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GLYCOSIDES FROM *STENOCHLAENA PALUSTRIS*

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Key Word Index—*Stenochlaena palustris*; Pteridaceae; leaves; glycoside; stenopaluside; 3-oxo-4,5-dihydro-α-ionyl β-D-glucopyranoside; cerebroside; 3-formylindole.

Abstract—A novel glycoside, (4*S**,5*R**)-4-[(9*Z*)-2,13-di-(*O*-β-D-glucopyranosyl)-5,9,10-trimethyl-8-oxo-9-tetradecene-5-yl]-3,3,5-trimethylcyclohexanone, namely stenopaluside, and a new cerebroside, 1-*O*-β-D-glucopyranosyl-(2*S**,3*R**,4*E*,8*Z*)-2-N-[(2*R*)-hydroxytetracosanoyl]octadecasphinga-4,8-dienine, were isolated from the leaves of *Stenochlaena palustris*, along with four known natural products, 3-oxo-4,5-dihydro-α-ionyl β-D-glucopyranoside, 3-formylindole, lutein, and β-sitosterol-3-*O*-β-D-glucopyranoside. The structures of the isolates were elucidated by spectroscopic and chemical methods. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Stenochlaena palustris (Burm.) Bedd., a scrambling fern, is distributed in a large part of the tropical areas from southern and northern India through Malaysia to Polynesia and Australia [1]. In the central district province of Papua New Guinea (PNG) and the Nicobar Islands, the tender leaves of *S. palustris* are used as a contraceptive by young people and are eaten as a vegetable by old people [2, 3]. No phytochemical studies on *S. palustris* have been reported except that a search for alkaloid-containing plants in New Guinea found it to be alkaloid-negative [4].

In our continuing search for bioactive compounds from plants used in the traditional medicine of PNG, we have investigated the leaves of *S. palustris* collected in the central district, near Port Moresby, PNG. The current report describes the isolation and structural elucidation of a novel glycoside, namely stenopaluside (**1**), a new cerebroside, 1-*O*-β-D-glucopyranosyl-(2*S**,3*R**,4*E*,8*Z*)-2-N-[(2*R*)-hydroxytetracosanoyl]octadecasphinga-4,8-dienine (**2**), and four known natural products, 3-oxo-4,5-dihydro-α-ionyl β-D-glucopyranoside (**3**), 3-formylindole (**4**), lutein (**5**), and β-sitosterol-3-*O*-β-D-glucopyranoside (**6**) from this plant.

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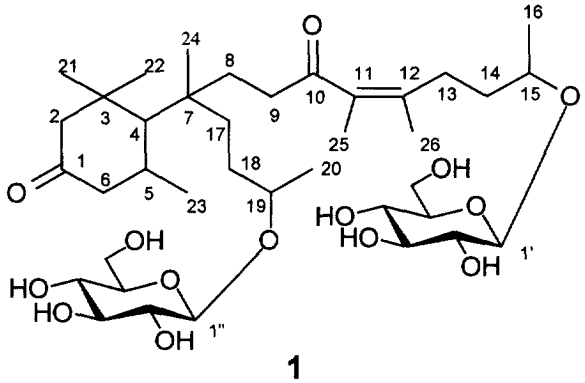
RESULTS AND DISCUSSION

The air-dried leaves of *S. palustris* were extracted with MeOH and 70% aqueous MeOH. The MeOH extract was partitioned between n-hexane and 90% aqueous MeOH. The alcoholic phase was further partitioned between $CHCl_3$ and 60% aqueous MeOH. The $CHCl_3$ phase was concentrated *in vacuo* and subjected to repeated chromatography to yield compounds **1**–**6**.

Compound **1** was isolated as a colourless amorphous solid. The positive FABMS of compound **1** gave a quasimolecular peak at *m/z* 747 $[M + H]^+$ corresponding to an empirical molecular formula of $C_{38}H_{66}O_{14}$. The UV spectrum of **1** gave an absorption maximum at λ 249 nm (log ε 4.47) indicative of the presence of a conjugated system. Its IR spectrum displayed the absorption bands attributable to hydroxyl groups (3406 cm^{-1}) and two carbonyl (1699 and 1651 cm^{-1}) functionalities. The ^{13}C NMR spectrum and DEPT experiments showed that the molecule contained 8 methyl groups, 10 methylenes, 14 methines and 6 quarternary carbons.

