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Pensteminoside, an unusual catalpol-type iridoid from *Penstemon gentianoides* HBK (Plantaginaceae) ☆

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Abstract

From the MeOH and ethyl acetate extracts of aerial parts of *Penstemon gentianoides* HBK (Plantaginaceae) an unusual iridoid of the catalpol-type, was isolated and characterized as pensteminoside: (8-*O*-*trans*-cinnamoyl, 6-hydroxy, 1-[*β*-D-glucopyranoside-6′-*O*-((4″-hydroxy)-cinnamoyl)]-catalpol) was isolated, along with the known iridoids: plantarelanoside and globularisicin, the flavone: luteolin and diosmetin, as well the phenylpropanoids, verbascoside and martynoside. Their structures were established by 1D and 2D NMR spectroscopic analyses.

Keywords: *Penstemon gentianoides*; Plantaginaceae; Iridoids; Flavonoids; Phenyl propanoids; Structural elucidation

1. Introduction

In continuation of our general screening program of Mexican flora with biological activities (Céspedes et al., 2005, 2006a,b), an examination of the extracts of *Penstemon gentianoides*, formerly Scrophulariaceae now Plantaginaceae (Judd et al., 2002) tribe Cheloneae (Albach et al., 2005), was initiated. Previous work on the aerial parts of *P. gentianoides* indicated that the ethyl acetate soluble fraction from the methanolic extract possessed significant antioxidant activity (Domínguez et al., 2005). On this basis, the phytochemical analysis of the ethyl acetate extract from the leaves of *P. gentianoides* was undertaken.

The Plantaginaceae family is characterized by a great number of iridoids (Boros and Stermitz, 1990), and the botanic and ecological characteristics of *P. gentianoides* (HBK) Poiret, Lindl. Don. (Plantaginaceae), were reported previously (Domínguez et al., 2005).

This plant has not been studied ethnobotanically or phytochemically, but is popularly used as an ethnomedicine which suggests that it is useful as an anti-inflammatory agent against different ailments. In Mexico, the dried roots and leaves are used as an anti-inflammatory infusion. In our phytochemical investigation of the leaves of this plant, we have isolated and elucidated the structure of a new catalpol-type iridoid pensteminoside **1**. Furthermore, we obtained six known compounds: Plantarenaloside **2** (Damtoft et al., 1981; Armandodoriano et al., 1982), globularisicin **3** (Chaudhuri and Sticher, 1981), luteolin **4** (Nissler et al., 2004), diosmetin **5** (Mabry et al., 1970), verbascoside **6** (Arciniegas et al., 1997; Pardo et al., 1993; Liu et al., 1998; Akdemir et al., 2004) and martynoside **7** (Teboring and Junior, 1989; Calis et al., 1984) (Fig. 1). All these compounds were identified by means of spectroscopic analyses, using ^{1}H NMR, ^{13}C NMR, and 2D NMR, with chemical correlations and comparisons to literature data. On this basis, the assignments of known

