Thioglucosides from the Seeds of *Raphanus sativus* L.

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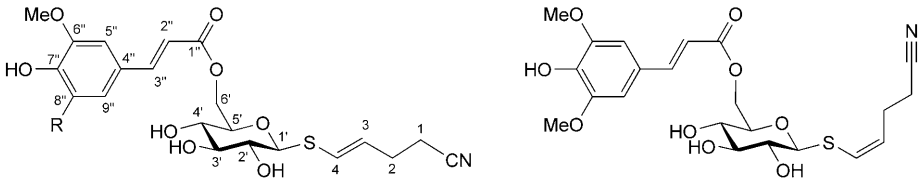
Three new thioglucosides, (4*E*)-5-{6-*O*-[(2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]-*β*-glucopyranosylsulfanyl}pent-4-enenitrile (**1**), (4*E*)-5-{6-*O*-[(2*E*)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoyl]-*β*-glucopyranosylsulfanyl}pent-4-enenitrile (**2**) and its (4*Z*)-isomer **3**, were isolated from the seeds of *Raphanus sativus* L. (radish), together with two known compounds. Their structures were determined by spectroscopic methods, including UV/VIS, 1D- and 2D-NMR, FAB- and HR-FAB-MS experiments.

Introduction. – *Raphanus sativus* L. (Cruciferaceae), commonly known as radish, is widely available throughout the world and consumed as a vegetable or condiment in human diets. Different parts of radish, including the roots, seeds, and leaves, are also being used for medicinal purposes [1–4]. In China, it has been used as a traditional Chinese herbal medicine for more than 1400 years, since being recorded in '*Tang Materia Medica*', the first Chinese pharmacopoeia [5]. From the seeds of *R. sativus* L., some glucosinolates have been isolated [6–8]. Glucosinolates and/or their breakdown products have recently attracted considerable interest because of their cancer-chemoprotective properties.

Herein, we report the isolation and identification of three new constituents (**1**–**3**) from the seeds of *R. sativus* L., which were obtained together with two known compounds, (*E*)-sinapic acid methyl ester (**4**) and (*E*)-ferulic acid ethyl ester (**5**)[1]).

Results and Discussion. – The thioglucoside **1** was obtained as a yellow oil. Its UV spectrum showed a maximum at 320 nm (log $\varepsilon = 3.80$). The molecular formula $C_{21}H_{25}NO_8S$ was determined by HR-FAB-MS (*m/z* 474.1225 ($[M+Na]^+$; calc. 474.1199)). The structure of **1** was established as (4*E*)-5-{6-*O*-[(2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]-*β*-glucopyranosylsulfanyl}pent-4-enenitrile by means of in-depth ^{1}H- and ^{13}C-NMR (*Table 1*) as well as 2D-NMR (^{1}H,^{1}H-COSY, HSQC, HMBC, NOESY) analyses (*Figure*).

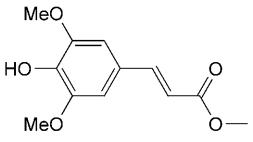
[1]) Most likely an artifact formed by esterification of ferulic acid with EtOH during extraction.



1 R = H
2 R = MeO

Arbitrary atom numbering

3



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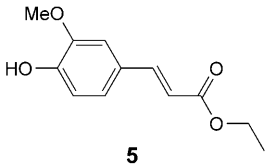

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The ^{1}H-NMR data of **1** showed the presence of an (*E*)-feruloyl (=(*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl) moiety, with the following typical signals [9]: three aromatic H-atoms forming an *ABX* system [δ(H) 7.09 (*dd*, *J* = 8.1, 1.5 Hz, 1H); 6.82 (br. *d*, *J* = 8.1 Hz, 1 H); 7.22 (*d*, *J* = 1.5 Hz, 1 H)]; two H-atoms of an (*E*)-configured C=C bond [δ(H) 7.65 (*d*, *J* = 15.9 Hz, 1 H); 6.43 (*d*, *J* = 15.9 Hz, 1 H)]; and an aromatic MeO group at δ 3.90 (*s*, 3 H). The ^{1}H-NMR spectrum also showed the presence of another (*E*)-configured C=C bond at high field [δ(H) 6.34 (br. *d*, *J* = 15.2 Hz, 1 H), 5.82 (*dt*, *J* = 15.2, 7.3 Hz, 1 H)] and two CH_2 groups [δ(H) 2.41–2.45 (*m*, 2 H), 2.31–2.36 (*m*, 2 H)].

