136. 24-Isopropylcholesterol and 22-Dehydro-24-isopropylcholesterol, Novel Sterols from a Sponge

by **Werner Hofheinz** and **Gottfried Oesterhelt**

Pharmazeutische Forschungsabteilung und Zentrale Forschungseinheiten der *F. Hoffmann-La Roche & Co. AG*, CH-4002 Basel

(25.IV.79)

Summary

24-Isopropylcholesterol (**1**) and 22-dehydro-24-isopropylcholesterol **2** have been isolated as the only sterols from an Australian sponge of the genus *Pseudaxinyssa*. Structures have been deduced from spectroscopic data.

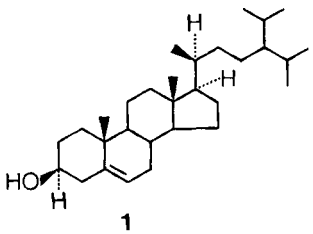
Sterols of sponges are characterized by a great variety of unusual structural features, such as modified ring systems and polyalkylated side chains [1]. They also frequently occur as mixtures of very complex composition. We now report the isolation of two novel sterols, **1** and **2**, as the only two sterols of a new species of the sponge-genus *Pseudaxinyssa* (class *Demospongia*, order *Axinellida*) which was collected at various localities on the *Australian Great Barrier Reef*.

The sterols were isolated by *Soxhlet* extraction of sun dried sponge samples with petrol ether and purified by chromatography on preparative silica gel plates. GC. analysis showed the presence of only two components, whose molecular formulae were obtained from high resolution mass spectra as $C_{30}H_{52}O$ and $C_{30}H_{50}O$. Catalytic hydrogenation transformed the mixture to one single sterol, $C_{30}H_{54}O$, proving that both sterols have the same carbon framework, one being mono-unsaturated, the other di-unsaturated. Since peaks in the mass spectrum of the mono-unsaturated component at *m/e* 273, 255, 231 and 213 gave a clear indication of the presence of a mono-unsaturated, intact steroid nucleus [2], the strong peak at *m/e* 271 in the spectrum of the di-unsaturated sterol had to be taken as evidence for the presence of one double bond in its side chain [3]. Mass spectra of the silylated sterols were very similar to those of β-sitosterol and stigmasterol, with prominent peaks at *m/e* 129 and *M*-129 which are typical for $\Delta 5$-sterols [2] [4]. It therefore could be concluded that both sterols possess normal cholesterol-like structures with three 'extra-carbon atoms' in the side chain. The nature of the side chains could be derived from NMR. spectra. Samples (0.2–0.3 mg) of pure sterols were obtained by GC. separation. The NMR. spectrum of sterol **1** shows in addition to the signals of five methyl groups [C(18) 0.68; C(19) 1.01; C(21) 0.94(*d*); C(26) and C(27) 0.85 ppm(*d*)] a six-proton doublett at 0.87 ppm indicating the presence of a second isopropyl group. A similar observation was made in the NMR. spectrum of sterol **2** (doublet at 0.77 ppm), which was, furthermore, almost superimposable onto the spectrum of

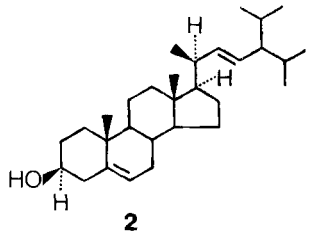
0018-019X/79/4/1307-03$01.00/0

stigmasterol, in particular with respect to the signals due to the *trans* double bond at C(22), C(23). The *trans* configuration was also evident from an IR. absorption at 965 cm^{-1}. The spectra of both sterols show, furthermore, a one-proton signal at 5.32/5.36 ppm, typical for the olefinic proton of Δ^5-sterols [5].

One *trans* double bond and an 'extra isopropyl group' can easily be accomodated in the side chain as given in structure **2**. The only alternative would be one with the isopropyl group at C(22) and a Δ^{23}-double bond. Such a structure is, however, highly unlikely as the fragmentation pattern of a Δ^{23}-isomer should be distinctly different [3]. A definite proof of structures **1** and **2** comes finally from an unambiguous synthesis [6].



1



2

24-Isopropylsterols have no precedent. Their presence in *Pseudaxinyssa* together with the unusual simplicity of the sterol mixture implies that this sponge has a highly specified ability to synthesize its sterols either *de novo* or by transformation of dietary sterols. This specificity is obviously not subject to the influence of environmental factors, as we have investigated three different samples collected at different reefs at different seasons, and observed very similar sterol compositions: 46% **1** + 53% **2**, 28% **1** + 72% **2**, resp. 30% **1** + 30% **2**. It therefore seems to be justified to consider these sterols as useful chemotaxonomic markers.

We wish to thank Dr. *C.R. Wilkinson*, Department of Zoology, Univ. of Queensland, Australia, for collecting the sponge samples and Prof. Dr. *P. Bergquist*, Department of Zoology, Univ. of Auckland, New Zealand, for the taxonomic classification. We are grateful to our colleagues of the spectroscopy department for providing spectra and for many helpful discussions.

