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FATTY ACID COMPOSITIONS OF TWO SIPHONACEOUS GREEN ALGAE FROM THE COAST OF KARACHI

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ABSTRACT

Two green seaweeds *Bryopsis pennata* Lamour. and *Valoniopsis pachynema* (Mart.) Bϕrg. collected from the coast of Karachi have been studied for their fatty acid compositions. Altogether 34 fatty acids were identified as methyl esters from them, out of which 9 were saturated and 25 unsaturated acids including 17 monoenoic, 5 dienoic and 4 trienoic acids. They were identified by gas chromatography-mass spectroscopy (GC-MS). Both seaweeds contained exactly the same saturated fatty acids, their unsaturated acids (67.58-71.81%) were greater in proportion than the saturated ones (28.17-32.38%). Tridecatrienoic (28.1%) and palmitic (13.93%) acids occurred in highest amount. Oleic acid was not uniformly present and occurred in small proportion (1.53%).

Introduction

Bryopsis pennata Lamouroux and *Valoniopsis pachyderm* (Martens) Bϕrgesen are filamentous, siphonaceous, green algae with no corsswall or septation present in the thalli and found attached to rocks forming small dense fluorescent bushes in sandy bottom pools in mid- to lower littoral rocks (Aliya and Shamed, 1996). The latter forms tufts on exposed rocks in the lower and sub- littoral zones, it also occurs as drift material in the form of spongy balls on the sandy shores.

Three species of *Bryopsis* and one of *Valoniopsis* have been reported from the coast of Karachi (Shameel and Tanaka, 1992) but none of them have ever been investigated phycochemically. These siphonaceous green algae provide nourishment to a variety of herbivorous fishes and crustaceans. The biological significance of naturally occurring fatty acids revealed that they are necessary for the animal metabolism and storage of energy. They influence the utilization and metabolism of carbohydrates, proteins, minerals and vitamins and serve as carrier for fat-soluble vitamins such as A, D, E and their precursors (Dewille, 1979). They influence immune response as linoleic acid has an effect on lymphatic system (Satiyavati et al., 1982), the humoral response could be improved by the high consumption of essential fatty acids (Wagner *et al.,* 1982). The present report describes the fatty acid composition of the two different orders of the class Bryopsidophyceae.

Materials and Methods

Algal material:

Specimens of *Bryopsis pennata* and *Valoniopsis pachynema* were collected from the mid-, lower and sub-littoral zones in epilithic as well as drift conditions. Collections were made from Manora, Buleji and Sandspit near Karachi during October 1989 to September 1991. Seaweeds were thoroughly washed with tap water to remove all epiphytes, animal castings, sand particles etc., dried in shade and cut into small pieces.

Extraction:

The dried seaweeds *(B. pennata* = 1.4 kg, *V. pachynema* = 1.5 kg) were soaked in methanol (MeOH) for about 1 month at room temperature. The MeOH extract was then evaporated under vacuum, which yielded a residue *(B. pennata* = 50 g, *V. pachynema* = 20 g).

Column chromatography:

The MeOH extracts of the algae were partitioned separately with ethylacetate (EtOAc) and water and this partitioning was done 3 times. The EtOAc portion was evaporated which yielded an extract *(B. pennata* = 4 g, *V. pachynema* = 19 g). This was then loaded on to silica gel columns (70-230 mesh) and fractions were eluted first with n-hexane and then polarity was increased by adding chloroform. Three different fractions of n-hexane:chloroform were analyzed for the two species each (95:5, 92:8 & 90:10 for *B. pennata;* 90:10, 80:20 & 75:25 for *V. pachynema).*

Esterification:

All six fractions were esterified with diazomethane, for this purpose 0.5 mg of each fraction was dissolved in MeOH and 0.5 mL of diazomethane was added. The reaction mixture was kept overnight at room temperature (28°C) and then evaporated under reduced pressure. The methylated fatty acid fractions were analyzed first by gas liquid chromatography (GLC) and finally gas chromatography-mass spectrometry (GC-MS). The techniques for GLC and GC-MS were the same as described previously (Aliya *et al.,* 1992).

Results and Discussion

A total of 34 different fatty add methyl esters *were* detected from green algae *Bryopsis* penman and *Valoniopsis pachynema.* The significant ions in the^{i}r mass spectra are as follows:

9-Methyl decenoate: GC-MS: *m/z* 184 (M^+, $C_{11}H_{20}O_2$, 10%), 127 (M^+ - 57, 5%), 113 (8%), 99 (9%), 85 (41%), 71(62%), 57 (100%).

