Iridoid Glucosides from *Veronica pectinata* var. *glandulosa*

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Z. Naturforsch. **58c,** 481–484 (2003); received January 20/March 10, 2003

A new highly oxygenated iridoid glucoside, urphoside B (**1**) was isolated from the *Veronica pectinata* var. *glandulosa* together with seven known iridoid glucosides, aucubin, catalpol, veronicoside, catalposide, verproside, amphicoside and 6-*O*-veratroyl catalpol. The planar as well as the stereo structures of the isolated compounds were determined by means of extensive 1D- and 2D-NMR spectroscopy and confirmed by HR-Mass.

Key words: Iridoid Glucoside, Chlorinated Iridoid Glucoside, Urphoside B

Introduction

Iridoids represent a large group of cyclopentano[*c*]pyran monoterpenoids and are found as natural constituents in a large number of plant families, usually as glucosides. Iridoid glucosides have biogenetic and chemotaxonomic importance since they provide a structural link between terpenes and alkaloids (Bruneton, 1995). A bicyclic H-5/H-9 β,β-*cis*-fused cyclopentanopyran ring system is the most common structural feature of these substances, however several enantiomeric iridoids are also exist in nature suggesting their complex stereochemistry (Boros and Stermitz, 1990; Foderaro *et al*., 1992).

In a continuation of our studies on the secondary metabolites of *Veronica* species, we have studied the iridoid glucosides from *V. pectinata* var. *glandulosa*. The genus *Veronica* L. (Scrophulariaceae), which is widely distributed in Europe and Asia, especially in the Mediterranean area, is represented by 79 species in Turkey, 26 of which are endemic (Davis, 1978). Some of the *Veronica* species are used as diuretic and for wound healing in traditional Turkish medicine (Baytop, 1984). *Veronica* species have been known to be rich in iridoid glucosides. Mainly aucubin, catalpol, benzoic and cinnamic acid esters of catalpol, mussaenoside and mussaenosidic acid esters were reported nearly the investigated *Veronica* species (Lahloub, 1983; Taskova *et al*., 1999, Harput *et al*., 2002a). In our previous research on *Veronica* species, we have isolated 10 iridoid glucosides including a new iridoid glucoside, urphoside A together with 4 new and 4 known phenylethanoid glycosides (Harput *et al*., 2002a, b; Saracoglu *et al*., 2002). In addition, *Veronica* species have showed that the water-soluble portion of their MeOH extract suppresses nitric oxide production in lipopolysaccharide-stimulated mouse peritoneal macrophages, while the chloroform soluble portion of the MeOH extracts are cytotoxic against KB and B16 cells (Harput *et al*., 2002c).

Material and Methods

General experimental procedures

Optical rotations were measured on JASCO DIP 140 digital spectrometer using a sodium lamp operating at 589 nm. The UV spectra (λ_{max}) were recorded on Shimadzu UV-240 spectrometer. NMR measurements were performed on a JEOL JNM-A 500 spectrometer in methanol-d_4 with tetramethylsilane (TMS) as an internal standard. FAB-MS was recorded in a NBA matrix in the positive ion mode on a JEOL JMS-DX300 spectrometer. HR-mass spectroscopy was measured in ESI positive ion mode on Bruker Daltonics APEXS II with a 7 T magnet. TLC plates using Silica gel 60 F_{254} and RP-18 F_{254} were obtained from Merck (Darmstadt, Germany). Medium pressure liquid chromatography (MPLC) was per-

formed by a Lobar glass column packed with reversed-phase material (Merck, Lichroprep RP-18, 40–63 μm).

Plant material

Veronica pectinata L. var. *glandulosa* RIEK ex M.A. (Scrophulariaceae) was collected from Beypazarı, Turkey. A voucher specimen (HUEF 99015) has been deposited in the Herbarium of Faculty of Pharmacy, Hacettepe University.

Extraction and isolation

The air dried aerial parts of *V. pectinata* var. *glandulosa* (80 g) were extracted with MeOH at 40 °C for 12 h (×2, 2 l). The combined extracts were evaporated under vacuum to give 13 g of crude extract. The MeOH extract was dissolved in H_2O (0.1 l). H_2O-insoluble material was removed by filtration. The filtrate was fractionated with $CHCl_3$ (×4, 100 ml), and the water fraction was lyophilized to yield 10 g dry weight. The water fraction was subjected to polyamide column chromatography eluted with H_2O, followed by increasing concentrations of MeOH to give six fractions: Frs. A–F (Fr. A, 4.9 g; Fr. B, 0.45 g; Fr. C, 0.28 g; Fr. D, 0.27 g; Fr. E, 0.41 g; Fr. F, 0.88 g). Fractions A–D, rich in iridoid glucosides, were further applied to a series of column chromatographies. An aliquot of Fr. A (500 mg) was chromatographed over silica gel by stepwise elution with $CHCl_3$:MeOH:H_2O (90:10:1–60:40:4 v/v/v), and then rechromatographed over MPLC. Eluting with increasing concentrations of MeOH (20 → 50%) yielded compounds **2** (2.2 mg) and **3** (2.0 mg). Fr. B (200 mg) was subjected to silica gel column chromatography eluting with $CHCl_3$:MeOH:H_2O (95:5:0.5 → 50:50:5 v/v/v) and compounds **4** (2.0 mg), **7** (38 mg) and **8** (2.3 mg) were isolated. Silica gel column chromatography of Fr. C (200 mg), eluting with $CHCl_3$:MeOH:H_2O (95:5:0.5 → 70:30:3 v/v/v), was resulted to the isolation of compound **1** (2.3 mg). Fr. D (150 mg) was also subjected to silica gel column chromatography eluting with the same solvent system and compounds **5** (5.5 mg) and **6** (10.0 mg) were isolated. Fraction E, rich in phenylethanoid glycosides were previously studied and four phenylethanoid glycosides were isolated (Saracoglu *et al.*, 2002). Fraction F, rich in flavonoid glycosides, is still under investigation.

