SYNTHETIC EXPERIMENTS IN THE BENZOPYRONE SERIES

Part XXIV. A Synthesis of Isogenistein

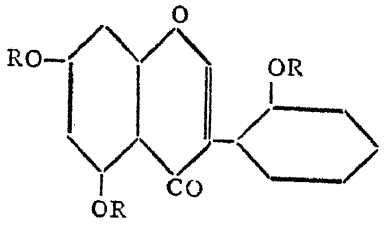
By T. R. Seshadri, F.A.Sc. and S. Varadarajan

(*From the Department of Chemistry, University of Delhi, Delhi*)

Received November 13, 1952

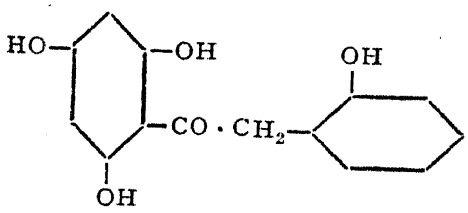
Among the benzopyrone derivatives containing the C_{15} skeleton, compounds having a hydroxyl group in the 2′-position are rare. Two such are the flavonols datiscetin[1] and morin.[2] As belonging to this type among isoflavones have been reported two substances isogenistein and 8-methyl isogenistein, isolated from soya beans.[3]

Isogenistein occurs as its glucoside isogenistin from which it is obtained by acid hydrolysis. Analytical values and molecular weight determination indicated that it had the formula $C_{15}H_{10}O_5$. It yielded a triacetate and a dimethyl ether. Degradation of isogenistein (I *a*) with alkali gave formic acid and 2:4:6:2′-tetrahydroxy phenyl benzyl ketone (II) which could be further degraded into phloroglucinol and *o*-hydroxy phenyl acetic acid (III). Based on these reactions, Okano and Beppu[3] assigned it the constitution of 5:7:2′-trihydroxy isoflavone (I *a*).

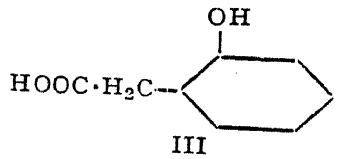


I

a, R = H
b, R = CH_3



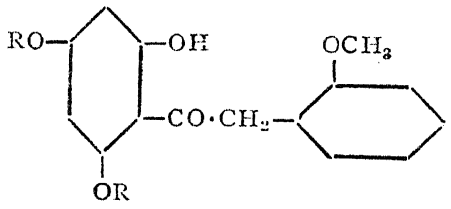
II



III

A synthesis of isogenistein has now been undertaken and it follows the same lines as the synthesis of genistein by Narasimhachari and Seshadri.[4] The starting point for the present work is 2:4:6-trihydroxy-2′-methoxy phenyl benzyl ketone (IV *a*) which has been made by the Hoesch condensation of phloroglucinol with *o*-methoxy phenyl acetonitrile. This nitrile was originally made by Pschorr, Wolfes and Buckow[5] from the difficultly accessible *o*-methoxy benzyl chloride by treatment with potassium cyanide. More

recently it has been prepared by the azlactone method[6] from *o*-methoxy benzaldehyde by Bergel, Haworth, Morrison and Rinderknecht[7] and this has been adopted for the present work. The above authors did not isolate and characterise the intermediate *o*-methoxy phenyl pyruvic acid and its oxime. It is found that the pyruvic acid is a liquid. The oxime is obtained as a solid but attempts to crystallise it yield a product melting at 69–70°. The melting point and the analytical values prove it to be identical with *o*-methoxy phenyl acetonitrile, showing that the oxime is very unstable and loses water and carbon dioxide even during crystallisation.



IV

a, R=H
b, R=CH_3

On methylation with two moles of dimethyl sulphate in acetone medium, the trihydroxy ketone (IV *a*) yields 2-hydroxy-4 : 6: 2′-trimethoxy phenyl benzyl ketone (IV *b*). It gives a blue colour with concentrated nitric acid and exhibits a wine red ferric reaction. These properties are in agreement with its structure. Condensation of the ketone (IV *b*) with ethyl formate and sodium[8] gives rise to a good yield of 5: 7: 2′-trimethoxy isoflavone (I *b*). It crystallises with one molecule of water which is very tenaciously held and is not lost even on drying at 120° *in vacuo* for 2 hours.* It melts at 180–81°. Okano and Beppu[3] did not prepare the trimethyl ether of the natural compound and no comparison is therefore possible.

