

## New Sphingolipids from *Abutilon pakistanicum*

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Pakistamides A and B (**1** and **2**, respectively), two new sphingolipids, have been isolated from the AcOEt-soluble sub-fraction of the MeOH extract of the whole plant of *Abutilon pakistanicum*. Their structures were assigned by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, and by DEPT and COSY, NOESY, HMQC, HMBC, EI-MS, and FAB-MS experiments.

**Key words:** *Abutilon pakistanicum*, Malvaceae, Sphingolipids, Pakistanamide A, Pakistanamide B

### Introduction

The genus *Abutilon* (Malvaceae) comprises about 150 species which are perennial herbs, shrubs and rarely small trees, distributed in sub-tropical regions of all over the world. Generally, *Abutilon* species contain considerable amounts of mucilage due to which they are used for the treatment of rheumatism and as demulcent and diuretic agents [1–3]. One of the species of the genus *Abutilon* is *Abutilon pakistanicum* which commonly grows in the southern parts of Pakistan. The literature survey revealed that steroids [4, 5], esters, flavonoid glycosides [6, 7], and iridoids [8] have so far been reported from this species. The ethnopharmacological and chemotaxonomic importance of the genus *Abutilon* led us to further investigate the chemical constituents of *A. pakistanicum*. We herein report the isolation and structural elucidation of two new sphingolipids named pakistamides A (**1**) and B (**2**).

### Results and Discussion

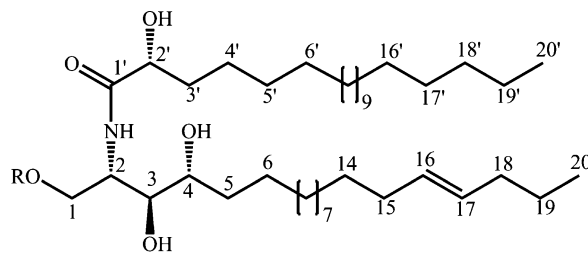
The MeOH extract of the whole plant of *A. pakistanicum* was suspended in water and divided into sub-fractions soluble in *n*-hexane,  $\text{CHCl}_3$ , AcOEt, *n*-BuOH, and  $\text{H}_2\text{O}$ . The AcOEt-soluble sub-fraction was subjected to repeated column chromatography over silica gel to afford compounds **1** and **2**.

Pakistanamide A (**1**) was obtained as a colorless gummy solid. The UV spectrum showed absorption maxima at 203 and 228 nm. The IR absorption bands at 3515–3340 and 1635  $\text{cm}^{-1}$  indicated the presence of hydroxyl, amine and olefinic functionalities, respectively. The characteristic IR absorptions at 1647 and 1545  $\text{cm}^{-1}$  suggested that compound **1** was a secondary amide derivative [9]. The molecular formula was established as  $\text{C}_{40}\text{H}_{79}\text{O}_5\text{N}$  by high-resolution EI-MS which showed  $[\text{M}]^+$  at  $m/z = 653.5950$ . Further peaks at  $m/z = 635.5848$ , 617.5741 and 699.5635 were due to the successive loss of water molecules.

In the  $^1\text{H}$  NMR spectrum (Table 1), the proton of the secondary amide nitrogen showed a doublet at  $\delta = 8.50$  (d,  $J = 9.0$  Hz). The proton signals at  $\delta = 5.58$  (dt,  $J = 16.0, 6.1$  Hz) and 5.50 (dt,  $J = 16.0, 5.9$  Hz) were attributed to a disubstituted double bond. The up-field region showed a broad signal for four methylene groups in the range  $\delta = 1.90$ –2.24 while the rest of the methylene protons resonated at  $\delta = 1.25$ –1.32 (br. s,  $26 \times \text{CH}_2$ ). A triplet for the terminal methyl groups was observed at  $\delta = 0.86$  (t,  $J = 7.1$  Hz, 6H). Two oxymethylene protons resonated at  $\delta = 4.41$  (dd,  $J = 4.5, 10.8$  Hz) and 4.50 (dd,  $J = 8.0, 10.8$  Hz), and three resonances for the oxymethine protons appeared at  $\delta = 4.61$  (dd,  $J = 3.5, 7.5$  Hz), 4.30 (dd,  $J = 4.9, 7.0$  Hz) and 4.23–4.25 (1H, m), confirming that compound **1** is a sphingolipid [9, 10]. On the basis of the coupling constant between the olefinic protons ( $J = 16.0$  Hz)

