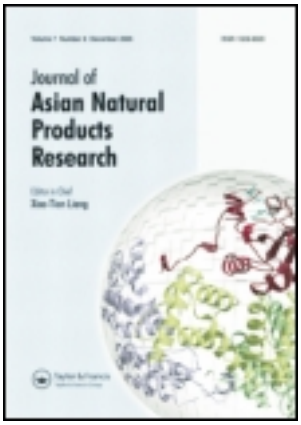

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Spiraeamide, new sphingolipid from *Spiraea brahuica*

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Spiraeamide (**1**), a new sphingolipid, has been isolated from the ethyl acetate-soluble fraction of the methanolic extract of the whole plant of *Spiraea brahuica*, along with marrubiin (**2**), 19-acetylmarrubenol (**3**), and 6-acetylmarruenol (**4**). Their structures were elucidated by ^{1}H and ^{13}C NMR spectra, and COSY, NOESY, HMQC, HMBC, EI-MS, and FAB-MS experiments.

Keywords: *Spiraea brahuica*; Rosaceae; sphingolipid; spiraeamide

1. Introduction

The genus *Spiraea* (Rosaceae) comprises over 90 species growing as shrubs in temperate regions of the northern hemisphere and in eastern Asia [1]. Various *Spiraea* species are effective remedy for the treatment of inflammation and malaria [2]. The fruits and roots of various *Spiraea* species are used as diuretic and detoxicant agents, and also for the treatment of cough, headache, and toothache [2,3]. *Spiraea brahuica* Boissier is distributed in Asia. In Pakistan, it abundantly grows in Ziarat valley of the province of Balochistan. No phytochemical or pharmacological work has so far been carried out on this species. The chemotaxonomic and ethnopharmacological significance of the genus *Spiraea* prompted us to carry out phytochemical studies on *S. brahuica*. As a result, we herein report the isolation and structural elucidation of a new sphingolipid named as spiraeamide (**1**), along with marrubiin (**2**), 19-acetylmarrubenol (**3**), and 6-acetlymarruenol (**4**), reported for the first time from this species.

2. Results and discussion

The methanolic extract of the whole plant of *S. brahuica* was suspended in water and successively fractionated into *n*-hexane, chloroform, ethyl acetate, and *n*-butanol-soluble fractions. The ethyl acetate-soluble fraction was subjected to column chromatographic techniques to afford compounds **1**–**4**, respectively.

Spiraeamide (**1**) was obtained as a white amorphous solid. The IR spectrum showed absorptions for hydroxyl and amine groups (3500–3340 cm^{-1}), olefinic (1640 cm^{-1}), and sec. amide (1646 and 1540 cm^{-1}) derivative [4]. The UV spectrum exhibited the absorption maxima at 204 and 230 nm. The HR-FAB-MS (positive) showed a quasi-molecular ion peak at *m/z* 844.6869 $[M + H]^+$ which deduced the molecular formula $C_{48}H_{94}NO_{10}$. In the ^{1}H NMR spectrum, the proton of sec. amide nitrogen showed a doublet at δ 8.47 (d, $J = 9.0$ Hz). The signals at δ 5.30 (1H, m) and 5.27 (1H, m) were attributed to protons of the disubstituted double bond. The upfield region

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showed a broad signal for the protons of four methylene groups in the range of δ 1.48–1.96, whereas rest of the methylene protons resonated at δ 1.18–1.22 (br. s, 28 × CH_2). A triplet for the terminal methyl groups was observed at δ 0.82 (6H, t, $J = 6.4$ Hz). The anomeric proton showed the signal at δ 4.22 (1H, d, $J = 7.5$ Hz), the oxymethine protons of the hexose unit resonated in the range of δ 3.36–3.17, and the oxymethylene protons appeared at δ 3.77 (1H, d, $J = 11.2$ Hz) and 3.68 (1H, d, $J = 11.2$ Hz). Two oxymethylene protons resonated at δ 4.01 (dd, $J = 4.6$, 10.7 Hz) and 3.74 (dd, $J = 7.8$, 10.7 Hz), and three oxymethine protons were observed at δ 3.95 (dd, $J = 3.6$, 7.6 Hz), 3.48 (dd, $J = 5.0$, 7.2 Hz), and 3.45–3.46 (1H, m), confirming that compound **1** is a glycoside of sphingolipid [4]. The ^{13}C NMR (BB and DEPT) spectrum showed the signal of an amide carbonyl carbon at δ 175.7 whereas the olefinic methines resonated at δ 130.2 and 129.2. The azomethine signal, characteristic of sphingolipid, appeared at δ 50.1, whereas an oxymethylene carbon resonated at δ 68.6 along with three resonances of oxymethine carbons at δ 74.2, 72.1, and 72.0. The anomeric carbon showed signal at δ 102.8 whereas the oxymethine and oxymethylene carbons of the hexose unit appeared in the range of δ 76.0–61.0. The methylenes of the aliphatic chains resonated in the range of δ 22.6–34.2 with the two terminal methyl carbons at δ 14.0.

