Novel Cerebrosides Isolated from the Fermentation Mycelia of *Tuber indicum*

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The four new cerebrosides **1**–**4** possessing a unique C_{18} 9-methylsphinga-4,8-dienine-related moiety and a cyclic octapeptide, **5**, possessing alternating proline and glycine moietes were isolated from the *Tuber indicum* fermentation mycelium. Their structures were established on the basis of a spectroscopic analysis including NMR and HR-ESI-MS, as well as an acidic methanolysis experiment. To the best of our knowledge, the cerebrosides identified in the present study are quite different from those isolated from *Tuber indicum* fruiting bodies. Additionally, it was the first time that a cyclic peptide was isolated from the *Tuber* genus.

Introduction. – Truffles, the hypogeous fruiting bodies belonging to the *Tuber* species, are a precious and expensive delicacy in the famous French and Italian cuisines. Because of the decrease in the natural production and worldwide increase of demand, submerged fermentation is viewed as a potential way to produce *Tuber* mycelia, which could be considered as an alternative resource for its fruiting bodies [1][2]. For a better understanding of this alternative resource, our group has identified and assayed some known compounds from the *Tuber* fermentation mycelia, such as volatile organic compounds [3][4], hydrosoluble nucleosides [5], and fatty acids [6]. In this study, we aim to phytochemically search for some new compounds with special chemical structures from the *Tuber* fermentation mycelium. Through solvent partition and repeated column chromatography, compounds **1**–**5** (*Fig. 1*) were isolated, and their structures were identified. Compounds **1**–**4**[1]) were cerebrosides, while **5** was a cyclopeptide. To the best of our knowledge, cerebrosides **1**–**4** are new compounds, and a cyclic peptide, *i.e.*, **5**, was isolated from the *Tuber* genus for the first time.

Results and Discussion. – An AcOEt extract (100 g) of the cultured mycelia was separated by column chromatography and semi-preparative HPLC to afford the new compounds **1**–**4**, which were supposed to have analogous chemical skeletons, besides the known cyclopeptide **5**.

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

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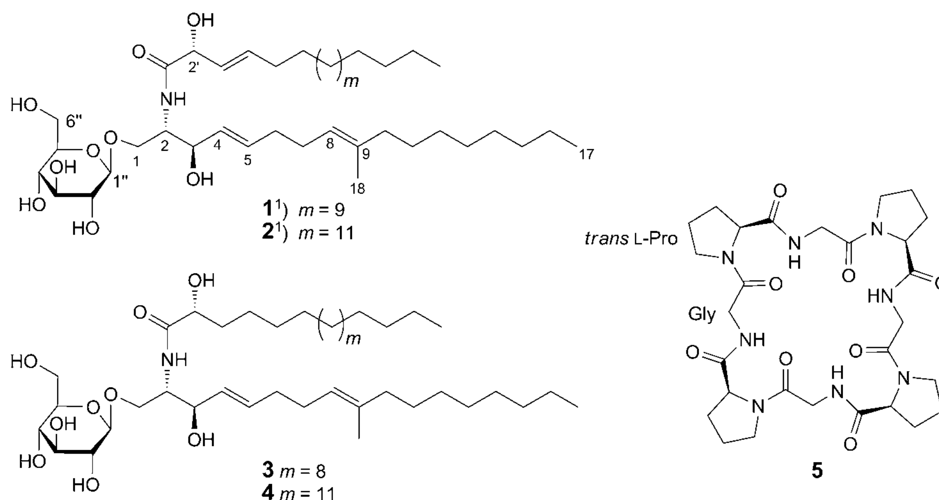

Fig. 1. *Compounds* **1**–**5** *isolated from the fermentation mycelia of* Tuber indicum

