

Nitrogenous glycerolipids from *Ulva fasciata* (Ulvaceae, Chlorophyta) of West Coast of India

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An inseparable mixture of nitrogenous glycerolipids was isolated from the Dragendorff positive fraction of the methanol extract of the marine green alga *Ulva fasciata*, collected from Okha (22°28' N, 69°05' E) at Gujarat (India) coast. Monoacylglyceryl-*N,N,N*-trimethylhomoserine having 16 : 0 and 18 : 2 fatty acid residues were found to be the predominant components of this fraction. This is the first report of their occurrence in this source.

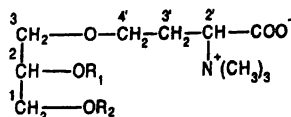
Nitrogenous glycerolipids isolated so far from different species of *Ulva* were identified as diacylglyceryl-*N,N,N*-trimethylhomoserine (DGTS), better known as betaine lipids for their quaternary ammonium moiety. Some amino acid betaines were also reported from *U. lactuca*¹. Occurrence of betaine lipids was found to be restricted to the non-flowering lower plants like ferns, mosses, algae, fungi, lichens etc. and it may have some taxonomic importance considering its presence in almost all green algae except *Chlorella*²⁻⁶. The molecular geometry and charge distribution of betaines have shown some similarities to the ubiquitous phospholipids, particularly phosphocholines. The amount of phosphocholine was found to be low in a number of organisms, producing betaine lipids. Therefore, the latter might act as a substitute for phosphocholine as a membrane constituent and/or an intermediate in cellular lipid metabolism⁴. Four *Ulva* species, namely, *U. pertusa*, *U. fenestrata*, *U. rigida* and *U. lactuca* have been studied and their betaine lipids were found to be present in the form of amino acid betaines and DGTS. Except a few reports on detailed spectroscopic studies^{2,7,8} characterization of these compounds have been done mainly on TLC plates using spray reagents, e.g. Dragendorff's or molybdenum-blue reagents^{4,6}.

In the present investigation on the lipid soluble compounds from *Ulva*, an acylglyceryltrimethylhomoserine fraction 1 has been isolated from the methanol extract of *U. fasciata* for the first time. The composition of this fraction was elucidated on the basis of GC-MS, ¹³C and ¹H NMR and IR spectral data.

Results and Discussion

Physicochemical properties and polar nature of 1 which decomposes on standing, suggest it to be a quaternary ammonium compound. Similar types of Dragendorff positive unstable polar compounds reported from various species of *Ulva*, have been identified as betaine lipids (diacylglyceryltrimethylhomoserine), phospholipids and sulfolipids^{9,10}. In the ¹³C NMR spectrum of 1, signals at 175.4 and 171.4 ppm stand for COO and OCOCH₂, respectively. The IR spectrum indicated the presence of ester group (1738 cm⁻¹). ¹H NMR spectrum indicated the presence of aliphatic chain (δ 1.30, br s, methylene envelop). Signals in δ 3–4 region are in agreement with those reported² for glyceryl and *N,N,N*-trimethylhomoserine protons. All these chemicals and spectral data showed that 1 is an acylated betaine lipid. According to literature reports, betaine lipids with different fatty acid moieties, e.g. 16 : 0, 18 : 1, 18 : 2, 18 : 3 etc., occur together as inseparable mixture. Therefore, 1 was subjected to ester hydrolysis and the acid part was converted to its methyl ester. GC-MS analysis showed that 16 : 0 acid is the major component along with 18 : 2 acid in minor quantity. Therefore, 1 may be regarded as a mixture of betaine lipids having various fatty moieties such as palmitic (16 : 0), linoleic (18 : 2, Δ^9 and Δ^{12} *cis*), linolelaidic (18 : 2, Δ^9 , Δ^{12} *trans*) acids. Scrutiny of the FAB mass spectrum (positive mode) of 1 showed the presence of more than one molecular ion peaks, indicating 1 to be a mixture of compounds. In fact, three peaks at *m/z* 474, 498 and 712 were identified as possible protonated

