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## Fatty Acid Composition of Lipids of Some of Marine Sponges from Orissa Coast

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Accepted 2 February 2001

Revised 22 January 2001

Received 11 September 2000

**The fatty acid composition of lipid constituents of seventeen species of marine sponges from the Orissa coast have been studied by GLC. Most abundant are saturated fatty acids. Among the unsaturated acids, oleic acid occurs in 40% of the species investigated. Six polyunsaturated fatty acids were noted in different species. Docosahexaenoic acid ( $\omega$ 3 PUFA) is present in two species in large quantities. The sponges, *Plakina monolopha* and *Callyspongia* sp. which have shown antimicrobial activity<sup>1</sup> are found to have >80% oleic acid.**

Polyunsaturated fatty acids (PUFA), especially some specific  $\omega$ 3 fatty acids ( $\alpha$ -linolenic acid, eicosapentaenoic acid and docosahexaenoic acid) and  $\omega$ 6 fatty acids (linoleic acid and arachidonic acid) play an important role in human and animal health<sup>2,4</sup>. Various applications of lipids, phospholipids, saturated and unsaturated fatty acids in pharmaceutical formulations have also been reported<sup>4-7</sup>. Marine sponges have been reported as source of various novel compounds such as steroids, terpenoids and antibiotics besides lipids<sup>8-15</sup> that include glycolipids, phospholipids and corresponding long chain saturated and unsaturated fatty acids. In our earlier investigation, seventeen marine sponges collected from the Orissa coast, have been studied for the antimicrobial activities of free fatty acid components of their lipids<sup>1</sup>. Composition of the identified fatty acids from these sponge species as determined by GLC analysis is presented in this paper.

Sponges were thoroughly washed and air-dried. Ten grams of each species was homogenised and successively extracted three times with chloroform-methanol (2:1, v/v) to isolate lipids<sup>16</sup>. Crude lipid extracts were purified by "folch" wash<sup>17</sup> to remove non-lipid contaminants. The chloroform phase was separated from the combined extract, dried over anhydrous sodium sulphate and concentrated under nitrogen atmosphere.

The pure lipids isolated above were saponified with 1 M alcoholic potassium hydroxide and free fatty acids were regenerated by treatment with 6 M HCl. Free fatty acids were then extracted with diethyl ether, washed with chilled water to remove traces of acid and then dried over anhydrous sodium sulphate. The solvent was recovered and fatty acids were dried under vacuum. The fatty acids so obtained were converted to corresponding methyl esters<sup>18</sup>. Fatty acids (10 mg) were dissolved in 4 ml of 5% hydrochloric acid in methanol and 0.5 ml benzene and then the mixture was refluxed in a silicone bath at 80-100° for 2 h. After cooling, the methyl esters were extracted with petroleum ether, simultaneously neutralised and dried over sodium sulphate - sodium bicarbonate mixture. The solvent was evaporated to dryness at reduced pressure at 40° in a water bath. These fatty acid methyl esters (FAME) were then analysed by GLC for identification.

Samples were analysed on a PYE-UNICAM GC (model no. GC - 104) equipped with FID and stainless steel column (1.8 m) packed with 10% DEGS using nitrogen as a carrier gas (40 ml/min) with a chart speed of 0.7 cm/min. Column temperature, detector temperature and injection port temperature were maintained at 190°, 210° and 210° respectively. The sample was applied in 1  $\mu$ l quantity. Identification of fatty acid components of

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TABLE 1: FATTY ACID COMPOSITION BY GLC