Compound **1** was a white amorphous powder, whose molecular formula was determined as $C_{43}H_{79}NO_9$ by HR-ESI-MS (*m/z* 776.6001 ($[M+Na]^+$, $C_{43}H_{79}NNaO_9^+$). The IR spectrum of **1** showed absorption bands of OH groups at 3396 cm^{-1}, a glycoside moiety (C–O) at 1083 cm^{-1}, a secondary amide group at 1536 and 1640 cm^{-1}, long aliphatic chains at 2921, 1468, and 721 cm^{-1}, and an amide carbonyl group at 1644 and 1678 cm^{-1}. The ^1H- and ^{13}C-NMR spectra (*Table*) including the DEPT data further indicated that **1** contained one amide carbonyl group (δ(C) 174.2), five olefinic CH groups (δ(C) 123.7, 127.8, 129.8, 133.3, and 133.6), one quaternary olefinic C-atom (δ(C) 135.6), a series of oxygenated C-atoms (δ(C) 61.5, 68.8, 70.4, 71.7, 72.9, 73.8, 76.7, 76.7, and 103.5), two terminal Me groups (δ(H) 0.88 (*t*, $J=13.8$ Hz, 6 H)), and one allylic Me group (δ(H) 1.58 (*s*). For a better understanding of the detailed planar structure of **1**, 2D-NMR experiments including HMBC and $^1H,^1H$-COSY were performed (*Fig. 2*). A series of HMBCs, including δ(C) 103.5 (C(1''))/δ(H) 3.18 (H–C(2'')), δ(C) 76.7 (C(3''))/δ(H) 3.18 (H–C(2'')), δ(C) 73.8 (C(4''))/δ(H) 3.26 (H–C(5'')), δ(C) 76.7 (C(5''))/δ(H) 3.66 and 3.85 (H_a–C(6'') and H_b–C(6'')), confirmed the hexose residue in the molecule. The cross-peak δ(C) 103.5 (C(1''))/ δ(H) 4.11 and 3.69 (H_a–C(1) and H_b–C(1)) further suggested that the hexose residue was attached to a long-chain base at C(1). The $^1H,^1H$-COSY cross-peaks, including δ(H) 5.46 (H–C(4))/5.70 (H–C(5)), 5.70 (H–C(5))/2.02 (CH_2(6)), 2.02 (CH_2(6))/5.13 (H–C(8)), and 2.02 (CH_2(7))/5.13 (H–C(8)), unambiguously assigned the position of the C=C bonds between C(4) and C(5) and C(8) and C(9) of the long-chain base. Additionally, the position of a Me group at C(9) was also evidenced by the HMBC spectrum, in which the allylic Me group at δ(H) 1.58 (Me(18)) was correlated with the olefinic C-atoms at δ(C) 123.7 (C(8)) and 135.6 (C(9)) and the CH_2 group at δ(C) 39.6 (C(10)). The assignment of an OH group at C(3) was supported by the HMBCs δ(H) 4.11 (H–C(3))/δ(C) 133.6 (C(4)) and 129.8 (C(5)). Finally, because the OH group at δ(H) 4.42 (H–C(2')) displayed an HMBC cross-peak with the C=O group at δ(C) 174.2

Table. *^{1}H- and ^{13}C-NMR Data* (600 and 125 MHz, resp.; CD_3OD) *of Compounds* **1**–**4**. δ in ppm, J in Hz.