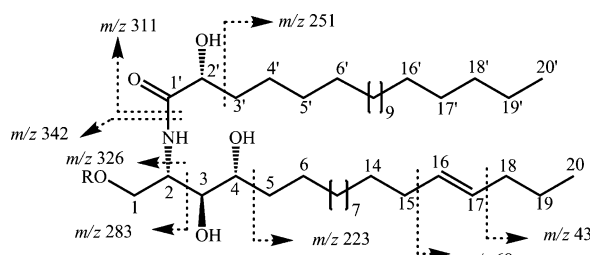
and the signals of the allylic methylenes at  $\delta = 33.3$  and  $33.1$ , a *trans*-configuration of the double bond was evident [11]. The  $^{13}\text{C}$  NMR spectrum (BB and DEPT) (Table 1) showed a signal of an amide carbonyl carbon at  $\delta = 175.2$  whereas the olefinic methines resonated at  $\delta = 130.8$  and  $130.4$ . The azomethine signal characteristic of a sphingolipid appeared at  $\delta = 52.7$  while an oxymethylene carbon resonated at  $\delta = 62.5$  along with three resonances of oxymethine carbons at  $\delta = 76.9$ ,  $73.7$  and  $73.0$ . The methylenes of the aliphatic chains resonated in the range of  $\delta = 22.9$ – $35.6$  with the two terminal methyl carbons at  $\delta = 15.7$ .

In the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum, the azomethine proton ( $\delta = 5.06$ – $5.09$ ) showed correlations with the oxymethylene protons H-1 at  $\delta = 4.50$  and  $4.41$  and the oxymethine proton H-3 at  $\delta = 4.30$  which further correlated with another oxymethine proton H-4 at  $\delta = 4.23$ – $4.25$  revealing the position of the two hydroxyl groups at C-3 and C-4, respectively. The position of the third hydroxyl group was confirmed as C-2' by HMBC correlations of the oxymethine proton H-2' at  $\delta = 4.61$  and the amide proton ( $\delta = 8.50$ ) to the amide carbonyl carbon ( $\delta = 175.2$ ). The olefinic proton at  $\delta = 5.50$  showed a COSY correlation with the signals at  $\delta = 1.90$ – $1.92$  and HMBC correlations with C-15 ( $\delta = 33.1$ ) and C-19 ( $\delta = 22.9$ ) suggesting the position of the double bond at C-16. The position of the double bond and the length of the fatty acid chain were further confirmed by methanolysis and fragment analysis. Acid methanolysis of compound **1** gave the fatty acid methyl ester which was characterized by mass spectrometry as methyl 2-hydroxyicosanoate ( $m/z = 342$  [M] $^+$ ) [12]. Its optical rotation  $[\alpha]_{\text{D}}^{25} = -2.4$  indicated the *R*-configuration [13]. Thus both chains of compound **1** were confirmed to be of equal length with the double bond located in the sphingosine base. The position of the olefinic bond was confirmed by the fragmentation peak in the EI-MS at  $m/z = 585$  due to the loss of  $\text{C}_5\text{H}_8$  by McLafferty rearrangement. The diagnostic fragment ion peaks at  $m/z = 311$  and  $342$  due to the cleavage of the amide bond confirmed the length of the fatty acid consisting of 20 carbons (Fig. 2). The stereochemistry of the sphingosine base was presumed to be  $2S, 3S, 4R$ , since the  $^{13}\text{C}$  and  $^1\text{H}$  NMR signals assigned to C-1, C-2, C-3, C-4, and H-2 were in good agreement with those sphingolipids composed of ( $2S, 3S, 4R$ )-phytosphingosines and ( $2R$ )-2-hydroxy fatty acids [14–16]. This was further supported by the NOESY spectrum. The azomethine proton at  $\delta = 5.06$ – $5.09$  (H-2) showed correlations with H-2' at  $\delta = 4.61$ , H-4 at  $\delta = 4.23$ – $4.25$  and  $\text{H}_{\beta-1}$  at  $\delta = 4.50$ . On the other hand, H-3 at  $\delta = 4.30$  showed correlations with  $\text{H}_{\alpha-1}$  at  $\delta = 4.41$  as well as with the amide proton at  $\delta = 8.50$ . On the basis of these evidences, the structure of pakistamide A (**1**) could be assigned as ( $2R$ )-2-hydroxy-*N*-[( $2S, 3S, 4R, 16E$ )-1,3,4-trihydroxy-16-icosen-2-yl]icosanamide (Fig. 1).



