SYNTHETICAL EXPERIMENTS IN THE FLAVONE, FLAVONOL AND ISOFLAVONE SERIES

A Thesis
submitted by

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of
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CONTENTS.

Page.

Part I : Coupling of Hydroxyflavones with Diazotized Aniline as a route to FLavones hydroxylated in the 5:6- and 5:8-positions 1

Experimental 24

Part II : A new Synthesis of Rhamnazin 78

Experimental 85

Part III : Experiments in the Isoflavone series. 90

Experimental 95

Summary 105

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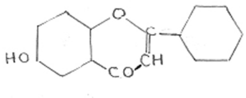
PART I

COUPLING OF HYDROXYFLAVONES WITH DIAZOTISED ANILINE AS A ROUTE TO FLAVONES HYDROXYLATED IN THE 5:6- AND 5:8- POSITIONS.

Examining the reactivity of natural colouring matters derived from phloroglucinol towards diazonium salts, A. G. Perkin[1] found that chrysin, apigenin, euxanthone and catechin gave disazo dyes. Mahal and Venkataraman[2] found that 6-hydroxyflavone (I) couples with diazotised p-nitraniline to form a monoazo dye formulated as (II) in view of the tendency for the fixation of the double bonds in the benzene ring of the flavone molecule as indicated in (III). It was then suggested[3] that a route to the synthesis of -- 5:6-dihydroxyflavone (IV) then becomes possible. It has now been confirmed[4] that 6-hydroxyflavone couples with diazonium salts in the 5-position, and 5:6-dihydroxyflavone has been synthesised by a method which promises to be of value for the synthesis of other



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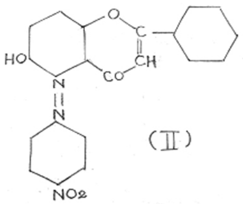

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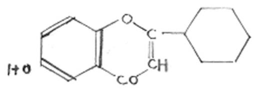

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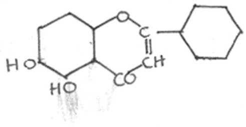
di- and polyhydroxyflavones and flavonols.

Coupling 6-hydroxyflavone (I), dissolved in alcohol and pyridine with the addition of ammonia, with diazotised aniline, a nearly quantitative yield of 5-benzeneazo-6-hydroxyflavone (V) was obtained. 6-Hydroxyflavone is sparingly soluble in aqueous caustic soda, and the sodium salt tends to separate at the low temperature necessary for coupling, although diazotised p-nitraniline on account of its greater reactivity in comparison with diazotised aniline couples readily with 6-hydroxyflavone even under these conditions. The dye (V) was reduced to 5-amino-6-hydroxyflavone (VI) by means of zinc dust in boiling acetic acid. Other reagents



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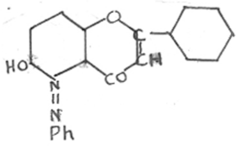

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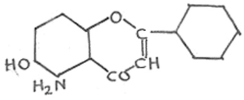
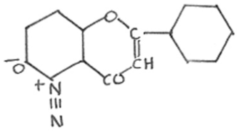


(VI)

for reduction as well as the hydrosulphite reduction of the alkali-soluble azo dye obtained by coupling diazotised suphanilic acid with 6-hydroxyflavone, were tried; but various difficulties were encountered in the isolation of the aminohydroxyflavone, and zinc-acetic acid procedure was found to be suitable.

When the amine (VI) was dissolved in hydrochloric acid and treated with sodium nitrite, the brownish yellow product obtained crystallized from alcohol in needles; the substance was insoluble in aqueous alkali, gave a yellow solution with concentrated sulphuric acid, no coloration with ferric chloride and on heating, decomposed at about 173°. From these reactions and the nitrogen content, the substance was regarded as the diazo-oxide (VII).[5,6] On boiling with a mixture of equal volumes of concentrated sulphuric acid and water, the diazo-oxide (VII) underwent hydrolysis, and gave a dihydroxyflavone, which agreed in all its properties with



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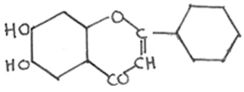

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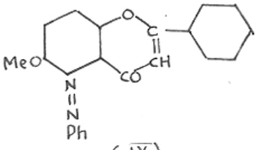
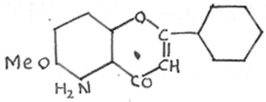


(IX)

5:6-dihydroxyflavone (IV) and not with the 6:7-isomer (VIII). 6:7-Dihydroxyflavone has been synthesised by Chadha and Venkataraman[7] by the Robinson reaction on 2:4:5-trihydroxyacetophenone; and 5:6-dihydroxyflavone by Baker[8] by the action of sodamide[9] on 2-benzoyloxy-5:6-dimethoxyacetophenone, followed by cyclisation and demethylation. Nakazawa[10] has synthesised 5:6-dihydroxyflavone by a route similar to Baker's. 2-Hydroxy-6-methoxyacetophenone was oxidised in alkaline solution to 2:5-dihydroxy-6-methoxyacetophenone. The dibenzoate of this compound was then converted to the β-diketone by the sodamide method.[9] Cyclisation and hydrolysis gave 5-methoxy-6-hydroxyflavone, which was demethylated by means of aluminium chloride in nitrobenzene[11] to 5:6-dihydroxyflavone.

When 5-benzeneazo-6-hydroxyflavone was refluxed in acetone solution with dimethyl sulphate and -- potassium carbonate, 5-benzeneazo-6-methoxyflavone (IX) was obtained. Reduction with zinc dust and acetic acid diluted with alcohol resulted in a product which gave a deep brown coloration with ferric chloride in alcoholic solution, thus indicating -- demethylation. However, reduction with Raney nickel and hydrogen at 40 pounds' pressure in alcoholic solution gave 5-amino-6-methoxyflavone (X). The diazonium salt obtained from this amine decomposed only in sulphuric acid of 80% strength, but the resulting product was 5:6-dihydroxyflavone instead of 5-hydroxy-6-methoxyflavone (XI).



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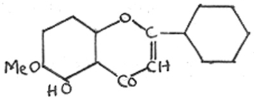

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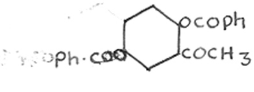

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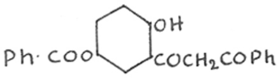

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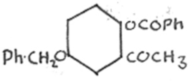

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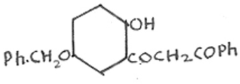
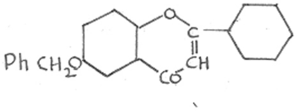


(XV)

6-Hydroxyflavone (I) required for coupling was prepared by the conversion of 2:5-dibenzoyloxyacetophenone (XII) to 2-hydroxy-5-benzoyloxydibenzoylmethane (XIII) by the action of potassium carbonate in toluene[12] or sodamide in benzene and cyclisation of the diketone (XIII), with simultaneous debenzoylation, by the action of cold concentrated sulphuric acid, Potassium carbonate was found in this case to be a better reagent than sodamide and excellent yields of 6-hydroxyflavone could be obtained. -- 6-Hydroxyflavone was first prepared by Kostanecki, Levi and Tambor.[13] Chadha and Venkataraman[7] prepared it by the Robinson reaction on quinacetophenone and later Bhalla, Mahal and Venkataraman[14] obtained it by converting 2-benzoyloxy-5-benzyloxyacetophenone (XIV) to 2-hydroxy-5-benzyloxydibenzoylmethane (XV) by the action of sodamide in ether, ring closure with sulphuric acid and debenzylation of the 6-benzyloxyflavone (XVI) thus produced.



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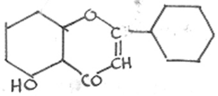

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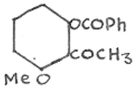

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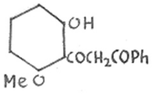

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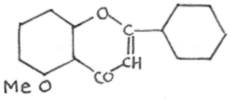

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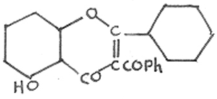
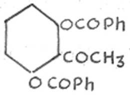


(XXI)

5-Hydroxyflavone (XVII), first prepared by Simonis and Danshevski (as its methyl ether),[15] then by Sugasawa[16] and later by Baker,[17] was recently obtained in good yields by Seshadri and co-workers[18] by converting 2-benzoyloxy-6-methoxyacetophenone (XVIII) to the corresponding diketone (XIX) using sodamide in toluene at 100°, cyclisation of the diketone to 5-methoxyflavone (XX) and demethylation of the latter. They also obtained it by debenzoylation of 3-benzoyl-5-hydroxyflavone[17] (XXI) with alcoholic sodium carbonate.

2-Acetylresorcin dibenzoate (XXII), when treated with sodium ethoxide in absolute alcohol,[19] gave 2-hydroxy-6-benzoyloxydibenzoylmethane (XXIII), which was --



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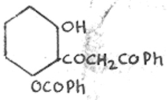

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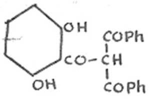


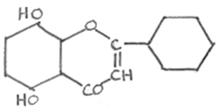
(XXIV)

converted into 5-hydroxyflavone by the action of glacial acetic acid containing fused sodium acetate. This method is more advantageous than Seshadri's method, since it does not involve monomethylation of 2-acetylresorcin prior to benzoylation nor does it involve demethylation after formation of the flavone.

2-Acetylresorcin dibenzoate (XXII), dissolved in ether or boiling benzene and treated with sodamide, gave a compound which is formulated as a triketone (XXIV), since it gave 3-benzoyl-5-hydroxyflavone[17] (XXI) by refluxing with glacial acetic acid and fused sodium acetate.

As study of the behaviour of 5-hydroxyflavone towards benzenediazonium chloride was undertaken, in the hope that a simple synthesis of primetin (XXV) might emerge. 5-Hydroxyflavone, dissolved in a mixture of pyridine and alcohol, coupled with --

diazotised aniline. Here it was found advantageous to add one mole of caustic soda per mole of hydroxyflavone, besides ammonia, to get a good yield of the azo dye, presumably (XXVI). This on reduction gave a primary amine assumed to be (XXVIII). The diazonium salt from (XXVIII) was unusually stable, but on slow boiling with 50% sulphuric acid until the concentration was about 80%, decomposition with evolution of nitrogen took place. The product, however, was -- 5:6-dihydroxyflavone and not 5:8-dihydroxyflavone. The constitution of the azo dye from 5-hydroxyflavone was therefore examined. When 4-benzeneazo-2-acetylresorcin (XXX) was heated with benzoic anhydride and



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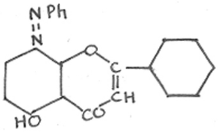

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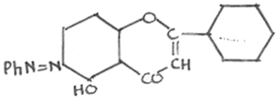
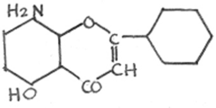


(XXVII)

sodium benzoate, the product was a benzeneazo-5-hydroxyflavone different from (XXVI) and formulated as (XXVII). Cyclisation of (XXX) in this direction so as to form (XXVII) and not (XXVI) is to be -- expected on account of chelation of the azo and *o* -hydroxyl groups as well as the steric hindrance of the reactivity of the hydroxyl group sandwiched between the azo and acetyl groups. The constitution



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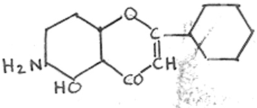

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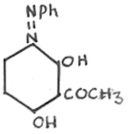
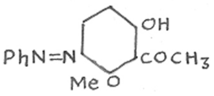


(XXX)

of (XXVII) and (XXVI) as the 6- and 8-benzeneazo derivatives of 5-hydroxyflavone was confirmed by the fact that (XXVI) could be methylated by means of dimethyl sulphate and potassium carbonate in acetone solution, while (XXVII) was completely -- resistant to methylation. Further, reduction of (XXVII) and diazotisation of the aminophenol (XXIX) thus obtained gave a diazo-oxide in the same manner as 5-amino-6-hydroxyflavone,[4] indicating the adjacent orientation of the amino and hydroxyl groups in (XXIX) and therefore the benzeneazo and hydroxyl groups in (XXVII). The amine (XXIX) agrees in its properties with 6-amino-5-hydroxyflavone prepared umambiguously from 6-acetyl-5-hydroxyflavone by Sugasawa,[20] except for a slight difference in the melting point (Sugasawa, 177°). Vigorous hydrolysis of the diazo-oxide with sulphuric acid gave 5:6-dihydroxyflavone. Sugasawa has stated that all his attempts to replace the amino by hydroxyl in 6-amino-5-hydroxyflavone were unsuccessful. He has obviously failed to observe the extraordinary stability of the diazo compound. It was found necessary to use boiling 80% sulphuric acid to hydrolyse the diazo-oxide.

Since the aminophenols (XXVIII) and (XXIX) were isomeric and not identical compounds, the former is 8-amino-5-hydroxyflavone, so that the azo dye (XXVI) behaves normally during reduction. The formation of 5:6-dihydroxyflavone and not 5:8-dihydroxyflavone from (XXVI) and (XXVII), can then be explained by postulating the opening of the pyrone ring under the drastic conditions of acid treatment of the diazonium salt, followed by cyclisation in the alternative direction. Such rearrangements have been noticed by Wesseley,[21] Baker[22] and Seshadri[23] during demethylation of flavones containing methoxyl groups in the 5:8-positions by hydriodic acid. It has also been observed that by boiling with 32% hydrochloric acid or 50-80% sulphuric acid, 8-amino-5-hydroxyflavone (XXVIII) is converted into the 6-amino compound (XXIX); and primetin is converted into 5:6-dihydroxyflavone.

2-Hydroxy-6-methoxyacetophenone[22] gave a mono-azo dye when coupled with benzenediazonium chloride. This dye is different from the compound obtained by methylating 4-benzeneazo-2-acetylresorcin (XXX) with 1 mole of dimethyl sulphate in acetone solution in the presence of potassium carbonate. The latter compound must be 2-hydroxy-4-methoxy-3-acetylazobenzene(XXXII). Hence the first compound must be



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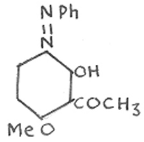

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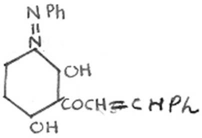
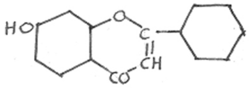


(XXXIII)

4-hydroxy-2-methoxy-3-acetyl azobenzene (XXXI). When (XXXI) was submitted to the Robinson reaction with benzoic anhydride, the result was unexpected. Instead of (XXVII) or its methyl ether, (XXVI) was obtained. Demethylation occurred, apparently by a direct attack of the anhydride on the methoxyl group, immediate cyclisation to (XXVI) then taking place. Demethylation during the Robinson reaction was observed by Baker[8] in the case of 2:5-dihydroxy-6-methoxyacetophenone, but the cyclisation of the flavone proceeded in the direction to be anticipated and the product was 5:6-dihydroxyflavone.

Attempts were made to oxidise 3'-benzeneazo-2':6'-dihydroxychalkone (XXXIII), obtained by the condensation of 4-benzeneazo-2-acetylresorcin with benzaldehyde, to the corresponding flavone, by the action of selenium dioxide in amyl alcohol or xylene. The experiments were unsuccessful, since the original compound was obtained unchanged from the reaction mixture.

7-Hydroxyflavone[12] (XXXIV) gave with benzenediazonium chloride a monoazo dye which was readily reduced to the amine. The diazonium compound -- obtained from this amine was found to be extremely stable and did not undergo hydrolysis even in boiling 80% sulphuric acid. Further, increase in the acid concentration resulted in the hydrolysis of the diazonium solution, but simultaneously charring took place and no crystallisable product could be isolated. However, the diazonium solution could be coupled with alkaline β-naphthol to produce an azo dye. In



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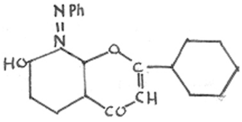

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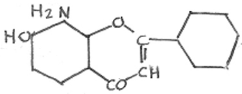
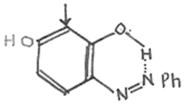


(XXXVI)

7-hydroxyflavone coupling should normally take place in the 8-position by analogy with the behaviour of 6-hydroxyflavone which has been shown to couple in the 5-position. The amine obtained from the dye (XXXV) is therefore formulated as (XXXVI).