Experimental Part

General remarks. GC. analyses were performed on a *Perkin-Elmer* 900 chromatograph with flame ionization detector fitted with 2 m glass columns (i.d. 2.2 mm) packed with either 5% SE 30 or 5%. OV-17 on gaschrom Q. Carrier gas Helium at 30 ml/min; injection port temperature 280°, detector temp. 270°. Separations were run at isothermal conditions at 225° (OV-17) or 250° (SE 30). GC./MS. coupling utilized a *Varian* MAT CH 7 mass spectrometer in combination with a *Perkin-Elmer* 990 chromatograph with a two-step *Watson-Biemann* separator. Mass spectra were done at 70 eV, 250°. Sterols were analyzed either free or as silyl ethers (prepared with bis(trimethylsilyl)acetamide). High resolution mass spectra were obtained with a *AEI* MS 902/DS 30; data given are *m/e* (% relative intensity). ^{1}H-NMR. spectra have been run on a *Bruker* HX-90 spectrometer with *Nicolet* Computer 1083 (90 MHz, internal reference, TMS = 0).

Isolation of sterols from *Pseudaxinyssa sp.*: Sponges were collected on *Dip reef*, on *Bowl reef*, and on *Grub reef* northeast of *Townsville*, Australia, and sun dried. In a typical experiment a 70 g sample of crushed sponge was extracted in a *Soxhlet* with petroleum ether (b.p. 60–80°). Evaporation of the solvent gave 0.5 g (0.7%) of a gum; 50 mg of the latter was applied onto one PLC. plate (*E. Merck*, precoated

silica gel HF_{254} plate). The plate was developed twice with methanol/dichloromethane 3:97. Sterols were located by spraying edge-strips with vanillin/sulfuric acid, and eluted with ethyl acetate. Stripping the solvent gave 8 mg crystalline sterols. Pure fractions of **1** and **2** (0.2–0.3 mg each) were obtained by repeated GC. runs on SE 30.

Data of **1**. Retention times, relative to cholesterol, after silylation: 1.88 (OV-17), 1.86 (SE 30). – ^{1}H-NMR. ($CDCl_3$): 5.36 (*m*, 1H); 3.53 (br. *m*, 1H); 1.01 (*s*, 3 H); 0.94 (*d*, $J=7$ Hz, 3 H); 0.87 (*d*, $J=6.7$ Hz, 6 H); 0.85 (*d*, $J=6.7$ Hz, 6 H); 0.68 (*s*, 3 H). – MS.: 428.4014 (*M*, $C_{30}H_{52}O$, calc. 428.4017; 4.8%), 413 (3), 410 (4), 395 (4), 343 (6), 317 (4), 273 (7), 255 (6), 231 (4), 213 (10). – MS. of silyl ether: 500 (12, *M*), 485 (6), 410 (29), 395 (18), 371 (45), 255 (14), 129 (100).

Data of **2**. Retention time, relative to cholesterol, after silylation: 1.66 (OV-17); 1.63 (SE 30). – ^{1}H-NMR. ($CDCl_3$): 5.32 (*m*, 1H); 5.01 (*m*, 2 H); 3.51 (*m*, 1H); 1.02 (*d*, $J=6.7$ Hz, 3 H); 1.00 (*s*, 3 H); 0.85 (*d*, $J=6.5$ Hz, 6 H); 0.77 (*d*, $J=6.6$ Hz, 6 H); 0.70 (*s*, 3 H). – IR. (KBr): 965 cm^{-1}. – MS.: 426.3846 (*M*, $C_{30}H_{50}O$, calc. 426.3860, 7.4%), 383 (17), 365 (32), 271 (15), 255 (38). – MS. of silyl ether: 498 (10, *M*), 483 (4), 455 (4), 408 (13), 369 (7), 365 (62), 255 (34), 129 (55), 97 (100).

Hydrogenation of sterol mixture: 28 mg sterol mixture was stirred in 10 ml acetic acid with 28 mg PtO_2 under H_2 for 24 h. After filtration and evaporation, the residue was recrystallized from aceton; yield 3 mg, m.p. 152–155°. – MS.: 430 (58, *M*), 415 (44), 233 (94), 215 (100).

REFERENCES

[1] *L. Minale, G. Cimino, S. De Stefano & G. Sodano,* Fortschr. Chem. org. Naturstoffe *33*, 1 (1976); *F.J. Schmitz* in 'Marine Natural Products', Vol. 1, P.J. Scheuer, Ed., Academic Press, New York, N.Y. 1978, pp. 241–297.

[2] *B.A. Knights,* J. Gas Chromatogr. *5*, 273 (1967).

[3] *S.G. Wyllie & C. Djerassi,* J. org. Chemistry *33*, 305 (1968).

[4] *C.J.W. Brooks, E.C. Horning & J.S. Young,* Lipids *3*, 391 (1968).

[5] *N.S. Bhacca & D.H. Williams,* 'Applications of NMR. Spectroscopy in Organic Chemistry', Holden-Day, Inc., San Francisco 1964.

[6] *W.C.M.C. Kokke, C.S. Pak, W. Fenical & C. Djerassi,* Helv. *62*, 1310 (1979).