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Sphingolipids from *Conyza canadensis*

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Abstract

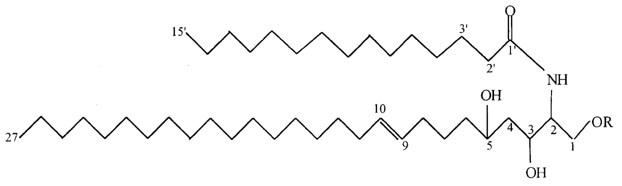
Sphingolipid **1** and its corresponding β-D-glucopyranoside derivative **2** have been isolated from the ethylacetate fraction of *Conyza canadensis* along with β-sitosterol **3**, stigmasterol **4**, β-sitosterol 3-*O*-β-D-glucoside **5** and harmine **6**, reported for the first time from this species. The structures of **1** and **2** were elucidated through spectroscopy including two-dimensional NMR.

Keywords: *Conyza canadensis*; Compositae; Sphingolipids

1. Introduction

Conyza canadensis Linn. belongs to the family Compositae (commonly called Canada fleabane) is an annual herb, distributed in Western Himalayas, Punjab, Kashmir and Pakistan (Chopra, 1982). It is astringent, stimulant, haemostatic and diuretic, also used in diarrhoea, dysentry, uterine haemorrhages, dropsy, gravel, cystitis, calculus, bronchial catarrh and haemoptysis (Sastri, 1952). Literature survey revealed that only two aromatic carboxylic acids have so far been reported from this species (Bohlmann et al., 1979). Phytochemical investigations on the ethylacetate soluble fraction of *C. canadensis* have now resulted in the isolation of new sphingolipids **1** and **2** along with β-sitosterol **3**, stigmasterol **4**, β-sitosterol 3-*O*-β-D-glucoside **5** and harmine **6**, reported for the first time from this species.

Sphingolipids are a biologically important class of compounds (Kolter and Sandhoff, 1999) some of which have been reported to exhibit antihepatotoxic (Kim et al., 1997), antitumor and immunostimulatory activities (Natori et al., 1994). These are mainly reported from different fungi (Lourenco et al., 1996) and sponges (Hattori et al., 1998; Hirsch and Kashman, 1989; Yunker and Scheuer 1978; Li et al., 1995; Lourenco et al., 1996; Gao et al., 2001a).



1 R = H
2 R = *β*-D-glucopyranoside

2. Results and discussion

Compounds **1**–**6** were isolated by column chromatography of the ethylacetate soluble fraction over silica gel, as described in Experimental. Compound **1** was obtained as colorless gummy solid and showed the molecular ion peak in HRMS at *m*/*z* 665.6362 corresponding to molecular formula $C_{42}H_{83}NO_4$ (calcd for $C_{42}H_{83}NO_4$ 665.6321). The IR spectrum showed the hydroxyl bands at 3340 and 3220 cm^{-1}, bands at 1620 and 1540 cm^{-1} due to the amide group and a band at 1660 cm^{-1} for the olefinic bond. The ^1H NMR spectrum of **1** (Table 1) showed the presence of two terminal methyls at δ 0.86, methylenes at δ 1.25 (*br s*, 18H) and δ 1.30 (*br s*, 18H), an amide proton signal at δ 8.55 (*d, J* = 8.8 Hz), signals of *trans*-olefinic bond at δ 5.48 (*br dt, J* = 16.8, 6.5 Hz) and 5.54 (*br dt, J* = 16.8, 6.5 Hz). The ^{13}C NMR spectrum of **1** showed a carbon signal at δ 177.0 (CONH), methine carbons at δ 130.8 and 130.6

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Table 1
^{1}H and ^{13}C NMR data of compounds **1** and **2**

