Sterol Constituents of *Daedalea quercina* L. (Fr.)

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In the course of our studies of higher terpenoids, we have examined the ethyl acetate extract of *Daedalea quercina* L. (Fr.) and isolated a mixed crystal of ergosterol and 5-dihydroergosterol, together with 5-dihydroergosterol (ergosta-7, 22-dien-3β-ol) and ergosterol peroxide (5, 8-epidioxyergosta-6, 22-dien-3β-ol).[1]

The sources of *D. quercina* were as follows; (1) Commercially supplied; living on beech; 380 g. (2) Commercially supplied; generally called "Baikisei" and used as a herb medicine; living on Prunus Mume Sieb. et Zucc.; 2.5 kg. (3) Living on beech. Collected in April and May, 1962, at Tanzawa in Kanagawa Prefecture; 15 kg.

The first sample gave a neutral alcoholic compound, named DQ-ol A_1. The ultraviolet spectrum of DQ-ol A_1 was very similar to that of ergosterol, but its intensities were slightly weaker. The concentrated extract of the second, "Baikisei," gave DQ-ol A_2, which was found to be identical with DQ-ol A_1. The mother liquor furnished, on chromatographic separation, DQ-ol B and DQ-ol C, which were later identified as 5-dihydroergosterol and ergosterol peroxide respectively. The third sample afforded DQ-ol A_3 which showed much weaker absorptions in the ultraviolet spectrum than those of DQ-ol A_1.

The constants of DQ-ol A_1 were found to be very similar to those of "neosterol," which was first considered by Wieland et al.[2] to be a new sterol isolated from yeast fat. Barton et al.[3] have established, however, that neosterol consists of 75.5% of ergosterol and 24.5% of 5-dihydroergosterol, and have found that "synthetic neosterol" and its benzoate can not be separated by recrystallization. Table I shows the constants of neosterol, "synthetic neosterol", DQ-ol A_1, and ergosterol.

From these data, it seems probable that DQ-ol A_1 is a mixed crystal of ergosterol and 5-dihydroergosterol, as in the case of neosterol, and that DQ-ol A_3 contains much more 5-dihydroergosterol. The chemical evidence for these conclusions is as follows:

a) On catalytic hydrogenation over platinum oxide in acetic acid, DQ-ol A_1 absorbed 1.89 mol. of hydrogen, while ergosterol absorbed 2.01 mol. of hydrogen under the same conditions.

b) DQ-ol A_3 acetate gave only 5-dihydroergosteryl acetate on hydrogenation over Raney nickel in benzene; 0.49 mol. of hydrogen was absorbed.

c) The photosensitized oxidation of DQ-ol A_3 afforded 5-dihydroergosterol in a 60% yield and a small amount of ergosterol peroxide.

d) 3β-Acetoxy-Δ^7- and -$\Delta^{5,7}$-steroids are well known to show their C-18 methyl signals at τ 9.43 and τ 9.37 respectively.[4] The NMR spectrum of DQ-ol A_3 acetate consisted of two signals of an approximately equal intensity at τ 9.36 and 9.46,*1 and the whole spectrum could be interpreted as the sum of those of ergosteryl acetate and of 5-dihydroergosteryl acetate.

e) The infrared spectra of DQ-ol A_1 and A_3 may be well interpreted as a sum of the spectra of ergosterol and 5-dihydroergsoterol.

Table II shows the estimated compositions of DQ-ol A_1 and of DQ-ol A_3.

TABLE I. CONSTANTS OF NEOSTEROL, "SYNTHETIC NEOSTEROL," DQ-OL A_1 AND ERGOSTEROL

Substance	M. p. °C	$[\alpha]_D$	ε at λ_{max} 271 mμ	282 mμ	293 mμ
Neosterol[2]	164—165	−105°	11400	11400	6900
"Synthetic neosterol"[3]	164—165	−107°	9500	10100	5000
DQ-ol A_1	160—161	−103°	9100	9450	5300
Ergosterol	162—163	−119°	11500	12100	7050

1) Cf. L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corporation, New York (1959), Chap. 4.
2) H. Wieland and M. Asano, *Ann.*, **473**, 300 (1929); H. Wieland, F. Rath and H. Hesse, ibid., **548**, 34 (1941).
3) D. H. R. Barton and D. J. Cox, *J. Chem. Soc.*, **1948**, 1357.
4) R. F. Zürcher, *Helv. Chim. Acta*, **46**, 2054 (1963).
*1 Run on a Japan Electron Optics Model 3H-60 high-resolution spectrometer at 60 Mc. in a $CDCl_3$ solution, using TMS as the internal standard.