Position	**1**		**2**		**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
Long-chain base:								
H_a–C(1)	4.11 (overlap)	68.8	4.09 (overlap)	70.4	4.09 (overlap)	69.9	4.07 (overlap)	68.6
H_b–C(1)	3.69 (*dd*, *J* = 10.3, 3.6)		3.70 (overlap)		3.69 (*dd*, *J* = 10.2, 3.6)		3.69 (*dd*, *J* = 10.2, 3.6)	
H–C(2)	3.94–3.96 (*m*)	53.4	3.97–4.01 (*m*)	55.3	3.97 (overlap)	54.8	3.97 (overlap)	53.5
H–C(3)	4.11 (overlap)	70.4	4.14 (overlap)	72.2	4.11 (overlap)	71.8	4.11 (overlap)	70.5
H–C(4)	5.46 (*dd*, *J* = 14.4, 7.2)	133.6	5.44 (overlap)	135.4	5.46 (*dd*, *J* = 15.0, 7.2)	134.9	5.46 (*dd*, *J* = 15.0, 7.2)	133.4
H–C(5)	5.70 (*dd*, *J* = 15.0, 6.0)	129.8	5.71–5.73 (*m*)	131.8	5..72 (*dd*, *J* = 15.6, 6.0)	131.3	5.72 (*dd*, *J* = 15.6, 6.0)	129.9
CH_2(6)	2.02 (overlap)	31.9	2.05 (overlap)	33.7	2.02 (overlap)	33.3	2.05 (overlap)	32.6
CH_2(7)	2.02 (overlap)	27.6	2.05 (overlap)	29.4	2.02 (overlap)	31.0	2.05 (overlap)	27.5
H–C(8)	5.13 (br.)	123.7	5.14 (br.)	125.5	5.12 (br.)	125.5	5.11 (br.)	123.6
C(9)		135.6		137.4		137.0		136.3
CH_2(10)	1.96 (*t*, *J* = 14.4)	39.6	1.97 (*t*, *J* = 15.0)	41.4	1.96 (*t*, *J* = 13.8)	40.9	1.96 (*t*, *J* = 15.0)	39.5
CH_2(11)	1.36–1.38 (*m*)	27.9	1.37–1.40 (*m*)	29.8	1.36–1.39 (*m*)	29.3	1.35–1.40 (*m*)	27.9
CH_2(12)–CH_2(16)	1.27 (overlap)	22.5–31.8	1.27 (overlap)	24.4–31.4	1.27 (overlap)	23.9–31.8	1.27 (overlap)	22.5–29.5
Me(17)	0.88 (*t*, *J* = 13.8)	13.2	0.89 (*t*, *J* = 13.8)	15.1	0.88 (*t*, *J* = 13.8)	14.6	0.88 (*t*, *J* = 14.4)	13.2
Me(18)	1.58 (*s*)	14.9	1.58 (*s*)	16.8	1.58 (*s*)	16.3	1.58 (*s*)	14.9
Acyl:								
C(1′)		174.2		175.3		177.4		176.0
H–C(2′)	4.42 (*d*, *J* = 5.4)	71.7	4.43 (*d*, *J* = 4.8)	73.7	3.97 (overlap)	73.1	3.97 (overlap)	71.8
H–C(3′)	5.49 (*dd*, *J* = 15.0, 6.0)	133.3	5.49 (*dd*, *J* = 15.0, 6.0)	135.3	2.04 (overlap)	36.0	2.05 (overlap)	34.7
H–C(4′)	5.82 (*dd*, *J* = 15.0, 6.6)	127.8	5.82 (*dd*, *J* = 15.0, 6.6)	129.7				
CH_2(5′ to *n*′) or CH_2(4′ to *n*′)[a])[b])	1.27 (overlap)	22.5–31.8	1.29 (overlap)	24.4–31.4	1.27 (overlap)	26.4–31.8	1.27 (overlap)	22.5–29.5
Me(*n*′ + 1)′[b])	0.88 (*t*, *J* = 13.8)	13.2	0.89 (*t*, 13.8)	15.1	0.88 (*t*, *J* = 13.8)	14.6	0.88 (*t*, *J* = 14.4)	14.9
Sugar moiety:								
H–C(1″)	4.25 (*d*, *J* = 7.8)	103.5	4.27 (*d*, *J* = 7.8)	105.4	4.25 (*d*, *J* = 7.8)	104.9	4.25 (*d*, *J* = 7.8)	103.5
H–C(2″)	3.18 (*dd*, *J* = 16.8, 7.8)	72.9	3.20 (*dd*, *J* = 16.8, 8.4)	74.7	3.17 (*dd*, *J* = 16.8, 7.8)	75.2	3.17 (*dd*, *J* = 16.8, 9.0)	72.0
H–C(3″)	3.34 (*dd*, *J* = 16.8, 8.4)	76.7	3.32–3.35 (*m*)	78.5	3.33 (*dd*, *J* = 16.8, 8.4)	78.1	3.34 (*dd*, *J* = 16.8, 8.4)	76.8
H–C(4″)	3.28 (overlap)	73.8	3.29 (overlap)	75.6	3.29 (overlap)	73.3	3.29 (overlap)	73.8
H–C(5″)	3.26 (overlap)	76.7	3.27 (overlap)	78.6	3.26 (overlap)	78.2	3.26 (overlap)	76.8
H_a–C(6″)	3.66 (*dd*, *J* = 12.0, 4.8)	61.5	3.66 (*dd*, *J* = 11.4)	63.3	3.65 (*dd*, *J* = 12.0, 4.8)	62.9	3.65 (*dd*, *J* = 12.0, 4.2)	61.5
H_b–C(6″)	3.85 (*d*, *J* = 12.0)		3.86 (*d*, *J* = 11.4)		3.85 (*d*, *J* = 10.8)		3.85 (*d*, *J* = 12.0)	

