5$ requires C, 65.7; H, 5.1%). An alcoholic solution of the substance gives a violet-brown colouration with ferric chloride.

2-Hydroxy-4:6-dimethoxyphenyl 2-methoxybenzyl ketone (VI):

The ketone (V)(2 g.) was dissolved in dry acetone (50 cc.) and treated with dimethyl sulphate (1.4 cc.; 2 mols.) and freshly ignited potassium carbonate (6 g.). After refluxing for 14 hours, acetone was removed and water was added. The precipitate (1.9 g.)

crystallized from alcohol in colourless needles, m.p. 116° (Found: C, 67.0; H, 5.8. $C_{17}H_{18}O_5$ requires C, 67.6; H, 5.9%). The alcoholic solution gives a deep red colour with ferric chloride.

5:7:2'-Trimethoxyisoflavone (VII):

To a solution of the ketone (VI)(1 g.) in freshly distilled ethyl formate (20 cc.), cooled to 0°, sodium dust (1.5 g.) was added. The mixture was left in the refrigerator for 22 hours, and then at room temperature for two hours, poured into ice and hydrochloric acid and allowed to stand. The solid that separated (1 g.) crystallized from water in colourless needles, m.p. 138° (Found: C, 69.0; H, 5.4. $C_{18}H_{16}O_5$ requires C, 69.2; H, 5.1%). Cyclization of the ketone (VI) to the isoflavone (VII) was indicated by the insolubility of the substance in aqueous caustic soda and the absence of ferric chloride colouration. The yield of this substance (VII) is low if the reaction is carried out at room temperature.

5:7:2'-Trihydroxyisoflavone (I):

The ether (VII)(1 g.) in dry benzene (50 cc.)

was added to a solution of freshly distilled aluminium bromide (5.12 g.); 6 mols.), in benzene (50 cc.), when immediate separation of a complex was noticed. The mixture was refluxed for 3 hours, poured over ice and hydrochloric acid, and extracted with ether. The ether layer was washed with water and then extracted with 1% caustic soda solution. On acidifying the alkaline extract a colourless precipitate was obtained (0.80 g.), which crystallized from dilute alcohol in colourless needles, m.p. 187° (Found: C, 66.3; H, 4.1; $C_{15}H_{10}O_5$ requires C, 66.7; H, 3.7%). With alcoholic ferric chloride it gives a brownish violet colour. The triacetyl derivative separated from dilute acetic acid as colourless needles, m.p.151° (Found: C, 64.0; H, 4.3. $C_{21}H_{16}O_8$ requires C, 63.6; H, 4.0%).

2:6-Dihydroxy-4-methoxy-m-tolyl 2-methoxybenzyl ketone (VIII):

The ketone (VIII) was prepared in the same manner as (V) from 2:6-dihydroxy-4-methoxytoluene (4 g.), o-methoxybenzyl cyanide (4 g.), fused zinc chloride (6 g.) and ether (150 cc.). It

crystallized from dilute alcohol in pale yellow needles (2.8 g.), m.p. 194° (Found: C, 67.5; H, 6.2. $C_{17}H_{18}O_5$ requires C, 67.6; H, 5.9%). The ferric reaction in alcohol is dark violet-brown.

2-Hydroxy-4:6-dimethoxy-m-tolyl 2-methoxybenzyl ketone (IX):

The ketone (VIII)(1.5 g.), dimethyl sulphate (0.5 cc., 1 mol.) and anhydrous potassium carbonate (5 g.) and acetone (50 cc.) were refluxed for 14 hrs. and worked up as usual. The product crystallized from dilute alcohol in colourless needles (1.4 g.), m.p. 150° (Found: C, 68.2; H, 6.4. $C_{18}H_{20}O_5$ requires C, 68.4; H, 6.5%). An alcoholic solution of the substance gives a greenish brown colour with ferric chloride.

