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STEROL COMPOSITION OF MARINE ALGAE FROM KARACHI COAST OF ARABIAN SEA

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ABSTRACT: During the course of chemical investigations of marine algae collected from Karachi coast of Arabian sea, five sterols named as sarangosterol (1), 23-methyl cholesta-5, 25-dien-3ß-ol (2) from *Endarachne binghamiae* (brown alga), sargasterol (3) from *Dictyota indica* (brown alga), cholesterol (4) from *Laurencia obtusa* (red alga) and clerosterol (5) from *Codium iyengarii* (green alga) have been isolated. Their structures were elucidated with the help of spectroscopic means.

KEY WORDS: Sterols - Marine algae - *Endarachne binghamiae* - *Dictyota indica* - *Laurenica obtusa* - *Codium iyengarii.*

INTRODUCTION

Among the three major divisions of algae (Chlorophyta, Phaeophyta and Rhodophyta), the members of Phaeophyta (brown algae) have been studied moderatly. Previously we reported hexadecanoic acid, 24-methylene cholesterol and D-mannitol from *Endarachne binghamiae* (Bano *et al.,* 1987) and secodolastane diterpenoids from *Dictyota indica* (Bano *et al.,* 1990). In this paper we report two sterols named as saran gosterol (**1**) and 24-methyl cholesta-5, 25-dien-3ß-ol (**2**) from *E. binghaniae* and a sterol, sargasterol (**3**) from *D. indica.* These three compounds have not been reported so far from their respective sources.

The red algae of the genus *Laurencia* have been investigated extensively for their halogenated metabolities specially sesquiterpenoids. In our previous work we have isolated only halogenated sesquiterpenoids from *L. pinnatifida* (Bano *et al.* 1988a, b, Ahmed and Ali, 1991). This time, during the search of sterol composition we found cholestrol (**4**) as a major metabolite isolated from *L. obtusa.*

The members of Cholorphyta (green algae) are rarely studied and found complex with reference to their chemical composition, which might be due to the presence of much chlorophyll. As a part of studies on marine natural products from Karachi coast of Arabian sea, we found a sterol named as clerosterol (**5**) from *Codium iyengarii.*

MATERIALS AND METHODS

EXTRACTION AND ISOLATION:

E. binghamiae (3 kg., wet wt.) was collected from Buleji, Karachi. It was extracted

with methanol. The methanolic extract was evaporated under vacuum to dryness and partitioned between ethyl acetate and water. The ethyl acetate fraction after evaporation yielded 2.3g of crude extract which was chromatographed on silica gel column. Elution with hexane:ether (6.5:3.5) yielded compound **1** (18.25 mg). Further elution with hexane:ether (7:3) afforded compound **2**(5.51 mg).

D. indica (1kg) was extracted with acetone. The acetone extract was evaporated under vacuum and the residue obtained was subjected to column chromatography on silica gel. The sargasterol (**3**) (13.37 mg) was eluted with hexane:ether (8:2) and purified by thick layer chromatography developed in hexane:ether:acetic acid (7.5:2.0:0.5).

L. obtusa (1.5kg, wet wt.) was collected from Manora coast of Karachi and extracted in a Soxhlet apparatus with chloroform and then with methanol. The chloroform extract was condensed under reduced pressure to afford gummy mass. This was subjected for column chromatography using solvent systems hexane, hexane:chloroform, chloroform, chloroform:methanol and finally with pure methanol. Elution with hexane:chloroform (1:1) yielded crystalline compound, identified as cholesterol (**4**).

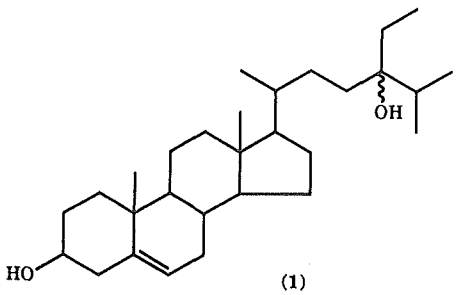
C. iyengarii was collected from Manora, Hawksbay and Buleji. The alga was washed with water and dried in shade. The air-dried alga (1.3 kg) was soaked in ethanol for one month. Ethanolic extract was evaporated under reduced pressure to obtain a gummy residue which was then partitioned with ethyl acetate and water. The ethyl acetate extract (8 g) after evaporation under reduced pressure was subjected to column chromatography on silica gel. The elution was carried out with mixtures of solvents in the order of increasing polarity starting with hexane, ether, chloroform and methanol. The fraction eluted with hexane:ether (9:1) gave pure compound **5**.

