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NOVEL MARINE STEROLS WITH MODIFIED BILE ACID SIDE CHAIN FROM THE SEA PEN PTILOSARCUS GURNEYI*

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In contrast to the extensive recent[1] work on natural products of gorgonians (order Gorgonacea) and soft corals (order Alcyonacea), members of the closely related sea pens (order Pennatulacea) have not been investigated. We now wish to report the isolation of novel steroids, possessing unusual side chain functionality and stereochemistry, from the sea pen Ptilosarcus gurneyi Gray.[2,3]

Repeated column chromatography over silica gel of the crude hexane extract yielded ~10-15 mg (0.02%) of a white solid, homogeneous by tlc. Gas chromatographic (GC) and GC-MS analysis indicated a mixture of six compounds which, as the acetate derivatives, were separated on a GC column (1% OV-25) into three fractions: A (20% of the mixture), B (80%) and C (<1%). Each fraction contained two components of molecular weights 430/432 (9/1),[4] 428/430 (7/3),[4] and 442/444 (1/1)[4] respectively.

A small amount of the 428/430 compounds (peak B) was collected by preparative GC and the partial structure 1 deduced from the spectral data (ir, $\nu_{max}^{CHCl_3}$ 1710 and 1645 cm^{-1}; uv, λ_{max}^{MeOH} 205 nm ($\varepsilon \sim 7000$); 100 MHz pmr($CDCl_3$), three proton singlet at δ 3.70 ($-CO_2CH_3$)).[5] The transoid nature of the double bond in 1 was clearly denoted by the 16 Hz coupling constant between H_A (δ 5.78, 1H, d, J = 16 Hz) and H_B (δ 6.91, 1H, dd, J = 16,9 Hz). The multiplicity of the δ 6.91 proton required that it was coupled to two vicinal protons, H_A and H_C. The pmr and mass spectral data provided convincing evidence that the 428 compound contains a steroid nucleus with 3β-acetoxy-Δ^5-functionality by the one proton signals for the C-6 olefinic proton at δ 5.35 (br t), the δ 4.80 (br m) signal for the C-3 α-proton, and by the two 3-proton singlets at δ 0.99 (C-19) and δ 0.65 (C-18). The absence of a molecular ion at m/e 428 and the fragment peaks appearing at m/e 368 [M^+ - AcOH (base peak)], m/e 255 [M^+ - (AcOH + side chain)], m/e 249 (B-ring cleavage + H transfer) and m/e 213 (D-ring cleavage + H transfer) are all in good agreement with the proposed Δ^5 steroid nucleus.[6]

The presence of four oxygens (acetate and carbomethoxyl groups) and the 428 molecular weight require a molecular formula of $C_{27}H_{40}O_4$. The steroid nucleus and partial structure 1 encompass all atoms except for CH_3; clearly the completed structure is 1 in which R is replaced by the steroid nucleus and R' is a methyl group (confirmed by a secondary methyl group pmr signal at δ 0.99 (3H, d, J = 6.0 Hz)).

Confirmation of this structural assignment (2 with unknown C-20 stereochemistry) was attempted by synthesis. The i-methyl ether aldehyde 3[7] was treated with the ylide carbomethoxymethylenetriphenylphosphorane, p-toluene sulfonic acid in 20% aqueous dioxane, and finally acetic anhydride in pyridine to yield methyl (E)-3β-acetoxy-$\Delta^{5,22}$-choladien-24-oate (4), m.p. 151.5 - 152°, which differed from the natural product in gas chromatographic retention and pmr spectral detail (see Table 1). The pmr data confirmed the nature of the steroid nucleus and side chain functionalities, thus suggesting that the difference between the natural product and 4 was a stereochemical one, most likely at C-20. That this assumption was correct was established by synthesis of the C-20 epimer of 4. The i-methyl ether aldehyde 3 was exposed to methanolic KOH at room temperature followed by $LiAlH_4$ reduction to yield a mixture from which the i-methyl ether alcohol 5 was obtained pure by column chromatography over tlc-mesh silica gel.[8] Quantitative reoxidation to the aldehyde without affecting the C-20 stereochemistry was effected with the Collin's reagent.[9] The complete side chain unit and the steroid nucleus functionalities were introduced as before to yield 2, m.p. 151.0 - 151.5°, which had identical R_f, gas chromatographic retention time and pmr spectral properties as those of the natural product (see Table 1).

Table 1

Compound	Retention time on 1% OV-25	100 MHz pmr data in δ				
		H_A	H_B	C-18	C-19	C-21
2 (natural)	23.7 min	5.78	6.91	0.65	0.99	0.99
2 (synthetic)	23.7 min	5.78	6.91	0.66	0.99	0.99
4 (synthetic)	28.2 min	5.76	6.87	0.74	1.01	1.08

Evidence indicating that the m/e 430 component of peak B is the 5,6-dihydro analog of 2 was provided by the mass spectral peaks at m/e 430 (M^+), m/e 257 [M^+ - (AcOH + side chain)] and m/e 215 (D-

ring cleavage + H transfer).[6] Singlet signals, 3H, in the pmr spectrum at δ 0.80 and δ 0.64 are in excellent agreement with calculated values for the C-19 and C-18 methyl groups of a 3β-acetoxy-5α,14α-steroid.[10]

With the limited data in hand at present, the 430/432 steroids in peak A of the GC chromatogram are believed to be the 22,23-dihydro and 5,6,22,23-tetrahydro analogs of 2 respectively. The 442/444 compounds in peak C are believed to be the 4-methyl homologs of the peak B components.