The (*Z*)-configuration of the double bond was assigned based on the significantly upfield shifted ^{13}C NMR signals for C-7′ and C-10′ at δ 26.0 and 25.3, respectively, and due to the relatively small coupling constant between H-8′ and H-9′ ($W_{1/2} = 3.5$ Hz) [5]. The larger coupling constant of the anomeric proton allowed us to assign β-configuration to the hexose unit.

In the ^{1}H-^{1}H-COSY spectrum, azomethine proton (δ 4.14–4.17) showed correlations with oxymethylene protons H-1′ at δ 4.01 and 3.74 and oxymethine proton H-3′ at δ 3.48 which further correlated with another oxymethine proton H-4′ at δ 3.45–3.46, revealing the position of two hydroxyl groups at C-3′ and C-4′, respectively. Methanolysis of compound **1** provided the glycone, which could be identified as a mixture of α- and β-anomers of methyl D-glucoside. The fatty acid methyl ester was characterized by mass spectrometry as methyl 2-hydroxyicosanoate (*m/z*: 342 $[M]^+$) [6]. Thus, the length of sphingosine base chain was of 22 carbons with double bond located in the base chain. In HMBC experiment, the oxymethine proton at C-4′ (δ 3.45–3.46) showed ^{2}J and ^{3}J correlations with methylene carbons at δ 32.3 and 27.2, allowing us to assign these to C-5′ and C-6′, respectively. The methylenic protons of C-6′ (δ 1.18–1.22) showed ^{2}J correlation with C-7′ (δ 26.0) and ^{3}J correlation with C-8′ (δ 130.3). The olefinic protons at δ 5.27 and 5.30 showed ^{3}J correlations with C-7′ (δ 26.0) and C-10′ (δ 25.3), respectively, allowing us to assign the double bond to C-8′. The fragmentation peaks in the EI-MS at *m/z* 471, 209, and 183 were due to the cleavage of vinylic bond by McLafferty rearrangement, further confirming the position of double bond at C-8′ (Figure 2). The attachment of *O*-β-D-glucose moiety was confirmed at C-1′ by downfield shift of C-1′ and confirmed through HMBC experiment; the anomeric proton at δ 4.22 shows ^{3}J correlation with C-1′ at δ 68.6. The chemical shifts of proton at δ 4.14–4.17 (H-2′) and the carbon signals at δ 68.6 (C-1′), 50.1 (C-2′), 74.2 (C-3′), 72.1 (C-4′), 175.7 (C-1), and 72.0 (C-2) were very close to the phytoceramides having 2*R*, 2′*S*, 3′*S*, and 4′*R*-stereochemistry [7], revealing the same configuration at C-2′, C-3′, C-4′, and C-2 in **1**, which was further supported by NOESY spectrum; the azomethine proton at δ 4.15–4.17 (H-2′) showed correlations with H-2 at δ 3.95, H-4′ at δ 3.45–3.46, and $H_β$-1′ at δ 4.01. On the other hand, the H-3′ at δ 3.48 showed correlations with $H_α$-1′ at 3.74 as well as the amide proton at δ 8.47. On the basis of

