

## Isolation of 24-Oxocholesterol from the Marine Brown Alga *Pelvetia canaliculata* (Phaeophyceae)

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It has been reported<sup>1,2</sup> that the main steroid in the brown seaweed *Pelvetia canaliculata* (L.) Dene. et Thur. is fucosterol (24-ethylidenecholest-5-en-3 $\beta$ -ol). From an ethereal extract of this alga we have isolated in addition to fucosterol small amounts of another  $\Delta^5$ -steroid, identified as 24-oxocholesterol (3 $\beta$ -hydroxycholest-5-en-24-one). This sterol has to our knowledge not previously been isolated from algae.

Fucosterol crystallized from methanol as long, white needles, m.p. 124°C. The identity was established by comparison of NMR and mass spectra and optical rotation with published data.<sup>3-5</sup>

24-Oxocholesterol crystallized in mixture with small amounts of other steroids after most of the fucosterol had been removed. The steroid mixture showed positive Liebermann-Burchard test and melted between 138 and 145°C. After acetylation and purification by chromatography 24-oxocholesteryl acetate crystallized from methanol-chloroform, m.p. 127–128°C. Its mass spectrum had the molecular ion peak at  $m/e$  442 (<1%) and base peak at  $m/e$  382 (M–60). The NMR spectrum was reminiscent of that of fucosterol,<sup>3</sup> apart from the lack of the signals from the ethylidene group. The IR spectrum revealed the presence of a non-conjugated carbonyl group in addition to that of the acetate function. After hydrolysis of the ester, 24-oxocholesterol crystallized from methanol, m.p. 133–134°C. Its IR spectrum exhibited a strong carbonyl band and its mass spectrum, very similar to that of 24-methyl cholesterol<sup>6</sup> (24-methylcholest-5-en-3 $\beta$ -ol, C<sub>28</sub>H<sub>48</sub>O), showed the molecular ion peak at  $m/e$  400 (100%). However, accurate mass measurement gave the molecular formula C<sub>27</sub>H<sub>44</sub>O<sub>2</sub>. Further, the occurrence of ions corresponding to  $\alpha$ -cleavage about C-24 ( $m/e$  71 and  $m/e$  43) and the presence of an ion assignable to McLafferty rearrangement caused by a carbonyl function at this position ( $m/e$  314) would be expected for 24-oxocholesterol.

For comparison 24-oxocholesterol was prepared from fucosterol by oxidation with osmium tetroxide followed by periodic acid as used by Bergmann *et al.*<sup>7</sup> for the oxidation of 24-methylene cholesterol. The mixed melting point of the two preparations of 24-oxocholesterol gave no depression, and the IR, NMR, and mass spectra were identical.

**Experimental.** Melting points are uncorrected and were determined on an Electrothermal melting point apparatus. Kieselgel 0.05–0.2 mm (Merck) was used for column chromatography. For analytical and preparative thin layer chromatography (TLC) chromatoplates were coated with Kieselgel G (Merck) or Kieselgel PF<sub>254+366</sub> (Merck). Light petroleum ether was of b.r. 40–60°C. IR spectra were made on a Perkin-Elmer 257 spectrophotometer and the NMR spectra recorded on a Varian A-60A spectrometer, with CDCl<sub>3</sub> (Merck) as solvent. Mass spectra were recorded with an AEI MS 902 instrument.

**Isolation of 24-oxocholesterol from *Pelvetia canaliculata*.** Dried (at 30–40°C for 48 h in a drying cabinet with forced air circulation) and ground *Pelvetia canaliculata* (1.4 kg), collected in the Trondheimsfjord in June, was extracted with diethyl ether in a Soxhlet extractor for 42 h. The extract was evaporated *in vacuo*, and the residue (63.6 g) was chromatographed crudely on a silica gel column (800 g) by successive elution with portions of ca. 4000 ml of light petroleum ether, benzene, ether, and methanol:ether (1:9). The dark green ether fraction (18.8 g) was rechromatographed on silica gel (300 g) with ether-benzene mixtures. The fraction eluted with ether:benzene (1:19, 6.0 g) was crystallized several times from methanol and afforded fucosterol.

