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A NEW HEXAHYDROXYSTEROL FROM A SOFT CORAL *SARCOPHYTON ELEGANCE* MOSER FROM THE COASTS OF GULF OF MANNAR IN THE INDIAN OCEAN

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ABSTRACT

A new hexahydroxysterol, (24S) -24-methylcholestane-1β, 3β, 5α, 6β ,25, 26-hexol 25-monoacetate (**5**) was isolated from a soft coral *Sarcophyton elegance* Moser collected from the coasts of the Gulf of Manner of Indian Ocean together with five known polyhydroxysterols **1**, **2**, **3**, **4** and **6.** The structures of the marine natural products, compounds **3**, **4, 5** and **6** were derived from spectral data of their acetyl derivatives **3a**, **4a**, **5a** and **6a** respectively.

KEYWORDS: Indian Ocean, Marine Natural Products, Polyhydroxysterols, *Sarcophyton Elegance* Moser, Soft Coral

INTRODUCTION

In the past few years, the majority of metabolites reported from coelenterates were terpenes or polyhydroxysterols. The soft corals of the genus *Sarcophyton* are rich sources of variation of polyhydroxysterols[1-3]. As a part of our continuing work on the steroid metabolites of the Indian Ocean[4-8], lipid extracts of the soft coral *Sarcophyton elegance* Moser were examined and isolated six polyhydroxysterols. Previously a little work has been done with the soft coral *Sarcophyton elegance*.

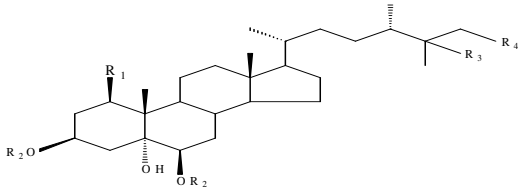
EXPERIMENTAL

The ^{1}H NMR spectrum was recorded on $CDCl_3$ on a Bruker VM-400 (400 MHz FT NMR) spectrometer and ^{13}C NMR on JEOL JNM GX-270 spectrometer using TMS as internal standard. Mass spectrum was determined on a JEOL D-300 (EI/CI) spectrometer. Elemental analysis was carried out on a CARLO ERBA EA 1108 – Instrument. The IR spectrum was recorded on a Perkin-Elmer 841 spectrometers. Melting points were determined on a Kofler hot stage and are uncorrected. UV spectra were recorded on Roy Spectronic 1201 double beam spectrophotometer.

Collection, Extraction and Purification:

The soft coral *Sarcophyton elegance* Moser was found to grow as large mats on the inter tidal rocks on the coasts of the Gulf of Mannar in the Indian Ocean, near Mandapam in Tamil Nadu state, India. After uprooting the organism, it was cut into thin slices and preserved in ethanol and brought to the laboratory for procasting. The material (1.3 kg dry weight) was repeatedly extracted with ethanol and solvent was removed under reduced pressure. The dry material after extraction was powdered and extracted with ethyl acetate and the residue obtained after stripping off the solvent was added to the ethyl acetate soluble portion of the extract. The crude extract was chromatographed over a column of silica gel and each fraction was then subjected to chromatography over the columns/layers of silica gel. It resulted in the isolation two pure polyhydroxysterols **1**, **2** and a sterol mixture. The sterol mixture contains four polyhydroxysterols and they were isolated after acetylation with acetic anhydride/pyridine as the initial fraction was inseparable mixture on silica gel.

The polyhydroxysterols **3**, **4**, **5, 6** were isolated and characterised by their acetyl derivatives **3a**, **4a**, **5a**, **6a** respectively. Among these, compound **5** is a new polyhydroxysterol and identified by its spectral data as a hexahydroxysterol, (24S)-24- methylcholestane-1β, 3β, 5α, 6β, 25, 26-hexol 25-monoacetate (**5**). The remaining five polyhydroxysterols (24S)-24-methylcholestane-3β, 5α, 6β, 25-tetrol 25-monoacetate(**1**)[1], (24S)-24-methylcholestane-3β, 5α, 6β, 25-tetrol (**2**)[9], (24S)-24-methyl cholestane-1β, 3β, 5α, 6β tetrol (**3**)[3], (24S)-24-methylcholestane-1β, 3β, 5α, 6β, 25-pentol 25-monoacetate(**4**)[2] and (24S)-24-methylcholestane-1β,3β,5α,6β,25-pentol (**6**)[2] were previously known and their structures were derived by comparison of spectral data with those of the known sterols. The present paper deals with the structure elucidation of hexahydroxysterol (**5**).