[a]) CH_2(5′ to *n*′) for **1** and **2**, and CH_2(4′ to *n*′) for **3** and **4**. [b]) *n*′ = 18 for **1**, *n*′ = 17 for **3**, and *n*′ = 20 for **2** and **4**.

Fig. 2. *Key features observed in the HMBC* (H → C) *and* ^{1}*H*,^{1}*H-COSY* (—) *plot of compound* **1**[1])

(C(1′)) and ^{1}H,^{1}H-COSY cross-peaks with the olefinic CH groups at δ(H) 5.49 (H–C(3′)) and 5.82 (H–C(4′)), a fatty 2-hydroxyall-3-enoic acid moiety was suggested to exist in the molecule. Based on the above conclusions, compound **1** was considered as a cerebroside with a constitution similar to asperamide B (=(2*R*,3*E*)-*N*-{(1*S*,2*R*,3*E*,7*E*)-1-[(β-D-glucopyranosyloxy)methyl]-2-hydroxy-8-methylnonadeca-3,7-dien-1-yl}-2-hydroxyhexadec-3-enamide) [7] and cerebroside A (=(2*R*,3*E*)-*N*-{(1*S*,2*R*,3*E*,7*E*)-1-[(β-D-glucopyranosyloxy)methyl]-2-hydroxy-8-methylheptadeca-3,7-dien-1-yl}-2-hydroxyhexydec-3-enamide) [8], both of which were isolated from the *Pachybasium* species. To establish the chain length of the fatty acid and sphingosine-related moieties (sphingenine = sphingosine = (2*S*,3*R*)-2-aminooctadec-4-ene-1,3-diol) and the configuration of the sugar moiety, **1** was subjected to acidic methanolysis to provide three fractions containing a fatty acid methyl ester **1a**, a sphingoid base **6**, and a methyl glycoside **7** (*Scheme*). The fraction containing **1a** was analyzed by GC/MS which revealed the characteristic fragment-ion peaks at *m*/*z* 326 (M^+) and 267 ($[M - COOMe]^+$), suggesting the presence of a C_{19} fatty acid moiety in **1**. The fraction

Scheme. *Methanolysis of Compounds* **1**–**4**

containing **6**, analyzed by LC/ESI-MS, showed the quasi-molecular ion of the main peak at *m*/*z* 298 ($[M+H]^+$), indicating that 18 C-atoms were contained in the sphingoid-base moiety of **1**. Compound **7** had an optical rotation $[\alpha]_D^{25} = +75.6$ ($c = 0.2$, MeOH), which was quite near to that of methyl β-D-glucoside ($[\alpha]_D^{25} = +77.4$ ($c = 0.2$, MeOH) [9], suggesting that the hexose contained in **1** was a β-D-glucose residue. The coupling constant $J = 7.8$ Hz (*Table*) for the anomeric proton H–C(1″) also supported this conclusion [7][10]. The configuration of **1** was deduced by comparing the NMR and optical rotation data with those of analogs in [7][9][11][12]. Except for the overlap of the proton signals of H_a–C(1) and H–C(3), H_a–C(1) (δ(H) 4.11 (overlap)) and other H-atoms, including H_b–C(1) (δ(H) 3.69 (*dd*, $J = 10.3$, 3.6 Hz)), H–C(2) ((δ(H) 3.94–3.96 (*m*)), H–C(3) (δ(H) 4.11 (overlap)), and H–C(2′) (δ(H) 4.42 (*d*, $J =$ 5.4 Hz)), presented chemical shifts and coupling constants very similar to those of asperamide B [7]. Furthermore, the specific optical rotation of **1** ($[\alpha]_D^{25} = -1.8$) was also very close to that of asperamide B ($[\alpha]_D^{25} = -1.2$) [7]. Therefore, **1** may contain the same (2*S*,2′*R*,3*R*)[1]) moiety as that present in asperamide B [7]. The geometry of the C=C bonds of **1** was established by comparing its NMR data with literature: the values of the vicinal coupling constants were $J = 15.0$ Hz for the protons of CH(4)=CH(5) and CH(3′)=CH(4′) compatible with (3′*E*,4*E*)[1]) configuration [12][13]. Additionally, according to previous experiences, an Me group at an (*E*)-C=C bond always appears at δ(C) 15.4, while that at to a (*Z*)-C=C bond appears at δ(C) 22.7 [9]. Thus, the chemical shift of Me(18) at δ(C) 14.9 (*Table*) indicated that the C(8)=C(9) bond was (*E*)-configured. Putting all these points together, the structure of **1** was established as (2*S*,3*R*,4*E*,8*E*)-2-{[(2*R*,3*E*)-2-hydroxynonadec-3-enoyl]amino}-9-methylheptadeca-4,8-diene-1,3-diol 1-(β-D-glucopyranoside)[1]).

