

## Minor sterols from the sponge *Ircinia ramosa* (Killer)

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**Abstract**-Three sterols, isolated from the lipid fraction of the sponge *Ircinia ramosa* were characterised as cholest-5-en-3 $\beta$ -ol-7-one (7-oxo cholesterol, 1), cholest-5-23-dien-3 $\beta$ ol-7-one (7-oxo desmosterol, 2) and 24E-ethyl cholest-5-en-3 $\beta$ -ol-7-one (7-oxo sitosterol, 3) respectively. Besides two minor sterols viz., 24E-ethyl cholest-5-en-3 $\beta$ , 7-diol (7-hydroxy sitosterol, 4) and 24-ethylcholest-5, 24(28)-dien-3 $\beta$ , 7-diol (7-hydroxy fucosterol, 5) were also tentatively identified on the basis of mass spectral data.

### INTRODUCTION

Marine organisms, especially, sponge, are rich sources of many sterols having unique structural features (SCHMITZ, 1978; MINALE and SODANO, 1976 and DJERASSI *et al.*, 1976). Purification and structure elucidation of individual sterols were difficult task till a few years ago. However, the recent advances in separation and purification techniques (HPLC, GLC, etc.) as well as the modern physical methods available for their structure determination (NMR, IR, UV and MS) have enabled natural products chemists to study even submilligram quantities of these compounds and arrive at their correct structure including stereochemistry.

We have isolated earlier four  $\Delta^5$ -3 $\beta$ -hydroxy sterols and their corresponding  $\Delta^4$ -3-oxo analogs from the less polar lipid fraction of the methanolic extract of the sponge *Ircinia ramosa* (PARAMESWARAN *et al.*, 1989). Herein we report the isolation and structure elucidation of five more polar dioxygenated sterols (1-5) from the same source (fig. 1). The structures of these compounds were assigned based on their spectral data, such as IR, UV-Vis, NMR and MS. The co-occurrence of these compounds in this species suggests enzymatic oxidation of the commonly occurring  $\Delta^5$ -3 $\beta$ -hydroxy sterols into their 7-oxo derivatives via 7-hydroxy intermediates.

### MATERIAL AND METHODS

IR spectra were recorded on a PE 1310 spectrophotometer and  $^1\text{H}$  NMR spectra in  $\text{CDCl}_3$  on a Bruker WP-200 instrument using TMS as the internal standard. CIMS was recorded on a Biospect instrument using ammonia as reagent gas in the positive mode and ammonium chloride added to the analyte in the negative mode to yield  $[\text{M}+\text{NH}_4]^+$  and  $[\text{M}+\text{Cl}]^-$  pseudomolecular ions, respectively. HPLC separation was carried out on a PE 5010 instrument with a refractomonitor model 1107 detector.

The animals (3 kg) were collected from the lagoon of Bangaram island in the Lakshadweep by skin diving. They were immediately soaked in MeOH, transported to the laboratory and processed. The concentrated methanolic extract (100 gms) was washed with water, dried ( $\text{Na}_2\text{SO}_4$ ), and concentrated to yield a residue (5 gms) which was subsequently loaded on a silica gel column and eluted with increasing proportions of EtOAc in pet. ether. The fraction eluting with 5 to 25% EtOAc in pet. ether was further chromatographed on a second silica gel column. The sterol fraction (monitored by TLC) were combined, concentrated (60 mgs) and finally separated on analytical HPLC (ODS, 5 $\mu$ , 250x4.6mm; MeOH-H $_2$ O=100:8, flow rate = 1ml/min.) to yield com-

pounds 1- 5 together with other less polar sterols reported earlier (PARAMESWARAN *et al.*, 1989).

