



Main Sterols from the Ophiuroids *Ophiocoma echinata*, *Ophiocoma wendtii*, *Ophioplocus januarii* and *Ophionotus victoriae*

CARMENZA DUQUE,* JORGE ROJAS,* SVEN ZEA,† ALEJANDRO J. ROCCATAGLIATA,‡
MARTA S. MAIER‡|| and ALICIA M. SELDES‡

*Departamento de Química, Facultad de Ciencias, Universidad Nacional de Colombia, AA 14490 Bogotá,
Colombia;

†Departamento de Biología, Facultad de Ciencias, Universidad Nacional de Colombia, AA 14490 Bogotá,
Colombia;

‡Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires,
Pabellón 2, Ciudad Universitaria, (1428) Buenos Aires, Argentina

Key Word Index—*Ophiocoma echinata*; *Ophiocoma wendtii*; *Ophioplocus januarii*; *Ophionotus victoriae*;
Ophiuroidea; Echinodermata; marine sterols; sterol composition.

Abstract—Sterol compositions of the cold water ophiuroids *Ophioplocus januarii* and *Ophionotus victoriae* and of the tropical ophiuroids *Ophiocoma echinata* and *Ophiocoma wendtii* are reported. The four sterol mixtures contain Δ^5 mono- and di-unsaturated common 3β -hydroxy-sterols. *Ophioplocus januarii* and *O. victoriae* contain 24-methylcholesta-5,24(28)-dien- 3β -ol and 24 ξ -ethylcholesta-5,24(28)-dien- 3β -ol in higher abundance than in *O. echinata*. These sterols were not found in *O. wendtii*. An interesting finding is the presence of $\Delta^{5,24(28)}$ -24-*n*-propylidenecholesterol in 7.6% in *Ophionotus victoriae*. © 1997 Elsevier Science Ltd

Introduction

Echinoderms represent a rich source of natural compounds, including lipids, quinoid pigments, triterpenoid and steroid glycosides as well as sterols and their derivatives (Stonik and Elyakov, 1988). At present the echinoderms are divided into five classes: the Crinoidea (sea lilies), the Holothuroidea (sea cucumbers or holothurians), the Echinoidea (sea urchins), the Asteroidea (starfishes) and the Ophiuroidea (brittlestars).

Starfishes and sea cucumbers contain appreciable quantities of cytotoxic oligoglycosides (D'Auria *et al.*, 1993) and Δ^7 -sterols instead of cholesterol or phytosterols of the Δ^5 -series. The presence of Δ^7 -sterols may be a result of an adaptation to the organism's own toxins because Δ^7 -sterols form complexes with cytotoxic saponins of echinoderms with greater difficulty than with cholesterol. By contrast sea lilies, brittlestars and sea urchins lack oligoglycosides and contain Δ^5 -sterols. Although several important papers and a general review of sterols from the phylum Echinodermata have been published (Goad, 1978; Kerr and Baker, 1991) only sporadic papers on the sterol content of ophiuroids (Ricchio *et al.*, 1985) have appeared in the literature.

In continuation of our studies on the metabolites of echinoderms (Roccatagliata *et al.*, 1995) we had occasion to examine the sterol content of the ophiuroids *Ophiocoma wendtii*, *Ophiocoma echinata*, *Ophioplocus januarii* and *Ophionotus victoriae*. Previous work on *O. wendtii*, *O. echinata* and *O. victoriae* (D'Auria *et al.*, 1995) involved the isolation of polyhydroxysteroids with sulfoxy substituents at C-3 and C-21. Recently, we

||Corresponding author (Fax: (0541) 782-0529).

have isolated a new sulfated alkene from *O. echinata* (Roccatagliata *et al.*, 1997) and four sulfated polyhydroxysteroids from *O. januarii*, which have shown antiviral activity against four pathogenic viruses in humans (Roccatagliata *et al.*, 1996).

