CEREBROSIDES OF THE FAR EASTERN SPONGE Hymeniacidon assimilis

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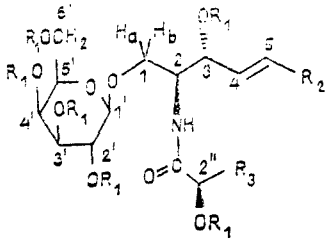
Cerebrosides have been isolated from chloroform–ethanol extract of the sponge Hymeniacidon assimilis. Their structures have been established with the aid of ^{1}H NMR spectroscopy, acid hydrolysis, and ozonolysis as the β-galactosides of the C_{16}-, C_{17}-, and C_{18}-erythro-Δ^4-sphingenines of normal, iso, and anteiso structures acylated in the amino groups with C_{21}-, C_{22}-, and C_{23}-α-hydroxy acids. The cerebrosides from H. assimilis differ in structure from the corresponding compounds isolated previously from sponges.

Although cerebrosides have been detected in glycolipid fractions of many marine organisms [1], there have been few structural studies of such substances isolated from marine invertebrates. It has been shown that in invertebrates cerebrosides may be synthesized that are either rarely found in terrestrial organisms or were previously completely unknown [2-4]. In addition, in some marine cerebrosides an antihistamine action has been detected [5], and in the ceramides related to them an antibacterial activity [6].

Continuing a study of the secondary metabolites of sponges, we have isolated cerebrosides from the Far Eastern sponge Hymeniacidon assimilis and have established their chemical structures.



I: R_1 = H; R_2 = A; R_3 = B

II: R_1 = Ac; R_2 = A; R_3 = B

A = $-(CH_2)_{8-12}-CH_3$;

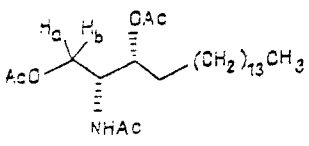
$-(CH_2)_{8-11}-CH(CH_3)-CH_3$;

$-(CH_2)_{8,9}-CH(CH_3)-C_2H_5$

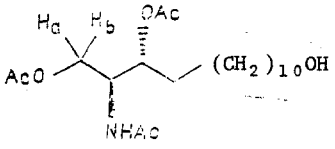
B = $-(CH_2)_{12,13;17-22}CH_3$;

$-(CH_2)_{10-12;15-19}-CH(CH_3)-CH_3$;

$-(CH_2)_{9-11;15-17}-CH(CH_3)-C_2H_5$



III



IV

A cerebroside fraction giving a single spot on TLC (general formula I) was obtained from a chloroform extract of the sponge after repeated column chromatography on silica gel. The acid hydrolysis of (I) with 2 N hydrochloric acid gave galactose, which was identified

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TABLE 1. Fatty Acid Composition of the H. assimilis Cerebrosides*

2-hydroxy acids (as methyl esters)	Amount, %	M+	2-hydroxy acids (as methyl esters)	Amount, %	M+
i-15:0	3,7	272	n-21:0	12,4	356
ai-15:0	1,0	272	i-22:0	6,7	370
n-15:0	0,1	272	ai-22:0	1,3	370
i-16:0	2,7	286	n-22:0	42,3	370
ai-16:0	3,2	286	i-23:0	7,8	384
n-16:0	1,1	286	ai-23:0	1,1	384
i-17:0	0,3	300	n-23:0	2,3	384
ai-17:0	2,5	300	i-24:0	0,3	398
i-20:0	0,3	342	n-24:1	0,8	396
n-20:0	2,0	342	n-24:0	3,6	398
i-21:0	2,0	356	n-25:0	1,3	412
ai-21:0	1,8	356			

*Components amounting to more than 0.1% are given; i) iso; ai) anteiso; n) normal acids.

TABLE 2. ^{1}H NMR Spectrum of the Peracetate of (II)

Chemical shift, ppm				Spin-spin coupling constants, Hz			
H	δ	H	δ	H, H	J	H, H	J
CH-1a	3,62 dd	CH-1′	4,45 d	1a, 1b	−10,1	1′,2′	7,9
CH-1b	3,94 dd	CH-2′	5,15 dd	1a, 2	4,7	2′,3′	10,5
CH-2	4,32 m	CH-3′	5,00 dd	1b, 2	4,2	3′,4′	3,4
CH-3	5,31 t	CH-4′	5,38 dd	2,3	7,3	4′,5′	1,5
CH-4	5,34 dd	CH-5′	3,91 dt	2,NH	9,1	5′,6′	6,9
CH-5	5,82 dt	CH-6′	4,13 d	3,4	7,3	2″,3″	6,5
NH	6,35 d	CH_3	0,88 t	4,5	14,6		
CH-2″	5,16 t	CH_3	0,89 d				
(CH_2)	1,25 br.s						
OCH_3	1,97—2,20 s						

