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8-EPILOGANIN, AN IRIDOID GLUCOSIDE FROM *ODONTITES VERNA**

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Key Word Index—*Odontites verna* subsp. *serotina*; Scrophulariaceae; iridoid glucosides; 8-epiloganin; ^{1}H NMR and ^{13}C NMR spectra.

Abstract—*Odontites verna* subsp. *serotina* contains besides odontoside, aucubin, mussaenoside, shanzhiside methyl ester and catalpol, a new iridoid glucoside, 8-epiloganin.

INTRODUCTION

In the course of our research on iridoids in the flora of Italy, we reported that the iridoid-containing fraction of *Odontites verna* subsp. *serotina* (Dumort) (Scrophulariaceae) [1] contained odontoside, aucubin, shanzhiside methyl ester, mussaenoside, catalpol and a new iridoid [2, 3]. In this paper we show that the new iridoid is 8-epiloganin.

RESULTS AND DISCUSSION

8-Epiloganin (**1**) was obtained as an amorphous compound with molecular formula $C_{17}H_{26}O_{10}$ and $[\alpha]_D$ −100.9°. On acid hydrolysis it gave glucose (1 mol) together with black products due to the decomposition of the aglycone. Its ^{1}H NMR spectrum showed the presence of an iridoid structure in which both C-10 and C-11 carbons were present: C-10 (δ 1.05, *d*, J = 7.0 Hz), methyl geminal with a proton; C-11, carbomethoxy group (δ 3.76, *s*) as confirmed by the signal of the olefinic H-3 which was highly deshielded (δ 7.42). Two hemiacetalic proton signals at δ 5.59 (*d*, J = 3.0 Hz) and 4.81 (*d*, J = 7.0 Hz) were assigned to H-1 and the anomeric proton of a β-D-glucopyranosyl moiety respectively. The proton noise decoupled ^{13}C NMR spectrum of **1** (Table 1) showed 17 lines which are assigned using the SFORD technique and utilizing the known chemical shift rules [4, 5] and ^{13}C NMR data of model compounds [6–10]. The assignments were in agreement with the information obtained from the ^{1}H NMR spectrum. In addition, the ^{13}C NMR spectrum showed that one secondary alcoholic function was present in the cyclopentane ring of **1** (δ 79.0, *d*). As expected, the acetylation of **1** in mild conditions gave only the pentaacetate **2**.

Enzymatic hydrolysis of **1** with β-glucosidase demonstrated that a β-D-glucose unit was linked at C-1 and afforded an aglycone which on immediate treatment with MeOH and strong acidic resin gave the two monomethyl acetals **3** and **4** (10:1). The isolation of these two cyclic acetals proved that the ring junction must be *cis* [11]. The most abundant product (**3**) had a negative M_D value (−85°) and on the basis of the criterion used for iridoid aglycones [12, 13], it was assigned the structure of the β-methyl aglycone of **1**, while the structure of the α-methyl aglycone was assigned to **4**, which had a positive M_D value (+27°). The comparison of the M_D values of **1** (−394°) with those of **3** and **4** demonstrated that the C-1 centre of **1**, like all known iridoids, had the *S*-configuration.

In the ^{1}H NMR spectrum of **3** (Experimental) the resonances of H-3, H-5, H-9, and H-1 were easily detected and their respective coupling constants were measured by

Table 1. ^{13}C NMR spectral data for **1**, **3**, **5**, **6** (20 MHz, TMS ($CDCl_3$) and dioxane (D_2O) as internal standard)

Carbon No.	**1** (D_2O)	**6** (D_2O)	**3** ($CDCl_3$)	**5** ($CDCl_3$)
1	96.5	97.5	100.9	100.7
3	152.2	151.7	151.3	151.3
4	114.0	113.9	112.2	111.6
5	29.4	30.7	30.7	31.3
6	39.6	41.3	40.9	38.3
7	79.0	75.0	78.8	81.0
8	43.5	41.0	44.4	42.1
9	41.8	45.8	42.1	42.1
10	14.0	12.9	14.1	14.0
11	170.7	170.7	171.2	170.9
OMe	52.6	52.6	51.1	51.2
1′	99.1	99.5	56.0	56.2
2′	73.5	73.5		MeCO
3′	76.6	76.6		21.3
4′	70.5	70.5		170.9
5′	77.1	77.2		
6′	61.6	61.6		

* Part 2 in the series "Iridoids in the Flora of Italy". For Part 1 see ref. [2]; the name vernoside was originally used for this compound, see ref. [3].

