CCCLIX.—*Synthetical Experiments in the* iso*Flavone Group. Part II. A Synthesis of Methylgenistein (Methylprunetol) Dimethyl Ether and the Constitution of Prunetol (Genistein).*

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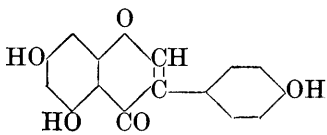
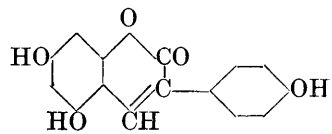
THE identity of genistein from *Genista tinctoria*, Linn. (Perkin and Newbury, J., 1899, **75**, 830; Horsfall and Perkin, J., 1900, **77**, 1312), with prunetol, obtained by demethylation of its monomethyl ether—prunetin (from *Prunus* bark;* Finnemore, *Pharm.*

* The botanical relations of the *Prunus* employed were not investigated by Finnemore, but Holmes (*Pharm. J.*, 1909, **28**, 191) states that it was intermediate between *P. avium* and *P. emarginata*.

J., 1910, **31**, 604)—was mooted as a possibility in Part I (J., 1925, **127**, 1981) and has now been proved by direct comparison (see p. 2716). Finnemore (*loc. cit.*) proposed the *iso*flavone constitution (I) for prunetol, whilst Bargellini * (*Gazzetta*, 1925, **55**, 949) has suggested its formulation as an α-pyrone (II).



(I.)



(II.)

Although the behaviour of genistein and prunetol in most respects can be interpreted equally well by either formula, the nature of the products obtained on methylation of genistein and prunetin affords strong evidence of the correctness of Finnemore's supposition. Only two of the three hydroxyl groups of genistein and only one of the two in prunetin can be methylated by means of methyl iodide and alcoholic potassium hydroxide, whilst a by-product in the former case is a methylgenistein dimethyl ether still containing a free phenolic hydroxyl group. Such protection of a hydroxyl group from the action of the methylating agent, and also the methylation on carbon, recall the similar behaviour of members of the flavone series, *e.g.*, luteolin: the phenomena are inexplicable on the basis of formula (II). The synthetical experiments now recorded supply a conclusive proof that (I) represents genistein and prunetol and consequently that *iso*flavone derivatives occur in nature.

The methods which have been employed are based on those described in Part I and, in spite of numerous attempts to devise a more practicable synthesis of *iso*flavones bearing no substituent in position 2, we still find it necessary to proceed through the 2-styryl*iso*flavones and the *iso*flavonecarboxylic acids derived from these in small yield by oxidation.

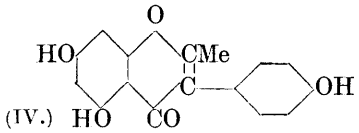
2 : 4 : 6-*Trihydroxyphenyl* p-*hydroxybenzyl ketone*,

$$C_6H_2(HO)_3\cdot CO\cdot CH_2\cdot C_6H_4\cdot OH \quad (III),$$

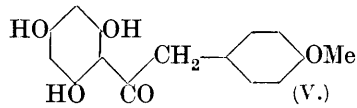
obtained from phloroglucinol and *p*-hydroxyphenylacetonitrile by an application of the Hoesch reaction, could be acetylated with formation of the acetyl derivative of 2-*methylgenistein* (IV) in poor yield, but could not be cinnamoylated with production of a chromone derivative. Much better results were obtained with the *methyl* ether (V) prepared from *p*-methoxyphenylacetonitrile

* In a letter dated September 27th, 1926, Professor Bargellini has informed us that he has synthesised the compound (II) and has found that its properties do not correspond with those of prunetol.