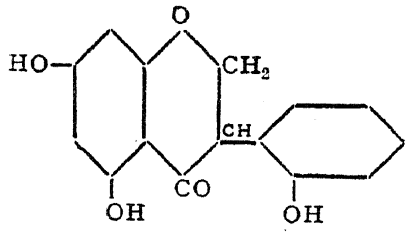
Demethylation of the trimethyl ether (I *b*) offered considerable difficulty. On boiling with acetic anhydride and hydriodic acid, it yields a resinous product from which a very small amount of crystalline compound melting at 222–23° could be isolated. This is soluble in aqueous sodium carbonate and gives an initial pink colour with alcoholic ferric chloride which changes to pale brown. These properties indicate that it is 5: 7: 2′-trihydroxy isoflavone (I *a*). Repetition of the experiment using larger amounts of acetic anhydride and hydriodic acid does not bring about any change in the results. Some complex changes besides demethylation seem to be taking place to a certain extent.

The alternative method of demethylation using aluminium chloride in benzene solution[9] has been next attempted. Here also due to the very low

* It has subsequently been found that the substance is not a hydrate of 5; 7; 2′-trimethoxy isoflavone, but is actually 2-hydroxy-5; 7; 2′-trimethoxy-isoflavanone.

solubility of the trimethyl ether (I *b*) in benzene, at first there was not much success. However, by employing a very large quantity of the hot solvent and by adding aluminium chloride to a constantly stirred mixture of the compound and the solvent and then refluxing for 2 hours, the demethylation has been successfully carried out. The product has been purified by dissolution in aqueous sodium carbonate. It also melts at 222–23° and is in every way identical with the product obtained by demethylation of the trimethyl ether (I *b*) with hydriodic acid. The identity of the products obtained by the two methods therefore definitely establishes that they are both 5 : 7 : 2′-trihydroxy isoflavone (I *a*). Okano and Beppu[3] however recorded the melting point of the natural compound as 302° and the ferric reaction to be violet. These differences definitely establish that the natural substance of Okano and Beppu cannot be 5 : 7 : 2′-trihydroxy isoflavone.

It is possible that the naturally occurring substance is an isoflavanone (V) which would account for the formation of 2 : 4 : 6 : 2′-tetrahydroxy phenyl benzyl ketone (II) by alkali degradation (see Part XXIII[10]). To test this possibility, the tetrahydroxy ketone (II) has now been synthetically obtained by the demethylation of 2-hydroxy-4 : 6 : 2′-trimethoxy phenyl benzyl ketone (IV *b*) with aluminium chloride in benzene solution. It is found to melt at 217–20° whereas the tetrahydroxy ketone obtained by Okano and Beppu[3] has been reported to melt at 182–83°.



V

In view of these differences, it can be said that the natural compound is not a simple isoflavone or isoflavanone but it is of a different nature.

EXPERIMENTAL

o-*Methoxy phenyl acetonitrile*

The starting material for this preparation, *viz.*, *o*-methoxy benzaldehyde was made according to the method of Dickinson and Marshall,[11] who reported its boiling point to be 242–45°. It is now found to distil at 236–37° as described by Shoesmith and Connor.[12] 2-Phenyl-4-(*o*-methoxy benzal)-oxazolone was prepared earlier from this aldehyde by Mauthner[13] who gave the melting point of the oxazolone as 165–66°. More recently Bergel *et al.*[7] have reported its melting point to be 156–57°. It has now been prepared by

heating together *o*-methoxy benzaldehyde (30 g.), hippuric acid (45 g.), fused sodium acetate (15 g.) and acetic anhydride (75 c.c.). It crystallised from alcohol as bright yellow prisms melting at 165–66°. Yield 42 g. It was hydrolysed by boiling with sodium hydroxide solution (22 g. in 130 c.c. of water) for an hour and *o*-methoxy phenyl pyruvic acid was separated from benzoic acid through its bisulphite compound. Yield 14 g. The pyruvic acid was a heavy yellow oil and it was converted into its oxime by heating with aqueous sodium hydroxide (150 c.c. of 8%). Attempts to crystallise the oxime from dilute alcohol yielded *o*-methoxy phenyl acetonitrile as thin colourless rectangular prisms melting at 69–70° alone or when mixed with an authentic sample of the nitrile, prepared according to the method of Bergel *et al.*,[7] by heating the oxime with acetic anhydride (Found: C, 73·1; H, 6·3; C_9H_9ON requires C, 73·5; H, 6·1%).

