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PHYCOCHEMICAL STUDIES ON STEROLS FROM THREE BROWN SEAWEEDS OF NORTHERN ARABIAN SEA

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ABSTRACT: Three brown algae *i.e. Dictyota hauckiana* Nizamuddin, *Levringia boergesenii* Kylin and *Spatoglossum variabile* Figari *et* De Notaris, collected from the coastal areas of Karachi, have been investigated for their sterol composition. Four sterols with cholesta skeleton including a new one, 17α-hydroxy-24β-vinyl cholesterol have been isolated from them. Their structures were elucidated with the help of spectroscopic analysis. The new sterol has been named as hauckiosterol after its first source of isolation.

KEY WORDS: Sterol - cholesta skeleton - *Dictyota hauckiana* - *Levringia boergesenii* - *Spatoglossum variabile* Phaeophyta - Karachi coast.

INTRODUCTION

During phycochemical investigations on brown seaweeds of northern Arabian Sea several new and novel natural products have been isolated *e.g.* 1,1,6,6-tetrachloro-3, 4-diphenyl hexane from *Dictyota dichotoma* (Hudson) Lamouroux (Ahmad *et al.*, 1990), dictinol, dictindiol and dictintriol from *Dictyota indica* Sonder (Ahmad *et al.*, 1991), *tris*-β-butyrolactone from *Colpomenia sinuosa* (Mertens *ex* Roth) Derbès *et* Solier (Shaikh *et al.*, 1991a) and stokerene from *Stokeyia indica* Thivy *et* Doshi (Atta-ur-Rahman *et al.*, 1991). The brown algae of Arabian Sea appeared to be very promising in this regard and have shown very interesting results during phycochemical studies (Dhargalkar *et al.*, 1980; Parekh *et al.*, 1984; Pullaiah *et al.*, 1985; Khafaji, 1986; Rao *et al.*, 1986). In the present study an attempt was made to investigate the sterol constitution of three brown seaweeds, during which a new sterol was isolated simultaneously from all of them.

MATERIALS AND METHODS

The yellow brown, membranous, dichotomously branched specimens of *Dictyota hauckiana* Nizamuddin with dentate margin having proliferations (4 kg) were collected as drift material during the months of October and November 1987 from the sandy beaches of Hawkesbay and Buleji. The dark brown, very mucoid, tomentose and cylindrical thalli of *Levringia boergesenii* Kylin with a dense covering of indeterminate cortical filaments (5 kg) were detached as epilithon from the lower littoral rocks at Buleji during October 1988. The chocolate brown, foliaceous, flat, cuneate and sub-dichotomously branched plants of *Spatoglossum variabile* Figari *et* De Notaris (1.5 kg) were picked up as drift from large rocky ledges of Manora and Buleji during April and June 1989. The large and healthy specimens, free from epiphytes and animal castings, were thoroughly washed with tap water, air dried in shade and finally milled.

The milled thalli were percolated with methanol and the extract was dried at reduced pressure. The residue obtained was partitioned between ethyl acetate and water. The ethyl acetate layer was separated and evaporated at vacuum to dryness. In *D. hauckiana* the gummy residue obtained (80 g) was subjected to column chromatography using solvent systems *n*-hexane, *n*-hexane:ether, ether, chloroform, chloroform:methanol and finally methanol. The fraction eluted with *n*-hexane:ether (70:30,v/v) furnished residue containing compound 4, which was further chromatographed over silica gel column. The fraction eluted with 2% methanol in chloroform yielded the pure compound 4 (21.04 mg). Its purity was checked on TLC by using *n*-hexane:Et_2O:HOAc(1:1:0.8).

In *S. variabile* the sterolic compounds were separated in very small quantities through preparative thin layer chromatography on silica gel plates using n-hexane:Et_2O:HOAc(1:1:0.8, v/v) as developing solvent. Compound 1 was obtained in pure form (20.35 mg) with *n*-hexane:ether (9:1), the fraction eluted with hexane:ether(4:1) was a mixture of compound 2 (6.8 mg) and compound 3 (8.1 mg), which were purified on preparative thin layer chromatography using *n*-hexane:ether (1:1). The fraction eluted with hexane:ether (7:3, v/v) was further chromatographed over silica gel column. The fraction eluted with 2% methanol in chloroform provided the pure compound 4 (5.25 mg). The two sterols (10.52 mg of compound 3 and 15.8 mg of compound 4) of *L. boergesnii* were separated and purified similarly.

