SYNTHETIC EXPERIMENTS IN THE BENZOPYRONE SERIES

Part XIII. Constitution of Prunetin and Its Synthesis

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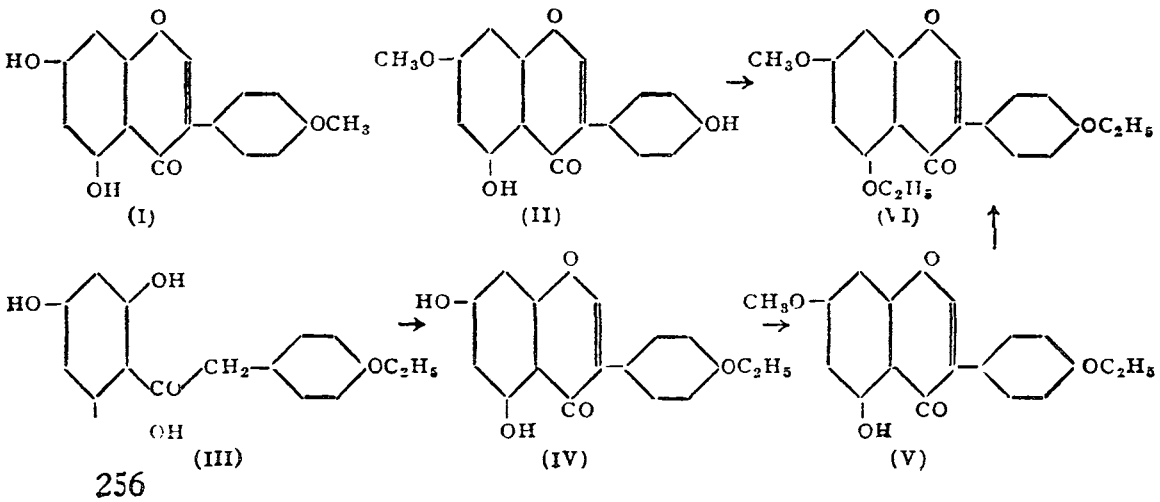
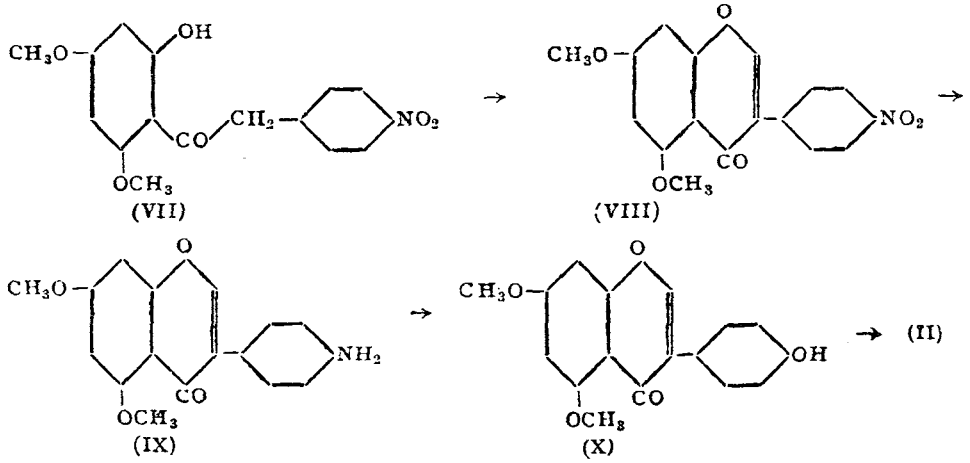
THAT prunetin was a monomethyl ether of the isoflavone genistein was established by Baker and Robinson.[1] Since it underwent partial methylation to yield a monomethyl ether,[2] it was clear that the resistant 5-hydroxyl was free in the molecule. Consequently the 7 or 4′ position should carry the methoxyl group. This point was unsettled till Shriner and Hull[3] synthesised the 4′-methyl ether (I) which differed from prunetin, but was found to agree with another naturally occurring monomethyl ether, biochanin-A.[4] By elimination, therefore, prunetin should be the 7-methyl ether of genistein (II). A stricter synthetic proof could be provided by completely ethylating prunetin and establishing the constitution of the diethyl ether by synthesis. This is now reported in this paper. By the condensation of phloroglucinol and *p*-ethoxy-phenyl-acetonitrile and converting the resulting ketone (III) into isoflavone, 4′-O-ethyl genistein (IV) is obtained. This is then partially methylated using one mole of dimethyl sulphate. As in all similar cases the methyl group goes into the 7-position leaving the resistant 5-hydroxyl free; the properties of the product agree with this constitution (V). Final complete ethylation yields 7-methoxy-5:4′-diethoxy isoflavone (VI) which is found to be identical with the diethyl ether of prunetin.

