

Catalytic Hydrogenation of Isoflavones. The Preparation of (\pm)-Equol and Related Isoflavans

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Abstract

Hydrogenation of daidzein, or preferably its *O,O*-diacetyl derivative, gives (\pm)-equol in good yield only over Pd/C prepared according to the Wessely and Prillinger method, whereas other Pd/C catalysts give mixtures of products. Hydrogenation of *O,O,O*-triacetylgenistein can be used to prepare isoflavan-4',5,7-triol.

The isoflavones formononetin (1) and daidzein (2) are converted in the sheep into ($-$)-isoflavan-4',7-diol (3) (($-$)-equol),¹ and the oestrogenic effect of subterranean clover has been attributed to ($-$)-equol produced in this manner.² Accordingly, a convenient method has been required for preparation of (\pm)-equol for biological studies. The method³ of Wessely and Prillinger for hydrogenation of daidzein (2) has been found to give a satisfactory yield of (\pm)-equol only if their special conditions⁴ for the preparation of the catalyst are observed. The Pd/C catalyst is first suspended in glacial acetic acid and shaken in an atmosphere of oxygen for several days before use. Hydrogenation of daidzein over this catalyst is extremely rapid and the conversion into (\pm)-equol is usually complete in 15-30 min. A defect of the method is the large amount of Pd/C required, but attempts to use less catalyst, or to recover the catalyst and re-treat with oxygen, have given mixtures of products. When commercial grades of Pd/C are used without prior treatment with oxygen the hydrogenation proceeds very slowly or not at all at atmospheric pressure. The low solubility of daidzein in glacial acetic acid can be overcome to a very limited extent by addition of a small proportion of diglyme, and it is more convenient to carry out the hydrogenation on the more soluble *O,O*-diacetyldaidzein.

Catalytic hydrogenation of *O,O*-diacetyldaidzein (4) in acetic acid over Pd/C which has not been treated with oxygen is facilitated by an increase in temperature and hydrogen pressure, but conditions for the preparation of (\pm)-equol without large amounts of other products have not been established. Even though there is a large amount of unchanged *O,O*-diacetyldaidzein, the second most abundant hydrogenation product, after hydrolysis of *O*-acetyl groups, has been identified as 3-cyclo-

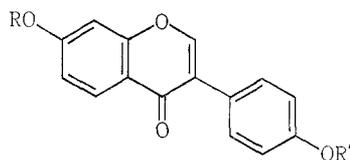
¹ Braden, A. W. H., Hart, N. K., and Lamberton, J. A., *Aust. J. Agric. Res.*, 1967, **18**, 335.

² Shutt, D. A., Weston, R. H., and Hogan, J. P., *Aust. J. Agric. Res.*, 1970, **21**, 713.

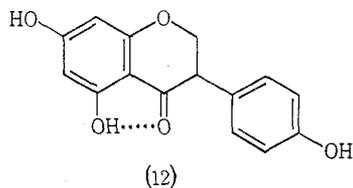
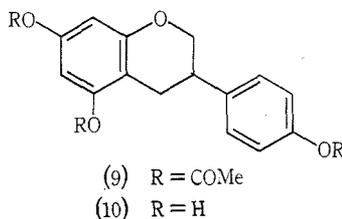
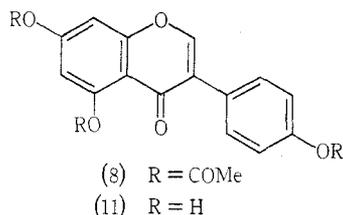
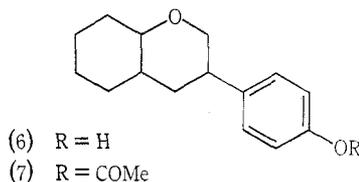
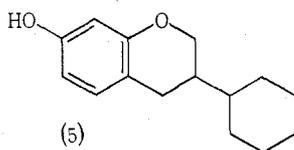
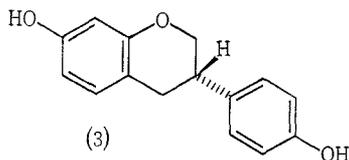
³ Wessely, F., and Prillinger, F., *Ber. Dtsch. Chem. Ges.*, 1939, **72**, 629.

