Ionone, Iridoid and Phenylethanoid Glycosides from *Ajuga salicifolia*

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From the aerial parts of *Ajuga salicifolia* (L.) Schreber, a new ionone glycoside (3β-hydroxy-7,8-dihydro-4-oxo-β-ionol-9-*O*-β-D-glucopyranoside) was isolated, along with the known compounds, corchoionoside C, 8-*O*-acetylmioporoside, ajugol, harpagide, 8-*O*-acetylharpagide, lavandulifolioside and leonosides A and B. This is the first report of the occurrence of ionone glycosides and 8-*O*-acetylmioporoside in *Ajuga* species. Ajugol, lavandulifolioside, leonoside A and B were isolated for the first time from *Ajuga salicifolia.* The structures were elucidated by means of 1D-, 2D-NMR spectroscopy, and HR-MALDI mass spectrometry.

Key words: Ajuga salicifolia, Lamiaceae, Ionone Glycosides

Introduction

In the flora of Turkey, the genus *Ajuga* (Lamiaceae) is represented by 11 species (Davis, 1982), some of which are traditionally used in wound healing, as diuretic, as well as against diarrhea and high fever (Baytop, 1984). There have been many phytochemical investigations on *Ajuga* species, focusing mainly on the isolation of phytoecdysteroids and diterpenes and their antifeedant and insect growth inhibiting activities (Camps and Coll, 1993; Camps *et al.*, 1981). Besides our investigations, there was only one report on *Ajuga salicifolia* (L.) Schreber, concerning the isolation of a diterpene (Bozov *et al.*, 1993), and in an chemotaxonomic investigation, the presence of catechin, flavonoid glycosides, and iridoid glycosides in this plant were described (Litvinenko *et al.*, 1970). Recently, we reported new and novel antileukemic and cytotoxic sterols from the aerial parts of *Ajuga salicifolia*, which was collected in Ankara, Turkey (Akbay *et al.*, 2002a; Akbay *et al.*, 2002b). Continuing our investigations, we isolated a new ionone glycoside, 3β-hydroxy-7,8-dihydro-4-oxo-β-ionol-9-*O*-β-D-glucopyranoside (**1**), together with the known compounds, corchoionoside C (**2**), 8-*O*-acetylmioporoside (**3**), ajugol (**4**), harpagide (**5**), 8-*O*-acetylharpagide (**6**), lavandulifolioside (**7**) and leonosides A (**8**) and B (**9**). This paper describes the isolation and structure elucidation of these compounds and emphasizes on their chemotaxonomic significance.

Material and Methods

General experimental procedures

VLC: *RP-18* HL, 40–63 μm (Chemie Uetikon), silica gel *60*, 40–63 μm (Merck). CC: silica gel *60*, 40–63 μm and 63–200 μm (Merck), Sephadex-LH-20. MPLC: Büchi 681 pump, 45 × 3.5 cm Büchi MPLC column packed with *RP-18* HL, 40–63 μm. HPLC: Merck-Hitachi L-6200 pump connected to a Rheodyne 7125 Injector, a Merck-Hitachi L-4000 UV detector, a Merck D-2500 Chromatointegrator, and a Knauer HPLC column (Spherisorb S10 ODS 2, 10 μm; 250 × 20 mm). TLC: Silica gel *60* F_{254} precoated aluminium plates (0.2 mm, Merck), *RP-18* F_{254} precoated plates (0.25 mm, Merck), Detection: 5% H_2SO_4 in EtOH and 1% vanillin in EtOH and heating at 100–110 °C for 5 min. Optical rotation: Perkin-Elmer 241 polarimeter. UV: UVIKON 930 spectrophotometer. HR-MALDI-MS: Ionspec Ultima FTMS spectrometer with 2,5-dihdyroxybenzoic acid (DHB) as matrix. ^{1}H–, ^{13}C NMR, DEPT-135, DEPT-90, [^{1}H,^{1}H]–COSY, [^{13}C,^{1}H]–HSQC, [^{13}C,^{1}H]–HMBC and [^{1}H,^{1}H]–NOESY experiments for compound **1** were measured on a Bruker DRX-600 at 295 K (operating at 600.13 MHz for ^{1}H, and

150.92 MHz for ^{13}C). ^{1}H–, ^{13}C NMR spectra for the other compounds were measured on a Bruker AMX-300 at 295 K (operating at 300.13 MHz for ^{1}H, and 75.47 MHz for ^{13}C). Chemical shifts δ were given in ppm and coupling constants *J* in Hz. The spectra were measured in CD_3OD for all compounds and also in D_2O for the iridoids to compare with the literature data. The spectra were referenced against residual non-deuterated solvent.

Plant material

Ajuga salicifolia (L.) Schreber was collected in Ankara, Beytepe in July 1998. The plant was identified by Prof. Zeki Aytac, Gazi University, Ankara (Turkey). A voucher specimen (HU-98014) was deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University (Ankara, Turkey).

