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GLYCOLIPIDS FROM *MIRABILIS HIMALAICA*

GUO-LIN ZHANG,* QI-YI XING and MING-ZHE ZHANG

Department of Chemistry, University of Beijing, Beijing 100871, People's Republic of China

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Key Word Index—*Mirabilis himalaica*; Nyctaginaceae; roots; glycolipids.

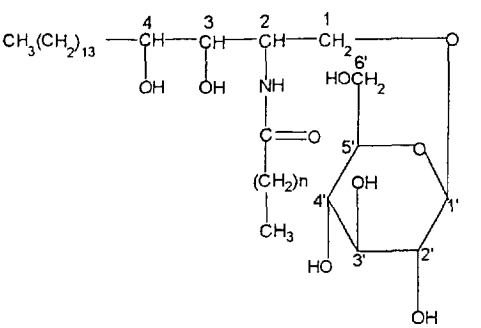
Abstract—Two new glycolipids, *N*-pentacosanosyl-β-D-glucopyranosyl-(1-1′)-phytosphingosine and its homologue, *N*-hexacosanosyl-β-D-glucopyranosyl-(1-1′)-phytosphingosine were isolated from roots of *Mirabilis himalaica*, along with the six known compounds, daucosterol, syringaresinol-4′-*O*-β-D-monoglucoside, 2,3-dihydroxypropyl (*Z*,*Z*)-9,12-octadecadienate, ursolic acid, β-sitosterol and oleanolic acid. Their structures were elucidated using spectroscopic methods.

INTRODUCTION

Plants belonging to the genus *Mirabilis* are used in Chinese medicine as a remedy for various diseases [1]. Some sterols [2], triterpenoids and their glucosides from the roots [3, 4], unsaturated acids from the seeds [5] and different xanthins from the flowers [6] of *M. jalapa* have been characterized. Because of the antiviral effects of proteins from *M. jalapa*, they have been studied intensively biologically and biochemically [7]. However, there are no reports about the constituents of *M. himalaica*, which grows in south western and north western China and is used to treat stomach disorders, nephritic oedema and gonorrhoea [8]. In our study of this species, two new glycolipids, *N*-pentacosanosyl-β-D-glucopyranosyl-(1-1′)-phytosphingosine (**1**) and *N*-hexacosanosyl-β-D-glucopyranosyl-(1-1′)-phytosphingosine (**2**), as well as the known compounds, daucosterol (**3**), syringaresinol-4′-*O*-β-D-monoglucoside (**4**), 2,3-dihydroxypropyl (*Z*,*Z*)-9,12-octadecadienate (**5**), ursolic acid (**6**), β-sitosterol (**7**) and oleanolic acid (**8**), were isolated and their structures elucidated by spectral methods including NMR, IR and FAB mass spectrometry. Because of the effects, including antitumour, marrow cell growth acceleration and immunopotency of sphingoglucolipids, which possess similar skeletons to those of **1** and **2** but bear galactopyranosyl instead of glucopyranosyl and different *N*-substituents, a number of such glucolipids have been isolated and synthesized before [9–12].

RESULTS AND DISCUSSION

Glycolipid **1** was isolated as white amorphous powder. On TLC it gave an orange colour when visualized with Dragendorff's reagent. The FAB mass spectrum (positive) showed a $[M]^+$ peak at *m/z* 843. IR revealed strong absorptions for hydroxyl groups (3450 cm^{-1}), amide (1632 and 1540 cm^{-1}) and hydroxyl groups of a sugar (1080–1040 cm^{-1}). A β-D-glucopyranosyl moiety was recognized from signals at δ 105.0, 75.3, 78.2, 71.9, 78.3 and 63.1 in the ^{13}C NMR, and signals at δ 3.20–3.90 (5H) in the ^1H NMR, as well as the ion peaks at *m/z* 680 $[M-glc]^+$ and 664 $[M-C_6H_{11}O_6]^+$ in the FAB mass spectrum. The identification of glucose in the aqueous solution after hydrolysis of **1** in 10% HCl supported this confirmation. The resonance at δ 177.6 is due to the carbonyl group of an amide. ^1H NMR signals at δ 0.87 (*t*, *J* = 7 Hz, 6H) were ascribed to two methyl groups and the signals at δ 1.20–1.50, 1.50–1.80, 1.90–2.10 (each *m*, 72H), to *ca* 36 —CH_2— groups. Thus, the presence of two aliphatic chains was concluded. One of the two chains, $CH_3(CH_2)_{23}CONH$— (A), could be deduced from the significant FAB mass spectral peaks at *m/z* 478 $[M-CH_3(CH_2)_{23}CO]^+$, 382 $[CH_3(CH_2)_{23}CONH_2+H]^+$, 317 $[M+H-CH_3(CH_2)_{23}CO-glc+$



