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IRIDOID GLUCOSIDES FROM *PEDICULARIS*

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Key Word Index—*Pedicularis* species; Scrophulariaceae; iridoid glucosides; aucubin; euphroside; plantarenaloside; shanzhiside methyl ester; gardoside methyl ester; ixoroside; 8-epiloganin; mussaenoside; pedicularioside; penstemoside; penstemonoside; boschnaloside.

Abstract—*Pedicularis palustris* contains the iridoid glucosides aucubin and gardoside methyl ester, and five hydroxy derivatives of 8-epi-deoxyloganin, together with boschnaloside and three hydroxy derivatives of boschnaloside. From *P. silvatica*, plantarenaloside, 8-epiloganin and euphroside have been isolated. Euphroside is also present in *P. lapponica*, in addition to aucubin and mussaenoside. Chemical evidence for the structure of euphroside is presented. Pedicularioside is shown to be identical with penstemoside, and the former name is thus redundant.

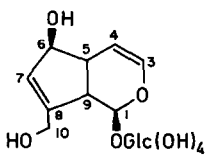
INTRODUCTION

The presence of iridoid glucosides in the genus *Pedicularis* has previously been demonstrated by paper chromatography [1]. Aucubin (**1**) was found in all four species investigated, catalpol in three, and an iridoid ester in one. As an extension of this study, we have surveyed the presence of iridoids in three species of *Pedicularis* and identified altogether eleven iridoids. The isolation and characterization of these are presented in the following.

RESULTS AND DISCUSSION

Fractionation of the alcoholic extract of the aerial parts of *P. palustris* L. yielded eleven iridoid glucosides (in order of decreasing polarity): aucubin (**1**) and euphroside (**2**), and in addition to these two major constituents ixoroside (**3**), shanzhiside methyl ester (**4**), gardoside methyl ester (**5**), plantarenaloside (**6**), 8-epiloganin (**7**), mussaenoside (**8**), penstemoside (pedicularioside) (**9**), penstemonoside (**10**) and boschnaloside (**11**).

While most of the compounds are well described in the literature [2–8], **2**, **9** and **10** had each been reported only once before; therefore particular care was taken in their identification. Our physical and spectral data of euphroside (**2**) and the tetraacetate **2a** are in good accord with those reported [9]. In addition, we have related the structure of **2** to that of ipolamiide (**13**). Thus reduction of **2** with sodium borohydride followed by acetylation gave **12a**, which proved to be identical with a sample of **12a** prepared by reduction of **13a** with lithium aluminium hydride, followed by acetylation. We earlier [8] discussed the structure of **9** which we named pedicularioside. The proof of the structure was based on NMR evidence as well as on the fact that **9a** was different from **14a** and **15a** prepared from hastatoside. After our paper was submitted [8], however, Chaudhuri *et al.* [11] presented the structures of two iridoids from *Penstemon barbatus* (Scrophulariaceae), namely penstemoside and penstemo-



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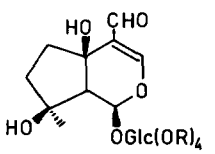

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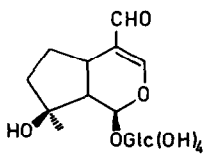

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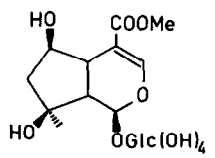

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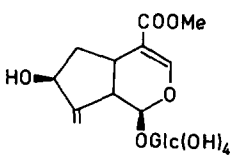

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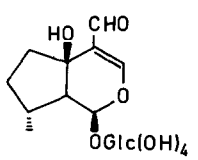

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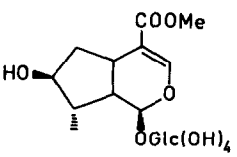

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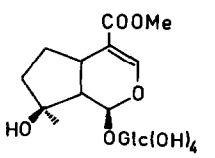

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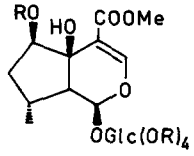

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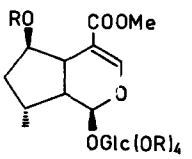

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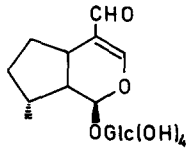

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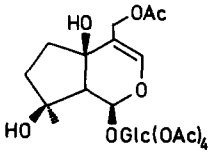
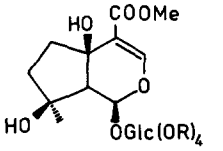


12a



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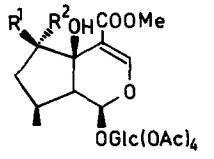
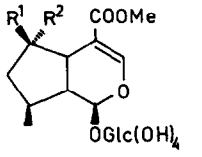
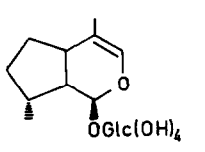


