382. *A New Synthesis of* iso*Flavones. Part II.** 5 : 7 : 2′-*Trihydroxy*iso*flavone.*

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5 : 7 : 2′-Trihydroxy*iso*flavone (I) has been synthesised from 2-methoxybenzyl 2 : 4 : 6-trihydroxyphenyl ketone (II; R = Me) by the ethoxalylation process described in the preceding paper. It is not identical with the " *iso*genistein " isolated by Okano and Beppu from soya bean. The intermediate 2-carbethoxy*iso*flavone is converted by hydrobromic acid into 5′ : 7′-dihydroxychromono(2′ : 3′-3 : 4)coumarin (VI) which contains the greater part of the pentacyclic nucleus of rotenone. Evidence is presented which very strongly suggests that the " *iso*genistein," " methylgenistein," and " methyl-*iso*genistein," which Okano and Beppu claim to have isolated from soya bean, are all genistein, and that the same authors' " tatoin," isolated from the same source, is daidzein.

Okano and Beppu (*J. Agric. Chem. Soc. Japan*, 1939, **15**, 645) isolated from soya bean a glycoside, which when hydrolysed gave a pale yellow, phenolic substance, $C_{15}H_{10}O_5$, m. p. 302°, giving a triacetate, m. p. 189°, and a dimethyl ether, m. p. 120—125°. This aglycone was stated to be 5 : 7 : 2′-trihydroxy*iso*flavone (I), because alkaline hydrolysis gave phloroglucinol, formic acid, *o*-hydroxyphenylacetic acid, and 2-hydroxybenzyl 2 : 4 : 6-trihydroxyphenyl ketone (II; R = H). Because of its supposed structural similarity to genistein (5 : 7 : 4′-trihydroxy*iso*flavone) it was named " *iso*genistein."

We have now synthesised 5 : 7 : 2′-trihydroxy*iso*flavone (I), and find that it is colourless, and is dimorphic, having m. p.s 187° and 222—223°; the higher-melting form is the more stable. The triacetyl derivative has m. p. 132—134°, and the dimethyl ether has m. p. 160°. It is thus very different from " *iso*genistein," a conclusion which we reported earlier (*Chem. and Ind.*, 1952, 1058) and which has independently been reached by Dr. S. Varadarajan and Professor T. R. Seshadri (personal communication, 28th November, 1952), by Karmarkar, Shah, and Venkataraman (*Proc. Indian Acad. Sci.*, 1952, **36**, *A*, 552), and by Dr. W. B. Whalley (personal communication, 2nd February, 1953).

The preparation of (I) was achieved by means of the new synthesis described in Part I. The benzyl phenyl ketone (II; R = H) could not be obtained from phloroglucinol and 2-hydroxybenzyl cyanide by the Hoesch reaction, probably because the cyanide formed a non-reactive cyclic imine hydrochloride (III) in presence of hydrogen chloride. 2-Methoxybenzyl cyanide was therefore condensed with phloroglucinol by the Hoesch reaction, giving 2-methoxybenzyl 2 : 4 : 6-trihydroxyphenyl ketone (II; R = Me), and this was then treated with ethoxalyl chloride in pyridine to give 2-carbethoxy-5 : 7-dihydroxy-2′-methoxy*iso*flavone (IV). Alkaline hydrolysis and decarboxylation then yielded 5 : 7-dihydroxy-2′-methoxy*iso*flavone (V), and final demethylation of (V) gave 5 : 7 : 2′-trihydroxy*iso*flavone (I). Monomethylation of (V) gave 5-hydroxy-7 : 2′-dimethoxy*iso*flavone.

