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Terpioside from the Marine Sponge *Terpios* sp., the First Glycosphingolipid Having an L-Fucofuranose Unit[‡]

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The new diglycosylceramide terpioside (**1a**) has been isolated from the marine sponge *Terpios* sp. Terpioside is a diglycosylated glycosphingolipid which is the first example of a natural glycosphingolipid having an L-fucofuranose unit. The structure of terpioside was elucidated by extensive spectroscopic analysis, whereas chemical degradation was used to establish the nature of the alkyl chains and the absolute configuration of the sugars and of the ceramide stereogenic centers.

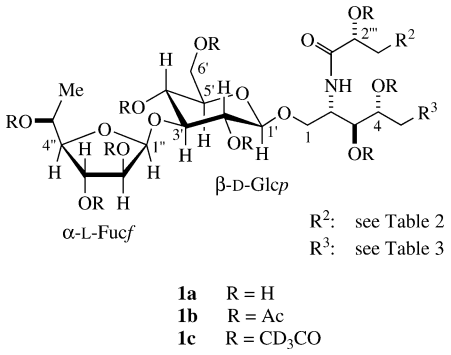
Introduction

Marine sponges are being shown to produce an increasingly large number of interesting biologically active glycosphingolipids, such as the immunosuppressive plakosides from *Plakortis simplex*[1] and the immunostimulating α-galactoglycosphingolipids[2–4] from sponges of the genera *Agelas* and *Axinella*.

As a part of our investigation focused on the search for new glycosphingolipids (GSLs) from marine invertebrates, we examined the extract of the sponge *Terpios* sp. (Suberitidae) collected from along the Florida coast. *Terpios* is an encrusting sponge of ecological importance, as revealed by a study carried out by the Marine Laboratory of the University of Guam[5] which found that *Terpios* sponges are efficient competitors of corals for space. They overgrow quickly on corals and, often, kill them. Indeed, *Terpios* sponges represent one of the most important causes of destruction of some coral reefs.

Although the chemistry of some genera of sponges from the Caribbean area, such as *Agelas* and *Axinella*, has been extensively studied, the *Terpios* genus has not yet been examined in depth. In fact, only a few papers describing the chemistry of *Terpios* species from the Pacific have been published so far, and these include reports on the isolation of nakiterpiosin, nakiterpiosinone,[6] and terpiodiene[7] from the Okinawan sponge *Terpios hoshinata* and a norcholestanol[8] from the Hawaian sponge *Terpios zeteki*.

In this paper we report the isolation and stereostructure elucidation of terpioside (**1a**), a new diglycosylated GSL from a Caribbean *Terpios* sp. which has a unique sugar moiety formed by a α-fucofuranoside linked to the 3-position of a β-glucopyranoside, the sugar residue linked to the ceramide (Scheme 1).



1a R = H
1b R = Ac
1c R = CD_3CO

Scheme 1.

Results and Discussion

Samples of *Terpios* sp. were collected from the tropical waters of Key Largo (Florida) and kept frozen until extraction. The specimens were extracted with chloroform and methanol, and the combined extracts were partitioned between water and BuOH. The organic phase was dried, and according to our standard procedure a glycolipid fraction was obtained by subsequent reversed-phase and normal-phase column chromatography. The glycolipid fraction was acetylated, and the peracetylated glycolipids were subjected to repeated HPLC on SiO_2 columns to give 62.6 mg of pure

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compound **1b**. Compound **1b** was deacetylated with MeOH/MeONa to yield the natural glycolipid **1a**.

The ESI mass spectrum of the natural GSL **1a** showed a series of sodiated pseudomolecular ion peaks at *m/z* = 986, 1000, 1014, 1028 1042, and 1056, which suggested a series of homologues differing in the size of the alkyl chains. A high-resolution measurement performed on the most abundant ion at *m/z* = 1028.7567 indicated the molecular formula $C_{55}H_{107}NO_{14}$ for the dominant homologue.

