Novel Sphingolipids from *Conyza canadensis*

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New sphingolipids, 1,3,5-trihydroxy-2-hexadecanoylamino-(6*E*,9*E*)-heptacosdiene (1), 1,3,5-trihydroxy-2-hexadecanoylamino-(6*E*,9*E*)-heptacosdiene-1-*O*-glucopyranoside (2), 1,3-dihydroxy-2-hexanoylamino-(4*E*)-heptadecene (3) have been isolated from *Conyza canadensis*, along with five known compounds, *p*-hydroxybenzoic acid, 3,5-dihydroxybenzoic acid, 3,5-dimethoxybenzoic acid, 3β-hydroxyolean-12-en-28-oic acid, and 3β-erythrodiol, isolated for the first time from this species. Their structures were determined by spectroscopic methods (^{1}H- and ^{13}C-NMR, IR and MS) and two- dimensional (2D)-NMR experiments.

Key words *Conyza canadensis*; compositae; sphingolipids

The genus *Conyza*, belonging to the family Compositae, comprises about seventeen species of annual, biennial or perennial herbs, particularly inhabiting temperate and mountainous regions.[1,2] *Conyza canadensis* LINN. is an annual herb, distributed in Western Himalayas, Punjab and Kashmir.[3] It is an astringent, stimulant, haemostatic and diuretic, and is also used to treat diarrhea, dysentery, uterine hemorrhages, dropsy, gravel, bronchial catarrh and cystitis. A literature survey revealed that vanillic acid, syringic acid[4] and (*E*)-lachnophyllum ester[1] have so far been reported from this species. In this paper we describe the isolation and structural elucidation of three new sphingolipids, **1**—**3**, along with the five known compounds, *p*-hydroxybenzoic acid, 3,5-dihydroxybenzoic acid, 3,5-dimethoxybenzoic acid,[5] 3β-hydroxyolean-12-en-28-oic acid,[6] and 3β-erythrodiol,[7] isolated for the first time from this species.

Results and Discussions

The MeOH extract of the shade-dried whole plant of *C. canadensis* was evaporated *in vacuo*, suspended in H_2O, and successively partitioned with *n*-hexane, EtOAc and *n*-BuOH. As a result of a series of chromatographic separations, eight compounds have been obtained from the ethylacetate-soluble fraction, as described in the Experimental section.

Compound **1** was isolated as a colorless gummy solid. The molecular formula was assigned as $C_{42}H_{82}NO_4$ by high-resolution fast atom bombardment (HR-FAB)-MS, showing a $[M+H]^+$ ion peak at *m/z* 664.6246 (Calcd for 664.6243). The IR spectrum revealed the presence of two hydroxyl groups (3340, 3220 cm^{-1}), an amide group (1620, 1540 cm^{-1}) and an olefinic group (1660 cm^{-1}). The ^{1}H-NMR spectrum of **1** (Table 1) showed the presence of two terminal methyls at δ 0.85 (6H, t, J=6.8 Hz), nine methylenes at δ 1.25 (18H, br s) and another nine methylenes at δ 1.30 (18H, br s), and an amide proton signal at δ 8.56 (1H, d, J=8.9 Hz). It further showed two *trans*-olefinic bonds at [δ 5.17 (1H, dd, J=15.6, 8.6 Hz); δ 5.05 (1H, dt, J=15.6, 8.0 Hz); δ 5.45 (1H, dt, J=16.0, 6.8 Hz); 5.58 (1H, dt, J=16.0, 6.8 Hz)]. The ^{13}C-NMR spectrum of **1** corroborated the presence of two methyl, thirty-two methylene, seven methine and one quaternary carbons, which revealed that **1** was a phytosphingosine-type sphingolipid.[8,9] The resonance of a two-proton triplet in ^{1}H-NMR at δ 2.43 (2H, t, J=7.0 Hz) due to methylene protons connected to the amide carbonyl indicated the *N*-acyl moiety in **1** was a non-hydroxy fatty acid. Methanolysis of **1** yielded methyl pentadecanoate detected by gas chromatography-mass spectrometry (GC-MS) and Co-TLC with an authentic sample (Aldrich Cat. No. 23,545-8). The presence of a pentadecanoyl moiety was also confirmed by the characteristic ion at *m/z* 225.2219 (Calcd for $C_{15}H_{29}O$: 225.2218), as well as significant fragment ions at *m/z* 225 $[CH_3(CH_2)_{13}CO]^+$, 242 $[CH_3(CH_2)_{13}CONH_2+H]^+$ and 420 $[M-H_2O-CH_3(CH_2)_{13}CO]^+$ in the electron impact (EI)-MS. Thus, the base is C-27 phytosphingosine containing three hydroxyls and an amino group. The presence of another two-proton triplet at δ 2.35 (2H, t, J=8.0 Hz) indicated the presence of methylene between two hydroxymethines. The pres-

