Galactocerebroside and other secondary metabolites of sea mouse

*Chloeia parva*¹–⁴,†

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Novel galactocerebrosides derived from 2-amino-4-alkene-1,3-diol along with fatty acid methyl esters (FAME), cholesterol, ceramides, 1-O-acylglycerols and 1-O-alkylglycerols have been isolated from a sea mouse *Chloeia parva*. The structure of galactocerebrosides is established from one-dimensional [¹H, homodecoupling and ¹³C (NDC and DEPT-135)] NMR spectral studies of the metabolite and its acetate as well as two-dimensional (COSY-90, X-II correlation optimized for ¹JC-H) NMR spectral studies of the latter. The remaining components isolated have been characterized from chemical studies and detailed NMR spectral analyses of parent materials and products derived thereof. The lengths of the alkyl and acyl chains in galactocerebrosides, FAME, ceramides, 1-O-acylglycerols and 1-O-alkylglycerols are established by gas chromatographic analysis of appropriate derivatives.

Past three decades have witnessed a tremendous surge of interests in marine natural product research throughout the globe.⁵ As a part of our search for bioactive components from marine sources a sea mouse, *Chloeia parva* was collected from the Eastern coast of the Bay of Bengal, adjoining West Bengal. This paper deals with the results of the chemical investigations including the structural elaboration of interesting and novel marine metabolites, galactocerebrosides. Cerebrosides are important membrane components in both plant and animal cells, and they appear to participate in cell regulatory functions and trans-membrane signaling. Such galactocerebrosides have earlier been reported⁴ from star fish (*Pentaceraster regula*⁶), sponge (*Chondropsis* sp.⁷,⁸, *Halichondria japonica*⁹, *Halichondria panicea*¹⁰, *Agelas longissima*¹¹–¹³, *Agelas clathrodes*¹⁴, *Agelas conifera*¹⁵, *Agelas dispar*¹⁶, *Agelas sp.*¹⁷, *Axinella sp.*¹⁸, *Plakortis simplex*¹⁹ and *Agelas mauritianus*²⁰,²¹), sea star (*Stellaster equestris*²² and *Astropecten latespinosis*²³), annelid (*Marphysa sanquinea*²⁴) and red algae (*Corallina pilulifera*²⁵) and continue to attract the attention of investigators because of their structural complexity and wide range of biological activities, e.g., coronary vasodialators, antihypertensive and histidine carboxylase inhibitors,⁷,⁸ cytotoxic,¹⁷,²³ immunosuppressive properties,¹⁹ antitumour and immunostimulatory,²⁰,²¹ active ingredients for the treatment of AIDS and HIV-related diseases²⁶ and inhibited the entry of HIV-I in neural cell line²⁷.

Results and discussion

The sea mouse *Chloeia parva* was crushed mechanically and extracted with CH₂Cl₂ followed by CH₂Cl₂-MeOH (1 : 1). The CH₂Cl₂ and CH₂Cl₂-MeOH (1 : 1) extracts were then subjected separately to column chromatography with silica gel and the residues from different fractions obtained therefrom were then applied to preparative tlc. The CH₂Cl₂-MeOH (1 : 1) extract of the organism afforded mainly galactocerebrosides (1), *Rf* ~0.3 in CHCl₃-MeOH (90 : 10), [α]D²⁵ ~1.4° (c 0.96, MeOH) in about 0.00035% yield (wet weight) while the CH₂Cl₂ extract yielded 1-O-acylglycerol (2) together with other ubiquitous compounds viz., ceramide (3), 1-O-alkylglycerol (4), cholesterol (5) and fatty acid methyl ester (FAME, 6). These compounds have been characterized from chemical studies, detailed NMR spectral analyses of both parent materials and derived products as well as from gas chromatographic analyses of appropriate derivatives for establishing alkyl chain lengths.

**Galactocerebroside**: The UV spectrum of the component 1 in aldehyde free ethanol showed no characteristics absorption for conjugated chromophore. The IR spectrum of the material displayed absorption bands for the presence of -OH and -NHCO- functions and methylene units. The detailed 1D (¹H-, ¹³C-, homodecoupling) and 2D (COSY and XHCORR)
NMR experiments of the metabolite and its pentaacetate were in consonance with formulations 1 and 7 respectively. The $^1$H spectrum of the component 1 in CDCl$_3$-CD$_3$OD displayed a signal at $\delta$ 5.59 (1H, d, $J$ 6.5 Hz, very slowly exchangeable by D$_2$O) for an amide proton (CONH-) that was coupled to a multiplet signal at $\delta$ 3.93 (1H) for an amide proton (CONH-) that was coupled to a multiplet signal at $\delta$ 3.93 (1H). The spectrum also showed few signals in the 3.3-4.2 ppm region and most of them were for the protons on oxygenated carbons as in a sugar moiety of a glycoside. Besides, the $^1$H spectrum contained the signals at $\delta$ 5.61 (1H, dt, $J$ 15.2 and 5.7 Hz) and 5.39 (1H, dd, $J$ 15.2 and 7.3 Hz) likely for the trans oriented olefinic protons, 4.15 (1H, d, $J$ 6.5 Hz) for the ketomethylene protons, 2.02 (2H, t, $J$ 7.5 Hz) for the ketomethylene protons, 1.88 (2H, m) for the allylic protons, 1.25 (huge, br.s) for a number of methylene protons of the long chain alkyl moiety and 0.74 (6H, t, $J$ 6.6 Hz) for the terminal methyls.

