Original article

Pakistamide C, a new sphingolipid from Abutilon pakistanicum

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Introduction

The genus Abutilon consists of about 150 species, which are distributed in the tropical and subtropical regions of Asia and other parts of the world (Nasir and Ali, 1972; Baquar, 1989). The species are distributed among small trees, shrubs and perennial herbs. Generally, Abutilon species were used for various disorders, like rheumatism, and used as diuretic and a demulcent, due to their considerable amount of mucilage content (Nasir and Ali, 1972; 1979; Baquar, 1989). Abutilon pakistanicum Jafri and Ali, Malvaceae, commonly grows in the southern parts of Pakistan. Different steroids (Ahmed et al., 1990; Hussain et al., 2008a), esters glycosides, flavonoids (Ahmed et al., 1991; Ali et al., 2010b), triterpenes, iridoids (Ali et al., 2010b), and sphingolipids (Ali et al., 2012) have been found in species from genus Abutilon. Sphingolipids are known to have a vast range of biological activities (Ahmed et al., 1990; Hussain et al., 2008b). Therefore, the chemotaxonomic and ethnopharmacological importance of this genus prompted us to carry out further phytochemical studies on Abutilon pakistanicum.

ARTICLE INFO

Article history:
Received 11 December 2013
Accepted 30 April 2014

Keywords:
Abutilon pakistanicum
Malvaceae
Sphingolipid
Pakistamide C

ABSTRACT

The Abutilon genus from the Malvaceae family is of medicinal importance, and members of this genus are distributed in the tropical and subtropical regions of Asia and other parts of the world. Abutilon pakistanicum Jafri & Ali is mainly found in Pakistan. It has been used by different systems of traditional medicines to treat different diseases. Pakistamide C, a new sphingolipid, has been isolated from the ethyl acetate soluble fraction of the methanolic extract of A. pakistanicum. Different spectroscopic techniques such as NMR (\textsuperscript{1}H, \textsuperscript{13}C, COSY, NOESY, HSQC, HMBC) and Mass spectrometry (EI-MS, and FAB-MS experiments) were used to elucidate the structure of pakistamide C.

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http://dx.doi.org/10.1016/j.bjp.2014.07.002
As a continuation of our research (Ibrahim et al., 2013a; 2013b) on the phytochemical characterization of medicinal plants, we isolated pakistamide C (1) from Abutilon pakistanicum. The isolation and structural characterization by spectrometric data of this new sphingolipid, pakistamide C are presented here.

Materials and methods

Plant material

Whole specimen of Abutilon pakistanicum Jafri & Ali, Malvaceae (8 kg) was collected at Karachi, Pakistan in June 2004. Identification of material was done by plant taxonomist, Prof. Dr. Surraiya Khatoon, at the Department of Botany, University of Karachi. A specimen voucher was deposited in the herbarium of the Department of Botany (voucher no. 697 KUH).

Extraction and isolation

The shade-dried plant material (8 kg) was grounded and soaked in MeOH (3 × 20 l) to get the crude methanolic extract (350 g). The extract (350 g) was then divided into n-hexane (65 g), chloroform (75 g), ethyl acetate (35 g), n-butanol (90 g), and water (60 g) soluble fractions using solvent-solvent extraction procedure.

Ethyl acetate fraction (35 g)

Ethyl acetate soluble fraction was subjected to column chromatography (CC) with silica gel as stationary phase and n-hexane/AcOEt, AcOEt and AcOEt/MeOH (with polarity increase) as mobile phase. That yielded six major sub-fractions (A, B, C, D, E, F).

Sub-fraction D

The fraction retrieved from the AcOEt layer (2.1 g), was subjected to chromatography on a silica gel column, and eluted with CHCl₃-MeOH (8:2).

Sub-fraction D2

The semipure compound was further purified by preparative TLC using the CHCl₃-MeOH (8.5:1.5) as eluent to afford compound 1 (14 mg).

