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A new secosteroid from the gorgonian *Subergorgia suberosa* Pallas of the Indian Ocean

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An Indian Ocean gorgonian *Subergorgia suberosa* Pallas furnishes a new secosteroid **4** along with a secosteroid **6**, a trihydroxysteroid **5**, a mixture of pregnane derivatives **2** and **3**, a mixture of eleven monohydroxysterols and subergorgic acid **1**, a tricyclicsesquiterpene acid.The structure of the new secosteroid has been established as 3,9-dioxo-9,11-secocholesta-5,7-dien-11-al by a study of its physical and spectral (UV, IR, ^1H and ^{13}C NMR and Mass) data.

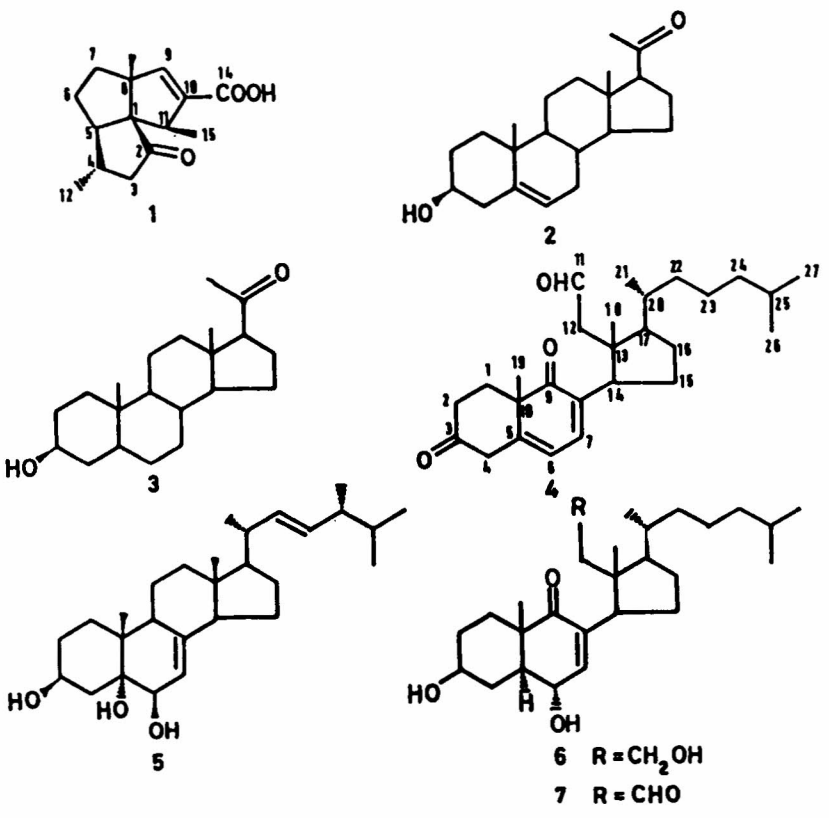
The gorgonian coral *Subergorgia suberosa* Pallas (Coelenterata, Gorgonacea, Subergorgiidae) is prominent in Indo-Paciic region and occurs quite widely in Indian waters. Chemical examination of this species collected from the Paciic Ocean was found to furnish a tricyclic sesquiterpene acid, subergorgic acid **1**, whose structure was determined by X-ray crystallographic methods[1]. In addition, four pregnane steroids have been isolated from the same species. In our continuing interest on the bioactive secondary metabolities of marine organisms of the Indian Ocean[2], we collected this species from Mandapam coast (9°16'N, 79°12'E) and the results of its chemical examination are reported here.

The initial methanolic extract of the organism was fractionated into ethyl acetate. The ethyl acetate soluble material was chromatographed over a column of silica gel eluting with solvents from hexane to ethyl acetate. Based on TLC monitoring identical, fractions were combined and further purified by chromatography or recrystallisation to furnish four pure compounds (**1, 4, 5** and **6**) besides mixture of inseparable monohydroxysterols and a mixture of two pregnane steroids (**2** and **3**).

Compound **1**, obtained as colourless cubes, m.p. 179-80°, $[\alpha]_D^{25} - 23°$, $C_{15}H_{20}O_3$, EIMS M^+ 248, was found to be subergorgic acid, identical in every respect with that reported earlier[1].