Undecadienoate: *m/z* 196 (M^+, $C_{12}H_{20}O_2$, 10%), 139 (M^+- 57, 5%), 125 (8%), 111 (15%), 97 (16%), 83 (22%), 69 (31%), 55 (21 %).

10-Undecenoate: 198 (M^+, $C_{12}H_{12}O_2$, 15%), 155 (M^+- 32, 5%), 141 (M^+-57, 7%), 127 (9%), 113 (9%), 99 (12%), 85 (46%), 71 (68 %), 57 (100%).

9-Dodecenoate: 212 (M^+, $C_{13}H_{22}O_2$, 15%), 155 (M+-57, 12%), 141 (14%), 127 (5%), 113 (7%, 99 (27%), 85 (100 %).

Tridecatrienoate: 222 (M^+, $C_{14}H_{22}O_2$, 25%), 165 (M^+-57, 7%), 151 (10%), 137 (13%), 123 (20%), 109 (26%), 95 (27%), 81 (42 %).

Tridecadienoate: 224 (M^+, $C_{14}H_{22}O_2$, 6%), 167 (M^+- 57, 3%), 153 (5%), 139 (4%), 125 (9%), 97 (22%), 83 (20%), 73 (3%).

9-Tridecenoate: 226 (M^+-$C_{14}H_{26}O_2$, 35%), 169 (M^+- 57, 9%), 155 (10%), 141 (10%), 127 (11%), 113 (12%), 99 (17%), 85 (58 %), 71(79%), 57 (100%).

7-Ethyl-3-methyl-2, 6-undecadienoate: 238 (M^+, $C_{15}H_{26}O_2$, 76%), 181(M^+-57, 4%), 167 (5%), 153 (8%), 139 (9%), 125 (28%), 111 (54%), 97 (87%), 83 (100%).

9-Tetradecenoate: 240 (M^+, $C_{15}H_{28}O_2$, 23%), 183 (M^+- 57, 5%), 169 (6%), 155 (7%, 141(7%), 127 (9%), 113 (11%, 99 (16%), 85 (53%), 71 (73%), 57 (100%).

***n*-Tetradecanoate**: 242 (M^+, $C_{15}H_{30}O_2$, 20%), 199 (M^+- 43, 15%), 185 (M^+-57, 3%), 171 (2%), 157 (4%), 143 (18%), 129 (7%), 115 (2%), 101 (7%), 83 (5%), 74 (100%).

3, 7, 11-Trimethyi-2, 6, 10-dodecatrienoate: 250 (M^+, $C_{16}H_{26}O_2$, 10%), 193 (M^+-57, 2%), 179 (3%), 167 (12%, 165 (5%), 151 (2%), 137 (5%), 123 (10%), 109 (20%), 95 (18%), 81 (17%), 58 (100%).

Pentadecenoate: 254 (M^+, $C_{16}H_{30}O_2$, 30%), 197 (M^+-57, 8%), 183 (8%), 169 (9%), 155 (9%), 141 (10%), 113 (12%), 99 (16%), 85 (55%), 71 (74%), 57 (100%).

***n*-Pentadecanoate**: 256 (M^+, $C_{16}H_{32}O_2$, 46%), 213 (M^+- 43, 16%), 199 (M^+-57, 6%), 185 (5%), 171 (6%), 157 (7%), 143 (20%), 129 (8%), 115 (2%), 101 (7%), 87 (60%), 74 (100%).

Hexadecadienoate: 266 (M^+, $C_{17}H_{30}O_2$, 32%), 209 (M^+- 57, 2%), 195 (2%), 181 (3%), 167 (4%), 153 (6%), 139 (6%), 125 (18%), 111 (35%), 97 (66%), 83 (90%), 69 (100%).

9-Hexadecenoate: 268 (M^+, $C_{17}H_{32}O_2$, 42%), 211 (M^+- 57, 12%), 197 (2%), 183 (12%), 155 (10%), 141 (15%), 127 (13%), 113 (16%), 99 (17%), 85 (57%), 71 (83%, 57 (100%).

***n*-Hexadecanoate**: 270 (M^+, CHMO2, 58%), 239 (M^+- 31, 17%), 227 (M^+-43, 23%), 213 (M^+-57, 3%), 199 (10%), 185 (10%), 171 (9%), 157 (3%), 143 (23%), 129 (10%), 115 (6%), 101 (8%), 87 (64%), 74 (100%).

