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STEROL AND FATTY ACID COMPOSITIONS OF A MARINE *ALGA BRYOPSIS PENNATA* (BRYOPSIDOPHYCEAE, CHLOROPHYTA)

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ABSTRACT

The green alga *Bryopsis pennata* Lamouroux, collected from the coast of Karachi, has been investigated for its sterol and fatty acid compositions. Four sterols and 19 fatty acids have been isolated from methanolic extract of this alga and identified by ^{1}H-NMR, El- & GC-MS techniques. The sterol having cholesta skeleton was found as major constituent (78.12%), while other three with ergosta skeleton occurred in traces (5-9%). Seven saturated fatly acids were present in greater quantity (72.58%) than 12 unsaturated ones (27.41%). The latter included 7 monoenoic, 2 dimwit:, 2 trienoic and 1 pentaenoic acids. Tricosanoic acid was found in the highest amount (30.24%), clopentenyl undecanoic and heneicosapentaenoic acids were the unique and rare fatty acids detected.

Introduction

Bryopsis pennata Lamouroux is an olive-green, siphonaceous, plumose, sparsely branched alga with distichous pinnules gradually narrowed towards the slightly constricted base. It grows in sandy bottom rock pools and as epilithon on mid to lower littoral rocks of Manora and Buleji in Pakistan (Shameel and Tanaka, 1992). Various species of *Byropsis* have been phycochemically investigated in different laboratories and provided interesting results. Cystine containing proteins and a wound healing protein was isolated from *B. hypnoides* (Burr and West, 1971; Burr, 1972), and immunochemical studies were made on ribulose-1, 5-biphosphate carboxylase/oxygenise of *B. maxima* (Kajikawa *et al.,* 1988). The sterol fractions of *B. hypnoides* were analysed by Dickson *et al* (1979), and those of *B. plumosa* were studied in detail by Dc Napoli *et al.* (1982). The latter species has further been investigated for its pigment composition (Fawley and Lee, 1990). Several studies were made on different aspects *of B. corticulans,* e.g. fructose-1,

6-biphosphatase activity (Yu, 1989), role of3phosphoglycerate in chloroplast (Yu and Pedersen, 1991) and chlorophyll-protein complex (Wu *et at*, 1991). The carotene contents of *B. maxima* were investigated by Tsujimoto *et al*, (1992). But no detailed phycochemical study was ever carried out on *B. pennata*. In this paper an attempt was made to analyse the sterol and fatty acid compositions of this seaweed.

Material and Methods

Algal material

The bunches of algal filaments were carefully detached from mid to lower litoral rocks and edges of the boulders at French Beach of Buleji near Karachi, Pakistan during april to July 1989. The healthy specimens, free from epiphytes and animal castings were selected and thoroughly washed to remove sand particles and attached detritus.

Isolation of sterols

The washed material was freeze dried and extracted with methanol (MeOH, 3x600 mL) at room temperature. Combined extract was evaporated to dryness and the residue was refluxed with 10% KOH in 80% ethanol (EtOH, 50 mL). After extraction with diethyl ether (Et_2O) the organic phase, taken to dryness, was chromatographed on a silica gel column using C_6H_6-Et_20 (4:1, v/v) as eluent. The sterol fraction was collected and after acetylation with Ac_2O-C_5H_5N v/v) for 12 hours at room temperature was fractionated on a $AgNO_3$-si gel (1:2) column (eluent:petrol-C_6H_6, 7:3 v/v).

The unsaponfiable fraction of the algal extract was chromatographed on a silica gel column and the sterol fraction was acetalated and analysed by GC-MS. The sterol acetates were fractionated by column chromatography on si-gel impregnated with $AgNO_3$. The various column fractions were monitored by GC and combined accordingly. The sterols were identified by ^{1}H-NMR and mass spectrometry (El-MS).

Extraction of fatty acids

The washed material was dried in shade. The dried material (300 g) was extracted with 90% aqueous MeOH at room temperature, the procedure was repeated three times and combined solvent mixture evaporated under reduced pressure to give a thick material weighing 5.23 g. It was saponified by refulxing at 100°C for 10 hours with 10% KOH (25 mL) and then concentrated under vacuum. Water and Et_2O were added to the reaction mixture and this treatment was also repeated thrice. The aqueous alkaline layer was acidified with HCl and then extracted with Et_2O. The ether fraction was washed three

times with H_2O and once with hydrogen carbonate to remove residual non-saponifiable impurities. The resultant Et_2O fraction was dried over anhydrous Na_2SO_4, and on evaporation of Et_2O a residue (14 mg) was obtained.