RESULTS AND DISCUSSION

The column chromatography of ethyl acetate extract of *E. binghamiae* yielded a fraction eluted with hexane:ether (6.5:3.5) was further purified on a silica gel column. The compound **1** was isolated during re-column chromatography with chloroform: methanol (9.8:0.2) in pure form. The HRMS of **1** showed a molecular ion peak at m/z 428.36452 corresponding to the molecular formula $C_{29}H_{48}O_2$. The presence of the peaks at m/z 255,231,213,145 and 119 which are common peaks in Δ^5 sterols (Ikekawa *et al.,* 1966) indicated that the sterol has the same ring system as cholesterol. Further, the peak at m/z 314 showed the cleavage of the bond between C-22 - C-23, together with a hydrogen transfer from the charge retaining moiety and the intense peak at m/z 271 appeared in the EIMS of **1**, corresponding to the loss of side chain together with two hydrogen atoms from the steroid nucleus (Wyllie and Djerassi, 1968).

The ^{13}C-NMR (broad band) spectrum of **1**, summarized in Table-1, which exhibited 29 carbon resonances. The DEPT spectrum indicated the presence of five

methyls, eleven methylene and nine methine signals. The remaining ^{13}C-NMR signals, in broad band spectrum, were due to the quaternary carbons. The methine signal at δ 71.84 was assigned to C-3 indicating the presence of a ß-hydroxyl group at this carbon (Holland *et al.* 1978). A multiplet at δ 3.51 ($W^1/_2 = 16.36$ Hz), in the ^{1}H-NMR spectrum, confirmed the presence of a ß-hydroxyl group at C-3 of a sterol (Ikekawa *et al.* 1966), while the carbon signals at δ 140.85 (-C=) and 121.75 (=CH) showed the presence of an endocyclic double bond between C-5 - C-6 in a sterol (Holland *et al.* 1978). A distorted triplet at δ 5.34 was correlated with C-6 methine signal at δ 121.75 in a hetero-Cosy (2D-NMR) spectrum.

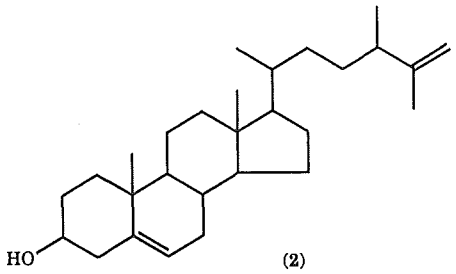


(1)

The ^{1}H and ^{13}C-NMR spectra suggested that **1** has the same ring system as cholesterol (Ikekawa *et al.,* 1966; Holland *et al.,* 1978). The methylene signal at δ 112.80 and methine signal at δ 142.65, in ^{13}C-NMR spectrum, which showed the presence of a vinyl group at C-24 (Guyot *et al.,* 1982). The vinyl protons give rise the three double-doublets at δ 5.12 (J = 1.52, 10.84 Hz), 5.18 (J = 1.52, 17.36 Hz) and 5.79 (J = 10.84, 17.36 Hz). The last signal correlated with the olefinic carbon signal appeared at δ 142.65 in the hetero-Cosy spectrum. The appearance of double doublet at δ 5.79, in ^{1}H-NMR spectrum, showed the absence of a proton at C-24. This information allowed us to place a hydroxyl group at C- 24 (Ikekawa *et al.,* 1966). This was further confirmed by a downfield quaternary carbon signal at δ 77.73 in ^{13}C-NMR spectrum which showed the presence of a quaternary hydroxyl group. Hence, on the basis of foregoing evidences the structure of **1** was elucidated as 24-hydroxy, 24-vinyl cholesterol. Some spectroscopic vlaues can also be matched with its 24-hydroperoxy isomer (Guyot *et al.*, 1982). This compound was previously reported as sarangosterol from brown alga *Sargassum ringgoldianum* (Ikekawa *et al.,* 1966).