in the form of the corresponding aldononitrile peracetate by the GLC—MS method. Methanolysis of the cerebroside fraction led to the methyl esters of α-hydroxy acids and to sphingosine bases. Fatty acid analysis was carried out with the aid of GLC—MS. The specific rotation of this fraction ($[\alpha]_D^{20}$ 3°) permitted the assumption that the acids had the R-configuration at C-2" [6]. The results of the fatty acid analysis are given in Table 1.

As follows from the figures given, saturated α-hydroxy acids predominated in (I), the main components being the 2-hydroxy-$C_{21:0}$ and the 2-hydroxy-$C_{22:0}$ acids of normal structure, and also the 2-hydroxy-$C_{21:0}$ and 2-hydroxy-$C_{23:0}$ acids of iso structure.

In the establishment of the structure of the sphingosine bases present in the composition of (I), we used ^{1}H NMR spectroscopy with application of the NOE, spin-decoupling, and INDOR procedures. For comparison, the ^{1}H NMR spectra of the peracetates of threo- and erythro-octadecasphingamines were analyzed. We obtained the latter from a commercial preparation and assigned them to the threo- and erythro-series on the basis of literature information from the GLC behavior of the corresponding N-acetyl-1,3-bistrimethylsilyl derivatives [7].

Tables 2 and 3 give the results of the ^{1}H NMR of derivative (II) and of the model compounds (III) and (IV). With the aid of these methods, in peracetate (II) the presence was shown of the structural fragment

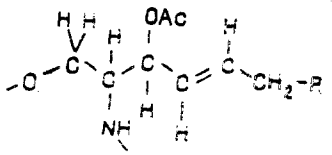

TABLE 3. ^{1}H NMR Spectra of the Model Compounds (III) and (IV)

II	Chemical shift, ppm, δ		Spin-spin coupling constants, Hz		
	III	VI	H, H	J, III	J, IV
CH-1a	4,02 dd	4,06 dd	1a,1b	−11,3	−11,6
CH-1b	4,05 dd	4.25 dd	1a, 2	6,1	3,9
CH-2	4,41 m	4,39 m	1b, 2	6,1	6,1
CH-3	5,07 dt	4,91 dt	2,3	3,4	5,5
NH	5,63 d	5,80 d	3.4	6,8	5,5
CH_3-18	0,88 t	0,88 t			
$(CH_2)_n$	1,25 br.s	1,25 br.s			
OCH_3	2,03 s	2,00 s			
	2,06 s	2,07 s			
	2,09 s	2,08 s			

TABLE 4. Composition of the Acetylated Derivatives of the Products of Ozonolysis of the Sphingosine Bases from the Cerebrosides of H. assimilis

(as its ascetate)	Amount in the mixture, %	Corresponding sphingosine base
n-C_{10}	0,5	Tetradecasphingenine
n-C_{11}	0,16	4-Pentadecasphingenine
i-C_{12}	6,91	14-Methyl-4-pentadecasphingenine
n-C_{12}	7,81	4-Hexadecasphingenine
i-C_{13}	10,0	15-Methyl-4-hexadecasphingenine
ai-C_{13}	63,29	14-Methyl-4-hexadecasphingenine
n-C_{13}	1,0	4-Heptadecasphingenine
i-C_{14}	2,50	16-Methyl-4-heptadecasphingenine
ai-C_{14}	1,06	15-Methyl-4-heptadecasphingenine
n-C_{14}	3,5	4-Octadecasphingenine
i-C_{15}	1,23	17-Methyl-4-octadecasphingenine

It was concluded that the corresponding sphingosine bases had a 4(5)-double bond with the E-configuration ($J_{4,5}$ = 14.6 Hz). The doublet signal at 4.45 ppm (J = 7.9 Hz) showed that the glycosidic bond in (II) had the β-configuration. A comparison of the SSCCs of the H-2 and H-3 protons in the spectra of all three compounds showed the erythro-configuration of the main sphingosine bases in (II). The presence in the ^{1}H NMR spectrum of (II), in addition to the triplet signal of methyl groups at 0.88 ppm, of a doublet signal at 0.89 ppm, as well, permitted the suggestion that the main sphingosine component in (I) had a methyl branch. In actual fact, the ozonolysis of (II) followed by reductive treatment of the ozonides gave the combined fatty alcohols which were identified in the form of acetates by GLC on a capillary column with OV-101. As follows from Table 4, the main sphingosine component had the structure of 14-methyl-4-hexadecasphingenine (more than 60% of the total fatty bases).