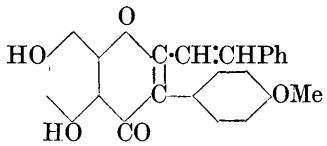
and phloroglucinol. Treatment of this ketone with a mixture of acetic anhydride and sodium acetate at 180°, followed by hydrolysis of the product, afforded 5 : 7-*dihydroxy*-4′-*methoxy*-2-*methyl*iso*flavone* (2-*methylgenistein* 4′-*methyl ether*) in satisfactory yield. 2-Methylgenistein, best obtained by demethylation of its 4′-methyl ether, closely resembles genistein (prunetol) in its properties. Cinnamoylation of (V) under closely-defined conditions yields the



(IV.)

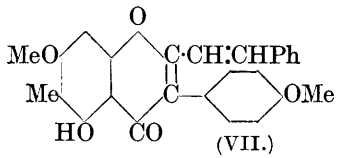


(V.)

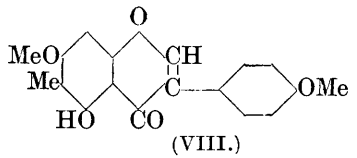


(VI.)

monocinnamoyl derivative (probably the 7-*cinnamate*) of 5 : 7-*dihydroxy*-4′-*methoxy*-2-*styryl*iso*flavone* (VI), which is itself obtained by the hydrolysis of the isolated primary product, again under conditions which do not allow of much variation. An excess of methyl iodide and sodium methoxide in boiling methyl-alcoholic solution converts (VI) into a C-*methyl* derivative of its 7-*methyl* ether (VII). We have assumed from analogies that the methyl



(VII.)



(VIII.)

group enters the position 6 in the phloroglucinol nucleus, but there is no clear proof that this is the case; the question of the situation of the methyl groups has, however, no bearing whatever on the validity of the argument here presented.

Attempts to oxidise the acetyl derivatives of (VI) and (VII) by potassium permanganate in pyridine solution gave benzoic acid only, but the oxidation of the acetyl derivative of (VII) by potassium permanganate in acetone solution gave benzoic and anisic acids along with an uncharacterised carboxylic acid which, after hydrolysis and decarboxylation by heating in glycerol, yielded the *iso*flavone (VIII). This was identical in all respects with methylgenistein dimethyl ether (Horsfall and Perkin, *loc. cit.*), with which it was directly compared.

EXPERIMENTAL.

Identity of Genistein and Prunetol.—We are greatly indebted to Professor A. G. Perkin for specimens of genistein and its dimethyl ether and to Mr. H. Finnemore for specimens of prunetol and prunetin and their derivatives. The genistein and prunetol differed but slightly in appearance; both specimens melted at 290—291° after softening and becoming red at about 284°. The behaviour of a mixture was the same. The specimens gave identical reactions: a pale yellow solution in alkalis; a violet coloration with ferric chloride in alcoholic solution, becoming brownish-green with excess of the reagent; a pale yellow solution in concentrated sulphuric acid which exhibited a weak bluish-green fluorescence in the light of an iron arc. The specimens of genistein dimethyl ether and prunetin methyl ether consisted of characteristic leaflets and could not be distinguished in appearance; both melted at 140—142°, as also did a mixture of the two. In sulphuric acid they gave identical pale yellow solutions exhibiting weak fluorescence and with alcoholic ferric chloride they developed the same reddish-purple coloration.

2 : 4 : 6-*Trihydroxyphenyl* p-*Hydroxybenzyl Ketone* (III).—A solution of *p*-hydroxyphenylacetonitrile (10 g.) and anhydrous phloroglucinol (9·4 g.) in dry ether (75 c.c.) was saturated with hydrogen chloride at 0° and again after 24 hours. The crystalline crust of *ketimine hydrochloride* was washed with ether and heated with water for 1 hour; the ketone (12 g.) then separated. It is very sparingly soluble in the usual organic solvents and crystallises from 50% methyl alcohol in microscopic needles which melt at 259° with reddening and decomposition (Found: C, 60·4; H, 5·0. $C_{14}H_{12}O_5,H_2O$ requires C, 60·4; H, 5·1%). The combined water is tenaciously held and in general properties the substance closely resembles its higher homologue, phloretin.