The fraction eluted with hexane:ether (7:3) from the same column was further chromatographed on a silica gel column. The compound **2** was eluted with hexane:chloroform (7.5:2.5). The ^{1}H-NMR spectrum of 2 showed three tertiary methyl signals at δ 0.68 (s), 1.00 (s) and 1.43 (s), and two secondary methyl signals at δ 0.88 (d, J = 7.11 Hz) and 0.90 (d, J = 6.60 Hz). A multiplet at δ 3.50 and a distorted triplet at δ 5.34 were characteristic signals for 3ß-hydroxy-5-ene sterols (Ikekawa *et al.,* 1966;

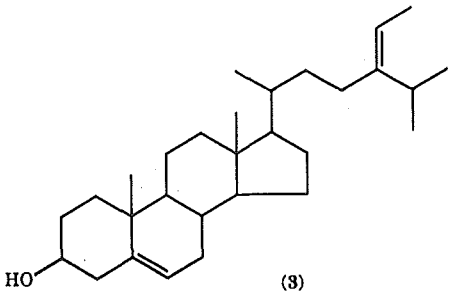
Guyot *et al.,* 1982). The signals at δ 4.65 (s) and 4.70 (s) indicated the presence of a methylene group in side chain of sterol. The downfield methyl signal at δ 1.43 and methylene signals at δ 4.65 and 4.70 allowed the placement of a double bond between C-25 and C-26 in a 24-methyl cholesterol skeleton.



(2)

The EIMS of 2 exhibited a molecular ion peak at m/z 398 corresponding to the molecular formula $C_{28}H_{46}O$. The mass fragments at m/z 271, 255, 213, 145 and 119 were characteristics of Δ^5 sterols (Ikekawa *et al.,* 1966; Wyllie and Djerassi, 1968) which showed that the ring system of sterol was same as cholesterol. The other mass fragments at m/z 356 and 314 showed the cleavage of bonds between C-24 - C-25, and C-22 - C-23, respectively (Wyllie and Djerassi, 1968). These spectral results suggested that the structure of 2 was 24-methyl cholesta-5, 25-dien-3ß-ol.

The compound 3 was obtained from the column chromatography of acetone extract of *D. indica.* The FDMS and EIMS of 3 showed the molecular ion peak at m/z 412 corresponding to the molecular formula $C_{29}H_{48}O$. The mass fragments appeared in EIMS of m/z 314 and 271 showed the cleavage of the bonds between C-22 - C-23, and C-17 - C-20, respectively (Wyllie and Djerassi, 1968). The remaining mass fragments indicated that the sterol 3 has also same ring system as cholesterol. This fact was confirmed by the assignments of H-3 (δ 3.51), H-6 (δ5.34), H-18 (δ0.68), H-19 (δ 1.00) and its ^{13}C-NMR spectrum (see Table-1).



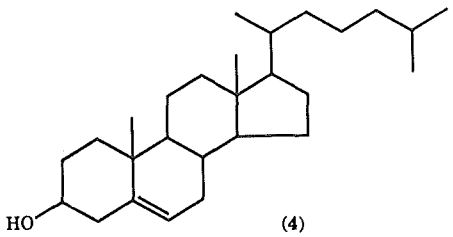
(3)

The ^{1}H-NMR spectrum of 3 indicated the presence of four secondary methyl signals. The downfield doublet at δ1.56 (J = 6.7 Hz) indicated that a methyl group is attached to a double bond while the downfield quartet at δ 5.14 allowed us to place the double bond between C-24 and C-28. These results indicate that 3 has same structure as reported for sargasterol. The ^{13}C-NMR and hetero-Cosy (2D-NMR) spectra

showed the complete agreement with the structure elucidated by ^{1}H and mass spectra.

The compound **4** was eluted with hexane chloroform when the chloroform extract of *L. obtusa* was loaded on silica gel column. The EI mass spectrum of **4** exhibited the molecular ion peak at m/z 386 corresponding to the molecular formula $C_{27}H_{46}O$. The other mass fragments observed at m/z 353, 273, 255, 213, 145 and 119 were same as cholesterol (Zaretskii *et al.,* 1967).

