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NEW GLYCOSPHINGOLIPIDS FROM MARINE ORGANISMS

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Abstract - The structure of several ceramides and cerebrosides, from three marine sponges and a soft coral collected in the Red Sea, are reported. Specifically, the structure determination of ptiloceramide, halicerebroside A and amphicerebrosides B-F was achieved following suitable degradations and NMR measurements.

The first recognition of glycolipids in a variety of marine invertebrates came in the early 1970's.[1] It was suggested that these glycolipids contain free and acylated cerebroside-like lipids. At the same time Karlsson[2] determined the structure of a cerebroside isolated from the sea star Asterias rubens, and it has been shown that the latter compound has a more complex structure than the relatively simple brain cerebrosides.[3] However, very few reports on the exact structures of other marine glycosphingolipids have been made until most recently.[4-7]

The latter reports of the isolation and characterization of new glycosphingolipids from the sea anemone Anemona sulcata,[4] the starfish Acanthaster planci,[5] the sponge Chondropsis sp.[6] and from the sea hare Aplysia kurodai[7] have led us to publish our results on related compounds.

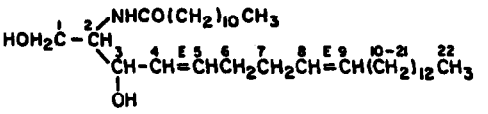
Spectroscopic (mainly NMR) as well as chromatographic methods[1] have shown that quite a few of the marine organisms we have investigated (sponges, tunicates and soft corals) contain ceramides and cerebrosides. Thus, a sphingosine derivative (1, Fig.1) was separated from Heteroxenia gardaquensis collected at the entrance to the Gulf of Suez.[8] This compound was found to be erythro-docosasphinga-4,8-dienine, identical with the most recently reported metabolite of Anemona sulcata.[4]

Another ceramide was isolated from Ptilocaulis spiculifer. After several chromatographies on Sephadex LH-20 eluted with hexane-chloroform 3:7 or chloroform-methanol 1:1, we isolated from the ethyl acetate extract of this sponge a fraction exhibiting antifungal activity (Candida albicans, MIC=12.5 μg/ml). Final purification of the active components, named ptiloceramides (2a), was achieved following acetylation and chromatography of the tetraacetyl derivatives (2b) on a silica gel column. This mixture of tetraacetyl ptiloceramides (2b) exhibited in the mass spectra (DCI, isobutane) a series of peaks at m/z: 852, 838, 824, 810 and 796(MH^+), suggesting five homologues (Fig.1) which were not further separated.

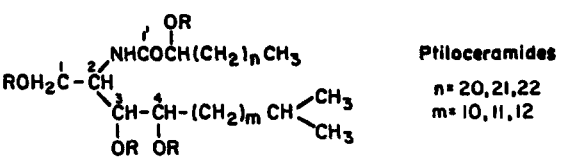
The sequence of the C-1 to C-5 moiety in 2b, which includes three hydroxyls and an amide functionality (Fig.1), was established by a COSY experiment which correlated all the corresponding protons, including the NH signal (δ_H 6.60-7.40ppm; a region which is characteristic for the NH in glycosphingolipids).

The multiplicity and chemical shift of the fourth CHOAc group (δ_H 5.11m;δ_C 74.0d) suggested it to be α to the amide carbonyl (on C-2"). Indeed, acid hydrolysis of ptiloceramide (2a, 10%HCl in MeOH) afforded a mixture of three n-C_{23}-C_{25} α-hydroxy methylesters which were identified by their mass spectrum (see Experimental). Obtaining of normal α-hydroxy esters was in full agreement with the isopropyl terminus on the isolated sphingosine portion (C_1-$C_{17(19)}$).[9] As no separation of the homologues has been undertaken, the m to n combinations were not disclosed. Worth mentioning is the structure of cerebroside 3, isolated by Endo from the sponge Chondropsis sp.[6], which also possesses the iso-propyl terminus as compound 2a (Fig.1).

Another sponge we have investigated, Haliclona sp., was found to be very sticky. The compound responsible for this feature was tracked down to the CH_2Cl_2-MeOH 95:5 extract which exhibited mild antitumor activity against P388 leukemia cells (84% inhibition in a concentration of 20 μg/ml). Almost 10% of this methanolic dichloromethane extract was composed of a single compound designated halicerebroside A (4a, Scheme 1). Compound 4a, which gives a highly viscous solution in chloroform, was purified on Sephadex LH-20 and TSK HW-40 columns, both eluted with chloroform-methanol.

