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Iridoid keto-glycosides from *Caryopteris* × *Clandonensis*

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Abstract

Two new iridoid keto-glycosides, clandonoside and 8-*O*-acetylclandonoside, together with their hydrates, were isolated from the stems of *Caryopteris* × *Clandonensis* besides the known harpagide and 8-*O*-acetylharpagide. The structure of clandonoside and 8-*O*-acetylclandonoside were established as harpagide-aglucone-1-*O*-β-D-ribohexo-3-ulopyranoside and 8-*O*-acetylharpagide-aglucone-1-*O*-β-D-ribohexo-3-ulopyranoside, respectively, by spectroscopic methods. This is the first report of a C-1 linked β-D-ribohexo-3-ulopyranose (β-D-ketohexose) iridoid. © 1999 Elsevier Science Ltd. All rights reserved.

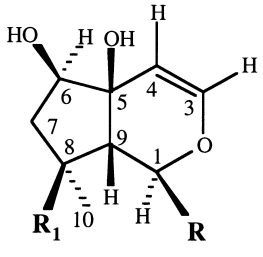
Keywords: Caryopteris × *Clandonensis*; Verbenaceae; Iridoid keto-glycosides; Hydrated keto-glucose; Clandonoside; 8-*O*-acetylclandonoside; Harpagide; 8-*O*-acetylharpagide

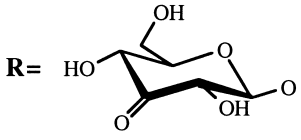
1. Introduction

Caryopteris × *Clandonensis* Simmonds, also called Bluebeard, is a hybrid between *C. incana* (Thumb.) Miq. and *C. mongholica* Bunge. It is a deciduous shrub with a silvery blue green foliage and lavender-blue flowers valued to gardeners because of its bloom in late summer and autumn, the time of year that few shrubs are flowering. So far, the only chemical investigations reported for this plant is one on α-caryopterone, a pyranojuglone (Matsumoto, Mayer, & Eugster, 1969) and one on the detection of acetylharpagide by chromatography (Kooiman, 1975). Here, we report the isolation and structural elucidation of two iridoid keto-glycosides, clandonoside (**2**) and 8-*O*-acetylclandonoside (**4**), together with the known iridoids harpagide (**1**) and 8-*O*-acetylharpagide (**3**). Clandonoside and its acetylated derivate are new iridoids containing a very uncommon carbohydrate moiety.

2. Results and discussion

The MeOH extract of dried stems of *C.*× *Clandonensis* gave a residue containing several iridoids (detected with vanillin sulfuric reagent as reddish spots). The major iridoid compounds **1** and **3** were

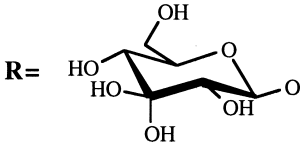




keto form (**A**)

R_1 = OH :
clandonoside (**2**)

R_1 = OAc :
8-O-acetylclandonoside (**4**)



hydrated form (**B**)
only present in aqueous solution

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isolated and identified respectively as harpagide and 8-*O*-acetylharpagide by comparing their spectral data with those in the literature (El-Naggar & Beal, 1980; Boros & Stermitz, 1990).

Compounds **2** and **4** were both obtained as a white amorphous powder. ^{1}H and ^{13}C NMR spectra (including HMQC, HMBC, COSY and NOESY) for the two compounds displayed patterns of peaks very similar to that of harpagide for **2** and 8-*O*-acetylharpagide for **4** except for the sugar moiety, indicating the identity of the aglycones. Signals due to the sugar of **2** and **4** were identical, thus **2** and **4** had the same carbohydrate moiety (Table 1).