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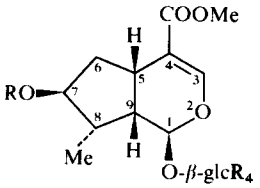
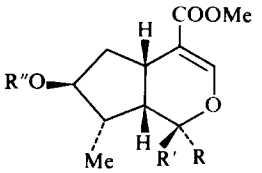
decoupling experiments. The multiplet at δ 3.87 was due to a proton (H-7) geminal with a hydroxyl group and its irradiation demonstrated that the secondary alcoholic function present in the cyclopentane ring must be located at C-7. In fact, this experiment simplified the resonance of H-8 but did not modify that of H-5. At the same time the signal pattern in the region δ 2.30–1.60, relative to the 2H-6 (AB part of an ABMX), was transformed into an eight-line system (AB part of an ABM) in which the coupling constants $J_{6\alpha,7}$, $J_{6\beta,7}$ were eliminated. The multiplet of H-8 was superimposed on the low field region of this system and, during the irradiation of H-7, appeared as a broad pentuplet with $J_{8,10}$ very similar to $J_{8,9}$. Proton noise decoupled and SFORD ^{13}C NMR spectra of **3** (Table 1) confirmed the assignments of the aglycone carbon in the ^{1}H NMR spectrum of **1**, except for C-8 and C-9. These carbons were differentiated between by comparison of the ^{13}C NMR data of **3** with those of its monoacetate **5** (Table 1): the acetylation of an alcoholic hydroxyl group results in a downfield α-shift and upfield β-shifts [4, 5]. The comparison between ^{13}C NMR spectra of **3** and **5** showed that the C-7 was deshielded by 2.2 ppm (α-effect) and the C-6 was shielded by 2.6 ppm (β-effect); the signals of C-8 and C-9 which were separate in the spectrum of **3** (δ 42.1 and 44.4) were superimposed (δ 42.1) in the spectrum of **5**. These data indicated that the signal at δ 44.4 in the spectrum of **3** had been shielded by 2.3 ppm and was attributed to C-8 (β-acetylation shift) while the signal at δ 42.1 had not been affected by the acetylation and was attributed to C-9. By analogy, the signals at δ 41.8 and 43.5 in the spectrum of **1** were attributed to C-9 and C-8 respectively. It was noted that the C-1 resonance absorbs at δ 100.9 in **3** (δ 96.5 in **1**) due to the replacement of the *O*-β-D-glucosyl residue with an *O*-methyl group. This variation was analogous to that observed for C-1 in the couple β,β-trehalose (δ 100.7)/methyl-β-D-glucopyranoside (δ 104.2) and it was the first time in which it was possible to verify this analogy between the acetalic carbon of the dihydropyrane ring of iridoids and the acetalic carbon of the tetrahydropyrane ring of glucopyranose.