The ^1H and ^{13}C NMR spectra (Table 1) of compound **1** established the presence of two almost equivalent terminal sugar residues. The ^{13}C NMR signals at δ 102.3 / 102.2, 75.2 (2C), 78.2 (2C), 71.9 (2C), 78.0 (2C), and 62.9 (2C) were attributed to the two sugar residues and suggested that they were both β-D-glucopyranose [5]. The ^1H NMR spectrum of **1** displayed the resonances of the anomeric pro-



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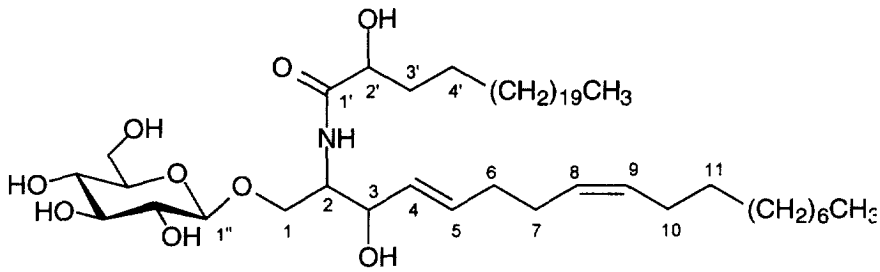

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tons at δ 4.35 (*d*, J = 7.7 Hz), and 4.34 (*d*, J = 7.8 Hz) supporting the β-configuration of the two sugar moieties.

Four quarternary carbon signals in the downfield region, δ 214.5, 201.6, 168.8 and 131.6, were assigned to two ketone groups and one tetrasubstituted double bond. The ^{13}C NMR chemical shifts of the double bond carbons (δ 168.8 and 131.6) strongly suggested an α,β-unsaturated carbonyl system in the molecule, the signal at δ 168.8 for the β-carbon and at δ 131.6 for the α-carbon, since these carbon resonances were significantly shifted downfield due to the electron-withdrawing power of the conjugated carbonyl group [6]. This strong shift of the β-carbon to lower field also indicated methyl substitution on the olefinic carbons [6, 7]. Furthermore, the HMBC spectrum revealed that the α-olefinic carbon was correlated to a methyl singlet at δ 1.76, and the β-olefinic carbon was also correlated to this methyl group and another methyl singlet at δ 1.21, giving a partial structure **a**.

The empirical formula $C_{38}H_{66}O_{14}$ required 6 degrees of unsaturation in the molecule, indicating that the compound should have one additional ring system besides the above mentioned groups which collectively accounted for five degrees of unsaturation. The DQF-COSY, TOCSY and HMQC experiments allowed the establishment of 4 further partial structures (**b**–**e**).

The presence of a cyclohexanonyl ring consisting of the partial structure **b**, the second carbonyl carbon (δ_C 214.5), an isolated methylene (C-2) and a dimethylated quarternary carbon (C-3) was deduced from the HMBC spectrum. The HMBC spectrum also revealed the connectivities of one site (C-8) of the partial structure **c** to a methylated quarternary carbon (C-7), and the other site (C-9) to the α,β-unsaturated carbonyl group (partial structure **a**) which in turn was connected to C-13 of the partial structure **d**. The chemical shifts of H-15 (δ 3.97), C-15 (δ 75.8), H-19 (δ 3.89) and C-19 (δ 75.9) indicated the positions 15 and 19 each to be linked with a sugar moiety. These glycosidic linkages were confirmed by the HMBC spectrum, where C-15 correlated to the anomeric proton at δ 4.35, and C-19 to the anomeric proton at δ 4.34. These data led to

