Chemistry of Natural Compounds, Bioorganic, and Biomolecular Chemistry

Glycosphingolipid with a branched sphingosine base from soft corals from the Andaman Islands

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New cerebroside with the structure of 1-(β-D-glucopyranosyloxy)-2-*N*-(2-hydroxyacyl)-*erythro*-2-amino-9-methyloctadeca-4*E*,8*E*-dien-3-ol (**1**) was isolated from the soft coral *Cladiella* sp. collected on the seaboard of the Andaman Islands (Indian Ocean). The acyl groups in compound **1** were identified as residues of D-2-hydroxy-$C_{20:0}$, -$C_{21:0}$, and -$C_{22:0}$ acids.

Key words: soft coral, *Alcyonaceae*, *Cladiella*, glycosphingolipids, cerebrosides, ^{1}H and ^{13}C NMR spectra.

Sea invertebrates serve as a rich source of new glycosphingolipids and often contain these compounds in large amounts comparable only with their content in human brain.[1] The number of glycosphingolipids isolated from sea organisms is rapidly increasing, many cerebrosides of sea origin exhibiting antitumor properties.[2—4]

As part of our continuing studies of natural compounds present in sea organisms from the Andaman Islands,[5] we isolated and structurally studied cerebroside **1**. This compound was found in a soft coral (the class *Anthozoa*, the order *Alcyonaceae*) identified as *Cladiella* sp. and was isolated by multiple column chromatography on silica gel.

The IR spectrum of compound **1** (KBr) has absorption bands at 3300—3400 (hydroxy groups), 1640 and 3250 (amide), and 1450, 2850, and 2900 cm^{-1} (the aliphatic chain). The mass spectrum (MALDI-TOF) of **1** shows a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 806.65 for the major component, which corresponds to the molecular formula $C_{45}H_{85}O_9N$. Two associated homologs give the corresponding peaks at *m/z* 820.68 and 834.69. The ^{13}C and ^{1}H NMR spectra of solutions of compound **1** in $CDCl_3$ or in a $CDCl_3$—CD_3OD mixture are indicative of the cerebroside nature of the compound isolated. In the ^{13}C NMR spectrum (CD_3OD), the polymethylene fragments give an intense signal at δ 30.8—31.2, while the terminal

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methyl groups give a signal at δ 14.9 (q). The signal of the amide carbonyl group at δ 177.6 (s) and the signal of the C<u>H</u>—NHCO fragment at δ 55.0 (d) are indicative of the presence of the acylated amino group. The signal of the hemiacetal carbon atom at δ 105.1 (d) and eight signals at δ 62.3—77.8 belonging to the carbon atoms bound to the oxygen atoms are evidence for the presence of the glycosyl residue bound to the sphingosine base. Finally, the presence of one disubstituted and one trisubstituted double bonds in the compound under study is confirmed by signals at δ 137.2 (s), 135.1 (d), 131.5 (d), and 125.2 (d). One of the substituents at the trisubstituted double bond proved to be the methyl group (δ 16.3, q).

The ^{1}H NMR spectral data confirm the presence of all the above-mentioned groups and fragments in compound **1**. This spectrum has a signal of the terminal methyl groups of the sphingosine base and of the fatty-acid residue at δ 0.89 (t), a signal of the polymethylene fragments at δ 1.27 (br.s), signals of the allylic methylene groups at δ 1.96 (t) and δ 2.06 (m), signals for the protons of the C<u>H</u>—O and C<u>H$_2$</u>—O groups at δ 3.20—4.50, and signals for the olefinic protons at δ 5.08—5.81. From the spectrum it is evident that branching at the ends of the aliphatic chains in the sphingosine and fatty-acid residues is absent. The characteristic signal at δ 4.27 (d, J = 7.7 Hz) is indicative of the presence of the monosaccharide unit bound to the remaining portion of the molecule through the 1,2-*trans*-glycoside bond. The ^{1}H NMR spectrum of compound **1**, unlike those of other cerebrosides, has a signal of the olefinic methyl group at δ 1.68 (br.s), which is evidence for the presence of methyl branching either in the sphingosine base or in the fatty-acid fragment.