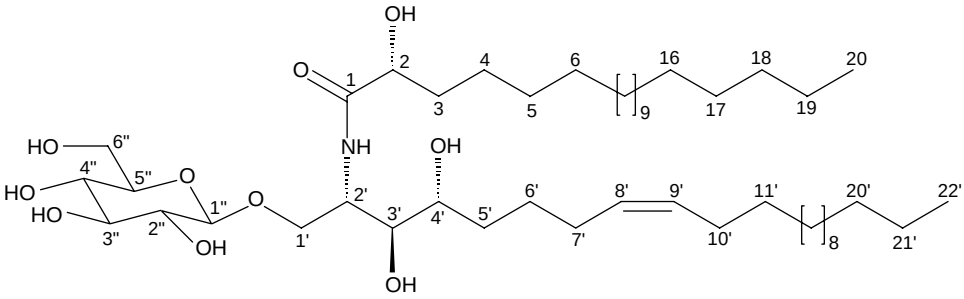

Figure 1. Structure of spiraeamide (**1**).

these evidences, the structure of spiraeamide (**1**) could be assigned as '(2*R*)-*N*-(2*R*,3*S*,4*R*,8*Z*)-3,4-dihydroxy-1-[(3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy-docosen-2-yl]-2-hydroxyicosanamide' (Figure 1).

The known compounds were identified as marrubiin (**2**) [8], 19-acetylmarrubenol (**3**) [9], and 6-acetylmarrubenol (**4**) [9] through the comparison of physical and spectral data with the reported data in the literature.

3. Experimental

3.1. General experimental procedures

Melting points were measured on a Gallenkamp apparatus (Loughborough, England) and are uncorrected. Optical rotations were measured on JASCO DIP-360 polarimeter (Jasco, Tokyo, Japan). UV spectra were recorded on Hitachi UV-3200 spectrophotometer (Hitachi, Tokyo, Japan) whereas the IR spectra were recorded on Shimadzu FT-IR-8900 spectrometer (Shimadzu, Kyoto, Japan) as KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-400 spectrometer in deuterated solvents. Two-dimensional NMR spectra were recorded on AM-400 spectrometer (Bruker BioSpin, Faellanden, Switzerland). The chemical shifts are reported in ppm (δ), relative to tetramethylsilane as an internal standard and scalar couplings are reported in Hz. Mass spectra (El and HR-EI) were obtained in an electron impact mode on Finnigan MAT-112 and MAT-113 spectrometers (Finnigan, Waltham, MA, USA), and FAB mass spectra were obtained on Jeol JMS HX 110 spectrometer (Jeol, Tokyo, Japan) and ions are reported in *m/z* (%). Column chromatography (CC) was conducted on silica gel (70–230 mesh, E. Merck, Darmstadt, Germany). HPLC was carried out on LC-908W-C-60 (Japan Analytical Industry Co. Ltd, Tokyo, Japan). TLC was conducted on pre-coated silica gel G-25-UV_{254} plates (E. Merck) and detection was obtained at 254 and 366 nm or by spraying ceric sulfate in 10% H_2SO_4 (heating).

3.2. Plant material

The whole plant of *S. brahuica* was collected from Ziarat valley of Balochistan province of Pakistan in 2008 and identified by Prof. Dr Rasool Bakhsh Tareen, Plant Taxonomist, Department of Botany, University of Balochistan, Quetta, where a voucher specimen has been deposited in the herbarium (Voucher specimen No. SB.RBT.08.BUH).

3.3. Extraction and isolation

The whole plant materials of *S. brahuica* (40 kg) were shade dried, ground, and extracted with MeOH (3 × 50 liters, 10 days each) at room temperature (rt). The combined methanolic extract was evaporated under reduced pressure at rt to yield a residue (700 g), which was suspended in water (1.5 liters) and successively fractionated with *n*-hexane (80 g), $CHCl_3$ (100 g), EtOAc (55 g),

Table 1. ^{1}H (CD_3OD, 400 MHz) and ^{13}C (CD_3OD, 100 MHz) NMR spectral data and HMBC correlations of spiraeamide (**1**).