Compound **6**, obtained as colourless needles, m.p. 125-27°, $[\alpha]_D^{25} - 7°$, $C_{27}H_{46}O_4$, EIMS M^+ 434, was recognised as a steroid from its ^1H NMR spectrum (cf. Table I). It showed hydroxylic absorption (3435 cm^{-1}) and an α,β-unsaturated six-membered ketone (1665 cm^{-1}) in its IR spectrum. The latter was supported by its UV maximum at 240 nm. It showed signals for all the 27 carbons in its ^{13}C NMR spectrum whose substitution pattern was revealed by the DEPT spectrum. It showed three oxygenated carbons accounting for two secondary hydroxyls at δ 69.9 and 69.0, and a primary hydroxyl at 58.5 (t), besides a carbonyl at 204.9 (s). Two olefinic carbons of a trisubstituted double bond were noticed at δ 149.3 (d) and 135.9 (s). One olefinic proton appeared at δ 7.0 (br, s) indicating that it might be the β-proton of an α,β-unsaturated carbonyl system. Of the five double bond equivalents required for the molecule, only two were accounted for in the α,β-unsaturated carbonyl system suggesting the molecule to be tricyclic and obviously, a secosteroid. The appearance of a fragment ion at m/z 321 in its mass spectrum arising by the loss of C_8H_{17} suggests that all the functionalities are in the tricyclic system of the steroid unit. A search in literature revealed that a secosteroid with similar functionalities has been isolated recently from *Spongia officinalis*[3]. On the basis of a comparison of physical and spectral characteristics of compound **6** with those of the literature its identity was established as 9,11-seco-3β,6α,11-trihydroxy-5α-cholest-7-en-9-one **6**. This is its first report from a gorgonian species.

Compound **4** was obtained as a pale yellow oil (50 mg, $[\alpha]_D^{25} + 15.0°$). Its molecular formula was established as $C_{27}H_{40}O_3$ from its elemental analysis and EIMS (M^+ 412). Presence of multiple carbonyl functionalities was indicated by peaks at 1720, 1700, 1665 cm^{-1} in its IR spectrum. A peak of medium intensity at 2870 cm^{-1} coupled with one of the carbonyl functions suggested the



presence of an aldehyde group. One of the remaining carbonyl frequencies at 1665 cm^{-1} might be taken for a conjugated keto functionality. The UV maximum at 303 nm indicated an extended conjugation and possibly with a homoannular diene system in a six-membered ketone moiety. The molecular formula as well as its ^1H NMR spectral data (cf. Table I) indicated the compound be a steroid derivative with cholestane side chain. A close examination of the physical and spectral data suggested it to be a new steroid derivative.

Its ^{13}C NMR spectrum exhibited all the 27 car-

Table I—^1H NMR spectral data of compounds **4**, **6** and **7**

Assignment	**4** (90 MHz, $CDCl_3$)	**6** (400 MHz, pyridine-d_5)	**7*** (400 MHz, pyridine-d_5)
H-3	—	3.88 (m)	3.89 (m)
H_{ax}-4	—	1.86 (br d, J = 12 Hz)	1.8 (ddd, J = 12.4, 12.4, 12.4 Hz)
H_{eq}-4	3.30 (br s)	2.99 (br d, J = 12 Hz)	2.98 (br dd, J = 12.4, 3.3 Hz)
H-6	6.55 (br s)	4.60 (d, J = 9.9 Hz)	4.61 (br d, J = 9.8 Hz)
H-7	6.90 (br s)	7.0 (br s)	7.07 (br s)
H_a-11	9.90 (t)	4.3 (br d, J = 9.9 Hz)	10.26 (br d, J = 3.8 Hz)
H_b-11	—	4.1 (br d, J = 9.9 Hz)	—
H_a-12	2.40 (br s)	1.95 (m)	2.30 (dd, J = 6.2, 3.8 Hz)
H_b-12	2.40 (br s)	1.65 (m)	2.16 (m)
H-14	3.80 (br s)	3.59 (t, J = 8.8 Hz)	3.84 (dd, J = 11.1, 8.1 Hz)
18-CH_3	0.85 (s)	0.80 (s)	0.74 (s)
19-CH_3	1.30 (s)	1.23 (s)	1.20 (s)
21-CH_3	1.00 (d, J = 6 Hz)	1.06 (d, J = 6 Hz)	0.97 (d, J = 6.8 Hz)
26-CH_3	0.90 (d, J = 6 Hz)	0.86(d, J = 6.6 Hz)	0.86 (d, J = 6.8 Hz)
27-CH_3	0.90 (d, J = 6 Hz)	0.85 (d, J = 6.6 Hz)	0.86 (d, J = 6.8Hz)

