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IRIDOID GLUCOSIDES FROM *PENSTEMON SECUNDIFLORUS* AND *P. GRANDIFLORUS*: REVISED STRUCTURE OF 10-HYDROXY-8-EPIHASTATOSIDE

HENRIK FRANZYK,* SØREN ROSENDAL JENSEN and FRANK R. STERMITZ†

Department of Organic Chemistry, The Technical University of Denmark, 2800 Lyngby, Denmark and †Department of Organic Chemistry, Colorado State University, Fort Collins, CO 80523, U.S.A.

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Key Word Index—*Penstemon secundiflorus*; *P. grandiflorus*; Scrophulariaceae; iridoid glucosides; 5,6β-dihydroxyadoxoside; revision of 10-hydroxy-8-epihastatoside; chemical correlations.

Abstract—Thorough large scale investigations of *Penstemon secundiflorus* ssp. *lavendulus* and of *P. grandiflorus* resulted in the isolation of the new iridoid glucosides 5,6β-dihydroxyadoxoside. The structure of 10-hydroxyepihastatoside has been revised to 10-hydroxyhastatoside using a chemical correlation. Also, the known iridoids (5αH)-6-epidihydrocornin, cornin, hastatoside, 8-epihastatoside, β-dihydrohastatoside, penstemoside, (5αH)-10-hydroxy-6-epidihydrocornin, 10-hydroxycornin and catalpol were isolated. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Penstemon secundiflorus Benth. is a highly variable Colorado *Penstemon* species. A population from Pueblo, Canon City, and eastern Arkansas River Valley has recently been returned to its previous designation *P. versicolor*, while the name *P. secundiflorus* was retained for the more widespread Colorado front range populations [1, 2]. A third, montane population, has recently been stated to be a "dwarf" variety so different that it may allow for its taxonomic separation as *P. secundiflorus* ssp *lavendulus* [2]. We have previously reported on the glycoside content (iridoids in particular) of the population near Pueblo, Colorado (*aka P. versicolor*), which remarkably was found to contain an iridoid glucoside with a *trans*-fused ring junction [3].

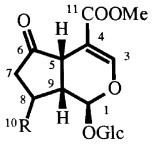
The iridoid content of a *P. secundiflorus* ssp *lavendulus* population has now been investigated for comparison with our findings for *P. secundiflorus* from near Fort Collins, Colorado [4]. Preliminary analytical scale examination of a number of different *Penstemon* species indicated that *P. grandiflorus* Nutt. from South Dakota might have a similar iridoid pattern and, thus, we also present the detailed results from this species.

*Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

The ethanol extracts of the two *Penstemon* species, *P. secundiflorus* ssp. *lavendulus* (fresh plant) and *P. grandiflorus* (dried plant) were partitioned between water and ether. The water-soluble part was subsequently treated with alumina and subjected to reverse phase vacuum liquid chromatography (RP-VLC) to remove mainly aromatics and salts/sugars, respectively. The partially purified extracts thus obtained contained almost exclusively iridoid glucosides. Many of these iridoids were obtained in pure or almost pure form by way of normal phase VLC using a high-capacity silica gel (TLC type). This proved a convenient method for a both fast and efficient separation of even large batches of crude iridoids (1–15 g) on relatively small columns (4–8.5 cm in both diameter and height). However, in some instances a final purification by reverse phase MPLC was necessary.

In both flowers and leaves of *P. secundiflorus* the following iridoids constituted the main components isolated: cornin (**1**), 10-hydroxycornin (**2**) and catalpol (**3**), while hastatoside (**4**), 10-hydroxyhastatoside (**5**), β-dihydrohastatoside (**6**), a novel dihydroxylated iridoid (**7**) and (5αH)-6-epidihydrocornin (**8**) [3] were less abundant. In addition, the leaves also contained another 5,9-*trans*-iridoid, 10-hydroxy-(5αH)-6-epidihydrocornin (**9**) [4], in small amounts. Due



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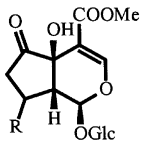
12 R = β-COOMe



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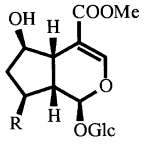

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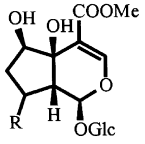

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7 R = β-CH_2OH

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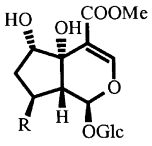

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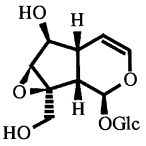