Methanolysis of the resulting cerebroside afforded a mixture of methyl esters of fatty acids identified by TLC and GLC-MS as methyl esters of 2-hydroxy-$C_{20:0}$, -$C_{21:0}$, and -$C_{22:0}$ acids (in a ratio of 45 : 15 : 40). The mass spectra of these derivatives have molecular ion peaks at m/z 342, 356, and 370, respectively. Based on the specific rotation of the mixture of methyl esters ($[\alpha]_D$ −2.0), they were assigned to the D series.[6]

Treatment of cerebroside **1** with an Ac_2O—Py mixture at ~20 °C for 24 h gave peracetate **1a**, which, unlike the starting compound, is readily soluble in organic solvents. Compound **1a** was studied in more detail by ^{1}H NMR spectroscopy (500 MHz) and by the COSY, HMQC, and HMBC experiments (Table 1).

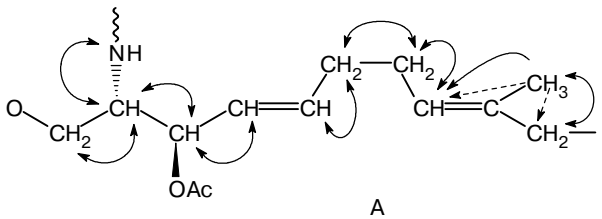
The sequences of the protons in the fragment A involving the C(1)—C(10) atoms and in the fragment B including the C(1″)—C(6″) atoms were established by NMR analysis. It was revealed that the 8(9)-double bond adopts the *E* configuration taking into account the upfield position of the signal of the C(9)<u>Me</u> methyl group typical of trisubstituted *E*-olefins.[7] Another double bond also has the *E* configuration as follows from the vicinal spin-spin coupling constant of the corresponding protons ($J_{4,5}$ = 15.3 Hz).

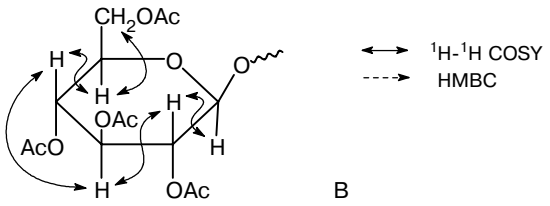
Taking into account that the chemical shifts and the spin-spin coupling constants in the ^{1}H NMR spectrum of the compound under study are similar to the corresponding values for the known 2,3-*erythro*-cerebroside peracetate isolated from the sponge *Hymeniacidon*

Table 1. Data of ^{1}H (500 MHz) and ^{13}C (75 MHz) NMR spectroscopy for cerebroside peracetate **1a** ($CDCl_3$)

Assignment[a]	δ_H, J/Hz	δ_C	Assignment[a]	δ_H, J/Hz	δ_C[b]
1	3.60 (dd, J = 10.2, 4.3); 3.93 (dd, J = 10.2, 3.9)	67.6 (t)	19	1.57 (d, J = 1.3)	16.4 (q)
2	4.30 (m)	54.1 (d)	NH	6.31 (d, J = 9.1)	—
3	5.31 (t, J = 7.3)	74.4 (d)	2′	5.14 (dd, J = 7.2, 4.6)	73.5 (d)
4	5.40 (dd, J = 15.3, 7.3)	125.0 (d)	3′	1.78 (m)	32.3 (t)
5	5.82 (m)	137.4 (d)	4′—(ω′ − 3)[c]	1.29 (m)	29.5 (t)
6	2.10 (m)	27.8 (t)	ω′ − 2[c]	1.29 (m)	32.3 (t)
7	2.10 (m)	33.0 (t)	ω′ − 1[c]	1.29 (m)	23.1 (t)
8	5.08 (m)	123.3 (d)	ω′ [c]	0.87 (t, J = 7.0)	14.5 (q)
9	—	136.6 (s)	1″	4.47 (d, J = 7.9)	101.0 (d) [101.6]
10	1.94 (br.t, J = 7.6)	40.1 (t)	2″	4.95 (dd, J = 9.5, 7.9)	71.6 (d) [71.3]
12	1.35 (m)	28.4 (t)	3″	5.18 (t, J = 9.5)	73.1 (d) [72.9]
13—15	1.29 (m)	29.5 (t)	4″	5.07 (t, J = 9.5)	68.7 (d) [68.5]
16	1.29 (m)	32.3 (t)	5″	3.68 (m)	72.4 (d) [71.9]
17	1.29 (m)	23.1 (t)	6″	4.14 (dd, J = 12.3, 2.3); 4.23 (dd, J = 12.3, 4.7)	62.2 (t) [62.1]
18	0.87 (t, J = 7.0)	14.5 (q)	OAc	1.99, 2×2.01, 2.03, 2.09, 2.17 (all s)	20.9—21.4 (quintet); 169.6, 169.7, 170.0, 170.1, 170.15 (all s)