*Data are taken from ref. 3.

Table II—^{13}C NMR spectral data of compounds **4**, **6** and **7**

Carbon No.	**4** ($CDCl_3$, 22.5 MHz)*	**6** (Py-d_5, 100 MHz)*	**7**† (Py-d_5, 100 MHz)
1	33.7	32.9	32.8
2	29.9	31.7s	31.7
3	201.9	69.9	69.8
4	27.4	34.4	34.3
5	152.0	50.0	49.8
6	128.1	69.0	68.8
7	139.1	149.3	150.0
8	153.0	135.9	133.0
9	185.9	204.9	205.1
10	45.1	45.5	45.3
11	197.9	58.5	203.9
12	48.6	42.7	50.9
13	47.7	46.7	46.4
14	45.0	43.0	43.5
15	27.4	27.6	26.7
16	26.3	26.6	26.4
17	51.4	50.8	52.0
18	16.9	17.7	16.4
19	12.4	16.6	16.2
20	34.5	35.1	35.3
21	19.5	19.4	19.4
22	35.4	35.8	35.7
23	24.7	24.9	24.5
24	39.4	39.8	39.6
25	27.9	28.3	28.2
26	22.5	22.7	22.7
27	22.7	22.9	22.9

*^{13}C assignments of compounds **4** and **6** are confirmed by DEPT experiments.

†*Data are taken from ref. 3.

bon signals (cf. Table II) and their substitution pattern was derived by DEPT spectrum. It also revealed the presence of three carbonyl functionalities [a six-membered ketone (δ 201.9), an aldehyde (δ 197.9), and a conjugated ketone (δ 185.9)]. In the olefinic region it exhibited four carbons at δ 152.0, 128.1, 139.1 and 153.0 accounting for two double bonds. The molecular formula requires eight double equivalents of which five were explained in the above functionalities suggesting the molecule to be tricyclic in nature and hence by inference to be a secosteroid.

