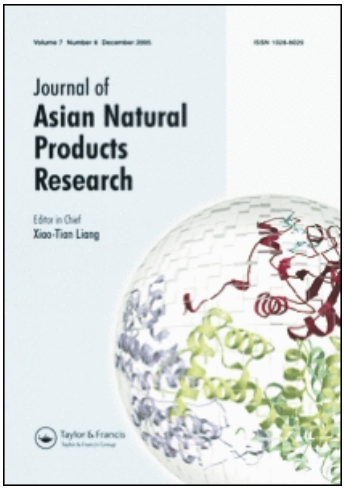

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Structure of barlericin, the neolignan diglycoside from *Barleria acanthoides*

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NOTE

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Barlericin, the new neolignan diglycoside, has been isolated from the *n*-butanol soluble sub-fraction of *Barleria acanthoides* along with dehydrodiconiferyl alcohol 12-*O*-β-D-glucopyranoside (**2**), reported for the first time from the genus *Barleria*. Their structures have been assigned on the basis of spectral studies.

Keywords: *Barleria acanthoides*; Acanthaceae; neolignan diglycoside; *barlericin*

1. Introduction

The genus *Barleria* (Acanthaceae) is represented in Pakistan by four species [1], including *Barleria acanthoides*, which commonly grows in the suburbs of the district of Karachi [2]. A variety of medicinal properties are attributed to this plant [3]. In continuation of our search for the discovery of plant-based drugs, phramacochemical studies have been undertaken on *B. acanthoides*. Its methanolic extract showed strong toxicity in brine shrimp lethality test [4]. On further fractionation, the major toxicity was observed in the *n*-butanolic sub-fraction. Our previous studies on this fraction resulted in the isolation of two superoxide-scavenging phenolic glycosides [5]. In continuation of these studies, we herein report the isolation and structure elucidation of a new neolignan diglycoside named as barlericin (**1**) along with dehydrodiconiferyl alcohol 12-*O*-β-D-glucopyranoside (**2**) [6], reported for the first time from the genus *Barleria* (Figure 1).

2. Results and discussion

The methanolic extract of *B. acanthoides* was divided into *n*-hexane, ethyl acetate, *n*-butanol, and water-soluble sub-fractions. The *n*-butanolic sub-fraction was subjected to a series of chromatographic techniques to obtain neolignan glycosides **1** and **2**.

Barlericin (**1**) was isolated as a gummy solid. The molecular formula was assigned as $C_{31}H_{40}O_{15}$ by HR-FAB-MS in negative mode, showing the $[M-H]^-$ peak at m/z 651.2291. The IR spectrum of **1** showed the presence of hydroxyl group (3420 cm^{-1}), an olefinic moiety (1610 cm^{-1}), and aromatic ring (1520 cm^{-1}). The UV spectrum showed absorption maxima at 219 (sh), 285, and 300 (sh) nm. The ^{13}C NMR spectrum (BB and DEPT) showed 31 signals comprising 2 methyl, 5 methylene, 16 methine, and 8 quaternary carbons as illustrated in Table 1. It showed very close similarity to dehydrodiconiferyl alcohol 12-*O*-β-D-glucopyranoside (**2**) [6] with additional signals due to a pentose moiety.

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Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data for compound **1** recorded in CD_3OD.

Position	δ_H	δ_C
2	5.52 (d, $J = 6.5$ Hz)	89.3
3	3.49 (ddd, $J = 5.5, 6.5, 7.0$ Hz)	55.1
4	–	130.2
5	6.99 (br s)	116.6
6	–	132.2
7	6.96 (br s)	111.9
8	–	145.5
9	–	149.1
10	6.62 (d, $J = 16.0$ Hz)	134.4
11	6.26 (ddd, $J = 16.0, 6.5, 5.9$ Hz)	124.4
12	4.49 (ddd, $J = 12.5, 6.5, 1.4$ Hz) 4.28 (ddd, $J = 12.5, 5.9, 1.4$ Hz)	70.9
13	3.79 (dd, $J = 11.0, 7.0$ Hz) 3.83 (dd, $J = 11.0, 5.5$ Hz)	64.7
1′	–	134.4
2′	6.94 (d, $J = 1.5$ Hz)	116.5
3′	–	149.4
4′	–	147.6
5′	6.77 (d, $J = 8.0$ Hz)	116.1
6′	6.82 (dd, $J = 8.0, 2.0$ Hz)	119.7
8-OCH_3	3.80 (s)	56.3
3′-OCH_3	3.87 (s)	56.6
1″	4.41 (d, $J = 7.5$ Hz)	101.9
2″	3.48 (m)	78.6
3″	3.93 (d, $J = 1.0$ Hz)	77.8
4″	3.27 (m)	71.7
5″	3.24 (m)	77.9
6″	3.67 (dd, $J = 10.0, 5.5$ Hz) 3.85 (dd, $J = 10.0, 7.0$ Hz)	62.7
1‴	5.38 (d, $J = 1.0$ Hz)	110.4
2‴	3.38 (m)	78.5
3‴	–	80.8
4‴	4.09 (d, $J = 9.5$ Hz) 3.72 (d, $J = 9.5$ Hz)	75.4
5‴	3.63 (m) 3.66 (m)	66.2

Thus, barlericin is a diglycoside of dehydrodiconiferyl alcohol.

