SYNTHETIC EXPERIMENTS IN THE BENZOPYRONE SERIES

Part XXII. A Synthesis of 8-Methyl Genistein

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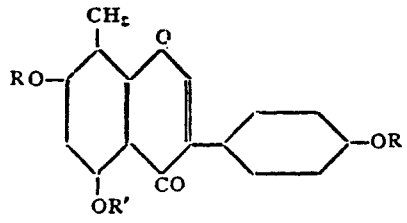
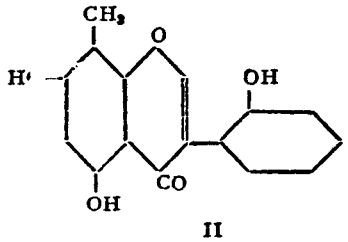
Received November 3, 1952

OKANO and BEPPU[1] reported the isolation of four isoflavones from soya beans. Three of these contain C-methyl groups and are the only examples of nuclear methylated isoflavones occurring in nature. They have deduced the constitutions of these three isoflavones as 8-methyl genistein (I *a*), 8-methyl isogenistein (II) and 8-methyl-5:4'-dihydroxy isoflavone (tatoin) (III) on the basis of degradation experiments.

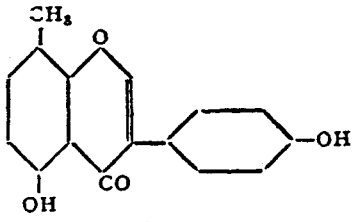


I



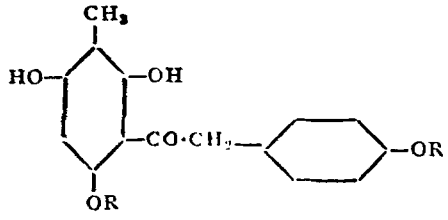
II

a, R = R' = H
b, R = CH_3, R' = H
c, R = R' = CH_3

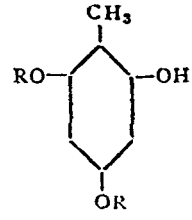


III

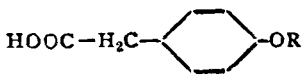
The constitution of (I *a*) was arrived at as follows. The analytical values and the molecular weight determination corresponded to the formula $C_{16}H_{12}O_5$. It yielded a triacetate, a dimethyl ether (I *b*) and a trimethyl ether (I *c*). On heating with alkali, it yielded formic acid and a tetrahydroxy ketone (IV *a*) which could be further degraded into C-methyl phloroglucinol (V *a*) and *p*-hydroxy phenyl acetic acid (VI *a*). The trimethyl ether (I *c*) on heating with alkali gave C-methyl phloroglucinol-α-dimethyl ether (V *b*) and *p*-methoxy phenyl acetic acid (VI *b*).



IV



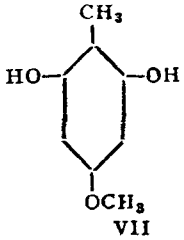
V



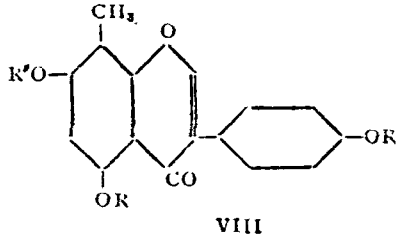
VI

a, R = H
b, R = CH_3

A synthesis of 8-methyl genistein (I *a*) was also reported by Shriner and Hull.[2] The starting point for their synthesis was C-methyl phloroglucinol α-methyl ether (VII).[3] On Hoesch condensation with *p*-methoxy phenyl acetonitrile, it yielded 2:4-dihydroxy-3-methyl-6:4′-dimethoxy phenyl benzyl ketone (IV *b*) which on treatment with ethyl formate in presence of sodium gave rise to 7-hydroxy-8-methyl-5:4′-dimethoxy isoflavone (*VIII a*). The trihydroxy isoflavone (I *a*) obtained by the demethylation of this substance with hydriodic acid was found by the above authors to have the same melting point as natural 8-methyl genistein. The melting point of the acetate was also in agreement with that reported for the triacetate of 8-methyl genistein by Okano and Beppu.[1] However no direct comparison of the natural and synthetic samples was made.