Materials and Methods

Animals. *Ophiocoma echinata* and *O. wendtii* were collected at Neguange Bay, Colombia and identified by Dr Sven Zea. *Ophioplocus januarii* was collected at San Antonio Oeste, Río Negro, Argentina and *O. victoriae* at Potter Cove near Jubany Base, South Shetland Islands, Antarctica. *Ophioplocus januarii* and *O. victoriae* were identified by Dr Alejandro Tablado from the Museo de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina where voucher specimens (MACN 31239 and MACN 31240) are preserved.

Extraction and isolation of sterols. The animals (1 kg, wet weight) were kept frozen until worked up. The frozen animals were chopped and extracted with MeOH (2×2l) during 12 h. The combined extracts were evaporated, and partitioned between hexane and H₂O. The crude sterol mixtures were detected exclusively in the hexane layer which was evaporated, leaving brownish oils (3.8 g from *O. echinata*, 6.4 g from *O. wendtii*, 4.6 g from *O. januarii* and 3.8 g from *O. victoriae*). Each oil was subjected to flash chromatography on silica gel using benzene:ethyl acetate (5:1) as the eluent to afford the crude sterol mixture (135 mg from *O. echinata*, 420 mg from *O. wendtii*, 192 mg from *O. januarii* and 450 mg from *O. victoriae*). Each sterol mixture was separated by HPLC. Enriched fractions and pure compounds were analyzed by GC-MS and ¹H NMR spectroscopy.

Analytical methods. HPLC was performed using a Merck-Hitachi D-6500 apparatus and a Merck-Hitachi L-4500 detector. High-performance liquid chromatography (HPLC) column: Ultrasphere ODS C-18 (10×250 mm; eluant: MeOH:H₂O (98:2)). The flow rate was 0.6 ml min⁻¹. For gas chromatography (GC), a Hewlett-Packard model 5890 gas chromatograph was used with a OV-101 capillary column (25 m×0.32 mm i.d.). Helium was used as the carrier gas at a flow rate of 1.3 ml min⁻¹. GC-mass spectrometry (MS) was performed on a VG Trio 2 (Fisons) mass spectrometer. ¹H NMR spectra were recorded on a Bruker AC-200 NMR spectrometer.

Results and Discussion

After purification of the sterol mixtures, they were separated using reversed-phase HPLC. The identification of the sterols was based on comparison of GC and HPLC retention times with authentic standards and on a careful analysis of the GC-MS spectral data. ¹H NMR spectroscopy (200.1 MHz) was also used for structural assignment of those sterols isolated in sufficient amount. Results are listed in Table 1 and the structures of the identified sterols are depicted in Fig. 1.

The results obtained from the four ophiuroids showed that they contained 3 β -hydroxy- Δ^5 -sterols. Neither stanols nor Δ^7 -sterols were found among the main constituents of the sterol mixtures. This observation is in accordance with the fact that the four ophiuroids lack oligoglycosides.

As shown in Table 1, the four sterol mixtures contained Δ^5 mono- and di-unsaturated sterols, the mono-unsaturated sterols accounting for *c.* 49% of the sterol composition in *O. echinata* and 43% in *O. wendtii*. On the contrary, the sterol mixtures of *O. januarii* and *O. victoriae* showed *c.* 70% of di-unsaturated sterols and (22*E*)-24*S*-methylcholesta-5,22-dien-3 β -ol (**5**) as the major sterol in both mixtures.

Among the di-unsaturated sterols isolated from the four ophiuroids, 22-dehydrocholesterol (**3**) is an important component (14–19%) in all of them. There are reports on the rare occurrence of **3** as the major sterol of the diatoms *Biddulphia sineusis* and *Nitzschia cylindrus* (Kerr and Baker, 1991) and as a minor component of molluscs and macroalgae. An interesting finding is the higher abundance of 24-norcholesta-5,22-dien-3 β -ol (**1**) in the sterol mixtures of the cold water ophiuroids *O. januarii* (4.1%) and *O. victoriae* (4.9%) with respect to the sterol mixtures of the tropical ophiuroids *O. echinata* (1.7%) and *O. wendtii* (1.5%). The presence of this sterol along with 24-methyl-27-norcholesta-5,22-dien-3 β -ol (**2**) in large quantities in the dinoflagellate *Gymnodinium simplex* (Goad and Withers, 1982) is indicative of the dietary origin of these norsterols