5:7:2'-Trimethoxy-8-methylisoflavone (X):

A solution of the ketone (IX)(2 g.) in freshly distilled ethyl formate (150 cc.), cooled to 0°, was gradually added to sodium dust (3 g.). An immediate reaction ensued, which soon became vigorous, the mixture turned reddish /brown in colour. After 4

hours the reaction mixture was poured over ice and hydrochloric acid with stirring. Next day the separated solid (1.7 g.) was filtered and crystallized from dilute alcohol; the colourless needles had m.p. 183-84° (Found: C, 70.2; H, 5.7. $C_{19}H_{18}O_5$ requires C, 69.9; H, 5.5%). Cyclization of the ketone (IX) to the isoflavone (X) was indicated by the absence of ferric reaction and the insolubility in aqueous caustic soda.

5:7:2'-Trihydroxy-8-methylisoflavone (II):

The trimethyl ether (X) was treated with aluminium bromide in benzene under the conditions described for the demethylation of (VII). The product crystallized from dilute alcohol in colourless needles, m.p.234° (Found: C, 68.0; H, 4.6. $C_{16}H_{12}O_5$ requires C, 67.6; H, 4.2%). The alcoholic solution of the substance gives an intense green colour with ferric chloride.

On acetylation with acetic anhydride and a few drops of pyridine in the usual manner the triacetyl derivative crystallized from alcohol in colourless needles, m.p. 105° (Found: C, 63.2;

H, 4.8; $C_{22}H_{18}O_8$, 1/2 H_2O requires C, 63.0; H, 4.5%). On drying at 120° and 2 mm. pressure over P_2O_5 for 4 hours, the m.p. rose to 139° (Found: C, 64.7; H, 4.8. $C_{22}H_{18}O_8$ requires C, 64.4; H, 4.4%).

2:6-Dihydroxy-4-methoxy-m-tolyl 4-nitrobenzyl ketone (XI):

This ketone (XI) was prepared in the same manner as (V) from 2:6-dihydroxy-4-methoxytoluene (2 g.), p-nitrobenzyl cyanide (2.1 g.), fused zinc chloride (2 g.) and ether (50 cc.). It crystallized from alcohol in colourless needles, m.p. 201° (Found: C, 60.8; H, 4.8; N, 4.5. $C_{16}H_{15}O_6$ requires C, 60.6; H, 4.7; N, 4.4%). It gives a brown colouration with ferric chloride.

7-Hydroxy-5-methoxy-8-methyl-4'-nitroisoflavone (XII):

The above ketone (0.1 g.) was dissolved in dry pyridine (2 cc.). Ethyl orthoformate (0.4 cc.) and piperidine (2 drops) were then added. After refluxing the solution for about five minutes the colour changed from brown to orange red. After about

an hour some solid separated. It was filtered and crystallized from glacial acetic acid in yellow needles. The substance decomposes above 320°. (Found: C, 62.0; H, 4.1; N, 4.6. $C_{17}H_{13}O_6N$ requires C, 62.4; H, 4.0; N, 4.3%). It did not give any colouration with alcoholic ferric chloride.

7-Hydroxy-5-methoxy-8-methyl-4'-aminoisoflavone (XIII):

7-Hydroxy-5-methoxy-8-methyl-4'-nitroisoflavone (XII)(0.2 g.) was added to a suspension of zinc dust (0.4 g.) and ethyl alcohol (35 cc.) To the refluxing solution was then added glacial acetic acid (4 cc.) during four hours, and the refluxing was continued for another two hours. The solution was then filtered hot and water was added after concentrating it to about 5 cc. The product which separated was filtered and dried (0.16 g.). It gave a positive test for the primary amine with alkaline β-naphthol. It crystallized from alcohol in cream coloured irregular prisms, m.p.264° (decomp.) (Found: N, 4.7. $C_{17}H_{15}O_4N$ requires N, 4.7%). It gives no colouration with alcoholic ferric chloride.

