

## Iridoid glucosides from *Caryopteris mongholica*

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Two new iridoid glucosides, 8-acetyl-6'-*O*-(*p*-coumaroyl) harpagide and 6'-*O*-(*p*-coumaroyl) antirrinocide, were isolated from the *Caryopteris mongholica*, together with two known iridoid glucosides, 8-acetylharpagide and harpagide. Their structures were elucidated, mainly by interpretation of their spectroscopic data (UV, IR, MASS,  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^1\text{H}/^1\text{H}$ -COSY, HMQC, HMBC and NOESY NMR) and chemical methods.

### 1. Introduction

*Caryopteris* plants are widely distributed in China. Most of them possess medicinal activities, such as antipyretic, detoxicant, expectorant and cough depressant properties and are being used as Chinese folk medicines [1]. *Caryopteris mongholica* Bunge, a small shrub with fragrant flowers found mainly distributed in the Northwest of China, has been used for the treatment of rheumatism in folk medicine. In a previous paper, a hypolactin-7-glucoside was reported from this plant [2]. In the course of our search for biologically active substances from this plant, we isolated two new iridoid glucosides together with two known iridoid glucosides. This paper describes the structure elucidation of these new iridoid glucosides.

### 2. Investigations, results and discussion

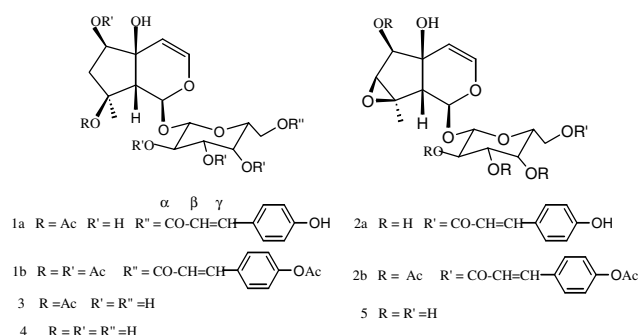
The dried powdered whole plants of *C. mongholica* were extracted with methanol at room temperature. The extract was fractionated successively by petrol, chloroform, ethyl acetate and *n*-butanol. The ethyl acetate extract was further separated by silica gel chromatography to give two new iridoid glucosides, 8-acetyl-6'-*O*-(*p*-coumaroyl) harpagide (**1a**) and 6'-*O*-(*p*-coumaroyl) antirrinocide (**2a**). The *n*-butanol extract was also purified by silica gel chromatography to give two known iridoid glucosides, 8-acetylharpagide (**3**) and harpagide (**4**).

Compounds **1a** and **2a** were isolated as an inseparable mixture which showed a single spot in TLC with several eluents. It showed UV bands at 315 and 222 nm and IR bands at 1706 and 1630  $\text{cm}^{-1}$  (conjugated ester), 1600 and 1500  $\text{cm}^{-1}$  (phenyl group), along with a strong hydroxyl absorbent band (3350  $\text{cm}^{-1}$ ). Acid hydrolysis of the mixture afforded glucose as the sole sugar. Alkaline hydrolysis of **1a** + **2a** yielded *p*-coumaroyl acid, besides a mixture of 8-acetylharpagide (**3**) and antirrinocide (**5**), which were identified by their physical and spectroscopic data and by direct comparison with authentic samples. These facts established that **1a** + **2a** were a mixture of the *p*-coumaroylates of 8-acetylharpagide and antirrinocide and that they occurred in ca. 1:1 ratio indicated by integration of the olefinic protons corresponding to the ester groups in the  $^1\text{H}$  NMR spectrum. Acetylation under mild conditions formed a mixture of hexa and penta acetate, **1b** + **2b**, which was separated on a column of silica gel. Two compounds, **1b** and **2b** were isolated in pure form.

