5α,8α-Epidioxycholest-6-en-3-β-ol from Three Cone Snails of the Indian Ocean

M. Aknin[1], I. Viracaoundin, R. Faure, and E.M. Gaydou*

Laboratoire de Phytochimie de Marseille et ESA 6009, Faculté des Sciences et Techniques de Saint Jérôme, Université d'Aix-Marseille III, Marseille Cedex 20, France

ABSTRACT: Three cone snail species (*Conus ebraeus, C. leopardus, C. tessulatus,* family Conidae) have been investigated for their sterolic fraction. Besides cholesterol, 5α,8α-epidioxycholest-6-en-3-β-ol has been isolated from each species and the structure determined using two-dimensional nuclear magnetic resonance. The occurrence of 5α,8α-epidioxycholest-6-en-3-β-ol in high amounts in the crude extract (0.5%), constitutes an interesting source of this compound.
JAOCS 75, 1679–1681 (1998).

KEY WORDS: Cholesterol, cones, *Conus ebraeus, Conus leopardus, Conus tessulatus,* 5α,8α-epidioxycholest-6-en-3-β-ol, sterols.

Cone snails are marine venomous predators, famous for their beautiful shells and to the biochemist for their *Conus* peptides. Research laboratories for physiological investigations currently use some of these molecules. While many reports have been done on conotoxins (1), few works have been published on the lipid fraction of this genus.

In continuing our current interest in sterols from marine sources (2–5), we have investigated the composition of the sterol fractions isolated from three cones widespread in tropical seas, *Conus ebraeus, C. leopardus,* and *C. tessulatus* (family Conidae in the class Gastropoda and the order Caenogastroda).

In this paper we report the isolation and structure determination of an unusual steroid, 5α,8α-epidioxycholest-6-en-3-β-ol found in each cone in high amounts.

EXPERIMENTAL PROCEDURES

Materials. The three species of cones, *C. ebraeus, C. leopardus,* and *C. tessulatus,* were collected near the Dzaoudzi beach (Mayotte Island, northwest of Madagascar, Indian Ocean) at 0.5–1 m depth. Cones were immediately frozen and kept in a freezer until chemical investigations. About forty cones of each species (2–3 cm long) were broken with a hammer and the animal flesh was washed with water and macerated with methanol/chloroform (1:2, vol/vol) in a Waring blender for 1 h. After standing overnight, the animal tissue was removed by filtration and the solution was concentrated at reduced pressure.

Isolation of sterols. The brown gum residue (2.5 g for *C. ebraeus,* 2.5 g for *C. leopardus,* 2.4 g for *C. tessulatus*) was fractionated over silica gel 60 (40 g, 230 mesh; E. Merck, Darmstadt, Germany) using a column 40 cm in length (20 mm i.d.). Elution was carried out by applying a stepwise gradient of *n*-hexane (750 mL), chloroform (750 mL), and methanol (750 mL). The chloroform fraction (1 g for *C. ebraeus,* 0.8 g for *C. leopardus,* 0.7 g for *C. tessulatus*) was chromatographed over silica gel (20 g). Fractions were monitored by thin-layer chromatography (TLC) as previously described for unsaponifiable matter (6). The fractions eluted with *n*-hexane/ethyl acetate (80:20, vol/vol) contained materials with R_f values similar to those of cholesterol (R_f 0.28, with *n*-hexane/ethyl acetate (1:1, vol/vol) having a characteristic red color spot using 50% sulfuric acid as visualizing reagent), were pooled (70 mg, 2.5% from crude extract for *C. ebraeus,* 70 mg, 2.8% for *C. leopardus,* 80 mg, 3.3% for *C. tessulatus*). The epidioxysterol fractions eluted with *n*-hexane/ethyl acetate (60:40, vol/vol) (R_f 0.44, with *n*-hexane/ethyl acetate (1:1, vol/vol) having a characteristic green color spot) were pooled (10 mg, 0.36% from crude extract for *C. ebraeus;* 12 mg, 0.48% for *C. leopardus;* 12 mg, 0.50% for *C. tessulatus*). Further purification of the epidioxysterol fraction using high-performance liquid chromatography (HPLC) (RP-18 column with 8% aqueous methanol mobile phase) revealed the presence of only one compound, 5α,8α-epidioxycholest-6-en-3-β-ol. Recrystallization from methanol yielded colorless needles, m.p. 149–150°C, lit. 150–151°C (7).

