Synthesis of Lichesterol [Ergosta-5,8(9),22-trien-3β-ol]

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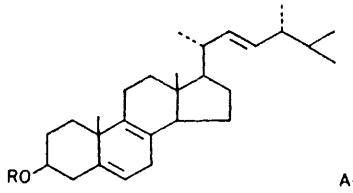
Ergosta-5,8(9),22-trien-3β-ol has been synthesized and its identity with lichesterol has been confirmed. Ergosteryl acetate was transformed into 7α-[*NN'*-bis(ethoxycarbonyl)hydrazino]ergosta-5,8(9),22-trien-3β-yl acetate which was converted into lichesterol by reduction with lithium in ethylamine.

LICHESTEROL is a sterol, isolated in 1973 from *Xantoria parietina*, to which the structure of ergosta-5,8(9),22-trien-3β-ol (1a) was assigned on the basis of spectroscopic evidence.[1] The presence of the sterol (1a) has subsequently been reported in yeast mutants,[2] in *Clorella ellipsoidea*,[3] and in *Candida albicans* mutants.[4]

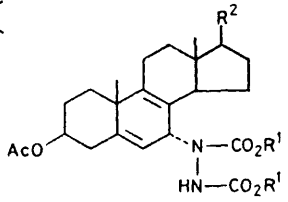
The biosynthetic origin of lichesterol is not clear since it could involve either a reversible $\Delta^8 \rightleftharpoons \Delta^7$ isomerase at the $\Delta^{5,7}$ level,[1] or a direct introduction of a Δ^5 bond into a $\Delta^{8(9)}$ sterol.[1,2a]

The last proposal is particularly noteworthy since it has been generally accepted that a 7(8) double bond is necessary for the introduction of Δ^5-unsaturation.[5] In order to resolve this point, and also to provide a final chemical proof[1] for the stereochemistry at C-24 of lichesterol, we have synthesized compound (1a).



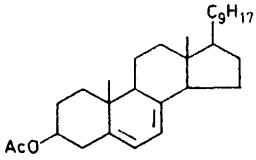
(1)

a; R = H
b; R = Ac

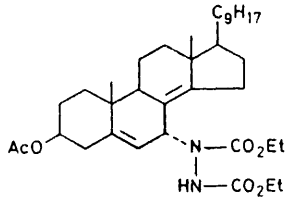


(2)

a; R^1 = Me, R^2 = C_9H_{17}
b; R^1 = Et; R^2 = C_9H_{17}
c; R^1 = Me, R^2 = C_8H_{17}
d; R^1 = Et, R^2 = C_8H_{17}



(3)



(4)

Steroid 5,7-dienes have been found to react[6] with esters of azodicarboxylic acid to give adducts of the ene reaction, such as compounds (2), containing a 5,8-diene system.

The adduct (2b), derived from ergosteryl acetate (3) appeared to be a useful intermediate for the ready synthesis of compound (1a) by cleavage of the C–N bond and insertion of a hydrogen atom at the 7α-position by a process which does not cause reconjugation of the 8(9) and 5(6) double bonds.

Thus, the acetate (3) was treated with diethyl azodicarboxylate[6] to afford two ene adducts to which the structures of 7α-[*NN'*-bis(ethoxycarbonyl)hydrazino]-ergosta-5,8(9),22-trien-3β-yl acetate (2b) and 7α-[*NN'*-bis(ethoxycarbonyl)hydrazino]ergosta-5,8(14),22-trien-3β-yl acetate (4) were assigned.

The more polar, major adduct (2b) was obtained by direct crystallization; in contrast, the more soluble adduct (4), was isolated only by preparative t.l.c., on silica gel, of the mother liquor of compound (2b) after removal of as much of the latter as possible.†

The assignment of structures (2b) and (4) is consistent with the observed chemical and physical properties of these compounds (see Experimental section), and with earlier work.[7]

Initial experiments on the base-catalysed cleavage of compound (2b), which could give a substituted hydrazine and hence the alcohol (1a) *via* aerial oxidation to give an allyldi-imide, yielded a complex reaction mixture from which no single product could be isolated. Meanwhile, a report appeared[8] showing that the ene adducts of some olefins treated with azodicarboxylate esters regenerated the starting olefins (*i.e.* underwent a π-bond migration) by alkaline hydrolysis followed by oxidation (for which many oxidizing agents were suitable). Hence, our oxidative approach was discontinued in favour of the reductive method described below.

