Proceedings of the Iowa Academy of Science

Volume 61 | Annual Issue Article 33

1954

Synthesis of Estrogenic Isoflavone Derivatives

Lester Yoder
Iowa Agricultural Experiment Station

Edmund Cheng
Iowa Agricultural Experiment Station

Wise Burroughs
Iowa Agricultural Experiment Station

Synthesis of Estrogenic Isoflavone Derivatives*

By Lester Yoder, Edmund Cheng and Wise Burroughs

Natural estrogens in plants have assumed considerable importance since the demonstration of their presence in subterranean clover by Bennetts (1) in 1946 and the identification of the isoflavone, genistein (V,4′,5,7-OH) as the probable substance responsible for some of the estrogenic activity by Curnow and Bennetts (2) in 1952. Earlier the isoflavones were found in soybean meal as genistein glucoside (3) (5), methyl genistein (V, 6′,5,7,-OH,8-CH_3), isogenistein (V,6′,5,7-OH), tatoin (V,4′,5-OH,8-CH_3) (4), daidzein (V,4′,7-OH) and formononetin (V,4′-OCH_3,7-OH) (5). Furthermore the isoflavone biochanin A (V,4′-OCH_3,5,7-OH) was isolated from chana seed by Bose and Siddique (6) and formononetin from subterranean clover by Bradbury and White (7).

A practical procedure for synthesizing a number of the isoflavone derivatives was in demand due to the fact that naturally occurring estrogens in feeds were believed involved in the coming practice of improving rations of farm animals by estrogen supplementation by Burroughs and others (8) at the Iowa Agricultural Experiment Station. Therefore comprehensive tests of estrogenic activity of the isoflavones needed to be carried out. These tests are reported in part elsewhere by Cheng et al. (9).

A number of partial procedures for the synthesis of isoflavones have been published. The synthesis involves the condensation of two intermediate aromatic phenolic derivatives. Of these only the substituted benzyl cyanides (I, Chart I) were not available commercially and published procedures for their synthesis proved difficult. However the preparation of these by Julian and Sturgis (10) Chart I, through the Gränacher (11) synthesis, was most promising as to yields, variety and availability of the starting materials, hydroxybenzaldehydes and rhodanine. The example given, i.e., the synthesis of 3,4-dimethoxyphenyl-acetonitrile starting with veratrol, does not apply well as published with the open hydroxybenzaldehydes necessary for synthesis of various estrogenic isoflavones.

For the condensation of the phenyl-acetonitriles with various phenols to produce the deoxybenzoins (III) the Hoesch (12)

*Journal Paper No. J-2508 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 1208.

hydrogen chloride synthesis proved satisfactory as shown in the general flow sheet depicted in Chart II.

All of these isoflavones have been synthesized, in small quantities and in poor yields, genistein by Baker and Robinson (13) and biochanin A by Shriner and Hull (14), and daidzein and formononetin by Wessely, Kornfeld and Lechner (15).

Most attempts at synthesis have been concerned with shortening the synthesis by ring closure of the deoxybenzoins to form the inner pyrone ring without protecting the hydroxyl groups not involved. Thus Baker and Robinson (13) synthesized genistein in 1928 by cinnamoylization of the exposed 6-hydroxy group of methylated deoxybenzoin then by dehydration and inner ring closure obtained the 2-styryl isoflavone. Shriner and Hull (14) shortened the synthesis by formylating the unmethylated unprotected hydroxydeoxybenzoin with sodium powder and ethyl formate. The yields of genistein were too poor by these procedures in our hands. Baker and coworkers had been oxidizing the styryl derivatives with permanganate at the double bond on the 2-methylene group to produce a 2-carboxyl which was found to evolve carbon dioxide at the melting point. Baker et al. (16) recently utilized this idea in a synthesis of genistein adaptable to a wide variety of substituted deoxybenzoins in which the 2-carboxyisoflavone was produced directly in a 60 percent yield with ethyl oxalyl chloride in a pyridine solution of the deoxybenzoin followed by mild saponification. Heating the acid through the melting point was said to produce a 98 per cent yield of the isoflavone but proper details of the procedure have not been published (17).

