Atriplisides A and B, Two New Glycosides from *Perovskia atriplicifolia*

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The new triterpenoidal and flavonone glycosides atriplisides A and B have been isolated from the ethyl acetate fraction of the whole plant of *Perovskia atriplicifolia*. Their structures were elucidated on the basis of spectroscopic data, especially 2D NMR techniques including ^{1}H-COSY, HMQC, HMBC, and NOESY.

Key words: Perovskia atriplicifolia, Atripliside A, Atripliside B, Triterpenoidal and Flavonone Glycosides

Introduction

The genus *Perovskia* belonging to the family Labiatae comprises seven species. One of these is *Perovskia atriplicifolia* Benth, commonly known as Russian sage, which is a shrubby plant found in central Asia, Pakistan, Afghanistan and Iran [1]. The plant has antibacterial activity and is also used as cooling medicine in the treatment of fever [2]. Previously essential oils, phenolic compounds, triterpenes and a steroid have so far been reported from this species [3, 4]. The ethanopharmacological and chemotaxanomic importance of the genus *Perovskia* prompted us to investigate the chemical constituents of *P. atriplicifolia*. As a result, we have isolated atriplisides A (**1**) and B (**2**), two new triterpenoidal and flavonone glycosides. Their structures were elucidated on the basis of spectroscopic data, especially 2D NMR techniques including ^{1}H-COSY, HMQC and HMBC.

Results and Discussion

The methanolic extract of the whole plant was divided into *n*-hexane, chloroform, ethyl acetate and water soluble fractions. Column chromatography of the ethyl acetate soluble fraction provided two new triterpenoidal and flavonone glycosides named as atriplisides A (**1**) and B (**2**), respectively.

Atripliside A (**1**) was obtained as a white powder and gave positive Liebermann Burchard and ceric sulphate tests for a triterpene. It also produced brisk effervescence with dilute sodium bicarbonate solution indicating the presence of a free carboxylic group. The molecular formula $C_{36}H_{58}O_{11}$ was established on the basis of negative HR-FABMS showing the $[M–H]^-$ peak at *m/z* = 665.3877 (calcd. 665.3885). Consequently **1** had an unsaturation index of 8. The IR spectrum indicated the presence of a hydroxyl group (3400 – 3500 cm^{-1}), a carboxylic group (1720 cm^{-1}) and a double bond (1650 cm^{-1}). The ^{13}C NMR and DEPT spectra showed 36 carbon atoms consisting of six methyl, ten methylene, twelve methine and eight quaternary carbon atoms. The notable features were the presence of the carboxylic group (δ_C = 181.1), one trisubstituted double bond (δ_C = 144.5, 123.0), oxymethine carbons (δ_C = 85.0, 69.5), an oxymethylene carbon (δ_C = 66.1) and six methyl groups (δ_C = 29.4, 26.5, 23.8, 18.4, 17.6 and 17.5 respectively). In addition the spectrum showed signals of a hexose moiety (anomeric carbon at δ_C = 102.4, five oxymethine carbons between δ_C = 78.2 – 71.6 and the oxymethylene carbon at δ_C = 62.0). The ^{1}H NMR spectrum showed a trisubstituted double bond at δ_H = 5.26 (t, *J* = 3.4 Hz), three oxymethine protons at δ_H = 3.77 (ddd, *J* = 10.8, 9.8, 4.0 Hz), δ_H = 3.30 (dd, *J* = 12.7, 4.6 Hz) and δ_H = 3.01 (d, *J* = 9.8 Hz). The oxymethylene protons were observed at δ_H = 4.01 (d, *J* = 11.2 Hz) and δ_H = 3.38 (d, *J* = 11.2 Hz). The singlets of the methyl groups were located at δ_H = 1.22, 1.15, 1.03, 0.98, 0.96 and 0.78. The signal of the anomeric proton of the hexose moiety was identified at δ_H = 4.29 (d, *J* = 7.8 Hz) in addi-

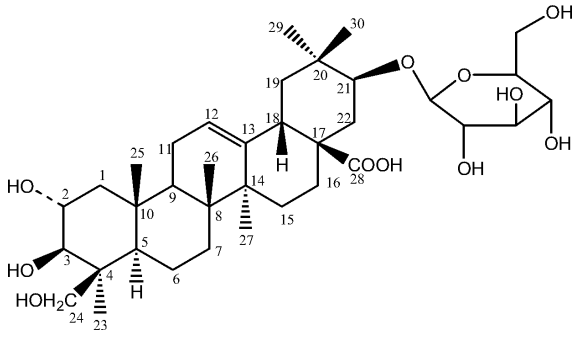

Fig 1. Structure of atripliside A (**1**).

