MINOR AND TRACE STEROLS IN MARINE INVERTEBRATES 55. THE ISOLATION, STRUCTURE ELUCIDATION AND SYNTHESIS OF ERGOSTA-5,24(28),25-TRIEN-3β-OL

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Received 8-5-85

ABSTRACT

The isolation, structure determination and synthesis of ergosta-5,24(28),25-trien-3β-ol, as well as the synthesis of its 28-^{14}C analog -- a possible biosynthetic precursor of several marine sterols -- is described.

The hitherto undescribed ergosta-5,24(28),25-trien-3β-ol could be a key intermediate in the biosynthesis (Scheme 1) of three intriguing marine sterols, (24R)(26R)24,26-dimethyl-26,27-cyclocholesten-3β-ol (petrosterol) in the sponge Petrosia ficiformis (2,3), 26,27-dimethyl-ergosta-5,24(28)-dien-3β-ol (xestosterol) in the sponge Xestospongia testudinaria (4) as well as (24R)24-ethyl-27-methylcholesta-5,25(26)-dien-3β-ol (strongylosterol) in the sponge Strongylophora durissima (5). However, a thorough analysis of these sponges did not furnish that sterol, thus leaving open the question of its natural occurrence. In this communication we report the first isolation of ergosta-5,24(28),25-trien-3β-ol as a naturally occurring sterol from the soft coral Sinularia gyrosa, which was collected in Guam.

The sterol mixture of Sinularia gyrosa was subjected to reverse phase HPLC (2 Altex-Ultrasphere columns in series, mobile phase methanol). The fraction corresponding to a peak with the shortest RRT 0.78 (cholesterol RRT 1.00) was collected. The isolated compound was a minor sterol (0.1%) of a sterol mixture of 15 sterols (Table 1). It

gave a single peak in the GC with RRT 1.57 (cholesterol RRT 1.00), and its mass spectrum with M^+ 396 showed a pattern typical of Δ^5-3 β-sterols (m/z 271, 255, 231, 213) with an extremely intense fragment ion at m/z 371 (M^+ - side chain), thus suggesting a C28 sterol with three degrees of unsaturation -- one in the nucleus and two in the side chain. The ^{1}H NMR spectrum showed, in addition to the C_6 proton at 5.055 ppm (1 H, m, 6-CH), the presence of two narrow doublets at 4.935 (2H, d, J = 8.72 Hz) and 5.074 (2H, d, J = 5.18 Hz), along with a doublet at 0.974 of the only secondary methyl group (C-21) and the singlets at 1.008 and 0.682 for the tertiary methyl groups (C-19 and C-18 respectively). Irradiation experiments showed that the olefinic protons are not coupled to any other protons in the molecule, thus indicating that they correspond to two methylidene groups with two geminal protons, each coupled in a conjugated diene system. This was confirmed by its UV spectrum (λ_{max} 227 nm). Of the possible structures fitting the above data, only structure 3 in Scheme 2 was consistent with the methyl singlet at 1.869 which required attachment of the side chain methyl group to one of the double bonds.

Final proof of the structure was achieved through the synthesis of the sterol as outlined in Scheme 2. This route was also used to synthesize the 28-^{14}C analog required for biosynthetic incorporation experiments to test the existence of this intermediate in the biosynthesis of petrosterol, xestosterol and strongylosterol (Scheme 1). The label at C-28 was introduced through the Wittig reaction of methyl-(^{14}C)-triphenylphosphonium iodide (6) and 6 β-methoxy-3 α,5-cyclocholest-25-en-24-one, followed by the removal of the i-methyl ether protecting group with p-toluenesulfonic acid.

Incubation experiments with the labelled sterol are under investigation and will be reported in due course.

EXPERIMENTAL

Sample collection. Sinularia gyrosa was collected in Guam by Prof. F.J. Schmitz and the sterol fraction was isolated by Dr. V.J. Lakshmi, both of the Department of Chemistry, University of Oklahoma, in a manner very similar to that described earlier (7).