The component 1 in its $^{13}$C spectrum showed oxymethylene carbon resonances at $\delta$ 103.6, 73.4, 72.2, 71.2, 69.0 along with the oxymethyl carbon resonance at $\delta$ 61.6 consistent with the presence of a pyranoside unit where $\delta$ 103.6 signal was due to the anomeric carbon. Again, the spectrum also displayed another oxymethylene carbon resonance at $\delta$ 74.8, carbon signals for the 1,2-disubstituted olefinic system at $\delta$ 134.1 and 128.9, and a carbon resonance at $\delta$ 68.7 for the oxymethyl (C-1) carbon in the sphingosine basic unit. The spectrum also had carbon resonances at $\delta$ 174.3 for the amide carbonyl as well as methine carbon resonance at $\delta$ 53.4 for CH-NH function and ketomethylene (-COCH$_2$-) carbon resonance at $\delta$ 36.4. Assignments of carbon resonances were made by using substituent chemical shift parameters applicable to olefinic and alkyl system$^{28-30}$. The signals for the terminal methyl carbon and inner methylene carbons of the long aliphatic chain at $\delta$ 13.6 and 29.5 respectively were also observed in the $^{13}$C spectrum of 1.

Treatment of the component 1 with Ac$_2$O-pyridine yielded a pentaacetate 7, $R$=0.5 in CHCl$_3$. The IR spectrum of the product showed new absorption bands at 1750 and 1240 cm$^{-1}$ (for acetate function) along with reduction in intensity of the broad band at 3400-3300 cm$^{-1}$ confirmed the changeover to the acetate 7. The formation of the pentaacetate upon acetylation was indicated by the generation of five acetate methyl signals at $\delta$ 2.16, 2.07, 2.04, 2.03 and 2.00 (3H each, s) in the $^1$H spectrum of the acetate 7. Four of the proton signals that appeared at $\delta$ 3.3-4.2 region of the $^1$H spectrum of the compound 1 resonated at a downfield position by about 1 ppm while two other by about 0.5 ppm on acetylation and eventually appeared at $\delta$ 5.39 (1H, dd, $J$ 3.9 and 3.5 Hz), 5.23 (1H, dd $J$ 7.8 and 2.5 Hz),
5.15 (1H, dd, J 10.7 and 7.8 Hz), 5.00 (1H, dd, J 10.5 and 3.3 Hz), 4.13 (1H, dd, J 11.3 and 6.5 Hz) and 4.10 (1H, dd, J 11.3 and 6.9 Hz) in the $^1$H NMR spectrum of the acetate. The conjugate display of an oxymethylene proton resonance at δ 4.44 (1H, d, J 7.8 Hz) in the $^1$H spectrum pictured out the presence of O-$\beta$-D-galactosyl unit as a part of the molecule. The $^1$H spectrum of 7 also showed signals for trans oriented olefinic protons at δ 5.78 (1H, dt, J 14.9 and 6.6 Hz) and 5.37 (1H, dd, J 15.1 and 7.4 Hz), a ketomethylene proton at δ 2.19 (2H, t, J 7.2 Hz), an allylic methylene protons at δ 2.04 (m), a number of methylene protons of the linear alkyl chain at δ 1.25 (huge, br.s) and protons of terminal methyls at δ 0.88 (6H, t, J 6.9 Hz) in addition to a signal at δ 5.75 (1H, d, J 7.4 Hz, slowly exchangeable by D$_2$O) for an amide proton. The $^1$H spectrum also displayed resonances for a pair of non-equivalent geminally related oxymethyl protons at δ 3.95 (1H, dd, J 10.2 and 3.6 Hz) and 3.58 (1H, dd, J 10.2 and 4.0 Hz) and two other multiplets at δ 4.30 (1H) and 1.59 (2H).

The proton network in 7 was established by homodecoupling and $^1$H-$^1$H COSY experiments. Further, two-dimensional $^{13}$C-$^1$H correlation experiment on the acetate 7 optimized for one-bond C-H coupling allowed identification of the corresponding protonated carbon resonances correlating with the signal/s of the proton/s attached with that carbon. The results are summarised in Table 1.