Nuclear magnetic resonance (NMR). ^1H (400 MHz) ^{13}C (100 MHz) NMR spectra of cholesterol and 5α,8α-epidioxycholest-6-en-3-β-ol were recorded on a Bruker AMX-400 (Wissembourg, France) using tetramethylsilane (TMS) as internal standard and $CDCl_3$ as solvent.

RESULTS AND DISCUSSION

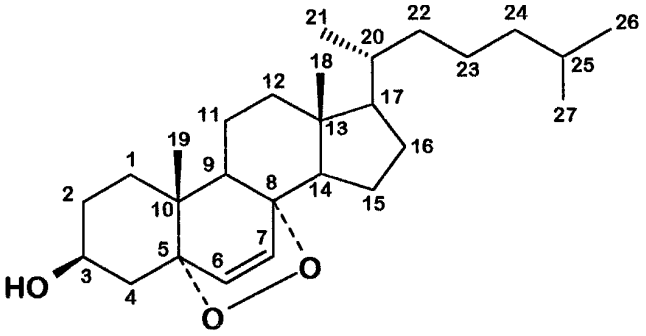
Examination of the chloroform-methanol extract of the flesh of the three cone species, *C. ebraeus, C. leopardus,* and *C.*

[1]Present address: IUFM de la Réunion, Saint-Denis, Ile de La Réunion.

*To whom correspondence should be addressed at Laboratoire de Phytochimie de Marseille et ESA 6009, Faculté des Sciences et Techniques de Saint Jérôme, Université d'Aix-Marseille III, avenue Escadrille Normandie Niemen, 13397 Marseille Cedex 20, France.
E-mail: emile.gaydou@iut-chimie.u-3mrs.fr

tessulatus, using TLC on silica gel, showed the presence of two major compound families in the sterol fraction. The fractionation and purification of this mixture revealed the presence of cholesterol in the less polar compound family, which was identified by comparison of the R_f value with that of an authentic standard cholesterol and by comparison of their ^{13}C NMR chemical shifts of the isolated compound with those given in the literature (8). The more polar sterol fraction (R_f 0.28, using *n*-hexane/ethyl acetate, 1:1), giving a green color spot on the TLC plate, was shown to be composed of only one compound using reversed-phase HPLC. The identification of this compound was achieved using NMR experiments. Structural elucidation and complete ^1H and ^{13}C assignment of 5α,8α-epidioxycholest-6-en-3-β-ol were established from the concerted application of homonuclear and both direct and long-range heteronuclear chemical shift correlation experiments using a previously reported strategy (7). Chemical shifts of proton and carbon resonances of 5α,8α-epidioxycholest-6-en-3-β-ol are given in Table 1. Assignments were done using ^1H hydrogen-correlated spectroscopy and heteronuclear multiple quantum bond connectivity experiments. Results obtained are in agreement with those given by Gunatilaka *et al.* (7) for ^1H, Wright *et al.* (10) for side chain ^{13}C chemical shifts, and Seo *et al.* (11) for ^{13}C of 5α,8α-epidioxysterol.

TABLE 1
^1H Nuclear Magnetic Resonance[a] (NMR) and ^{13}C NMR[b] of 5α,8α-Epidioxycholest-6-en-3-β-ol Isolated from Cone Snails



Carbon number	δ_H[c] [mult., *J* (Hz)]	δ_C[c] (mult.)
1	H-1_{ax}: 1.93 (*m*)	34.75 (CH_2)
	H-1_{eq}: 1.67(*ddd,* 13.9, 3.6, 3.6)	
2	1.51 (*m*)	30.16 (CH_2)
3	3.94 (*m*)	66.52 (CH)
4	H-4_{ax}: 1.89	37.00 (CH_2)
	H-4_{eq}: 2.09 (*dd,* 13.9, 4.8)	
5		82.24 (C)
6	6.21 (*d,* 8.5)	135.46 (CH)
7	6.48 (*d,* 8.5)	130.83 (CH)
8		79.54 (C)
9	1.46 (*m*)	51.10 (CH)
10		36.97 (C)
11	1.49 (*m*)	23.48 (CH_2)
12	1.09	39.49 (CH_2)
	1.21	
13		44.80 (C)
14	1.53 (*m*)	51.63 (CH)
15	0.98 (*m*)	20.68 (CH_2)
16	1.34 (*m*)	28.31 (CH_2)
17	1.18 (*m*)	56.46 (CH)
18	0.77 (*s*)	12.69 (CH_3)
19	0.85 (*s*)	18.24 (CH_3)
20	1.36 (*m*)	35.29 (CH)
21	0.87 (*d,* 6.5)	18.64 (CH_3)
22	1.30 (*m*)	36.00 (CH_2)
23	1.20 (*m*)	23.86 (CH_2)
24	1.09	39.49 (CH_2)
	1.21	
25	1.39 (*m*)	28.06 (CH)
26	0.83 (*d,* 6.6)	22.89 (CH_3)
27	0.84 (*d,* 6.6)	22.62 (CH_3)