The ^{13}C- NMR spectrum of **1** (*Table 1*) showed six aromatic C-atoms at δ(C) 150.8, 149.5, 127.7, 124.3, 116.6, and 111.7, four olefinic resonances at δ(C) 147.0, 115.4, 130.5, 124.4, one C=O group at δ(C) 169.0, a MeO group at δ(C) 56.5, and a CN group at δ(C) 120.6. Additionally, the ^{1}H- and ^{13}C-NMR spectra showed the signals of a sugar moiety: δ(H) 4.47 (*d*, *J* = 9.6 Hz, 1 H), 4.33 (*dd*, *J* = 12.0, 6.2 Hz, 1 H), 4.52 (*dd*, *J* = 12.0, 1.8 Hz, 1 H), 3.51–3.60 (*m*, 1 H), 3.38–3.39 (*m*, 2 H), 3.28–3.30 (*m*, 1 H), and at δ(C) 64.7, 71.5, 74.0, 79.5, 79.4, and 87.1, respectively. Based on the HSQC, HMBC, ^{1}H,^{1}H-COSY, and NOESY data (*Figure*, *Table 1*), a β-glucopyranosyl moiety was identified. The absolute configuration of the sugar was most likely D, but clear-cut experimental evidence was absent.

In the HMBC spectrum of **1**, the correlation of H_a–C(6′) at δ(H) 4.33 with C(1″) at δ(C) 169.0 suggested that the C=C group of the feruloyl moiety was esterified with the 6′-OH function of the sugar; and a correlation of H–C(4) at δ(H) 6.34 with C(1′) at δ(C) 87.1 was also observed. Moreover, in the ^{13}C-NMR spectrum, the anomeric signal at δ(C) 87.1 indicated attachment to an S-atom, as in other 1-thio-β-D-glucosides [10]. The positions of the MeO and OH group on the aromatic ring were determined by NOESY experiments (*Figure*).

Compound **2** was obtained as a yellow oil. The UV spectrum showed a maximum at 329 nm (log ε = 3.81). The HR-FAB mass spectrum exhibited the quasi-molecular ion peak at *m*/*z* 504.1341 ([*M* + Na]$^+$; calc. 504.1304), indicating the molecular formula

Table 1. *^{1}H-, ^{13}C-, and 2D-NMR Data for* **1**. At 500/125 MHz, resp., in CD_3OD; δ in ppm, J in Hz. Arbitrary atom numbering (see chemical formula).

	δ(C)	δ(H)	^{1}H,^{1}H-COSY	HMBC (C → H)
CH_2(1)	17.6	2.41–2.45 (*m*)	2	2
CH_2(2)	30.0	2.31–2.36 (*m*)	1, 3, 4	1, 3, 4
H–C(3)	130.5	5.82 (*dt*, *J* = 15.2, 7.3)	2, 4	1, 2
H–C(4)	124.4	6.34 (br. *d*, *J* = 15.2)	2, 3	2
H–C(1′)	87.1	4.47 (*d*, *J* = 9.6)	2′	4
H–C(2′)	74.0	3.28–3.30 (*m*)	1′, 3′	4′
H–C(3′)	79.4	3.38–3.39 (*m*)	2′	2′, 4′
H–C(4′)	71.5	3.38–3.39 (*m*)	5′	3′
H–C(5′)	79.5	3.51–3.60 (*m*)	6′	3′, 4′
H_a–C(6′)	64.7	4.33 (*dd*, *J* = 12.0, 6.2)	5′, 6′	5′
H_b–C(6′)		4.52 (*dd*, *J* = 12.0, 1.8)		
C(1″)	169.0			6′, 3″
H–C(2″)	115.4	6.43 (*d*, *J* = 15.9)	3″	3″
H–C(3″)	147.0	7.65 (*d*, *J* = 15.9)	2″	5″, 9″
C(4″)	127.7			2″, 8″
H–C(5″)	111.7	7.22 (*d*, *J* = 1.5)	9″	3″, 9″
C(6″)	149.5			5″, 8″, MeO
C(7″)	150.8			5″, 8″, 9″
H–C(8″)	116.5	6.82 (br. *d*, *J* = 8.1)	9″	
H–C(9″)	124.3	7.09 (*dd*, *J* = 8.1, 1.5)	5″, 8″	3″, 5″
5″-MeO	56.5	3.90 (*s*)		
CN	120.6			1, 2