14a R^1 = H, R^2 = OAc
15a R^1 = OAc, R^2 = H



16 R^1 = H, R^2 = OH
17 R^1 = OH, R^2 = H



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noside. Despite the somewhat differing physical data for **9** and **9a** (see Experimental) found by us and by the Swiss group [11], the ^{1}H NMR spectra of pedicularioside (**9**) and of a sample of penstemoside provided by Professor Sticher were completely identical. Therefore we wish to withdraw the name pedicularioside as it is now redundant. The NMR spectrum of **10** indicated that it contained one hydroxyl group, shown, by decoupling experiments, to be located at C-6. Furthermore, **10** was different from both **16** and **17** prepared from cornin [12], and hence must contain an 8α-methyl group. The β-configuration of the 6-hydroxyl group in **10** followed from ^{13}C NMR [8]: (i) the low chemical shift of C-1 (96.5 ppm) and the small value of the $\delta_{C\text{-}3} - \delta_{C\text{-}4}$ shift difference (42.9 ppm), and (ii) the fact that the ^{13}C NMR spectrum of **10** showed a better overall similarity with that of **17** than with that of **16** (cf. [8]). Finally, comparison of an ^{1}H NMR spectrum of **10** with a spectrum of penstemonoside provided by Professor Sticher proved the identity of the two.

Euphroside (**2**) was the major constituent of *P. silvatica* L., while **6** and **7** were found in only small amounts. In *P. lapponica* L., aucubin (**1**), euphroside (**2**) and mussaenoside (**8**) were present in almost equal amounts.

It is noteworthy that euphroside seems to be characteristic of the genus *Pedicularis*. It was probably recorded as catalpol in the paper chromatographic investigation of Kooiman [1] since no reference compound was available at that time.

In the light of biosynthetic experiments conducted in this laboratory [13–15], it seems likely that all the iridoid glucosides in the above *Pedicularis* species are derived from 8-epi-iridodial glucoside (**18**).

EXPERIMENTAL

Microanalyses were performed at the Novo Microanalytical Laboratory, Bagsværd, Denmark. Vouchers have been deposited at The Botanical Museum, Copenhagen, Denmark.

P. palustris L. subsp. *opsiantha* (E. L. Ekm.) E. Almq. was collected at Uggerby Beach, Denmark (voucher IOK-8/80). Whole fresh plants (1.05 kg) were extracted with EtOH and the extract was treated as described earlier [16, 17] to give a crude mixture of glucosides. Separation was performed by repeated chromatography on reversed-phase silica gel (Merck Lichroprep RP-8) using H_2O–MeOH mixtures (4:1 to 1:1), if necessary followed by prep. TLC using $CHCl_3$–MeOH mixtures. The following compounds were obtained:

Aucubin (**1**, 2.2 g, 0.2%): identified by ^{1}H NMR and by the mp (127°) of the hexaacetate (lit. [18]: 128°).

Euphroside (**2**, 3.5 g, 0.35%): a foam $[\alpha]_D^{20} - 167°$ (MeOH; *c* 0.63); ^{1}H NMR and ^{13}C NMR (the latter published in ref. [8]) were essentially as reported [9]. Acetylation gave the tetraacetate (**2a**), mp 211° (corr.); $[\alpha]_D^{20} - 124°$ ($CHCl_3$; *c* 0.9) (lit. [9]: mp 209–210°; $[\alpha]_D^{20} - 126°$ ($CHCl_3$; *c* 0.7); ^{1}H NMR as reported [9]; ^{13}C NMR (22.6 MHz, $CDCl_3$): δ190.1 (C-11), 169.5–170.8 (4 × acetyl-C=O), 157.5 (C-3), 125.4 (C-4), 95.9 (C-1'), 95.0 (C-1), 78.8 (C-8), 72.2 (C-5, C-5' and C-3'), 70.9 (C-2'), 68.2 (C-4'), 61.5 (C-6'), 59.5 (C-9), 40.0 (C-7), 36.6 (C-6), 23.9 (C-10) and 20.6 (4 × acetyl-Me).

Ixoroside (**3**, 27 mg, 0.003%): ^{1}H NMR as reported [2] (the fraction contained two unidentified iridoids as impurities).

Shanzhiside methyl ester (**4**, 144 mg, 0.01%): ^{1}H NMR as reported [19].

Gardoside methyl ester (**5**, 40 mg, 0.004%): ^{1}H NMR as reported [10].

Plantarenaloside (**6**, 346 mg, 0.035%); ^{1}H and ^{13}C NMR as reported [20, 21].