	Compound **1**		Compound **2**	
Position	^{13}C[a] (DEPT)	^{1}H[b] multiplicity *J* (Hz)	^{13}C[a] (DEPT)	^{1}H[c] multiplicity *J* (Hz)
1	62.0 (CH_2)	4.41 *dd* (10.7, 4.8)	70.4 (CH_2)	4.32 *dd* (10.3, 3.3)
		4.94 *dd* (10.5, 4.4)		4.60 *dd* (10.3, 6.2)
2	53.0 (CH)	5.11 *m*	51.7 (CH)	5.03 *m*
3	76.8 (CH)	4.33 *m*	75.9 (CH)	4.35 *m*
4	35.7 (CH_2)	2.40 *m*	35.6 (CH_2)	2.36 *m*
5	73.0 (CH)	4.52 *m*	72.4 (CH)	4.50 *m*
6	33.2 (CH_2)	1.99 *m*	33.0 (CH_2)	1.83 *m*
7	33.0 (CH_2)	1.45 *m*	32.9 (CH_2)	1.38 *m*
8,11	34.1 (CH_2)	1.95–2.01 *m* (4H)	33.8 (CH_2)	2.02–2.21 *m* (4H)
	33.8 (CH_2)		33.3 (CH_2)	
9	130.8 (CH)	5.54 (*br dt* 16.8, 6.5)	130.9 (CH)	5.34 (*br dt* 16.5, 6.2)
10	130.6 (CH)	5.48 (*br dt* 16.8, 6.5)	130.7 (CH)	5.40 (*br dt* 16.5, 6.2)
12	33.0 (CH_2)	1.60 *m*	32.1 (CH_2)	1.58 *m*
13	32.9 (CH_2)	1.37 *m*	30.5 (CH_2)	1.42 *m*
14	30.5 (CH_2)		29.0 (CH_2)	
15	29.6 (CH_2)		28.3 (CH_2)	
16–24	29.9 (CH_2)	1.30 (*br s* 18H)	30.0 (CH_2)	1.22 (*br s* 18H)
25	29.5 (CH_2)		29.5 (CH_2)	
26	17.6 (CH_2)	1.48 *m*	19.4 (CH_2)	1.50 *m*
27	14.2 (CH_3)	0.86 *t* (7.0)	14.3 (CH_3)	0.77 *t* (6.8)
NH		8.55 *d* (8.8)		8.45 *d* (8.6)
1′	177.0 (C)		175.8 (C)	
2′	32.1 (CH_2)	2.20 *t* (7.0)	30.1 (CH_2)	1.89 *t* (6.3)
3′	30.2 (CH_2)	1.34 *m*	29.9 (CH_2)	1.30 *m*
(4′–12′)	29.9 (CH_2)	1.25 *br s* (18H)	30.0 (CH_2)	1.13 *br s* (18H)
13′	22.9 (CH_2)	1.20 *m*	23.6 (CH_2)	1.35 *m*
14′	19.6 (CH_2)	1.65 *m*	21.0 (CH_2)	1.72 *m*
15′	13.8 (CH_3)	0.86 *t* (7.0)	13.9 (CH_3)	0.77 *t* (6.8)
1...			105.5 (CH)	4.78 *d* (7.7)
2...			75.1 (CH)	3.85 *m*
3...			78.1 (CH)	3.91 *m*
4...			71.5 (CH)	3.95 *m*
5...			78.5 (CH)	3.78 *m*
6...			62.6 (CH_2)	4.27 *dd* (11.5, 4.5)
				4.07 *dd* (10.0, 3.5)

[a] ^{13}C NMR carried out at 100 MHz.
[b] ^{1}H NMR carried out at 400 MHz.
[c] ^{1}H NMR carried out at 500 MHz.

(C=C), three methines at δ 53.0 (CHNH), 76.8 (CHOH), 73.0 (CHOH) and one methylene at δ 62.0 (CH_2OH). This suggested that **1** was a phytosphingosine-type sphingolipid (Natori et al., 1994; Gao et al., 2001b).

The presence of a two protons triplet in ^{1}H NMR at δ 2.2 due to methylene protons connected to the amide carbonyl indicated that *N*-acyl moeity in **1** was a non-hydroxy fatty acid. Methanolysis (Gao et al., 2001b) of **1** yielded methyl pentadecanoate (**1a**) detected by GC–MS. The existence of the pentadecanoyl moeity was also confirmed by the characteristic ions at *m*/*z* 225 $[CH_3(CH_2)_{13}CO]^+$, 242 $[CH_3(CH_2)_{13}CONH_2+H]^+$, and 422 $[M-H_2O-CH_3(CH_2)_{13}CO]^+$ in the EIMS. Therefore, the base is C_{27}-phytosphingosine containing three hydroxyls and an amino group. Its structure could be established through characteristic fragment ions in EIMS (Fig. 1) and further confirmed through ^{1}H–^{1}H COSY, HMQC and HMBC correlations. Based on these cummulative evidence, the compound **1** was assigned the structure 1,3,5-trihydroxy-2-hexadecanoylamino-9-(*E*)-heptacosene.

Compound **2** was also obtained as a gummy solid showing $[M+H]^+$ peak in positive mode HRFABMS at *m*/*z* 828.6934 corresponding to the molecular formula $C_{48}H_{94}NO_9$ (calcd for $C_{48}H_{94}NO_9$ 828.6928). The IR spectrum was similar to **1** while the ^{1}H NMR spectrum showed additional peaks due to the sugar moeity [anomeric proton at δ 4.78 (*d*, $J=7.7$ Hz), four protons geminal to the hydroxyl group between δ 3.85–3.95 and hydroxymethylene protons at δ 4.07 and 4.27]. The ^{13}C NMR spectrum also revealed the presence of sugar

