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PHYCOCHEMICAL STUDIES ON *SPATOGLOSSUM VARIABILE* (DICTYOTALES, PHAEOPHYTA)

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Abstract

From methanolic extract of *Spatoglossum variabile* Figari *et* De Notaris, collected from the coast of Karachi, a saturated fatty acid (Palmitic acid), 4 sterols (cholesterol; 24-methylene cholesterol; 24-methyl cholesterol and myriosterol) and a spatane diterpenoid (19-acetoxy-5, 15, 18-trihydroxy spata-13, 16-diene) have been isolated and identified using GC-MS and ^{1}H-NMR techniques. This seaweed is the second source for the occurrence of myriosterol.

Introduction

Phycochemistry is the study of the natural products and the chemical constituents occurring in algae, from a biological point of view (Shameel, 1990 a). The biological evaluation of marine algae belonging to Dictyotales have shown antibacterial, antiviral and cytotoxic activity (Gerwick *et al.*, 1981). A spatol isolated from *Spatoglossum schmittii* and *S. howleii* was found to inhibit synchronous cell division in the fertilized sea urchin egg and human cancer cell cleavage *in vitro* (Gerwick & Fenical, 1983). Mehta & Parekh (1978) isolated mannitol, Dhargalkar *et al.* (1980) and Qasim (1986) studied protein and carbohydrate contents and Gerwick *et al.*, (1981) and Gerwick & Fenical (1983) detected three diterpenes of spatane class and two other derivatives from different species of *Spatoglossum*.

Spatoglossum variabile Fagari *et* De Notaris is a common marine seaweed, which occurs as epilithon on mid-to sub-littoral rocks from Manora to Ras Malan seashore waters in Pakistan (Shameel, 1990b). A sterol designated as myriosterol has been reported (Ahmad *et al.*, 1990). The present paper describes the results of a phyco-chemical investigation on *S. variabile*.

Materials and Methods

Healthy thalli of *Spatoglossum variabile* (1.5 kg fresh weight) were collected from the coast of Buleji, near Karachi in April and June 1987. The thalli were homogenized and percolated three times with MeOH. The methanolic extract was evaporated

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under reduced pressure and the brownish syrupy residue (13.2 g) fractionated with EtOAc in aqueous phase. On evaporation of EtOAc this fraction (2.7 g) was subjected to silica gel column chromatography and eluted in increasing order of polarity with hexane, diethyl ether, $CHCl_3$ and MeOH.

The fraction eluted with *n*-hexane-diethyl ether (9:1) gave a crystalline compound (10 mg), which after recrystallization with Me_2CO was found to be a fatty acid. Another fraction eluted with *n*-hexane-diethyl ether (7:3) afforded a mixture of sterolic compounds as exhibited on chromogenic reaction with Liebermann-Buchardt test. Sterols were separated in very small quantities through preparative layer chromatography on silica gel plates using hexane-diethyl ether-HOAc (1:1:0.8 %) as developing solvent. In this way four sterols were separated. Therafter the said column with $CHCl_3$: MeOH (9:1) furnished a dark brownish gummy mass. It was repeatedly subjected to thin layer chromatography on silica gel plates using $CHCl_3$:MeOH (98:2), which ultimately yielded a pure compound, a diterpenoid.

The GC-MS was performed on a GC-Hewlett Packard with 11/73 DEC computer data system and a 1.2 m x 4 mm packed glass capillary column coated with gas chrom Q (100-120 mesh, OV 101 1%). The column temperature was programmed between 70-250°C with a rate of increase of 80°C per minute. The carrier gas (helium) flow rate was 32 ml/min and the injector temperature was 250°C. The ^{1}H-NMR spectra were obtained in $CDCl_3$ as a solvent and TMS as the internal reference on Bruker WM 400 instrument equipped with an Aspect 2000 computer. The details of the spectral techniques have been reported in Hayee-Memon *et al.*, (1991).

Results

The methanolic extract of *S. variabile* followed by fractionation and chromatographic separation resulted in the isolation of 6 natural products containing 1 saturated fatty acid, 4 sterols and 1 diterpenoid (Table 1). Mass and fragmentation pattern and the spectral data were obtained, on the bases of which they have been identified as follows:

Palmitic acid [1]: MS m/z (abundance), 256 (M^+, $C_{10}H_{32}O_2$, 18%), 228 (8%), 213 (M^+-43, 8%), 199 (M^+-57, 7%), 186 (5%), 172 (18%), 158 (15%), 144 (31%), 129 (73%), 115 (55%), 101 (27%) 83 (13%), 77 (100%). **^{1}H-NMR** ($CDCl_3$,δ): 2.34 (t), 0.85 (distort t), 1.25 (s).

