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Acylated iridoid glucosides from *Veronica anagallis-aquatica*

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Abstract

Three new (**1**–**3**) and four known iridoid glucosides (**4**–**7**) as well as a known phenylethanoid glycoside (**8**) were isolated from the aerial parts of *Veronica anagallis-aquatica* and their structures were determined as 6′-*O*-benzoyl-8-epiloganic acid named aquaticoside A (**1**), 6′-*O*-*p*-hydroxybenzoyl-8-epiloganic acid named aquaticoside B (**2**), 6′-*O*-benzoyl-gardoside named aquaticoside C (**3**), veronicoside (**4**), catalposide (**5**), verproside (**6**), verminoside (**7**) and martynoside (**8**) on the basis of 1D and 2D NMR spectral analysis.

Keywords: *Veronica anagallis-aquatica*; Scrophulariaceae; Acylated iridoid glucosides; Aquaticosides A, B and C

1. Introduction

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). In addition, *V. anagallis-aquatica* are boiled in milk to obtain poultice which is applied to abdomen for abdominal pain and its aqueous extract has been used as bath to alleviate rheumatic pain in different places in Turkey (Fujita et al., 1995). *Veronica* species have been known to be rich in iridoid glucosides. Mainly aucubin, catalpol, benzoic and cinnamic acid esters of catalpol were reported from almost all the investigated *Veronica* species, however mussaenoside, mussenosidic acid esters and geniposidic acid are only found in a few species (Afifi-Yazar et al., 1981; Grayer-Barkmeijer, 1973; Taskova et al., 1998, 2002; Harput et al., 2002, 2003). As a part of our systematic studies on *Veronica* species, the present study was conducted on *V. anagallis-aquatica*. Previously, the presence of 7-*O*-*p*-hydroxybenzoyl-8-epiloganic acid, 7-*O*-*p*-hydroxybenzoyl-gardoside, several catalpol derivative iridoid glucosides and a bis-sesquiterpene, aquaticol have been described from the titled plant (Lahloub, 1992; Lahloub et al., 1993; Su et al., 1999). In a continuation of the studies on *Veronica* species, we report here the isolation and structure elucidation of three new aromatic esters of iridoid glucosides (**1**–**3**) and several catalpol derivatives from *V. anagalis-aquatica*.

2. Results and discussion

The methanolic extract of *V. anagallis-aquatica* was fractionated by polyamide column chromatography after removing chlorophylls. A combination of RP-MPLC and Si-gel column chromatographies led to the isolation of three new (**1**–**3**) and four known iridoid glucosides (**4**–**7**) in addition to a known phenylethanoid glycoside (**8**) (Fig. 1). The NMR data of compounds **4**–**8** were in excellent agreement with those reported for veronicoside (**4**), catalposide (**5**), verproside (**6**), verminoside (**7**) and martynoside (**8**) (El-Naggar and Beal, 1980; Calis et al., 1988; Ozipek et al., 2000).

Compound **1** was isolated as an amorphous powder with a negative optical rotation ($[\alpha]_D^{24} - 77.4°$, $c = 0.30$; MeOH). The molecular formula was determined as

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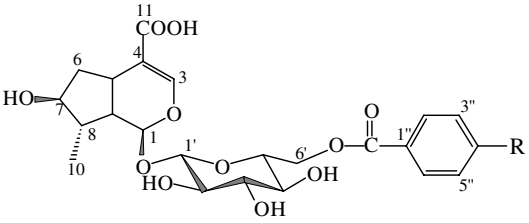
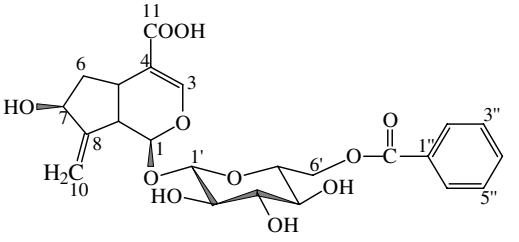
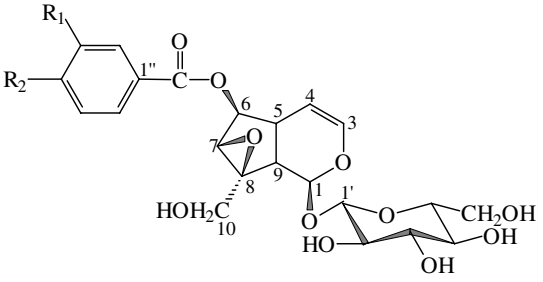