The four 2-carboxyisoflavones (IV) isolated in this total synthesis have not been described heretofore. They embody a chromophore grouping which imparts a yellow color diminishing in intensity with the lack of the 5-hydroxy group in the isoflavones (IV).

We have now developed this scheme to produce satisfactory yields of the isoflavones, genistein, daidzein, biochanin A, and formononetin.

Synthesis of Intermediates

1. *Preparation of phenyl-acetonitriles (I).* When R = CH_3 the procedure of Julian and Sturgis (10) produced satisfactory yields from rhodanine (Pract) and the corresponding aldehydes. However note (c) should be observed.

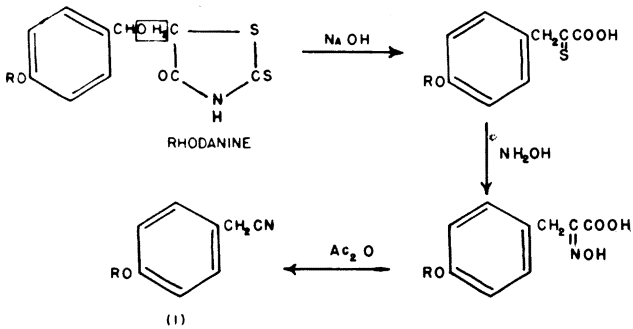

Chart I. Synthesis of phenylacetonitriles.

When R = H a number of modifications of the published procedure were necessary.

(a) The aldehyde-rhodanine condensation mixture was poured into only 1 to 1.5 l. of water and the filter cake washed with methanol.
(b) In the alkali cleavage of aldehyde-rhodanine the solution with the alkali was heated only 7 min. at 90-100°. The filter cake from the acidified solution was water washed, suctioned well and although still moist, was ready for conversion into the oxime in the next step. The oximine pyruvic acid crystallized sufficiently pure from the vacuum distilled alcohol reaction residue. The overall yield to the oximine was 70 per cent.
(c) The dehydration of the oximino-pyruvic acid to p-hydoxyphenylacetonitrile with acetic anhydride had to be carried out at not more than 10° above the decomposition point and with occasional cooling in an ice bath while stirring with a thermometer. The temperature was raised to 85° to test for the end of the reaction. The acetic anhydride distillation residue even then consisted partially of p-acetoxyphenylacetonitrile. The ester fraction was saponified by stirring the water-washed distillation residue at 40° with 10 per cent sodium hydroxide until solution was complete. Acidification of the cold solution with dilute hydrochloric acid precipitated the p-hydroxyphenyl-acetonitrile which could be dried and recrystallized from isopropyl ether in almost quantitative yield. When R = CH_3 oil was formed which had to be distilled at 150 to 160° at 20 mm.

2. *Preparation of the deoxybenzoins (III).* The hydrogen chloride (12) condensation of the nitriles with phloroglucinol or resorcinol succeeded without the use of anhydrous zinc chloride according to Baker and Robinson (13). It was modified further and carried out as shown in Chart II as follows:

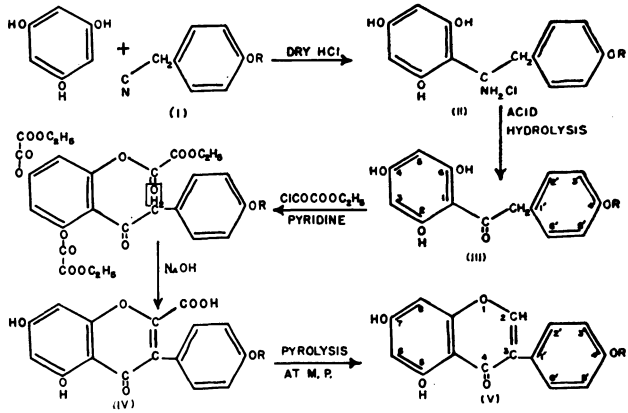

Chart II. Synthesis of isoflavones.