VII



VIII

a, R = CH_3, R′ = H
b, R = H, R′ = CH_3

Elsewhere it has been pointed out that the origin of C-hydroxy and C-methyl groups in flavonoids have certain common features and some of the methods of nuclear oxidation are applicable for nuclear methylation also.[4, 13] These considerations could be conveniently tested using the naturally occurring C-methyl isoflavones as examples. Nuclear oxidation of isoflavones has been recently reported[5] and nuclear methylation has been under study. In the course of this work it was found necessary to prepare the C-methyl isoflavones by conventional methods for purposes of reference.

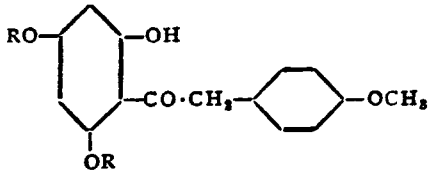
The preparation C-methyl phloroglucinol (V *a*) and its α-monomethyl ether (VII) used in the synthesis of Shriner and Hull[2] involve a number of

steps of decreasing yields. Direct nuclear methylation at the ketone stage seemed to offer a better method and a synthesis of 8-methyl genistein, based on such a reaction, has now been undertaken.

The ketone required for this purpose is 2:4:6-trihydroxy-4'-methoxy phenyl benzyl ketone (IX *a*). This was made earlier by Baker and Robinson[6] by the condensation of *p*-methoxy phenyl acetonitrile with phloroglucinol in presence of hydrogen chloride. The required nitrile has been prepared by a number of methods. A review of the earlier literature has been given by Shriner and Hull.[2]

For the preparation of nitriles of this type, the method of Baker and Robinson[7] is of general applicability. The preparation of *p*-methoxy phenyl acetonitrile itself has been very briefly mentioned by Baker and Eastwood.[8] The details of the preparation are now described and further, *p*-methoxy phenyl pyruvic acid oxime, which was not described earlier has now been isolated and characterised.

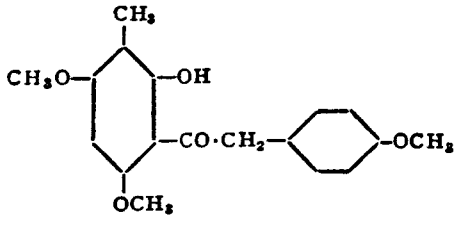
The nuclear methylation of the ketone (IX *a*) has been carried out by heating an acetone solution of the ketone with excess of methyl iodide and potassium carbonate. Two products are obtained and these are separated by fractional crystallisation from methanol. The less soluble fraction which is obtained in an yield of 15% is 2-hydroxy-3-methyl-4:6:4'-trimethoxy phenyl benzyl ketone (X). That this compound has the constitution (X) and not the alternative (XI) is based on analogy with nuclear methylation of phloracetophenone.[3] It does not give any blue or green colour with nitric acid, showing that it contains another nuclear substituent. This conclusion is based on a detailed examination of this colour reaction by Rao and Seshadri[9] who have pointed out that a green or blue colour is given by ketones of type (XII) and that it is not answered by ketones of type (XIII). The more soluble fraction which gives blue colouration with nitric acid consists of 2-hydroxy-4:6:4'-trimethoxy phenyl benzyl ketone (IX *b*).[10, 11]



