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Note

Steroids from marine sponges *Suberites vestigium* and *Chrotella australiensis*[†]

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The sponges *Suberites vestigium* and *Chrotella australiensis* have been examined for steroids. Both the sponges contain C₂₇₋₂₉ mono and diunsaturated sterols, in addition sponge *C. australiensis* contains cholest-4-ene-3-one and 24-ethyl cholest-4-ene-3-one. Batyl alcohol and its higher homologue have also been identified in *S. vestigium*. This is first report of steroids from these sponges.

In continuation of our investigation on bioactive metabolites from marine organisms, we report herein the isolation and identification of steroids from the sponges *Suberites vestigium* and *Chrotella australiensis*. The crude methanolic extract of *S. vestigium* exhibited *in vitro* antihistaminic activity and *C. australiensis* was found to be anti-viral. The sponges were extracted with methanol and crude extract fractionated with chloroform, ethyl acetate and butanol. Chloroform fraction of *S. vestigium* yielded sterols 1-4 and glyceryl ethers, *n*-batyl alcohol and its higher homologue whereas the chloroform fraction of *C. australiensis* gave steroids 5-10. Characterisation of the constituents of these sponges is based on their spectral data and comparison with the literature values. These sponges are hitherto unexamined for their chemical constituents.

Compound 1: Its ¹H NMR spectra showed a multiplet at δ 3.6 and a broad singlet at δ 5.35, the spectrum further displayed signals for two secondary methyls at δ 0.68 (3H, s, C-18) and 1.02 (3H, s, C-19) characteristic of 5-ene-3β-hydroxysterols¹, a secondary methyl at δ 0.85 (3H, d, C-21) and a primary methyl at δ 0.79 (3H, t, C-29). It also showed a tertiary methyl at 1.62 (3H, s, C-26) and broad singlets at δ 4.7 and 4.62 for methylene protons.

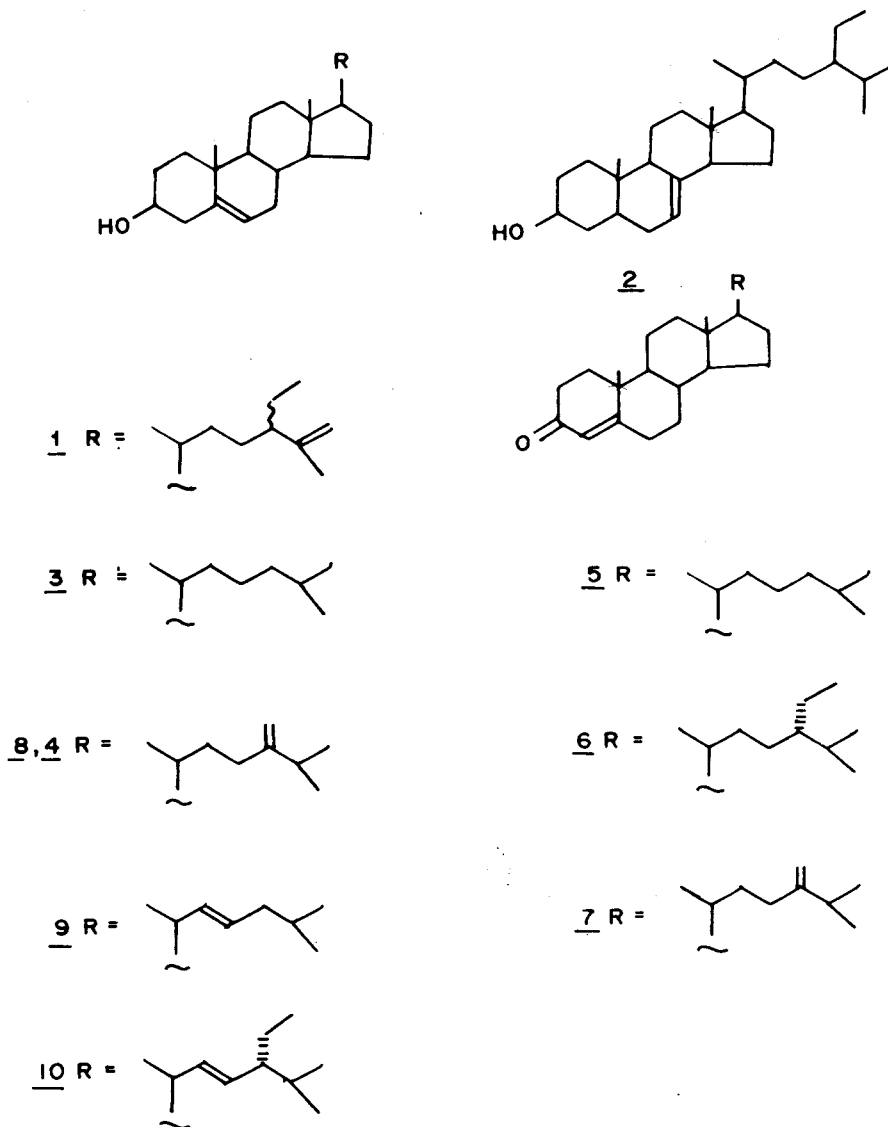
The mass spectrum of 1 displayed besides M⁺ at 412, fragments at m/z 329 [M-C₆H₁₁], 273 [M-Sc] and 301 [M⁺-Sc+2H]. A single molecular ion peak at m/z 412 and a strong fragment at 271 indicated the presence of two double bonds, one being in the nucleus and the other in the side chain. A prominent peak at m/z 314 is consistent with Δ²⁵⁽²⁶⁾ double bond. The presence of common cholesterol nucleus was evident from the diagnostic ions at m/z 273, 255, 231 and 213. All the data led us to conclude that the compound 1 is 24-ethyl-cholest-5, 25(27)-diene-3β-ol or clerosterol².

