STEROL COMPOSITION OF THE MOLLUSK *AULACOMYA ATER*

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Extensive examination of the sterols of mollusks has pointed out that bivalves are unique in containing a great diversity of Δ^5 (1-3) and $\Delta^{5,7}$ (4-6) sterols. In view of this and as part of our investigation on natural products isolated from Argentine marine invertebrates (7), we report herein the identification of the sterols obtained from the bivalve mollusk *Aulacomya ater* (cholga). The specimens were collected at the south Atlantic shore (San Jose Gulf), where they live on sand beds extending from the shore to a depth of 50 m.

The mixture was analyzed by glc on a capillary column and was quantified by automatic integration of the glc peaks by the addition of known quantities of stigmasterol, which was used as the internal standard. The components of the mixture were characterized by glc/ms of the free sterol mixture and of their acetylated derivatives (table 1). Mass spectrometric analysis of the trimethylsilyl ether derivatives allowed unequivocal assignments of Δ^5 and Δ^7 sterols (8,9), which were done in all the cases by comparison with authentic samples. As shown in table 1, we identified from the sterol mixture various Δ^5 and $\Delta^{5,22}$ compounds characteristic of mollusks, cholesterol being the most abundant of them.

24-Methyl-cholesta-5,24(28)-dien-3β-ol (10) is also among the common sterols found in marine invertebrates.

The unusual 24-norcholesta-5,22-dien-3β-ol, first reported by Idler *et al.* (11) from a scallop, is now recognized as being widespread in the marine environment. The glc showed the presence of a leading shoulder on the peak from 22-*trans*-cholesta-5,22-dien-3β-ol, with relative retention time corresponding to 22-*trans*-24-methyl-27-norcholesta-5,22-dien-3β-ol (occelasterol) and/or 22-*cis*-cholesta-5,22-dien-3β-ol. It is known that these two compounds present identical retention times and mass spectra (12), making impossible a safe distinction between them by these methods. Moreover, their mass spectra are quite similar to that of the *trans*-Δ^{22} isomer. Mass spectrometric analysis of the leading side of the glc peak showed the presence of compound(s) with mass spectra identical to that of the *trans*-Δ^{22} isomer. The occurrence of the *cis*-Δ^{22} sterol as a natural product has been questioned (12). The presence of cholest-7-en-3β-ol is unusual in this class of mollusks, which contains predominantly Δ^5 sterols (6). It is interesting to note the appearance of the steroidal hydrocarbon whose mass spectral analysis points to a triunsaturated cholestane (13), cholesta-2,4,6-triene or cholesta-1,4,6-triene. The imprecise determination is due to the fact that it is not possible to distinguish between the mass spectra of both compounds because of the normal isomerization that occurs when polyunsaturated hydrocarbons are analyzed by electron impact mass spectrometry (14). Otherwise, these known compounds (15-17) are, to our knowledge, unprecedented as natural products and we do not know whether they exist as natural compounds or are artifacts of the extraction procedure.

These mollusks are plankton feeders, and, as it has been shown with other bivalves (18), some of the sterols reported here may come from direct uptake or by bioconversion of sterols from dietary sources.

TABLE 1. Sterol composition of *Aulacomya ater*.

Sterols	Ms characteristic fragments	rrt[a]	Estimated %	
			by glc	by SIM
Cholestatriene	366(M^+),351,253,247, 211,143,135,119	0.78	1.35	
24-Norcholesta-5,22-dien-3β-ol	370(M^+),355,352,300, 271,255,213,97,55	0.81	3.19	
22-*cis*-Cholesta-5,22-dien-3β-ol and/or 22-*trans*-24-Methyl-27-norcholesta-5,22-dien-3β-ol	384(M^+),366,351,300, 273,271,255,213,111, 69,55	0.92	13.80	
22-*trans*-Cholesta-5,22-dien-3β-ol	384(M^+),366,351,300, 273,271,255,213,111, 69,55	0.94		
Cholest-5-en-3β-ol	386(M^+), 371,368,301, 275,273,255,231,213, 145,43	1.00	48.66	
22-*trans*-24-Methylcholesta-5,22-dien-3β-ol	398(M^+),380,365,300, 271,255,213,69,55	1.04	11.36	
Cholest-7-en-3β-ol	386(M^+), 371,273,255, 231,229,213,147,145, 105,43	1.09	2.07	
24-Methylcholest-5-en-3β-ol	400(M^+),385,382,367, 315,289,273,213,105, 43	1.11		8.00
24-Methylcholesta-5,24(28)-dien-3β-ol	398(M^+),383,380,314, 299,281,271,255,229, 213,55	1.11		7.20
22-*trans*-24-Ethylcholesta-5,22-dien-3β-ol	412(M^+),397,394,379, 351,300,273,271,255, 69,55	1.15	1.71	
24-Ethylcholesta-5-en-3β-ol	414(M^+),396,381,368, 329,303,255,213,91,55	1.23	2.69	

[a]Relative retention times (rrt) of the free sterols to cholest-5-en-3β-ol.

