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THREE GLUCOSINOLATES FROM SEEDS OF *BRASSICA JUNCEA*

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Key Word Index—*Brassica juncea*; Brassicaceae; seeds; glucosinolates; *p*-hydroxybenzyl glucosinolate; 9-(methylsulfonyl)nonyl glucosinolate; 8-(methylsulfonyl) octyl glucosinolate.

Abstract—Three native glucosinolates have been isolated from seeds of *Brassica juncea* using mild methods for their separation. The compounds were identified by various NMR techniques. © 1997 Elsevier Science Ltd. All rights reserved

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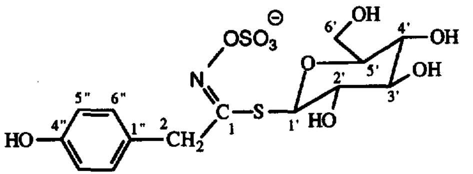
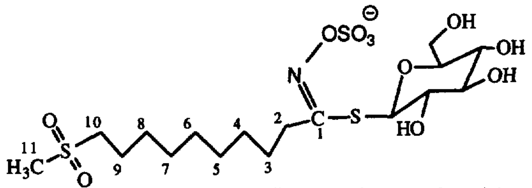
INTRODUCTION

Brassica juncea (L.), commonly called brown mustard or Indian mustard, grows in Mediterranean countries but also in the United States. Black mustard (*B. nigra*) used since antiquity is now rarely cultivated, having been replaced by brown mustard. The seeds from these species have traditionally been used, particularly in the form of plasters, as rubefacients and counter-irritants. When consumed in large doses they have an emetic action but they are principally known as condiments [1–3]. Both species produce burning sensations on the tongue and the properties reported for *B. nigra* in the literature are usually the same as those of *B. juncea* [4, 5].

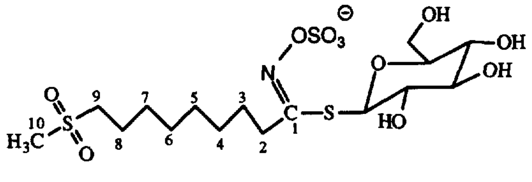
In the present work we isolated from *B. juncea* three glucosinolates. The main one is *p*-hydroxybenzyl glucosinolate (**1**), the principal glucosinolate of white mustard (*Sinapis alba*), with two minor new glucosinolates, 9-(methylsulfonyl)nonyl glucosinolate (**2**) and 8-(methylsulfonyl)octyl glucosinolate (**3**). We have been able to detect only traces of sinigrin (prop-2-enyl glucosinolate) in our sample. We report the structural elucidation of these compounds on the basis of their spectral data.



p-hydroxybenzyl glucosinolate (**1**)



9-(methylsulfonyl)nonyl glucosinolate (**2**)



8-(methylsulfonyl)octyl glucosinolate (**3**)

RESULTS AND DISCUSSION

The methods of extraction and purification are described in the Experimental.

The mobility of compound **1** on silica gel TLC using chloroform–methanol–water as mobile phase is similar to sinigrin, R_f 0.5. The R_f values of compounds **2** and **3** are 0.6 and 0.7, respectively. UV spectra show λ_{max} at 229 nm for compound **1** and 225 nm for compounds **2** and **3**. IR spectra show, for the three products, absorption bands at 3420 cm^{-1} (OH), 2970 and 2920 (CH), 1640 (C=N), 1058 (C-O), 880-800 (sulfonic acid) and 650 (C-S). Compounds **2** and **3** also show two absorption bands at 1 140 cm^{-1} and 1310 cm^{-1} for the sulfone group (*R*-SO_2-*R′*), while

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Table 1. ^{13}C NMR, ^{1}H NMR and ^{13}C–^{1}H HMQC correlations for compounds **1–3** (400 MHz in CD_3OD)