Carbons	δ_C	δ_H	$^{1}H-^{13}C$ HMBC correlations
NH	–	8.47 (1H, d, $J = 9.0$ Hz)	C-1, C-1′, C-2′
1	175.7	–	–
2	72.0	3.95 (1H, dd, $J = 3.6$, 7.6 Hz)	C-1′, C-3′, C-4′
3	34.2	1.73–1.76 (1H, m), 1.48–1.51 (1H, m)	C-1′, C-2′, C-4′, C-1′, C-2′, C-4′
4	27.1	1.18–1.22 (2H, br. s)	//
5–18	29.3–31.8	1.18–1.22 (28H, br. s)	//
19	22.6	1.18–1.22 (2H, br. s)	//
20	14.0	0.82 (3H, t, $J = 6.4$ Hz)	C-18′, C-19′
1′	68.6	4.01 (1H, dd, $J = 4.6$, 10.7 Hz), 3.74 (1H, dd, $J = 7.8$, 10.7 Hz)	C-2, C-3, C-1″, C-2, C-3, C-1″
2′	50.1	4.14–4.17 (1H, m)	C-1, C-1′, C-3, C-4
3′	74.2	3.48 (1H, dd, $J = 5.0$, 7.2 Hz)	C-1, C-2, C-4
4′	72.1	3.45–3.46 (1H, m)	C-2, C-3, C-5
5′	32.3	1.80–1.82 (1H, m), 1.61–1.63 (1H, m)	C-4, C-5, C-7, C-4, C-5, C-7
6′	27.2	1.18–1.22 (2H, br. s)	//
7′	26.0	1.95 (2H, m)	C-5, C-6, C-8, C-9
8′	130.2	5.30 (1H, m)	C-6, C-7, C-10
9′	129.2	5.27 (1H, m)	C-7, C-10, C-11
10′	25.3	1.95 (2H, m)	C-9, C-11
11′–19′	29.5–30.1	1.18–1.22 (18H, br. s)	//
20′	31.8	1.18–1.22 (2H, br. s)	//
21′	22.6	1.18–1.22 (2H, br. s)	//
22′	14.0	0.82 (3H, t, $J = 6.4$ Hz)	C-20, C-21
1″	102.8	4.22 (1H, d, $J = 7.5$ Hz)	C-1, C-2″
2″	73.2	3.17–3.19 (1H, m)	C-1″, C-3″
3″	76.0	3.34–3.36 (1H, m)	C-2″, C-4″
4″	69.5	3.30–3.33 (1H, m)	C-3″, C-6″
5″	75.9	3.21–3.23 (1H, m)	C-6″
6″	61.0	3.77 (1H, d, $J = 11.2$ Hz), 3.68 (1H, d, $J = 11.2$ Hz)	C-5″, C-5″

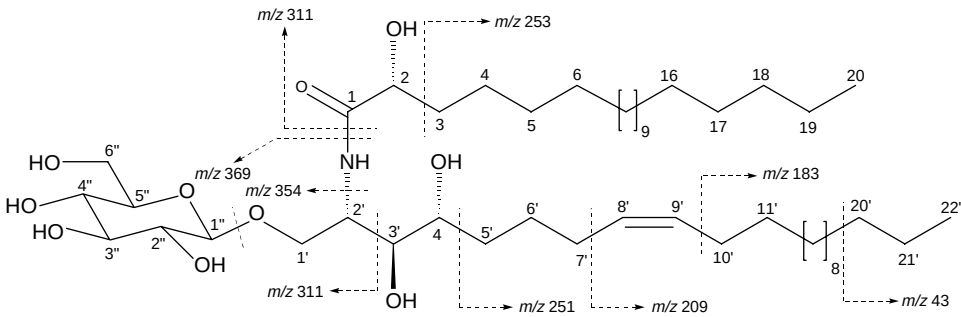

Figure 2. Mass fragmentation pattern of spiraeamide (**1**).

n-BuOH (40 g) soluble fractions. The EtOAc-soluble fraction (55 g) was subjected to CC over silica gel eluting with *n*-hexane–$CHCl_3$, $CHCl_3$, and $CHCl_3$–MeOH in increasing order of polarity to obtain 10 sub-fractions. The sub-fraction obtained with $CHCl_3$ (2.6 g) was rechromatographed over silica gel and eluted with *n*-hexane–EtOAC (9.0:1.0) to provide compound **2** (50 mg). The sub-fraction obtained with $CHCl_3$–MeOH (9.9:0.1; 2.4 g) was rechromatographed over silica gel and eluted with the same solvent system. The semi-pure fraction so obtained was purified through preparative TLC using $CHCl_3$–MeOH (9.4:0.6) as eluent to provide compounds **3** (40 mg) and **4** (60 mg), respectively. The sub-fraction obtained with $CHCl_3$–MeOH (8.5:1.5; 0.7 g) was rechromatographed over silica gel eluting with $CHCl_3$–MeOH (8.8:1.2) to afford a semi-pure fraction, which was purified through preparative TLC using $CHCl_3$–MeOH (8.0:2.0) as eluent to afford compound **1** (35 mg). Its purity was checked by HPLC over reverse-phase C_{18} silica gel column, eluting with 90% MeOH in water.