6β-Methoxy-3α,5-cyclo-cholest-25(26)-en-24-one (2). A solution of 200 mg (0.54 mmol) of 6β-methoxy-3α,5-cyclocholan-24-al 1 (prepared from cholenic acid methyl ester by a known procedure (8)) in 5 mL anhydrous ether was added slowly to the Grignard solution from 72 mg (3 mmol) of magnesium and 360 mg (3 mmol) of 2-bromopropene in ether. The mixture was stirred overnight and worked up in the usual manner. The crude alcohol was oxidized using Collins reagent. The usual workup followed by purification through preparative TLC (silica gel, 8:2 hexane/ethyl acetate as eluent) afforded the ketone 2 (80 mg; 34% yield) from aldehyde 1. NMR 0.714 (3H, s, 18-CH), 0.930 (3H, d, J = 6.44 Hz, 21-CH), 1.017 (3H, s, 19-CH), 1.869 (3H, s, 27-CH), 3.319 (3H, s, 6-OCH), 5.748 and 5.948 (2H, 2s, 26-CH); mass spectrum, m/z (relative intensity) 412 (65, M), 397 (34), 380 (22), 365 (7), 357 (62), 285 (65), 253 (100).

Ergosta-5,24(28-^{14}C),25-trien-3β-ol (3). 0.5 mL of anhydrous ether and 15.4 mg (0.037 mmol) of methyl-(^{14}C)-triphenylphosphonium iodide were placed in a dry 5 mL reactvial; 0.030 mL (0.072 mmol) of an approximately 2.7 M n-butyllithium solution in hexane was introduced through the valve which was then closed and the mixture stirred for 3 h at room temperature. The ketone 2 (30 mg, 0.073 mmol) in 1 mL dry ether was added and the mixture stirred for 5 days. The mixture was then diluted with ether and filtered through a small silica gel column in a pasteur pipette. After evaporation of the solvent, the product was purified by thin layer chromatography (silica gel, 95:5 hexane/ethyl acetate as eluent) and the i-methyl-ether protecting group removed by hydrolysis with p-toluenesulfonic acid in 20% aqueous dioxane to afford 3. Specific activity 42.9 mCi/mmol. NMR 0.683 (3H, s, 18-CH), 0.975 (3H, d, J = 6.47, 21-CH), 1.008 (3H, s, 19-CH), 1.902 (3H, s, 27-CH), 4.937 and 4.963 (2H, 2s, 26-CH), 5.055 and 5.072 (2H, 2s, 28-CH). The NMR and mass spectra as well as GC and HPLC retention times of the unlabelled sample were identical with those of the natural specimen.

The above procedure for the synthesis of the labelled compound was used on the same scale for the synthesis of the unlabelled compound using unlabelled methyltriphenylphosphonium iodide.

ACKNOWLEDGMENTS

Financial support for this research was provided by NIH grants GM 06840 and GM 28352. Use of the 300 MHz NMR spectrometer was made possible by NSF grant CHE 81-09064. (We thank Annemarie Wegmann-Szente for mass spectral determinations .) Use of the 500 MHz NMR spectrometer was made possible by NIH grant RR 00711. We thank Drs. F.J. Schmitz and V.J. Lakshmi of the University of Oklahoma for providing the sterol fraction of S. gyrosa.

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APPENDIX

Petrosterol: (24R)(26R)24,26-Dimethyl-26,27-cyclocholesten-3β-ol.
Xestosterol: 26,27-Dimethylergosta-5,24(28)-dien-3β-ol.
Strongylosterol: (24R)24-Ethyl-27-methylcholesta-5,25(26)-dien-3β-ol.