	1		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
Glucose moiety						
1′	82.6	4.5 (d ^{3}J 9.6) 1H	83.7	4.81 (d ^{3}J 9.8)	83.7	Same data as **2**
2′	74.1	1H 3.1 superimposed on H-3′	74.1	3.25 (dd ^{3}J 9.7–8.6)	74.1	Same as **2**
3′	79.3	1H	79.5	3.39 (d ^{3}J 8.6)	79.5	Same as **2**
4′	71.1	1H 3.2 superimposed on H-5′	71.1	3.33 (partially under peak solvent)	71.1	Same as **2**
5′	82.1	1H	82.3	3.38 (d ^{3}J 8.5: d ^{3}J H-6′A 1.0; d ^{3}J H-6′B 4.2)	82.3	3.3 (partially under peak solvent)
6′	62.7	6′A 3.8 (d ^{2}J 12.3) 2H 6′B 3.6	62.7	6′A 3.83 (d ^{2}J 12.2) 6′B 3.65 (d ^{3}J H-5′4.2; ^{4}J H-4′1.5 W coupling)	62.7	Same as **2**
Aliphatic moiety						
1	161.2–		161.9–		161.9–	
2	38.9	2A 4.1 (d ^{2}J 15.7) 2H 2B 3.9	33.5	2.68 (dd ^{3}J 8.7–7.0) 2H	33.5	Same data as **2**
3			28.5	1.76 (d ^{3}J 7.0) 2H	28.4	1.76 (dd ^{3}J 8.0–7.0)
4			30.0		29.9	
5			30.0	1.37 (m) 8H	29,9	1.45 (m) 6H
6			30.1		29.9	
7			30.2		29.2	1.47 (dd ^{3}J 8.0–7.0)
8			29.3	1.45 (dd ^{3}J 7.2–7.1) 2H	23.3	1.80 (dd ^{3}J 8.0–7.0)
9			23.3	1.82 (dd ^{3}J 8.1–7.2) 2H	55.2	3.12 (dd ^{3}J 8.0–8.0)
10			55.2	3.1 (dd ^{3}J 8.1–7.8) 2H	40.5	2.95 (s) 3H
11			40.5	2.94 (s) 3H	–	
Aromatic moiety						
1″	127.8–					
2″–6″	130.3	7.2 (d ^{3}J 8.5) 1H				
3″–5″	116.6	6.7 (d ^{3}J 8.5) 1H				
4″	157.6–					

compound **1** exhibits absorption due to C=C aromatic stretch at 1 590 cm^{-1} [6]. The negative FAB-mass spectrum of compound **1** showed a $[M]^-$ at m/z 424 and the negative electrospray mass spectra of compounds **2** and **3** showed, respectively, peaks corresponding with $[M]^-$ at m/z 522 and 508.

The ^{13}C NMR chemical shifts (Table 1) for the common part of the three products are characteristic of glucosinolates, namely, the quaternary carbon C-1 at 161 ppm and the glucosyl resonances, with the anomeric carbon C-1′ at 83 ppm and the C-5′ at 82 ppm [7–9]. In addition, the signals for compound **1** indicated the presence of a *p*-hydroxybenzyl group (symmetrical pattern for C-2″-6″ and C-3″-5″). The assignment of carbons for the compounds **2** and **3** were achieved by HMQC and HMBC two-dimensional shift correlations.

The ^{1}H NMR data of the aglycone moieties of compounds **2** and **3** show the presence of a chain of methylenes (1.3–1.8 ppm) and a methyl singlet (2.94 ppm for **2** and 2.95 ppm for **3**). The anomeric protons for the three products are well resolved and their coupling constants of 9.8 Hz, reveal that the sugars are β-linked glucopyranoses.

The presence of the sulfonyl group in compounds **2** and **3** was confirmed by their M_r values, 522 and 508, and by IR spectra (absorption bands at ν 1 140 and 1 310 cm^{-1}). The ^{13}C–^{1}H HMBC NMR spectra (data not shown) confirmed the structures for compounds **2** and **3** but we did not observe any correlation between the terminal methyls and the last methylene of the side-chains in these compounds.

From these data we can confirm that these products are glucosinolates; The major one, *p*-hydroxybenzyl glucosinolate, has never been described in *B. juncea* seeds. Moreover, the two other products, 9-(methylsulfonyl)nonyl and 8-(methylsulfonyl)octyl glucosinolates are new compounds. The analogous methylsulfinyl (sulfoxide) compounds have been described in *Arabis* [10].

EXPERIMENTAL

General. IR: KBr disks. MS: FAB and ESI in MeOH. HMQC, HMBC 2D-shift correlations, ^{1}H and *J* mod. ^{13}C NMR: 400 and 100 MHz, respectively, in CD_3OD, using the solvent peaks at 3.31 and 49.0 ppm as refs. FAB-MS: 9 kV (MeOH, glycerol). ESI-MS: 3.5 kV (MeOH).