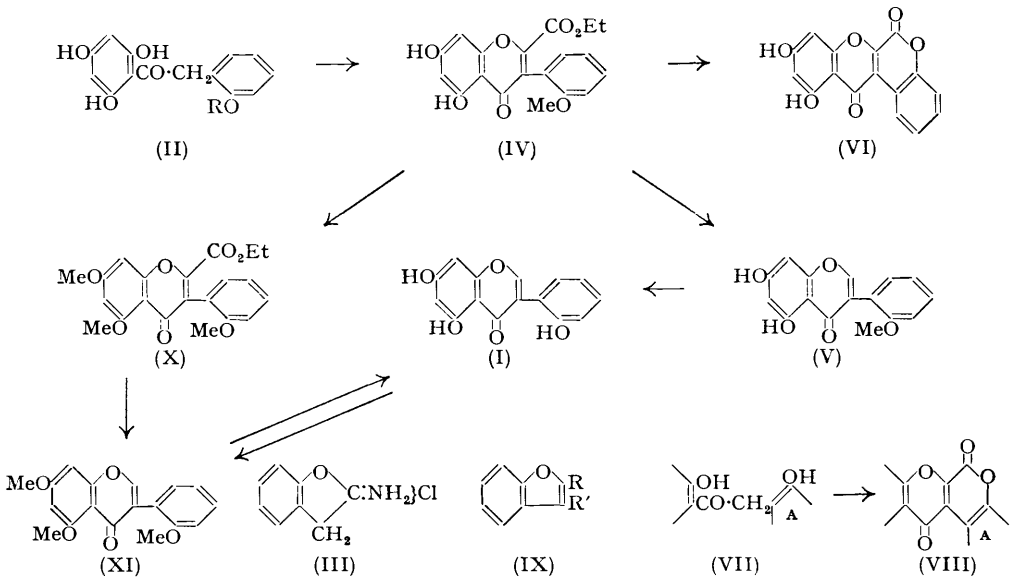
The action of hydrobromic acid–acetic acid upon 2-carbethoxy-5 : 7-dihydroxy-2′-methoxy*iso*flavone (IV) caused hydrolysis of the ester group, demethylation, and lactonis-

* Part I, preceding paper.

ation, giving the bright yellow lactone (VI) of 2-carboxy-5 : 7 : 2′-trihydroxy*iso*flavone [5′ : 7′-dihydroxychromono(2′ : 3′-3 : 4)coumarin]. This substance is of interest in connection with the problem of the synthesis of compounds of the rotenone type, as it contains four of the five fused rings found in rotenonone, the non-degraded oxidation product of rotenone (Butenandt, *Annalen*, 1928, **464**, 257).

After our work was completed we found that a partial synthesis involving ethoxalylation, and formation of an *iso*flavone nucleus and a chromono(2′ : 3′-3 : 4)coumarin had been previously carried out. La Forge (*J. Amer. Chem. Soc.*, 1932, **54**, 3377) treated derritol, partial formula (VII), with ethoxalyl chloride in pyridine at room temperature for 48 hours, and obtained rotenonone, partial formula (VIII). In the light of the present work this unexpected result clearly involved *C*-ethoxalylation, cyclisation to the 2-carbethoxy-*iso*flavone derivative, and interaction of the ester group with the phenolic group of nucleus A.

The demethylation of 5 : 7-dihydroxy-2′-methoxy*iso*flavone (V) was effected by hydrobromic acid in acetic acid and by aluminium chloride in benzene. The latter was used in order to avoid the possibility of hydrolysis of the heterocyclic ring and subsequent closure to give a benzofuran, either 3-(2 : 4 : 6-trihydroxybenzoyl)benzofuran (IX; R = H, R′ = 2 : 4 : 6-trihydroxybenzoyl), or 3-formyl-2-(2 : 4 : 6-trihydroxyphenyl)benzofuran



(IX; R = 2 : 4 : 6-trihydroxyphenyl, R′ = CHO). Such a change occurs when 2′-methoxyflavones are demethylated with hydrogen iodide (Philbin and Wheeler, *Chem. and Ind.*, 1952, 449), and Dr. R. Winter (Dissertation, Bristol, 1950) has found that 5 : 7 : 8-trihydroxy-2-methyl*iso*flavone is converted into 5 : 6 : 7-trihydroxy-2-methyl*iso*flavone by prolonged treatment with hydrobromic acid. In view of the difference between our final product and the " *iso*genistein " described by Okano and Beppu, it was desirable to obtain additional proof that no fundamental change had occurred during demethylation. Hence the intermediate compound 2-carbethoxy-5 : 7-dihydroxy-2′-methoxy*iso*flavone (IV) was methylated to 2-carbethoxy-5 : 7 : 2′-trimethoxy*iso*flavone (X), then hydrolysed and decarboxylated to 5 : 7 : 2′-trimethoxy*iso*flavone (XI), and this substance was identical with the trimethyl ether of 5 : 7 : 2′-trihydroxy*iso*flavone (I), thus confirming the structure of the latter. A further proof of the *iso*flavone structure of (I) is that the ultra-violet absorption spectra curves of genistein and of (I) are almost identical (see Experimental section). Partial demethylation of 5 : 7 : 2′-trimethoxy*iso*flavone (XI) with hydrobromic acid gave 5 : 2′-dihydroxy-7-methoxy*iso*flavone.