Heptadecatrienoate: 278 (M^+, $C_{18}H_{30}O_2$, 5%), 221 (M^+ - 57, 3%), 207 (5%), 193 (5%), 179 (8%), 165 (6%), 151 (8%), 137 (15%), 123 (50%), 109 (28%), 95 (75%, 81 (43%), 67 (30%).

Heptadecenoate: 282 (M^+, $C_{18}H_{34}O_2$, 6%), 225 (M^+- 57, 4%), 211 (4%), 197 (4%), 183 (5%), 169 (5%), 155 (7%), 141 (6%), 127 (7%), 113 (11%), 99 (13%), 85 (51%), 71 (86%), 57 (100%).

***n*-Heptadecanoate**: 284 (M^+, $C_{18}H_{36}O_2$, 52%), 241 (M'- 43, 18%), 227 (M^+-57, 6%), 199 (7%), 185 (8%,171 (4%), 157 (20%), 143 (10%), 129 (4%), 115 (6%), 101 (55%), 88 (100%).

Octadecadienoate: 294 (M^+, $C_{19}H_{34}O_2$, 5%), 237 (M^+- 57, 6%), 223 (3%), 209 (2%), 195 (6%), 181 (4%), 167 (7%), 153 (7%), 139 (8%), 125 (17%), 111 (27%), 97 (42%), 83 (45%), 71(61%), 57 (100%).

9-Octadecenoate: 296 (M^+, $C_{19}H_{36}O_2$, 25%), 239 (M^+ - 57, 32%), 222 (M^+ -74, 45%), 180 (25%%), 110 (22%), 96 (35%), 82 (35%), 71 (20%).

***n*-Octadecanoate**: 298 (M^+, $C_{19}H_{38}O_2$, 5%), 225 (M^+- 43, 3%), 241 (M^+-57, 63%), 227 (2%), 199 (10%), 185 (4%), 157 (3%), 143 (22%), 129 (8%), 101 (7%), 88 (62%), 74 (100%).

Nonadecenoate: 310 (M^+, $C20H_{38}O_2$, 35%), 253 (M^+- 57, 7%), 239 (8%), 225 (7%), 211 (6%), 197 (8%, 183 (8%), 169 (9%), 155 (8%), 141 (7%), 127 (12%), 113 (15%), 99 (18%), 85 (58%), 71 (74%), 57 (100%).

9-Eicosenoate: 324 (M^+, $C_{21}H_{44}O_4$, 35%), 747 (M^+-57, 8%), 253 (8%), 239 (8%), 225 (9%), 211 (8%), 197 (8%), 183 (10%), 169 (6%), 155 (11%), 141 (12%), 127 (14%), 113 (15%), 99 (20%), 85 (58%), 171 (78%), 57 (100%).

***n*-Eicosaaoate**: 326 (M^+, $C_{21}H_{42}O_2$, 90%), 283 (M^+- 43, 22%), 269 (M^+-57, 3%), 255 (2%), 241 (5%), 227 (7%), 213 (2%), 185 (6%), 157 (2%), 143 (18%), 129 (8%), 101 (6%), 87 (42%), 73 (100%).

Henetcosenoate: 338 (M^+, $C_{22}H_{42}O_2$, 25%), 281 (M^+-57, 6%), 267 (8%), 253 (8%), 239 (7%), 225 (9%), 211 (8%), 197 (8%), 183 (7%), 169 (9%), 155 (10%), 141 (11%), 127 (12%), 111 (16%), 97 (22%), 85 (58%), 71 (78%), 57 (100%).

11-Docosenoate: 352 (M^+, $C_{23}H_{44}O_2$, 22%), 295 (M^+- 57, 5%), 281 (6%), 267 (6%), 253 (8%), 239 (9%), 225 (8%), 211 (8%), 197 (7%), 183 (8%), 169 (8%), 155 (8%), 141 (10%), 127 (12%), 113 (13%), 99 (20%), 85 (55%), 71 (76%%), 57 (100%).

***n*-Docosanoate**: 354 (M^+, $C_{23}H_{46}O_2$, 90%), 311 (M^+-43, 20%), 297 (M^+-57, 2%), 283 (2%), 269 (5%), *255 (8%),* 227 (2%), 199 (8%), 185 (5%), 171 (3%), 157 (2%), 143 (18%), 129 (6%), 115 (2%), 101 (4%), 87 (35%), 73 (48%).

