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ISOLATION AND STRUCTURE OF 26,27-CYCLOAPLYSTEROL (PETROSTEROL) A CYCLOPROPANE-CONTAINING MARINE STEROL

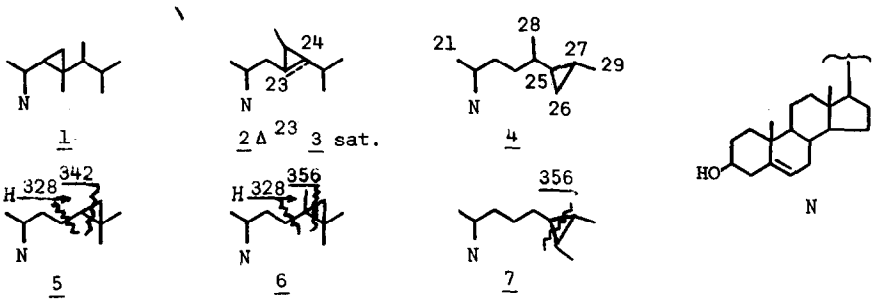
B. N. Ravi[a], W. C. M. C. Kokke[a,b], Claude Delseth[b] and Carl Djerassi[b*]

Joint Contribution from (a) Scripps Institution of Oceanography, La Jolla, Ca. 92093 and (b) Department of Chemistry, Stanford University, Stanford, Ca. 94305.

One of the most intriguing features of marine chemistry is the occurrence of sterols with cyclopropyl-containing side chains, typified by gorgosterol (1)[1] and its relatives[2] with a 22-23 cyclopropane ring and calysterol (2)[3] with a 23-28 cyclopropene ring. Current speculations[4] suggest that these two types are not only structurally, but also biogenetically distinct. The recent report[5] of the isolation of "petrosterol" (23,28-cyclostigmast-5-en-3β-ol) (3) seemed particularly significant, since it closely resembled calysterol (2) and could possibly be its biosynthetic precursor. Its structure was based[5] on spectral analysis and the hydrogenolytic opening to the known β-sitostanol (24-ethylcholestan-3β-ol). Recently, we investigated the sterol mixture of a partially identified Pacific sponge (Halichondria species) collected by R. Walker and C. Ireland at Canton Island (2°50'S/171°42'W) and isolated as the principal component (ca. 75%) a new sterol with properties virtually identical to those reported[5] for petrosterol. However our high resolution mass spectral and 360 MHz NMR spectral measurements discussed below led us to the conclusion that our sterol possessed structure 4, i.e. 26,27-cycloaplysterol, and therefore was different from petrosterol.[5] We have now learned[6] that X-ray analysis of petrosterol p-bromobenzoate showed the original structure 3 to be incorrect and that it is in fact 26,27-cycloaplysterol (4) with the 24R,25S,27R configuration (a stereochemical feature which we had not elucidated).

Reverse-phase HPLC[7] analysis of the total sterol mixture indicated the presence of at least 7 sterols. The main component (75%), $C_{29}H_{48}O$, m.p. 120-121°C, was shown by high resolution mass spectrometry to have the usual cholesterol nucleus [e.g. A/B ring fission at M-85 (m/e 327) and M-111 (m/e 301) as well as ring D fission peaks at m/e 231 and 213 ($-H_2O$); side chain loss peaks at m/e 273 and 255 ($-H_2O$) as well as 271 (additional loss of 2 H) and 253 ($-H_2O$)]. The loss of the side chain together with two hydrogen atoms is typical[8] of sterols with unsaturation in the side chain, but is also displayed by cyclopropane-containing sterols.[1,9] Of particular relevance was a group of weak (5-10% rel. intensity) but highly significant peaks at m/e 370 (loss of C_3H_6), 355 (loss of $C_3H_6 + CH_3$), 352 (loss of $C_3H_6 + H_2O$) and 337 (loss of $C_3H_6 + CH_3 + H_2O$), which we associate with fission of the 25-26 and 25-27 bonds (see 4) - a behavior typical[9] of cyclopropanes. Decisive evidence in favor of structure 4 came from an analysis of the 360 MHz NMR spectrum, the relevant assignments (aside from usual C-3H and C-6H) being supported by extensive

decoupling experiments. Methyl signals: 0.683 (s,3H,C-18); 0.891 (d, J=6.5Hz,3H,C-28); 0.920 (d, J=6.5Hz,3H,C-21); 1.004 (d, J=6Hz,3H,C-29); 1.010 (s,3H,C-19). Cyclopropyl proton signals: 0.05-0.18 (m,2H,C-26); 0.40-0.50 (m,1H,C-27); 0.55-0.65 (m,2H,C-24 + C-25). Off-resonance CMR spectral examination verified the presence of CH_2 in a cyclopropane ring. Direct comparison of our sterol in terms of GC mobility, NMR and mass spectral measurements with petrosterol[5,6] demonstrated their identity and we are therefore retaining the trivial name first proposed by the Italian workers.[5] Their reported conversion[5] to sitostanol was based only on GC mobility and it is quite conceivable that one of the possible hydrogenolysis products of 4 may have an identical R_f value and thus have led to mistaken identification.



GC-MS analysis of certain minor HPLC fractions demonstrated the presence of two new sterols ($C_{28}H_{46}O$ and $C_{29}H_{48}O$). Their mass spectra below m/e 314 were very similar to that of petrosterol (4), but instead of the latter's M-42 (C_3H_6) peak, they exhibit unique M-56 (C_4H_8) peaks as well as m/e 328 (typical[10] of 24-alkyl-Δ^{25}-sterols and hence most likely also of related cyclopropanes[9]). A poor 360 MHz NMR spectrum (0.2 mg. of 50% enriched $C_{28}H_{46}O$ contaminated by cholesterol, cholesta-5,22-dien-3β-ol and 24ξ-methylcholesta-5,22-dien-3β-ol) showed the presence of cyclopropane signals. These data are compatible with structure 5, which would be of great biosynthetic interest. Insufficient $C_{29}H_{48}O$ sterol was available for NMR examination, but the m/e 356 (M-C_4H_8) and m/e 328 peaks point towards 6, with 7 being less likely (cf. absence of m/e 328 in 4). A more detailed investigation of the trace sterols of this relatively inaccessible sponge is definitely indicated.

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