Results and Discussion

The methanol extract of *V. pectinata* var. *glandulosa* was suspended in water and partitioned with chloroform. The water-soluble portion of the extract was subjected to polyamide column chromatography to afford six main fractions. Repeated chromatography of the polyamide fractions resulted in the isolation of eight compounds (**1–8**) in pure form. Compound **1** was isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{23}$ −122°, c = 0.05; MeOH). The molecular formula of **1** was determined to be $C_{23}H_{29}O_{13}Cl$ by high resolution (HR)-ESI-MS. Its UV absorption [λ_{max} 210, 299 and 331 (sh)] as well as ^{1}H- and ^{13}C-NMR spectra indicated the presence of a nonconjugated enol-ether system and an aromatic acid (Table I). Inspection of the ^{13}C-NMR spectral data revealed the presence of one glucopyranosyl unit, one

Table I. ^{13}C and ^{1}H NMR (CD_3OD, 125 MHz for ^{13}C and 500 MHz for ^{1}H NMR spectral data and selected HMBC correlations for compound **1***.

C/H	DEPT	δ_C	δ_H	J (Hz)	HMBC (C→H)
1	CH	92.9	5.70 d	(3.7)	H-1′, H-3
3	CH	141.2	6.31 dd	(6.3/2.1)	H-1, H-4
4	CH	105.7	5.26 dd	(6.3/3.5)	H-3
5	CH	36.8	2.90 m		H-6
6	CH	85.3	5.10 dd	(12.2/7.3)	H-4, H-7
7	CH	69.7	4.20 d	(7.3)	H-5, H-9
8	C	81.0			H_2-10
9	CH	48.5	2.63 dd	(10.5/3.7)	
10	CH_2	63.6	4.10 d	(11.6)	H-7, H-9
			4.83 d	(11.6)	
1′	CH	99.6	4.66 d	(7.9)	H-1
2′	CH	74.8	3.20 t	(9.1)	H-1′, H-3′
3′	CH	78.0	3.36 t	(9.3)	H-2′
4′	CH	71.7	3.28†		
5′	CH	78.2	3.28†		
6′	CH_2	62.9	3.67 dd	(12.0/6.0)	
			3.83 dd	(12.0/2.1)	
1″	C	122.7			H-2″, H-5″
2″	CH	113.7	7.57 d	(1.8)	H-6″
3″	C	148.8			H-2″, H-5″, OCH_3
4″	C	153.3			H-2″, H-6″
5″	CH	116.1	6.89 d	(8.5)	H-6″
6″	CH	125.4	7.60 dd	(8.5/1.8)	H-2″
C=O	C	167.6			H-6, H-2″
OCH_3	CH_3	56.5	3.91 s		

* The ^{13}C and ^{1}H NMR assignments were based on HMQC, HMBC, COSY and NOESY experiments.
† Signal patterns unclear due to overlapping.

trisubstituted aromatic ring with one methoxy group, and a carbonyl function in addition to nine carbon signals belonging to the aglycone moiety. The gross structure was determined from ^{1}H-NMR and ^{1}H-^{1}H-shift correlation spectroscopy (^{1}H-^{1}H COSY) experiments. Construction of the iridoid skeleton started with the acetal proton at δ 5.70 (d, J = 3.7, H-1). This acetal proton was coupled to the methine proton at δ 2.63 (dd, J = 10.5/3.7 Hz, H-9), which in turn was coupled to the second methine proton at δ 2.90 (m, H-5). H-5 was further coupled to an olefinic proton at δ 5.26 (dd, J = 6.1/3.5 Hz), which in turn was coupled to another olefinic proton δ 6.31 (dd, J = 6.3/2.1 Hz). These vicinally coupled olefinic protons were ascribed to H-4 and H-3, respectively, confirming the presence of an iridoid moiety with nonconjugated enol-ether system. These assignments were confirmed by the $^2J_{CH}$ and $^3J_{CH}$ correlations in the heteronuclear multiple bond correlation (HMBC) spectrum of **1** (Table I).

