Pergamon

PII: S0031-9422(96)00578-X

Phytochemistry, Vol. 44, No. 4, pp. 691–693, 1997
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0031-9422/97 \$17.00+0.00

A PHENYLPROPANOID GLYCOSIDE FROM *BALLOTA NIGRA*

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(*Received* 28 *May* 1996)

Key Word Index—*Ballota nigra*; Lamiaceae; phenylpropanoid glycoside; ballotetroside.

Abstract—A new phenylpropanoid glycoside, ballotetroside, has been isolated from the aerial parts of *Ballota nigra*. On the basis of chemical and spectral data, its structure has been established as (3,4-dihydroxyphenyl)-2-ethyl[α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranosyl-(1 → 3)]-β-D-apiofuranosyl-(1 → 6)-4-*O*-caffeoyl-β-D-glucopyranoside. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Ballota nigra L. is a perennial herb widely distributed in Europe where flowered aerial parts are commonly employed in medicine as a neurosedative drug [1]. In a continuation of our phytochemical study of this species [2, 3], we describe herein the isolation and structural determination of a novel phenylpropanoid glycoside, ballotetroside (**1**), obtained from the more polar fractions of the hydroalcoholic extract of the aerial parts.

RESULTS AND DISCUSSION

Ballotetroside (**1**) was obtained as an amorphous powder, whose UV spectrum indicated its polyphenolic nature. The electrospray (ES)-mass spectrum gave a pseudomolecular ion $[M+Na]^+$ *m/z* 911, suggesting the molecular formula $C_{39}H_{52}O_{23}$, in good agreement with the observation of 39 signals on the ^{13}C NMR spectrum. The IR spectrum showed absorption bands typical of hydroxyl (3380 cm^{-1}), α,β-unsaturated ester (1690 and 1630 cm^{-1}) and aromatic rings (1600, 1520 and 815 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) exhibited signals accounting for one 3,4-dihydroxyphenylethanol unit and one (*E*)-caffeic acid unit. The tetrasaccharide structure of **1** was indicated by four anomeric doublets at δ 4.32 ($J = 6$ Hz), 4.37 ($J = 7$ Hz), 4.92 ($J = 2$ Hz) and 5.45 ($J = 1$ Hz), suggesting the presence of α-L-arabinose, β-D-glucose, α-L-rhamnose and β-D-apiose moieties, respectively. In agreement with these statements, acid hydrolysis of **1** yielded (*E*)-caffeic acid, arabinose, glucose, rhamnose and apiose. A well-resolved triplet at δ 4.94 ($J = 8$ Hz) was assignable to an ester-bearing methine proton. Clear cross-peaks in the 2D DQF-COSY spectrum of **1** were observed for the glucose moiety between H-1 (δ 4.37) and H-2 (δ 3.37), H-2 and H-3 (δ 3.77) and H-3 and H-4 (δ 4.94), indicating that this latter position was the acylation site of (*E*)-caffeic acid on the tetrasaccharidic core.

Acetylation of **1** using acetic anhydride in pyridine at room temperature afforded the peracetyl derivative **2**, exhibiting a pseudomolecular ion $[M+Na]^+$ *m/z* 1457 in the ES-mass spectrum. Thorough study of the ^1H–^1H COSY spectrum of **2** permitted assignment of each of the proton resonances of the methylene and methine signals of the sugar part of the molecule (Table 1). High field resonances observed for the signals of H-3 Glc (δ 3.97), CH_2-6 Glc (δ 3.58–3.67) and H-2 Rha (δ 3.90) indicated that these positions were glycosidation sites [4], whereas arabinose and apiose were terminal sugars. A ^{13}C–^1H HETCOR experiment allowed us to assign unambiguously all the ^{13}C resonances of the four sugars units. Finally, cross-peaks observed between H-3 Glc and C-1 Rha, CH_2-6 Glc and C-1 Api, and H-1 Ara and C-2 Rha, in the ^{13}C–^1H COLOC spectrum of **2** permitted us to determine unambiguously the sugar sequence. Thus, the structure of ballotetroside (**1**) was established as (3,4-dihydroxyphenyl)-2-ethyl [α-L-arabinopyranosyl-(1 → 2)-α-L-rhamnopyranosyl-(1 → 3)]-β-D-apiofuranosyl-(1 → 6)-4-*O*-caffeoyl-β-D-glucopyranoside. In good agreement with this statement, mild hydrolysis of **1** [5] furnished forsythoside B [6], verbascoside [7] and caffeic acid, successively.