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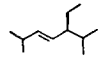
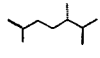
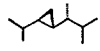
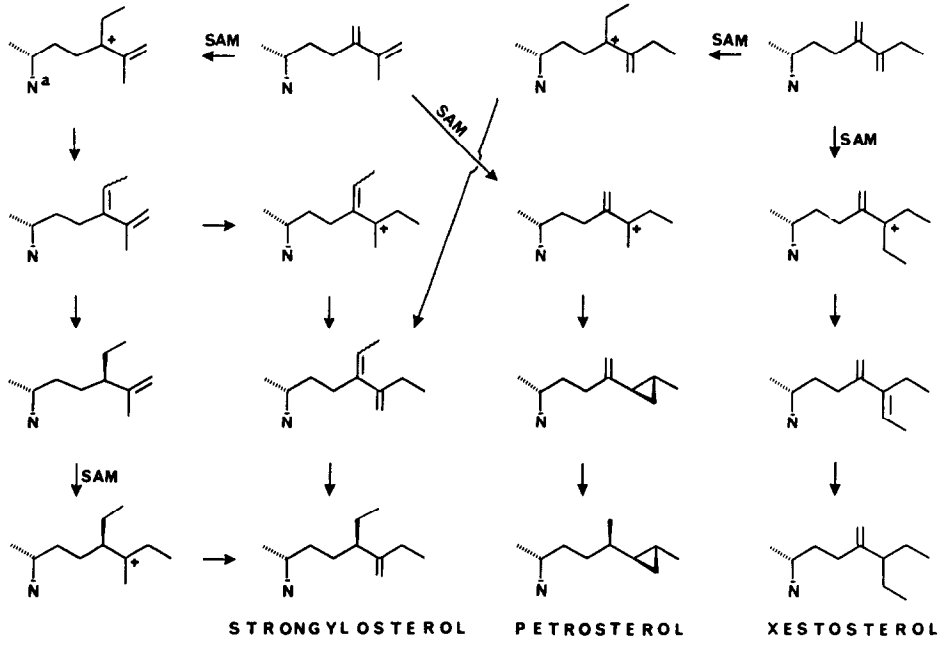
TABLE 1. STEROLS ISOLATED FROM THE SOFT CORAL SINULARIA GYROSA

	SIDE CHAIN	NUCLEUS[c]	M^+	MOBILITY GC[a]	MOBILITY HPLC[b]	%
1.		Δ^5	396	1.57	0.78	0.3
2.		Δ^5	384	0.97	0.81	0.3
3.		Δ^5	398	1.32	0.84	0.2
4.		Δ^5	398	1.35	0.87	47.0
5.		Δ^5	398	1.13	0.91	0.4
6.		Δ^5	398	1.13	0.93	11.0
7.		Δ^0	400	1.35	0.96	0.5
8.		Δ^5	386	1.00	1.00	7.0
9.		Δ^5	412	1.30	1.04	0.7
10.		Δ^5	412	1.40	1.10	0.2
11.		Δ^5	400	1.31	1.12	14.0
12.		Δ^5	412	1.70	1.19	1.0
13.		Δ^5	414	1.62	1.21	0.4
14.		Δ^5	426	2.30	1.49	15.0
15.		Δ^0	428	2.28	1.64	4.0

[a]Gas chromatographic retention times are relative to cholesterol (1.00) on 3% OV-17 column; oven temperature 260° C.

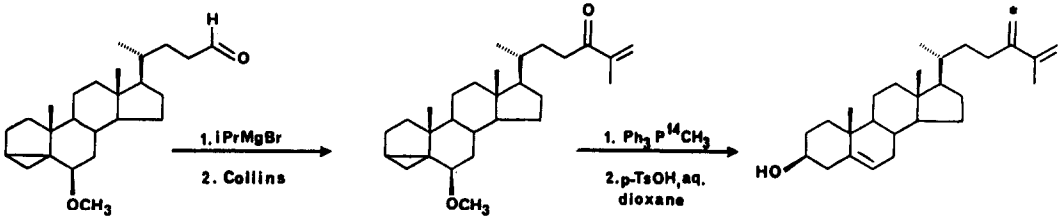
[b]Relative to cholesterol(1.00) using two Altex Ultrasphere ODS columns and methanol as eluent.

[c]Δ^0 refers to a 5α saturated Δ^5 nucleus.



[a] N refers to a Δ^5-3β-ol sterol nucleus.

SCHEME 1



SCHEME 2