The ^{1}H NMR spectrum was also similar to dehydrodiconiferyl alcohol showing an ABX coupling system at δ 6.77 (1H, d, $J = 8.0$ Hz), 6.82 (1H, dd, $J = 8.0$, 2.0 Hz), and 6.94 (1H, d, $J = 2.0$ Hz) along with two unresolved broad singlets of aromatic protons at δ 6.96 and 6.99, respectively.

The singlets of two methoxyl protons were observed at δ 3.80 and 3.87. The *trans*-olefinic protons resonated at δ 6.26 (1H, dt, $J = 16.0, 6.5$ Hz) and 6.62 (1H, d, $J = 16.0$ Hz), while a C_3 unit attached to the aromatic ring gave signals at δ 3.49 (1H, ddd, $J = 5.5$, 6.5,7.0 Hz, H-3), 3.79 (1H, dd, $J = 11.0$, 7.0 Hz, H-13a), 3.83 (1H, dd, $J = 11.0$, 5.5 Hz, H-13b), and 5.52 (1H, d, $J = 6.5$ Hz, H-2). The oxymethylene protons resonated at δ 4.28 (1H, ddd, $J = 12.5$, 6.5, 1.4 Hz, H-12a) and 4.49 (1H, ddd, $J = 12.5$, 5.9, 1.4 Hz, H-12b). The presence of two sugar units was evident by the appearance of two anomeric protons at δ 5.38 (1H, d, $J = 1.0$ Hz) and 4.41 (1H, d, $J = 7.5$ Hz) in addition to signals due to three oxymethylene and five oxymethine protons, as illustrated in Table 1.

The sugar moieties were suggested to be a glucose and an apiose from the ^{13}C NMR spectrum. Acid hydrolysis produced aglycone that could be identified

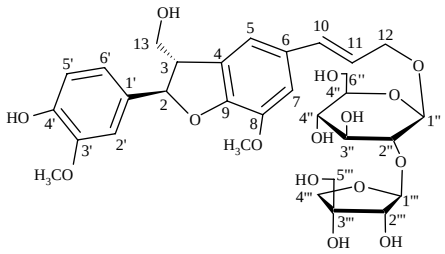

Figure 1. Structure of barlericin (**1**).

as (2*R*,3*S*)-(−)-dehydrodiconiferyl alcohol by comparison of physical and spectral data with those reported in literature [6]. The correlations observed in COSY, HMQC, and HMBC experiments revealed the connectivity of the sugars and dehydrodiconiferyl alcohol (Figure 2). The long-range H–C correlations from the anomeric protons at δ 4.41 to C-12 at δ 70.9, and the methine proton at δ 3.48 to the anomeric carbon at δ 110.4 showed that the glucose was connected at the C-12 of the dehydrodiconiferyl alcohol, and the apiose was connected at the C-2″ of the glucose moiety. The anomeric configuration of glucose was β from the coupling constant of the anomeric proton signal (δ 4.41, $J = 7.5$ Hz), and that of apiose was also β from the ^{13}C NMR spectral data of the C-1 and C-2 of apiose [7]. No NOE interaction was observed between H-2 and H-3 in conformity to their relative *trans* orientation. Compound **1** had a minus CD curve with a minimum at 284 nm, being similar to the curve for (−) dehydrodiconiferyl

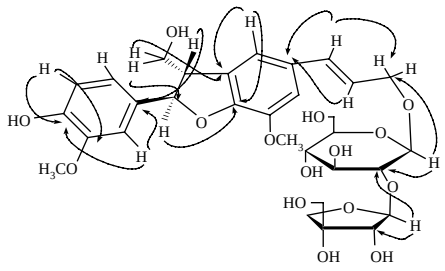

Figure 2. Important HMBC (→) correlations of (**1**).

alcohol. Thus, the absolute stereochemistry at the C-2 and C-3 positions in **1** was confirmed as *R* and *S*, respectively. Therefore, the structure of barlericin (**1**) was assigned as (2*R*,3*S*)-(−) dehydrodiconiferyl alcohol 12-*O*-β-D-apiofuranosyl(1 → 2)-β-D-glucopyranoside.

Compound **2** could be identified as dehydrodiconiferyl alcohol 12-*O*-β-D-glucopyranoside through comparison of physical and spectral data with those reported in literature [6].