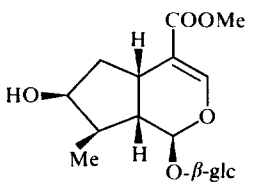
The reported data indicated that **1** was an isomer of loganin (**6**). The similarity between **1** and **6** was evident from their ^{1}H NMR spectra (Experimental) which only differed significantly in the signals of H-7 (δ 4.0–3.7 in **1**, 3.87 in **3**, 4.14 in **6**) and H-9 (δ 2.73 in **1**, 2.3–1.4 in **6**). These data suggested **1** had a different configuration at C-7 and/or C-8 to **6**. The absolute configuration of the C-7 centre of **1** was shown to be the same as in **6** (i.e. *S*) by application of Horeau's method on the methyl acetal **3**. Therefore, because the configurations of the C-1, C-5, C-7 and C-9 centres of **1** and **6** are alike, the C-8 centre of **1** must have the opposite configuration to the C-8 centre of **6**. The configuration of the methyl group of C-10 in **1** was shown to be the opposite of that in **6** by comparison of the ^{13}C NMR spectra (Table 1) with those of model compounds. Thus the 8-epimeric pairs pulchelloside I (**7**)/pulchelloside (II) (**8**) [14] and plantarenaloside (**9**) [15–17]/stansioside (**10**) [18] showed a shift difference of +4.9 ppm and +5.0 ppm, respectively for C-9. The corresponding difference in the pair **1**/**6** was found to be +4.0 ppm (Table 1). In addition, ^{13}C NMR data of **1** and **6** were significantly in accord with those of **7** and **8** which have the same relative configurations at C-7 and C-8. In fact, the shielding effects observed for C-7 (δ 2.6) and C-8 (δ 2.3) in the couple **7**/**8** were comparable to those found in the couple **1**/**6** (δ 4.0 for C-7 and δ 2.5 for C-8). Also, very



1 R = H
2 R = Ac



3 R = R" = H, R' = OMe
4 R = OMe, R' = R" = H
5 R = H, R' = OMe, R" = Ac



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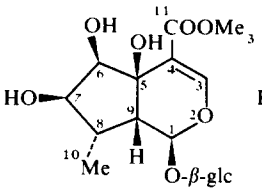

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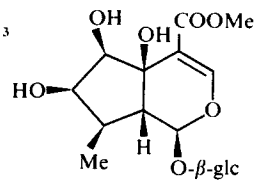

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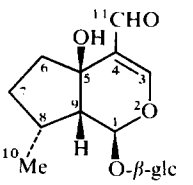

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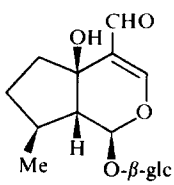

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similar were the chemical shifts of C-10: δ 13.5 in **7**, 14.0 in **1**; δ 12.5 in **8**, 12.9 in **6**: shielding effect on C-10 in the couple **7/8**, 1.0 ppm and in the couple **1/6** of 1.1 ppm. It was also found that there was good agreement between the chemical shifts of C-7 and C-8 of **1** and **6** and the corresponding shifts of *trans*- and *cis*-2-methyl-cyclopentanols [19]: δ 79.0 (C-7), 43.5 (C-8) in **1**; 79.8 (C-1), 42.2 (C-2) in *trans*-2-methyl-cyclopentanol; 75.0 (C-7), 41.0 (C-8) in **6**; 75.2 (C-1), 39.8 (C-2) in *cis*-2-methyl-cyclopentanol. It was noted that in *trans*-2-methyl-cyclopentanol the methyl group resonates at δ 18.3 (13.7 in *cis*) while in **1** and in **7** C-10 resonates at $\delta \sim 13$. This was evidently due to a diaxial interaction between the α-methyl groups at C-8 and the C-1 carbon. All the reported data were consistent with **1** having the structure and configuration of 8-epiloganin.

We are now examining the five iridoids which were more polar than aucubin because we think that when the structures of all the iridoids of *O. verna* are known, it will be of interest to examine their biogenetic relationships.

EXPERIMENTAL

PC: Schleicher & Schüll No. 2043b Mgl. Spray reagents: 2 N H_2SO_4, vanillin (2 g vanillin, 4 ml conc HCl, 100 ml MeOH), benzidine (0.5 g benzidine, 20 ml HOAc, 80 ml EtOH) and resorcin (5 g resorcin, 4 ml conc H_2SO_4, 296 ml EtOH). Evapns of volatile material were performed under red. pres.