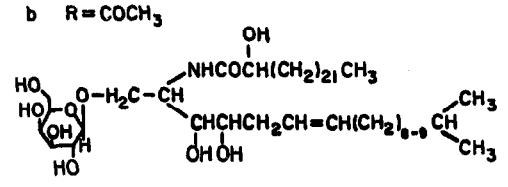


1. Erythro-docosasphinga-4,8-dienine



Ptiloceramides
n= 20,21,22
m= 10,11,12

2a. R=H
b R=COCH$_3$

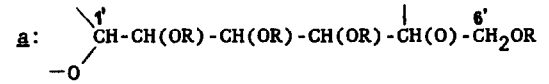


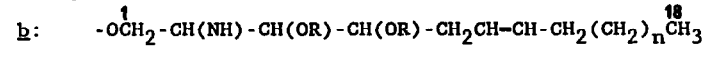
3

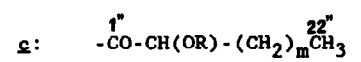
(β-galactose)

Figure 1

For NMR measurements the heptaacetate of 4a (4b) was prepared under the usual conditions (Ac_2O/Pyr., rt overnight). 2D-NMR studies of 4b, including a COSY[10], a RELAY[11] (Fig. 2) and a one bond HETCOR[12] experiments, proposed the following partial structures (a-c):

a: 

b: 

c: 

The following correlations have been observed in a COSY experiment; for a between: 1'/2', 2'/3', 3'/4', 4'/5' & 5'/6'; for b between: 1/2, 2/NH&3, 3/4, 4/5, 5/6, 6/7, 7/8,

8/9 & 17/18 and for c between: 2"/3", 3"/4", 21"/22"; and in a RELAY experiment we observed the additional following correlations between protons: for a: 1'/3', 2'/4', 3'/5', 4'/6' and for b: 1/3, 3/5, 5/7, 6/8 (see Fig.2).

Reductive ozonolysis of 4b (O_3, $NaBH_4$) furnished n-1-dodecanol (5) and compound 6 (Scheme 1). The n-dodecanol (5) confirmed the 6(7) position of the double bond, in 4, as proposed in sub-structure b, and the complete NMR analysis of segment 6 (see Experimental) determined the β-glucosyl ceramide structure.

Acid hydrolysis of 4a (4 hrs reflux in 10% methanolic HCl) afforded three compounds: $CH_3(CH_2)_{19}CH(OH)CO_2CH_3$, methyl glucopyranoside[14] and sphingosine 7 (Scheme 1). The sugar portion was chromatographically purified after acetylation and yielded mainly compund 8a. Alkaline removal of the acetate groups (10% NH_4OH in MeOH), followed by acidic cleavage of the methyl glycoside, yielded D-glucose, $[\alpha]_D$=+50° (lit. +52°)[7]. The structure of the sphingosine, erythro-octadeca-sphinga-6-enine (7), was established by its NMR spectrum and the fragmentations in the mass spectrum (see Experimental) and was found to be in full accordance with the structure of comound 6. Compound 4a has many similarities with 3 (Fig.1) and also with the most recently reported acanthacerebrosides.[5]