Table 1. ^{1}H-NMR Spectral Data of Compounds **1** and **2**

Proton No.	**1**[a]	**2**[a]
1	4.42 (1H, dd, 11.5, 5.0)	4.43 (1H, dd, 11.0, 4.3)
	4.49 (1H, dd, 11.3, 4.9)	4.51 (1H, dd, 11.0, 6.5)
2	5.13 (1H, m)	5.12 (1H, m)
3	4.33 (1H, m)	4.30 (1H, m)
4	2.35 (2H, t, 8.0)	2.28 (2H, t, 7.8)
5	4.62 (1H, m)	4.57 (1H, m)
6	5.17 (1H, dd, 15.6, 8.6)	5.19 (1H, dd, 15.5, 8.4)
7	5.05 (1H, dt, 15.6, 8.0)	5.09 (1H, dd, 15.5, 7.9)
8	2.78 (2H, m)	2.59 (2H, m)
9	5.45 (1H, dt, 16.0, 6.8)	5.48 (1H, dt, 15.2, 6.0)
10	5.58 (1H, dt, 16.0, 6.8)	5.56 (1H, dt, 15.2, 6.0)
11	2.30 (2H, m)	2.21 (2H, m)
12	1.95 (2H, m)	2.10 (2H, m)
13	1.70 (2H, m)	1.67 (2H, m)
14	1.20 (2H, m)	1.70 (4H, m)
15	1.75 (2H, m)	
16—24	1.30 (18 H, br s)	1.30 (18H, br s)
25	1.67 (2H, m)	1.41 (2H, m)
26	1.24 (4H, m)	1.20 (2H, m)
26, 15′	0.85 (6H, t, 6.8)	0.86 (6H, t, 7.0)
NH	8.56 (1H, d, 8.9)	8.58 (1H, d, 8.8)
2′	2.43 (2H, t, 7.0)	2.33 (2H, t, 7.0)
3′	2.26 (2H, m)	2.30 (2H, m)
4′—12′	1.25 (18H, br s)	1.25 (18H, br s)
13′	1.62 (2H, m)	1.70 (4H, m)
14′	1.24 (4H, m)	1.36 (2H, m)
Glc 1″		4.88 (1H, d, 7.8)
2″		3.02 (1H, m)
3″		3.98 (1H, m)
4″		4.02 (1H, m)
5″		3.85 (1H, m)
6″		4.39 (1H, dd, 12.0, 5.0)
		4.20 (1H, dd, 11.5, 4.5)

a) Measured in pyridine. Glc: β-D-glucopyranosyl.

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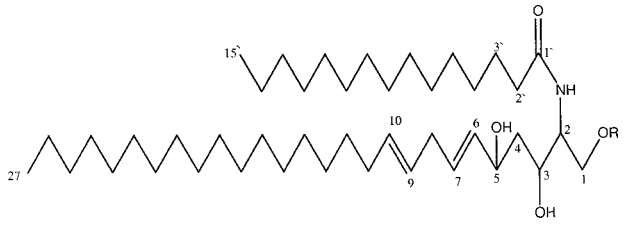

Fig. 1. Compounds **1** and **2**

1, R=H; **2**, R=β-D-Glucopyranoside.

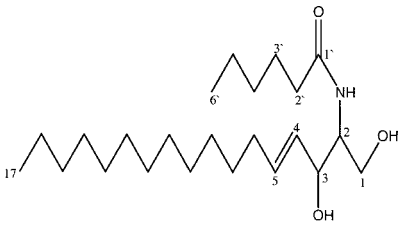

Fig. 2. Compound **3**

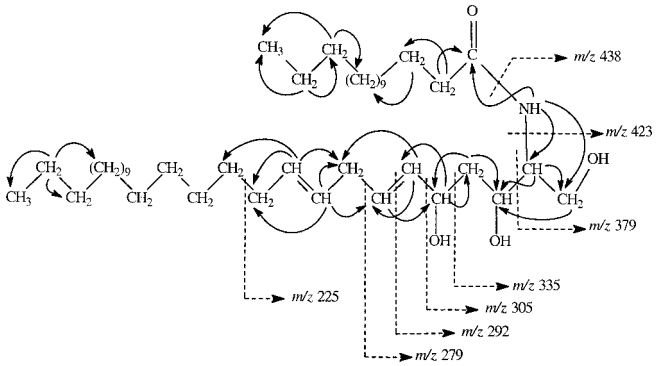

Fig. 3. Mass Fragmentation Pattern and Important HMBC Correlations of Compound **1**

