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MINOR AND TRACE STEROLS IN MARINE INVERTEBRATES 38[1]: ISOLATION, STRUCTURE ELUCIDATION AND PARTIAL SYNTHESIS OF PAPAKUSTEROL, A NEW BIOSYNTHETICALLY UNUSUAL MARINE STEROL WITH A CYCLOPROPYL-CONTAINING SIDE CHAIN.

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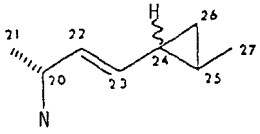
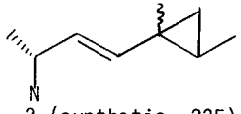
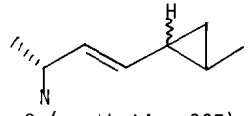
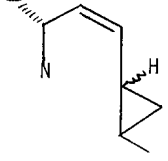
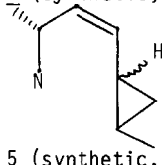
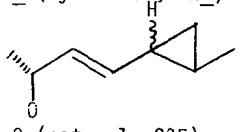
Summary: A new cyclopropyl-containing sterol papakusterol (22-dehydro-24,26-cyclocholesterol), arising by a hitherto unknown biosynthetic process, was isolated from six "deep sea" gorgonians and its structure elucidated by ^{1}H-NMR analysis and partial synthesis. Traces of the corresponding $\Delta^{5,7}$-diene have also been encountered.

In our search for new marine sterols we considered it important to examine "deep sea" organisms where photochemically induced biosynthetic processes are inhibited because of lack of light. Since the collection of these species has now become possible by the use of a mini-submarine,[2] we investigated the sterol fraction of six gorgonians[3] collected at a depth of up to 1200 ft. Aside from conventional sterols,[4] all six samples contained a new sterol 1, named papakusterol (from the Hawaiian "papaku" for ocean floor) (M^+ = 382.326, $C_{27}H_{42}O_1$) in yields varying from 6 to 16%, which was purified by reverse phase HPLC (Altex Ultrasphere ODS-5) with r.r.t. 0.56 (cholesterol = 1 in abs. MeOH), and apparently homogeneous by GC analysis (OV-17 column at 260°, r.r.t. = 1.07 with cholesterol = 1). Its mass spectrum displayed the typical pattern of Δ^5-3β-hydroxyl sterols[5] (m/z 213, 231, and 253) and a base peak at m/z 271 ($C_{19}H_{27}O_1$) which strongly suggested[6] the presence of unsaturation in the side chain. In fact, the ^{1}H-NMR spectrum (Table 1) showed the presence of a trans double bond in the side chain in addition to the C-6 olefinic signal (5.34 ppm). Moreover, the sterol displayed the characteristic pattern of cyclopropyl protons, one of which is coupled with a methyl group (0.62-0.68 ppm), thus leading to the proposed structure 1. An accurate analysis of the ^{1}H-NMR of 1 uncovered a slight doubling of some peaks (mainly C-21 and C-22 signals), which suggested that the natural material consists of a 3:1 mixture of two of the four possible isomers of 1.[7]

In order to verify our structural assignment, the four isomers 2-5 were synthesized by Wittig condensation (THF, nBuLi, 12 hrs. reflux) of the aldehyde 6[8] with trans-2-methylcyclopropylmethyl triphenylphosphonium bromide (7)[9] followed by reverse phase HPLC[10] to yield 2 (m.p. 108-110°), 3 (m.p. 118-120°), 4 (m.p. 113-114°), and 5 (m.p. 100-101°). The ^{1}H-NMR spectra (see

Table 1. Selected ^1H 360 NMR chemical shift values ($CDCl_3$)