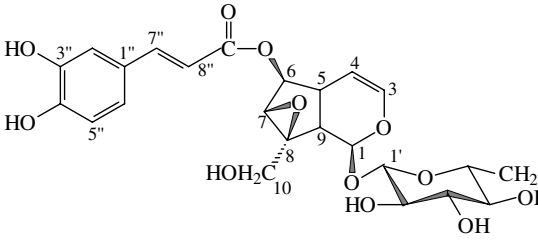
R	
H	(**1**) Aquaticoside A
OH	(**2**) Aquaticoside B



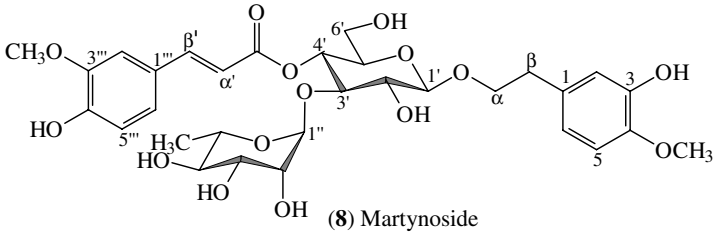
(**3**) Aquaticoside C



R_1	R_2	
H	H	(**4**) Veronicoside
H	OH	(**5**) Catalposide
OH	OH	(**6**) Verproside



(**7**) Verminoside



(**8**) Martynoside

Fig. 1. Isolated compounds from *V. anagallis-aquatica* (**1**–**8**).

$C_{23}H_{28}O_{11}$ based on its HR-ESI-FTMS. Its UV spectrum (MeOH) showed λ_{max} at 234, 299 and 331 nm indicating the presence of a conjugated enol–ether system and an aromatic acid moiety. The ^{13}C NMR spectrum (Table 1) showed 23 resonances, including six for a sugar unit, seven for a benzoyl moiety and the remaining ten for a 4-substituted iridoid aglycone moiety. The gross structure of compound **1** was determined from 1D and 2D NMR (COSY, HMQC, HMBC, NOESY) experiments. Its NMR data were very similar to those of 7-*O*-*p*-hydroxybenzoyl-8-epiloganic acid (Table 1) (Lahloub et al., 1993). The differences were the presence of a benzoyl group signals instead of a *p*-hydroxybenzoyl moiety and the appearance of a downfield shift of C-6′ (δ 64.90) as well as an upfield shift of C-5′ (δ 75.65) instead of the downfield shift of C-7 (δ 82.9) in 7-*O*-*p*-hydroxybenzoyl-8-epiloganic acid. These observations suggested the esterification of the glucose moiety at C-6′ (OH). The HMBC correlation of the H_2-6′ (δ 4.56 *dd* and 4.71 *dd*) to the ester carbonyl function (δ 167.81) also confirmed the attachment of the benzoyl group to C-6′ (OH). Therefore compound **1** was identified as 6′-*O*-benzoyl-8-epiloganic acid and the trivial name, aquaticoside A was proposed.