Results and discussion

MeOH extract of Abutilon pakistanicum Jafri & Ali, Malvaceae, was suspended in water and fractionated into n-hexane, CHCl₃, AcOEt, n-BuOH, and H₂O fractions. The AcOEt-soluble fraction was subjected to repeated silica gel column chromatography, which yielded compound 1.

The compound pakistamide C (1) was a gummy colorless solid. Absorption peaks for UV spectrum were observed at 203 and 230 nm. The IR showed absorption bands at 3510-3345, 1543, 1640, and 1645 cm⁻¹ for the characteristic peaks of amine, hydroxyl and olefinic functional groups and secondary amide (Gao et al., 2001).

The molecular formula C₄₂H₈₃NO₅ was deduced from the EI-MS, FAB-MS, and high-resolution EI-MS spectra with a molecular ion peak at m/z 681.6289 (calculated for C₄₂H₈₃NO₅, 681.6282). Further peaks appeared at m/z 663.6192, 645.6083 and 627.5979, yielded from consecutive water losses (Fig. 1).

The ¹H NMR spectrum showed (Table 1), a doublet at δ 8.48 (d, J = 8.9 Hz), assigned to the proton attached to nitrogen of the amide group. The substituted double bond signals were

![Figure 1 – Mass fragmentation pattern of 1.](image-url)
observed at δ 5.58 (dt, J = 16.0, 6.1 Hz) and 5.50 (dt, J = 16.0, 5.9 Hz). Protons of upfield signals determined four methylene groups at δ 1.90–2.24, while the rest of the methylene protons resonated at δ 1.21–1.33 (br, s, 28 × CH₂). Methyl protons have a triplet at δ 0.85 (t, J = 7.0 Hz, 6H), which confirms the defined sequence of terminal groups.

The two oxymethylene protons resonated at δ 4.42 (dd, J = 4.4, 10.6 Hz) and δ 4.48 (dd, J = 7.8, 10.6 Hz), while oxymethine protons resonated at δ 4.52 (dd, J = 4.0, 7.6 Hz), 4.32 (dd, J = 5.0, 7.1 Hz) and 4.24–4.26 (1H, m). These signals confirm the basic skeleton to be a sphingolipid (Gao et al., 2001; Muralidhar et al., 2003). The coupling constant of olefinic protons (J = 16.0 Hz) confirmed the trans configuration of the double bond (Jung et al., 1996). The 13C NMR spectrum (BB and DEPT) (Table 1) showed signal for an amide carbonyl carbon at δ 175.1, whereas the olefinic methines resonated at δ 130.8 and δ 130.4. The four methine carbon signals appeared at δ 52.8 (CHNH), δ 77.0 (CHOH), δ 73.5 (CHOH) and δ 72.4 (CHOH), while an oxymethylene carbon resonated at δ 62.5. The aliphatic methylenic carbons resonated in the range of δ 22.8–35.8, while the two terminal methyl carbons were observed at δ 15.5.

The 1H-1H COSY spectrums showed the correlation between the oxygenated methylene hydrogens at δ 4.48 and 4.42 with the azomethine hydrogen at δ 5.07–5.09. This relates with the oxymethylene proton H-3 at δ 4.32, which further cross peaks showed another oxymethylene proton H-4 at δ 4.24–4.26 confirming the position of C-3 and C-4 for the two hydroxyl groups, respectively (Fig. 2). The third hydroxyl group position was assigned at C-2’, in which H-2’ at δ 4.52 showed HMBC correlation with amide carbonyl carbon (δ 175.1).

The COSY spectrum showed the correlations of olefinic protons at δ 5.58 and δ 5.50 with δ 2.00–2.02 and 1.90–1.92. Also, H-17 at δ 5.50 showed correlation with C-15 (δ 33.2) and C-19 (δ 22.8) in HMBC spectrum, thus confirming the position of the double bond at C-16. The fragmentation pattern and methanolation confirmed the position of the double bond and the length of the fatty acid chain.

