



## CAFFEOYL ESTERS OF THREONIC ACID AND ITS LACTONE FROM *VIGUIERA PAZENSIS*

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Received August November 11, 2008. In final form February 2, 2009.

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### Abstract

Two caffeoyl esters of sugar derivatives: 3-*O*-caffeoyl-2-*C*-methyl-*D*-threono-1,4-lactone, 4-*O*-caffeoyl-2-*C*-methyl-*D*-threonic acid, along with the previously known compounds: 2-*C*-methyl-*D*-threono-1,4-lactone, caffeic acid, carabrone, 5,7,3'-trihydroxy-4'-methoxyflavone, and 5,7,3'-trihydroxy-6,4'-dimethoxyflavone were isolated from the aerial parts of *Viguiera pazensis*. Their structures were elucidated by application of various spectroscopic methods, including 1D and 2D NMR spectroscopy.

**Keywords:** *Viguiera pazensis*; Asteraceae; 3-*O*-caffeoyl-2-*C*-methyl-D-threono-1,4-lactone; 4-*O*-caffeoyl-2-*C*-methyl-D-threonic acid; 2-*C*-methyl-D-threono-1,4-lactone.

## Resumen

Dos ésteres de derivados de azúcares con ácido cafeico: 3-*O*-cafeoil-2-*C*-metil-D-treono-1,4-lactona, ácido 4-*O*-cafeoil-2-*C*-metil-D-treónico, junto con los compuestos previamente conocidos: 2-*C*-metil-D-treono-1,4-lactona, ácido cafeico, carabrona, 5,7,3'-trihidroxi-4'-metoxiflavona y 5,7,3'-trihidroxi-6,4'-dimetoxiflavona fueron aislados de la parte aérea de *Viguiera pazensis*. Las estructuras fueron determinadas utilizando una combinación de métodos espectroscópicos, incluyendo espectroscopia de RMN en una y dos dimensiones.

**Palabras clave:** *Viguiera pazensis*; Asteraceae; 3-*O*-caffeoyl-2-*C*-methyl-D-threono-1,4-lactone; 4-*O*-caffeoyl-2-*C*-methyl-D-threonic acid; 2-*C*-methyl-D-threono-1,4-lactone.

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## Introduction

The large genus *Viguiera* (Asteraceae) [1] seems to be characterized by the occurrence of sesquiterpene lactones and diterpenes [2, 3, 4, 5, 6, 7, 8].

In the present study, the EtOH extract of the aerial parts of *Viguiera pazensis* yielded two caffeoyl esters of sugar derivatives: 3-*O*-caffeoyl-2-*C*-methyl-D-threono-1,4-lactone (**1**) and 4-*O*-caffeoyl-2-*C*-methyl-D-threonic acid (**2**), together with the known compounds: 2-*C*-methyl-D-threono-1,4-lactone (**3**) [9, 10, 11], caffeic acid, carabrone [12], 5,7,3'-trihydroxy-4'-methoxyflavone (diosmetin) [13], and 5,7,3'-trihydroxy-6,4'-dimethoxyflavone (desmethoxycentaureidin) [14].

In previous articles about this species, [15, 16] was reported the isolation of other typical secondary metabolites of the genus *Viguiera*.

The structures of the new compounds were determined using a combination of spectroscopic techniques, including multinuclear and multidimensional NMR spectroscopy. The identity of the known compounds was established by comparison of their physical and spectroscopic data with those reported in the literature.

## Experimental

### *General experimental procedures.*

<sup>1</sup>H NMR and 2D NMR experiments were measured on a Bruker Avance DMX-500 NMR spectrometer operating at 500.13 MHz (<sup>1</sup>H) and 125.76 MHz (<sup>13</sup>C) using standard Bruker software. High-resolution ESI-MS spectra were recorded using an Agilent 6520 Accurate-Mass Q-TOF mass spectrometer, with an ESI source in the negative ion mode; the samples were injected in a solution of MeOH containing 0.1% formic acid at a flow rate of 200 µl/min. IR spectra were recorded on KBr disks, using an IR-FT Bruker model IFS-88 spectrometer. UV spectra were registered in a Beckman spectrophotometer. Optical rotation was measured in a Jasco J-810.

