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FATTY ACID COMPOSITION OF THREE SPECIES OF *HYPNEA* (GIGARTINALES, RHODOPHYTA) FROM KARACHI COAST

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ABSTRACT: *Hypnea musciformis* (Wulf.) Lamour., *H. pannosa* J. Ag. and *H. valentiae* (Turn.) Mont., collected from the northern Arabian Sea coast of Pakistan, have been investigated for their fatty acid compositions through GC-MS. Palmitic acid was present in largest quantity (55-57%) and oleic was the major (7.6-8.4%) unsaturated fatty acid. Pentacosanoic and hexacosenoic acids are being reported for the first time from any species of *Hypnea*. The three species differed remarkably due to their habitat ecology.

KEY WORDS: Fatty acid - *Hypnea* spp. - Rhodophyta - Karachi.

INTRODUCTION

Phycochemistry is a term recently used by Shameel (1990). Various species of *Hypnea* Lamour, have often been subjected to a broad phycochemical investigation, regarding *e.g.* sterols (Combaut *et al.*, 1984), carbohydrates (Mahran *et al.*, 1985) and nucleosides (Kazlauskas, 1983). This genus is represented by three species at northern Arabian Sea coast of Pakistan. They grow abundantly on lower and sub-littoral rocks during October to March at Karachi and the adjacent coastal areas of Lasbela (Shameel, 1987). The present investigation reports a phycochemical study of fatty acids isolated and identified from *Hypnea musciformis* (Wulf.) Lamour., *H. pannosa* J. Ag. and *H. valentiae* (Turn.) Mont., collected from the coast of Karachi.

MATERIALS AND METHODS

Fresh thalli of *H. musciformis, H. pannosa* and *H. valentiae* were collected from lower littoral rocks as well as drift material from the rocky ledges at Buleji and Paradise Point, during the months of October to March in 1986 and 1987. The healthy specimens, free from epiphytes and animal castings were selected, thoroughly washed and dried in the shade.

EXTRACTION:

Dried thalli (*ca.* 500g) of each species were extracted with hexane:chloroform (1:1, v/v) to give a total soluble extract, which on evaporation under reduced pressure afforded a reddish black residue weighing about 1 g.

SAPONIFICATION:

Saponification of the extract was carried out by refluxing at 100°C for 6 hours with 15% KOH in 50% ethanol (25ml). The resulting mixture was concentrated under vacuum and distilled water was added to make up the volume and finally ethylacetate was added. The partitioning procedure between distilled water and ethylacetate was

repeated three times. The total combined ethylacetate fraction was acidified with 1N HCl (pH 4-5) and then extracted again with ethylacetate. The procedure was repeated several times to furnish a total of 1.5 litre ethylacetate extract, which on evaporation under reduced pressure yielded 0.5 g of the material.

ESTERIFICATION:

The extract so obtained was subjected to methylation with diazomethane. About 2 ml of diazomethane was added to 0.25 g fatty acid fraction and the reaction mixture was left at room temperature for 24 hours until dissolved, and the aliquotes were directly injected into a gas chromatograph-mass spectrometer (GC-MS).

GAS CHROMATOGRAPH MASS SPECTROMETERY:

The GC-MS of the methylated fatty acid fractions was performed on a GC-Hewlett Packard with 11/73 DEC computer data system and a 1.2m x 4mm packed glass capillary column coated with gas chrome Q (100-120 mesh, OV 101, 1%). The column temperature was programmed between 70-250°C with a rate of increse of 80 °C per minute. The carrier gas (helium) flow rate was 32 ml/min. and the injector temperature was 250 °C.

RESULTS AND DISCUSSION

Altogether 11 saturated and 4 unsaturated fatty acid methyl esters have been identified from lipid fractions of *H. musciformis, H. pannosa* and *H. valentiae* (Table I). Both the quantity and variety of saturated fatty acids (86-92%) were appreciably greater than those of unsaturated fatty acids (8-14%). Similar observations have also been made in other red seaweeds of Karachi (Shameel, 1990). However, Kato and Ariga (1982) observed the other way round in the red algae of Japan, which indicates geographical differences in the phycochemistry of seaweeds.

Palmitic acid was present in largest quantity (55-57%) in all the three species. This is quite usual as it was also found in greatest amount in several red seaweeds (Qasim, 1986; Shameel, 1990). Myristic and margaric acids were the next abundant acids (14-15%) in *H. pannosa*. Myristic acid was also found in great amount (38%) in *Scinaia fascicularis* (BØrg.) Huisman (Hayee-Memon *et al.*, 1991b), while margaric acid has been detected in large quantity (28-29%) in *Dermonema abbottiae* Afaq., Nizam. *et* Shameel (Afaq-Husain *et al.*, 1991) and *Gracilaria foliifera* (Forssk.) BØrg. (Hayee -Memon *et al.*, 1991a). All the other acids were present in small quantity (less than 9%) in the three species of *Hypnea*.