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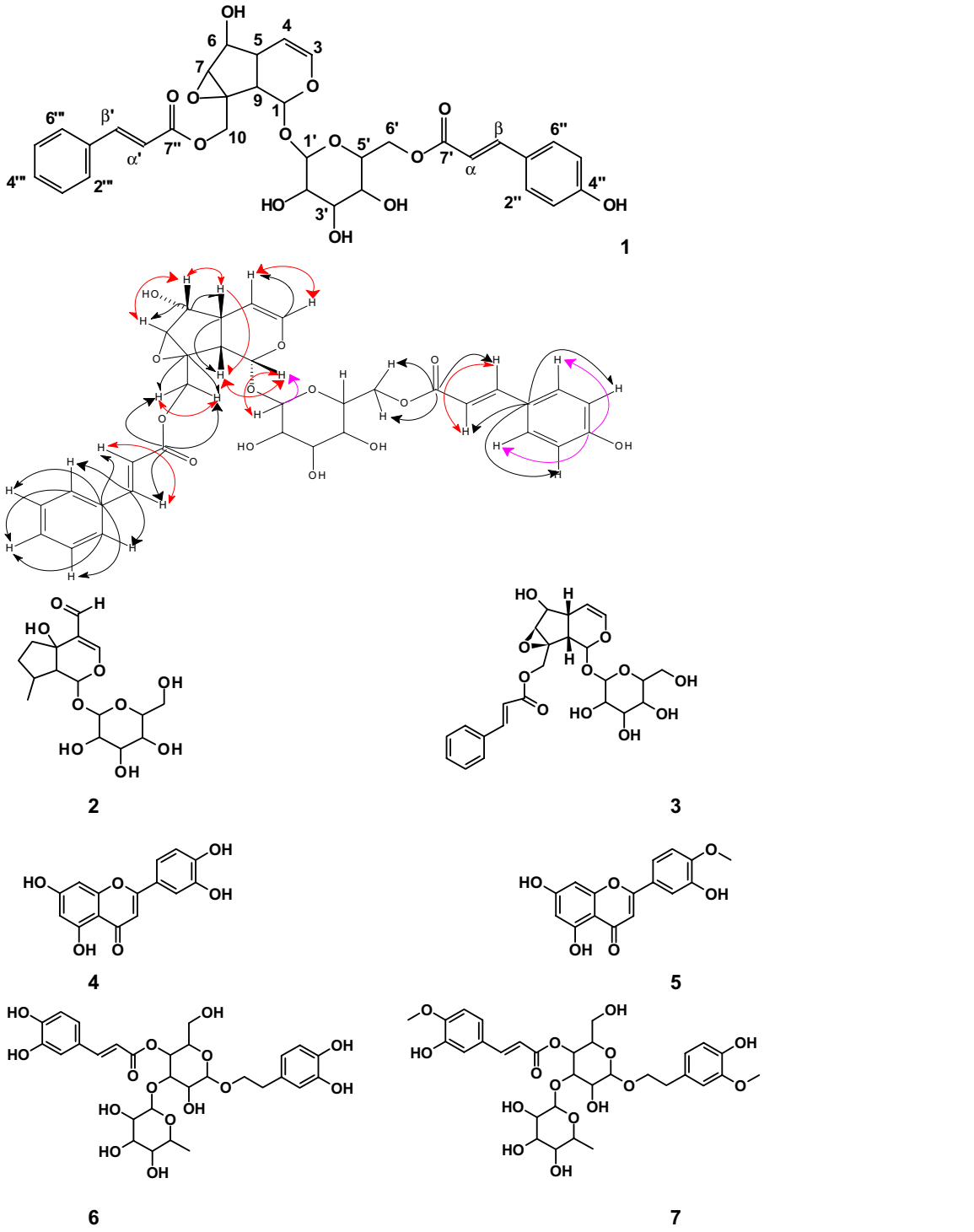

Fig. 1. Chemical structures of pensteminoside (**1**), its numbering and the results of correlations COLOC (→), FLOCK (⟶) and NOESY (⟶) experiments, plantarenaloside (**2**), globularisicin (**3**), luteolin (**4**), diosmetin (**5**), verbascoside (**6**) and martynoside (**7**).

compounds were made. This is the first phytochemical report of *P. gentianoides*.

2. Results and discussion

The new catalpol-type iridoid (Pensteminoside **1**) was isolated as a brown powder and was slightly hygroscopic. The UV spectrum exhibited absorption bands characteristic of an iridoid system and cinnamoyl chromophores (Nguyen et al., 2005; Calis et al., 2001). The IR spectrum also revealed the presence of hydroxyl (3263, 3207 cm^{-1}), (2935–2561 cm^{-1}), unsaturated bonds (2500–1900 cm^{-1}), aromatic (711–624 cm^{-1}). The molecular formula was determined to be $C_{33}H_{34}O_{13}$on the basis of FAB and high resolution MS (*m*/*z* 639); moreover, HR ESI-MS of **1**

exhibited a pseudomolecular ion peak $[M+Na]^+$ at *m/z* 661.19035 suggesting a molecular formula of $C_{33}H_{34}O_{13}$, which was confirmed by the observation of two methylenes, 26 methines and five quaternary carbon resonances in the ^{13}C NMR and DEPT spectra (Table 1). The UV and IR signals are typical for catalpol-type structure.