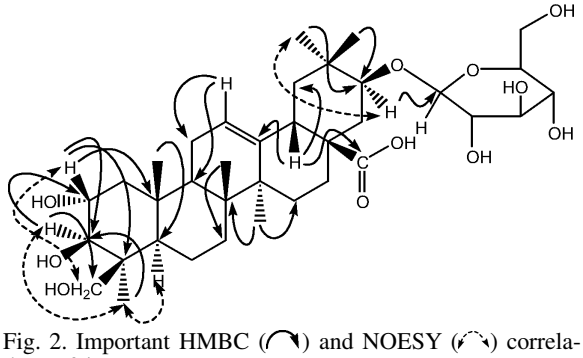

Fig. 2. Important HMBC (↷) and NOESY (⇢) correlations of **1**.

tion to further oxymethine protons ranging from δ_H = 3.36 – 3.15 and oxymethylene protons at δ_H = 3.81 (dd, J = 11.6, 5.1 Hz) and δ_H = 3.68 (dd, J = 11.6, 2.4 Hz).

The above data indicated that **1** is a derivative of oleanolic acid (Fig. 1). The comparatively upfield oxymethine proton at δ_H = 3.01 showed ^{1}H-COSY correlation to another oxymethine proton at δ_H = 3.77; the latter in turn showed additional correlations to two further protons at δ_H = 1.94 and 0.89, respectively, allowing us to assign the oxymethine protons to C-3 and C-2 which could further be confirmed through HMBC correlations (Fig. 2). The larger coupling constants of both H-3 and H-2 allowed to assign equatorial configurations to the corresponding hydroxyl groups. The oxymethylene protons at δ_H = 4.01 and δ_H = 3.38 showed ^2J correlation with C-4 (δ_C = 44.4) and ^3J correlations with both C-3 (δ_C = 69.5), C-5 (δ_C = 57.1) and the methyl group at δ_C = 23.8, thereby confirming the presence of the hydroxymethyl group at C-4. Its configuration could be deduced through ^{13}C NMR. In case of oxidation at C-24, the CH_3-23 resonates at δ_C = 23 – 25 ppm while in case of oxidation at C-23, the CH_3-24 is observed at δ_C = 14 – 16 ppm [5]. In case of **1** the methyl group attached to C-4 resonated at δ_C = 23.8 in conformity to its presence at C-4 in α configuration. The remaining oxymethine proton at δ_H = 3.30 was assigned to C-21 based on HMBC correlations. The protons of both the methyl groups attached to C-20 (δ_C = 29.4 and 18.4) showed ^2J correlations with C-20 (δ_C = 37.4) and ^3J correlations with C-19 (δ_C = 48.2) and C-21 (δ_C = 84.0). The oxymethine proton at δ_H = 3.30 showed ^2J correlations with C-20 (δ_C = 37.4) and C-22 (δ_C = 40.4) as well as ^3J correlations with C-29 (δ_C = 29.4), C-30 (δ_C = 18.4) and C-17 (δ_C = 48.0). The larger coupling constant of H-21 allowed us to assign an α and axial configuration to it.

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1**[a].

Position	^{1}H (multiplicity, J (Hz))	^{13}C (DEPT)
1	089, 1.94	47.8
2	377 (ddd, 10.8, 9.8 & 4.0)	69.5
3	3.01 (d, 9.8)	85.0
4	–	44.4
5	0.97 (m)	57.1
6	1.39, 1.64	19.8
7	1.32, 1.4	34.2
8	–	40.5
9	1.63	48.2
10	–	39.0
11	1.71, 2.00	25.2
12	5.26 (t, 3.4)	123.0
13	–	144.5
14	–	42.0
15	1.72	28.8
16	1.90, 1.94	24.8
17	–	48.0
18	2.89 (dd, 14.4, 4.0, 1H)	42.0
19	1.19 (d, 14.4)	48.2
20	–	37.4
21	3.30 (dd, 12.7, 4.6 1H)	84.0
22	1.71, 2.12	40.4
23	1.22 (s)	23.8
24	3.38 (d, 11.2), 4.01 (d, 11.2)	66.1
25	0.78 (s)	17.5
26	0.96 (s)	17.6
27	1.15 (s)	26.5
28	–	181.1
29	1.03 (s)	29.4
30	0.98 (s)	18.4
Glc-1	4.29 (d, 7.8)	102.4
Glc-2	3.15 (t, 8.2)	75.5
Glc-3	3.31 (m)	78.2
Glc-4	3.36 (m)	71.6
Glc-5	3.25 (m)	77.6
Glc-6a	3.68 (dd, 11.6, 2.4)	62.0
Glc-6b	3.81 (dd, 11.6, 5.1)	–

[a] Solvent: [D_5]pyridine; ^{1}H NMR carried out at 400 MHz; ^{13}C NMR carried out at 100 MHz.