### Plant material

*Viguiera pazensis* was collected in March 2003, Department of Cachi, Salta Province, Argentina. The plant material was identified by Ing. Lázaro J. Novara. A voucher specimen (N°11953), is deposited at the Museum of the Facultad de Ciencias Naturales, Universidad Nacional de Salta, Argentina.

### Extraction and isolation

The air-dried aerial parts of *V. pazensis* (677.2 g) were exhaustively extracted in a Soxhlet using hexane and 96% EtOH, for a period of 12 h. The EtOH extract was concentrated at reduced pressure. The resulted residue was successively treated with CHCl<sub>3</sub> and EtOAc. The CHCl<sub>3</sub> extract (1.85 g) was then fractionated by silica gel VLC, eluting with benzene and AcOEt (100 mL) of increasing polarity (10 %) to afford from benzene-AcOEt (3:2-1:1) solvent system: carabrone (3.2 mg), diosmetin (1.5 mg) and desmethoxycentaureidin (1.2 mg).

The AcOEt extract (2.1 g) was subjected to silica gel C-18 reversed-phase (23 g) CC (2 x 18 cm) eluted with 100 mL of MeOH-H<sub>2</sub>O (7:3) as eluent, to give three fractions. F1 was submitted to silica gel flash chromatography (CHCl<sub>3</sub>-MeOH, 10:1) followed by Sephadex LH-20 to yield 2-*C*-methyl-*D*-threono-1,4-lactone (**3**) (6.2 mg). F2 was purified by Sephadex LH-20 and silica gel flash chromatography (CHCl<sub>3</sub>-MeOH, 10:1) to gave 4-*O*-caffeoyl-2-*C*-methyl-*D*-threonic acid (**2**) (25.0 mg) and caffeic acid (1.1 mg). F3 was purified by Sephadex LH-20 to afford 3-*O*-caffeoyl-2-*C*-methyl-*D*-threono-1,4-lactone (**1**) (28.1 mg).

### 3-*O*-caffeoyl-2-*C*-methyl-*D*-threono-1,4-lactone (**1**)

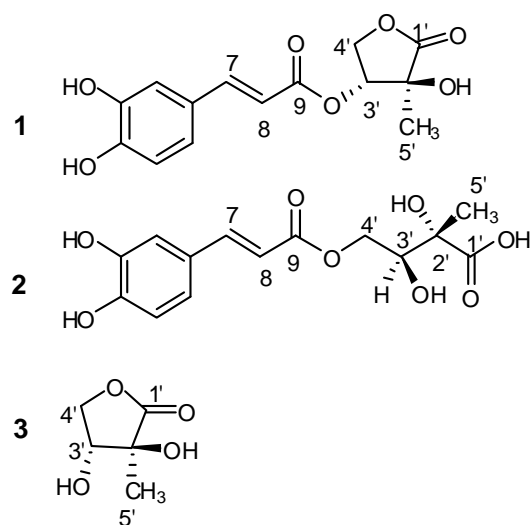
Amorphous powder,  $[\alpha]_D^{18.6} - 63.76^\circ$  (c 0.80, MeOH). UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 213 (4.62), 245 (4.51), 302 (4.61), 332 (4.73); IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3398 (OH), 1784 (C=O lactone), 1703 (C=O ester), 1639, 1599, 1282, 1178, 1111. <sup>1</sup>H (500.13 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C (125.76 MHz) NMR spectroscopic data, see Table 1. HR-ESI-MS  $m/z$  293.06680 [M-H]<sup>-</sup> (Calcd. for C<sub>14</sub>H<sub>13</sub>O<sub>7</sub> 293.06613).

Hydrolysis of **1** with 2 N HCl: Compound **1** (18 mg) in 2 N HCl (2 mL) was left for 1 hr at 90 °C. After neutralization with 1 N NaOH, the solution was concentrated and the residue was extracted with AcOEt.