	CH-18	CH-19	CH-21	CH-27	CH-22	CH-23	CH-25	CH-26a	CH-26b
			0.992(75%) d,J=6.5		*5.284 dd J=8.3;15.1			0.370 ddd	0.442 ddd J=4,4,8
	0.676	1.004		*1.037 d J=5.9		4.904 dd J=8.4;15.2	*0.63-0.68 m		
1 (natural, 22E)			0.995(25%) d,J=6.5		5.266 dd J=8.5,15.2				
	0.678	1.005	0.991 d J=6.5	*1.038 d J=5.9	*5.283 dd J=8.3	4.910 dd J=8.3;15.3	*0.62-0.68 m	0.369 ddd J=4,4,8	0.442 ddd J=4,4,8
2 (synthetic, 22E)									
	0.676	1.006	0.997 d J=6.3	*1.039 d J=5.9	*5.268 dd J=8.2;15.2	4.908 dd J=8.4;15.4	*0.62-0.68 m	0.367 ddd	0.457 ddd J=4,4,8
3 (synthetic, 22E)									
	0.746	1.020	1.022 d J=6.2	*1.073 d J=5.9	*5.049 J=10.3	4.594 t J=10	*0.67 m	0.442 m 2H	
4 (synthetic, 22Z)									
	0.748	1.020	1.013 d J=5.6	*1.069 J=5.9	*5.049 t J=10.3	4.601 t J=10.1	*0.66 m	0.463 bt 2H J=6.4	
5 (synthetic, 22Z)									
			1.021(60%) J=6						
	0.615	0.941		1.041 d J=6	5.3 dd	4.92 dd	0.6-0.7 m	0.38 m	0.46 m
8 (natural, 22E)			1.023(40%) J=6						

*Assigned by spin decoupling

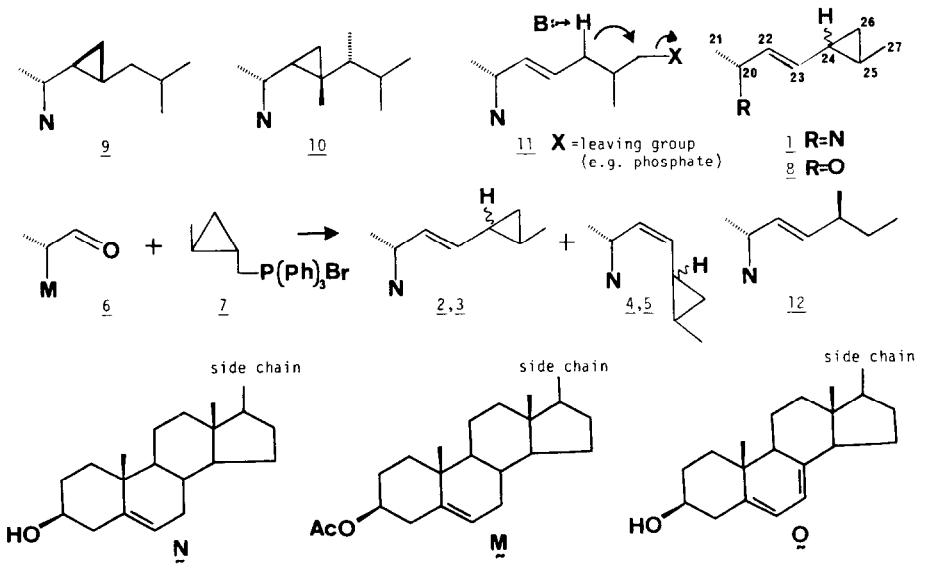
Table 1) of 2 and 3 were identical with that of the natural mixture 1, thus leaving only the absolute configuration at positions 24 and 25 to be determined.

One of the gorgonians (Acanthagorgia sp.) contained in trace amounts another new sterol (HPLC r.r.t. = 0.47 in abs. MeOH, GC r.r.t. = 1.35 with cholesterol = 1), which on the basis of the mass spectrum (M^+ = 380) and of the ^{1}H-NMR spectrum (see Table 1; C-6 and C-7 olefinic signals at 5.39 and 5.57 ppm) was assigned the structure of 7-dehydropapakusterol (8). Just as with 1, its 7-dehydro analog exists as a mixture (60:40) of two stereoisomers.

