

CLEROSTEROL FROM *ENHYDRA FLUCTUANS*

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**Key Word Index**—*Enhydra fluctuans*; Compositae; sterols; clerosterol (stigmasta-5,25-dien-3- $\beta$ -ol); sitosterol glucoside.

**Plant:** *Enhydra fluctuans* Lour. (Voucher specimen No. C-2/73, deposited at the Department of Botany, Bangalore University). **Source:** United Chemical and allied Products, Calcutta. **Uses:** Medicinal [1]. **Previous work:** Whole plant [2–6].

**Present work:** Air-dried and milled *Enhydra fluctuans* (whole plant) was extracted with light petrol (60–80°), followed by 90% EtOH. Petrol was evaporated from the first extract and residue saponified at 100° under N<sub>2</sub>. The non-saponifiable portion was taken in light petrol and chromatographed over alumina (Brockmann grade II), eluting with increasing benzene in light petrol. Light petrol–C<sub>6</sub>H<sub>6</sub> (2:3) eluted the sterol fraction which was acetylated (C<sub>5</sub>H<sub>5</sub>N–Ac<sub>2</sub>O) and chromatographed over 15% AgNO<sub>3</sub> impregnated Si gel eluting as before. Three fractions were obtained, the first yielding stigmasteryl acetate, the second clerosteryl acetate (mixed with a small quantity of stigmasteryl acetate) and the third stigmasta-5,22,25-trien-3- $\beta$ -yl acetate. Further purification of the second fraction by preparative TLC on 30% AgNO<sub>3</sub> impregnated Si gel [developing solvent: petrol–C<sub>6</sub>H<sub>6</sub> (3:2)] yielded pure clerosteryl acetate (stigmasta-5,25-dien-3- $\beta$ -yl acetate), mp 126–127° (lit. [7] 126–126.5°),  $[\alpha]_D^{25}$  –42.8° (c, 0.92 in CHCl<sub>3</sub>). IR  $\nu_{\max}$  cm<sup>–1</sup> 1750 (acetate), 1645 and 890 (>C=CH<sub>2</sub>). Saponification yielded clerosterol, mp 130–131° (lit. [8] 130–133°),  $[\alpha]_D^{25}$  –41.7° (c, 0.92 in CHCl<sub>3</sub>). IR  $\nu_{\max}$  cm<sup>–1</sup> 3650–3400 (OH), 1645 and 890 (>C=CH<sub>2</sub>). The acetate and the parent sterol were identical in all respects (mp,

mmp,  $[\alpha]_D$  and IR) with samples of clerosteryl acetate and clerosterol isolated from *Codium fragile* [7].

The EtOH extract was concentrated, diluted with H<sub>2</sub>O and extracted successively with Et<sub>2</sub>O and EtOAc. The latter extract yielded sitosterol O- $\beta$ -D-glucoside, mp 304–305°; tetraacetate, mp 168–170°,  $[\alpha]_D^{25}$  –32.8° (c, 0.95 in CHCl<sub>3</sub>). The identities of the glucoside and its acetate were confirmed by direct comparison with authentic samples (mp, mmp, and IR).

**Comments:** This is the first report of the occurrence of clerosterol in the Compositae. Its existence, along with stigmasta-5,22,25-trien-3- $\beta$ -ol and stigmasterol may be of significance from the point of view of biosynthesis of these phytosterols.

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