The fatty acid fraction so obtained was then esterified with diazomethane. To a quantity of 5 mg fatty acid fraction 1 mL of diazomethane was added and the reaction mixture was left overnight in a deep-freezer. It was then evaporated under reduced pressure and the residue was dissolved in $CHCl_3$, the aliquotes were directly injected into gas liquid chromatograph (GLC) and gas chromatograph-mass spectrometer (GC-MS). The details of this technique were the same as reported earlier (Siddiqui, 1993).

Results

Four sterols were identified from the methanolic extract of *Bryopsis pennam.* Their molecular formulae and weights have been presented in Table 1, and their structures are given in Fig. 1. Following are the significant spectral data especially the mass fragmentation pattern, on the basis of which these sterols were identified:

Cholesta-5, 20, 24-trien-β-ol: EI-MS: *m/z* 382 (M^+, $C_{27}H_{42}O$), 367 (M^+-CH_3), 364 (M^+-H_2O), 349 (M^+-CH_3-H_2O), 313 (M^+-CH_3-H_9), 283 (M^+-CH_3-CH_3-C_6H_{11}-H), 271, 253, 213, 200, 161, 147, 133, 120, 95, 71, 57.

Ergosta-8, 14, 22-trien-3β-ol: *m/z* 396 (M^+, $C_{28}H_{44}O$), 381 (M^+-CH_3), 364 (M^+-CH_3-OH), 288 (M^+-SC), 275, 273, 255, 212, 199, 185 (M^+-SC-42-ROH), 173, 160, 147, 133, 120, 105, 91, 81, 57.

Ergosta-5, 7, 22-trien-3β-ol: 396 (M^+, $C_{28}H_{44}O$), 381 (M^+-CH_3), 288, 271 (M^+-SC), 255, 239, 213, 185 (M^+-SC-42-ROH), 174 (M^+-CH_3-CH_2OH), 161, 147, 107, 91, 81, 57.

Ergosta-5, 8, 22-trien-3β-ol: 396 (M^+, $C_{28}H_{44}O$), 381 (M^+-CH_3), 379 (M^+ -OH), 364 (M^+-CH_3-OH), 275 (M^+-SC), 227 (M^+-ROH- SC-C_2H_2), 213, 185, 161, 147, 133, 121, 105, 81, 57, 55.

The GC-MS data of the methylated fatty acids showed the presence of nineteen acids. The significant ions in the mass spectra of these fatty esters are given below:

9, 10-Dodecenoate methyl ester: GC-MS: *m/z* 212 (M^+,$C_{13}H_{24}O_2$, 1%), 196 (M^+-16, 3%), 182 (M^+-30, 3%, 154 (4%), 144 (5%), 126 (7%), 113 (10%), 99 (2%), 85 (28%), 71 (60%), 57 (100%).

Tridecatrienoate: *m/z* 222 (M^+,$C_{14}H_{22}O_2$, 3%), 195 (M^+-27, 1%), 167 (M^+-55, 2%), 153 (1%), 139 (2%), 125 (5%), 111 (10%), 97 (25%), 83 (35%), 69 (78%, 55 (100%).

Tridecadrenoate: 224 (M^+, $C_{14}H_{24}O_2$, 9%), 205 (M^+ -19, 5%), 187 (M^+-37, 9%), 159 (12%), 145 (13%), 133 (17%), 113 (16%, 98 (15%), 85 (44%), 71 (87%), 57 (100%).

Tridecenoate: 226 (M^+, $C_{14}H_{26}O_2$, 2%), 211 (M^+-15, 1%), 197 (M^+-29, 2%), 183 (1%), 155 (3%), 141 (4%), 127 (5%), 113 (10%), 99 (15%), 85 (45%), 71 (65), 57 (100%).

9, 10-Tetradecenoate: 240 (M^+, $C_{16}H_{28}O_2$, 7%), 213 (M^+-27, 8%), 199 (M^+-41, 13%), 165 (23%), 144 (20%), 129 (23%), 115 (24%), 96 (25%). 83 (53%), 69 (47%), 55 (100%).