1: n = 23
2: n = 24

* Author to whom correspondence should be addressed.

H]$^+$ and 299 [M $-$ $C_6H_{11}O_6$ $-$ $CH_3(CH_2)_{23}CO$]$^+$. In the ^{13}C NMR, the signals at δ 73.4 (C-4), 76.0 (C-3), 70.4 (C-1) and 52.1 (C-2), and ^{1}H NMR signals at δ 5.25 5.40 (*m*, 2H, 2-H and 1′-H), 4.27 (*d*, J = 7 Hz, 2H, 1-H), 4.0 (*m*, 2H, 3-H and 4-H) suggested that β-D-glucopyranosyl was connected to the second chain, which bears two hydroxyl groups, as well as the amide (A). The IR and ^{1}H NMR spectra of **1** are almost the same as those of the stereoisomers of cornuta-glycolipids, *N*-tricosanosyl-β-D-glucopyranosyl-(1-1′)-phytosphingosine [13]. However, the [M]$^+$ of **1** is at *m/z* 843, instead of *m/z* 815 in cornuta-glycolipids, *N*-tricosanosyl-β-D-glucopyranosyl-(1-1′)-phytosphingosine [13]. However, the [M]$^+$ of **1** is at *m/z* 843, instead of *m/z* 815 in cornuta-glycolipids. The FAB-mass spectrum of **1** showed a strong ion peak at *m/z* 478 [M $-$ $CH_3(CH_2)_{23}CO$]$^+$ just as those of cornuta-glycolipids, supporting the presence of phytosphingosine in **1**. Thus, **1** was elucidated as *n*-pentacosanosyl-β-D-glucopyranosyl (1-1′)-phytosphingosine. This compound has not been reported before.

Glycolipid **2**, a white amorphous powder, also reacted with Dragendorff's reagent to give an orange spot. Its IR showed absorptions at 3560 cm^{-1} (—OH), 1631 and 1547 cm^{-1} (—CONH—) and 1000–1100 cm^{-1} (—OH in sugar). D-Glucose was detected after hydrolysis of **2** in 15% HCl. The ^{1}H NMR and ^{13}C NMR of **2** are almost the same as those of **1**. However, the integrals of the signals at δ 1.20–1.50, 1.50–1.80 and 1.90–2.10 in the ^{1}H NMR of **2** is *ca* 74 protons (37 × —CH_2—) instead of 72 protons as in **1**. The FAB mass spectrum of **2** gave the quasi-molecular ion peak at *m/z* 858 [M + 1]$^+$. Other peaks at *m/z* 696 [M + H $-$ glc + H]$^+$, 478 [M $-$ $CH_3(CH_2)_{24}CO$]$^+$ and 316 [M + 1 $-$ ($C_6H_{11}O_5$ $-$ $CH_3(CH_2)_{24}$)CO]$^+$ confirmed the presence of β-D-glucopyranosyl and the side-chain, $CH_3(CH_2)_{24}CO$, which contains one "—CH_2—" more than that in **1**. Consequently, **2** was identified as a homologue of **1**.

EXPERIMENTAL

General. MPS: uncorr. IR: KBr discs. NMR: 400 MHz (^{1}H) and 100 MHz (^{13}C), TMS as int. standard. FAB-MS: glycerol as matrix. CC: silica gel, 160–200 mesh.

