SYNTHETIC EXPERIMENTS IN THE BENZOPYRONE SERIES

Part XIX. Methyl Ethers of Genistein*

By N. Narasimhachari, T. R. Seshadri, F.A.Sc.
and
S. Sethuraman
(From the Department of Chemistry, Delhi University)

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Of the three possible monomethyl ethers of genistein the 4′-methyl ether was synthesised by Shriner and Hull[1] and soon after this synthesis it was also found to occur in nature as biochanin-A.[2] Another earlier known naturally occurring monomethyl ether, prunetin was therefore considered to be the 7-methyl ether of genistein and this constitution was confirmed by later synthetic work. Narasimhachari and Seshadri[3] prepared its diethyl ether and showed that it was identical with 7-methoxy-5: 4′-diethoxy isoflavone obtained by independent synthesis. The synthesis of prunetin itself was first reported by Iyer, *et al.*,[4] using 2-hydroxy-4: 6-dimethoxy-4′-nitrophenyl-benzyl ketone and passing through a number of stages. A simplified synthesis was given by Kotake and Fukui[5] who employed 2: 4′-dihydroxy-4: 6-dimethoxy-phenyl-benzyl ketone for the isoflavone condensation. An even simpler method however, is the direct partial methylation of genistein to prunetin reported by Narasimhachari and Seshadri[3] and this is also significant biogenetically.

The schemes of synthesis adopted by Iyer, *et al.*[4] and Kotake and Fukui[5] seem to have been based on the assumption that there should be difficulty in the partial demethylation of isoflavones just as in the case of flavones, that it should be particularly difficult to demethylate the 4′-position without affecting the 7-position and that even for the partial hydrolysis of the 5-methyl ether group leaving out the 7-methoxyl group, mild conditions are necessary. In a previous part of this series[6] this question has been specially examined and the experimental results (preparation of isoformononetin and 7-methoxy-5-hydroxy isoflavone) were described leading to the conclusion that the 7-methoxyl is markedly far more resistant than the others enabling even the use of hydriodic acid for the partial demethylation. In further support

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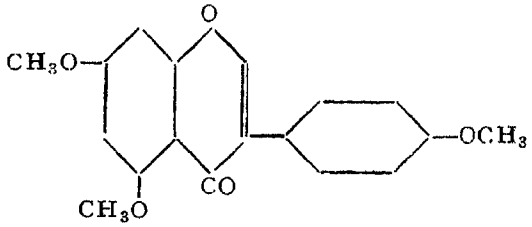
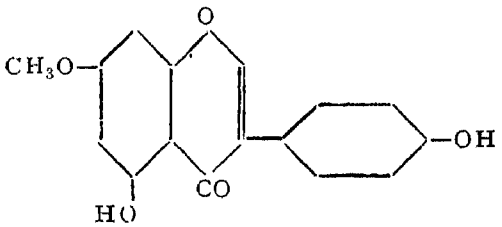
of this contention the demethylation of genistein trimethyl ether (I) has now been carried out using hydrobromic acid and hydriodic acid and good yields of prunetin (II) obtained. This now provides the simplest synthetic method of preparing this substance.

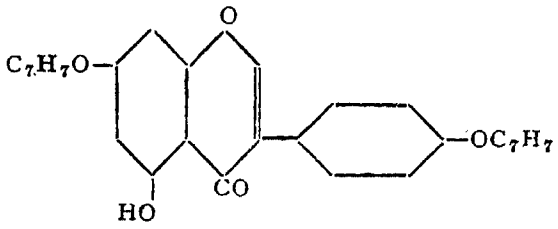


(I)



(II)

The third methyl ether of genistein now named isoprunetin (III) (5-O-methyl genistein) has also now been made. For this purpose genistein is first benzylated to yield the dibenzyl ether (IV). The reactions of this compound are in agreement with the presence of a free 5-hydroxyl group. Subsequent methylation (V) and debenzylation yields isoprunetin (III). The identity of this compound is established by its complete ethylation. The product is found to be identical with 5-methoxy-7: 5′-diethoxy isoflavone (VI) prepared through 7: 4′-diethyl ether of genistein (VII) as intermediate. This diethyl ether of genistein was first reported by Perkin and Horsfall[7]