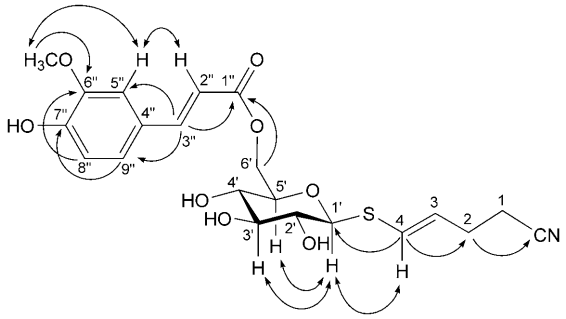

Figure. *Key HMBC* (→) *and NOESY* (↔) *correlations for* **1**

$C_{22}H_{27}NO_9S$. The ^{1}H- and ^{13}C-NMR spectra of **1** and **2** were very similar. The difference consisted in an additional MeO group at the aromatic moiety in the case of **2** (sinapoyl *vs.* feruloyl moiety). Based on the HSQC, HMBC, ^{1}H,^{1}H-COSY, and NOESY data (*Table 2*), the structure of the thioglucoside **2** was elucidated as (4*E*)-5-{6-*O*-[(2*E*)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoyl]-β-glucopyranosylsulfanyl}pent-4-enenitrile.

The ^{1}H-NMR data of thioglucoside **2** (*Table 2*) showed the signals of an (*E*)-olefin at δ(H) 6.46 (*d*, *J* = 16.0, 1 H) and 7.65 (*d*, *J* = 16.0 Hz, 1 H), of a 1,3,4,5-tetrasubstitued benzene ring at δ(H) 6.94 (*s*, 2 H), and of two MeO groups at δ(H) 3.90 (*s*, 6 H). The

Table 2. *^{1}H- and ^{13}C-NMR Data for* **2** *and* **3**. At 500/125 MHz, resp., in CD_3OD; δ in ppm, J in Hz. Arbitrary atom numbering (see chemical formulae).

	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	2.43–2.46 (*m*)	17.6	2.49–2.53 (*m*)	16.9
CH_2(2)	2.33–2.36 (*m*)	29.9	2.42–2.49 (*m*)	26.0
H–C(3)	5.82 (*dt*, *J* = 15.1, 7.0)	130.6	5.71 (*dt*, *J* = 10.0, 7.0)	128.4
H–C(4)	6.34 (*dt*, *J* = 15.1, 1.0)	124.4	6.40 (*d*, *J* = 10.0)	125.2
H–C(1′)	4.47 (*d*, *J* = 9.7)	87.1	4.47 (*d*, *J* = 10.5)	87.0
H–C(2′)	3.28–3.30 (*m*)	74.0	3.28–3.31 (*m*)	74.3
H–C(3′)	3.38–3.40 (*m*)	79.4	3.37–3.42 (*m*)	79.4
H–C(4′)	3.38–3.40 (*m*)	71.5	3.37–3.42 (*m*)	71.4
H–C(5′)	3.56–3.61 (*m*)	79.5	3.56–3.61 (*m*)	79.6
H_a–C(6′)	4.51 (*dd*, *J* = 2.1, 12.0)	64.8	4.51 (br. *d*, *J* = 12.0)	64.7
H_b–C(6′)	4.34 (*dd*, *J* = 6.2, 12.0)		4.33 (*dd*, *J* = 12.0, 5.8)	
C(1″)		169.0		169.0
H–C(2″)	6.46 (*d*, *J* = 16.0)	115.9	6.44 (*d*, *J* = 16.0)	115.7
H–C(3″)	7.65 (*d*, *J* = 16.0)	147.2	7.63 (*d*, *J* = 16.0)	147.3
C(4″)		126.6		126.6
H–C(5″,9″)	6.94 (*s*)	107.1	6.92 (*s*)	107.0
C(6″,8″)		149.6		149.5
C (7″)		139.8		139.7
6″,8″-MeO	3.89 (*s*)	56.5	3.88 (*s*)	56.9
CN		120.6		120.8

^{13}C-NMR spectrum showed six aromatic resonances at δ(C) 126.6, 107.1 (2 C), 149.6 (2 C), and 139.8, two olefinic reosonances at δ(C) 115.9, 147.2, a C=O group at δ(C) 169.0, and two equivalent MeO C-atoms at δ(C) 56.5, in agreement with a sinapoyl (= (*E*)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoyl) moiety [11].