molecular ion peaks. Peaks at 496, 520 and 734 correspond to the respective $(M+Na)^+$ ions. The first two peaks at m/z 474 and 498 can not occur from diacylglyceryltrimethylhomoserine, which is a commonly occurring betaine lipid. In 1H NMR spectrum, the signals for the hydrogen linked with C-2 of glycerol appeared at δ 3.94, indicating the presence of a free hydroxyl group⁹. The integration ratio of the terminal methyl protons (total number of protons appearing at δ 0.89 and 0.99) to those of the ester linked methylene of glycerol moiety (two non-equivalent protons at δ 4.17 and 4.07) is more than 3 : 1 and less than 3 : 2. This indicates that the fatty acid present per glycerol unit is less than two, and **1** is a mixture of betaines where diacylated compounds are present in a relatively smaller quantity. These along with the fact that two different fatty acids (16 : 0 and 18 : 2) were detected in the fatty acid methyl ester analysis leads one to conclude that **1** is mainly a mixture of monoacylglycerylbetaines. Considering m/z 712 $(M+H^+)$ as another molecular ion peak, it can also be concluded that a small amount of diacylated betaine is present in this mixture. The multiplet at δ 5.01 in 1H NMR seems to be due to the C-2 methine proton of a diacylated glycerol derivative.



Compound A : $R_1 = H, R_2 = COCH_2(CH_2)_{15}CH_3$

Compound B : $R_1 = H, R_2 = CO(CH_2)_7CH=CHCH_2CH_2CH_2CH_2CH_2CH_3$

Compound C : $R_1 = R_2 = COCH_2(CH_2)_{15}CH_3$

Compounds **1a** ($C_{26}H_{51}O_6N$, mol. wt. 473), **1b** ($C_{28}H_{51}O_6N$, mol. wt. 497) and **1c** ($C_{42}H_{82}O_7N$, mol. wt. 711) have been proposed as the three components of **1** having different fatty acid moieties with glycerol backbone and homoserine functionality. Their structures have been assigned by comparing with standard and reported compounds¹¹. Compound **1a** is 1-palmitoylglyceryl-3-*O*-4'-(*N,N,N*-trimethyl)homoserine, **1b** is 1-linoleyl-1-linolelaidylglyceryl-3-*O*-4'-(*N,N,N*-trimethyl)homoserine and **1c** is 1,2-dipalmitoylglyceryl-3-*O*-4'-(*N,N,N*-trimethyl)homoserine, compound **1a** being the major component.

Experimental

U. fasciata (Ulvaceae, Chlorophyta) was collected from Okha (lat. 22°28' N, long. 69°05' E) on the West Coast of India, in December 1995.

IR spectrum was recorded on a BIO-RAD FTS 40 spectrophotometer, NMR spectra (MeOD) on a Bruker DRX-300 FT spectrometer using TMS as internal standard

and the FAB-MS on a JEOL SX 102/DA-6000 spectrometer/data system using xenon as the FAB (6 kV, 10 mA) gas. *m*-Nitrobenzyl alcohol was used as the matrix. GC-MS was performed on a Shmadzu QP-2000 machine at 70 eV, using ULBON HR-1 capillary column (0.25 mm \times 50 m, film thickness 0.25 micron) and the other conditions of the experiment were as follows : column length, 50 m; injector temp., 250°; detector temp., 280°; temp. program 100° (7 min) to 250° at 50°/min; flow rate 2 ml/min.