5 : 7 : 4′-*Trihydroxy*-2-*methyl*iso*flavone* (2-*methylgenistein*) (IV) is most readily prepared by demethylation of its 4′-methyl ether (see below) by means of boiling hydriodic acid (*d* 1·7). It was first obtained in the following manner. A mixture of the above-described tetrahydroxydeoxybenzoin (5 g.), sodium acetate (5 g.), and acetic anhydride (25 c.c.) was heated at 190—200° for 48 hours; the dark product then obtained by decomposition and washing with water was dissolved in a very small amount of acetic acid. Crystals separated slowly and after a long time these were freed from adhering oil by contact with porous porcelain and thrice crystallised from acetic acid (charcoal). The thin, pinkish prisms, m. p. 171° (Found: C, 65·3; H, 4·7. $C_{20}H_{16}O_7$ requires C, 65·2; H, 4·4%), were insoluble in cold, aqueous sodium hydroxide, but gave a violet

coloration with alcoholic ferric chloride. The substance is doubtless 5-*hydroxy*-7 : 4′-*diacetoxy*-2-*methyl*isoflavone, since several substituted 5-hydroxybenzochromones are already known which are insoluble in aqueous alkalis and yet give a positive ferric chloride reaction. The parent substance, obtained by hydrolysis with aqueous sodium carbonate, crystallised from very dilute alcohol in colourless needles containing $1H_2O$, m. p. 258—259° (slight decomp.) (Found in anhydrous substance: C, 67·2; H, 4·4. $C_{16}H_{12}O_5$ requires C, 67·6; H, 4·3%). Ferric chloride added to an alcoholic solution gives a violet and then a brownish-green coloration. Solutions in aqueous sodium hydroxide and in concentrated sulphuric acid are pale yellow and the latter exhibits a weak bluish-green fluorescence. In all these properties and in its solubility relations this compound exhibits a noteworthy resemblance to genistein. The O-*triacetyl* derivative was readily obtained in the usual manner (therefore the formation of a diacetyl derivative which is noted above is almost certainly due to hydrolysis in the isolation process rather than to incomplete acetylation) and crystallised from alcohol, in which it was sparingly soluble, in colourless, silky needles, m. p. 214° (Found: C, 64·4; H, 4·5. $C_{22}H_{18}O_8$ requires C, 64·4; H, 4·4%).

2 : 4 : 6-*Trihydroxyphenyl* p-*Methoxybenzyl Ketone* (V).—*p*-Methoxyphenylacetonitrile (20 g.) (obtained in excellent yield by methylation of *p*-hydroxyphenylacetonitrile with methyl sulphate and aqueous potassium hydroxide at about 50°) and phloroglucinol (20 g.), when treated as in the similar case described above, gave 30 g. of the almost pure ketone. This crystallises from 50% methyl alcohol in minute, colourless prisms, m. p. 192—193° (Found: C, 62·0; H, 5·7. $C_{15}H_{14}O_5,H_2O$ requires C, 61·7; H, 5·5%). The substance is only slowly dehydrated at 140°; ferric chloride added to an alcoholic solution develops a reddish-purple coloration; and the solutions in alkalis are yellow.

5 : 7-*Dihydroxy*-4′-*methoxy*-2-*methyl*isoflavone (4′-*Methyl Ether of IV*).—The acetylation of the foregoing ketone was carried out in the usual way, the mixture being heated at 180° for 20 hours. A *diacetyl* derivative was obtained in almost quantitative yield after treatment of the reaction product with water; this crystallised from ethyl alcohol in colourless needles, m. p. 208—209° (Found: C, 65·7; H, 4·9. $C_{21}H_{18}O_7$ requires C, 66·0; H, 4·7%). The parent substance, obtained by hydrolysis, crystallises from not too much alcohol in prisms, m. p. 205° (Found: C, 68·2; H, 4·8. $C_{17}H_{14}O_5$ requires C, 68·4; H, 4·7%). This *iso*flavone gives colourless solutions in aqueous alkalis and the addition of ferric chloride to an alcoholic solution produces a reddish-violet and

