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IRIDOID GLYCOSIDES FROM *PENSTEMON CONFERTUS*

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Key Word Index—*Penstemon confertus*; Scrophulariaceae; iridoid glycosides; 7-hydroxyebuloside; dihydroserruloside; confertoside; NMR

Abstract—Three ester iridoids of the valeriana type have been isolated from dried leaves of *Penstemon confertus*. Their structures were elucidated by ^{1}H NMR and ^{13}C NMR spectroscopy as the known 7-hydroxyebuloside and two new iridoid glycosides, dihydroserruloside (dihydropenstemide-aglucone-11-*O*-β-D-ribohexos-3-uloside) and confertoside (dihydropenstemide-aglucone-11-*O*-β-D-glucosyl-1,4-β-D-alloside). This is the second report of β-D-ribohexopyranoside-3-ulose (β-D-keto-glucose) attached to an iridoid aglycone. Confertoside is the first iridoid disaccharide with 1,4-β-D-allosyl-β-D-glucose as the carbohydrate moiety.

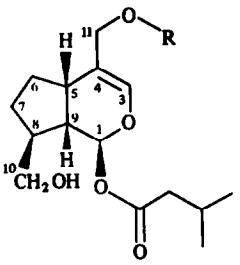
INTRODUCTION

Previously we reported the isolation of dihydropenstemide (**1**) [1] from leaves of *Penstemon confertus* Dougl. Dihydropenstemide, an ester iridoid of the valeriana type, is the main iridoid compound of this plant. Further investigation resulted in the isolation of three additional iridoid glycosides [2] of the same type: 7-hydroxyebuloside (**2**), dihydroserruloside (**3**) and confertoside (**4**). 7-Hydroxyebuloside was first isolated from *Sambucus ebulus* L. (Caprifoliaceae) [3]. Dihydroserruloside and confertoside are new iridoids containing very rare carbohydrate moieties.

RESULTS AND DISCUSSION

The methanol extract of dried leaves of *P. confertus*, after removal of chlorophyll, gave a residue containing several iridoids (detected with vanillin–sulphuric acid as mostly brown spots). Separation of the extract by CC, DCCC, and preparative TLC yielded dihydropenstemide (**1**), 7-hydroxyebuloside (**2**), dihydroserruloside (**3**) and confertoside (**4**). The major iridoid compound (**1**) was isolated and identified as dihydropenstemide [1] earlier.

7-Hydroxyebuloside (**2**) (purple spot with vanillin–sulphuric acid) was obtained as an amorphous powder. Its molecular formula ($C_{21}H_{34}O_{10}$) was confirmed by FAB mass spectroscopy (*m/z* 469 $[M+Na]^+$). Its ^{13}C NMR spectrum proved the presence of isovaleric acid attached to C-1 (δ93.51) and of β-D-glucose attached to C-11 (δ69.76). Two absorptions at δ139.42 and 117.19 were assigned to C-3 and C-4. The signals at δ75.13 and 13.32 indicated the β-configuration of the hydroxyl function at C-7 and of the methyl group at C-8, respectively, in good agreement with the data reported for 7-hydroxyebuloside [3]. Comparison of the ^{1}H NMR data of **2** with those of 7-hydroxyebuloside [3] confirmed the identity of both substances. Dihydroserruloside (**3**) was obtained as a yellowish, amorphous powder (chloroform–methanol). It was very unstable and decomposed after the ^{1}H and ^{13}C NMR spectra were recorded. Its ^{13}C NMR spectrum showed signals for 21 carbons. Two absorptions at δ93.15 and 70.08 were assigned to C-1 and C-11, respectively, indicating esterification and glycosidation. Comparison of the ^{13}C NMR data of **3** and **1** revealed very good agreement of the signals of the iridoid skeleton. Major differences were found for the sugar moiety. The signal at δ179.80, arising from a carbonyl function, indicated the presence of a keto sugar as it occurred in serruloside



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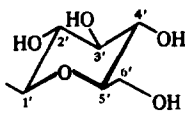

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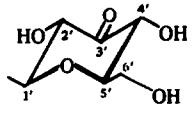

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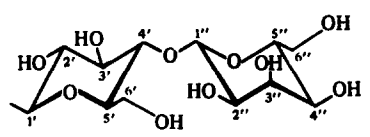

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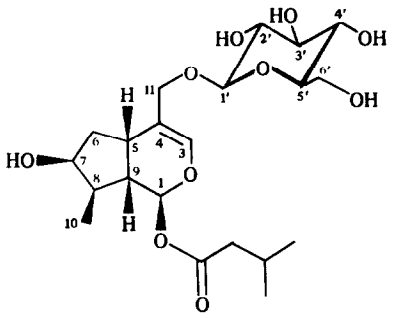