2:4:6-*Trihydroxy-2'-methoxy phenyl benzyl ketone* (*IV a*)

To a solution of dry phloroglucinol (12 g.) and *o*-methoxy phenyl acetonitrile (12 g.) in dry ether (150 c.c.) was added powdered, fused zinc chloride (2 g.). The mixture was cooled to 0° and a stream of dry hydrogen chloride passed in for 5 hours. On leaving overnight at 0°, the ketimine hydrochloride separated as a dark red oil. The upper layer of ether was decanted off. The ketimine hydrochloride was washed twice with dry ether and heated with water (150 c.c.) on a boiling water-bath for 4 hours. On cooling, a pale yellow solid separated. It was filtered and crystallised from dilute alcohol. 2:4:6-Trihydroxy-2'-methoxy phenyl benzyl ketone was obtained as pale yellow prisms melting at 168–70°. Yield 11·5 g. It gave a deep pink ferric reaction in alcoholic solution and dissolved in dilute sodium carbonate to give a pale yellow solution (Found: C, 61·9; H, 5·6; loss on drying at 120° for 2 hours *in vacuo*, 5·9; $C_{15}H_{14}O_5$, H_2O requires C, 61·7; H, 5·5; loss of H_2O, 6·2%).

2-*Hydroxy*-4:6:2'-*trimethoxy phenyl benzyl ketone* (*IV b*)

The above trihydroxy ketone (2 g.) in dry acetone (150 c.c.) was treated with dimethyl sulphate (1·4 c.c., 2 moles) and potassium carbonate (7 g.) and the mixture refluxed for four hours. The potassium salts were filtered and washed with warm acetone. The solvent was distilled off from the filtrate and the residue crystallised from alcohol twice. 2-Hydroxy-4:6:2'-trimethoxy phenyl benzyl ketone separated as sheaves of thin colourless plates melting at 116–18°. Yield 1·45 g. It gave a wine red colour with ferric chloride in alcoholic solution. It was insoluble in aqueous sodium hydroxide (10%). It gave a blue colour with concentrated nitric acid (Found: C, 67·8; H, 6·3; $C_{17}H_{18}O_5$ requires C, 67·5; H, 6·0%).

*5:7:2′-Trimethoxy isoflavone (I b)**

A suspension of the above ketone (1·5 g.) in ethyl formate (25 c.c.) was treated with powdered sodium (1 g.) at 0° and the mixture left in the refrigerator for 48 hours. Ice and hydrochloric acid (10 c.c.) were then added and the excess of ethyl formate distilled under reduced pressure. The precipitate was collected and crystallised twice from alcohol, when 5:7:2′-trimethoxy isoflavone came out as colourless rectangular rods melting at 180–81°. Yield 1·1 g. It was sparingly soluble in alcohol, benzene and ethyl acetate. It did not give any colour with alcoholic ferric chloride. No blue or green colour was produced when concentrated nitric acid was added to the substance (Found in a sample dried at 110° *in vacuo*: C, 65·9; H, 5·9; $C_{18}H_{16}O_5$, H_2O requires C, 65·5; H, 5·5%).

5:7:2′-Trihydroxy isoflavone (Isogenistein) (I a)

(i) To a suspension of well powdered 5:7:2′-trimethoxy isoflavone (1 g.) in hot benzene (200 c.c.), powdered anhydrous aluminium chloride (4·3 g.) was added in small lots with constant shaking. The complex began to separate as a greenish yellow solid. The mixture was refluxed on a water-bath for 4 hours. Benzene was then distilled off and the residue cooled and treated with ice and hydrochloric acid (10 c.c.). On leaving overnight, a colourless solid separated. It was filtered and treated with aqueous sodium carbonate. Undissolved impurities were filtered off and the filtrate on acidification yielded 5:7:2′-trihydroxy isoflavone. When crystallised thrice from dilute alcohol, it was obtained as short pale yellow needles sintering at 183° and melting at 222–23°. Yield 0·4 g. It gave a deep pink colour with alcoholic ferric chloride which changed to pale brown on the addition of excess of the reagent (Found: C, 64·8; H, 4·2; loss on drying at 120° in vacuum for 6 hours, 3·7; $C_{15}H_{10}O_5$, $\frac{1}{2}H_2O$ requires C, 64·5; H, 3·9; loss of H_2O, 3·2%).