When the acetate (2b) was treated with lithium in ethylamine, at −20 °C a compound was obtained, in 60% yield, to which the structure of ergosta-5,8(9),22-trien-3β-ol (1a) was assigned on the basis of spectroscopic data (see Experimental section).

Comparison of the properties of this compound (as the acetate) with those of the natural lichesterol[1,4] established the identity of the two compounds.

This simple synthesis of compound (1a) is the final proof of the 24*R*-configuration of lichesterol and the first synthesis of a $\Delta^{5,8}$ diene steroid.

† Treatment of ergosteryl acetate with dimethyl azodicarboxylate gave, according to ref. 6,7α-[*NN'*-bis(methoxycarbonyl)-hydrazino]ergosta-5,8(9),22-trienyl acetate(2a), without any $\Delta^{5,8(14)}$ isomer. It seems unlikely that the discrepancy with our results is due to the different azodicarboxylate used in the reaction. In fact, we have observed (in contrast with Van der Gen *et al.*) that treatment of cholesteryl acetate with diethyl azodicarboxylate gave both 7α-[*NN'*-bis(ethoxycarbonyl)hydrazino]cholesta-5,8-dien-3β-yl acetate (2d) and 7α-[*NN'*-bis(ethoxycarbonyl)-hydrazino]cholesta-5,8(14)-dien-3β-yl acetate.

In fact, the method proposed by Tsuda *et al.*[9] for obtaining cholesta-5,8-dien-3β-yl benzoate by dehydrobromination of 7α-bromocholesteryl benzoate was shown by Barton *et al.*[2a] to be irreproducible.

EXPERIMENTAL

I.r. spectra were recorded as KBr discs with a Perkin-Elmer 257 spectrometer. ^{1}H N.m.r. spectra were determined with deuteriochloroform as solvent and tetramethylsilane as internal reference on Varian HA-100 and Varian XL 100 spectrometers. Routine optical rotations were recorded with a Perkin-Elmer 141 spectropolarimeter for 1% solutions in chloroform. The mass spectra were determined on a MAT Varian 112S spectrometer by direct inlet. The progress of all reactions was monitored by t.l.c. on silica gel G (HF_{254}) microplates or by g.l.c. (2 m silanized glass column of 3% SE 30 on Gas Chrom Q support, operating at 220—240 °C).

Reaction of Diethyl Azodicarboxylate with Ergosteryl Acetate (3).—To a solution of ergosteryl acetate (3) (1 g), dissolved in sodium-dried benzene (10 ml), diethyl azodicarboxylate (1 g) was added and the solution was refluxed under nitrogen for 4 h. Removal of the solvent and of the excess of ester under reduced pressure gave a crude solid which, on crystallization from hexane, yielded 7α-[NN′-*bis*(*ethoxycarbonyl*)*hydrazino*]*ergosta*-5,8(9),22-*trien*-3β-*yl acetate* (2b) (0.76 g), m.p. 135—137 °C, $[\alpha]_D^{20}$ −66°; $\nu_{max.}$ 3 470, 1 755, 1 715, and 1 705 cm^{-1}; δ 6.13 (1 H, m, NH), 5.52 (1 H, m, 6-H), 5.18—5.32 (3 H, overlapping, 7-H, 22-H, and 23-H), 4.05—4.85 (5 H, overlapping, 3-H, and 2 × CO_2CH_2Me), 2.00 (3 H, s, AcO), 1.21 (3 H, s, 19-H), and 0.66 (3 H, s, 18-H); *m/e* 436 (M^+ − $C_6H_{12}N_2O_4$, 3%), and 377 (100%) (Found: C, 70.6; H, 9.2; N, 4.5. $C_{36}H_{56}N_2O_6$ requires C, 70.5; H, 9.2; N, 4.6).