Genus-species	Percentage of fatty acid composition																
	C <sub>8:0</sub>	C <sub>10:0</sub>	C <sub>12:0</sub>	C <sub>14:0</sub>	C <sub>16:0</sub>	C <sub>16:1</sub>	C <sub>18:0</sub>	C <sub>18:1</sub>	C <sub>18:2</sub>	C <sub>18:3</sub>	C <sub>20:0</sub>	C <sub>20:1</sub>	C <sub>20:4</sub>	C <sub>20:5</sub>	C <sub>22:0</sub>	C <sub>22:4</sub>	C <sub>22:6</sub>
<i>Acanthella cavernosa</i> (Dendy)	-	0.004	0.03	2.64	55.40	-	38.57	-	-	-	-	-	-	1.99	-	-	-
<i>Acanthella elongata</i> (Dendy)	-	0.09	0.09	0.05	0.11	-	39.19	31.54	-	28.59	-	-	-	-	-	-	-
<i>Azorica pfeifferae</i> Carter	-	-	-	-	37.40	0.28	-	-	-	-	62.10	-	-	-	-	-	-
<i>Callyspongia fibrosa</i> (Ridley & Dendy)	-	-	-	0.56	13.90	9.80	8.60	-	-	-	1.86	-	-	-	-	-	65.10
<i>Callyspongia</i> sp.	-	1.61	1.64	1.87	1.88	-	-	80.05	1.62	-	2.65	1.83	-	-	-	-	-
<i>Epipolasis</i> sp.	-	-	-	0.02	1.73	-	-	-	-	-	61.72	-	-	-	-	36.53	-
<i>Heteronema erecta</i> Keller	-	15.98	-	9.55	36.10	18.16	8.69	1.92	-	1.78	6.83	-	-	-	-	-	-
<i>Hyattella cribriformis</i> (Hyatt)	-	0.39	-	-	63.90	-	-	-	-	-	-	-	17.47	-	-	-	17.70
<i>Oceanapia</i> sp.	-	0.004	-	0.06	0.09	-	0.07	-	-	-	28.40	-	-	-	-	-	71.30
<i>Petrosia testudinaria</i> (Lamarck)	-	0.003	0.09	32.22	-	38.70	-	26.44	-	-	-	-	-	-	0.38	-	-
<i>Phakellia dendyi</i> Bergquist	-	0.17	-	59.20	36.94	-	3.02	-	-	-	-	-	-	-	-	-	-
<i>Plakina monolopha</i> Schulze	0.74	1.40	1.38	0.31	-	2.03	0.60	82.17	0.69	-	2.43	1.23	-	-	-	-	-
<i>Plakortis simplex</i> (Schulze)	-	0.43	0.47	0.69	39.32	-	17.60	36.26	0.48	-	0.84	0.66	-	0.19	-	-	-
<i>Psammaplysilla purpurea</i> (Carter)	-	0.09	0.04	98.70	0.81	-	-	-	-	-	-	-	-	-	-	-	-
<i>Raspailia</i> sp.	-	0.42	0.42	1.31	41.33	-	9.80	30.80	0.36	1.65	-	-	1.72	0.18	-	11.22	-
<i>Spirastrella inconstans</i> (Dendy)	-	0.64	0.60	0.44	95.80	-	2.24	-	-	-	-	-	-	-	-	-	-
<i>Spirastrella vagabunda</i> Ridley	-	0.06	0.09	99.00	0.64	-	-	-	-	-	-	-	-	-	-	-	-

Means of duplicate analysis; Values represent percent of total acid.

samples was done by comparing with the retention time of standard fatty acid methyl esters (FAMES) procured from Sigma Chemical Co., USA. Relative peak area percentage of individual sample was recorded. The study has been restricted to saturated and unsaturated fatty acids from C<sub>8</sub> to C<sub>22</sub>.

Percentage composition of identified fatty acid components from individual sponge species is compiled in Table 1. Among the saturated fatty acids, tetradecanoic acid (myristic acid, C<sub>14:0</sub>) occurs in fifteen out of seventeen species. It is dominant in the sponge *Spirastrella*

*vagabunda* (99%), *Psammaplysilla purpurea* (98.7%) and in *Phakellia dendyi* Bergquist (59.2%). Hexadecanoic acid (Palmitic acid, C<sub>16:0</sub>) is present in fifteen species, being dominant in *Spirastrella inconstans* (95.8%) and in *Hyattella cribriformis* (63.9%). Octadecanoic acid (stearic acid, C<sub>18:0</sub>) is present in ten species and is found dominant in *Acanthella elongata* (39.1%) and *Acanthella cavernosa* (38.57%). Arachidic acid (C<sub>20:0</sub>) is present in eight species with high percentage in *Azorica pleifferae* (62.1%) and *Epipolasis* sp (61.72%). Interestingly, the percentage content of saturated fatty acids having chain length C<sub>8:0</sub>, C<sub>10:0</sub>, C<sub>12:0</sub> and C<sub>22:0</sub> have been observed to be very poor (<2%) in the species examined, except in one species, *H. erecta* Keller which contained 16% of C<sub>10:0</sub> acid (decanoic acid).

Among the monounsaturated fatty acids (MUFA), cis-9-hexadecanoic acid (palmitoleic acid, C<sub>16:1</sub>) is present in five species and is maximum in *Petrosia testudinaria* (39%). Cis-9-octadecenoic acid (oleic acid, C<sub>18:1</sub>) is present in seven species and is dominant in two species *Plakina monolopha* (82.17%) and *Callyspongia* sp (80.05%). This acid is present in 36%, 32%, 31% and 26% in *P. simplex*, *A. elongata*, *Raspailia* sp and *P. testudinaria* respectively. Cis-11-eicosenoic acid (C<sub>20:1</sub>) is present in three species (<2%).