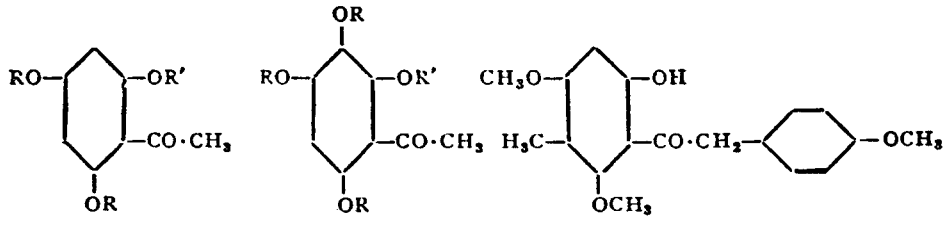
IX

a, R = H

b, R = CH_3



X



XII XIII XI

R = alkyl

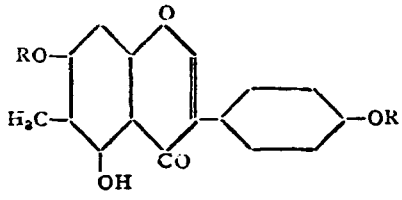
R′ = H or alkyl

Treatment of the C-methyl ketone (X) with ethyl formate and sodium at 0°,[12] gives rise to a good yield of 8-methyl-5: 7: 4′-trimethoxy isoflavone (I *c*). It melts at 181–83°. Okano and Beppu[1] have however reported that 8-methyl genistein trimethyl ether had a melting point 154–55°. On demethylation with hydriodic acid, (I *c*) yields 8-methyl-5: 7: 4′-trihydroxy isoflavone (I *a*). This is found to be soluble in aqueous sodium carbonate showing that all the methoxyl groups including the one at the 7-position have been demethylated. When crystallised from dilute alcohol, it has a melting point 231–32°. The melting point does not rise on further crystallisation. Okano and Beppu[1] have however given the melting point of natural 8-methyl genistein as 298°. Shriner and Hull[2] have also reported the melting point of their synthetic sample to be 296–300°. Okano and Beppu[1] have stated that the natural isoflavone gives a violet or violet brown colour with alcoholic ferric chloride, whereas the synthetic product obtained in the present work gives a deep green colour with this reagent. Shriner and Hull[2] have not recorded this colour reaction with their substance.

It may be relevant to draw a comparison between 8-methyl genistein and other nuclear methylated chromones, in the ferric reactions exhibited by them. As described in an earlier publication,[13] all the C-methyl chromones from *Eugenia caryophyllata*, eugenitin, isoeugenitol and isoeugenitin give a blue colour with ferric chloride. In the absence of a C-methyl group, as exemplified by nor-eugenin and eugenin, only a wine red colour is developed. Genistein itself gives a pink ferric reaction and in the case of prunetin and genistein-7: 4′-dimethyl ether, it is brownish violet. Hence the production of a deep green colour with ferric chloride by the synthetic 8-methyl genistein is more in agreement with expectations for this structure than the violet or violet brown colour as given by the natural compound.

Besides the differences mentioned above, there is considerable difference in the melting points of the acetates also. The acetate prepared in this work

by the acetic anhydride-pyridine method on crystallisation from acetic acid melts at 213–15° whereas Okano and Beppu[1] and Shriner and Hull[2] report the melting point of the acetate to be 184° and 184–85° respectively. The acetate obtained in the present work has also been deacetylated with alcoholic hydrochloric acid. The resulting hydroxy compound has a melting point 231–32°, showing that it is a pure entity.



XIV

a, R = H

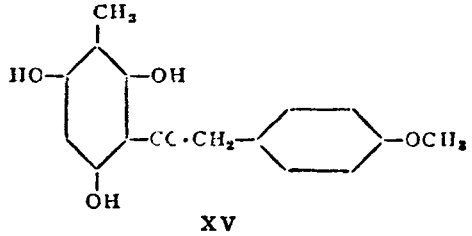
b, R = CH_3

There exists however a possibility that during the treatment of 8-methyl-5: 7: 4′-trimethoxy isoflavone (I *c*) with hydriodic acid, besides demethylation, isomeric change into 6-methyl-5: 7: 4′-trihydroxy isoflavone (XIV *a*) has also taken place. That this has not happened has been proved in two ways. The trimethyl ether (I *c*) has been demethylated with anhydrous aluminium chloride in benzene solution. It has recently been pointed out by Narasimhachari and Seshadri[14] that this reagent in benzene medium is capable of causing demethylation of the very resistant 7-methoxyl group in isoflavones. A large amount of the solvent has to be employed for the demethylation of (I *c*) due to the low solubility of the compound. That this reagent causes demethylation without isomeric change is well established.[15] The product is found to melt at 231–32° and is in every way identical with the trihydroxymethyl isoflavone obtained by demethylation of (I *c*) with hydriodic acid. Secondly, the hydriodic acid demethylation product has been methylated with excess of dimethyl sulphate and potassium carbonate in acetone solution. The resulting trimethyl ether is identical with the original 8-methyl-5: 7: 4′-trimethoxy isoflavone (I *c*). These two experiments definitely establish the absence of any rearrangement during demethylation. In an earlier publication[5] it has been shown that closely related isoflavones having a hydroxyl or methoxyl in the 8-position instead of a methyl group also do not undergo ring isomeric change when boiled with hydriodic acid.

The discrepancies are difficult to explain. The present synthesis seems to be quite unambiguous and hence it is possible that the natural compound does not have the constitution of 8-methyl genistein, as deduced by Okano

and Beppu.[1] But the discrepancies between the products of the present synthesis and those of Shriner and Hull[2] are more difficult to explain. The earlier method is certainly an acceptable one and hence there is difficulty in explaining the differences. Their method has therefore been reexamined and forms the subject-matter of a forthcoming publication.

