

Sterols from the Lakshadweep sponge, *Ircinia ramosa* (Killer)

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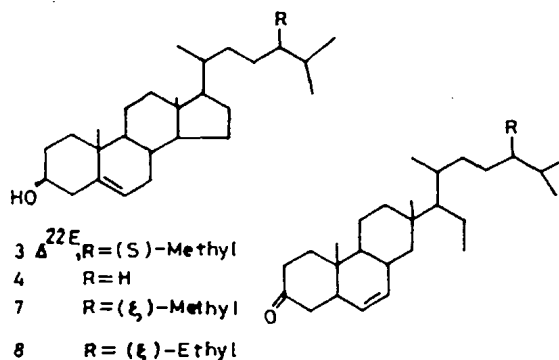
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Four monohydroxy sterols, viz. (22*E*, 24*S*)-24-methylcholest-5,22-dien-3 β -ol (**3**), cholesterol (**4**), 24 ξ -methylcholesterol (**7**) and 24 ξ -ethylcholesterol (**8**) and the corresponding Δ^4 -3-ketones, viz. (22*E*, 24*S*)-24-methylcholest-4,22-dien-3-one (**1**), cholest-4-en-3-one (**2**), 24 ξ -methylcholest-4-en-3-one (**5**) and 24 ξ -ethylcholest-4-en-3-one (**6**) have been isolated from the sponge *Ircinia ramosa*. Their structures have been determined by comparison of their spectral data with those reported in literature.

The sterols from the sponge, *Ircinia ramosa* were examined in this laboratory as part of the ongoing Indo-US collaborative programme on bioactive substances from the organisms of the Indian Ocean. Reverse phase HPLC of the sterol-rich fraction on HPLC (see Experimental) yielded 24*S*-methylcholest-4,22-dien-3-one (**1**, Rt 28.2 min), cholest-4-en-3-one (**2**, Rt 30.0 min), 24*S*-methylcholest-5,22-dien-3 β -ol (**3**, Rt 33.6 min), cholesterol (**4**, Rt 35.4 min), 24 ξ -methylcholest-4-en-3-one (**5**, Rt 36.2 min), sitosterone (**6**, Rt 41.4 min), 24 ξ -methylcholesterol (**7**, Rt 43.8 min) and 24 ξ -ethylcholesterol (**8**, Rt 51.6 min). Their structures were determined by comparing their spectral data with those reported in literature^{1,2}.

Compounds **1**, **2**, **5** and **6** (M^+ : 396, 384, 398 and 412 respectively) were shown to be Δ^4 -3-ketones from their IR, UV, PMR and mass spectral data. The compounds **3**, **4**, **7** and **8** (M^+ : 398, 386, 400 and 414 respectively) showed the presence of hydroxyl groups but no carbonyl groups as evident from their IR spectra. Their PMR data were characteristic of 3 β -hydroxy, Δ^5 -sterols.

Δ^4 -3-ketones occur rarely in marine organisms. They were first reported from the sponge *Stelletta charella* alongwith other sterols¹. Subsequently Kokke *et al.* also isolated three such compounds from the dinoflagellate *Pyrocists lunula*³. The co-occurrence of Δ^5 -3 β -hydroxysterol and the corresponding Δ^4 -3-ketones suggests that the sponge *I. ramosa* contains 3 β -hydroxy dehydrogenase, Δ^5 -isomerase and Δ^4 -reductase enzymes, essential for the conversion of sterol into steroidal hormones⁴.



- 3 Δ^{22E} , R=(S)-Methyl
- 4 R=H
- 7 R=(ξ)-Methyl
- 8 R=(ξ)-Ethyl

- 1 Δ^{22E} R=(ξ)-Me
- 2 R=H
- 5 R=(ξ)-Methyl
- 6 R=(ξ)-Ethyl

Experimental

IR spectra were recorded on a PE 1310 spectrophotometer and ¹H and ¹³C NMR spectra in CDCl₃ on a Bruker (IBM) WP-200 instrument using TMS as an internal standard. CIMS was recorded on a Biospect instrument using ammonia as reagent gas in positive mode. NH₄Cl was used in the negative mode to get [M + Cl⁻] pseudo molecular ion peaks. HPLC separation was carried out on a PE 5010 instrument.

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 g) was partitioned between CHCl₃ and 10% MeOH in water. The CHCl₃ layer was washed with water, dried (Na₂SO₄) and concentrated to yield a residue (5 g). The residue was loaded over silica gel column and eluted with increasing proportions of EtOAc in petroleum ether. The fraction (eluting with 5 to 25% EtOAc in pet. ether) was further purified on a second silica gel column. The sterol fractions (monitored by TLC) were combined, concentrated (60 mg) and further purified on analytical HPLC (ODS 5u, MeOH: H₂O = 100:8, flow rate = 1 ml/min.) to yield compounds 1-8.

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