The Structure of ' Cholestane-3,4,6-trione '

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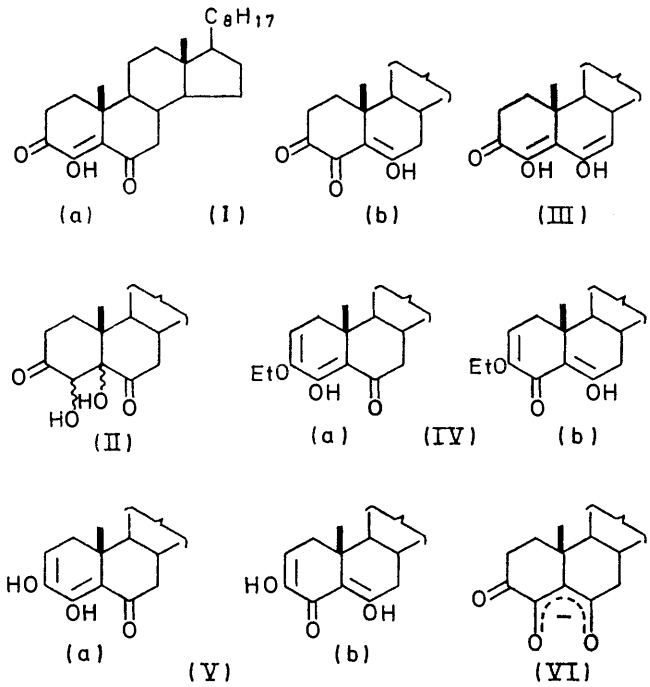
The compound described as ' cholestane-3,4,6-trione ' by Windaus and Kuhr has been shown to be the 3-ethyl ether of the corresponding dienol. Although relatively stable to acid, the enol ether may be hydrolysed to yield the triketone, a mixture of two stable tautomers, which may be separated readily by fractional crystallisation. Structures have been assigned to the two tautomers on spectroscopic evidence.

DURING work on the structure determination of the isoxazole obtained on photorearrangement of 6-nitrocholesteryl acetate,[1] we required cholestane-3,4,6-trione for comparison with a degradation product. Windaus and Kuhr [2] were the first to report the synthesis of the triketone; from the u.v. absorption of their compound they suggested that it existed as the enol (Ia). Their route involved oxidation of cholest-4-ene-3,6-dione by permanganate to give a 4,5-dihydroxy-3,6-diketone (II), which was treated with hydrogen chloride in ethanol to afford the supposed enol (Ia). Fieser and his coworkers [3] later repeated this synthesis of ' cholestane-

[1] J. T. Pinhey and E. Rizzardo, *Chem. Comm.*, 1965, 362.
[2] A. Windaus and E. Kuhr, *Annalen*, 1937, **532**, 52.
[3] L. F. Fieser and M. Fieser, ' Steroids,' Reinhold, New York, 1959, p. 307.

3,4,6-trione' and showed that the same compound resulted from a similar treatment of the 4,5-dihydroxycholestane-3,6-dione (II) obtained from oxidation of cholest-4-ene-3,6-dione with hydrogen peroxide–osmium tetroxide by Butenandt and Wolz.[4] In addition, they suggested that the u.v. absorption [$\lambda_{max.}$ 276 and 338 nm (ε 4600 and 6800)] of the 'triketone' indicated that in solution there was an equilibrium mixture of species (I) and (III), the latter giving rise to the long-wavelength band.



In our hands the dihydroxy-diketone (II), prepared by the method of Butenandt and Wolz, gave a single product in high yield when heated under reflux with hydrogen chloride in ethanol; the m.p. and u.v. absorption were almost identical with the published data.[2,3,5] However, analysis of the compound agreed with the formula $C_{29}H_{46}O_3$, and the mass spectrum indicated a molecular weight of 442, 28 mass units higher than that required for the triketone. The n.m.r. spectrum established that the product was the enol ether (IVa and/or b). The hydrogen-bonded proton of the enolised β-diketone system appeared as a sharp singlet at δ 15·5, the olefinic proton H-2 gave rise to a double doublet at δ 5·53 (*J* 3·4 and 7·0 Hz), and the ethyl ether function produced a quartet at δ 3·82 (*J* 7 Hz) and a triplet at δ 1·40 p.p.m. (*J* 7 Hz). The u.v. absorption indicated that a tautomeric mixture of (IVa) and (IVb) was probably present in solution, the long-wavelength band being due to the former and the band at 276 nm arising from (IVb).

