A new isoquinoline alkaloid with anti-microbial properties from Berberis jaeschkeana Schneid. var. jaeschkeana

Muhammad Alamzeba*, M. Rafiullah Khanb, Mamoon-Ur-Rashidc, Saqib Alidl and Ashfaq Ahmad Khanb

“Institute of Chemical Sciences, University of Swat, Swat 19130, Pakistan; bPhytopharmaceutical and Neutraceutical Research Laboratories (PNRL), Institute of Chemical Sciences, University of Peshawar, Peshawar, Pakistan; cDepartment of Chemistry, University of Poonch Rawalakot, AJK, Poonch, Pakistan; dUniversity of Swabi, Swabi, Pakistan

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One new isoquinoline alkaloid named berberidione (1) along with four new source alkaloids berberine (2), palmatine (3), jatrorrhizine (4) and chondrofoline (5) and three new source non-alkaloids syringic acid (6), β-sitosterol (7) and stigmasterol (8) was isolated and characterised from different fractions of Berberis jaeschkeana Schneid var. jaeschkeana. All the structures were determined from 1D and 2D spectroscopic data. Crude extract, sub-fractions and isolated compounds showed excellent anti-microbial properties. The toxicity level for the alkaloids was found to be very low on THP-1 cells.

Keywords: Berberis jaeschkeana; berberidione; anti-microbial

1. Introduction

Members of the family Berberidaceae are known to have isoquinoline, bisbenzylisoquinoline, aporphine, protopine, protoberberine and benzyl isoquinoline types of alkaloids (Shamma et al. 1973). The genus Berberis is the largest genus of Berberidaceae having 450–500 species, mostly shrubs and distributed in Asia, Europe and America (Chopra et al. 1956; Ahrendt 1961). Members of the genus Berberis are used for the treatment of many diseases around the world. These diseases include fever, malaria, jaundice, hepatitis, wound healing, eye and ear diseases (Watt 1889; Kirtikar & Basu 1933; Chopra et al. 1958; Asif et al. 2007).
Thus considering the phytochemical and biological importance of the genus *Berberis*, we decided to explore the phytochemistry and bioactivities of *Berberis jaeschkeana* Schneid var. *jaeschkeana* for the first time.

2. Results and discussion

Methanolic extract from the bark of roots (4 kg) of *B. jaeschkeana* Schneid var. *jaeschkeana* has resulted in the isolation of eight compounds. They include one new isoquinoline alkaloid, four new source alkaloids and three new source non-alkaloids. The structures of the new and new source compounds were established from ID and 2D spectroscopic data and comparison with the literature.

2.1. Berberidione (1)

Compound 1 was isolated as pale yellow sticky solid which turns brown on exposure to light, UV (MeOH) \( \lambda_{\text{max}} \) (log e): 300 nm (5.84), 260 (5.87) nm. Its molecular formula was established to be C\(_{18}\)H\(_{15}\)NO\(_8\) with 12 degrees of unsaturation on the basis of positive HR-ESI-MS (m/z 374.0996 [M + H]\(^+\); calcd 374.0878). The IR spectrum for compound 1 displayed absorption bands for OH stretching (3341 cm\(^{-1}\)), aromatic H-stretching (3031 cm\(^{-1}\)) and carbonyl stretching (1723 and 1720 cm\(^{-1}\)). The \(^1\)H NMR spectrum of compound 1 (Table S1) showed three aromatic protons (\( \delta \) 7.53, 1H, s, H-1; 7.41, 1H, s, H-9 and 6.68, 1H, s, H-4), three methylene groups (\( \delta \) 6.03, 2H, s, H-14, 3.57, 2H, dt, \( J = 6.8, \) 2.8 Hz, H-6 and 2.94, 2H, t, \( J = 6.8 \) Hz, H-5) and a methoxy singlet (\( \delta \) 3.97, 3H, s, 10-OCH\(_3\)).

The \(^13\)C NMR for compound 1 (Table S1) showed 3 methine carbons (\( \delta \) 107.97, 107.27 and 107.07), 3 methylene carbons (\( \delta \) 101.43, 40.28 and 28.42), 1 methoxy carbon (\( \delta \) 56.50) and 11 quaternary carbon atoms. The methoxy hydrogens showed a strong three bond away correlation with C-10 which helped in adjusting and finalising the position of the methoxy group. Methylene protons at position 14 showed two three bond away correlation with C-2 and C-3. H-4 correlated with C-3, C-2, C-4a and C-5 according to the HMBC–NMR. The methylene protons at position 5 correlated with C-6 and C-13b, while the methylene protons at position 6 correlated with C-5, C-8 and C-13b. The position of the two carbonyl carbons was confirmed from the HMBC–NMR spectrum of compound 1.

In the HMBC–NMR spectrum, H-1 and H-6 showed strong three bond away correlations with \( \delta \) 166.64 (C-13a), while H-10 showed a two bond away correlation with \( \delta \) 170.32 (C-8), which confirmed the position of the two carbonyl groups. The COSY spectrum for compound 1 showed only one \(^1\)H–\(^1\)H correlations between H-5 and H-6. Compound 1 may have been formed by the breakdown of alkaloid berberine. Compound 1 possibly breaks down at position 8 when exposed to light, which explains its unstable nature when exposed to light. Based on all these information, the structure of berberidione (Figure 1) was established.