11-Δ-2, 3-cyclopentenyl-*n*-undecanoate: 252 (M^+, $C_{16}H_{28}O_2$, 30%), 220 (M^+-32, 13%), 178 (M^+-74, 2%), 167 (2%), 153 (3%), 139 (7%), 125 (8%), 111 (18%), 97 (37%), 83 (50%), 69 (35%), 58 (55%), 43 (100%).

***n*-Pentadecanoate**: 256 (M^+, $C_{16}H_{32}O_2$, 20%), 225 (M^+-31, 15%), 207 (M^+-49, 17%), 165 (60%), 149 (37%), 137 (40%), 123 (58%), 109 (42%), 95 (55%), 81 (60%), 55 (80%), 42 (100%).

***n*-Hexadeeanoate**: 270 (M^+, $C_{17}H_{34}O_2$, 15%), 239 (M^+-31, 9%), 227 (M^+-43, 5%), 199 (3%), 185 (5%), 169 (6%), 155 (8%), 141 (7%), 127 (10%), 113 (5%), 99 (6%), 85 (40%), 71 (100%).

Heptadecatrienoate: 278 (M^+, $C_{18}H_{30}O_2$, 15%), 251 (M^+-27, 20%), 223 (M^+-55, 28%), 198 (17%), 170 (15%), 140 (10%), 115 (22%), 97 (31%), 82 (61%), 71 (100%).

Heptadecadienoate: 280 (M^+, $C_{18}H_{32}O_2$, 10%), 248 (M^+-32, 5%), 206 (M^+ -74, 10%), 169 (40%), 149 (50%), 113 (10%), 99 (20%), 85 (40%), 71 (100%).

Heptadecenoate: 282 (M^+, $C_{18}H_{34}O_2$, 2%), 267 (M^+-15, 5%), 253 (M^+-29, 13%), 239 (5%), 225 (10%), 210 (6%), 183 (3%), 141 (4%), 127 (5%), 113 (6%), 99 (10%), 85 (35%), 71 (49%), 57 (100%).

***n*-Heptaderanoate**: 284 (M^+, $C_{18}H_{36}$,O_2, 10%), 253 (M^+-31, 7%), 241 (M^+-43, 5%), 185 (5%), 171 (3%), 157 (10%), 143 (5%), 129 (11%), 101 (50%), 87 (100%)

Nonadecenoate: 310 (M^+, $C_{20}H_{38}O_2$, 1%), 278 (M^+-32, 3%), 253 (M^+-57, 5%), 239 (3%), 192 (1%), 169 (6%), 141 (4%), 113 (5%), 85 (28%), 71 (49%), 57 (100%).

n-Nonadecanoate: 312 (M^+, $C_{20}H_{40}O_2$, 25%), 281 (M^+-31, 2%), 269 (M^+-43, 5%), 255 (31%), 227 (3%), 213 (10%), 199 (2%), 175 (3%), 157 (12%), 143 (10%), 129 (15%), 115 (24%), 101(50%), 87 (100%).

Heneicosapentaenoate: 330 (M^+, $C_{22}H_{34}O_2$, 10%), 309 (M^+-31, 5%), 256 (M^+-74, 3%), 239 (4%), 225 (2%), 211 (3%), 197 (5%), 169 (6%), 155 (1%), 141 (7%), 127 (5%), 99 (3%), 85 (30%), 71(50%), 57 (100%).

Heneicosenoate: 338 (M^+, $C_{22}H_{42}O_2$, 7%), 295 (M^+-43, 5%), 281 (M^+-57, 10%), 267 (10%), 253 (5%), 239 (18%), 225 (20%), 197 (5%), 183 (2%), 143 (3%), 127 (7%), 113 (5%), 99 (8%), 85 (28%), 71(55%), 57 (100%).

***n*-Docosanoate**: 354 (M^+, $C_{23}H_{46}O_2$, 5%), 323 (M^+-31, 5%), 309 (M^+-45, 6%), 281 (8%), 267 (18%), 239 (4%), 195 (5%, 169 (3%), 113 (8%), 99 (15%), 85 (100%).

Tricosenoate: 366 (M^+, $C_{24}H_{46}O_2$, 7%), 352 (M^+ -14, 2%), 309 (M^+-57, 12%), 298 (17%), 253 (7%), 183 (5%), 169 (1%), 154 (5%), 141 (6%), 113 (8%), 85 (49%), 57 (100%).

