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IRIDOID GLUCOSIDES FROM THREE *VERONICA* SPECIES

RILKA TASKOVA, NEDJALKA HANDJIEVA,*† DIMITAR PEEV and SIMEON POPOV†

Institute of Botany, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria; † Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

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Key Word Index—*Veronica bellidioides*, *V. alpina*, *V. kellererii*; Scrophulariaceae; iridoid glucosides; aromatic glucosides; new iridoid glycoside; 6′-*O*-menthiafoloylmussaenosidic acid.

Abstract—A new iridoid glucoside, 6′-*O*-menthiafoloylmussaenosidic acid, was isolated from the methanolic extract of *Veronica bellidioides* along with 11 known iridoid glucosides and the aromatic glucosides, picein and 4-*O*-glucopyranosylzingeron. The structure of the new iridoid was elucidated on the basis of its spectral data. Geniposidic acid and 4-*O*-glucopyranosylzingeron were found for the first time in the genus *Veronica* and verminoside in *V. bellidioides*. Nine known iridoids of which the major ones were mussaenoside and aucubin were found in *V. alpina*, and nine known iridoids with amphicoside as the major one were found *V. kellererii*.

INTRODUCTION

The genus *Veronica* L. is represented in Bulgaria by 38 species [1]. Iridoid glucosides, phenylethanoids, flavonoids, acetophenones, phenolic acids and alkaloids are found in different *Veronica* species [2–12]. *Veronica bellidioides* L. contains aucubin, catalpol, catalpol esters of benzoic and cinnamic acid derivatives [9–12], mussaenosidic acid esters, picein, acetophenone, ehrenoside [11], bellidoside and verbellidoside [9]. To our knowledge, no phytochemical studies on *V. alpina* L. and *V. kellererii* Deg. et Urum. have been reported.

The present paper reports on the iridoid glucosides in *V. bellidioides*, *V. alpina* and *V. kellererii*, which belong to section *Veronicastrum* Benth. *V. bellidioides* (2n = 4x = 36) and *V. alpina* (2n = 2x = 18) (subsection *Alpinae* Römpp) are very sparsely distributed in the high mountain regions of the country, while the endemic *V. kellererii* (2n = 2x = 16 (subsection *Diffusae* Römpp) is a rare plant found only on Mount Pirin and Mount Rila.

RESULTS AND DISCUSSION

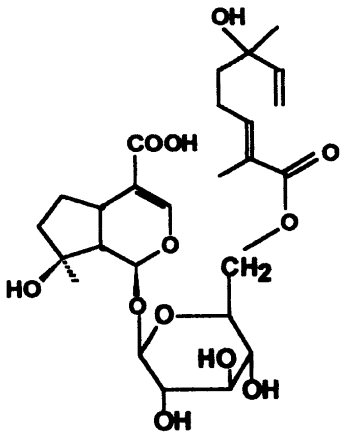
Dried aerial parts of the three species were extracted with methanol. The extracts were partitioned between water and dichloroethane. The water-soluble parts treated with charcoal and eluted with water–methanol, methanol–acetone and methanol–dichloroethane mixtures. Purification by column and low pressure reversed phase chromatography allowed the isolation of pure compounds.

Two samples of *V. bellidioides*, collected in different years, from the same natural population were studied. TLC and HPLC showed no differences between the samples. Fourteen pure compounds were isolated and identified as 11 known iridoid glucosides in addition to a new compound (**1**) and the known aromatic glucosides, picein (**15**) and 4-*O*-glucopyranosylzingeron (**16**). The last compound was found earlier in *Pinus contorta* [13], *P. sylvestris* [14] and *Vitis vinifera* [15] and now in the genus *Veronica*.

The major constituent was picein (**15**), followed by veronicoside (**4**) and catalpol (**3**). Minor components were minecoside (**10**), mussaenoside (**13**), catalposide (**5**), amphicoside (**7**), speedoside (**11**), welloside (**12**), verminoside (**9**), aucubin (**2**), geniposidic acid (**14**), 4-*O*-glucopyranosylzingeron (**16**) and the new compound **1**. The known isolated iridoid glucosides were identified by spectral data [2–6, 16, 17]. Verminoside (**9**) and geniposidic acid (**14**) were isolated for the first time from *V. bellidioides*. Speedoside (**11**) and welloside (**12**) have been reported in a congress abstract [12], but neither experimental nor spectral data were given and to our knowledge a full paper has not appeared. The ^{13}C NMR data for **11** and **12** are given in Table 1 and the ^{1}H NMR in the Experimental.