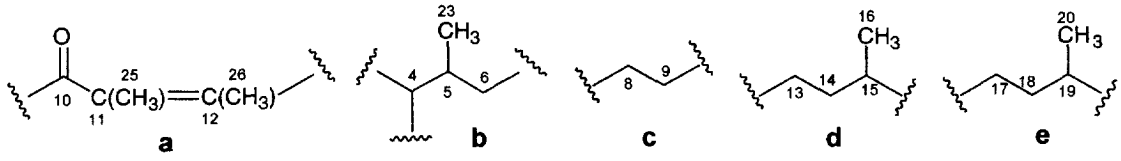

Table 1. ^{1}H and ^{13}C NMR data and ^{1}H-^{1}H correlations of compound **1** (CD_3OD, 300.13 MHz for ^{1}H, 75.47 MHz for ^{13}C)

Position	δ_C	δ_H (multi., J in Hz)	^{1}H-^{1}H correlations
1	214.5		
2	57.1	ax 2.38 (*d*, 13.3)	2_{eq}
		eq 1.97 (*dd*, 13.3, 2.1)	2_{ax}, 6_{eq}
3	40.4		
4	53.6	1.16 (*m*)	5
5	37.7	1.80 (*m*)	4, 6_{eq}, 6_{ax}, 23
6	50.9	eq 2.22 (*ddd*, 12.9, 5.3, 2.1)	6_{ax}, 5, 2_{eq}
		ax 2.15 (*t*, 12.9)	6_{eq}, 5
7	37.6		
8	38.4	1.82 (2H, *t*, 6.9)	9
9	35.1	2.45 (2H, *t*, 6.9)	8
10	201.6		
11	131.6		
12	168.8		
13	27.9	a 2.53 (*m*)	13b, 14
		b 2.30 (*m*)	13a, 14
14	37.2	1.67 (2H, *m*)	13a, 13b, 15
15	75.8	3.97 (br *q*, 6.1)	14, 16
16	19.8	1.23 (3H, *d*, 6.1)	15
17	25.9	a 1.69 (*m*)	
		b †	
18	40.5	1.67 (2H, *m*)	
19	75.9	3.89 (*m*)	18, 20
20	27.2*	1.21 (3H, *d*, 6.4)	19
21	30.0	1.07 (3H, *s*)	
22	21.1	0.77 (3H, *s*)	
23	21.5	1.09 (3H, *d*, 6.4)	5
24	27.2	1.20 (3H, *s*)	
25	19.8*	1.21 (3H, *s*)	
26	11.7	1.76 (3H, *s*)	
1′	102.3	4.35 (*d*, 7.7)	2′
2′	75.2	3.17 (*m*)	1′, 3′
3′	78.2	3.36 (*m*)	2′, 4′
4′	71.9	3.31 (*m*)	3′, 5′
5′	78.0	3.28 (*m*)	4′, 6′a, 6′b
6′	62.9	a 3.87 (*d*, 11.6)	6′b, 5′
		b 3.66 (*dd*, 11.6, 3.9)	6′a, 5′
1″	102.2	4.34 (*d*, 7.8)	2″
2″	75.2	3.17 (*m*)	1″, 3″
3″	78.2	3.36 (*m*)	2″, 4″
4″	71.9	3.31 (*m*)	3″, 5″
5″	78.0	3.28 (*m*)	4″, 6″a, 6″b
6″	62.9	a 3.87 (*d*, 11.6)	6″b, 5″
		b 3.66 (*dd*, 11.6, 3.9)	6″a, 5″

* May be interchangeable.
† Overlapped by the intense methyl signals at ca. 1.20 ppm.

the extended partial structures **i**, **ii** and **iii** (Fig. 1), accounting for all elements of the molecular formula. The linkage shown in structure **1** is the unique combination form for these extended partial structures, and hence the planar structure of compound **1** was established. This was confirmed by a weak correlation between C-4 and H-17 at δ 1.69 observed in the HMBC spectrum.