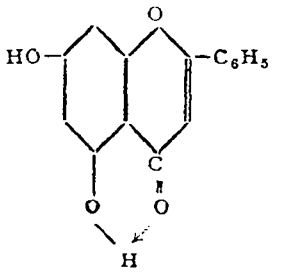


A synthesis of prunetin has recently been announced by Iyer, Shah and Venkataraman[5] using the following steps.

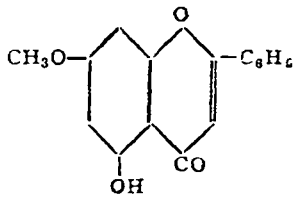


A simpler method which would appear to be significant for the study of biogenesis is described in this paper; it is based on the following considerations. In Part XII[6] of this series it was pointed out that among the monomethyl ethers of anthoxanthins of different types, the most numerous have the methoxyl group in the 7-position. This would suggest that they are probably formed in nature by direct methylation of this position. Two alternatives have then to be considered. Either the hydroxyl in this position is definitely more reactive than those in other positions or there exists a mechanism which protects the other groups from methylation. Very little seems to be known about the second alternative except that the 5-hydroxyl group is chelated and therefore definitely resistant to methylation. Regarding the first alternative the position may be stated as follows. Partial monomethylation of hydroxy flavones has so far been successful only in the case of chrysin (XI)[7], the simplest of dihydroxy flavones; the success in this case is easily understood, since in chrysin the two hydroxyl groups differ considerably in reactivity. Tectochrysin (XII) can be conveniently made in the laboratory by this partial methylation. But when more hydroxyl groups are present particularly in the side phenyl nucleus, the difference between these and the 7-hydroxyl is far less marked and the success of partial monomethylation in the flavone group does not seem to be so definite. Perkin and Horsfall[8] made attempts to prepare a monomethyl ether of luteolin by direct methylation by means of methyl iodide and alkali and recorded that they could get only the 7: 4′-dimethyl ether (XIII) in a small yield. Thus in this case the 4′-hydroxyl is as reactive as the 7-hydroxyl. Examining carefully other related groups of anthoxanthins some of them seemed

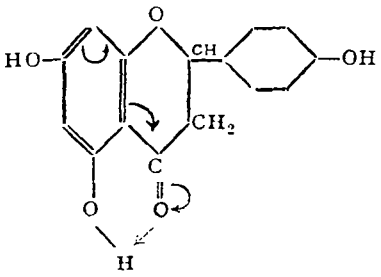
to offer greater possibilities. For example, Shinoda and Sato[9] recorded that they could methylate naringenin (XIV) with restricted quantities of diazomethane to yield sakuranetin (XV). Our earlier attempt[10] to repeat this by the more convenient method employing dimethyl sulphate and potassium carbonate in dry acetone solution was not successful because no suitable procedure for the purification of the product and removing unchanged naringenin and its dimethyl ether was available. This has now been worked out using solubility in dilute aqueous sodium carbonate (5%) and aqueous sodium hydroxide (2%). Actually the explanation of this partial methylation and of the successful separation of the mixture involves one and the same principle. Of the three hydroxyls in naringenin (XIV), the one in the 5-position is resistant owing to chelation with the neighbouring carbonyl. There exists appreciable difference between the other two hydroxyl groups also; the one in the 7-position is more acidic because of conjugation



(XI)



(XII)