A solution of anhydrous phloroglucinol (9.4 g.) and p-hydroxyphenyl-acetonitrile (10 g.) in 75 ml. of dry ether in an Erlenmeyer flask was cooled to 0° in an ice bath and a slow stream of hydrogen chloride passed into the solution to saturation. The ketimine hydrochloride (II, Chart II) separated on the bottom. The flask was stoppered and set in a refrigerator for 12 hrs. then the mixture again saturated with hydrogen chloride and kept in the refrigerator for 24 hrs. more. The ether was decanted and the crust washed with ether. The insoluble ketimine hydrochloride was then heated on the steam bath with 400 ml. of 2 per cent hydrochloric acid until hydrolized to the deoxybenzoin (III) which crystallized on cooling. Yield 12 g. A further 2 g. were obtained from the ether extract. The total yield was 65 per cent. Recrystallized from 50 per cent methanol, it melted at 277°.

(a) The analogue (III, 4′-OCH_3, 2,4,6-OH) deoxybenzoin made by the same procedure using p-methoxyphenyl-acetonitrile instead of p-hydroxyphenyl-acetonitrile melted at 191°.
(b) The corresponding analogues (III, 4′-OH,4,6-OH) (III, 4′-OCH_3, 4,6-OH) using resorcinol instead of phloroglucinol in the above procedure, melted at 188° and 154° respectively.

Synthesis of the Isoflavones

1. *Formation of the pyrone ring of the isoflavones (V).* Deoxybenzoin (III, 4′,2,4,6-OH), 2.6 g., 0.01 mole, prepared as in procedure 2, was dissolved in 14 ml. dry pyridine and 5.5 ml., 0.04 mole, of ethyl oxalyl chloride (18) added dropwise with stirring at a reaction temperature of 40 to 50°. At the end of the reaction the mixture was warmed while stirring to 65° for 20 min. then cooled, triturated with ether and the mixture stirred into 50 ml. of

ice water containing an excess of hydrochloric acid and transferred to a separatory funnel. Two more extractions with ether were combined and washed with dilute hydrochloric acid and water then dried with sodium sulfate. The ether was distilled and the residue combined with any crystals gathered from the aqueous phase by filtration and treated in 10 times their weights of water with enough 50 per cent sodium hydroxide solution to maintain a pH of 12 for 3 hrs. The oxalyl ester was thus completely saponified. Addition of con. hydrochloric acid to a pH of 2 liberated the water insoluble yellow 2-carboxylic acid (IV) of genistein, the isoflavone (V,4′,5,7-OH). It crystallized on standing. The yield was 2.5 g. or 80 per cent. Recrystallized from 50 per cent methanol, it melted at 320° (decomp.).

Anal. Calcd for $C_{16}H_{10}O_7$: C, 61.15; H, 3.28. Found C, 60.84; H, 3.47.

(a) The deoxybenzoin (III, 4′,4,6-OH) 0.01 mole, by procedure 1 required only 0.03 mole of ethyl oxalyl chloride to produce the less colored 2-carboxylic acid of daidzein. It melted at 293° (decomp.).

Anal. Calcd. for $C_{16}H_{10}O_6$-$2H_2O$: C, 57.49; H, 4.22. Found C, 58.64; H, 4.41.

(b) The deoxybenzoin (III, 4′OCH_3,2,4,6-OH) 9.01 mole, by procedure 1 required 0.03 mole of ethyl oxalyl chloride to produce the slightly colored 2-carboxylic acid of biochanin A. Recrystallized from methanol, it melted at 275° (decomp.). Anal. Calcd. for $C_{17}H_{12}O_7$-CH_3OH: C, 60.00; H, 4.47. Found C, 59.55; H, 4.56.

(c) The deoxybenzoin (III, 4′-OCH_3,4,6-OH), 0.01 mole, by procedure 1 required 0.02 mole of ethyl oxalyl chloride to produce almost colorless 2-carboxylic acid of formononetin. Recrystallized from methanol, it melted at 263-4° (decomp). Anal. Calcd. for $C_{17}H_{12}O_6$-CH_3OH: C, 62.78; H, 4.68. Found C, 60.86; H, 4.63.

2. *Pyrolysis of the 2-carboxylic acids (IV) of the isoflavones.* The oven dried 2-carboxy-4′,5,7-trihydroxyisoflavone, 2.5 g., was tamped into the bottom of a 50 ml. centrifuge tube which was plunged into an alloy bath heated 5° above the melting point of the acid, i.e. 325°. After evolution of carbon dioxide had ceased in less than 5 min. the tube was removed and air cooled. The glassy melt was triturated into solution in a minimum of boiling ethanol and passed through a column of half its weight in Norite.