The ^{1}H NMR spectrum of the peracetyl derivative **1b** exhibited (i) an intense aliphatic chain signal at δ = 1.25 ppm, (ii) several signals due to oxymethine and oxymethylene groups between δ = 5.4 and 3.4 ppm, and (iii) an amide NH doublet at δ = 6.76 ppm. These signals are indicative of a GSL structure. The ^{1}H NMR spectrum also showed in the methyl region a triplet at δ = 0.87 ppm (ethyl terminus) and a doublet at δ = 0.85 ppm (isopropyl terminus), the intensities of which were not in an integral ratio relative to those of other signals in the spectrum. This showed that the alkyl chains differ not only in the length, but also in the branching of the alkyl chains.

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of terpioside peracetate **1b** ($CDCl_3$).

Pos.	δ_H [ppm] (mult., *J* [Hz])[a]	δ_C [ppm] (mult.)[b]
1a	3.80 (dd, 10.4, 2.9)	66.0 (CH_2)
1b	3.61 (dd, 10.4, 3.9)	
2	4.27 (m)	47.9 (CH)
2-N*H*	6.78 (d, 9.0)	–
3	5.12 (dd, 8.4, 3.2)	71.7 (CH)
4	4.89 (ddd, 10.0, 3.2, 3.2)	73.0 (CH)
5	1.59 (m)	28.5 (CH_2)
6a	1.30 (m)	25.6 (CH_2)
6b	1.19 (m)	
1′	4.32 (d, 8.0)	100.1 (CH)
2′	4.81 (dd, 9.5, 8.0)	72.0 (CH)
3′	3.83 (t, 9.5)	79.3 (CH)
4′	4.97 (t, 9.5)	69.6 (CH)
5′	3.53 (ddd, 9.5, 3.9, 3.9)	72.3 (CH)
6′	4.12 (m)	62.3(CH_2)
1′′	5.23 (d, 4.6)	100.8 (CH)
2′′	5.06 (dd, 6.3, 4.6)	75.2 (CH)
3′′	5.28 (t, 6.3)	73.5 (CH)
4′′	3.79 (d, 6.3)	80.4 (CH)
5′′	4.96 (q, 6.6)	69.6 (CH_2)
6′′	1.21 (d, 6.6)	15.5 (CH_3)
1′′′	–	169.7 (C)
2′′′	5.17 (dd, 6.8, 4.9)	73.6 (CH)
3′′′	1.83 (m)	31.8 (CH_2)
4′′′	1.32 (m)	24.7 (CH_2)
Ac CH_3	2.22, 2.09, 2.08, 2.06, 2.10, 2.05, 2.03	21.0–20.6 (CH_3)
CO	–	170.9–169.1 (C)

[a] Additional ^{1}H signals: δ = 1.24 (br., alkyl chain protons), 0.87 (t, *J* = 7.0 Hz, *n*-chain Me groups), 0.85 (d, *J* = 6.5 Hz, iso-chain Me groups) ppm. [b] Additional ^{13}C signals: δ = 31.9 (CH_2, ω-2), 22.7 (CH_2, ω-1), 22.7 (CH_3, iso-chain Me groups), 14.1 (CH_3, ω) ppm.

Planar Structure

Extensive NMR analysis was used to establish the planar structure of terpioside. The ceramide portion of the molecule is that commonly found in glycolipids from marine sponges, that is, it is composed of a trihydroxylated saturated sphinganine and an α-hydroxy fatty acid residue. Starting from the amide NH doublet at δ = 6.78 ppm (2-NH) we were able to assign all the protons of the polar part of the sphinganine up to 6-H_2 by analysis of the COSY spectrum (see Table 1). The α-hydroxy substitution of the fatty acid residue was revealed by the absence in the ^{1}H NMR spectrum of **1b** of the characteristic triplet at $\delta \approx$ 2.3 ppm for the fatty acid α-protons, whereas the spectrum displayed an acetoxymethine proton resonance at δ = 5.17 ppm (2′′′-H) coupled with a methylene resonance at δ = 1.83 ppm (3′′′-H), in turn coupled with proton resonances in the broad signal, relative to alkyl chain protons, at δ = 1.24 ppm. In addition, the signal at δ = 5.17 ppm showed an intense correlation peak with the amide NH doublet in the ROESY spectrum.