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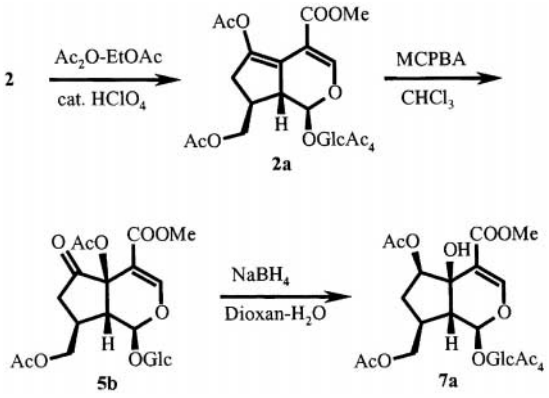

to the relative scarcity of extract available from the stems and roots, these were investigated only by ^1H and ^{13}C NMR. The stems exhibited an iridoid pattern very similar to the leaves, while the roots had **4** and **5** as major components and **1**, **2** and **7** were seen as minor constituents; traces of some unidentified iridoids were also observed.

Likewise, *P. grandiflorus* was examined with respect to its iridoid content in different plant parts; in this case the fruits and leaves/stems were worked up separately. The leaves/stems had **1** and **2** as the major iridoids, and **4**, **5**, **6** and **7** were present in lower amounts while the fruits in addition to these also contained small amounts of 8-epihastatoside (**10**) [3] and penstemoside (**11**) [5]. Notably, neither catalpol (**3**) nor any 5,9-*trans*-iridoids were found in this species.

Previously, a compound identified as 10-hydroxy-8-epihastatoside had been isolated from *P. secundiflorus* [3], but only as its pentaacetate. The stereochemistry at C-8 was based on NMR experiments showing an nOe between the C-10 methylene protons and H-1, indicating both to be on the same side of the bicyclic ring system (i.e. 8α-configuration). In the present work, we obtained a glucoside (**5**; giving a pentaacetate, **5a**, identical to the reported compound [3]) which was compared with similar compounds (see Table 1) by ^{13}C NMR. Thus, two known pairs **1**/**4** and **12**/**13** [6] of 6-ketoiridoids with varying 8β-substituents showed very similar difference spectra ($\Delta = \delta_{5\text{-OH}} - \delta_{5\text{-deoxy}}$, where the two δ-values may be for any of two analogous aglucone carbon atoms in a given 5-OH/5-deoxy pair). Moreover, the chemical shift of C-5 was significantly more low-field (2–3 ppm) in both **4** and **13** than in the known 8α-compound, **10** [3]. This observation prompted us to compare the above two pairs with **2**/**5** and indeed the difference spectrum as well as the chemical shift of C-5 of the 5-hydroxylated compound pointed strongly to a similar configuration at C-8 in all three pairs. Thus, to accommodate the ^{13}C NMR data, **5** appeared to be 10-hydroxyhastatoside and not the originally assigned 8-epimer. In order to settle this problem unequivocally, a chemical correlation between **2** [7] and **5** was undertaken. First, acetylation of **2** under acidic conditions afforded the peracetylated 5,6-enol-acetate derivative **2a**, which then was subjected to oxidation by *m*-chloroperbenzoic acid (MCPBA) [6] to afford a moderate yield of 10-hydroxyhastatoside hexaacetate (**5b**). Acetylation of the above natural **5** gave the known pentaacetate **5a** [3], while prolonged acetylation for 4.5 days yielded a 3:1-mixture of a hexaacetate and unreacted **5a**; this hexaacetate proved identical (NMR) to **5b** prepared from **2**. Thus, it has been shown that **5** is 10-hydroxyhastatoside and not 10-hydroxy-8-epihastatoside as previously assumed.

Also, a novel compound **7** was isolated from both *P. secundiflorus* ssp *lavendulus* and *P. grandiflorus*. From the ^{13}C NMR spectrum (see Table 2) it was readily identified as a usual C-4 substituted iridoid glucoside showing 17 signals, of which 6 signals were assigned to a β-glucopyranosyl moiety. Of the remaining signals assignable to the aglucone, two appeared at relatively low field (δ 76.2 and δ 74.4) inferring that two hydroxylated carbons were present in the cyclopentane ring. The ^1H NMR

Table 1. Comparison of ^{13}C NMR data (D_2O) for 5-deoxy/5-OH pairs of 6-ketoiridoids