The new source compounds (Figure 2) were identified as berberine (2) (Blasko et al. 1988), palmatine (3) (Wafio et al. 1999), jatrorrhizine (4) (Thuy et al. 2006), chondrofoline (5) (Panichpol et al. 1977; Mambu et al. 2000), syringic acid (6) (Chen et al. 1999), \( \beta \)-sitosterol (7) (Slomp & Mackellar 1962) and stigmasterol (8) (Sadikun et al. 1996).

The structures of all the new source compounds were determined by \(^1\)H NMR, \(^13\)C NMR, COSY, HSQC and HMBC spectra by comparison with the literature.

3. Experimental

3.1 General experimental procedures

ID and 2D NMR spectra were recorded on Bruker Avance DRX-400, Bruker Corporation, Germany, UV spectra with Thermo Spectronic Unicam UV-300 Spectrophotometer, Thermo
3.2. Plant material

*B. jaeschkeana* Schneid var. *jaeschkeana* was collected from Azad Kashmir Pakistan during and was identified by Prof. Dr Tanveer Akhtar (Chairperson Botany Department University of Azad Jammu and Kashmir). A voucher specimen bearing number 9615-B has been deposited in the herbarium of the Botany Department University of Peshawar.

3.3. Extraction and isolation

Bark of roots (4 kg) was first dried at room temperature and was then pulverised with heavy duty grinder. The powdered plant material was soaked in commercial-grade methanol at room temperature for 10 days. The dilute crude extract obtained was first filtered and concentrated under reduced pressure with rotary evaporator to yield a dark brownish black residue (296 g).

The residue was then treated with 5% aqueous HCl solution, filtered and allowed to stand overnight to afford Fr. A (107 g), which was a mixture of mainly berberine and other protoberberine alkaloids. The filtrate was then extracted with dichloromethane (800 mL × 4) to isolate the non-alkaloidal components, which on concentration afforded Fr. B (16 g). The acidic filtrate was then basified with NH₃ to pH 9 and extracted with ethyl acetate to afford Fr. C (59 g). The remaining aqueous solution was termed as Fr. D, which was a mixture of quaternary alkaloids.

3.3.1. *Berberidione (1)*

Amorphous orange yellow solid turns to brown on exposure to light (unstable on exposure to light); UV (MeOH) \( \lambda_{\text{max}} \) (log ε): 300 nm (5.84), 260 (5.87) nm.

IR \( \tilde{\nu} \): 3341 (O–H, st.), 1723, 1720 (C=O, st.) cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CDCl\(_3\)):

\( \delta \) 7.53 (1H, s, H-1), 6.68 (1H, s, H-4), 2.94 (2H, t, J = 6.8 Hz, H-5), 3.57 (2H, dt, J = 6.8 and 2.8 Hz, H-6), 7.41 (1H, s, H-9), 6.03 (2H, s, H-14) and 3.97 (3H, s, 10-OMe). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)):

\( \delta \) 107.07 (C-1), 146.96 (C-2), 151.01 (C-3), 107.27 (C-4), 133.75 (C-4a), 28.42 (C-5), 40.28 (C-6), 170.32 (C-8), 119.05 (C-8a), 107.97 (C-9), 146.63 (C-10), 141.65 (C-11), 139.71 (C-12), 142.01 (C-12a), 166.64 (C-13a), 122.37 (C-13b), 101.43 (C-14) and 56.50 (10-OMe). ESI-MS: 374.0996 [M + H]\(^+\) (caled for C\(_{18}\)H\(_{16}\)NO\(_8\); 374.0878).

Figure 1. Structure of compound 1.
3.4. Microbial culture preparation

The anti-microbial properties of the crude extract, fractions and pure compounds were tested against five strains of bacteria namely *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Staphylococcus epidermidis*. These organisms were placed in Mueller–Hinton agar (MHA) in the refrigerator at 4°C prior to subculture. The bacterial strains were collected from the stock culture of Phytopharmaceutical and Neutraceutical Research Laboratories (PNRL), Institute of Chemical Sciences, University of Peshawar Pakistan.
3.5. Anti-microbial activity

The anti-bacterial properties of the crude extract, fractions and pure compounds were evaluated by using the modified agar-well diffusion method (Figure 3). MHA was used as medium. The cultures were taken in triplicates at incubation temperature of 37°C for 24 to 72 h. The broth culture (0.6 mL) of the test organism was placed in a sterile Petri-dish and 20 mL of the sterile molten MHA was added. Streptomycin was used as standard anti-microbial agent at a concentration of 2 mg/mL. Inoculation was done for 1 h. Incubation was done at 37°C for 24 h and the diameters of the zone of inhibition of microbial growth were measured in millimetres.

3.6. Cytotoxicity determination

The toxicity of the alkaloids including compound 1 was evaluated by using THP-1 cells method (Gutowska et al. 2010).

4. Conclusion

This is the first comprehensive report on the phytochemical and biological evaluation of B. jaeschkeana Schneid var. jaeschkeana. It can be concluded from this study that B. jaeschkeana could be used as a new source of several classes of biologically active compounds.

Supplementary material

Supplementary material relating to this paper is available online.

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