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SINAPINYL BUT-3-ENYLGLUCOSINOLATE FROM *BOREAVA ORIENTALIS*

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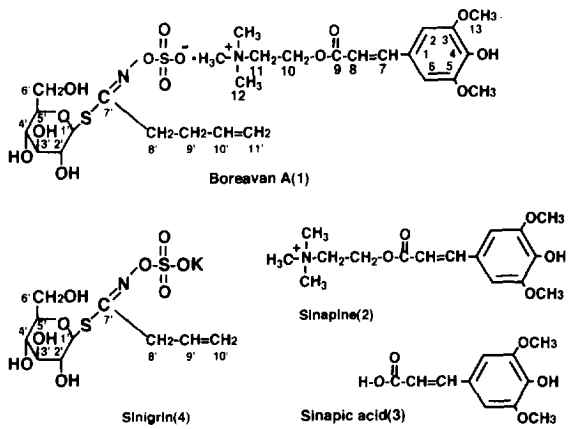
Key Word Index—*Boreava orientalis*; Cruciferae; fruits; glucosinolate salt; boreavan A.

Abstract—A new glucosinolate salt, named boreavan A, has been isolated from fruits of *Boreava orientalis*. Its structure has been established as sinapinyl but-3-enylglucosinolate on the basis of chemical and spectral evidence, including 2D-shift correlation and DEPT NMR experiments.

INTRODUCTION

In previous papers [1, 2], we reported the isolation and identification of hydroxybenzoic acids, their glucosides and a sinapic acid ester from fruits of *Boreava orientalis*. Vanillic and 2,3-dihydroxybenzoic acids were found to have a role as antioxidants.

In continuation of our investigation on the constituents of the fruits of *B. orientalis*, we have isolated a new glucosinolate salt, designed as boreavan A (**1**). In this paper, we report the structural elucidation of this compound obtained from the methanol extract on the basis of UV, mass, ^{1}H and ^{13}C NMR spectral data, including 2D-shift correlation and DEPT experiments.



RESULTS AND DISCUSSION

Extraction was carried out as described in the Experimental. The butanol- and water-soluble fraction of the extract was subjected Sephadex LH-20 and MCI gel (CHP-20) column chromatography to obtain **1**.

The silica gel TLC behaviour of **1** was similar to that of sinalbin [3]; enzymatic hydrolysis yielded sinapine and D-glucose. Its IR spectrum showed absorption bands at 3394 (OH), 2926 (CH), 1713 (C=O), 1428, 1155, 1113 (S=O) and 1056 (C–O) cm^{-1}. The UV spectrum showed a maximum at 332 nm, the bathochromic shift to 345 nm on addition of sodium hydroxide indicating the presence of an aromatic ring with a free hydroxyl group.

Electron impact–mass spectrometry of **1** showed the presence of peaks at *m/z* 224, 207, 180, 165 and 137. The presence of these peaks indicated the existence of sinapic acid. The negative ion FAB mass spectrum showed the presence of a $[M-H]^-$ at *m/z* 681 and a fragment ion due to a gluconapine (but-3-enylglucosinolate) moiety at *m/z* 372 [4].

The ^{13}C NMR spectrum of **1** indicated the presence of methyls, methylenes and an aromatic ring (Table 1). The chemical shift values were in good agreement with those of sinapine (**2**) and sinapic acid (**3**). The signals at δ83.6 and 82.2 were assigned to the anomeric carbon and C-5 of the glucose moiety; these have characteristic chemical shift values for glucose core in glucosinolates. Additionally, the values of the signals for C-7′ and the carbons of the D-glucose moiety are independent of those of the side-chain (C-8′, 9′, 10′ and 11′) (Table 1). These results are similar to those for sinigrin (**4**) and other glucosinolates [5]. The chemical shift value of the side-chain was not affected by the counter-ion previously reported [6].