8-*Epiloganin* (**7**, 10 mg, 0.001%): ^{1}H NMR as reported [5].

Mussaenoside (**8**, 184 mg, 0.02%): ^{1}H NMR as reported [3].

Penstemoside (= *pedicularioside* [8]) (**9**, 81 mg 0.008%): a foam, $[\alpha]_D^{20} - 171°$ (MeOH; *c* 0.5) (lit. [11]: $[\alpha]_D^{20} - 100°$ (MeOH; *c* 0.7)). ^{1}H NMR (400 MHz, D_2O): δ7.57 (*s*, H-3), 5.77 (*s*, H-1), 4.69 (*d*, *J* = 8.2 Hz, H-1'), 4.25 (*t*, *J* = 4.3 Hz, H-6), 3.69 (*s*, OMe), 2.57 (*m*, H-8 and H-9), 1.76 (*ddd*, *J* = 13.8, 6.9 and 3.8 Hz, H-7), 1.45 (*ddd*, *J* = 13.8, 7.5 and 4.7 Hz, H-7), 0.84 (*d*, *J* = 6.8 Hz, 10-Me), identical to a spectrum of an authentic sample; ^{13}C NMR (67.9 MHz, D_2O): δ169.1 (*s*, C-11), 155.5 (*d*, C-3), 112.3 (*s*, C-4), 96.3 (*d*, C-1), 76.3 (*d*, C-6), 73.2 (*s*, C-5), 52.6 (*q*, OCH_3), 49.2 (*d*, C-9), 39.6 (*t*, C-7), 30.5 (*d*, C-8), 16.2 (*q*, C-10) and 99.4, 77.1, 76.2, 73.3, 70.4, 61.5 (C-1' to C-6'), essentially as that reported in ref. [11], except for solvent effects. (Found: C, 47.2; H, 6.4. Calc. for $C_{17}H_{26}O_{11} \cdot 1\frac{1}{2} H_2O$: C, 47.1; H, 6.7%.)

Penstemoside pentaacetate (**9a**): mp (EtOH) 148° (corr.); $[\alpha]_D^{20} - 115°$ ($CHCl_3$; *c* 0.5) (lit. [11]: $[\alpha]_D^{20} - 105°$ ($CHCl_3$; *c* 0.9); ^{1}H NMR (270 MHz, $CDCl_3$): δ7.46 (*s*, H-3), 5.54 (*d* (*br*), *J* = 1.8 Hz, H-1), 5.46 (*dd*, *J* = 2.3 and 4.2 Hz, H-6), 3.76 (*s*, OMe), 3.34 (*s*, OH), 2.69 (*m*, H-9 and H-8), 2.08, 2.08, 2.01, 1.98, 1.91, (5 × OAc), 1.87 (*ddd*, $J_{7a,6}$ = 2.2 Hz, $J_{7a,8}$ = 6.6 Hz, H-7a), 1.36 (*ddd*, $J_{7b,6}$ = 4.4, $J_{7b,7a}$ = 13.6, $J_{7b,8}$ = 9.2 Hz, H-7b), 0.99 (*d*, $J_{8,10}$ = 6.4 Hz, 10-Me), ^{13}C NMR data are given in ref. [8].