Plant material. Roots of *M. himalaica* (Edgew) Heimeri were collected in Ganlan Autonomous Region (Gansu Province, China), in August, 1991, and identified in Lanzhou Medical College, where a voucher specimen is deposited.

Extraction and isolation. Powdered dried roots (3 kg) were soaked in 95% EtOH (3 × 10 l). After removing solvents under red. pres., 140 g residue was obtained. This was divided into 4 frs by CC with $CHCl_3$–MeOH (10:1). **5** (25 mg) was isolated by purification of Fr. 1 by CC ($CHCl_3$–Me_2CO, 10:1). Fr. 2 was subjected to further CC ($CHCl_3$–Me_2CO, 5:1) to yield **6** (50 mg), **7** (30 mg) and **8** (20 mg). From fr. 3, **1** (40 mg) and **2** (35 mg) were obtained by CC ($CHCl_3$–MeOH–MeCN, 7:1:1). Fr. 4 was sepd. by CC ($CHCl_3$–MeOH, 5:1) to yield **3** (80 mg) and **4** (30 mg).

N-*Pentacosanosyl-β-D-glucopyranosyl-(1-1′)-phytosphingosine* (**1**). White powder, mp >180° (dec). $[\alpha]_d^{25}$ + 19° (pyridine; *c* 0.1). IR ν_{max} cm^{-1}: 3450, 2922, 2840, 1632, 1540, 1440, 1070. FAB-MS: 843 [M]$^+$, 680 [M $-$ glc]$^+$, 664 [M $-$ $C_6H_{11}O_6$]$^+$, 478 [M $-$ $CH_3(CH_2)_{23}CO$]$^+$, 382 [$CH_3(CH_2)_{23}CONH_2$ + H]$^+$, 317 [M + H $-$ $CH_3(CH_2)_{23}CO$ $-$ glc + H]$^+$, 299 [M $-$ $C_6H_{12}O_6$ $-$ $CH_3(CH_2)_{23}CO$]$^+$. ^{1}H NMR (CD_3OD): δ 7.75 (*d*, 1H, —NH—), 5.32 (*d*, 1H, J = 6 Hz, 1′-H), 5.29 (*p*, 1H, J = 7 Hz, 2-H), 4.27 (*d*, 2H, J = 8 Hz, 1-H), 4.0 (*m*, 2H, 3-H and 4-H), 3.85 (*d*, 1H, J = 9 Hz), 3.80 (*dd*, 1H, J = 7, 1 Hz), 3.68 (*dd*, 1H, J = 5, 2Hz), 3.59 (*t*, 1H, J = 5 Hz), 3.50 (*m*, 1H), 3.36 (*m*, 1H), 3.20 (*t*, 1H, J = 7 Hz), 0.87 (*t*, 6H, J = 7 Hz, 2 × CH_3). ^{13}C NMR (CD_3OD,DEPT): δ 70.4 (C-1), 52.1 (C-2), 76.0 (C-3), 73.4 (C-4), 105.0 (C-1′), 75.3 (C-2′), 78.2 (C-3′), 71.9 (C-4′), 78.3 (C-5′), 63.1 (C-6′), 15.2 ($\underline{C}H_3$), 177.6 (C=O), 34.3, 33.5, 33.4, 33.1, 31.3, 31.2, 31.0, 30.9, 28.8, 27.7, 27.6, 27.1, 26.7, 24.2, 24.1 and 24.5 (—CH_2).