TABLE II. ERGOSTEROL IN DQ-OL A*2

Estimated from the data in:	DQ-ol A_1	DQ-ol A_3	DQ-ol A_3 acetate
$[\alpha]_D$	74%	30%	—
UV	79%	35%	50%
Hydrogenation	80%	—	49%

Experimental*3

Isolation.—*DQ-ol A_1*.—Chopped, air-dried fungus (100 g.) was extracted with ethyl acetate (800 cc.). After filtration, the ethyl acetate extract was concentrated to a small volume and crude crystals (330 mg.), m. p. 153—160°C, were separated out; these gave white needles after filtration through the column of alumina, m. p. 160—161°C (from ethyl acetate), $[\alpha]_D$ −103° (*c* 1.72), λ_{max}^{EtOH} 271 mμ (ε 9100), 282 mμ (ε 9450) and 293 mμ(ε 5300), ν_{OH} 3622.1 cm^{-1}*4 (in CCl_4 soln., secondary hydroxyl group), ν^{Nujol} (cm^{-1}) 1664, 1605, 1050, 968, 846(sh.), 835, 802.

Found: C, 82.93, 83.06, 82.09; H, 11.39, 11.41, 11.18. Calcd. for $C_{28}H_{44}O\cdot\frac{1}{2}H_2O$: C, 82.90; H, 11.18: for $C_{28}H_{46}O\cdot\frac{1}{2}HO$: C, 82.61; H, 11.62%.

DQ-ol A_2.—Powdered, air-dried fungus "Baikisei" (2.5 kg.) was extracted with ethyl acetate (10 l.). The extract, worked up as above, gave crude crystals; these in turn yielded DQ-ol A_2 (11 mg.), as white needles, m. p. 160—161°C, virtually identical with that of DQ-ol A_1.

The dark brown residue of the mother liquor was dissolved in benzene and adsorbed on alumina (600 g.). The column was eluted with (i) benzene, (7 l.), (ii) benzene-ether (9 : 1) (7 l.), (iii) benzene-ether (3 : 1) (5 l.), (iv) benzene-ether (1 : 1) (3 l.) and (v) ether (4 l.). Fraction (ii) gave 400 mg. of DQ-ol B (characterized as its acetate; m. p. 172—174°C, identical with that of 5-dihydroergosteryl acetate). Fraction (v) gave 80 mg. of DQ-ol C, m. p. 171—173°C, identical with that of an authentic sample of ergosterol peroxide.

DQ-ol A_3.—Fifteen kilograms of *Daedalea quercina* L. (Fr.) was extracted by the same method. After purification, DQ-ol A_3 (4.51 g.), m. p. 162—163°C, $[\alpha]_D$ −54° (*c* 1.53), λ_{max}^{EtOH} 271 mμ (ε4550), 282 mμ (ε 4600) and 293 mμ(ε 2700) was obtained. (Mixed m. p. with DQ-ol A_1: 158—162°C). The infrared spectrum of DQ-ol A_3 is virtually identical with that of DQ-ol A_1, except for the two peaks at 835 and 802 cm^{-1}, which are weaker than in the case of A_1.

On acetylation DQ-ol A_3 gave its acetate, m. p. 172—174°C (from methanol), λ_{max}^{EtOH} 271 mμ (ε 6100), 282 mμ(ε 6400) and 293 mμ(ε 3800).

The Photosensitized Oxidation of DQ-ol A_3.—A hot solution (about 60°C) of DQ-ol A_3 (500 mg.) in 95% ethanol (200 cc.), containing eosin (3 mg.), was aerated with a dry air-stream for 9 hr. under irradiation by a 200-watt lamp. After cooling, white crystals (305 mg.) were separated from the reaction mixture. Recrystallization from ethanol gave 5-dihydroergosterol, m. p. 172—173.5°C; identified with an authentic sample prepared by the hydrogenation of ergosterol.

After the concentration of the mother liquor, a small amount of the crystalline product was obtained. Its infrared spectrum was completely superimposable upon that of ergosterol peroxide.

The Hydrogenation of DQ-ol A_3 Acetate.—DQ-ol A_3 acetate (440 mg.) in thiophene-free benzene was hydrogenated over Raney nickel at room temperature. Within 10 min., 13.2 cc. of hydrogen was absorbed. The reaction mixture was then worked up in the usual manner, and the crystallization of the product from ethyl acetate gave 5-dihydroergosteryl acetate (350 mg.), m. p. 173°C, $[\alpha]_D$ −17.6° (*c* 2.7)

Under the same conditions, ergosteryl acetate (440 mg.) absorbed 27.2 cc. of hydrogen to yield 5-dihydroergosteryl acetate (320 mg.), m. p. 174°C, $[\alpha]_D$ −16.2° (*c* 1.85).

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*2 The other component is 5-dihydroergosterol.

*3 All the m.p.'s are measused in the capillary and are uncorrected. The specific rotations are for chloroform solutions.

*4 Run on a Perkin-Elmer 112G spectrometer.