PERGAMON

Phytochemistry 54 (2000) 807–809

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Iridoids from *Caryopteris* x *clandonensis*

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Received 11 October 1999; received in revised form 14 February 2000

Abstract

In continuation of our phytochemical studies on *Caryopteris* x *clandonensis* (Lamiaceae), three further iridoids were isolated from the methanolic extract of the stems. Their structures were established by 1D and 2D NMR and MS analysis as a C-6 epimer of 8-*O*-acetylharpagide (6-*epi*-8-*O*-acetylharpagide), a derivative of harpagide which contained the unusual feature of a 3′,4′ *seco*-glycopyranosyl moiety (clandonoside II) and a methyl cetal of 8-*O*-acetylharpagide aglucone hydrate named clandonensine.

Keywords: *Caryopteris* x *clandonensis*; Lamiaceae; Verbenaceae; Iridoids; *Seco*-glycoside; 6-*epi*-8-*O*-acetylharpagide; Clandonoside II; Clandonensine; 8-*O*-acetylharpagide

1. Introduction

The genus *Caryopteris*, as recently delimited, comprises seven eastern Asian species and a horticultural hybrid, *C.* x *clandonensis* Simmonds (Cantino et al., 1999) Traditionally included in the family Verbenaceae, this genus has been transferred, with a large part of the former Verbenaceae, to the Lamiaceae (Cantino et al., 1992).

In early work on the constituents of the methanolic extract of dried stems of *Caryopteris* x *clandonensis*, six harpagide type iridoids were isolated, i.e. the well known harpagide and 8-*O*-acetylharpagide; clandonoside and 8-*O*-acetylclandonoside together with their hydrates, which were keto-glycosides (Hannedouche et al., 1999).

In continuation of our investigations on the iridoid composition of *C.* x *clandonensis*, we isolated three further new minor iridoids. Their structures were established as 6-*epi*-8-*O*-acetylharpagide (**1**), a derivative of harpagide, which contained the unusual feature of a 3′,4′ *seco*-glycopyranosyl moiety, named clandonoside II (**2**) and a methyl cetal of 8-*O*-acetylharpagide aglucone hydrate named clandonensine (**3**).

2. Results and discussion

6-*epi*-8-*O*-acetylharpagide (**1**) was obtained as a white amorphous powder with *Mr* 406 (DCI mass spectrum: 424 $[M + NH_4]^+$) compatible with the molecular formula of 8-*O*-acetylharpagide. The ^1H NMR spectrum of **1** (Table 1) supported an iridoid structure closely related to this one. The major differences were the chemical shifts of the signals belonging to H-6 at δ 4.27 (*dd*, $J = 12.5$, 6.6 Hz) and the AB-system assignable to H-7a and H-7b, indicating a difference of stereochemistry at C-6 between these two compounds. The H-6 α-configuration of 8-*O*-acetylharpagide was well known and confirmed by an NOE between H-6 and H-4. For **1**, this NOE enhancement was not observed but one existed between H-6 and H-9, thus assigning a β configuration for H-6. The structure of 6-*epi*-8-*O*-acetylharpagide was therefore proposed for **1** on the basis of the above data.

Clandonoside II (**2**) was obtained as a white amor-

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0031-9422/00/$ - see front matter
PII: S0031-9422(00)00069-8