	1*	**4***	Δ_{4-1}	**10†**	**12**	**13**	Δ_{13-12}	**2¶**	**5**	Δ_{5-2}
C-1	97.0	95.2	−1.8	95.7	96.9	95.3	−1.6	97.6	95.5	−2.1
C-3	154.4	157.4	+3.0	157.4	154.6	157.5	+2.9	154.9	157.6	+2.7
C-4	104.5	105.6	+1.1	107.4	104.0	105.4	+1.4	104.2	105.9	+3.5
C-5	43.5	74.6	+31.1	71.7	44.0	74.0	+30.0	44.2	74.3	+30.1
C-6	218.9	215.4	−3.5	215.6	215.5	212.0	−3.5	219.3	214.3	−5.0
C-7	43.8	40.9	−2.9	41.4	37.9	‖		38.4	35.5	−2.9
C-8	29.7	26.5	−3.2	25.1	39.8	36.2	−3.6	36.7	33.3	−3.4
C-9	45.0	52.2	+7.2	46.0	40.7	47.1	+6.4	39.9	47.2	+7.3
C-10	20.0	19.5	−0.5	17.5	176.3	175.4	−0.9	64.6	64.1	−0.5
C-11	170.0	168.2	−1.8	168.2	169.2	167.9	−1.3	170.0	168.2	−1.8
11-OMe	53.0	52.8	−0.2	52.8	52.9	52.8	−0.1	53.1	52.8	−0.3
C-1′	100.0	100.3		99.9	100.3	100.2		100.2	100.2	
C-2′	73.5	73.3		73.2	§	§		73.6	73.2	
C-3′	76.4	76.2		76.1	§	§		76.6	76.2	
C-4′	70.4	70.4		70.4	§	§		70.4	70.4	
C-5′	77.2	77.3		77.2	§	§		77.2	77.2	
C-6′	61.5	61.5		61.5	§	§		61.5	61.5	

*Essentially as in Ref. [6], but supplemented with sugar data.
†Data taken from Ref. [3].
§Not reported in Ref. [6].
‖Not reported in Ref. [6] due to ^2H exchange.
¶The signals at δ 38.4, 61.5 and 64.6 were shown to be methylenes by DEPT.

spectrum (see Experimental) combined with 1D ^1H decoupling experiments showed that these hydroxy groups could only be located at C-5 and C-6. Comparison (Table 2) of the ^{13}C NMR difference spectra [8] of the pair β-dihydrocornin (**14**) and **6** and of the pair 6β-hydroxyadoxoside (**15**) and **7** revealed a remarkably close fit for these two pairs. Hence, **7** was most likely 5,6β-dihydroxyadoxoside and acetylation under mild conditions yielded the expected hexaacetate **7a**. Final proof was obtained by performing a $NaBH_4$ reduction of **5b**, which surprisingly afforded **7a** as the sole product. This may be explained by a strong steric hindrance due to the neighboring 5-*O*-acetyl group so attack of hydride only occurs from below (i.e. adds to the 6α-position) followed by a fast migration of this acetyl group to the initially formed 6-alkoxy ion.

EXPERIMENTAL

General procedures

M.p.s uncorr.; ^1H NMR (300 and 500 MHz): glucosides in D_2O using the solvent peak (4.75 ppm) as internal standard; acetates in $CDCl_3$ (7.27 ppm); ^{13}C NMR: C-6′ was set to 61.5 ppm [8] in D_2O, while solvent peak (77.0) was used in $CDCl_3$; mass spectra (chemical ionization with NH_3 as reagent gas) were obtained on a VG Trio-2 (with direct inlet at 150°C); Prep TLC: 20 × 40 cm plates coated with 1 mm layers of silica gel PF_{254} (Merck); bands were detected in UV light (254 nm); reverse phase MPLC: Merck Lobar RP C-18 columns size B and C using H_2O–MeOH mixtures as eluents and peaks were detected by UV (240 nm). RP-VLC (reverse phase vacuum liquid chromatography) was per-

Table 2. Comparison of ^{13}C NMR data (D_2O) for 5-deoxy/5-OH pairs of 6β-OH-iridoids

	14	**6**	Δ_{6-14}	**15***	**7**	Δ_{7-15}
C-1	96.5	96.0	−0.5	97.3	97.0	−0.3
C-3	153.0	154.8	+1.8	153.5	156.1	+2.6
C-4	110.1	112.2	+2.1	109.4	110.6	+1.2
C-5	41.8	73.3	+31.5	41.0	74.4	+33.4
C-6	77.7	76.2	−1.5	76.9	76.2	−0.7
C-7	41.8	39.8	−2.0	35.7	33.4	−2.3
C-8	34.0	30.8	−3.2	42.6	39.0	−3.6
C-9	47.1	54.8	+7.7	41.6	49.4	+7.8
C-10	19.9	19.6	−0.3	65.9	65.9	0.0
C-11	170.5	169.4	−1.1	170.5	169.2	−1.3
11-OMe	52.7	52.8	+0.1	52.6	52.7	+0.1
C-1′	99.4	99.7		99.5	99.9	
C-2′	73.5	73.3		73.4	73.3	
C-3′	76.5	76.2		76.4	76.3	
C-4′	70.4	70.5		70.4	70.4	
C-5′	77.2	77.3		77.2	77.2	
C-6′	61.5	61.5		61.5	61.5	

*Values for the signals of C-5, C-8 and C-9 may be interchanged [9].