The ^{1}H, ^{1}H–^{1}H and ^{13}C–^{1}H 2D COSY NMR data are presented in Table 2. The ^{1}H NMR spectrum of **1** showed the presence of the typical proton signals of a sinapoyl moiety, which were two aromatic methoxyls, a singlet equivalent to two aromatic protons and an olefinic proton attributable to a *E*-double bond. Furthermore, this spectrum showed signals assigned to a choline

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Table 1. ^{13}C NMR spectral data of compound **1** and reference compounds (100 MHz, CD_3OD)*

C	**1** (δ)	DEPT	**2** (δ)	**3** (δ)	**4** (δ)
Sinapine moiety					
1	126.4	C	126.4	126.9	
2	107.2	CH	107.1	107.1	
3	149.4	C	149.1	149.3	
4	139.8	C	139.1	139.7	
5	149.4	C	149.1	149.3	
6	107.2	CH	107.1	107.1	
7	148.2	CH	148.2	147.3	
8	114.9	CH	114.7	116.6	
9	167.9	C	168.5	171.1	
10	58.9	CH_2	59.0	—	
11	66.2	CH_2	66.0		
12	54.7	CH_3	54.8		
13	57.0	CH_3	57.1	57.0	
Glucose moiety					
1′	83.6	CH			83.2
2′	74.1	CH			74.2
3′	79.5	CH			79.4
4′	71.1	CH			71.2
5′	82.2	CH			82.0
6′	62.5	CH_2			62.7
7′	160.8	C			161.2
Side-chain moiety					
8′	33.0	CH_2			37.9
9′	32.4	CH_2			134.3
10′	138.3	CH			118.6
11′	116.0	CH_2			

*2, Sinapine; 3, sinapic acid; 4, sinigrin.

moiety, *O*-Me and *N*-Me groups at δ4.64, 3.78, 3.85 and 3.25, and proton signals of the side-chain in the glucosinolate moiety at δ2.76, 2.48, 5.91, 5.10 and 4.98. Assignments of these signals were determined from ^1H–^1H and ^{13}C–^1H 2D COSY experiments as shown in Table 2.

In the side-chain of the glucosinolate moiety, the signals are sufficiently resolved to identify the protons in the side-chain. These signals have been characterized by the major splitting, although much fine structure is also apparent [4]. However, assignments for the glucose core could not be achieved by a combination of ^1H–^1H and ^{13}C–^1H 2D COSY. These spectra yielded useful information about the anomeric proton of the glucose core in the glucosinolate. The anomeric proton (δ4.82) of the glucose core was not influenced by the presence of the side-chain; the coupling constant was 9.76 Hz in deuterium oxide. This indicated that the component sugar was β-linked glucose.

From these data, it is apparent that the molecular structure of **1** is a glucosinolate salt. It was named boreavan A, which to the best of our knowledge is a new natural product.

EXPERIMENTAL

Plant material. Fruits on *B. orientalis* (Jaud. and Spach) were collected in Sept. 1990 at Ankara (Turkey). A voucher specimen is retained in the 'Ankara Universitesi Eczacilik Facultesi Herbaryume'.

Analysis and separation. TLC-1 was carried out on cellulose plates (Merck) using EtOH–EtOAc–H_2O (15:1:1) and TLC-2 on silica gel 60 F_{256} (Merck) using

Table 2. ^1H NMR, ^1H–^1H 2D COSY NMR and ^{13}C–^1H 2D COSY NMR spectral data for compound **1** (400 MHz, CD_3OD)

H		^1H–^1H 2D COSY, correlated H	^{13}C–^1H 2D COSY, correlated C
1			
2 6	6.94 *br s* (2H)		107.2
3			
4			
5			
7	7.68 *d* (J = 15.9 Hz, 1H)	H-8	148.2
8	6.45 *d* (J = 15.9 Hz, 1H)	H-7	114.9
9			
10	4.65 *br t* (J = 4.6 Hz, 2H)	H-11	58.9
11	3.78 *br t* (J = 4.6 Hz, 2H)	H-10	66.2
12	3.25 *s* (9H)		54.7
13	3.85 *s* (6H)		57.0
1′	4.82 *d* (J = 9.76 Hz*, 1H)		83.6
2′	3.20		
6′	3.87		
8′	2.76 *q* (1H)	H-9′	33.0
9′	2.48 *m* (1H)	H-8′, 10′	32.4
10′	5.91 *ddt* (J = 17.0, 10.3, J = 6.4 Hz, 1H)	H-9′, H-11′	138.3
11′	5.10 *dm* (J = 17.0, 1H) 4.98 *dm* (J = 10.3, 1H)	H-10′	116.0

*Overlapping in MeOH but observed in D_2O.