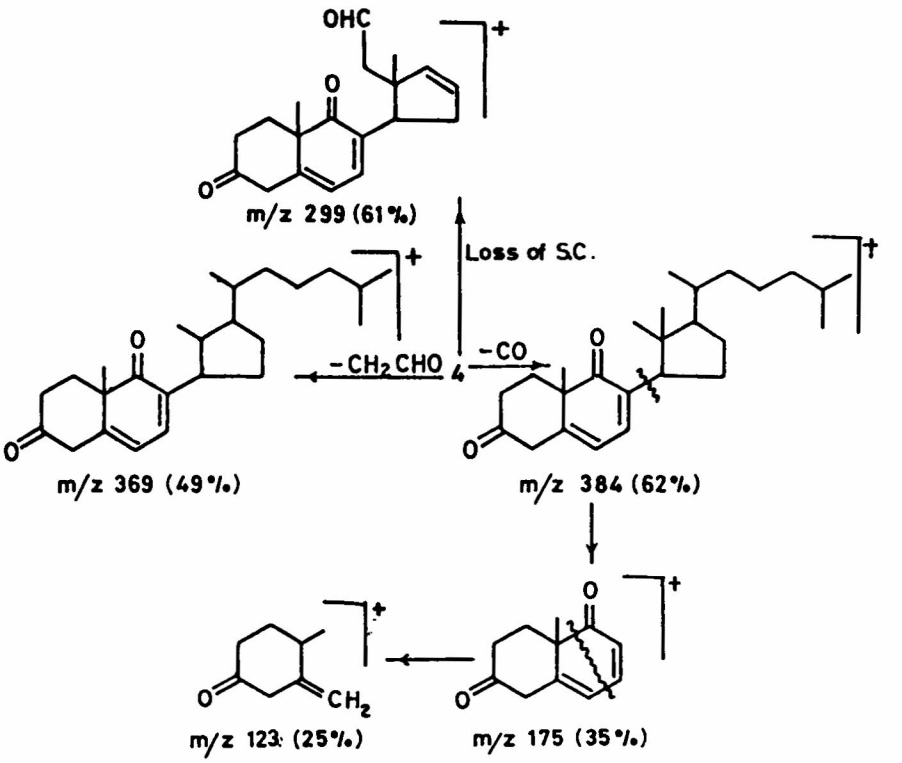
In its mass spectrum, the ion m/z 299 arising by the loss of cholesterol side chain (C_8H_{17}) was very prominent (61%) suggesting that all the functionalities of the molecule must be in the tricyclic basic unit. In all probability it might be a ring-C secosteroid, as a few similar steroids have already been reported from sponges[3] and soft corals[4]. For the two double bonds in the molecules, only two olefinic protons could be noticed that too lowfield at δ 6.55 abd 6.90 as broad singlets, suggesting that they were part of an extended conjugated system present in the molecule. As indicated by UV spectrum these two could form a homoannular diene system with extended ketone and this system fits in well with 5,7-dien-9-one. From the biogenetic considerations, 9-keto compound would have formed by the cleavage of 9,11-double bond and which incidentally indicates that the C-11 oxygenated carbon formed by the cleavage of the double bond must be present as aldehyde. The only remaining keto carbonyl might be present at C-3. From the foregoing information the structure of compound **4** could be deduced as 3,9-dioxo-9,11-secocholesta-5,7-dien-11-al.

A ring-C secoaldehyde **7** with two hydroxyls at C-3 and C-6 intact has been reported from *Spongia officinalis*[3] along with the corresponding alcohol **6**. It is coincidence that this alcohol has now been isolated from this species also (compound **6** described above). The ^{13}C NMR spectral data of compound **4** agreed closely with those of the literature aldehyde **7**[3], except in the carbons with structural differences to support its structure **4**.

The mass spectral fragmentation (Scheme I) further supported its structure. All the ions, in particular, the ions at m/z 299 (61%), 177 (38%) and 123 (25%) formed on the expected lines are in conformity with the structure.

Compound **5**, obtained as colourless needles (m.p. 221-23°, $[\alpha]_D^{25}$ –64.0°), was found to be a trihydroxysteroid identical in every respect with (22*E*, 24*R*)-24-methylcholesta-7,22-diene-3β,5α,6β-triol **5**. This appears to be its first report from the gorgonians while it was previously isolated from the sponge species, *Spongionella gracillis*[4], *Sclerophytum sp*[4], *Lobophytum crassum*[4] and *Sinularia sp*[5].

Evaporation of the latter fractions of benzene eluants from the column left a solid which on repeated crystallization from chloroform-methanol afforded colourless needles, m.p. 147-49°. Although it was homogeneous on TLC, its EIMS (M^+ 316 and 318) indicated it to be a mixture of two steroids of molecular compositions $C_{21}H_{32}O_2$ and $C_{21}H_{34}O_2$ respectively and possibly pregnane derivatives, one being the dihydro derivative of the other. The IR spectrum showed carbonyl (1720 and 1700 cm^{-1}) as well as hydroxyl (3240 cm^{-1}) functionalities. Its ^1H NMR confirmed it to be a mixture of two compounds by showing close pairs



Scheme I

of peaks for methyls and acetyl groups as required for pregnenalone derivatives. An olefinic proton was observed at δ 5.35 as in 5-enes and the carbinolic protons appeared at δ 3.6 ($W_{1/2}$ = 20 Hz) accounting for the 3β-hydroxysterols. It was thus found to be a mixture of 3β-hydroxy-pregn-5-en-20-one **2** and 3β-hydroxy-pregnan-20-one **3**.