Compound 2: M⁺ 414; it contains cholestane nucleus with saturated side chain and the nuclear double bond at 7-position as evident from ¹H NMR spectra which showed characteristic broad singlet at δ 5.17 for H-7 and the high field resonances for C-18 and C-19 methyl protons at δ 0.57 and 0.68 respectively³. The structure of 2 was established as 24-ethyl-cholest-7-ene-3β-ol.

Sterols 3 and 4 with molecular ion peak at 386 and 398 were characterised as cholesterol and 24-methylene-cholesterol respectively, from the comparison of their spectral data (EIMS and ¹H NMR) with literature values⁴.

Chromatography of more polar materials present in the chloroform fraction resulted in the isolation of glyceryl ethers. The ¹H NMR spectrum was suggestive of the presence of batyl alcohol and its homologue which exhibited signals at δ 0.87 (3H, t), 1.25 [(CH₂)_n] and 3.4-3.9 (m). ¹³C NMR spectra showed signals at δ 72.44, 71.8, 70.48 and 64.27 for four carbons directly attached to oxygen in the molecule, at δ 39.4-18.8 for the methylenes of aliphatic chain and at δ 13.9 for the terminal methyl of the aliphatic chain. The presence of batyl alcohol and its homologue was further confirmed by fragmentation pattern of the mass spectrum of the mixture which showed molecular ion peaks at M⁺ 344 and 358.

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The chloroform solubles of the sponge *C. australiensis* yielded Δ^4 -3-keto-steroids **5-7** and some common sterols **8-10**. A prominent peak and often base peak usually occurs at m/z 124 (B-ring cleavage), other important peaks are ($M^+ - 42$) due to loss of ketone and ($M^+ - 42$ -side chain). The presence of Δ^4 -3-ketene moiety was further evidenced by the presence of signals at δ 198.89, 170.99 and 123.64 in ^{13}C NMR spectrum as well as absorptions at 1728, 1620, 1273 cm^{-1} in the IR spectra. ^1H NMR spectra depicted olefinic protons at δ 5.7 (4-H) and 1.20 (19- CH_3). These data established the presence of nuclear Δ^4 -3-ketone moiety and suggested strongly that the compound **5**, with M^+ 384 and characteristic peaks at m/z 342 and 229, was cholest-4-ene-3-one and the compound **6**, with M^+ 412 and peaks at m/z 370 and 229, was identified as 24-ethyl-cholest-4-

ene-3-one, the $24S$ configuration followed from the triplet in the ^1H NMR of C-29 methyl at δ 0.858 ($J=7.4$). This fraction also contained 24-methylene-cholest-4-ene-3-one **7**, as evident from the mass spectrum of the compound with M^+ 396 and fragments at m/z 271 and 124.

Δ^4 -3-one steroids are of rare occurrence and were first reported in the sponge *Stelletta clarella* along with other sterols⁵. Subsequently, Kokke *et al.*⁶ also isolated three such compounds from the dinoflagellate *Procytis lunula* and Parameshwaran *et al.*⁷ reported from sponge *Ircinia ramosa*. Δ^4 -ene-3-one steroids have also been reported from the red alga *Gracilaria textorii*⁸.

Compound **8** exhibited molecular ion peak at m/z 398 and a triplet at m/z 299, 300 and 301, characteristic of Δ^{24} double bond. Its ^1H NMR depicted

olefinic protons at δ 5.3 (6-H) and two broad singlets at δ 4.76 and 4.62 for methylene protons establishing the structure of **8** as 24-methylenecholesterol.

The mass spectrum of compound **9** showed in addition to the molecular ion peak at 384, the usual ion corresponding to the loss of side chain at m/z 273, 366 due to loss of H_2O from the molecule and at 371 [$M^+ - H_2O - CH_3$]. These data in conjunction with the 1H NMR signals at δ 5.4, 3.6 and 0.67 for protons at 22, 3 and 19 positions respectively, suggested the structure of **9** as 22-*trans*-cholesta-5,22-diene-3 β -ol.

Compound **10** was identified as 22-*trans*-24*S*-ethylcholesta-5,22-diene-3 β -ol on the basis of its mass spectrum which showed molecular ion peak at M^+ 412 and prominent fragments at m/z 397, 394, 351, 300, 273 and 271. The 24*S* configuration followed from the triplet at δ 0.853 from its 1H NMR spectrum⁹.

The occurrence of Δ^4 -3-keto steroids and the corresponding Δ^5 -3 β -hydroxy sterols is of particular significance because it suggests that the sponge *C. australiensis* contains 3 β -hydroxydehydrogenase, Δ^5 -isomerase and Δ^4 -reduction system. These enzymes are essential for conversion of sterols to steroid hormones.

Experimental

1H NMR spectra were recorded on Bruker WM 400 and 300 MHz (chloroform, TMS) FTNMR spectrometer; mass spectra on Jeol D300 mass spectrometer at 70 eV; and IR spectra on Shimadzu FTIR-8001 (KBr pellets). Compounds were repeatedly purified over silica gel (60-120 mesh) column and their purity was checked on TLC using different solvent systems. Final purification was done on HPLC using ODS-5 μ column with MeOH as solvent at the rate of 1 ml/min.

Collection, extraction and isolation

The sponges *S. vestigium* and *C. australiensis* were collected from Mandapam and Goa coast respectively, stored in methanol and transported to laboratory. The solvent was drained off and concentrated under reduced pressure. The crude methanolic extract was suspended in 20% aq. methanol and extracted with chloroform, ethyl acetate and *n*-butanol. The chloroform fraction was chromatographed over silica gel using increasing amount of ethyl acetate in pet. ether.

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