3.4. *Spiraeamide (1)*

White amorphous solid; $[\alpha]_D^{25}$ + 25; UV λ_{max} (MeOH) nm (log ϵ): 204 (2.2), 230 (3.4); IR ν_{max} (KBr) cm^{-1}: 3500–3340 (OH/NH), 1646, 1540 (HN–C = O), 1640 (C = C); ^1H (CD_3OD, 400 MHz) and ^{13}C (CD_3OD, 100 MHz). For NMR spectral data see Table 1; EI-MS *m/z* (rel. int. %): 681 ([M-glucose]$^+$, 5), 663 ([M-glucose–H_2O]$^+$, 9), 645 ([M-glucose–$2H_2O$]$^+$, 15), 627 ([M-glucose–$3H_2O$]$^+$, 12), 615 (11), 471 (18), 391 (20), 369 (19), 354 (13), 320 (15), 311 (40), 251 (30), 209 (25), 183 (20), 97 (75), 83 (80), 55 (100). HR-FAB-MS: *m/z* 844.6869 [M + H]$^+$ (calcd for $C_{48}H_{94}NO_{10}$, 844.6878; Figure 2).

3.5. *Methanolysis of 1*

A solution of compound **1** (3 mg) in MeOH (4 ml) containing 1 N HCl (2 ml) was refluxed for 4 h, concentrated under reduced pressure, diluted with H_2O and extracted with hexane. Evaporation of the hexane fraction provided methyl 2-hydroxyicosanoate (2.1 mg): EI-MS *m/z* 342 ([M]$^+$, 15), 283 (35), 111 (30), 97 (75), 69 (80), 55 (100). The aqueous layer was then neutralized by addition of Ag_2CO_3, and concentrated *in vacuo*. The residue was purified by CC to afford a mixture of the α- and β-anomers of methyl D-glucoside. These were identified by TLC ($CHCl_3$–MeOH–H_2O, 12:7:1): R_f 0.65 (β) and 0.63 (α), optical rotation $[\alpha]_D^{25}$ + 76.8 (*c* 0.03, MeOH), as well as EI-MS [*m/z* 194 (M$^+$)]. The sphingosine base could not be isolated due to lack of material.

References

[1] L. Lingdi and C. Alexander, *Flora of China* **9**, 47 (2005).

[2] X.S. Zhang and D. Wang, *Chinese Medicine Dictionary* (Shanghai Science and Technology Publishing House, Shanghai, p. 1117 and 1978 1986).
[3] Z.W. Xie, *Quanguo Zhongcaoyao Huibian (A Collection of Chinese Herbal Drugs)*, 2nd ed., (People's Hygenic Publishing House, Beijing, p. 514 and 767 1996).
[4] J.M. Gao, Z.J. Dong, and K. Liu, *Lipids* **36**, 175 (2001).
[5] J.H. Jung, C.-O. Lee, Y.C. Kim, and S. Kang, *J. Nat. Prod.* **59**, 319 (1996).
[6] K. Pan, M. Inagaki, N. Ohno, C. Tanaka, R. Higuchi, and T. Miyamoto, *Chem. Pharm. Bull.* **58**, 470 (2010).
[7] L.P. Sandjo, A. Tchoukoua, H.N. Ntede, M. Yemloul, E. Perspicace, F. Keumedjio, F. Couty, G. Kirsch, and T. Ngadjui, *J. Am. Oil Chem. Soc.* **87**, 1167 (2010).
[8] W. Knöss and J. Zapp, *Planta Med.* **64**, 357 (1998).
[9] G. Savona, F. Piozzi, L.M. Aranguez, and B. Rodriguez, *Phytochemistry* **18**, 859 (1979).