The mother-liquor was evaporated under reduced pressure and the glass residue was purified by preparative t.l.c. (20% EtOAc–toluene) to give compound (2b) (90 mg) and the non-crystalline 7α-[NN′-*bis*(*ethoxycarbonyl*)*hydrazino*]-*ergosta*-5,8(14),22-*trien*-3β-*yl acetate* (4) (200 mg); $\nu_{max.}$ 3 470, 1 755, and 1 705 cm^{-1}; δ 6.23 (1 H, m, NH), 5.18—5.35 (4 H, overlapping, 6-H, 7-H, 22-H, and 23-H), 4.05—4.85 (5 H, overlapping, 3-H and 2 × CO_2CH_2Me), 2.00 (3 H, s, AcO), and 0.90 (6 H, s, 18- and 19-H); *m/e* 436 (M^+ − $C_6H_{12}N_2O_4$, 3%) and 377 (100%) (Found: C, 70.4; H, 9.3; N, 4.5. $C_{36}H_{56}N_2O_6$ requires C, 70.5; H, 9.2; N, 4.6%).

Synthesis of Ergosta-5,8(9),22-*trien*-3β-*ol* (1a).—The acetate (2b) (0.5 g), dissolved in ethylamine (20 ml), was treated with lithium (0.2 g) and the mixture was shaken at −20 °C for 30 min after the initial appearance of a blue colour. After the usual work-up the compound (1a) (195 mg) was obtained and was found to be identical with lichesterol, m.p. 132—134 °C (from acetone–methanol), $[\alpha]_D^{20}$ −34° (lit.,[1] m.p. 114—115 °C, $[\alpha]_D$ −26.6°; the following characteristics are identical); $\nu_{max.}$ (KBr) 3 400, 965, and 805 cm^{-1}; δ 5.43 (1 H, m, 6-H), 5.20 (2 H, m, 22-H and 23-H), 3.55 (3 H, m, 3-H) 2.52 (2 H, m, 7-H), 2.36 (1 H, m, 4α-H), 2.28 (1 H m, 4β-H), 1.19 (3 H, s, 19-H), 0.67 (3 H, s, 18-H), 1.02 (3 H, d, *J* 7 Hz, 21-H), 0.91 (3 H, d, *J* 7 Hz, 28-H), 0.83 (3 H, d, *J* 7 Hz, 27-H), and 0.81 (3 H, d, *J* 7 Hz, 26-H); *m/e* 396 (M^+, 66%), 363 (M^+ − Me − H_2O, 100%), 337 (7%), 271 (M^+ − side chain, 24%), 253 (M^+ − side chain − H_2O, 23%), 217 (11%), and 211 (10%) (Found: C, 84.9; H, 11.2. $C_{28}H_{44}O$ requires C, 84.8; H, 11.0%).

Acetylation of lichesterol (1a) with acetic anhydride–pyridine afforded ergosta-5,8(9),22-trien-3β-yl acetate (1b), m.p. 138—140 °C; $[\alpha]_D^{20}$ −40°; $\nu_{max.}$ 1 740, 965, and 805 cm^{-1}; δ 5.44 (1 H, m, 6-H), 5.19 (2 H, m, 22-H and 23-H), 4.62 (1 H, m, 3-H), 2.52 (2 H, m, 7-H), 2.42 (1 H, m, 4α-H), 2.34 (1 H, m, 4β-H), 1.20 (3 H, s, 19-H), and 0.67 (3 H, s, 18-H); *m/e* 438 (M^+, 6%), 378 (M^+ − AcOH, 100%), 363 (85%), 253 (42%), and 211 (18%) (Found: C, 82.3; H, 10.7. $C_{30}H_{46}O_2$ requires C, 82.1; H, 10.6%). All the characteristics are identical with those reported.[1,2a]

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