TABLE 1. STEROL COMPOSITION OF *O. echinata*, *O. wendtii*, *O. januarii* AND *O. victorinae*

Sterol	RRT*		%			
	HPLC	GC	<i>O. echinata</i>	<i>O. wendtii</i>	<i>O. januarii</i>	<i>O. victorinae</i>
(22 <i>E</i>)-24-Norcholesta-5,22-dien-3 β -ol (1)	0.62	0.68	1.7	1.5	4.1	4.9
24-Methyl-27-norcholesta-5,22-dien-3 β -ol (2)	0.72	0.90	2.2	0.9	5.2	1.0
22-Dehydrocholesterol (3)	0.77	0.89	19.8	18.3	18.2	14.1
24-Methylcholesta-5,24(28)-dien-3 β -ol (4)	0.79	1.38	0.8	—	3.8	9.2
(22 <i>E</i>)-24 <i>S</i> -Methylcholesta-5,22-dien-3 β -ol (5)	0.82	1.10	17.3	21.3	29.0	23.1
(22 <i>E</i>)-24 <i>R</i> -Methylcholesta-5,22-dien-3 β -ol (6)	0.88	1.11	4.3	11.5	—	2.2
24 ξ -Ethylcholesta-5,24(28)-dien-3 β -ol (7)	0.94	1.45	2.8	—	8.5	6.7
24 ξ - <i>n</i> -Propylcholesta-5,24(28)-dien-3 β -ol (8)	0.96	1.80	—	—	—	7.6
Cholest-5-en-3 β -ol (9)	1.00	1.00	38.3	27.5	17.2	18.2
24 ξ -Methylcholest-5-en-3 β -ol (10)	1.07	1.39	3.2	2.5	2.2	—
24 ξ -Ethylcholesta-5,22-dien-3 β -ol (11)	1.09	1.45	2.3	3.5	2.0	1.2
24 ξ -Ethylcholest-5-en-3 β -ol (12)	1.14	1.70	7.3	13.0	9.8	11.9

*Relative retention time (RRT) of the sterols referred to cholesterol.

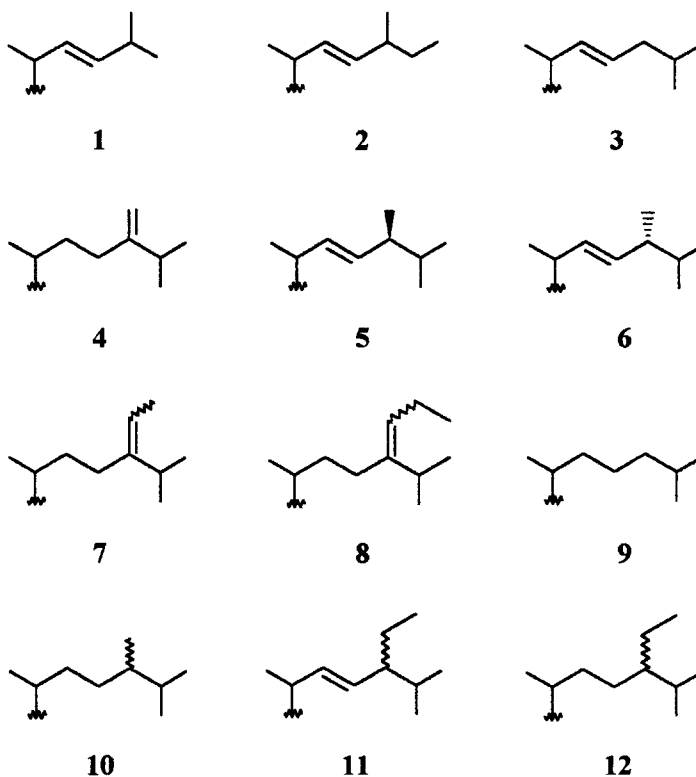


FIG. 1. Δ^5 -STEROLS IDENTIFIED FROM *O. echinata*, *O. wendtii*, *O. januarii* AND *O. victorinae*.

frequently found in marine invertebrates. 24-Methylcholesta-5,24(28)-dien-3 β -ol (**4**) is more abundant in *O. januarii* (3.8%) and *O. victoriae* (9.2%) than in *O. echinata* (0.8%) and has not been found in *O. wendtii*. *De novo* synthesis of cholesterol from mevalonate was demonstrated in the brittlestar *Ophiocomina nigra* (Goad, 1978) although C₂₈ and C₂₉ sterols were not radioactive, confirming the dietary origin of these sterols.