The two sugar units were revealed by the presence of two characteristic resonances in the anomeric region of the ^{13}C NMR spectrum at δ = 100.1 and 100.8 ppm which were associated through the HMQC spectrum with the corresponding anomeric protons resonating as doublets at δ = 4.32 (*J* = 8.0 Hz, 1′-H) and 5.23 ppm (*J* = 4.6 Hz, 1′′-H). These protons were used in the analysis of the COSY and TOCSY spectra as starting points for the sequential assignment of all the protons in each monosaccharide unit. Data from a TOCSY experiment were particularly useful in the assignment of four oxymethine protons [δ = 4.81, 3.83, 4.97, and 3.53 ppm (2′-H, 3′-H, 4′-H, and 5′-H)] and a couple of oxymethylene protons to the sugar having the anomeric proton (δ = 4.32 ppm), suggesting it is a hexose. Evidence that this sugar unit is linked to the ceramide primary hydroxy group was provided by the HMBC correlation peak of 1′-H with C-1 and the ROESY correlation peak between 1′-H and 1b-H. In addition, the deshielded chemical shift of the oxymethine protons at the 2′-, 4′-, and 6′-positions indicated that the relevant hydroxy groups are acetylated. In contrast, the high-field chemical shift of the C-3′ proton signal (δ = 3.83 ppm) suggested glycosylation at this position, whereas the high-field chemical shift of the C-5′ proton signal (δ = 3.51 ppm) confirmed that this carbon atom is involved in the pyranose acetal function.

Similarly, the second sugar spin system was recognized from the TOCSY and COSY experiments as a 6-deoxyhexose. This was indicated by a doublet arising from three protons [δ = 1.21 ppm (6′′-H_3)], which pointed to the presence at the 6-position of a methyl group instead of the usual –CH_2OH group. The high-field chemical shift of the 4′′-H signal (δ = 3.79 ppm), compared with the low-field chemical shift of the 5′′-H signal (δ = 4.96 ppm), clearly indicated that C-4′′ links an oxygen atom involved in an acetal function instead of an ester function; thus, the sugar must be in the furanose form and not in the more common pyranose form. This second sugar unit is linked to C-3 of the first sugar as confirmed by an HMBC correlation between C-1′′ and 3′-H and a ROESY correlation between 1′′-H and 3′-H.

Stereostructure and Alkyl Chains

The study of the values of the coupling constants is a useful method for elucidating the relative configurations of all the chiral centers of sugars in the pyranose form and, consequently, for establishing the nature of the sugar. In our case, the first hexose unit was readily recognized as a β-glucopyranoside because of the large coupling constants between all the pairs of vicinal ring protons (see Table 1), which pointed to their axial orientation.

As for the furanose sugar unit, the ROESY spectrum showed correlation peaks between 1′′-H/4′′-H, 2′′-H/4′′-H, and 3′′-H/5′′-H, which indicated that 1′′-H, 2′′-H, and 4′′-H lie on the same side of the five-membered ring, whereas 3′′-H and the $CHOHCH_3$ group at C-4′′ are on the other side. The configuration at C-5 could not be established on the basis of the ROESY data, so the sugar could be either an α-fucofuranoside (6-deoxy-α-galactofuranoside) or a 6-deoxy-β-altrofuranoside. Degradation analysis (see below) showed the former to be the case.