Tricosenoate: 366 (M^+, $C_{24}H_{46}O_2$, 20%), 309 (M^+-57, 6%), 295 (7%), 281 (7%), 267 (8%), 253 (8%), 239 (9%), 225 (9%), 211 (9%), 197 (10%), 183 (8%), 169 (9%), 155 (10%), 141 (10%), 127 (10%), 113 (12%), 99 (15%), 85 (53%), 71 (73%), 57 (100%).

***n*-Tetracosanoate**: 382 (M^+, $C_{25}H_{50}O_2$, 8%), 325 (M^+- 57, 5%), 208 (M^+-74, 4%), 194 (22%), 180 (5%), 166 (7%), 152 (21%), 138 (12%), 124 (21%), 110 (22%), 96 (42%), 82 (55%), 68 (90%), 54 (100%).

Pentacosenoate: 394 (M^+, $C_{26}H_{50}O_2$, 14%), 337 (M^+ - 57, 5%), 323 (5%), 309 (6%), 295 (6%), 281 (7%), 267 (6%), 253 (6%), 239 (6%), 225 (7%), 211 (8%), 197 (7%), 183 (8%), 169 (8%), 155 (9%), 141(10%), 127 (11%), 113 (13%), 99 (20%), 85 (75%), 71 (76%), 57 (100%).

***n*-Hexacosanoate**: 410 (M^+, $C_{27}H_{54}O_2$, 90%), 367 (M^+ - 43, 15%), 353 (M^+-57, 25%), 255 (3%), 199 (6%), 185 (3%), 143 (15%), 129 (6%), 101 (3%), 87 (30%), 73 (4%).

Nonacosatrienoate: 446 (M^+, $C_{30}H_{54}O_2$, 5%) 389 (M^+, 57, 6%), 372 (M^+-74, 7%), 303 (3%), 275 (2%), 247 (2%), 219 (8%), 207 (5%), 191 (3%), 163 (4%), 149 (5%), 135 (10%), 107 (3%), 90 (3%).

Nonacosenoate: 450 (M^+, $C_{30}H_{58}O_2$, 12%), 393 (M^+- 57, 20%), 379 (5%), 365 (10%), 351 (3%), 337 (5%), 323 (4%), 309 (4%), 295 (6%), 281 (6%), 267 (7%), 253 (5%), 239 (7%), 225 (8%), 211 (8%), 199 (8%), 183 (8%), 169 (5%), 155 (10%), 141 (10%), 127 (11%), 113 (16%), 99 (19%), 85 (54%), 71(73%), 57 (100%).

Valoniopsis pachynema appeared to be much more diverse in its fatty acid composition as compared to the other alga. It exhibited 30 different fatty acids, out of which nine were found to be saturated and 21 unsaturated including 14 monoenoic, four dienoic and three trienoic acids (Table 1). The analysis of *B. pennata* revealed only 20 different fatty acids, out of which nine were saturated and 11 unsaturated including eight monoenoic, two dienoic and one trienoic. It is interesting to note that in both the plants nine saturated fatty acids were identified and all nine were exactly the same acids, although both seaweeds belong to different orders of Chlorophyta. In both algae the number and quantity of unsaturated fatty acids (67.58-71.81%) is much more than those of saturated ones (28.17-32.38%), which is contrary to some previous reports on green seaweeds of Karachi (Qasim, 1986; Shameel, 1990, 1993). But other reports on fatty acids of green algae like *Caulerpa* and *Codium* (Aliya et al, 1992, 1993) have revealed similar pattern where unsaturated fatty acids were found in larger amount than saturated ones. It appears that the biosynthesis of unsaturated fatty acids in these seaweeds is much more active than those of saturated ones.

Palmitic acid occurred in *V. pachydema* in highest amount (13.93%) which is the usual case in green seaweeds (Shameel and Khan, 1991), and it was also detected in highest quantity in *Valonia utricularis* (37.3%), which belongs to the same family (Pohl et at, 1968; Pohl and Zurheide, 1979). In *B. pennata* tridecatrienoic acid was present in highest amount (28.1%), this is contrary to the previous reports on this genus which showed highest percentage of palmitate and oleate (Dembitsky et al., 1991). In *B. hypnoides* from the shores of Black Sea near Sospol in Bulgaria 18:3 (n-3) was the most abundant (34.9%) fatty acid (Stefanov et al., 1988). An appreciable proportion (10.1%) of 16:4 (n-3) was detected in *B. plumosa* from northeast of the Black Sea at the Bays of Feodosia and Karadagh (Dembitsky et al., 1991) but tetraenoic and higher polycnoic fatty acids could not be detected in *V. pachynema* or *B. pennata*.

