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Aitchisonides A and B, new iridoid glucosides from *Aitchisonia rosea*

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Aitchisonides A (**1**) and B (**2**), new iridoid glucosides, were isolated from the *n*-butanolic fraction of *Aitchisonia rosea* along with deacetylasperulosidic acid (**3**) and nepetanudoside B (**4**), which were reported for the first time from the genus *Aitchisonia*. Their structures have been assigned on the basis of spectral analysis including ^{1}H and ^{13}C NMR spectra and by DEPT, 2D COSY, NOESY, and HMBC experiments.

Keywords: *Aitchisonia rosea*; Rubiaceae; aitchisonides A and B; iridoid glucosides

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1. Introduction

The family Rubiaceae comprises 450 genera and well over 6500 species largely of tropical and subtropical distribution, but some also grow in temperate regions and few are arctic in distribution [1]. In Pakistan, it is represented by 33 genera and 87 species [2]. *Aitchisonia* has only one species named as *Aitchisonia rosea.* The plant grows in Pakistan, Iran, and Afghanistan. In Pakistan, it is mainly found in the province of Balochistan. The literature survey revealed that no phytochemical or pharmacological studies have so far been carried out on *A. rosea*. The methanolic extract of this plant showed strong toxicity in brine shrimps lethality test. On subsequent fractionation, the maximum toxicity was observed in the *n*-butanolic subfraction. This prompted us to carry out investigations of the *n*-butanolic subfraction resulting in the isolation and structural elucidation of two new iridoid glucosides named as aitchisonides A (**1**) and B (**2**) (Figure 1) along with deacetylasperulosidic acid (**3**) and nepetanudoside B (**4**), which were reported for the first time from the genus *Aitchisonia*.

2. Results and discussion

The *n*-butanol-soluble subfraction of the methanolic extract of *A. rosea* was subjected to a series of chromatographic techniques to obtain two new iridoid glucosides. The latter were named aitchisonides A (**1**) and B (**2**) isolated as gummy solid. The UV spectra of all the isolated compounds showed absorption maxima at 233–235 nm, which is characteristic for iridoids [3].

The HR negative fast atom bombardment mass spectrometry (FAB-MS) of aitchisonide A (**1**) established the molecular formula $C_{19}H_{26}O_{12}$, showing an $[M-H]^-$ peak at *m/z* 445.1334 (calcd for $C_{19}H_{25}O_{12}$, 445.1346). The IR spectrum

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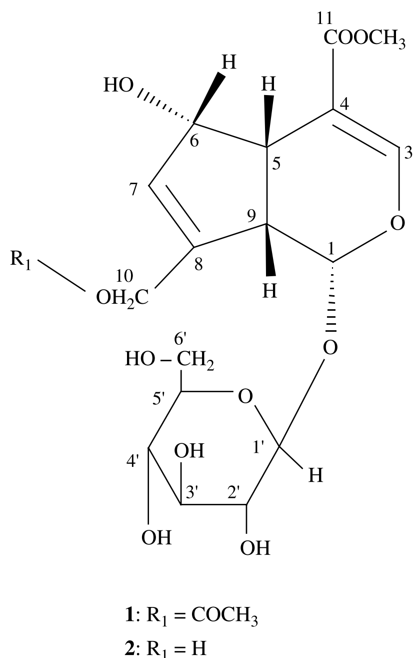

Figure 1. Structures of compounds **1** and **2**.

showed absorption bands due to hydroxyl group (3404 cm^{-1}), ester carbonyls (1730 and 1706 cm^{-1}), and double bond (1635 cm^{-1}). The ^{13}C NMR spectrum of **1** showed 19 carbon signals comprising 2 methylene, 11 methine, 2 methyl, and 4 quaternary carbons. It showed signals of ester moieties at δ 172.5 and 169.4, respectively. The downfield oxymethine carbon, which resonated at δ 100.6, could be ascribed to the anomeric carbon of a sugar moiety. Further oxymethine carbons were observed at δ 101.3, 78.6, 77.9, 75.4, 74.9, and 71.5, respectively. The two oxymethylene carbons were observed at δ 66.2 and 63.0. The signals at δ 155.3, 145.9, 131.9, and 108.1 showed the presence of two olefinic bonds.

