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ANALYSIS OF FATTY ACIDS FROM *CODIUM IYENGARII (BRYOPSIDOPHYCEAE)*

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ABSTRACT

The green seaweed *Codium iyengorii* Bϕrg., collected from the coast of Karachi, has been studied for its fatty acid composition. The acids were converted into methyl esters and identified by GC-MS, which reveled the presence of 11 saturated, 10 monarthenoic and 2 ethinoic acids. Palmitic acid wrs present in the highest amount, while dodccenoic acid was in largest quantity among unsaturated fatty acids. Malone, hepranoic, tridecynoic, hexadecymoic, trkoscnoic, trieosanoic and heptacosznoic acids am being repotted for the first time from any species of *Codium* as well as any seaweed of Pakistan.

Introduction

Codium iyengani Bϕrgesen is a spongy, cylindrical, much elongated and regularly dichotomous, dark green alga. It grows attached firmly to the lower littoral rocks at Karachi and the adjacent coastal areas. This alga was initially described from Karachi as *Codium elongatum* (Turner) C. Agardh (Anand, 1940; Salim, 1965) and later on reported as *C. ivengani* from Lasbela coast (Shamed, 1987). It is found in abundance along the coast of Karachi during October and March. Recently a few investigations have been conducted on its total fat, glycerides, phospholipids and sterols ((Hsim, 1986), protein, carbohydrate and lipid contents (Qari, 1988), preliminary screening of fatty acids (Shamed, 1990), amino-acid composition (Qasim, 1991) and steroidal saponins (Ahmad et *at.,* 1991). *Codium fragile* (Sur.) Harlot is of particular interest to phycochemists and its sterols (Rubinstein and Goad, 1974), fatty acid composition (Khotimchonko and Svetoshev, 1983) and chemical constituents (Kim and Yong, 1989) have been studied. Other species of *Codium* have also been investigated phycochemically (Fattorusso et al, 1980; De Napoli *et al,* 1982; Riguera *et al.,* 1985). No detailed phycochemical study has so far been carried out on *C. iyengani.* In this paper an attempt has been made to investigate its fatty acid composition in detail.

Materials and Methods

Algal material

Specimens of *Codium iyengarii* were collected both as drift material as well as epilithon from the littoral rocks of Manora and Buleji, near Karachi during October 1988 and March 1990. Seaweeds were thoroughly washed with tap water to remove sand particles, epiphytes and animal castings and later on dried in shade and chopped into small pieces.

Extraction

The dried seaweeds (1.3 kg) were soaked in EtOH for about 1 month at room temperature. The ethanolic extract so obtained was evaporated under vacuum, which yielded about 53 g of thick and dark green residue, out of which 20g of the extract was used for saponification and 33g for column chromatography.

Saponification

A 150 ml quantity of EtOH:H_2O (1:1, v/v) containing 10% KOH was added to 20 g of extract and the mixture was saponified by refluxing at 100°C for 12 hours. The mixture was then concentrated under reduced pressure and thereafter water and Et_2O were added, shaken vigorously and the Et_2O layer was separated. The Et_2O layer was evaporated and the residue used as fraction A.

Column chromatography

The ethanolic extract of *C iyengarii* (33 g) was partitioned with hexane and water, the partitioning with hexane was repeated 3 times. The hexane extract was evaporated under pressure, which yielded 8 g of thick greenish residue. This residue was loaded on to a silica gel column (70-230 mesh) and fractions were eluted fast with hexane and then polarity was increased by adding the quantity of ether. The fraction B was eluted from hexane:ether (80:20, v/v), fraction C from hexane:ether (75:25, v/v) and fraction D from hexane:ether (70:30, v/v), all fractions were thick and oily.

Esterification

All the 4 lipid fractions (A-D) were esterifled with diazomethane, 0.5 mg of each fraction was dissolved in MeOH and 03 ml of diazomethane was added. The reaction mixture was kept overnight at room temperature (28°C) and was then evaporated under reduced pressure. The methylated fatty acid fractions were analysed first by GLC and finally by GC-MS.