***n*-Tricosanoate**: 368 (M^+, $C_{24}H_{48}O_2$, 75%), 353 (M^+-15, 6%), 297 (M^+-71, 31%), 253 (8%), 169 (1%), 155 (5%), 141 (6%), 127 (7%), 113 (8%, 99 (5%), 85 (45%. 71 (65%), 57 (100%).

Seven saturated and twelve unsaturated fatty acids were identified from the methanolic extract of *B. pennata*. Their relative retention time (R.R.T.) and relative percentages (rel. %age) are given in Table 2.

Discussion

Four sterols were isolated from the methanolic extract of *Bryopsis pennata.* One of them with cholesta skeleton was found as major constituent (78.12%), while three others with ergosta skeleton were present in traces (5-9%, Table 1). Ergosterol has been detected in *Chlorella candida and C. nocturna,* ergost-7-enol in C. *ellipsoidea* and *C. saccharophila* and ergost-5-enol in C. *ernersonii* and *C. fusca* (Patterson, 1971). *Chlorella ellipsoidea* also accumulates 5α-ergosta-8, 14-dien-3β-ol in the presence of a sterol inhibitor (Dickson *et al,* 1972). A variety of sterols with ergosta skeleton are found in green algae. Sterols with cholesta skeleton were detected in *Chaetomorpha crassa* (Ikekawa *et al,* 1968), *Oocystis polymorpha* (Orcutt and Richardson 1970), *Bryopsis coniculans. B. mucosa* and *B. plumosa* (Dickson *et al,* 1979; De Napoli *et al,* 1982), *Ulva penusa* and several other green algae (Goodwin, 1974) as major constituents. Green algae

usually exhibit great variation in their sterol composition as compared with other algal phyla.

Altogether 19 fatty acids were found, table 2 shows the details of various acids present in the methanolic extract of *B. pennata.* Seven saturated and twelve unsaturated acids could be detected, the former were found in greater quantity (72.58%) than the latter (27.41%). Similar observations were made on nine green seaweeds of Karachi (Shameel and Khan, 1991). Tricosanoic acid was found in the highest amount among all the acids (30.24%), which was even greater than the total quantity of unsaturated fatty acids (27.41%), while palmitic acid was present in a small amount (6.05%). This is contrary to the previous observations, palmitic acid was found as major component (77-88%) in twelve littoral green seaweeds of Karachi coast (Shameel, 1993) Behenic acid was detected in smallest quantity in *B. penna*ta (2.02%). It is a rare fatty acid, and out of eighteen (Qasim, 1986) and nine investigated species of Karachi seaweeds it was only found in small amount (1.43%) in *Ulva fasciata* (Shameel, 19%). Even an earlier study on *U. fasciata* did not reveal its presence (Usmanghani *et al.,* 1985). Tricosanoic acid has also been detected in *Codium flabellatum* and *C. iyengarii* but in minor quantities (2.07-4.75%, Aliya *et al.,* 1991, 1992).

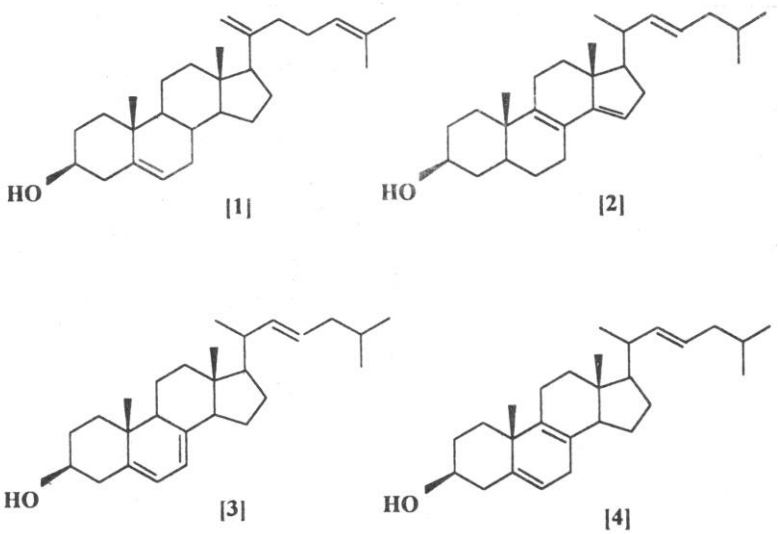

Fig. l. Sterols isolated from *Blyopsis pennata:* [1] = cholesta-5, 20, 24-trien-3β-ol, [2] = ergosta-8, 14, 22-trien-3β-ol, [3] = ergosta-5, 7, 22-trien-3β-ol, [4] = ergosta-5, 8, 22-lien-3β-ol.