Acid hydrolysis of **1** provided the sugar moiety which could be identified as D-glucose by the sign of its optical rotation and by comparing the reten-

tion time of its trimethylsilyl (TMS) ether with that of the standard in gas chromatography. The larger coupling constant of the anomeric proton allowed us to assign the β configuration to the glucose moiety. The downfield shift of H-21 indicated the glycosylation at this position which could be confirmed by a strong ^3J correlation between H-21 and the anomeric carbon (δ_C = 102.4). Thus the structure of atripliside A (**1**) could be assigned as 21-*O*-β-D-glucopyranosyl-2α, 3β, 24-tetrahydroxyolean-12-en-28-oic acid. The NOESY correlations and ^{13}C NMR assignments made with the help of HMQC were in complete agreement with the assigned structure (Fig. 1, Table 1).

Atripliside B (**2**) was obtained as a yellow gummy solid, $[\alpha]_D^{25}$ = 43.4° (*c* = 0.12, CH_3OH). It gave violet coloration with $FeCl_3$. The HRFABMS of **2** in negative ion mode gave the $[M–H]^-$ peak at *m/z* = 609.1800 corresponding to the molecular formula $C_{28}H_{33}O_{15}$ (calcd. 609.1819). It further showed prominent fragments at *m/z* = 447 and 301 due to the successive losses of sugar units. The HREIMS gave the peak of the aglycone at *m/z* = 302 followed by retro Diels-Alder fragments at *m/z* = 152 and 149 confirming the presence of two hydroxyl groups in ring A along with one methoxyl and one hydroxyl group in ring B. The IR spectrum showed the presence of hydroxyl groups (3445 cm^{-1}), a carbonyl group (1675 cm^{-1}) and a methoxyl group (1580, 1115 cm^{-1}). It developed a magenta color with Mg/HCl, and the UV spectrum exhibited characteristic absorption maxima of flavonones at 212, 285 and 325 nm [6].

The ^{13}C NMR spectra (BB and DEPT) showed twenty eight signals comprising of two methyl, two methylene, sixteen methine and eight quaternary carbons (Table 2). The signals at δ_C = 79.8, 43.1 and 197.0 were typical of C-2, C-3 and C-4 of the flavonone skeleton [7]. Apart from further peaks of the aromatic carbons, the spectrum showed the signals of two anomeric carbons at δ_C = 101.5 ($C_{glc}-1$) and δ_C =102.0 ($C_{rha}-1$), oxymethine carbons ranging between δ_C = 71.2–78.4, oxymethylene carbons at δ_C = 67.4 ($C_{glc}-6$) and a methyl group at δ_C = 18.6 ($C_{rha}-6$). This data suggested that atripliside B (**2**) is a glycoside of flavonone with L-rhamnose at the terminal position. On acid hydrolysis it provided a mixture of sugars which could be identified as L-rhamnose and D-glucose, respectively. The ^{1}H NMR spectrum showed the characteristic AMX system

Table 2. ^{1}H and ^{13}C NMR spectral data and important HMBC correlations of compound **2**[a].