then a brownish-green coloration. The colourless solution in concentrated sulphuric acid exhibits a very weak bluish-green fluorescence. On methylation with methyl sulphate and aqueous potassium hydroxide, a mixture of the mono- and di-methyl ethers is produced; these separate together from alcohol, the former in feathery crystals and the latter in quadrilateral plates. The two forms were sorted out and separately crystallised from alcohol. 5-*Hydroxy*-7 : 4′-*dimethoxy*-2-*methyl*isoflavone crystallises in colourless needles, m. p. 197—199° (Found: C, 69·6; H, 5·5. $C_{18}H_{16}O_5$ requires C, 69·2; H, 5·2%). It was also obtained by the use of methyl iodide. The reactions of the substance closely resemble those of genistein dimethyl ether.

5 : 7 : 4′-*Trimethoxy*-2-*methyl*isoflavone, m. p. 175—176° (Found: C, 70·0; H, 5·8. $C_{19}H_{18}O_5$ requires C, 69·9; H, 5·6%), does not give a ferric chloride reaction in alcoholic solution. Its pale yellow solution in concentrated sulphuric acid exhibits a blue fluorescence.

5 : 7-*Dihydroxy*-4′-*methoxy*-2-*styryl*isoflavone (VI).—An efficiently stirred mixture of anhydrous 2 : 4 : 6-trihydroxyphenyl *p*-methoxybenzyl ketone (10 g.) and cinnamic anhydride (100 g.) was heated (oil-bath at 180—200°) for 2 hours; powdered sodium cinnamate (10 g.) was then slowly introduced and the heating continued for 6 hours. The pasty product was scraped out while hot, ground under water, washed with much water and with aqueous sodium carbonate, and dried at a moderate temperature. The chocolate-coloured powder was added to alcohol (250 c.c.) and concentrated sulphuric acid (5 c.c.), and decomposition of the excess of cinnamic anhydride effected by gentle warming for 1½ hours followed by boiling for 1 hour. After cooling, the solid was collected and washed with alcohol. It crystallised from 1 litre of glacial acetic acid (charcoal) in thin, bright yellow, microscopic prisms, m. p. 230—231° (yield 8 g.) (Found: C, 76·6; H, 4·9. $C_{33}H_{24}O_6$ requires C, 76·7; H, 4·7%). This substance appears to be 5-*hydroxy*-7-*cinnamoyloxy*-4′-*methoxy*-2-*styryl*isoflavone (hydrolysis yielded 83·5% of the theoretical amount of cinnamic acid), the situation of the cinnamoyl group being inferred from the insolubility of the compound in aqueous sodium hydroxide, even on boiling, and from the green coloration developed in alcoholic solution with ferric chloride. (This reaction is weak on account of the extremely sparing solubility of the substance in alcohol.) The cinnamate (10 g.) was treated with a boiling mixture of alcohol (200 c.c.), potassium hydroxide (4 g.), and water (10 c.c.) for ¾ hour, after which water (200 c.c.) was introduced and the liquid saturated with carbon dioxide. The bright yellow precipitate was isolated; the substance crystallised from a large volume of alcohol in yellow

needles, m. p. 278—279°, containing 1EtOH (yield about 7 g.) (Found in material dried at 100°: C, 74·9; H, 5·0. $C_{24}H_{18}O_5$ requires C, 74·6; H, 4·7%). An alcoholic solution becomes reddish-brown on the addition of ferric chloride. The solubility of the substance in cold aqueous sodium hydroxide is slight, but on heating, a viscous, orange solution is produced; this froths readily and on cooling deposits colloidal flocks that become crystalline on the addition of alcohol. The *diacetyl* derivative, obtained in the usual manner, crystallises from acetic acid in yellow, microscopic needles, m. p. 225—226° (Found: C, 69·3, 69·3; H, 5·0 5·0; $C_2H_4O_2$, by direct loss on fusion, 6·5. $C_{28}H_{22}O_7$,0·5$C_2H_4O_2$ requires C, 69·6; H, 4·8; $C_2H_4O_2$, 6·0%). Several attempts to oxidise this diacetate so as to produce a substituted *iso*flavone-carboxylic acid were fruitless.