Homodecoupling as well as COSY-90 experiments established that a doublet signal at δ 4.44 (1H, J 7.8 Hz) was coupled with a double-doublet signal at δ 5.15 (1H, J 10.7 and 7.8 Hz) which in turn was coupled with a double-doublet signal at δ 5.00 (1H, J 10.5 and 3.3 Hz). The latter signal was again coupled to a signal at δ 5.39 (1H, dd, J 3.9 and 3.5 Hz). The double-doublet signal at δ 5.39 (1H) was further coupled with a multiplet signal at δ 3.90 (1H) and the latter was related to each of the mutually coupled geminally coupled proton signals at δ 4.13 (1H, dd, J 11.3 and 6.5 Hz) and 4.10 (1H, dd, J 11.3 and 6.9 Hz). The aforesaid observations were in conformity with the presence of an O-$\beta$-galactopyranoside moiety in the acetate 7 as well as in the parent compound 1. It has further been noted that a signal at δ 4.30 (1H, m) was coupled to an amide proton signal resonating at δ 5.75 (1H, d, J 7.4 Hz), a pair of geminal oxymethyl proton signals at δ 3.95 (1H, dd, J 10.2 and 3.6 Hz) and 3.58 (1H, dd, J 10.2 and 4.0 Hz) and an acetoxymethylene proton signal at δ 5.23 (1H, dd, J 7.8 and 2.5 Hz). The latter signal had coupling interaction with olefinic proton signal at δ 5.37 (1H, dd, J 15.1 and 7.4 Hz) that in turn was coupled with a trans oriented olefinic proton resonating at δ 5.78 (1H, dt, J 14.9 and 6.6 Hz). The latter signal was further coupled to allylic methylene protons resonate at δ 2.04 (m). Consequently, a basic sphingosine moiety of a ceramide system viz., -OCH$_2$-CH(NHCOR)CH(OAc)CH=CHCH$_2$- was present in 7. Again, the signal at δ 2.19 (2H, t, J 7.2 Hz) coupled to a signal at δ 1.59 (2H, m) indicating the presence of a unit like -COCH$_2$CH$_2$- in the acetate 7. The formation of the pentaacetate 7 was also confirmed from the $^{13}$C NMR spectrum of 1. In the $^{13}$C spectrum there were additional methyl signals at δ 21.0 (IC), 20.7 (IC), 20.5 (2C) and 20.4 (IC) and carbonyl signals at δ 172.7, 170.2, 170.1, 169.9, 169.8 and 169.4 accountable for five acetate functions along with an amide carbonyl group. Other carbon signals of the acetate 7 appeared more or less at the similar positions to that of the parent compound 1.

The composition of the acyl part and also sphingosine basic unit were ascertained by GC analysis of suitable FAME and Alc-OTMS derivatives prepared from it. Reaction involved methanalysis of galactocerebroside (1) by refluxing with dry methanol and concentrated H$_2$SO$_4$. Subsequent work-up afforded FAME which on GC analysis over 10% DEGS column afforded the composition of the FAME and thus also of the acyl part in cerebroside 1. The acyl part was composed mainly from the fatty acids 14 : 0 (4.4%), 16 : 0 (28.0), 18 : 0 (16.8), 16 : 1u9 (9.2), 18 : 1u9 (13.6), 18 : 2u6 (2.0), 18 : 3u3 (6.3) and 22 : 2u6 (5.3). For the determination of carbon chain length of the basic sphingosine unit the acidic aqueous extract left after removal of FAME by solvent extraction was stirred with a bit of NaNO$_2$ for half an hour and then refluxed with NaIO$_4$ in nitrogen atmosphere. Usual work-up yielded a fatty aldehyde that on reduction with KBH$_4$ followed by trimethylsilylation with trimethylchlorosilane afforded trimethylsilylated alcohol (Alc-OTMS). GLC analysis of Alc-OTMS over 3% SP 2100 column indicated the presence of C$_{16}$ Alc-OTMS as almost exclusive constituent. Further computation led to the conclusion that a C$_{18}$ basic sphingosine unit was present in the novel cerebroside 1 which was incidentally a mixture of at least eight major compounds.

Ceramide, 1-O-acylglycerol and 1-O-alkylglycerol:

Further resolution of the residue from CHCl$_3$-MeOH (95 : 5) eluates of the silica chromatogram of CH$_2$Cl$_2$ extract of the organism Chloea parva by preparative tlc afforded a ceramide fraction which was identified as N-acyl-erythro-2-amino-4E, 8E-octadecadiene-1,3-diol (3). Levorotation of the ceramide fraction indicates that it belongs to the $\pm$-2(25S,3R)-erythro series$^{31,32}$. The residues from late CHCl$_3$-MeOH (95 : 5) and earlier CHCl$_3$-MeOH (90 : 10) eluates of the above chromatogram of CH$_2$Cl$_2$ extract were
Table I. $^{13}$C-$^1$H, homodecoupling and $^1$H-$^1$H correlation studies of the pentaacetate $^{7b}$