Position	^{1}H (multiplicity, *J* (Hz))	^{13}C (DEPT)	HMBC (H-C)
2	5.47 (dd, 12.3, 2.0)	79.8	C-2′, C-4, C-6′
3-ax	3.20 (dd, 17.0, 12.3)	43.1	C-1′, C-4, C-10
3-eq	2.80 (dd, 17.0, 2.9)	43.1	–
4	–	197.0	–
5	–	163.4	–
6	6.6 (d, 2.0)	96.4	C-5, C-8, C-10
7		166.4	–
8	6.5 (d, 2.0)	97.7	C-6, C-7
9	–	164.4	–
10	–	104.3	–
1′	–	132.1	–
2′	7.5 (d, 1.6)	115.3	C-1′, C-2, C-4′, C-6′
3′		148.4	–
4′		149.1	–
5′	6.9 (d, 8.1)	112.3	C-1′, C-3, 4′-OMe
6′	7.1 (dd, 8.1, 1.6)	118.1	C-2, C-4′
OMe-4′	3.72 (s)	55.8	C-4′
OH-5	12.00 (1H, s)	–	–
OH-3′	9.52 (1H, s)	–	–
Glc-1	4.9 (d, 7.6)	101.5	C-7, C_{glc}-2
Glc-2	4.25 (m)	74.5	C_{glc}-1
Glc-3	4.32 (dd, 8.9, 8.7)	78.4	–
Glc-4	4.12 (m)	71.2	C_{glc}-5
Glc-5	4.16 (m)	77.5	–
Glc-6a	4.61 (dd, 10.4, 4.9)	67.4	C_{rha}-1, C_{glc}-5
Glc-6b	4.19 (dd, 10.4, 2.5)	67.4	C_{rha}-1
Rha-1	5.18 (brs)	102.4	C_{rha}-3, C_{rha}-5, C_{glc}-6
Rha-2	4.65 (m)	72.1	C_{rha}-1, C_{rha}-4
Rha-3	4.52 (dd, 9.1, 3.3)	72.7	–
Rha-4	4.21 (d, 4.7)	74.0	–
Rha-5	4.28 (m)	69.8	C_{rha}-4, C_{rha}-6
Rha-6	1.56 (d, 6.0)	18.6	C_{rha}-5

[a] Solvent: [D_5]pyridine; ^{1}H NMR carried out at 400 MHz; ^{13}C NMR carried out at 100 MHz.

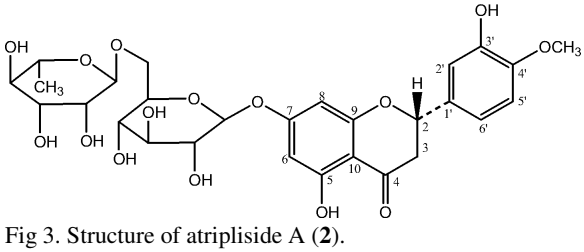

Fig 3. Structure of atripliside A (**2**).

of the flavonone skeleton with resonances at δ_H = 5.47 (1H, dd, *J* = 12.3, 2.9 Hz, H-2), δ_H = 3.20 (1H, dd, *J* = 17.0, 12.3 Hz, H-3_{ax}) and δ_H = 2.80 (1H, dd, *J* = 17.0, 2.9 Hz, H-3eq) [7]. The protons of the 1,3,4-trisubstituted ring B were observed at δ_H = 7.5 (1H, d, *J* = 1.6 Hz, H-2′), δ_H = 7.1 (1H, dd, *J* = 8.1, 1.6 Hz, H-6′) and δ_H = 6.9 (1H, d, *J* = 8.1 Hz, H-5′). The *meta* coupled protons of ring A at δ_H = 6.6 (1H, d, *J* = 2.0 Hz, H-6) and δ_H = 6.5

(1H, d, J = 2.0 Hz, H-8) allowed us to assign oxygen functionalities to C-5 and C-7, respectively. The signal of the chelated hydroxyl group appeared at δ_H = 12.00 (1H, s) while the methoxyl group gave a signal at δ_H = 3.72 (3H, s). The anomeric protons were observed at δ_H = 4.9 (1H, d, J = 7.6 Hz, $H_{glc}-1$) and δ_H = 5.18 (1H, brs, $H_{rha}-1$). This allowed us to assign β and α configurations to the sugar units [6]. The methyl protons of the rhamnose moiety appeared at δ_H =1.56 (3H, d, J = 6.0 Hz) while oxymethylene protons of the D-glucose moiety resonated at δ_H = 4.61 (1H, dd, J = 10.4, 4.9 Hz, $H_{glc}-6\alpha$) and 4.19 (1H, dd, J = 10.4, 2.5 Hz, $H_{glc}-6b$). The signals of the oxymethine protons appeared between δ_H = 4.11 – 4.65. The downfield shift of the oxymethylene carbon of the glucose moiety by about 5.00 ppm indicated the 1-6 linkage between L-rhamnose and D-glucose. The point of attachment of the sugar moiety was shown to be C-7 through HMBC correlations. The positions of the hydroxyl and methoxyl groups were further confirmed by HMBC. The absolute configuration at C-2 was assigned '*S*' on the basis of the CD spectrum which showed similar Cotton effects as reported in the literature for related flavonoids [8, 9]. Thus the structure of atripliside B (**2**) was assigned as (2*S*)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-(3,4,5-trihydroxy-6-{[(3,4,5-trihydroxy-6-methyltetrahydro-2*H*-pyran-2-yl)oxy]methyl}tetrahydro-2*H*-pyran-2-yl)-2,3-dihydro-4*H*-chromen-4-one. The ^{1}H COSY, HMQC and HMBC correlations were in complete agreement with the assigned structure (Fig. 3, Table 2).