H. valentiae exhibited the greatest variation of fatty acids by possessing nine saturated and three unsaturated acids, while in *H. pannosa* five saturated and one unsaturated fatty acids could be detected. Myristic, palmitic, margaric, stearic and oleic were the most common fatty acids found in all the three species of *Hypnea*; pentadecylic, nonadecylic and behenic were present in two species only; while the remaining acids were least common and could be detected in only one species. Oleic acid was present in largest quantity (7.6-8.4%) among unsaturated acids. It is the major unsaturated fatty acid of several red algae (Shameel, 1990).

Table I: Relative percentages of fatty acids analysed as methyl esters from *Hypnea*.

Common name	Systematic name	Mol. form./(M^+)	*H. musci-formis*	*H. pan-nosa*	*H. valen-tiae*
A. SATURATED FATTY ACID METHYL ESTERS:			89.54	91.58	86.06
Methyl undecylate	Methyl-*n*-undecanoate	$C_{12}H_{24}O_2$/(200)	3.36	-	-
Methyl tridecylate	Methyl-*n*-tridecanoate	$C_{14}H_{28}O_2$/(228)	5.80	-	-
Methyl myristate	Methyl-*n*-tetradecanoate	$C_{15}H_{30}O_2$/(242)	4.32	14.83	3.58
Methyl pentadecylate	Methyl-*n*-pentadecanoate	$C_{16}H_{32}O_2$/(256)	3.95	-	4.87
Methyl palimitate	Methyl-*n*-hexadecanoate	$C_{17}H_{34}O_2$/(270)	56.71	50.41	54.98
Methyl margarate	Methyl-*n*-heptadecanoate	$C_{18}H_{36}O_2$/(284)	3.16	13.58	5.57
Methyl stearate	Methyl-*n*-octadecanoate	$C_{19}H_{38}O_2$/(298)	5.92	6.84	3.25
Methyl nonadecylate	Methyl-*n*-nonadecanoate	$C_{20}H_{40}O_2$/(312)	-	5.92	4.96
Methyl arachidate	Methyl-*n*-eicosanoate	$C_{21}H_{42}O_2$/(326)	-	-	2.17
Methyl behenate	Methyl-*n*-docosanoate	$C_{23}H_{46}O_2$/(354)	6.32	-	3.92
Methyl pentacosanoate	Methyl-*n*-pentacosanoate	$C_{26}H_{52}O_2$/(396)	-	-	2.76
B. UNSATURATED FATTY ACID METHYL ESTERS:			10.40	8.38	13.91
Methyl tetradecatrienoate	Methyl-tetradecatrienoate	$C_{15}H_{24}O_2$/(236)	-	-	3.21
Methyl palmitoleate	Methyl-9,10-hexadecenoate	$C_{17}H_{32}O_2$/(268)	2.85	-	-
Methyl oleate	Methyl-9,10-octadecenoate	$C_{19}H_{36}O_2$/(296)	7.55	8.38	8.13
Methyl hexacosenoate	Methyl-17,18-hexacosenoate	$C_{27}H_{52}O_2$/(408)	-	-	2.57

The saturated and unsaturated fatty acids detected from red seaweeds of Karachi were only upto C_{19} (Qasim, 1986). Behenic acid, which was recently detected from *Dermonema abbottiae* (Afaq-Husain *et al.*, 1991), was also found in *H. musciformis* and *H. valentiae* in appreciable amount (4-6%). The presence of pentacosanoic and hexacosenoic acids in *H. valentiae* is a unique feature. Such long chain fatty acids have not so far been detected from any marine alga of Karachi. Furthermore, they have been detected for the first time from any species of *Hypnea*. The fatty acid compositions of *H. musciformis* and *H. valentiae* were more or less similar, while *H. pannosa* differed remarkably from the other two species.

It appears that different species of *Hypnea* exhibit prominent differences in the fatty acid composition and this may be due to their habitat ecology. *H. musciformis* is purely a sub-littoral alga and grows in more or less uniform conditions (Shameel, 1987), while the other two species grow on lower littoral rocks and are subjected to diurnal exposure, which has a direct influence on their general metabolism. *H. pannosa* is epilithic as cushions of interwoven filaments on lower littoral rocks faced to rough conditions of the open sea and thus is often exposed to very severe conditions. Therefore, the three species of *Hypnea* differ in their phycochemistry.

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