It is known that resorcinol couples so readily that even with 1 mole of benzene diazonium chloride, about 10% of disazo dye is formed. Using 2 mols. of the diazonium salt, two disazo dyes are obtainable depending on the pH of the reaction mixture. At pH 5.8, the product is 2:4-bisbenzeneazoresorcinol. 4-Benzeneazoresorcinol (XXXVII) is first formed and the entry of the second azo group in the 2-position is due to chelation indicated in (XXXVII) as a result of which one of the Kekulé forms makes a larger contribution to the resonance of the benzene ring; coupling then takes place at the end of the double bond attached to the hydroxylated carbon atom. Under strongly alkaline conditions, the hydrogen bond is broken, and coupling then takes place, as in -- resorcinol itself, in the o-, p-position to the two hydroxyl groups as indicated in (XXXVIII).



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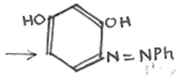

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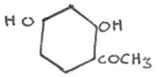
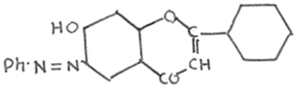


(XXXIX)

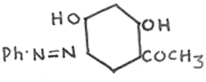
Resorcinol may be coupled in one operation or in progressive stages with 3 mols. of a diazonium salt. The second coupling proceeds to completion very readily, but not the third. With benzenediazonium chloride, the trisazo dye may be obtained in about 25% yield; but better yields of the trisazo dyes may be obtained by using **diazo** salts of higher coupling energy, but the third coupling cannot be carried out quantitatively.[24]

When resacetophenone (XXXIX) was coupled with 1 mole of benzenediazonium chloride at pH 8-9, the product was a mixture of dyes. By treatment with 5% caustic soda, alkali soluble and alkali insoluble parts could be separated. The former was a monoazo dye, while the alkali-insoluble substance was a disazo dye which could only be 3:5-bisbenzeneazoresacetophenone, since analysis indicated no displacement of the acetyl group. When the coupling was carried out under strongly alkaline conditions the disazo dye was obtained together with a little monoazo dye. The latter was identical with that obtained by -- coupling at pH 8-9. The orientation of the benzeneazo group in the monoazo dye was then investigated.

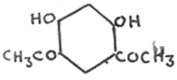
By carrying out the Robinson reaction on the monobenzeneazoresacetophenone using benzoic anhydride and sodium benzoate, a flavone was obtained in poor yield. This compound was different from the azo dye obtained by direct coupling of 7-hydroxyflavone with benzenediazonium chloride. Since the latter is probably 8-benzeneazo-7-hydroxyflavone (XXXV), the Robinson reaction product is considered to be 6-benzeneazo-7-hydroxyflavone (XL). It would therefore appear that, under the conditions employed, resacetophenone couples in the 5-position as shown in (XLI).



(XL)



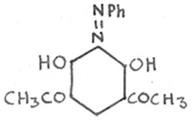
(XLI)



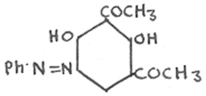
(XLII)

Further experiments were carried out to elucidate the position of coupling in resacetophenone.

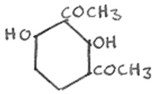
Monobenzeneazoresacetophenone dibenzoate was prepared by heating the dye (XLI) with benzoyl -- chloride in pyridine. By the action of potassium carbonate in boiling toluene, the dibenzoate yielded a compound which behaved like a diketone; but the corresponding flavone could not be obtained by treatment with cold concentrated sulphuric acid or boiling 20% alcoholic sulphuric acid or boiling -- glacial acetic acid containing fused sodium acetate.



(XLIII)



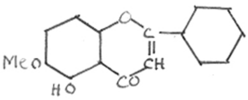
(XLIV)



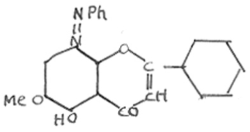
(XLV)

4:6-Diacetylresorcin (XLII) can only couple in the 2-position, and therefore, the dye obtained by coupling it with diazotised aniline must be 2-benzeneazo-4:6-diacetylresorcin (XLIII), since analysis indicated no displacement of acetyl groups.[24] The compound obtained by carrying out the Fries rearrangement on <u>mono</u>benzeneazoresacetophenone diacetate was found to be different from 2-benzeneazo-4:6-diacetylresorcin (XLIII). Hence the former compound can only be 6-benzeneazo-2:4-diacetylresorcin (XLIV). A sample of 6-benzeneazo-2:4-diacetyl resorcin was prepared by coupling 2:4-diacetylresorcin[25] (XLV) with benzenediazonium chloride and was found to be identical with the Fries rearrangement product from monobenzeneazoresacetophenone diacetate. This conclusively shows that resacetophenone couples in the 5-position.

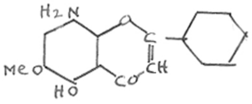
5-Hydroxy-6-methoxyflavone (XLVI) was prepared by methylating 5:6-dihydroxyflavone with one mole of dimethyl sulphate in acetone solution in the presence of anhydrous potassium carbonate; and also by partially demethylating 5:6-dimethoxyflavone with aluminium chloride in ether at room temperature.[22] (XLVI) gave a monoazo dye when --



(XLVI)

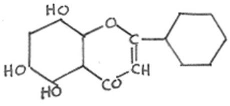


(XLVII)

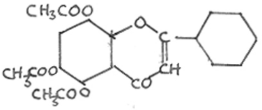


(XLVIII)

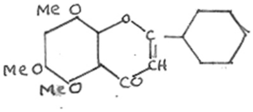
coupled with benzenediazonium chloride. This can only be 8-benzeneazo-5-hydroxy-6-methoxyflavone (XLVII). The dye was reduced to the amine (XLVIII). Refluxing the amine for 24 hours with 32% hydrochloric acid[26] gave a product which did not contain nitrogen. It crystallised from dilute alcohol in sheaves of orange-yellow needles melting at 234-236°. It analyses correctly as a trihydroxyflavone and must therefore be 5:6:8-trihydroxyflavone (XLIX). It gave a red solution with aqueous caustic soda and a brownish violet colour with alcohol-ferric chloride. It also gave a triacetyl and a trimethyl derivative formulated as (L), and (LI) respectively. During hydrochloric acid treatment the amine (XLVIII) undergoes hydrolysis as well as demethylation in the 6-position. Gardenin



(XLIX)



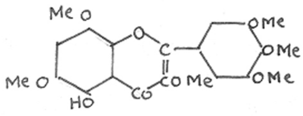
(L)



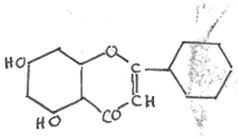
(LI)

Gardenin is the first example of a naturally occurring flavone with hydroxyl groups in the 5, 6, and 8-positions. It is present in the -- resinous exudation from the leaf-bud of <u>Gardenia lucida</u>. Roxb., It was reported about 70 years ago by Stenhouse[27] and recently shown to be 5-hydroxy-3:6:8:3':4':5'-hexamethoxyflavone (LII) by Bose[28] and Balakrishna and Seshadri.[29]

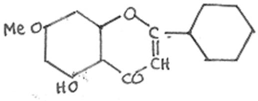
Chrysin (LIII), prepared by the method of Robinson and Venkataraman[30] was partially methylated to form tectochrysin (LIV) by refluxing



(LII)

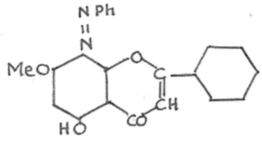


(LIII)

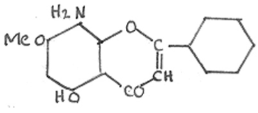


(LIV)

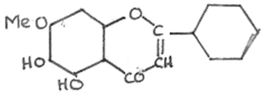
with 1 mole of dimethyl sulphate in acetone solution in the presence of potassium carbonate. Tectochrysin (LIV) coupled with benzenediazonium chloride yielding a monoazo dye, formulated as 8-benzeneazo-tectochrysin (LV), by analogy with the behaviour of 5-hydroxyflavone towards benzenediazonium chloride. Further it could be methylated to a benzeneazodimethoxyflavone by means of dimethyl sulphate and potassium carbonate in acetone. Reduction of (LV) with zinc dust in alcohol-acetic acid yielded the amine (LVI). The



(LV)

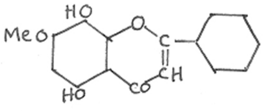


(LVI)

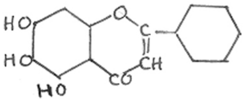


(LVII)

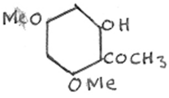
diazonium salt from (LVI) was even more difficult to decompose than the salt from the amine (XXVI), prolonged boiling with 80% sulphuric acid being necessary. Under these conditions, some general decomposition was unavoidable and the product which gave all the characteristic colour reactions of baicalein, could not be isolated in pure crystalline form. However, on boiling (LVI) with 32% hydrochloric acid for 24 hours,[26] baicalein-7-methyl ether (LVII), agreeing in all the properties with the substance described by Sastri and Seshadri[34] was obtained in good yield. Thus during treatment with hydrochloric acid there is hydrolysis of the amine, accompanied by a rearrangement of 5:8-dihydroxy-7-methoxyflavone (LVIII) to 5:6-dihydroxy-7-methoxyflavone (LVII). Demethylation of (LVII) with hydriodic acid yielded baicalein (LIX). This compound was first isolated from a natural source



(LVIII)

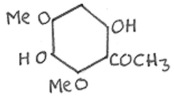


(LIX)

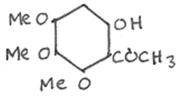


(LX)

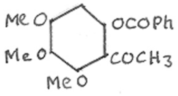
by Shibata, Nakamura and Iwata,[32] but synthesised earlier by Bargellini.[33] A more recent and less ambiguous synthesis is due to Sastri and Seshadri.[34] They oxidised 2-hydroxy-4:6-dimethoxyacetophenone (LX) to 2:5-dihydroxy-4:6-dimethoxyacetophenone (LXI) with alkaline persulphate. Partial methylation of the latter to 2-hydroxy-4:5:6-trimethoxyacetophenone (LXII), Baker-Venkataraman transformation of its benzoate (LXIII), and cyclisation of the diketone (LXIV) led to baicalein-trimethyl ether



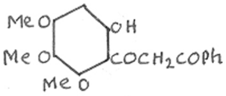
(LXI)



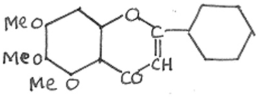
(LXII)



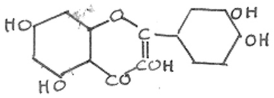
(LXIII)



(LXIV)



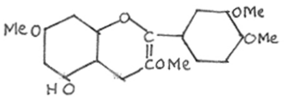
(LXV)



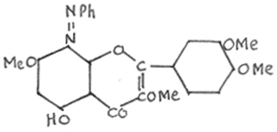
(LXVI)

(LXV), which gave baicalein (LIX) on demethylation with hydriodic acid.

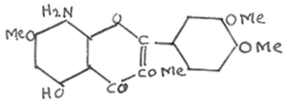
Attempts have been made in the present work to couple quercetin (LXVI) with benzenediazonium chloride, but as anticipated, the diazonium salt underwent rapid decomposition with evolution of nitrogen. Quercetin 3:7:3':4'-tetramethyl ether (LXVII) was prepared by methylating quercetin in acetone with 4 mols. of dimethyl sulphate. The tetramethyl ether coupled smoothly with benzenediazonium chloride in a mixture of alcohol and pyridine and a monoazo dye considered to be 8-benzeneazo-quercetin-3:7:3':4'-tetramethyl ether (LXVIII) was obtained. This azo dye, like 8-benzeneazo-tectochrysin (LV), underwent methylation with dimethyl sulphate and potassium carbonate in



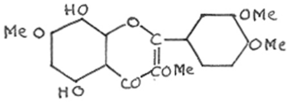
(LXVII)



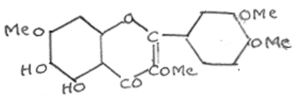
(LXVIII)



(LXIX)



(LXX)



(LXXI)

acetone. The dye (LXVIII) could be reduced to the amine (LXIX), the diazonium salt of which was extremely stable and could not be hydrolysed even in boiling 80% sulphuric acid, as in the case of 8-amino-7-hydroxyflavone. Boiling with hydrochloric acid also did not give any homogeneous product which could be purified by crystallisation. Hence it was found impossible to obtain the -- corresponding hydroxymethoxyflavone (LXX) or (LXXI).

EXPERIMENTAL

Quinacetophenone :

Hydroquinone diacetate (22 g.) was mixed with finely powdered aluminium chloride (56 g.) and heated in an oil-bath, raising the temperature rapidly to 120° and then to 180°, over a period of 2 hrs. It was maintained at this temperature for 30 mins. The reaction product was cooled, treated with crushed ice and hydrochloric acid, and the yellow product washed with water. It crystallised from alcohol in yellowish green needles (11 g.) melting at 204°

Quinacetophenone dibenzoate (XII) :

Quinacetophenone (6 g.) was dissolved in pyridine (12 c.c.) and benzoyl chloride (11.40 g., 2 mols.) added. The mixture was heated on a water-bath for 1 hr., and the reaction product poured into ice-cold dilute hydrochloric acid. On crystallising the product from alcohol, colourless prisms (11 g.) were obtained and melted at 110-111°.

2-Hydroxy-5-benzoyloxydibenzoylmethane (XIII) :

Quinacetophenone dibenzoate (10 g.) was dissolved in warm dry toluene (60 c.c.) and refluxed in an oil-bath for 6 hrs. after the addition of freshly ignited potassium carbonate (30 g.), the flask being fitted with a reflux-condenser and calcium chloride tube. The yellow solid was filtered, washed with hot toluene and sucked dry. The dry powder was stirred up in water and again filtered and washed with water. The solid thus obtained was triturated with dilute hydrochloric acid to remove traces of potassium carbonate, filtered ~~and~~ washed with water and dried. The crude product (8.8 g.) could be directly cyclized to 6-hydroxyflavone by treatment with cold concentrated sulphuric acid. The crude diketone was crystallised twice from benzene and the lemon-yellow needles melted at 201-202° (Found: C, 73.6; H, 4.9. $C_{22}H_{16}O_5$ requires C, 73.3; H, 4.4%) A brown colour develops when a drop of ferric chloride is added to its alcoholic solution. With aqueous sodium hydroxide a yellow colour is obtained which turns brown on standing. The same compound (1.2 g.) could be obtained by refluxing quinacetophenone dibenzoate (2 g.) dissolved in dry benzene (20 c.c.) and finely powdered sodamide (3 g.) for six hrs. The yellow product was filtered, sucked dry and stirred into ice cold dilute acetic acid.

6-Hydroxyflavone (I) :

Crude 2-hydroxy-5-benzoyloxydibenzoylmethane (8.8 g.) was dissolved in cold concentrated sulphuric acid (80 c.c.) and left overnight. The solution was poured over crushed ice, the grey solid filtered, washed with water and crystallised from alcohol. The pale yellow needles (5 g.) melted at 236°. The acetate, prepared by heating the flavone with acetic anhydride and pyridine, crystallized from alcohol in colourless woolly needles and melted at 158°.