Compound **1** gave NMR spectra characteristic of a 4-substituted iridoid esterified with a monoterpenoid acid. The ^{13}C NMR spectrum (Table 1) contained 26 signals, of which 16 were assigned to a mussaenosidic acid moiety and the remaining 10 were consistent with

* Author to whom correspondence should be addressed.



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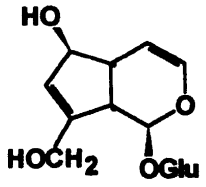

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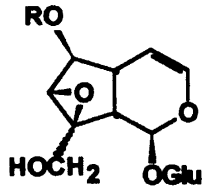
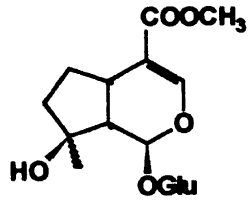


	R
3	H
4	benzoyl
5	p-hydroxybenzoyl
6	3,4-dihydroxybenzoyl
7	vanilloyl
8	3,4-dimetoxybenzoyl
9	caffeoyl
10	isoferuloyl
11	4-O-glucopyranosyl caffeoyl
12	4-O-glucopyranosyl feruloyl



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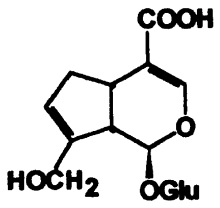

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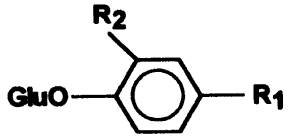
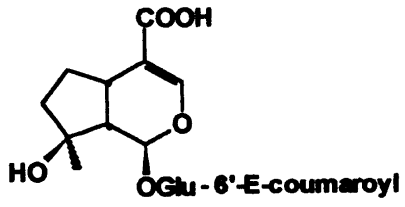


	R_1	R_2
15	$COCH_3$	H
16	$CH_2CH_2COCH_3$	OCH_3



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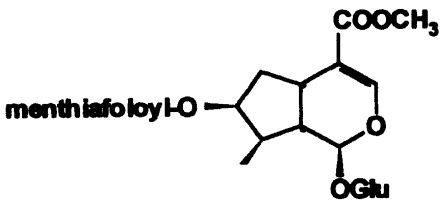

18

a menthiafoloyl substituent. All signals arising for the aglycone and glucosidic moiety were very similar to those of albidoside (**17**) [18], while the signals for the menthiafoloyl moiety were similar to those of jashelmoside A (**18**) [19]. The downfield shift of C-6′ (δ 63.6) and H_2-6′ (δ 4.41 and 4.34), and the upfield shift of C-5′ (δ 74.8) indicated esterification of the glucose moiety at C-6′ as ladroside from *V. officinalis* [5]. Therefore, the structure of compound **1** was identified as 6′-*O*-menthiafoloylmussaenosidic acid.

Table 1. ^{13}C NMR spectral data of 6′*O*-menthiafoloylmussaenosidic acid (**1**) (62.9 MHz, D_2O), speedoside (**11**) (62.9 MHz, D_2O–CD_3OD, 1:1), welloside (**12**) (62.9 MHz, D_2O), and the model compounds albidoside (**17**) and jashemsloside A (**18**) (CD_3OD)