Compound **3** was identified as the (*Z*)-isomer of **2**, and obtained as a yellow oil. The $[M+Na]^+$ signal appeared at *m*/*z* 504.1331 (calc. 504.1304) in the HR-FAB mass spectrum, consistent with the molecular formula $C_{22}H_{27}NO_9S$. The UV spectrum of **3** showed a maximum at 329 nm (log ε = 3.81). The configuration of the C(3)=C(4) bond was confirmed to be (*Z*) based on a ^{1}H-NMR coupling constant *J* of 10.0 Hz (*Table 2*). Thus, from these data, in combination with ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY experiments, compound **3** was identified as (4*Z*)-5-{6-*O*-[(2*E*)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoyl]-β-glucopyranosylsulfanyl}pent-4-enenitrile.

The two known compounds, (*E*)-sinapic acid methyl ester (= methyl (2*E*)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoate; **4**) and (*E*)-ferulic acid ethyl ester (= ethyl (2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate; **5**) were identified by comparison with the spectroscopic data reported in the literature [11][12]. Note that **5** is most likely an artifact due to esterification of ferulic acid in the presence of hot EtOH (extraction procedure).

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical Group, Co.*), C_{18} reverse-phase (RP) silica gel (250 mesh; *Merck*), and *Sephadex LH-20* (*Pharmacia)*. TLC: Precoated silica gel GF_{254} plates (*Qingdao Haiyang Chemical Group*). HPLC: *Waters LC 515* system. UV Spectra: *Hitachi U-2010* apparatus; λ_{max} (log ε) in nm. NMR Spectra: *Bruker ARX-500* spectrometer, at 500 or 125 MHz for ^{1}H and ^{13}C, resp.; δ in ppm rel. to Me_4Si, *J* in Hz. HR-FAB-MS: *Autospec UltimaETOF* mass spectrometer; in *m*/*z*.

Plant Material. Seeds of *Raphanus sativus* L. were collected in Hubei Province, P. R. China, in September 2005, and identified by Prof. *Hong Zhao*, Department of Medicine College, Dalian University. A voucher specimen (No. 20050015) was deposited at the School of Bioengineering, Dalian University, P. R. China.

Extraction and Isolation. The powdered seeds of *R. sativus* L. (15 kg) were extracted with petroleum ether (PE; 3×10 l) at r.t. for 3 d. The defatted residue was extracted with 95% EtOH at reflux, and then filtered by gauze. The EtOH extract was concentrated on a rotary evaporator, the residue was suspended in H_2O, and extracted successively with PE, AcOEt, and BuOH. The AcOEt-soluble fraction was evaporated, the residue (100 g) was separated by CC (SiO_2; $CHCl_3$/MeOH 1:0, 100:1, 50:1, 30:1, 15:1, 10:1, 5:1, 2:1, 0:1). The fraction eluted with $CHCl_3$/MeOH 15:1 was further separated by HPLC on an *ODS* column (8 μm, 250×10 mm) at a flow rate of 3.0 ml/min, with UV detection at 330 nm, eluting with H_2O/MeCN 2:8 to afford **1** (10 mg), **2** (20 mg), and **3** (9 mg).

*(4*E*)-5-{6-*O*-[(2*E*)-3-(4-Hydroxy-3-methoxyphenyl)prop-2-enoyl]-β-glucopyranosylsulfanyl}pent-4-enenitrile* (**1**). Yellow oil. UV (MeOH): 320 (3.80). ^{1}H- and ^{13}C-NMR: see *Table 1*. FAB-MS: 474 ($[M+Na]^+$), 318, 302, 177. HR-FAB-MS: 474.1225 ($[M+Na]^+$; $C_{21}H_{25}NNaO_8S^+$; calc. 474.1199).

*(4*E*)-5-{6-*O*-[(2*E*)-3-(4-Hydroxy-3,5-dimethoxyphenyl)prop-2-enoyl]-β-glucopyranosylsulfanyl}-pent-4-enenitrile* (**2**). Yellow oil. UV (MeOH): 330 (3.81), 279 (sh). ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 481 (M^+), 369, 351, 224, 207, 175. HR-FAB-MS: 504.1341 ($[M+Na]^+$, $C_{22}H_{27}NNaO_9S^+$; 504.1304).

*(4*Z*)-5-{6-*O*-[(2*E*)-3-(4-Hydroxy-3,5-dimethoxyphenyl)prop-2-enoyl]-β-glucopyranosylsulfanyl}-pent-4-enenitrile* (**3**). Yellow oil. UV (MeOH): 330 (3.81), 279 (sh). ^{1}H- and ^{13}C-NMR: see *Table 2*. FAB-MS: 504 ($[M+Na]^+$), 274, 207. HR-FAB-MS: 504.1331 ($[M+Na]^+$, $C_{22}H_{27}NNaO_9S^+$; 504.1304).

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