In the other direction, the proton at C-5 was correlated to the oxymethine proton at δ 5.10 (dd, J = 12.2/7.3 Hz, H-6), which in turn was coupled to another methine proton at δ 4.20 (d, J = 7.3 Hz, H-7). The absence of any other homonuclear coupling observed for H-7 and H-9 indicated a totally substituted C-8 (δ 81.0, s). HMBC correlations between C-8/H_2-10, C-10/H-9 and C-10/H-7 showed the attachment of a hydroxymethyl group at C-8. The chemical shift value and coupling constant of C-10 (δ_C 63.6; δ_H 4.10, 4.83 d, J = 11.6 Hz) required a tertiary hydroxyl function at the C-8 position. ^{1}H- and ^{13}C-NMR spectral data of compound **1** showed good correlation with those urphoside A (Harput *et al*., 2002a). Main difference between compound **1** and urphoside A is the presence of carbon signal at δ 69.7 instead of δ 84.4 which representing C-7. This upfield shift (Δδ −14.7 ppm) suggested different substitution pattern for 7th position. Previously reported 7-Cl substituted iridoid glucosides, glutinoside, rehmaglutin B and D, in the case of 6 acylated condition, have showed δ 70.3, 70.1 and 67.5 for C-7 (Yoshikawa *et al*., 1986; Kitagawa *et al*., 1986). These data suggested chlorine substitution for C-7 in **1**. Its HR-ESI-MS spectrum also exhibited expected chlorine-characteristic sodiated ion $[M+Na]^+$ at *m/z* 571.1186 confirming the molecular formula $C_{23}H_{29}O_{13}ClNa$. Signals in the region of δ 3.20–4.66 with a characteristic anomeric proton resonance at δ 4.66 (d, J = 7.9 Hz), as well as HMBC and nuclear

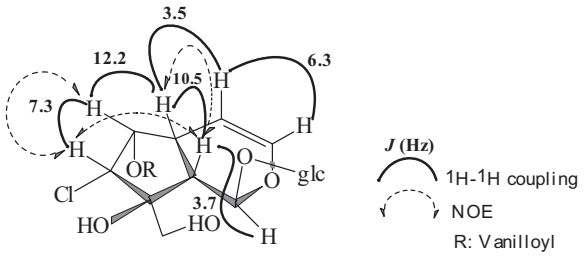

Fig. 1. Selected ^{1}H-^{1}H coupling constants and NOEs detected for compound **1**.

Overhouser effect (NOE) correlations between C-1/H-1′ and H-1/H1′ suggested that **1** contains a β-glucopyranoside unit at the C-1 position of the aglycone. The signals at δ 6.89–7.60 observed as an ABX system suggested the presence of trisubtituted acyl moiety. The signal at δ 3.91 (3H, s, OCH_3), which correlated C-3″ signal of the aromatic ring in HMBC spectrum, indicated the presence of a vanilloyl group. The HMBC correlation of the ester carbonyl (δ 167.6) to the H-6 signal of the aglycone and a downfield shift in H-6 proton signal (δ 5.10, dd) confirmed the attachment of the vanilloyl group to C-6.

To determine the relative stereochemistry of the chiral centers in **1**, NOE and ^{1}H-NMR decoupling experiments were performed (Fig. 1). NOE correlations between H-5, H-9, H-6 and H-7 suggested the same axial direction for these protons. Since H_2-10 protons correlated only with each other, the orientation of the hydroxymethyl group was assign to be α.

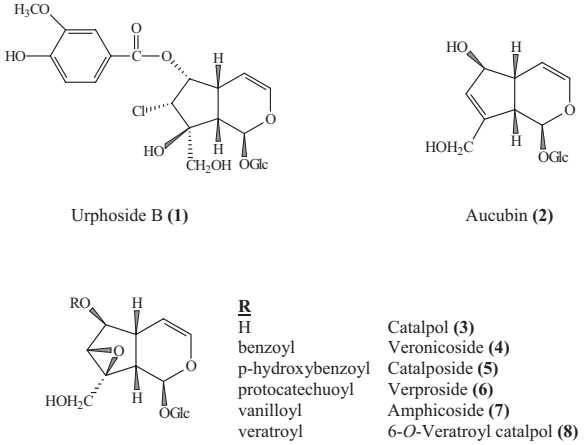

Fig. 2. Iridoid glucosides isolated from *Veronica pectinata* var. *glandulosa* **(1–8)**.

$^3J_{HH}$ coupling constants and dihedral angles of **1** also confirmed its stereochemical structure. To the best of our knowledge, compound **1** is described here for the first time and named urphoside B.

In addition to this compound, seven known iridoid glucosides, aucubin (**2**), catalpol (**3**) veronicoside (**4**), catalposide (**5**), verproside (**6**), amphicoside (**7**) and 6-*O*-veratroyl catalpol (**8**) were isolated (Fig. 2) and their structures were identified by the comparison of their spectral data with those reported in the literature (El-Naggar and Beal, 1980).

Acknowledgements

Authors are grateful to Prof. Z. Aytac of the Faculty of Sciences, Gazi University, Ankara, Turkey, for the authentication of the plant specimen. We also thank to Ms. S. Kato of the Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan, for recording NMR spectra and Mr. Tseng from Nihon Bruker Daltonics for measuring HR-ESI experiments.

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