C	**1**	**17**	**18**	**11**	**12**
Aglycone moiety					
1	95.1 *d*	95.6	97.3	96.4 *d*	95.2 *d*
3	148.8 *d*	151.4	152.5	142.7 *d*	142.3 *d*
4	116.5 *s*	114.1	113.1	104.1 *d*	102.8 *d*
5	32.5 *d*	33.3	32.5	37.1 *d*	36.7 *d*
6	30.0 *t*	30.9	40.4	81.6 *d*	81.4 *d*
7	39.8 *t*	40.1	78.6	61.6 *d*	60.5 *d*
8	81.1 *s*	81.0	40.9	68.0 *s*	67.2 *s*
9	51.5 *d*	52.2	47.1	43.2 *d*	43.0 *d*
10	24.4 *q*	25.0	13.7	61.6 *t*	61.0 *t*
11	170.9 *s*	169.0	169.2		
OMe			51.8		
Sugar moiety					
1	98.8 *d*	99.8	100.1	102.5 *d*	101.7 *d*
2	73.6 *d*	74.8	74.6	74.5 *d*	74.5 *d*
3	76.4 *d*	75.7	77.8	77.4* *d*	77.3* *d*
4	70.8 *d*	71.7	71.5	71.2 *d*	71.3 *d*
5	74.8 *d*	75.7	78.2	77.9* *d*	78.1* *d*
6	63.9 *t*	64.2	62.7	62.4 *t*	62.4 *t*
1				100.5 *d*	99.6 *d*
2				74.5 *d*	74.4 *d*
3				77.1* *d*	77.3* *d*
4				71.0 *d*	70.9 *d*
5				77.9* *d*	77.8* *d*
6				62.2 *t*	62.1 *t*
Menthiafoloyl moiety					
1	170.9 *s*		169.1		
2	127.9 *s*		128.7		
3	145.6 *d*		144.0		
4	24.2 *t*		27.5		
5	40.7 *t*		41.7		
6	74.4 *s*		73.5		
7	144.6 *d*		145.8		
8	113.2 *t*		112.5		
9	12.5 *q*		12.5		
10	27.0 *q*		24.5		
Aromatic moiety					
1		127.0		131.0 *s*	130.2 *s*
2		131.2		117.1 *d*	112.4 *d*
3		116.9		148.7 *s*	150.4 *s*
4		161.8		147.4 *s*	149.7 *s*
5		116.9		117.9 *d*	116.5 *d*
6		131.2		123.2 *d*	123.9 *d*
α		114.9		117.1 *d*	116.9 *d*
β		146.9		147.4 *d*	147.0 *d*
C═O		169.0		168.9 *s*	169.1 *s*
OMe					56.8 *q*

The major constituents of *V. alpina* isolated were mussaenoside (**13**) and aucubin (**2**), followed by minecoside (**10**) and catalpol (**3**) together with little veronicoside (**4**), amphicoside (**7**), catalposide (**5**), verminoside (**9**) and speedoside (**11**). The lowest iridoid concentration was found in this sample.

Two samples of *V. kellererii* collected at different sea levels (1900 and 2200 m s. m.), were studied. TLC and HPLC showed no differences between the samples. The major constituent amphicoside (**7**) was accompanied by low concentrations of verproside (**6**), 6-*O*-veratroylcatalpol (**8**), veronicoside (**4**), catalpol (**3**) and traces of catalposide (**5**), verminoside (**9**), aucubin (**2**) and geniposidic acid (**14**).

Veronica bellidioides, *V. alpina* and *V. kellererii* are easily distinguished by chemical means on the basis of

the high concentrations of picein, veronicoside and catalpol in *V. bellidioides*, of mussaenoside and aucubin in *V. alpina* and amphicoside in *V. kellererii*.

EXPERIMENTAL

General procedure

^{1}H NMR (250 MHz) and ^{13}C NMR (62.9 MHz) in D_2O, CD_3OD. Reverse phase LPLC: Merck Lobar C-18 column size C, H_2O–MeOH mixts were used as eluents. Plant materials were collected at florescence: *Veronica bellidioides* L. on Mount Rila (peak of Mussala, 2920 m s. m., 1995 and 1996), *V. alpina* on Mount Rila (Marichini ezera, 2700 m s. m., 1996) and *V. kellererii* on Mount Pirin (Djamdjievi scali, 1900 m s. m. and under the peak of Vihren, 2200 m s. m., 1996) by R. Taskova and Dr D. Peev. The voucher specimens were deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences.

Isolation of glucosides

Dry above-ground parts were extracted twice with MeOH and the concd extract partitioned between $Cl(CH_2)_2Cl$–H_2O. The aq. phase was concd, treated with charcoal and eluted with H_2O–MeOH mixtures, MeOH–Me_2CO (1:1) and MeOH–$Cl(CH_2)_2Cl$ (1:1).

