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STUDIES ON THE FATTY ACIDS OF *DICTYOTA* SPECIES FROM PAKISTAN

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ABSTRACT

An analysis of the fatty acids extracted from three brown algae *Diclyata dichototna* (Huds.) Lamour., *D. Aauckiana* Ninzam and *D. Indica* Sond. is reported. Altogether twelve saturated and unsaturated fatty ands were identified as methyl esters by GC-MS, out of which pentadcrylic, palmitic and margaric acids occur in all the three species, while alien are of selective distribution. *D. indica* slightly differed in its fatty and composition as compared to abet species.

Introduction

Around ten species of *Dictyota* occur along the coast of Pakistan (Shameel, 1990a). Its three species, *D. dichotoma* (Hudson) Lamouroux, *D. hauckiona* Nizamuddin and *D. indica* Sonder in Kützіog, are conspicuous phytobenthos on sand covered rocks as well as pools in mid- to sub-littoral zones of Karachi seashore, where they occur in abundance and appear as a rich source of food for the herbivorous invertebrates and fish species (Fenical, 1978).

The plants of *Dictyota* have received much attention and 90 diterpenes of different skeletal classes have been isolated from some 20 different species (Teixiera *et a*l., 1986). The literature search has revealed that apart from *D. dichotoma* (Johns *a at,* 1979) no phycochemical work has so *f*ar been done on the fatty acids of any species *of Dictyota,* phycochemistry is a term recently used by Shameel (1990b). In this paper an analysis of fatty acids extracted from the above mentioned three species of *Dictyota* is presented.

Materials and Methods

Plant material

Large specimens of *Dictyota* dichotoma, *D. hauckiana* and *D. indica* were collected from mid- to sub-littoral rocks during the months of May to July 1987 and 1988 at Hawkes Bay and Buleji, near Karachi, Pakistan. The healthy seaweeds were selected, cleaned with tap water to remove sand particles and animal castings, dried in shade and milled.

Extraction

About 1.5 kg of the milled seaweeds were extracted three times by percolation at room temperature with $CHCl_3$: hexane (1:1, v/v) for 20 days. The combined extract so obtained was evaporated under vacuum to give 50 g of thick dark brown residue.

Saponification

A quantity of 150 ml of ethanol-water (1:1.v/v) containing 10% KOH was added to the residue and reaction mixture was saponified by re0uxing at 100°C for 8 h. The mixture was concentrated under reduced pressure and thereafter water and Et_2O were added and this procedure was repeated three times. The unsaponifiable matter was partitioned and th*ereby* separated. The aqueous alkaline fraction was acidified with 6N HCI (pH 5-6) and then fractionated several times with Et_2O. The total Et_2O fraction was dried over anhydrous Na_2SO_4 and on evaporation of Et_2O a residue (3.25 g) was obtained.

Estenfication

The fatty acid component was esterified with diazomethane. The fatty acid part weighing 03 g was dissolved in MeOH and 0.5 ml of diazomethane was added. The reaction mixture was kept overnight at room temperature (28°C) and was then evaporated under reduced pressure. The aliquotes were d[i]rectly injected into the gas chromatograph attached to mass spectrometer (GC-MS).

Gas chromatography-mass spectrometry

The GC-MS of the methylated fraction was performed on GC-Hewlett Packard with 11/73 DEC computer and a 1.2 m x 4 mm packed glass column gas chrom Q (110-120 mesh, OV 101, 1%). The column temperature was programmed between 70 - 250 °C with a rate or increase of 8°C per minute. The injector temperature was 250°C, the carrier gas (helium) flow rate was 32 ml/min.

Results and Discussion

The GC-MS data of the methylated fatty acids revealed the presence of 8 saturated and 4 unsaturated acids, their mass spectral assignments are given below:

Methyl laurate (Dodeeanoic methyl ester): GC-MS, rats 214 (M^+, $C_{15}H_{30}O_2$), 171 (M^+ - 43), 143, 129, 115, 101, 87, 74 (100%).

Methyl myristate (Tetradeeanoic methyl ester): GC-MS, m/z 242 (M^+, $C_{15}H_{30}O_2$), 211 (M^+ -31), 199 (M^+ -43), 185, 178, 157, 143, 129, I11, 87, 74 (100%).

Methyl pentadeeyiate (Pentadeeaook methyl ester): GC-MS, m/z 256 (M^+, $C_{16}H_{32}O_2$), 213 (M^+-43), 199, 185, 171,157,143, 129,115, 101, 87, 73 (100%).

Methyl palmltale (Hexadecanoic methyl ester): GC-MS, m/z 270 (M^+, $C_{17}H_{34}O_2$), 239 (M^+ -31), 227 (M^+ -43), 213,199, 185, 171, 157, 143, 129, 101, 87, 74 (100%).

Methyl margarate (Heptadecaook methyl ester): GC-MS, m/z 284 (M^+, $C_{18}H_{36}O_2$), 241 (M^+ -43), 227, 213, 199, 185, 171, 157, 143, 115, 101, 87, 73 (100%).

Methyl stearate (Octaderaoole methyl ester): GC-MS, m/z 298 (M^+, $C_{19}H_{38}O_2$), 267 (M^+ -31), 255 (M^+ -43), 227, 213, 199, 185, 171, 143, 129, 115, 101, 87, 73 (100%).

Methyl nonadecylate (Nonadecanolc methyl ester): GC-MS, m/z 312 (M^+, $C_{20}H_{40}O_2$), 281 (M^+ -31), 267 (M^+ -43), 241, 227, 213, 185, 171, 157, 129, 87, 73 (100%).