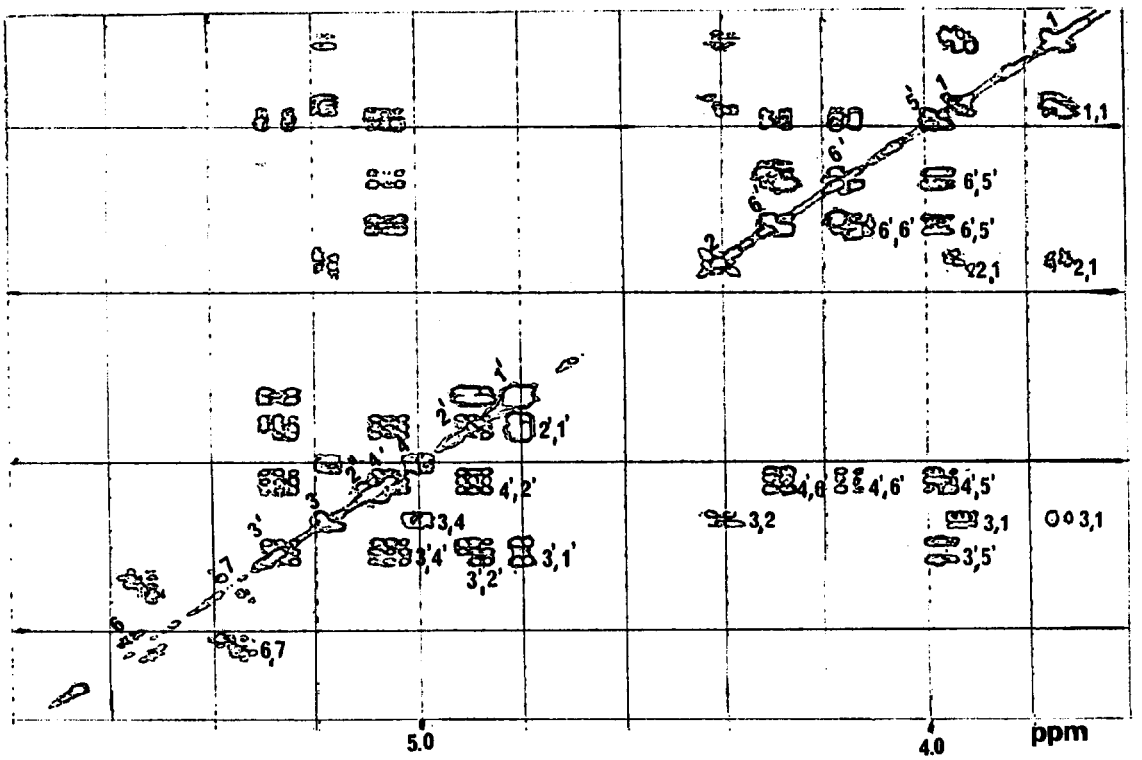
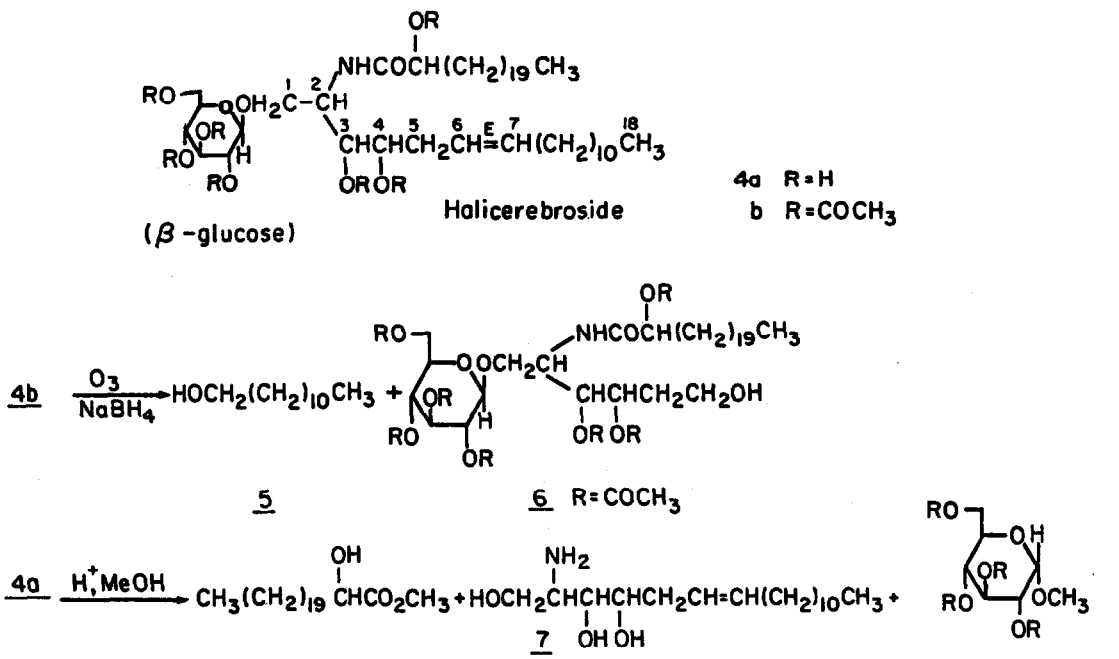


Figure 2. Partial RELAY[11] spectrum of compound 4b in d_6-acetone.

Another very sticky sponge (immediately after being taken out of the water) is Amphimedon viridis. As with the Haliclona sp., the methanolic-chloroform extract afforded a highly viscous mixture, responsible for the stickiness. To facilitate the separation of



Scheme 1. Halicerebroside A and its degradation products 8a R=H
8b R=COCH3

this mixture and for NMR structure elucidation, the crude extract was acetylated and submitted to repeated chromatographies on Sephadex LH-20 and silica gel columns.

The last two columns were found to be superior to reverse phase, cyano and amino bonded silica columns which failed to separate the mixture. Out of five acetylated compounds, designated amphicerebrosides B-F (compounds 9b-13b, Fig.3), only one (11b) was obtained in a pure state. All the others could only be obtained in an enriched form. However, by comparing and substracting the NMR spectra of the mixtures, individual spectroscopic data were obtained and their structure elucidated.

Comparison of the NMR data of acetylated amphicerebroside B (9b, Table 1) with that of hepta-acetyl halicerebroside A (4b) clearly pointed to the identity of the two long chains (C_1"-C_{22}" and C_3-C_{20}) in the two compounds and to differences in the sugar moiety. The presence of an amino-monosaccharide, suggested by the chemical shifts[13] of the latter moiety, was confirmed following acetylation of the acidic hydrolysis product 16a, vide infra, with Ac_2O in MeOH to give the mono acetamide.

Acid hydrolysis of 9b (10% HCl in MeOH, 4hr) afforded methyl 2-amino-2-deoxy-glucopyranoside (16a), compound 14 and methyl α-hydroxy-doeicosanoate (Fig.4). As before, the monosaccharide was purified after acetylation (see Experimental). The NMR spectrum of 9b clearly pointed out the β-anomeric linkage of the glycoside.

Amphicerebroside C heptaacetate (10b) differs from 9b only in the location of the double bond which is here in the 5(6) rather than the 6(7) position in 9b. This was determined by the NMR spectra which included a COSY experiment.