(Found: C, 52.5; H, 6.0. Calc. for $C_{27}H_{36}O_{16}$: C, 52.6; H, 5.9%.)
Penstemonoside (**10**, 20 mg, 0.002%) could not be purified completely, but deacetylation of the pentaacetate (**10a**, 5 mg) with NaOMe in MeOH for 1 hr followed by purification by HPLC gave pure **10** (*ca* 1 mg) as seen by ^{1}H NMR (400 MHz, D_2O): δ7.41 (*s*, H-3), 5.53 (*d*, J = 2.4 Hz, H-1), 4.70 (*d*, J = 8.5 Hz, H-1'), 4.18 (*m*, H-6), 3.68 (*s*, OMe), 2.82 (*d* (*br*), J = 9.0 Hz, H-5), 2.65 (*dt*, J = 2.4, 9.2 and 9.2 Hz, H-9), 2.51 (*m*, H-8), 1.71 (*dd* (*br*), J = 13.9 and 7.4 Hz, H-7), 1.45 (*ddd*, J = 13.9, 9.9 and 4.8 Hz, H-7), 0.96 (*d*, J = 7.4 Hz, 10-Me), identical to a spectrum (360 MHz) of an authentic sample; ^{13}C NMR (67.9 MHz, D_2O): δ169.1 (C-11), 153.5 (C-3), 110.9 (C-4), 96.5 (C-1), 77.5 (C-6), 52.6 (OMe), 41.6, 41.4, 41.0 (C-5, C-7, C-9), 32.9 (C-8), 16.2 (C-10), 99.1, 77.0, 76.4, 73.4, 70.3, 61.5 (C-1' to C-6'). Acetylation gave penstemonoside pentaacetate (**10a**), crystals, mp (EtOH) 154.5–156° (corr.); $[\alpha]_D^{20}$ −109° ($CHCl_3$; *c* 0.5) (lit. [11]: $[\alpha]_D^{20}$ −133° ($CHCl_3$; *c* 0.8)); ^{1}H NMR (90 MHz, $CDCl_3$): δ7.37 (*d*, J = 1 Hz, H-3), 5.32 (*d*, J = 2 Hz, H-1), 5.22 (*m*, H-6), 3.69 (*s*, OMe), 2.92 (*d* (*br*), $J_{5,9}$ = 8 Hz, H-5), 2.65 (*m*, H-9), 1.87–2.09 (5 × OAc), 1.03 (*d*, J = 7 Hz, 10-Me); ^{13}C NMR (22.6 MHz, $CDCl_3$): δ166.5 (C-11), 151.6 (C-3), 109.6 (C-4), 94.1 (C-1), 78.7 (C-6), 51.3 (OMe), 40.7, 38.9, 38.5 (C-5, C-7, C-9), 31.9 (C-8), 15.9 (C-10), 95.4, 72.3, 72.0, 70.4, 68.0, 61.5 (C-1' to C-6'). (Found: C, 53.8; H, 6.1. Calc. for $C_{27}H_{36}O_{15}$: C, 54.0; H, 6.0%.)
Boschnaloside (**11**, 6 mg, 0.0006%): ^{1}H NMR as reported [7].

Reduction of euphroside tetraacetate. **2a** (204 mg) was dissolved in MeOH (10 ml) and $NaBH_4$ (182 mg) was added with stirring. After 20 min, excess HOAc was added and the solvents were removed. Acetylation of the crude product gave a mixture (219 mg), which after prep. TLC yielded **12a** (140 mg). Crystallization (Et_2O) gave pure **12a**, mp 159–160°; $[\alpha]_D^{20}$ −131° ($CHCl_3$; *c* 0.9); ^{1}H NMR (90 MHz, $CDCl_3$): δ6.32 (*s* (*br*), H-3), 5.52 (*d*, J = 1.5 Hz, H-1), 4.63 (AB system, J = 13 Hz, 11-CH_2), 2.76 (*s*, OH), 2.50 (*s* (*br*), H-9), 1.99–2.10 (5 × OAc), 1.24 (*s*, 10-Me). (Found: C, 52.9; H, 6.1. $C_{26}H_{36}O_{15}$ requires: C, 53.0; H, 6.2%.)

Reduction of ipolamide tetraacetate. A stirred suspension of $LiAlH_4$ (330 mg) in THF (50 ml) was cooled to −70° and a soln of **13a** (323 mg) in THF (15 ml) was added during 20 min. After stirring for 36 hr at −23°, excess reagent was decomposed with EtOH (2 ml). Addition of saturated aq. Na_2SO_4 gave a ppt. which was removed by filtration. The filtrate was neutralized with HOAc, reduced to dryness, redissolved in H_2O and adsorbed on charcoal (6 g) followed by washing with H_2O. The iridoid fraction (60 mg) was eluted with MeOH and acetylated to give crude **12a** (87 mg). Recrystallization gave a product indistinguishable from that obtained above.

P. sylvatica L. was collected at Børsmose, Henne, Denmark (voucher IOK-55/79). Frozen aerial parts of the plant (0.85 kg) were treated as above. The mixture of glucosides was separated by CC and prep. TLC to give euphroside (**2**, 5.5 g, 0.6%), plantarenaloside (**6**, 272 mg, 0.03%), and 8-epiloganin (**7**, 57 mg, 0.01%), all identified by ^{1}H NMR.

P. lapponica L. was collected at Rondvassbu, Otta, Norway (voucher IOK-18/80). Dry aerial parts (6.5 g) were treated as above to give a mixture of euphroside (**2**) and aucubin (**1**) (7 mg, *ca* 1:1 mixture, each *ca* 0.05%), identified by ^{1}H NMR, and mussaenoside (**8**, 4 mg, 0.06%), identified by ^{1}H NMR.

Acknowledgements—We thank Dr. B. Løjtnant for collecting and identifying *P. palustris*, and Dr. Alfred Hansen for the identification of *P. silvatica* and *P. lapponica*. Professor Sticher, ETH, is thanked for a sample of penstemoside and for an NMR spectrum of penstemonoside. Finally, we thank NOVO Industri A/S and The Danish Natural Science Research Council for access to NMR facilities.

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