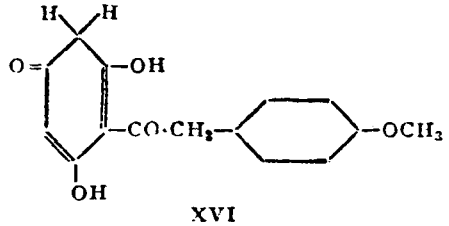
It is possible that during the preparation of the important intermediate, 2-hydroxy-3-methyl-4:6:4'-trimethoxy phenyl benzyl ketone (X) by nuclear methylation, instead of one C-methyl group, two such groups might have entered. Hence the above ketone (X) has now been prepared in another manner also, which leaves no doubt about its constitution. C-Methyl phloroglucinol (V *a*)[2] has been condensed with *p*-methoxy phenyl aceto-nitrile. A good yield of 2:4:6-trihydroxy-3-methyl-4'-methoxy phenyl benzyl ketone (XV) is obtained. This on methylation with two moles of dimethyl sulphate and excess of anhydrous potassium carbonate in acetone solution gives rise to 2-hydroxy-3-methyl-4:6:4'-trimethoxy phenyl benzyl ketone (X), identical in every way with the product obtained by nuclear methylation of 2:4:6-trihydroxy-4'-methoxy phenyl benzyl ketone (IX *a*). In the above methylation of the ketone (XV), it is the hydroxyl group in the 6-position (para to the methyl) that is methylated and not the one in the 2-position (ortho to the methyl group) follows from similar methylations of simpler ketones.[3, 16] It is also well known that dimethyl sulphate, unlike methyl iodide, does not cause any nuclear methylation and therefore the product of methylation of 2:4:6-trihydroxy-3-methyl-4'-methoxy phenyl benzyl ketone (XV) should contain only one C-methyl group and have the constitution (X).



XV

In this connection should be noted the observation of Curd and Robert-son[3] who found that treatment of C-methyl phloracetophenone with excess of methyl iodide gave only 2-hydroxy-3-methyl-4:6-dimethoxy aceto-phenone, *i.e.*, no further nuclear methylation took place. In the present work also, 2:4:6-trihydroxy-3-methyl-4'-methoxy phenyl benzyl ketone (XV) is found to yield the dimethyl ether (X) only, on heating with excess of methyl iodide and potassium carbonate in acetone solution and no further nuclear methylation takes place.

No explanation has been put forward so far for the absence of further nuclear methylation in C-methyl phloracetophenone derivatives. According to the mechanism of nuclear methylation of polyhydroxy carbonyl compounds,[17] this reaction requires the formation of the isomeric carbonyl form (XVI) and for this purpose a double bond should exist between the 3- and 4-positions of the ketone (IX *a*). This is facilitated by the fixation caused by the chelate ring. Consequently no double bond seems to function between the 4- and 5-positions, the 5-position does not get activated and no nuclear methylation takes place. This scheme assumes that the chelate structure is fixed and does not change from one position to another under the conditions of the experiment.



XVI

But this does not explain all observed facts. For example, C-methyl phloroglucinol (V *a*) yields the ketone (XV) which when methylated yields the same dimethyl ether (X) as obtained by the alternative method and not (XI). In (XV), ordinarily the more acidic hydroxyl group in the 6-position (para to the methyl group) could be expected to enter into chelation with the neighbouring carbonyl, in which case, it will be resistant to methylation. It is actually not so; the 6-hydroxyl group gets methylated easily and the 2-hydroxyl is again resistant and chelated. Obviously some other factor is playing an important part in the reactivity of (XV). This should be the tautomeric effect already explained.[13] The formation of the *p*-quinonoid structure being more favoured, the 6-hydroxyl group is not so readily available for chelation and hence the chelate ring is formed with the alternative 2-hydroxyl group.

Partial methyl ethers of 8-methyl genistein have also now been prepared. The technique of partial demethylation has been used for this purpose. Hydriodic acid under controlled conditions has been used for the demethylation of the 5- and 4'-methoxyl groups.[18] This reagent is preferable to hydrobromic acid which often causes resinification. Using it, 8-methyl-5:7:4'-trimethoxy isoflavone (I *c*) has been demethylated at 110° and the product, 8-methyl-5:4'-dihydroxy-7-methoxy isoflavone (8-methyl prunetin) (VIII *b*) is obtained in a good yield. It is insoluble in aqueous sodium carbonate. It gives a brilliant green colour with alcoholic ferric chloride indicating the

presence of a free hydroxyl group at the 5-position. The diacetate has also been prepared by the acetic anhydride-pyridine method.