[a] The numbers of the H or C atoms are given.
[b] The ^{13}C NMR spectrum of methyl-β-D-glucopyranoside peracetate is given in brackets.
[c] The terminal atoms of the chain of the acyl substituent are labelled as ω′, ω′ − 1, ω′ − 2, and ω′ − 3 (*e.g.*, for $C_{20:1}$ fatty acid − C(20), C(19), C(18), and C(17), respectively).





assimilis,[8] the relative stereochemistry at the C(2) and C(3) atoms can be considered as *erythro*. Based on the results obtained, the sphingosine base in compound **1** was identified as *erythro*-2-amino-9-methyloctadeca-4*E*,8*E*-diene-1,3-diol. The absolute configuration of this compound remains to be established.

Based on the results of acid hydrolysis of the mixture of methyl glycosides obtained upon methanolysis, the NMR spectral data, and the results of measurements of the optical rotation, the monosaccharide unit in cerebroside **1** was identified as D-glucose.

Analysis of the spectral data for compound **1a** provided support for this conclusion. Actually, the chemical shifts and the spin-spin coupling constants for the protons and the chemical shifts of the carbon atoms of the monosaccharide unit in the NMR spectra are identical with the corresponding values for methyl-β-D-glucopyranoside peracetate (see Table 1).

The fact that the glucose residue is bound at position 1 of the sphingosine base follows from the NMR spectra, in particular, from the NOE experiments. Thus, NOE on the H(1) proton was observed at δ 3.60 on irradiation of the signal of the H(1′) proton at the anomeric carbon atom. Based on our results, it was established that cerebroside isolated occurred as a mixture of 2-(2-hydroxydodecasanoylamino)-, 2-(2-hydroxyuncosanoylamino)-, and 2-(2-hydroxyeicosanoylamino)-1-(β-D-glucopyranosyloxy)-*erythro*-9-methyloctadeca-4*E*,8*Z*-dien-3-ols. Its structure can be described by general formula **1**.

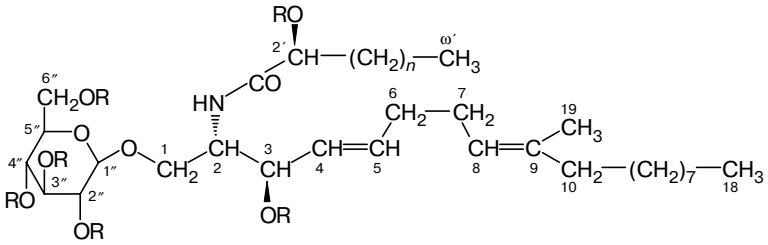
Cerebroside **1** was also found in one *Cladiella* sp. specimen and in a *Sinularia* sp. specimen collected in the same region.

Previously,[9] related β-D-glucoside containing the same branched sphingosine base was isolated from *Schizophyllum commune* as a compound inducing maturation of carposome of this fungus. However, unlike cerebroside isolated in this study, glycosphingolipid from *S. commune* contains 2-hydroxypalmitic acid as the major fatty-acid component. Similar cerebroside from the sea anemone *Metridium senile*[10] also differs from **1** by the lengths of the fatty-acid residues. The major aliphatic acids involved in this cerebroside were identified as D-2-hydroxy-$C_{16:0}$ and -$C_{20:0}$ acids.