Ballotetroside (**1**) is to our knowledge the first example of a tetrasaccharidic phenylpropanoid glycoside [8, 9].

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Table 1. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data of compounds **1** (CD_3OD) and **2** ($CDCl_3$)(δ, *J* in Hz)

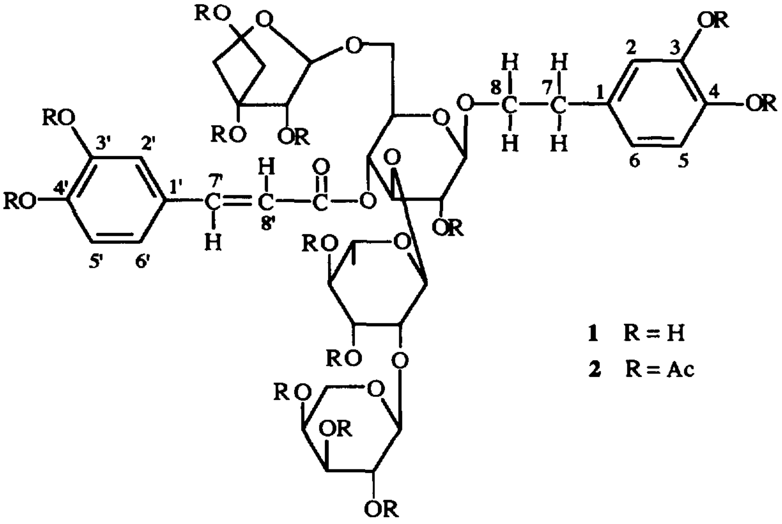
	1		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
Aglycone				
1	—	131.4	—	137.6
2	6.70*d*(2)	117.1	7.01*d*(1)	123.7
3	—	145.9	—	141.7
4	—	144.5	—	140.4
5	6.67*d*(8)	116.3	7.07*d*(5)	123.0
6	6.58*dd*(8, 2)	121.3	7.07*dd*(5, 1)	127.1
7	2.79*t*(7)	36.5	2.84	35.2
8	3.75 and 4.00	72.3	3.60 and 4.15	69.5
Caffeic acid				
1′	—	127.6	—	132.6
2′	7.08*d*(2)	115.3	7.36*d*(2)	122.8
3′	—	146.7	—	142.5
4′	—	149.6	—	143.8
5′	6.78*d*(8)	116.5	7.21*d*(8)	124.0
6′	6.97*dd*(8, 2)	123.2	7.39*dd*(8, 2)	126.3
7′	7.59*d*(16)	148.0	7.68*d*(16)	144.5
8′	6.28*d*(16)	114.7	6.34*d*(16)	117.7
CO	—	168.1	—	164.9
Glucose				
1	4.37*d*(7)	104.1	4.35*d*(8)	100.3
2	3.37	75.9	4.95–5.04	73.1
3	3.77	82.3	3.97*t*(9)	76.6
4	4.94*t*(8)	71.1	4.95–5.04	69.4
5	3.69	74.3	3.58–3.67	73.6
6	3.54 and 3.78	68.3	3.58–3.67	66.9
Rhamnose				
1	5.45*d*(1)	101.9	4.97*d*(2)	99.2
2	3.96	82.6	3.90	76.2
3	3.65	71.7	4.88*dd*(10, 4)	70.5
4	3.29	74.0	4.78*t*(10)	70.7
5	3.52	70.3	3.67–3.75	66.6
6	1.07*d*(7)	18.4	1.03*d*(7)	17.4
Apiose				
1	4.92*d*(2)	110.9	5.00*d*(3)	106.0
2	3.87	78.1	5.31*d*(3)	76.0
3	—	80.6	—	83.8
4	3.74 and 3.92	75.0	4.09 and 4.18*d*(11)	72.4
5	3.55	65.6	4.54 and 4.69*d*(13)	63.1
Arabinose				
1	4.32*d*(6)	107.3	4.32*d*(7)	102.0
2	3.58	72.7	5.13*dd*(10, 7)	68.7
3	3.53	74.2	4.95–5.04	69.5
4	3.78	69.8	5.17–5.22	67.3
5	3.80	67.3	3.52 and 3.92*dd*(12, 3)	62.5

EXPERIMENTAL

General. NMR spectra were recorded at 300 MHz (^{1}H) and 75 MHz (^{13}C), using TMS as int. standard. ^{1}H–^{1}H COSY, ^{13}C–^{1}H HETCOR and ^{13}C–^{1}H COLOC were performed using standard microprograms.