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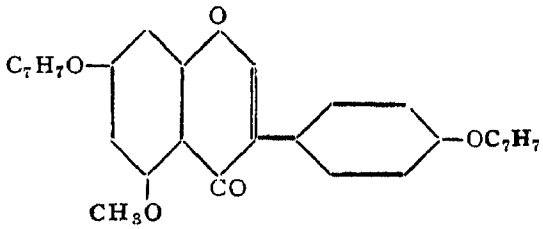

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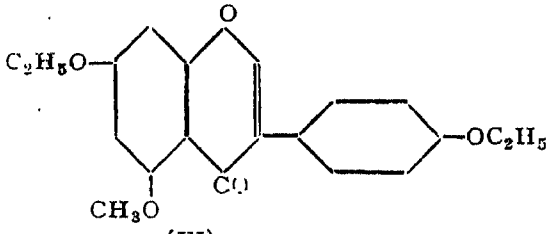

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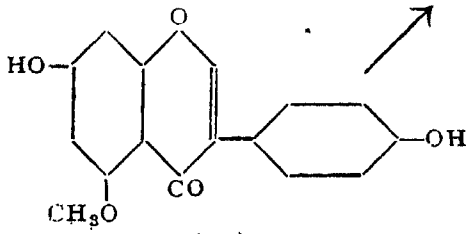

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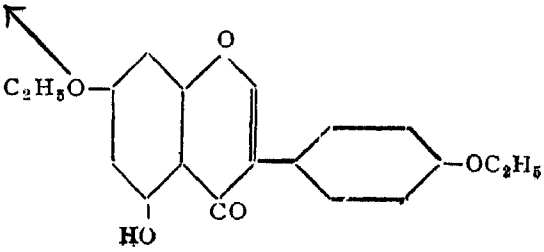

(VII)

who expressed that they had difficulties in getting it pure by using alcoholic potash and ethyl iodide for ethylation. The melting point recorded by them is somewhat low. It has now been obtained more easily by ethylating genistein with ethyl iodide (2 moles) and potassium carbonate in acetone solution. Further methylation of it yields 5-methoxy-7: 4′-diethoxy isoflavone (VI).

In their examination of the bark of *Prunus puddum* Chakravarti and Bhar[1] obtained a genistein monomethyl ether having a somewhat lower melting point than prunetin; its acetate was also found to have a lower melting point than prunetin acetate. Further they reported that it did not undergo partial methylation yielding its monomethyl ether. They could only get its dimethyl ether which was identical with genistein trimethyl ether. Consequently they came to the conclusion that it was a new substance having the constitution of the 5-monomethyl ether of genistein and gave it the name prunusetin. Narasimhachari and Seshadri[9] pointed out that this conclusion was erroneous because the substance gave a brownish violet colouration with ferric chloride and because it was insoluble in aqueous sodium carbonate. They further showed that by careful purification the melting points of the substance and its acetate could be raised to those of prunetin and its acetate respectively and that partial methylation could be accomplished by using one mole of dimethyl sulphate and anhydrous potassium carbonate in anhydrous acetone medium. The product, a dimethoxy compound had the same melting point and properties as monomethyl prunetin. They stated therefore that there was no doubt that prunusetin was essentially prunetin though slightly impure and the new name was not necessary. In spite of these evidences, Chakravarti and Sen[10] expressed the feeling that their original claim might be correct. Subsequently our experiments[4] on ethylation using this sample of prunetin from *Prunus puddum* was reported and the diethyl ether was found to be identical with 7-methoxy-5: 4′-diethoxy isoflavone. To these evidences we may now add the results reported in this paper on the preparation and properties of the 5-methyl ether of genistein; there is absolutely no agreement between the recorded properties of prunusetin and of the 5-methyl ether of genistein (isoprunetin) whereas there is close resemblance between prunusetin and prunetin. In our earlier paper[9] we have emphasised that there should be marked difference between the 5-methyl ether of genistein and prunetin, the 7-methyl ether. The former was expected to have a higher melting point, to be soluble in sodium carbonate and not give ferric chloride colour. All these expectations are found to be correct as could be seen from the data presented in the following table.