Concentration of hexane-benzene (1:3) fractions left a residue, which though homogeneous on TLC, was found to be a mixture of monohydroxysterols from its ^{1}H NMR spectrum. The mixture was as such acetylated with acetic anhydride and pyridine and the acetate (m.p. 126-28°) was found to consist of eleven monohydroxysterols from its GC-MS analysis. Comparison of their individual mass fragmentations[6-12] and relative retention times[13] with the reported data in the literature, these were identified as cholesta-5,24-dien-3β-ol, cholest-7-en-3β-ol, cholest-5-en-3β-ol, cholest-8-en-3β-ol, 24-methylcholesta-5,25-dien-3β-ol, 24-methylcholesta-7,25-dien-3β-ol, cholesta-7,24(28)-dien-3β-ol, 24-methylcholest-5-3β-ol, 24-ethylidenecholest-5-en-3β-ol (fucosterol),24-ethylidenecholest-5-en-3β-ol (Δ^5-avenasterol) and 24-propylidenecholest-5-en-3β-ol. These are listed in Table III with their characteristic relative retention times (RR_t) and major mass fragmentation ions.

Experimental Section

General. Melting points were determined on a VEB-analytic Dreader HMK hot plate and are uncorrected. IR spectra were recorded on a Perkin-Elmer-841 IR spectrometer in KBr or $CHCl_3$ solution. UV spectra were recorded on a Milton Roy spectronic 1201 spectrophotometer in $CHCl_3$ or MeOH. ^{1}H NMR spectra were recorded on a Bruker at 400 MHz or a JEOL JNM EX-90 at 90 MHz and ^{13}C NMR spectra on JEOL JNM Ex-90 spectrometer at 22.5 MHz in $CDCl_3$ or pyridine-d_5 using TMS as internal standard. GCMS analysis was performed on a Shimadzu QP-2000 instrument at 70 eV using ULBON HR-1 equivalent to OV-17, fused silica capillary column (0.25 mm × 50 M) with film thickness 0.25 micron. Silica gel (100-200 mesh) was used for column chromatography and silica gel-G (acme) for TLC. All the spots were visualised by spraying 5% sulphuric acid in methanol.

Collection, extraction and isolation. The gorgonian species was collected from the Mandapam Coast in April 1991, and identified as *Subergorgia suberosa* Pallas by Dr P A Thomas, Scientist CMFRI, Trivandrum, India. The voucher specimens were deposited at NIO, Goa and at the Department of Organic Chemistry, Andhra Univers-

Table III—GC-MS Data of acetyl derivative of monohydroxysterol mixture

Name (Acetates of)	R_t (min)	RR^*_t	Mol. formula	Major mass fragmentation peaks	Ref.
Cholesta-5, 24-dien-3β-ol (Demosterol)	32.50	0.89	$C_{29}H_{46}O_2$	366 (50), 351 (10), 255 (40), 213 (15), 145 (20), 120(25)	6
Cholest-7-en-3β-ol (Lathosterol)	36.25	0.99	$C_{29}H_{48}O_2$	368 (70), 353 920), 255(45), 213 (26), 94 (75)	6
Cholest-5-en-3β-ol, (Cholesterol)	36.00	1.00	$C_{29}H_{48}O_2$	368 (70), 353 (25), 255 (50), 213 (30), 120 (74)	6
Cholest-8-en-3-β ol (Zymosterol)	38.76	1.07	$C_{29}H_{48}O_2$	368 (80), 353 (25), 255 (30)	7
24-Methylcholesta-5,25-dien-3β-ol	40.80	1.13	$C_{30}H_{48}O_2$	380 (45), 365 (5), 255 (35), 69 (85)	8
24-Methylcholesta-7,25-dien-3β-ol	42.15	1.16	$C_{30}H_{48}O_2$	380 (50), 255 (40), 94 (40), 69 (90)	11
Cholesta-7, 24(28)-dien-3β-ol	44.85	1.23	$C_{30}H_{48}O_2$	380 (60), 365 (10), 296(60), 83 (70),	12
24-Methylcholest-5-en-3β -ol (Campesterol)	45.00	1.24	$C_{30}H_{50}O_2$	382 (65), 367 (20), 255 (20), 120 (25)	9
24-Ethylidenecholest-5-en-3β -ol (Fucosterol)	46.18	1.27	$C_{31}H_{50}O_2$	394 (40), 379 (5), 296 (10), 255 (20), 55 (100)	9
24-Ethylidenecholest-5-en- 3β-ol (Δ^5- avenasterol)	52.50	1.45	$C_{31}H_{50}O_2$	394 (50), 296 (100), 281 (20), 255 (5)	9
24-Propylidenecholest-5-en-3β-ol	57.32	1.58	$C_{32}H_{52}O_2$	408 (45), 296 (100), 281 (15), 253 (10), 69 (60)	10