On methylation with one mole of dimethyl sulphate, 8-methyl prunetin (VIII *b*) yields its monomethyl ether, 8-methyl-5-hydroxy 7: 4'-dimethoxy isoflavone (I *b*). Its properties are in agreement with this formulation. It exhibits a deep green ferric reaction which is comparable to the blue colour given by the corresponding chromone, isoeugenitin.[13]

A C-monomethyl-O-dimethyl genistein was described by Perkin and Horsfall,[19] who obtained it by the methylation of genistein with methyl iodide. This had a melting point 200–02° and it gave an initial violet colour changing to olive green with alcoholic ferric chloride.[6] The melting point of 8-methyl-5-hydroxy-7: 4'-dimethoxy isoflavone (I *b*) obtained in the present work is 164–66°. The melting point of this substance and its ferric reaction are different from those reported for the methyl ether of Perkin and Horsfall.[19] Therefore the latter substance should be isomeric and not identical with (I *b*) and hence it should be 6-methyl-5-hydroxy-7: 4'-dimethoxy isoflavone (XIV *b*) as suggested by Baker and Robinson.[6] It may however be mentioned that the 8-position is normally the reactive one in chromones. This nuclear methylation in which the 6-position gets substituted is therefore to be regarded as abnormal.

The structure assigned to the nuclear methylation product of genistein is supported by other considerations also. Perkin and Horsfall[19] demethylated this substance and obtained a C-methyl trihydroxy isoflavone, which had a melting point of 278°. This should therefore be different from 8-methyl genistein synthesised in the present work, which melts at 231–32. Hence the trihydroxy methyl isoflavone of Perkin and Horsfall should be 6-methyl genistein (XIV *a*). A comparison of the melting points of these C-methyl genistein derivatives with those of the related C-methyl chromones[13] also supports the above conclusion. These are presented in the following table.

Substance	M.P.
2: 8-Dimethyl-5-hydroxy-7-methoxy chromone (Isoeugenitin) ..	148–49°
2: 6-Dimethyl-5-hydroxy-7-methoxy chromone (Eugenitin) ..	161–62°
2: 8-Dimethyl-5: 7-dihydroxy chromone (Isoeugenitol)	235–37°
2: 6-Dimethyl-5: 7-dihydroxy chromone (Eugenitol)	274–76°
8-Methyl-5-hydroxy-7: 4'-dimethoxy isoflavone	164–66°
6-Methyl-5-hydroxy-7: 4'-dimethoxy isoflavone	200–02°
8-Methyl-5: 7: 4'-trihydroxy isoflavone (8-Methyl genistein) ..	231–32°
6-Methyl-5: 7: 4'-trihydroxy isoflavone (6-Methyl genistein) ..	276–78°

In all these cases the 6-methyl compounds melt much higher than their 8-methyl isomers and therefore, the product obtained by nuclear methylation of genistein should be the 6-methyl derivative (XIV *b*).

EXPERIMENTAL

p-*Methoxy phenyl acetonitrile*

2-Phenyl-4-anisal oxazolone[20] was obtained by heating together anisaldehyde (25 c.c.), hippuric acid (44 g.), fused sodium acetate (14 g.) and acetic anhydride (80 c.c.). Yield 45 g. The oxazolone (45 g.) was heated with aqueous sodium hydroxide (23 g. dissolved in 135 c.c. of water) for one hour. The solution was cooled, diluted with water (120 c.c.) and saturated with sulphur dioxide. The precipitated benzoic acid was filtered after 15 hours and the filtrate heated with concentrated hydrochloric acid (135 c.c.) for one hour on a water-bath. The precipitated *p*-methoxy phenyl pyruvic acid amounted to 15 g. The separation of benzoic acid from the pyruvic acid was originally effected by Cain *et al.*,[21] by fractionation of their ethyl esters. The above method is more convenient.

The oxime of the acid was prepared by heating the acid (15 g.) with aqueous sodium hydroxide (150 c.c. of 8%) and hydroxylamine hydrochloride (12 g.) for 15 minutes at 50° and acidifying the alkaline solution after 15 hours. Yield 15 g. It crystallised from dilute methanol as colourless prisms melting at 149–51° (decomp.) (Found: C, 57·1; H, 5·2; $C_{10}H_{11}O_4N$ requires C, 57·4; H, 5·3%). Baker and Eastwood[8] also prepared this compound but did not isolate and characterise it.