Gas liquid chromatography (GLC)

The unknown fatty add fractions were analysed by GLC along with methyl ester standards on a Shimadzu GC-9A model gas chromatogrph, equipped with a Shimadzu C-R6A Chromatopac integrator. The column length was 2 m, whereas inner diameter of the column was 3 mm and outer diameter 5 mm. The column material used was GP 3% SP-2310/2% SP-2300 on 100/120 Chromosorb WAW. The column initial temperature was 150 °C, while the final temperature was 250°C with a rate of increase of 8°C/min. The detector as well as injector temperature was 300°C, nitrogen flow rate was 30 ml/min.

Gas chromatography - mass spectrometry (GC-MS)

The GC-MS of fatty acid methyl esters was performed on a Hewlett Packard GC with a 11173 DEC computer system and a 1.2 m x 4 mm packed glass capillary column, coated with gas chrome Q (100-120 mesh, OV 101, 1%). The column temperature was programmed from 170°C to 250°C with a rate of increase of 8°C/min. The injector temperature was 250°C, the carrier gas (He) flow rate was 32 ml/min.

Results and Discussion

The analysis of the fatty acid methyl esters of *Codium iyengarii* through GC-MS revealed the presence of 23 different fatty acids. The significant ions in their mass spectra are as follows:

Dimethyl-propanedioate: GC-MS, m/z 132 (M^+, $C_5H_8O_4$, 10%), 89 (M^+ -43, 90%), 73 (100%).

Methyl-n-heptanoate: GC-MS, m/z 144 (M^+, $C_8H_{16}O_2$, 3%), 113 (M^+-31, 6%), 101 (M^+ -43, 8%), 87 (78%), 73 (100%).

Methyl-10-undecenoate: GC-MS, m/z 198 (M^+, $C_{12}H_{22}O_2$, 35%), 166 (M^+ -32, 2%), 141 (M^+ -57, 17%), 127 (16%), 113 (15%), 99 (20%), 85 (80%), 71(100%).

Methyl-9-dodecenoate: GC-MS, m/z 212 (M^+, $C_{13}H_{24}O_2$, 85%), 155 (M^+ -57, 40%), 141 (42%), 127 (50%), 113 (62%), 99 (85%), 85 (90%), 71 (100%).

Methyl-12-tridecynoate: GC-MS, m/z 224 (M^+, $C_{14}H_{24}O_2$, 4%), 167 (M^+ -57, 7%), 153 (6%), 139 (7%), 125 (48%), 111 (17%), 97 (24%), 83 (46%), 73 (100%).

Methyl-tridecenoate: GC-MS, m/z 226 (M^+, $C_{14}H_{26}O_2$, 5%), 94 (M^+ -32, 10%), 169 (M^+ -57, 12%), 155 (7%), 141 (20%), 127 (12%), 113 (22%), 99 (23%), 85 (70%), 71 (100%).

Methyl-9-tetradecenoate: GC-MS, m/z 240 (M+, $C_{15}H_{28}O_2$, 18%), 183 (M^+ -57, 10%), 169 (11%), 155 (10%), 141 (9%), 127 (10%), 113 (12%), 99 (18%), 85 (73%), 71 (109%).

Methyl-n-tetradecanoate: GC-MS, m/z 242 (M^+, $C_{15}H_{30}O$ 5%), 199 (M^+ -43, 10%), 185 (20%), 171 (5%). 157 (25%), 143 (20%), 129 (10%), 115 (13%), 101 (85%), 83 (70%), 73 (100%).

Methyl-pentadecenoate: GC-MS, m/z 254 (M^+, $C_{16}H_{30}O_2$, 7%) 222 (M^+ -32, 2%), 180 (M^+ -74, 5%), 166 (5%), 152 (17%), 138 (7%), 124 (36%), 110 (20%), 96 (8%), 82 (42%), 73 (100%).

Methyl-9-hexadecenoate: GC-MS, m/z 268 (M^+, $C_{17}H_{32}O_2$, 20%), 236 (M^+ -32, 3%), 211 (M^+ -57, 10%), 197 (7%), 183 (4%), 169 (2%), 155 (6%), 141 (10%), 127 (12%), 113 (40%), 99 (17%), 85 (80%), 71 (100%).

Methyl-n-hexadecanoate: GC-MS, m/z 270 (M^+, $C_{17}H_{32}O_2$, 60%), 239 (M^+ 31, 22%), 227 (M^+ -43, 45%), 213 (70%), 199 (25%), 185 (20%), 171 (25%), 157 (84%), 143 (70%), 129 (30%), 115 (38%), 101 (85%), 83 (80%), 73 (100%).