Comparison of the two siphonaceous green seaweeds shows certain similarities like having same number and same range of saturated fatty acids while some very prominent differences are also seen which may be due to different environmental and ecological factors in which these seaweeds grow. Oleic acid was not uniformly present in them and occurred in small amount (1.53%).

Table 1
Fatty acids analysed as methyl esters from *Bryopsis pennata* and *Valoniopsis pachynema*

S.No.	Systematic name	Common name	Molecular formula	Mol. wt.	Relative percentage *Bryopsis pennata*	*Valoniopsis pachynema*
Saturated						
1.	*n*-Tetradecanoate	Myristate	$C_{15}H_{30}O_2$	242	2.95	2.11
2.	*n*-Pentadecanoate	Pentadecylate	$C_{16}H_{32}O_2$	256	1.87	2.39
3.	*n*-Hexadecanoate	Palmitate	$C_{17}H_{34}O_2$	270	13.93	11.23
4.	*n*-Heptadecanoate	Margarate	$C_{18}H_{36}O_2$	284	1.50	2.67
5.	*n*-Octadecanoate	Stearate	$C_{19}H_{38}O_2$	298	4.13	6.35
6.	*n*-Eicosanoate	Arachidate	$C_{21}H_{42}O_2$	326	1.31	1.26
7.	*n*-Docosanoate	Behenate	$C_{23}H_{46}O_2$	354	0.24	1.30
8.	*n*-Tetracosanoate	Lignocerate	$C_{25}H_{50}O_2$	382	1.13	4.23
9.	*n*-Hexacosanoate	Cerotate	$C_{27}H_{54}O_2$	410	1.11	0.84
Unsaturated						
10.	9-Decenoate	--	$C_{11}H_{20}O_2$	184	2.30	--
11.	Undecadienoate	--	$C_{12}H_{20}O_2$	196	1.23	--
12.	10-Undecenoate	Undecylenate	$C_{12}H_{22}O_2$	198	2.85	--
13.	9-Dodecenoate	Lauroleate	$C_{13}H_{24}O_2$	212	1.39	--
14.	Tridecatrienoate	--	$C_{14}H_{22}O_2$	222	--	28.10
15.	Tridecadienoate	--	$C_{14}H_{24}O_2$	224	6.24	--
16.	9-Tridecenoate	Decylacrylate	$C_{14}H_{26}O_2$	226	1.92	--
17.	7-Ethyl-3-methyl-2-, 6-undecadienoate	--	$C_{15}H_{26}O_2$	238	1.64	11.82
18.	9-Tetradecenoate	Myristoleate	$C_{15}H_{28}O_2$	240	3.95	9.24
19.	3,7,11-Trimethyl-2,6, 10-dodecatrienoate	--	$C_{16}H_{26}O_2$	250	1.88	--
20.	Pentadecenoate	--	$C_{16}H_{30}O_2$	254	2.71	2.40
21.	Hexadecadienoate	--	$C_{17}H_{30}O_2$	266	--	3.78
22.	9-Hexadecenoate	Palmitoleate	$C_{17}H_{32}O_2$	268	9.78	5.08
23.	Heptadecatrienoate	--	$C_{18}H_{30}O_2$	278	1.48	--
24.	Heptadecenoate	Heptadecylenate	$C_{18}H_{34}O_2$	282	--	1.38
25.	Octadecadienoate	Linoleate	$C_{19}H_{34}O_2$	294	6.43	--
26.	9-Octadecenoate	Oleate	$C_{19}H_{36}O_2$	296	--	1.53
27.	Nonadecenoate	Nonadecylenate	$C_{20}H_{38}O_2$	310	2.29	--

Table continued . . .

S.No.	Systematic name	Common name	Molecular formula	Mol. wt.	Relative percentage *Bryopsis pennata*	*Valoniopsis pachynema*
28.	9-Eicosenoate	Gadoleate	$C_{21}H_{40}O_2$	324	4.55	--
29.	Heneicosenoate	--	$C_{22}H_{42}O_2$	338	7.15	1.31
30.	11-Docosenoate	Cetoleate	$C_{23}H_{44}O_2$	352	4.03	1.29
31.	Tricosenoate	--	$C_{24}H_{46}O_2$	366	3.60	--
32.	Pentacosenoate	--	$C_{26}H_{50}O_2$	394	3.76	1.65
33.	Nonacosatrienoate	--	$C_{30}H_{54}O_2$	446	1.51	--
34.	Nonacosenoate	--	$C_{30}H_{58}O_2$	450	1.12	--

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