⁴ Wessely, F., and Prillinger, F., *Sitzungsber. Akad. Wiss. Wien, Math.-Naturwiss. Kl., Abt. 2A*, 1938, **147**, 273.

hexyl-3,4-dihydro-2*H*-1-benzopyran-7-ol (5), formed by saturation of ring C and hydrogenolysis of an *O*-acetyl group. A minor product (c. 10% of the amount of (5)) has been shown to be 4-(perhydro-1-benzopyran-3-yl)phenol (6), of unestablished stereochemistry, formed by complete reduction of ring A.



	R	R'
(1)	H	Me
(2)	H	H
(4)	COMe	COMe



Similar hydrogenation experiments have been carried out with *O,O,O*-triacetylgenistein (8). Although the minor products have not been subjected to such detailed investigation in this instance, isoflavan-4',5,7-triyl triacetate (9) was isolated in 60% yield, and converted into isoflavan-4',5,7-triol (10). When genistein (11) was subjected to hydrogenation a complex mixture of products was obtained. 4',5,7-Trihydroxyisoflavan-4-one (12) was isolated from the mixture.

Experimental

(a) (±)-Equol by a Modified Wessely-Prillinger Method

Palladium-on-charcoal (10%, 30 g) was suspended in glacial acetic acid (600 ml) and shaken under oxygen for 2-3 days. The suspension of catalyst was added to a solution of *O,O*-diacetyl-*daidzein* (6.7 g) in glacial acetic acid (1.3 l.). On shaking with hydrogen at atmospheric pressure, uptake was rapid and after 30 min the solution was filtered through Celite and the layer of Pd/C washed with hot acetic acid (1 l.). The combined filtrates were evaporated; the residue was taken up in CHCl_3 and washed with NaHCO_3 (5%) in water. Evaporation of the CHCl_3 solution gave

crystalline *O,O*-diacetylsquol which was suspended in a solution of NaOH (7 g) in ethanol (40 ml) and water (5 ml). After heating at 100° for 5 min the solution was acidified (HCl) and diluted with a little hot water to give (±)-squol as colourless crystals, 4.1 g, m.p. 158–160° (lit.³ 158°).

(b) *Catalytic Hydrogenation of O,O-Diacetyldaidzein over Untreated Pd/C*

In a typical experiment *O,O*-diacetyldaidzein (12 g) was hydrogenated at 95° and 6890 kPa in acetic acid (400 ml) over Pd/C (5%, 12 g) for 10 h. The products were chromatographed on silica gel and *O,O*-diacetyldaidzein (1.5 g) was recovered. The remainder was hydrolysed (KOH/EtOH) and the products (6 g) chromatographed on silica gel. Elution with chloroform gave 3-cyclohexyl-3,4-dihydro-2H-1-benzopyran-7-ol (5) (1.2 g), m.p. 118–119° (Found: C, 77.7; H, 8.8. C₁₅H₂₀O₂ requires C, 77.6; H, 8.6%). *m/e* 232 (M, base peak). P.m.r. (CDCl₃) δ 1.0–2.0, broad m, 12H, cyclohexyl, C3–H; 2.4–2.7, m, 2H, C4–H; 3.6–4.4, m, 2H, C2–H; 6.2–7.0, m, 3H, Ar–H. The material recovered from the mother liquors after crystallization of (5) contained another product which was isolated by preparative t.l.c. on silica gel plates developed in chloroform/4% methanol. The faster-moving material was shown to be (5), while the lower R_F band gave 4-(perhydro-1-benzopyran-3-yl)phenol (6) as a colourless gum. The 4'-*O*-acetyl derivative (7) was obtained as colourless crystals, m.p. 55–60°, from methanol/water (Found: C, 74.0; H, 7.8. C₁₇H₂₂O₃ requires C, 74.4; H, 8.1%). *m/e* 274 (M, 15% of base peak). P.m.r. (CDCl₃): δ 1.0–2.2, br, 11H; 2.55, s, 3H, OAc; 2.6–4.3, m, 4H; 6.96, 2H, d, C3'–H, C5'–H; 7.20, 2H, d, C2'–H, C6'–H. After the isolation of (5) and (6), continued elution of the column with chloroform/1% methanol gave (±)-squol (2.5 g).

