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Lipids and Fatty Acids of the Gastropod Mollusc *Cerethidea obtusa*

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ABSTRACT

The gastropod mollusc Cerithidea obtusa *was examined for its lipids and fatty acids. The non-polar lipids identified were hydrocarbons, steryl esters, triglycerides, free fatty acids and sterols. The major phospholipid was phosphatidyl choline. The predominant fatty acids were myristic, palmitic, stearic, oleic, linolenic, arachidonic and eicosapentaenoic acids. Combinations of chromatographic techniques have been employed for the qualitative and quantitative determination of the lipids and fatty acids of* C. obtusa.

INTRODUCTION

The molluscs constitute one of the more important invertebrate groups in the animal kingdom and are divided taxonomically into seven classes of which *Gastropoda*, *Bivalvia* and *Cephalopoda* comprise a major marine fishery resource and are commercially important world-wide (Joseph,

1982). In addition to their commercial value, current interest in the role of dietary polyunsaturated fatty acids in human health, particularly that of eicosapentaenoic acid (20:5ω3) in the amelioration of certain cardiovascular diseases (Dyerberg *et al.*, 1975, 1978; Dyerberg & Bang, 1979) also focuses attention on the molluscs, as well as other marine fishery products, which are excellent sources for polyunsaturates.

Sunderbans mangrove environment constitutes a complex ecosystem at the confluence of the deltaic estuarine complex of the river Hooghly and Bay of Bengal. The enormous salt marshes and mud flats of this mangrove system support a luxuriant benthic macrofauna in which molluscs, perhaps, constitute the major population. Fatty acid distribution in various lipid classes of the gastropod *Cerethidea obtusa*, one of the dominant gastropod animals of this mangrove area, has been determined. The mollusc is edible and consumed by the local people.

MATERIALS AND METHODS

The gastropod molluscs *C. obtusa* were collected from Prentice Island, between latitudes 21·43° and 21·46°N and longitudes 88·18° and 88·19°E of the Sunderbans mangrove forest, West Bengal, India.

Extraction

The lipids were extracted according to the procedure of Bligh & Dyer (1959). From fifty animals (360 g), flesh (80 g) was taken out of the shells and homogenised with 336 ml of methanol–chloroform (2:1 v/v) for 2 min. The homogenate was filtered under suction and the residue was re-homogenised with the same volume of methanol–chloroform–water (2:1:0·8 v/v/v). After filtration the residue was further extracted, as in the first step. The combined filtrate was diluted with 328 ml of chloroform–water (1:1 v/v); the mixture was shaken thoroughly and the phases were allowed to separate. The lower chloroform layer, which contained the total lipid, was evaporated to dryness in a rotary evaporator at 35°C and weighed. The isolated lipids were dissolved in chloroform–methanol (2:1 v/v) and stored at 0°C (Kates, 1972).

Column chromatography

The lipid extract was subjected to column chromatography using a silicic acid (Mallinckrodt, 300 mesh) column (Rouser *et al.*, 1967). The lipids were separated into non-polar lipids (chloroform eluates), traces of

glycolipids (acetone eluates) and phospholipids (methanol eluates). The non-polar lipids and phospholipids were estimated by weighing.

Thin-layer chromatography (TLC)

Thin-layer chromatography was performed on 20 × 20 cm or 14 × 20 cm chromatoplates covered with silica gel G (0·25–0·50 mm thickness). The solvent system for non-polar lipid was petroleum ether (60–80°C)–diethyl ether–acetic acid (80:20:1 v/v/v) (Mangold, 1969). Phospholipids were separated using a solvent system of chloroform–acetone–methanol–acetic acid–water (6:8:2:1:1 v/v) (Rouser *et al.*, 1969). Phospholipids were estimated according to Barlette (1959). Spots of separated lipid materials were detected by iodine vapour and by specific spray reagents. Rhodamine 6G (Marinetti, 1964) spray reagent was used for all lipids and the Dittmer–Lester (Dittmer & Lester, 1964) reagent was used for phospholipids. All the lipids were identified by comparing the R_f with those of authentic standards. Non-polar lipid components were estimated by preparative TLC and weighing.

Saponification of lipids and esterification of fatty acids

Fatty acids and non-saponifiables were separated after saponification of the lipids (Misra *et al.*, 1984). Fatty acids were methylated using diazomethane (Schlenk & Gallerman, 1960).

Gas–liquid chromatography

A Pye Unicam (model 104) gas chromatograph, equipped with flame ionisation detector and dual glass column (1·8 m × 0·3 cm), was used. Fatty acid methyl esters were analysed on 10% DEGS supported on 80–100 mesh Diatomite C (Misra *et al.*, 1983). Catalytically reduced (Ghosh & Dutta, 1972) methyl ester samples were also analysed on the same column, under identical operational conditions of the instrument.

