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TRANS-FUSED IRIDOID GLYCOSIDES FROM *PENSTEMON MUCRONATUS*

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Key Word Index—*Penstemon mucronatus*; Scrophulariaceae; iridoid glycosides; isolation; taxonomy.

Abstract—Two new *trans*-fused iridoid glycosides (5αH)-6α-8-epidihydrocornin and (5αH)-6α-8-hydroxy-8-epiloganin, were isolated from *Penstemon mucronatus*, along with cornin, penstemoside and three hastatosides. The *trans*-fused iridoids are only the second and third known among over 900 described *cis*-fused iridoid glycosides. Two pairs of iridoids, identical except for the stereochemistry at C-8, were found. Structures were determined by spectroscopic methods. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

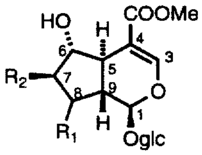
Although all previously described iridoid glycosides contain *cis*-fused ring structures, (5αH)-6-epidihydrocornin (**1**), an iridoid containing a *trans*-fused cyclopentanopyrano ring system, was isolated from *Penstemon secundiflorus* [1, 2]. We now report two additional *trans*-fused iridoids, this time from *P. mucronatus* N. Holmgren.

RESULTS AND DISCUSSION

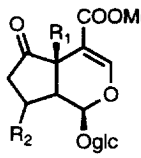
Dried aerial parts (53.8 g) of *P. mucronatus* from northwestern Colorado yielded 1.87 g of a purified iridoid glycoside mixture after extraction, differential solvent purification and VLC. Additional purifications by HPLC yielded the known iridoids hastatoside (**2**; 418 mg), 8-epihastatoside (**3**; 134 mg), β-dihydrohastatoside (**4**; 176 mg), penstemoside (**5**; 135 mg), and cornin (**6**; 12 mg). The mixtures also yielded the two new iridoids (5αH)-6α-8-epidihydrocornin (**7**; 45 mg) and (5αH)-6α-hydroxy-8-epiloganin (**8**; 6 mg). The known iridoids were identified by spectral comparison with standard isolated samples [2]. The new compounds were identified as follows.

(5αh)-6α-8-Epidihydrocornin (7)

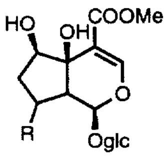
HRFABMS of **7**, $[\alpha]_D^{22}$ −73° (*c* 2.0, MeOH), established the molecular formula as $C_{17}H_{26}O_{10}$ (FABMS, $[M + H]^+$ 391.1609; calc 391.1604). The ^{13}C NMR spectrum showed 17 resonances, including six for the glucose moiety, two for the C-4 carbomethoxy group, and nine for the iridoid skeleton (Table 1). A C-10 methyl was indicated by the ^{13}C resonance at δ 17.1 and the δ 1.01 doublet in the ^1H NMR spectrum (Table 1). The δ 75.4 ^{13}C resonance suggested a carbon bearing an OH and HMQC correlated this resonance with the δ 4.04 ^1H resonance. This proton was coupled to the δ 2.64 ^1H resonance, assignable to H-5, and hence the OH was at C-6. Structures such as dihydrocornin,



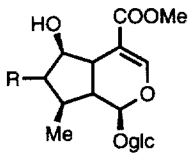
1: R_1 = β-Me, R_2 = H
7: R_1 = α-Me, R_2 = H
8: R_1 = α-Me, R_2 = OH



2: R_1 = OH, R_2 = β-Me
3: R_1 = OH, R_2 = α-Me
6: R_1 = H, R_2 = β-Me



4: R = β-Me
5: R = α-Me



9: R = β-OH
10: R = α-OH

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Table 1. NMR data for (5αH)-6α, 8-epidihydrocornin, **7**, (D_2O).

H or C	δ_H	δ_c
1	5.69 *d* (9.7)	102.7
3	7.51 *d* (2.4)	157.0
		109.4
5	2.64 *ddd* (13.0, 8.9, 2.3)	41.4
6	4.04 *ddd* (8.9, 6.5, 5.2)	75.4
7	2.54 *ddd* (13.9, 8.9, 5.3)	41.4
	1.24 *ddd* (13.9, 6.5, 2.7)	
8	2.25 *m*	30.0
9	1.91 *ddd* (13.1, 9.7, 7.1)	45.2
10	1.01 *d* (7.1)	17.1
OMe	3.69 *s*	52.9
C=O		171.6
1′	4.82 *d* (8.1)	100.7
2′	3.28 *dd* (9.2, 8.0)	73.6
3′	3.46 *dd* (9.2, 8.7)	76.8
4′	3.39 *dd* (9.8, 8.7)	70.4
5′	3.43 *m*	77.3
6′	3.85 *dd* (12.5, 1.8)	61.5
	3.68 *dd* (12.5, 5.1)	

