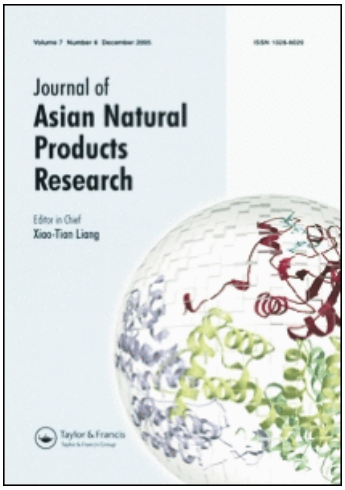

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Duan, Li-Xin , Feng, Bao-Min , Chen, Fang , Liu, Jing-Yang , Li, Fan , Wang, Yong-Qi and Pei, Yue-Hu(2007) 'Sinapic acid derivatives from the seeds of *Raphanus nussatirus* L.', Journal of Asian Natural Products Research, 9: 6, 557 — 561
To link to this Article: DOI: 10.1080/10286020600883468
URL: http://dx.doi.org/10.1080/10286020600883468

Journal of Asian Natural Products Research, Vol. 9, No. 6, September 2007, 557–561

Sinapic acid derivatives from the seeds of *Raphanus nussatirus* L.

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(Received 18 January 2006; revised 22 May 2007; in final form 1 June 2007)

A new disulfide glycoside, raphthioglucoside (**1**), and a new sinapic acid derivative, sinapic acid 5-hydroxymethylfurfural ester (**2**), together with sinapic acid (**3**) have been isolated from the seeds of *Raphanus nussatirus* L. The structures of compounds **1**-**3** were determined based on chemical analysis and spectroscopic methods (UV, 1D and 2D NMR, HRFABMS, HREIMS and elemental analysis).

Keywords: *Raphanus nussatirus* L; Disulfide glycoside; Sinapic acid derivatives

1. Introduction

The seeds of *Raphanus nussatirus* L., a traditional Chinese herbal medicine, have been used for expectorant, anti-cough and antiasthmatic purposes. It was well known that Brassicaceae family contained glucosinolates and a few glucosinolates have been isolated from the seeds of *Raphanus nussatirus* L [1–3]. During our investigation of the chemical constituents of the seeds of *Raphanus nussatirus* L., a disulfide glycoside (**1**), with a disulfide bond at the anomeric carbon and different from those glucosinolates, a new sinapic acid derivative (**2**) and sinapic acid (**3**) have been isolated from the seeds of *Raphanus nussatirus* L.

2. Results and discussion

Compound **1** was obtained as colourless needles from CH_3OH, mp 83–84°C. The UV (CH_3OH) spectrum showed maximum absorption at 329.0 nm. The FAB mass spectra exhibited ion peak at *m/z* 447 $[M - H]^-$ and 449 $[M + H]^+$. Its molecular formula of $C_{18}H_{24}O_9S_2$ was determined by HRFABMS at the ion peak *m/z* 449.0947 $[M + H]^+$.

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Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020600883468

The ^{1}H NMR data of compound **1** give signals of trans alkene at δ 6.48 (1H, d, $J = 15.9$ Hz), 7.61 (1H, d, $J = 15.9$ Hz), 1, 3, 4, 5-tetrasubstitued benzene ring at δ 7.03 (2H, s), two methoxyl groups at δ 3.90 (6H, s) and a phenolic hydroxyl group at δ 7.77 (1H, s), which are characteristic of a sinapic acid moiety. The ^{13}C NMR showed the presence of 18 carbon atoms. Six benzene carbons at δ 126.0, 106.8 × 2, 148.8 × 2, 139.4, two alkene carbons at δ 115.7, 146.2, a carbonyl carbon at δ 167.2 and two methoxyl carbons at δ 56.7 × 2, which are also in agreement with sinapic acid. Additionally, the ^{1}H NMR and ^{13}C NMR spectra gave signals of a sugar moiety at δ_H 4.49 (1H, d, $J = 8.8$ Hz), 4.31 (1H, dd, $J = 12.0$, 6.4 Hz), 4.55 (1H, dd, $J = 12.0$, 1.6 Hz), 3.57 (1H, m), 3.64 (1H, m), 3.50 (1H, m), 3.44 (1H, m) and δ_C 64.4, 71.1, 72.4, 79.2, 91.6. Based on the HMQC, HMBC, ^{1}H–^{1}H COSY and NOESY spectral data (figure 2), it was elucidated as β-D-glucopyranosyl moiety. The HMBC correlations of H-6″ at δ 4.31 and 4.54 and C-1 at δ 167.2 suggested the carbonyl group of sinapic acid was esterified with the C-6″ hydroxyl group of the sugar.