Further evidence that the previous workers were dealing with the enol ether (IVa and/or b) was provided by the u.v. spectrum of a solution in ethanol containing potassium hydroxide [$\lambda_{max.}$ 257 and 357 nm (ε 5700 and 10,000)], which on acidification reverted to that of a solution in neutral ethanol. This behaviour is similar to that reported by Meyer[5] for the previously produced compound, but differs markedly from that found for the actual triketone (see later). The analysis figures of Windaus and Kuhr are satisfactory for both the triketone and its ethyl enol ether.

The required triketone was produced readily by heating the dihydroxy-diketone (II) with hydrogen chloride in anhydrous dioxan, or slowly by treatment with aqueous sulphuric acid of the enol ether (IVa and/or b), which was relatively stable to hydrolysis. By either method the triketone was produced as a mixture of stable tautomers [(Va and/or b) and (Ia and/or b)] which were readily separated by fractional crystallisation. The less soluble dienol tautomer (Va and/or b) showed i.r. absorption at 3350, 1640, 1590, and 1570 cm^{-1}, consistent with the proposed structure, whereas the keto-enol tautomer (Ia and/or b) displayed bands in this region at 1713, 1630, and 1570 cm^{-1}. The u.v. spectra were also consistent with these two structures. Compound (Va and/or b) had u.v. absorption [$\lambda_{max.}$ 277 and 340 nm (ε 5000 and 7500)] similar to that of the 3-ethyl ether (IVa and/or b), whereas tautomer (Ia and/or b) displayed a single absorption maximum at 318 nm (ε 8600). In the n.m.r. spectra of (Va and/or b) and (Ia and/or b) the exchangeable protons of the β-diketone systems gave rise to sharp singlets at δ 14·07 and 14·22 p.p.m., respectively. The spectrum of (Ia and/or b) exhibited no other signals downfield from δ 3·0 whereas that of (Va and/or b) showed another exchangeable proton signal at δ 6·07 p.p.m. due to the 3-hydroxy-group and a double doublet at δ 5·83 (*J* 4 and 7 Hz) assigned to H-2.

In neutral solution both tautomers were stable and each could be recovered quantitatively from refluxing methanol. However, in the presence of acid a relatively slow equilibration did occur. The equilibrium mixture, prepared by keeping compound (Va and/or b) for 2 h in dioxan containing hydrochloric acid, was shown by n.m.r. spectroscopy to contain *ca.* 40% of (Va and/or b) and 60% of (Ia and/or b). Treatment of either compound with hydrogen chloride in anhydrous ethanol afforded the enol ether (IVa and/or b). When treated with sodium hydroxide, a mixture of (Va and/or b) and (Ia and/or b) afforded a sodium enolate which was assigned the structure (VI) by virtue of its i.r. absorption (Nujol) in the carbonyl region at 1690 and 1610 cm^{-1}, and its n.m.r. spectrum ($CDCl_3$), which exhibited no signals downfield from the methylene envelope. Acidification of the salt gave only tautomer (Ia and/or b).

EXPERIMENTAL

M.p.s were taken with a Kofler hot-stage apparatus. Unless otherwise stated, u.v. spectra were determined for solutions in ethanol with a Perkin-Elmer 4000A Spectracord, and i.r. spectra for Nujol mulls with a Perkin-Elmer

[4] A. Butenandt and H. Wolz, *Ber.*, 1938, **71**, 1483.
[5] A. S. Meyer, *J. Org. Chem.*, 1955, **20**, 1240.

221 spectrometer. N.m.r. spectra were recorded on either a Varian A60 or a HA100 instrument (solutions in deuteriochloroform; tetramethylsilane as internal reference), and mass spectra were obtained with an A.E.I. MS902 instrument. Analyses were carried out by the Australian Microanalytical Service, University of Melbourne.