EXPERIMENTAL

Fresh tissue of *Aulacomya ater* specimens (104 g) was homogenized in ethanol (400 ml) and filtered. The remaining solid was extracted twice with ethanol (100 ml each) and the residue reextracted once with ethyl acetate (100 ml). The combined organic extracts were taken to dryness and the residue was dissolved in ethyl acetate (200 ml), washed with water, dried over magnesium sulphate, and evaporated. The syrup (1.7 g) was chromatographed on a silica gel column, eluting with mixtures of toluene-ethyl acetate of increasing polarity. The crude sterol mixture (0.151 g) was recrystallized from ethanol giving a crystalline mixture. This was analyzed by glc using a Hewlett-Packard 5840 gas chromatograph equipped with a 12 m × 0.2 mm fused silica capillary column coated with methyl silicone fluid (Hewlett-Packard).

The steroids were chromatographed between 240° and 280° at a rate of 10°/min, with helium as the carrier gas. Quantification was made by automatic integration of the glc peaks before and after the addition of a known amount of stigmasterol used as the internal standard. Glc detector relative responses were determined with an artificial mixture of the most common sterols (C-27, C-28, and C-29). The identity of the steroids were assigned by glc/ms using a Varian-Mat CH7-A mass spectrometer coupled to a Varian 1440 gas chromatograph and interfaced to a Varian-Mat Data System 166 computer.

Analysis of their acetate (acetic anhydride-pyridine, 1:1) and trimethylsilyl ether derivatives (hexamethyldisilazane - trimethylchlorosilane - pyridine, 3:3:10) were performed in the same conditions. The relative amounts of 24-methylcholest-5-en-3β-ol and 24-methyl-cholesta-5,24(28)-dien-3β-ol were discerned by single ion

monitoring (SIM) of the sterols base peaks in the mass chromatogram of the mixture. Data accuracy were checked by SIM quantification of artificial mixtures of the two sterols.

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LITERATURE CITED

1. D.R. Idler and P. Wiseman, *J. Fish. Res. Board Can.*, **29**, 385 (1972).
2. S. Teshima, G.W. Patterson, and S.R. Dutky, *Lipids*, **15**, 1004 (1981).
3. L.J. Goad, "The sterols of marine algae and invertebrate animals," in: "Biochemical and Biophysical Perspectives in Marine Biology," Vol. 3, D.C. Malins and J.R. Sargent, Eds. Academic Press, London, 1976.
4. D.R. Idler and P. Wiseman, *J. Fish. Res. Board Can.*, **21**, 1035 (1972).
5. S. Teshima and G.W. Patterson, *Comp. Biochem. Physiol.*, **68B**, 171 (1981).
6. L.J. Goad, "The sterols of marine invertebrates: composition, biosynthesis and metabolites," in: "Marine Natural Products," P.J. Scheuer, Ed., Academic Press, New York, 1978.
7. M.S. Romero and A.M. Seldes, *Experientia*, **39**, 258 (1983).
8. C.J.W. Brooks, E.C. Horning, and J.S. Young, *Lipids*, **3**, 391 (1968).
9. J. Diekman and C. Djerassi, *J. Org. Chem.*, **32**, 1005 (1967).
10. D.R. Idler and U.H.M. Fagerlund, *J. Am. Chem. Soc.*, **77**, 4142 (1955).
11. D.R. Idler, P.M. Wiseman, and L.M. Safe, *Steroids*, **16**, 451 (1970).
12. M. Kobayashi and H. Mitsuhashi, *Steroids*, **24**, 399 (1974).
13. C.J.W. Brooks and B.S. Middleditch, in: "Modern Methods of Steroid Analysis," E. Heftmann, Ed. Academic Press, Inc., New York, 1973.
14. J. Schmutz, H. Schaltegger, and M. Sanz, *Helv. Chim. Acta*, **34**, 1111 (1951).
15. D.H. Gould, K.H. Schaaf, and W.L. Rnigh, *J. Am. Chem. Soc.*, **73**, 1263 (1951).
16. F. Hunziker, F.X. Mullner, K.G. Reuteler, and H. Schaltegger, *Helv. Chim. Acta*, **38**, 1316 (1955).
17. H. Budzikiewicz, D. Djerassi, and D.H. Williams. In: "Mass Spectrometry of Organic Compounds," Holden-Day, Inc., San Francisco, 1967.
18. C.J. Berenberg and G.W. Patterson, *Lipids*, **16**, 276 (1981).

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