In ^{13}C NMR spectrum, the anomeric carbon signal was at δ 91.6, which indicated that the anomeric carbon was connected with sulfur atom, similar to 1-thio-β-D-glucosides [4]. Moreover, the methyl signals at δ 2.48 and 24.8 also showed the connection with a disulfide bond [5], and this was further confirmed by the fragment ion peak at m/z 369 [M-S_2CH_3] in positive FABMS and the fragment ion peaks at m/z 401 [M-SCH_3], 367 [M-SCH_3-H_2S] in negative FAB MS. The total S content was 13.04% by using a sulfur elemental analyzer, thus the structure of compound **1** was deduced as figure 1.

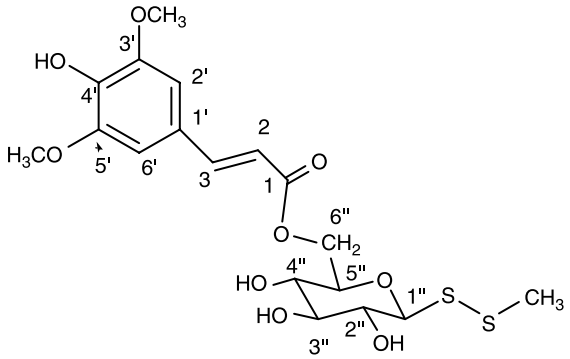
Compound **2** was obtained as yellow needles from CH_3OH, mp 105 ~ 106°C. The UV (CH_3OH) spectrum showed maximum absorption at 330.0 nm. Its molecular formula of $C_{17}H_{16}O_7$ was determined by HREIMS at the ion peak m/z 332.0896 $[M + H]^+$. The ^{1}H NMR and ^{13}C NMR spectra revealed the presence of a sinapic acid moiety at δ_H 8.99 (1H, s, 4′-OH), 7.06 (2H, s), 7.62 (1H, d, $J = 15.9$ Hz), 6.60 (1H, d, $J = 15.9$ Hz), 3.77 (6H, s) and δ_C 166.0, 148.0 × 2, 146.4, 138.5, 123.8, 113.9, 106.4 × 2, 56.1 × 2. Additionally, the ^{1}H NMR spectrum showed the presence of aldehyde proton at δ 9.61 (1H, s) and signals of a 2, 5-substitued furfural ring at δ 7.54 (1H, d, $J = 3.5$ Hz), 6.85 (1H, d, $J = 3.5$ Hz) and a methylene at δ 5.28 (2H, s), similar to those of 5-hydroxymethylfurfural [6]. The HMBC correlations of H-6″ at δ 5.28 and C-1 at δ 166.0, C-5″ at δ 155.6 and C-4″ at δ 112.9 exhibited that sinapic acid was esterified with 5-hydroxymethylfurfural. The assignments of the NMR data were completed by analyzing ^{13}C NMR, HMQC and HMBC spectra. The structure of compound **2** was concluded as figure 1.

The structure of sinapic acid (**3**) was established based on the spectral data by comparison with those reported in the literature [7].

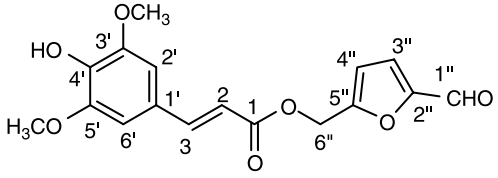
3. Experimental

3.1 General experimental procedures

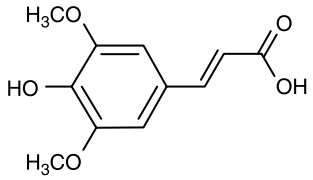
Melting point was measured on a Yamaco- micro-melting and is uncorrected. All NMR spectra were recorded on Bruker-ARX-400 spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz), using TMS as an internal standard. The HRFABMS was determined by MAT-95 mass spectrometer. The total S contents were analyzing by EA1108 Elemental Analyzer. HPLC was performed using Shimadzu LC-8A on Shimadzu PRC. Column chromatography



Compound **1**



Compound **2**



Compound **3**

Figure 1. Structures of **1**, **2**, **3**.

was performed on silica gel G (200 ~ 300 mesh, Qingdao Haiyang Chemical Factory) and reversed-phase silica gel (Chromatorex C_{18} and C_8).

3.2 Plant material

The seeds of *Raphanus nussatirus* L. were collected in Hebei Province, China, in September 2003 and identified by professor Hong Zhao of Dalian University. A voucher specimen is deposited in College of Bioengineering of Dalian University with the No.20030015.