4,5-Dihydroxycholestane-3,6-dione (II).—To a solution of cholest-4-ene-3,6-dione (0·4 g) in dry ether (20 ml) was added osmium tetroxide (0·05 g) followed by 80% hydrogen peroxide (1 ml). After 0·5 h the product was collected and crystallised from ethyl acetate–ethanol to give 4,5-dihydroxycholestane-3,6-dione (0·35 g), m.p. 246—248° (lit.,[4] 243—245°), $\nu_{max.}$ 3500, 3400, and 1710 cm^{-1}, δ 4·72 (1H, s, 4-H), 3·84 [1H (exch. with D_2O), s, OH], 3·43 [1H (exch. with D_2O), s, OH], 1·09 (3H, s, 10-CH_3), and 0·66 p.p.m. (3H, s, 13-CH_3).

Enol (IVa *and/or* b) *of 3-Ethoxycholest-2-ene-4,6-dione*.—4,5-Dihydroxycholestane-3,6-dione (0·3 g) was heated at reflux for 2·5 h in absolute ethanol (100 ml) containing hydrogen chloride (5 g), and the solution was then concentrated to *ca.* 20 ml. The product which separated from the cooled solution crystallised from ethanol to yield the *enol* of 3-ethoxycholest-2-ene-4,6-dione as pale yellow plates (0·27 g), m.p. 148—149° (Found: C, 79·0; H, 10·6. $C_{29}H_{46}O_3$ requires C, 78·7; H, 10·5%), $\nu_{max.}$ 1600 cm^{-1}, $\lambda_{max.}$ 276 (ε 5100) and 339 nm (ε 7400), δ 1·12 (3H, s, 10-CH_3) and 0·68 p.p.m. (3H, s, 13-CH_3), *m/e* 442 (15%), 427 (100), and 165 (6).

Dienol (Va *and/or* b) *and Keto-enol* (Ia *and/or* b) *of Cholestane-3,4,6-trione*.— 4,5-Dihydroxycholestane-3,6-dione (0·39 g) in anhydrous dioxan (100 ml) containing hydrogen chloride (7 g) was heated under reflux for 0·5 h. The solvent was removed under reduced pressure and the residue was fractionally crystallised from methanol. The less soluble *dienol tautomer* (Va and/or b) was obtained as pale yellow plates (0·10 g), m.p. 157—159° (Found: C, 78·0; H, 10·2. $C_{27}H_{42}O_3$ requires C, 78·2; H, 10·2%), $\lambda_{max.}$ (EtOH–KOH) 376 nm (ε 9200) [giving on acidification $\lambda_{max.}$ 318 nm (ε 8800)], δ 1·14 (3H, s, 10-CH_3) and 0·69 p.p.m. (3H, s, 13-CH_3), *m/e* 414 (27%), 399 (100), 386 (4), and 371 (29).

The more soluble *keto-enol tautomer* (Ia and/or b) crystallised from methanol as pale yellow needles (0·11 g), m.p. 140—142° (Found: C, 78·0; H, 10·3. $C_{27}H_{42}O_3$ requires C, 78·2; H, 10·2%), $\lambda_{max.}$ (EtOH–KOH) 376 nm (ε 9800) [giving on acidification $\lambda_{max.}$ 318 nm (ε 9000)], δ 1·24 (3H, s, 10-CH_3) and 0·72 p.p.m. (3H, s, 13-CH_3). The mass spectrum was identical to that of (Va and/or b).

Acidic Hydrolysis of the Ethyl Ether (IVa *and/or* b).—A solution of the enol ether (IVa and/or b) (0·1 g) in tetrahydrofuran (30 ml) and 25% sulphuric acid (10 ml) was heated under reflux for 10 h. Extraction with ether and work-up of the extract gave a residue which on fractional crystallisation from methanol afforded cholestane-3,4,6-trione tautomer (Va and/or b) (0·02 g), m.p. 156—159°, and tautomer (Ia and/or b) (0·02 g), m.p. 140—142°. Both were identical (mixed m.p.s and i.r. spectra) with the respective compounds already obtained.

Acidification of the Sodium Salt of Cholestane-3,4,6-trione.—To a mixture of the tautomers (Va and/or b) and (Ia and/or b) (0·05 g) in methanol (15 ml) was added methanolic 5% sodium hydroxide until the solution was alkaline. Addition of water (7 ml) precipitated a yellow-green solid which decomposed at 195—205°. Acidification of this material with dilute sulphuric acid and extraction with ether afforded tautomer (Ia and/or b) (0·03 g), m.p. and mixed m.p. 140—142° (i.r. spectrum identical).

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