[a]400 MHz.
[b]100 MHz.
[c]δ values from trimethylsilane ($CDCl_3$ solution).

This molecule is reported for the first time in cones to our knowledge. The occurrence of various 5α,8α-epidioxysterols has been described in various sponges (7,12–14), in tunicates (7,15), in sea hares (7,16), a common pillar coral (7), and more recently in a bivalve (17). The presence of only one 5α,8α-epidioxysterol, probably resulting from the biological cholesterol conversion and absence of the $\Delta^{5,7}$ corresponding diene as in the bivalve *Meretrix meretrix,* suggests that in such marine invertebrates 5α,8a-epidioxy sterols act as substrate in several enzyme systems (18).

While a large variety of side chain 5α,8α-epidioxysterols have been characterized from marine organisms, such compounds have been also found in lower terrestrial organisms (19–22). Various 5α,8α-epidioxysterols have been reported to have antiallergic and antiviral activity (23), antitumor activity in certain tumor cells (24,25), and PLA_2 inhibitory activity (11). Since the content of 5α,8α-epidioxycholest-6-en-3-β-ol in *C. ebraeus, C. leopardus,* and *C. tessulatus* is relatively high, cones may be a convenient source of this 5α,8α-epidioxysterol.

ACKNOWLEDGMENT

We thank J.-C. Martin, ARSPAL, St.-Denis, Réunion Island for his help in collection and cone identifications.

REFERENCES

1. Myers, R.A., L.J. Cruz, J.E. Rivier, and B.M. Olivera, *Conus* Peptides as Chemical Probes for Receptors and Ions Channels, *Chem. Rev. 93*:1923–1936 (1992).
2. Aknin, M., K. Dogbevi, A. Samb, J.M. Kornprobst, E.M. Gaydou, and J. Miralles, Fatty Acid and Sterol Compositions of Eight Brown Algae from the Senegalese Coast, *Comp. Biochem. Physiol. 102B*:841–843 (1992).
3. Aknin, M., R. Moellet-Nzaou, J.M. Kornprobst, E.M. Gaydou, A. Samb, and J. Miralles, Sterol Composition of Twelve *Chlorophyceae* from the Senegalese Coast and Their Chemotaxonomic Significance, *Phytochemistry 31*:4167–4169 (1992).
4. Costantino, V., E. Fattorusso, A. Mangoni, M. Aknin, and E.M. Gaydou, Novel 3-β-Methoxysteroids from the Senegalese Sponge *Microscleroderma spirophora*, *Steroids 59*:181–184 (1994).
5. Aknin, M., E.M. Gaydou, N. Boury-Esnault, V. Costantino, E. Fattorusso, and A. Mangoni, Nor-Sterols in *Axinella proliferans*