The ^1H and ^{13}C NMR spectroscopy data were consistent with a C-10 iridoid monoglucoside moiety. The complete assignment of all proton and carbon resonances were based on the analyses of COSY, HETCOR, FLOCK, COLOC and NOESY experiments (Fig. 1 and Table 1). Analysis of the ^1H NMR spectrum (500 MHz, acetone d_6) shows a doublet at δ_H4.78 (1H, *d*, $J = 8$ Hz, H-1′) characteristic of an anomeric proton suggesting the presence of one *β*-glucopyranosyl unit. Moreover, the vicinally coupled olefinic protons at δ_H6.32 (1H, *dd,* $J = 1.5$, 6 Hz) and δ_H5.02 (1H, *dd,* $J = 5$, 6 Hz) were assigned to H-3 and H-4, respectively, typical of an iridoid catalpol unit; additionally the chemical shifts at δ_H2.24 (*dddd,* $J = 2$, 3, 5, 7.5 Hz, H-5) and δ_H 2.52 (*dd,* $J = 7.5$, 10 Hz, H-9) showed that the fused rings are *cis*, which was confirmed by NOESY experiments.

The ^{13}C NMR (DEPT) (Table 1) spectrum of **1** displayed signals for: two methylene (δ_C 63.74 C-10, δ_C 63.49 C-6′) and ten methines, with two of the latter characteristic carbons of an iridoid of the catalpol-type δ_C38.88 and δ_C43.29 (C-5 and C-9); one anomeric δ_C100 (C-1′); six oxygenated carbons (C-2′, C-3′, C-4′, C-5′, C-6 and C-7) and one characteristic carbon at downfield δ_C 95.25 (C-1) supporting a glucose moiety, as well as, four quaternary carbons including one aromatic hydroxylated (C-1″, C-1‴, C-4″, and C-8) and two carbonyl esters, six olefinic (C-3, C-4, C-*α*, C-*β*, C-*α*′ and C-*β*′) carbons and nine aromatic carbons (δ_C 129.81–116.78 ppm, Table 1).

An additional analysis showed four *trans* olefinic protons according to the coupling constants observed as two AB systems. One was centered at δ_H 6.50 and δ_H 7.67 (*d*, $J = 16$ Hz, C-*α*′ and C-*β*′) and the other at δ_H 6.38 and δ_H 7.63 (*d*, $J = 16.5$ Hz, C-*α*′ and C-*β*′). These belong to two *trans*-cinnamoyl moieties which were confirmed by assignment of the remaining signals that corresponded to nine aromatic protons between δ_H 6.83 and δ_H 7.62. Four aromatic protons (δ_H 7.54 *d* and δ_H 6.83 *d*, AA′BB′ system $J = 8.5$, each 2H, H-2″, H-6″ and H-3″, H-5″) were charac-

Table 1
^1H and ^{13}C spectroscopy data of new iridoid catalpol-type

Assignments		δ C^{13} (ppm) 300 MHz	δ H^1 (ppm) *J* (Hz) 500 MHz	COLOC	FLOCK	NOESY
Aglycone	1	95.25	4.95 *d*10		H-9, H-1′	H-1′, H-9, H-5
	3	141.53	6.32 *dd*1.5, 6	H-4	H-4	H-4
	4	103.89	5.02 *dd*5, 6		H-5, H-6	H-3, H-9, H-5
	5	38.88	2.24 *dddd*2, 3, 5, 7.5	H-9		H-9
	6	79.33	3.89 *d*8.5	H-5, H-7	H-9, H-7	H-5, H-7
	7	62.01	3.46 *m*			H-6
	8	62.77		H-10		
	9	43.29	2.52 *dd*7.5, 10	H-5		H-5, H-1
	10	63.74	a 4.13, b 5.07*d*12.5			H-10
Glucosyl	1′	100.00	4.78 *d*8		H-2′, H-1	H-2′
	2′	74.64	3.29 *t*8.5	H-4′		
	3′	77.66	3.46 *m*	H-5′, H-2′, H-4′	H′2, H-4′	
	4′	70.96	3.46 *m*			
	5′	75.33	3.58 *m*			
	6′	63.49	4.45 *dd*3, 8.5			
Hydroxycinnamoyl	1″	127.07	–	α,H3″, H-5″	α,H3″, H-5″	
	2″	131.10	7.54 *d*8.5		*β*	
	3″	116.78	6.83 *d*8.5			
	4″	160.62	–		H-6″, H-2″, H-3″, H-5″.	
	5″	116.78	6.83 *d*8.5			
	6″	131.10	7.54 *d*8.5		*β*	
	α	115.61	6.38 *d*16		*β*	H-*β*
	β	145.62	7.63 *d*16	H-2″, H-6″		H-α
	COO 7′	167.55	–	*β*, H-6′	*β*′	
Cinnamoyl	1‴	135.53	–	α′, 5‴, 3‴		
	2‴	129.81	7.62 *dd*2.5, 6	H-4‴		
	3‴	129.13	7.40 *m*	*β*′		
	4‴	131.10	7.40 *m*			
	5‴	129.13	7.40 *m*	*β*′		
	6‴	129.81	7.62 *dd*2.5, 6	H-4‴		
	α′	119.07	6.50 *d*16		*β*′	H-*β*′
	β′	145.53	7.67 *d*16	H2‴, H6‴		H-α′
	COO 7″	166.74	–	*β*′ H-10a, H-10b		