*RR_t with respect to cholesteryl acetate whose retention time under the same experimental conditions was 36.25 min.

ity, Visakhapatnam with registration number AUI-027. Specimens (1.75 kg) were washed with fresh water, soaked in methanol and brought to the laboratory. The combined methanolic extract (98 g) was concentrated to which methanol was added. It was heated, cooled to room temperature and then kept overnight in a refrigerator. The fat deposits (14 g) were separated by filtration and the filtrate was extracted with ethyl acetate. The combined ethyl acetate extract on concentration left a residue (32 g) which on extensive chromatography over silica gel column using solvents of increasing polarity from n-hexane through benzene to ethyl acetate, afforded four pure compounds along with a mixture of monohydroxysterols and a mixture of pregnane derivatives.

Subergorgic acid 1. Colourless cubes from hexane-chloroform, yield 500 mg, m.p. 179-80°, $[\alpha]_D^{25}$ −23.0 (*c* 0.7, $CHCl_3$); R_f 0.56 (benzene-EtOAc 9:1); $C_{15}H_{20}O_3$ (M^+, m/z 248); UV ($CHCl_3$): 211 nm; IR ($CHCl_3$): 3100, 1730, 1690, 1643, 1284. cm^{-1}; ^1H NMR ($CDCl_3$): δ 11.6 (1H, br s, H-14), 6.41 (1H, s, H-9), 3.01 (1H, q, *J*= 7 Hz, H-11), 2.33 (1H, dd, *J*= 16.7, 6.7 Hz, H_3),2.01 (1H, dd, *J*= 8.8, 6.4 Hz, H-5), 2.01(1H, dd, *J*= 16.7, 12.6 Hz, H-3), 1.22 (3H, s,13-CH_3), 1.13 (3H, d, *J*=7.3 Hz, 15-CH_3), 1.12 (3H, d, *J*=6.7 Hz, 12-CH_3); ^{13}C NMR ($CDCl_3$):δ 68.5 (s, C-1) 217.7 (s, C-2), 48.7 (t, C-3), 62.6 (d, C-4), 51.5 (d, C-5), 38.2 (t, C-6), 28.2 (t, C-7), 61.7 (s, C-8), 152.1 (d, C-9),136.6 (s, C-10), 33.2 (d, C-11), 23.3 (q, C-12), 19.8(q, C-13), 169.6 (s, C-14), 17.6 (q, C-15); MS: m/z 248 (M^+),230 ($M^+ - H_2O$), 215 ($M^+ - H_2O - CH_3$).

Mixture of 3β-hydroxypregn-5-en-20-one and 3β-hydroxypregnan-20-one (2 and 3). Colourless needles from chloroform-methanol, yield 30 mg; R_f 0.32 (benzene-EtOAc; 9:1); IR ($CHCl_3$:3240, 1720, 1700, 1640 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.35 (1H, br s), 3.6(2H, m), 0.69 (3H, s), 0.65 (3H, s), 1.0 (3H, s), 0.82 (3H, s), 2.13 (3H, s) and 2.15 (3H, s), MS: Two series of mass fragmentations were found by inspection at m/z 316, 301, 298, 283, 255 and at m/z 318, 303, 300, 285 and 257.

3,9-Dioxo-9,11-secocholesta-5,7-diene-11-al 4. Colourless oil, yield 50 mg, $[\alpha]_D^{25}$ + 15.0° (*c* 0.23, $CHCl_3$); R_f 0.7 (benzene-EtOAc; 2.3). Anal: Calcd for $C_{27}H_{40}O_3$: C, 78.64; H, 9.7. Found: C, 78.01; H, 10.12%; UV ($CHCl_3$): 303 nm; IR ($CDCl_3$): 2870, 1720, 1700, 1665 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$): see Tables I and II; MS: m/z 412 (M^+), 397, 384, 369, 299, 175, 123, 81, 69, 55.

