STEROLS FROM THE MARINE SPONGES *ORINA ARCOFERUS* AND *GEODIA MEGASTRELLA*

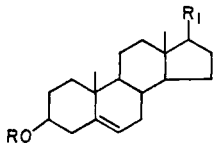
JOHN F. KINGSTON, EARL BENSON, BRIAN GREGORY and ALEX G. FALLIS

Department of Chemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3X7

As part of our investigation of local marine natural products, we have examined a number of marine invertebrates inhabiting the cold waters off Newfoundland and Labrador (1). Warm water sponges have proved a rich source of various natural products including sesquiterpenes (2), sesterterpenes (3), purines (4) and steroids (5). Sponges of the *Choristida* and *Haplosclerida* orders have received relatively little attention (6). We report herein the isolation and identification of the sterols isolated from the sulphur sponge *Geodia megastrella* (Class *Desmospongiae*, Order *Choristida*) collected from a depth of 400 fathoms off the Labrador coast (Hamilton Bank) and the glass sponge *Orina arcoferus* (Class *Desmospongiae*, Order *Haplosclerida*) from the same general area.

Fractionation of the acetone extract of freeze-dried animals by high speed liquid chromatography on silica gel afforded, from the chloroform eluent, a lipid fraction containing the sterol components. This mixture was acetylated and further fractionated into four bands by tlc on silver nitrate impregnated silica gel. In each case the major component was shown to be 24-methylenecholesteryl acetate (**8**) (7) located in the first band (lowest R_f) and identified by direct comparison (mp, ^1H nmr, ms) with an authentic sample. The acetate was hydrolyzed ($KOH/EtOH/H_2O$) to the free sterol, a sample of which was purified by glc.

The second band included all the other steryl acetates containing a Δ^5 and sidechain double bond at either Δ^{22} or Δ^{24}. As indicated in the table these were separated by glc on both the acetate and free sterol and the mass spectra and retention times compared with authentic samples. Occelasterol (**3**) and *cis*-22-dehydrocholesterol (**2**) cannot be distinguished by



1. R = H or Ac, R_1 =
2. R = H or Ac, R_1 =
3. R = H or Ac, R_1 =
4. R = H or Ac, R_1 =
5. R = H or Ac, R_1 =
6. R = H or Ac, R_1 =
7. R = H or Ac, R_1 =
8. R = H or Ac, R_1 =
9. R = H or Ac, R_1 =
10. R = H or Ac, R_1 =
11. R = H or Ac, R_1 =
12. R = H or Ac, R_1 =

glc, and their mass spectra are the same as the *trans* Δ^{22} isomer (**4**) (8). At least two of these three sterols were present in each of the sponges ex-

Table I. Glc Retention Times of *O. arcoferus* and *G. megastrella* Sterols and their Acetates.

	RELATIVE RETENTION TIME STEROL			RELATIVE RETENTION TIME ACETATE			ESTIMATED % (a)	
	AUTHENTIC SAMPLE	*O. arcoferus*	*G. megastrella*	AUTHENTIC SAMPLE	*O. arcoferus*	*G. megastrella*	*O. arcoferus*	*G. megastrella*
24-NORCHOLESTA-5,22-DIEN-3β-OL (e) (1) (10)		0.67	0.66		0.67	0.67	11	0.5
cis-22-DEHYDROCHOLESTEROL (b,e) (2) (8) OCCELASTEROL (b,e) (3) (9)		0.91	0.90		0.91	0.91	(c) 13	3
trans-22-DEHYDROCHOLESTEROL (e) (4) (8)		~0.94	~0.94		~0.94	~0.94	(c)	
CHOLESTANOL	1.00	1.00	1.00	1.00	1.00	1.00	2	
CHOLESTEROL (5)	1.00	1.00	1.00	1.00	1.00	1.00	17	9
24-METHYLCHOLESTA-5,22-DIEN-3β-OL (d) (BRASSICASTEROL, SPONGESTEROL) (6) (11)	1.15	1.16	1.15	1.15	1.14	1.15	13	3.5
24-METHYLCHOLESTEROL (d) (DIHYDROBRASSICASTEROL, CAMPESTEROL) (7)	1.34	1.35	1.36	1.32	1.32	1.32	3	1
24-METHYLENECHOLESTEROL (8) (7)	1.35	1.36	1.35	1.35	1.35	1.36	27	72
24-ETHYLCHOLESTA-5,22-DIEN-3β-OL (d) (PORIFERASTEROL, STIGMASTEROL) (9)	1.43	—	—	1.44	—	—		
24-ETHYLCHOLESTEROL (d) (CLIONASTEROL, β-SITOSTEROL) (10)	1.65	1.66	1.65	1.63	1.63	1.62	5	3
FUCOSTEROL (11) (12)	1.79	1.79	1.77		1.73	1.71	4	4
ISOFUCOSTEROL (12) (12)				1.80				

Footnotes for Table 1 on next page.

amined. It is not clear if the *cis* Δ^{22} sterol (**2**) is actually a natural product as its existence has recently been questioned (9).