teristic of a 4-hydroxy cinnamoyl moiety, this being corroborated by a downfield signal at δ_C 160.62 in ^{13}C NMR spectrum (C-4″, Table 1). Additionally, in the FLOCK experiment this carbon (C-4″), was coupled with δ_H 7.54 (H-2″ and H-6″) and δ_H 6.83 (H-3″ and H-5″) protons. Furthermore, δ_C127.07 (C-1″), had a coupling with δ_H 6.83 (2H, *d*, $J = 8.5$, H-3″ and H-5″) and 6.38 (1H, *d*, $J = 16$ Hz, H-α) that confirmed the 4″-hydroxy position. Five additional aromatic protons at δ_H 7.40 (3H, *m*, H-3‴, H-4‴ and H-5‴) and 7.62 (2H, *dd*, $J = 2.5$, 6 Hz, H-2‴ and H-6‴) allowed us to deduce the presence of a cinnamoyl group (Fig. 1, Table 1).

The location of the β-glucosyl unit, 4″-hydroxycinnamoyl and cinnamoyl groups were determined using COLOC and FLOCK experiments (Fig. 1). The position of the sugar linkage to the aglycone was established to be at the C-1 of the catalpol unit by long-range coupling between δ_C 95.25 and the proton signal at δ_H4.78 (H-1′). COLOC correlations were observed between both the carbonyl ester group (δ_C 167.55, C-7′) and the olefinic proton at δ_H7.63 (1H, *d*, $J = 16$ H-β), with glucose methylene group at δ_H4.45 (2H, *dd*, $J = 4$, 7, H-6') defined the acylation of 4″-hydroxy-*trans*-cinnamoyl at C-6′ (Table 1).

The carbonyl group at δ_C 166.74 (C-7′) of the *trans*-cinnamoyl moiety in the COLOC spectrum displayed long-range correlations with the doublet at δ_H 7.67 (1H, *d*, $J = 16$ Hz, H-β′), with the two methylene protons at δ_H 4.13 and δ_H 5.07 (2H, *d*, $J = 12.5$ Hz, H-10a and H-10b), respectively. Likewise, there was an interaction between the methylene H-10 and the carbon resonance at δ_C 63.74 indicating that the esterification site of this moiety with catalpol unit is at C-8.

Thus this compound was identified as 8-*O*-*trans*-cinnamoyl, 6-hydroxy, 1-[β-D-glucopyranoside-6′-*O*-((4″-hydroxy)-cinnamoyl)]- catalpol (**1**), that we have named as pensteminoside (Fig. 1). To the best of our knowledge, there are no previous reports of this molecule in the literature.

3. Experimental

3.1. General experimental procedures

IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer, whereas ^1H and ^{13}C NMR spectra were recorded at 300 and 500 MHz, and 75 and 125 MHz, respectively, on Varian VXR-300S and VXR-500S spectrometers. Chemical shifts (ppm) are given relative to TMS, with $CDCl_3$, MeOH-d_4, and acetone-d_6 from Aldrich Chemical Co. used as solvents. Coupling constants are in Hz. ESI low resolution: Instrument: double focussing sectorfield mass spectrometer. Manufacturer: Thermofinnigan MAT (Bremen, Germany), model: MAT95XLT, resolution: 3000 (10% valley definition), scan: 50–1200 amu (2 s/decade), ES-Device: modified microESI supplied by the manufacturer (the original fused silica sample transfer capillary was replaced by stainless steel tubing of similar dimensions (SMS Service für Massenspektrometrie GmbH, Idstein, Germany)). The non-conductive ferrule that holds the capillary was replaced by a graphite ferrule. Concentration: approx. 50 μg/mL, solvent: MeOH (unless otherwise stated), flow: approx. 1 μL/min, typical sprayvoltage pos. mode: 1.3–1.8 kV, typical sprayvoltage neg. mode: 1.1–2.3 kV. EI accurate mass method: peak matching, resolution: 10000 (10% valley definition), mass calibrant: PPG (appropriate amount mixed with sample). In addition, EIMS data were determined on a JEOL JMS-AX505HA mass spectrometer at 70 eV. FABMS were obtained on a JEOL JMS-SX102A mass spectrometer operated with an acceleration voltage of 10 kV. Samples were desorbed from a nitrobenzyl alcohol matrix using 6 KeV Xenon atoms. UV spectra of pure compounds were determined on a Shimadzu UV-160 instrument. Optical rotation was measured on a JASCO DIP-360 spectropolarimeter. Melting points were obtained on a Fisher–Johns hot-plate apparatus and are uncorrected.