	R_1	R_2	R_3	R_4
1.	H	H	O A c	H
2.	H	H	O H	H
3.	O H	H	D o u b l e	B o n d
3 a.	O A c	A c	D o u b l e	B o n d
4.	O H	H	O A c	H
4 a.	O A c	A c	O A c	H
5.	O H	H	O A c	O H
5 a.	O A c	A c	O A c	O H
6.	O H	H	O H	H
6 a.	O A c	A c	O H	H

Figure 1

Acetylation of Sterol Mixture

The inseparable sterol fraction obtained from the chromatography of crude extract of the soft coral was re-crystallized from chloroform-methanol (8:2) yields a crystalline sterol mixture (385mg). The sterol mixture (220mg), dry pyridine (10ml) and acetic anhydride (10ml) was stirred at room temperature over night. The excess reagents were removed in vacuo and the residue was partitioned between water and ether. The ether extract was dried over anhydrous $MgSO_4$ and the solvent stripped off under reduced pressure, it yielded a mixture of acetylated derivatives as low melting solid (190mg). After repeated chromatography, it yielded four acetylated sterols **3a**, **4a**, **5a** and **6a**. Out of these **3a**, **4a**, and **6a** were known sterols[2, 3].

(24S)-24-methylcholestane-1β,3β,5α,6β,25,26-hexol 1,3,6,25-tetraacetate(**5a**):Low melting solid, $[\alpha]_D^{25}$ +21.9°(c,0.5,$CHCl_3$); Anal. found C 66.31%, H 8.98%; $C_{36}H_{58}O_{10}$ requires C 66.46%, H 8.82%; **IR** ($CHCl_3$): ν_{max} 3550-3400,2990,1735,1370,1250 and 1035 cm^{-1}; **^{1}H NMR** ($CDCl_3$):δ 5.15-5.26(2H,m,1α &3α-H),4.69(1H,br s,6α-H),0.67(3H,s,18-Me),1.24 (3H,s,19-Me), 0.90[a](3H,d,J=7.0 Hz,21-Me), 3.94(2H,m,26-H_2), 1.39(3H,s,27-Me), 0.81[a] (3H,d,J=6.9 Hz,28-Me), 2.00(6H,s,-OCOC$\underline{H}_3$x2), 2.04(3H,s,-OCOC$\underline{H}_3$), 2.09(3H,s,-OCOC$\underline{H}_3$). [[a]Methyl proton signal assignments may be interchanged]; **^{1}H NMR** (d_5-pyridine):δ 4.05 (1H,d,J=11.7 Hz,26-H_a), 4.07(1H,d,J=11.7 Hz, 26-H_b),5.35(1H,br s, 6α-H), 5.76(1H,m,3α-H)5.83(1H,m,1α-H); **^{13}C NMR** ($CDCl_3$):δ 75.2(C-1),33.0 (C-2), 67.6(C-3), 36.4(C-4) 75.3 (C-5),76.5(C-6),31.3(C-7),31.0(C-8),44.8(C-9),43.1(C-10), 22.9(C-11), 40.1(C-12),41.9(C-13), 55.4(C-14),

24.4 (C-15), 27.9 (C-16), 56.1 (C-17), 11.9 (C-18), 10.2 (C-19), 36.2 (C-20), 18.9 (C-21) 34.1 (C-22),2 7.9 (C-23), 40.1 (C-24), 86.0(C-25), 68.4(C-26), 18.6(C-27), 14.4(C-28), 170.0 (-O<u>C</u>OCH$_3$), 170.1 (-O<u>C</u>OCH$_3$), 170.1 (-O<u>C</u>OCH$_3$), 170.4 (-O<u>C</u>OCH$_3$), 21.2 (-OCO<u>C</u>H$_3$), 21.4 (-OCO<u>C</u>H$_3$), 21.7 (-OCO<u>C</u>H$_3$), 22.4 (-OCO<u>C</u>H$_3$); **MS:**m/z(%)[M$^+$-AcOH] 590(1), 572(3), 558(2), 512(9), 494(5), 470(4), 454(7), (2),445 (11), 435(30), 392 (5), 387(5), 386(13), 327(5),326(9), 309(11), 268(23), 267(9), 266(23), 249(11), 248(21), 241(21), 208(32), 95(33), 55(39) & 43(100).

RESULTS AND DISCUSSIONS

The ^{13}C NMR spectrum showed thirty five carbon resonances, out of which three resonances at δ 170.4, 170.1 and 170.0 are attributed to four acetoxyl carbonyl carbons and six carbon resonances at δ 86.0, 76.5, 75.3, 73.2, 68.4 and 67.6 are attributed to six oxygen bearing carbon atoms. The presence of four acetoxy carbonyl carbons satisfies four degrees of un-saturation, whereas the molecular formula requires eight and hence a normal tetracyclic skeleton could be assumed for this steroid. The presence of four acetoxyl groups satisfies eight oxygen atoms in the molecule and the remaining two oxygen atoms may be present as tertiary/hindered hydroxyl groups.