Compound 11b was, as mentioned above, the only compound that was completely purified. Confirmation of its structure, the dihydro derivative of either 9b or 10b, was obtained upon catalytic hydrogenation of the latter compounds to afford 11b.

Table 1. ^{1}H and ^{13}C NMR data of amphicerebroside B heptaacetate (9b)[a].

C No	δ_C(m)	δ_H[b](m, J in Hz)	C No	δ_C(m)	δ_H[b](m, J in Hz)
1	66.8t	3.77 (m), 3.67 (m)	3"-21"	29.5t[d]	1.30 (brs)d
2	47.4d	4.36 (m)	22"	13.3q	0.86 (t, J=7.0)
3	68.7d	5.07 (t, J=9.3)	1'	99.7d	4.78 (d, J=8.3)
4	72.3d	4.92 (dt, J=9.3,3.8)	2'	54.9d	3.69 (m)
5	36.5t	2.00[c]	3'	71.9d	5.26 (t, J=9.8)
6	124.1d	5.31 (dt, J=14.1,8.3)	4'	71.9d	5.04 (m)
7	134.5d	5.45 (dt, J=14.1,7.6)	5'	71.5d	3.73 (ddd, J=9.3,4.9
8	32.4d	2.02[c]			2.4)
9-17	29.5t[d]	1.30 (brs)[d]	6'	62.0t	4.25 (dd, J=9.4,4.9)
18	22.5d	1.50 (m)			4.12 (dd, J=9.4,2.4)
19,20	22.5q(x2)	0.85 (d, J=6.6)	NH-2		6.68 (d, J=9.0)
C=O	171.5s(x7)		NH-2'		6.09 (d, J=7.5)
2"	72.3d	4.90 (m)			

a. The compound was dissolved in $CDCl_3$; 360.13 MHz for ^{1}H and 90.53 MHz for ^{13}C.
b. Correlations between protons and carbons were established from a CHCORR experiment (J=140Hz). c. Overlapping with the methyls of the acetate groups.
d. Several methylenes of a long chain.

The NMR spectra of compounds 12b and 13b, amphicerebrosides E & F heptaacetates, pointed to the same structures as compounds 9b and 10b respectively, except for the anomeric C-O bond which, according to the 4.9Hz coupling constant of H-1' with H-2', has to be of the α configuration in amphicerebrosides E and F(12 & 13). Acidic hydrolysis in methanol and selective acetylation of the amine moiety in the sugar molecule furnished methyl 2-acetamido-2-deoxy-D-glucopyranoside[14].

The five heptaacetyl amphicerebrosides, 9b-13b, exhibited low antifungal activity against C.albicans.

After identifying the amphicerebrosides B-F from Amphimedon viridis we are fairly convinced that the same compounds are also present in small amounts in the extract of the Haliclona sp. sponge.

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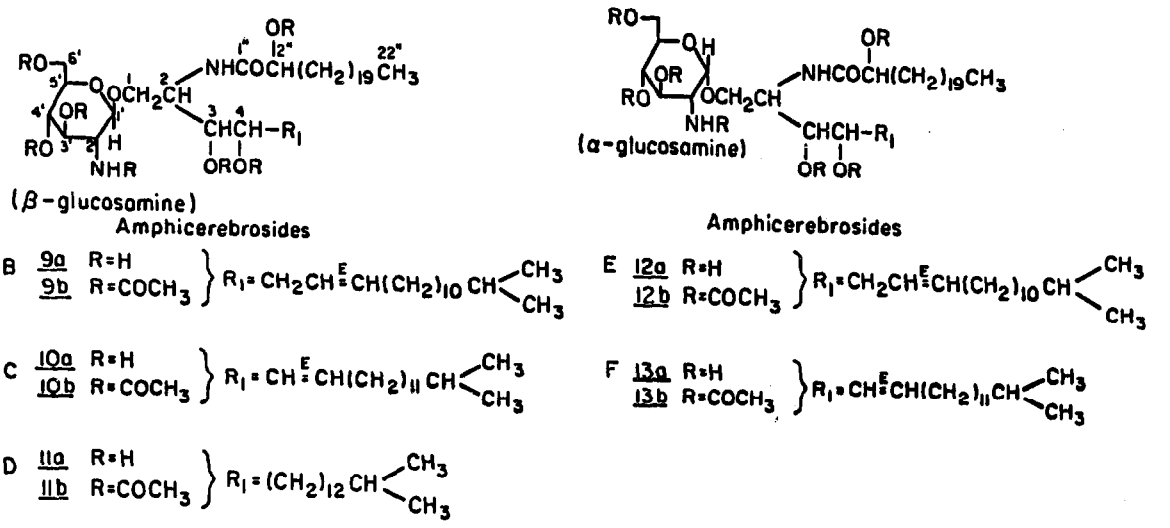

Figure 3. The structures of amphicerebrosides B-F (9-13).