Table 3. ^{13}C NMR data ($CDCl_3$) of iridoid acetates (including DEPT)

	2a*	**5a†**	**5b§**	**7a¶**
C-1	97.3	93.6	92.5	95.9
C-3	154.0	153.0	154.8	153.1
C-4	105.4	108.0	103.6	111.0
C-5	111.3	73.0	77.1	74.0
C-6	142.8	207.9	202.6	77.9
C-7	36.0	35.1	36.3	30.6
C-8	37.1	30.1	31.0	35.5
C-9	44.5	47.0	44.3	48.8
C-10	65.5	65.4	65.3	67.8
C-11	164.9	165.6	164.3	166.2
11-OMe	51.6	51.9	51.7	51.8
C-1′	102.7	96.0	95.1	96.4
C-2′	70.8	70.5	70.1	70.6
C-3′	72.0	72.0	72.4	72.0
C-4′	68.0	68.0	67.9	68.1
C-5′	72.4	72.4	72.5	72.3
C-6′	61.5	61.5	61.5	61.5
CH$_3$C=O	20.8	20.7	20.9	21.3
	20.7	20.6	20.8	20.7
	20.6	20.4	20.6	20.6
	20.5		20.1	20.4
CH$_3$*C*=O	170.8	170.7	170.7	170.8
	170.5	170.6	170.6	170.5
	170.1	170.2	170.3	170.1
	169.3	169.7	169.4	169.8
	169.1	169.3	169.2	169.3
	168.3			

*Two close signals at δ 20.8, while δ 20.5 had double intensity.
†Essentially as in Ref. [3]; δ 20.6 and 20.7 had double intensity.
§Two close signals at δ 170.6, while δ 20.8 and 20.6 had double intensity.
¶Signal at δ 170.5 had double intensity.

formed using C-18 coated silica gel.; normal phase VLC (referred to as VLC) was performed using Sigma silica gel type H (10–40 μm), the columns were prewashed with hexanes and eluted with $CHCl_3$–MeOH mixtures. Plant material of *Penstemon secundiflorus* var. *lavendulus* (voucher FRS-530) was collected on June 14, 1997 in Chaffee County, Colorado (four miles south of Buena Vista off county road 329). Plants from this location were identified by W. A. Weber, University of Colorado at Boulder; *P. grandiflorus* (voucher FRS-528) was collected in June 1997 in Fall River County, South Dakota (8.3 miles south of US18 on SH17 at Hot Springs) and identified by C. Reed, Botanist, Hot Springs, South Dakota. The aerial parts of *P. secundiflorus* with a small amount of roots attached were separated into (i) flowers including the top of the stem, (ii) leaves, (iii) stems and (iv) roots, while *P. grandiflorus* was divided into fruits and leaves/stems.

Isolation procedure for P. secundiflorus

The flowers (76 g) were homogenized with 95% aq. EtOH (2 × 250 ml). The filtrates were concd and the residue partitioned in Et_2O–H_2O (2:1, 450 ml). The aq. layer was washed with Et_2O (150 ml) and concd to give a crude extract (11.1 g). Leaves (64 g) and stems (46 g) similarly afforded crude extract (6.5 and 2.7 g, respectively). Roots (7.5 g) were ground dry and then stirred overnight with 95% aq. EtOH (150 ml). Upon filtration the plant material was homogenized with 95% EtOH (150 ml). The filtrate was concd and the residue partitioned in Et_2O–H_2O; the aq. layer was concd to give a crude extract (0.70 g).

The crude from the flowers (11.1 g) was loaded (in 40 ml H_2O) onto an RP-VLC column (6 × 8.5 cm), which was eluted with H_2O (400 ml) and then with MeOH (2 × 400 ml). Concn of the MeOH-eluates gave a residue, which was redissolved in MeOH and filtered through act.C/Celite. Concn of the filtrate gave a residue (2.51 g) almost free of sugars. The crude leaf-, stem- and root-extracts were treated as above to give similar residues (4.43, 0.51 and 0.16 g, respectively).