After concentration of the hot solution and dilution with a volume of hot water the isoflavone crystallized in colorless flat needles melting at 295° in a yield of 2.0 g. of genistein.

(a) When this procedure was applied to 2-carboxy-4′,7-dihydroxyisoflavone of daidzein the insolubility of the prolysis product necessitated solution in ethanol at the rate of 0.5 g. per 200 ml. solvent. However the norited solution could be concentrated by boiling to 50 ml. to crystallize daidzein as flat colorless needles on cooling. It melted at 330°.
(b) The 2-carboxy-4′,7-dihyroxyisoflavone did not appear to melt since the melting point of daidzein is so high that pyrolysis at 300° had to be carried out blindly for 5 min.
(c) Biochanin A from 2-carboxy-4′-methoxy-5,7,-dihydroxyisoflavone by the above procedure formed clumps of curved crystals by crystallization from ethanol diluted with hot water and melted at 215°.
(d) Formononetin from 2-carboxy-4′-methoxy-7-hydroxyisoflavone by the above procedure formed plates from ethanol which melted at 255°.

All of the melting points agree reasonably well with those of the literature.

Summary

Detailed procedures are given for the total synthesis of the naturally occurring isoflavone derivatives, genistein, daidzein, formononetin and biochanin A. p-Hydroxy- and p-methoxybenzyl cyanides were prepared through the condensation of the benzaldehydes with rhodanine by modifications of the Gränacher thio- and oximino-pyruvic acid synthesis. The isoflavone rings were formed through the Hoesch condensation of the benzylcyanides with phloroglucinol or resorcinol and inner pyrone ring formation by the Baker-Olis reaction with ethyl oxalyl chloride to yield the 2-carboxyl derivatives. Pyrolysis at the melting points evolved carbon dioxide to yield the isoflavones desired.

Literature Cited

1. Bennetts, H. W., Austral. Vet. J., *22*, 70 (1946).
2. Curnow, D. H. and Bennett, H. W. Abstract of papers from 6th International Grassland Congress (1952).
3. Walter, D. D., J. Am. Chem. Soc., *63*, 3273 (1941).
4. Okano, K. and Beppu, J., J. Agri. Chem. Soc. Japan, *15*, 645 (1939).
5. Waltz, E. Ann., *489*, 118 (1931).
6. Bose, J. and Siddiqui, S. J., Sci. & Ind. Research, *4*, 231 (1945). C.A., *40*, 2832 (1946).
7. Bradbury, R. B. and White, D. E., J. Chem. Soc., 3447, (1951); 871 (1953).
8. Burroughs, W., Culbertson, C. C., Kastelic, J., Hammond, W. E., and Cheng, E., Iowa Agric. Exp. Sta. A.H. Leaflet No. 189 (1954).
9. Cheng, E., Story, C. D., Yoder, L., Hale, W. H., and Burroughs, W., Science, *118*, 164 (1953); J. Animal Sci., *12*, 507 (1953).
10. Julian, P. L., and Sturgis, B. M., J. Am. Chem. Soc., *57*, 1126 (1935).

11. Gränacher, Ch., Helvetica Chim. Acta, *5,* 610 (1922); Gränacher, Ch., Gerö, M., Ofner, A., Kloppenstein, A., and Schlatter, E. *ibid., 6,* 458 (1923).
12. Hoesch, K., Ber., *48,* 1122 (1915).
13. Baker, W. and Robinson, R., J. Chem. Soc. (a) 2715 (1926) (b) 3115 (1928).
14. Shriner, R. L., and Hull, C. J., J. Org. Chem., *10,* 228, 288 (1945).
15. Wessely, F., Kornfeld, L., and Lechner, F., Ber., *66,* 685 (1933).
16. Baker, W., Olis, W. D., Binns, P. J. L., Chadderton, J., Dunstan, J., Harborne, J. B., and Weight, D., Nature, *169,* 706 (1952).
17. Baker, W., Harborne, J. B., and Olis, W. D., Chemistry and Industry, 1058 (1952).
18. Weygand, C., Organic Preparations 105 (1945). Interscience Publishers, New York.

Iowa Agricultural Experiment Station
Ames, Iowa