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(penstemide-aglucone-11-*O*-β-D-ribohexos-3-uloside) isolated from *Penstemon serrulatus* Menz. [4]. The chemical shifts of C-1′ to C-6′ of **3** were in good agreement with the ^{13}C NMR data reported for the sugar moiety of serruloside and confirmed the structure of **3** to be dihydroserruloside (dihydropenstemide-aglucone-11-*O*-β-D-ribohexos-3-uloside). The ^{1}H NMR data of **3** also demonstrated the identity of the iridoid skeletons of **1** and **3**. The doublet of δ4.38, corresponding to H-1′, $J_{1',2'}$ = 7.9 Hz, proved the β-configuration of D-ribohexopyranoside-3-ulose, which has now been found for the second time attached to an iridoid aglycone.

Confertoside (**4**), white, crystalline powder (chloroform–methanol), mp 100–101°, whose molecular formula $C_{27}H_{44}O_{15}$ was confirmed by FD mass spectroscopy (*m/z* 648 $[M+K+H]^+$). Its ^{13}C NMR spectrum in CD_3OD also indicated the presence of 27 carbons. The chemical shifts of the iridoid skeleton (15 carbons) showed very good correspondence to the data of the dihydropenstemide-aglucone part recorded from **1** and **3**. The remaining 12 signals were assigned to the carbohydrate part in glycosidic linkage to the hydroxyl function at C-11. Two absorptions at δ103.40 and 102.61 referred to C-1′ and C-1″, respectively. The ^{13}C–^{1}H coupling constants of the anomeric carbons (159 Hz, C-1′ and 164 Hz, C-1″) indicated the β-configuration of both sugar moieties [5]. The signals of C-1′ to C-6′ were assigned to β-D-glucose with the glycosidic linkage of the second sugar moiety to the hydroxyl group at C-4′ (typical glycosidation effects: C-4′ shifted downfield δ9.81, C-3′ and C-5′ shifted upfield δ1.41 and 1.55, respectively). The resonances at δ72.17, 73.05, 68.81, 75.78, and 62.89 resulted from a β-D-allopyranosyl moiety (C-2″ to C-6″). The ^{13}C NMR data of **4**, recorded in D_2O, confirmed the structure of the carbohydrate moiety. The signals of C-1″ to C-6″ showed excellent agreement with the data reported for the β-D-allopyranosyl moiety in allosyl-epoxydecaloside and allosyl-decaloside [6]. The chemical shifts of C-1′ to C-6′ corresponded very well with the ^{13}C NMR data reported for the β-D-glucopyransoyl moiety in β-lactoside [7]. The ^{1}H NMR spectrum of **4** in CD_3OD also proved that **4** derived its structure from dihydropenstemide–aglucone. Doublets at δ4.31 and 4.71 corresponding to H-1′ and H-1″, respectively, $J_{1',2'}$ = 7.9 Hz and $J_{1'',2''}$ = 8.0 Hz, confirmed the β-configuration of both sugar moieties. The ^{1}H NMR spectrum recorded in D_2O showed very good agreement of the signals for H-1″ to H-6″ with the reported ^{1}H NMR data for β-D-allose [8]. The triplet at δ4.17 and the double doublets at δ3.48 and 3.64 referred to H-3″, H-2″, and H-4″. The structure of confertoside as dihydropenstemide-aglucone-11-*O*-β-D-glucosyl-1,4-β-D-alloside was confirmed by all relevant experimental data. Iridoid glycosides with an allose moiety attached at C-11 have been reported from *Viburnum opulus* L. [9] and *V. betulifolium* Batal. [10] (Caprifoliaceae) and from two species of *Mentzelia* (Loasaceae) [6]. In the opulus iridoids III and IV xylosylallose is attached to an iridoid moiety. Confertoside is the first iridoid found with allosylglucose in glycosidic linkage at C-11.

EXPERIMENTAL

Penstemon confertus Dougl. was grown from seeds supplied by the Botanical Garden Vancouver, and was identified by Dr P. Junior. A voucher specimen (30/85) has been deposited at the Institut für Pharmazeutische Biologie, Philipps-Universität Marburg, F.R.G..

Isolation procedure. Dried, powdered leaves (68 g) were refluxed twice with 500 ml MeOH for 30 min. After concn of the combined extracts *in vacuo*, 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). The residue (4.75 g) was chromatographed on silica gel (160 g) with $CHCl_3$–MeOH (49:1 to 1:1), to afford 155 fractions of 150–200 ml (for further detail see ref. [1]).