^{1}H NMR HOD referenced to 4.73 ppm.
^{13}C NMR C-6′ referenced to 61.5 ppm.
Coupling constants in Hertz.

penstemonoside, or some isomer of these would fit the data. There were two anomalous resonances in the spectra which correlated with the previously known *trans*-fused iridoid (**1**) [1], rather than with *cis*-fused iridoids. Thus, the C-1 resonance was at δ 102.7 (δ 103 for **1**, compared to δ 96–100 for *cis*-fused iridoids) and the allylic coupling of the H-3/H-5 resonances was 2.4 Hz (2.1 Hz for **1**, compared to 0.5–1.5 Hz for *cis*-fused iridoids). The 9.7 Hz $J_{1,8\beta}$ is also diagnostice for *trans*-fused iridoids. A literature survey of NMR data for 15 *cis*-fused iridoids with 8β-Me groups showed $J_{1,8\beta}$ to typically be 1–3 Hz, with a maximum of 5.2 Hz in a single case. NOE enhancements were seen for H-6 → H-9/H-10/H-1/H-5, H-7β → H-7α/H-8 and H-7α → H-7β/H-10. This means that H-9, H-8 and H-6 are all on the β-face while H-1, H-5 and the C-10 methyl are on the α-face as depicted in **7**. According to models, there is greater rigidity and less flexibility in the *trans*-fused system than in the usual *cis*-fused iridoids and hence the NOE data should be conclusive.

(5αh)-6α-hydroxy-8-Epiloganin (**8**)

HRFABMS of **8**, $[\alpha]_D^{22}$ −57 (*c* 0.27, MeOH), established the molecular formula as $C_{17}H_{26}O_{11}$ (FABMS, [M + H] + 407.1557; calc. 407.1553), which suggested that an additional OH was present in **8** as compared to **7**. Consistent with this was the occurrence of two ^{13}C NMR resonances at δ 82.9 and 87.4 (Table 2) instead of the single resonance at δ 75.4 for **7** (Table 1). The formula would be consistent with a 6β-hydroxyloganin (**9**) structure, but **9** has the C-6 and C-7 resonances at δ 79.5 and 75.1, respectively [3]. On the other hand, there is a close correspondence with the δ 84.6 and 83.4 resonances reported for 6β-hydroxy-7-epiloganin, **10** (Table 2) [4]. The data therefore suggests similar, probably isomeric, structures for **8** and **10**. Irradiation of H-1 yielded an enhancement of the H-10 protons, suggesting the 8-epi stereochemistry, but the irradiation also yielded an enhancement of H-5. This would indicate another iridoid with the 5αH stereochemistry. Consistent with this were reciprocal NOE enhancements between the H-10 protons and H-5 when either was irradiated. The 2.1 Hz coupling constant between H-3 and H-5 (1.2 Hz for **10**) was also consistent with the 5αH formulation as were the C-1 carbon resonance at δ 101.8 (δ 96.1 for **10**) and the 9.3 Hz $J_{1,8\beta}$. H-6 irradiation gave an enhancement of H-9 and irradiation of H-7 gave enhancements of H-5 and H-10. These NOE experiments therefore established the stereochemistry for the final (5αH)-6α-hydroxy-8-epiloganin structure **8**.

The iridoid glucosides of *P. mucronatus* thus put it into the very small group of Rocky Mountain and nearby plains species which have cornin, hastatosides and/or *trans*-fused iridoids. All are in Section Anularis (= Coerulei). No species outside this section have been reported to contain such iridoids, but some others within the section (*P. angustifolius*, *P. cyathorphorus*) lack these compounds. *P. mucronatus* is said to vegetatively resemble *P. cyathophorus* and *P. osterhoutii* from nearby sites in western Colorado [5], but the iridoid content would make it more closely related to *P. nitidus*, *P. secundiflorus* [2, 6], and *P. grandiflorus* [6] of the Rocky Mountain front range.