TABLE

	Prunetin (7-O-methyl genistein)	Prunusetin	Isoprunetin (5-O-methyl genistein)
1. Melting point	240–42°	237–38°	284–86°
2. Acetate	224–26°	220–22°	168–70°
3. Ferric chloride	Violet	Violet	Nil
4. Solubility in sodium carbonate	Insoluble	Insoluble	Soluble

EXPERIMENTAL

Demethylation of genistein trimethyl ether

(1) *With hydrobromic acid.*—Genistein trimethyl ether[3] (0·5 g.) was dissolved in glacial acetic acid (10 c.c.) and the solution treated with aqueous hydrobromic acid (d. 1·51; 10 c.c.). The mixture was heated in a boiling water-bath for 3 hours. It was then cooled, diluted with water and the solid product filtered and washed with water. It readily dissolved in cold aqueous sodium hydroxide; the solution was filtered from a small resinous impurity and the clear alkaline solution acidified. The product crystallised from alcohol as colourless needles melting at 238–40°. Yield 0·35 g. It was identical in every respect with prunetin and a mixed melting point with an authentic sample[9] was undepressed. The acetate crystallised from ethyl acetate as rhombohedral prisms melting at 223–25° alone or in admixture with a sample of prunetin acetate.

(2) *With hydriodic acid.*—On demethylation with hydriodic acid in acetic anhydride solution at 140° for one hour (*cf.* Narasimhachari and Seshadri[11]) a mixture of prunetin and genistein was obtained and the separation could be effectively carried out using sodium carbonate in which the former is insoluble. From 1 g. of the trimethyl ether 0·25 g. of prunetin could be obtained.

By carrying out the demethylation at 120° for half an hour[6] an almost quantitative yield of prunetin was obtained. After the product was washed with aqueous sodium carbonate it crystallised from alcohol as colourless prisms melting at 238–40° (mixed m.p. with prunetin undepressed) and gave an acetate identical with prunetin acetate (m.p. and mixed m.p. 223–25°). The carbonate solution on acidification gave only a trace of genistein.

Genistein-7: 4'-dibenzyl ether

Genistein (1·5 g.) was refluxed in acetone solution (250 c.c.) with benzyl chloride (1·3 c.c.) and anhydrous potassium carbonate (8 g.) for 20 hours. Acetone was then distilled off and the residue treated with water. The solid that separated was filtered and washed with water. 7: 4'-O-dibenzyl genistein crystallised from ethyl acetate as colourless tiny prisms melting at 190–92°. It gave a red colour with ferric chloride and was sparingly soluble in aqueous sodium hydroxide. It was not easily soluble in alcohol and acetone. Yield, 1·5 g. (Found: C, 76·9; H, 5·2; $C_{29}H_{22}O_5$ requires C, 77·3; H, 4·9%).

7: 4'-*Dibenzyloxy-5-methoxy isoflavone*

The above compound (1·5 g.) was methylated by refluxing with dimethyl sulphate (0·5 c.c.) and anhydrous potassium carbonate (2 g.) in acetone (200 c.c.) for 20 hours. The solvent was then distilled off and water added to the residue. The colourless solid that separated was filtered, washed with water and crystallised from ethyl acetate. 7: 4'-Dibenzyloxy-5-methoxy isoflavone was obtained in the form of colourless rectangular plates and needles and melted at 189–90°. It gave no colour with ferric chloride and was insoluble in aqueous sodium hydroxide (Found: C, 77·2; H, 5·2; $C_{30}H_{24}O_5$ requires C, 77·6; H, 5·2%).