The ^{1}H NMR spectrum showed two olefinic protons at δ 7.64 (s, 1H) and 6.02 (br s, 1H). The anomeric proton of the hexose moiety was observed at δ 4.71 (d, $J = 7.8$ Hz, 1H). The larger coupling constant confirmed the β-glucosidic linkage. Further signals of the hexose moiety were observed at δ 3.23–3.38 along with oxymethylene protons at δ 3.85 (dd, $J = 11.9$, 1.6 Hz, 1H) and 3.64 (dd, $J = 11.9$, 5.7 Hz, 1H). The oxymethine protons of the iridoid moiety resonated at δ 5.05 (d, $J = 7.0$ Hz, 1H) and 4.80 (m, 1H), while the oxymethylene protons were observed at δ 4.95 and 4.79 (d, $J = 14.6$ Hz, each 1H). The two methyl groups were observed as singlets at δ 3.60 and 2.33, respectively. Acid hydrolysis gave a mixture of products from which the sugar moiety could be isolated and identified as D-glucose through the sign of its optical rotation and comparison of the retention time of its tetramethylsilane (TMS) ether with standard in gas chromatography (GC). The positions of carbomethoxy group, hydroxyl, and acetoxymethyl moieties were assigned to C-4 (δ 108.1), C-6 (δ 75.4), and C-10 (δ 66.2) on the basis of HMBC experiment of **1** (Figure 2). The complete agreement of NMR parameters of the cyclopentane ring with those of daphylloside [4] confirmed the relative positions of the substituents. The position of the sugar moiety was confirmed at C-1 based on HMBC

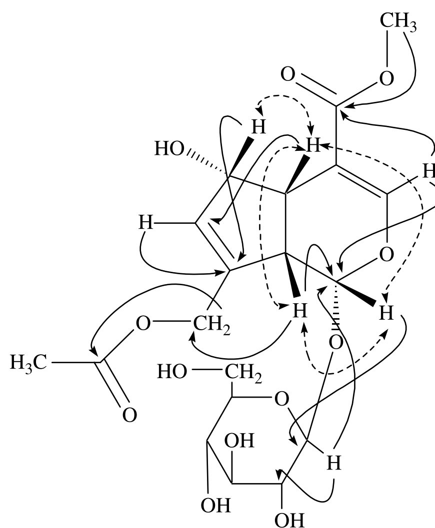

Figure 2. Important HMBC (⟶) and NOESY (◂- - -▸) correlations.

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spectrum; the anomeric proton at δ 4.71 showed ^{2}J correlations with C-2′ (δ 74.9) and ^{3}J correlations with C-1 (δ 101.3). The structure of **1** is similar to that of daphylloside. In so far, however, as the two compounds widely differ in optical rotations, **1** is apparently a stereoisomer of daphylloside. The stereochemistry was derived through a series of decoupling experiments and NOE interactions. The similarity of NMR chemical shifts of the five-member ring with those of daphylloside indicated β- and pseudo-equatorial orientation of H-6, β- and pseudo-axial orientation of H-5 and β- and pseudo-equatorial disposition of H-9 [5]. All these protons showed correlations with each other in the NOESY spectrum, revealing their relative *cis* orientation. Irradiation of the olefinic proton at δ 6.02 simplified the multiplet of H-6 into a doublet showing pseudo-equatorial and pseudo-axial coupling with H-5 ($J = 3.0$ Hz). Strong NOE interaction further confirmed β-configuration of both H-6 and H-5. The *cis* fusion of the ring was also confirmed by a strong NOE interaction between H-5 and H-9. Moreover, irradiation of the signal of H-6 at δ 4.80 collapsed the multiplet of H-5 into a doublet ($J = 2.9$ Hz) due to pseudo-axial and pseudo-equatorial coupling with H-9. The larger glucose moiety at C-1 was most likely in a more favored equatorial configuration, which was confirmed by pseudo-axial and pseudo-equatorial coupling of a magnitude of 3.3 Hz between β- and pseudo-axial H-1 and H-9. This could also be confirmed by strong NOE interaction between 1-βH and 5-βH. From the above evidence, structure of **1** could be assigned as 10-acetoxy-6α-hydroxy-4-carbomethoxy-5βH,9βH-iridoid-1α-*O*-β-D-glucopyranoside(1-epi-daphylloside).