The pure sterols obtained were subjected to NMR and mass scanning. The electron impact mass spectra (EIMS) were recorded on MAT-112 spectrometer coupled with PDP 11/34 computer system. High resolution mass spectrometry (HRMS) was performed on Jeol JMS-HX 110 spectrometer. Nuclear magnetic resonance spectra (^{1}H-and ^{13}C-NMR) were recorded in $CDC1_3$ on Bruker AM-300 spectrometer operating at 300 MHz for ^{1}H- and 75 MHz for ^{13}C-nuclei, respectively.

The broad band and DEPT spectra were recorded on the same instrument at 75 and 100 MHz. The distortionless enhancement polarization transfer (DEPT) experiments were carried out with $\theta = 45^\circ$, 90° and 135°. The two-dimensional COSY-45 experiments were acquired at 300 MHz with a sweep width of 4000 Hz (2k data points) in $\omega 2$ and 200 Hz (256 t_1 values zero-filled to 1k) in $\omega 1$. The heteronuclear 2D ^{1}H-^{13}C chemical shift correlation experiments were carried out at 300 MHz with a sweep width of 12820 H_2 (2k data points in $\omega 1$ and 1024 H_2 (256 t_1 values zero-filled to 2k) in $\omega 2$. In both the 2D experiments a 2 seconds relaxation delay was used and 16 transients were performed for each t_1 value. Chemical shifts were reported in ppm relative to TMS.

RESULTS AND DISCUSSION

Altogether four sterols named as 1,2,3 and 4 were isolated from *Dictyota hauckiana, Levringia boergesenii* and *Spatoglossum variabile*. They were identified and elucidated as follows:

The EIMS of compound 1 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 assumption of being cholesterol was confirmed by the ^{1}H-NMR spectrum of