ence of two olefinic bonds was confirmed by the two-proton multiplet at δ 2.78, which indicated that this methylene is flanked by two double bonds. Further structure and the position of double bonds could be established through characteristic fragment ions in EI-MS (Fig. 3), and was confirmed through the two-dimensional shift correlation ^{1}H–^{1}H COSY, heteroatom multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) correlations (Fig. 3). Based on this cumulative evidence, compound **1** was assigned the structure 1,3,5-trihydroxy-2-hexadecanoylamino-(6*E*,9*E*)-heptacosdiene.

Compound **2** was also a gummy solid, assigned the molecular formula $C_{48}H_{92}NO_9$ by HR-FAB-MS, which showed a $[M+H]^+$ peak at *m*/*z* 826.6775 (Calcd for 826.6771). The IR spectrum of **2** was similar to **1**, while the ^{1}H-NMR spectrum showed additional peaks due to a sugar moiety [anomeric proton at δ 4.88 (1H, d, J=7.8 Hz), and four protons geminal to the hydroxyl group between δ 3.02—4.02]. The ^{13}C-NMR spectrum also revealed the presence of a sugar moiety [anomeric carbon at δ 105.7, a hydroxyl containing methine carbons at δ 75.2, 77.9, 71.8 and 78.1, and a further signal at δ 62.6 (CH_2OH)]. This suggested that **2** is a glycoside of **1**. This was also confirmed by EI-MS, which showed a prominent peak at *m*/*z* 645 due to the elimination of a sugar moiety.

Table 2. ^{13}C-NMR Spectral Data of Compounds **1** and **2**

Carbon No.	**1**[a)]	**2**[a)]
1	62.0	70.2
2	53.0	52.5
3	76.9	76.8
4	35.7	35.9
5	72.9	73.1
6	131.5	132.3
7	134.3	135.1
8	34.2	35.1
9	130.8	130.5
10	130.9	130.7
11	32.1	31.5
12	29.9	31.1
13	29.6	28.5
14	28.8	28.0
15	27.4	25.8
16—24	30.0	29.9
25	25.8	26.0
26	17.9	18.5
27	14.5	14.8
1′	177.5	176.8
2′	33.8	34.1
3′	32.9	33.4
4′—12′	30.0	29.9
13′	22.6	23.2
14′	19.7	20.1
15′	13.8	14.0
Glc 1″		105.7
2″		75.2
3″		77.9
4″		71.8
5″		78.1
6″		62.6

a) Measured in pyridine. Glc: β-D-glucopyranosyl.

Further fragmentation was similar to **1**. The ^{13}C-NMR signals of the sugar moiety corresponded to β-D-glucopyranoside. The methanolysis provided, in addition to other products, an anomeric mixture of methyl glucosides, which was confirmed by comparison of the retention time of TMS ether with that of the standard. Their specific rotations indicated a δ-configuration. The position of the glucose moiety was evident by a downfield chemical shift of the hydroxymethylene carbon at δ 70.2 in the ^{13}C-NMR spectrum, by 8.2 ppm, and this was confirmed by HMBC, in which an additional correlation was observed between the anomeric proton (δ 4.88) and the hydroxymethylene carbon (δ 70.2). Thus, compound **2** was confirmed as β-D-glucopyranoside of **1**.

Compound **3** was also obtained as a colorless gummy solid. The molecular formula was assigned as $C_{23}H_{46}NO_3$ by HR-FAB-MS, showing a $[M+H]^+$ ion at *m*/*z* 384.3479 (Calcd for 384.3477). The IR spectrum was similar to **1**, and the ^{1}H-NMR spectrum showed the presence of two terminal methyls resonating at δ 0.87 (6H, t, J=6.8 Hz), six methylenes at δ 1.24 (12H, br s), an amide proton signal at δ 8.54 (1H, d, J=8.7 Hz), and the signal of a *trans*-olefinic bond at δ 5.24 (1H, dd, J=15.5, 8.5 Hz) and 5.01 (1H, dt, J=15.5, 8.0 Hz). The ^{13}C-NMR spectrum of **3** corroborated the presence of two methyl, sixteen methylene, four methine, and one quaternary carbons. Methanolysis of **3** yielded methylhexanoate **3a** detected by GC-MS and Co-TLC with an authentic sample (Aldrich Cat. No 25,994-2). The presence of a hexanoyl moeity was also confirmed by the characteristic ion at *m*/*z*

