[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF INDIANA UNIVERSITY]

ISOFLAVONES. III. THE STRUCTURE OF PRUNETIN AND A NEW SYNTHESIS OF GENISTEIN[1]

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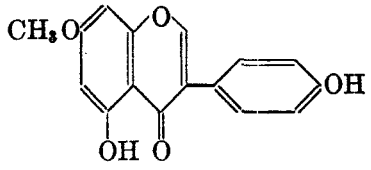
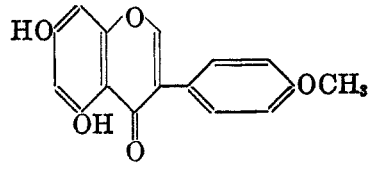
Prunetrin is a glucoside isolated by Finnemore (1) in 1910 from the bark of a species of wild cherry closely related to *Prunus emarginata.* Acid hydrolysis of prunetrin produced glucose and the aglycon, prunetin. The provisional formula (I) was suggested by Finnemore (1) because alkaline degradation formed *p*-hydroxyphenylacetic acid and a phenol which liberated methyl iodide on treatment with hydriodic acid. The demethylation product of prunetin was



I



II

shown by Baker and Robinson (2) to be identical with genistein, an isoflavone isolated from dyer's broom, *Genista tinctoria* by Perkin and Newberry (3) in 1899. Genistein has also been isolated from soybeans by Walz (4), Okano and Beppu (5), and by Walter (6). The structure of genistein has been established as 4′,5,7-trihydroxyisoflavone (Formula VIII) as the result of both degradation (7) and synthesis (8).

Since a number of the natural glycosides of isoflavones have the sugar residue attached to the 7-hydroxyl group, Baker (9) reports that Robinson suggested that the alternative formula, II, for prunetin is one which must be considered. Neither of the compounds possessing structures I or II has been synthesized.

In the present investigation the isoflavone shown by formula II was synthesized and found to differ from prunetin. Hence, Finnemore's structure I appears to be the correct representation for prunetin.

The first stage in the synthesis of the isoflavone II is the preparation of the substituted desoxybenzoin of formula VI. Three methods were studied. One was the Hoesch reaction between phloroglucinol (III) and homoanisonitrile (IV) leading to the ketimine hydrochloride (V), which was hydrolyzed to 2,4,6-trihydroxy-α-*p*-methoxyphenylacetophenone (VI). This method, previously used by Baker and Robinson (2), gave better yields than a Fries rearrangement of 3,5-dihydroxyphenyl homoanisate or direct acylation of phloroglucinol in nitrobenzene solution with homoanisoyl chloride in the presence of anhydrous aluminum chloride.

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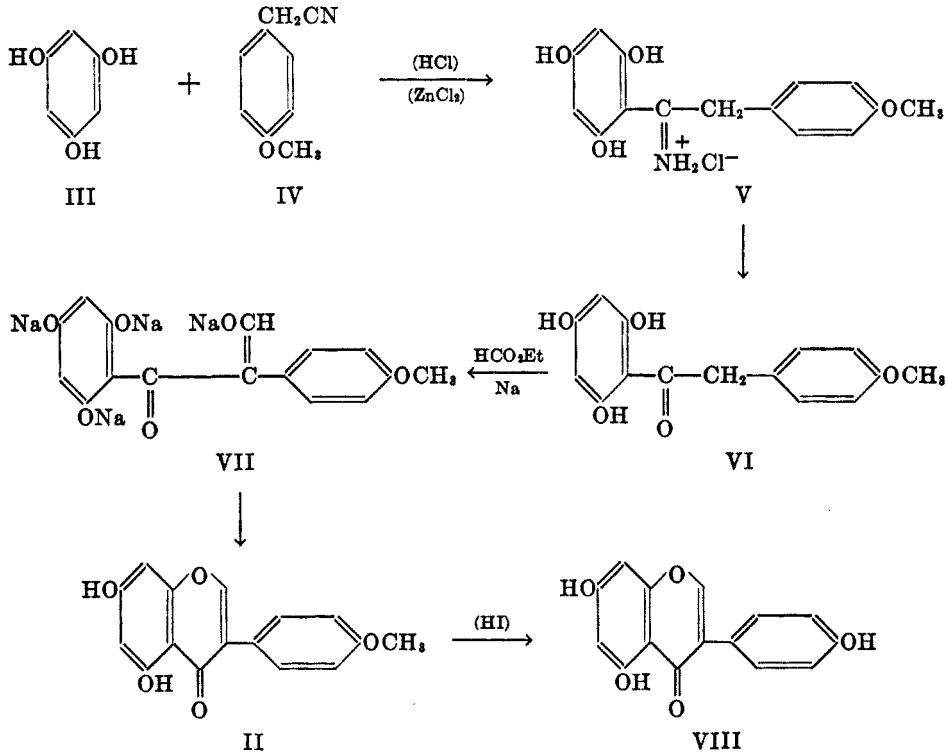
The second step in the synthesis was the condensation of the ketone VI with ethyl formate and sodium. Acidification of the intermediate (VII) produced



the isoflavone of structure (II). This compound melted at 214–215° whereas prunetin melts at 242°. Demethylation of II by means of hydriodic acid gave genistein, 4′,5,7-trihydroxyisoflavone (VIII). This series of reactions thus provides an additional synthesis of genistein by a route different from that used by Baker and Robinson (8).

