

ISO-FLAVONES OF SOYABEANS

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SOYABEANS were shown by Walz¹ to contain genistein (5 : 7 : 4'-trihydroxy isoflavone) and daidzein (7 : 4'-dihydroxy isoflavone). Later Okano and Beppu² claimed to have isolated 5 : 7 : 2'-trihydroxy isoflavone ("iso-genistein") and 5 : 7 : 4'-trihydroxy-8-methyl isoflavone ("methyl genistein") and 5 : 7 : 2'-trihydroxy-8-methyl-isoflavone ("methyl-iso-genistein"). Compounds of these constitutions have recently been synthesised by Seshadri and Varadarajan³ and have been found to differ from the natural products in melting points and colour reactions with alcoholic ferric chloride. The derivatives and degradation products also are found to be different. The work of Baker *et al.*,⁴ Karmarkar *et al.*,⁵ and of Whalley⁶ have independently led to the same conclusion. Baker *et al.*⁴ have made the suggestion that the samples reported as methyl genistein, methyl iso-genistein and iso-genistein by Okano and Beppu were all genistein of varying degrees of purity. This was considered to be more likely because the elementary analysis is not inconsistent with this suggestion and some of the supposed hydrolysis products were not satisfactorily characterised. One significant point may be emphasised here with regard to the claims of Okano and Beppu of having isolated 8-methyl genistein and 8-methyl isogenistein. It is now established that all 6 or 8 C-methyl chromone derivatives⁷ give a characteristic deep green colour with alcoholic ferric chloride while those without the C-methyl group give a violet or brown colour. Synthetic 8-methyl genistein and 8-methyl isogenistein give deep green colour whereas the samples of Okano and Beppu are reported to give violet red or brown colour.

Baker *et al.*⁴ have further suggested that the product "tatoin", supposed to be 5 : 4'-dihydroxy-8-methyl isoflavone which was isolated by Okano and Beppu from Soyabeans and by Bhandari, Bose and Siddiqui⁸ from Soybean germs may prove to be identical with daidzein (7 : 4'-dihydroxy isoflavone), because the melting points of dimethyl daidzein is 162-63°^{9,10,11} the same as that recorded by Okano and Beppu for the so-called dimethyl tatoin (165°) and the melting point of the diacetate of daidzein (182°) is also the same as the melting point of diacetate of tatoin (185°) recorded by Okano and Beppu. Earlier Walz¹ had recorded the lower m.p. of 153° for dimethyl daidzein and this might have given room

for the claims of Okano and Beppu. This m.p. has been corrected later as mentioned above.

In view of the marked differences noted by Seshadri and Varadarajan³ between the synthetic products and the natural ones isolated by Okano and Beppu,² it was considered necessary to attempt their isolation from soyabeans. Work was started in this connection long before the suggestion of Baker for explaining the discrepancy was published. The soyabeans employed in the present investigation were of the yellow variety (Pusa selection) called "Glycine Max", kindly supplied by the Indian Agricultural Research Institute, New Delhi. The ground beans (3 lb.) were extracted first with petroleum ether which removed only oily matter. Subsequently extraction was done with hot ethyl alcohol. The alcoholic extract was distilled under reduced pressure to about 75 c.c. and finally extracted with petroleum ether to remove the remaining oily impurities. It was then repeatedly extracted with ether. The ethereal extract on distillation gave a small quantity of a crude product (A). It was crystallised twice from methyl alcohol when it gave a small quantity of a product (B) melting at 316-17° with decomposition. This had all the properties recorded for "tatoin" by Okano and Beppu.² The aqueous alcoholic residue left after extraction with petroleum ether and ether was boiled with 7 per cent. sulphuric acid for 2 hours. There was no solid product, neither could anything be obtained by ether extraction.

Later extraction of the germs was done adopting the procedure of Siddiqui *et al.*⁸ About 60 lb. of soyabeans were germinated and the germs extracted, and about 300 mg. of a crude product (C) was obtained. It was repeatedly crystallised from methyl alcohol when a small quantity of a crystalline product (D) (50 mg.) was obtained. It melted at 316-17° C., mixed melting with the above sample (B) was undepressed.

At first the crude products (A) and (C) were subjected to circular paper chromatography following the procedure of Krishnamurthy and Seshadri (unpublished work) for isoflavones. The irrigating solvent was alcohol-water (1 : 3) mixture. When the chromatogram was developed with alcoholic ferric chloride it gave a green ring having circular R_f 0.63. This agreed with that of an authentic sample of genistein.