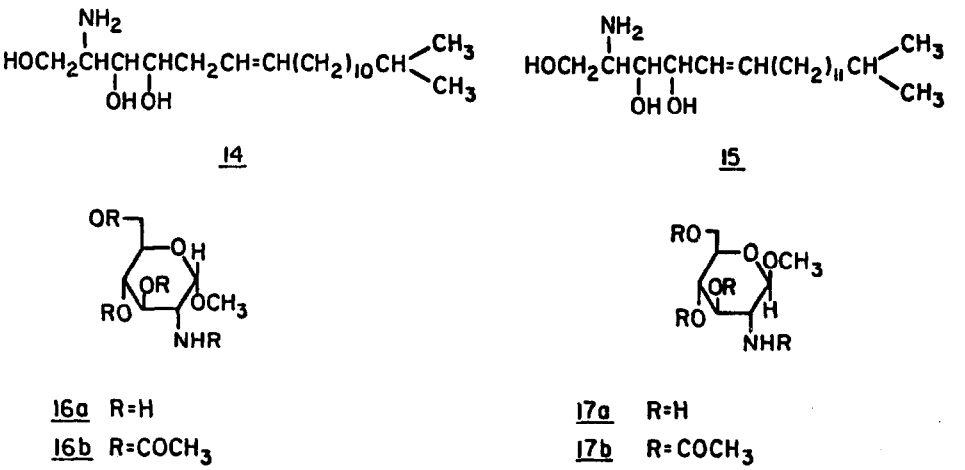

Figure 4. Degradation products of amphicerebrosides B-F

Experimental: For general information see Tetrahedron 43 (14), 3257 (1987). Ptiloceramide 2a was isolated from Ptilocaulis spiculifer collected in the Bahamas. The sponge (50gr) was freeze-dried, ground and extracted with ethyl acetate (2 x 250 ml). This extract was evaporated in vacuo (2gr) and then chromatographed on a Sephadex LH-20 column with hexane-chloroform 3:7 or chloroform-methanol 1:1. The bio-active fractions (in vitro activity, against Candida albicans up to MIC = 12.5 μg/ml) were combined (150mg) and

acetylated with a mixture of pyridine-Ac_2O (1:1, 10ml) at rt overnight. After evaporation the residue was chromatographed on a silica gel column, eluted with petroleum ether/ethyl acetate to afford 2b (100mg). Tetraacetyl ptiloceramide 2b: an oil, CIMS m/z 852, 838, 824, 810 and 796 (MH^+); ν_{max} ($CHCl_3$): 3500-3300, 2940, 1740, 1608, 1220cm^{-1}; δ_H($CDCl_3$): 6.64d(NH,J=9.0), 5.11m(H-3, H-2'), 4.95dt(H-4,J=9.8,3.2), 4.44m(H-2), 4.35dd(H-1,J=11.7,6.4), 4.02dd(H-1,J=11.7), 2.19s (Ac-2'), 2.09s, 2.06s, 2.03s(3Ac), 1.82m(H-3'), 1.65m(2H,H-5), 1.50m(H-isopropyl), 1.30m(H-3'), 1.29 (long chain), 0.88t(3H,J=6.3), 0.85d(6H,J=6.6); δ_C($CDCl_3$, C No.): 181.3s, 171.2s, 170.8s, 169.9s(X2), 74.0d(2'), 72.7d(4), 72.5d(3), 62.4t(1), 47.9d(2), 31.9t(3'), 31.8t(5), 29.2t (the long chain), 28.7t, 25.5t, 24.9t, 22.6q, 22.6d, 20.9q(X4), 14.0q.

Isolation of halicerebroside A (4a): Freeze-dried Haliclona sp. (40gr), collected in the straits of the Gulf of Eilat (the Red Sea) in July 1986, was ground and extracted with chloroform and chloroform/methanol 95:5. Evaporation of the 5% methanolic extract yielded a viscous dark oil (2.4gr). Repeated chromatographies on Sephadex LH-20 and TSK HW-40 columns, both prepared and eluted with chloroform/methanol 1:1, afforded halicerebroside A (4a, 300mg). Compound 4a: a sticky oil, CIMS m/z 816 (MH^+, Methane); ν_{max}($CHCl_3$): 3350, 2920, 1670, 1210, 1070 cm^{-1}; δ_H($CDCl_3$+CD_3OD): 7.66d(1H,J=8.5), 5.41dt(2H,J=15.2,6.5), 4.23d(1H,J=7.6), 4.12-3.22m(11H, the sugar and ceramide protons), 2.26dd(1H,J=7.3,3.0), 2.08m(1H), 1.92t(2H,J=6.2), 1.71m(1H), 1.50m(1H), 1.18 (the long chain), 0.80t(6H,J=6.5); δ_C($CDCl_3$+CD_3OD): 175.3s, 133.5d, 125.2d, 102.9d, 75.7d(X2), 72.8d, 71.4d, 71.1d, 69.3d(X2), 68.1d, 60.7d, 49.7d, 35.2t, 33.8t.