n-BuOH–*n*-PrOH–HOAc–H_2O (3:1:1:1). The developed TLC-2 plate was dried and sprayed with 25% TCA in $CHCl_3$. After heating for 10 min at 140°, the plate was sprayed with a 1:1 mixture of 1% aq. potassium hexacyanoferrate and 5% aq. $FeCl_3$ [2]. The glucosinolate was visualized with I_2 vapour. NMR spectra were determined at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR in CD_3OD with MeOH as int. standard. FAB-MS were recorded at ambient temp. and 7 kV, EI-MS at 70 eV. Ground fruits were stirred with hexane, air-dried and extracted with MeOH. The concd extract was applied to a column of Sephdex LH-20 (50 g) and MCI gel (CHP 20p, 100 g, 75–150 μ, Mitsubish-kasei Corporation). The column was eluted with H_2O and elution monitored by TLC (R_f 0.63). Frs containing the desired product were combined and dissolved in H_2O. The crude compound was purified by rechromatography on Sephadex LH-20 (50 cm × 2.5 cm i.d. column).

Sinapinyl but-3-enylglucosinolate (*boreavan A*) (**1**). Colourless amorphous powder. HR negative ion FAB-MS *m/z*: 681.20190 $[M-H]^-$ $C_{27}H_{41}N_2O_{14}S_2$ required 681.19993. $[\alpha]^{24}$ −8.5 (MeOH; *c* 0.01). UV λ_{max}^{EtOH} nm (ε): 332 (1311); + NaOH 345. ^{1}H NMR see Table 2. ^{13}C NMR see Table 1. EI-MS *m/z* (rel. int.): 223 (5.3), 206 (4.2), 179 (17.3), 164 (8.7), 136 (7.5), 71 (30.7), 63 (81.9) and 35 (100). Negative ion FAB-MS (glycerol): *m/z* 681 ($[M-H]^- = C_{27}H_{41}N_2O_{14}S_2$), 372 (gluconapine but-3-enylglucosinolate) = $C_{11}H_{18}NO_9S_2$), 97, 80. IR ν_{max}^{KBr} cm^{-1}: 3394 (OH), 2926 (C–H), 1713 (C=O), 1632, 1605, 1515 (aromatic ring), 1428, 1341, 1257, 1155, 1113 (S=O), 1056 (C–O), 821 (S–O).

Enzymatic hydrolysis of **1**. Purified sinapyl glucosinolate (5 mg) was incubated in H_2O (1 ml; pH 7) with thioglucosidase (Sigma) at 37° for 1 day. The mixt. was dild to 20 ml with H_2O, filtered through a Millipore UF membrane C3 LCC and the effluent analysed by GC and TLC-2. FID-GC analysis was performed by temp. prog. (150–220°, 3° min^{-1}) using a glass column (2 m × 3 mm i.d.) packed with 2% OV-17 on shimalite-W. Carrier gas: N_2 (30 ml min^{-1}), injection and detector temps 300°. Identification was carried out by comparison of R_t (GC: 16.0 and 19.0 min) and MS data with those of D-glucose.

Sinapine (**2**), *sinapic acid* (**3**) *and sinigrin* (**4**). Sinigrin (Sigma), sinapic acid (Sigma) and sinapine (isolated from seed of *Brassica hirta*) were identified by comparing IR, ^{1}H NMR, ^{13}C NMR and MS with those of reference compounds.

Sinapine (**2**). Colourless needles, mp 176–177.5°. UV λ_{max}^{EtOH} nm: 241, 333; + NaOH 261, 402. ^{1}H NMR (CD_3OD): δ 3.28 (9H, *s*, N–C$\underline{H}_3$ × 3), 3.88 (6H, *s*, O–C$\underline{H}_3$ × 2), 3.86–3.81 (2H, N–C$\underline{H}_2$CH$_2$–O), 4.68–4.63 (2H, N-CH$_2$C$\underline{H}_2$–O), 6.45 (1H, *d*, J = 16.11 Hz, C$\underline{H}$=CHCO), 6.93 (2H, *s*, aromatic − 2′, 6′), 7.66 (1H, *d*, J = 16.11 Hz, CH=C$\underline{H}$CO). ^{13}C NMR see Table 1. EIMS *m/z*: 223, 206, 179 (base peak), 164, 136. Positive ion FAB-MS (glycerol): *m/z* 310 ($[M]^+ = C_{16}H_{24}NO_5$), 251 ($C_{13}H_{15}O_5$), 104 ($C_5H_{14}NO$). IR ν_{max}^{KBr} cm^{-1}: 3464 (OH), 3356, 2064, 1712 (C=O), 1640 (C=C), 1604, 1518, 1458 (aromatic C=C), 1430 (N–CH$_3$), 1336 (OCH$_3$), 1300, 1270, 1220, 1168, 984, 952.

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