The above flower-residue (2.51 g in 10 ml H_2O) was poured onto an Al_2O_3-column (4 × 4 cm, *ca.* 50 g, prewashed with water). Elution with H_2O (200 ml) afforded after concn an iridoid residue (1.56 g) with the verbascoside (detected by NMR prior to this treatment) and most other aromatic glycosides removed. Likewise, separate Al_2O_3-treatments (using 50, 20 and 10 g, respectively) of the leaf residue (4.43 g), stem residue (0.51 g) and root residue (0.16 g) gave similar iridoid residues (3.24, 0.36 and 0.08 g, respectively).

An aliquot of the Al_2O_3-treated flower extract (0.45 g) in EtOH–MeOH (4:1, 5 ml) was subjected to normal phase VLC (4 × 4 cm). Gradient elution: hexane, $CHCl_3$ and then $CHCl_3$–MeOH mixtures (10:1 to 5:1). This gave a 1:7-mixture of **8** and **1** (41 mg), **1** (44 mg), an impure fr. containing **1** and **4** (32 mg), impure **6** (36 mg), **2** (96 mg), a 1:1-mixture of **2** and **5** (57 mg) and a 5:1-mixture of **3** and **7** (135 mg).

The Al_2O_3-treated leaf extract (3.24 g) was similarly fractionated by VLC (4.5 × 6.5 cm). Gradient elution with hexane, $CHCl_3$, followed by $CHCl_3$–MeOH mixtures (8:1 to 4:1) afforded successively a 1:2-mixture of **8** and **1** (421 mg), **1** (648 mg), a 1:5-mixture of **1** and **4** (286 mg), a complex mixture (167 mg) of **4**, **6**, **9** and **2**, fr. A (980 mg), fr. B (262 mg) and fr. C (277 mg). Repeated VLC of fr. A (980 mg) yielded **9** (104 mg), **2** (691 mg) and fr. D (130 mg). Repeated VLC of fr. C (277 mg) gave a 1:1-mixture of **2** and **5** (109 mg) and fr. E (145 mg). MPLC of fr. B (262 mg) eluting with H_2O and then H_2O–MeOH (25:1 to 6:1) yielded fr. F (140 mg) and **7** (6:1, 30 mg). MPLC of an aliquot of fr. E (101 mg) yielded **5** (6 mg) and **7** (64 mg). Prep. TLC ($CHCl_3$–MeOH 3:1, reeluting the bands with MeOH) of fr. D and F (270 mg) yielded **2** (141 mg) and **5** (78 mg) as the slower moving band.

The partially purified stem and root extracts were examined by ^1H and ^{13}C NMR to determine their relative content of iridoid constituents. Stems: **1**, **2** and **4** (*ca.* 2:2:1) were major constituents while **5**

and **7** were minor components and only traces of **8** and **9** were observed. Roots: **4** and **5** (*ca.* 2:1) were major components, while **1**, **2** and **7** (*ca.* 2:1:1) were minor constituents. Traces of some unidentified iridoids were also present.

Penstemon grandiflorus

Dried leaves and stems (270 g) were homogenized in 95% EtOH (1.2 l) and the cake of plant material was washed with MeOH (0.6 l). Concn of the combined filtrates yielded a crude residue (20.0 g); a second extraction afforded additional extract (13.0 g). Fruiting inflorescences (335 g) were extracted as above (with half the amounts of solvents) to give a total crude extract (18.6 g). Separate partitioning of the two crudes between Et_2O and H_2O gave water-soluble extracts from the leaf/stem and inflorescence (27.3 and 11.4 g, respectively).

Treatment of the above two extracts with Al_2O_3 (200 g each) yielded leaf/stem and inflorescence residues (22.9 and 6.9 g, respectively) almost free of aromatic glycosides. The leaf/stem residue (22.9 g) was subjected to RP-VLC (6 × 8.5 cm) in two runs, each eluted with H_2O (250 + 200 ml) and then MeOH (700 ml). The second H_2O-frs were concd to yield a mixture of polar iridoids (4.84 g), whereas the MeOH-frs afforded a mixture of all iridoids present (14.0 g). Likewise, RP-VLC of the inflorescence residue (6.9 g) gave a mixture (3.42 g, from MeOH-eluate) almost free of sugars.