Veronica bellidioides. Dry above-ground parts 16 g and 30 g. The MeOH extracts (1.8 g and 8.8 g) were combined and the MeOH fr. (1.2 g) separated on silica gel eluted with $CHCl_3$–MeOH–H_2O (60:15:4, 60:22:4) to give veronicoside (**4**, fr. 11, 21 mg), mussaenoside (**13**, frs 18–22, 59 mg), catalposide (**5**, frs 23–26, 29 mg), and a mixture of aucubin and catalpol (**2**+**3**, frs 59–61, 25 mg). Fractions 12–16 (175 mg), after additional separation using a B-size Lobar column and eluting with H_2O–MeOH (4:6–3:7), gave picein (**13**, frs 2–3, 73 mg) and amphicoside (**7**, frs 5–6, 7 mg). Fractions 46–50 (40 mg) were purified on a B-size column with MeOH–H_2O (1:1–7:3) to give 6′-*O*-menthiafoloylmussaenosidic acid (**1**, frs 25–37, 12 mg). The MeOH–Me_2CO fr. (1 g) was chromatographed on silica gel to give verminoside (**9**, frs 36–38, 9 mg). The MeOH–$Cl(CH_2)_2Cl$ fr. (2.5 g) was separated on silica gel. Additional separation on silica gel followed by purification on a Lobar column B of (a) frs 3–4 (51 mg) yielded 4-*O*-glucopyranosylzingeron (**16**, frs 5–8, 18 mg), (b) frs 6–7 (600 mg) gave minecoside (**10**, frs 41–49, 59 mg), (c) fr. 12 gave welloside (**12**, frs 34–39, 19 mg) and speedoside (**11**, frs 36–43, 68 mg), and (d) fr. 13 (235 mg) gave geniposidic acid (**14**, frs 33–43, 32 mg).

V. alpina. Dry above-ground parts 18 g. The MeOH extract (5.2 g) gave the MeOH fr. (0.35 g) and a MeOH–Me_2CO fr. (0.36 g) which yielded veronicoside (**4**, frs 18–20, 6 mg), mussaenoside (**13**, frs 24–30, 78 mg), catalposide (**5**, frs 35–40, 22 mg) and aucubin and catalpol (**2**+**3**, frs 42–44, 53 mg). The MeOH–$Cl(CH_2)_2Cl$ fr. (1 g) afforded **4** (frs 11–13, 17 mg), **5** (frs 25–26, 25 mg), **9** (frs 29–30, 12 mg) and **11** (frs 42–44, 8 mg). Fractions 17–21 (99 mg) afforded, after separation on a Lobar column, **7** (frs 15–17, 5 mg), **5** (frs 18–19, 5 mg), **10** (frs 25–31, 50 mg) and **4** (frs 33–37, 3 mg).

V. kellererii. Dry above-ground parts 15 g and 31 g. The MeOH extracts (1.9 g and 5.8 g) were combined and separated as described above. The 50% MeOH fr. (0.6 g) yielded **7** (fr. 2, 28 mg), **6** (fr. 3, 23 mg) and **2**+**3** (fr. 6, 46 mg). Additional separation of frs 12–16 (97 mg) on a Lobar column gave **14** (frs 22–23, 28 mg). The MeOH fr. (0.4 g) gave **8** (fr. 5, 4 mg), **4** (fr. 6, 4 mg), **7** (frs 8–9, 26 mg), **5** (fr. 35, 130 mg) and **6** (frs 22–34, 161 mg). The MeOH–$Cl(CH_2)_2Cl$ fr. (1.8 g) yielded **8** (19 mg), **7** (69 mg), **5** (6 mg) and **9** (6 mg).

6″-O-*Menthiafoloylmussaenosidic acid* (**1**). ^{1}H NMR (250 MHz, D_2O): 7.14 (*s*, H-3), 6.76 (*tq*, $J = 7.5$ and 1.0 Hz, H-3″), 5.86 (*dd*, $J = 17.5$ and 10.9 Hz, H-7″), 5.15 (*d*, $J = 2.3$ Hz, H-1), 5.11 (*dd*, $J = 17.6$ and 1.0 Hz, H-8″ *trans*), 5.05 (*dd*, $J = 11$ and 1.0 Hz, H-8″ *cis*), 4.72 (*d*, $J = 7.9$ Hz, H-1′), 4.40 (*dd*, $J = 12$ and 5.8 Hz, H-6′$_a$), 4.34 (*dd*, $J = 12$ and 2.7 Hz, H-6′$_b$), 3.61 (*m*, H-5′, 3.42 (*m*, H-2′ and H-3′), 3.26 (*t*, $J = 8.8$ and 8.2 Hz, H-2′), 3.04 (*ddd*, H-5), 2.10–2.20 (H-6$_b$, H-9, H_2-4″), 1.63 (H-6$_a$, H-7$_a$, H_2-5″), 1.30 (H-7$_b$), 1.73 (*s*, H-9″), 1.21 (*s*, H-10 and H-10″); ^{13}C NMR: Table 1.