The importance of papakusterol (1) resides in its biosynthetic origin. One of the unique structural features of marine sterols is the existence of various cyclopropyl-containing side chains ranging from the simple 22(R),23(R)-methylenecholesterol (9)[11] to the highly substituted gorgosterol (10).[12] While the intimate details of the cyclopropyl ring formation in 9 and 10 have not yet been established, it has now been demonstrated (in the case of 10)[13] that the extra carbon atom is derived from methionine. Papakusterol (1) is unusual in that no bioalkylation can be involved in its formation. Either it arose from a squalenoid precursor, which already possesses a cyclopropane ring, or it is the result of the displacement of a C-26 functionalized 22-dehydrocholesterol precursor of type 11. The generation of an allylic cyclopropyl ring in the biosynthesis of presqualene[14] may be a precedent.

An even more intriguing aspect of the papakusterol side chain substitution pattern is that by fission of the 25-26 bond one generates the unusual 27-norergostane type side chain (12)[4,15] which is also unique to marine sterols and for which no unambiguous biosynthetic route has as yet been demonstrated.[16]

Scheme I



The biosynthesis of papakusterol (1) is not connected with its "deep sea" origin (>1200 ft). Rather, it appears to be a dietary (planktonic) constituent, since it appears to occur also in the soft coral Sarcophytum glaucum[17] (depth <100 ft.).

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NOTES AND REFERENCES

1. For preceding paper see: T. B. Tam Ha, W. C. M. C. Kokke and C. Djerassi, Steroids, in press
2. Specimens were collected aboard the minisubmarine Makali'i, off Makapuu, Oahu, in depths between -300 and -350 m, between Dec. 1981 and June 1982. All specimens were frozen as soon as Makali'i surfaced. Work up consisted of EtOH extraction, solvent partition and BioSil chromatography of the lipids.
3. Identification of animals is incomplete. Photographs and specimens are retained pending identification.
4. The two main sterols present in the organisms were cholesterol (30%) and 22-dehydrocholesterol (15%), with different amounts of other C-8, C-9, and C-10 side chain standard sterols: (occelasterol, brassicasterol, crinasterol, chalinasterol, campesterol, cholestanol and sitosterol).
5. S. G. Wyllie, B. A. Amos and L. Tokes, J. Org. Chem. 42, 725 (1977).
6. S. G. Wyllie and C. Djerassi, J. Org. Chem. 33, 305 (1968).
7. Since the synthon 7 is trans-oriented, the two isomers must be 24(R),25(R)- or 24(S),25(S)-isomers.
8. M. Fryberg, A. C. Oehlschlager and A. M. Unrau, Tetrahedron 27, 1261 (1971).
9. The Wittig reagent 7 (m.p. 130-132°) was prepared in 50% yield from trans-2-methylcyclopropane carboxylic acid (Aldrich) through the known 2-methylcyclopropylmethyl bromide.
10. Isomers 2 and 3 showed the same indistinguishable HPLC and GC r.r.t.; their difficult separation was achieved by repeated reverse phase HPLC (two Altex Ultrasphere ODS 5μ, (10 mm I.D. x 25 cm) in series, MeOH/H_2O 92:8).
11. P. A. Blanc and C. Djerassi, J. Am. Chem. Soc. 102, 7113 (1980); and ibid., 103, 7036 (1981).
12. N. C. Ling, R. L. Hale and C. Djerassi, J. Am. Chem. Soc. 92, 5281 (1970).
13. W. C. M. C. Kokke and C. Djerassi, to be published.
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15. M. Kobayashi and H. Mitsuhashi, Tetrahedron 30, 2147 (1974); for stereochemistry, see Y. Hirano and C. Djerassi, J. Org. Chem. 47, 2420 (1982).
16. C. Djerassi, N. Theobald, W. C. M. C. Kokke, C. S. Pak and R. M. K. Carlson, Pure & Appl. Chem. 51, 1815 (1979) and references cited therein.
17. M. Kobayashi and H. Mitsuhashi, private communication.

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