The unsaturated fatty acids present in *B. pennata* included seven monoenoic, two dienoic, two trienoic ad one pentaenoic acids. Among all the investigated species of Karachi seaweeds (Qasim, 1986; Shameel, 1990), it is for the first time that a pentacnoic acid was detected. The highest degree of unsaturation known was of tetraenoic acids. Among unsaturated fatty acids heptadecatrienoic acid was found in highest proportion (4.84%), while oleic acid was altogether absent. It is also contrary to the previous results, oleic acid was observed to be the most commonly occurring unsaturated fatty acid (2.3-9,3%) of green seaweeds of Karachi (Shamed and Khan, 1991; Shamed, 1993). The fatty acid composition *of B. pennata* agrees with the general observation made by Pohl and Zurheide (1979), that marine macroscopic Chlorophyta primarily synthesize C_{16} and C_{18} fatty acids and C_{20} and C_{22} fatly acids are usually much less abundant.

Several unique and *rare* compounds were isolated and identified from *Bryopsis pennata*. All the four sterols isolated were not known from any of the investigated seaweeds of Karachi. Similarly cyclopentenyl undecanoic and heneicosapentaenoic acids were not previously detected in any of the seaweed species investigated from the coast of Karachi. The former is *a very* rare natural product.

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Table 1
Sterol composition of the methanolic extract of *Bryopsis pennata*

Systematic name	Mol. form.	Mol. wt.	Str. No.*	Rel. %age
Cholesta-5, 20, 24-trien-3β-ol	$C_{27}H_{42}O$	382	[1]	78.12
Ergosta-8, 14, 22-trien-3β-ol	$C_{28}H_{44}O$	396	[2]	9.26
Ergosta-5, 7, 22-trien-3β-ol	$C_{28}H_{44}O$	396	[3]	7.43
Ergosta, 5-8, 22-trien-3β-ol	$C_{28}H_{44}O$	396	[4]	5.17

*For structures see fig. no.1.

Table 2
Fatty acid composition of *Bryopsis pennata* analysed as methyl esters

Systematic name	Common name	Molecular formula	Mol. wt.	R.R.T.	Rel. %age
Saturated fatty acid methyl esters:					**72.58**
11-Δ2, 3-Cyclopentenyl-*n*-undecanoate	Hydnocarpate	$C_{16}H_{28}O_2$	252	0.78	12.10
n-Pentadecanoate	Pentadecylate	$C_{16}H_{32}O_2$	256	2.24	8.06
n-Hexadecanoate	Palmitate	$C_{17}H_{34}O_2$	270	1.00	6.05
n-Heptadecanoate	Margarate	$C_{18}H_{36}O_2$	284	0.50	4.03
n-Nonadecanoate	Nonadecylate	$C_{20}H_{40}O_2$	312	0.75	10.08
n-Docosanoate	Behenate	$C_{23}H_{46}O_2$	354	1.20	2.02
n-Tricosanoate	–	$C_{24}H_{48}O_2$	368	1.50	30.24
Unsaturated fatty acid methyl esters:					**27.41**
9, 10-Dodecenoate	Lauroleate	$C_{13}H_{24}O_2$	212	1.11	0.40
Tridecatrienoate	–	$C_{14}H_{22}O_2$	222	0.53	0.81
Tridecadienoate	–	$C_{14}H_{24}O_2$	224	1.51	3.22
Tridecenoate	Decylacrylate	$C_{14}H_{26}O_2$	226	1.28	2.02
9, 10-Tetradecenoate	Myristoleate	$C_{15}H_{28}O_2$	240	1.61	2.82
Heptadecatrienoate	–	$C_{18}H_{30}O_2$	278	0.98	4.84
Heptadecadienoate	–	$C_{18}H_{32}O_2$	280	1.20	4.03
Heptadecenoate	Heptadecylenate	$C_{18}H_{34}O_2$	282	0.89	0.81
Nonadecenoate	Nonadecylenate	$C_{20}H_{38}O_2$	310	1.24	0.40
Heneicosapentaenoate	–	$C_{22}H_{34}O_2$	330	1.39	2.02
Heneicosenoate	–	$C_{22}H_{42}O_2$	338	1.01	2.82
Tricosenoate	–	$C_{24}H_{46}O_2$	366	1.52	3.22

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