Compound **2** was also isolated as an amorphous powder with the negative optical rotation ($[\alpha]_D^{24} - 58.1°$, $c = 0.17$; MeOH) and formulated as $C_{23}H_{28}O_{12}$ based on its HR-ESI-FTMS. The ^1H and ^{13}C NMR spectra of compound **2** closely resembled those of **1** (Tables 1and 2). The only difference was the presence of the signals due to a *p*-hydroxybenzoyl group instead of a benzoyl moiety. From these data compound **2** was clearly determined as 6′-*O*-*p*-hydroxybenzoyl-8-epiloganic acid and named aquaticoside B.

The molecular formula of compound **3** ($[\alpha]_D^{24} + 11.5°$, $c = 0.16$; MeOH) was determined to be $C_{23}H_{26}O_{11}$ on the basis of the HR-ESI-FTMS. Due to a close relationship between compounds **1** and **3** ($\Delta m/z = 2$, lacking of two hydrogen), its spectral data was compared with those of **1**. The ^1H and ^{13}C NMR spectra of **3** exhibited expected signals for the β-glucopyranosyl unit, benzoyl moiety and 4-conjugated iridoid skeleton (Tables 1 and 2). The presence of an additional olefinic proton signals at δ 5.19 (*br.s*) and 5.25 (*t*, $J = 1.8$ Hz) pointed the occurrence of an exomethylene group in the structure of compound **3**. The NMR data of its iridoid part resembled those of 7-*O*-*p*-hydroxybenzoylgardoside (Lahloub et al., 1993) while the acyl part and esterification point were identical to those of **1** (Table 1). Concerning the HMBC correlations between H_2-6′ (δ 4.54 *dd* and 4.69 *dd*) and the ester carbonyl carbon (δ 167.80), compound **3** was determined as 6′-*O*-benzoyl-gardoside and the trivial name aquaticoside C was proposed.

The significance of the iridoid glycosides to clarify the evolution and taxonomy of the genus *Veronica* have been demonstrated previously (Grayer-Barkmeijer, 1973; Taskova et al., 2002). According to these results, aucubin, catalpol and catalpol esters of benzoic, *p*-hydroxybenzoic, protocatechuic, vanillic, veratroic and caffeic acids have showed a wide distribution in the genus *Veronica*, while 4-substitute iridoids, such as loganin, geniposidic acid, mussaenoside, 6′-*O*-menthiafoloylmussaenosidic acid and alpinoside have been found in a limited number. The catalpol derivatives isolated in this study have all been reported from *V. anagallis-aquatica* previously (Grayer-Barkmeijer, 1973; Lahloub et al., 1993; Taskova et al., 2002).

Since the biosynthesis of the iridoids in the Scrophulariaceae is *via* 8-epiloganin, the presence of this compound or the corresponding acid have been expected in *Veronica* species and it has already been reported from *V. alpina*, *V. bellidioides*, *V. officinalis*, *V. allionii* and *V. aphylla* (Grayer-Barkmeijer, 1973). On the other hand, 7-*O*-*p*-hydroxybenzoyl-8-epiloganic acid and the

Table 1
^{13}C NMR spectral data (CD_3OD) for compounds **1**–**3**, and model compounds (δ in ppm)

C	DEPT	**1** (125 MHz)	**2** (125 MHz)	7-*O*-*p*-Hydroxybenzoyl-8-epiloganic acid[a] (75.5 Hz)	**3** (125 MHz)	7-*O*-*p*-Hydroxybenzoyl-gardoside[a] (75.5 Hz)
Aglycone						
1	CH	95.99	95.92	95.8	96.77	96.3
3	CH	151.55	150.91	152.3	151.59	153.1
4	C	115.00	116.25	114.2	113.99	112.0
5	CH	32.16	32.24	31.7	32.52	33.7
6	CH_2	41.45	41.44	39.2	41.12	39.0
7	CH	78.99	79.08	82.9	73.92	78.6
8	CH[b]	45.44	45.41	43.3	152.92	149.0
9	CH	43.20	43.22	43.3	44.81	44.8
10	CH_3[c]	14.34	14.34	14.4	113.09	116.7
11	C	172.00	172.00	171.1	173.00	171.3
Glucose						
1′	CH	99.75	99.76	99.8	100.18	100.0
2′	CH	74.84	74.85	74.8	74.78	74.9
3′	CH	77.94	77.96	78.0	77.90	76.8
4′	CH	71.93	71.99	71.7	71.97	71.8
5′	CH	75.65	75.73	78.4	75.59	78.1
6′	CH_2	64.90	64.58	62.9	64.96	61.1
Acyl						
1″	C	131.38	131.23	126.6	131.42	122.5
2″	CH	130.58	132.86	132.7	130.59	132.8
3″	CH	129.65	116.25	116.2	129.66	116.2
4″	CH[d]	134.36	163.67	163.6	134.36	163.6
5″	CH	129.65	116.25	116.2	129.66	116.2
6″	CH	130.58	132.86	132.7	130.59	132.8
C=O	C	167.81	167.95	167.9	167.80	167.8