The 11,12 double bond was found to be in the *Z*-configuration, as evidenced by the ^{13}C resonance signal of the β-methyl carbon appearing at relatively higher field (δ_C 11.7 ppm). Generally, on the basis of steric interactions a pronounced upfield shift will be observed for the β-methyl carbon in *cis*-orientation to an α-methyl group [8]. The γ-effects are usually found for carbons in gauche or eclipsed orientations, and such an effect is greater for *cis*-vicinal methyls than for a β-methyl *cis* to

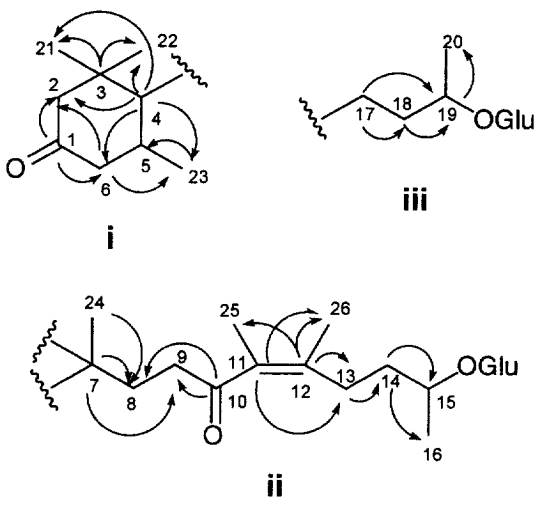

Fig. 1. Extended partial structures **i**, **ii** and **iii** of **1**. Arrows indicate key long-range correlations observed in the HMBC spectrum.

a carbonyl function [8]. This *cis* alkene bond geometry was also confirmed by a NOE interaction between the α- and β-methyl protons observed in the ROESY spectrum.

In the ^{1}H NMR spectrum of **1**, the presence of a large diaxial coupling (12.9 Hz) between H-6 and H-5 indicated that the secondary methyl group at C-5 was equatorial. The four-bond long-range correlation between C-7 and H-6_{eq} observed in the HMBC spectrum, which could be explained by a W-shape steric relationship between these two atoms, together with the consideration of molecular models suggested that the side chain at C-4 was also equatorial. The above data of compound **1** do not allow the assignment of the relative stereochemistry at chiral centres C-7, C-15 and C-19. Compound **1** was therefore determined as (4*S**,5*R**)-4-[(9*Z*)-2,13-di-(*O*-β-D-glucopyranosyl)-5,9,10-trimethyl-8-oxo-9-tetradecene-5-yl]-3,3,5-trimethylcyclohexanone, and designated as stenopaluside.

The assignments of all the proton and carbon signals of compound **1** are listed in Table 1. It is noteworthy that the chair conformation of the cyclohexanone ring fixes H-2_{eq} and H-6_{eq} in a W-shape (Fig. 2), resulting in a doublet of doublets at

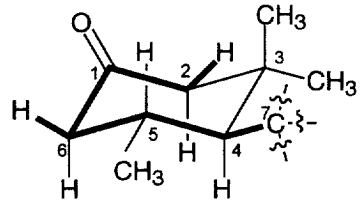

Fig. 2. Relative stereochemistry of cyclohexanone ring of **1**. Solid lines indicate the observed W-shape correlations from the DQF-COSY and HMBC spectra.

Table 2. ^1H and ^{13}C NMR data of compound **2** (C_5D_5N, 300.13 MHz for ^1H, 75.47 MHz for ^{13}C)

position	δ_C	δ_H (mult., J in Hz)
1	70.2	4.69 (*dd*, 10.3, 5.7)
		4.25 (*m*)
2	54.7*	4.77 (*m*)
3	72.5†	4.74 (*m*)
4	132.2	5.96 (*m*)‡
5	132.1	5.96 (*m*)‡
6	32.9	2.18 (*m*)
7	27.4	2.18 (*m*)
8	130.7	5.48 (*t*, 4.1)
9	129.4	5.48 (*t*, 4.1)
10	27.6	2.06 (*m*)
11	26.0	1.77 (*m*)
12–17	32.1, 30.1, 29.9, 29.6, 23.0	1.28 (*m*)
18	14.3	0.86 (3H, *t*, 6.9)
2-NH		8.34 (*d*, 8.2)
3-OH		6.85 (*d*, 4.3)
1′	175.7	
2′	72.3	4.57 (*m*)
3′	35.7	2.06 (*m*)
4′-23′	32.1, 30.1, 29.9, 29.6, 23.0	1.28 (*m*)
24′	14.3	0.86 (3H, *t*, 6.9)
2′-OH		7.61 (*d*, 4.8)
1″	105.7	4.91 (*d*, 7.7)
2″	75.2	4.02 (*m*)
3″	78.6	4.22 (*m*)
4″	71.6	4.22 (*m*)
5″	78.5	3.90 (*m*)
6″	62.7	4.50 (*d*, 11.7)
		4.36 (*m*)

* When measured in $CDCl_3$-CD_3OD (2:1), δ_{C^-} = 53.7.
† When measured in $CDCl_3$-CD_3OD (2:1), δ_C is also 72.5.
‡ In $CDCl_3$-CD_3OD (2:1) two signals at δ_H 5.46 (*dd*, 15.4, 7.0) and 5.71 (*dt*, 15.4, 5.7) were observed for H-4 and H-5.