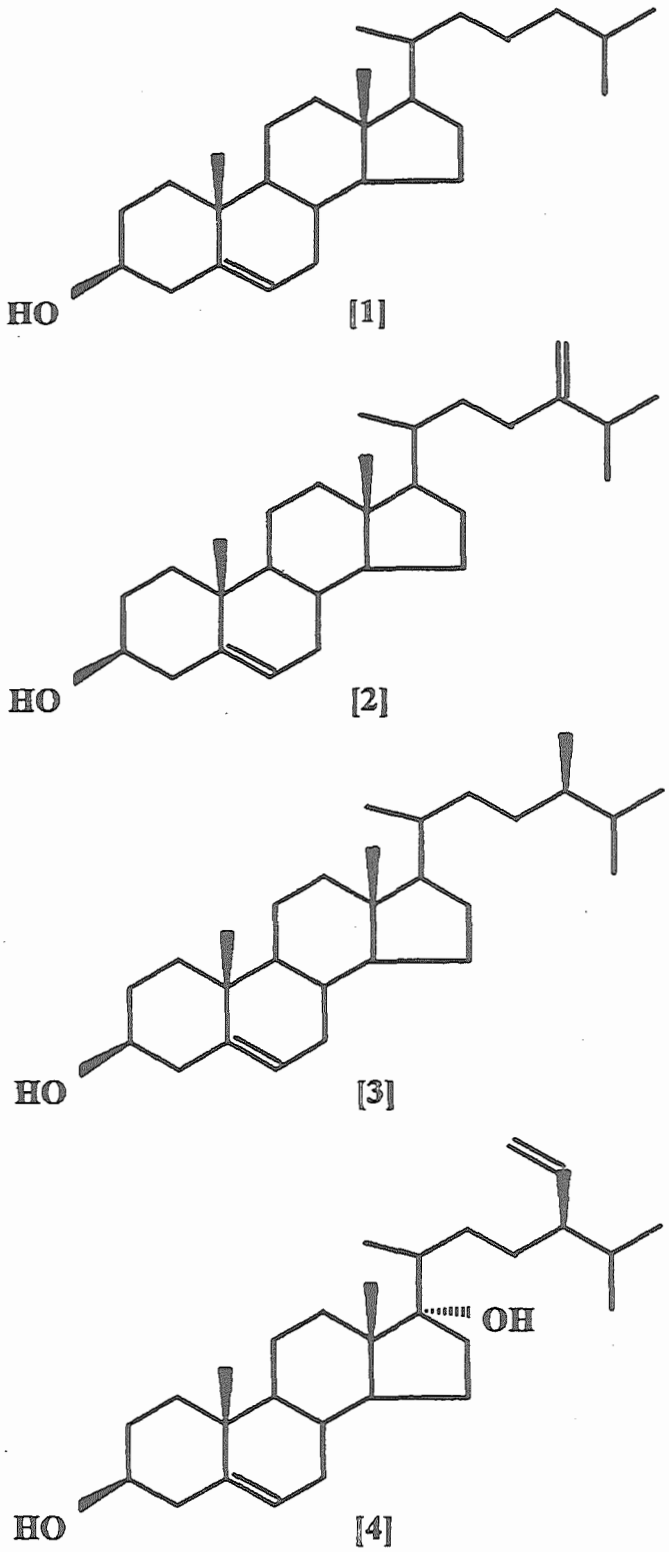

Fig.1. Sterols isolated from three brown algae: [1]=Cholesterol, [2]=Ostreasterol, [3]=24-Methyl cholesterol, [4]=Hauckiosterol.

compound 1, which indicated two tertiary methyl signals appeared at δ 0.68(s) and 1.00(s) assigned for H-18 and H-19 respectively and three secondary methyl signals appeared at δ 0.90 (d,J=6.42 Hz, 3H) and 0.85 (d, J=6.21 Hz, 6H) assigned for H-21, H-26 and H-27 respectively. A multiplet at δ 3.50 (1H,W1/2=15.2 Hz) and a distorted triplet appeared at δ 5.33 were characteristic signals of H-3 and H-6 (Ikekawa *et al.*, 1966) and showed a complete agreement of the structure of compound 1 with that of cholesterol ([1]in Fig.1).

The compound 2 showed the molecular ion peak at *m/z* 398 corresponding to the molecular formula $C_{28}H_{46}O$. The other fragments appeared at *m/z* 314, 271, 255 and 213, which correspond to the fragmentation pattern of a sterol with cholesta skeleton (Ikekawa *et al.*, 1966; Wyllie and Djerassi, 1968). The fragment at *m/z* 314 indicated the presence of ethylene group at C-24 (Wyllie and Djerassi, 1968). The ^{1}H-NMR spectrum showed signals at δ 0.69 (s,H-18), 1.01 (s,H-19), 0.95 (d,J=6.42 Hz, H-21), 0.88 (d,J=6.93 Hz, H-26 and H-27). The carbinylic (H-3) proton appear as a multiplet at δ 83.51, while the olefinic proton resonance appeared at δ 5.30 (distorted triplet) corresponding to H-6, and the peaks at δ 4.60 (s) and 4.70 (s) were assigned to H-28. The EIMS and ^{1}H-NMR spectra suggested that compound 2 was ostreasterol ([2]in Fig. 1).

The EIMS of compound 3 showed a molecular ion peak at *m/z* 400 corresponding to the molecular formula $C_{28}H_{48}O$. The peaks observed at *m/z* 255, 213, 145 and 119 were characteristic peaks of Δ^5 sterols (Ikekawa *et al.*, 1966). The peak appeared at *m/z* 271 was due to the loss of side chain. The other mass fragments at *m/z* 385 and 314 indicated the presence of additional methyl group at C-24 (Wyllie and Djerassi, 1968). The ^{1}H-NMR spectrum of compound 3 showed two quarternary methyl signals appearing at δ 0.68 and 1.00, which were assigned for H-18 and H-19. The doublets appeared at δ 0.87 (J=7.05 Hz, 6H) and 0.90 (J=7.80 Hz, 3H) were assigned for H-26, H-27 and H-21 respectively. While the additional methyl signal at δ 0.79 (d, J=7.05 Hz) was assigned for H-28. A multiplet at δ 3.57 and a distorted triplet and δ 5.34 appeared due to H-3 and H-6 protons. In the light of foregoing evidences the structure of compound 3 was identified as 24-methyl cholesterol ([3] in Fig.1).