5-O-*Methyl genistein* (*isoprunetin*)

A suspension of the dibenzyl methyl ether (1 g.) in glacial acetic acid (10 c.c.) was treated with concentrated hydrochloric acid (10 c.c.) and the mixture heated in a boiling water-bath for one hour. It was filtered hot to separate the unchanged benzyl ether and the filtrate diluted with water. The solid product that separated on cooling was filtered and washed with water and small quantities of petroleum ether. It was then dissolved in aqueous sodium carbonate and the solution filtered. The clear filtrate when acidified gave 5-O-methyl genistein as a colourless solid. It was crystallised from ethyl acetate-alcohol mixture when it separated in the form of colourless rectangular plates and prisms melting at 284–86°. It was acetylated with acetic anhydride and pyridine and the acetate on crystallising from ethyl acetate was obtained as colourless rectangular plates melting at 169–70° (Found: C, 65·0; H, 4·0; OCH_3 8·1; $C_{20}H_{16}O_7$ requires C, 65·2; H, 4·3; OCH_3 8·4%). The pure acetate was deacetylated with alcoholic hydrochloric acid and the product crystallised from ethyl acetate-alcohol mixture. 5-O-Methyl genistein separated in the form of colourless

rectangular plates and prisms melting at 284–6°. It dissolved readily in aqueous sodium carbonate and sodium hydroxide and gave no colour with ferric chloride (Found: C, 65·6; H, 4·8; OCH_3 9·9; $C_{16}H_{12}O_5$, $\frac{1}{2}H_2O$ requires C, 65·5; H, 4·5%; OCH_3 10·6%). Yield 0·2 g.

5-*Methoxy*-7: 4′-*diethoxy isoflavone*

(1) *Ethylation of isoprunetin.*—5-O-Methyl genistein (0·2 g.) was refluxed in acetone solution with ethyl iodide (0·5 c.c.) and anhydrous potassium carbonate (2 g.) for 6 hours. On filtering the mixture and distilling off the solvent from the filtrate the ethyl derivative was obtained as a colourless solid. It crystallised from ethyl acetate as colourless stout rhombic prisms and melted at 120–21°. It was identical with 7: 4′-diethoxy-5-methoxy isoflavone obtained from genistein according to the method described below and the mixed melting point was not depressed.

(2) *Methylation of* 7: 4′-O-*diethyl genistein.*—Genistein 7: 4′-diethyl ether was prepared by ethylating genistein with ethyl iodide (2 moles) and potassium carbonate in acetone solution. It crystallised from alcohol as colourless lens-shaped crystals melting at 139–40° (Perkin and Horsfall[7] reported 132–34°). It gave a red colour with ferric chloride and was sparingly soluble in aqueous alkali (Found: C, 69·8; H, 5·6; $C_{19}H_{18}O_5$ requires C, 69·9; H, 5·5%). Its acetate crystallised from alcohol as colourless needles melting at 171–2° (Perkin and Horsfall[7] gave m.p. 168–70°).

Genistein-7: 4′-diethyl ether was boiled with excess of dimethyl sulphate and potassium carbonate in acetone solution for 20 hours; 5-Methoxy-7: 4′-diethoxy isoflavone crystallised from ethyl acetate as colourless stout rhombic prisms melting at 120–21°. It was insoluble in aqueous alkali and gave no colour with ferric chloride (Found: C, 71·0; H, 5·9; $C_{20}H_{20}O_5$ requires C, 70·6; H, 5·9%).

Summary

It is shown that the most convenient method of preparing prunetin is by the partial demethylation of genistein trimethyl ether with hydrobromic or hydriodic acid under controlled conditions. The 5-methyl ether of genistein (isoprunetin) has now been synthesised by the methylation of 7: 4′-O-dibenzyl genistein and subsequent debenzylation. Its properties are very different from those of prunetin and it yields a diethyl ether which could be independently prepared from genistein. The present work conclusively proves that prunusetin cannot be 5-O-methyl genistein (isoprunetin) and should be the same as prunetin.

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