The IR spectrum of aitchisonide B (**2**) showed the absorption bands due to hydroxyl group (3424 cm^{-1}), conjugated ester (1710 cm^{-1}), and double bond (1630 cm^{-1}). The negative HR-FAB-MS established the molecular formula $C_{17}H_{24}O_{11}$, showing an $[M-H]^-$ peak at *m/z* 403.1236. Comparison of NMR spectral data showed a striking resemblance between **1** and **2**, and the only notable difference was the absence of the acetyl signals in the spectra of **2**. It was confirmed by the chemical shifts of both the oxymethylene carbon and oxymethylene protons, which showed upfield shifts. Therefore, compound **2** is a C-10 deacetylated derivative of **1**. The NMR parameters of the cyclopentane ring showed complete agreement with those of deacetyldaphylloside [4]. The NOESY and $^{1}H-^{1}H$ correlation spectroscopy spectra again established an all-*cis* β-configuration at H-5, H-6, H-9, and H-1. The NOE interactions were also similar to those of **1**. Thus, the structure of aitchisonide B (**2**) could be assigned as 10-hydroxy-6α-hydroxy-4-carbomethoxy-5βH,9βH-iridoid-1α-*O*-β-D-glucopyranoside(1-epi-10-deacetyldaphylloside).

Although iridoid glucosides are frequently reported from the family Rubiaceae, this is the first instance of their occurrence in the genus *Aitchisonia* which may be of chemotaxonomic importance. None of the isolated compounds could be subjected to pharmacological screening due to paucity of material.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCO DIP-360 digital polarimeter using a 10 cm tube. The UV and IR spectra were recorded on Hitachi-UV-3200 and JASCO 320-A spectrometers, respectively. The ^{1}H, ^{13}C NMR, and 2D NMR spectra were recorded on a Bruker AMX-400 spectrometer with TMS as an internal standard. Chemical shifts (δ) are in ppm, relative to TMS as an internal standard, and scalar coupling constants (J) are reported in Hertz. FAB and HR-FAB-MS (neg. ion mode, matrix: glycerol) were recorded on a JEOL

JMS-HX110 mass spectrometer. Column chromatography was carried out on various adsorbents including silica gel 230–400 mesh (E. Merck, Darmstadt, Germany), Diaion HP-20 ion exchange resin (Nippon Rensui Co., Mitsubishi Chemical Corporation, Tokyo, Japan), and polyamide 6 pulver 0.05–0.16 mesh (Serva, Heidelberg, Germany). Thin layer chromatography was performed on precoated silica gel F_{254} plates (E. Merck); the detection was done at 254 nm and by spraying with ceric sulfate reagent. Preparative 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*

Whole plant of *A. rosea* Hemsley was collected in August 2006 from Ziarat valley, Balochistan (Pakistan) and identified by Dr Rasool Baksh Tareen, Plant Taxonomist, Department of Botany, University of Balochistan, where a voucher specimen (2186) has been deposited.

3.3 *Extraction and isolation*

The shade-dried whole plant material (30 kg) of *A. rosea* was extracted at room temperature with MeOH (3× 50 liters). The combined methanolic extract (1 kg) was divided into *n*-hexane (300 g), EtOAc (200 g), and *n*-BuOH-soluble subfraction (20 g). The *n*-BuOH-soluble fraction was dissolved in H_2O and loaded on a column of Diaion HP-20 and the elution was successively carried out with water and mixtures of H_2O and MeOH in decreasing order of polarity. The fraction obtained from MeOH–H_2O (1:1) was chromatographed over polyamide and eluted with $CHCl_3$ and MeOH in increasing order of polarity, leading to three major subfractions I–III. Subfraction I obtained from $CHCl_3$–MeOH (9.8:0.2) was a binary mixture, which was separated through HPLC with a flow rate of 3.5 ml/min using MeOH–H_2O (1:1) as the eluent, affording **2** (15 mg, R_t 24 min) and **1** (12 mg, R_t 30 min). Subfraction II obtained from $CHCl_3$–MeOH (9:1) was chromatographed over silica gel and eluted with $CHCl_3$–MeOH (8.8:1.2) to afford deacetylasperulosidic acid **3** (20 mg). Subfraction III obtained from $CHCl_3$–MeOH (8.5:1.5) was first fractionated by HPLC

Table 1. ^{1}H NMR spectral data of compounds **1** and **2** (400 MHz, CD_3OD).