The compound 4 provided the following spectral data (only significant parts are given):

HRMS: *m/z* 428.3650 (M^+, $C_{29}H_{48}O_2$), 410.3550 (M^+-H_2O, $C_{29}H_{46}O$), 367.2989 ($C_{26}H_{39}O$), 328.2738 ($C_{23}H_{36}O$), 314.2597 ($C_{22}H_{34}O$), 271.2065 ($C_{19}H_{27}O$), 253.1980 ($C_{19}H_{25}$), 213.1627 ($C_{16}H_{21}$), 145.1009 ($C_{11}H_{13}$), 119.0861 ($C_{19}H_{11}$). **EIMS:** *m/z* 428 (M^+, $C_{29}H_{48}O_2$, 20%), 410 (M^+ -H_2O, 38%), 395 (M^+-CH_3-H_2O,42%), 344 (M^+ -C_6H_{12}, 51%), 301 (M^+ -C_9H_{17}-2H, 34%), 285 (M^+ -C_9H_{17}-H_2O, 38%), 271 (40%), 253 (32%), 213 (30%), 145 (37%), 119 (42%), 95 (54%), 81 (100%). **^{1}H-NMR:** δ 3.51 (m, W1/2=15.15 Hz, H-3), 5.33 (t, H-6), 0.67 (s, H-18), 0.99 (s, H-19), 0.94 (d,J=6.52 Hz, H-21), 0.87 (d, J=6.60 Hz, H-26), 0.84 (d, J=6.60 Hz, H-27), 5.73 (ddd, J=2.52, 11.37, 17.80 Hz, H-28), 5.13 (dd, J=1.53, 17.80 Hz, H-29), 5.25 (dd, J=1.26, 11.37 Hz, H-29) ppm. **^{13}C-NMR:** ppm 37.33 (C-1), 31.63 (C-2), 71.88 (C-3), 42.27 (C-4), 140.82 (C-5), 121.71 (C-6), 31.94 (C-7), 31.98 (C-8), 50.23 (C-9), 36.56 (C-10), 21.14 (C-11), 39.84 (C- 12), 43.00 (C-13), 56.84 (C-14), 24.34 (C-15), 31.53 (C-16), 89.06 (C-17), 12.00 (C-18), 19.41 (C-19), 38.76 (C-20), 18.92

(C-21), 29.36 (C-22), 34.17 (C-23), 56.07 (C-24), 32.06 (C-25), 17.71 (C-26), 18.79 (C-27), 137.27 (C-28), 116.23 (C-29).

The HRMS of compound 4 exhibited molecular ion peak at *m/z* 428.3650 corresponding to the molecular formula $C_{29}H_{48}O_2$. The other fragments observed at *m/z* 410.3550, 367.2989, 328.2738, 314.2597, 271.2065, 253.1980, 213.1627, 145.1009 and 119.0861 correspond to the fragmentation pattern of a sterol with cholesta skeleton (Wyllie and Djerassi, 1968). The peak at *m/z* 271 appearing in the mass spectrum (EIMS) corresponded to the loss of side chain and the fragments at *m/z* 213, 145 and 119 indicated the absence of an additional hydroxy group in ring A, B and C in cholesterol skeleton (Ikekawa *et al.*, 1966), while the peak at *m/z* 253 showed that the second hydroxy group is present in ring D. The accurate position of second hydroxy group was determined by ^{13}C-NMR spectrum.