The molecular formula of **2** was determined as $C_{45}H_{83}NO_9$ by HR-ESI-MS (*m*/*z* 782.6108 ($[M+H]^+$, $C_{45}H_{84}NO_9^+$)). No significant difference was found in the IR and NMR spectra of **1** and **2** (*Table* and *Exper. Part*). This indicates that **2** is an analogue of **1**. Compounds **1** and **2** only differed from each other in the different number of C-atoms in the aliphatic fatty acid chain. The acidic methanolysis of **2** (*Scheme*) revealed the characteristic fragment **6** arsing from of the sphingoid-base moiety by LC/ESI-MS (*m*/*z* 298 ($[M+H]^+$), while the characteristic fragment **2a** arising from the fatty acid moiety was determined by GC/MS (*m*/*z* 354 (M^+) and 295 ($[M-\text{COOMe}]^+$)). These results indicated that **2** contained the same sphingosine-related moiety as **1**, while 21 C-atoms were present in its fatty acid moiety. Therefore, compound **2** was established as (2*S*,3*R*,4*E*,8*E*)-2-{(2*R*,3*E*)-2-hydroxyheneicos-3-enoyl]amino}-9-methylheptadeca-4,8-diene-1,3-diol 1-(β-D-glucopyranoside)[1]).

The molecular formula of **3** was determined as $C_{42}H_{79}NO_9$ by HR-ESI-MS (*m*/*z* 742.5345 ($[M+H]^+$, $C_{42}H_{80}NO_9^+$)). The ^{1}H- and ^{13}C-NMR spectra of **3** (*Table*) were almost the same as that of **1** and **2**, except for the absence of one C=C bond. The acidic methanolysis (*Scheme*) showed that compound **3** contained the same sphingosine-related and sugar moiety as **1** and **2** and that the fatty acid moiety was derived from a saturated aliphatic acid. GC/MS Analysis of **3a** revealed characteristic ions at *m*/*z* 314 (M^+) and 255 ($[M-\text{COOMe}]^+$), confirming proved the existence of a saturated fatty acid moiety, *i.e.*, 2-hydroxyoctadecanoyl, in **3**. Therefore, compound **3** was established as (2*S*,3*R*,4*E*,8*E*)-2-{(2*R*)-2-hydroxyoctadecanoyl]amino}-9-methylheptadeca-4,8-di-ene-1,3-diol 1-(β-D-glucopyranoside)[1]).