5-Benzeneazo-6-hydroxyflavone (V) :

A solution of benzenediazonium chloride, prepared from aniline (2 g.; 2.5 mols.) hydrochloric acid (32%; 6·0 c.c.), alcohol (30 c.c.) and sodium nitrite (1.8 g. in 10 c.c. water) was gradually added at 0° to a solution of 6-hydroxyflavone (2 g.) in alcohol (50 c.c.), pyridine (14 c.c.) and ammonia (d 0.88; 2 c.c.). During addition of the diazonium solution and for six hours thereafter, the mixture was mechanically stirred and maintained at 0°. On leaving overnight in the refrigerator, the dark red precipitate was collected, boiled with a mixture of equal volumes of hydrochloric acid (32%) and water for a few minutes, filtered, washed and crystallised from alcohol. The dark red, elongated plates (2 g.) melted at 191-192° (Found: N, 8.4; $C_{21}H_{14}O_3N_2$ requires N, 8.2%).

<u>5-Amino-6-hydroxyflavone (VI)</u> :

5-Benzeneazo-6-hydroxyflavone (1 g.) was dissolved in boiling acetic acid (30 c.c.) and to the boiling solution zinc dust (2 g.) was added in small lots and the mixture was refluxed for 20 mins. The deep red solution became brown in colour. Zinc dust (0.5 g.) was again added, and the solution refluxed for 20 mins. longer. The reaction mixture was filtered and the zinc dust extracted thrice with 5 c.c. portions of hot acetic acid. The filtrate was cooled, diluted with water and allowed to stand in the refrigerator. The yellowish brown precipitate was filtered, washed with water, sucked dry and dissolved in hot alcohol (Norit). The filtered alcoholic solution was diluted with water and allowed to stand in the refrigerator. The orange-brown clusters of needles were collected and dried in vacuo. The substance (0.5 g.) melted at 235° after shrinking at 200° (Found: N, 5.7. $C_{15}H_{11}O_3N$ requires N, 5.5%). The brown alcoholic solution turns dark greenish brown on the addition of ferric chloride. The substance dissolves in caustic soda giving an orange brown colour. It gives a pale brown colour with concentrated sulphuric acid. It gradually goes into a pale brown solution by boiling with N-hydrochloric acid.

Flavone-5-diazo-6-oxide (VII) :

5-Amino-6-hydroxyflavone (0.5 g.) was boiled with N-hydrochloric acid (20 c.c.), till most of it went into solution. The solution was filtered, and the residue twice extracted with 20 c.c. portions of the acid. A small amount of undissolved resinous matter was rejected. The combined hydrochloric acid extracts were cooled to room temperature and sodium nitrite (2 g.) was added in small lots, the reaction flask being rotated during addition. On leaving the reaction mixture in the refrigerator for 30 minutes, the yellowish brown precipitate was filtered, washed with water and dried. There was no appreciable change in the yield by carrying out the sodium nitrite addition at 10° or 50°. On crystallisation from alcohol, glistening brown needles were obtained, which decomposed at 173° (Found: C, 68.2; H, 3.1; N, 10.4. $C_{15}H_8O_3N_2$ requires C, 68.2; H, 3.0%; N, 10.6%). The substance does not respond to Lassaigne's sodium fusion test for nitrogen. It is insoluble in aqueous soda, and dissolves in concentrated sulphuric acid with a yellow colour. The alcoholic solution gives no colour with ferric chloride. The method of preparation, the colour of the substance, its instability to heat and other properties are characteristic of the diazo-oxides.

5:6-Dihydroxyflavone (IV) :

To a boiling mixture of concentrated sulphuric acid (10 c.c.) and water (10 c.c.), the flavone diazo-oxide (0.1 g.) was added in small lots. It immediately went into solution with frothing, a reddish brown solution being formed. Boiling was continued for five more minutes, the solution allowed to cool, and diluted with water. On letting stand in the refrigerator, the yellowish brown precipitate was filtered, washed free from acid and twice crystallised from dilute alcohol. The honey-yellow needles (0.03 g.) melted at 189-90° (Found: C, 70.9; H, 4.3. $C_{15}H_{10}O_4$ requires C, 70.8; H, 3.9%). The alcoholic solution of the substance gives an intense olive green colour with ferric chloride; an orange red precipitate with lead acetate in alcohol; and a red insoluble sodium salt when aqueous sodium hydroxide is added. The yellow solution in concentrated sulphuric acid exhibits no fluorescence.

5:6-Diacetoxyflavone :

5:6-Dihydroxyflavone (0.05 g.) was dissolved in acetic anhydride (2 c.c.) and fused sodium acetate (0.5 g.) added. The mixture was refluxed for 2 hours, poured over crushed ice, the greyish brown solid collected, washed with water and crystallised from alcohol (norit). The pale yellow prisms melted at 164-165° (Found: C, 67.8; H, 4,3. $C_{19}H_{14}O_6$ requires C, 67.4; H, 4.2%).

5-Benzeneazo-6-methoxyflavone (IX) :

5-Benzeneazo-6-hydroxyflavone (1 g.) was dissolved in anhydrous acetone (20 c.c.) and refluxed for 12 hrs. with dimethyl sulphate (1.25 g.), and freshly - ignited potassium carbonate (4 g.). The reaction product was poured into water, acetone removed on the boiling water-bath and the reddish brown solid crystallized from alcohol in brownish red plates (0.65 g.) which melted at 161° (Found: N, 8.2. $C_{22}H_{16}O_3N_2$ requires N, 8.0%).

Reduction of 5-benzeneazo-6-methoxyflavone with zinc dust in alcohol-acetic acid medium :

The methylated azo dye (0.35 g.) was dissolved in a boiling mixture of alcohol (18 c.c.) and acetic acid (3 c.c.). To the red solution zinc dust (0.5 g.) was added and the mixture refluxed for 15 minutes, filtered hot, the zinc dust washed twice with 2 c.c. portions of alcohol, and the filtrate cooled and diluted with water. The orange-red precipitate was crystallized from dilute alcohol. The orange-red micro needles (0.20 g.) melted from 200-212°. A deep brown colour is given when a drop of ferric chloride is added to its alcoholic solution, indicating the presence of a free hydroxyl group, hence showing that demethylation has occurred during reduction.

Reduction of 5-benzeneazo-6-hydroxyflavone with hydrogen under pressure in the presence of Raney nickel as catalyst :

The methylated azo dye (0.55 g.) was dissolved in alcohol (30 c.c.), Raney nickel (0.5 g.) added and then shaken in a Parr hydrogenator with hydrogen at 40 lbs. pressure for 6 hrs. The yellow solution was filtered from the catalyst and washed twice with 5 c.c. portions of alcohol. On dilution with water, the filtrate became turbid and soon a yellow solid was deposited. On filtration and crystallization of the solid from dilute alcohol, bright yellow plates (0.3 g.) melting at 148°, with previous shrinking were obtained (Found: N, 4.8. $C_{16}H_{13}O_3N$ requires N, 5.2%). The compound in alcoholic solution develops no colour when ferric chloride is added. It is 5-amino-6-methoxyflavone (X).

Diazotisation and hydrolysis of 5-amino-6-methoxyflavone:

The amine (0. 1 g.) was dissolved in warm mixture of water (4 c.c.), concentrated sulphuric acid (0.5 c.c) and acetic acid (4 c.c.). The mixture was cooled in ice and gradually treated with powdered sodium nitrite (0.1 g.) with continuous stirring. After 20 mins., excess nitrous acid was destroyed by addition of urea and the solution poured into a boiling mixture of concentrated sulphuric acid (10 c.c.) and water (10 c.c.) After the addition, a drop of the boiling mixture was added every 5 minutes to alkaline β-naphthol. A deep red colour was developed as long as the solution contained the diazonium salt. The solution gradually decreased in volume due to evaporation of water and when the volume was about 15 c.c. there was a vigorous effervescence due to escape of nitrogen from the reaction mixture accompanied by the simultaneous change of colour from reddish-brown to a deeper brown, and then, the solution failed to give a red colour with alkaline β-naphthol, indicating complete hydrolysis of the diazonium salt. The solution was cooled, diluted with water and the brown product crystallized from dilute alcohol. It melted at 185° with previous shrinking. It exhibited all the properties of 5:6-dihydroxyflavone.

2-Hydroxy-6-benzoyloxydibenzoylmethane (XXIII) :

2-Acetylresorcin dibenzoate (2 g.) (prepared according to Baker[17]), was added to a solution of sodium ethoxide, obtained by dissolving sodium (0.6 g.) in absolute alcohol (40 c.c.). During addition and afterwards, the mixture was frequently shaken until the ester completely went into solution (1 hr.). There was a rapid change of colour of the solution from pale yellow to deeper yellow and then to green. The flask was corked and left overnight at room temperature. The reaction mixture set into a gel which was dark brown in colour with a greenish tinge. It was acidified with ice-cold dilute acetic acid. On dilution, the brown solution became turbid and soon shining crystals were deposited. After 4 hrs., the crystals were filtered and crystallized from dilute alcohol in plates and needles (0.71 g.) and melted at 124° (Found: C, 72.9; H, 4.3. $C_{22}H_{16}O_5$ requires C, 73.3; H, 4.4%). Its alcoholic solution develops a reddish brown colour with a drop of ferric chloride.

The filtrate after separation of the crude diketone deposited a brown precipitate (0.1 g.) which on crystallisation from alcohol gave needles which melted at 157°. This was 5-hydroxyflavone, since its melting point remained undepressed when mixed with an authentic sample of 5-hydroxyflavone.

5-Hydroxyflavone (XVII) : 2-Hydroxy-6-benzoyloxydibenzoylmethane (1.4 g.) was refluxed for 5 hrs. with glacial acetic acid (15 c.c.) containing fused sodium acetate (3 g.). The product was poured into water and the brown precipitate crystallised from alcohol in needles (.1 g.) and melted at 157°.

2:6-Dihydroxytribenzoylmethane (XXIV):

Method (I) : 2-Acetylresorcin dibenzoate (2 g.) was dissolved in dry ether (40 c.c.) in a pressure bottle. Finely divided sodamide (3 g.) was added and the bottle stoppered and mechanically shaken for 6 hours. The light green precipitate was filtered and cautiously added to ice-cold dilute acetic acid. The yellow precipitate was collected and crystallized first from benzene and then from ethyl acetate in long yellow plates which melted at 180-181° (Found: C, 73.4; H, 4.4. $C_{22}H_{16}O_5$ requires C, 73.3; H, 4.4%).

Method (II) : 2-Acetylresorcin dibenzoate (2 g.) was dissolved in dry benzene (20 c.c.), finely powdered sodamide (3 g.) added, and the mixture refluxed in an oil-bath for 6 hrs. The pale green product was filtered washed with benzene sucked dry, and gradually stirred into ice-cold dilute acetic acid. The yellow precipitate (1 g.) was crystallized first from benzene and then from ethyl acetate in long yellow plates and melted at 181°. The melting point was not depressed by mixing with the product obtained by method (I) but when mixed with 3-benzoyl-5-hydroxyflavone prepared according to Baker, the melting point was 160-165°. Its alcoholic solution gives a deep purple colour with a brown tinge when a drop of ferric chloride is added.

3-Benzoyl-5-hydroxyflavone (XXI) :

The triketone from the previous experiment (0.5 g.) was refluxed for 6 hrs. with glacial acetic acid (5 c.c.) containing fused sodium acetate (1 g.) The reaction product was poured into water and the brown solid crystallized from a mixture of alcohol and acetic acid in yellow pillars (0.4 g.) which melted at 178-179°. The melting point remained undepressed when mixed with 3-benzoyl-5-hydroxyflavone prepared according to Baker[17].

8-Benzeneazo-5-hydroxyflavone (XXVI) :

A solution of benzenediazonium chloride, prepared as usual from aniline (2 g.; 2.5 mol.), in alcohol (30 c.c.) was gradually added at 0-6° to a solution of 5-hydroxyflavone (2 g.) in alcohol (80 c.c.), pyridine (14 c.c.), sodium hydroxide (2 c.c. of 20% aqueous solution) and ammonia (d,.88; 2 c.c.). During addition of the diazonium solution and for 6 hours thereafter, the mixture was mechanically stirred and maintained at 0-6°. It was then left overnight in the refrigerator and the brown precipitate that separated was collected, boiled with hydrochloric acid (16%) for a few minutes, the solid filtered and crystallized from acetic acid in orange plates (1.5 g.) which melted at 214-215° (Found: N, 8.15. $C_{21}H_{14}O_3N_2$ requires N, 8.2%). Its alcoholic solution gives a reddish brown colour on addition of a drop of ferric chloride and becomes deep red on addition of aqueous sodium hydroxide. Acetylation of the dye as usual gave the acetyl derivative which crystallized from alcohol in orange yellow needles which melted at 186° (Found: N, 7.5. $C_{23}H_{16}O_4N_2$ requires N, 7.3%).

8-Benzeneazo-5-methoxyflavone :

8-Benzeneazo-5-hydroxyflavone (1 g.) dissolved in anhydrous acetone (20 c.c.) was refluxed with dimethyl sulphate (1.25 g.) and freshly ignited potassium carbonate (4 g.). The product of reaction was poured into water, acetone removed on the boiling water-bath and the brown solid crystallised from glacial acetic acid in narrow orange-coloured plates which melted at 206-208°. The melting point was 172-180° when mixed with 8-benzeneazo-5-hydroxyflavone (Found: N, 7.9. $C_{22}H_{16}O_3N_2$ requires N, 8.0%). Its alcoholic solution gives no colour when a drop of ferric chloride is added nor does the yellow colour of the solution deepens when aqueous sodium hydroxide is added.

8-Amino-5-hydroxyflavone (XXVIII) :

8-Benzeneazo-5-hydroxyflavone (1 g.) suspended in a boiling mixture of alcohol (80 c.c.) and acetic acid (10 c.c.) was treated under reflux with zinc dust (2 g.) added over a period of 1 minute with shaking of the flask. There was a vigorous reaction, the red solid gradually dissolved and the red solution became orange-yellow in colour. Boiling was continued 10 mins. longer and then the hot solution was filtered, the zinc dust was extracted thrice with 5 c.c. portions of hot alcohol. The filtrate was cooled and diluted with water. The compound that separated was filtered and crystallised from dilute alcohol in orange-red rods

(0.6 g.) which melted at 204-205° (Found: N, 5.7. $C_{15}H_{11}O_3$ N requires N, 5.5%). The orange yellow alcoholic solution turns dark greenish brown on addition of a drop of ferric chloride. The compound is insoluble in aqueous caustic soda but gives a yellow solution with concentrated sulphuric acid.

5-Hydroxyflavone-8-diazonium sulphate and 5:6-dihydroxyflavone :

8-Amino-5-hydroxyflavone (0.1 g.) was suspended in water (10 c.c.) and concentrated sulphuric acid (0.5 c.c.) On boiling the mixture, the orange compound became white and gradually dissolved. The mixture was filtered and the small amount of residue extracted again with water (10 c.c.) and concentrated sulphuric acid (0.5 c.c.) and the solution filtered. The combined filtrates on cooling deposited the amine sulphate in microcrystalline needles. The suspension was cooled and sodium nitrite (0.04 g.) in water (1 c.c.) was added with stirring. The crystals first became reddish brown, but completely went into solution in 10 minutes time, giving a clear, pale brown solution. After 20 minutes, the excess nitrous acid was destroyed by the addition of urea and the diazonium solution gradually added to a boiling mixture of water (20 c.c.) and concentrated sulphuric acid (10 c.c.). After the addition, a drop of the boiling mixture was tested every 5 minutes with alkaline β -naphthol and produced a deep red colour, as long as the solution contained the diazonium salt. The volume of the boiling mixture gradually decreased due to removal of water. When it reached a volume of about 15 c.c. there was a vigorous effervescence due to escape of nitrogen, the orange-yellow colour of the solution -- changed to brown, and then, the solution failed to give a red colour with alkaline β -naphthol indicating -- complete hydrolysis of the diazonium salt. The mixture was cooled and added to ice and water. The brownish yellow precipitate was collected, washed with water and crystallized from dilute alcohol (Norit) in yellow needles (0.03 g.). It melted at 188-189°. The melting point was not depressed by mixing with an authentic sample of 5:6-dihydroxyflavone prepared from 6-hydroxyflavone.[4]

On acetylation as usual with acetic anhydride and pyridine, 5:6-diacetoxyflavone was obtained. It melted at 166-167° alone or when mixed with 5:6-diacetoxyflavone obtained from 6-hydroxyflavone.[4]

5-Hydroxyflavone-8-azo-β-naphthol : Was prepared by coupling alkaline β-naphthol with diazotised 8-amino-5-hydroxyflavone. The compound crystallized from nitrobenzene in elongated plates and melted at 288-290° (Found: N, 6.4. $C_{25}H_{16}O_4N_2$ requires N, 6.9%).