N-*Hexacosanosyl-β-D-glucopyranosyl-(1-1′)-phytosphingosine* (**2**). White powder, mp >180° (dec). $[\alpha]_d^{25}$ − 22° (pyridine, *c* 0.1). IR ν_{max} cm^{-1}: 3450, 2956, 2922, 2871, 2852, 1631, 1547, 1467, 1457, 1100, 1074 FAB-MS: 858 [M + 1]$^+$, 696 [M + 2H $-$ glc]$^+$, 478 [M $-$ $CH_3(CH_2)_{24}CO$]$^+$, 316 [M + H $-$ $C_6H_{11}O_5$ $-$ $CH_3(CH_2)_{24}CO$]$^+$. ^{1}H NMR (CD_3OD): δ 7.85 (*d*, 1H, J = 9 Hz, —N$\underline{H}$—), 5.32 (*m*, 2H, 1′-H and 2-H), 4.28 (*d*, 2H, J = 8 Hz, 1-H), 4.04 (*m*, 2H, 3-H and 4-H), 3.84 (*d*, 1H, J = 10 Hz). 3.80 (*dd*, 1H, J = 7, 1 Hz), 3.63 (*dd*, 1H, J = 11, 1 Hz), 3.55 (*t*, 1H, J = 6 Hz), 3.50 (*m*, 1H), 3.36 (*m*, 1H), 3.20 (*t*, 1H, J = 8 Hz), 0.90 (*m*, 2 × $C\underline{H}_3$). ^{13}C NMR (CD_3OD, DEPT): δ 70.4 (C-1), 52.4 (C-2), 76.4 (C-3), 73.7 (C-4), 105.3 (C-1′), 75.6 (C-2′), 78.5 (C-3′), 72.2 (C-4′), 78.6 (C-5′), 63.3 (C-6′), 15.3 (—CH_3), 177.7 (C=O), 34.6, 34.4, 34.1, 33.8, 32.7, 31.6, 31.4, 33.8, 32.7, 31.6, 31.4, 31.2, 31.1, 29.1, 29.0, 28.9, 27.8, 27.6, and 27.3 (—$\underline{C}H_2$—).

Daucosterol (**3**). White powder, mp 287–289°. ^{13}C NMR, mp, MS and IR identical to ref. [14].

Syringaresinol-4′-O-β-D-monoglucoside (**4**). Yellow powder, mp >250° (dec). $[\alpha]_d^{18}$ = −42.2° (pyridine, *c* 0.5). IR, UV [15] and ^{13}C NMR [16] same as those reported.

2,3-*Dihydroxypropyl* (Z,Z)-9,12-*octadecadienate* (**5**). White powder, mp 14–15°. IR, MS, ^{1}H NMR [17]. EIMS *m/z* (rel. int.): 354 [M]$^+$, 346 [M $-$ H_2O]$^+$, (5), 325 [M $-$ C_2H_5]$^+$ (5), 264 [$CH_3(CH_2)_{16}COOH$]$^+$ (48), 262 (100), 221 (23). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.30 (*m*, 4H), 4.13 (*d*, 2H, J = 5 Hz, 1′-H), 3.90 (*m*, 1H, 2′-H), 3.67 (*dd*, 1H, J = 12, 3 Hz, 3′-H), 3.57 (*dd*, 1H, J = 12, 6 Hz, 3′-H), 2.80 (*m*, 2H, 11-H), 2.34 (*t*, 2H, J = 7 Hz, 2-H), 2.05 (*m*, 4H, 8- and 14-H), 1.70 (*m*, 2H, 3-H), 1.25 (*br*, 14H), 0.87 (*t*, 3H, J = 7 Hz, 18-H). ^{13}C NMR ($CDCl_3$, DEPT, 75 Hz): δ 174.2 (C, C=O), 129.9, 129.6, 128.0 and 127.8 (—$\underline{C}H$=), 70.1 (CH, C-2′), 64.9 (CH_2, C-1′), 63.3 (CH_2), C-3′), 34.0,

29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 29.0, 27.1, 25.5, 24.8 and 22.6 (CH_2), 14.0 (CH_3).

Ursolic acid (**6**). White powder, mp 280–283°. IR, MS and ^{1}H NMR identical to lit [18].

β-Sitosterol (**7**). White powder, mp 137–138°. ^{1}H NMR, ^{13}C NMR, IR and MS superimposable on those of an authentic sample.

Oleanolic acid (**8**). White powder, mp 308–310°. IR, mp, ^{1}H NMR [17] ^{13}C NMR [19] identical to those reported.

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