Methanolation yielded the fatty acid methyl ester, characterized by mass spectrometry as methyl 2-hydroxydocosanoate (m/z 370 [M]+) (Inagaki et al., 2006). Thus confirming the sphingosine base length composed of 20 carbons with double bond located in the base chain.

The olefinic bond position was long-established by the fragmentation peak in the EI-MS at m/z 613 due to the loss of C₂H₅ by McLafferty rearrangement. The peaks at m/z 339 and 342 result from the cleavage of the amide bond, further confirmation for the length of the fatty acid consisting of 20 and 22 carbons, respectively (Fig. 1). The 13C and 1H NMR data along with specific rotation of compound 1 were compared with previously reported data of sphingolipids, which confirmed the stereochemistry of the sphingosine base and the fatty acid. The stereochemistry was assigned to be (2’R) for 2’-hydroxy fatty acid and (2S, 3S, 4R) for phytosphingosines moiety (Loukaci et al., 2000; Zhang et al., 2007; Sandjo et al., 2010).

The stereochemistry at different stereocenters was further confirmed through NOESY spectrum, which showed correlation of the azomethine proton at δ 5.07–5.09 (H-2) with H-4 at δ 4.24–4.26 and H-2’ at δ 4.52 (Fig. 3). While H-3 at δ 4.32 showed through space coupling with the amide proton at δ 8.48 and H-1α at δ 4.42. So, on the basis of different spectrosopic data as mentioned above the structure of pakistamide C (1) was unambiguously established as (R)-2-hydroxy-N-((2S,3S,4R,E)-1,3,4-trihydroxyicos-16-en-2-yl) docosanamide.

**Pakistamide C (1)**

(R)-2-hydroxy-N-((2S,3S,4R,E)-1,3,4-trihydroxyicos-16-en-2-yl) docosanamide, colorless gummy solid. [α]D = +25.0 (c = 0.05, MeOH). UV ʎmax (MeOH) nm: 202 (1.5), 230 (4.0). IR (KBr) cm⁻¹: 3510-3338 (OH/NH), 2920 (CH), 1647, 1545 (HN–C = O), 1636 (C = C); 730 (aliphatic Cs). 1H and 13C NMR: see Table 1. EI-MS: m/z (%): 681 (7) [M]+, 663 (10) [M–H 2O]+, 645 (10) [M–2H 2O]+, 627 (15) [M–3H 2O]+, 613 (10), 342 (17), 339 (16), 283 (10), 281 (40), 223 (65), 68 (55), 43 (80). HREI-MS: m/z 681.6266 (calcd. 681.6271 for C₄₂H₈₃NO₅[M]+). FAB-MS: m/z 682.6338 (calcd. 682.6344 for C₄₂H₈₃NO₅ + H+).

**Methanolation of 1**

Compound 1 (2 mg) was dissolved in MeOH (10 ml). This solution was taken in a 50 ml round bottom flask. After the addition of few drops of 5% HCl, the reaction mixture was refluxed for 2 h at 80°C. On completion of the reaction, the
solution was diluted with water, and extracted with n-hexane. The n-hexane layer was then separated and concentrated for further analysis using GC-MS, which agrees with the data reported for methyl 2-hydroxydocosanoate (m/z 370 [M]+) [13].

### Authors’ contributions

BA, HN, KH, IH, MSHA and MI contributed in collecting the plant sample, running the laboratory work, analysis and drafting of the data. MI, NH and IH contributed to the analysis of the spectroscopic data and writing of the manuscript. BA, TG and MI designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

### Conflicts of interest

The authors declare no conflicts of interest.

### Acknowledgements

We are thankful to Higher Education Commission of Pakistan, for financial support. The authors are grateful to H. E. J. Research institute of chemistry, University of Karachi, for providing spectro-support in carrying out NMR studies.

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