Isolation of iridoid-containing fraction. O. verna subsp. *serotina* was collected in October 1979 in Villa Doria-Pamphilj (Roma, Italy) when it was in flower. Voucher specimens of the plant were identified by Dr. Anna Francesconi, Istituto di Botanica dell'Università di Roma. Fresh aerial parts of the plant (4 kg) were extracted with 90% EtOH (8 l. × 2) at room temp. for 3 days. PC in *n*-BuOH–HOAc–H_2O (63:10:27) showed the presence of 11 iridoids with R_f values of 0.69 (odontoside), 0.58 (mussaenoside), 0.56 [8-epiloganin (**1**)], 0.40 (shanzhiside methyl ester), 0.30 (unknown I), 0.25 (aucubin), 0.22 (catalpol), 0.11 (unknown II), 0.09 (unknown III), 0.07 (unknown IV), 0.05 (unknown V). The ethanolic extract was concd to an aq. suspension which was treated with decolorizing charcoal (1 kg). The resulting suspension was stratified on a Gooch funnel (20 cm ϕ) containing a layer of charcoal (100 g). Monosaccharides were eluted with H_2O (30 l.), disaccharides with 5% EtOH (5 l.), aucubin and most of the iridoids with the lowest R_f values with 30% EtOH (25 l., fraction A), aucubin and most of the iridoids with the highest R_f values with 50% EtOH (8 l., fraction B) and 80% EtOH (8 l., fraction C). Fraction C (6.5 g) was chromatographed on cellulose (300 g) in *n*-BuOH satd with H_2O (BH) to give odontoside (1 g), mussaenoside and **1** (250 mg), aucubin, shanzhiside methyl ester and I (0.8 g), and aucubin (0.8 g). Fraction B (9.5 g), was chromatographed on cellulose (400 g) in BH, to give odontoside (0.5 g), mussaenoside and **1** (1 g), shanzhiside methyl ester (3 g), aucubin, shanzhiside methyl ester and I (2 g), and aucubin (3 g). Fraction A (9 g) was chromatographed on cellulose (400 g) in *n*-BuOH–MeOH–H_2O (70:5:25) to give aucubin (2.5 g), catalpol (1.8 g), II (0.3 g), II and III (0.1 g), III (0.25 g), III and IV (0.1 g), IV (0.35 g), IV and V (0.12 g) and V (0.3 g). Fractions containing mussaenoside and **1**, were each rechromatographed on Si gel in $CHCl_3$–MeOH (8:2) to give **1** (0.8 g). Amorphous powder; $[\alpha]_D^{25}$ −100.9° (MeOH; *c* 1.7). UV λ_{max}^{MeOH} nm (log ε): 236 (4.1); IR ν_{max}^{KBr} cm^{-1}: 3390, 2910, 2870, 1690, 1635, 1440, 1300, 1185, 1155, 1075; ^1H NMR (90 MHz, D_2O): δ 7.45 (1 H, *s* (*br*), H-3), 5.59 (1 H, *d*, $J_{1,9}$ = 3.0 Hz, H-1), 4.81 (1 H, *d*, $J_{1'2'}$ = 7.5 Hz, H-1'), 4.1–3.7 (H-7 covered by glucosylic signals), 3.76 (3 H, *s*, COOMe), 3.11 (1 H, *m*, H-5), 2.73 (1 H, *dt*, $J_{5,9}$ = 8.5, $J_{8,9}$ = 8.5, $J_{1,9}$ = 3.0 Hz, H-9), 2.30 (*m*, H-8), 2.4–1.7 (*m*, H-6), 1.05 (3 H, *d*, $J_{8,10}$ = 7.0 Hz, H-10). (Found: C, 51.16; H, 6.84. $C_{17}H_{26}O_{10}$ requires: C, 52.30; H, 6.71%).

Penta-O-acetyl derivative of **1** (**2**). **1** (50 mg) was treated with dry pyridine (0.5 ml) and Ac_2O (1.0 ml) for 1.5 hr at room temp. After addition of MeOH (3 ml), the soln was left for 20 min, then evapd to give crude **2** (62 mg) which when chromatographed on Si gel (6 g) in Et_2O gave pure **2** (45 mg) as an amorphous powder. (Found: C, 52.94; H, 6.14. $C_{27}H_{36}O_{15}$ requires: C, 53.99; H, 6.04%).