Position	**1**	**2**
1	5.05 (d, 7.0)	5.05 (d, 6.9)
3	7.64 (s)	7.64 (s)
5	3.04 (m)	3.02 (m)
6	4.80 (m)	4.79 (d, 5.6)
7	6.02 (br s)	6.01 (s)
9	2.60 (m)	2.57 (m)
10	4.95 (d, 14.6), 4.79 (d, 14.6)	4.21 (d, 15.6), 4.45 (d, 15.6)
11-OCH_3	3.60 (s)	3.73 (s)
10-$OCOCH_3$	2.33 (s)	–
1′	4.71 (d, 7.8)	4.71 (d, 7.8)
2′	3.23 (m)	3.23 (m)
3′	3.38 (m)	3.37 (m)
4′	3.27 (m)	3.28 (m)
5′	3.27 (m)	3.33 (m)
6′	3.64 (dd, 11.9, 5.7), 3.85 (dd, 11.9, 1.6)	3.60 (dd, 11.9, 5.5), 3.85 (dd, 11.9, 2.0)

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Table 2. ^{13}C NMR spectral data for compounds **1** and **2** (100 MHz, CD_3OD).

Position	**1**	**2**
1	101.3	101.5
3	155.3	155.3
4	108.1	108.2
5	42.4	42.7
6	75.4	75.4
7	131.9	129.8
8	145.9	151.5
9	46.3	45.8
10	66.2	61.6
11	169.4	169.4
12	172.5	–
11-OCH_3	52.8	52.8
10-$OCOCH_3$	20.7	–
1′	100.6	100.5
2′	74.9	74.9
3′	78.6	78.5
4′	71.5	71.6
5′	77.9	77.8
6′	63.0	62.8
1	101.3	101.5
3	155.3	155.3
4	108.1	108.2

into two fractions A and B. The purification of fraction B on HPLC using CH_3CN-H_2O (1:1) as the eluent afforded nepetanudoside B (**4**) (15 mg, R_t 27 min).

3.3.1 *Aitchisonide A (1)*

Gummy solid; $[\alpha]_D^{20}$ -100 ($c = 0.02$, CH_3OH); UV CH_3OH λ_{max}, nm ($\log \varepsilon_{max}$): 232 (3.5); IR (KBr) ν (cm^{-1}): 3404, 1730, 1706, 1635. For ^1H and ^{13}C NMR spectral data, see Tables 1 and 2. HR-FAB-MS *m/z*: 445.1334 $[M-H]^-$ (calcd for $C_{19}H_{25}O_{12}$, 445.1346).

3.3.2 *Aitchisonide B (2)*

Gummy solid; $[\alpha]_D^{25}$ -112 ($c = 0.02$, CH_3OH); UV CH_3OH λ_{max}, nm ($\log \varepsilon_{max}$): 232 (3.3); IR (KBr) ν (cm^{-1}): 3424, 1710, 1630. For ^1H and ^{13}C NMR spectral data, see Tables 1 and 2. HR-FAB-MS *m/z*: 403.1236 $[M-H]^-$ (calcd for $C_{17}H_{23}O_{11}$, 403.1240).

The structures of deacetylasperulosidic acid (**3**) and nepetanudoside B (**4**) were confirmed by comparisons of their physical and spectral data with those reported in the literature [4,6].

3.3.3 *Acid hydrolysis of compounds 1 and 2*

Compounds **1** and **2** (each 4 mg) in MeOH (5 ml) containing 1 N HCl (4 ml) were refluxed for 4 h, concentrated under reduced pressure, and diluted with H_2O (8 ml). It was extracted with ethyl acetate, and the residue from the organic phase was a mixture of products, which was not worked up due to the paucity of material. The aqueous phase was concentrated and D-glucose was identified by the sign of its optical rotation ($[\alpha]_D$ $+52.2$ for **1** and $+52.8$ for **2**). It was further confirmed by comparing the retention time of its TMS ether with the standard sample in GC.

References

[1] A. Perveen and M. Qaiser, *Pak. J. Bot.* **39**, 999 (2007).
[2] S.I. Ali and E. Nasir, *Flora of Pakistan* (Department of Botany, University of Karachi, Karachi, 1989), Vol. 190, p. 1.
[3] H. Kirmizibekmez and O. Sticher, *J. Nat. Prod.* **64**, 60 (2001).
[4] L.O. Demirezer, F. Gurbuz, Z. Guvenalp, and K. Stroch, *Turk. J. Chem.* **30**, 525 (2006).
[5] S.E. Drewes, M.M. Horn, and J.D. Connolly, *Phytochemistry* **47**, 991 (1998).
[6] Y. Takeda, T. Yagi, T. Matsumoto, and G. Honda, *Phytochemistry* **42**, 1085 (1996).

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