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IRIDOID GLYCOSIDES FROM *PENSTEMON RICHARDSONII*

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Key Word Index—*Penstemon richardsonii*; Scrophulariaceae; iridoid glycosides; penstemide; penstebioside; penstemide-aglucone-11-*O*-β-cellobioside; ^{1}H NMR and ^{13}C NMR spectra.

Abstract—Five ester iridoids of the valeriana type have been isolated from dried leaves of *Penstemon richardsonii*. The structures have been elucidated by FAB MS, ^{1}H NMR and ^{13}C NMR spectroscopy and by enzymatic cleavage of the glycosidic linkages. Besides the known iridoids penstemide, penstemide-aglucone, 8-epi-valerosidatum and serrulatoside, a new iridoid, penstebioside (penstemide-aglucone-11-*O*-β-cellobioside) was found. This is the second report of an iridoid aglycone attached to cellobiose.

INTRODUCTION

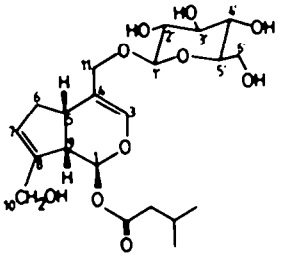
Previous investigations [1] have shown the occurrence of numerous iridoid glycosides in several species within the genus *Penstemon*. Besides widely distributed compounds such as aucubin and catalpol, typical for the Scrophulariaceae, the presence of valeriana-type ester iridoids in *Penstemon* is remarkable. The first report of these ester iridoids was that of Jensen *et al.* [2], who reported the isolation of penstemide (**1**) from *P. deustus* Dougl.

Preliminary studies by TLC of *P. serrulatus* Menz. and *P. richardsonii* Dougl. showed the occurrence of compounds related to penstemide. Further investigation resulted in the isolation of five iridoid glycosides from *P. richardsonii* [3]. The occurrence of the known compounds penstemide (**1**), penstemide-aglucone, 8-epi-valerosidatum and serrulatoside as well in *P. richardsonii* as in *P. serrulatus* [4] confirms the close relationship of both species in the taxonomic classification of Keck [5], who quoted both in the subgenus *Saccanthera*, subsection *Serrulati*. Besides these known substances, a novel iridoid disaccharide (penstebioside) has been isolated from *P. richardsonii* and has been identified as penstemide-aglucone-11-*O*-β-cellobioside (**2**).

RESULTS AND DISCUSSION

The residue from the methanolic extract of the dried leaves of *P. richardsonii*, after removal of chlorophyll, was fractionated by column chromatography. On TLC the iridoids could be detected with vanillin–sulphuric acid as blue and purple spots. Separation of the extract by CC, DCCC and preparative TLC yielded the known compounds penstemide-aglucone, penstemide, 8-epi-valerosidatum and serrulatoside (listed in order of increasing polarity), and the new iridoid glycoside penstebioside (**2**). Diglycosides of iridoids mostly contain different hexoses or desoxyhexoses besides glucose [6, 7]. Cellobiose linked to an iridoid aglycone seems to be rare and was first isolated from *Odontites verna* (Bell.) Dum. [8].

Penstebioside (**2**) was obtained as a yellowish amorphous powder (chloroform–methanol), mp 84–86°. Enzymatic cleavage [9] with β-glucosidase yielded penstemide and penstemide-aglucone. This suggested that **2** might be the diglucoside of penstemide-aglucone. The molecular formula $C_{27}H_{42}O_{15}$ was confirmed by FAB mass spectroscopy (*m/z* 645 $[M + K]^+$, 629 $[M + Na]^+$). The ^{13}C NMR spectrum also indicated the presence of 27 carbons. Two absorptions at 93.24 and 69.99 ppm were assigned to C-1 and C-11, respectively, indicating esterification and glycosidation. The ^{13}C NMR data also showed that four carbons of the iridoid skeleton are olefinic: C-3



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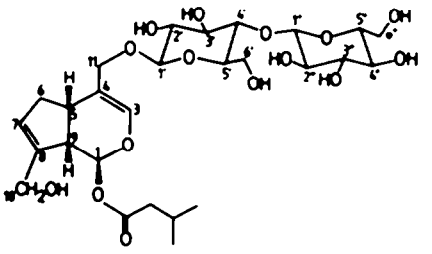

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and C-4 (140.90 and 116.21 ppm), C-7 and C-8 (129.40 and 144.04 ppm). Comparison of the ^{13}C NMR spectra of **1** and **2** confirmed the presence of an additional β-glucopyranosyl moiety in the molecule. The signals of C-3' to C-5' showed typical glycosidation effects (C-4' downfield 9.21 ppm, C-3' and C-5' upfield 1.71 and 1.67 ppm, respectively), indicating that the glycosidic linkage of the second β-glucopyranosyl moiety is to the hydroxyl group at C-4'. The ^{13}C ^{1}H coupling constants of the anomeric carbons (162 Hz, C-1' and 163 Hz, C-1") indicated the β-configuration of both sugar moieties [10]. The signals of carbons C-1' to C-6' and C-1" to C-6" are in excellent agreement with the reported ^{13}C NMR data for methyl-β-cellobioside [11] (Table 1).