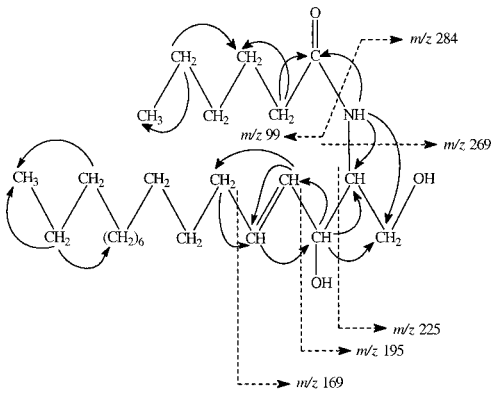

Fig. 4. Mass Fragmentation Pattern and Important HMBC Correlations of Compound **3**

99.0811 (Calcd for $C_6H_{11}O$: 99.0809), as well as significant fragment ions at *m/z* 99 $[CH_3(CH_2)_4CO]^+$, 116 $[CH_3(CH_2)_4CONH_2+H]^+$, and 266 $[M-H_2O-CH_3(CH_2)_4CO]^+$ in the EI-MS. Therefore, the base is C-17 phytosphingosine containing two hydroxyls and an amino group. Further HMBC interactions shown in (Fig. 4) were in accordance with the assigned structure of **3** as 1,3-dihydroxy-2-hexanoylamino-(4*E*)-heptadecene.

The configuration at the chiral centers of **1**—**3** cannot be established without chemical transformation that would require much more material.

Experimental

General optical rotations were measured with a JASCO DIP-360 digital polarimeter. IR spectral data were taken on a JASCO 302-A spectrophotometer with KBr. The NMR spectra were run on Bruker spectrophotometers: 400 MHz and 125 MHz instruments. Chemical shifts, δ in ppm relative to $SiMe_4$ as an internal standard, and coupling constants *J* are described in Hz. EI- and FAB-MS were recorded on JEOL JMX-HX-110 and JMS-DA-500 mass spectrometers, *m/z* (rel. int). Silica gel 60, 70—230 mesh and 200—440 mesh (both from E. Merck), were used for column and flash chromatography, respectively. Silica gel plates (Si 60 F_{254}, E. Merck) were used for TLC.

Plant Material The whole plant of *Conyza canadensis* Linn. (Compositae) was collected from Karachi (Pakistan), in July, 2000 and air-dried. The identity of the plant was verified by Dr. Javed Zaki, Plant Taxonomist, Department of Botany, University of Karachi, Pakistan. A voucher specimen was deposited in the herbarium of the University of Karachi, Pakistan.

Extraction and Isolation The shade dried plant (30 kg) was extracted with MeOH three times at room temperature and filtered. The filtrate was evaporated *in vacuo* to give a dark green residue, which was suspended in water and partitioned successively with *n*-hexane, EtOAc and *n*-BuOH. The EtOAc-soluble fraction was chromatographed on a column of silica gel using *n*-hexane–EtOAc in increasing order of polarity to give five major fractions A, B, C, D and E. The major fraction A, which was eluted with *n*-hexane–EtOAc (8 : 2), was subjected to column chromatography over silica gel using various mixtures of *n*-hexane and ethylacetate. The fraction which was eluted with *n*-hexane–EtOAc (6 : 4) was a mixture of two major UV active components. Repeated preparative TLC over silica gel using *n*-hexane–EtOAc (2 : 8) as an eluent provided compounds **4** (19 mg) and **5** (12 mg), respectively. The fractions which were eluted with *n*-hexane–EtOAc (5.5 : 4.5) showed one major spot on TLC. It was rechromatographed over flash silica elution with *n*-hexane–EtOAc (4 : 6) yielding compound **6** (18 mg). The fraction B obtained from *n*-hexane–EtOAc (6 : 4) gave a mixture of two compounds which were purified by chromatography over silica gel using *n*-hexane–EtOAc (1 : 1) and (4 : 6) as eluents, respectively, to yield **7** (15 mg) and **8** (14 mg). The fraction C, which was eluted with *n*-hexane–EtOAc (4 : 6), gave one major spot and was further purified by chromatography over silica gel using *n*-hexane–EtOAc (3 : 7) as an eluent to obtain **3** (5 mg). The fraction D, which was eluted from *n*-hexane–EtOAc (2 : 8), gave two major spots and was rechromatographed over flash silica elution with EtOAc and EtOAc–MeOH (9 : 1) providing **1** (7 mg) and **2** (8 mg), respectively. The known compounds **4**—**8** were characterized through comparison of physical and spectral data with that in the literature.