4-Benzeneazo-2-acetylresorcin (XXX) :

A solution of benzenediazonium chloride prepared by the usual method from aniline (2.4 g.; 1 mole) in alcohol (15 c.c.) was gradually added to a solution of 2-acetylresorcin (4 g.; 1 mole) in alcohol (50 c.c.) and pyridine (15 c.c.). During addition of the diazonium solution and for 2 hours thereafter, the mixture was mechanically stirred and kept at 0°. It was acidified with hydrochloric acid, filtered and washed with water. The orange-red dye was boiled with hydrochloric acid (16% solution) for a few minutes (to remove pyridine), filtered and crystallized from alcohol. The orange needles (4.5 g.) melted at 152-153° (Found: N, 11.5. $C_{14}H_{12}O_3N_2$ requires N, 10.9%). An orange-red colour is developed when a drop of ferric chloride is added to its alcoholic solution. Refluxing the dye with acetic anhydride and pyridine gave the diacetyl derivative which crystallized from alcohol in pale orange needles and melted at 126° with shrinking at 122° (Found: N, 8.1. $C_{18}H_{16}O_5N_2$ requires N,8.2%).

2-Hydroxy-4-methoxy-3-acetylazobenzene (XXXII):

4-Benzeneazo-2-acetylresorcin (0.5 g.) was dissolved in dry benzene (10 c.c.) and refluxed for 8 hours with dimethyl sulphate (0.25 g.; 1 mole) and freshly ignited potassium carbonate (1 g.). The reaction mixture was diluted with water and the benzene layer extracted four times with 5% aqueous caustic soda. On acidification of the alkaline extracts an orange solid was obtained which crystallized from dilute alcohol in needles which melted at 90° (Found: N, 10.1. $C_{15}H_{14}O_3N_2$ requires N, 10.4%). Its alcoholic solution gives a pale reddish-brown colour with a drop of ferric chloride and it dissolves in concentrated sulphuric acid giving a light orange solution.

After extraction with caustic soda, the benzene layer was evaporated to dryness and crystallized from alcohol, when orange plates, melting at 110° with previous shrinking, were obtained (Found: N, 10.3. $C_{16}H_{16}O_3N_2$ requires N, 9.9%). Its alcoholic solution does not give any characteristic colour on addition of ferric chloride. This is the dimethyl ether.

4-Hydroxy-2-methoxy-3-acetylazobenzene (XXXI):

A solution of benzenediazonium chloride, prepared as usual from aniline (0.6 g., 1 mole) and alcohol (15 c.c.), was gradually added to a solution of 2-acetylresorcin monomethyl ether (1.1 g.) in alcohol (40 c.c.) and pyridine (6 c.c.). During addition and for 4 hours thereafter, the solution was mechanically stirred and maintained at 0°. The mixture was left overnight in the refrigerator and the light orange precipitate collected, treated with hydrochloric acid (to remove pyridine), washed with water and crystallized from alcohol in orange needles (1 g.) and melted at 100° with shrinking at 97° (Found: N, 10.7. $C_{15}H_{14}O_3N_2$ requires N, 10.4%). Its alcoholic solution gives a deep brown colour with ferric chloride.

6-Benzeneazo-5-hydroxyflavone (XXVII):

4-Benzeneazo-2-acetylresorcin (4 g.) was intimately mixed with benzoic anhydride (60 g.) and sodium benzoate (10 g.). The mixture was heated at 180-190° for 10 hrs. The reddish brown product was dissolved by refluxing on a water bath with alcohol (30 c.c.), potassium hydroxide (34 g. in 40 c.c. water) was gradually added, and the red solution was refluxed for 40 minutes. The alcohol was removed under reduced pressure, the semi-solid mass diluted with water and saturated with carbon dioxide. The reddish brown precipitate was filtered, washed with water and crystallized from acetic acid in orange-yellow rods (3 g.) and melted at 210°. The melting point was lowered to 178-188° when mixed with compound (XXVI) (Found: N, 8.4. $C_{21}H_{14}O_3N_2$ requires N, 8.2%). Its alcoholic solution gives a reddish brown colour with a drop of ferric chloride. By refluxing the above compound with acetic anhydride and pyridine, the acetyl -- derivative was obtained. It crystallized in light orange needles and melted at 195° (Found: N, 7.6. $C_{23}H_{16}O_4N_2$ requires N, 7.3%).

6-Benzeneazo-5-hydroxyflavone was subjected to methylation under the same conditions used for methylating compound (XXVI). The resulting product on crystallisation from acetic acid gave orange rods melting at 206°. The mixed melting point with the original 6-benzeneazo-5-hydroxyflavone was also 206° indicating that methylation has not taken place.

6-Amino-5-hydroxyflavone (XXIX) :

6-Benzeneazo-5-hydroxyflavone (1 g.) was suspended in a mixture of boiling alcohol (60 c.c.)

and acetic acid (10 c.c.). To the mixture under reflux, zinc dust (2 g.) was added. A vigorous reaction took place and the orange-red solution changed to a deep grey colour with a brownish tinge. More zinc dust (0.5 g.) was added, the mixture was refluxed for 10 minutes more, filtered hot, extracted thrice with 5 c.c. portions of hot alcohol, and the filtrate diluted with water after cooling to room temperature. The yellow precipitate was collected and crystallized by dissolving in hot alcohol (Norit), diluting to incipient turbidity and cooling. The yellow elongated plates (0.6 g.) melted with previous shrinking at 181°. Sugasawa describes the amine as golden-yellow plates melting at 177°[20]. (Found: N, 5.7. $C_{15}H_{11}O_3N$ requires N, 5.5%). The alcoholic solution turns intense reddish-brown when a drop of ferric chloride is added. With concentrated sulphuric acid, a yellow solution is obtained. It does not dissolve in aqueous caustic soda.

<u>Diazotisation of 6-amino-5-hydroxyflavone and conversion into 5:6-dihydroxyflavone</u> :

6-Amino-5-hydroxyflavone (0.3 g.) was boiled with water (30 c.c.) and sulphuric acid (98%; 3 c.c.)

The yellow crystals changed to white and then dissolved. On cooling, colourless crystals of the amine sulphate were deposited. On addition of sodium nitrite (0.5 g.), the white crystals gradually disappeared and were replaced by a yellow precipitate. This was filtered, washed with water and dried. It could not be suitably crystallized; therefore the crude substance was analysed (Found: N, 9.6. $C_{15}H_8O_3N_2$ requires -- N, 10.6%). This is undoubtedly flavone-6-diazo-5-oxide.

After preparing the diazo compound as above, excess nitrous acid in the reaction mixture was destroyed by addition of urea, and the solution containing a suspension of the diazo-oxide was gradually added to a boiling mixture of water (30 c.c.) and sulphuric acid (98%; 30 c.c.). The brownish yellow solution was kept boiling and a drop tested every 5 minutes with alkaline β-naphthol. When, as a result of gradual evaporation of water, the mixture reached a volume (about 45 c.c.) corresponding to 80% concentration of sulphuric acid, there was a vigorous effervescence due to escape of nitrogen and the mixture changed to a dark orange brown, and at this point no test could be obtained with alkaline β-naphthol. The mixture was cooled, poured into water and the brown precipitate collected. Crystallisation from dilute alcohol (Norit) yielded yellow needles (0.1 g.) which melted at 188-189°. The melting point was not altered when mixed with an authentic sample of 5:6-dihydroxyflavone.[4] On acetylation in the usual way, 5:6-diacetoxyflavone melting at 166° was obtained. No depression in melting point was noticed when mixed with 5:6-diacetoxyflavone.[4]

Action of hydrochloric acid (16%) on 8-amino-5-hydroxyflavone (XXVIII):

8-Amino-5-hydroxyflavone (0.1 g.) was refluxed with hydrochloric acid (16%; 20 c.c.) for 20 hours. The deep brown precipitate was filtered and washed with water. The compound thus obtained melted at 230-235°. On treatment with sodium bicarbonate solution, a precipitate was obtained which crystallized from dilute alcohol in yellow elongated plates (0.06 g.) and -- melted at 178°. The acidic filtrate from the experiment also gave some more of the compound on treating with sodium bicarbonate. The melting point was scarcely altered by mixing with a sample of 6-amino-5-hydroxyflavone (XXIX). The compound melting at 230-235° is the hydrochloride of the amine.

Action of hydrochloric acid (16%) on 6-amino-5-hydroxyflavone (XXIX) :

6-Amino-5-hydroxyflavone (0. 1 g.) was refluxed with hydrochloric acid (16%; 20 c.c.) for 20 hrs., neutralized with sodium bicarbonate, and the solid obtained, crystallized from dilute alcohol in yellow elongated plates (0.05 g.) melting at 181°. The melting point was not depressed by admixture with original 6-amino-5-hydroxyflavone. Hence, no change had taken place during the hydrochloric acid treatment of 6-amino-5-hydroxyflavone.

Primetin (5:8-dihydroxyflavone) (XXV):

It was obtained by the method described by Seshadri through alkaline persulphate oxidation of 5-hydroxyflavone.[18]

Action of hydrochloric acid on primetin :

Primetin (0.05 g.) was dissolved in alcohol (1 c.c.) and hydrochloric acid (32%; 3 c.c.) and the mixture refluxed for 20 hrs. The product was diluted with water, and the yellow precipitate crystallized from dilute alcohol in needles melting at 189°. The compound gave all reactions of 5:6-dihydroxyflavone and the melting point was not altered when mixed with 5:6-dihydroxyflavone obtained from 6-hydroxyflavone.[4]

Action of sulphuric acid (80%) on primetin :

Primetin (0.05 g.) was refluxed with sulphuric acid (98%; 10 c.c.) and water (5 c.c.) for 45 minutes, after which it was cooled, diluted with water and the yellow precipitate crystallized from dilute alcohol. The yellow needles shrank at 170° and melted at 180-185°. It gives all the reactions of 5/ 5:6-dihydroxyflavone.

Robinson reaction on 4-hydroxy-2-methoxy-3-acetylazobenzene using the anhydride and sodium salt of benzoic acid : 8-Benzeneazo-5-hydroxyflavone (XXVI):

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4-Hydroxy-2-methoxy-3-acetylazobenzene (2.3 g.) was intimately mixed with benzoic anhydride (36 g.) and sodium benzoate ~~mixe~~ (12 g.). The mixture was heated at 180-190° for 10 hours, cooled, powdered and dissolved in boiling alcohol (200 c.c.), potassium hydroxide (22 g. in 40 cc. water) was gradually added, and the red solution refluxed for 40 minutes on the water-bath. The alcohol was removed under reduced pressure, the semi-solid was diluted with water and saturated with carbon dioxide. The orange-yellow solid was filtered, washed with water and crystallized from acetic acid. The orange plates (1.9 g.) melted at 215°. The melting point was scarcely depressed by admixture with 8-benzeneazo-5-hydroxyflavone obtained by coupling 5-hydroxyflavone with benzenediazonium chloride.

3-Benzeneazo-2:6-dihydroxyphenylstyryl ketone (XXXIII):

4-Benzeneazo-2-acetylresorcin (1.06 g.) and benzaldehyde (1.3 g., 3 mols.) were mixed with absolute alcohol (20 c.c.) and then potassium hydroxide (15 g. in 20 c.c. water) was added. After shaking the flask, it was stoppered and left overnight at room temperature. The reaction product was added to crushed ice and hydrochloric acid. The bright red precipitate was collected and crystallized from glacial acetic acid. The deep red plates (0.7 g.) melted at 178° (Found: N, 7.9. $C_{21}H_{16}O_3N_2$ requires N, 8.1%).

Action of selenium dioxide on the above chalkone:

The chalkone (0.5 g.) was refluxed with selenium dioxide (1 g.) in either xylene or amyl alcohol (20 c.c.) for 12 hours. The amyl alcohol or xylene was steam distilled and the red solid extracted with acetic acid. The extracts on concentration and cooling gave a compound which melted at 177°. The melting point was not altered by mixing with the original chalkone.

8-Benzeneazo-3-benzoyl-5-hydroxyflavone :

A solution of benzene diazonium chloride, prepared as usual from aniline (0.3 g.; 2.5 mols.) in alcohol (8 c.c.) was gradually added to a solution of 3-benzoyl-5-hydroxyflavone (0.3 g.) in alcohol (15 c.c.), pyridine (2 c.c.), sodium hydroxide (0.3 c.c. of 20% solution) and ammonia (d,0.88; 0.3 c.c.). During addition of the diazonium solution and for 4 hours thereafter, the reaction mixture was mechanically stirred and kept at 0°. Leaving overnight in the refrigerator, a brown precipiate which had separated was filtered and crystallized from acetic acid. The orange needles (0.15 g.) melted at 247-249° (Found: N, 6.6. $C_{28}H_{18}O_4N_2$ requires N, 6.3%). Acetylation in the usual manner gave orange plates of the acetyl derivative and melted at 184° (Found: N, 5.9. $C_{30}H_{20}O_5N_2$ requires N, 5.7%).

8-Benzeneazo-7-hydroxyflavone (XXXV):

A solution of benzenediazonium chloride prepared as usual from aniline (2 g.; 2.5 mols.), was gradually added to a solution of 7-hydroxyflavone (2 g. prepared according to Baker[12]) in sodium hydroxide (20 c.c. of 0.5 N solution) and sodium acetate (7.2 g. in 20 c.c. water). During addition and for 4 hours thereafter, the reaction mixture was mechanically stirred and kept at 0°, the conditions being maintained just alkaline to phenolphthalein by addition of caustic soda solution. The mixture was left overnight in the refrigerator and the red precipitate collected, washed with water and crystallized from glacial acetic acid in orange needles (1.9 g.) which melted at 215° (Found: N, 8.4. $C_{21}H_{14}O_3N_2$ requires N, 8.2%).

<u>8-Amino-7-hydroxyflavone (XXXVI)</u>:

Benzeneazo-7-hydroxyflavone (1 g.) was dissolved in alcohol (50 c.c.) and glacial acetic acid (8 c.c.). To the boiling solution, zinc dust (3 g.) was added. There was a vigorous reaction and the red solution changed to brownish yellow, After refluxing for 10 minutes, zinc dust (0.5 g.) was added and the mixture filtered, the zinc dust being thrice extracted with 5 c.c. portions of hot alcohol. The filtrate was cooled, and diluted. The yellow precipitate was crystallized from dilute alcohol in yellow needles (0.65 g.) and melted at 244° (Found: N, 5.4. $C_{15}H_{11}O_3N$

requires N, 5.5%). The yellow alcoholic solution turns green on addition of a drop of ferric chloride. A brown solution is formed when aqueous caustic soda is added as also with concentrated sulphuric acid. 7-Hydroxyflavone-8-azo-β-naphthol was prepared by treating diazotised 8-amino-7-hydroxyflavone with alkaline β-naphthol. The compound crystallized from nitrobenzene in intense red plates having a green reflux and melted at 287-289° (d) (Found: N, 7.00. $C_{25}H_{16}O_4N_2$ requires N, 6.9%).