Therefore, cerebroside isolated by us belongs to a group of glycosphingolipids containing the sphingosine base branched at position 9, which are very rare in occurrence, and differs form other representatives of this group by the acid-fatty composition.

Experimental

The melting points were determined on a Boetius stage. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-250 spectrometer (250 and 62.9 MHz, respectively) and on a Bruker DPX-300 spectrometer (300 and 75 MHz, respectively) with Me_4Si as the internal standard. In addition, the ^{1}H NMR spectra were measured on a Bruker DRX-500 spectrometer at 500 MHz. The optical rotation was measured on a Perkin—Elmer 141 polarimeter. The laser desorption mass spectra (MALDI-TOF) were obtained on a Bruker Biflex III mass spectrometer using an N_2 laser (337 nm). Specimens of the compounds were dissolved in MeOH (1 mg mL^{-1}). Analysis was carried out using aliquots (1 μL) with 2,5-dihydroxybenzoic acid as the matrix. TLC was carried out on glass plates with a fixed silica gel L layer (300 mesh, Chemapol). GLC was performed on a Perkin—Elmer Sigma 2000 gas chromatograph equipped with a capillary column (25 m × 0.2 mm) packed with OV-101 at 100—250 °C (5 deg min^{-1}) using helium as the mobile phase. The GLC-mass spectrometric analysis was performed on an LKB-9021 instrument equipped with a capillary column (25 m × 0.32 mm) packed with SE-54 at 110—250 °C (5 deg min^{-1}). Helium was used as the mobile phase; the ionizing voltage was 70 eV.



R = H (**1**), Ac (**1a**)

n = 17—19

Biological material. Specimens of soft corals were collected by divers at a depth of 5—10 m on the seaboard of the Andaman Islands (the Hori and Diglipur islands, latitude 13°20′ N, longitude 93°02′ E) on March 1993. A specimen with typical morphological features was placed in a plastic container with EtOH and was used for subsequent identification. The identification of the animals was performed by Dr. A. B. Malyutin (the Institute of Sea Biology of the Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia). The specimens are stored (No. MF-VA/39) at the Department of Organic Chemistry of the Andra University (India).

Isolation of cerebroside 1. Crushed soft corals (the weight of the animals was 1.78 kg) were exhaustively extracted with a 4 : 1 $CHCl_3$—MeOH mixture at ~20 °C. The combined extracts were concentrated to dryness *in vacuo* and the residue (20 g) was repeatedly chromatographed on a column with silica gel (500 g, 100—200 mesh, Acme) using the AcOEt—hexane system (10 : 1 → 1 : 1). Cerebroside **1** was obtained in a yield of 17 mg, m.p. 208—210 °C. The product (obtained after column chromatography using the 4 : 1 AcOEt—hexane mixture) is poorly soluble in $CHCl_3$ and in most of other organic solvents. MS (MALDI) for the major component: found *m/z* 806.65 $[M + Na]^+$, $C_{45}H_{85}O_9NNa$. Calculated M + Na = 806.61. Peracetate **1a** was obtained upon treatment of compound **1** with a 1 : 1 Ac_2O—Py mixture (24 h, ~20 °C) as a colorless amorphous powder, $[\alpha]_D$ −23.8 (*c* 4.40, $CHCl_3$).

In addition, ethyl arachidate (0.75 g), new sterol 4α,23-dimethyl-24-ethylcholestan-3β-ol (0.5 g), batyl alcohol (1.25 g), 24-methylenecholest-5-ene-3β,16β-diol (45 mg), 24-methylene-3-*O*-α-L-fucopyranosylcholest-5-ene-3β,16β-diol, and new glycoside, *viz.*, 2-hydroxy-3-(octadecyclooxy)propyl-β-D-arabinopyranoside[11] (55 mg), were isolated.