The ^{1}H NMR spectrum in $CDCl_3$ showed the seven tertiary methyl signals at δ 0.67 (3H, s), 1.24 (3H, s), 1.39 (3H, s), 2.00 (6H, s), 2.04 (3H, s), 2.09 (3H, s) and two secondary methyl signals at δ 0.81 (3H, d, J=6. 9 Hz) and 0.90 (3H, d, J=7. 0 Hz). The signals at δ 2.00, 2.04 and 2.09 are assigned to the four acetoxyl methyls and the remaining methyls suggest the C_{28} stroll with a methyl on C-24. The ^{1}H NMR spectrum also showed three single proton low field signals at 5.15-5.26 (2H,m) [two single protons signals merged] and 4.69 (1H,br s) which are assigned to three single protons present on three acetoxyl bearing carbon atoms. The ^{1}H NMR spectrum also showed a proton signal at δ 3.94 (2H, m) characteristic of oxygenated methylene protons.

A close inspection of the ^{13}C NMR and ^{1}H NMR spectral data of the compound **5a** indicate the presence of 1β,3β,5α,6β-tetrahydroxyl pattern in the steroid nucleus. Oxygenation at 3β,5α,6β is supported by the characteristic broad singlet of the 6α-proton[10] at δ 4.69 and the deshielded nature of the 3α-proton at δ 5.15-5.26 due to the 1,3-diaxial interaction with 5α-hydroxyl group[11]. The down field methyl proton signal at δ 1.24 assigned to 19-methyl group suggests that one of the acetoxyl may be on C-1, since only one acetoxyl at this position would cause the deshielding effect of the vicinal 19-methyl signal. Further the corresponding 1α-methine appears at δ 5.15-5.26 which is significantly lower than its usual position and this may be ascribed to the influence of the 5α-hydroxyl group through 1,3-diaxial interaction. The position of the three acetoxyl groups and one tertiary hydroxyl group may be on C-1, C-3, C-6 and C-5 respectively.

The position of the remaining one acetoxyl and one hydroxyl group are yet to be fixed. The mass spectrum of the compound showed prominent fragments due to cleavage of the side chain at m/z 447 (M$^+$-s chain, 2H), 387 (M$^+$-s chain, 2H, AcOH), 327 (M$^+$-s chain, 2H, 2 AcOH), 267 (M$^+$-s chain, 2H, 3 AcOH) and 249 (M$^+$-s chain, 2H, 3 AcOH, H_2O) suggest that the remaining tertiary acetoxyl and primary hydroxyl groups may be present in the side chain.

The signals at δ 4.05 (1H, d,J=11.7 Hz) and 4.07 (1H, d,J=11.7 Hz) in the ^{1}H NMR spectrum in d_5-pyridine are assigned to –CH_2OH group present in the molecule. The signal at δ 1.39 (3H, s) in the ^{1}H NMR spectrum is assigned to 27-methyl protons. The chemical shift and the multiplicity of this methyl proton signal suggests the acetoxyl group on C-25. The ^{1}H NMR spectrum of the compound **6a** (24-methylcholestane-1β,3β,5α,6β,25-pentol type sterol) contained a six-proton singlet at δ 1.38 whereas in compound **5a** these signals are replaced by a three proton singlet at δ 1.38 and two proton signal at δ 4.94 (2H,m). Based on the above observations the acetoxyl and hydroxyl groups in the side chain may be

present on C-25 and C-26 respectively. Thus, the compound may be described as 24-methylcholestane-1β,3β,5α,6β,25, 26-hexol 1,3,6,25-tertraacetate. On biogenetic grounds the configuration at C-24 expected to have 24 (S) -configuration[12]. Hence, the structure of the compound may be described as (24S)-24-methylcholestane-1β,3β,5α,6β,25,26-hexol 1,3,6, 25-tertraacetate(**5a**) and that of the natural sterol is described as (24S)-24-methylcholestane-1β,3β,5α,6β,25,26-hexol 25-monoacetate(**5**). This is the new addition to the list of polyhydroxyl sterols.

CONCLUSIONS

In marine animals, the soft corals of the genus *Sarcophyton* are rich sources of variation of polyhydroxysterols. Isolation of a hexahydroxysterol from natural sources is rare. We report here the isolation and characterization of a new hexahydroxysterol (24S) -24-methylcholestane-1β,3β,5α,6β,25,26-hexol 25-monoacetate (**5**) as its tetra acetyl derivative (**5a**) along with other polyhydroxysterols.

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