Experimental Section

General

IR spectra were recorded on a Jasco-320 A spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-400 spectrometer with tetramethylsilane (TMS) as an internal standard. The 2D NMR experiments were recorded on a Bruker AMX 500 NMR spectrometer. Optical rotations were measured on a Jasco DIP-360 digital polarimeter using a 10 cm tube. Mass spectra were recorded on Finnigan MAT 12 and MAT 312 spectrometers. TLC was performed on precoated silica gel F_{254} plates; the detection was done at 254 nm and by spraying with ceric sulphate solution. Silica gel (E. Merck, 230 – 400 mesh) was used for column chromatography. Melting points were determined on a Gallenkamp apparatus and are uncorrected.

Collection and identification

The whole plant of *Perovskia atriplicifolia* was collected from Quetta valley (Pakistan) and identified by Prof. Rasool Bakhsh Tareen, Department of Botany, University of Baluchistan, where a voucher speciment has been deposited.

Extraction and isolation

The shade dried plant material (20 kg) was extracted with methanol (3 × 50 L) at r. t. The extract was evaporated to yield the residue (780 g) which was divided into *n*-hexane- (65 g), chloroform- (60 g), ethyl acetate- (48 g), *n*-butanol- (70 g) and water- (41 g) soluble fractions. The EtOAc-soluble fraction was subjected to column chromatography over silica gel eluting with $CHCl_3$, $CHCl_3$/MeOH and MeOH in increasing order of polarity. The fraction which eluted with $CHCl_3$/MeOH (9.5 : 0.5) was subjected to preparative TLC ($CHCl_3$/MeOH, 9 : 1) to afford **1** (19 mg). The fraction which eluted with $CHCl_3$/MeOH (9 : 1) was further subjected to column chromatography using $CHCl_3$/MeOH (8.5 : 1.5) as eluent to obtain **2** (15 mg).

*Atripliside A (**1**):* White amorphous powder, $[\alpha]_D^{20}$ = +45.5° (MeOH, c = 0.12 mg mL^{-1}). – IR (KBr): ν = 3500 – 3400, 1720, 1650, 1382 cm^{-1}. – ^{1}H, ^{13}C NMR see Table 1. – HRFABMS: m/z = 665.3877 (calcd. 665.3885 for $C_{36}H_{57}O_{11}$, $[M-H]^-$).

*Atripliside B (**2**):* Yellow gummy solid, $[\alpha]_D^{20}$ = −43.4° (MeOH, c = 0.12 mg mL^{-1}). – IR (KBr): ν = 3445, 1675, 1580, 1115 cm^{-1}. – ^{1}H, ^{13}C NMR see Table 2. – HRFABMS: m/z = 609.1800 (calcd. 609.1819 for $C_{28}H_{33}O_{15}$, $[M-H]^-$). – EIMS: m/z (%) = 302 (20) [M-sugar units]$^+$, 152 (43), 149 (47).

*Acid hydrolysis of **1** and **2***

A solution of **1** or **2** (4 mg) in methanol (4 mL) and 2 M HCl (3 mL) was refluxed for 4 h. The solutions were concentrated under reduced pressure, diluted with water (5 mL) and extracted with ethyl acetate. The sugar residue from the aqueous phase in case of **1** was identified as D-glucose by the sign of its optical rotation $[\alpha]_D$ = +52°, comparing the retention time of its TMS ether with that of the standard in gas chromatography and Co-TLC with an authentic sample of D-glucose using the solvent system *n*-BuOH/EtOAc/HOAc/H_2O (12 : 2 : 2 : 2). TLC was run three times in the same direction and spots were visualized with aniline phthalate.

In case of **2** the sugar residue was subjected to preparative TLC using the solvent system EtOAc/MeOH/H_2O/HOAc (1 : 2 : 2 : 2), and the separated sugars identified as L-rhamnose and D-glucose through Co-TLC with authentic samples and the sign of the optical rotation $[\alpha]_D^{25}$ = +7.9° for L-rhamnose and $[\alpha]_D^{25}$ = +52.2° for D-glucose, respectively.

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