The mother liquor (ca. 0.5 g) from the crystallization of fucosterol was chromatographed once more on silica gel (25 g) and eluted with ether-benzene. The fraction eluted with 4% ether in benzene (0.18 g) contained one major and two minor components as revealed by TLC (silver nitrate on silica gel (1:5), benzene:ethyl acetate (7:3)). The mixture was acetylated with acetic anhydride in pyridine at room temperature overnight, and the product chromatographed on a column of silver nitrate on silica gel (1:5, 20 g). The fraction eluted with benzene:light petroleum ether (3:2, ca. 30 mg) was purified by preparative TLC on Kieselgel PF<sub>254+366</sub> with benzene as eluent, and furnished, after crystallization from methanol-chloroform, 24-oxocholesteryl acetate (21 mg) m.p. 127–128 (also the unstable form<sup>7</sup> m.p. 115–116°C was observed);  $\nu_{\max}$  (KBr) 1732

and 1705  $\text{cm}^{-1}$ ;  $m/e$  442 (<1 %), base peak  $m/e$  382 (M-60), 367, 296, 281, 261, 255, 228, 213;  $\tau$  9.32 (s, 3H, C-18),  $\tau$  8.97 (s, 3 H, C-19),  $\tau$  8.91 (d,  $J \sim 7$  cps, 9H, C-21, C-26, C-27),  $\tau$  7.97 (s, 3H, acetate),  $\tau \sim 5.4$  (m, 1H, C-3), and  $\tau \sim 4.6$  (diffuse triplet, 1H, C-6).

24-Oxocholesterol was obtained by hydrolysis of 24-oxocholesteryl acetate with 5 % KOH in methanol at room temperature overnight, m.p. 133–134°C after crystallization from methanol, mixed with synthetic material (see below) m.p. 135–137°C;  $\nu_{\text{max}}$  (KBr) 1710  $\text{cm}^{-1}$ ;  $M^+$  found 400.3356, calc. for  $\text{C}_{27}\text{H}_{44}\text{O}_2$  400.3341,  $m/e$  400 (100 %), 385 (11 %), 382 (28 %), 367 (10 %), 315 (16 %), 314 (30 %), 299 (9 %), 289 (18 %), 281 (10 %), 273 (9 %), 271 (15 %), 255 (14 %), 246 (7 %), 231 (9 %), 229 (9 %), 213 (20 %), 71 (40 %), 43 (84 %).

24-Oxocholesterol from fucosterol. Fucosterol (42 mg) was treated with osmium tetroxide (25 mg) in dry benzene and a few drops of pyridine at room temperature overnight. The product was refluxed with sodium sulfite (0.25 g) in alcohol for 2 h. After filtration through Celite, the solution was evaporated and the glycol (38 mg) was oxidized with periodic acid (34 mg) in ethanol and pyridine at room temperature overnight. Extraction with ether gave 34 mg of 24-oxocholesterol, m.p. 137–138°C after crystallization from methanol;  $\nu_{\text{max}}$  (KBr) 1710  $\text{cm}^{-1}$ ;  $m/e$  400 (100 %), 385 (10 %), 382 (23 %), 367 (10 %), 315 (16 %), 314 (25 %), 299 (9 %), 289 (16 %), 281 (8 %), 273 (9 %), 271 (14 %), 255 (14 %), 246 (6 %), 231 (10 %), 229 (10 %), 213 (19 %), 71 (42 %), 43 (96 %);  $\tau$  9.32 (s, 3H, C-18),  $\tau$  8.98 (s, 3H, C-19),  $\tau$  8.91 (d,  $J \sim 7$  cps, 9H, C-21, C-26, C-27),  $\tau$  8.33 (s, 1H, OH),  $\tau \sim 6.5$  (m, 1H, C-3),  $\tau \sim 4.65$  (diffuse triplet, 1H, C-6).

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*Note added in proof.* After this note had been submitted for publication we became aware of a short communication by Knights, B.A. *Phytochemistry* 9 (1970) 903. Dr. Knights reported *inter alia* the presence of 24-oxocholesterol in the brown alga *Ascophyllum nodosum*, but suggested that it is an artefact arising from fucosterol by aerial oxidation during storage. Although in our case we cannot with certainty exclude this possibility, we think

it unlikely in view of the great care taken to avoid uncontrolled oxidation during the various operations.

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## The Synthesis of a Mixed Disulfide of Glutathione and 3-Carboxy-4-nitrobenzenethiol ("Reduced Ellman's Reagent")

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We have previously prepared and studied mixed disulfides of glutathione and naturally occurring aliphatic thiols.<sup>1-4</sup> In these investigations, as well as in studies made in other laboratories, it has been found that among a number of aliphatic disulfides, only the mixed disulfide of coenzyme A and glutathione gives an activity with glutathione reductase, which exceeds 1 % of the activity obtained with glutathione disulfide.<sup>5,6</sup> Since very little is known about the interaction between glutathione reductase and its disulfide substrate, it is of interest to obtain glutathione sulfonyl derivatives other than glutathione