Compound **1b** has the molecular formula  $\text{C}_{36}\text{H}_{42}\text{O}_{18}$  based on FAB-MS data ( $m/z$  684 [ $\text{M}-\text{H}_2\text{O}-\text{AcOH}$ ] $^+$ ) and on counting carbons and hydrogens from the data of its  $^1\text{H}$  and  $^{13}\text{C}$  NMR DEPT spectra. The IR of compounds **1b** showed the typical absorption of an enol ether system of

an iridoid at 1630  $\text{cm}^{-1}$  [3], an ester function at 1706  $\text{cm}^{-1}$  and a phenyl function at 1600, 1510  $\text{cm}^{-1}$ . The UV maximum of **1b** at 281 nm revealed the presence of an  $\alpha,\beta$ -unsaturated ester [4]; a shoulder at 220 nm was attributable to a conjugated ether system [3]. The  $^1\text{H}$  NMR spectra of **1b** (Table) ( $\text{CDCl}_3$ ) was strongly reminiscent of that of 8-acetylharpagide (**3**) [5–6] and in addition contained signals due to a trans-*p*-coumaroyl group [ $\delta$  6.48 and 7.73 (each 1 H, d,  $J$  = 16 Hz), and 7.13 and 7.58 (each 2 H, d,  $J$  = 8.7 Hz)]. The  $^{13}\text{C}$  NMR spectrum of **1b** (Table) showed the presence of a methyl, a methylene, five methines, two quaternary carbons having an oxygen function and six acetalic carbons in addition to the signals due to a trans-*p*-coumaroyl ester of 8-acetylharpagide (**3**) which was isolated at the same time. In fact, the  $^{13}\text{C}$  NMR signals assignable to the aglycone portion were identical to those of **3**. The location of the *p*-coumaroyl group was elucidated to be at C-6' from the analytical result of HMBC: C- $\alpha$  of the *p*-coumaroyl moiety was correlated with H-6' of sugar moiety. This assignment was further supported by the fact that the signal assignable to C-6' of **1a** + **2a** resonated downfield (1.84 ppm), whereas the signal assignable to C-5' was shifted upfield (1.93 ppm), compared to that of **3** in the  $^{13}\text{C}$  NMR spectrum. On the basis of these findings, the structure of **1b** was elucidated as 8-acetyl-6'-*O*-(*p*-coumaroyl) harpagide hexaacetate. Compound **1a** should be 8-acetyl-6'-*O*-(*p*-coumaroyl) harpagide. Its molecular formula was deduced as  $\text{C}_{26}\text{H}_{32}\text{O}_{13}$  based on FAB-MS data ( $m/z$  553 [ $\text{M}+1$ ] $^+$ ) and the NMR spectra data of **1a** + **2a**.

The structure of compound **2b** was deduced from the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data which were similar to those of **5** [7 to 8] except for the signals of the coumaric acid residue. The presence of coumarate was indicated by the typical signals in the  $^1\text{H}$  NMR spectrum (Table). As the signals for H-6' of the glucose part were downfield, the relative position of the coumarate was established. Compound **2b** was proven to be 6'-*O*-(*p*-coumaroyl) antirrinocide pentaacetate, with a molecular formula of  $\text{C}_{34}\text{H}_{38}\text{O}_{17}$ . Thus compound **2a**



**Table:  $^1\text{H}$  NMR (400 Hz) and  $^{13}\text{C}$  NMR data (100 Hz) of compounds **1a–2b** (in  $\text{CD}_3\text{OD}$  or  $\text{CDCl}_3\text{TMS}$  as int. standard)\***