Cold water ophiuroids *O. januarii* and *O. echinata* contained 24 ξ -ethylcholesta-5,24(28)-dien-3 β -ol (**7**) in higher abundance, 8.5 and 6.7%, respectively, than in *O. echinata* (2.8%) and was not detected in *O. wendtii*.

Finally, only the Antarctic ophiuroid *O. victoriae* showed the presence of $\Delta^{5,24(28)}$ -24-*n*-propylidenecholesterol (**8**) in 7.6% abundance. This sterol is characteristic of *Chryso-phyte* algae of the order of *Sarcinochrysidales* which are known to have high concentrations of 24-*n*-propylsterols (Moldovan *et al.*, 1990).

Brittlestars exhibit a variety of feeding methods, including predation, deposit feeding, scavenging and suspension feeding; some species are capable of feeding by more than one process. Thus, the differences in the sterol compositions of the cold water ophiuroids with respect to the tropical ones could be attributed in part to different habitats and feeding habits.

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References

- D'Auria, M. V., Minale, L. and Riccio, R. (1993) Polyoxygenated steroids of marine origin. *Chem. Rev.* **93**, 1839–1895.
- D'Auria, M. V., Gomez Paloma, L., Minale, L., Riccio, R. and Zampella, A. (1995) On the composition of sulfated polyhydroxysteroids in some ophiuroids and the structure determination of six new constituents. *J. Nat. Prod.* **58**, 189–196.
- Goad, L. J. (1978) The sterols of marine invertebrates: composition, biosynthesis and metabolites. In *Marine Natural Products*, ed. P. J. Scheuer, pp. 76–172. Academic Press, New York.
- Goad, L. J. and Withers, N. (1982) Identification of 27-nor-(24*R*)-24-methylcholesta-5,22-dien-3 β -ol and brassicasterol as the major sterols of the marine dinoflagellate *Gymnodinium simplex*. *Lipids* **17**, 853–858.
- Kerr, R. G. and Baker, B. J. (1991) Marine sterols. *Nat. Prod. Rep.* **8**, 465–497.
- Moldovan, J. M., Fago, F. J., Lee, C. Y., Jacobson, S. R., Watt, D. S., Slougui, N.-E., Jeganathan, A. and Young, D. C. (1990) Sedimentary 24-*n*-propylcholestanes, molecular fossils diagnostic of marine algae. *Science* **247**, 309–312.
- Riccio, R., D'Auria, M. V. and Minale, L. (1985) Unusual sulfated marine steroids from the ophiuroid *Ophioderma longicaudum*. *Tetrahedron* **41**, 6041–6046.
- Roccatagliata, A., Maier, M. and Seldes, A. M. (1995) A novel marine steroid sulfate from the starfish *Luidia ludwigi*. *J. Nat. Prod.* **58**, 1941–1944.
- Roccatagliata, A. J., Maier, M. S., Seldes, A. M., Pujol, C. A. and Damonte, E. B. (1996) Antiviral sulfated steroids from the ophiuroid *Ophioplocus januarii*. *J. Nat. Prod.* **59**, 887–889.
- Roccatagliata, A. J., Maier, M. S., Seldes, A. M., Zea, S. and Duque, C. (1997) A new sulfated alkene from the ophiuroid *Ophiocoma echinata*. *J. Nat. Prod.* **60**, 285–286.
- Stonik, V. A. and Elyakov, G. B. (1988) Secondary metabolites from echinoderms as chemotaxonomic markers. In *Biorganic Marine Chemistry*, ed. P. J. Scheuer, pp. 43–86. Springer, Berlin.