Thus, the cerebrosides from H. assimilis are β-galactosides of 4-sphingenines acylated in the amino group by α-hydroxy acids. Their main structural feature is a predominance in the sphingosine fragment of a C_{17}-fatty base with the anteiso structure. The cerebrosides from H. assimilis differ in structure from the sphingosine bases of the corresponding compounds from the sponges Chondrilla nucula [6] and Chondropsis sp. [5]. Moreover, the cerebrosides from Chondrilla nucula, in contrast to the galactosides that we have isolated, are β-glucosides.

EXPERIMENTAL

The sponge Hymeniacidon assimilis was collected on Kashevarov Bank (central part of the Sea of Okhotsk) from a depth of 147 m by beam trawl in August, 1986 during the second voyage of the Scientific Research Ship "Academician Oparin." The animals were determined by V. M. Koltun.

Specific rotations were measured on a Perkin-Elmer 141 polarimeter at room temperature. IR spectra were obtained on a Perkin-Elmer 983 instrument in KBr and in chloroform solutions.

^{1}H NMR spectra were recorded on a Bruker WM-250 instrument at 250 MHz. The signals in the NMR spectra are given in the values of the δ scale, the solvent being deuterochloroform and the internal standard tetramethylsilane.

The GLC analysis of the aldononitrile derivatives of the carbohydrates and the methyl esters of the α-hydroxy acids and also of the acetates of the alcohols was carried out on Perkin-Elmer Sigma 2000 and Shimadzu 9A chromatographs with a Shimadzu Chromatopak C-R3A data-processing system.

To analyze the peracetates of the aldononitrile derivatives of the monosaccharides we used a 3 mm × 3 m column with 3.1% of Silar-5CP on Chromaton N-AW-DMCS (0.100-0.125 mm). The carrier gas was argon (35 ml/min), and the separation temperature 230°C. The methyl esters of α-hydroxy acids and the acetates of alcohols were chromatographed on a 0.25 mm × 25 m column with the immobilized phase OV-101 using helium as the carrier gas with a flow splitter ratio of 1:60 at a separation temperature of 240°C.

The chromato-mass spectrometric analysis of the peracetates of aldononitriles and methyl esters of α-hydroxy acids was carried out on a LKB-2091 mass spectrometer coupled with a Packard 438A chromatograph, an injector of the "falling needle" type, and a capillary quartz column 25 m long with the phase SE-54 at column temperatures of 230 and 240°C, respectively, using helium as the carrier gas (1 ml/min), and at an ionizing energy of 70 eV.

For thin-layer chromatography we used type L silica gel (Czechoslovakia), 5/40 μm, with the addition as fixing agent of 5% of gypsum, and for column chromatography the 40/100 μm product. Koch–Light racemic C_{18}-sphinganine was used as standard.

The chromatographic analysis of the threo- and erythro-N-acetyl-1,3-bistrimethylsilyl derivatives of C_{18}-sphinganine and of the sphingosine bases was performed on a Pye Unicam 104 chromatograph with a 0.5 × 150 cm column containing 3% of SE-30 at 230°C.

Isolation of Cerebroside (I). An ethanol–chloroform (1:1) extract of the sponge H. assimilis was evaporated to dryness. The residue was distributed between 90% ethanol and hexane. The aqueous ethanolic layer was diluted with water to an ethanol concentration of 70% and was extracted with chloroform several times. The chloroform extracts were combined and evaporated. The residue was chromatographed repeatedly on a silica gel column in the chloroform–ethanol (8:1) system. This gave 30 mg of a colorless amorphous substance (0.02% on the dry weight of the sponge); $[\alpha]_D^{20}$ +7° (c 0.1; ethanol). IR spectrum (KBr), cm^{-1}: 3379, 2918, 2851, 1627, 1543, 1463, 1169, 1121, 1087, 969, 852, 874.

The acetylation of the cerebroside (I) was carried out with a mixture of acetic anhydride and pyridine (1:1) at room temperature for 16 h. The solution was concentrated in vacuum and the residue was chromatographed on a column of silica gel in the hexane–ethyl acetate (2:1) system. This gave the colorless amorphous substance (II). IR spectrum ($CHCl_3$), cm^{-1}: 3433, 2928, 2855, 1754, 1677, 1519, 1463, 1370, 1243, 1203, 1173, 1133, 1080. Details of the ^{1}H NMR spectrum are given in Table 2.