Methyl arachidate (Eicosanoic methyl ester): GC-MS, m/z 326 (M^+, $C_{21}H_{42}O_2$), 295 (M^+ -31), 283 (M^+ -43), 256, 277, 207,171,143, 111, 101, 99, 87, 74 (100%).

Methyl lauroleate (Dodecenoic methyl ester): GC-MS, m/z 212 (M^+, $C_{13}H_{24}O_2$), 180 (M^+ -32), 138 (M^+ -74), 125, 111, 97, 74 (100%).

Methyl oleate (Octadecenoic methyl ester): GC-MS, m/z 296 (M+, $C_{19}H_{36}O_2$), 264 (M^+ -32), 222 (M^+ -74), 180, 123, 111, 97, 83, 69, *55* (100%).

Methyl heptadecadienoate (Heptadeca-9,12-dienoic methyl ester): GC-MS, m/z 280 (M^+, $C_{18}H_{32}O_2$), 248 (M^+ -32), 206 (M^+ -74), 185, 171, 143, 129, 101, 97, 87, 74 (100%).

Methyl eicosatrienoate (Ekosa-8, 11, 14-trienok methyl ester): GC-MS, m/z 320 (M^+, $C_{21}H_{36}O_2$), 288 (M^+ -32), 246 (M^+ -74), 288 199,171,157,143, 129, 115, 101, 87, 73 (100%).

The relative percentages of total fatty acids of the three *Diclyota* species are given in Table 1. The total fatty add pattern shows a relatively high level of saturated as compared to unsaturated components. Similar observations have also been made in other brown seaweeds (Qasim, 1986; Shamed, 1990b). Palmitic add was found to occur in largest amount in all the three species. This is not unusual, as hexadecanoic add commonly occurs in Dictyolalean algae (Shaikh *et al.*, 1990, 1991).

Pentadecylic, palmitic and margaric, all being saturated fatty acids, are common in the three species of *Dktyota* The occurrence of margaric acid is unusual, as it has so far

been only reported from *Iyengaria stellata* a member of Scytosiphonales (Usmanghani *et al*, 1987), while the first two acids were found in several brown algae (Qasim, 1986; Shamed 1990b; Shaikh *et al.*, 1991). Myristic and oleic acids were commonly found in *D. hauckiana* and *D.* indica and arachidic acid in *D. dichotoma* and *D.* indica, no acid was common in *D. dichotoma* and *D. hauckfana.* Laurie, stearic and lauroleic acids were only found in *D. dichotoma,* nonadecylic add only in D. hauckiana and heptadecadienoic and eicosatrienoic adds only in *D. indices.* This indicates a selective distribution of most of the fatty acids within *Dicyota* species.

Table 1

Relative percentages of total fatty acids of *Dictyota* species analysed as methyl esters

Common name	Systematic name	Molecular formula	Mol. wt.	*D. dichotoma*	*D. hauckiana*	*D. indica*
Saturated Fatty Acids						
Me laurate	Dodecanoic Me-ester	$C_{13}H_{26}O_2$	214	6.88	---	---
Me myristate	Tetradecanoic Me-ester	$C_{15}H_{30}O_2$	242	---	6.27	6.53
Me pentadecylate	Pentadecanoic Me-ester	$C_{16}H_{32}O_2$	256	16.12	15.38	2.92
Me palmitate	Hexadecanoic Me-ester	$C_{17}H_{34}O_2$	270	20.83	21.37	19.43
Me margarate	Heptadecanoic Me-ester	$C_{18}H_{36}O_2$	284	16.29	18.31	21.15
Me stearate	Octadecanoic Me-ester	$C_{19}H_{38}O_2$	298	15.79	---	---
Me nonadecylate	Nonadecanoic Me-ester	$C_{20}H_{40}O_2$	312	---	18.15	---
Me arachidate	Eicosanoic Me-ester	$C_{21}H_{42}O_2$	326	19.82	---	11.00
Unsaturated Fatty Acids						
Me lauroleate	Dodecenoic Me-ester	$C_{13}H_{24}O_2$	212	4.20	---	---
Me oleate	Octadecenoic Me-ester	$C_{19}H_{36}O_2$	296	---	19.77	20.29
Me heptadecadienoate	Heptadeca-9, 12-dienoic Me-ester	$C_{18}H_{32}O_2$	280	---	---	3.09
Me eicosatrienoate	Eicosa-8, 11, 14-trienoic Me-ester	$C_{21}H_{36}O_2$	320	---	--	15.13

Johns *et al.* (1979) have reported 18 fatty acids, ranging from C12:0 to C22:06 from *D. dichotoma* on the basis of retention time measurements. The fatty acids comparable in both the studies are pentadecyhc, palmitic, margaric, stearic and arachidic acids, while the detection of lauric and lauroleic acids is an addition to the previous information. The long chain fatty adds are present in the investigated *Dictyota* species. They may be requ[i]red to confer some distinctive physical properties upon the membranes in addition to serve as the precursors of prostanoids (Christie 1987).

D. indica appears to differ slightly in its fatty add distribution than the other two species. It possesses comparatively less saturated acids and more unsaturated ones than the others, as it revealed the presence of di- and tri-unsaturated fatty acids. Oleic acid was found in the largest proportion among unsaturated acids. Some other brown seaweeds studied from this area indicated the presence of this acid in comparatively large amount (Oasim 1986, Shamed 19906). The three investigated species of *Dictyota* do not show anatomical variation, but remarkably differ from one another in size and general morphology. This may be an indication of slight differences in their fatty add composition.

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