7:4'-Dihydroxy-5-methoxy-8-methylisoflavone (XIV):

The above substance (XIII)(0.16 g.) was dissolved in a mixture of water (2 cc.) and conc. sulphuric acid (3 cc.). The solution was diazotized with sodium nitrite (0.2 g.), at 0° and after destroying the excess of the nitrous acid by urea, it was poured in a boiling solution of sulphuric acid (5 cc.) and water (15 cc.). The boiling was continued for about five minutes. The solution diluted, cooled and the precipitate was collected. It crystallized from dilute acetic acid in thick pale yellow rectangular rods, m.p.300-2° (decomp.) Its alcoholic solution gives no colouration with ferric chloride.

5:7:4'-Trihydroxy-8-methylisoflavone (III):

The above compound (XIV), m.p. 300-2° (decomp.) was refluxed with acetic acid (1 cc.), acetic anhydride (1 cc.) and hydriodic acid (d. 1.7, 1 cc.) for three hours, cooled, and the excess of iodine was destroyed by adding sodium bisulphite solution. The precipitate was collected and was passed through a florex column, using benzene as a solvent. The

eluted compound was then crystallized from dilute alcohol in pale yellow prismatic needles, m.p.231-32° (Found: in material dried at 140°/0.2 mm. for 3 hours: C, 67.2; H, 4.6. $C_{16}H_{12}O_5$ requires C, 67.6; H, 4.2%). The substance gave an intense green colouration with ferric chloride (Seshadri et al.[16] m.p.231-32°. Whalley[8] m.p. 252°).

3:5-Dicarbomethoxy-2:6-dihydroxyphenyl benzyl ketone (XV):

Dimethyl resorcinol-4:6-dicarboxylate (5 g.) in dry nitrobenzene (75 cc.) at 5-10° was treated with powdered aluminium chloride (10 g.). Phenylacetyl chloride (5 cc.) was gradually added, the reaction mixture stirred for 3 hours and left at room temp. for 24 hours. It was then heated on a steam bath for an hour and poured over ice and hydrochloric acid. After steam distillation of nitrobenzene and unreacted ester, the product (5.75 g.) was filtered and crystallized thrice from dilute alcohol. The colourless needles had m.p.131° (Found: C, 63.2; H, 4.6. $C_{18}H_{16}O_7$ requires C, 62.8; H, 4.6%). An alcoholic solution of the substance gives a blood red ferric

colour. The 2:4-dinitrophenylhydrazone prepared as usual crystallized from dilute acetone in yellow plates, m.p. 215° (Found: N, 10.3. $C_{24}H_{20}O_{10}N_4$ requires N, 10.6%).

2:6-Dihydroxyphenyl benzyl ketone (XVI):

The ester (XV)(1 g.) was treated with boiling 10% alcoholic caustic soda (200 cc.) for 3 hours, alcohol was distilled off, and the solution acidified and extracted with ether. The ether extract was extracted with sodium bicarbonate solution, the aqueous extract acidified and filtered. The precipitate was a carboxylic acid, but decarboxylation occurred during crystallization from water. The precipitate was decarboxylated by boiling with water for 3 hours, and the product (XVI) (0.5 g.) crystallized from dilute alcohol in faint yellow plates, m.p.170° (Found: C, 74.0; H, 5.7. $C_{14}H_{12}O_3$ requires C, 73.7; H, 5.3%). The substance gives a green colour with alcoholic ferric chloride.

5-Hydroxyisoflavone (IV):

The ketone (XVI)(1 g.), dry pyridine (20 cc.),

piperidine (1 cc.) and ethyl orthoformate (1.9 g., 3 mols.) were refluxed for 8 hours, and poured into dilute hydrochloric acid. The separated solid was filtered (1 g.) and crystallized from dilute alcohol. The colourless needles had m.p. 102° (Found: C, 75.6; H, 4.4. $C_{15}H_{10}O_3$ requires C, 75.6; H, 4.3%). The alcoholic solution gives a dark violet colour turning immediately to dark olive with ferric chloride. The substance dissolves in aqueous caustic soda very slowly. The substance dissolves in aqueous caustic soda very slowly with a faint yellow colour. Acetylation by refluxing with acetic anhydride and pyridine for 2 hours gave the acetyl derivative which crystallized from dilute acetic acid in colourless needles, m.p. 144° (Found: C, 72.6; H, 4.6. $C_{17}H_{12}O_4$ requires C, 72.9; H, 4.3%).