| $^{13}$C Signal position (δ) and multiplicity | $^{13}$C Signal assignment | Proton resonance (δ) showing $^{1}$J$^{13}$C-H correlation | Assignments in homodecoupling and COSY correlation |
|---------------------------------------------|---------------------------|--------------------------------------------------------|--------------------------------------------------|
| **Methyl**                                  |                           |                                                        |                                                  |
| 14 0                                       | -CH$_2$CH$_3$             | 0.88, t (6.9)                                           | -CH$_2$CH$_3$ 1.25                                |
| 20 4                                       | -OCOCH$_3$               | 2.06, s                                                 | -OCOCH$_3$                                          |
| 20 5                                       | 2 x -OCOCH$_3$           | 2.03, s, 2.04                                          | -OCOCH$_3$                                          |
| 20 7                                       | -OCOCH$_3$               | 2.07, s                                                 | -OCOCH$_3$                                          |
| 21 0                                       | -OCOCH$_3$               | 2.16, s                                                 | -OCOCH$_3$                                          |
| **Methylene**                              |                           |                                                        |                                                  |
| 22 6                                       | -CH$_2$CH$_2$CH$_3$      | 1.25 br s                                               | -CH$_2$CH$_3$CH$_3$ 0.88, 1.59, 2.04                |
| 25 7                                       | C-3'                     | 1.59, m                                                 | H$_2$-3'                                            |
| 29 3                                       | (CH$_2$)$_2$             | 1.25, br s                                              | (CH$_2$)$_2$                                         |
| 29 7                                       | (CH$_2$)$_2$             | 1.25, br s                                              | (CH$_2$)$_2$                                         |
| 31 9                                       | -CH$_2$CH$_2$CH$_3$      | 1.25, br s                                              | -CH$_2$CH$_2$CH$_3$                                 |
| 32 3                                       | C-6                      | 2.04, m                                                 | H$_2$-6                                             |
| 36 8                                       | C-2'                     | 2.19, t (7.2)                                           | H$_2$-2'                                            |
| 61 3                                       | C-6"                    | 4.13, dd (11.3, 6.9), 4.10, dd (11.3, 6.9)             | H$_4$-6", 3.90, 4.10                                 |
| 67 0                                       | C-1                      | 3.95, dd (10.2, 3.6), 3.58, dd (10.2, 4.0)             | H$_3$-1, 3.58, 4.30                                  |
| **Methylene**                              |                           |                                                        |                                                  |
| 50 7                                       | C-2                      | 4.30 m                                                  | H-2                                                 |
| 67 1                                       | C-4"                    | 5.39, dd (3.9, 3.5), 4.10, dd (10.7, 7.8)              | H-4", 3.90, 5.00                                     |
| 69 1                                       | C-2"                    | 5.15, dd (10.7, 7.8)                                   | H-2"                                              |
| 70 9                                       | C-5"                    | 3.90, m                                                 | H-5"                                                |
| 71 0                                       | C-3"                    | 5.00, dd (10.5, 3.7)                                   | H-3"                                                |
| 73 9                                       | C-3                      | 5.23, dd (7.8, 2.5)                                    | H-3                                                 |
| 101 1                                      | C-1"                    | 4.44, d (7.8)                                          | H-1"                                                |
| 124 7                                      | C-4                      | 5.37, dd (15.1, 7.4), 5.78, dd (14.9, 6.6)             | H-4                                                 |
| 136 9                                      | C-5                      | 5.78, dt (14.9, 6.6)                                   | H-5                                                 |
| **Quaternary**                             |                           |                                                        |                                                  |
| 169 4, 169 8, 169 9, 170 1, 170 2 and 172 7 |                           |                                                        | 5 x -OCOCH$_3$ and -CONH-                           |

$^{7b}$Values in parentheses indicate $^1$H-$^1$H coupling constant in Hertz (Hz)

Further fractionated by preparative tlc to afford 1-O-acylglycerol (2) and 1-O-alkylglycerol (4). Treatment of the former with Ac$_2$O-pyridine yielded the diacetate 2A and the same metabolite furnished the acetone 2B with aceto in presence of anhydrous copper sulphate. The diacetate 4A was obtained from 1-O-alkylglycerol by the action of Ac$_2$O-pyridine. The $^1$H NMR spectra and extensive homodecoupling experiments of all these compounds were in support of the structures adduced. The $^{13}$C NMR spectra lent further confirmation to the structures 2 and 4 of the metabolites isolated.

### Experimental

The crude residues were fractionated in each case by column chromatography (silica gel, 60–120 mesh, Tara Chemicals, Kolkata), preparative thin layer chromatography (silica gel, E Merck) and final purification by chromatography on a short column of silica gel (100–200 mesh, E Merck). Spots were detected by staining with iodine vapour. All chromatography experiments were monitored by micro-tlc. Gas chromatography (GC) experiments were carried out on a Hewlett Packard M5890, Series II gas chromatograph fitted with a Hewlett Packard integrator M3394A.
The UV spectra were recorded in spectral alcohol (ethanol) on a Hitachi U2000 spectrophotometer and IR spectra were examined in KBr disc on a Perkin Elmer-782 spectrophotometer. NMR (1H, 13C, 1H-1H COSY and 13C-1H XHCORR) experiments were recorded on a Bruker AM 300L supercon spectrometer equipped with ASPECT 3000 computer fitted with an array processor using programme version DISR87.1 or DISR94.1 in CDCl₃, unless otherwise stated, as solvent at 300.13 MHz for proton and at 75.47 MHz for carbon. Multiplicity of the carbon signals was determined from DEPT-135 experiments. The chemical shift values are in δ (ppm) downfield from TMS. Deutero-solvent signal served as an internal standard in carbon spectral measurements; δTMS = δ (CDCl₃) + 77.0 ppm. Standard procedures were used for two-dimensional NMR experiments. Optical rotations were measured in a Perkin Elmer M241 electronic polarimeter at 25°C.

Animal material: The marine organism (a sea mouse) Chloea parva (Phylum : Annelida, Class : Polychaeta, Family : Amphipomidae) was collected from the eastern coast of Bay of Bengal near Digha (latitude 21°37'N, longitude 87°31'30"E, which is about 180 km west of Kolkata), West Bengal and stored in a freezer until extraction.

Extraction and isolation: The raw organism (3 kg) frozen in liquid N₂ was crushed and extracted with CH₂Cl₂ (2 x 4L) and then with CH₂Cl₂-MeOH (1 : 1) (2 x 4L). Solvents were removed under reduced pressure to afford the respective extracts (~4 and 2 g, respectively).