Methyl-heptadecenoate: GC-MS, m/z 282 (M^+, $C_{18}H_{34}O_2$, 2%), 250 (M^+ -32,7%), 208 (M^+ -74, 2%), 194 (8%), 180 (2%), 165 (6%), 151 (4%), 137 (7%), 123(13%), 109 (20%), 95 (30%), 81 (33%), 73 (100%).

Methyl-n-heptadecanoate: GC-MS, m/z 284 (M^+, $C_{18}H_{36}O_2$, *5%)*, 241 (M^+ -43%, 25%), 227 (65%), 213 (10%), 199 (20%), 185 (22%), 171 (27%), 157 (10%), 143 (70%), 129 (30%), 115 (15%), 101 (30%), 87 (90%), 73 (100%).

2-Methoethyl-4-hexadecynoate: GC-MS, m/z 296 (M^+, $C_{l8}H_{32}O_3$, 95%), 222 (M^+ -74, 12%), 208 (10%), 194 (18%), 180 (20%), 165 (17%), 151 (22%), 137 (56%), 126 (85%), 115 (70%), 101 (75%), 87 (82%), 73 (100%).

Methyl-9-octadecenoate: GC-MS, m/z 296 (M^+, $C_{18}H_{32}O_2$, 25%), 264 (M^+ -32, 85%), 222 (M^+ -74, 42%), 208 (9%), 194 (6%), 180 (30%), 166 (15%), 152 (16%), 137(20%), 125 (22%), 111 (38%), 97 (68%), 83 (85%), 71 (100%).

Methyl-n-octadecanoate: 1GC-MS, m/z 298 (M^+, $C_{19}H_{38}O_2$, 60%), 267 (M^+ -31, 15%) 255 (M^+ -43, 30%), 241 (10%), 227 (7%), 213 (10%), 199 (20%), 185 (15%), 171 (17%)

157 (22%) 143 (60%), 129 (40%), 115 (35%), 101 (85%), 87 (80%), 73 (100%).

Methyl-nonadecenoate: GC-MS, m/z 310 (M^+, $C_{20}H_{38}O_2$, 90%), 236 (M^+ -74, 38%), 222 (85%), 208 (22%), 194 (35%), 180 (80%), 166 (70%), 152 (90%), 137 (85%), 123 (80%), 109 (85%), 95 (80%), 81 (85%), 71 (100%).

Methyl-n-eicoeanoate: GC-MS, m/z 326 (M^+, $C_{21}H_{42}O_2$, 6%), 283 (M^+ -43, 3%), 241 (7%), 227 (10%), 213 (15%), 199 (18%), 185 (20%), 171 (25%), 157 (27%), 143 (50%), 129 (45%), 115 (45%), 101 (80%), 87 (35%), 73 (100%).

Methyl-n-henelcosanoate: GC-MS, m/z 340 (M^+, $C_{22}H_{44}O_2$, 12%), 309 (M^+ -31, 2%), 297 (M^+-43, 3%), 255 (4%), 241 (5%), 227 (6%), 213 (5%), 199 (7%), 185 (6%), 171 (6%), 157 (12%), 143 (13%), 129 (11%), 115 (12%), 101 (42%), 87 (11%), 73 (100%).

Methyl-n-docosanoate: GC-MS, m/z 354 (M^+ $C_{23}H_{46}O_2$, 35%), 323 (M^+ -31, 5%), 311 (M^+ -43, 6%), 297 (3%), 255 (4%), 241 (2%), 227 (2%), 213 (3%), 199 (7%), 185 (2%), 171 (7%), 157 (11%), 143 (7%), 129 (25%), 115 (14%), 101(27%), 87 (40%), 73 (100%).

Methyl-tricoseaoate: GC-MS, m/z 366 (M^+, $C_{24}H_{46}O_2$, 5%), 334 (M^+ -32, 3%), 292 (M^+ -74, 6%), 278 (2%), 264 (3%), 250 (3%), 236 (4%), 222 (5%), 208 (62%), 194 (8%), 180 (44%), 166 (6%), 152 (25%), 138 (5%), 124 (5%), 110 (15%), 96 (20%), 82 (12%), 73 (100%).