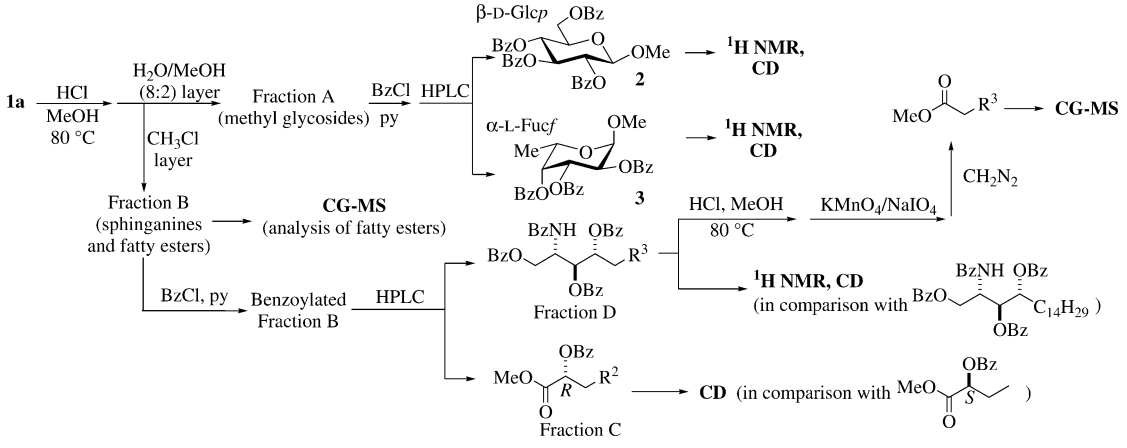
The remaining structural features of terpioside were established by chemical degradation. The natural glycolipid **1a** (300 μg) was subjected to acidic methanolysis, and the reaction products were separated, perbenzoylated, and analyzed by CD and GC-MS (Scheme 2). This procedure, set up in our laboratory and described in detail in a previous paper,[4] allowed us to establish the absolute configuration of each sugar and of the hydroxy acid, and the relative and absolute configurations of the phytosphingosine, as well as to identify the alkyl chains of fatty acids (Table 2) and sphingosines (Table 3).

Table 2. Fatty acyl composition of terpioside **1a**.

Fatty acid methyl ester	Composition [%]
Methyl 2-hydroxy-20-methylhenicosanoate (iso-C_{22})	3.6
Methyl 2-hydroxydocosanoate (*n*-C_{22})	1.7
Methyl 2-hydroxy-21-methyldocosanoate (iso-C_{23})	17.0
Methyl 2-hydroxy-20-methyldocosanoate (ante-iso-C_{23})	4.2
Methyl 2-hydroxytricosanoate (*n*-C_{23})	4.4
Methyl 2-hydroxy-22-methyltricosanoate (iso-C_{24})	2.2
Methyl 2-hydroxy-21-methyltricosanoate (ante-iso-C_{24})	5.3
Methyl 2-hydroxytetracosanoate (*n*-C_{24})	22.3
Methyl 2-hydroxy-23-methyltetracosanoate (iso-C_{25})	15.5
Methyl 2-hydroxy-22-methyltetracosanoate (ante-iso-C_{25})	5.0
Methyl 2-hydroxypentacosanoate (*n*-C_{25})	11.7
Methyl 2-hydroxy-24-methylpentacosanoate (iso-C_{26})	3.2
Methyl 2-hydroxy-23-methylpentacosanoate (ante-iso-C_{26})	1.8
Methyl 2-hydroxyhexacosanoate (*n*-C_{26})	2.1

In particular, analysis of the perbenzoylated methyl glycoside fraction led to the isolation of the 6-deoxyglycoside **3**, which was tentatively identified as tri-*O*-benzoyl-α-fucopyranoside on the basis of its ^{1}H NMR spectrum. An authentic sample of tri-*O*-benzoyl-α-fucopyranoside was prepared from L-fucose by acidic methanolysis and subsequent perbenzoylation, and its ^{1}H NMR and CD spectra were recorded. The spectra were identical to those of compound **3**, thus confirming that the outer sugar of terpioside is a fucose and showing that its absolute configuration is L.

Once the structure of the peracetyl derivative **1b** had been elucidated, 30 mg of the compound were deacetylated using MeOH in MeONa to give the natural glycolipid **1a** in quantitative yield. The one- and two-dimensional NMR



Scheme 2. Microscale degradation analysis of terpioside (**1a**).

Table 3. Sphinganine composition of terpioside **1a**.