On the other hand when the chromatogram was developed with ammonical silver nitrate another pink ring with R_f 0.9 became visible and this was found to agree with the R_f value given by an authentic sample of daidzein under similar conditions. It therefore appeared that these were mixtures containing genistein and daidzein. Sample (C) which was available in larger quantity was separated by subjecting it to partial methylation using two moles of dimethyl sulphate. Under these conditions the dimethyl ether of daidzein and genistein would be produced and they could be separated by extraction with alkali in which the latter would be soluble by virtue of the free 5-hydroxyl (resistant) it would still possess. Experimental details are given below.

The mixture (230 mg.) was dissolved in anhydrous acetone (50 ml.). Dimethyl sulphate (2 mol.; 0.16 ml.) and anhydrous potassium carbonate (1 g.) were added and the mixture gently refluxed for 8 hours. The solvent was then distilled off and water added to the residue. It was then extracted with ether. The ethereal layer was repeatedly extracted with sodium hydroxide solution (3 per cent.). The ether solution was marked (E) and the alkaline solution (F). The alkaline solution (F) was acidified with hydrochloric acid. It was re-extracted with ether, the ether distilled off and the residue crystallised from a mixture of ethyl acetate and petroleum ether when a colourless product was obtained. It gave a green colour with alcoholic ferric chloride solution, and melted at $233-35^\circ$ with previous sintering at $210-15^\circ$. It appeared to be a mixture containing mono- and dimethyl ethers of genistein. It was therefore completely methylated with excess of dimethylsulphate and potassium carbonate in anhydrous acetone medium and the trimethyl ether finally crystallised from ethyl acetate petroleum ether mix-

ture. It melted at $163-64^\circ$, yield 150 mg. Mixed melting point with an authentic sample of trimethyl genistein was undepressed. The ether solution (E) was distilled and the residue crystallised from a mixture of ethyl acetate petroleum ether when dimethyl daidzein melting at $162-63^\circ$ was obtained; yield 70 mg. Mixed melting point with an authentic sample was undepressed. Approximate composition of the mixture was 70 per cent. genistein and 30 per cent. daidzein.

The fairly sharp melting samples (B) and (D) agreeing with the reported properties of 'tatoin' were finally examined by chromatography. Both of them gave two rings (1) R_f 0.63 with alcoholic ferric chloride, and (2) R_f 0.9 with ammonical silver nitrate. Thus they also proved to be mixture though diadzein appeared to be the major component.

The above experiments definitely show that the soyabeans contain only genistein and daidzein and give strong support to the contention that '8-methyl genistein', 'isogenistein' and '8-methyl-isogenistein' are only genistein of varying degrees of purity, and that 'tatoin' is only daidzein containing as impurity genistein.

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 2. Okano and Beppu, *J. Agri. Chem. Soc. Japan*, 1939, **15**, 645.
 3. Varadarajan and Seshadri, *Proc. Ind. Acad. Sci.*, 1953, **37A**, 145, 508, 514, 526.
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 6. Whalley, *J.A.C.S.*, 1953, **75**, 1059.
 7. Mukerjee, Seshadri and Varadarajan, *Proc. Ind. Acad. Sci.*, 1953, **37A**, 127; Mukerji and Seshadri, *Ibid.*, 1953, **38A**, 207.
 8. Bhandari, Bose and Siddiqui, *Jour. Sci. Ind. Res.*, 1949, **8B**, 217.
 9. Lechner and Dinjaski, *Akad. Wiss. Wien*, 1933, **142**, **2B**, 411.
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SYMPOSIUM ON ELECTROCHEMICAL PROCESSES

WITH the approval of the Council of Scientific and Industrial Research, it has been decided to hold a symposium on "Electrochemical Processes and Their Applications to Indian Industries", in the Central Electrochemical Research Institute, Karaikudi, sometime during the month of February 1954.

Even if the intending participants are not in a position to be present at the symposium, they are requested to forward their papers to the Convener of the Symposium who will arrange for getting them read and discussed at the

symposium. The symposium includes sections on electrothermal processes, metallurgical and non-metallic industries, electric furnace technology, electrolytic processes, inorganic and organic chemical industries, electric batteries, electrodeposition and allied processes, electric discharges and their application, electroanalyses and applications. It is open to the contributors to deal with the fundamental and theoretical or applied aspects of any of the subjects which generally fall within the scope of the above sections.