The dry oxime (15 g.) was heated carefully with acetic anhydride (10 c.c.) for 15 minutes at 100° and the excess of acetic anhydride decomposed with water. The acetic acid was nearly neutralised with aqueous sodium hydroxide and the nitrile extracted with ether. The ether solution was successively washed with aqueous sodium bicarbonate and water, dried over sodium sulphate and distilled. The residual oily *p*-methoxy phenyl acetonitrile (10 g.) was dried in a vacuum desiccator and used for Hoesch condensations.

Nuclear methylation of (IX a)

A solution of 2:4:6-trihydroxy-4'-methoxy phenyl benzyl ketone[6] (3 g.) in acetone (150 c.c.) was treated with methyl iodide (6·5 c.c.) and ignited potassium carbonate (15 g.) and the mixture refluxed in a water-bath for 3 hours. The solution was filtered off and the potassium salts were washed with hot acetone repeatedly. Acetone was distilled off from the combined filtrate. The residue was treated with water (50 c.c.) when potassium iodide dissolved. The insoluble methylated product was filtered,

washed with water and crystallised from methanol twice. 2-Hydroxy-3-methyl-4 : 6 : 4′-trimethoxy phenyl benzyl ketone (X) separated as colourless rectangular rods melting at 114–15°. Yield 0·45 g. Its alcoholic solution gave a pinkish brown colour with ferric chloride. It did not give any blue or green colour with concentrated nitric acid. It was sparingly soluble in methanol, ethyl alcohol and benzene and easily in ethyl acetate and acetone (Found: C, 68·8; H, 6·5; $C_{18}H_{20}O_5$ requires C, 68·4; H, 6·3%).

The alcoholic mother liquors on concentration and dilution with a small amount of water gave a crystalline solid. It was recrystallised twice from methanol when it was obtained as thin colourless rectangular prisms melting at 88–89° and giving a blue colour with conc. nitric acid. Yield 1·4 g. A mixed melting point with a sample of 2-hydroxy-4 : 6 : 4′-trimethoxy phenyl benzyl ketone (IX *b*)[11] was not depressed.

2 : 4 : 6-*Trihydroxy-3-methyl-4′-methoxy phenyl benzyl ketone* (*XV*)

To a solution of C-methyl phloroglucinol (V *a*)[2] (9 g.) and *p*-methoxy phenyl acetonitrile (10 g.) in dry ether (200 c.c.) was added fused zinc chloride (2 g.) and a stream of dry hydrogen chloride was passed for 5 hours through the ether solution cooled in an ice-bath. On leaving overnight, the ketimine hydrochloride separated as a heavy dark red oil. The ether layer was decanted off and the ketimine hydrochloride washed with dry ether twice and heated with water (150 c.c.) for 2 hours in a boiling water-bath. On cooling to 0°, a yellow solid separated. It was filtered and washed with water. When crystallised from dilute alcohol twice, 2 : 4 : 6-trihydroxy-3-methyl-4′-methoxy phenyl benzyl ketone crystallised as short pale yellow needles melting at 220–21°. Yield 10 g. (Found: C, 66·4; H, 5·5; $C_{16}H_{16}O_5$ requires C, 66·7; H, 5·6%). It was easily soluble in alcohol, acetone and ethyl acetate and insoluble in benzene and petroleum ether. With ferric chloride in alcoholic solution it gave a deep pink colour.

Methylation

The above ketone (1 g.) in dry acetone (100 c.c.) was heated under reflux with dimethyl sulphate (0·7 c.c.) and potassium carbonate (3 g.) for 4 hours. The potassium salts were filtered off and washed with acetone. The residue left after distilling off acetone, crystallised from methanol as colourless rectangular rods melting at 114–15° alone or when mixed with a sample of 2-hydroxy-3-methyl-4 : 6 : 4′-trimethoxy phenyl benzyl ketone (X) described earlier. Yield 0·8 g. The same product was obtained by carrying out the methylation using excess of methyl iodide in place of dimethyl sulphate.