The molecular formula of **4** was determined as $C_{45}H_{85}NO_9$ by HR-ESI-MS (*m/z* 784.6273 ($[M+H]^+$, $C_{45}H_{86}NO_9^+$)). As shown in the *Table*, the ^{1}H- and ^{13}C-NMR spectra indicated that **4** was an analog of **1**–**3**. In addition to that, compound **4** contained only two C=C bonds. The acidic methanolysis (*Scheme*) showed that compounds **3** and **4** were different from each other only in the chain length of the fatty acid moiety. The identified characteristic ion of **4a** in the GC/MS (*m/z* 356 (M^+) and 297 ($[M-COOMe]^+$)) further indicated that the fatty acid moiety contained 21 C-atoms. Therefore, the structure of **4** was established as (2*S*,3*R*,4*E*,8*E*)-2-{(2*R*)-2-hydroxyheneicosanoyl]amino-9-methylheptadeca-4,8-diene-1,3-diol 1-(β-D-glucopyranoside)[1]).

Thus from the *T. indicum* fermentation mycelia, four novel cerebrosides were isolated, and their structures were identified. Their common characteristic is that they all contained a C_{18} 9-methylsphinga-4,8-dienine-related and β-D-glucopyranosyl moiety (sphinga-4,8-dienine = (2*S*,3*R*,4*E*,8*E*)-2-aminooctadeca-4,8-diene-1,3-diol). They differed from each other in their fatty acid moiety with either a different number of C=C bonds or with a different chain length. Although some similar cerebrosides or ceramides have been isolated from *T. indicum* fruiting bodies, their reported structures are different from those of the cerebrosides investigated in this study by the number and position of C=C bonds and by the chain length of the sphingoid-base or fatty acid moiety [14]. Besides cerebrosides, the cyclic octapeptide **5** composed of L-proline and glycine was also isolated, and its structure was identified as cyclo(L-Pro-Gly)$^+$ based on the previously described spectral data [15]. To the best of our knowledge, it is the first time that a cyclic peptide has been found in the *Tuber* genus. Furthermore, it is very interesting that although *Tuber indicum* belongs to a higher fungus, compounds **1**–**5** were more similar to the constituents isolated from lower organisms, including the unique marine protist *Thraustochytrium globosum* [16], the marine sponge *Prosuberites laughlini* [17], the marine fungus *Aspergillus niger* EN-13 [7], an imperfect fungus *Pachybasium* sp. [8], and the basidiomycetes *Polyporus ellisii* and *Cortinarius umidicola* [12].

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Experimental Part

General. All solvents of anal. grade were purchased from the *BoDi Chemical Factory* (Tianjin, China). Colum chromatography (CC): silica gel (flash CC; SiO_2; (200–300 mesh; *Qingdao Marine Chemical Factory* (Qingdao, China)); *Sephadex-LH-20* gel (*Amersham Pharmacia Biotech China Ltd.* (Shanghai, China)). TLC: SiO_2 *G* (*Qingdao Marine Chemical Factory*); pre-coated SiO_2 *G* plates (*Merck Inc.* (Darmstadt, Germany)). Prep. medium-pressure liquid chromatography (MPLC): *Büchi-B-608*

system (Flawil, Switzerland). Prep. HPLC: *Waters-600* instrument coupled with a *2487* multiple-wave detector; *ODS* column (10 × 250 mm, 5 μm; *Agela Technologies Inc.* (Beijing, China)); 100% MeOH as mobile phase (4 ml/min). Optical rotations: *Perkin-Elmer-314* polarimeter. IR (KBr) Spectra: *Nicolet-Nexus-670* FT-IR spectrometer; $\tilde{\nu}$ in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR Spectra: *Varian-Mercury* NMR instrument (600 and 400 MHz); δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-ESI-MS: *Bruker-Bio-TOF-Q-plus* mass spectrometer (*Bruker Daltonics Ltd.*, Coventry, UK). GC/ESI-MS: *Shimadzu-GC/MS-QP-2010-plus* mass spectrometer (Tokyo, Japan) and *Agilent-7890A* GC system equipped with a *5975C* quadrupole MS detector (*Agilent Technologies, Inc.*, CA, USA). LC/ESI-MS: *Agilent-1200* HPLC system equipped with an ion-trap MS (*Agilent Technologies, Inc.*, CA, USA).

Mycelia Material. The strain of *Tuber indicum* was purchased from the Mianyang Institute of Edible Fungi (Sichuan, China). Fermentation mycelia were cultured in our laboratory according to the procedure described previously [1][2]. The harvested mycelia were rinsed with deionized water to remove the culture medium. The clean mycelia was stored at -20° before it was used.