Isolation of **2**. Separation of fractions nos 53–83 (1.67 g) by DCCC with $CHCl_3$–MeOH–H_2O (5:6:4), descending flow, resulted in 200 × 11 ml fractions. Prep. TLC of fractions nos 37–46 (33 mg) in EtOAc–EtOH–H_2O (35:13:2) yielded 19 mg **2** as an amorphous powder. $C_{21}H_{34}O_{10}$ requires 446, FAB-MS: *m/z* 469 $[M+Na]^+$; ^{13}C NMR (100 MHz, CD_3OD): see Table 1; ^{1}H NMR (400 MHz, CD_3OD): δ6.32 (*br s*, H-3), 5.88 (*d*, *J* = 4.5 Hz, H-1), 4.27 (*d*, *J* = 7.8 Hz, H-1′), 4.15 (AB centre, *J* = 11.6 Hz, 2H-11), 4.07 (*m*, H-7), 3.86 (*dd*, A<u>B</u>X, *J* = 2.0, 11.8 Hz, H-6′), 3.66 (*dd*, A<u>B</u>X, *J* = 5.4, 11.8 Hz, H-6′), 3.19 (*dd*, *J* = 7.8, 9.0 Hz, H-2′), 2.99 (*m*, H-5), 2.06 (*m*, H-6α), 2.02 (*m*, H-9), 1.77–1.87 (*m*, 2H, H-6β, H-8), 1.08 (*d*, *J* = 6.9 Hz, 3H-10), 2.22 (–CH_2–), 2.08 (–CH<), 0.96 (*d*, *J* = 6.6 Hz, 2 × Me).

Isolation of **3**. After concn of the combined fractions nos 21–52 and 108–155 of the CC *in vacuo*, the residue (1.2 g) was subjected to DCCC ($CHCl_3$–MeOH–H_2O (5:6:4), descending flow, which afforded 233 × 12 ml fractions. By prep. TLC with EtOAc–EtOH–H_2O (35:13:2) as eluant 21 mg **3** were isolated from the fractions nos 21–28. $C_{21}H_{32}O_{10}$, amorphous powder, which decomposed after the ^{1}H NMR and ^{13}C NMR spectra were recorded. ^{13}C NMR (100 MHz, CD_3OD): see Table 1; ^{1}H NMR (400 MHz, CD_3OD): δ6.38 (*br s*, H-3), 5.97 (*d*, *J* = 4.7 Hz, H-1), 4.38 (*d*, *J* = 7.9 Hz, H-1′), 4.22 (AB centre, *J* = 11.6 Hz, 2H-11), 4.21 (*dd*, *J* = 1.7, 10.0 Hz, H-4′), 4.12 (*dd*, *J* = 1.7, 7.9 Hz, H-2′), 3.94 (*dd*, A<u>B</u>X, *J* = 2.2, 12.1 Hz, H-6′), 3.78 (*dd*, A<u>B</u>X *J* = 5.0, 12.1 Hz, H-6′), 3.53 (*d*, *J* = 6.0 Hz, 2H-10), 2.81 (*m*, H-5), 1.98–2.02 (*m*, 2H, H-8, H-9), 1.93 (*m*, H-7), 1.81 (*m*, H-7), 1.70 (*m*, H-6), 1.38 (*m*, H-6), 2.22 (–CH_2–), 2.07 (–CH<), 0.96 (*d*, *J* = 6.6 Hz, 2 × Me).

Isolation of **4**. The residue (241 mg) of polar fractions nos 113–135 from the second DCCC run was chromatographed on silica gel (40 g) developed with EtOAc–EtOH (49:1 to 4:1) to afford 225 × 20 ml fractions. After concn of the combined fractions nos 144–200 in vacuo 75 mg **4** were obtained. $C_{27}H_{44}O_{15}$ requires 608, FD-MS: *m/z* 648 $[M+K+H]^+$, mp 100–101° (uncorr.), $[\alpha]_D^{20}$ – 57° (MeOH; *c* 0.147). ^{13}C NMR (100 MHz, D_2O (dioxan), and 25 MHz, CD_3OD): see Table 1; ^{1}H NMR (400 MHz, CD_3OD): δ6.37 (*br s*, H-3), 5.96 (*d*, *J* = 4.7 Hz, H-1), 4.71 (*d*, *J* = 8.0 Hz, H-1″), 4.31 (*d*, *J* = 7.8 Hz, H-1′), 4.15 (AB