5 : 7 : 2′-Trihydroxy*iso*flavone is œstrogenic, and is active in mice at a dose of 4 mg.;

it therefore possesses about one-quarter of the activity of the isomeric 5 : 7 : 4′-trihydroxy-*iso*flavone (genistein) (Bradbury and White, *J.*, 1951, 3447). We are indebted to Dr. R. B. Bradbury of the Commonwealth Scientific and Industrial Research Organisation and Dr. D. H. Curnow of the University of Western Australia for these tests.

We suggest that the name " *iso*genistein " of the material, m. p. 302°, isolated by Okano and Beppu from soya bean should lapse, and as there are many possible isomers of genistein there is no adequate reason for transferring it to 5 : 7 : 2′-trihydroxy*iso*flavone.

The iso*Flavones of Soya Bean.*—Soya beans have been proved to contain genistein and daidzein (7 : 4′-dihydroxy*iso*flavone) (Walz, *Annalen*, 1931, **489**, 118), and in addition Okano and Beppu (*loc. cit.*) claim to have isolated 5 : 7 : 2′-trihydroxy*iso*flavone (" *iso*genistein "), and 5 : 7 : 4′- (" methylgenistein ") and 5 : 7 : 2′-trihydroxy-8-methyl*iso*flavone (" methyl*iso*genistein "). The present paper shows that " *iso*genistein " is not (I), and, although Shriner and Hull (*J. Org. Chem.*, 1945, **10**, 228) claim to have synthesized " methylgenistein " the work of W. B. Whalley (paper presented at the 122nd Meeting of the American Chemical Society, September 14—19, 1952) has shown that these two *C*-methyl*iso*flavones are not identical with the materials isolated by Okano and Beppu from soya bean.

It has now been observed that there is a strong resemblance between the physical properties of the three products isolated by Okano and Beppu and those of genistein as shown in the Table. Indeed, with the exception of the curiously high melting point of

	M. p.	Triacetyl derivative	Dimethyl ether	Trimethyl ether	Glycoside
Genistein	300°	195—198°	140—142°	162—163°	256°
" Methylgenistein "	298	184	125—134	154—155	—
" Methyl*iso*genistein "	301—302	188	242 (142 ?)	152—153	255
" *iso*Genistein "	302	189	120—125	—	265

242° given for " methyl*iso*genistein dimethyl ether " which appears likely to be an error for 142°, the agreement is so remarkable as to render it almost certain that " methylgenistein," " methyl*iso*genistein," and " *iso*genistein " were all genistein of slightly varying degrees of purity. This seems the more likely, because some of the supposed hydrolysis products were not satisfactorily characterised and the elementary analyses are consistent with this suggestion.

A further product " tatoin," supposed to be 5 : 4′-dihydroxy-8-methyl*iso*flavone, has been isolated from soya bean by Okano and Beppu (*loc. cit.*), and by Bhandari, Bose, and Siddiqui (*J. Soc. Ind. Res., India*, 1949, **8B**, 217). The structure has not yet been proved by synthesis, and the annexed comparison suggests that " tatoin " may prove to be identical with daidzein (7 : 4′-dihydroxy*iso*flavone). The recorded analyses of " tatoin " and its derivatives agree equally well with the formulæ $C_{15}H_{10}O_4$ and $C_{16}H_{12}O_4$ for the parent compound.

	M. p.	Diacetyl derivative, m. p.	Monomethyl ether, m. p.	Dimethyl ether, m. p.
" Tatoin " [a]	318°	185°	160—163° [e]	165
,, [b]	316—317	185	—	—
Daidzein	315—320 [c]	182 [c]	7- 218—220 [d]	153 [c]
			4′- 255—257 [d]	

[a] Okano and Beppu (*loc. cit.*). [b] Bhandari, Bose, and Siddiqui (*loc. cit.*). [c] Walz (*loc. cit.*). [d] Agkoramurthy, Narasimhachari, and Seshadri (*Proc. Indian Acad. Sci.*, 1951, **33**, *A*, 257). [e] Tatoin monomethyl ether, prepared by monomethylation of " tatoin," with diazomethane, is likely to be a mixture, and a mixture of the two monomethyl ethers of daidzein might well melt as low as 160—163°.

Experimental

Analyses are by Mr. B. S. Noyes, Bristol.