The identity of the isolated fatty acid was further confirmed by comparing it with previously obtained spectral data for palmitic acid (Bano *et al.*, 1987). The sterolic fraction was submitted to preparative TLC. The scrapped off sterol bands were repeatedly subjected to chromatography, purified, recrystallized and consequently the following 4 sterols were identified.

Cholesterol [2]: MS m/z (abundance), 386 (M^+, $C_{27}H_{46}O$, 92%), 371 (M^+-CH_3, 33%), 368 (M^+ -H_2O, 64%), 353 (M^+ -CH_3 -H_2O, 37%), 273 (M^+ -C_8H_7 -side chain, 17%), 252 (M^+ -side chain-C_{16} -C_{17}, 8%), 231 (M^+ -side chain-ring D cleavage, 18%), 121 (16%), 107 (25%).

Table 1. Natural products isolated from *Spatoglossum variabile*.

Common Name	Systematic Name	Molecular Formula	Mol. Wt.	Quantity (mg/kg wet wt.)
Palmitic acid	Hexadecanoic acid	$C_{16}H_{32}O_2$ [1]	256	6.66
Cholesterol	Cholest-5-en -3β-ol	$C_{27}H_{46}O$ [2]	386	13.56
24-Methylene cholesterol	24-Methylene-cholest-5-en-3β-ol	$C_{28}H_{46}O$ [3]	398	4.56
24-Methyl cholesterol	24-R-Methyl-cholest-5-en-3β-ol	$C_{28}H_{48}O$ [4]	400	5.40
Myriosterol	3β,17-Dihydroxy -stigmasta-5, 28 diene	$C_{29}H_{48}O$ [5]	428	3.50
Spatane diterpenoid	19-Acetoxy-5, 15, 18--trihydroxy-spata-13, 16-diene	$C_{22}H_{34}O$ [6]	378	2.33

24-Methylene cholesterol [3]: MS m/z (abundance), 398 (M^+, $C_{28}H_{46}O$, 10%), 383 (M^+ -CH_3, 30%), 365 (M^+ -CH_3 -H_2O, 18%), 314 (M^+ -C_6H_{12}, 20%), 271 (M^+-C_9H_{17} -2H, 18%), 225 (M^+ -C_9H_{17} -H_2O, 16%), 16%), 213 (M^+ -$C_{12}H_{23}$ -H_2O, 24%), 171 (12%), 145 (34%), 81 (76%).

24-Methyl cholesterol [4]: MS m/z (abundance), 400 (M^+, $C_{28}H_{48}O$, 60%), 385 (M^+ -CH_3, 4%), 382 (M^+ -H_2O, 19%), 367 (M^+ -CH_3 -H_2O, 12%), 315 (86%), 300 (86%), 273 (M^+ -side chain, 7%), 271 (34%), 255 (48%), 231 (43%), 207 (15%), 107 (11%).

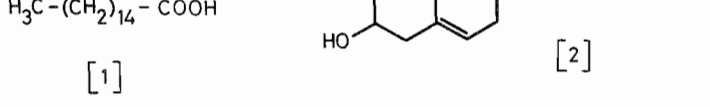
Myriosterol [5]: MS m/z (abundance), 428 (M^+, $C_{29}H_{48}O_2$, 46%), 410 (M^+ -H_2O, 11%), 392 (M^+ -20H, 52%), 367 (M^+ -C_3H_7 -H_2O, 15%), 314 (M^+ -C_7H_{12} -H_2O, 27%), 271 (14%), 255 (20%), 231 (12%), 213 (18%), 145 (10%), 119 (12%). **^{1}H-NMR** ($CDCl_3$, δ) = 0.67 (s), 0.84 (d, J = 6.7 Hz), 0.86 (d, J = 6.5 Hz), 0.94 (d, J = 6.5 Hz), 0.99 (s), 3.5 (m), 5.13 (dd, J = 17.8, 1.5 Hz).

Identification of 4 sterols isolated was also based on no difference in mixture melting point with an authentic sample and on direct comparison on TLC plate with the reference sample (Ahmad *et al.*, 1990). Ultimately a novel compound, a diterpenoid was isolated.

19-Acetoxy-5, 15, 18-trihydroxy-spata-13, 16-diene [6]:

MS m/z (abundance), 378 (M^+, $C_{22}H_{34}O_5$, 52%), 300 (M^+ -HOAc-H_2O, 18%), 287 (M^+, C_{19}, $H_{27}O_2$, 26%), 269 (M^+, $C_{19}H_{25}O$, 12%), 211 (M^+, $C_{16}H_{19}$, 27%), 159 (M^+, $C_{12}H_{15}$, 38%), 145 (M^+, $C_{11}H_{13}$, 23%), 135 (M^+, $C_{10}H_{15}$, 17%), 105 (M^+, C_8H_9, 34%), 81 (M^+, C_5H_5O, 43%). **^{1}H-NMR** ($CDCl_3$, 400 MHz, δ): 3.70 (C_5-*H*, d, J = 3.99 Hz), 2.26-2.35 (C_6-*H*, ddd, J = 13.1, 13.1, 4.2 Hz), 2.93-2.99 (C_7-H, m), 0.96-0.99 (C_{11}-*H*, d, J = 6.33 Hz), 1.06 (C_{12}-*H*, s), 5.29 (C_{14}-*H*, s) 4.41 (C_{15}-*H*, d, J = 6.18 Hz), 4.43 (C_{16}-*H*, d, J = 6.18 Hz), 5.70 (C_{17}-*H*, m), 3.93-4.08 (C_{19}-*H*, d, J = 11.07 Hz), 1.29 (C_{20}-*H*, s), 2.09 (OAc, s).