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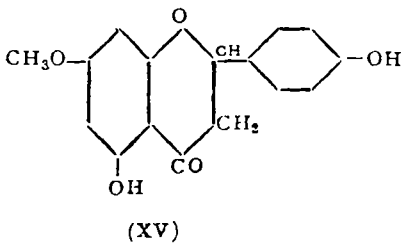
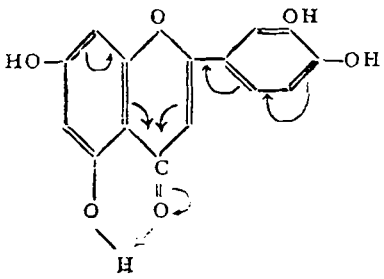
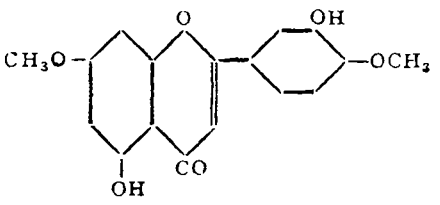


(XV)





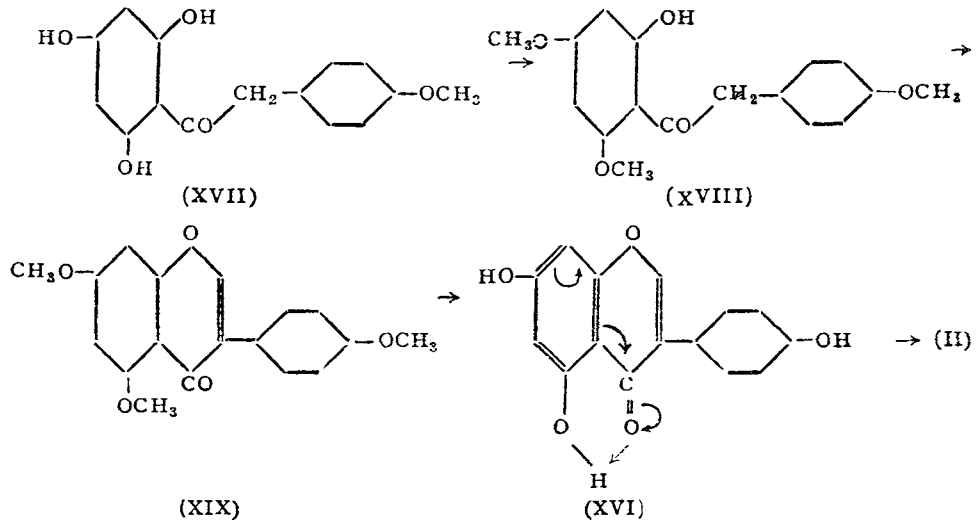
(XIII)

with the carbonyl (vinylogy) whereas the one in the side-phenyl nucleus is not conjugated. As a consequence the 7-hydroxyl is more readily methylated and further its presence renders the compound soluble in aqueous sodium carbonate.

The same considerations become applicable to the isoflavone genistein (XVI) though the structural causes are different. In the flavanones the oxygen ring has no ethylenic double bond conjugating the side-phenyl ring with the carbonyl group whereas in the isoflavones the side-phenyl nucleus is in the α-position with reference to the C = O group and thus conjugation is averted. Consequently a simplified synthesis of prunetin by the partial methylation of genistein should be possible. This has now been achieved and the results are reported in this paper. Earlier Walz[11] seems to have reported the isolation of a monomethyl ether of genistein by direct methylation with methyl iodide. Its melting point was recorded as 189–91° and its structure as 5 : 7-dihydroxy-4′-methoxy isoflavone. But it does not agree with the description of biochanin-A with which it should be identical according to his surmise or of prunetin as could be expected from the considerations discussed above. Detailed study of this product was not recorded by Walz. It was most probably a mixture. In our experiments using dimethyl sulphate no detectable quantity of biochanin-A (4′-methyl-genistein) was met with.

Genistein required for our experiments was obtained by the demethylation of prunetin and also by synthesis in the following manner. Phloroglucinol was condensed with *p*-methoxy-phenyl-acetonitrile and the resulting ketone[1] (XVII) partially methylated using two moles of dimethyl sulphate to yield the 2-hydroxy-4 : 6 : 4′-trimethoxy ketone (XVIII). This trimethoxy ketone was prepared earlier by Robertson, *et al.*[12] from phloroglucinol-dimethyl ether and *p*-methoxy-phenyl acetonitrile. It was finally converted into genistein-trimethyl ether (XIX). The isoflavone condensation proceeds much better in this case than with (XVII) as originally adopted by Shriner and Hull.[3] Final demethylation yielded genistein in good yield.