The identification of **4** as cholesterol was supported by the ^{1}H-NMR spectrum which showed two tertiary methyl signals, at δ 0.67 (s) and 1.00 (s) for H-18 and H-19, respectively and three secondary methyl signals, appeared at δ 0.91 (3H, d,J = 6.56 Hz) and 0.86 (6H, d,J = 6.64 Hz) for H-21, H-26 and H-27, respectively. A multiplet at δ 3.51 (1H, $W^{1}/_{2}$ = 15.20 Hz) and a distorted triplet appeared at δ 5.35 were charac teristic signals of H-3 and H-6 in cholesterol (Ikekawa *et al.,* 1966; Rubinstein *et al.,* 1976).



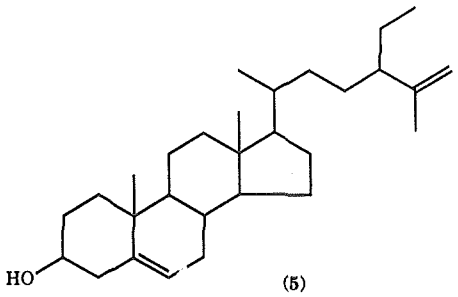
(4)

The ^{13}C-NMR (broad band) spectrum of **4** showed 27 carbon signals. The DEPT spectrum indicated the presence of five methyls, eleven methylene and eight methine carbon signals. The remaining three carbon signals, in broad band spectrum were due to the quaternary carbons. All the carbon signals, appeared in ^{13}C-NMR spectrum, showed complete agreement with the structure of **4** as cholesterol.

Compound **5** was obtained in pure form, through the silica gel column which was loaded by ehtyl acetate extract of *C. iyengarii*. The ^{13}C-NMR spectrum of **5** showed the presence of twenty nine carbon signals. The DEPT spectrum showed five methyls, twelve methylene and eight methine signals, the remaining four signals in the broad band spectrum were due to quaternary carbon atoms. The ^{13}C-NMR spectrum exhibited two sets of olefinic carbon signals at δ 147.6, 111.4 and 140.8, 121.7 due to a terminal double bond ($C=CH_2$) and trisubtituted double bond (C = CH-) funtionalities. The ^{13}C- NMR showed the methyl group resonances at δ 11.85, 12.06, 17.82, 18.67 and 19.40. It further showed the methine signal at δ 71.80. Comparison of the ^{13}C-NMR data with other sterols showed the presence of cholestane skeleton (Johnson and Jankowski, 1978). This was further supported by ^{1}H-NMR spectrum

Table-1: ^{13}C-NMR data of compounds 1,3 and 5

Carbon no.	1	3	5
1	37.30	37.29	37.29
2	31.71	31.70	31.61
3	71.84	71.85	71.80
4	42.35	42.34	42.26
5	140.85	140.85	140.83
6	121.75	121.76	121.74
7	31.94	31.94	33.70
8	31.95	31.95	31.93
9	50.18	50.18	50.17
10	36.53	36.54	36.52
11	21.10	21.11	21.11
12	39.80	39.80	39.82
13	42.40	42.38	42.34
14	56.80	56.80	56.80
15	24.29	24.34	24.30
16	28.23	28.26	28.18
17	55.88	55.84	56.10
18	11.87	11.86	11.85
19	19.40	19.41	19.40
20	36.17	36.45	35.35
21	18.81	18.87	18.67
22	29.13	25.74	33.70
23	34.61	35.25	29.43
24	77.73	147.80	49.55
25	35.95	34.80	147.60
26	16.47	22.14	17.82
27	17.55	22.25	111.43
28	142.65	115.62	26.53
29	112.89	13.18	12.06



(5)

which showed the methylene group resonances at δ 4.71 and 4.62 as broad singlets. A multiplet at δ 3.50 and a distorted triplet at δ 5.34 is the characteristic pattern of the Δ^5 - steroids (Findlay and Petil, 1985). The ^{1}H-NMR spectrum further showed three tertiary methyl group signals at δ 0.66, 0.99 and 1.55, a secondary methyl group resonance as doublet at δ 0.89 (J = 6.6 Hz) and a primary methyl group signal as triplet at δ 0.79. The mass spectrum of **5** displayed the molecular ion peak at m/z 412 and other fragment peaks at 394 $(M\text{-}H_2O)^+$,314 (M-part of side chain $C_7H_{14})^+$,271 (M-side chain + 2H)$^+$. The presence of the common cholesterol nucleus was attested by the diagonostic ions at m/z 371, 314, 255 and 213. All these results suggest the structure of **5** as clerosterol.

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