The isolated diterpenoid is a member of the class "Spatane". The ^{1}H-NMR quite conclusively showed four methyl groups at δ 0.96, 0.99, 4.07 and 4.30 and an OAc group at δ 2.09. Consequently comparison of the MS and NMR spectral assignments with the data reported in the literature (Gerwick *et al.*, 1981) allowed the verification of the isolated diterpene.



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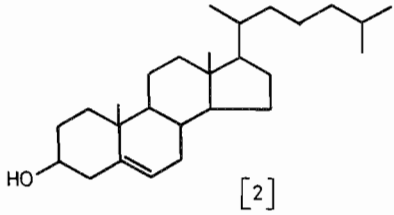

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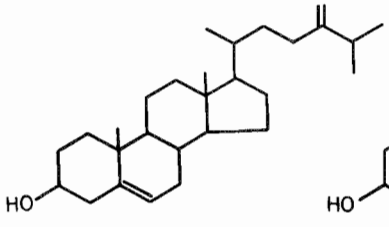

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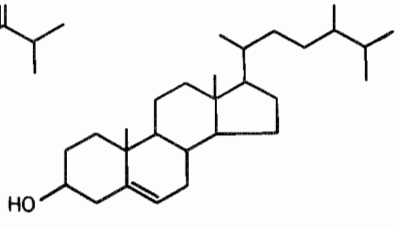

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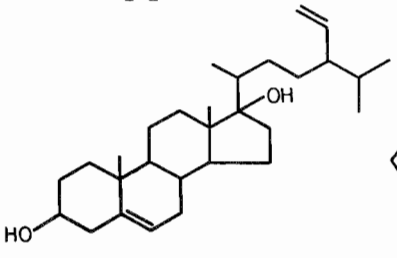

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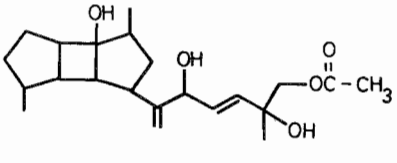

[6]

Discussion

S. variabile yielded appreciable quantity of palmitic (hexadecanoic) acid (Table I). It is the most common saturated fatty acid present in aquatic animals and marine benthic algae (Qasim, 1986; Shameel, 1990 a) and has been rported from several brown seaweeds of Karachi coast (Bano *et al.,* 1987; Usmanghani *et al.*, 1987; Shaikh *et al.*, 1991 a, b). It is usually present in largest quantity and is the only fatty acid which crystallizes.

Fucosterol is usually the major sterol of Phaeophyta, while some brown seaweeds possess the related sargasterol or saringosterol in large quantities (Wood, 1988). *S. variabile* yielded cholesterol in appreciable amount alongwith small quantities of 24-methylene cholesterol, 24-methyl cholesterol and myriosterol (Table I). Cholesterol, a major sterol of Rhodophyta and often reported from Phaeophyta (Goad, 1978), was also found as a major component in *Iyengaria stellata* (Usmanghani *et al.*, 1987 b). However 24-methylene cholesterol and 24-methyl cholesterol have been reported in small amount from several brown seaweeds (Wood, 1988, Usmanghani *et al.*, 1987 b, Bano *et al.*, 1987, Shaikh *et al.*, 1991 b). Myriosterol is a recently discovered sterol, detected for the first time in *Myriogloia sciurus* (Ahmad *et al.*, 1990), and *S. variabile* is the second source of its occurrence.

Small amount of 19-acetoxy-5,15,18-trihydroxy-spata-13, 16-diene was also isolated from *Spatoglossum variabile*. It was for the first time isolated from *Stoechospermum marginatum* (Gerwick *et al.*, 1981) and later on also reported from *Spatoglossum schmittii* and *S. howleii* (Gerwick & Fenical, 1983.

Members of the "spatane" class of diterpenes have been isolated from *Dilophus marginatum, Spatoglossum schmittii* and *Stoechospermum marginatum* (Shaikh *et al.*, 1990). The spatane skeleton, which is diterpene analogue of the bourbonene skeleton, can be considered as resulting from further cyclization of *cis*-fused pachydictyol skeleton spatol, a potent inhibitor of cell division.

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