Diazotisation of 8-amino-7-hydroxyflavone and hydrolysis of the diazonium salt :

The amine (0.2 g.) was dissolved in a boiling mixture of concentrated sulphuric acid (1 cc.) and water (20 cc.) and filtered. White crystals of the amine sulphate were deposited on cooling the filtrate. To the ice-cooled solution, sodium nitrite (0.2 g. in 2 cc. water) was gradually added; the white crystals disappeared and were replaced by a brown precipitate. After 30 minutes the excess nitrous acid was destroyed by the addition of urea and the diazonium solution gradually added to a boiling mixture of concentrated sulphuric acid (20 cc.)

and water (20 cc.). Boiling of the reaction mixture was continued, a drop being tested every 5 minutes with alkaline β-naphthol. Due to evaporation of water the acid gradually became concentrated; soon the boiling of the mixture stopped and a slight smell of sulphur dioxide indicated that charring was taking place; but even at this stage, the mixture continued to give a red coloration with alkaline β-naphthol. The mixture was cooled and diluted with water. A small quantity of a brown fluffy precipitate appeared and was filtered. Its alcoholic solution gave an intense green colour with ferric chloride. The material was too small for further study.

<u>Coupling resacetophenone with benzenediazonium chloride in alcohol and pyridine (pH 8-9):</u>

Benzenediazonium chloride prepared as usual from aniline (0.6 g.; 1 mol.) in alcohol (10 cc.) was gradually added to resacetophenone (1 g.) dissolved in alcohol (25 cc.) and pyridine (5 cc.). During addition and for 3 hours thereafter, the mixture was mechanically stirred and kept at 0°. It was left overnight in the refrigerator and the orange precipitate filtered. It was extracted with 5% aqueous caustic soda and then washed with water. The alkali extracts on acidification gave an orange precipitate which crystallized from alcohol in orange-red plates (0.5 g.) and melted at 200° (Found: N, 11.5. $C_{14}H_{12}O_3N_2$ requires N, 10.9%). This is a mono-benzeneazoresacetophenone. It gives a brown colour with alcoholic ferric chloride. The acetyl derivative was obtained by refluxing the dye for 4 hours with acetic anhydride and pyridine. It crystallized from alcohol in red plates and melted at 106-107° (Found: N, 8.4. $C_{18}H_{16}O_5N_2$ requires N, 8.2%).

The alkali insoluble portion crystallises from acetic acid in red clusters of needles (0.2 g.) and melted at 224-225° (Found: N, 15.7. $C_{20}H_{16}O_3N_4$ requires N, 15.5%). This is 3:5-bisbenzeneazoresacetophenone.

Coupling of resacetophenone in the presence of sodium carbonate:

Benzenediazonium chloride prepared from aniline (0.6 g.; 1 mol.), hydrochloric acid (32%; 2 cc.), water (10 cc.) and sodium nitrite (0.5 g. in 2 cc. water), was gradually added to a solution of resacetophenone (1 g.) in water (40 cc.), sodium carbonate (4 g.) and sodium acetate (2.4 g.). During addition and for 1 hour thereafter the solution was mechanically stirred and kept at 0°. The red precipitate on crystallisation from alcohol gave a compound (0.6 g.) which melted at 198°, the melting point being scarcely depressed by mixing with <u>mono</u>benzeneazo - resacetophenone obtained from the previous experiment.

<u>Coupling resacetophenone in the presence of sodium hydroxide</u> :

Benzenediazonium chloride prepared as usual from aniline (0.37 g.; 1 mol.) was treated with sodium acetate (1.2 g.) to neutralise the mineral acidity and then added to a solution of resacetophenone (0.6 g.) in sodium hydroxide (10 cc. of 10%) solution and 30 cc. water). During addition and for 2 hours thereafter, the mixture was mechanically stirred and maintained at 0°. The reaction product was filtered and washed with water. The filtrate on acidification yielded a red compound which crystallized from alcohol in plates (0.2 g.) and melted at 198-200°. The melting point remained undepressed when mixed with <u>mono</u>benzeneazoresacetophenone from the previous experiment.

The residue on crystallization from glacial acetic acid gave red needles (0.4 g.) and melted at 224-225°. The melting point was not depressed on admixture with pure *bis*benzeneazoresacetophenone.

*Mono*benzeneazoresacetophenone dimethyl ether :

*Mono*benzeneazoresacetophenone (2 g.) in anhydrous acetone solution was refluxed for 14 hours with freshly ignited potassium carbonate (8 g.) and dimethyl sulphate (3 g.). The red precipitate crystallized from alcohol in orange-red plates (1.5 g.) and melted at 162-163° (Found: N, 9.75. $C_{16}H_{16}O_3N_2$ requires N, 9.9%). It is insoluble in caustic soda and does not give any characteristic coloration with alcoholic ferric chloride.

Reduction of monobenzeneazoresacetophenone :

*Mono*benzeneazoresacetophenone (2 g.) was suspended in water (20 c.c.) and sodium hydroxide (5 cc. of 20% solution) added. The mixture was heated to ~~a~~ boiling and sodium hydrosulphite was added in small lots, keeping the condition alkaline, until test for hydrosulphite could be obtained on vat paper even 5 minutes after addition of one lot of hydrosulphite. Boiling was continued for 15 minutes more and then the product was cooled and acidified with acetic acid. The precipitate was ether extracted and the ether layer dried over anhydrous sodium sulphate. On removal of ether under vacuum a greenish yellow product was obtained. It was crystallized from water containing a little hydrosulphite in yellow elongated plates (1 g.) and melted at 131°. For analysis it was vacuum dried, since heating it even in a steam-oven converted it into an amorphous material without any sharp melting point (Found: N, 8.1. $C_8H_9O_3N$ requires N, 8.4%).

Robinson reaction on monobenzeneazoresacetophenone using the anhydride and sodium salt of benzoic acid:

Monobenzeneazoresacetophenone (0.6 g.), powdered benzoic anhydride (7 g.) and sodium benzoate (3 g.) were intimately mixed and heated at 180-190° for 10 hours. The reaction mass was cooled, dissolved in hot alcohol (100 c.c.) and then potassium hydroxide (3.5 g. in 10 cc. water) was gradually added. The mixture was refluxed for 30 minutes, the greater portion of alcohol removed under reduced pressure, the product diluted with water and saturated with carbon dioxide. The precipitate that separated was filtered and crystallized from a mixture of alcohol and acetic acid. The brownish yellow needles (0.2 g.) melted at 239° with previous shrinking (Found: N, 8.3. $C_{21}H_{14}O_3N_2$ requires N, 8.2%). The colour of its alcoholic solution is not changed by addition of a drop of ferric chloride. An orange yellow colour is developed on dissolving the compound in concentrated sulphuric acid. Its alcoholic solution becomes orange-red on addition of aqueous caustic soda.

Reduction of the compound from the previous experiment:

The compound from the previous experiment (0.2 g.) was added to alcohol (20 c.c.) and acetic acid (4 c.c.). To the mixture, under reflux, zinc dust (0.5 g.) was added and the mixture refluxed for 10 minutes. The red mixture became brownish yellow. The reaction mixture was filtered, the zinc dust extracted thrice with 3 cc. portions of alcohol, and the filtrate cooled and diluted with water. The yellowish brown precipitate, obtained by keeping the mixture in the refrigerator, was crystallized from dilute alcohol in minute needles which melted at 269° (Found: N, 6.1. $C_{15}H_{11}O_3N$ requires N, 5.6%).

Its alcoholic solution turns deep brown on addition of a drop of ferric chloride. It dissolves in aqueous caustic soda giving a yellow solution. It dissolves in concentrated sulphuric acid with a light yellow colour. Its solution in dilute hydrochloric acid undergoes diazotisation as indicated by the formation of a deep red colour with alkaline β-naphthol.

Benzoylation of monobenzeneazoresacetophenone :

Monobenzeneazoresacetophenone (1 g.) was suspended in dry pyridine (10 c.c.) and benzoyl chloride (1.8 g.;2.5 mol.) added. The mixture was refluxed for 10 hours at 180-190°. The reaction product was poured into a mixture of ice and hydrochloric acid and ether extracted. The ether layer was washed successively with dilute hydrochloric acid, sodium carbonate, ice-cold 5% caustic soda and finally with water until free from alkali. The ether was dried with calcium chloride and the ether removed. The reddish brown pasty solid separated from alcohol in orange needles and melted at 118-120° (Found: N, 6.4. $C_{28}H_{20}O_5N_2$ requires N, 6.03%). Its orange alcoholic solution does not develop a deeper colour on addition of ferric chloride. It gradually dissolves in aqueous caustic soda at room temperature, giving a yellow solution.

Rearrangement of the ester obtained in the previous experiment, with anhydrous potassium carbonate in toluene :

The ester (1 g.) was dissolved in toluene (30 c.c.) and refluxed for 8 hours with freshly ignited potassium carbonate (4 g.). The reaction product was filtered, the potassium carbonate layer washed with hot benzene and sucked dry. This was added to water and the solid obtained stirred up with dilute hydrochloric acid. The orange-red solid was filtered and crystallized from acetic acid in orange pillars (0.6 g.) and melted at 217° (Found: N, 6.4. $C_{28}H_{20}O_5N_2$ requires N, 6.03%). No colour is developed when ferric chloride is added to its alcoholic solution. Its alcoholic solution becomes orange red by addition of aqueous soda. It gives an orange yellow solution with concentrated sulphuric acid. The compound could be recovered unchanged from concentrated sulphuric acid or after refluxing for 7 hours with glacial acetic acid containing fused sodium acetate, or for 2 hours with 20% alcoholic sulphuric acid.

2-Benzeneazo-4:6-diacetylresorcin (XLIII) :

A solution of benzenediazonium chloride from aniline (0.2 g.) was added at 0° to a mechanically stirred solution of 4:6-diacetylresorcin (0.2 g.) in caustic soda (5 c.c. of 5% solution) and water (25 c.c.). After 2 hours the mixture was acidified with hydrochloric acid and the red precipitate crystallized from acetic acid in orange-red needles and melted at 202° (Found: N, 9.7. $C_{16}H_{14}O_4N_2$ requires N, 9.4%).

Fries' rearrangement on monobenzeneazoresacetophenone diacetate :

Monobenzeneazoresacetophenone diacetate (0.4 g.) was heated with finely powdered anhydrous aluminium chloride (1 g.). The temperature was rapidly raised to 120° and then gradually to 180° in 2 hours time. It was maintained at 180° for 1 hour and then cooled. The product was treated with ice and dilute hydrochloric acid. The red precipitate was heated with aqueous caustic soda (10%) for 1 hour and then the solution acidified with hydrochloric acid. The red precipitate was dissolved in chloroform and passed through a column of alumina. The chromatogram was eluted with chloroform. The dye dissolved and came down along with the chloroform at a very slow rate indicating that it has been strongly adsorbed by the alumina. A very small amount of deep red substance remained at the top of the column. The chloroform from the solution was distilled off and the red crystalline mass crystallized from a number of alcohol and acetic acid in deep red elongated plates and melted at 145° (Found: N, 9.7. $C_{16}H_{14}O_4N_2$ requires N, 9.4%).

6-Benzeneazo-2:4-diacetylresorcin (XLV):

A solution of benzenediazonium chloride was prepared from aniline (0.05 g.) and added to a solution of 2:4-diacetylresorcin (0.05 g., prepared according to Limaye[25]) in caustic soda (3 c.c. of 5% solution) and water (20 c.c.) with mechanical stirring and cooling with ice. After 2 hours, the mixture was acidified with hydrochloric acid and the red precipitate crystallized from alcohol in red elongated plates and melted at 143° alone or when mixed with the Fries rearrangement product obtained from the previous experiment.

5:6-Dimethoxyflavone :

5:6-Dihydroxyflavone (1 g.) in dry acetone (30 c.c.) was refluxed for 15 hours with freshly ignited potassium carbonate (8 g.), and dimethyl sulphate (2 g.). The product was poured in water and excess acetone removed on the water bath. The precipitate that was obtained crystallized from alcohol in colourless plates (0.8 g.) and melted at 196°.

5-Hydroxy-6-methoxyflavone (XLVI) :

Method I : 5:6-Dimethoxyflavone (0.5 g.) was suspended in dry ether (30 c.c.) and anhydrous aluminium chloride (6 g.) added in small lots. It was well shaken for 5 minutes and left corked for 48 hours at room temperature (30°). Ether was decanted off from the orange precipitate, to which water was then added. The precipitate was collected and refluxed for 30 minutes with acetic acid (10 c.c.) and hydrochloric acid (32% of 4 c.c.). The mixture was diluted with water and the yellow precipitate crystallized from dilute alcohol in yellow needles (0.3 g.) and melted at 129-130°.

Method II : 5:6-Dihydroxyflavone (1 g.) in dry acetone (30 c.c.) was refluxed for 15 hours with freshly ignited potassium carbonate (2 g.) and dimethyl sulphate (0.62 g.; 1 mol.) on a water bath. The reaction product was filtered and the potassium carbonate extracted twice with hot acetone. The brownish yellow solid obtained by removal of acetone was dissolved in ether and shaken with 5% aqueous caustic potash. The ether layer was removed and the red potassium salt of 5-hydroxy-6-methoxyflavone extracted with ether to remove traces of 5:6-dimethoxyflavone. The potassium salt was filtered from the alkaline liquid and washed with water. It was treated with dilute hydrochloric acid and the yellow precipitate crystallized from dilute alcohol in needles (0.6 g.) and melted at 129-130°.

The potassium carbonate layer yielded 5:6-dihydroxyflavone (0.3 g.) on treatment with water.

8-Benzeneazo-5-hydroxy-6-methoxyflavone (XLVII) :

Benzenediazonium chloride prepared as usual from aniline (1 g.) in alcohol (40 c.c.) was gradually added at 0° to a solution of 5-hydroxy-6-methoxyflavone

(0.8 g.) in alcohol (60 c.c.), pyridine (12 c.c.), sodium hydroxide (0.6 c.c. of 5N solution) and ammonia (d 0.88; 2.0 c.c.). During addition of the diazonium solution and for 6 hours thereafter the solution was mechanically stirred and then left overnight in the refrigerator. The orange precipitate was filtered, treated with hydrochloric acid and crystallized from acetic acid in dull orange elongated plates (0.5 g.) and melted at 232-234° (Found: N, % 7.8. $C_{22}H_{16}O_4N_2$ requires N, 7.9%). A reddish brown colour is developed when a drop of ferric chloride is added to its alcoholic solution. It gives an orange solution with alcoholic caustic soda and a scarlet red colour with concentrated sulphuric acid.

8-Amino-5-hydroxy-6-methoxyflavone (XLVIII) :

8-Benzeneazo-5-hydroxy-6-methoxyflavone (0.5 g.) in a boiling mixture of alcohol (60 c.c.) and acetic acid (10 c.c.) was treated with zinc dust (2 g.) added during the period of 1 minute. The mixture was refluxed for 15 minutes, then filtered, the zinc dust extracted thrice with 5 c.c. portions of alcohol, and the filtrate diluted after cooling. The reddish brown precipitate on crystallization from dilute alcohol gave reddish brown elongated plates (0.3 g.) and melted at 210° (Found: N, 5.2. $C_{16}H_{13}O_4N$ requires N, 4.9%). A green colour is developed when a drop of ferric chloride is added to its alcoholic solution which changes to greenish brown and then deep brown. It dissolves in concentrated sulphuric acid with a yellow colour.

Hydrolysis of 8-amino-5-hydroxy-6-methoxyflavone

5:6:8-Trihydroxyflavone (XLIX) :

The amine (0.05 g.) was refluxed with hydrochloric acid (32%; 10 c.c.) for 24 hours. By this time a drop of the reaction mixture, treated with sodium nitrite, failed to give a deep red colour with alkaline β-naphthol, indicating absence of the amino group. The yellowish-brown solid (0.02 g.) was filtered dried and directly acetylated by refluxing with acetic anhydride (2 c.c.) and pyridine (0.5 c.c.) for 2 hours. The product was poured on crushed ice and the precipitate crystallized from alcohol in shining rectangular plates and melted at 208-210° (Found: C, 63.2; H, 4.3. $C_{21}H_{16}O_8$ requires C, 63.6; H, 4.04%). This is 5:6:8-triacetyxyflavone (L). It gives no characteristic coloration with alcoholic ferric chloride.