The third band contained mainly the acetates of Δ^5 sterols with a saturated side chain. The fourth band was mainly cholestanyl acetate but showed traces of the acetates of C_{28} and C_{29} stanols, which were not present in sufficient quantity for glc collection and identification.

The two sponges contained similar mixtures of sterols but in considerably different amounts, sterols accounting for 1.18% of the dry weight of *O. arcoferus* and only 0.096% of the dry weight of *G. megastrella*.

EXPERIMENTAL

The glass sponge (*Orina arcoferus* Class *Desmospongiae*, Order *Haplosclerida*) was collected at a depth of ~85 fathoms in the Bradore Trough on the Hamilton Bank (Labrador), and the sulfur sponge (*Geodia megastrella* Class *Desmospongiae*, Order *Choristida*) was collected using a deep water trawl (400 fathoms) on the South Hamilton Bank in the North Hawke Channel. The later sample was elliptical in shape 92 x 66 cm, therefore, the entire investigation was conducted with one animal.

The sponges were cut in pieces and freeze dried. They were pulverized in a mortar and pestle and extracted twice with acetone. The oily extracts so obtained were fractionated by high speed liquid chromatography (Waters Porasil A, 75-125 μ, 122 cm x 7 mm i.d.) with chloroform as eluent. Thus *Orina arcoferus* (239 g, freeze dried) gave 8.32 g of crude extract; further purification of 365 mg of this extract afforded 124 mg of sterols (sterol yield from freeze dried sponge, 1.18%). Similarly, *Geodia megastrella* (167 g, freeze dried) afforded 776 mg of crude extract, which provided 160 mg of sterols (0.096%).

The sterol fractions were acetylated (acetic anhydride, pyridine, 23°, 16 h) and separated into four major bands by preparative tlc on silver nitrate impregnated silica gel ($AgNO_3$:silica gel PF 254 and 366 1:5 W/W, developed twice with benzene-petroleum ether, 2:3). Portions of these bands were hydrolyzed (8% aq KOH/EtOH, 4 h) to afford the free sterols. These sterols and the sterol acetate mixtures were independently analyzed and collected by prep. glc (1.5% OV-17 on 100/120 mesh Gas Chrom Q, 3 m x 6 mm i.d., 80 ml/min He, temp. 270° sterols, 282° acetates) as summarized in table 1. The mass spectra of the pure sterols and acetates collected from the glc were recorded (Hitachi Perkin Elmer RMU6E, 70 eV) and compared with those of authentic samples and/or published spectra.

ACKNOWLEDGMENT

We thank IBM Canada, Memorial University of Newfoundland, and the National Research Council of Canada for financial support of this research, R. Bowring for collecting the sponges, H. M. Reiswig and E. L. Bousfield for their taxonomic identification, and E. Bullock for his encouragement.

The following individuals generously provided samples of authentic sterols or their acetates: L. J. Goad, R. H. Thompson, and P. J. Scheuer.

Received 12 March 1979.

LITERATURE CITED

1. For previous work see J. F. Kingston, B. Gregory and A. G. Fallis, *J. Chem. Soc. Perkin I*, 1979, in press.
2. B. J. Burreson, P. J. Scheuer, J. Finer and J. Clardy, *J. Am. Chem. Soc.*, **97**, 4763 (1975).
3. F. Cafieri, E. Fattorusso, C. Santacroce and L. Minale, *Tetrahedron*, **28**, 1579 (1972).
4. E. Cullen and J. P. Devin, *Can. J. Chem.*, **53**, 1690 (1975).
5. F. J. Schmitz, in P. J. Scheuer, Marine Natural Products, Chemical and Biological Perspectives Vol I. Academic Press, New York, N.Y. 1978, pp. 241–297.

Footnotes for Table 1

(a) The remaining percentages consist of a number of sterols present in too small a quantity to be identified.
(b) These two compounds cannot be distinguished by glc or ms.
(c) The presence of at least two of these compounds was indicated by a leading shoulder on the glc peak. Collection on the leading and trailing sides of the peak afforded compounds with identical ms.
(d) 24-α and β isomers cannot be distinguished by these methods.
(e) Authentic samples were not available for these compounds. Glc and ms data were in agreement with the literature values (8–10, 12).

Relative retention times are reported to accuracies of ±0.02.

6. M. de Rosa, L. Minale and G. Sodano, Comp. Biochem. Physiol., **46B**, 823 (1973).
7. T. R. Erdman and R. H. Thomson, *Tetrahedron*, **28**, 5163 (1972).
8. D. R. Idler and P. Wiseman, Comp. Biochem. Physiol., **38A**, 581 (1971).
9. M. Kobayashi and H. Mitsuhashi, *Steroids*, **24**, 399 (1974).
10. D. R. Idler, P. M. Wiseman and L. M. Safe, *Steroids*, **16**, 451 (1970).
11. I. Rubinstein and L. J. Goad, *Phytochem.*, **13**, 485 (1974).
12. B. A. Knight and C. J. W. Brooks, *Phytochem.*, **8**, 463 (1969).