2-*Methoxybenzyl Cyanide.*—This was prepared from *o*-methoxybenzaldehyde *via* the azlactone by Bergel, Haworth, Morrison, and Rinderknecht (*J.*, 1944, 263), but the method was only very briefly described, and only the azlactone intermediate was characterised.

o-Methoxybenzaldehyde (80 g.), hippuric acid (120 g.), anhydrous sodium acetate (40 g.), and acetic anhydride (220 c.c.) were heated on a steam-bath for ½ hr. and cooled. Alcohol

(50 c.c.) was added and the azlactone collected, and washed with alcohol and then with hot water (yield, 124 g., 75%). The azlactone (50 g.) was boiled for 1 hr. with sodium hydroxide (25 g.) in water (150 c.c.), and the cooled solution diluted with water (150 c.c.) and saturated with sulphur dioxide. The filtered solution, now free from benzoic acid, was heated for 1 hr. on a steam-bath in an open basin with concentrated hydrochloric acid (75 c.c.), and cooled. o-*Methoxyphenylpyruvic acid* was collected (17 g., 50%), and crystallised from benzene in rhombic plates, m. p. 157° (Found : C, 62·5; H, 5·2. $C_{10}H_{10}O_4$ requires C, 61·8; H, 5·2%). It gave a red solution in concentrated sulphuric acid. The crude acid (15 g.) was added to a warm solution of hydroxylamine hydrochloride (12 g.) in 8% aqueous sodium hydroxide, the mixture acidified after 24 hr., and the *oxime* (15 g., 92%) collected. It separated from ethyl acetate in prisms, m. p. 157° with evolution of carbon dioxide (Found : N, 6·9. $C_{10}H_{11}O_4N$ requires N, 6·7%). The oxime (20 g.) was warmed with acetic anhydride (20 c.c.) until evolution of carbon dioxide ceased (20 min.), then poured into water, and the 2-methoxybenzyl cyanide (14 g., 98%) crystallised from methanol, giving rhombic plates, m. p. 69·5° (Found : C, 73·1; H, 6·3; N, 9·8. Calc. for C_9H_9ON : C, 73·5; H, 6·2; N, 9·5%).

2-*Methoxybenzyl* 2 : 4 : 6-*Trihydroxyphenyl Ketone* (II; R = Me).—A solution of 2-methoxybenzyl cyanide (5·1 g.) and phloroglucinol (10 g.) in dry ether (150 c.c.) was saturated with hydrogen chloride at 0° in presence of powdered zinc chloride (0·5 g.) and kept at 0° for 6 days. After addition of dry ether, the liquid layer was decanted, and the solid ketimine hydrolysed with water (500 c.c.) (steam-bath, 2 hr.). 2-*Methoxybenzyl* 2 : 4 : 6-*trihydroxyphenyl ketone* separated from aqueous ethanol as needles (7·1 g., 74%), m. p. 167—169° (Found : C, 65·9; H, 5·1; OMe, 11·5. $C_{14}H_{11}O_4$·OMe requires C, 65·7; H, 5·1; OMe, 11·3%). Its alcoholic solution gave a purple colour with a trace of ferric chloride.

2-*Carbethoxy*-5 : 7-*dihydroxy*-2′-*methoxy*iso*flavone* (IV)—Ethoxalyl chloride (60 c.c.) was added at 0° with shaking to a solution of 2-methoxybenzyl 2 : 4 : 6-trihydroxyphenyl ketone (37 g.) in pyridine (250 c.c.), and after 24 hr. the mixture was poured into water and extracted with chloroform (3 × 500 c.c.), and the organic layer washed with dilute hydrochloric acid (4 × 200 c.c.), dried ($MgSO_4$), and evaporated. The solid was crystallised from aqueous methanol, giving 2-*carbethoxy*-5 : 7-*dihydroxy*-2′-*methoxy*iso*flavone* as deep yellow needles (40 g., 84%), m. p. 154—156° (Found : C, 63·9; H, 4·6. $C_{19}H_{16}O_7$ requires C, 64·0; H, 4·5%). It gave an intense red-brown ferric chloride colour. The *diacetyl* derivative separated from ethanol in colourless prisms, m. p. 139—140° [Found : C, 62·7; H, 4·8; (OMe) (OEt), 17·7. $C_{20}H_{12}O_7$(OMe)(OEt) requires C, 62·7; H, 4·6; (OMe)(OEt), 17·3%].