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on an ATAGO AP-300 digital polarimeter using a 200 mm tube. The UV and IR spectra were recorded on Hitachi-UV-3200 and Jasco-320-A spectrometers. Circular dichroism spectra were recorded on a JASCO-810 spectropolarimeter. EI-MS and HR-FAB-MS (neg. mode, matrix: glycerol) were recorded on JEOL JMS-HX110 and JMS-DA 5000 mass spectrometers. The ^{1}H and ^{13}C NMR, COSY, HMQC, and HMBC spectra were recorded on a Bruker AM-400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR, respectively. The chemical shifts were reported in ppm (δ) units, and the coupling constants (J) were reported in Hertz. Column chromatography was carried out on various adsorbents including diaion HP-20 ion exchange resin (Nippon Rensui Co., Tokyo, Japan) and Sephadex LH-20 (Amersham Biosciences Limited, Amersham, Sweden) and silica gel (230–400 mesh, E. Merck, Darmstadt, Germany). Thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F_{254} plates (20 × 20 cm, 0.2 mm thick; E. Merck), and visualization was achieved at 254 nm as well as by spraying with ceric sulfate reagent. High-performance liquid chromatography (HPLC) was used for final purification on a recycling preparative HPLC (LC-908W-C-60, Japan

Analytical Industry Co. Ltd, Tokyo, Japan) using a column of ODS-M-80 (4 μm, 250 × 20 mm; Japan Analytical Industry Co. Ltd).

3.2 Plant material

The whole plant of *B. acanthoides* Vahl was collected in 2007 from Karachi, Pakistan, and identified by Dr Suraiya Khatoon, Plant Taxonomist, Department of Botany, University of Karachi, Pakistan, where a voucher specimen (KUH-3969) has been deposited.

3.3 Extraction and isolation

The shade-dried plant material (20 kg) of *B. acanthoides* was extracted at room temperature with MeOH (3 × 50 liter × 10 days each). The combined methanolic extract (300 g) was divided into *n*-hexane (100 g), ethyl acetate (60 g), *n*-butanol (23 g), and water-soluble (95 g) sub-fractions. The *n*-butanolic sub-fraction was dissolved in water and column chromatographed over diaion HP-20, eluting successively with H_2O, MeOH–H_2O (1:1), MeOH–H_2O (1:2), and MeOH. The fraction that was eluted with MeOH–H_2O (1:2) (3.2 g), was further chromatographed over Sephadex LH-20. The elution was carried out with MeOH–H_2O (1:1), collecting 30 fractions of 50 ml each. The fractions 7–14 showing similar TLC profiles were combined and designated as Fraction A (22 mg). Similarly, the fractions 23–30 also showed similar TLC profiles, which were combined and designated as Fraction B (30 mg). Fraction A was chromatographed over silica gel and eluted with EtOAc–MeOH (1:1), collecting 10 fractions of 20 ml each. The last five fractions provided a semi-pure compound (18 mg). These were combined and subjected to recycling HPLC using solvent system MeOH–H_2O (1:1) (flow rate 3 ml/min) to obtain compound **1** (14.2 mg, R_t 18 min).

Fraction B was rechromatographed over silica gel eluting with EtOAc–MeOH (7:3) and the resulting eluate (15 mg) was subjected to recycling HPLC using solvent system MeOH–H_2O (1:1) (flow rate 3.0 ml/min) to furnish compound **2** (11 mg, R_t 24 min).

3.3.1 Barlericin (*1*)

Gummy solid; $[\alpha]_D^{20} = -8$ ($c = 0.128$, CH_3OH). UV λ_{max} nm (log ε): 219 (sh), 285, 300 (sh). IR (KBr) ν cm^{-1}: 3425, 1610, 1520, 1076, 1030. ^1H and ^{13}C NMR spectral data are shown in Table 1. CD (MeOH) $\Delta\varepsilon_{284}$ −8.767, $\Delta\varepsilon_{234}$ + 6.816, $\Delta\varepsilon_{213}$ −7.622, and $\Delta\varepsilon_{205}$ + 14.496. HR-FAB-MS *m/z* 651.2291 $[M-H]^-$ (calcd for $C_{31}H_{39}O_{15}$, 651.2289).

3.4 Acid hydrolysis of compound 1

Compound **1** (4 mg) in MeOH (5 ml) containing 1 N HCl (4 ml) was refluxed for 4 h, concentrated under reduced pressure and diluted with H_2O (8 ml). It was extracted with ethyl acetate, and the aglycone obtained through preparative TLC was crystallized from acetone–hexane as colorless crystals, mp 140–141°C. Its color reactions, melting point, and spectral data showed complete agreement with those reported in the literature for (2*R*,3*S*)-(−)-dehydrodiconiferyl alcohol [8].

The aqueous phase was a mixture of glycone products that could not be worked up due to paucity of the material.

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