5-*Acetoxy*-7 : 4′-*dimethoxy*-2-*styryl*-6-*methyl*isoflavone (*Acetyl* derivative of VII).—A mixture of dihydroxymethoxystyryl*iso*flavone (8 g.), methyl alcohol (200 c.c.), sodium methoxide (from 2·3 g. of sodium), and an excess of methyl iodide was refluxed for 48 hours (more sodium methoxide and methyl iodide were added after 24 hours). The product, after being heated with dilute aqueous sodium hydroxide, in which it was insoluble, crystallised from much alcohol in thin, bright yellow prisms, m. p. 217—218°. 5-*Hydroxy*-7 : 4′-*dimethoxy*-2-*styryl*-6-*methyl*isoflavone (VII) gives a green coloration with ferric chloride, and with acetic anhydride yields the *acetyl* derivative, which crystallises from ethyl alcohol in very fine, pale yellow needles, m. p. 218—219° (Found: C, 73·7; H, 5·5. $C_{28}H_{24}O_6$ requires C, 73·7; H, 5·3%). All the above styryl derivatives give bright yellow solutions in sulphuric acid and these exhibit a weak, green fluorescence.

Methylgenistein (*Methylprunetol*) *Dimethyl Ether* (VIII).—(A) Crabtree and Robinson (J., 1918, **113**, 868) indicated that *C*-methylation of resacetophenone by methyl iodide precedes *O*-methylation and is favoured by an increase in the concentration of alkali-metal alkyloxide in the solution. Accordingly we methylated prunetol by refluxing it for 12 hours with a methyl-alcoholic solution of sodium methoxide (10 mols.) and an excess of methyl iodide; the only isolable product was methylprunetol (methylgenistein) dimethyl ether (compare Perkin and Horsfall, *loc. cit.*) which, after two crystallisations from alcohol, formed tiny, colourless needles or prismatic needles, m. p. 200—201·5° (Found: C, 69·3; H, 5·4. Calc. for $C_{18}H_{16}O_5$: C, 69·2; H, 5·2%).

(B) Finely-powdered potassium permanganate (5·6 g., a 20% excess) was gradually added during 4 hours to a solution of acetoxydimethoxymethylstyryl*iso*flavone (5 g.) in pure acetone (600

c.c.) cooled at −10° to −15°, which was continuously stirred and through which a stream of carbon dioxide was led. Towards the end of the operation a slight rise of temperature was permitted. Water was added and, after removal of acetone and a trace of benzaldehyde in a current of steam, the filtered liquid was concentrated to a small volume, saturated with carbon dioxide and filtered. On acidification, a mixture of tarry material and some white crystals was precipitated and from this a small quantity of benzoic acid was sublimed. The crystalline constituent, of which a further quantity was obtained by extraction of the tar with boiling water, proved to be anisic acid. The residue was dissolved in aqueous sodium bicarbonate and the solution boiled, filtered, and acidified. The brown, crystalline precipitate was collected, dried at 100° (to a viscous mass), and heated with anhydrous glycerol at 250° for ½ hour. The product was mixed with water and sodium bicarbonate and extracted with ether. The brown residue, after removal of the solvent from the combined extracts, crystallised very readily from alcohol in minute, colourless needles, m. p. 198—200·5° (after three crystallisations; yield, 0·05 g.) (Found: C, 69·0; H, 5·3%).

A mixture of the products obtained in (A) and (B) had m. p. 199—201° and the melt crystallised at once on cooling. The specimens were indistinguishable in appearance, in crystalline form, and in their solubility relations and reactions. The pale yellow solution in sulphuric acid was devoid of fluorescence and the addition of ferric chloride to an alcoholic solution developed a violet coloration, which became olive-green with an excess of the reagent.

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