Sphinganine	Composition [%]
(2*S*,3*S*,4*R*)-2-Amino-16-methyl-1,3,4-heptadecanetriol (iso-C_{18})	4.5
(2*S*,3*S*,4*R*)-2-Amino-15-methyl-1,3,4-heptadecanetriol (ante-iso-C_{18})	15.5
(2*S*,3*S*,4*R*)-2-Amino-1,3,4-octadecanetriol (*n*-C_{18})	4.1
(2*S*,3*S*,4*R*)-2-Amino-16-methyl-1,3,4-octadecanetriol (ante-iso-C_{19})	70.1
(2*S*,3*S*,4*R*)-2-Amino-1,3,4-nonadecanetriol (*n*-C_{19})	5.8

spectra of the natural GSL **1a** were recorded and analyzed. The information provided by the COSY and HMQC NMR spectra of **1a** confirmed all the structural features determined for its peracetylated derivative and allowed the assignment of all the resonances in its ^{1}H and ^{13}C NMR spectra (see Exp. Sect.).

To check that no acetyl groups were present in the natural terpioside **1a** before the peracetylation reaction used for its isolation, we performed the acetylation reaction on the glycolipid fraction using trideuterioacetic anhydride and then purified the mixture to give the deuterio derivative **1c**. The ^{1}H NMR spectrum of **1c** was identical to that of **1b**, except that no acetyl methyl singlet was present.

Conclusions

The lipophilic extract of Caribbean *Terpios* sp. was analyzed and a new GSL, terpioside, was isolated. The structure of terpioside was determined by the combined use of spectroscopic analysis and microscale chemical degradation.

Although glycolipids containing L-fucose in the pyranose form (including the important Lewis GSLs expressed by some human cancer cells) are relatively common, terpioside is the first natural GSL containing an L-fucose in the furanose form in the sugar part of the molecule. Terpioside is also the first GSL reported from sponges of the genus *Terpios*.

The alkyl chains of terpioside ceramide are also worthy of note. Methyl-branched alkyl chains are commonly found in glycosphingolipids obtained from sponges, but they are usually part of the sphinganines, whereas the fatty acid residues are generally unbranched. In contrast, in terpioside iso and ante-iso fatty acids comprise more than 50 mol-% of the total fatty acid residues.

These findings extend the variety of GSLs isolated from marine sponges and represent an additional proof of the chemical diversity of marine sponge chemistry.

Experimental Section

General Remarks: High-resolution ESI-MS data were recorded with a Micromass QTOF Micro mass spectrometer; sample were dissolved in MeCN/H_2O (1:1) with 0.1% TFA. ESI-MS data were recorded with an Applied Biosystem API 2000 triple-quadrupole mass spectrometer. The spectra were recorded by infusion of the samples into the ESI source using MeOH as the solvent. Optical rotations were measured at 589 nm with a Perkin-Elmer 192 polarimeter using a 10-cm microcell. ^{1}H and ^{13}C NMR spectra were determined with a Varian Unity Inova spectrometer at 500.13 and 125.77 MHz, respectively; chemical shifts are referenced to the residual solvent signal ($CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.0 ppm; [D_5]-pyridine: δ_H = 8.73, 7.56, and 7.21 ppm; δ_C = 149.9, 135.6, and 123.6 ppm). For an accurate measurement of the coupling constants, the one-dimensional ^{1}H NMR spectra were transformed at 64K points (digital resolution: 0.09 Hz). Homonuclear ^{1}H connectivities were determined by COSY experiments. Through-space ^{1}H connectivities were evidenced through a ROESY experiment with a mixing time of 500 ms. The reverse multiple-quantum heteronuclear correlation (HMQC) spectra were recorded by using a pulse sequence with a BIRD pulse 0.5 s before each scan to suppress the signal originating from protons not directly bound to ^{13}C; the interpulse delays were adjusted for an average $^1J_{CH}$ of 142 Hz. The gradient-enhanced multiple-bond heteronuclear correlation (HMBC) experiment was optimized for a $^3J_{CH}$ of 8.3 Hz. GC-MS spectra were performed with a Hewlett-Packard 5890 gas chromatograph with an MSD HP 5970 MS mass-selective detector, a split/splitness injector, and a fused-silica column (25 m × 0.20 mm HP-5; cross-linked 25% Ph Me silicone, 0.33-mm film thickness). The temperature of the column was varied after a delay of 3 min from the injection from 150 to 280 °C with a gradient of 10 °C min^{-1}; quantitative determination was based on the area of the GLC peaks. High-performance liquid chromatography (HPLC) was achieved with a Varian Prostar 210 apparatus equipped with a Varian 350 refractive index detector or a Varian 325 UV detector.