The acetyl derivative (0.015 g.) was hydrolysed by heating with alcohol (5 c.c.) and hydrochloric acid (32%; 1 c.c.) for 30 minutes. The solution was diluted, excess alcohol removed under vacuum and the orange-yellow product crystallized from dilute alcohol in sheaves of needles and melted with decomposition at 234-236° (Found: C, 67.4; H, 4.0. $C_{15}H_{10}O_5$ requires C, 66.7; H, 3.7%). It gives a brown colour with alcoholic ferric chloride and a red solution with aqueous caustic soda which becomes paler and finally yellow on standing.

<u>5:6:8-Trimethoxyflavone</u> :

Crude 5:6:8-Trihydroxyflavone (0.02 g.) was refluxed with anhydrous acetone (10 c.c.), freshly ignited potassium carbonate (1 g.) and dimethyl sulphate (0.25 g.) for 8 hours. The product was poured in water, excess acetone removed on the water bath and the brown solid crystallized from dilute alcohol in yellow rods which melted at 148° (Found: C, 69.4; H, 5.6. $C_{18}H_{16}O_5$ requires C, 69.2; H, 5.1%). Its alcoholic solution develops no characteristic colour with ferric chloride.

8-Benzeneazotectochrysin (LV) :

Benzenediazonium chloride prepared from aniline in alcohol was gradually added to a solution of tectochrysin (0.8 g.), pyridine (12 c.c.) alcohol (60 c.c.), sodium hydroxide (0.75 c.c. of 5N solution) and ammonia (d 0.88; 1.2 c.c.). During addition of the diazonium solution and for 4 hours thereafter, the mixture was kept at 0-6° and mechanically stirred. On leaving overnight in the refrigerator, an orange precipitate had separated. This was filtered, treated with hydrochloric acid (32%) in which it partly dissolved and diluted with water. The red precipitate was crystallized from glacial acetic acid in orange needles (0.6 g.) and melted at 232° (Found: N, 7.6. $C_{22}H_{16}O_4N_2$ requires N, 7.8%). It gives an orange solution with concentrated sulphuric acid and a brown colour when a drop of ferric chloride is added to its alcoholic solution.

8-Benzeneazo-5:7-dimethoxyflavone :

8-Benzeneazotectochrysin (0.1 g.), in dry acetone solution, was refluxed with dimethyl sulphate (0.5 g.) and freshly ignited potassium carbonate (2 g.) for 15 hours on a water bath. The reaction product was poured into water, excess acetone removed on the water bath and the brown solid crystallized from alcohol in orange needles and melted at 207-208° (Found: N, 7.8. $C_{23}H_{18}O_4N_2$ requires N, 7.3%). The compound in alcoholic solution does not give the brown colour, characteristic of hydroxyl group, when a drop of ferric chloride is added, and the colour of its alcoholic solution does not deepen on addition of aqueous caustic soda.

8-Aminotectochrysin (LVI):

8-Benzeneazotectochrysin (1 g.) was suspended in alcohol (60 c.c.) and glacial acetic acid (10 c.c.) To the boiling mixture, zinc dust (2.0 g.) was added, and the mixture refluxed for 10 minutes with frequent shaking. The red colour of the dye gradually disappeared, and an orange solution resulted. This was filtered, the zinc dust extracted thrice with 5 c.c. portions of boiling alcohol and the filtrate was cooled and diluted with water. The orange-yellow precipitate was crystallized from dilute alcohol in fine brownish yellow needles (0.7 g.) and melted at 197° with previous shrinking (Found: N, 4.7. $C_{16}H_{13}O_4$ N requires N,4.9%). A brown coloration develops when a drop of ferric chloride is added to to its alcoholic solution. With aqueous sodium hydroxide the amine does not dissolve but turns red. It dissolves in hot hydrochloric or sulphuric acids giving white salts on cooling. On addition of sodium nitrite, the salts gradually dissolve leaving a trace of a slight fulffy precipitate. A brownish red colour is developed on addition of a drop of it to alkaline β-naphthol.

7-Methylether of baicalein (LVII):

8-Aminotectochrysin (0.8 g.) was refluxed for 24 hours with hydrochloric acid (25%; 45 c.c.). The reaction product was filtered hot and the residue washed with hot water. The yellow precipitate on crystallisation from alcohol gave yellow crystalline plates (0.4 g.) and melted at 219° (Found: C, 67.4; H, 4.3. $C_{16}H_{12}O_5$ requires C, 67.6; H, 4.3%). A deep green colour similar to that produced with 5:6-dihydroxyflavone is given when a drop of ferric chloride is added to its alcoholic solution. With aqueous sodium hydroxide it develops an orange-red colour which changes to a greenish brown precipitate. With sodium amalgam in absolute alcohol, a transient orange colour is given which is rapidly replaced by a dark greenish blue colour; after some minutes a brown precipitate is formed. With concentrated sulphuric acid, it gives a yellow non-fluorescent solution. It gives an orange-yellow precipitate with alcoholic lead acetate. All these properties agree with those recorded by Sastri and Seshadri[31] for the compound.

5:6-Diacetoxy-7-methoxyflavone :

7-Methylether of baicalein (0.1 g.) was refluxed with acetic anhydride (5 c.c.) and pyridine (0.5 c.c.) for 4 hours. The product was poured over crushed ice and the white precipitate crystallized from alcohol in almost colourless plates and melted at 240°; Seshadri quotes melting point 239-240° (Found: C, 65.3; H, 4.5 $C_{20}H_{16}O_7$ requires C, 65.2; H, 4.3%).

5:6:7-Trimethoxyflavone:

7-Methylether of baicalein (0.1 g.) was refluxed for 8 hours with dry acetone (20 c.c.), dimethyl sulphate (0.5 c.c.) and freshly ignited potassium carbonate (1 g.). On dilution with water and removal of excess acetone on the water-bath, the trimethylether separated as a crystalline solid which was crystallized from dilute alcohol in narrow rectangular plates and melted at 165°. Seshadri gives melting point 165-166°.

Baicalein (LIX) :

7-Methylether of baicalein (0.1 g.) was refluxed with hydriodic acid (d 1.5; 4 c.c.) and acetic anhydride (4 c.c.) for 4 hours. The product was poured into saturated sodium bisulphite solution and the mixture heated for 30 minutes on the water-bath. The yellow solid was collected and crystallized from dilute alcohol in yellow plates and melted at 264°. It gives all properties described by Seshadri and others[32] (Found: C, 66.6; H, 3.7. $C_{15}H_{10}O_5$ requires C, 66.7; H, 3.7%).

Quercetin 3:7:3':4'-Tetramethyl ether:

Dry quercetin (2 g.) dissolved in anhydrous acetone (40 c.c.) was refluxed with freshly ignited potassium carbonate (8 g.) and dimethyl sulphate (3.2 g for 20 hours on a water bath. The reaction product was poured in water, acidified with hydrochloric acid, acetone removed on the water-bath and the grey precipitate crystallized from a mixture of alcohol and acetic acid in yellow needles (1.1 g.) and melted at 155-157°.

8-Benzeneazoquercetin-3:7:3':4'-Tetramethyl ether (LXVI

A solution of benzenediazonium chloride prepared as usual from aniline (1 g.; 4 mol.) in alcohol (30 c.c was gradually added to a solution of quercetin 3:7:3':4'-tetramethylether (1 g.) in alcohol (80 c.c.), pyridine (10 c.c.), sodium hydroxide (3 c.c. of 1 N solution) and ammonia (d,0.88; 0.8 c.c.). During addition of the diazonium solution and for 4 hours thereafter the mixture was mechanically stirred and maintained at 0°. On leaving overnight in the refrigerator, a deep red precipitate separated. This was filtered, treated with hydrochloric acid and crystallized from acetic acid in deep orange needles (0.8 g.) and melted at 184° (Found: N, 6.3 $C_{25}H_{22}O_7N_2$ requires N, 6.06%). A brown colour is developed when a drop of ferric chloride is added to its alcoholic solution. It gives a red solution with alcoholic caustic soda and an orange red solution with concentrated sulphuric acid.

8-Benzeneazoquercetin-3:5:7:3':4'-pentamethyl ether:

The dye (0.1 g.) from the previous experiment in dry acetone (15 c.c.) was refluxed with freshly ignited potassium carbonate (2 g.) and dimethyl sulphate (0.6 g.) for 15 hours on a water bath. The reaction product was poured in water, excess acetone removed by heating on a water-bath. The orange residue was crystallized from alcohol containing little acetic acid. The orange hairy needles melted at 214°. Its alcoholic solution does not develop any characteristic colour on addition of ferric chloride and its orange alcoholic solution does not deepen in colour on addition of caustic soda (Found: N, 6.4. $C_{26}H_{24}O_7N_2$ requires N, 6.0%).

Reduction of 8-benzeneazoquercetin-3:7:3':4'-tetramethyl ether :

The dye (0.5 g.) was suspended in a mixture of alcohol (40 c.c.) and acetic acid (7 c.c.). To this mixture under reflux, zinc dust (2 g.) was added in small lots during 1 minute's time, and then boiling continued for 10 minutes. The suspension of the dye gradually disappeared and a brownish yellow solution resulted.The mixture was filtered, the zinc dust extracted thrice with 5 c.c. portions of hot alcohol and the filtrate cooled. The orange-yellow crystalline solid obtained on dilution of the filtrate was ~~x~~ recrystallized from alcohol in orange-yellow needles (0.35 g.) and melted at 212° (Found: N, 3.9. $C_{19}H_{19}O_7N$ requires N, 3.75%). The yellow alcoholic solution became deep brown on addition of ferric chloride. A lemon yellow solution is obtained by dissolving it in concentrated sulphuric acid. It is insoluble in aqueous caustic soda. Its suspension in dilute sulphuric acid gets diazotized on addition of sodium nitrite as indicated by a deep red colour developed upon addition to alkaline β-naphthol.

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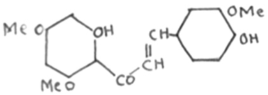
PART II.

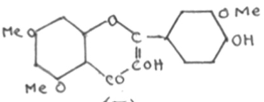
A NEW SYNTHESIS OF RHAMNAZIN.

Among the methods which have been described for the synthesis of partially methylated hydroxyflavonols with a free hydroxyl in the 3-position, two convenient procedures are the preferential demethylation of the 3-methoxyl group by means of aluminium chloride[1] or hydrobromic acid[2] and the oxidation of *o*'-hydroxychalkones with alkaline hydrogen -- peroxide.[3] Since there are several naturally occurring flavonols (e.g. rhamnazin, rhamnocitrin) in which a hydroxyl group in the 2-phenyl ring is unmethylated, the utility of Algar and Flynn's method[3] for their synthesis have been examined. It has been found that in the oxidation of *o*'-hydroxychalkones to flavonols by hydrogen peroxide a free hydroxyl in the 4-position does not interfere. The method has been used for synthesising 3:4'-dihydroxy-7-methoxyflavone and rhamnazin[4].

Condensation of phloracetophenone-4:6-dimethyl ether with vannilin gave the chalkone (I) which on



(I)



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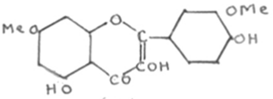
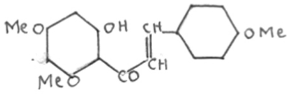


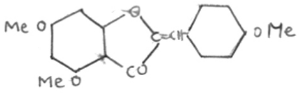
(III)

oxidation with alkaline hydrogen peroxide yielded the flavonol (II), the 5-methyl ether of rhamnazin (III). Rhamnazin (III) was then obtained by the action of aluminium chloride in nitrobenzene on (II) at about 100°. Kuhn, Low and Trischmann have synthesised rhamnazin by a much longer procedure, involving the condensation of 2:6-dihydroxy-4-methoxyphenyl benzoyloxymethylketone with 3-methoxy-4-benzoyloxybenzoic anhydride.[5] Rao and Seshadri synthesised it in a nearly similar way by condensing 2-hydroxy-ω:4:6-trimethoxyacetophenone with the anhydride and sodium salt of 3-methoxy-4-benzoyloxybenzoic acid and subjecting 4'-hydroxy-3:5:7:3'-tetramethoxyflavone thus obtained to partial demethylation with aluminium chloride in nitrobenzene solution.[6]

The formation of the flavonol (II) from the chalkone (I) is noteworthy in view of the observation of Geissman and Fukushima[7] that the oxidation of the chalkone (IV) gave as the predominant product the --



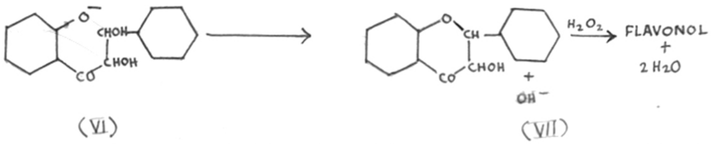
(IV)



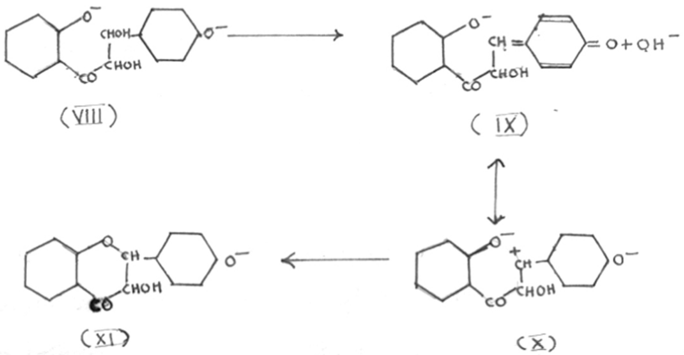
(V)

benzalcoumaranone (V), the corresponding flavonol being formed only in very small amount. They have shown that this change in the course of the Algar-Flynn reaction is the effect of the 6'-methoxy group in (IV); oxidation of analogous chalkones, prepared by the condensation of phloracetophenone dimethyl ether with benzaldehyde, veratraldehyde and piperonal, led in every case to the benzalcoumaranone, together with a trace of the flavonol only in the case of the veratraldehyde derivative. When the chalkone is the benzal, anisal or veratral or piperonal derivative of 2-hydroxy-, 2-hydroxy-4-methoxy-, 2-hydroxy-3:4-dimethoxy-, or 2-hydroxy-3:4:5-trimethoxyacetophenone, the flavonol is obtained and not the benzalcoumaranone.[3,8,9,10]

Reichel and Steudel[11] have outlined a mechanism of the oxidation of 2'-hydroxychalkone to flavonol which involves the addition of hydrogen peroxide ion to the chalkone ion, rearrangement of the adduct to the glycol (VI), the displacement of the β-hydroxyl as a hydroxyl ion and cyclisation to 3-hydroxyflavanone (VII) by an attack of the anionic oxygen on the --

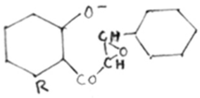


β-carbon atom, and finally the oxidation of (VII) to flavonol. Murakami and Irie[8] have demonstrated the formation of (VII) from 2'-hydroxychalkone under mild conditions of treatment with alkaline hydrogen

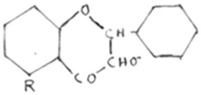


peroxide. A 4-hydroxyl group in a chalkone, as in (I) is evidently favourable factor for the reaction leading to the formation of the 3-hydroxyflavanone and therefore the flavonol. Taking 2':4-dihydroxychalkone as the simplest example, the repulsion of the β-hydroxyl in the glycol (VIII) as a hydroxyl ion is facilitated by the resonance of the phenolate ion as indicated in (IX). Cyclization of (IX) to the anion (XI) of 3:4'-dihydroxyflavanone, through the resonance structure (X), is then readily understood.