(c) *Catalytic Hydrogenation of O,O,O-Triacetylgenistein*

O,O,O-Triacetylgenistein (4.5 g) was dissolved in acetic acid (100 ml) and hydrogenated over Pd/C (5%, 4.5 g) at 95° and 9990 kPa for 22 h. The product was chromatographed on silica gel and elution with chloroform gave isoflavan-4',5,7-triyl triacetate (9) 2.7 g, colourless crystals from methanol, m.p. 137.5–139° (Found: C, 65.4; H, 5.3. C₂₁H₂₀O₇ requires C, 65.6; H, 5.2%). M 384. P.m.r. (CDCl₃): δ 2.24, 6H, s, 2×OAc; 2.28, 3H, s, OAc; 3.99, 1H, t, *J* 11 Hz, C2–H_{ax}; 4.32, 1H, dd, *J* 11, 4 Hz and *J* 2 Hz long-range coupling to a C4-proton, C2–H_{eq}; 6.50, 6.56, AB doublets, *J* 2 Hz, C5, C8–H; 7.07, 7.25, AA'BB' system, *J*_{AB} 9 Hz, C2',C3',C5',C6'–H. Hydrolysis of compound (9) (7.7 g) in ethanol (50 ml) with NaOH (3 g) at 90° for 30 min afforded isoflavan-4',5,7-triol (10) (1.1 g), colourless crystals from water, 209–212° after preliminary melting and resolidification at 90° (Found: C, 62.4; H, 5.9. C₁₅H₁₄O₄·2H₂O requires C, 61.2; H, 6.2%). M 258. P.m.r. (Me₂SO) δ 3.82, 1H, t, *J* 11 Hz, C2–H_{ax}; 4.03, 1H, dd, *J* 11, 4 Hz, C2–H_{eq}; 5.66, 5.87, AB doublets, *J* 2 Hz, C6,C8–H; 6.68, 7.05, AA'BB' system, *J*_{AB} 9 Hz, C2',C3',C5',C6'–H. Even after prolonged drying, microanalysis indicated some retention of water. When genistein (11) (1.0 g) itself was dissolved in acetic acid (350 ml) and hydrogenated at 95° and 6890 kPa for 14 h, a mixture of products was obtained. These were chromatographed on silica gel to give 4',5,7-trihydroxyisoflavan-4-one (12) (200 mg) as colourless crystals from CHCl₃/MeOH, m.p. 221–222° (Found: C, 65.9; H, 4.5. Calc. for C₁₅H₁₂O₅: C, 66.2; H, 4.4%). M 272. P.m.r. (Me₂SO) δ 3.94, 1H, t, *J* 7 Hz, C3–H; 4.48, 2H, d, *J* 7 Hz, both C2–H; 5.84, 2H, s, C6,C8–H; 6.67 and 7.04, AA'BB' system, *J*_{AB} 9 Hz, C2',C3',C5',C6'–H. (lit.⁵ m.p. of 4',5,7-trihydroxyisoflavan-4-one monohydrate, 215.5–217°).

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⁵ Inoue, N., *Nippon Kagaku Zasshi*, 1958, **79**, 1537.