Extraction and Isolation. First, the mycelia (*ca.* 3 kg) was extracted by EtOH, and then the extract was divided into three fractions by extracting with hexane, AcOEt, and 95% EtOH. The AcOEt fraction (100 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 99 : 1, 90 : 10, 85 : 15, 80 : 20, and 70 : 30): *Fractions A – F* (TLC monitoring). *Fr. D* (*ca.* 5.0 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 85 : 15): **5** (15.0 mg) and a mixture of cerebrosides (*ca.* 35.3 mg). The cerebroside mixture was further separated by semi-prep. HPLC: **1** (15.2 mg), **2** (10.2 mg), **3** (3.2 mg), and **4** (5.1 mg).

(2R,3E)-N-{(1S,2R,3E,7E)-1-[(β-D-Glucopyranosyloxy)methyl]-2-hydroxy-8-methylhexadeca-3,7-dien-1-yl}-2-hydroxynonadec-3-enamide (**1**): Amorphous powder. $[\alpha]_D^{25} = -1.8$ ($c = 0.2$, MeOH). IR (KBr): 3396, 2921, 2985, 1640, 1536, 1467, 1082. ^1H- and ^{13}C-NMR: *Table.* HR-ESI-MS: 776.6001 ($[M + Na]^+$, $C_{43}H_{79}NO_9Na^+$; calc. 776.5653).

(2R,3E)-N-{(1S,2R,3E,7E)-1-[(β-D-Glucopyranosyloxy)methyl]-2-hydroxy-8-methylhexadeca-3,7-dien-1-yl}-2-hydroxyheneicos-3-enamide (**2**): Amorphous powder. $[\alpha]_D^{25} = -2.0$ ($c = 0.2$, MeOH). IR (KBr): almost identical to that of **1**. ^1H- and ^{13}C-NMR: *Table.* HR-ESI-MS: 782.6108 ($[M + H]^+$, $C_{45}H_{84}NO_9^+$; calc. 782.6146).

(2R)-N-{(1S,2R,3E,7E)-1-[(β-D-Glucopyranosyloxy)methyl]-2-hydroxy-8-methylhexadeca-3,7-dien-1-yl}-2-hydroxyoctadecanamide (**3**): Amorphous powder. $[\alpha]_D^{25} = +0.8$ ($c = 0.2$, MeOH). IR (KBr): almost identical to that of **1**. ^1H- and ^{13}C-NMR: *Table.* HR-ESI-MS: 742.5345 ($[M + H]^+$, $C_{42}H_{80}NO_9^+$; calc. 742.5833).

(2R)-N-{(1S,2R,3E,7E)-1-[(β-D-Glucopyranosyloxy)methyl]-2-hydroxy-8-methylhexadeca-3,7-dien-1-yl}-2-hydroxyheneicosanamide (**4**): Amorphous powder. $[\alpha]_D^{25} = +1.3$ ($c = 0.2$, MeOH). IR (KBr): almost identical to that of **1**. ^1H- and ^{13}C-NMR: *Table.* HR-ESI-MS: 784.6273 ($[M + H]^+$, $C_{45}H_{86}NO_9^+$; calc. 784.6303).

Cyclo(glycyl-L-prolylglycyl-L-prolylglycyl-L-prolylglycyl-L-prolyl) (*Cyclo(L-Pro-Gly)₄*; **5**): Colorless amorphous powder. $[\alpha]_D^{25} = -60.2$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3204, 3113, 1678, 1644, 1457, 1304, 1296, 1005, 794, 500. ^1H-NMR: 1.86 (*m*); 2.01 (*m*); 2.32 (*m*); 3.50 (*dd*, $J = 9.3$, 4.5); 3.57 (*dd*, $J = 9.6$, 5.7); 3.83 (*dd*, $J = 8.4$, 2.1); 4.02 (overlap). ^{13}C-NMR: 22.6; 28.7; 45.6; 46.9; 58.8; 163.7; 170.1. HR-ESI-MS: 617.3036 ($[M + H]^+$, $C_{28}H_{41}N_8O_8^+$; calc. 617.3047).

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