Collection, Extraction, and Isolation: Specimens of *Terpios* sp. were collected in December 2005 from along Key Largo coast (Florida) and identified by Prof. S. Zea (University of Colombia). They were frozen immediately after collection and kept frozen until extraction. The sponge (220 g of dry weight after extraction) was homogenized and extracted with methanol (3 × 1 L), and then with chloroform (3 × 1 L); the combined extracts were partitioned between H_2O and *n*BuOH. The organic layer was concentrated in vacuo and afforded 20 g of a dark green oil, which was purified by chromatography on a column packed with RP-18 silica gel. A fraction eluted with $CHCl_3$ (5.9 g) was further purified by chromatography on an SiO_2 column, giving a fraction [820 mg; eluent: EtOAc/MeOH (7:3)] mainly composed of glycolipids. This fraction was peracetylated with Ac_2O in pyridine for 12 h. The acetylated glycolipids were subjected to HPLC separation on an SiO_2 column [eluent: *n*-hexane/EtOAc (6:4)], thus affording 62 mg of terpioside peracetate **1b**.

Terpioside Peracetate 1b: Colorless oil, $[\alpha]_D^{25}$ = –8 (*c* = 0.15, $CHCl_3$). ESI-MS (positive ion mode, MeOH): *m*/*z* = 1434, 1420, 1406, 1392, 1378, 1364 ([M + Na]$^+$ series). ^{1}H and ^{13}C NMR: see Table 1. Composition of the fatty acids: see Table 2. Composition of the sphinganines: see Table 3.

Deacetylation of 1b: Compound **1b** (30 mg) was dissolved in MeOH (950 μL) and a 0.4 M solution of MeONa in MeOH (50 μL) was added. The reaction was allowed to proceed at 25 °C for 18 h and then the reaction mixture was dried under nitrogen and the residue partitioned between water and chloroform. After removal of the solvent, the organic layer gave 22 mg of the native glycosphingolipid **1a**.

Terpioside (1a): Colorless amorphous solid, $[\alpha]_D^{25}$ = –25 (*c* = 0.15, MeOH). HR-MS (ESI, positive ion mode, MeOH): calcd. for $[C_{55}H_{107}NNaO_{14}]^+$ 1028.7589; found 1028.7567 [M + Na]$^+$. ^{1}H NMR ([D_5]pyridine): δ = 0.86 (*n*- and iso-chain Me groups), 1.28 (large band, alkyl chains), 1.54 (d, *J* = 6.5 Hz, 3 H, 6′′-H), 1.65 (m, 1 H, 6-H$_b$), 1.69 (m, 1 H, 4′′′-H$_b$), 1.75 (m, 1 H, 4′′′-H$_a$), 1.88 (m, 1 H, 5-H$_b$), 1.90 (m, 1 H, 5-H$_a$), 1.99 (m, 1 H, 3′′′-H$_b$), 2.22 (m, 1 H, 5-H$_a$), 3.75 (m, 1 H, 5′-H), 3.95 (ddd, *J* = 7.8, 7.8, 3.7 Hz, 1 H, 2′-H), 4.08 (ddd, *J* = 9.2, 9.2, 3.5 Hz, 1 H, 4′-H), 4.15 (m, 1 H, 4-H), 4.16 (overlapped, 4′′-H), 4.23 (overlapped, 3′-H), 4.24 (overlapped, 6′-H$_b$), 4.26 (m, 1 H, 3-H), 4.36 (overlapped, 5′′-H), 4.56 (m, 1 H, 2′′′-H), 4.47 (dd, *J* = 10.8 and 7.0 Hz, 1 H, 1-H$_a$), 4.65 (dd, *J* = 10.8, 3.9 Hz, 1 H, 1-H$_b$), 4.85 (d, *J* = 7.9 Hz, 1 H, 1′-H), 5.24 (m, 2-H), 6.08 (br. s, 1 H, 4-OH), 6.14 (d, *J* = 4.5 Hz, 1 H, 1′′-H), 6.25 (br. s, 1 H, 2′′-OH), 6.43 (br. s, 1 H, 6′-OH), 6.53 (br. s, 1 H, 5′′-OH), 6.86 (br. s, 1 H, 3-OH), 7.10 (br. s, 1 H, 4′-OH), 7.36 (br. s, 1 H, 2′-OH), 7.45 (br. s, 1 H, 3′′-OH), 7.73 (br.