8-Methyl-5 : 7 : 4'-trimethoxy isoflavone (I c)

To pulverised sodium (1 g.) cooled to 0° was added with vigorous stirring, a suspension of 2-hydroxy-3-methyl-4 : 6 : 4'-trimethoxy phenyl benzyl ketone (1 g.) in freshly distilled ethyl formate (20 c.c.) in the course of 30 minutes. The ketone was completely transferred to the reaction flask by washing with some more ethyl formate. The stirring was continued for an hour and the flask was tightly corked and left in the refrigerator. The red viscous liquid slowly set into a colourless solid mass. The cork was removed from time to time to release the pressure developed in the reaction flask. After keeping for 48 hours at 0°, pieces of ice (60 g.) were added and unreacted ethyl formate was distilled off under reduced pressure. The brown solid was filtered after cooling the mixture to 0° and washed with water. It was crystallised from methanol twice when it separated in the form of colourless thick prisms melting at 181–83°. Yield 0·6 g. It did not give any colour with alcoholic ferric chloride. It was sparingly soluble in methanol, ethyl alcohol and benzene and easily in ethyl acetate and acetone (Found: C, 70·0; H, 5·3; $C_{19}H_{18}O_5$ requires C, 69·9; H, 5·5%).

8-Methyl-5 : 7 : 4'-trihydroxy isoflavone (8-Methyl genistein) (I a)

(i) The above trimethyl ether (I *c*) (0·85 g.) was powdered well and suspended in dry benzene (200 c.c.). Well ground anhydrous aluminium chloride (2·5 g.) was added with stirring and the mixture refluxed in a water-bath for 2 hours. The aluminium chloride complex separated as a liquid. Benzene was then distilled off completely. The residual dark viscous complex was cooled and treated with crushed ice (80 g.) and concentrated hydrochloric acid (10 c.c.) and stirred vigorously. The colourless precipitate thus obtained was filtered and washed with water. It was treated with dilute sodium carbonate solution and the undissolved oily matter was separated by filtration. The filtrate was poured into excess of hydrochloric acid. The colourless precipitate was collected and crystallised from dilute alcohol when it separated as pale yellow prisms melting at 231–32°. Yield 0·5 g. The melting point did not rise on further crystallisation. It gave a deep green colour with ferric chloride in alcoholic solution. A solution of the substance in concentrated sulphuric acid became red on the addition of a drop of concentrated nitric acid (Found: C, 65·1; H, 4·7; loss on drying *in vacuo* at 120° for 8 hours, 3·5; $C_{16}H_{12}O_5$, $\frac{1}{2}H_2O$ requires C, 65·5; H, 4·4; H_2O loss, 3·1%); Found in dried sample: C, 67·8; H, 4·6; $C_{16}H_{12}O_5$ requires C, 67·6; H, 4·2%).

(ii) 8-Methyl-5 : 7 : 4'-trimethoxy isoflavone (0·8 g.) was heated with hydriodic acid (20 c.c., *d.*, 1·7) in an oil-bath at 150° for 4 hours. To the

cooled solution was added a saturated aqueous solution of sodium bisulphite (80 c.c.). On leaving overnight, a pale yellow solid separated. It was filtered and washed with water. It was macerated with aqueous sodium carbonate (10%). Almost the whole of it dissolved. A small amount of undissolved matter was filtered and discarded. The filtrate was acidified with hydrochloric acid and the nearly colourless precipitate filtered and washed with water. 8-Methyl-5: 7: 4′-trihydroxy isoflavone crystallised from dilute alcohol in the form of very pale yellow irregular prisms melting at 231-32°. Yield 0·4 g. A mixed melting point with the sample obtained in (i) above was not depressed.

Remethylation

The trihydroxy compound (0·3 g.) obtained by hydriodic acid demethylation of 8-methyl-5: 7: 4′-trimethoxy isoflavone was dissolved in dry acetone (80 c.c.) and heated under reflux with excess of dimethyl sulphate (0·6 c.c.) and potassium carbonate (3 g.) for 30 hours. The solution was separated by filtration from the potassium salts, which were then washed with acetone. The solvent was distilled off from the combined filtrate and the residue treated with water (100 c.c.). After 48 hours, the precipitate was filtered and crystallised from alcohol. It came out as colourless prisms melting at 181–83°. It did not give any colour with alcoholic ferric chloride and a mixture of this substance with a sample of 8-methyl-5: 7: 4′-trimethoxy isoflavone (I *c*) also melted at 181–83°.