H	1b	2b	C	1a	1b	2a	2b
1	6.02 s	5.99 s	1	94.2	94.0	94.1	94.1
3	6.35 d (6.5)	6.31 d (6.0)	3	143.5	141.6	142.8	141.4
4	5.49 d (6.5)	5.16 d (6.0)	4	107.7	107.0	107.2	106.7
6	5.39 d (3.9)	4.97 d (3.8)	5	72.9	71.4	73.6	73.1
7	1.94 d (16)	3.51 s	6	78.4	77.6	77.5	78.0
	2.36 d (15.9)						
9	3.17 s	3.18 s	7	45.8	43.3	66.0	63.1
10	1.46 s	1.49 s	8	87.7	86.0	65.9	62.7
1'	4.88 d (7.8)	4.94 d (7.8)	9	55.5	54.5	53.1	52.0
2'–4'	5.04–5.30	4.96–5.31	10	22.1	22.1	17.6	17.0
5'	3.85 m	3.82 m	C=O	173.3	173.3		
6'	4.38 dd (12.3, 4.7)	4.33 dd (12.3, 4.2)	Me	22.5	22.4		
	4.41 dd (12.3, 2.6)	4.39 dd (21.2, 2.3)					
2''	7.58 d (8.7)	7.58 d (8.7)	1'	99.8	96.4	99.4	96.2
3''	7.13 d (8.7)	7.14 d (8.7)	2'	74.6	71.0	74.3	70.8
5''	7.13 d (8.7)	7.14 d (8.7)	3'	77.5	72.0	77.5	72.3
6''	7.58 d (8.7)	7.58 d (8.7)	4'	71.6	68.7	71.5	68.5
$\alpha$	6.48 d (16.2)	6.42 d (16.0)	5'	75.6	72.0	75.4	72.3
$\beta$	7.73 d (16.0)	7.70 d (15.6)	6'	64.7	62.1	63.9	61.7
			1''	127.1	132.1	127.1	131.4
			2''	131.1	129.3	131.1	129.4
			3''	116.7	122.1	116.8	122.2
			4''	161.1	152.1	161.0	152.2
			5''	116.7	122.1	116.7	122.2
			6''	131.1	129.3	131.1	129.4
			$\alpha$	146.7	144.4	146.7	144.8
			$\beta$	115.1	117.5	115.1	117.2
			C=O	168.8	166.3	166.8	166.3

\* Assignment from  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC, HMBC and NOESY

should be 6'-*O*-(*p*-coumaroyl) antirrinoside. Its molecular formula was deduced as  $\text{C}_{24}\text{H}_{28}\text{O}_{12}$  based on FAB-MS ( $m/z$  509  $[\text{M} + 1]^+$ ) and the NMR spectra data of **1a + 2a**.

### 3. Experimental

#### 3.1. Equipment

M.p.s.: X-4 microscope (the fourth instrument of Beijing) uncorr. Optical rotation: polarimeter 241 (Perkin Elmer) solvent  $\text{CHCl}_3$  and MeOH, IR-spectra were recorded on a Nicolet-5DX. IR spectrometer.  $^1\text{H}$ ,  $^{13}\text{C}$  and 2D NMR spectra were recorded at 400 MHz, solvent  $\text{CD}_3\text{OD}$  and  $\text{CDCl}_3$ , using TMS as int. Standard. EIMS and FAB-MS were determined on a MS50 (A.E.I. Brunner) and a ZAB-HS mass spectrometer.

#### 3.2. Plant material

The plant material was collected in July 1997 at Lanzhou district Gansu of P.R. China and was identified by Prof. Guo-liang Zhang, Department of Biology, Lanzhou University of P.R. China. A voucher specimen (NO. 9701) has been deposited at the Lab. of Natural Products, Department of Chemistry, Lanzhou University, Lanzhou, P.R. China.

#### 3.3. Extraction and isolation

Air-dried and powdered whole plants of *C. mongholica* (3 kg) were exhaustively extracted with MeOH at RT. The extract was concentrated under reduced pressure. The residue was suspended in  $\text{H}_2\text{O}$ , extract with petroleum,  $\text{CHCl}_3$ , EtOAc and BuOH, respectively. The EtOAc extract (20 g) was obtained and chromatographed on a silica gel column (200–300 mesh 400 g) with a  $\text{CHCl}_3$ –MeOH gradient as developing solvent. Combination of the appropriate fractions (monitored by TLC analysis) led to five fractions. From fr. 4 ( $\text{CHCl}_3$ –MeOH, 30:1), a crude material was obtained and purified by rechromatography on a silica gel column (300–400 mesh with  $\text{CHCl}_3$ –MeOH (30:1) to give compound **1a + 2a** (120 mg) (single spot on TLC). The BuOH extract was chromatograph on silica gel column (200–300 mesh) (400 g) with a  $\text{CHCl}_3$ –MeOH– $\text{H}_2\text{O}$  gradient as developing solvent, to give five fractions. Of which fr. 2 ( $\text{CHCl}_3$ –MeOH– $\text{H}_2\text{O}$ , 20:1:0.1) was rechromatographed on silica gel to give 8-acetyl-harpagide (**3**) (200 mg). Harpagide (**4**) (80 mg) was obtained from fr. 3 ( $\text{CHCl}_3$ –MeOH– $\text{H}_2\text{O}$ , 10:1:0.1) and was purified by the procedure reported for compound **3** above.