After collection, the algae (7 kg) was washed with water and soaked in methanol (2.5 dm³). The algal material was homogenized and extracted repeatedly with methanol at room temperature until the extract was colorless. The methanol extract was evaporated under reduced pressure to obtain a dark green gummy residue. A part (6.0 g) of this extract was subjected to repeated flash column chromatography (230–400 mesh silica gel, SRL, India); 90% methanol-toluene to methanol eluates rich in one major spot (silica gel TLC, detection by iodine and Dragendorff), were pooled and evaporated to give a gummy product (0.194 g). Repeated filtration of this fraction on active neutral alumina (Grade IV, SRL, India) afforded **1** as a transparent oily liquid (0.080 g); ν_{max} 3470 (O-H), 2913 (C-H alkane), 2850 (C-H), 1738 (C=O ester), 1627 (C=O CO₂), 1467 (C-H bending), 1362 (C-H bending, C=O stretching CO₂), 1312, 1179, 1120 (C-O-C), 963, 876, 825, 721 cm⁻¹; 1H NMR δ (300 MHz, *d*₄-MeOH) 0.89 (distorted t, 6.6 Hz, terminal methyl protons), 0.99 (m), 1.30 (br s), 1.61 (m, H-3''), 2.11 (m, H-3' and H-8', H-14' protons of **1b**), 2.26 (m, H-3'), 2.34 (t, 5.2 Hz, H-2''), 2.81 (m, H-11'' of **1b**), 3.23 (br s, N⁺-CH₃), 3.30; 3.44–3.61 (two overlapping contours in COSY, H-3 and H-4'), 3.68 (m, H-4'), 3.86 (dd, 12 and 3 Hz, H-2'), 3.94 (H-2 of **1a** and **1b**), 4.17 (dd, 12 and 3 Hz, H-1), 4.06 (dd, 9 and 6 Hz, H-1), 5.01 (m, H-2 of **1c**) and 5.35 (m, olefinic protons of **1b**); ^{13}C NMR δ (75 MHz, *d*₄-MeOH) 14.1 (terminal methyl carbons), 14.7, 19.5, 23.7 (C-3''), 25.5 (C-11'' of **1b**), 26.0, 26.4, 26.5, 27.8 (C-8'', C-14'' of **1b**; *Z*-form), 28.1, 29.0, 30.2, 30.4, 30.6, 30.7, 31.8, 32.9 (C-8'', C-14'' of **1b**; *E*-form), 33.0 (C-3'), 34.9 (C-2''), 35.1, 52.5 (N⁺-CH₃), 61.6 (C-1 of **1c**), 66.5 (C-1 of **1a** and **1b**), 68.4 (C-3 of **1a** and **1b**), 69.5 (C-2 of **1a** and **1b**), 70.5 (C-3 of **1c**), 73.3 (C-4'), 74.4 (C-1 of **1c**), 76.9 (C-2'), 79.5, 128.2 (C-10'', C-12'' of **1b**; *Z*-form), 128.9 (C-10'', C-12'' of **1b**; *E*-form), 129.9 (C-9'' of **1b**; *Z*-form), 130.5 (C-9'' of **1b**; *E*-form), 130.8 (C-13'' of **1b**; *Z*-form), 131.1 (C-13'' of **1b**; *E*-form), 171.4 (C-1') and 175.4 (C-1'); FAB-MS m/z (%) 734 (3.2), 712 (1.2), 685 (0.8), 663 (2.4), 522 (14), 520 (8), 497 (48), 496 (100), 474 (32), 459 (10), 409 (62), 393 (8), 312 (10), 271 (38), 240 (14), 174 (12), 95 (42), 81 (46), 69 (66), 55 (80) and 23 (36).

Methylation of the fatty acid mixture obtained by saponification of 1 : A mixture of **1** (25 mg) and 2% aq. methanolic NaOH (20 ml) was refluxed for 2 h with a little

benzene. The reaction mixture was then cooled, diluted with water (15 ml) and methanol and evaporated by keeping it on a steaming water-bath. The residue was extracted with ether (5 ml \times 2). The aqueous layer was acidified with dil. HCl and re-extracted with ether (5 ml \times 3). The ethereal solution was washed with water and dried (Na_2SO_4) to afford the crude fatty acid mixture. This mixture was then treated with 10% solution of acetyl chloride in dry methanol and the reaction mixture was kept overnight. The reaction mixture was partitioned between water and ether. The ether layer was washed with 10% aq. NaHCO_3 , dried (Na_2SO_4). The crude fatty acid methyl ester thus obtained after removal of the solvent was chromatographed over silica gel to afford a mixture of methyl ester (15 mg). GC-MS spectra of this fatty acid methyl ester fraction showed the presence of two fatty acids 16 : 0 and 18 : 2 compared to the profile of standard fatty acids. ^1H NMR δ (300 MHz, d_4 -MeOH) : 0.90 (distorted t, 6.6 Hz, terminal methyl protons), 0.97 (t), 1.29 (br s), 1.60 (m, H-3''), 2.11 (m, allylic protons), 2.31 (m, α -methylene protons), 2.81 (m, doubly allylic methylene protons), 3.65 (s, COOCH_3), 3.31, 5.35 (m, olefinic protons).

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