Plant material. Aerial parts of *B. nigra* L. were collected in 1993 in the vicinity of Angers (France) and identified in comparison with an authentic sample cultivated in the Botanical Garden of the University of Lille (France). A voucher specimen (No F.B.B.n.5) is kept in the Herbarium of the Department of Pharmacognosy of that University.

Extraction and purification. Dried powdered aerial parts (1 kg) were exhaustively lixiviated with 50% EtOH (10 l). The extract was concd *in vacuo* and the syrupy residue (120 g) was submitted to CC on silica gel 70–230 mesh (1 kg) eluted with EtOAc and mixts of EtOAc–MeOH of increasing polarity. The frs con-



1 R = H
2 R = Ac

taining **1**, eluted with EtOAc–MeOH (1 : 9), were pooled and concd *in vacuo* to afford a residue (7 g), which was rechromatographied on a column of silica gel H (EtOAc–MeOH–H_2O, 20 : 5 : 3 to yield compound **1** (400 mg). The purity of **1** confirmed by TLC on silica gel G F254 precoated plates and the solvent previously used for elution in CC; **1** was detected by UV fluorescence and after spraying with 2-aminoethyldiphenylborinate reagent.

Ballotetroside **1**. $[\alpha]_D^{20}$ $-33°$ (MeOH; c 0.9). UVλ_{max}^{MeOH} nm: 203, 222 (sh), 244 (sh), 291, 330. IR ν^{KBr} cm^{-1}; 3380, 1690, 1630, 1600, 1520, 815. ES-MS (m/z): 911 $[M+Na]^+$. ^1H NMR (CD_3OD) and ^{13}C NMR (CD_3OD): Table 1.

Acid hydrolysis of **1**. A soln of **1** (20 mg) in 5% HCl (10 ml) was refluxed for 3 hr. H_2O was then added and the mixt. extracted with Et_2O. The aq. layer was neutralized with $BaCO_3$ and the ppt. filtered off. The filtrate was concd and the sugars (glucose, rhamnose, apiose and arabinose) were identified by TLC plate using BuOH–iso-PrOH–H_2O (5 : 3 : 1). Concn of the Et_2O layer followed by prep. TLC gave caffeic acid (2 mg) (TLC, UV and MS).

Partial acid hydrolysis of **1**. Partial hydrolysis of **1** (0.5% in MeOH) was carried out on four TLC plates (5 μl of solution of **1** and 5 μl 3N HCl). Each plate was heated at 100° during 5, 10, 15 and 30 min, and then developed with HCO_2H–HOAc–H_2O–EtOAc (11 : 11 : 27 : 100). Hydrolysis products were identified after spraying with 2-aminoethyldiphenylborinate reagent by comparison with authentic samples of forsythoside B [1-arabinosyl] and verbascoside [1-arabinosyl-apiosyl].

Acetylation of **1**. Treatment of **1** (200 mg) with Ac_2O (5 ml) and pyridine (5 ml) at room temp. overnight followed by CC on silica gel H ($CHCl_3$–EtOAc–Me_2CO, 15 : 3 : 2) afforded the peracetylated derivative **2** (250 mg) as an amorphous powder. $[\alpha]_D^{20}$ $-56°$ ($CHCl_3$; c 0.7). UVλ_{max}^{MeOH} nm 201, 214 (sh), 285, 331. IR ν^{KBr} cm^{-1}: 1740, 1640, 1500, 1370, 1220. ES-MS (m/z): 1457 $[M+Na]^+$. ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$): Table 1.

Acknowledgements—The authors wish to thank Adrapharm for financial support (Grant No. 179), V. Cassado (University of Lille) for technical assistance and Dr G. Baudouin (University Paris V) for her kind interest in this work.

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