1. R = H
2. R =  $\beta$ -D-Glucopyranoside

Fig. 1. Structures of pakistamides A (**1**) and B (**2**).



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Fig. 2. Mass fragmentation of pakistamides A (**1**) and B (**2**).

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Pakistamide B (**2**) was obtained as a colorless amorphous powder. The IR and UV spectra were similar to that of **1**. The HR-FAB-MS (positive mode) showed a quasi molecular ion  $[\text{M}+\text{H}]^+$  at  $m/z = 816.6565$  indicating a molecular formula  $\text{C}_{47}\text{H}_{90}\text{O}_{10}\text{N}$ . The  $^1\text{H}$  NMR spectrum (Table 1) was similar to that of **1** except the additional signal of an anomeric proton at  $\delta = 5.51$  (d,  $J = 8.0$ ) and further oxymethine protons of a hexose unit resonating in the range of  $\delta = 4.00$ – $4.30$ . The oxymethylene protons appeared at  $\delta = 4.32$  and  $4.51$ . The larger coupling constant of the anomeric proton allowed us to assign the  $\beta$ -configuration to the hexose moiety. The  $^{13}\text{C}$  NMR spectra (BB and DEPT) were also

C	Compound 1		Compound 2	
	$\delta_C$	$\delta_H$ (mult., <i>J</i> in Hz)	$\delta_C$	$\delta_H$ (mult., <i>J</i> in Hz)
1	62.5	4.41 (dd, 4.5, 10.8)	70.1	4.55 (dd, 4.7, 10.9)
2	52.7	4.50 (dd, 8.0, 10.8)	53.4	4.75 (dd, 8.0, 10.9)
3	76.9	5.06–5.09 (m)	77.1	5.20–5.23 (m)
4	73.7	4.30 (dd, 4.9, 7.0)	73.9	4.35 (dd, 5.0, 7.2)
5	34.1	4.23–4.25 (m)	34.4	4.22–4.26 (m)
6–14	29.5–30.1	1.91–1.93 (m)	29.5–30.1	1.92–1.94 (m)
15	29.5–30.1	2.20–2.24 (m)	29.5–30.1	2.21–2.24 (m)
16	33.1	1.25–1.32 (br, s)	33.1	1.25–1.32 (br, s)
17	33.1	1.99–2.01 (m)	33.1	1.99–2.01 (m)
18	130.8	5.58 (dt, 6.1, 16.0)	131.1	5.60 (dt, 7.0, 16.0)
19	130.4	5.50 (dt, 5.9, 16.0)	130.8	5.53 (dt, 6.2, 16.0)
20	33.3	1.90–1.92 (m)	33.4	1.89–1.91 (m)
NH	22.9	1.25–1.32 (m)	22.7	1.25–1.32 (m)
1'	15.7	0.86 (t, 7.1)	15.1	0.85 (t, 7.5)
2'	–	8.50 (9.0)	–	8.51 (8.5)
3'	175.2	–	175.2	–
4'	73.0	4.61 (dd, 3.5, 7.5)	73.2	4.63 (dd, 3.5, 7.5)
5'–18'	35.7	2.20–2.23 (m)	35.8	2.22–2.24 (m)
19'	–	2.00–2.02 (m)	–	2.01–2.04 (m)
Me 20'	26.7	1.25–1.32 (br, s)	26.5	1.25–1.32 (br, s)
1''	29.4–30.4	1.25–1.32 (br, s)	29.4–30.4	1.25–1.32 (br, s)
2''	22.9	1.25–1.32 (br, s)	22.7	1.25–1.32 (br, s)
3''	15.7	0.86 (t, 7.1)	15.1	0.85 (t, 7.5)
4''	–	–	105.1	5.51 (d, 8.0)
5''	–	–	74.7	4.14–4.16 (m)
6''	–	–	78.7	4.00–4.03 (m)
	–	–	71.0	4.21–4.23 (m)
	–	–	77.9	4.28–4.30 (m)
	–	–	62.2	4.32 (d, 11.9)
	–	–	–	4.51 (d, 11.9)

Table 1.  $^1\text{H}$  NMR (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz) spectral data of compounds **1** and **2** recorded in  $\text{C}_5\text{D}_5\text{N}$ .