[a] Data from Lahloub et al. (1993)
[b] C for **3**.
[c] CH_2 for **3**.
[d] C for **2** and model compounds.

Table 2
^{1}H NMR spectral data for compounds **1**–**3** in CD_3OD (δ in ppm, 500 MHz)

H	**1**		**2**		**3**	
Aglycone						
1	5.27 *d*	(5.5)	5.28 *d*	(5.5)	5.18 *d*	(5.2)
3	7.37 *s*		7.34 *s*		7.36 *d*	(1.2)
5	3.05 *dt*	(7.6, 8.2)	3.06 *dt*	(7.3, 8.2)	3.19 *dt*	(7.0, 7.6)
6	1.72 *dt*	(7.0, 13.7)	1.78 *dt*	(7.0, 14)	1.83 *dt*	(6.4, 12.8)
	2.02 *m*		2.03 *m*		1.96 *m*	
7	3.73 *m*		3.76 *m*		4.31 *t*	(5.8)
8	2.07 *m*		2.07 *m*			
9	2.47 *dt*	(5.5, 8.2)	2.48 *dt*	(5.2, 8.2)	2.92 *ddd*	(1.8, 5.2, 7.3)
10	1.02 *d*	(7.3)	1.03 *d*	(7.3)	5.19 *br.s*	
					5.25 *t*	(1.8)
Glucose						
1′	4.76 *d*	(7.9)	4.74 *d*	(7.9)	4.74 *d*	(7.9)
2′	3.28 *dd*	(7.3, 9.5)	3.29 *dd*	(7.9, 9.1)	3.30 *dd*	(7.9, 9.1)
3′	3.46[a]		3.45[a]		3.46[a]	
4′	3.46[a]		3.45[a]		3.46[a]	
5′	3.67 *m*		3.64 *t*	(7.0)	3.66 *m*	
6′	4.56 *dd*	(6.4, 11.9)	4.49 *dd*	(6.4, 11.9)	4.54 *dd*	(6.4, 11.9)
	4.71 *dd*	(2.4, 11.9)	4.65 *dd*	(2.4, 11.9)	4.69 *dd*	(2.4, 11.6)
Acyl						
2″	8.08 *dd*	(1.2, 8.5)	7.93 *d*	(8.8)	8.07 *dd*	(1.2, 8.5)
3″	7.52 *t*	(7.8)	6.86 *d*	(8.8)	7.52 *t*	(7.9)
4″	7.64 *t*	(8.2)			7.65 *t*	(7.6)
5″	7.52 *t*	(7.8)	6.86 *d*	(8.8)	7.52 *t*	(7.9)
6″	8.08 *dd*	(1.2, 8.5)	7.93 *d*	(8.8)	8.07 *dd*	(1.2, 8.5)

[a] Signal patterns are unclear due to overlapping.

structurally similar compound 7-*O*-*p*-hydroxybenzoylgardoside were isolated from *V. anagallis-aquatica* growing in Egypt (Lahloub, 1992; Lahloub et al., 1993), however there was no report for the presence of loganic acid, gardoside and their esters in the same species from Bulgaria (Taskova et al., 2002). Although, the isolation of the esters of 8-epiloganic acid and gardoside from *V. anagallis-aquatica* in this study was recorded for the second time however, the esterification sites are different than those reported in the literature, which may be useful from the view point of chemotaxonomy of the genus *Veronica*.