Plant material. Seeds of *B. juncea* (L.) Coss and Czern were obtained from Gifrer Barbezat (F69 France). A voucher specimen is conserved in the Faculté de Pharmacie, Université Paul Sabatier, Laboratoire de Pharmacognosie 35, chemin des Maraîchers F-31062 France.

Extraction and isolation. Isolation of **1**. Pulverized dried seeds (*ca* 50 g) were added to 500 ml 100% boiling MeOH stirred magnetically for 30 min, then cooled and filtered. The filtrate was then evapd under red. pres. to dryness (6.5 g). A 3 g portion of this extract was dissolved in 10 ml of MeOH and loaded on the top of a low-pressure (8 bar) SDS 6–35 μm silica gel column (460 × 26 mm) conditioned in $CHCl_3$–MeOH (13:9); 200 × 4 ml frs were collected and the glucosinolates were visualized on a silica Merck F_{254} TLC developed with $CHCl_3$–MeOH–H_2O (13:9:2), under UV (254 nm) and by spraying with 10% H_2SO_4–thymol 1% in EtOH soln (R_f 0.5). Glucosinolate-containing frs were pooled and evapd to dryness. The residue was then dissolved in 2 ml of MeOH and a second low-pressure (5 bar) column (230 × 26 mm) separation was carried out to obtain frs providing 30 mg of pure product. These frs were also monitored by HPLC ($k' = 2.76$) using a MeOH (5 mM tetraheptylammonium bromide as ion pairing agent)/phosphate buffer (pH 7.0; 0.01 M), (3:2) as mobile phase (1 ml min^{-1}, 120 bar)) on a C18 (125 × 4.5 mm) column using spectrophotometric detection at 229 nm [11].

Isolation of **2** *and* **3**. Frs containing compounds **2** and **3**, as revealed by HPLC, were combined and evapd to dryness. They were then deposited on a C18 cartridge (Mega bond elut, size 60 CC/10 GRM Varian) dissolved in deionized H_2O (*ca* 4 ml) and filtered using a Vac Elut SPS 24 filtration system (Analytichem International) under low pressure at a flow rate of 0.5 ml min^{-1}. The cartridge was washed successively by 25 × 4 ml of H_2O, collected in 25 different tubes. Each tube was monitored by HPLC (conditions as previously described) and the frs containing compound **2** ($k' = 5.4$) and compound **3** ($k' = 3.72$) were combined and lyophilized to obtain *ca* 5 mg of each pure product. Sinigrin was detected by addition of authentic material (Sigma) using HPLC ($k' = 2.50$).

Hydroxybenzyl glucosinolate: 1-*thio*-β-D-*glucopyranose* 1-[-*hydroxy*-N-(*sulfoxy*)*benzeneethanimidate*] **1**. $C_{14}H_{18}NO_{10}S_2$. Amorphous powder. UV λ_{max}^{MeOH} nm: 229. IR, ^1H and ^{13}C NMR: see text. Negative FAB-MS *m/z* (rel. int.): $[M]^-$ 424(54), 297(27), 284(85), 279(90), 277(36), 265(84), 261(56), 238(100), 216(62).

9-(*Methylsulfonyl*)*nonyl glucosinolate*: 1-*thio*-β-D-*glucopyranose* 1-[-10-(*methylsulfonyl*)-N-(*sulfoxy*) *decanimidate*] **2**. $C_{17}H_{32}NO_{11}S_3$. Amorphous powder. UV λ_{max}^{MeOH} nm: 225. IR, ^1H and ^{13}C NMR: see text. Negative ESI-MS *m/z* (rel. int.): $[M]^-$ 522 (100), 501(10), 424(5), 381(3), 293(3), 250(5), 205(5).

8-(*Methylsulfonyl*)*octyl glucosinolate*: 1-*thio*-β-D-*glucopyranose* 1-[-9-(*methylsulfonyl*)-N-(*sulfoxy*)*nonanimidate*] **3**. $C_{16}H_{30}NO_{11}S_3$. Amorphous powder. UV λ_{max}^{MeOH} nm: 226. IR, ^1H and ^{13}C NMR: see text. Negative ESI-MS *m/z* (rel. int.): $[M]^-$ 508 (100), 457 (10), 266 (25), 252(47), 161(15).

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