s, 1 H, 2′′′-OH), 8.53 (d, J = 9.5 Hz, 1 H, 2-NH) ppm. ^{13}C NMR ([D$_5$]pyridine): δ = 14.3 (CH_3, n-chain Me groups), 20.6 (CH_3, C-6′′), 22.8 (CH_3, iso-chain Me groups), 22.9 (CH_2, n-chain ω-1 CH_2 groups), 25.6 (CH_2, C-4′′′), 26.3 (CH_2, C-6), 30.5–29.5 (several CH_2, alkyl chains), 32.1 (CH_2, n-chain ω-2 CH_2 groups), 33.8 (CH_2, C-5), 35.3 (CH_2, C-3′′′), 51.4 (CH, C-2), 61.9 (CH_2, C-6′), 65.9 (CH, C-5′′), 61.9 (CH_2, C-6′), 69.2 (CH, C-4′), 72.1 (CH, C-4; CH, C-2′′′), 74.9 (CH, C-3′′), 77.9 (CH, C-5′), 79.2 (CH, C-2′′), 84.1 (CH, C-3′), 86.4 (CH, C-4′′), 105.1 (CH, C-1′), 103.1 (CH, C-1′′), 105.3 (CH, C-1′), 175.3 (C, C-1′′′) ppm. Composition of the fatty acids: see Table 2. Composition of the sphinganines: see Table 3.

Methanolysis of 1a: Compound **1a** (100 μg) was dissolved in 1 N HCl in 91% MeOH (500 μL), and the solution obtained was kept in a sealed tube at 80 °C for about 12 h. The reaction mixture was dried under nitrogen and partitioned between $CHCl_3$ and H_2O/MeOH (8:2). The aqueous layer was concentrated to give a mixture of methyl glycosides (fraction A), whereas the organic layer contained a mixture of α-hydroxy acid methyl esters and sphinganines (fraction B).

Methyl Tri-*O*-benzoyl-α-L-fucopyranoside (3): L-Fucose (2.0 mg) was subjected to acidic methanolysis as described above. The resulting methyl glycosides were benzoylated with benzoyl chloride (50 μL) in pyridine (500 μL) at 25 °C for 16 h. The reaction was then quenched with MeOH; after 30 min, the mixture was dried under nitrogen. Methyl benzoate was removed by keeping the residue under vacuum with an oil pump for 24 h. The residue was purified by HPLC [column: Luna SiO_2, 5 μ; eluent: n-hexane/iPrOH (99:1); flow: 1 mL min^{-1}; UV detector: 280 nm] affording the glycoside **3** (t_R = 6.6 min). ^{1}H NMR ($CDCl_3$): δ = 1.29 (d, J = 6.5 Hz, 3 H, 6-H), 3.47 (s, 3 H, OMe), 4.39 (br. q, J = 6.5 Hz, 1 H, 5-H), 5.24 (d, J = 3.6 Hz, 1 H, 1-H), 5.64 (dd, J = 10.7, 3.6 Hz, 1 H, 2-H), 5.76 (br. d, J = 3.4 Hz, 1 H, 4-H), 5.95 (dd, J = 10.7, 3.4 Hz, 1 H, 3-H), 7.24 (t, J = 7.7 Hz, 2 H, benzoyl *meta* protons), 7.37 (t, J = 7.4 Hz, 2 H, benzoyl *meta* protons), 7.42 (t, J = 7.6 Hz, 1 H, benzoyl *para* proton), 7.53–7.45 (overlapping signals, 3 H, benzoyl protons), 7.61 (t, J = 7.5 Hz, 1 H, benzoyl *para* proton), 7.79 (d, J = 7.9 Hz, 2 H, benzoyl *ortho* protons), 7.98 (d, J = 7.9 Hz, 2 H, benzoyl *ortho* protons), 8.11 (d, J = 8.0 Hz, 2 H, benzoyl *ortho* protons) ppm. CD (MeCN): λ_{max} = 237 ($\Delta\varepsilon$ = –42), 222 ($\Delta\varepsilon$ = +14) nm.