### 4-*O*-caffeoyl-2-*C*-methyl-*D*-threonic acid (**2**)

Amorphous powder,  $[\alpha]_D^{18.6} + 15.05^\circ$  (c 0.40, MeOH). UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 206 (4.23), 218 (4.25), 245 (4.05), 300 (4.15), 325 (4.25); IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3387 (OH), 1690 (C=O acid and ester, broad), 1630, 1523, 1449, 1283, 1182, 1117. <sup>1</sup>H (500.13 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C (125.76 MHz) NMR spectroscopic data, see Table 1. HR-ESI-MS  $m/z$  311.07732 [M-H]<sup>-</sup> (Calcd. for C<sub>14</sub>H<sub>15</sub>O<sub>8</sub> 311.07669).

Hydrolysis of **2** (16 mg) with 2 N HCl: same as for compound **1**.



## Discussion

Compound **1**, was obtained as a powder, with an  $[\alpha]_D^{18.6} - 63.76^\circ$  (c 0.80, MeOH). Negative mode HR-ESI-MS revealed a molecular formula of  $C_{14}H_{14}O_7$  from the pseudomolecular ion  $C_{14}H_{13}O_7$  corresponding to the  $[M-H]^-$  peak at  $m/z$  293.06680, and additional negative ions at  $m/z$  179.03443 and 135.04453, assigned to the losses of ( $[M-H]^-$  - [-O-threonolactone]) and ( $[M-H]^-$  - [-O-threonolactone + C=O]). The presence of a saturated  $\gamma$ -lactone moiety was observed in the IR spectrum by the carbonyl group signal at  $1784\text{ cm}^{-1}$ . The combined analysis of the IR (see experimental), the  $^1\text{H}$  NMR and the  $^{13}\text{C}$  NMR spectra (Table 1) suggested the presence of a caffeoyl group attached to the C-3 hydroxyl group of a 2-methyl-2,3,4-trihydroxy- $\gamma$ -butirolactone moiety. These evidences were further supported by COSY, HSQC and HMBC NMR spectral analysis. The lactone moiety was clearly evidenced by the chemical shifts and coupling constants of an AMX system corresponding to H-4'a, H-4'b and H-3' (Table 1). The high proton chemical shift of H-3' at  $\delta$  5.30 ( $\delta_C$  77.1) in  $\text{CD}_3\text{OD}$ , suggested that the caffeoyloxy group was attached to C-3'. This was confirmed in the HMBC experiment by the correlation between H-3' and C-9 (caffeoyl carbonyl group at  $\delta_C$  167.7). In addition, the three-bond proton-carbon cross-correlation peaks for C-1' ( $\delta_C$  178.2) with both H-3' and H-4' evidenced the ring closure.

The lactone group corresponds to the 2-C-methyl-D-threonolactone. The structure of this sugar lactone was confirmed by hydrolysis of **1** with 2 N HCl, showing the spectral data (IR and NMR) identical to those of literature [10, 11]. Based on the above evidences, the structure of compound **1** is proposed to be 3-O-caffeoyl-2-C-methyl-D-1,4-threonolactone.

Compound **2** was isolated as a powder,  $[\alpha]_D^{18.6} + 15.05^\circ$  (c 0.40, MeOH). Its IR spectrum showed a broad absorption band at  $1690\text{ cm}^{-1}$  assigned to the vibration of the carbonyl group. The negative mode HR-ESI-MS showed an  $[M-1]^-$  at  $m/z$  311.07732 (pseudomolecular ion  $C_{14}H_{15}O_8$ ) suggesting a molecular formula of  $C_{14}H_{16}O_8$ , indicating the addition of an  $\text{H}_2\text{O}$  molecule respect to compound **1**. The IR,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (Table 1) suggested the presence of a caffeoyl ester attached to a 2-methyl-2,3,4-trihydroxybutyric acid. The HMBC experiment showed common correlations for carbonyl C-9 (caffeoyl group) at  $\delta_C$  169.2 with H-7, H-8, H-4'a and H-4'b, clearly indicating that the caffeoyloxy group was

attached to C-4', evidences that together with the difference of 18 Da in the molecular weight respect to compound **1** strongly support the opening of the lactone ring. These data led us to unequivocally propose the esterification to be at 4-hydroxyl group of the 2-methyl-2,3,4-trihydroxy- $\gamma$ -butyric acid.

**Table 1:**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of compounds **1**, **2** and **3** ( $\text{CD}_3\text{OD}$ , TMS as internal standard).