Partial methylation of genistein (XVI) with one mole of dimethyl sulphate proceeded satisfactorily. From the resulting mixture genistein was removed by macerating with cold aqueous sodium carbonate. The insoluble portion was treated with cold aqueous sodium hydroxide (2%) in which prunetin dissolved leaving out the higher methylation products. It was recovered by acidification and recrystallised. A yield of 20% could be obtained. The synthetic product agreed with natural prunetin from *Prunus puddum*[2] in every respect and yielded identical derivatives. The diethyl



ether was compared with synthetic 5:4′-diethoxy-7-methoxy isoflavone and found to be identical.

Experimental

2:4:6-*Trihydroxy-phenyl-4′-ethoxy-benzyl ketone* (*III*):

A solution of phloroglucinol (5g.), *p*-ethoxy-phenyl-acetonitrile[13] (5 g.) and zinc chloride (1 g.) in dry ether (100 c.c.) was cooled in ice, saturated with dry hydrogen chloride for 4 hours and left in the ice-chest overnight. Ether was then decanted off and the pale yellow solid ketimine hydrochloride dissolved in water and heated on a boiling water-bath for 1 hour. On cooling, the ketone separated as a colourless crystalline solid. It crystallised from dilute methyl alcohol as colourless prisms melting at 208–10° (Found: in a sample dried at 110° *in vacuo*: C, 62·4; H, 6·1; $C_{16}H_{16}O_5$, H_2O requires C, 62·7; H, 5·9%.)

5:7-*Dihydroxy-4′-ethoxy isoflavone* (IV):

Powdered sodium (1 g.) was cooled in ice and treated with an ice-cold solution of the above ketone (2 g.) in ethyl formate (10 c.c.) with shaking. After keeping in the refrigerator for 48 hours, pieces of ice were added and the excess of ethyl formate was removed under reduced pressure. The residue was extracted with ether and the ether layer separated and washed with aqueous bicarbonate. On evaporating the resulting ether solution a pale yellow solid was left behind. By acidifying the aqueous layer and extracting with ether more of the substance could be obtained; but it was less pure. Total yield 0·5 g. 5:7-Dihydroxy-4′-ethoxy isoflavone crystallised from ethyl acetate-petroleum ether mixture as colourless prisms melting

at 238–40°. It gave a pink colour with ferric chloride and dissolved in aqueous sodium carbonate (Found: C, 64·2; H, 5·3; $C_{17}H_{14}O_5$, H_2O requires C, 64·5; H, 5·1%.)

Partial methylation to 5-hydroxy-7-methoxy-4′-ethoxy isoflavone (V):

The above dihydroxy compound (IV) (0·5 g.) was methylated by refluxing with dimethyl sulphate (0·2 c.c.) and anhydrous potassium carbonate (0·5 g.) for 6 hours in acetone solution. It crystallised from alcohol as colourless needles melting at 142–4°. It gave a deep red colour with ferric chloride and was sparingly soluble in aqueous sodium hydroxide (Found: C, 68·8; H, 5·3; $C_{18}H_{16}O_5$ requires C, 69·2; H, 5·1%.)

Ethylation to 7-methoxy-5:4′-diethoxy isoflavone (VI):

The 5-hydroxy compound (V) was refluxed in dry acetone solution with excess of ethyl iodide and anhydrous potassium carbonate for 20 hours. The ethyl derivative crystallised from alcohol as colourless needles melting at 116–7°, gave no colour with ferric chloride and was insoluble in aqueous alkali (Found: C, 70·2; H, 5·7; $C_{20}H_{20}O_5$ requires C, 70·6; H, 5·9%.)

Ethylation of prunetin to diethyl-prunetin:

A sample of natural prunetin was ethylated with excess of ethyl iodide in acetone solution as described above. Prunetin diethyl ether crystallised from alcohol as colourless needles melting at 116–7°. It was identical in its properties with 7-methoxy-5:4′-diethoxy isoflavone (VI) and the mixed melting point was undepressed.