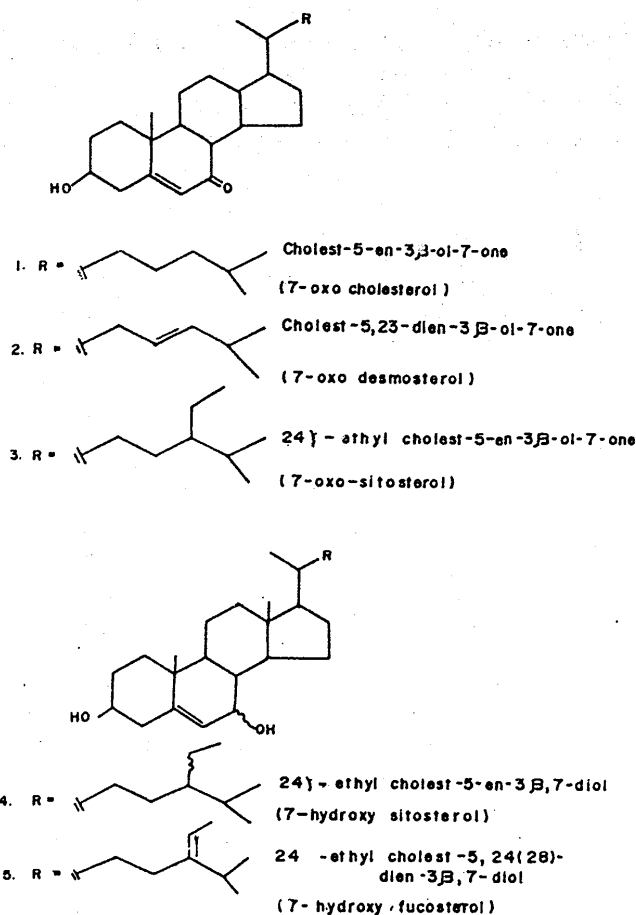


Fig. 1. Sterols isolated from *Ircinia ramosa*

## RESULTS AND DISCUSSION

Reverse phase HPLC separation of the polar steroidal mixture yielded three peaks A-C ( $R_t$ s 11.3, 12.4 and 17.4 mins, respectively). The compound eluting under peak B was shown to be a conjugated ketone from its IR ( $1660\text{ cm}^{-1}$ ) and UV-Vis (232nm) spectra. The  $^1\text{H}$  NMR spectrum of this compound compared well with that of  $\Delta^5$ -3 $\beta$ -hydroxy-7-oxo sterols (ROMERO and SELDES, 1986 and AMANN *et al.*, 1987). Conclusive evidence for the structure was provided by its mass spectrum which showed the molecular ion peak at  $m/e$  400, corresponding to the molecular formula  $\text{C}_{27}\text{H}_{44}\text{O}_2$  and the major fragment ions at  $m/e$  205, 192, 187, 179 and 161, characteristic of 3 $\beta$ -hydroxy-5-en-7-one moiety (see Fig 2, Biemann, 1966). From the above results the compound was conclusively characterised as cholest-5-en-3 $\beta$ -ol-7-one (7-oxo cholesterol 1). The mass spectrum of the above compound also exhibited a minor peak at  $m/e$  398, corresponding to the formula  $\text{C}_{27}\text{H}_{42}\text{O}_2$ . The additional double bond of this compound is located at C-23 in the side chain, as indicated by the major fragment ions at  $m/e$  315 $[\text{M}-\text{C}_6\text{H}_{11}]^+$  and 297 $[\text{M}-\text{C}_6\text{H}_{11}-\text{H}_2\text{O}]^+$ . Thus this compound was identified as cholest-5, 23-dien-3 $\beta$ -ol-7-one (7-oxo desmosterol, 2). The IR and UV-Vis spectra of the compound eluting under peak C was similar to that of compound 1. The mass spectral data indicated the molecular formula to be  $\text{C}_{29}\text{H}_{48}\text{O}_2$ , the ethyl analog of compound 1. Its  $^1\text{H}$  NMR spectrum confirmed the presence of an ethyl group in the side-chain,

at C-24. Thus this compound was characterised as 24E-ethyl cholest-5-en-3 $\beta$ -ol-7-one (7-oxo sitosterol 3).