The polar leaf/stem mixture (4.84 g) was subjected to normal-phase VLC eluting with hexanes, $CHCl_3$ and then $CHCl_3$–MeOH mixtures (10:1 to 4:1) giving successive frs of impure **2** (313 mg) and a 9:1-mixture of **2** and **5** (1.59 g). The less polar and more polar frs were combined to fr. G (1.58 g). Similarly, VLC of the less polar leaf/stem mixture (14.0 g) eluting with $CHCl_3$–MeOH (12:1 to 8:1) afforded almost pure **1** (3.60 g), fr. H (2.06 g), almost pure **2** (3.13 g), while eluting with MeOH gave fr. I (3.27 g). Purification of fr. G and fr. I by MPLC afforded a 2:1-mixture of **2** and **5** (0.64 g) and then almost pure **7** (0.41 g). Also, MPLC of fr. H yielded **2** (47 mg), a 3:2-mixture of **4** and **1** (0.57 g), and **6** (0.52 g).

Similarly, VLC of the inflorescence residue (3.42 g) gave impure **1** (9:1, 0.52 g), fr. J (0.31 g), **2** (0.50 g) and then fr. K (0.41 g), while elution with MeOH gave impure **5** and **7** (0.83 g) in a 1:2-ratio. MPLC of fr. J gave **2** (70 mg), **10** (40 mg), **4** (30 mg), **1** (20 mg), **11** (30 mg) and **6** (30 mg); likewise fr. K yielded a 1:5-mixture of **5** and **2** (250 mg), **2** (10 mg), **7** (50 mg), almost pure **4** (20 mg), **1** (10 mg) and **6** (20 mg).

10-*Hydroxyhastatoside* (**5**)

Amorphous, $[\alpha]_D^{21}$ −277° (*c* 0.5, MeOH), ^{1}H NMR (500 MHz, D_2O): δ 7.82 (1H, *br s*, H-3), 5.99 (1H, *br s*, H-1), 3.68 (3H, *s*, 11-OMe), 3.65 (2H, *m*, 2 × H-10), 2.74 (1H, *dd*, *J* = 19.4 and 10.8 Hz, H-7a), 2.46 (1H, *br d*, *J* = 10.2 Hz, H-9), 2.18 (1H, *m*, H-8), 2.07 (1H, *dd*, *J* = 19.4 and 7.2 Hz, H-7b), 4.79 (1H, *d*, *J* = 8.5 Hz, H-1′), 3.91 (1H, *dd*, *J* = 12.3 and 2.2 Hz, H-6a′), 3.71 (1H, *dd*, *J* = 12.3 and 5.9 Hz, H-6b′), 3.49 (1H, obsc. by H-3′, H-5′), 3.48 (1H, *t*, *J* = 9.0 Hz, H-3′), 3.39 (1H, *br t*, *J* = 9.2 Hz, H-4′), 3.28 (1H, *dd*, *J* = 9.0 and 8.5 Hz, H-2′). ^{13}C NMR: see Table 1. Found C, 46.7; H, 5.8. $C_{17}H_{24}O_{12}\cdot H_2O$ requires: C, 46.6; H, 6.0%.

Acetylation of 10-*hydroxyhastatoside pentaacetate* (**5a**)

Acetylation of **5** for 2 h gave 10-hydroxyhastatoside pentaacetate (**5a**) [3], which was re-acetylated in pyridine–Ac_2O (1:1) for 4.5 days at room temp. yielding a 3:1-mixture of hexaacetate and unreacted **5a**, of which the former was shown to be identical (NMR) with compound **5b** chemically derived from **2** (see below).

5,6β-*Dihydroxyadoxoside* (**7**)

M.p. 137–138°C (from EtOH), $[\alpha]_D^{21}$ −110° (*c* 0.4 MeOH), ^{1}H NMR (500 MHz, D_2O): δ 7.66 (1H, *s*, H-3), 5.77 (1H, *d*, *J* = 2.0 Hz, H-1), 4.32 (1H, *dd*, *J* = 5.1 and 3.1 Hz, H-6), 3.74 (3H, *s*, 11-OMe), 3.68-3.61 (2H, *m*, 2 × H-10), 2.37 (1H, *dd*, *J* = 8.7 and 2.0 Hz, H-9), 2.00 (1H, *m*, H-7a), 1.93 (1H, *m*, H-8), 1.40 (1H, *br ddd*, *J* = 14.2, 4.5 and 3.1 Hz, H-7b), 4.75 (1H, obsc. by HOD-signal, H-1′), 3.91 (1H, *dd*, *J* = 12.3 and 2.0 Hz, H-6a′), 3.72 (1H, *dd*, *J* = 12.3 and 5.6 Hz, H-6b′), 3.49 (1H, *m*, H-5′), 3.49 (1H, *t*, *J* = 9.1 Hz, H-3′), 3.40 (1H, *br t*, *J* = 9.5 Hz, H-4′), 3.29 (1H, *dd*, *J* = 9.0 and 8.1 Hz, H-2′). ^{13}C NMR: see Table 2. Found C, 46.5; H, 6.2. $C_{17}H_{26}O_{12}\cdot H_2O$ requires: C, 46.4; H, 6.4%.