Methyl-n-tricosanoate: GC-MS, m/z 368 (M^+, $C_{24}H_{48}O_2$, 10%), 294 (M^+ -74, 5%), 280 (10%), 265 (7%), 251 (5%), 237 (5%), 223 (4%), 209 (5%), 195 (8%), 181 (10%), 167 (10%), 153 (10%039 (15%025 (35%011 (54%), 97 (90%), 83 (90%), 73 (100%).

Methyl-n-beptacosanoate: GC-MS, m/z 424 (M^+, $C_{28}H_{56}O_2$, 2%), 393 (M^+ -31, 7%), 381 (M^+ -43, 5%), 367 (3%), 339 (40%), 311 (25%), 297 (25%), 283 (35%), 269 (60%), 255 (23%), 241 (25%), 227 (50%), 213 (72%), 199 (60%), 185 (40%), 171 (42%), 157 (80%), 143 (85%), 129 (77%), 115 (80%), 101 (85%), 87 (90%), 73 (100%).

Around 11 saturated, 10 monoethenoic and 2 ethinoic tatty acids were identified. The relative retention time (RRT) and relative percentage of occurrence of their methyl esters are given in Table 1. The identity of fatty acids was confirmed by matching of the mass spectra of the unknown compounds with those of the NBS mass spectra library (Defies and Milne, 1978). The fractions A, C and D showed a relatively high level of saturated fatty adds as has commonly been observed in seaweeds, but fraction B contained a high level of unsaturated acids. This is quite astonishing, as no data has uptil now been reported from any seaweed species showing a high level of unsaturated fatty acids (Qasim, 1986; Lie Ken Jie, 1989).

Codium iyengarii revealed the presence of 11 different saturated fatty acids and provided a series of interesting results. Palmitic acid was present in largest quantity among all the fatty acids detected, saturated as well as unsaturated. It is quite usual, as it occurs in largest amount in various green (Shamed and Khan, 1991), brown (Shamed *et al*., 1991) and red seaweeds (Qasim, 1986). The detection of malonic and heptanoic acids is very interesting. It is the first report of occurrence of fatty acids with low molecular weight from a Karachi seaweed. Malone acid is also the first known dicarboxylic acid from marine algae of this region. Similarly the detection of tricosanoic and heptacosanoic acids is the first report of occurrence of fatty acids with high molecular weight. Uptil now no fatty acid lower than C8 and higher than C_{22} was known in any of the seaweed species from Karachi (Shamed, 1990). This increases the range of saturated fatty acids found in Karachi seaweeds. It is interesting that octanoic acid, which was detected by Qasim (1986) in this species, was not found in our specimens.

Table 1

Fatty acids of *Codium iyengarii* analysed as methyl esters

Systematic name	Common name	Molecular formula	Mol. wt.	RRT*	Relative percentage
Fraction A					
Unidentified	---	---	---	0.35	2.89
Dimethyl-propanedioate	Dimethyl malonate	$C_5H_8O_4$	132	0.96	3.13
Methyl-*n*-heptanoate	Methyl heptate	$C_8H_{16}O_2$	144	1.24	5.06
Unidentified	---	---	---	1.44	3.85
Methyl-9-dodecenoate	Methyl lauroleate	$C_{13}H_{24}O_2$	212	2.05	3.37
Methyl-12-tridecynoate	---	$C_{14}H_{24}O_2$	224	2.17	1.68
Methyl-9-tetradecenoate	Methyl myris-toleate	$C_{15}H_{28}O_2$	240	2.35	6.02
Methyl-pentadecenoate	---	$C_{16}H_{30}O_2$	254	2.53	1.68
Methyl-9-hexadecenoate	Methyl palmi-toleate	$C_{17}H_{32}O_2$	268	2.80	5.30
Methyl-*n*-hexadecanoate	Methyl palmitate	$C_{17}H_{34}O_2$	270	2.96	13.01
Methyl-*n*-heptadecanoate	Methyl margarate	$C_{18}H_{36}O_2$	284	3.08	19.75
12-Methoxy-methyl-4-hexadecynoate	---	$C_{18}H_{32}O_3$	296	3.30	24.00

(Table 1 Continued)