Peak A was found to be a mixture of sterols from its  $^1\text{H}$  NMR spectrum. Its IR and UV spectra indicated the absence of any carbonyl groups. However, the quantity available was insufficient for further purification and detailed structural investigation. Its mass spectrum showed two prominent peaks as  $m/e$  430 and 428, corresponding to the molecular formula  $\text{C}_{29}\text{H}_{50}\text{O}_2$  and  $\text{C}_{29}\text{H}_{48}\text{O}_2$ , respectively. The major fragment ions at  $m/e$  289, 271, 247 and 229 compared to the corresponding values of 287, 269, 245 and 227 observed for compound 1-3, indicated that they are dihydroxy sterols with both the hydroxyl groups present with the ring system. Based on biogenetic considerations, they may be assigned to C-3 and C-7, thereby, completing the pathway  $\Delta^5$ -3 $\beta$ -hydroxy sterols  $\longrightarrow$   $\Delta^5$ -3 $\beta$ ,7-dihydroxy sterols  $\longrightarrow$   $\Delta^5$ -3 $\beta$ -hydroxy-7-oxo sterols. Thus, these two compounds have been tentatively characterised as 24E-ethyl cholest-5-en-3 $\beta$ , 7-diol 4 and 24-ethyl cholest-5, 24(28)-dien-3 $\beta$ , 7-diol 5 respectively.

Sponges are known to oxidise  $\Delta^5$ -3 $\beta$ -hydroxy sterols into the corresponding  $\Delta^4$ -3-oxo derivatives *in vivo* (PARAMESWARAN *et al.*, 1989). The isolation of compounds 1-5 indicates the presence of another enzymatic oxidation sequence of sterols in this sponge, yielding 7-oxo steroidal derivatives via the corresponding hydroxyl intermediates. Similar 7-oxygenated sterols have previously been reported from another sponge, *Patingera magellanica* (ROMERO and SELDES, 1986).

*Cholest-5-en-3 $\beta$ -ol-7-one (7-oxo cholesterol 1)* :  $R_t=12.4$  mins.; IR (neat): 3400, 2940, 2920, 2860, 1700, 1660, 1460, 1380, 1260, 1050 and 740  $\text{cm}^{-1}$ ; UV (hexane) 232nm; PCIMS: 418[M+NH $_3$ ] $^+$ , 401[M+H] $^+$ , 382[M-H $_2$ O] $^+$ ; NCIMS: 435[M+ $^{35}\text{Cl}$ ] $^+$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 5.76(1H,s,6-H), 3.68(1H,m,3-H), 1.2(3H,s,19-H), 0.916(3H,d, J=6.5Hz, 21-H), 0.86(6H,d,J=6.8Hz,26 and 27-H) and 0.70(3H,s,18-H); EIMS:400(M $^+$ ,6.13%),385([M-15] $^+$ ,1.09%),382([M-18] $^+$ ,1.77%),367([M-33] $^+$ ,2.94%),287([M-sc] $^+$ ,4.59%),269([M-sc-H $_2$ O] $^+$ ,3.48%),245([M-sc-42] $^+$ ,6.52%),227([M-sc-42-H $_2$ O] $^+$ ,3.88%),211(2.98%),205(E,3.57%),192(D,6.76%),179(F,1.71%),187(E-H $_2$ O,7.55%),174(D-H $_2$ O,7.01%) and 161(F-H $_2$ O,14.54%). (For fragments A-F, see Fig. 2).