When comparing the ^{1}H NMR spectra of **1** and **2** in CD_3OD, no differences were found for the signals of the protons of the iridoid skeleton. Doublets at 4.31 and 4.39 ppm corresponding to H-1' and H-1", respectively, $J_{1',2'} = 7.9$ Hz (in each case), confirmed the β-configuration of the D-glucose. The ^{1}H NMR spectrum recorded in D_2O showed excellent agreement with the data reported for cellobiose [12]. All the experimental data confirmed the structure of **2** as penstemide-aglucone-11-*O*-β-cellobioside.

EXPERIMENTAL

P. richardsonii Dougl. was grown from seeds supplied by Alpengarten, Meyrin, Switzerland, and was identified by Dr P. Junior. A voucher specimen (27/85) has been deposited at the Institut für Pharmazeutische Biologie, Philipps-Universität Marburg, F.R.G.

Isolation procedure. Air-dried, powdered leaves (82 g) were refluxed twice with 500 ml MeOH for 30 min. After evaporation of the solvent under red pres., the residue was dissolved in 200 ml MeOH-H_2O (1:1). Chlorophyll was separated by addition of $Pb(OAc)_2$, and the surplus lead was removed with Na_2HPO_4. After centrifugation, the iridoids were extracted with $CHCl_3$–*i*-PrOH (3:2) (500 ml and 3 × 300 ml) and chromatographed on silica gel (200 g) developed with $CHCl_3$–MeOH (9:1 to 1:9). The residue, 4.75 g, afforded 54 × 110 ml fractions and 89 × 180 ml fractions.

Isolation of **2**. Fractions Nos 89–143 (1.17 g) consisted of a mixture of more polar iridoids, with serrulatoside as the main component and **2** as a minor component. DCCC with $CHCl_3$–MeOH–H_2O (5:6:4), descending flow, gave 230 × 12 ml fractions, the last 34 of which gave 36 mg **2**. FAB MS *m/z*: 645 [M + K]$^+$, 629 [M + Na]$^+$, 527 [M + Na − isovaleric acid]$^+$. ^{13}C NMR (100 MHz, CD_3OD): see Table 1. ^{1}H NMR (400 MHz, CD_3OD): δ 6.42 (*s* (*br*), H-3), 5.82 (*s* (*br*), H-7), 5.77 (*d*, *J* = 7.3 Hz, H-1), 4.39 (*d*, *J* = 7.9 Hz, H-1"), 4.31 (*d*, *J* = 7.9 Hz, H-1'), 4.20 (AB centre, *J* = 11.5 Hz, 2H-11), 4.12 (in AB of 11, 2H-10), 3.17 (*m*, H-5), 2.81 (*t* (*br*), *J* = 7.5 Hz, H-9), 2.69 (*m*, H-6), 2.26 (*m*, H-6), 2.26 (-CH_2-), 2.09 (-CH<), 0.96 (*d*, *J* = 6.6 Hz, 2 × Me); (400 MHz, D_2O): δ 6.51 (*s* (*br*), H-3), 5.90 (*s* (*br*), H-7), 5.89 (*d*, *J* = 6.5 Hz, H-1), 4.52 (*d*, *J* = 7.8 Hz, H-1"), 4.50 (*d*, *J* = 8 Hz, H-1'), 4.24 (AB centre, 2H-11), 4.21 (in AB of 11, 2H-10), 3.98 (*dd*, *J* = 12.3 Hz, H-6'), 3.92 (*dd*, *J* = 12.3 Hz, H-6"), 3.82 (*dd*, *J* = 4.9 Hz, H-6'), 3.74 (*dd*, *J* = 5.8 Hz, H-6"), 3H centred at 3.64 (H-3', H-4', H-5'), 3.52 (*dd*, *J* = 9.6, 8.8 Hz, H-3"), 3.42 (*dd*, *J* = 9.6, 8.8 Hz, H-4"), 2H centred at 3.33 (*J* not determined; H-2', H-2"), 3.17 (*m*, H-5), 2.99 (*t* (*br*), H-9), 2.70 (*m*, H-6), 2.35 (*m*, H-6), 2.36 (-CH_2-), 2.08 (-CH<), 0.96 (*d*, *J* = 6.6 Hz, 2 × Me).

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (100 MHz, CD_3OD, δ ppm) and methyl-β-cellobioside [11]

C	1	2	Methyl-β-cellobioside
1	93.20	93.24	
3	140.72	140.90	
4	116.18	116.21	
5	37.94	37.88	
6	38.03	38.03	
7	129.41	129.40	
8	144.06	144.06	
9	47.15	47.22	
10	61.11	61.10	
11	69.88	69.99	
1'	103.39	104.57	104.5
2'	75.15	74.89	74.2
3'	77.92	76.51	76.4
4'	71.74	80.95	80.3
5'	78.12	76.45	75.9
6'	62.84	62.06	61.8
1"		103.29	103.9
2"		74.92	74.6
3"		78.10	77.5
4"		71.42	71.2
5"		77.91	77.2
6"		62.47	62.4
C=O	173.11	173.12	
-CH_2-	44.13	44.15	
-CH<	26.68	26.48	
-Me (2 ×)	22.63	22.61	

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