Table 1. ^{13}C NMR spectral data of compounds 1–3 (100 MHz, CD_3OD, δ ppm), 4 (25 MHz, CD_3OD; 100 MHz, D_2O; δ ppm), allosyldecaloside [6] and β-lactoside [7]

C	1	2	3	4 (CD_3OD)	4 (D_2O)	Allosyl-decaloside	β-Lac-toside
1	93.15	93.51	93.15	93.25	93.21	98.5	
3	140.64	139.42	140.96	140.59	140.21	141.6	
4	115.15	117.19	114.90	115.28	115.04	113.5	
5	36.90	33.56	36.94	36.94	35.77	44.7	
6	30.88	40.58	30.85	30.89	30.03	81.0	
7	28.14	75.13	28.13	28.14	27.69	136.1	
8	43.87	42.09	43.87	43.96	42.69	134.2	
9	44.95	46.71	44.98	45.03	43.67	47.7	
10	66.48	13.32	66.47	66.47	65.89		
11	69.65	69.76	70.08	69.76	69.91	70.1	
1′	103.52	103.53	104.90	103.40	102.01	99.7	103.99
2′	75.17	75.19	78.37	74.50	73.79	73.5	73.48
3′	77.92	78.03	179.80	76.51	75.32	76.5	75.33
4′	71.77	71.81	73.75	81.58	79.85	70.4	79.42
5′	78.18	78.23	78.37	76.63	75.52	77.1	75.65
6′	62.88	62.91	62.67	62.30	61.03	61.5	61.00
1″				102.61	101.46	99.4	103.87
2″				72.17	71.11	71.2	71.85
3″				73.05	72.02	72.0	73.69
4″				68.81	67.57	67.7	69.44
5″				75.78	74.66	74.4	76.23
6″				62.89	61.87	62.1	61.88
C=O	173.47	173.44	173.46	173.47	175.99		58.03 (OMe)
$-CH_2-$	44.21	44.26	44.20	44.25	43.81		
–CH<	26.77	26.81	26.78	26.75	26.25		
–Me	22.63	22.61	22.62	22.61	22.33		

centre, J = 11.5 Hz, 2H-11), 4.05 (*t*, J = 3.0 Hz, H-3″), 3.88 (*dd*, A BX, J = 2.6, 12.1 Hz, H-6′), 3.85 (*dd*, A BX, J = 1.8, 11.8 Hz, H-6″), 3.76 (*dd*, AB X, J = 5.9, 12.1 Hz, H-6′), 3.64 (*dd*, AB X, J = 5.9, 11.8 Hz, H-6″), 3.52 (*d*, J = 6.2 Hz, 2H-10), 3.35 (*dd*, J = 3.0, 8.0 Hz, H-2″), 3.25 (*dd*, J = 7.8, 9.2 Hz, H-2′), 2.81 (*m*, H-5), 1.98–2.02 (*m*, 2H, H-8, H-9), 1.93 (*m*, H-7), 1.81 (*m*, H-7), 1.68 (*m*, H-6), 1.39 (*m*, H-6), 2.22 ($-CH_2-$), 2.07 (–CH<), 0.96 (*d*, J = 6.6 Hz, 2 × Me); ^{1}H NMR (400 MHz, D_2O, DSS as int. standard): δ6.44 (*d*, J = 0.9 Hz, H-3), 5.96 (*d*, J = 4.7 Hz, H-1), 4.73 (*d*, J = 8.3 Hz, H-1″), 4.49 (*d*, J = 7.9 Hz, H-1′), 4.24 (*br s*, 2H-11), 4.17 (*t*, J = 3.2 Hz, H-3″), 3.95 (*dd*, A BX, J = 2.1, 12.2 Hz, H-6′), 3.89 (*dd*, A BX, J = 1.8, 11.9 Hz, H-6″), 3.82 (*dd*, AB X, J = 4.9, 12.2 Hz, H-6′), 3.70 (*dd*, AB X, J = 5.8, 12.2 Hz, H-6″), 3.64 (*dd*, J = 3.2, 10.4 Hz, H-4″), 3.56 (*dd*, J = 6.7, 11.1 Hz, 1H-10), 3.54–3.65 (4H, H-3′ to H-5′, 1H-10), 3.48 (*dd*, J = 3.2, 8.2 Hz, H-2″), 3.31 (*dd*, J not determined, H-2′), 2.82 (*m*, H-5), 2.01–2.10 (*m*, 2H, H-8 and H-9), 1.95 (*m*, H-7), 1.82 (*m*, H-7), 1.66 (*m*, H-6), 1.39 (*m*, H-6), 2.32 ($-CH_2-$), 2.07 (–CH<), 0.94 (*d*, J = 6.7 Hz, 2 × Me).

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