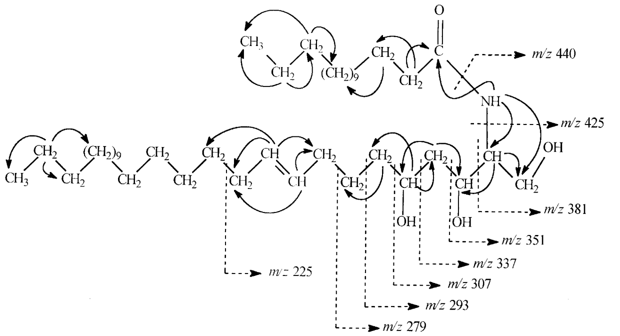

Fig 1. Mass fragmentation pattern and important HMBC correlations of compound **1**.

moeity [anomeric carbon at δ 105.5 and the hydroxyl containing methine carbons at δ 75.1, 78.1, 71.5, and 78.5 and a further signal at δ 62.6 (CH_2OH)]. This suggested that **2** is a glycoside of **1**. The ^{13}C NMR signals of sugar moeity corresponded to β-D-glucopyranoside. It was also confirmed by EIMS which showed prominent peak at m/z 667 due to the elimination of glucosyl moeity. Further fragmentation pattern was similar to **1**. The position of glucose moeity was evident by downfield chemical shift of hydroxymethylene carbon at δ 70.4 in ^{13}C NMR spectrum by 8 ppm and confirmed by HMBC experiments in which the additional correlation was observed between the anomeric proton (δ 4.78) with the hydroxymethylene carbon (δ 70.4). Thus compound **2** was confirmed as the β-D-glucopyranoside derivative of **1**.

3. Experimental

3.1. General

Optical rotations were measured on a Jasco DIP-360 polarimeter. IR spectra were recorded in KBr disk on a Jasco 320-A spectrophotometer. EI and FABMS were recorded on JMS HX 110 and JMS-DA 500 mass spectrometers with a data system. The ^{1}H, ^{13}C NMR, COSY, HMQC and HMBC spectra were recorded at 500 and 400 MHz. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are in Hz.

3.2. Plant material

C. canadensis Linn. (Compositae), whole plant was collected in July 2000, from Karachi (Pakistan) and identified by Dr. Javed Zaki, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen has been deposited.

3.3. Extraction and isolation

The dried plant material (30 kg) was extracted thrice with MeOH at room temperature. Evaporation of solvent from the combined extracts in vacuo gave a dark residue which was suspended in water and successively extracted with *n*-hexane, ethylacetate and *n*-butanol. The ethylacetate extract (200 g) was subjected to column chromatography over silica gel (70–230 mesh), successively eluting with *n*-hexane, *n*-hexane–ethylacetate, ethylacetate, ethylacetate–methanol and finally methanol in increasing order of polarity. The fraction which eluted with *n*-hexane–ethylacetate (8:2) was a mixture of two components. It was subjected to CC (flash silica 230–400 mesh) using *n*-hexane–ethylacetate (7.5:2.5) as eluent to afford **3** and *n*-hexane–ethylacetate (6:4) to obtain **4**. The fraction which eluted with *n*-hexane–ethylacetate (1:1) was rechromatographed over flash silica eluting with *n*-hexane–ethylacetate (4:6) to provide **5**. The fraction which eluted with *n*-hexane–ethylacetate (2:8) showed one major spot on TLC. It was rechromatographed over flash silica eluting with *n*-hexane–ethylacetate (1:9) to yield the compound **6**. The fraction which eluted with ethylacetate was rechromatographed over flash silica eluting with ethylacetate and ethylacetate–methanol (9:1) to obtain **1** (12 mg) and **2** (10 mg), respectively.

3.4. Compound 1

Colorless gummy solid, $[\alpha]_D^{25}$ +17.85° (pyridine; c 0.028); IR (KBr) ν_{max} cm^{-1}; 3340 (OH), 1660 (C=C), 1620 (C=O); EIMS data and important HMBC correlations are illustrated in Fig. 1. ^{1}H and ^{13}C NMR, see Table 1.

3.5. Methanolysis of 1

Compound **1** (10 mg) was refluxed with 1.5 mL of 0.9N HCl in 82% aq. methanol for 16 h (Gao et al., 2001b). The reaction mixture was cooled and extracted with *n*-hexane. The *n*-hexane layer was concentrated and silica gel column chromatography [*n*-hexane-ethylacetate (9:1–7:3)] gave the fatty acid methyl ester **1a** which could be identified as methyl pentadecanoate through GC–MS showing the molecular ion peak at m/z 256 and an intense peak at m/z 197 $[M-COOMe]^+$.

3.6. Compound 2

Colorless gummy solid, $[\alpha]_D^{25}$ +62.5° (pyridine; c 0.024); IR (KBr) ν_{max} cm^{-1}; 3340 (OH), 1660 (C=C), 1620 (C=O); EIMS data and important HMBC correlations are illustrated in Fig. 1. ^{1}H and ^{13}C NMR, see Table 1.

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