#### 3.4. 8-acetyl-6'-*O*-(*p*-coumaroyl)harpagide (**1a**) and 6'-*O*-(*p*-coumaroyl)antirrinoside (**2a**)

Amorphous powder; IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3356–3264, 2932, 1706, 1632, 1630, 1604, 1515, 1441, 1365, 1327, 1232, 1165, 1072, 1017, 957; UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm: 222, 315;  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  6.29 (1 H, d,  $J = 6$  Hz, 3-H), 5.92 (1 H, d,  $J = 6$  Hz, 1-H), 4.90 (1 H, dd,  $J = 6.2$  Hz, 4-H), 3.70 (1 H, d,  $J = 4.5$  Hz, 6-H), 9-H: 2.86, brs and 2.32, brs; 10-Me: 1.42, s and 1.30, s; OAc: 2.02, s. The signals of the *p*-coumaroyl group appear at  $\delta$  6.31 and 7.59, 6.78, 7.34 respectively;  $^{13}\text{C}$  NMR: Table.

#### 3.5. Acetylation of 8-acetyl-6'-*O*-(*p*-coumaroyl) harpagide (**1a**) + 6'-*O*-(*p*-coumaroyl) antirrinoside (**2a**)

100 mg of **1a + 2a** treated with pyridine–Ac $_2$ O (1:1) RT for 12 h, afforded after usual work up and CC, eluting with *n*-hexane–EtOAc (2:1), 20 mg of **1b** and 15 mg of **2b**.

#### 3.6. 8-Acetyl- 6'-*O*-(*p*-coumaroyl) harpagide hexaacetate (**1b**)

Amorphous powder, m.p. 208–210 °C (EtOH);  $[\alpha]_D^{25} -35^\circ$  ( $\text{CHCl}_3$ , c 0.72); UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm: 220 and 281; IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3521, 2960, 1760, 1646, 1602, 1509, 1435, 1374, 1246, 1219, 1163, 1037, 960. EIMS  $m/z$  (%): 684  $[\text{M} - \text{H}_2\text{O} - \text{AcOH}]^+$  (0.04), 477  $[\text{glc} + \text{ester tetraacetate}]^+$  (0.83), 384  $[\text{684-5AcOH}]^+$  (0.01), 227  $[\text{Agly}, \text{C}_{11}\text{H}_{15}\text{O}_5]^+$  (0.06), 211  $[\text{C}_{11}\text{H}_{15}\text{O}_4]^+$  (0.27), 193  $[\text{211-H}_2\text{O}]^+$  (0.23), 189  $[\text{ester acetate}]^+$  (29.5), 147  $[\text{189-C}_2\text{H}_3\text{O}]^+$  (44.8), 119  $[\text{147-CO}]^+$  (4.02), 93  $[\text{119-C}_2\text{H}_2]^+$  (1.19), 77  $[\text{93-O}]^+$ , 43 (100).

#### 3.7. 6'-*O*-(*p*-coumaroyl) antirrinoside pentaacetate (**2b**)

Amorphous powder, m.p. 198–200 °C (EtOH);  $[\alpha]_D^{25} 25^\circ$  ( $\text{CHCl}_3$ , c 0.12); UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm: 218 and 284; IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3520, 2922, 1753, 1638, 1601, 1508, 1433, 1371, 1222, 1162, 1038, 957, 909. EIMS  $m/z$  (%): 580  $[\text{M} - \text{H}_2\text{O} - 2\text{AcOH}]^+$  (0.01), 477  $[\text{glc} + \text{ester tetraacetate}]^+$  (0.71), 391  $[\text{580-189}]^+$  (0.21), 189  $[\text{esteracetate}]^+$  (43.34), 183  $[\text{Agly}, \text{C}_9\text{H}_{11}\text{O}_4]^+$  (0.22), 167  $[\text{C}_9\text{H}_{11}\text{O}_3]^+$  (0.75), 149  $[\text{167-H}_2\text{O}]^+$  (1.33), 147  $[\text{189-C}_2\text{H}_3\text{O}]^+$  (65.37), 119  $[\text{147-CO}]^+$  (5.36), 77  $[\text{119-C}_2\text{H}_2\text{O}]^+$  (1.99), 43 (100).