Isolation of amphicerebrosides B-F (9a-13a): Freeze-dried Amphimedon viridis, collected in the straits of the Gulf of Eilat in July 1982 (110gr), was ground and extracted with hot chloroform and chloroform/methanol 75:25. The latter extract (5.5gr) was acetylated (as described for 2a) and then purified by repeated chromatographies on Sephadex LH-20 eluted with chloroform/petroleum ether 7:3 and on Silica gel columns eluted with petroleum ether/10-30% acetone, to afford heptaacetyl amphicerebrosides B-F(9b-13b). The pairs of heptaacetyl amphicerebrosides 9b & 10b and 12b & 13b were eluted together as 1:1 mixtures, followed by compound 11 (Rf=0.35, 0.34 and 0.33, Silica gel, petroleum ether/acetone 3:1, visualized with I_2, respectively).

Amphicerebroside B heptaacetate (9b): an amorphous powder, ν_{max}($CHCl_3$): 3400, 2900, 1740, 1670, 1250, 1050 cm^{-1}; ^1H and ^{13}C NMR: Table 1.

Amphicerebroside C heptaacetate (10b): an amorphous powder, ν_{max}($CHCl_3$): 3400, 2900, 1740, 1670, 1250, 1050 cm^{-1}; δ_H($CDCl_3$): 6.66d(NH-2,J=9.1), 5.78dd(H-6,J=15.5,7.4), 5.36dd(H-5,J=15.5, 7.0), 4.81d(H-1',J=8.2), 4.35m(H-4), 2.15m(H-7); δ_C($CDCl_3$, C No.): 136.6d(6), 128.8d(5), 99.7d(1'), 86.9d(4), 32.2t(7), 32.1t, 31.2t, 29.0t (long chain), 24.6t,22.0t, 13.3q(X2); all the other resonance lines are identical with those of compound 9b (Table 1).

Amphicerebroside D heptaacetate (11b): an amorphous powder, ν_{max}($CHCl_3$): 3350, 2910, 1740, 1670, 1250 cm^{-1} ; δ_H($CDCl_3$) : 1.65m(H-5), 1.30brs(H-6,overlaps with the long chain

protons); $\delta_C(CDCl_3$,C No.): 31.9t(5), 29.5t(6); all the other resonance lines are identical with those of compound 9b (Table 1).

Amphicerebroside E heptaacetate (12b) : an amorphous powder, $\nu_{max}(CHCl_3)$: 3400, 2900, 1740, 1670, 1250, 1050 cm^{-1} ; $\delta_H(CDCl_3)$: 7.22d(NH-2,J=7.0), 6.14d(NH-2',J=7.8), 5.36t(H-3',J=9.5), 4.85d(H-1',J=4.9), 3.50m(H-2'); $\delta_C(CDCl_3$, C No.): 99.1d(1'), 72.0d(3'), 55.0d(2'), 47.9d(2); all the other resonance lines are identical with those of compound 9b (Table 1).

Amphicerebroside F heptaacetate (13b) : an amorphous powder, $\nu_{max}(CHCl_3)$: 3400, 2900, 1740, 1670, 1250, 1050 cm^{-1} ; $\delta_H(CDCl_3)$: 7.22d(NH-2,J=7.0), 6.14d(NH-2',J=7.8), 5.78dd(H-6',J=15.5, 7.4) 5.36m(H-5 and 3'), 4.85d(H-1',J=4.9), 3.50m(H-2'); $\delta_C(CDCl_3$, C No.): 136.6d(6), 128.8d(5), 99.1d(1'), 86.9d(4), 72.0d(3'), 55.0d(2'), 47.9d(2), 32.2t(7); all the other resonance lines are identical with those of compound 9b (Table 1).

Acid hydrolysis of ptiloceramide (2a): Compound 2a(30mg), dissolved in 10%HCl-MeOH(5ml) was refluxed for 4 hours. The solution was then neutralized with Ag_2CO_3(S), filtered, most of the MeOH evaporated and the residue extracted with ethyl acetate (2x15ml). The organic layer was dried over anhy. $MgSO_4$ and evaporated to dryness to yield a mixture of n-α-hydroxy methyl esters (8mg). EIMS m/e 412, 398, 384(M^+,28,26 & 13%, n-22, 21,20), 353, 339, 325(M-Ac,17,19,9%).