Speedoside (**11**). ^{1}H NMR (250 MHz, D_2O): 7.47 (*d*, $J = 15.8$, β), 6.38 (*d*, $J = 1.6$, H-3), 6.22 (*d*, $J = 15.8$, α), 4.29 (*d*, $J = 13.3$, H$_a$-10), 3.90 (*d*, $J = 13.3$, H$_b$-10), 3.56 (*d*, $J = 1.7$, H-7), 2.68 (*m*, H-5, H-9); ^{13}C NMR: Table 1.

Welloside (**12**). ^{1}H NMR (250 MHz, D_2O): 7.69 (*d*, $J = 15.9$, β), 6.39 (*d*, $J = 1.6$, H-3), 6.50 (*d*, $J = 15.9$, α), 6.39 (*d*, $J = 1.6$, H-3), 5.15 (*d*, $J = 9.2$, H-1), 4.20 (*d*, $J = 13.3$, H-10$_a$), 3.90 (*s*, OMe), 3.85 (*d*, $J = 13.3$, H-10$_b$), 3.72 (*d*, $J = 1$, H-7), 2.68 (*m*, H-5, H-9); ^{13}C NMR: Table 1.

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REFERENCES

1. Peev, D., *Genus Veronica L. in Flora Rep. Bulgaricae*, Vol. 10, ed. S. I. Kozhuharov and B. A. Kuzmanov. Academic Press, Sofia, 1995, p. 145.
2. Sticher, O. and Affifi-Yazar, Ü., *Helv. Chem. Acta*, 1979, **62**, 530.
3. Sticher, O. and Affifi-Yazar, Ü., *Helv. Chem. Acta*, 1979, **62**, 535.
4. Affifi-Yazar, Ü. and Sticher, O., *Helv. Chem. Acta*, 1980, **63**, 1905.
5. Affifi-Yazar, Ü. and Sticher, O., *Helv. Chem. Acta*, 1981, **64**, 16.
6. Lahloub, M.-F., Saad, H.-E. A. and Halim, A. F., *Mans. J. Pharm. Sci*, 1991, **7**, 390.
7. Lahloub, M.-F., *Mans. J. Pharm. Sci*, 1992, **8**, 56.

8. Lahloub, M.-F., Zaghlaul, M. G., Affifi, M. S. and Sticher, O., *Phytochemistry*, 1993, **33**, 401.
9. Lahloub, M.-F. and Sticher, O., Abstract, Gesamtkongress der Pharmazentischen Wissenschaften, Muenchen, Deutschland, April 17–20, 1983, p. 122.
10. Lahloub, M.-F., Calis, I. and Sticher, O., *Pharm. Tijdschr. Belg.*, 1984, **61**, 378.
11. Lahloub, M.-F., Gross, G. A., Sticher, O., Winkler, T. and Schulten, H. R., *Planta Med.*, 1986, **52**, 352.
12. Lahloub, M.-F. and Sticher, O., *Planta Med.*, 1989, **55**, 626.
13. Higushi, R. and Donnelly, M. X., *Phytochemistry*, 1977, **16**, 1587.
14. Andersson, R. and Lundren, L., *Phytochemistry*, 1988, **27**, 559.
15. Roscher, R. and Winterhalter, P., *J. Agric. Food Chem.*, 1993, **41**, 1452.
16. El-Naggar, L. J. and Beal, J. L., *J. Nat. Prod.*, 1980, **43**, 649.
17. Boros, Ch. A. and Stermitz, F. R., *J. Nat. Prod.*, 1990, **53**, 1055.
18. Calis, I., Ersöz, Tayfun, Saracoglu, I. and Sticher, O., *Phytochemistry*, 1993, **32**, 1213.
19. Damtoft, S., Franzyk, H. and Jensen, S. R., *Phytochemistry*, 1997, **45**, 743.