Table 2. NMR data for (5αH)-6α-hydroxy-8-epiloganin (**8**) and 6β-hydroxy-7-epiloganin (**10**) (D_2O)

	8		**10***
H or C	δ_H	δ_c	δ_c
1	5.68 *d* (9.3)	101.8	96.1
3	7.51 *d* (2.1)	157.1	152.7
		108.9	110.8
5	2.73 *ddd* (12.9, 9.6, 1.8)	40.2	37.8
6	3.85 *m*	82.9	84.6
7	3.73 *dd* (4.7, 1.6)	87.4	83.4
8	2.04 *m*	39.8	40.0
9	2.18 *m*	42.8	43.3
10	1.06 *d* (7.6)	14.2	16.2
OMe	3.69 *s*	52.9	52.8
C=O		171.4	
1′	4.81 *d* (8.0)	100.7	99.4
2′	3.29 *dd* (9.2, 8.0)	73.6	73.5
3′	3.43 *dd* (9.2, 8.7)	76.7	76.4
4′	3.38 *dd* (9.8, 8.7)	70.4	70.4
5′	3.43 *m*	77.3	77.2
6′	3.83 *m*	61.5	61.5
	3.69 *m*		

^{1}H NMR HOD referenced to 4.73 ppm.
^{13}C NMR C-6′ referenced to 61.5 ppm.
Coupling constants in Hertz.
*Ref. [4]

EXPERIMENTAL

Plant material

Penstemon mucronatus (voucher REK III-32-20, Colorado State University Herbarium; identified by W.A. Weber, University of Colorado Museum, Boulder) was collected on June 1, 1995 along Deer Park Road at 2.82, 3.01 and 3.47 mi. from the intersection of Deer Park Road with State Highway 40 in Moffat Co., Colorado.

Isolation

Ground, air-dried, aerial parts (53.80 g) were macerated in MeOH three times over 24 h and the extracts combined and evaporated *in vacuo* to yield a crude extract of 9.97 g. The extracts were then reconstituted in H_2O and shaken with Et_2O. The aq layer was dried *in vacuo* and triturated with MeOH. This was then filtered to yield a crude iridoid glycoside/sugar residue of 8.40 g This residue was dissolved in 210 ml of H_2O and stirred with 42 g of activated charcoal for 15 min. The mixture was filtered with Celite and washed with 810 ml of H_2O. The H_2O was discarded. The charcoal was washed with 800 ml of MeOH, which was evaporated to yield 2.88 g of residue A. The charcoal was then stirred with 300 ml of 95% EtOH for 25 min. This mixture was filtered over Celite, the charcoal washed with 500 ml of 95% EtOH and evaporated to yield 1.91 g of residue B.

Residue A was dissolved in EtOH and fractionated by VLC in a 60 ml fritted funnel (50 ml fractions; TLC grade silica gel) with $CHCl_3$–MeOH. Fractions 9–13 ($CHCl_3$–MeOH, 10:1 to 9:1) were combined and evaporated to yield 952 mg of a complex mixture of iridoids. This mixture was rechromatographed using HPLC (gradient elution of 15–25% MeOH/H_2O) to yield **2** (193 mg), **3** (46 mg), **4** (14 mg), **5** (7 mg), **6** (12 mg) and **7** (25 mg). Fractions 14–18 ($CHCl_3$–MeOH, 9:1) were combined and evaporated to yield 431 mg of a mixture containing (^1H NMR) **2**, **3**, **4**, and **5** in the ratios 5:1:4:3.6. Fractions 19–24 ($CHCl_3$–MeOH, 9:1 to 8:1) were combined and evaporated to yield a 151 mg mixture of iridoids which was rechromatographed (HPLC; gradient elution of 15–25% MeOH/H_2O) to yield **2** (3 mg), **4** (6 mg), **5** (11 mg), and **8** (6 mg).

Residue B was dissolved in EtOH and fractionated by VLC in a 60 ml fritted funnel (50 ml fractions; TLC grade silica gel). The column was eluted with 200 ml of $CHCl_3$–MeOH (19:1) and then 100 ml of $CHCl_3$–MeOH (3:1). These eluents were combined and evaporated to yield a 344 mg residue which was rechromatographed (HPLC; gradient elution of 15–25% MeOH/H_2O) to yield **2** (62 mg), **3** (57 mg), **4** (33 mg), **5** (4 mg), and **8** (20 mg).

Ground, air-dried, roots (30.0 g) were macerated in MeOH three times over 24 h and the extracts combined and evaporated *in vacuo* to yield 2.48 g of residue. This was reconstituted in H_2O and extracted with Et_2O. The aq. layer was evaporated *in vacuo* and triturated with MeOH. The filtered MeOH was evaporated to yield a residue of 2.16 g, which was dissolved in 54 ml of H_2O and stirred with 11 g of activated charcoal for 25 min. This mixture was filtered with Celite and washed with 500 ml of H_2O. The H_2O was discarded. The charcoal was then stirred with 100 ml of 95% EtOH for 30 min, filtered over Celite, and washed with 200 ml of 95% EtOH. The EtOH was evaporated to yield 785 mg of residue shown by ^1H NMR to be composed of **2**, **3**, **4** and **5** in a 2.6:1.7:1:1.5 ratio.

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