Acetylation of compound 4a to 4b: Compound 4a (50mg) was acetylated with Ac_2O-Pyridine 1:1 (2ml) at rt, as described for 2a to yield compound 4b (40mg), TLC: R_f=0.6(Silica gel, chloroform/methanol 9:1, visualized with vanillin). $\nu_{max}(CHCl_3)$: 3350, 2920, 1740, 1670, 1360, 1030cm^{-1}; δ_H (acetone-d_6): 7.29d(1H,NH-2,J=9.2), 5.53dt(1H,H-6,J=15.3,6.8), 5.36dt(1H,H-7,J=15.8,6.6), 5.26t(1H,H-3',J=9.5), 5.18dd(1H,H-3,J=8.3,3.5), 5.08t(1H,H-2'',J=6.2), 5.04t(1H,H-4',J=9.8), 4.98dt(1H,H-4,J=9.9,3.5), 4.88t(1H,H-2',J=8.0), 4.79d(1H,H-1',J=8.0), 4.40m(1H,H-2), 4.29dd (1H,H-6',J=12.3,4.8), 4.14dd(1H,H-6',J=12.3,2.4), 3.96ddd(1H,H-5',J=10.1,4.8,2.4), 3.92dd (1H,H-1,J=10.8,5.6), 3.73dd(1H,H-1,J=10.8,3.7), 2.51m(1H,H-5), 2.31m(1H,H-5), 2.11s(3H,Ac), 2.10s(3H,Ac, 2.06s(3H,Ac), 2.04s(3H,Ac), 2.01s(3H.Ac), 1.99brs(5H,Ac,H-8), 1.96s(3H,Ac), 1.83m (2H,H-3"), 1.31 (the long chain), 0.91brt(6H,J=6.5); δ_C (acetone-d_6, C No.): 168.6s(X8), 133.0d(6), 124.5d(7), 99.5d(1'), 73.1d(2"), 72.2d(4,3'), 71.2d(3), 70.7d(2'), 67.9d(4'), 66.8t(1), 61.3t (6'), 47.6d(2), 31.9t(3"), 31.2t(8,5), 28.5 (long chain), 19.4q (X7), 13.0q(18,40).

Reductive ozonolysis of 4b: A saturated solution of O_3 in CH_2Cl_2(10ml) at -78°C was added to compound 4b (45mg) in CH_2Cl_2 (1ml). After one hour, methanol (10ml) was added and the ozonide was reduced with $NaBH_4$ (40mg,rt, for two hours). The excess of the reagent was destroyed with acetic acid followed by water (20ml). The aq. layer was extracted with CH_2Cl_2 (3x15ml) and the combined organic layer dried over anhy. $MgSO_4$. The residue (42mg) was chromatographed on a silica gel column to furnish n-1-dodecanol (5,5mg) and compound 6 (28mg). TLC: Rf - 0.6 and 0.4 for 5 and 6 respectively (silica gel, heptane/acetone 2:1, visualized with vanillin). Compound 5: an oil, CIMS m/z 185(M-1,5%), 169(M-OH,12%), 155(M-CH_2OH,14%), 141(M-C_4H_5O,5%), 71(C_5H_{11},100%); $\delta_H(CDCl_3)$:

3.57t(2H,J=6.6), 1.49m(2H), 1.19 (the long chain), 0.82t(3H,J=6.5). Compound 6: an oil, CIMS m/z 960(MH^+, 60%), 901(M-OAc, 49%), 390($C_{14}H_{19}O_9$, 100%); δ_H ($CDCl_3$): 7.05d(NH,J=9.2), 5.23dd(1H,J=9.2,2.6), 5.11t(1H,J=9.5), 5.09m(3H), 4.86t(1H,J=8.0), 4.40d(1H,J=8.0), 4.23m(2H), 4.08dd(1H,J=12.3,2.3), 3.79dd(1H,J=10.5,2.8), 3.62m(2H), 3.55dd(1H,J=10.5,3.4), 3.51dt(1H,J= 10.3,3.4), 2.12s(Ac), 2.01s(Ac), 2.00s(Ac), 1.97s(2Ac), 1.95s(Ac), 1.92s(Ac), 1.75m(2H), 1.18t (the long chain), 0.80t(3H,J=6.6).

Acid hydrolysis of 4a: Compound 4a (100mg), dissolved in 10% aq. HCl/MeOH (10ml), was refluxed for four hours. Water was then added and the aq. phase extracted with ethyl acetate (3x15ml). The organic layer was dried over anhy. $MgSO_4$ and evaporated to furnish an oil (67mg) which was purified on a Silica gel column eluted with petroleum ether/ethyl acetate 4:1 and ethyl acetate/methanol 9:1 to afford methyl α-hydroxy-doeicosanoate (15mg) and sphingosine 7 (10mg). The aq. layer was neutralized with Ag_2CO_3(S) and filtered over celite, the solution was lyophilized and the residue acetylated, as described for 2a. Chromatography of the product on a Silica gel column eluted with petroleum ether/ethyl acetate 1:4, mainly afforded the α-anomer 8a (10mg). Alkaline hydrolysis of 8a(5mg) in 10% aq. NH_4OH-MeOH(2ml,rt, overnight) followed by acid hydrolysis (10% aq. NH_4OH-MeOH (2ml, rt overnight) followed by acid hydrolysis (10% H_2SO_4,1ml, 100°, 1hr) yielded D-glucose,$[\alpha]_D^{20}$ +50°(H_2O,lit. +52°)[7]. Methyl-α-hydroxy-doeicosanoate : an oil, EIMS m/e 370(M^+,2%), 355(M-CH_3,1%), 311(M-$C_2H_3O_2$,3%); δ_H ($CDCl_3$): 3.29t(1H,J=7.5), 1.77m(24), 1.24(the long chain), 0.88t(3H,J=6.3). Sphingosine 7: an oil, DCIMS m/z 316(MH^+), 298(M-OH, 20%), 195(M-$C_4H_{10}NO_3$,10%).