The CH₂Cl₂ and CH₂Cl₂-MeOH (1 : 1) extracts of the organism were subjected separately to column chromatography over silica gel (60–120 mesh, 120 g) using solvents of increasing polarity. The eluates of each silica chromatogram were tested by running on a glass column (1.8 mm × 2 mm) at 196°C employing inlet temperature at 250°C, FID detector at 250° and nitrogen flow rate 30 ml per min. FAME fraction composed of methyl esters of hexadecanoic acid (palmitic acid, 31.7%), octadecanoic acid (stearic acid, 28.5%), eicosanoic acid (arachidic acid, eicosic acid, 7.1%), docosanoic acid (docosic acid, 2.5%), tetracosanoic acid (3.0%), pentacosanoic acid (5.4%) as well as the unsaturated fatty acid 18 : 1ω9-octadecenoic acid (oleic acid, 17.4%) along with traces of other saturated and unsaturated fatty acids (< 2.0% each).

Ceramide [2S,3R]-N-acyl-2-aminosphinga-4,8-diene-1,3-diol)³¹,³² (3) : Colourless amorphous mass (28 mg), [α]°D -4.1° (c 0.29, CHCl₃); Rf 0.5 in CHCl₃-MeOH (95 : 5); IR νmax 3500–3100, 2955, 2920, 2850, 1645, 1625, 1550, 1465, 1375, 1055 and 970 cm⁻¹; δH 6.32 (1H, d, J 6.2 Hz, NH), 5.75 (1H, dt, J 15.3 and 5.8 Hz, H-5), 5.55 (1H, dd, J 15.3 and 5.7 Hz, H-4), 5.42 (1H, dt, J 15.2 and 5.9 Hz, H-9), 5.38 (1H, dt, J 15.2 and 5.9 Hz, H-8), 4.30 (1H, m, H-3), 3.94 (1H, m, H-2), 3.92 (1H, dd, J 9.6 and 3.4 Hz, H-4), 3.70 (1H, brd, J 9.7 Hz, Hb-1), 2.23 (2H, t, J 7.3 Hz, H-2'), 2.10 (4H, m, H-6 and H-2'), 1.96 (2H, m, H-2'), 1.63 (2H, m, H-2'), 1.25 (huge, brs, x CH₃) and 0.78 (6H, t, J 6.2 Hz, 2 x -CH₂CH₃) (δC 14.0 (2 x CH₃), 22.6 (2 x -CH₂CH₃), 25.9 (C-3'), 29.5, 29.6 and 29.7 (x CH₂), 31.9 (2 x -CH₂CH₂CH₂CH₃), 32.1 (C-7), 32.3 (C-6), 32.5 (C-10), 36.8 (C-2'), 54.8 (C-2'), 62.4 (C-1'), 79.5 (C-4'), 86.7 (C-1), 69.0 (C-4'), 71.2 (C-2'), 72.2 (C-5'), 73.4 (C-3'), 103.6 (C-1'), 128.9 (C-4), 134.1 (C-5) and 174.3 (C-1').

1-O-Acylglycerol (2) : Colourless semi-solid mass (42 mg), Rf 0.3 in CHCl₃-MeOH (95 : 5); IR νmax 3500–3200, 2910, 2840, 1730, 1465, 1380, 1125, 1180 and 1040 cm⁻¹; δH 5.35 (0.4H, m, olefinic H), 4.18 (1H, dd, J 11.6 and 4.6 Hz, Hₙ-1), 4.14 (1H, dd, J 11.7 and 6.0 Hz, Hₙ-2), 3.92 (1H, m, H-2), 3.69 (1H, dd, J 11.5 and 3.7 Hz, Hₙ-3), 3.59 (1H, dd, J 11.5 and 6.0 Hz, Hₙ-3), 2.31 (2H, t, J 7.2 Hz, -COCH₂CH₂), 2.01 (0.8H, m, -CH₂CH(CH=CHCH₂)₂), 1.60 (2H, m, -COCH₂CH₂CH₂-). 1.25 [several, br.s, -(CH₂)₅]- and 0.87 (0.4H, m, -(CH₂)₅). δC 13.8 (CH₃), 22.6 (CH₂CH₃), 25.0 (COCH₂CH₃), 29.7 ((CH₂)₅), 31.9 (CH₂CH₂CH₃ and CH₂CH=CHCH₂), 34.3 (COCH₂), 63.7 (C-3), 65.4 (C-1), 70.6 (C-2), 129.8 and 130.1 (-0.2C each, olefinic carbons) and 174.0 (CO). Methanolation of the 1-O- acylglycerol fraction by boiling in methanol-sulfuric acid and subsequent extraction of the diluted reaction mixture with ether afforded a FAME which was resolved over a glass column (1.8 mm × 2 mm) of 10% DEGS in liquid phase supported on chromosorb W (HP) isothermally at 196°C employing inlet temperature at 250°C, FID detector at 250° and nitrogen flow rate 30 ml per min. FAME fraction composed of methyl esters of hexadecanoic acid (palmitic acid, 31.7%), octadecanoic acid (stearic acid, 28.5%), eicosanoic acid (arachidic acid, eicosic acid, 7.1%), docosanoic acid (docosic acid, 2.5%), tetracosanoic acid (3.0%), pentacosanoic acid (5.4%) as well as the unsaturated fatty acid 18 : 1ω9-octadecenoic acid (oleic acid, 17.4%) along with traces of other saturated and unsaturated fatty acids (< 2.0% each).