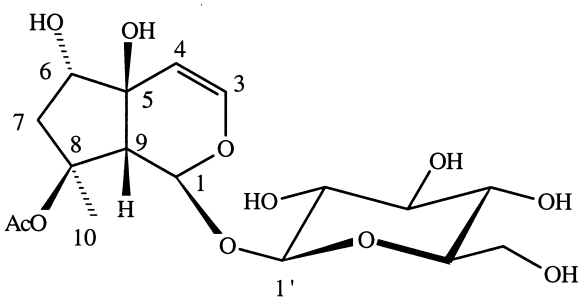
phous powder. ^{1}H and ^{13}C NMR spectra for **2** (Table 1) displayed patterns of peaks very similar to that of harpagide, except for the sugar moiety. The ^{13}C NMR spectrum showed 16 resonances, including nine for the harpagide aglucone and seven for the substituent. Quasi-molecular ion peaks in the positive ESI mass spectrum at m/z 445 $[M + Na]^+$ and m/z 461 $[M + K]^+$ were consistent with a molecular formula of $C_{17}H_{26}O_{12}$. Signals at δ 102.8, 75.1, 79.2 and 65.0 were, respectively connected to the pair of doublets of the methine at δ 5.26 and 4.45 (J = 3.3 Hz), to the methine at δ 4.55 (*dd*, J = 4.6 and 3.8 Hz) and to the methylene at δ 3.65–3.68 as is shown in the HMQC spectrum. These chemical shifts suggested a modified carbohydrate moiety. The presence of two COOMe was supported by the observation of two singlets at δ 3.76 and 3.78 in the ^{1}H NMR spectrum and by ^{13}C resonances at δ 55.5, 55.6 and 175.0. Thus, the two doublets (J = 3.3 Hz) at δ 5.26 and 4.45 were, respectively assignable to H-1′ and H-2′. HMBC spectrum analysis showed that H-2′ correlated with the carbonyl at δ 175.0. Furthermore, H-1′ correlated in the NOESY spectrum with the proton at δ 4.55 (*dd*, J 4.6 and 3.8 Hz) attributable to H-5′. The latter proton also correlated with the H-6′ methylene and with the carbonyl at δ 175.0 in the HMBC spectrum. This data allowed us to propose for **2**, the structure of harpagide-aglucone-l-*O*-3′,4′-*seco*-glycopyranoside named clandonoside II. To the best of our knowledge, this compound is the first natural product derivative, and moreover, the first iridoid with such *seco*-appendage. Since this plant has already been shown to contain the compound clandonoside with a 3-ketohexose moiety (Hannedouche et al., 1999) **2** is most likely formed by oxidative ring cleavage of the latter. Such *seco*-glycoside substitution is extremely rare and occur, to our knowledge, only in some saponins isolated from *Beta vulgaris* L. for example (Lavaud et al., 1996; Massiot et al., 1994). The nature of the asymmetric centres on the *seco*-glycoside remains to be determined.

Compound **3** was obtained as an amorphous white powder. Quasi-molecular ion peaks in the positive ESI mass spectrum at m/z 299 $[M + Na]^+$ and m/z 315 $[M + K]^+$, indicated a molecular mass of 276, suggesting the molecular formula to be $C_{12}H_{20}O_7$, which was supported by ^{13}C NMR data. No signals for a glycosidic moiety in the NMR spectra were observed (Table 1), indicating that **3** was a genine. ^{1}H and ^{13}C NMR spectra of **3** displayed a pattern of peaks very similar to that of 8-*O*-acetylharpagide aglucone, except for position 3 and 4 ($\delta_{C\text{-}3}$ 89.2, $\delta_{C\text{-}4}$ 40.4) indicating the saturation of the $C_{3–4}$ bond, with a hydroxy substitution at C-3. We also observed the presence of a methoxy group (δ_C 57.8 and δ_H 3.41, *s*, 3H). However, this methoxy group was C-1 linked, as shown in the HMBC spectrum by the correlation

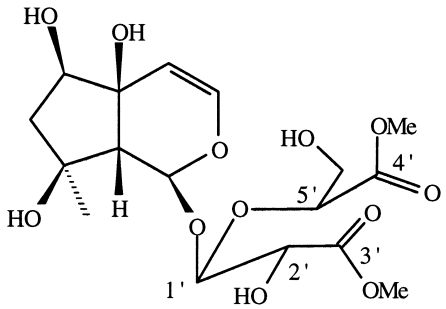
Table 1
^{1}H and ^{13}C NMR data for compounds **1**, **2** and **3** in D_2O (δ in ppm, J in Hz)