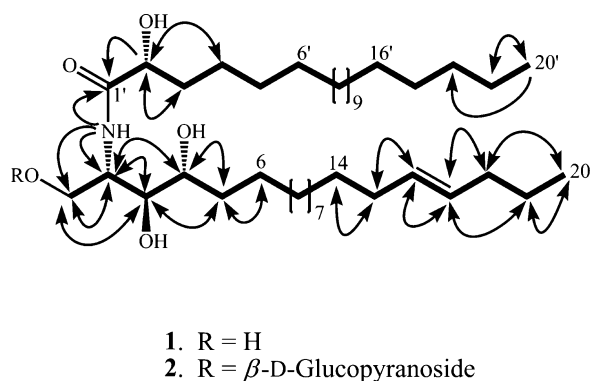


Fig. 3. Important HMBC ( $\rightarrow$ ) and  $^1\text{H}$ - $^1\text{H}$  COSY ( $\dashrightarrow$ ) correlations of pakistamides A (**1**) and B (**2**).

similar to that of **1** except the slight downfield shift of C-1 ( $\delta = 70.1$ ) and additional signals due to the presence of the hexose moiety (the anomeric carbon resonated at  $\delta = 105.1$ , and the oxymethine and oxymethylene carbons were observed in the range of  $\delta = 62.2$ – $78.7$ ). The hexose moiety could be

identified as D-glucose through acid methanolysis which also provided methyl 2-hydroxyicosanoate. The attachment of an *O*- $\beta$ -D-glucose moiety was confirmed to be at C-1 by an HMBC experiment, the anomeric proton at  $\delta = 5.51$  showing a  $^3J$  correlation with C-1 at  $\delta = 70.1$ . The NMR data (Table 1 and Fig. 3), were in complete agreement with the assigned structure of pakistamide B (**2**) (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,16*E*)-3,4-dihydroxy-1-(3,4,5-trihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-2-yl)oxy-16-icosen-2-yl]icosanamide (Fig. 1).

## Experimental Section

### General experimental procedures

Column chromatography (CC) was carried out using silica gel (230–400 mesh, E. Merck, Darmstadt/Germany). Thin layer chromatography (TLC) was performed with precoated silica gel F<sub>254</sub> plates (E. Merck, Darmstadt/Germany), and detection was done at 254 and 366 nm, and by spraying with ceric sulfate in 10%  $\text{H}_2\text{SO}_4$  (heating). Optical rotations were recorded on a Jasco P-2000 polarimeter. The UV

spectra were recorded on a Hitachi UV-3200 spectrophotometer while the IR spectra were recorded from KBr pellets on a Jasco 302-A spectrometer. EI-MS were measured on a Finnigan MAT 312 mass spectrometer, and FAB-MS were measured on a JEOL JMS-HX-110 mass spectrometer with glycerol as a matrix. Ions are given in  $m/z$ . The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker AMX-500 spectrometer in  $\text{C}_5\text{D}_5\text{N}$ . The 2D ( $^1\text{H}$ - $^1\text{H}$  COSY, HMQC, HMBC, NOESY) NMR spectra were recorded on the same instrument. Chemical shifts ( $\delta$ ) are given in ppm relative to tetramethylsilane as an internal standard, and scalar coupling constant ( $J$ ) are reported in Hertz.

#### Plant material

The plant material of *A. pakistanicum* Jafri and Ali (Malvaceae) (8 kg) was collected from Karachi in June 2004 and identified by Prof. Dr. Surraiya Khatoon, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen has been deposited in the herbarium (voucher no. 697 KUH).

#### Extraction and isolation

The shade-dried plant material of *A. pakistanicum* (8 Kg) was cut into small pieces and extracted with MeOH (3 × 20 L). The combined methanolic extract was evaporated under reduced pressure at room temperature to yield a residue (350 g), which was divided into sub-fractions soluble in *n*-hexane (65 g),  $\text{CHCl}_3$  (75 g), AcOEt (35 g), *n*-BuOH (90 g), and water (60 g). The AcOEt-soluble fraction (35 g) was subjected to column chromatography (CC) over silica gel eluting with *n*-hexane-AcOEt, AcOEt and AcOEt-MeOH in increasing order of polarity. The fraction which was obtained with AcOEt (2.1 g), was re-chromatographed over silica gel and eluted with  $\text{CHCl}_3$ -MeOH. The fractions obtained with  $\text{CHCl}_3$ -MeOH (8.5 : 1.5) provided a semi-pure sample which was further purified through preparative TLC using  $\text{CHCl}_3$ -MeOH (8.0 : 2.0) as eluent to afford compound **1** (28 mg). The fraction which eluted with AcOEt-MeOH (9.5 : 0.5) (0.7 g) was re-chromatographed over silica gel eluting with  $\text{CHCl}_3$ -MeOH (8.0 : 2.0) to afford compound **2** (25 mg).