3.2. Chemicals and solvents

All reagents used were analytical grade. Methanol, CH_2Cl_2, silica gel GF_{254} analytical chromatoplates, silica gel grade 60, (70–230, 60 Å) were used for cc, *n*-hexane and EtOAc were purchased from Merck-Mexico, S.A., Mexico. Column chromatography was also carried out on Silica Gel G (Merck, Darmstadt, Germany).

3.3. Plant material

P. gentianoides (HBK) Poiret, Lindl. Don. was collected on the highest hills (above 3000 m) within the Park "Los Dinamos", near Mexico City, in October 2002. The plants were identified botanically by Professor Francisco Ramos (Instituto de Biología, UNAM) and voucher specimens were deposited at the Herbarium of the Biology Institute at UNAM (MEXU), Gen: 7508, G-No. 7. The collected plants were air-dried and prepared for extraction.

3.4. Extraction and isolation

Leaves of *P. gentianoides* were dried (1.2 kg), milled and extracted with MeOH 100% (5 L) at room temperature. The MeOH extract (180 g) (**A**) was dried and redissolved in MeOH–H_2O (6:4), then partitioned with *n*-hexane (**C**), CH_2Cl_2 (**D**) and EtOAc (**E**), leaving a residue (**B**). The EtOAc partition (46.22 g) was further fractionated into eight fractions (**I–VIII**), by open cc using silica gel (type G, 10–40 μm, Sigma-Aldrich), (Dominguez et al., 2005). Elution was carried out with *n*-hexane–EtOAc in different ratios and adding MeOH to increase the polarity of the gradient until 100% MeOH was reached. All fractions were analyzed by TLC using ceric sulfate as the development system.

Fraction **III** was subjected to cc as above using *n*-hexane, EtOAc, and MeOH successively as eluents, gradually increasing the polarity of the gradient. Seven sub-fractions were obtained. Fractions 6–12 (*n*-hexane–EtOAc 1:1) contained compounds **4** (19 mg) and **5** (200 mg), as yellow powders. These compounds were separated by vacuum cc with silica gel G 10–40 μ using a CH_2Cl_2–Me_2CO elutant system (6:4). From the same fraction, compound **1** (48.5 mg) was eluted with EtOAc–MeOH (8:2) and precipitated with cold acetone. Fraction **IV** was purified using Sephadex LH-20: *n*-hexane–CH_2Cl_2–MeOH 2:1:1 to yield compound **3** (110 mg). Fraction **V** was purified on a silica gel G column with CH_2Cl_2–MeOH (9:1) as eluting system to yield compound **7** (40 mg), and with CH_2Cl_2–MeOH (8:2) system was obtained compound **6** (60 mg). Furthermore, compound **6** was purified by silica gel TLC using CH_2Cl_2–MeOH (7:3) as elutant. Compound **2**was isolated from the MeOH crude extract by cc using EtOAc–MeOH (8:2) as elutant.

4. Data

Pensteminoside 1: UV λ (MeOH) nm: 216, 231 and 277. $[\alpha_D]$: −0.058 (582 nm), 35.0 mg/mL. IR (cm^{-1}): 3263, 3207, 2935, 2561, 1725, 1457, 1024, 712, 624. MS: (FAB) (HRMS) (*m*/*z*): 639 $[M^+]$ ($C_{33}H_{34}O_{13}$), 613, 569, 525, 481, 437, 393, 391, 349, 307 (25%), 289 (15%), 273, 242, 165, 154 (100%), 139 (67%), 107 (20%), 89 (19%), 77 (18%), 55, 39. ^1H NMR and ^{13}C NMR data are in Table 1. HR ESI-MS *m*/*z*: 661.19035 $[M+Na]^+$. Calculated value 638.62516.

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