3. Experimental

3.1. General

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 spectrophotometer. NMR measurements were performed on a JEOL JNM-A 500 spectrometer in methanol-d_4 with tetramethylsilane (TMS) as an internal standard (^{1}H 500 MHz; ^{13}C 125 MHz). HR-ESI-FTMS was measured on Bruker Daltonics, APEX III Instrument. Medium pressure liquid chromatography (MPLC) was performed on a Labomatic (18.5 mm × 352 mm) and Buchi (25 mm × 460 mm) glass column, packed with Lichroprep RP-18 (40–63 μm, Merck), using Lewa M5 peristaltic and Buchi B-684 pumps. Column chromatography was carried out on silica gel (Merck, Kieselgel 60, 60-230 mesh), polyamide (Fluka, 50–60 μm).

3.2. Plant material

Veronica anagallis-aquatica L. was collected from Beytepe Campus of Hacettepe University, Ankara, Turkey in June, 2002. Voucher specimen (HUEF 02021) is deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University.

3.3. Isolation of the compounds

The air dried aerial parts of *Veronica anagallis-aquatica* (453 g) were extracted with MeOH at 40 °C for 12 h (3×, 3 l). The MeOH solution was evaporated under vacuum to give MeOH extract (75 g). The MeOH extract was dissolved in 90% MeOH (0.2 l) and extracted with hexane (10×, 0.1 l) to remove chlorophyll. The aq. layer was conc. to give the crude extract (68 g). The extract was subjected to polyamide column chromatography eluting with H_2O, followed by increasing concentrations of MeOH to give six main fractions: Frs. A–F (Fr. A, 12.4 g; Fr. B, 29 g; Fr. C, 2.6 g; Fr. D, 2.2 g; Fr. E 3.2 g; Fr. F. 1.54 g). Fr. B, rich in iridoid glucosides, was applied to the medium pressure liquid

chromatography (MPLC) using reversed phase material with increasing concentrations of MeOH in H_2O (0→100% MeOH) to yield Frs. B_1–B_7. Frs. B_1–B_7 were further applied to a series of silica gel column chromatography eluting with $CHCl_3$/MeOH (99:1→65:35) and compound **6** (200.2 mg) from Fr. B_1 (250 mg), compound **5** (213,1 mg) and compound **2** (10.3 mg) from Fr. B_2 (500 mg), compound **7** (2.2 mg) from Fr. B_3 (120 mg), compound **3** (8.0 mg) from Fr. B_4 (35 mg), compound **1** (15.5 mg) from Fr. B_5 (64 mg), compound **8** (2.5 mg) from Fr. B_6 (31 mg) and compound **4** (59.5 mg) from Fr. B_7 (350 mg) were isolated in pure forms. Isolation studies of the other fractions of polyamide column have still been continuing.

3.4. Aquaticoside A (*1*)

Amorphous powder; $[\alpha]_D^{24}-77.4°$ ($c = 0.30$, MeOH); UV (MeOH) λ_{max} nm: 234, 299 and 331; ^{13}C NMR: see Table 1; ^{1}H NMR: see Table 2; HR-ESI-FTMS *m/z*: 503.1518 $[M+Na]^+$ (calcd. for $C_{23}H_{28}O_{11}Na$: 503.1524).