δ_H 1.97 (J = 13.3, 2.1) for H-2_{eq} and a doublet of doublets of doublets at δ_H 2.22 (J = 12.9, 5.3, 2.1) for H-6_{eq}.

Compound **2** is a white amorphous powder. The positive FABMS of compound **2** showed ions at m/z 848 $[M + Na]^+$, 826 $[M + H]^+$, and 808 $[M + H\text{-}H_2O]^+$ in the high mass region, corresponding to the molecular formula $C_{48}H_{91}NO_9$. The IR spectrum of **2** displayed hydroxyl (3407 cm^{-1}) and secondary amide (1639 and 1542 cm^{-1}) absorption bands. The ^1H and ^{13}C NMR spectral data of **2** (Table 2) showed the presence of sugar, amide, and long-chain aliphatic moietes. In the ^1H NMR spectrum of **2**, an intense signal at δ 1.28 and a tortured triplet at δ 0.86 (6H, J = 6.9 Hz) assigned for two terminal methyls indicated it to have either two long aliphatic chains or one branched aliphatic chain. All of these primary spectral data strongly suggested that compound **2** was a glycosphingolipid [9]. This was supported by the observations that a proton attached to a nitrogen appeared at δ 8.34 in the ^1H NMR spectrum, and that a tertiary carbon at δ 54.7 and a quarternary carbon at δ 175.7 were correlated with this nitrogen proton in the HMBC spectrum.

The ^{13}C NMR signals at δ 105.7, 75.2, 78.6, 71.6, 78.5 and 62.7, as well as the anomeric proton resonance at δ 4.91 (*d*, J = 7.7), suggested that the sugar in **2** was a β-D-glucopyranose [5]. This deduction was confirmed by Co-TLC with the authentic methyl-β-D-glucopyranoside after methanolysis of **2**.

The presence of a significant FABMS fragment peak at m/z 646 $[M + H\text{-}H_2O\text{-hexose}]^+$, formed by elimination of the glucose and a water unit from the molecule, supported a Mr weight of 825 for compound **2**. The EIMS of the fatty acid methyl ester obtained by methanolysis of **2** exhibited a base peak at m/z 398 $[M]^+$ and a ion at m/z 339 $[M\text{-}COOCH_3]^+$. This established the Mr of the fatty acid portion to be 384. The structure of the acyl moiety was determined as 2-hydroxy-tetracosanoyl by the ^1H NMR spectrum of the methyl ester. Consideration of the molecular formula ($C_{48}H_{91}NO_9$) indicated the base moiety in this glycosphingolipid to be C_{18}-sphinga-4,8-dienine.