Sponge from the Indian Ocean, *Comp. Biochem. Physiol. 113B*: 845–848 (1996).
6. Gaydou, E.M., J.P. Bianchini, and J.V. Ratovohery, Triterpene Alcohols, Methylsterols, Sterols and Fatty Acids in Five Malagasy Legume Seed Oils, *J. Agric. Food Chem. 31*:833–836 (1983).
7. Gunatilaka, A.A.L., Y. Gopichand, F.J. Schmitz, and C. Djerassi, Minor and Trace Sterols in Marine Invertebrates. 26. Isolation and Structure Elucidation of Nine New 5α,8α-Epidioxysterols from Four Marine Organisms, *J. Org. Chem. 46*: 3860–3866 (1981).
8. Kalinowski, H.O., S. Berger, and S. Braun, *Carbon-13 NMR Spectroscopy,* J. Wiley, New York, 1988, p. 434.
9. Bombarda, I., J. Smadja, E.M. Gaydou, J.-Y. Conan, and R. Faure, Structure Elucidation of Oxidation-Reduction Products of Isolongifolene, *J. Agric. Food Chem. 42*:138–142 (1994).
10. Wright, J.L.C., A.G. McInnes, S. Shimizu, D.G. Smith, and J.A. Walter, Identification of C-24 Alkyl Epimers of Marine Sterols by ^{13}C Nuclear Magnetic Resonance Spectroscopy, *Can. J. Chem. 56*:1898–1903 (1978).
11. Seo, Y., J.R. Rho, and J. Shin, Isolation of Two Steroids from the Marine Polychaete Worm *Perinersis aibuhitensis*, *Ocean Res. 18*:83–87 (1996).
12. Fattorusso, E., S. Magno, C. Santacroce, and D. Sica, Sterol Peroxides from the Sponge *Axinella cannabina, Gazz. Chim. Gazz. Ital. 104*:409–413 (1974).
13. Sheikh, Y.M., and C. Djerassi, Steroids from Sponges, *Tetrahedron 30*:4095–4103 (1974).
14. Malorni, A., L. Minale, and R. Riccio, Steroids from Sponges: Occurrence of Steroidal $\Delta^{4,7}$-3,6-Diketones in the Marine Sponge *Raphidostila incisa*, *Nouv. J. Chim. 2*:351–354 (1978).
15. Guyot, M., and M. Durgeat, Occurrence of 9(11)-Unsaturated Sterol Peroxides in Tunicates, *Tetrahedron Lett. 22*:1391–1392 (1981).
16. Miyamoto, T., M. Honda, S. Sugiyama, R. Higushi, and T. Komori, Studies on the Constituents of Marine Opisthobranchia. III. Isolation and Structure of Two 5α,8α-Epidioxysterols and a Cholesteryl Ester Mixture from the Albumen Gland of *Aplysia juliana*, *Liebigs Ann. Chem.*:589–592 (1988).
17. Goswami, A., S. Saha, S. Muhopadhyay, and S.C. Pakrashi, Marine Products from Bay of Bengal. Part III: Sterol Composition of Four Mollusks and a Sea Pen, *Indian J. Mar. Sci. 24*:238–239 (1995).
18. Bates, M.L., W.W. Reid, and J.D. White, Duality of Pathways in the Oxidation of Ergosterol to Its Peroxide *in vivo*, *J. Chem. Soc., Chem. Commun.*:44–45 (1976).
19. Weete, J.D., Sterols of the Fungi. Distribution and Biosynthesis, *Phytochemistry 12*:1843–1864 (1973).
20. Arditti, J., R. Fisch, R. Ernst, M.H. Fisch, and B.H. Flick, Ergosterol Peroxide from *Rhizoctonia repens*; Composition, Conformation, and Origin, *J. Chem. Soc. Chem. Commun.*: 1217–1218 (1972).
21. Fisch, M.H., R. Ernst, B.H. Flick, J. Arditti, D.H.R. Barton, P.D. Magnus, and I.D. Menzies, Identity of Ergosterol 5β-8β-Peroxide, *Ibid.*:530 (1973).
22. Serebrayakov, E.P., A.V. Simolin, V.F. Kucherov, and B.V. Rozynov, New Metabolites of *Fusarium moniliforme* (*Gibberella fujikurvi*), *Tetrahedron 26*:5215–5223 (1970).
23. Lindequist, U., A. Lesnau, E. Teuscher, and H. Pilgrim, Antiviral Activity of Ergosterol Peroxide, *Pharmazie 44*:579–580 (1989).
24. Kahlos, K., L. Kangas, and R. Hiltunen, Ergosterol Peroxide, an Active Compound from *Inonotus radiatus*, *Planta Medica 55*: 389–390 (1989).
25. Valisolalao, J., B. Luu, and G. Ourisson, Chemical and Biochemical Study of Chinese Drugs. Part VIII. Cytotoxic Steroids from *Polyporus versicolor*, *Tetrahedron 39*:2779–2785 (1983).

[Received April 2, 1998; accepted July 16, 1998]