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SUMMARY

Part I:

Isoflavones have been reviewed in brief with reference to their occurrence, properties and reactions.

Synthetical methods have been discussed with particular reference to the recent developments in this field.

Part II :

As a preliminary to the synthesis of muningin, 6-hydroxy-5:7-dimethoxyisoflavone (I) has been synthesized by the cyclization of 2:5-dihydroxy-4:6-dimethoxyphenyl benzyl ketone with ethyl orthoformate. The ketone was prepared by the interaction of 2:6-dimethoxyhydroquinone and the boron trifluoride complex of phenylacetic acid. Demethylation of (I) with hydriodic acid gave 5:6:7-trihydroxyisoflavone

Unsuccessful attempts were made to prepare the analogues of (I) from p-nitro-, p-benzyloxy-, p-tosyloxy-, and p-phenylazophenylacetic acid.

Nitration of 8-bromo-5:7-dimethoxy-4'-nitroisoflavone resulted in the formation of a dimethoxy-trinitroisoflavone. Mononitration in the 6-position would have led to muningin.

5:6:7:4'-Tetramethoxyisoflavone (II; the dimethyl ether of muningin) was synthesized by the ethyl orthoformate cyclization of the appropriate deoxybenzoin, which was obtained from antiarol and the boron trifluoride complex of p-methoxyphenylacetic acid. Partial demethylations of the isoflavone (II) to 5-hydroxy-6:7:4'-trimethoxyisoflavone, 5:6-dihydroxy-7:4'-dimethoxyisoflavone, and 5:6:4'-trihydroxy-7-methoxyisoflavone (III) have been effected. Total demethylation of (II) gave 5:6:7:4'-tetrahydroxyisoflavone.

Treatment of (III) in acetone with benzoyl chloride (2 mols.) and potassium carbonate gave 6:4'-dibenzoyloxy-5-hydroxy-7-methoxyisoflavone, methylation of which yielded 6:4'-dibenzoyloxy-5:7-dimethoxyisoflavone (IV), m.p. of which was not depressed by mixing with O-dibenzoyl muningin prepared from natural muningin by using potassium carbonate, benzoyl chloride and acetone. Hydrolysis of (IV) with methanolic caustic potash, at 30° and

acidification gave 6:4'-dihydroxy-5:7-dimethoxy-isoflavone, identical with muningin.

Ethyl orthoformate cyclization of the appropriate ketones, prepared by the boron trifluoride method, gave muningin diethyl ether and muningin 4'-methyl ether. Selective demethylation of the latter resulted in the formation of 5:6-dihydroxy-7:4'-dimethoxyisoflavone.

Part III:

The wide applicability of boron trifluoride as a condensing agent in the synthesis of phenolic ketones has been demonstrated by the preparation of ketones from various phenols and substituted phenylacetic acids.

The scope and limitations of ethyl orthoformate in the synthesis of isoflavones has been discussed. Ethyl orthoformate has been shown to be generally useful except for the cyclization of deoxybenzoins having a phloroglucinol nucleus. A nitro group in the 4-position of the benzyl group in a deozybenzoin favours the cyclization. By using this method a series of known and unknown isoflavones have been synthesized.

Part IV:

5:7:2'-Trihydroxyisoflavone, 5:7:2'-trihydroxy-8-methylisoflavone, and 5:7:4'-trihydroxy-8-methylisoflavone have been synthesized, and have been found to be different respectively from "isogenistein", "methylisogenistein" and "methylgenistein" isolated by Okano and Beppu from soya bean.

As a preliminary to the synthesis of 5:4'-dihydroxy-8-methylisoflavone, which Okano and Beppu have stated to be the probable structure of tatoin, another constituent of soya bean, 5-hydroxyisoflavone has been synthesized. For this purpose the necessary 2-phenylacetylresorcinol was prepared by a new method in which dimethyl resorcinol-4:6-dicarboxylate was condensed with phenylacetyl chloride and the ester then hydrolysed and decarboxylated.

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Candidate