3.3 Extraction and isolation

The seeds powder of *Raphanus nussatirus* L. (15 kg) was extracted with petroleum ether at room temperature for 3 days. The residue was extracted with boiled EtOH (95%), then the gauze-filtered extract was concentrated *in vacuo* and the residue was extracted with petroleum ether, EtOAC and *n*-butanol successively. The EtOAC soluble fraction was evaporated and the residue (100.0 g) was separated into several fractions by silica gel column

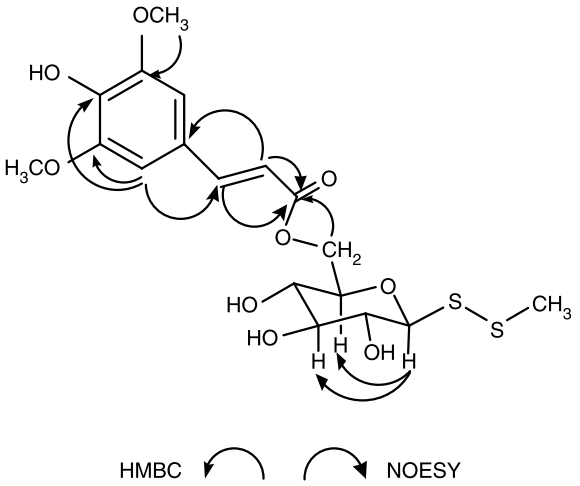


Figure 2. Main HMBC and NOESY correlations of compound **1**.

chromatography, eluting with $CHCl_3$:CH_3OH. The fraction eluted with $CHCl_3$:CH_3OH (15:1) was separated by HPLC (ODS column, 8μM, 250 × 10 mm, flow rate 3.0 ml/min, UV 254 nm) eluting with H_2O:CH_3OH (3:7) to afford **1** (70 mg). Compounds **2** (60 mg) and **3** (25 mg) were obtained from the fraction eluted with $CHCl_3$: CH_3OH (20:1).

Compound 1: Colourless needles (CH_3OH) with mp 83 ~ 84°C, $UV_{\lambda max}$ 229.0 nm. HRFABMS: positive ion peak $[M + H]^+$ at *m/z* 449.0947 (calcd for $C_{18}H_{25}O_9S_2$, 449.0958). Negative ion peak $[M - H]^-$ at *m/z* 447, 401, 367. The elemental analyzing of sulfur was 13.04%. ^1H NMR (500 MHz, CD_3COCD_3) and ^{13}C NMR (125 MHz, CD_3COCD_3) data see table 1.

Compound 2: Colourless needles (CH_3OH) with mp 105 ~ 106°C, $UV_{\lambda max}$ 330.0 nm, 279.0 nm (sh). HREIMS: $[M + H]^+$ at *m/z* 332.0896 (calcd for $C_{17}H_{16}O_7$, 332.0891). EIMS at *m/z* 332$[M]^+$, 223, 207, 109. ^1H NMR (500 MHz, DMSO-d_6) δ 9.61 (1H, s, H-1″), 8.99 (1H, s, 4′-OH), 7.62 (1H, d, $J = 15.9$ Hz, H-3), 7.54 (1H, d, $J = 3.5$ Hz, H-3″), 7.06

Table 1. The ^1H NMR and ^{13}C NMR data of compound **1**.

	δc	*HMQC*	*HMBC*
1	167.2		H-6″, H-2, H-3
2	115.7	H-2 (6.48, 1H, d, $J = 15.9$ Hz)	H-3
3	146.2	H-3 (7.61, 1H, d, $J = 16.0$ Hz)	H-2′, 6′
1′	126.0		H-2, H-3, H-2′, 6′
2′, 6′	106.6	H-2′, 6′ (7.03, 2H,s)	H-2′, 6′
3′, 5′	148.8		H-OCH_3, H-2′, 6′
4′	139.4		H-2′, 6′
1″	91.6	H-1″ (4.59, 1H, d, $J = 8.8$ Hz)	H-2″, H-5″
2″	72.4	H-2″ (3.57, 1H, m)	H-3″, H-1″
3″	79.2	H-3″ (3.50, 1H, m)	H-4″, H-2″, H-5″, H-1″
4″	71.1	H-4″ (3.44, 1H, m)	H-2″, H-5″, H-6″
5″	79.2	H-5″ (3.64, 1H, m)	H-4″, H-1″
6″	64.4	H-6″ (4.31, 1H, dd, $J = 12$, 1.6 Hz; 4.55, 1H, dd, $J = 12$, 6.4 Hz)	H-4″
—OCH_3	56.7	H-OCH_3 (3.90, 3H, S)	
CH_3	24.8	2.48 (3H, s)	
Ar-OH		(7.78, 1H, s)	

Measured in CD_3COCD_3.

(2H, s, H-2′,6′), 6.85 (1H, d, $J = 3.5$ Hz, H-4″), 6.60 (1H, d, $J = 15.9$ Hz, H-2), 5.28 (2H, s, H-6″), 3.77 (6H, s, CH_3O-). ^{13}C NMR (125 MHz, DMSO-d_6) δ 178.5 (C-1″), 166.0 (C-1), 155.6 (C-5″), 152.4 (C-2″), 148.0 (C-3′,5′), 146.4 (C-3), 138.5 (C-4′), 124.2 (C-3″), 123.8 (C-1′), 113.9 (C-2), 112.9 (C-4″), 106.4 (C-2′,6′), 57.4 (C-6″), 56.1 (CH_3O—).

Acknowledgements

This work was financially supported by the National Nature Science Foundation of China (NO. 90209041). The authors were grateful to Dr. Wangwei for NMR and HRFABMS measurements.

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