(22*E*, 24*R*)-24-Methylchoesta-7,22-dien-3β,

5α, 6β-triol 5. Colourless needle from chloroform-methanol, yield 98 mg, $[\alpha]_D^{25} - 64.0°$ (*c* 1.44, pyridine); R_f 0.51 (benzene-EtOAc; 2:3); IR (KBr): 3450, 1610,1050, 980 cm^{-1}; ^1H NMR (pyridine-d_5): δ 5.75 (1H, br s, H-7), 5.2 (1H, dd, J= 15, 7.5 Hz, H-23),5.0 (1H, br s, H-22), 4.85 (1H, m, H-3), 4.35 (1H, br s, H-6), 3.05 (1H, dd, J= 12.5, 12 Hz, H-4), 1.55 (3H, s, 19-CH_3), 1.06 (3H, d, J= 7 Hz, 21-CH_3), 0.96 (3H, d, J= 6.5 Hz, 28-CH_3), 0.88 (3H, d, J= 7 Hz, 27-CH_3), 0.87 (3H, d, J= 7 Hz, 26-CH_3), 0.68 (3H, s, 18-CH_3).

9,11-Seco-3β, 6α, 11-trihydroxy-5α-cholest-7-en-9-one 6. Colourless needles from chloroform-methanol,yield 128 mg, $[\alpha]_D^{25} - 7°$ (*c* 0.24, $CHCl_3$); R_f 0.42 (benzene-EtOAc; 3:7); UV (MeOH): 240 nm; IR (KBr): 3435, 1665, 1615, 915 cm^{-1}; ^1H NMR and ^{13}C NMR (pyridine-d_5); see Tables I and II.

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References

1 Groweiss A, Fenical W, Cun-Heng H, Clardy J, Zhongde W, Zhongnian Y & Kanghou L, *Tetrahedron Lett*, **1985**, 2379.

2 Anjaneyulu A S R, Raju K V S & Prakash C V S, *Nat Prod Lett*, 3, **1993**, 149.
Anjaneyulu A S R & Sagar K S, *J Chem Res (S)*, **1995**, 142.
Anjaneyulu A S R, Sagar K S & Venugopal M J R V, *Tetrahedron*, **51, 1995**, 10997.
Anjaneyulu A S R, Venkateswara Rao G, Sagar K S, Ravi Kumar K & Chandra Mohan K, *Nat Prod Lett*, **7, 1995**, 183.
Anjaneyulu A S R, Venugopal M J R V & Kameswara Rao N S, *J Chem Res (S)*, **1996**, 50.

3 Migliuolo A, Piccialli V & Sica D, *Steroids*, 57, **1992**, 344.

4 Anjaneyulu V, Nageswara Rao K & Suresh Babu, *Indian J Chem*, 33B, **1994**, 144.

5 Kobayashi M, Murali Krishna M, Haribabu B & Anjaneyulu V, *Chem Pharm Bull*, 41, **1993**, 87.

6 Galli G & Maroni S, *Steroids*, 10, **1967**, 189.

7 Tarchini C, Rohmer M & Djerassi C, *Helv Chim Acta*, 62, **1979**, 1210.

8 Theobald N, Wells R J & Djerassi C, *J Am Chem Soc*, 100, **1978**, 7677.

9 Knights B A & Brooks C J W, *Phytochemistry*, 8, **1969**, 463.

10 Rohmer M, Kokke W C M C, Fenical W & Djerassi C, *Steroids*, 35, **1980**, 219.

11 Neal A C, Prahl F G, Eglinton G, O'Hara S C M & Coner E D S, *J Mar Biol Assoc (UK)*, 66, **1986**, 1.

12 Sato S, Ikikawa N, Kanazawa A & Ando T, *Steroids*, 36, **1980**, 65.

13 Itoh T, Tani H, Fukushima K, Tamura T & Matsumoto T, *J Chromatography*, 234, **1982**, 65.