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STEREOCHEMISTRY OF 7,24(28)-STIGMASTADIEN-3β-OL AND THE FUCOSTEROLS

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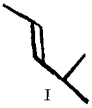
A recent communication described a stigmastadien-3β-ol as a component of the sterols of pumpkin ("Kürbis") seed (1). Its identity with the oat seed sterol Δ^7 avenasterol (2-4) was implied, and from a band in the IR spectrum of its acetate it was assigned the same configuration at the side-chain double bond as in fucosterol. It should, perhaps, first be noted that the 5,24(28)-stigmastadien-3β-ol (Δ^5 avenasterol (2)) also present in oat seed was gas-chromatographically different from fucosterol, but identical with *iso*fucosterol (3,5).

We wish to report that the main sterol of *Vernonia anthelmintica* seed, present to about 70% of the sterol mixture, and by comparison of published data the sterol of pumpkin seed (1) are indeed identical with Δ^7 avenasterol. However, we believe that the configuration at the C 24-28 double bond is not the same as in fucosterol but as in its stereoisomer about this bond.

The main sterol, as acetate, of *Vernonia anthelmintica* seed was purified by chromatography on silica impregnated with silver nitrate. After recrystallization its purity exceeded 95% by GLC. M.p. 153-156°, $[\alpha]^{22}_{578}$ + 8.3° (c 0.5 in $CHCl_3$), cf. Δ^7 avenasteryl acetate, m.p. 155°, $[\alpha]^{29}_{D}$ + 7° ± 2° (c 1.0 in $CHCl_3$) (ref. 2). The ratio of retention times before and after hydrogenation with Adams' catalyst was 1.16, indicating a double bond at C 24 (ref. 6). Mass spectrum: parent peak 454, M/e 356, 296, 255. NMR spectrum (obtained with a Varian HA-100): CH_3-18, singlet, δ 0.53; CH_3-19, singlet, δ 0.81; CH_3-21, broadened doublet, ca. δ 0.95; CH_3-26 and CH_3-27, doublet (J = 7 Hz), δ 0.97; CH_3-29, broadened doublet (J = 7 Hz), δ 1.59; CH_3CO, singlet, δ 2.00; H-25, septet (J = 7 Hz), δ 2.82; H-3 (axial), broad complex, δ 4.69; H-7 and H-28, complex, ca. δ 5.15. The work of Zürcher was decisive in determining the position of the double bond in the ring. Using parameters provided (7) it was possible to estimate δ-values for the CH_3-18 (δ 0.550) and CH_3-19 (δ 0.817) accurate to $\pm$ 0.02 ppm. Finally, the retention time on GLC was the same as Δ^7 avenasteryl acetate, assigned the 7,24(28)-stigmastadienyl structure from combined GLC-mass spectroscopic studies (3,4). We thank Dr B.A. Knights for this comparison.

Of significance concerning the stereochemistry at the side-chain double bond is the fact that in the sterol and its acetate from *Vernonia anthelmintica* the C-25 proton resonance is at δ 2.82, and from pumpkin seed at δ 2.84 (1). In fucosterol this proton resonates at δ 2.2, and in Δ^5 avenasterol (*iso*fucosterol) at δ 2.8 (ref. 8). This difference indicates the two different stereochemical environments about the C 24-28 double bond. For *iso*fucosterol Knights has undertaken an X-ray analysis to decide the orientation at this bond. However, NMR data for *cis*(II) and *trans*(I) 4-methyl-2-pentene (9) indicate that the isopropyl methine in the *trans* isomer (I) absorbs at a significantly higher field ($\delta \sim 2.3$) than in the *cis* (δ = 2.63). Applying this analogy to fucosterol and the avenasterols shows that the side chain orientation is as I for the former and II for the latter.



I

II

Consequently the nomenclature of the American Petroleum Institute and the National Bureau of Standards for cis/trans 3,4-dimethyl-2-pentene used in the IR assignment of the fucosterols (5) should be reversed, contrary to a conclusion reached by Rummens on other grounds (10).

We have also isolated stigmasteryl acetate (about 10%) from the acetylated mixture obtained from Vernonia anthelmintica seed, and obtained evidence for another C_{29} sterol (about 10%) with two conjugated double bonds, probably in the 8,14-positions, but with a saturated side chain. Although an early publication (11) describes brassicasterol as present, we have failed to find it in our samples.

In view of current biochemical interest in the introduction of double bonds into the side chain of phytosterols (12) these above correlations, and the high percentage and ready isolation of 7,24(28)-stigmastadien-3β-ol from Vernonia anthelmintica seed may deserve emphasis.

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