Methanolysis Compounds **1** and **3** (3 mg) were separately treated with 5% methanolic HCl at 90 °C for 1 h.[10] The fatty acid methyl ester produced was extracted with *n*-hexane and analyzed by GC-MS. In each case a single peak was obtained (*m/z* 256 for methyl pentadecanoate from **1** and *m/z* 130 for methyl hexanoate from **3**). The identity was further confirmed through Co-TLC with authentic samples.

The process was repeated with **2** (5 mg), which again yielded methyl pentadecanoate. The remaining MeOH layer was neutralized by adding a small excess of Ag_2CO_3. After centrifugation, the supernatant was evaporated to dryness under a nitrogen stream at room temperature. The residue was subjected to column chromatography on silica gel using $CHCl_3$–MeOH–H_2O 97 : 3 : 0.5) to give a methyl glycoside and a long chain base. The former was treated with *N*-trimethylsilylimidazole, and the derivative was analyzed by GC (fused silica capillary column Bonded Supelcowax 10, 0.53 mm×30 m). The peaks were identical with those of authentic methyl glucoside derivatives.

1,3,5-Trihydroxy-2-hexadecanoylamino-(6*E*,9*E*)-heptacosdiene (**1**): A colorless gummy solid, IR (KBr) cm^{-1}: 3340, 1660, 1620. EI-MS (Fig. 3); HR-FAB-MS *m/z*: 664.6246 $[M+H]^+$. (Calcd for $C_{42}H_{82}NO_4$: 664.6243). $[\alpha]_D^{25}$ −26.2° (*c*= 0.10, pyridine). ^{1}H- and ^{13}C-NMR spectral data are given in Tables 1 and 2, respectively.

1,3,5-Trihydroxy-2-hexadacanoylamino-(6*E*,9*E*)-heptacosdiene-1-*O*-glucopyranoside (**2**): A colorless gummy solid, IR (KBr) cm^{-1}: 3340, 1660, 1620. HR-FAB-MS *m/z*: 826.6775 $[M+H]^+$ (Calcd for $C_{48}H_{92}NO_9$: 826.6771). $[\alpha]_D^{25}$ −33.2° (*c*=0.12, pyridine). ^{1}H- and ^{13}C-NMR spectral data are given in Tables 1 and 2, respectively.

1,3-Dihydroxy-2-hexanoylamino-(4*E*)-heptadecene (**3**): A colorless gummy solid, IR (KBr) cm^{-1}: 3340, 1660, 1620. ^{1}H-NMR (pyridine, 400 MHz) δ: 8.54 (1H, d, *J*=8.7 Hz, NH), 5.24 (1H, dd, *J*=15.5, 8.5 Hz, H-4), 5.09 (1H, m, H-2), 5.01 (1H, dt, *J*=15.5, 8.0 Hz, H-5), 4.49 (1H, dd, *J*=10.9, 4.8 Hz, H-1a), 4.38 (1H, dd, *J*=10.9, 4.5 Hz, H-1b), 4.26 (1H, t, 8.6 Hz, OH-3), 2.50 (2H, t, *J*=7.4 Hz, H-2′), 2.41 (2H, m, H-6), 2.20 (2H, m, H-7), 2.15 (2H, m, H-3′), 1.98 (2H, m, H-4′), 1.75 (2H, m, H-8), 1.58 (4H, m, H-5′, 15), 1.40 (2H, m, H-16), 1.24 (12H, br s, H-9-14), 0.87 (6H, t, *J*=6.8 Hz, CH_3-6′, 17). ^{13}C-NMR (pyridine, 125 MHz) δ: 175.5 (s, C-1′), 134.3 (d, C-5), 131.2 (d, C-4), 75.5 (d, C-3), 52.1 (d, C-2), 62.1 (t, C-1), 33.4 (t, C-6), 32.3 (t, C-2′), 30.3 (t, C-7), 29.9 (t, C-9-14), 25.2 (t, C-4′), 21.0 (t, C-8), 19.9 (t, C-15), 19.2 (t, C-5′), 17.5 (t, C-16), 14.3 (q, C-17), 13.5 (q, C-6′). EI-MS (Fig. 4); HR-FAB-MS (positive) *m/z*: 384.3479 (Calcd for $C_{23}H_{46}NO_3$: 384.3477). $[\alpha]_D^{25}$ −19.1° (*c*=0.021, pyridine).

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