Methyl acetals **3** *and* **4**. **1** (0.1 g) was hydrolysed by treatment with β-glucosidase (EC 3.2.1.21) (50 mg) in H_2O (3 ml) at 27° for 3 hr. The mixture was extracted with EtOAc (25 ml × 4) and the organic solvent removed under red. pres. at 30°. The residue (60 mg) was dissolved in dry MeOH (3 ml) was stirred for 12 hr at room temp. with 0.5 g of strongly acid cation exchanger (Ionenaustaucher I Merck). The catalyst was removed by filtration and washed with MeOH (×2). The filtrate plus washings were evapd to give a colourless oil (57 mg) which was chromatographed on Si gel (6 g) in C_6H_6–Et_2O (1:9) to give **4** (4 mg) and then **3** (40 mg). **3**: $[\alpha]_D^{25}$ −35° (MeOH; *c* 0.15); ^1H NMR (90 MHz, $CDCl_3$): δ 7.40 (1 H, *s* (*br*), H-3), 4.74 (1 H, *d*, $J_{1,9}$ = 5.0 Hz, H-1), 3.87 (1 H, *m*, H-7), 3.69 (3 H, *s*, COOMe-4), 3.46 (3 H, *s*, OMe-1), 3.08 (1 H, *m*, H-5), 2.49 (1 H, *dt*, $J_{5,9}$ = $J_{8,9}$ = 8.0, $J_{1,9}$ = 5.0 Hz, H-9), 2.16 (1 H, *m*, H-8), 2.3–1.6 (AB part of the ABMX system, J_{AB} = 13.0, $J_{A,M}$ = 7.0, $J_{A,X}$ = 6.1 Hz, H-6), 1.05 (3 H, *d*, $J_{8,10}$ = 7.0 Hz, H-10). (Found: C, 58.30; H, 7.63. $C_{12}H_{18}O_5$ requires: C, 59.49; H, 7.49%). **4**: $[\alpha]_D^{25}$ +11° (MeOH; *c* 0.1).

Determination of the configuration of C-7 (*Horeau's method*). **3** (60 mg) was treated with racemic α-phenylbutyric anhydride (180 mg) and dry pyridine (300 mg) for 1.5 hr at room temp. After all **3** had reacted (TLC; Si gel, Et_2O), the soln was diluted with cold Et_2O then, keeping the temp. at 0°, it was washed with 2 M HCl and extracted with cold satd $NaHCO_3$ (×2). The alkaline extracts were washed with Et_2O, acidified with 2 M HCl and extracted with Et_2O (×2). Removal of the solvent gave α-phenylbutyric acid (69 mg, 100% yield 65 mg). The measured $[\alpha]_D$ −0.8° corresponded to a 20% optical yield (calc. for 100% optical purity, $[\alpha]_D$ −4.37°).

Mono-O-acetyl derivative (**5**). **3** (130 mg) was acetylated as previously described to give 140 mg of crude **5** which was chromatographed on Si gel (14 g) in C_6H_6–MeOtBu (19:1) to give **5** (125 mg) as a viscous oil.

Loganin (**6**). The following data are given to supplement the lack of data about this compound. ^1H NMR (90 MHz, D_2O): δ 7.44 (1 H, *s* (*br*), H-3), 5.41 (1 H, *d*, $J_{1,9}$ = 3.0 Hz, H-1) 4.80 (1 H, *d*, $J_{1'2'}$ = 7.5 Hz, H-1'), 4.14 (1 H, *m*, H-7), 3.73 (3 H, *s*, COOMe-4), 3.06 (1 H, *m*, H-5), 2.3–1.4 (4 H, H-6, H-8, H-9), 1.06 (3 H, *d*, $J_{8,10}$ = 7.0 Hz, H-10).

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