2-Hydroxy-4:6-dimethoxy-phenyl-4′-methoxy-benzyl ketone (XVIII):

2:4:6-Trihydroxy-phenyl-4′-methoxy-benzyl ketone[1] (4 g.) was refluxed in acetone solution with dimethyl sulphate (3 c.c.) and anhydrous potassium carbonate (6 g.) for 10 hours. Acetone was then distilled off and the residue treated with water and extracted with ether. The ether solution was extracted thrice with aqueous sodium hydroxide and the combined alkali extract was cooled and acidified. The colourless solid that separated out crystallised from alcohol in the form of colourless needles melting at 88–89° (see Robertson, *et al.*[12]). It gave a reddish brown colour with ferric chloride and a blue colour with concentrated nitric acid.

5:7:4′-Trimethoxy isoflavone (genistein trimethyl ether) (XIX):

Pulverised sodium (1 g.) was cooled in ice and treated with an ice-cold solution of the hydroxy-trimethoxy ketone (XVIII) in ethyl formate (10 c.c.) slowly with shaking. The mixture was left in the ice-chest for 48 hours

with occasional shaking. Pieces of ice were then added and ethyl formate removed under reduced pressure. The isoflavone that separated out was filtered and more of it was obtained by acidifying the aqueous solution. The combined product was washed with aqueous sodium hydroxide and crystallised from alcohol, when it separated in the form of colourless rectangular tablets melting at 160–61° identical with genistein trimethyl ether.[1, 2] Yield 1·2 g.

Partial methylation of genistein to prunetin:

Genistein (1 g.) was dissolved in dry acetone (100 c.c.), dimethyl sulphate (0·3 c.c., less than one mole) and anhydrous potassium carbonate (1 g.) added and the mixture refluxed for 4 hours. Acetone was then distilled off and the residue treated with water. The solid that separated out being insoluble in the carbonate solution was collected and washed with water. One crystallisation gave a product melting indefinitely between 210–20°. This was treated with 5% aqueous sodium hydroxide and the turbid solution filtered. On acidifying the alkaline solution a pale yellow solid was obtained (0·2 g.) which, after two crystallisations from alcohol came out as almost colourless needles melting at 238–40°. It gave a violet colour with ferric chloride and dissolved readily in aqueous sodium hydroxide. It agreed in its properties with prunetin and a mixed melting point with an authentic sample was undepressed. Acidification of the carbonate solution gave genistein (0·3 g.). (Found: C, 67·3; H, 4·4; OCH_3 10·6; $C_{16}H_{12}O_5$ requires C, 67·6; H, 4·2; OCH_3 10·9%.)

The acetate prepared from the synthetic sample agreed with the derivative prepared from natural prunetin; m.p. and mixed m.p. 224–26°. The diethyl ether obtained by complete ethylation crystallised from alcohol as colourless needles and melted at 116–7° alone or in admixture with the synthetic 7-methoxy-5:4′-diethoxy isoflavone.

Methylation of naringenin (*sakuranetin*) (*XV*):

Naringenin (1 g.) was dissolved in acetone (30 c.c.), treated with dimethyl sulphate (0·3 c.c.) and anhydrous potassium carbonate (1 g.) and the mixture refluxed on a water-bath for 3 hours. Acetone was then distilled off and the residue treated with water. The solid that separated out being insoluble in aqueous potassium carbonate was filtered and crystallised from alcohol. The product melted at 98–100° even after two crystallisations. It was then treated with cold 2% aqueous sodium hydroxide and the turbid solution filtered. After cooling, the alkali solution was acidified with cold hydrochloric acid (1:1). The pale yellow solid that separated was filtered

and crystallised twice from aqueous alcohol when it was obtained as colourless prismatic needles melting at 152–4°. It agreed in all its properties with sakuranetin and a mixed melting point with a sample obtained from *Prunus puddum* was undepressed.

Summary

The constitution of prunetin as the 7-methyl ether of genistein is established by preparing its diethyl ether and showing that it is identical with 7-methoxy-5:4′-diethoxy isoflavone obtained by independent synthesis. Prunetin itself has been synthesised by a method involving partial methylation of genistein using one mole of dimethyl sulphate. The theoretical considerations are discussed. The same procedure leads to a convenient preparation of sakuranetin.

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