#### 3.8. 8-acetylharpagide (**3**)

Amorphous powder; IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$  3410, 1713, 1653;  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  6.36 (1 H, d,  $J = 6.3$  Hz, 3-H), 5.92 (1 H, s, 1-H), 4.89 (1 H, d,  $J = 6.0$  Hz, 4-H), 4.45 (1 H, d,  $J = 7.8$  Hz, 1'-H), 1.98 (3H, s, OAc), 1.38 (3 H, s, 10-H).

### 3.9. Harpagide (4)

Amorphous powder; IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3400, 1655;  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  6.25 (1 H, d,  $J = 6.4$  Hz, 3-H), 6.02 (1 H, s, 1-H), 4.77 (1 H, d,  $J = 6.4$  Hz, 4-H), 2.77 (1 H, s, 9-H), 4.46 (1 H, d,  $J = 8.0$  Hz, 1'-H), 1.08 (3 H, s, 10-H).

### 3.10. Alkaline hydrolysis

Three drops of 0.1 N NaOH were added to a solution of **1a** + **2a** (20 mg) in MeOH (3 ml). The mixture was refluxed for 1 h, then diluted with  $\text{H}_2\text{O}$ , neutralized with dil. HCl, and extracted with  $\text{CHCl}_3$ . The  $\text{CHCl}_3$  layer was evaporated to dryness and identified as *p*-coumaric acid by direct comparison with an authentic sample. The  $\text{H}_2\text{O}$  layer was concentrated and the residue was separated by prep. TLC (silica gel; solvent  $\text{CHCl}_3$ –MeOH, 7:3) to give 8-acetylharpagide (**3**) (3 mg),  $[\alpha]_{\text{D}}^{25} -112^\circ$  (MeOH, *c* 0.13) and antirrhinoside (**5**) (2 mg),  $[\alpha]_{\text{D}}^{25} -68^\circ$  (MeOH, *c* 0.1). These identification were confirmed by comparison of the TLC behavior and  $^1\text{H}$  NMR spectrum of the products with those of authentic samples.

### 3.11. Acid hydrolysis

Compound **1a** + **2a** (4mg) was refluxed with 2 M HCl in MeOH (2 ml) for 4 h. The reaction mixture was evaporated under reduced pressure and the residue was extracted with  $\text{Et}_2\text{O}$ . The  $\text{H}_2\text{O}$  layer was neutralized with alkali solution and concentrated under reduced pressure. The residue was compared with standard sugar by silica gel TLC with *n*-propanol–MeOH– $\text{H}_2\text{O}$  (16:1:3), which showed the sugar to be glucose.

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### References

- 1 The Encyclopedia of Traditional Chinese Medicine p. 774. Shanghai Science and Technology Press, Shanghai 1985
- 2 Zapesochayaya, G. G.; Pangarova, T. T.: *Khim. Priir. Soedin.* **9**, 554 (1973)
- 3 Rimpler, H.: *Planta Med.* **33**, 313 (1978)
- 4 Loew, P.; Szczepanski, V.; Cosia, C. J.; Arigoni, D.: *J. Chem. Soc. Chem. commun.* 1276 (1968)
- 5 Lichiti, H.; Von Wartburg, A.: *Helv. Chim. Acta.* **49**, 1552 (1966)
- 6 Scarpati, M. L.; Guiso, M.; Panizzi, L.: *Tetrahedron Lett.* **39**, 3439 (1965)
- 7 Scarpati, M. L.; Guiso, M.; Esposito, P.: *Gazz. Chim. Ital.* **98**, 177 (1968)
- 8 Chaudhuri, R. K.: *Helv. Chim. Acta.* **62**, 1603 (1979)

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