5 : 7-*Dihydroxy*-2′-*methoxy*iso*flavone* (V).—The preceding ester (40 g.) in acetone (400 c.c.) was heated at 100° for 4 hr. with 5% aqueous sodium carbonate (400 c.c.). Evaporation of the solvent and acidification gave 5 : 7-dihydroxy-2′-methoxy*iso*flavone-2-carboxylic acid, which, after drying at 110°, formed a yellow powder (32 g., 87%), giving a reddish-brown ferric chloride reaction.

This acid (370 mg.) was decarboxylated in small portions at *ca.* 290° for 2—3 min., and the crude melt crystallised from aqueous ethanol, giving 5 : 7-*dihydroxy*-2′-*methoxy*iso*flavone* (V) as colourless needles (250 mg., 78%), m. p. 195—197° (Found : C, 67·3; H, 4·0. $C_{16}H_{12}O_5$ requires C, 67·6; H, 4·3%). Alternatively the acid (1·5 g.) was decarboxylated and directly acetylated, giving 5 : 7-*diacetoxy*-2′-*methoxy*iso*flavone*, which crystallised from methanol as needles (0·90 g., 53%), m. p. 192—193° (Found : C, 65·3; H, 3·9; OMe, 8·1. $C_{19}H_{13}O_6$·OMe requires C, 65·2; H, 4·4; OMe, 8·4%).

5 : 7 : 2′-*Trihydroxy*iso*flavone* (I).—(*a*) The *iso*flavone (V) (200 mg.) was heated (3 hr.) at 140—150° with hydrobromic acid (2·3 c.c.; *d* 1·5) and acetic acid (2·3 c.c.), giving, after dilution, 5 : 7 : 2′-*trihydroxy*iso*flavone* (I). This separated from aqueous ethanol as colourless, fibrous needles (52 mg., 27%), m. p. 187° (Found : C, 66·5; H, 4·2. $C_{15}H_{10}O_5$ requires C, 66·7; H, 3·7%). This *iso*flavone is dimorphic, and when crystallised later from the same solvent or from benzene, was obtained as clusters of prismatic needles, m. p. 222—223°. A mixture of the two forms sintered at *ca.* 187°, but melted at 222—223°. Its alcoholic solution gives a purple-brown ferric chloride reaction. The *triacetyl* derivative separates from aqueous methanol as colourless needles, m. p. 132—134° (Found : C, 63·5; H, 4·0. $C_{21}H_{16}O_8$ requires C, 63·6; H, 4·0%).

(*b*) 5 : 7-Diacetoxy-2′-methoxy*iso*flavone (150 mg.), acetic acid (2·5 c.c.), and hydrobromic acid (2·5 c.c.) were heated at 140° for 2·5 hr. giving the *iso*flavone (I) (52 mg., 47%).

(*c*) The *iso*flavone (V) (200 mg.) in dry benzene (40 c.c.) and powdered aluminium chloride (1 g.) were boiled for 4 hr. The benzene was evaporated, the aluminium complex decomposed with hydrochloric acid at 0°, and the product crystallised from aqueous ethanol and then

from chloroform–light petroleum, giving a solid (156 mg., 82%), m. p. 185—186° alone or mixed with a specimen prepared by method (*a*).

5-Hydroxy-7 : 2′-dimethoxyisoflavone.—The *iso*flavone (V) (80 mg.), dry benzene (10 c.c.), potassium carbonate (1 g.), acetone (5 c.c.), and methyl sulphate (0·03 c.c.) were kept at 100° for 2 hr. After filtration, the solvents were evaporated, leaving the solid *5-hydroxy-7 : 2′-dimethoxyisoflavone*; this separated from ethanol as needles (72 mg., 86%), m. p. 160° [Found : C, 68·6; H, 5·2; OMe, 20·1. $C_{15}H_8O_3(OMe)_2$ requires C, 68·4; H, 4·7; OMe, 20·8%].

5 : 7 : 2′-Trimethoxyisoflavone (XI).—(*a*) 2-Carbethoxy-5 : 7-dihydroxy-2′-methoxy*iso*flavone (IV) (14·4 g.), methyl sulphate (10 c.c.), and potassium carbonate (10 g.) in acetone (144 c.c.) were heated on a steam-bath for 18 hr., more potassium carbonate and methyl sulphate (10 c.c.) being added after 3 hr. Addition of water gave *2-carbethoxy-5 : 7 : 2′-trimethoxyisoflavone* (X) which separated from ethanol as a microcrystalline powder, m. p. 142—144° [Found : C, 65·8; H, 5·0; (OEt)(OMe)$_3$, 36·3. $C_{16}H_6O_3(OEt)(OMe)_3$ requires C, 65·6; H, 5·2; (OEt)(OMe)$_3$, 35·9%].