Galactocerebroside (1) : Colourless semi-solid mass (80 mg), Rf 0.3 in CHCl₃-MeOH (90 : 10), [α]D° -1.4° (c 0.29, CHCl₃); Rf 0.5 in CHCl₃-MeOH (95 : 5); IR νmax 3500–3100, 2920, 2855, 1640, 1620, 1540, 1465, 1455, 1370, 1070, 1035 and 900 cm⁻¹; δC 13.6 (2 x CH₃), 22.4 (2 x -CH₂CH₃), 25.7 (C-3'), 29.1 (C-7), 29.5 (x CH₃), 31.7 (2 x -CH₂CH₂CH₃), 32.3 (C-6), 36.4 (C-2'), 53.4 (C-2), 61.6 (C-6''), 68.7 (C-1), 69.0 (C-4''), 71.2 (C-2''), 72.2 (C-5''), 73.4 (C-3''), 74.8 (C-3), 103.6 (C-1''), 128.9 (C-4), 134.1 (C-5) and 174.3 (C-1').
74.4 (C-3), 129.0 (C-9), 129.3 (C-4), 131.3 (C-8), 133.4 (C-5) and 173.9 (C-1'). Methanolation of ceramide fraction by boiling in MeOH-H2SO4 and subsequent extraction of the diluted reaction mixture with ether afforded a FAME fraction composed of hexadecanoic acid (palmitic acid, 47.5%), octadecanoic acid (stearic acid, 30.3%), eicosanoic acid (arachidic acid, eicosenoic acid, 2.6%), docosanoic acid (docosanoic acid, 4.2%), tetracosanoic acid (3.9%) as well as the unsaturated fatty acid 23 : 1-tricosenoic acid (8.2%) along with traces of other saturated and unsaturated fatty acids (< 2.0% each).

1-O-Alkylglycerol (4): Colourless amorphous mass (60 mg); Rf 0.4 in CHCl3-MeOH (95: 5); IR νmax 3500–3200, 2910, 2840, 1460, 1380, 1180 and 1040 cm⁻¹; δH 5.40 (0.2H, m, olefinic methylene), 3.85 (m, H-2), 3.72 (1H, dd, J 11.3 and 3.8 Hz, H-3), 3.64 (1H, dd, J 11.4 and 5.2 Hz, H-3), 3.53 (1H, dd, J 9.0 and 3.5 Hz, Hα-1), 3.50 (1H, dd, J 9.0 and 3.2 Hz, Hβ-1), 3.46 (2H, t, J 6.6 Hz, H-20), 2.01 (0.4H, m), 1.59 (m, H2-2'), 1.25 (huge, br.s, x CH2) and 0.85 (3H, t, J 6.7 Hz, -CH2CH3); δC 14.0 (CH3), 22.6 (-CH2CH2CH3), 26.1 (C-3'), 29.5 and 29.7 (x CH2), 31.9 (CH2CH2CH3), 64.2 (C-3), 70.5 (C-1'), 71.8 (C-1') and 72.5 (C-2). The material 4 was converted to ditrimethylsilyl ether and resolved over a glass column (1.8 m x 2 mm) of 3% DEGS with subsequent acidification with aqueous HCl (1N; 20 ml) was followed by extraction with CHCl3 (3 x 25 ml). The material obtained from organic layer was purified by column chromatography to yield an amorphous pentaacetate 7 (20 mg); Rf 0.5 in CHCl3; IR νmax 3400–3300, 2955, 2920, 2845, 1750, 1680, 1505, 1465, 1370, 1240, 1170, 1070 and 1045 cm⁻¹.

Fatty acid methyl esters (6 and 6A): Colourless semi-solid mass (50 mg), Rf 0.75 in petrol-CHCl3 (20 : 80); IR νmax 2910, 2840, 1730, 1465, 1380, 1225, 1180 and 1040 cm⁻¹; δH 5.35 (0.45H, m, olefinic protons), 3.66 (3H, s, 2.80 (0.1H, m, diallylic methylene protons), 2.30 (2H, t, J 7.5 Hz, COCH2CH2), 2.01 (0.8H, CH2CH=CHCH2), 1.59 (2H, m, COCH2CH2CH3), 1.25 (huge, br.s, x CH2) and 0.88 (3H, t, J 6.6 Hz, CH3); δC 14.0 (CH3), 22.6 (CH2CH3), 25.0 (CH2CH2COOCH3), 29.5 and 29.7 (x CH2), 32.0 (CH2CH2CH3 and CH2CH=CHCH2), 34.0 (CH2COOCH3), 49.5 (OCH3) and 173.9 (CO). Gas chromatographic analysis established the material being consisted mainly of the saturated fatty acids (6): hexadecanoic acid (palmitic acid, 25.0%), octadecanoic acid (stearic acid, 16.2%), eicosanoic acid (arachidic acid, 2.0%) as well as the following unsaturated fatty acids (6A) viz., 16 : 1ω9-hexadecenoic acid (28.5%), 18 : 1ω9-octadecenoic acid (oleic acid, 16.5%), 18 : 3ω3-octadecatrienoic acid (3.4%), 20 : 4ω6-eicosatetraenoic acid (3.0%) and 22 : 4ω6-docosatetraenoic acid (2.5%) along with traces of other saturated and unsaturated fatty acids (< 2.0% each).