Peak identification and quantification

Fatty acid methyl ester peaks were identified by:

(1) Using cod liver oil fatty acid methyl ester as a secondary standard (Ackman & Burgher, 1965).
(2) Semilogarithmic plot of relative retention times (RRT) against carbon chain lengths of the fatty acids of cod liver oil and fitting the logarithm

of RRT of the fatty acids of samples into these plots (Ackman *et al.*, 1963).
(3) Comparison of equivalent chain length (ECL) values with those published (Ackman, 1963; Ackman & Burgher, 1965).
(4) Comparison of the chromatograms of the hydrogenated and non-hydrogenated samples.

Quantifications were done by triangulation, determination of peak area and internal normalisation technique.

RESULTS AND DISCUSSION

The total lipid, non-saponifiable matter, iodine value of total lipid and other lipid components of *C. obtusa* are presented in Table 1. The non-saponifiable matter was high (28%), compared to that of *B. boddaerti*, another benthic animal of this ecosystem (Misra *et al.*, 1983). The high non-saponifiable content was due to the presence of appreciably large amounts of free sterols and sterol esters in the non-polar lipid (Table 1). All the values reported were the averages of three analyses, errors being mostly within $\pm 2{\cdot}0\%$.

The non-polar lipid was 59% and phospholipid was 40% of the total lipid, glycolipid (1·0%) being present in traces.

Analysis of the phospholipid fraction by TLC revealed the presence of phosphatidyl choline as the major component, being over 95% of the total phospholipid. Other minor constituent phospholipids were: phosphatidyl ethanolamine (3%), phosphatidyl serine (1%) and lysophosphatidyl choline (1%).

The fatty acid compositions of total lipid, sterol ester, triglyceride and phospholipid are presented in Table 2. The components having identical

TABLE 1
Compositions of Various Lipids of *C. obtusa*

Lipids	*% w/w*	*Lipids*	*% w/w*
Total lipid[a]	1·2	Hydrocarbon[b]	4·4
Non-saponifiable[b]	28·0	Sterol ester[b]	5·7
Non-polar lipid[b]	59·0	Triglyceride[b]	23·0
Phospholipid[b]	40·0	Free fatty acids[b]	2·4
		Sterol[b]	20·0

Iodine value of total lipid (Wij's) = 90·0.
[a] Expressed as % w/w of wet tissue.
[b] Expressed as % w/w of total lipid.

TABLE 2
Fatty Acids Composition (Relative Area %) of *C. obtusa* Lipids

Fatty acids[a,b]	*Total lipid*	*Sterol ester*	*Triglyceride*	*Phospholipid*	*ECL*[c]
14:0	8·8	12·8	8·0	6·0	14·0
14:1	4·7	2·7	4·0	4·0	14·5
15:0	0·3	0·5	0·5	0·5	15·0
16:0	27·8	27·6	25·5	24·0	16·0
16:1	1·3	0·5	1·5	1·5	16·6
17:0	0·5	0·9	0·5	1·0	17·4
18:0	5·5	9·5	6·0	4·0	18·0
18:1	5·0	5·0	8·5	9·0	18·5
18:2ω6	4·2	3·5	4·5	5·0	19·4
20:0	0·7	1·5	0·5	—	20·0
18:3ω3	13·6	10·8	13·5	14·0	20·4
20:1	1·5	1·2	1·0	2·0	20·4
18:4ω3	0·5	0·5	0·5	0·5	21·3
20:2ω9	0·8	—	0·5	0·5	20·85
20:3ω9	2·5	2·0	2·3	2·8	21·6
22:0	0·4	1·0	0·4	—	22·0
22:1	0·5	0·5	1·0	0·5	22·7
20:4ω6	4·5	3·0	8·0	5·5	22·8
20:5ω3	10·7	10·0	11·0	12·0	24·0
22:4ω6	1·0	1·0	0·5	1·0	24·7
22:5ω6	0·9	1·0	1·0	1·5	25·5
22:5ω3	0·7	1·0	1·0	0·7	26·0
22:6ω3	3·7	3·5	2·8	4·0	26·2

[a] First and second figures represent carbon chain length: number of double bonds.
[b] The ω values represent the number of carbon atoms between the methyl group and the centre of the double bond nearest to it.
[c] ECL = Equivalent chain length.

