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A NEW CYCLO-THIOGLUCOSIDE FROM THE SEEDS OF *RAPHANUS SATIVUS* L.

Bao-Min Feng,^{a,b} Li-Xin Duan,^{a,c} Ling Tang,^{a,b} Yue-Hu Pei,^c and Yong-Qi Wang^{a, b,*}

^a College of Bioengineering, Dalian University, Economic and Development Zone, Dalian 116622, China

^b Liaoning Key Laboratory of Bioorganic Chemistry, Dalian University, Economic and Development Zone, Dalian 116622, China

^c School of Traditional Chinese Medicines of Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, China

E-mail: dalianwyq@163.com; Fax: +86-411-87403834

Abstract – A cyclo-thioglucoiside named as raphanuside was isolated from the seeds of *Raphanus sativus* L.. The structure of raphanuside was determined on the basis of extensive spectroscopic means including HR-FAB-MS, 1D and 2D NMR experiments.

INTRODUCTION

Raphanus sativus L. (Cruciferaee), commonly known as radish is widely available throughout the world and consumed as a vegetable or condiment in human diets. The seeds of *R. sativus* L., a traditional Chinese herbal medicine, have been used for expectorant, anti-cough and antiasthmatic purposes. It was well known that Brassicaceae plants contained organic sulfides and a lot of organic sulfides have been isolated from the this kind of seeds.¹⁻⁴ During our previous investigation on its chemical constituents, 3 thioglucoisides have been obtained.⁵ Continuous research has led to the isolation of raphanuside (**1**), and the isolation process and structural characterization was reported in this paper (Figure 1).

RESULTS AND DISCUSSION

Raphanuside (**1**) was obtained as white amorphus powder, mp 145~147 °C (CH₃OH), $[\alpha]_D^{20} +64.5$ (CH₃OH). HR-FAB-MS showed *m/z* as 374.1018 and the molecular formula was deduced to be C₁₆H₂₂O₈S (calc. 374.1035). ¹H-NMR displayed a symmetrical 1, 3, 4, 5-tetrasubstituted phenyl group

(δ_{H} 6.70, s, H-2', 6'), two methoxyl groups (δ_{H} 3.84, s, 3', 5'-OCH₃), a -CH-CH₂- fragment (δ_{H} 4.60, dd, $J=10.5, 2.0$, H-8; 2.76, dd, $J=14.0, 2.0$, H-7e; 3.08, dd, $J=14.0, 10.5$, H-7a) and a glucose fragment (δ_{H} 4.

Table 1. ¹H and ¹³C NMR data (CD₃OD) of raphanuside (1)

Position	δ_{C}	δ_{H}
1	76.9	4.47, 1H, d, $J=8.5$
2	85.5	3.45, 1H, brd, $J=8.5$
3	76.6	3.52, 1H, m
4	71.8	3.43, 1H, m
5	83.2	3.43, 1H, m
6	62.8	3.67, 1H, ddd, $J=12.0, 3.8, 1.2$; 3.87, 1H, brd, $J=12.0$
7	36.3	2.76, 1H, dd, $J=14.0, 2.0$, H-7e; 3.08, 1H, dd, $J=14.0, 10.5$, H-7a
8	81.9	4.60, 1H, dd, $J=10.5, 2.0$
1'	133.1	
2', 6'	104.8	6.70, 2H, s
3', 5'	149.2	
4'	136.4	
CH ₃ O	56.8	3.84, 6H, s