Systematic name	Common name	Molecular formula	Mol. wt.	RRT*	Relative percentage
Methyl-*n*-docosanoate	Methyl behenate	$C_{23}H_{46}O_2$	354	4.15	5.06
Methyl-*n*-tricosanoate	---	$C_{24}H_{48}O_2$	368	4.19	5.06
Fraction B					
Methyl-10-undecenoate	---	$C_{12}H_{22}O_2$	198	1.00	12.54
Unidentified	---	---	---	1.32	6.08
Methyl-9-dodecenoate	Methyl lauroleate	$C_{13}H_{24}O_2$	212	1.54	31.93
Methyl-tridecenoate	---	$C_{14}H_{26}O_2$	226	2.09	22.43
Methyl-9-tetradecenoate	Methyl myris-toleate	$C_{15}H_{28}O_2$	240	2.77	8.77
Unidentified	---	---	---	3.02	7.98
Methyl-tricosenoate	---	$C_{24}H_{46}O_2$	366	3.86	10.26
Fraction C					
Methyl-*n*-tetradecanoate	Methyl myristate	$C_{15}H_{30}O_2$	242	0.98	1.45
Methyl-*n*-hexadecanoate	Methyl palmitate	$C_{17}H_{34}O_2$	270	1.17	3.69
Methyl-heptadecenoate	---	$C_{18}H_{34}O_2$	282	1.29	2.37
Unidentified	---	---	---	1.34	6.46
Methyl-*n*-heptadeca-noate	Methyl margarate	$C_{18}H_{36}O_2$	284	1.38	10.68
Methyl-*n*-octadecanoate	Methyl stearate	$C_{19}H_{38}O_2$	298	1.48	9.23
Melthyl-*n*-nonadecenoate	---	$C_{20}H_{38}O_2$	310	1.54	13.19
Methyl-*n*-eicosanoate	Methyl arachidate	$C_{21}H_{42}O_2$	326	1.58	11.47
Unidentified	---	---	---	1.63	11.87
Methyl-*n*-heneicosanoate	---	$C_{22}H_{44}O_2$	340	1.69	2.90
Methyl-*n*-docosanoate	Methyl behenate	$C_{23}H_{46}O_2$	354	1.77	9.76
Methyl-*n*-heptacosanoate	---	$C_{28}H_{56}O_2$	424	1.82	10.42
Unidentified	---	---	---	1.89	6.46
Fraction D					
Methyl-*n*-tetradecanoate	Methyl myristate	$C_{15}H_{30}O_2$	242	0.97	3.09
Methyl-*n*-hexadecanoate	Methyl palmitate	$C_{17}H_{34}O_2$	270	1.45	51.50
Methyl-*n*-heptadecanoate	Methyl margarate	$C_{18}H_{36}O_2$	284	1.59	4.63
Methyl-9-octadecenoate	Methyl oleate	$C_{19}H_{36}O_2$	296	1.78	9.79
Methyl-*n*-octadecanoate	Methyl stearate	$C_{19}H_{38}O_2$	298	1.86	6.18

(Table 1 Continued)

Systematic name	Common name	Molecular formula	Mol. wt.	RRT*	Relative percentage
Unidentified	---	---	---	2.09	6.18
Unidentified	---	---	---	2.17	6.70
Methyl-*n*-docosanoate	Methyl behenate	$C_{23}H_{46}O_2$	354	2.49	11.80

*RRT = Relative retension time with respect to methyl undecenoate of fraction B.

The data of unsaturated fatty acids also divulged some interesting results. Tridecynoic and hexadecynoic acids are the first known aliphatic triple bonded or ethinoic acids and 2-methoxy hexadecynoic acid is the first detected substituted fatty acid from a Karachi seaweed (Qasim, 1986; Shamed, 1990). Dodecenoic acid was present in largest quantity among unsaturated acids (Table 1), while oleic acid was detected in relatively small quantity. It is unusual, as oleic acid normally occurs in largest amount among green seaweeds of Karachi (Shamed and Khan, 1991). The occurrence of tricosenoic acid is the first report, as no monoethenoic acid higher than C22 was known so far from Karachi seaweeds (Shamed, 1990). This enhances the range of monoethenoic acids.

It is quite interesting that no di-, tri- or polyethenoic acid was detected in *Codium iyengarii.* These acids were also not detected in C. *dwarkense* Bϕrg., C. *flabellamm* Silva *et* Nizam. (Shamed and Khan, 1991) and C. *fragile* (Khotimchonko and Svetoshev, 1983), while they are common in the members of Caulerpales and other orders of green seaweeds (Menzel and Wild, 1989). It appears that the seaweeds of Codiales differ in their fatty add composition than the members of the allied order Caulerpales.

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