The preceding crude ester (*ca.* 15 g.) was heated on a steam-bath for 3 hr. with 5% aqueous sodium carbonate (100 c.c.) and ethanol (100 c.c.). The ethanol was removed by distillation, and acidification then gave the related carboxylic acid (10 g., 70% yield from IV) as a white powder. The acid was decarboxylated at *ca.* 260° for 10 min., and the product crystallised from aqueous ethanol (charcoal), washed with aqueous sodium hydrogen carbonate (yield 6·8 g., 77%; m. p. 130—134°), and crystallised from light petroleum (b. p. 100—120°), giving *5 : 7 : 2′-trimethoxyisoflavone* (XI) as colourless needles, m. p. 138—139° [Found : C, 69·45; H, 5·0; OMe, 27·3. $C_{15}H_7O_2(OMe)_3$ requires C, 69·2; H, 5·1; OMe, 29·8%].

(*b*) 5 : 7 : 2′-Trihydroxy*iso*flavone (I) (116 mg.), acetone (20 c.c.), methyl sulphate (1 c.c.), and potassium carbonate (1 g.) were boiled for 20 hr., giving finally 5 : 7 : 2′-trimethoxy*iso*flavone, m. p. 138—139°, in 68% yield.

5′ : 7′-Dihydroxychromono(2′ : 3′-3 : 4)coumarin (VI).—2-Carbethoxy-5 : 7-dihydroxy-2′-methoxy*iso*flavone (IV) (300 mg.), acetic acid (5 c.c.), and hydrobromic acid (5 c.c.; *d* 1·5) were heated at 140° for 2 hr. and poured into water. The solid (225 mg., 90%) crystallised from pyridine, giving *5′ : 7′-dihydroxychromono(2′ : 3′-3 : 4)coumarin* (VI) as orange-yellow plates which deepened in colour at *ca.* 300°, but did not melt below 360° (Found: C, 65·3; H, 2·9. $C_{16}H_8O_6$ requires C, 64·9; H, 2·7%). It was insoluble in aqueous sodium hydrogen carbonate, and its alcoholic solution gave a brown colour with ferric chloride. The *dibenzoyl* derivative separated from acetone as colourless plates (93%), m. p. 248° (Found: C, 71·3; H, 3·1. $C_{30}H_{16}O_8$ requires C, 71·4; H, 3·2%).

5 : 2′-Dihydroxy-7-methoxyisoflavone.—5 : 7 : 2′-Trimethoxy*iso*flavone (XI) (3 g.), hydrobromic acid (30 c.c.; *d* 1·5), and acetic acid (30 c.c.) were boiled for 6 hr. and poured into water. The dark, resinous precipitate was chromatographed on alumina in acetone, and the eluate yielded a crystalline product which separated from ethanol as colourless plates (132 mg.), m. p. 175—177° (Found: C, 67·6; H, 3·6. $C_{16}H_{12}O_5$ requires C, 67·6; H, 4·3%). 5 : 2′-*Dihydroxy-7-methoxyisoflavone* gives a purple ferric chloride reaction. The *diacetyl* derivative separates from aqueous ethanol in needles, m. p. 108—110° (Found: C, 65·7; H, 4·2. $C_{20}H_{16}O_7$ requires C, 65·2; H, 4·4%).

Demethylation of the trimethyl ether (XI) (2 g.) with hydriodic acid (15 c.c.; *d* 1·7) and acetic anhydride (10 c.c.) for 1 hr. at 150° gave a mixture of partially demethylated products (1·52 g.), m. p. 164—170°. Some of this material (0·6 g.) was demethylated further with aluminium chloride in benzene, and gave 5 : 7 : 2′-trihydroxy*iso*flavone (0·4 g.), m. p. 184—186° (cf. Whalley, *loc. cit.*).

Ultra-violet absorption characteristics.

Compound	$\lambda_{min.}$ (log ε) (mμ)	$\lambda_{max.}$ (log ε) (mμ)	Inflections (log ε) (mμ)
5 : 7 : 4′-Trihydroxy*iso*flavone (genistein) ...	231 (4·04)	263 (4·50)	325 (3·71)
5 : 7 : 2′-Trihydroxy*iso*flavone	234 (4·17)	261 (4·43)	325 (3·78)
5 : 2′-Dihydroxy-7-methoxy*iso*flavone	234 (4·18)	259 (4·45)	282 (4·08) 320 (3·68)

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