The ^{13}C-NMR (broad band) spectrum of compound 4 exhibited 29 carbon resonances. The DEPT spectrum indicated the presence of five methyl, eleven ethylene and nine methine signals. The remaining ^{13}C-NMR signals in broad band spectrum were due to the quarternary carbons. The methine signal appeared at δ 71.88 was assigned for C-3 and indicated the presence of a hydroxy group at this carbon (Holland *et al.*, 1978). A multiplet at δ 3.51 (W1/2=15.15 Hz) in ^{1}H-NMR spectrum confirmed the presence of a hydroxy group at C-3 (Ikekawa *et al.*, 1966). The carbon signals at δ 140.82 (-C=) and 121.71 (=CH) showed the presence of endocyclic double bond between C-5 and C-6 (Holland *et al.*, 1978).

A distorted triplet at δ 5.33 correlated with C-6 methine signal in a hetero-COSY (2D-NMR) spectrum. The remaining olefinic carbon signals at δ 137.27 (HC=) and 116.23 (=CH_2) point out the presence of a vinyl group at C-24. The ^{1}H-NMR signals appeared at δ 5.13 (dd, J=1.53, 17.80 Hz) and 5.25 (dd, J=1.26, 11.37 Hz) showed the correlation with ethylene signal of C-29, while the signal at δ 5.73 (ddd, J=2.52, 11.37, 17.80 Hz) indicated the correlation with the ^{13}C-NMR signal at δ 137.27 in hetero-COSY (2D-NMR) spectrum and showed the presence of a quarternary hydroxy group. The downfield ^{13}C-chemical shifts of C-13 at δ 42.99, C-16 and at δ 31.53 and C-20 at δ 38.76 allowed to place the quarternary hydroxy group at C-17.

In view of the above data the structure of compound 4 was found to be 17α-hydroxy-24β-vinyl cholesterol ([4] in Fig.1). It is a new natural product, not previously known from any living source. It has been named hauckiosterol, as it was isolated for the first time from *D. hauckiana* and later on also found to be present in *L. boergesenii* and *S. variabile*.

The percent yields (w/w) of the isolated sterols are given in Table I. Cholesterol, though detected only in *S. variabile*, was found in largest quantity among all the sterols. The other three sterols are 24-derivatives of cholesterol. The cholesterol and its 24-derivatives occur quite frequently in brown seaweeds (Shaikh, 1993). Cholesterol, ostreasterol and 24-methyl cholesterol have been detected in *Stoechospermum marginatum* (C. Agardh) Kützing (Shaikh *et al.*, 1990), *Padina tetrastromatica* Hauck (Shaikh *et al.*, 1991b), *Laminaria digitata* and *L. faeroensis* (Patterson, 1971). Fucosterol, which is supposed to be the major sterol of Phaeophyta (Goodwin, 1974), was not detected in any investigated seaweed.

Table I. Percent yield (w/w) of sterols isolated from three brown seaweeds.

Comp. No.	Common name	Systematic name	Molecular formula	Mol. wt.	*Dictyota hauckiana*	*Levringia boergesenii*	*Spatoglossum variabile*
1.	Cholesterol	Cholest-5-en-3β-ol	$C_{27}H_{46}O$	386	-	-	1.35
2.	Ostreasterol	24-Methyl-ene cholesterol	$C_{28}H_{46}O$	398	-	-	0.45
3.	24-Methyl cholesterol	24-Methyl-cholest-5-en-3β-ol	$C_{28}H_{48}O$	400	-	0.32	0.54
4.	Hauckios-terol*	17α-Hydro-xy-24β-vinyl-cho-lesterol	$C_{29}H_{48}O$	428	0.53	0.21	0.35

*New natural product.

All of the four isolated sterols were present only in *S. variabile*, which appeared to be quite rich in sterol contents, two of them were detected in *L. boergesenii* while *D. hauckiana* contained only one. Hauckiosterol was detected in all the three investigated species, it was present in largest amount in *D. hauckiana* and smallest in *L. boergesenii.* It is interesting that it was simultaneously detected in three brown algae, two of them belong to the order Dictyotales and the other one to Chordariales. Hence this new sterol appears to have a wide range of occurrence in Phaeophyta. It cannot be used as chemotaxonomic marker.

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