Acid hydrolysis of a mixture of 9b & 10b: A mixture of compounds 9b & 10b (70mg) was dissolved in a 10% aq. HCl/MeOH solution (10ml), and refluxed for four hours. Water (5ml) was then added and the aq. solution extracted with ethyl acetate (3 X 10ml) to furnish methyl-α-hydroxy-doeicosanoate (10mg) and a mixture of spingosines 14 & 15 (8mg, in a 1:1 ratio). The aq. layer was treated with Ag_2CO_3(S), as described for 4a, then acetylated and the residue after evaporation chromatographed on a Silica gel column eluted with ethyl acetate, to mainly afford methyl-2-acetamido-2-deoxy-3,4,6-triacetate-α-D-glucopyranoside (16b,8mg). Methyl-2-acetamido-2-deoxy-3,4,6-triacetate-α-D-glucopyranoside (16b): an amorphous powder, EIMS m/e 361(M^+,3%), 302(M-Ac,8%), 242(M-2Ac,37%), 199(M-$C_4H_8O_2$,25%), 181(M-3Ac,24%); δ_H($CDCl_3$) : 5.53d(NH-2',J=10.7), 5.30t(H-3',J=10.2), 5.11t(H-4', J=9.5), 4.61d(H-1',J=8.4), 4.31dd(H-6',J=12.3,4.6), 4.19dd(H-6',J=12.3,2.4), 3.91dt(H-2',J=10.2, 8.6), 3.73ddd (H-5',J=9.5,4.6,2.4), 3.53s, 2.20s, 2.15s, 2.10s, 2.05s. Alkaline hydrolysis of 16b: Compound 16b in 10%NH_4OH-MeOH (2ml) at rt, overnight, afforded 16a (5mg). Acetylation of the amino group, with 10%Ac_2O-MeOH solution, (1ml) at rt, overnight, furnished methyl-2-acetamido-2-deoxy-D-glucopyranoside, $[\alpha]_D^{25}$ +114° (H_2O, lit. + 119°)[14].

Methyl-α hydroxy-doeicosanoate: an oil, EIMS m/e 370(M^+,2%), 355(M-CH_3,1%), 311(M-$C_2H_3O_2$,3%), δ_H($CDCl_3$): 3.29t(1H,J=7.5), 1.77m(2H), 1.24 brs (the long chain), 0.88t(3H,J=6.3).

Sphingosines 14 & 15 : an oil, DCI MS m/z 344(MH^+), 326(M-OH,10%), 300(M-C_3H_7,5%), 223(M-$C_4H_{10}NO_3$,8%).

Hydrogenation of a mixture of compounds 9b & 10b to give 11b: A mixture of 9b+10b(12mg) dissolved in ethyl alcohol (2ml) was hydrogenated in the presence of a catalytic amount of PtO_2 at 25° for 3hrs., under 3 atm. The solvent was then evaporated to afford compound 11b(8mg).

Hydrolysis of 11b: Hydrolysis under the same conditions as described for 9b & 10b, afforded methyl-α-hydroxy-doeicosanoate and sugar 16a.

Acid hydrolysis of 12b & 13b : Hydrolysis of 12b & 13b under the same conditions as described for 9b and 10b, furnished methyl-α-hydroxy-doeicosanoate (12mg) and sphingosines 14 & 15 (9mg, in a 1:1 ratio) and upon acetylation of the aq. layer, mainly methyl-2-acetamido-2-deoxy-3,4,6-triacetate-β-D-glucopyranoside (17b,8mg). Compound 17b, EIMS m/e 361(M^+,3%), 302(M-Ac,6%), 242 (M-2Ac,37%), 181(M-3Ac,24%); $\delta_H(CDCl_3)$: 5.72d(NH-2',J=10.3), 5.14t(H-3',J=9.6), 4.76d(H-1', J=8.4), 4.38dt(H-2',J=10.3,3.6), 4.23dd(H-6',J=12.3,4.5), 4.14dd(H-6',J=12.3,2.4), 3.95ddd(H-5', J=9.6,4.5,2.4), 3.43s(OCH_3), 2.10-2.05s(X4). Alkaline hydrolysis of 17b: Compound 17b (8mg) in 10% NH_4OH-MeOH (2ml) at rt overnight, afforded 17a (5mg). Hydrolysis of 17a (10% aq. H_2SO_4,1ml, 1hr, 100°C) afforded D-glucosamine. Acetylation of the amino group of 17a (10% Ac_2O-MeOH,1ml, rt, overnight) furnished N-acetyl-D-glucosamine, $[\alpha]_D^{25}$ +54° (H_2O, lit. +55°).

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