Ionone, Iridoid and Phenylethanoid Glycosides from Ajuga salicifolia

Pınar Akbay\textsuperscript{a}, İlhan Çalış\textsuperscript{b}, Jörg Heilmann\textsuperscript{a}, and Otto Sticher\textsuperscript{a,*}

\textsuperscript{a} Department of Applied BioSciences, Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology (ETH) Zurich, CH-8057 Zürich. Fax: +41-1-6356882.
\textsuperscript{b} Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara

* Author for correspondence and reprint request

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From the aerial parts of \textit{Ajuga salicifolia} (L.) Schreber, a new ionone glycoside (3β-hydroxy-7,8-dihydro-4-oxo-β-ionol-9-0-β-D-glucopyranoside) was isolated, along with the known compounds, corchoionoside C, 8-O-acetylimioporoside, 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-acetylimioporoside in \textit{Ajuga} species. Ajugol, lavandulifolioside, leonoside A and B were isolated for the first time from \textit{Ajuga salicifolia}. The structures were elucidated by means of 1D-, 2D-NMR spectroscopy, and HR-MALDI mass spectrometry.

\textit{Key words: Ajuga salicifolia, Lamiaceae, Ionone Glycosides}

Introduction

In the flora of Turkey, the genus \textit{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 \textit{Ajuga} species, focusing mainly on the isolation of phytocystero-

oids and diterpenes and their antifeedant and insect growth inhibiting activities (Camps and Coll, 1993; Camps \textit{et al.}, 1981). Besides our investigations, there was only one report on \textit{Ajuga salicifolia} (L.) Schreber, concerning the isolation of a diterpene (Bozov \textit{et al.}, 1993), and in a chemotaxonomic investigation, the presence of catechin, flavonoid
glycosides, and iridoid glycosides in this plant were described (Litvinenko \textit{et al.}, 1970). Recently, we reported new and novel antileukemic and cytotoxic steroids from the aerial parts of \textit{Ajuga salici-

folia}, which was collected in Ankara, Turkey (Akbay \textit{et al.}, 2002a; Akbay \textit{et al.}, 2002b). Continuing our investigations, we isolated a new ionone glyco-

side, 3β-hydroxy-7,8-dihydro-4-oxo-β-ionol-9-0-β-D-glucopyranoside (1), together with the known
compounds, corchoionoside C (2), 8-O-acetylimi-

poroside (3), ajugol (4), harpagide (5), 8-O-acetyl-

harpagide (6), lavandulifolioside (7) and leono-
sides A (8) and B (9). This paper describes the isolation and structure elucidation of these com-
pounds and emphasizes on their chemotaxono-
mic significance.

Material and Methods

\textbf{General experimental procedures}

VLC: \textit{RP}-18 HL, 40–63 \textmu m (Chemie Uetikon), silica gel 60, 40–63 \textmu m (Merck). CC: silica gel 60, 40–63 \textmu m and 63–200 \textmu m (Merck), Sephadex-

LH-20. MPLC: Böchi 681 pump, 45 × 3.5 cm Böchi MPLC column packed with \textit{RP}-18 HL, 40–63 \textmu m. HPLC: Merck-Hitachi L-6200 pump connected to a Rheodyne 7125 Injector, a Merck-Hitachi L-

4000 UV detector, a Merck-Hitachi L-

2500 Chromato-

integrator, and a Knauer HPLC column (Spheri-
sorb S10 ODS 2, 10 \textmu m; 250 × 20 mm). TLC: Silica gel 60 \textit{F} \textsubscript{254} precoated aluminium plates (0.2 mm, Merck), \textit{RP}-18 \textit{F} \textsubscript{254} precoated plates (0.25 mm, Merck). Detection: 5% H\textsubscript{2}SO\textsubscript{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 spec-
trometer with 2,5-dihydroxybenzoic acid (DHB) as matrix. \textsuperscript{1}H, \textsuperscript{13}C NMR, DEPT-135, DEPT-90, \textsuperscript{1}H–, \textsuperscript{13}C HSQC, \textsuperscript{13}C,\textsuperscript{1}H–HMBC and \textsuperscript{1}H,\textsuperscript{1}H–NOESY experiments for compound 1 were measured on a Bruker DRX-

600 at 295 K (operating at 600.13 MHz for \textsuperscript{1}H, and
150.92 MHz for $^{13}$C). $^{1}$H- and $^{13}$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$_2$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 5%) by HPLC (RP-18, flow 5 ml/min, H$_2$O-ACN-MeOH (78:15:7)). 40 g of the methanol extract were subjected to VLC (RP-18, H$_2$O-MeOH 100:0 → 0:100) to give eight main fractions. Fr. 2 (356 mg), eluted with 5% MeOH, was subjected to MPLC (H$_2$O → 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$_2$Cl$_2$-MeOH-H$_2$O (90:10:1) → (40:60:4). The three fractions, rich in phenylethanoids, were further fractionated separately by CC (silica gel, CH$_2$Cl$_2$-MeOH-H$_2$O (90:10:1) → (60:40:4) affording 7 (240 mg), 8 (67 mg) and 9 (233 mg).

**Spectroscopic data**

$\beta$-hydroxy-7,8-dihydro-4-oxo-$\beta$-ionol-9-0-$\beta$-d-glucopyranoside (1) was obtained as a colorless amorphous powder, [α]$_{D}$ $^0$ = -30.0$ ^{\circ}$ (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 248 (2.80) nm; $^1$H NMR (CD$_2$OD, 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$_2$OD, 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): 411.184 [M + Na]$ ^{+}$ (calculated for C$_{19}$H$_{32}$O$_{8}$Na, 411.1985).

**Results and Discussion**

Sequential percolation of the powdered aerial parts of _A. salicifolia_ with petroleum ether, dichloromethane, ethyl acetate, methanol and methanol-water (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-
O-acetylmioroside (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-acetylmiorapagide (6) (Assaad and Lahoub, 1988; Takeda, et al., 1987), lavandulifoliside (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 δC 104.2 and δH 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 δH 1.31 and 1.26 as singlets indicated the presence of geminal dimethyl groups (H₃-11, and H₃-12). The resonances at δH 1.83 (s), and δH 1.32 (d, J = 6.3) were attributed to the vinyl methyl (H₃-13), and to H₃-10, respectively. The 13C NMR spectrum displayed two oxymethine (δC 70.4, 77.8; C-3, C-9, respectively) signals which were consistent with the resonances at δH 4.30 (dd, J = 5.5, 13.9; H-3), and δH 3.93 (m; H-9) in the 1H NMR spectrum. The chemical shifts of the quaternary carbons at δC 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-acetylmioroside (3) in Ajuga species. To date, 8-O-acetylmioroside 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-acetylmiorapagide (6) from Ajuga salicifolia. Lavandulifoliside (7) (Çals et al., 1992; Didry et al., 1999; Çals et al., 1991), and ajugol (4) (Àkcos 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 leonosides A (8) and B (9) are reported for the first time from Ajuga salicifolia.

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