	<b>1</b>		<b>2</b>		<b>3</b>	
	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$
<b>1</b>	127.4	-	127.6	-		
<b>2</b>	115.3	7.06 (d, 1.8)	115.2	7.04 (d, 1.9)		
<b>3</b>	146.9	-	146.6	-		
<b>4</b>	150.0	-	149.5	-		
<b>5</b>	116.4	6.78 (d, 8.2)	116.3	6.78 (d, 8.1)		
<b>6</b>	123.2	6.97 (dd, 8.2, 1.8)	122.7	6.95 (dd, 8.1, 1.9)		
<b>7</b>	148.2	7.60 (d, 15.8)	146.8	7.60 (d, 15.9)		
<b>8</b>	113.6	6.30 (d, 15.8)	114.9	6.30 (d, 15.9)		
<b>9</b>	167.7	-	169.2	-		
<b>1'</b>	178.2	-	179.2	-	180.0	-
<b>2'</b>	74.7	-	76.9	-	75.7	
<b>3'</b>	77.1	5.30 (dd, 5.0, 3.2)	75.1	4.0 (m)	75.4	4.17 (5.4, 4.4)
<b>4'a</b>	71.0	4.69 (dd, 10.2, 5.0)	66.2	4.45 (dd, 11.0, 1.5)	72.7	4.49 (dd, 9.3, 5.4)
<b>4'b</b>		4.19 (dd, 10.2, 3.2)		4.20 (dd, 11.0, 7.5)		3.97 (dd, 9.3, 4.4)
<b>5'</b>	18.1	1.42 (s)	23.1	1.43 (s)	17.8	1.35 (s)

$^1\text{H}$  500.13 MHz ( $J$  values are in parentheses and reported in Hz, chemical shift are given in ppm).

$^{13}\text{C}$ : 125.76 MHz. Assignment were confirmed by HSQC and HMBC experiments.

The acid group of sugar moiety in compound **2** corresponds to the 2-*C*-methyl-D-threonic acid, this residual structure was confirmed by the spectral data of the 2-*C*-methyl-D-threono-1,4-lactone, obtained by induced lactonization from hydrolysis of **2** with 2 N HCl. From the above evidences the structure of compound **2** is proposed to be 4-*O*-caffeoyl-2-*C*-methyl-D-threonic acid.

The structural characterization of compound **3**, 2-*C*-methyl-D-threono-1,4-lactone (2*S*, 3*R*) with an  $[\alpha]_{\text{D}}^{18.5} - 23.23^\circ$  (c 0.3466, MeOH) and  $[\alpha]_{\text{D}}^{18.5} - 10.32^\circ$  (c 0.3466, H<sub>2</sub>O),  $^1\text{H}$  and  $^{13}\text{C}$  NMR (see Table 1), was carried out by comparison with literature data [9, 10], and by the characteristic chemical shift of the 2-*C*-methyl group in  $\text{CD}_3\text{OD}$  of the D-threonolactone (**3**) at  $\delta_{\text{C}}18.0$  [11].

Therefore, the present work, to the best of our knowledge, constitutes the first report of the natural occurrence of the caffeoyl esters of the threonolactone and of the threonic acid. The free threonolactone (**3**) was also isolated, which was previously reported only as a synthetic product [9, 17, 18, 10, 11].

## Conclusions

The natural occurrence of caffeoyl sugar erythronolactone has only been reported from *Bidens pilosa* (Asteraceae), together with the corresponding caffeoyl erythronic acids [19]. This lactone moiety was characterized as erythronolactone, previously reported as a natural product in higher plants [20, 21, 22, 23, 24, 25], and was thought to be a plant growth regulator [26], while to our knowledge there are no biological studies on its diastereomer **3**. The occurrence of this type of caffeoyl esters in two very distantly related genera of the Asteraceae is worthy of note for chemotaxonomic and ecological studies.

## Acknowledgements

This work was supported with funds from the Consejo de Investigación, Universidad Nacional de Salta, Argentina. Work at the Universidad Nacional de Córdoba was supported by grants from CONICET, FONCYT and SECyT-UNC, Argentina. We thank NSF (CHE-0130903) for partially supporting the NMR instrumentation at Carnegie Mellon University (USA).

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