Extraction and isolation

The dried and powdered aerial parts (1 kg) of *A. salicifolia* were extracted with petroleum ether, dichloromethane, ethyl acetate, methanol and methanol-water (1:1 v/v), respectively (sequential percolation with ca. 10–15 l of each solvent). After a TLC control, dichloromethane and ethyl acetate extracts were combined (24 g), and fractionated by VLC (silica gel 60, hexane → ethyl acetate → methanol), yielding 5 main fractions. Fr. 4 (2.7 g) was applied to CC (silica gel 60, hexane → ethyl acetate → methanol). The fraction (600 mg) eluted with EtOAc-MeOH 95:5 was further separated by VLC (*RP-18*, H_2O–ACN 100:0 → 0:100). Compound **1** (1.6 mg), **2** (2.7 mg) and **3** (1.8 mg) were isolated from subfraction 2 (38.7 mg) by HPLC (*RP-18*, flow 5 ml/min, H_2O–ACN–MeOH (78:15:7)).

40 g of the methanol extract were subjected to VLC (*RP-18*, H_2O–MeOH 100:0 → 0:100) to give eight main fractions. Fr. 2 (356 mg), eluted with 5% MeOH, was subjected to MPLC (H_2O → 70% MeOH). Two main fractions were purified on a Sephadex LH-20 column (MeOH) yielding compounds **4** (2.7 mg) and **5** (30 mg). The same procedure was applied to Fr. 4 (747 mg). Elution with 30% MeOH (VLC) furnished compound **6** (20 mg). Fr. 5 (8.4 g), obtained with 50% MeOH, was submitted to VLC (silica gel, CH_2Cl_2–MeOH–H_2O (90:10:1) → (40:60:4). The three fractions, rich in phenylethanoids, were further fractioned separately by CC (silica gel, CH_2Cl_2–MeOH–H_2O (90:10:1) → (60:40:4) affording **7** (240 mg), **8** (67 mg) and **9** (233 mg).

Spectroscopic data

3β-hydroxy-7,8-dihydro-4-oxo-β-ionol-9-*O*-β-D-glucopyranoside (**1**) was obtained as a colorless amorphous powder, $[\alpha]_D^{25}$ – 30.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 248 (2.80) nm; ^{1}H NMR (CD_3OD, 600.13 MHz) δ 4.38 (1H, d, *J* = 7.8 Hz, H-1′), 4.30 (1H, dd, *J* = 5.5 and 13.9 Hz, H-3), 3.93 (1H, m, H-9), 3.89 (1H, dd, *J* = 1.9 and 11.9 Hz, H-6′a), 3.69 (1H, dd, *J* = 5.4 and 11.9 Hz, H-6′b), 3.37 (1H, dd, *J* = 8.7 and 9.1 Hz, H-3′), 3.31 (1H, m, H-4′), 3.28 (1H, m, H-5′), 3.20 (1H, dd, *J* = 7.8 and 9.1 Hz, H-2′), 2.53 (1H, m, H-7a), 2.37 (1H, m, H-7b), 2.04 (1H, dd, *J* = 5.5 and 12.6, H-2a), 1.83 (3H, s, H_3-13), 1.78 (1H, t, *J* = 12.6 and 13.2, H-2b), 1.68 (2H, m, H-8), 1.32 (3H, d, *J* = 6.3, H_3-10), 1.31 (3H, s, H_3-11), 1.26 (3H, s, H_3-12); ^{13}C NMR data (CD_3OD, 150.92 MHz) δ 201.5 (C-4), 167.2 (C-6), 129.7 (C-5), 104.2 (C-1′), 78.3 (C-3′), 77.9 (C-5′), 77.8 (C-9), 75.4 (C-2′), 71.7 (C-4′), 70.4 (C-3), 62.8 (C-6′), 47.0 (C-2), 38.9 (C-1), 36.4 (C-8), 30.0 (C-12), 27.5 (C-7), 25.7 (C-11), 21.9 (C-10), 12.0 (C-13); HR-MALDI-MS (pos. mode): 411.1984 $[M + Na]^+$ (calculated for $C_{19}H_{32}O_8Na$, 411.1995).

Results and Discussion

Sequential percolation of the powdered aerial parts of *A. salicifolia* with petroleum ether, dichloromethane, ethyl acetate, methanol and methanol-H_2O (1:1 v/v) yielded the crude extracts. After TLC control, dichloromethane and ethyl acetate extracts were combined and subjected to subsequent VLC, CC, and HPLC which led to the isolation of two ionone glycosides (**1**, **2**) and 8-*O*-acetylmioporoside (**3**). The fractionation of the methanolic extract by vacuum liquid chromatography (VLC) afforded 8 fractions. The fractions 2, 4 and 5 were further fractionated with open column chromatography on silica gel and Sephadex LH-20, MPLC and HPLC on *RP-18* resulting in the isolation of three iridoid (**4**, **5**, **6**) and three phenylethanoid (**7**, **8**, **9**) glycosides.

