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-acetylimiporoside, ajugol, harpagide, 8-O-acetyl-harpagide, lavandulifolioside and leonosides A and B. This is the first report of the occurrence of ionone glycosides and 8-O-acetylimiporoside 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 phytocysteroiids 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 a 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-acetylimiporoside (3), ajugol (4), harpagide (5), 8-O-acetyl-harpagide (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 Chromatograph, and a Knauer HPLC column (Spherisorb S10 ODS 2, 10 μm; 250 × 20 mm). TLC: Silica gel 60 F254 precoated aluminium plates (0.2 mm, Merck), RP-18 F254 precoated plates (0.25 mm, Merck), Detection: 5% H2SO4 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-dihydroxybenzoic acid (DHB) as matrix. 1H-, 13C NMR, DEPT-135, DEPT-90, [1H,1H]-COSY, [13C,1H]-HSQC, [13C,1H]-HMBC and [1H,1H]-NOESY experiments for compound 1 were measured on a Bruker DRX-600 at 295 K (operating at 600.13 MHz for 1H, and
150.92 MHz for $^{13}\text{C}$). $^{1}\text{H}$–, $^{13}\text{C}$ NMR spectra for the other compounds were measured on a Bruker AMX-300 at 295 K (operating at 300.13 MHz for $^{1}\text{H}$, and 75.47 MHz for $^{13}\text{C}$). Chemical shifts $\delta$ were given in ppm and coupling constants $J$ in Hz. The spectra were measured in CD$_3$OD for all compounds and also in D$_2$O 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. 10–151 of each solvent). After a TLC control, dichloromethane and ethyl acetate extracts were combined (24 g), and fractionated by VLC (silica gel 60, hexane-acetate extracts were combined (24 g), and fractionated by VLC (silica gel 60, hexane-acetate extracts were combined and subjected to subsequence VLC, CC, and HPLC which led to the isolation of three iridoid glycosides. 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 pheylenethanoids (7, 8, 9) glycosides.

The known compounds 2–9 were identified as corchoinoside C (2) (Yoshikawa, et al., 1997), 8-
O-acetylmoporisode (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) (Çals et al., 1992), respectively, by comparing their 1H and 13C 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 C₁₉H₃₂O₈. The 1H- and 13C 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 1H NMR spectrum, the signals at δ_H 1.31 and 1.26 as singlets indicated the presence of geminal dimethyl groups (H₃-11, and H₃-12). The resonances at δ_H 1.83 (s), and δ_H 1.32 (d, J = 6.3) were attributed to the vinyl methyl (H₃-13), and to H₂-10, respectively. The 13C 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 1H 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. 1H-1H-COSY correlations allowed us to determine the two spin systems of the aglycone. In 13C, 1H-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 H₃-11, H₃-12, 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, H₃-13 confirmed the position of the carbonyl group. The stereochemistry at C-3 was established based on a NOESY experiment. The NOE observed between H₃-11 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-acetylmoporisode (3) in Ajuga species. To date, 8-O-acetylmoporisode 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) (Çals et al, 1992; Didry et al., 1999; Çals et al., 1991), and ajugol (4) (Akcos et al., 1998; Çals 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 leonoisides A (8) and B (9) are reported for the first time from Ajuga salicifolia.

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Davis P. H. (1982), Flora of Turkey and the East Aegean Islands, Edinburgh, p. 42.