3.5. Aquaticoside B (*2*)

Amorphous powder; $[\alpha]_D^{24}-58.1°$ ($c = 0.17$, MeOH); UV (MeOH) λ_{max} nm: 234, 299 and 331; ^{13}C NMR: see Table 1; ^{1}H NMR: see Table 2; HR-ESI-FTMS *m/z* 519.1460 $[M+Na]^+$ (calcd. for $C_{23}H_{28}O_{12}Na$: 519.1473).

3.6. Aquaticoside C (*3*)

Amorphous powder; $[\alpha]_D^{24}+11.5°$ ($c = 0.16$, MeOH); UV (MeOH) λ_{max} nm: 236, 299 and 331; ^{13}C NMR: see Table 1; ^{1}H NMR: see Table 2; HR-ESI-FTMS *m/z* 501.1357 $[M+Na]^+$ (calcd. for $C_{23}H_{26}O_{11}Na$: 501.1367).

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References

Afifi-Yazar, F.U., Sticher, O., Uesato, S., Nagajima, K., Inouye, H., 1981. Ladroside, a new iridoid glucoside from *Veronica officinalis* L. (Scrophulariaceae) and the elucidation of the absolute configuration at C(8) of mussaenoside. Helv. Chim. Acta 64, 16–24.

Baytop, T., 1984. Therapy with Medicinal Plants in Turkey (Past and Present). Publications of Istanbul University, No: 3255, Istanbul, p. 423.

Calis, I., Saracoglu, I., Kitagawa, S., Nishibe, S., 1988. Phenylethanoid glycosides isolated from *Rhynchocorys stricta* (Scrophulariaceae). Doga, Tu. J. Med. Pharm. 12, 234–238.

Davis, P.H., 1978. In: Flora of Turkey and East Aegean Islands, vol. 6. University Press, Edinburgh. pp. 689–753.

El-Naggar, L.J., Beal, J.L., 1980. Iridoids. A review. J. Nat. Prod. 43, 649–707.

Fujita, T., Sezik, E., Tabata, M., Yesilada, E., Honda, G., Takeda, Y., Tanaka, T., Takaishi, Y., 1995. Traditional medicine in Turkey VII. Folk medicine in Middle and West Black Sea Regions. Econ. Bot. 49, 406–422.

Grayer-Barkmeijer, R.J., 1973. A chemotaxonomic study of *Veronica*: iridoid glucosides. Biochem. Syst. 1, 101–110.

Harput, U.S., Nagatsu, A., Ogihara, Y., Saracoglu, I., 2003. Iridoid glucosides from *Veronica pectinata* var. *glandulosa*. Z. Naturforsch. 58c, 481–484.

Harput, U.S., Saracoglu, I., Nagatsu, A., Ogihara, Y., 2002. Iridoid glucosides from *Veronica hederifolia*. Chem. Pharm. Bull. 50, 1106–1108.

Lahloub, M.-F., 1992. A new iridoid glucoside from *Veronica anagallis-aquatica* var. *anagalloides*. Alexandria J. Pharm. Sci. 6, 134–135.

Lahloub, M.-F., Zaghloul, M.G., Afifi, M.S., Sticher, O., 1993. Iridoid glucosides from *Veronica anagallis-aquatica*. Phytochemistry 33, 401–405.

Ozipek, M., Saracoglu, I., Calis, I., Kojima, K., Ogihara, Y., 2000. Catalpol derivative iridoids from the roots of *V. multifida*. Hacettepe Univ. J. Faculty Pharmacy 20, 1–6.

Su, B., Zhu, Q., Jia, Z., 1999. Aquaticol, a novel bis-sesquiterpene from *Veronica anagallis-aquatica*. Tetrahedron Lett. 40, 357–358.

Taskova, R., Handjieva, N., Peev, D., Popov, S., 1998. Iridoid glucosides from three *Veronica* species. Phytochemistry 49, 1323–1327.

Taskova, R., Peev, D., Handjieva, N., 2002. Iridoid glucosides of the genus *Veronica* s.l. and their systematic significance. Plant Syst. Evol. 231, 1–17.