	δH			δC		
Position	**1**	**2**	**3**	**1**	**2**	**3**
1	5.95 *s*	6.05 *s*	5.38 *s*	95.8	97.1	103.0
3	6.51 *dd* (6.4, 1.0)	6.42 *d* (6.4)	5.30 *dd* (9.9, 2.3)	145.3	143.5	89.2
4α	5.11 *d* (6.5)	4.97 *dd* (6.4, 1.4)	1.40 *dd* (14.0, 10.0)	104.0	109.3	40.4
4β	–	–	1.85 *d* (13.9)	–	–	–
5	–	–	–	74.6	73.9	82.4
6	4.27 *dd* (12.5, 6.6)	3.80 *d* (4.2)	3.69 *d* (4.5)	77.8	79.0	77.8
7a	1.57 *dd* (13.5, 12.5)	1.98 *dd* (15.7, 4.2)	2.17 *dd* (16.2, 4.5)	46.1	48.0	47.6
7b	2.24 *dd* (13.5, 6.6)	2.14 *d* (15.7)	2.26 *d* (16.2)	–	–	–
8	–	–	–	86.6	79.7	89.9
9	2.70 *s*	2.54 *s*	2.35 *s*	56.7	59.4	55.0
10	1.44 *s*	1.24 *s*	1.49 *s*	23.5	26.6	24.2
C=O	–	–	–	176.8	–	176.8
OAc	2.03 *s*	–	2.01 *s*	24.3	–	24.4
1′	4.71 *dd* (8.0, 1.0)	5.26 *d* (3.3)	–	100.1	102.8	–
2′	3.25 *ddd* (9.3, 8.2, 1.1)	4.45 *d* (3.3)	–	75.2	75.1	–
3′	3.44–3.50	–	–	78.1	175.0	–
4′	3.38 *dd* (9.9, 1.0)	–	–	72.3	175.0	–
5′	3.44–3.50	4.55 *dd* (4.6, 3.8)	–	79.0	79.2	–
6′a	3.71 *dd* (12.4, 5.6)	3.65 *dd* (11.4, 3.9)	–	63.3	65.0	–
6′b	3.91 *d* (12.4)	3.68 *dd* (11.3, 4.5)	–	–	–	–
O–Me	–	3.76, 3.78 *s*	3.41 *s*	–	55.5, 55.6	57.8

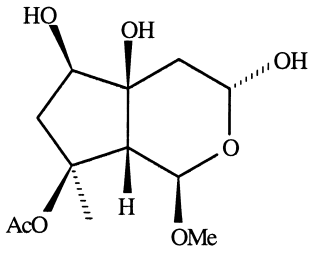
between H-1 and methoxyl carbon. The methoxy group was shown to be in the β-position by comparison with the chemical shifts of H-1 and H-9 with those of 8-*O*-acetylharpagide (Hannedouche et al., 1999). In the ^{1}H NMR spectrum, the hemicetal proton signal at δ 5.30 (*dd*, J = 9.9 and 2.3 Hz) could be assigned to H-3. The AB-system seen at δ 1.40 (*dd*, J = 14.0 and 10.0 Hz) and 1.85 (*d*, J = 13.9 Hz) derived from the methylene group at C-4. Assuming a chair conformation for the six-membered ring as shown by the axial/equatorial position of H-9/H-1, the down field signal at δ 1.85 was assignable to H-4 in β-position and the shielded signal at δ 1.40 to H-4α, in axial position. H-3 was in a *trans*-diaxial position as shown by its large coupling constant (J = 9.9 Hz) to H-4α. Thus, H-3 must be in β-position. This data led to the conclusion that compound **3** was 1-β-methoxy-3,4-dihydro-3α-hydroxy-8-*O*-acetylharpagide aglucone, named here clandonensine.



6-*epi*-8-*O*-acetylharpagide (**1**)



clandonoside II (**2**)



clandonensine (**3**)

3. Experimental

General procedures were the same as reported earlier (Hannedouche et al., 1999). **1** (11 mg), **2** (5 mg) and **3** (7 mg) were isolated by reverse phase chromatography (C18 cartridge) with H_2O–MeOH gradient.

3.1. 6-epi-8-O-Acetylharpagide *1*

$C_{17}H_{26}O_{11}$; white amorphous powder; $[\alpha]_D$ −36° (MeOH, *c* 0,0035); UV λ_{max} nm: 204; positive DCI-MS *m/z*: 424 $[M + NH_4]^+$; ^{1}H NMR, ^{13}C NMR: Table 1.

3.2. Clandonoside II *2*

$C_{17}H_{26}O_{12}$; white amorphous powder; UV λ_{max} nm: 204; positive ESI-MS *m/z*: 445 $[M + Na]^+$; 461 $[M + K]^+$; ^{1}H NMR, ^{13}C NMR: Table 1.

3.3. Clandonensine *3*

$C_{12}H_{20}O_7$; white amorphous powder; UV λ_{max} nm: 203; positive ESI-MS *m/z*: 299 $[M + Na]^+$; 315 $[M + K]^+$; ^{1}H NMR, ^{13}C NMR: Table 1.

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