47, d, $J=8.5$, H-1; 3.87, brd, $J=12.0$, H-6; 3.67, ddd, $J=12.0, 3.8, 1.2$, H-6; 3.52, m, H-3; 3.45, brd, $J=8.5$, H-2; 3.43, m, H-4, 5). The analysis of ¹³C-NMR and HSQC revealed 16 carbon signals including two *sp*² methines (δ_{C} 104.8, C-2', 6'), four *sp*² quaternary carbons (δ_{C} 149.2, C-3', 5'; 136.4, C-4'; 133.1, C-1'), six *sp*³ methines (δ_{C} 85.5, C-2; 81.9, C-8; 83.2, C-5; 76.9, C-1; 76.6, C-3; 71.8, C-4), two *sp*³ methylenes (δ_{C} 62.8, C-6; 36.3, C-7) and two methyl carbons (δ_{C} 56.8, 3', 5'-OCH₃). Glucose fragment was further confirmed by HSQC and HMBC analysis and the data was listed in Table 1. Moreover, in ¹³C-NMR spectrum, the anomeric carbon signal of the glucose fragment was at δ_{C} 76.9, which indicated that the anomeric carbon was connected with a sulfur atom, similar to 1-thio- β -D-glucosides,⁶ and this was further confirmed from its molecular formula by the HR-FAB-MS. The assignments of the hydrogen signals to the carbon signals were finished by HSQC analysis (Table 1). In HMBC, correlation between δ_{H} 2.76 (H-7e) and the anomeric carbon (δ_{C} 76.9), that between δ_{H} 4.60 (H-8) and δ_{C} 85.5 (C-2), 133.1 (C-1'), and that between δ_{H} 6.70 (H-2', 6') and δ_{C} 81.9 (C-8) determined the linkage of the fragments as shown in Figure 1. H-1 was determined to be α according to the J value between H-1 and H-2 and the

stereochemistry of H-8 was established as β based on the NOESY spectrum. Thus the structure of **1** was deduced to be as shown in Figure 1, and named raphanuside.

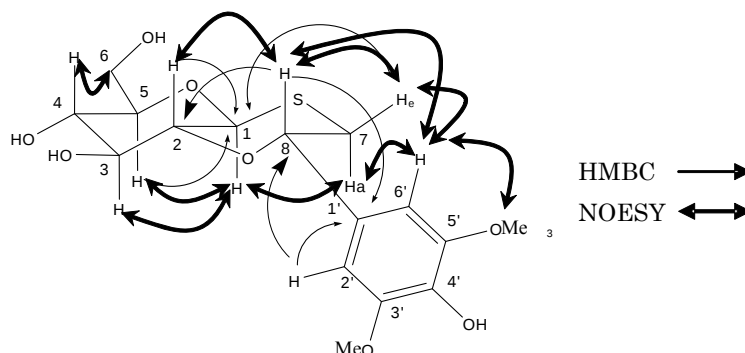


Figure 1: HMBC and NOESY correlations for raphanuside (**1**)

EXPERIMENTAL

General Experimental Procedures

Optical rotation was measured on a Horiba SEPA-300 polarimeter. NMR spectra was recorded on a Bruker-ARX-500 spectrometer in CD₃OD, δ in ppm and J in Hz. The HR-FAB-MS spectra was recorded with a Autospec-UltimaETOF spectrometer. HPLC was carried out on Waters LC 515 with 2487 detector. Silica gel (200–300 mesh, Qingdao Marine Chemical Group, China) and Sephadex LH-20 (Pharmacia) were used for Column chromatography. Fractions were monitored by TLC (pre-coated silica gel GF₂₅₄ plates made by Qingdao Marine Chemical Group, China).

Plant Material

The seeds of *Raphanus sativus* L. were collected in Hubei Province, China, in September 2005 and identified by Prof. W. Bao, School of Traditional Chinese Medicines, Shenyang Pharmaceutical University. A voucher specimen is deposited in college of Bioengineering of Dalian University with the No.20050015.

Extraction and Isolation

The seeds powder of *R. sativus* L. (15 kg) was left in petroleum ether (3×10L) at rt for 3 days. The defatted residue was extracted with EtOH (95%) under reflux and then filtered by gauze. The EtOH extract was concentrated by rotary evaporation and the residue suspended in H₂O and extracted with petroleum ether, EtOAc and *n*-BuOH successively. The *n*-BuOH soluble fraction was evaporated and the residue (100.0 g) was separated into several fractions by column chromatography on silica gel, eluting with CHCl₃: MeOH (1:0, 100:1, 50:1, 30:1, 15:1, 10:1, 5:1, 2:1, 0:1). The fraction eluted with CHCl₃:MeOH (10:1) was separated by HPLC (ODS column, 8 μ M, 250×10mm, flow rate 3.0ml/min, UV 330 nm) eluting with H₂O:MeCN (3:7) to afford **1** (7 mg).

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