or close ECL values were quantified from the totals of each carbon chain length obtained from the chromatogram of hydrogenated sample. As an example, the total of all the 18-carbon chain fatty acids in the total lipid, except 18:3ω3, was 15·2% (Table 2). The chromatogram of the hydrogenated sample showed the presence of 28·8% of stearic acid (C-18), indicating that the extra 13·6% must have been contributed by 18:3ω3. So, the combined peak due to 18:3ω3 and 20:1, which was 15·1%, must have contained 1·5% of 20:1. A representative chromatogram of the fatty acid methyl ester of total lipid is shown in Fig. 1. The ECL values of critical pairs, which could not be determined, have been reported from our

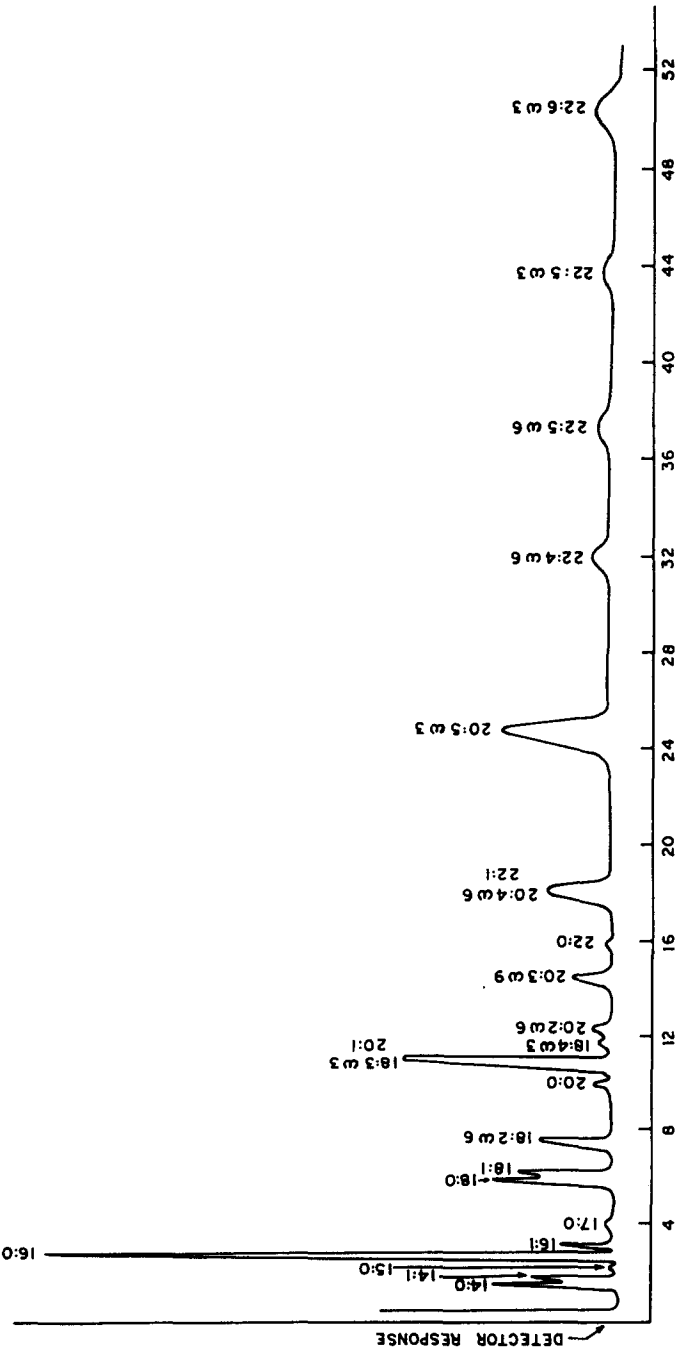

Fig. 1. Gas–liquid chromatogram of the fatty acid methyl esters of total lipid of *C. obtusa* analysed on 10% DEGS column. Oven temperature, 188°C. Detector and injection port temperature, 230°C. Nitrogen flow rate, 60 ml/min.

previous work (Misra *et al.*, 1983). Qualitatively, all the lipids have identical fatty acid compositions, with quantitative variations. In all the lipids, proportions of unsaturated fatty acids were higher than those of the saturated ones. The highest proportion of unsaturated fatty acids was in phospholipid (73%), the lowest being in sterol ester (53·2%).

Among the saturated fatty acids, major components were: myristic (14:0), palmitic (16:0) and stearic (18:0) acids. Predominant unsaturated fatty acids were: oleic (18:1), linoleic (18:2ω6), linolenic (18:3ω3), arachidonic (20:4ω6) and eicosapentaenoic (20:5ω3) acids. The double bond positions of the monoenoic acids could not be ascertained because, in the present study, a conventional packed GLC column was used, whereby the isomeric monoenes had identical retention times and were eluted together. The fatty acid composition of *C. obtusa* lipids was found to be typical of those of various gastropod molluscs, as reviewed by Joseph (1982).

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