5,6β-*Dihydroxyadoxoside hexaacetate* (**7a**)

M.p. 109–110°C (from EtOH–hexane), $[\alpha]_D^{21}$ −73.5° (*c* 0.5, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.49 (1H, *s*, H-3), 5.60 (1H, *dd*, *J* = 5.0 and 3.3 Hz, H-6), 5.50 (1H, *d*, *J* = 3.4 Hz, H-1), 4.15–4.09 (2H, *m*, 2 × H-10), 3.78 (3H, *s*, 11-OMe), 2.50 (1H, *dd*, *J* = 7.1 and 3.4 Hz, H-9), 2.16 (1H, m, H-8), 1.99 (1H, obsc. by AcO's, H-7a), 1.56 (1H, *br dt*, *J* = 14.7 and 2× 3.3 Hz, H-7b), 5.25 (1H, *t*, *J* = 9.7 Hz, H-3′), 5.08 (1H, *t*, *J* = 9.7 Hz, H-4′), 5.01 (1H, *dd*, *J* = 9.7 and 8.0 Hz, H-2′), 4.83 (1H, *d*, *J* = 8,0 Hz, H-1′), 2.12, 2.10, 2.07, 2.04, 2.01 and 1.95 (each 3H, *s*, 6 × AcO). ^{13}C NMR: see Table 3. CIMS (NH_3 as reagent gas) *m*/*z* $[M + NH_4]^+$ 692, 657, 632, 615, calcd for $C_{29}H_{38}O_{18}$, 674.61. Found C, 51.2; H, 5.7. $C_{29}H_{38}O_{18}$ requires: C, 51.6; H, 5.7%.

Acid-catalyzed acetylation of 10-*hydroxycornin* (**2**)

Pure **2** (210 mg) was treated with Ac_2O (2 ml) in EtOAc (6 ml) with a cat. amount of 72% $HClO_4$ (1 drop) added. After 5 min at 0°C followed by 1.5 h at room temp., ice (*ca.* 2 g) and satd $NaHCO_3$ (1 ml) were added to the dark mixture. When the ice had melted, the mixture was diluted with EtOAc (50 ml) and washed with satd $NaHCO_3$ (30 ml) and brine (15 ml). Drying (Na_2SO_4) and concn gave a residue (358 mg), which was purified by VLC. Elution with hexanes and then hexanes–Me_2CO mixtures (10:1 to 5:1) afforded the (5,6-enol)hexaacetate **2a** (214 mg, 63%).

10-*Hydroxycornin* (5,6-*enol*) *hexaacetate* (**2a**)

Unstable syrup, $[\alpha]_D^{21}$ −18° (*c* 0.8, $CHCl_3$), ^1H NMR (500 MHz, $CDCl_3$): δ 7.35 (1H, *br s*, H-3), 5.14 (1H, *d*, *J* = 9.2 Hz, H-1), 4.26 (1H, *dd*, *J* = 11.2 and 4.0 Hz, H-10a), 4.10 (1H, *dd*, *J* = 11.2 and 6.7 Hz, H-10b), 3.70 (3H, *s*, 11-OMe), 2.75 (1H, *ddd*, *J* = 14.5, 7.5 and 1.5 Hz, H-7a), 2.68 (1H, *m*, H-9), 2.48 (1H, *m*, H-7b), 2.46 (1H, *m*, H-8), 5.23 (1H, *t*, *J* = 9.3 Hz, H-3′), 5.12 (1H, *t*, *J* = 9.7 Hz, H-4′), 5.02 (1H, *dd*, *J* = 9.1 and 7.8 Hz, H-2′), 4.92 (1H, *d*, *J* = 7.8 Hz, H-1′), 4.21 (H, *dd*, *J* = 12.5 and 4.4 Hz, H-6a′), 4.17 (1H, *dd*, *J* = 12.5 and 2.7 Hz. H-6′), 3.73 (1H, *ddd*, *J* = 10.0, 4.4 and 2.7 Hz, H-5′), 2.15, 2.09, 2.06, 2.04, 2.02 and 2.01 (each 3H, *s*, 6 × AcO). ^{13}C NMR: see Table 3. CIMS (NH_3 as reagent gas) *m*/*z* $[M + NH_4]^+$ 674, 632, 615, calcd for $C_{29}H_{36}O_{17}$, 656.59.