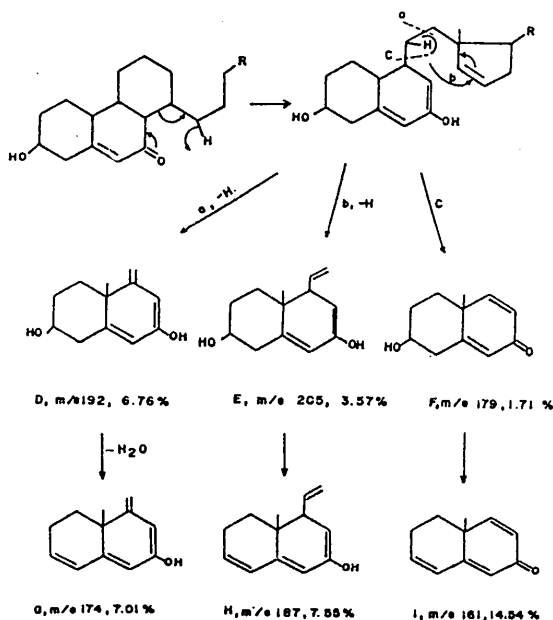


Fig. 2. Mass fragmentation of *Cholest-5-en-3 $\beta$ -ol-7-one*

*Cholest-5,23-dien-3 $\beta$ -ol-7-one (7-oxo desmosterol 2)*

EIMS: 398(M<sup>+</sup>, 3%), 350([M-CH<sub>3</sub>-CH<sub>3</sub>-H<sub>2</sub>O])<sup>+</sup>, 337([M-CO-H<sub>2</sub>O-CH<sub>3</sub>)<sup>+</sup>, 315([M-C<sub>6</sub>H<sub>11</sub>)<sup>+</sup>, 1.2%), 297([M-C<sub>6</sub>H<sub>11</sub>-H<sub>2</sub>O], 1.14%) etc.

*24E-ethyl cholest-5-en-3 $\beta$ -ol-7-one (7-oxo sitosterol 3)*

R<sub>t</sub>=17.4 mins.; IR(neat): 3400, 3040, 2940, 2920, 2860, 1700, 1660, 1460, 1380, 1260, 1180, 1060 and 740 cm<sup>-1</sup>; UV(hexane): 232 nm; PCIMS: 446[M+NH<sub>3</sub>)<sup>+</sup>, 429[M+H]<sup>+</sup> and 410 [M-18]<sup>+</sup>; NCIMS: 463 [M+<sup>35</sup>Cl]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$ 5.76(1H,s,6-H), 3.68(1H,m,3-H), 1.2(3H,s,19-H), 0.915(3H,d,J=6,8Hz, 21-H), 0.835(3H,t,J=7Hz, 29-H), 0.82(3H,d,J=6.9Hz, 26-H), 0.80(3H,d, J=6.6Hz, 27-H) and 0.69(3H,s,18-H).

*24E-ethyl cholest-5-en- 3 $\beta$ ,7-diol (7-hydroxy sitosterol 4)*

R<sub>t</sub>=11.3 mins.; EIMS: 430(M<sup>+</sup>, 5.4%), 412([M-18]<sup>+</sup>, 7.56%), 397 ([M-33]<sup>+</sup>, 3.21), 394[M-2H<sub>2</sub>O]<sup>+</sup>, 379[M-2H<sub>2</sub>O-CH<sub>3</sub>)<sup>+</sup>, 289[M-sc]<sup>+</sup>, 271([M- sc-H<sub>2</sub>O]<sup>+</sup>, 3.16%), 247([M-sc-42]<sup>+</sup>, 8.05%), 229([M-sc-42-H<sub>2</sub>O]<sup>+</sup>, 10.19%), 211([229-18], 6.43%) etc.

*24-ethylcholest-5,24(28)-dien-3 $\beta$ , 7-diol (7-hydroxyfucosterol 5)*

EIMS: 428(M<sup>+</sup>, 2.43%), 410([M-H<sub>2</sub>O]<sup>+</sup>, 395[M-33]<sup>+</sup>, 331([M-C<sub>7</sub>H<sub>13</sub>)<sup>+</sup>, 2.23%), 317[M-C<sub>8</sub>H<sub>15</sub>)<sup>+</sup>, 300, 299[M-C<sub>8</sub>H<sub>15</sub>-H<sub>2</sub>O]<sup>+</sup>, 271[M-sc-H<sub>2</sub>O]<sup>+</sup> etc.

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