

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

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ABSTRACT: Three cone snail species (*Conus ebraeus*, *C. leopardus*, *C. tessulatus*, family Conidae) have been investigated for their sterolic fraction. Besides cholesterol, 5 α ,8 α -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 5 α ,8 α -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*, 5 α ,8 α -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 Caenogastropoda).

In this paper we report the isolation and structure determination of an unusual steroid, 5 α ,8 α -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 ham-

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mer 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, 5 α ,8 α -epidioxycholest-6-en-3- β -ol. Recrystallization from methanol yielded colorless needles, m.p. 149–150°C, lit. 150–151°C (7).

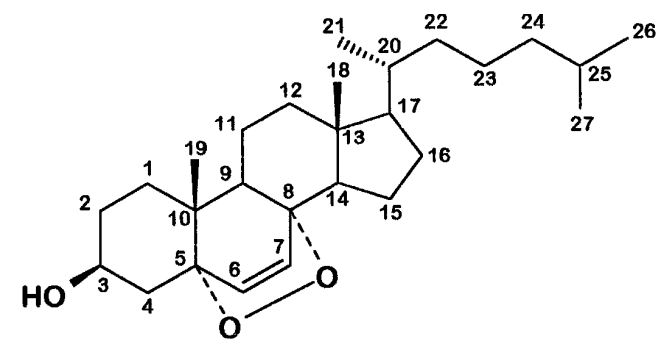
Nuclear magnetic resonance (NMR). ¹H (400 MHz) ¹³C (100 MHz) NMR spectra of cholesterol and 5 α ,8 α -epidioxycholest-6-en-3- β -ol were recorded on a Bruker AMX-400 (Wissembourg, France) using tetramethylsilane (TMS) as internal standard and CDCl₃ as solvent.

RESULTS AND DISCUSSION

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

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

TABLE 1
 ^1H Nuclear Magnetic Resonance^a (NMR) and ^{13}C NMR^b
of $5\alpha,8\alpha$ -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 (<i>m</i>) H-1 _{eq} : 1.67(<i>ddd</i> , 13.9, 3.6, 3.6)	34.75 (CH ₂)
2	1.51 (<i>m</i>)	30.16 (CH ₂)
3	3.94 (<i>m</i>)	66.52 (CH)
4	H-4 _{ax} : 1.89 H-4 _{eq} : 2.09 (<i>dd</i> , 13.9, 4.8)	37.00 (CH ₂)
5		82.24 (C)
6	6.21 (<i>d</i> , 8.5)	135.46 (CH)
7	6.48 (<i>d</i> , 8.5)	130.83 (CH)
8		79.54 (C)
9	1.46 (<i>m</i>)	51.10 (CH)
10		36.97 (C)
11	1.49 (<i>m</i>)	23.48 (CH ₂)
12	1.09 1.21	39.49 (CH ₂)
13		44.80 (C)
14	1.53 (<i>m</i>)	51.63 (CH)
15	0.98 (<i>m</i>)	20.68 (CH ₂)
16	1.34 (<i>m</i>)	28.31 (CH ₂)
17	1.18 (<i>m</i>)	56.46 (CH)
18	0.77 (<i>s</i>)	12.69 (CH ₃)
19	0.85 (<i>s</i>)	18.24 (CH ₃)
20	1.36 (<i>m</i>)	35.29 (CH)
21	0.87 (<i>d</i> , 6.5)	18.64 (CH ₃)
22	1.30 (<i>m</i>)	36.00 (CH ₂)
23	1.20 (<i>m</i>)	23.86 (CH ₂)
24	1.09 1.21	39.49 (CH ₂)
25	1.39 (<i>m</i>)	28.06 (CH)
26	0.83 (<i>d</i> , 6.6)	22.89 (CH ₃)
27	0.84 (<i>d</i> , 6.6)	22.62 (CH ₃)

^a400 MHz.

^b100 MHz.

^c δ values from trimethylsilane (CDCl₃ solution).

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 $5\alpha,8\alpha$ -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 $5\alpha,8\alpha$ -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 $5\alpha,8\alpha$ -epidioxysterol.

This molecule is reported for the first time in cones to our knowledge. The occurrence of various $5\alpha,8\alpha$ -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 $5\alpha,8\alpha$ -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 $5\alpha,8\alpha$ -epidioxy sterols act as substrate in several enzyme systems (18).

While a large variety of side chain $5\alpha,8\alpha$ -epidioxysterols have been characterized from marine organisms, such compounds have been also found in lower terrestrial organisms (19–22). Various $5\alpha,8\alpha$ -epidioxysterols have been reported to have antiallergic and antiviral activity (23), antitumor activity in certain tumor cells (24,25), and PLA₂ inhibitory activity (11). Since the content of $5\alpha,8\alpha$ -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 $5\alpha,8\alpha$ -epidioxysterol.

ACKNOWLEDGMENT

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

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