The presence of marine sterols possessing short side chains has recently been reported[11] and is a topic of current interest.[12] It is likely that they arise from the biological oxidation of cholesterol. On the other hand, no bile acid-type sterols have been isolated from marine sources other than fish bile and these possess the standard saturated bile acid side chain of "normal" (i.e. 4) C-20 stereochemistry. The natural occurrence of the methyl ester of an unsaturated bile acid side chain with inverted C-20 stereochemistry raises intriguing biosynthetic questions which we hope to answer. The C-20 stereochemistry had not undergone a change during the isolation procedure since no isomerization of 2 to 4 was detected under refluxing methanolic HCl conditions.

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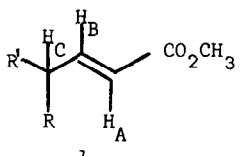

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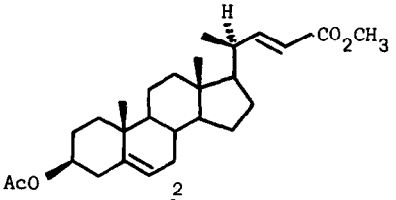

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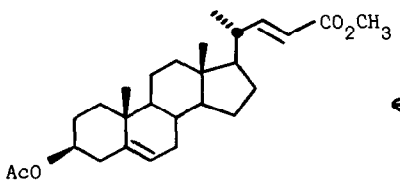

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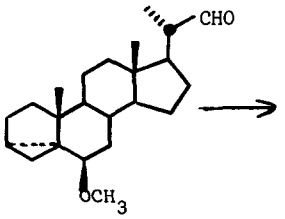

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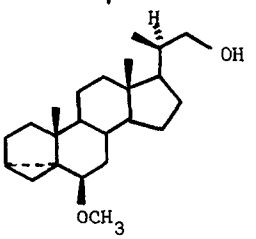

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REFERENCES

* Part 3 in the series "Minor and Trace Sterols in Marine Invertebrates". For part 2 see S. Popov, R. M. K. Carlson, A-M. Wegmann, and C. Djerassi, Tetrahedron Lett. 3491 (1976).

1. a) L. S. Ciereszko and T. K. B. Karns, in "Biology and Geology of the Coral Reef", ed. O. A. Jones and R. Endeon, Vol. II, Academic Press, New York, 1973, Ch. 6; b) E. Premuzic, Fort. Chem. Org. Nat., 29, 417 (1972); c) J. T. Baker and V. Murphy, "Handbook of Marine Science Compounds from Marine Organisms", Vol. I, CRC Press, Cleveland, Ohio, 1976.

2. The specimens used in this study were collected in Monterey Bay by dredge in August 1973, oven dried at <50°C and extracted twice with hexane.

3. D. J. Vanderah, presented in part at the Pacific Conference on Chemistry and Spectroscopy, Phoenix, Arizona, November 8, 1976.

4. Relative ratios determined from the M^+ - AcOH peaks in the mass spectra.

5. R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds", 2nd ed, John C. Wiley and Sons, Inc., New York, New York, 1963.

6. Z. V. Zaretskii, "Mass Spectroscopy of Steroids", Israel Universities Press, Jerusalem, Israel, 1976.

7. a) M. Fryberg, A. C. Oehlschlager, and A. M. Unrau, Tetrahedron, 27, 1261 (1971); b) J. A. Steele and E. Mosettig, J. Org. Chem., 28, 571 (1963); c) G. D. Anderson, T. J. Powers, C. Djerassi, J. Fayos, and J. Clardy, J. Amer. Chem. Soc., 97, 388 (1975).

8. Satisfactory high resolution analyses were obtained on 2, 4, and 5. The pure compounds were homogeneous by tlc and gas chromatographic (1% OV-25) analysis.

9. a) R. Ratcliff and R. Rodehorst, J. Org. Chem., 4000 (1970); b) J. C. Collins, W. W. Hess, and F. J. Frank, Tetrahedron Lett., 3363 (1968).

10. N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry", Holden-Day, Inc., San Francisco, Ca., 1964.

11. S. Popov, R. M. K. Carlson, A. Wegmann, and C. Djerassi, Steroids, 28, 699 (1976).

12. C. Djerassi, R. M. K. Carlson, S. Popov, and T. Varkony, paper presented at Nato Conference on Marine Natural Products, October 17, 1976, Jersey, U.K., to be published by Plenum Press.