Three poly unsaturated fatty acids (PUFA), linoleic acid (C<sub>18:2</sub>), arachidonic acid (C<sub>20:4</sub>) and docosatetraenoic acid (C<sub>22:4</sub>) belonging to ω6 family are identified in four, two and two species respectively. *H. cribriformis* has reported C<sub>20:4</sub> acid to the extent of 17% whereas the C<sub>22:4</sub> acid is found in *Epipolasis* sp and *Raspailia* sp to the extent of 36.53% and 11.22% respectively.

The presence of three polyunsaturated fatty acids of ω3 family is noted in different species. α-Linolenic acid (C<sub>18:3</sub>) is present in three species with the maximum content of 28.59% in *A. elongata*. Eicosapentaenoic acid (C<sub>20:5</sub>) is identified in three species in <2%. The PUFA, docosahexaenoic acid (C<sub>22:6</sub>) is present in large quantities in two species viz., *Oceanapia* sp (71.3%) and *Callyspongia fibrosa* (65.1%) with relatively less abundance in *H. cribriformis* (18%). It is noteworthy that *Raspailia* sp has reported five PUFAs which are C<sub>18:2</sub> (<1%), C<sub>18:3</sub> (<2%), C<sub>20:4</sub> (<2%), C<sub>20:5</sub> (<1%) and C<sub>22:4</sub> (11%) and one MUFA C<sub>18:1</sub> (31%).

The antibacterial activity of the fatty acid components of *Plakina monolopha* and *Callyspongia* sp. against human pathogenic organisms *V. cholerae*, *P. aeruginosa* and *C. albicans* has been reported by us earlier<sup>1</sup>. The present GC analysis shows the fatty acid mixtures of these two species contained large quantities oleic acid (C<sub>18:1</sub>, 80% and 82%). The antimicrobial activities of C<sub>18</sub> mono and poly unsaturated fatty acids is well documented in literature<sup>19-21</sup>. Hence, the inhibitory activity of the fatty acid components of the two sponges might be partly due to the presence of higher oleic acid content.

#### ACKNOWLEDGEMENTS

Authors thank the Director, R.R.L., Bhubaneswar for facilities, DOD for funding collections; National Institute of Oceanography (NIO), Visakhapatnam for geophysical scanning and Eastern Naval Command (Indian Navy) for SCUBA diving and collection of sponges. Authors are grateful to RSIC, Bose Institute, Calcutta for providing GLC facilities.

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## Spectrophotometric Methods for the Determination of Cefotaxime Sodium in Dosage forms

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Accepted 7 February 2001

Revised 23 January 2001

Received 22 September 2000

Two simple and sensitive spectrophotometric methods (A and B) in the visible region have been developed for the determination of cefotaxime sodium (CFTS) in bulk and in dosage forms. Method A is based on the reaction of CFTS with nitrous acid under alkaline conditions to form a stable violet colored chromogen with absorption maximum of 560 nm and method B is based on the reaction of CFTS with 1,10-phenanthroline and ferric chloride to form a red colored chromogen with the absorption maximum of 520 nm. The color obeyed Beer's law in the concentration range of 100-500 µg/ml for method A and 1.6-16 µg/ml for method B, respectively. When pharmaceutical preparations containing CFTS were analysed, the results obtained by the proposed methods are in good agreement with the labeled amounts and are comparable with the results obtained using a UV spectrophotometric method.

CFTS<sup>1</sup> chemically known as (6R, 7R)-7-[2-(2-Amino-4-thiazolyl) glyoxylamido]-3-(hydroxy methyl)-8-oxo-5-thia-1-aza bicyclo [4,2,0] oct-2-ene-2-carboxylic acid  $\alpha$ -(o-methyl oxime) acetate monosodium salt, is a third generation cephalosporin antibiotic used in the management of mild to moderate infections caused due to susceptible microorganisms. Many methods were reported for its determination such as HPLC<sup>2-7</sup>, microbiological<sup>8-10</sup> and spectrophotometry<sup>11-14</sup>. A review of the literature reveals that considerable attention has not been paid to the colorimetric determination of this drug. In the present work, the reaction of CFTS with nitrous acid under alkaline

conditions to form a stable violet colored chromogen (method A) and the reaction of CFTS with 1,10-phenanthroline and ferric chloride to form a stable red colored chromogen (method B) were used for the determination of CFTS.

A stock solution of CFTS (1 mg/ml) was prepared by dissolving 100 mg of the drug in 100 ml of distilled water. Working standard solutions were obtained by appropriate dilution of the stock solution. Solutions of sodium nitrite (1% w/v), hydrochloric acid (0.5 N), sodium hydroxide (5% w/v), 1,10-phenanthroline (0.01 M), ferric chloride (0.003 M) and ortho-phosphoric acid (0.2 M) were prepared in distilled water. All chemicals used were of

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