The known compounds **2–9** were identified as corchoionoside C (**2**) (Yoshikawa, *et al.*, 1997), 8-

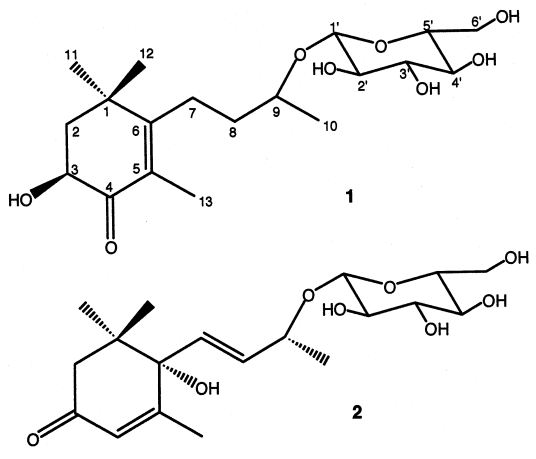

Fig. 1.

O-acetylmioporoside (**3**) (Jacke and Rimpler, 1983; Lammel and Rimpler, 1981), ajugol (**4**) (Agostini *et al.*, 1982; Nishimura, *et al.*, 1989), harpagide (**5**) (Yu *et al.*, 1998), 8-*O*-acetylharpagide (**6**) (Assaad and Lahloub, 1988; Takeda, *et al.*, 1987), lavandulifolioside (**7**) (Basaran *et al.*, 1988; Akcos *et al.*, 1998), leonosides A (**8**) and B (**9**) (Çalıs *et al.*, 1992), respectively, by comparing their ^{1}H and ^{13}C NMR data with previously published data.

Compound **1** was obtained as a colorless amorphous powder. The HR-MALDI-mass spectrum of compound **1** showed a pseudomolecular ion peak at *m/z* 411.1984 $[M + Na]^+$, compatible with the molecular formula $C_{19}H_{32}O_8$. The ^{1}H- and ^{13}C NMR spectra of **1**, together with DEPT mode measurement showed the presence of a β-D-glucopyranosyl moiety from the signals at δ_C 104.2 and δ_H 4.38 (1H, d, J = 7.8). They showed also the existence of an aglycone with 13 carbon atoms, which were sorted as 4 methyls, 3 methylenes, 2 methines, 4 quaternary carbons. In ^{1}H NMR spectrum, the signals at δ_H 1.31 and 1.26 as singlets indicated the presence of geminal dimethyl groups (H_3-11, and H_3-12). The resonances at δ_H 1.83 (s), and δ_H 1.32 (d, J = 6.3) were attributed to the vinyl methyl (H_3-13), and to H_3-10, respectively. The ^{13}C NMR spectrum displayed two oxymethine (δ_C 70.4, 77.8; C-3, C-9, respectively) signals which were consistent with the resonances at δ_H 4.30 (dd, J = 5.5, 13.9; H-3), and δ_H 3.93 (m; H-9) in the ^{1}H NMR spectrum. The chemical shifts of the quaternary carbons at δ_C 201.5, 129.7, and 167.2 exhibited the presence of a carbonyl group conjugated to an endocyclic double bond. ^{1}H, ^{1}H-COSY correlations allowed us to determine the two spin systems of the aglycone. In ^{13}C, ^{1}H-HMBC experiment, the long range correlation between C-9 and H-1′ showed the position of the glycosidation. The HMBC correlation between C-6 and H_3-11, H_3-12, H-2, H-7a, H-7b assigned the connection of the two spin systems. The long range correlation between C-4 and H-2, H-3, H_3-13 confirmed the position of the carbonyl group. The stereochemistry at C-3 was established based on a NOESY experiment. The NOE observed between H_3-11 and H-3 showed that these protons were at the same side of the plane. Therefore, the structure of **1** was established as 3β-hydroxy-7,8-dihydro-4-oxo-β-ionol-9-*O*-β-D-glucopyranoside, which is a new ionone glycoside to the literature.

Our phytochemical investigations on the aerial parts of *Ajuga salicifolia* provided chemotaxonomically significant data. This is the first report of the occurrence of ionone glycosides (**1**, **2**) and 8-*O*-acetylmioporoside (**3**) in *Ajuga* species. To date, 8-*O*-acetylmioporoside was only isolated from *Clerodendrum* spec. (Verbenaceae) (Lammel and Rimpler, 1981). Here is the first report of this compound from the family Lamiaceae. In 1970, Litvinenko *et al.* reported harpagide (**5**) and 8-*O*-acetylharpagide (**6**) from *Ajuga salicifolia.* Lavandulifolioside (**7**) (Çalıs *et al.*, 1992; Didry *et al.*, 1999; Çalıs *et al.*, 1991), and ajugol (**4**) (Akcos *et al.*, 1998; Çalıs *et al.*, 1991) were previously isolated from many genera in Lamiaceae, the latter also from *Ajuga reptans* (Guiso *et al.*, 1974). In this paper, compounds **4** and **7**, together with leonosides A (**8**) and B (**9**) are reported for the first time from *Ajuga salicifolia.*

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