Absolute Stereochemistry of Methyl Glycosides from Compound 1a: Fraction A from the methanolysis of compound **1a** was benzoylated with benzoyl chloride (20 μL) in pyridine (200 μL) at 25 °C for 16 h. The reaction was then quenched with MeOH; after 30 min, the mixture was dried under nitrogen. Methyl benzoate was removed by keeping the residue under vacuum with an oil pump for 24 h. The residue was purified by HPLC [column: Luna SiO_2, 5 μ; eluent: n-hexane/iPrOH (99:1); flow: 1 mL min^{-1}]. The chromatogram showed two peaks: methyl tetra-*O*-benzoyl-α-D-glucopyranoside (**2**), identified by comparison of its ^{1}H NMR and CD spectra with those reported in the literature,[4] and methyl tri-*O*-benzoyl-α-L-fucopyranoside (**3**), identified by comparison of its ^{1}H NMR and CD spectra with those of the authentic samples prepared from L-fucose.

Analysis of Fatty Acid Methyl Esters: Fraction B from the methanolysis of compound **1a** was analyzed by GLC-MS and its components identified by comparison of their retention times and mass spectra with those of authentic samples. The results are compiled in Table 2.

Analysis of Fraction B: Fraction B from the methanolysis of compounds **1a** was benzoylated as described above and the crude product of the reaction was purified by HPLC [column: Luna SiO_2, 5 μ; eluent: n-hexane/iPrOH (99:1); flow: 1 mL min^{-1}]. The chromatogram shows two peaks, which were identified as a mixture of homologues of benzoylated fatty acid methyl esters (fraction C, t_R = 4.0 min) and a mixture of perbenzoylated sphinganines (fraction D, t_R = 6.9 min) on the basis of their respective ^{1}H NMR spectra.

Methyl (*R*)-2-Benzoyloxyalkanoate: Fraction C from the mixture of homologues. CD (MeCN): λ_{max} = 229 ($\Delta\varepsilon$ = –3.4) nm. The ^{1}H NMR spectrum was identical to that reported in the literature.[4]

(2*S*,3*S*,4*R*)-1,3,4-*O*-Benzoyl-2-benzoylamino-1,3,4-alkanetriol: Fraction D from the mixture of homologues. CD (MeCN): λ_{max} = 233 ($\Delta\varepsilon$ = –1), 222 ($\Delta\varepsilon$ = +2) nm. The ^{1}H NMR spectrum was identical (apart from the methyl region) to that of an authentic sample of D-*ribo*-phytosphingosine perbenzoate.[9]

Oxidative Cleavage and GC-MS Analysis of Sphinganines: Fraction D was debenzoylated by acidic methanolysis as described above and subjected to oxidative cleavage with $KMnO_4$/$NaIO_4$ as described in the literature.[2] The resulting carboxylic acids were methylated with CH_2N_2. The esters obtained were analyzed by GC-MS, and the results are compiled in Table 3, expressed in terms of original sphinganines.

Supporting Information (see footnote on the first page of this article): ^{1}H, ^{13}C, COSY, ROESY, TOCSY, HSQC, and HMBC NMR spectra of compound **1b**; ^{1}H, ^{13}C, and COSY NMR spectra of compound **1a**; ^{1}H NMR and CD spectra of compound **3**.

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