MCPBA-oxidation of **2a**

The above **2a** (199 mg) was dissolved in $CHCl_3$ (3 ml) and *m*-chloroperbenzoic acid (MCPBA, 82 mg, 1.1 eq. with assumed 70% activity). After 24 h (shielded from light), an additional amount of MCPBA (20 mg) was added and upon stirring for a further 8 h, a white ppt had formed. The mixture was diluted with CH_2Cl_2 (50 ml) and washed with satd $NaHCO_3$ (2 × 25 ml). Drying (Na_2SO_4) and concn of the org. layer gave a residue (177 mg), which was purified by VLC eluting with hexanes and then hexane–Me_2CO mixtures (5:1 to 2.5:1) to yield 10-hydroxyhastatoside hexaacetate (**5b**, 73 mg).

10-*Hydroxyhastatoside hexaacetate* (**5b**)

M.p. (decomp.) 188–190°C (from Me_2CO–hexane), $[\alpha]_D^{21}$ −249° (*c* 0.4, $CHCl_3$), ^1H NMR (500 MHz, $CDCl_3$): δ 7.66 (1H, *br s*, H-3), 5.76 (1H, *d*, *J* = 1.6 Hz, H-1), 4.22-4.16 (2H, *m*, 2 × H-10), 3.73 (3H, *s*, 11-OMe), 3.35 (1H, *dd*, *J* = 11.2 and 1.6 Hz, H-9), 2.57 (1H, *dd*, *J* = 19.0 and 9.6 Hz, H-7a), 2.44 (1H, *dd*, *J* = 19.0 and 10.0 Hz, H-7b), 2.21 (1H, *m*, H-8), 5.21 (1H, *t*, *J* = 9.5 Hz, H-3′), 5.13 (1H, *t*, *J* = 9.7 Hz, H-4′), 5.02 (1H, *dd*, *J* = 9.5 and 8.3 Hz, H-2′), 4.86 (1H, *d*, *J* = 8.3 Hz, H-1′), 4.32 (1H, *dd*, *J* = 12.5 and 4.3 Hz, H-6a′), 4.16 (1H, *dd*, *J* = 12.5 and 2.3 Hz, H-6b′), 3.76 (1H, *ddd*, *J* = 10.0, 4.3 and 2.3 Hz, H-5′), 2.12, 2.10, 2.10, 2.04, 2.01, 1.96 (18 H, 6 × OAc). ^{13}C NMR: see Table 3. Found C, 51.6; H, 5.3. $C_{29}H_{36}O_{18}$ requires: C, 51.8; H, 5.4%.

$NaBH_4$-reduction of **5b**

The above **5b** (73 mg) was dissolved in dioxane (10 ml) and then $NaBH_4$ (8 mg in 1 ml H_2O) was added. Upon stirring at room temp. for 45 min, 5% HOAc (2 ml) was added and the mixture subsequently diluted with EtOAc (75 ml). The mixture was then washed with H_2O (50 ml) and satd $NaHCO_3$–brine (1:1, 50 ml). Drying (Na_2SO_4) and concn of the org. layer yielded almost pure **7a** (61 mg), which by NMR was shown to be identical to the sample prepared above from the natural compound.

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REFERENCES

1. Kelso, T. and Lawton, J., *Bull. Am. Penstemon Soc.*, 1996, **55**(2), 14.
2. Weber, W. A. and Wittman, R. C., *Colorado Flora: Eastern Slope*. University Press of Colorado, Niwot, 1996, p. 362.
3. Foderaro, T. A. and Stermitz, F. R., *Phytochemistry*, 1992, **31,** 4191.
4. Krull, R. E., Stermitz, F. R., Franzyk, H. and Jensen, S. R., *Phytochemistry*, **49**(6), 1605.
5. Berg, T., Damtoft, S., Jensen, S. R., Nielsen, B. J. and Rickelt, L. F., *Phytochemistry*, 1985, **24,** 491.
6. Jensen, S. R. and Nielsen, B. J., *Phytochemistry*, 1980, **19,** 2685.
7. Teborg, D. and Junior, P., *Planta Med.*, 1991, **57,** 184.
8. Damtoft, S., Jensen, S. R. and Nielsen, B. J., *Phytochemistry*, 1981, **20,** 2717.
9. Gardner, D. R., Narum, J., Zook, D. and Stermitz, F. R., *J. Nat. Prod.*, 1987, **50,** 485.