Acetylation of galactocerebrosides 1: Galactocerebroside 1 (25 mg) dissolved in pyridine (1 ml) was mixed with distilled Ac2O (1 ml), warmed on water bath for brief period and kept at room temperature for 24 hours. Dilution of the reaction mixture with cold H2O (50 ml) and subsequent acidification with aqueous HCl (1N; 20 ml) was followed by extraction with CHCl3 (3 x 25 ml). The material obtained from organic layer was purified by column chromatography to yield an amorphous pentaacetate 7 (20 mg); Rf 0.5 in CHCl3; IR νmax 3400–3300, 2955, 2920, 2845, 1750, 1680, 1505, 1465, 1370, 1240, 1170, 1070 and 1045 cm⁻¹.

FAME and Alc-OTMS from galactocerebrosides 1: The galactocerebroside fraction (8 mg) was refluxed with dry MeOH (2 ml) and conc. H2SO4 (2 drops) for half an hour. Then the reaction mixture was cooled, diluted with water and extracted with CHCl3 (3 x 10 ml). Usual work-up of the organic layer followed by solvent removal afforded FAME fraction that was purified by column chromatography on silica gel (100–200 mesh). Finally the purified FAME (3 mg) so produced gas chromatographed over 10% DEGS column and found to be consisted of methyl esters of 14 : 0 (4.4%), 16 : 0 (28.0), 18 : 0 (16.8), 16 : 1ω9 (9.2), 18 : 1ω9 (13.6), 18 : 2ω6 (2.0), 18 : 3ω3 (6.3) and 22 : 2ω6 (5.3) fatty acids.

The aqueous phase was treated with a bit of NaN02 and stirred for half an hour, then refluxed with NaN04.
(5 mg) for an hour under N₂ atmosphere. The reaction mixture was cooled, extracted with CHCl₃ (3 × 10 ml). Usual work-up and removal of solvent afforded a material which was as such dissolved in MeOH (1 ml) and stirred with KBH₄ (-5 mg) in the cold on a magnetic stirrer for an hour, diluted with water (25 ml) and extracted with CHCl₃ (3 × 10 ml). Usual work-up of CHCl₃ layer and removal of solvent yielded fatty alcohol (3 mg) as a colourless semi-solid mass. Rf 0.5 in CHCl₃-MeOH (99 : 1) after purification by column chromatography (silica gel, 100–200 mesh). The fatty alcohol thus produced was dissolved in 50 µl of dry pyridine to which 45 µl of hexamethyldisilizane (HMDS) and 30 µl of trimethylchlorosilane were added. The vial was vigorously shaken for 30 second and the reaction was allowed to complete at 80° for 20 minutes and the content was directly injected into the column (3% OV 17). GLC analysis of the product allowed determination of the carbon chain length (as C₁₈) of the basic sphingosine unit.

1-O-Acylglycerol diacetate : 1-O-Acylglycerol (2) (10 mg) in pyridine (0.5 ml) was treated with distilled Ac₂O (0.5 ml) and kept as such at room temperature overnight. Work-up of the reaction mixture and purification as in the preparation of 1-O-alkylglycerol diacetate yielded the diacetate 2A as a colourless amorphous mass (10 mg), Rf 0.6 (CHCl₃); IR νmax 2910, 2840, 1730, 1470, 8H cm⁻¹; δ H 5.34 (0.4H, m), 5.25 (1H, m, H-2), 4.32 (1H, dd, J 11.9 and 4.3 Hz, H₃-3), 4.27 (1H, dd, J 11.9 and 4.2 Hz, H₅-3), 4.17 (1H, dd, J 12.0 and 5.8 Hz, H₇-2), 4.13 (1H, dd, J 12.0 and 5.9 Hz, H₅-2), 2.32 (2H, t, J 7.5 Hz, H₂-2'), 2.08 (3H, s), 2.07 (3H, s), 2.02 (0.8H, m), 1.60 (2H, m), 1.25 (several, br. s) and 0.87 (3H, t, J 6.3 Hz).

1-O-Acylglycerol acetone : 1-O-Acylglycerol (2) (10 mg) was treated with dry acetone (5 ml) and anhydrous CuSO₄ (10 mg) and kept at room temperature overnight. The organic layer was directly spotted on to preparative tlc plate and developed with CHCl₃. Acetone 2B was obtained as a glassy mass (8 mg), Rf 0.7 (CHCl₃); δ H 5.34 (0.4H, m), 4.30 (1H, m, H-2), 4.17 (1H, dd, J 11.5 and 4.7 Hz, H₃-1), 4.08 (1H, dd, J 11.6 and 6.2 Hz, H₅-1), 4.07 (1H, dd, J 8.2 and 6.4 Hz, H₂-3), 3.72 (1H, dd, J 8.4 and 6.2 Hz, H₇-3), 2.34 (2H, t, J 7.6 Hz, H₂-2'), 2.00 (0.8H, m), 1.61 (2H, m), 1.43 (3H, s), 1.42 (3H, s), 1.25 (several, br. s) and 0.89 (3H, t, J 6.3 Hz).

1-O-Alkylglycerol diacetate : 1-O-Alkylglycerol (4) (5 mg) in pyridine (0.5 ml) was mixed with Ac₂O (0.5 ml) and kept as such at room temperature overnight. The mixture was acidified with aqueous HCl (1N; 10 ml) and extracted with CHCl₃ (3 × 20 ml). Usual work-up of CHCl₃ layer and solvent evaporation yielded a semi-solid mass which was purified by column chromatography yielding the diacetate 4A as an amorphous mass (4 mg), Rf 0.4 in CHCl₃-MeOH (98 : 2); ¹H NMR δ 5.18 (1H, m), 4.33 (1H, dd, J 12.0 and 3.6 Hz), 4.16 (1H, dd, J 11.9 and 6.4 Hz), 3.54 (2H, d, J 5.2 Hz), 3.43 (2H, m), 2.08 (3H, s), 2.06 (3H, s), 1.25 (br. s) and 0.85 (3H, t, J 7.2 Hz).