Acid Hydrolysis of Cerebroside (I). A mixture of 2 mg of (I) and 0.5 ml of 2 N HCl was heated at 100°C for 2 h. Then it was extracted with benzene and the aqueous layer was neutralized with Dowex resin (HCO_3^-). The resin was separated off by filtration and was washed with water. The aqueous layer and the wash-waters were combined and were concentrated in vacuum to dryness. The residue was dissolved in 0.5 ml of dry pyridine, an excess of hydroxylamine hydrochloride was added, and the mixture was heated at 100°C for 1 h. After the addition of 0.5 ml of acetic acid to the solution, the resulting mixture was heated at 100°C for another 1 h. The reaction mixture was evaporated and the residue was analyzed by the GLC and GLC–MS methods. The peracetate of the aldononitrile derivative of galactose was identified.

Methanolysis of the Peracetate of Cerebroside (II). A mixture of 13 mg of (II), 1 ml of 6 N HCl, and 3 ml of methanol was heated in a sealed tube at 100°C for 7 h. After cooling, the aqueous-methanolic layer was extracted several times with hexane. The combined hexane extracts were evaporated. This gave 4.5 mg of a mixture of acids which was analyzed by the GLC and GLC–MS methods. α-Hydroxy acids were identified (see Table 1), $[\alpha]_D^{20}$ −3° (c 0.3; $CHCl_3$).

The aqueous-methanolic layer was made alkaline with 2 N KOH to pH 9, after which it was extracted several times with chloroform. The chloroform extracts were evaporated to

dryness, giving 5.0 mg of an amorphous substance which was analyzed by the GLC and GLC–MS methods. The sum of the sphingosine bases was identified in the form of the N-acetyl-1,3-bistrimethylsilyl derivatives having characteristic peaks in the mass spectra with m/z 311 ($C_{18}\Delta^4$); 297 ($C_{17}\Delta^4$); and 283 ($C_{16}\Delta^4$) [8].

Ozonolysis of Cerebroside (I). Ozone was passed through a solution of 18 mg of (I) in a mixture of 2 ml of ethanol and 1 ml of methylene chloride for 4 h at a temperature of –60°C until a permanent blue coloration appeared. An excess of $NaBH_4$ was added to the resulting mixture and it was stirred at room temperature for 1 h. After acidification with acetic acid and evaporation with methanol, a residue was obtained which was chromatographed in the hexane–ethyl acetate (2:1) system. The resulting amorphous substance was acetylated with a mixture of acetic acid and pyridine (1:1) at room temperature for 16 h, and the reaction mixture was evaporated in vacuum to dryness. The alcohol acetates were analyzed by capillary GLC on the phase OV-101. Identification was carried out with the use of standards and by the determination of equivalent chain lengths [9]. The results of the analysis are given in Table 4.

Preparation of the threo- and erythro-N-Acetyl-1,3-bistrimethylsilyl-C_{18}-sphingenines. A solution of 10.6 mg of commercial racemic C_{18}-sphinganine in a mixture of 2 ml of acetic anhydride and pyridine (1:1) was left at room temperature for 16 h. Then the reaction mixture was evaporated to dryness. The residue was chromatographed repeatedly by preparative TLC in a fixed layer of silica gel in chloroform. This gave 5.3 mg of (III) and 5.8 mg of (IV). Details of the ^1H NMR spectra of (III) and (IV) are given in Table 3.

The isolated triacetates (III) and (IV) were dissolved in dry methanol containing 15% of ammonia, and the solutions were left at room temperature for 2 h and were then evaporated to dryness. Each of the N-acetyl derivatives so obtained was treated with 100 μl of N,O-bis-(trimethylsilyl)trifluoroacetamide (BTA), and the mixture was heated in sealed tubes at 60°C for 1 h. The derivatives so obtained were identified by GLC and GLC–MS from their retention times [7] on a column with SE-30 as threo-N-acetyl-1,3-bis(trimethylsilyl)octadecasphingenine (III) and erythro-N-acetyl-1,3-bis(trimethylsilyl)octadecasphingenine (IV).

SUMMARY

A cerebroside fraction has been isolated from the sponge Hymeniacidon assimilis. The cerebrosides obtained consisted of β-galactosides of erythro-4-sphingenines of normal, iso, and anteiso structures acylated in the amino group with α-hydroxy acids having chain lengths mainly of from 21 to 23 carbon atoms.

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