The olefinic protons at 4,5 appeared as a multiplet centred at δ 5.96 (2H) in C_5D_5N, while in $CDCl_3$-CD_3OD (2:1) a double triplet at δ 5.71 (1H, J = 15.4, 5.7 Hz) and a double doublet at δ 5.46 (1H, J = 15.4, 7.0 Hz) were observed. The 4,5 alkene bond was therefore determined to be *trans*. A triplet at δ 5.48 (2H, J = 4.1 Hz) in the ^1H NMR spectrum indicated the 8,9 double bond to be *cis*. The geometry of these two double bonds was also supported by the chemical shifts of C-6 (δ 32.9), C-7 (δ 27.4) and C-10 (δ 27.6), since the chemical shift values for the *trans* vinylic methylene are around δ 33 and for the *cis* form around δ 27 [9, 10]. For characterization of the relative stereochemistry of the base part, compound **2** was subjected to ^{13}C NMR measurement in $CDCl_3$-CD_3OD (2:1) as a solvent and compared with the ^{13}C NMR spectral data of glucosyl-erythro-ceramide and glucosyl-threo-ceramide [11]. A 2*S**,3*R**-configuration (D-erythro) was deduced by the chemical shifts of C-2 (δ 53.7) and C-3 (δ 72.5), in agreement with those reported for glucosyl-erythro-ceramide (δ 53.8, 72.6) [11] and synthetic N-octadecanoyl-D-erythro-sphingosine (δ 54.7, 73.1) [12]. The optical rotation ($[\alpha]_D^{25}$ −3.0°, *c* 0.115, $CHCl_3$) of the methyl-2-hydroxy-tetracosanoate obtained from methanolysis of **2** indicated it to be the R isomer [13]. Thus, compound **2** was identified as 1-*O*-β-D-glucopyranosyl-(2*S**,3*R**,4*E*,8*Z*)-2-N-[(2*R*)-hydroxy-tetracosanoyl] octadecasphinga-4, 8-dienine.

In addition to the isolation of these two new glycosides, four known natural products were also obtained from the same extract. Compound **3** was determined as 3-oxo-4,5-dihydro-α-ionyl β-D-glucopyranoside on the basis of the detailed NMR (^1H, ^{13}C, DQF-COSY, HMQC and HMBC) studies; the ^1H and ^{13}C-NMR data for the aglycone moiety are in good agreement with the previously published data [14, 15]. 3-Formylindole (**4**), lutein (**5**), and β-sitosterol-3-*O*-β-D-glucopyranoside (**6**) were identified by MS, ^1H, and ^{13}C NMR spectral analysis and comparison with the reported data of authentic

samples [16–19]. All of these compounds are reported for the first time from this plant.

The occurrence of compounds **1**, **3** and **4** in *S. palustris* may be of significance in several respects. Compounds **1** and **3** have an identical trimethylated cyclohexanone ring and different aliphatic side chains in the molecules. 3-Oxo-4, 5-dihydro-α-ionyl β-D-glucopyranoside, a C_{13} norisoprenoid, is considered to be formed by oxidative cleavage of carotenoids and subsequent transformation reactions at the plant pH [20]. Whether stenopaluside (**1**) is biosynthetically related to **3** or carotenoids in the plant is not yet known. In fact, the presence of carotenoids in ferns was described more than 10 years ago [21], and a very common carotenoid, lutein (**5**), has been isolated from *S. palustris* in the current work, but so far no other reports about the occurrence of such glycosides in ferns have appeared. Although 3-formylindole has been isolated from a variety of natural sources, including plants [16], red algae [22], and microorganisms [23], this is the first indole to be found in ferns.

EXPERIMENTAL

General

Optical rotations: in $CHCl_3$ or MeOH; UV: MeOH; IR: KBr pellets; EIMS: Hitachi-Perkin-Elmer-RMUGM mass spectrometer at 70 eV; FABMS: ZAB 2-SEQ spectrometer in the positive mode using 3-NOBA as matrix; NMR: Bruker AMX-300 spectrometer operating at 300.13 MHz for ^1H and at 75.47 MHz for ^{13}C, using the solvents as internal standard; Vacuum liquid chromatography (VLC): Merck silica gel (particle size 15 μm, vacuum by water aspiration); HPLC: Spherisorb S5 ODS II column (250 × 16 mm, particle size 5 μm, Knauer) with a Merck-Hitachi L-6200 Intelligent pump and Merck-Hitachi L-4000 UV detector.

Plant material

Leaves of *S. palustris* were collected near Port Moresby, central district province, PNG, in March 1991. The plant was identified by Dr. P. Hovenkamp, University of Leiden, The Netherlands, where a voucher specimen with the identification No. ETH 91/1127-03-91 is deposited.

Extraction and isolation

Air-dried and powdered leaves of *S. palustris* (1.52 kg) were successively percolated with MeOH and 70% MeOH at room temperature. The MeOH extract was concentrated *in vacuo*, and the resulting residue was partitioned between n-hexane and 90% aq MeOH. The alcoholic phase was further partitioned between $CHCl_3$ and 60% aq MeOH. After removal of solvents *in vacuo*, the residue of the $CHCl_3$ phase (19.4 g) was chromatographed on a vacuum column and eluted with $CHCl_3$ containing increasing amounts of MeOH. The collected fractions were evaporated *in vacuo* and examined by TLC. The homogenous fractions, showing similar spots on TLC, were put together to give 14 combined fractions (C_1-C_{14}) for further separation.