The molecular mass of **2** (*m*/*z* 362) and **4** (*m*/*z* 404), were two units less than those of **1** and **3** (respectively 364 and 406) which implied an unsaturation in the sugar moiety. The signal at δ 207.7 in the ^{13}C NMR spectrum, arising from a carbonyl function, suggested the presence of a keto sugar. Correlation observed in the HMBC spectrum between this carbonyl and two deshielded protons attributable to H-2′ (δ 4.32) and H-4′ (δ 4.37) indicated that sugar moiety is a 3-keto-hexose. The chemical shifts of C-1′ to C-6′ and H-1′ to H-6′ of **2** and **4** were in good agreement with the data reported in the literature and confirmed the structure of the carbohydrate as ribohexo-3-ulopyranose (Junior, 1984; Gering, Junior, & Wichtl, 1987; Iwagawa & Hase, 1989; Morris, Hoppe, & Kiely, 1989). The sugar moiety is C-1 linked, as it shown in the HMBC spectrum by the correlation between C-1 and H-1′. The β-configuration of this keto-hexose was deduced from the coupling constant ($J = 8.2$ Hz) of the anomeric proton at δ 4.8 (Junior, 1984; Gering et al., 1987; Iwagawa & Hase, 1989). The stereochemistry of **2** and **4** was deduced from the NOESY spectra by comparison with those of harpagide (**1**) and 8-*O*-acetylharpagide (**3**). All NOE enhancements were identical for the four aglycones. These data led to the conclusion that compound **2** was harpagide-aglucone-1-*O*-β-D-ribohexo-3-ulopyranoside and compound **4** was 8-*O*-acetylharpagide-aglucone-1-*O*-β-D-ribohexo-3-ulopyranoside, respectively named clandonoside (**2**) and 8-*O*-acetylclandonoside (**4**).

In addition, NMR spectra recorded in D_2O showed a split of the signals and more specially those of the 3-keto-hexose (the split did not appear in ^{1}H NMR spectra recorded in CD_3OD), which implicated a modification for the sugar in D_2O. It has already been shown that some keto-sugars in aqueous solution exist as the hydrated form (Morris et al., 1989; Andersen, Lundt, Marcussen, Søtofte, & Yu, 1998). Concerning the splith signals, the quaternary carbon at δ 97.0 arising from a $>C(OH)_2$ group correlating with two protons attributable to H-2′ (δ 3.47) and H-4′ (δ 3.34) suggested the presence of the hydrated form (**B**) of the ribohexo-3-ulopyranose (**A**). These results were confirmed by ESI mass spectra of **2** and **4**, which, when recorded in a mixture of water and methanol, showed peaks at 18 units more than those attributable to **2A** and **4A**.

In order to determine the conformation of the sugar moiety, the coupling constants for all the protons involved were calculated. The large coupling constant values for $^{3}J_{\text{H-1′, H-2′}}$ and $^{3}J_{\text{H-4′, H-5′}}$ (8.2 and 10 Hz,

Table 1
^{1}H NMR and ^{13}C NMR data for compounds **2A**, **2B**, **4A** and **4B** in D_2O (δ in ppm, *J* in Hz)

Position	δ H				δ C			
	2A	**2B**	**4A**	**4B**	**2A**	**2B**	**4A**	**4B**
1	5.76, d (1.0)	5.71 d (1.0)	6.12, d (1.2)	6.05, d (1.2)	95.7	95.5	96.8	96.6
3	6.34, d (6.5)	6.34, d (6.5)	6.42, d (6.4)	6.41, d (6.4)	143.9	143.9	145.0	145.0
4	5.03, dd (6.5, 1.4)	5.03, dd (6.5, 1.4)	4.98, dd (6.3, 1.7)	4.98, dd (6.3, 1.7)	109.2	109.0	107.6	107.5
5	–	–	–	–	73.8	73.8	75.0	75.1
6	3.80, dd (4.4, 4.6)	3.80, dd (4.4, 4.6)	3.82, t (4.3)	3.82, t (4.3)	79.0	79.0	78.9	78.9
7a	1.81, dd (14.2, 4.2)	1.81, dd (14.2, 4.2)	2.00, ddd (15.7, 4.3, 1.8)	2.00, ddd (15.7, 4.3, 1.8)	48.1	48.1	47.0	47.0
7b	1.98, dd (14.1, 4.8)	1.98, dd (14.1, 4.8)	2.14, dd (15.7, 4.0)	2.14, dd (15.7, 4.0)				
8	–	–	–	–	79.7	79.7	90.6	90.6
9	2.59, s	2.54, s	2.90, s	2.84, s	59.6	59.6	55.7	55.8
10	1.23, s	1.22, s	1.43, s	1.42, s	26.7	26.7	24.0	24.0
C=O	–	–	–	–	–	–	177.0	176.9
OAc	–	–	2.07, s	2.03, s	–	–	24.4	24.4
1′	4.85, d (8.2)	4.80, d (8.2)	4.87, d (8.2)	4.81, d (8.2)	102.2	100.4	102.6	100.7
2′	4.32, dd (8.2, 1.6)	3.34, d (8.2)	4.30, dd (8.2, 1.7)	3.33, d (8.2)	78.7	76.0	78.7	76.0
3′	–	–	–	–	208.7	97.0	208.7	97.0
4′	4.37, dd (10.3, 1.6)	3.47, d (10.0)	4.38, dd (10.3, 1.7)	3.47, d (10.0)	74.7	73.0	74.7	73.0
5′	3.59, ddd (10.3, 4.8, 2.0)	3.57, ddd (10.0, 5.7, 2.1)	3.55–3.61, m	3.55–3.61, m	78.9	78.0	78.9	78.0
6′a	3.83, dd (12.5, 4.8)	3.69, dd (12.3, 5.8)	3.83, dd (12.3, 4.7)	3.71, dd (12.4, 5.7)	63.3	63.6	63.2	63.6
6′b	3.98, dd (12.5, 2.0)	3.88, dd (12.3, 2.0)	4.01, dd (12.7, 2.1)	3.90, dd (12.4, 2.2)				