8-*Methyl*-5: 7: 4′-*triacetoxy isoflavone*

8-Methyl-5: 7: 4′-trihydroxy isoflavone (0·2 g.) was acetylated with acetic anhydride (5 c.c.) and few drops of dry pyridine at 140°. The triacetate was crystallised twice from acetic acid. It was obtained in the form of colourless plates and prisms melting at 213–15°. It was easily soluble in hot acetic acid (Found: C, 64·0; H, 4·7; $C_{22}H_{18}O_8$ requires C, 64·4; H, 4·4%).

The above acetate (0·12 g.) was deacetylated with a mixture of alcohol (10 c.c.) and concentrated hydrochloric acid (10 c.c.) by heating in a water-bath for 15 minutes. The crystalline precipitate obtained by dilution of the solution with water was recrystallised from dilute alcohol, when it separated as pale yellow prisms melting at 231–32° alone or when mixed with a sample of 8-methyl-5: 7: 4′-trihydroxy isoflavone (8-methyl genistein).

8-*Methyl*-5: 4′-*dihydroxy*-7-*methoxy isoflavone* (8-*Methyl prunetin*) (*VIII b*)

8-Methyl-5: 7: 4′-trimethoxy isoflavone (I *c*) (1·2 g.) was dissolved in acetic anhydride (8 c.c.) and to the cooled solution was added with stirring

hydriodic acid (15 c.c., d., 1·7). The mixture was heated in an oil-bath at 110–15° for 30 minutes. It was then cooled and treated with a saturated aqueous solution of sodium bisulphite (80 c.c.). A dark sticky solid was precipitated and it became greenish yellow solid on keeping overnight. It was filtered, washed with water and thoroughly macerated with aqueous sodium carbonate (10%). Only a very small amount dissolved. The insoluble portion was filtered, washed with water and crystallised from alcohol twice. 8-Methyl prunetin separated as thick yellow rectangular prisms melting at 207–09°. Yield 0·7 g. It gave a brilliant green colour with alcoholic ferric chloride and was soluble in dilute sodium hydroxide (Found: C, 68·9; H, 5·0; $C_{17}H_{14}O_5$ requires C, 68·5; H, 4·7%).

The diacetate prepared by acetylation with acetic anhydride and pyridine, crystallised from alcohol as colourless rods and rectangular prisms melting at 161–62°.

8-*Methyl*-5-*hydroxy*-7 : 4′-*dimethoxy isoflavone* (*I b*)

8-Methyl prunetin (VIII *b*) (0·3 g.) in dry acetone (100 c.c.) was treated with dimethyl sulphate (0·11 c.c., 1 mole) and ignited potassium carbonate (1·5 g.) and the mixture refluxed for 4 hours. The acetone solution was then filtered off and the solvent distilled off from the filtrate. The residue was treated with water and the precipitate filtered after 24 hours. When crystallised from alcohol twice, 8-methyl-5-hydroxy-7 : 4′-dimethoxy isoflavone was obtained as clusters of colourless needles melting at 164–66°. It gave a deep green colour with alcoholic ferric chloride (Found: C, 68·8; H, 5·1; $C_{18}H_{16}O_5$ requires C, 69·2; H, 5·1%).

SUMMARY

Okano and Beppu isolated a new isoflavone from soya bean and from its degradation reactions, they assigned to it the constitution of 8-methyl genistein. A synthesis of this compound starting from C-methyl phloroglucinol α-monomethyl ether was also reported by Shriner and Hull.

An easier method of synthesis of a compound of this structure has now been described, in which the main stage is the nuclear methylation of 2 : 4 : 6-trihydroxy-4′-methoxy phenyl benzyl ketone. The constitution of the product of this reaction is established by an independent synthesis from C-methyl phloroglucinol. Though this synthesis of 8-methyl genistein involves demethylation of its trimethyl ether with hydriodic acid, the absence of any rearrangement during this step has been established particularly by remethylation. The products of the present synthesis are found to be very different from the natural compound and its derivatives described by Okano

and Beppu and therefore it is considered that the natural compound cannot have the constitution assigned to it.

Partial demethylation of 8-methyl genistein trimethyl ether with hydriodic acid gives rise to 8-methyl prunetin which on partial methylation yields 8-methyl-5-hydroxy-7 : 4'-dimethoxy isoflavone. This is different from the C-monomethyl-O-dimethyl genistein obtained by Perkin and Horsfall by nuclear methylation of genistein. Therefore the product of Perkin and Horsfall may have the methyl group in the 6-position as suggested by earlier workers.

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