Fraction C_3 (230 mg) was further fractionated by VLC eluted with a gradient solvent system of CH_2Cl_2 and MeOH, controlled on TLC, to yield six subfractions. After crystallization, the first and second subfractions furnished 3-formylindole (**4**, 2.5 mg) and lutein (**5**, 3.5 mg), respectively.

Fraction C_8 (1190 mg) was separated again on a vacuum column with CH_2Cl_2-EtOAc (1:1) containing increasing portions of MeOH as eluent, and β-sitosterol-3-*O*-β-D-glucopyranoside (**6**, 160 mg) precipitated from three of the seven resulting subfractions.

Fraction C_9 (2930 mg) was also subjected to a vacuum column using increasing amounts of MeOH in CH_2Cl_2-EtOAc (1:1) as eluent. The fractions were combined into nine subfractions based on their TLC behaviour. The fifth subfraction (125 mg) was further fractionated by RP-HPLC with MeOH-H_2O (70:30) as mobile phase to give 3-oxo-4,5-dihydro-α-ionyl β-D-glucopyranoside (**3**, 4.4 mg) and stenopaluside (**1**, 11 mg). From the sixth subfraction, 21 mg of the cerebroside (**2**) precipitated

Stenopaluside (4S,5R*)-4-[(9Z)-2,13-di-(O-β-D-glucopyranosyl)-5,9,10-trimethyl-8-oxo-9-tetradecene-5-yl]-3,3,5-trimethylcyclohexanone* (**1**)

$[\alpha]_{D^{20}}$ −23.4° (*c* 0.092, MeOH); UV λ_{max}^{MeOH} nm (log ε): 249 (4.47). IR υ_{max}^{film} cm^{-1}: 3406 (OH), 2965, 2931, 1699 (C=O), 1651 (C=O), 1605, 1379, 1355, 1077, 1035; ^1H and ^{13}C NMR: Table 1.

Cerebroside

1-*O*-β-D-glucopyranosyl-(2*S*',3*R*',4*E*,8*Z*)-2-N-[(2*R*)-hydroxy-tetracosanoyl] octadecasphinga-4,8-dienine (**2**). $C_{48}H_{91}NO_9$; $[\alpha]_{D^{25}}$ +7.9° (*c* 0.088, MeOH); IR υ_{max}^{KBr} cm^{-1}: 3407 (OH), 2920, 2851, 1639, 1542, 1468, 1077, 1047; FABMS *m/z*: 848 $[M + Na]^+$, 826 $[M + H]^+$, 808 $[M + H\text{-}H_2O]^+$, 646 $[M + H\text{-}H_2O\text{-hexose}] +$; ^1H and ^{13}C NMR: Table 2.

Methanolysis of **2**

A soln of **2** (2.5 mg) in 2N HCl-MeOH (1.5 mL) was refluxed for 5 hr. H_2O was added to the reaction mixture, which was then extracted with n-hexane. The n-hexane layer was washed with H_2O and concentrated *in vacuo* to yield the, methyl-2-hydroxytetracosanoate, $[\alpha]_{D^{25}}$ −3.0° (*c* 0.115, $CHCl_3$). EIMS *m/z* (rel. int.): 398 $[M]^+$ (100), 339 $[M\text{-}COOCH_3]^+$ (44); ^1H NMR (300 MHz, $CDCl_3$): δ 0.89 (3H, *t*, *J* = 7.0 Hz, H-24), 1.26 (*m*, long chain CH_2), 3.80 (3H, *s*, $COOCH_3$), 4.20 (*m*, H-2).

The aq layer was evaporated under reduced pressure and examined on TLC (silica gel) developed with $CHCl_3$-MeOH-H_2O (16:9:2), sprayed with thymol-H_2SO_4 reagent, showing the presence of methyl-β-D-glucopyranoside.

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