respectively) revealed that the protons at 1′, 2′, 4′ and 5′ must be close to trans diaxial. Therefore, the keto-sugar and its hydrated form must have a chair-like conformation. To our knowledge, this is the first report treating of hydrated β-D-ribohexo-3-ulopyranose iridoids.

So far, keto-glycoside iridoids have only been reported from three plant sources, namely *Penstemon serrulatus*, *P. confertus* (Scrophulariaceae) (Junior, 1984; Gering et al., 1987) and *Viburnum suspensum* (Caprifoliaceae) (Iwagawa & Hase, 1989), comprising three compounds with this sugar moiety attached to C-11 of the iridoid aglucone. The presence of a sugar moiety different from β-D-glucopyranosyl (or a derivative thereof) at the 1-position of an iridoid glucoside is extremely rare (Boros & Stermitz, 1991). The occurrence of **1** and **2** in the genus *Caryopteris* has apparently only been shown by chromatography (Kooiman, 1975; von Poser, Toffoli, Sobral, & Henriques, 1997), and the present work has confirmed the presence of these compounds in the genus.

3. Experimental

3.1. Instrumentation

^{1}H NMR (400 MHz) and *J* mod. ^{13}C NMR (100 MHz) were obtained with D_2O as solvent. Chemical shifts are given in δ (ppm) with HDO (^{1}H, δ 4.77) as int. standard; ESI-MS: 3.5 kV (MeOH–H_2O).

3.2. Plant material

Caryopteris × *Clandonensis* Simmonds were purchased from a commercial supplier in Toulouse, France. Identification was made by Pr I. Fourasté (Lab. de Biodiversité Végétale, Faculty of Pharmaceutical Sciences, Toulouse) and a voucher specimen (CC-9709) was deposited at the Pharmacognosy Department of the Faculty of Pharmaceutical Sciences, University of Toulouse (France).

3.3. Isolation of clandonoside **2** and 8-*O*-acetylclandonoside **4**

The dried and powdered stems (500 g) were extracted with MeOH (10 l) at room temp. After evap of the MeOH, the residue (50 g) was taken up in H_2O and extracted with $CHCl_3$. The aq. phase was chromatographied on Amberlite XAD-4 with H_2O and MeOH to give a crude MeOH iridoid extract (5 g). The purification was achieved by further CC on silica-gel (70–200 μm) with a gradient of $CHCl_3$–MeOH. Final purification was achieved on reverse phase chromatography (C18 cartridge) with H_2O–MeOH gradient affording **1** (250 mg), **2** (20 mg), **3** (400 mg) and **4** (10 mg).

3.3.1. Clandonoside **2**

$C_{15}H_{22}O_{10}$; white amorphous powder; UV λ_{max} nm: 204; positive ESI-MS *m/z*: 385 $[M+Na]^+$, 401 $[M+K]^+$; ^{1}H NMR, ^{13}C NMR: Table 1.

3.3.2. Clandonoside hydrate **2B**

$C_{15}H_{24}O_{11}$; positive ESI-MS *m/z*: 403 $[M+Na]^+$, 419 $[M+K]^+$; ^{1}H NMR; ^{13}C NMR: Table 1.

3.3.3. 8-*O*-acetylclandonoside **4**

$C_{17}H_{24}O_{11}$; white amorphous powder; UV λ_{max} nm: 203; positive ESI-MS *m/z*: 427 $[M+Na]^+$; 443 $[M+K]^+$; ^{1}H NMR, ^{13}C NMR: Table 1.

3.3.4. 8-*O*-acetylclandonoside hydrate **4B**

$C_{17}H_{26}O_{12}$; positive ESI-MS *m/z*: 445 $[M+Na]^+$; 451 $[M+K]^+$; ^{1}H NMR, ^{13}C NMR: Table 1.

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