EXPERIMENTAL

2,4,6-Trihydroxy-α-p-methoxyphenylacetophenone. (Method A). A mixture of 10 g. of anhydrous phloroglucinol, 10 g. of homoanisonitrile (10), 75 ml. of anhydrous ether, and 4 g. of fused zinc chloride was placed in a 150-ml. Erlenmeyer flask and saturated for 3 hours at 0° with dry hydrogen chloride. The mixture was then stoppered and allowed to stand for two days in the coldest part of the refrigerator. At the end of this time 200 ml. of ether was added to the mixture. The ketimine hydrochloride precipitated as a red gummy oil which would not crystallize. The excess ether was carefully decanted from the oil and the crude ketimine hydrochloride added to one liter of 1% sulfuric acid. The mixture was then refluxed for one hour in order to hydrolyze the ketimine hydrochloride. Upon cooling the solution, the crude ketone precipitated partly as an oil which solidified upon standing overnight to form yellowish needles. The precipitate was filtered and recrystallized from 700 ml. of 50% methanol with the aid of Norit. The yield of recrystallized 2,4,6-trihydroxy-α-p-methoxyphenylacetophenone was 17.2 g. (92%) of small nearly colorless crystals melting at 192–193°. This value agrees with that reported previously by Baker and Robinson (2).

(Method B). In a 50-ml. Erlenmeyer flask fitted with a thermometer and an air condenser were placed 4.1 g. (0.015 mole) of 3,5-dihydroxyphenyl homoanisate, 6.6 g. (0.050 mole) of anhydrous aluminum chloride, and 50 ml. of nitrobenzene. The reactants were mixed by shaking, and the flask was then placed in an oil-bath at 60° and heated rapidly until the temperature of the mixture reached 150°. The reaction vessel was kept at this temperature for two hours, and then removed from the oil-bath. When the mixture was cold it was added to a stirred mixture of 50 ml. of cold water and 15 ml. of concentrated hydrochloric acid. The excess nitrobenzene was removed from the mixture by steam distillation and the crude 2,4,6-trihydroxy-α-*p*-methoxyphenylacetophenone recrystallized from 50% methanol with the aid of Norit. The yield of 2,4,6-trihydroxy-α-*p*-methoxyphenylacetophenone was 3.1 g. (75%); m.p. 191–193°.

(Method C). A mixture of 6.3 g. of phloroglucinol (0.05 mole), 19.9 g. (0.15 mole) of anhydrous aluminum chloride, and 50 ml. of nitrobenzene was warmed in a water-bath in a 125-ml. three-necked round-bottomed flask equipped with a stirrer, dropping-funnel, and a hydrogen chloride gas trap. After the phloroglucinol had dissolved, 9.2 g. of homoanisoyl chloride (0.05 mole) was dropped into the mixture over the course of about 15 minutes. The mixture was kept at 100° for two hours. It was then cooled in an ice-bath and with vigorous stirring there was added 25 ml. of concentrated hydrochloric acid diluted with 25 ml. of water and 50 g. of crushed ice. Stirring was continued for one-half hour and the nitrobenzene removed by steam distillation. The residue was concentrated to 200 ml. and cooled. The tarry material and partially crystalline material were filtered off and recrystallized from 50% methanol. After one recrystallization the yield of 2,4,6-trihydroxy-α-*p*-methoxyphenylacetophenone was 6.5 g. (50%) of crystals melting at 191–193°.

4'-Methoxy-5,7-dihydroxyisoflavone. To 1 g. (0.043 mole) of powdered sodium at 0° was added 2.0 g. of 2,4,6-trihydroxy-α-*p*-methoxyphenylacetophenone dissolved in 30 ml. of redistilled ethyl formate. The mixture was stirred for ten hours at 0° and then allowed to stand overnight in the refrigerator. Twenty grams of crushed ice was added and the mixture stirred for four hours or until the excess ethyl formate had evaporated. The solid material was then filtered off, dissolved in pyridine and precipitated with water. Repeated precipitation gave 0.8 g. (29%) of yellowish needles melting at 213–215°. Recrystallization from ethanol gave long white needles melting at 214.5–215°.

Anal. Calc'd for $C_{16}H_{12}O_5$: C, 67.60; H, 4.25.
Found: C, 67.16; H, 4.36.

Genistein (4',5,7-trihydroxyisoflavone). To 0.5 g. of 4'-methoxy-5,7-dihydroxyisoflavone contained in a 25-ml. Erlenmeyer flask fitted with a reflux condenser was added 10 ml. of hydriodic acid (sp. gr. 1.7). The mixture was refluxed for four hours. At the end of this time the excess acid was neutralized with 30% potassium hydroxide solution and the mixture then made slightly acidic with acetic acid. Upon cooling, 0.2 g. of white needles separated. Recrystallization from dilute ethanol gave white needles which melted at 285–293° with decomposition.

Anal. Calc'd for $C_{15}H_{10}O_5$: C, 66.67; H, 3.71.
Found: C, 66.43; H, 3.61.

SUMMARY

4'-Methoxy-5,7-dihydroxyisoflavone has been synthesized and found to be different from prunetin. Demethylation of this synthetic isoflavone produced genistein.

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