The influence of a substituent in the 6'-position of a chalkone as in (IV), in favouring the formation of benzalcoumaranone (e.g., V), has



(XII)



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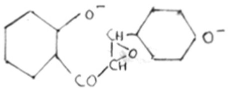
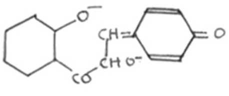
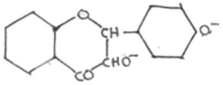


(XIV)

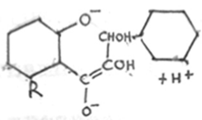
been explained by Geissman and Fukushima[7] by a mechanism in which the oxide (XII) is assumed to be the first stage, whether the product is a flavonol or a benzalcoumaranone, although numerous attempts made by them as well as by earlier workers to prepare the oxides of o'-hydroxychalkones have been unsuccessful. They regard (XIII) as the next stage when the final product is a flavonol.

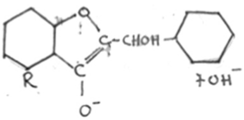
In the case of a chalkone with a hydroxyl group in the 4-position, the conversion of the oxide (XIV) to (XVI) will be facilitated by the resonance of the phenolate ion and the opening of the oxide ring as in the structure (XV).

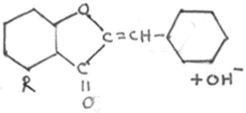

(XV)


(XVI)

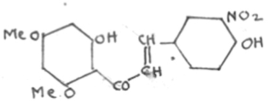
The mechanism suggested by Reichel and Steudel in which the glycol (VI) is a stage in the formation of the flavonol is adequate for -- explaining the formation of the benzalcoumaranones when the 6'-position is occupied by a methoxyl or other group (R) as a result of which there is a steric inhibition of the resonance involving the anionic oxygen and the *o*-carbonyl group in the ion (VI). Resonance of the carbonyl group indicated in


(XVII)

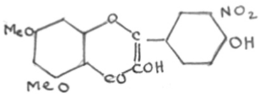

(XVIII)


(XIX)

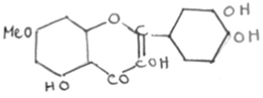
structure (XVII) will result in the repulsion of a proton by the α-carbon atom. Cyclisation to (XVIII) will then follow by an attack of the anionic oxygen of the phenol on the α-carbon atom with the displacement of a hydroxyl ion, and (XVIII) will finally be transformed into the stable benzalcoumaranone (XIX) with the further displacement of a hydroxyl ion.



(XX)



(XXI)



(XXII)

The chalkone (XX) was prepared by the condensation of phloracetophenone-4:6-dimethyl ether with 3-nitro-4-hydroxybenzaldehyde. On oxidation with alkaline hydrogen peroxide, the chalkone (XX) gave a flavonol, formulated as 3'-nitro-4'-hydroxy-5:7-dimethoxyflavonol (XXI). Reduction of (XXI), diazotization and hydrolysis and demethylation with aluminium chloride in nitrobenzene should lead to rhamnetin (XXII). Experiments on these lines are in progress.

EXPERIMENTAL.

2-Hydroxy-4:6-dimethoxyphenyl 4-hydroxy-3-methoxystyryl ketone (I) :

Phloracetophenone-4:6-dimethyl ether (2 g.), vannilin (2 g.), alcohol (20 c.c.) and 50% aqueous potassium hydroxide (10 c.c.) were refluxed for 1 hr. on the water-bath, and then left overnight at room temperature. The mixture was again refluxed for 1 hr., cooled and acidified with hydrochloric acid. The yellow precipitate crystallised from alcohol in orange-yellow needles (1.2 g.), m.p. 179-180° (Found: C, 65.9; H, 5.4. $C_{18}H_{18}O_6$ requires C, 65.5; H, 5.4%). The substance gives a brown colour with ferric chloride added to its alcoholic solution; a pale reddish-brown solution with concentrated sulphuric acid; and an orange-yellow solution with aqueous sodium hydroxide.

3:4'-Dihydroxy-5:7:3'-trimethoxyflavone (II) :

The chalkone (I)(1.0 g.) was dissolved in 20% aqueous sodium hydroxide (20 c.c.) and water (50 c.c.) and cooled to 0°. Hydrogen peroxide (2.5 c.c. of 30% solution) was added to the orange-red solution and the mixture left overnight in the refrigerator when it became orange in colour. On acidification with hydrochloric acid the yellow precipitate was collected and crystallised from alcohol. The narrow, rectangular yellow plates (0.3 g.) had m.p. 242-243° (Found: C, 62.4; H, 4.2. $C_{18}H_{16}O_7$ requires C, 62.8; H, 4.7%). The substance gives a brown colour with alcoholic ferric chloride; a yellow solution with a bright green fluorescence in sulphuric acid; and a yellow solution in aqueous caustic soda. The acetyl derivative, prepared in the usual manner, crystallized from alcohol in colourless needles, m.p. 198-200° (Found: C, 61.5; H, 4.6. $C_{22}H_{20}O_9$ requires C, 61.5; H, 4.9%). On methylating the flavonol (II) with excess of dimethyl sulphate, the pentamethyl ether of quercetin was obtained. It melted at 146° and the m.p. was undepressed when mixed with an authentic sample of quercetin pentamethyl ether obtained from quercetin.

Rhamnazin (III) :

The 5-methyl ether (II)(0.1 g.) was dissolved in nitrobenzene (2 c.c.) and anhydrous aluminium Chloride (0.2 g.) in nitrobenzene (2 c.c.) added. The mixture was heated on a boiling-water bath for 1 hr., the reaction product cooled and treated with petroleum ether (40-60°). The brown precipitate was filtered, washed twice with petroleum ether and dried. It was then added to dilute hydrochloric acid and heated for an hour on the water bath. The yellow precipitate was washed with water and crystallised from glacial acetic acid. The stout yellow needles (0.06 g.) had m.p. 214-215° and agreed in all its properties with rhamnazin[12,13] (Found: C, 61.9; H, 4.5. $C_{17}H_{14}O_7$ requires C, 61.8; H, 4.2%).

2-Hydroxy-4:6-dimethoxyphenyl-3-nitro-4-hydroxystyryl ketone (XX) :

Phloracetophenone-4:6-dimethyl ether (2 g.), 3-nitro-4-hydroxybenzaldehyde (2 g.), prepared according to Paal,[14] alcohol (50 c.c.) and 50% aqueous potassium hydroxide (40 c.c.) were mixed with cooling in ice and then left at room temperature for 3 days. The mixture was refluxed for 1 hour, again left overnight at room temperature and then acidified with dilute hydrochloric acid. The orange precipitate crystallized from acetic acid in orange-yellow needles (1.4 g.) m.p. 213-214° (Found: N, 3.9. $C_{17}H_{15}O_7N$ requires N, 4.1%). It gives a pale-brown colour with alcoholic ferric chloride. The crystals turn red in contact with concentrated sulphuric acid and then dissolve, giving a yellow solution.

3'-Nitro-3:4'-dihydroxy-5:7-dimethoxyflavone (XXI):

The chalkone from the previous experiment (1 g.) was dissolved in 20% aqueous sodium hydroxide (20 c.c.) and water (50 c.c.) and cooled to 0°. Hydrogen peroxide (2.5 c.c. of 30% solution) was added to the reddish brown solution and the mixture left overnight in the refrigerator when it became deep reddish-brown. The reaction mixture was acidified with hydrochloric acid and the yellow precipitate crystallized from acetic acid in yellow hairy needles (0.31 g.) and melted at 287° (Found: C, 56.7; H, 3.7; N, 4.3. $C_{17}H_{13}O_8N$ ------requires C, 56.8; H, 3.6; N, 3.9%). It gives an intense greenish-brown color with alcoholic ferric chloride and a yellow solution with a brownish tinge with concentrated sulphuric acid.

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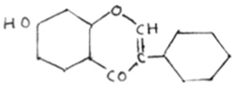
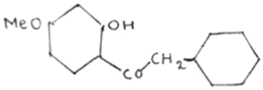
PART III

EXPERIMENTS IN THE ISOFLAVONE SERIES.

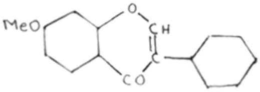
Several members of the isoflavone series have been synthesized by the action of sodium and ethyl formate on the suitably substituted *o*-hydroxyphenyl benzyl ketones.[1,2] In most of the cases, the hydroxyl groups other than the one *ortho* to the carbonyl group were protected by methylation or benzylation prior to the isoflavone condensation. Thus 7-hydroxyisoflavone (I) was obtained by treating 2-hydroxy-4-methoxyphenyl benzyl ketone (II) with sodium and ethyl formate and demethylating 7-methoxyisoflavone (III) thus produced.[2] Recently Shriner and Hull[3] have reported the synthesis of genistein 4'-methyl ether (IV) by treating 2:4:6-trihydroxyphenyl 4-methoxybenzyl ketone (V) with sodium and ethyl formate. Prunetin, isolated by Finnemore from Prunus bark was shown by Baker and Robinson to be the 4'- or 7-methyl ether of genistein(VI).[4,5] Shriner and Hull found that (IV) was different in its properties from prunetin, so that prunetin is to be regarded as



(I)

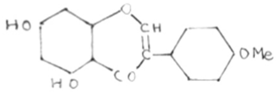


(II)

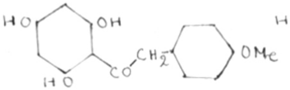


(III)

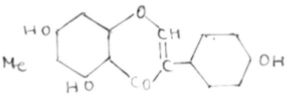
genistein 7-methyl ether (VII). Biochanin-A, one of the compounds obtained by Siddiqui from sprouted gram (<u>Cicer Arietinum</u>, Linn.) was formulated as the 4'-methyl ether (IV) of genistein.[6,7] The melting point of the compound agrees with that quoted by Shriner and Hull for (IV) obtained by them synthetically. However, Shriner and Hull's work cannot be regarded as conclusive.



(IV)

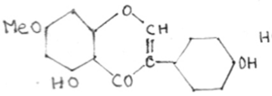


(V)

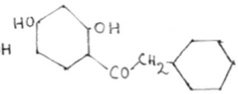


(VI)

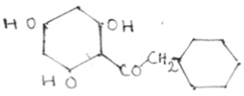
Although Shriner and Hull's synthesis of (IV) indicates that protection of hydroxyl groups is unnecessary for carrying out the ethyl formate reaction, it appeared desirable to investigate this point further from the general point of view of the synthesis of partially methylated polyhydroxyisoflavones. 2:4-Dihydroxyphenyl benzyl ketone (VIII) reacted smoothly with sodium and ethyl formate, yielding 7-hydroxyisoflavone



(VII)

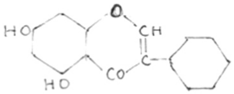


(VIII)

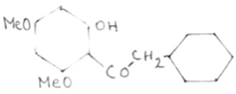


(IX)

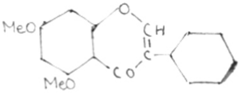
(I) in 50% yield. Performing the same experiment with 2:4:6-trihydroxyphenyl benzyl ketone (IX), 5:7-dihydroxyflavone (X) could not be obtained. A deep reddish brown product without sharp melting point, which could not be crystallized, was obtained. On the other hand, 2-hydroxy-4:6-dimethoxyphenyl benzyl ketone (XI), obtained by partial methylation of (IX), underwent smooth condensation with sodium and ethyl formate, yielding 5:7-dimethoxyisoflavone (XII). Partial demethylation with hydrobromic acid in acetic acid gave 5-hydroxy-7-methoxyisoflavone; and treatment with hydriodic acid yielded 5:7-dihydroxyisoflavone (X).



(X)

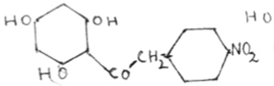


(XI)



(XII)

2:4:6-Trihydroxyphenyl 4-nitrobenzyl ketone (XIII) was prepared according to Yamashita.[8] The corresponding amine (XIV) was prepared by the reduction of (XIII) with Raney nickel and hydrogen at 40 lbs. pressure.



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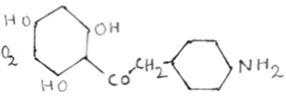

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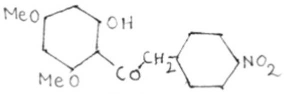
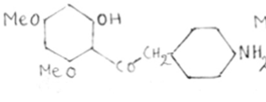


(XV)

That the carbonyl group remained intact during the reduction was ascertained by the fact that the same compound was obtained by the Hoesch reaction on phloroglucinol and *p*-acetamidobenzyl cyanide; -- deacetylation had taken place during hydrolysis of the ketimine hydrochloride. The dimethyl ether (XV) could be obtained from (XIII) only by the action of diazomethane in ether; other method of methylation involving the use of potassium carbonate or caustic soda, gave rise to resinous substances. Reduction of (XV) with Raney nickel and hydrogen gave 2-hydroxy-4:6-dimethoxyphenyl 4-aminobenzyl ketone (XVI). The latter could be converted into the acetamido derivative (XVII) by treatment with one mole of acetyl chloride in toluene solution at room temperature. The amine (XVI) by dissolving in hot dilute sulphuric acid and treating with sodium nitrite, underwent diazotization and simultaneous hydrolysis, and 2-hydroxy-4:6-dimethoxyphenyl 4-hydroxybenzyl ketone (XVIII) was thus obtained.



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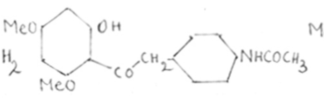

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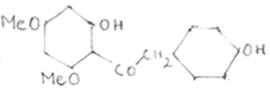
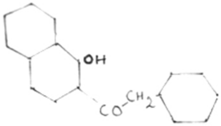


(XVIII)

The latter compound was also prepared according to the method of Zemplen and Bognar[9] by the Hoesch reaction on phloroglucinol dimethyl ether and p-hydroxybenzyl cyanide. Attempts to prepare the isoflavones from (XIII), (XV), (XVII) and (XVIII) by the action of sodium and ethyl formate, with a view to synthesize (VII), have so far been unsuccessful. Attempts to synthesize 2-hydroxy-4:6-dimethoxyphenyl 4-benzyloxybenzyl ketone and to convert it to (VII) through the obvious stages are in progress. A new and unambiguous synthesis of the isomer (IV) in view of the failure in the present work to convert (IX) directly into (X) has also been undertaken.

2-Phenylacetyl-1-naphthol (XIX) gives α-naphthaisoflavone (XX) in 5% yield by refluxing with ethyl



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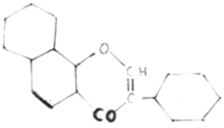

(XX)

orthoformate, zinc chloride and acetic anhydride.[10] Carrying out the same reaction with 2-hydroxy-4:6-dimethoxyphenyl 4-nitrobenzyl ketone (XV), the O-acetyl derivative was obtained. The same reaction on (XVIII) gave a resinous product from which no homogeneous substance could be isolated.

EXPERIMENTAL.