(ii) To a solution of 5:7:2′-trimethoxy isoflavone (1 g.) in acetic anhydride (25 c.c.), hydriodic acid (37 c.c., d. 1·7) was added cautiously and the mixture heated at 140–50° in an oil-bath for 2 hours. It was then cooled and treated with an aqueous solution of sodium bisulphite (120 c.c.). After 48 hours, the dark brown solid was filtered and purified by dissolution in aqueous sodium carbonate (10%). The trihydroxy isoflavone crystallised from dilute alcohol (charcoal) as short pale yellow needles sintering at 183° and melting at 222–23° alone or when mixed with a sample obtained in (i) above. Yield 70 mg.

---

* Please see footnote in an earlier page.

2:4:6:2′-*Tetrahydroxy phenyl benzyl ketone* (*II*)

To a solution of 2-hydroxy-4:6:2′-trimethoxy phenyl benzyl ketone (1 g.) in dry benzene (25 c.c.) was added anhydrous aluminium chloride (3 g.). The mixture was refluxed for 2 hours. The residue left on distilling off benzene was treated with ice and hydrochloric acid and the tetrahydroxy ketone so obtained was purified by dissolution in aqueous sodium carbonate. It was crystallised twice from alcohol and then twice from a mixture of ethyl acetate and benzene. It separated as short colourless needles melting at 217–20° (decomp.). Yield 0·3 g. It was easily soluble in alcohol and ethyl acetate and sparingly soluble in benzene and petroleum ether (Found in a sample dried at 120° *in vacuo* for 6 hours: C, 65·0; H, 4·9; $C_{14}H_{12}O_5$ requires C, 64·6; H, 4·6%).

Summary

One of the substances occurring in soya beans has been considered by Okano and Beppu to be 5:7:2′-trihydroxy isoflavone (isogenistein), based on degradation studies. A compound of this structure has now been made starting from 2:4:6-trihydroxy-2′-methoxy phenyl benzyl ketone. Partial methylation, treatment with ethyl formate and sodium followed by demethylation yield the required isoflavone. Aluminium chloride in benzene solution is found to be a much better reagent than hydriodic acid for the above demethylation. The products of the present synthesis are found to be different from the natural compound and its derivatives. Therefore the natural compound cannot be an isoflavone of this constitution. The main degradation product, considered to be 2:4:6:2′-tetrahydroxy phenyl benzyl ketone by Okano and Beppu differs considerably from a synthetic product of this constitution. Hence the natural compound cannot be a simple isoflavone or isoflavanone and should have a different structure.

References

1. Kalff and Robinson .. *J.C.S.*, 1925, 1968.
2. Robinson and Venkataraman .. *Ibid.*, 1929, 61.
3. Okano and Beppu .. *J. Agri. Chem. Soc. Japan*, 1939, **15**, 645.
4. Narasimhachari and Seshadri .. *Proc. Ind. Acad. Sci.*, 1950, 32A, 256.
5. Pschorr, Wolfes and Buckow .. *Ber.*, 1900, **33**, 166.
6. Baker and Robinson .. *J.C.S.*, 1929, 357.
7. Bergel, Haworth, Morrison and Rinderknecht .. *Ibid.*, 1944, 263.
8. Venkataraman *et al.* .. *Ibid.*, 1934, 513 and 1770.
9. Seshadri *et al.* .. *Proc. Ind. Acad. Sci.*, 1947, **24A**, 213; 1947, **25A**, 432; 1949, **29A**, 72; 1952, **35A**, 34, 82 and 202.
10. Seshadri and Varadarajan .. *Ibid.*, 1953, **37A**, 508.
11. Dickinson and Marshall .. *J.C.S.*, 1929, 1496.
12. Shoesmith and Connor .. *Ibid.*, 1929, 2231.
13. Mauthner .. *Ann.*, 1909, **370**, 374.