#### Pakistamide A, (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,16*E*)-1,3,4-trihydroxy-16-icosen-2-yl]icosanamide (**1**)

Colorless gummy solid. –  $[\alpha]_{\text{D}}^{25} = +28.7$  ( $c = 0.03$ , MeOH). – UV (MeOH):  $\lambda_{\text{max}}$  ( $\lg \epsilon_{\text{max}}$ ) = 203(2.1), 228(3.5) nm. – IR (KBr):  $\nu = 3515$ –3340, 3105–2925, 1647, 1635, 1545  $\text{cm}^{-1}$ . –  $^1\text{H}$  and  $^{13}\text{C}$  NMR: see Table 1. – MS (EI):  $m/z$  (%) = 653 (5)  $[\text{M}]^+$ , 635 (8)  $[\text{M}-\text{H}_2\text{O}]^+$ , 617 (10)  $[\text{M}-2\text{H}_2\text{O}]^+$ , 599 (11)  $[\text{M}-3\text{H}_2\text{O}]^+$ , 585 (14), 342 (15), 327 (13), 311 (18), 251 (30), 223 (70), 69 (65), 43 (75). – HRMS (EI):  $m/z = 653.5950$  (calcd. 653.5958 for  $\text{C}_{40}\text{H}_{79}\text{O}_5\text{N}$ ,  $[\text{M}]^+$ ).

#### Pakistamide B, (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,16*E*)-3,4-dihydroxy-1-(3,4,5-trihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-2-yl)oxy-16-icosen-2-yl]icosanamide (**2**)

Colorless gummy solid. –  $[\alpha]_{\text{D}}^{25} = +54.2$  ( $c = 0.06$ , MeOH). – UV (MeOH):  $\lambda_{\text{max}}$  ( $\lg \epsilon_{\text{max}}$ ) = 204(2.5), 230(3.2) nm. – IR (KBr):  $\nu = 3520$ –3335, 3100–2925, 1645, 1631, 1540  $\text{cm}^{-1}$ . –  $^1\text{H}$  and  $^{13}\text{C}$  NMR: see Table 1. – MS (EI):  $m/z$  (%) = 653 (2)  $[\text{M}-\text{glucose}]^+$ , 635 (6)  $[\text{M}-\text{glucose}-\text{H}_2\text{O}]^+$ , 585 (16), 342 (13), 326 (10), 311 (21), 251 (25), 223 (65), 69 (25), 43 (75). – HRMS ((+)-FAB):  $m/z = 816.6557$  (calcd. 816.6565 for  $\text{C}_{47}\text{H}_{90}\text{O}_{10}\text{N}$ ,  $[\text{M}+\text{H}]^+$ ).

#### Methanolysis of **1** and **2**

A solution of compound **1** or **2** (3 mg) in MeOH (4 mL) containing 1 N HCl (2 mL) was refluxed for 4 h, concentrated under reduced pressure, diluted with  $\text{H}_2\text{O}$ , and extracted with *n*-hexane. Evaporation of the *n*-hexane fraction provided methyl 2-hydroxyicosanoate (2.1 mg): colorless oil,  $[\alpha]_{\text{D}}^{25} = -2.4$  ( $c = 0.05$ ,  $\text{CHCl}_3$ ). – MS (EI):  $m/z = 342$  (15)  $[\text{M}]^+$ , 283 (35), 111 (30), 97 (75) 69 (80), 55 (100). In the case of **2** the same fatty acid methyl ester was obtained. The aqueous layer was then neutralized by addition of  $\text{Ag}_2\text{CO}_3$ , and concentrated *in vacuo*. The residue was purified by column chromatography to afford a mixture of the  $\alpha$ - and  $\beta$ -anomers of methyl D-glucoside. These were identified by TLC ( $\text{CHCl}_3$ -MeOH- $\text{H}_2\text{O}$  12 : 7 : 1):  $R_f = 0.65$  ( $\beta$ ) and 0.63 ( $\alpha$ ), optical rotation  $[\alpha]_{\text{D}}^{25} = +76.8$  ( $c = 0.03$ , MeOH), as well as by EI-MS ( $m/z = 194$   $[\text{M}]^+$ ). The sphingosine base could not be isolated due to lack of material.

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