Action of sodium and ethyl formate on 2:4-dihydroxyphenyl benzyl ketone: 7-Hydroxyisoflavone (I) :

The ketone (0.5 g.), prepared according to Chapman and Stephen,[11] in freshly distilled ethyl formate (15 cc.) was gradually added to finely pulverized sodium (0.5 g.), covered with dry ether (2 c.c.). A vigorous reaction took place which was complete in 4 hours as indicated by the slackening and then stoppage of hydrogen evolution. The mixture was first light yellow, then deep yellow, orange-brown, and finally brown. The reaction mixture was cautiously poured into ice and dilute hydrochloric acid. A brownish yellow semi solid mass separated, which however soon solidified. This was filtered dried and dissolved in concentrated sulphuric acid (15 cc.) After 3 hours the sulphuric acid solution was poured over crushed ice and the brown solid crystallized from dilute alcohol in white plates (0.25 g.) and melted at 210°. No depression in the melting point was observed when mixed with an authentic sample of 7-hydroxyisoflavone.[2]

Dimethylation of 2:4:6-trihydroxyphenyl benzyl ketone: 2-Hydroxy-4:6-dimethoxyphenyl benzyl ketone (XI) :

2:4:6-Trihydroxyphenyl benzyl ketone (2.45 g.), prepared according to Chapman and Stephen,[11] was refluxed with dry acetone (30 c.c.) freshly ignited potassium carbonate (4 g.) and dimethyl sulphate (2.5 g., 2 mols.) for 14 hours. The product was poured into water, excess acetone removed on the water-bath, the precipitate collected and crystallized from alcohol in colourless square plates (1.85 g.) and melted at 116°. (Found: C, 70.5; H, 5.7. $C_{16}H_{16}O_4$ requires C, 70.6; H, 5.9%). It gives a wine-red colour with alcoholic ferric chloride.

Action of sodium and ethyl formate on 2-hydroxy-4:6-dimethoxyphenyl benzyl ketone: 5:7-Dimethoxyisoflavone (XII):

The ketone (1 g.) dissolved in freshly distilled ethyl formate (24 c.c.) was gradually added to finely divided sodium (1 g.), preserved under dry ether (5 c.c.) at room temperature. There was at first a very slow reaction but soon it became vigorous. It took 4 hours for the sodium to go completely into solution. The light reddish brown reaction product was added to ice and dilute hydrochloric acid and excess ethyl formate allowed to evaporate. The sticky solid was filtered, dried and dissolved in concentrated sulphuric acid (10 c.c.). After 4 hours the sulphuric acid solution was poured over crushed ice and the grey solid filtered off and crystallized twice from dilute alcohol. The white plates (0.5 g.) melted at 120° (Found: C, 72.7; H, 4.7. $C_{17}H_{14}O_4$ requires C, 72.3; H, 4.9%).

Action of boiling hydrobromic acid in acetic acid on 5:7-dimethoxyisoflavone: 5-Hydroxy-7-methoxyisoflavone:

5:7-Dimethoxyisoflavone (0.2 g.) was dissolved in acetic anhydride (2 c.c.) and hydrobromic acid (d, 1.5; 2 c.c.). The mixture was refluxed for 6 hours. The reaction product was poured into water and the precipitate which separated was collected and crystallized from 50% alcohol in elongated plates which melted at 139-140° (Found: C, 72.0; H, 4.1. $C_{16}H_{12}O_4$ requires C, 71.6; H, 4.5%). The colourless alcoholic solution turns pale yellow when aqueous caustic soda is added and brown when ferric chloride is added.

Demethylation of 5:7-dimethoxyisoflavone with hydriodic acid in acetic acid: 5:7-Dihydroxyisoflavone (X):

5:7-Dimethoxyisoflavone (0.15 g.) was added to hydriodic acid (d, 1.7; 1 c.c.), acetic anhydride (1 c.c.) and glacial acetic acid (1 c.c.) The mixture was refluxed for 4 hours, cooled, diluted with water and treated with sodium sulphite to destroy free iodine. The white solid was collected and crystallized from dilute alcohol in needles which melted at 193-194°. (Found: C, 70.8; H, 3.9. $C_{15}H_{10}O_4$ requires C, 70.9; H, 3.9%). The compound in alcoholic solution gives a violet colour with a drop of ferric chloride; it dissolves in aqueous caustic soda giving a yellow solution. By refluxing the compound with acetic anhydride and pyridine, 5:7-diacetoxyflavone was obtained. It crystallized from alcohol in white hairy needles melting at 180° (Found: C, 67.0; H, 4.1. $C_{19}H_{14}O_6$ requires C, 67.5; H,4.1%).

Hoesch reaction on phloroglucinol and p-acetamidobenzyl cyanide : 2:4:6-Trihydroxyphenyl 4-aminobenzyl ketone (XIV):

Dry phloroglucinol (2 g.), p-acetamidobenzyl cyanide (2 g.), (prepared according to Gabriel[12]), dry ether (30 c.c.) and powdered fused zinc chloride (1 g.) was cooled in ice and a stream of dry hydrochloric acid gas passed through it for 4 hours. It was then left in the refrigerator for 4 days. Ether was decanted off from the yellowish brown ketimide hydrochloride and the latter once washed with ether. It was then added to water (100 c.c.) and refluxed for 2 hours, filtered and cooled

The solid that separated was treated with sodium bicarbonate, and the product thus obtained crystallized from dilute alcohol. The pale yellowish plates melted at 197°. (Found: N, 5.0. $C_{14}H_{13}O_4N$ requires N, 5.4%). It gives a violet brown colour with alcoholic ferric chloride. A crystalline precipitate of the hydrochloride is obtained on addition of hydrochloric acid. On cooling in ice and addition of sodium nitrite, a deep yellow solution is obtained. An orange-yellow precipitate gradually separated from this solution. On addition of a drop to alkaline β-naphthol, a deep red solution is obtained, indicating the presence of an amino group.

Reduction of 2:4:6-trihydroxyphenyl 4-nitrobenzyl ketone: 2:4:6-Trihydroxyphenyl 4-aminobenzyl ketone (XIV):

The ketone (1 g.) was dissolved in alcohol (60 c.c.) containing Raney nickel catalyst (1 g.) and shaken in a Parr hydrogenator with hydrogen at 40 lbs. pressure for 6 hours. The reaction product was filtered, the catalyst washed with alcohol and the filtrate diluted with water after removing the major portion of alcohol on the water-bath. A precipitate (0.6 g.) was obtained When crystallized from dilute alcohol it came out in yellow plates melting at 190°. No depression in the melting point was observed when mixed with the compound from the previous experiment.

Methylation of 2:4:6-trihydroxyphenyl 4-nitrobenzyl ketone: 2-Hydroxy-4:6-dimethoxyphenyl 4-nitrobenzyl ketone (XV):

The ketone (2 g.) was dissolved in ether (100 c.c.) and mixed at 0° with an etherial solution of diazomethane prepared from nitrosomethyl urea (4 g.). The mixture was kept in the refrigerator for 48 hours, ether was decanted off from the crystals that had separated. On recrystallization from alcohol, almost colourless needles (1.25 g.) melting at 148° were obtained (Found: C, 60.7; H, 4.8; N, 4.6. $C_{16}H_{15}O_6N$ requires C, 60.6; H, 4.7; N, 4.4%). The compound is insoluble in sodium carbonate but gives a violet solution with sodium hydroxide. It gives a brown coloration with alcoholic ferric chloride.

2-Hydroxy-4:6-dimethoxy 4-aminobenzyl ketone (XVI):

2-Hydroxy-4:6-dimethoxyphenyl 4-nitrobenzyl ketone (2 g.) was suspended in alcohol (50 c.c.) containing Raney nickel catalyst (2 g.) and shaken in a Parr hydrogenator with hydrogen at 40 lbs. pressure for 8 hours. The reaction mixture was filtered, the catalyst washed ~~/~~ with alcohol and the filtrate diluted with water after removing the major portion of alcohol by distillation. The pale brown precipitate crystallized from dilute alcohol in yellow needles, m.p. 103-104°

(Found: N, 5.4. $C_{16}H_{17}O_4N$ requires N, 4.9%). It gives a brown coloration with alcoholic ferric chloride. It dissolves in hot dilute hydrochloric acid and on cooling the solution its hydrochloride separates in needles melting at 198-200° (Found: N, 3.8. $C_{16}H_{18}O_4NCl$ requires N, 4.3%). From 2 g. nitro compound 1.25 g. amine hydrochloride was obtained.

2-Hydroxy-4:6-dimethoxyphenyl 4-acetamidobenzyl ketone (XVII):

2-Hydroxy-4:6-dimethoxyphenyl 4-aminobenzyl ketone (0.1 g.) was dissolved in hot toluene (2 c.c.) and cooled. Acetyl chloride (0.025 g., 1 mol.) was added, when immediately, a white crystalline precipitate separated. After 10 minutes the precipitate was collected and crystallized from dilute alcohol in white needles which melted at 159-160° (Found: N, 4.5. $C_{18}H_{19}O_5N$ requires N, 4.2%). It gives a violet brown colour with ferric chloride and is insoluble in hot dilute hydrochloric acid.

Diazotization and hydrolysis of 2-hydroxy-4:6-dimethoxyphenyl 4-aminobenzyl ketone: 2-Hydroxy-4:6-dimethoxyphenyl 4-hydroxybenzyl ketone (XVIII): The amine hydrochloride from The amine hydrochloride from the previous experiment (2.75 g.) was suspended in a mixture of concentrated sulphuric acid (8 c.c.) and water (80 c.c.) in a 250 c.c. three necked flask fitted with a thermometer, dropping funnel and water-condenser. The contents were heated to boiling; when the amine hydrochloride completely went into solution, sodium nitrite (0.688 g., 1 mol.) in water (7 c.c.), was added dropwise in the course of 10 minutes. There was a vigorous reaction and the mixture became reddish brown with a dark oil floating on the top. When the addition of sodium nitrite was over, water (16 c.c.) was added and the mixture heated to boiling, and then cooled. The flask was left overnight at room temperature. The dark solid was filtered off, taken up in hot alcohol and cooled. The alcoholic solution was filtered and evaporated to dryness on a water-bath. The residue was extracted 4 times with 25 c.c. portions of ether. The yellow ether solution was norited, filtered and the ether removed. The yellow residue (0.8 g.) melted at 98° with previous shrinking. On crystallizing from dilute alcohol it separated in needles melting at 109-110°. No depression in the melting point was observed when the compound was mixed with an authentic sample of 2-hydroxy-4′:6-dimethoxyphenyl 4-hydroxybenzyl ketone prepared according to Zemplen and Bognar[7] (Found: C, 66.4; H, 5.7. $C_{16}H_{16}O_5$ requires C, 66.7; H, 5.6%).

Action of ethyl orthoformate, zinc chloride and acetic anhydride on 2-hydroxy-4:6-dimethoxyphenyl 4-nitrobenzyl ketone :

A mixture of the ketone (0.45 g.), acetic anhydride (0.5 g.), zinc chloride (0.5 g.) and ethyl orthoformate (4.5 c.c.) was refluxed for 6 hours and then poured into water. The reddish brown precipitate obtained by leaving the mixture overnight was treated with concentrated sulphuric acid, the acid solution diluted with ice and water and the reddish brown solid (0.25 g.) was collected and crystallized from dilute acetic acid. The pale reddish needles melted at 208-210° (Found: C, 59.8; H, 4.4. The acetate $C_{18}H_{17}O_7N$ requires C, 60.2; H, 4.7%). It gives no characteristic colour with alcoholic ferric chloride.

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SUMMARY.

Part I

Perkin obtained disazo dyes by coupling natural colouring matters from resorcinol and phloroglucinol with benzenediazonium chloride. Mahal and Venkataraman observed the formation of a monoazo dye by coupling 6-hydroxyflavone with diazotized p-nitraniline. Such azo dyes have now been used as intermediates for the synthesis of polyhydroxyflavones. It has been found that 6-hydroxyflavone couples with benzenediazonium chloride in the 5-position. The azo dye has been reduced to 5-amino-6-hydroxyflavone, which on treatment with nitrous acid gave flavone-5-diazo-6-oxide. The latter was then hydrolyzed to 5:6-dihydroxyflavone with boiling sulphuric acid.

A monoazo dye has been prepared from 5-hydroxyflavone. An isomeric compound has been obtained by carrying out the Robinson flavone condensation on 4-benzeneazo-2-acetylresorcin, benzoic anhydride and sodium benzoate. Amines obtained by reduction of both the dyes gave 5:6-dihydroxyflavone on diazotization and hydrolysis, although one of them (8-amino-5-hydroxyflavone) should have yielded primetin (5:8-dihydroxyflavone). This is attributed to a rearrangement of 5:8-dihydroxyflavone to 5:6-dihydroxyflavone by boiling sulphuric acid. It has been shown that the dye obtained by coupling 5-hydroxyflavone with benzenediazonium chloride is the 8-benzeneazo compound, whereas the product obtained by the Robinson reaction on 4-benzeneazo-2-acetylresorcin is 6-benzeneazo-5-hydroxyflavone.

7-Hydroxyflavone gave a monoazo dye which on reduction yielded the corresponding amine. The diazonium compound from this amine was extremely stable even in boiling 80% sulphuric acid, and further increase in the acid concentration resulted in decomposition. In the course of this work it has been shown that resacetophenone couples in the 5-position when only one mole of benzenediazonium chloride is used.

5-Hydroxy-6-methoxyflavone has been coupled with benzenediazonium chloride. The monoazo dye was reduced to the amine and converted into the corresponding -- trihydroxyflavone (5:6:8) by refluxing with hydrochloric acid.

Tectochrysin has been converted into 8-benzeneazotectochrysin, and thence to 8-aminotectochrysin, which on boiling with hydrochloric acid yielded 7-methyl ether of baicalein. A rearrangement of 5:8-dihydroxy-7-methoxyflavone to 5:6-dihydroxy-7-methoxyflavone therefore takes place.

Quercetin 3:7:3':4'-tetramethyl ether gave a monoazo dye with benzenediazonium chloride. The dye has been reduced to the amine, but all attempts to obtain the corresponding dihydroxymethoxyflavone failed, as in the case of 8-amino-7-hydroxyflavone. Conditions have been worked out for the preparation of 5-hydroxyflavone and 6-hydroxyflavone in good yields.

Part II.

The Algar-Flynn oxidation of o'-hydroxychalkones to flavonols by means of alkaline hydrogen peroxide has been shown to be applicable to chalkones with a hydroxyl group in the 4-position, irrespective of the presence of a methoxyl group in the 6'-position. Rhamnazin 5-methyl ether, and rhamnazin by partial demethylation with aluminium chloride, have thus been synthesized. Geissman and Fukushima have shown that 2'-hydroxy-6'-methoxychalkones give benzalcoumaranones, the flavonols not being formed or only as minor products. The influence of the 4-hydroxyl group on the course of the reaction is discussed. 3'-Nitro-4'-hydroxy-5:7-dimethoxyflavonol has been prepared by the action of alkaline hydrogen peroxide on the appropriate chalkone. Reduction, diazotization, hydrolysis and partial demethylation should lead to rhamnetin. Experiments on these lines and on alternative synthesis of rhamnetin from 4-hydroxy-3-acetyl-2'-hydroxy-4':6'-dimethoxychalkone are in progress.

Part III.

Experiments have been carried out to determine the effect of free hydroxyl groups in the phenyl or benzyl portion of phenyl benzyl ketones on the sodium and ethyl formate reaction for the synthesis of isoflavones. 2:4-Dihydroxyphenyl benzyl ketone yielded 7-hydroxyisoflavone by the action of sodium and ethyl formate, but 2:4:6-trihydroxyphenyl benzyl ketone did not give 5:7-dihydroxyisoflavone. However, protection of the 4- and 6-hydroxyl groups by methylation was found to facilitate the isoflavone synthesis, since 2-hydroxy-4:6-dimethoxyphenyl benzyl ketone could be smoothly converted into 5:7-dimethoxyisoflavone. 5:7-Dihydroxyisoflavone and its 7-methyl ether have been obtained by demethylation of the latter with hydriodic acid and hydrobromic acid respectively. Attempts have been made to prepare the isoflavones from 2:4:6-trihydroxyphenyl 4-nitrobenzyl ketone, 2-hydroxy-4:6-dimethoxyphenyl 4-nitrobenzyl ketone, 2-hydroxy-4:6-dimethoxyphenyl 4-acetamidobenzyl ketone and 2-hydroxy-4:6-dimethoxyphenyl 4-hydroxybenzyl ketone, with a view to synthesize prunetin; they were unsuccessful.

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