Determination of the fatty-acid composition of cerebroside 1. Cerebroside (1 mg) was heated in a sealed tube with a 1 *M* HCl solution in 82% MeOH (1 mL) at 100 °C for 7 h. The reaction mixture was extracted with hexane (3×1 mL). The hexane layer was separated and passed through a small column (0.2×2 cm) with silica gel. The column was washed with a 1 : 1 hexane—AcOEt mixture (10 mL). The eluates were combined, concentrated *in vacuo* ($[\alpha]_D$ −4.0 (*c* 1.50, $CHCl_3$)), and analyzed by GLC and TLC. The TLC analysis on Sorbfil plates (the 2 : 1 hexane—$CHCl_3$ mixture) showed a spot with R_f 0.12, which is smaller than that of the standard methyl stearate (R_f 0.75). Based on the results from GLC and GLC-MS, the major components of the hexane extract were identified as D-2-hydroxy-$C_{20:0}$, -$C_{21:0}$, and -$C_{22:0}$ acids. MS (EI, 70 eV), *m/z* (I_{rel} (%)): 342 $[M]^+$ (60), 324 (5), 310 (7), 297 (8), 283 (90), 264 (7), 145 (15), 127 (20), 111 (60), 97 (100), 83 (100), 71 (70), 57 (100); 356 $[M]^+$ (70), 338 (1), 324 (4), 311 (7), 297 (86), 127 (35), 111 (70), 97 (95), 83 (90), 57 (100); 370 $[M]^+$ (80), 352 (2), 338 (5), 325 (6), 311 (80), 292 (8), 159 (7), 145 (15), 127 (20), 111 (50), 97 (90), 83 (85), 71 (80), 57 (100).

The aqueous-methanolic layer was treated with a 2 *M* KOH solution to pH 9 and the sphingosine bases were extracted with $CHCl_3$ (3×1 mL). The solution was neutralized with a 2 *M* HCl solution and concentrated to dryness *in vacuo*. The residue was heated with a 2 *M* HCl solution (1 mL) at 100 °C for 1 h. Then the mixture was again concentrated to dryness. Glucose was identified by GLC (comparison with a reference compound) as the corresponding aldonitrile peracetate, which was prepared according to a known procedure.[12] Based on the optical rotation of crude glucose ($[\alpha]_D$ +46.0 (*c* 1.05, H_2O)), the compound was assigned to the D series.

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References

1. L. R. Bjorkman, K.-A. Karlsson, and K. Nilson, *Comp. Biochem. Physiol.*, 1972, **43B**, 409.
2. Y. Hirsh and Y. Kashman, *Tetrahedron*, 1989, **45**, 3897.
3. T. Natori, Y. Koezuka, and T. Higa, *Tetrahedron Lett.*, 1993, **34**, 5591.
4. T. Natori, M. Morita, K. Akimoto, and Y. Koezuka, *Tetrahedron*, 1994, **50**, 2771.
5. V. Anjaneyulu, T. N. Makarieva, S. G. Ilyin, A. S. Dmitrenok, P. Radhika, P. V. Subbarao, V. V. Nesterov, M. Yu. Antipin, and V. A. Stonik, *J. Nat. Prod.*, 2000, **63**, 109.
6. D. H. S. Horni and M. Y. Y. Pretorius, *J. Chem. Soc.*, 1954, 1460.
7. F. W. Wehrli and T. Wirthlin, *Interpretation of Carbon-13 NMR Spectra*, 2nd ed., Willey, Chichester, 1983.
8. T. N. Makar′eva, V. A. Denisenko, V. I. Svetashev, M. V. Vysotskii, and V. A. Stonik, *Khim. Prirod. Soedin.*, 1989, 634 [*Chem. Nat. Compd.*, 1989 (Engl. Transl.)].
9. G. Kawai and Y. Ikeda, *Biochim. Biophys. Acta*, 1983, **754**, 243.
10. K.-A. Karlsson, H. Leffler, and B. E. Samuelson, *Biochim. Biophys. Acta*, 1979, **574**, 79.
11. V. Anjaneyulu, P. V. Subba Rao, P. Radhika, H. Laatsch, and R. N. Asolkar, *Indian J. Chem.*, 2001, **40B**, 405.
12. V. M. Easterwood and B. S. L. Heef, *Svensk. Pappers*, 1969, **23**, 768.

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