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References

1. A. Patra, K. K. Mandal and A. Majumdar, "Abstracts, Fifteenth National Symposium on Organic Chemistry", Centre of Advanced Studies on Natural Products, Department of Chemistry, University of Calcutta, 1994.

2. A. Patra, K. K. Mandal and A. Majumdar, "Abstracts, National Symposium on Recent Advances in Structure, Synthesis and Function of Biomolecules", Bose Institute, Kolkata, 1999.

3. A. Patra, "Abstracts : 37th Annual Convention of Chemists", Indian Chemical Society, Hardwar, 2000.

4. K. K. Mandal, Ph.D. (Sc.) Thesis, University of Calcutta, 2001.

5. D. J. Faulkner, Nat. Prod. Rep., 2002, 19, 1 and references cited therein; J. W. Blunt, B. R. Copp, M. H. G. Munro, P. T. Northcote and M. R. Prinsep, Nat. Prod. Rep., 2003, 20, 1.

6. U. Venkannababu, S. P. S. Bhandari and H. A. Garg, Liebigs Ann., 1997, 1245.

7. T. Nakagawa, M. Endo and M. Nishihama, Jpn. Kokai Tokyo Koho JP : 61, 57, 594 (86 57594) (1986) (Chem. Abstr., 1994, 105, 108506).

8. M. Endo, M. Nakagawa, Y. Hamamoto and M. Ishihama, Pure Appl. Chem., 1986, 58, 387.

9. A. Hayashi, Y. Nishimura and T. Matsubara, Biochem. Biophys. Acta, 1991, 1083, 179.

10. D. G. Nagle, W. C. McClatchey and W. H. Gerwick, J. Nat. Prod., 1992, 55, 1013.

11. F. Cafieri, E. Fattorusso, Y. Mahajnah and A. Mangoni, Liebigs Ann. Chem., 1994, 1187.

12. F. Cafieri, E. Fattorusso, A. Mangoni and O. Taglialatela-Seifati, Gazz. Chim. Ital., 1996, 126, 711.

13. F. Cafieri, E. Fattorusso, A. Mangoni and O. Taglialatela-Seifati, Liebigs Ann., 1995, 1477.
14. V. Costantino, E. Fattorusso and A. Mangoni, *Liebigs Ann.*, 1995, 1471.
15. V. Costantino, E. Fattorusso and A. Mangoni, *Liebigs Ann.*, 1995, 2133.
16. V. Costantino, E. Fattorusso, A. Mangoni, M. Di Rosa, A. Ianaro and P. Maffià, *Tetrahedron*, 1996, 52, 1573.
17. G. R. Pettit, J. Xu, D. E. Gingrich, M. D. Williams, D. L. Doubek, J. -C. Chapuis and J. M. Schmidt, *J. Chem. Soc., Chem. Commun.*, 1995, 915.
18. V. Costantino, E. Fattorusso, A. Mangoni, M. Aknin and E. M. Gaudon, *Liebigs Ann. Chem.*, 1994, 1181.
19. V. Costantino, E. Fattorusso, A. Mangoni, M. Di Rosa and A. Ianaro, *J. Am. Chem. Soc.*, 1997, 119, 12465.
20. T. Natori, Y. Koezuka and T. Higa, *Tetrahedron*, 1993, 34, 5591.
21. T. Natori, M. Morita, K. Akimoto and Y. Koezuka, *Tetrahedron*, 1994, 50, 2771.
22. R. Higuchi, Y. Harano, M. Mitsuyuki, R. Isobe, K. Yamada, T. Miyamoto and T. Komori, *Liebigs Ann. Chem.*, 1996, 593.
23. R. Higuchi, S. Matsumoto, M. Fujita, T. Komori and T. Sasaki, *Liebigs Ann.*, 1995, 545.
24. N. Noda, R. Tanaka, K. Miyahara and T. Kawasaki, *Tetrahedron Lett.*, 1992, 33, 7527.
25. R. Ishida, H. Shirahama and T. Matsumoto, *Chem. Lett.*, 1993, 9, 229.
26. N. Washida, K. Fuji, N. Yoshida, S. Yanagidaira, G. Hanagata and T. Kobayashi, *Jpn. Kokai Tokyo Koho JP*: 02, 250, 834 (90 250834) (*Chem. Abstr.*, 1991, **114**, 108968).
27. K. Yamada, Y. Harada, T. Miyamoto, R. Isobe and R. Higuchi, *Chem. Pharm. Bull.*, 2000, 48, 157.
28. G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance Spectroscopy for Organic Compounds", Wiley Interscience, New York, 1972.
29. J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, 1972.
30. F. W. Wehrli, A. P. Marchand and S. Wehrli, "Interpretation of Carbon-13 NMR Spectra", 2nd. ed., John Wiley, New York, 1988.
31. A. Majumdar, Ph.D. (Sc.) Thesis, University of Calcutta, 1997.
32. J. Shin and Y. Seo, *J. Nat. Prod.*, 1995, 58, 948.