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

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**Abstract** – A cyclo-thioglucoside 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. (Cruciferaceae), 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 thioglucosides 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 ℃ ($CH_3OH$), $[\alpha]_D^{20}$ +64.5 ($CH_3OH$). HR-FAB-MS showed *m/z* as 374.1018 and the molecular formula was deduced to be $C_{16}H_{22}O_8S$ (calc. 374.1035). $^1$H-NMR displayed a symmetrical 1, 3, 4, 5-tetrasubstituted phenyl group

($\delta_H$6.70, s, H-2', 6'), two methoxyl groups ($\delta_H$3.84, s, 3', 5'-$OCH_3$), a -CH-$CH_2$- fragment ($\delta_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 ($\delta_H$ 4.

Table 1. $^1$H and $^{13}$C NMR data ($CD_3OD$) of raphanuside (**1**)

| Position | δc | $\delta_H$ |
|---|---|---|
| 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 | |
| $CH_3O$ | 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 $^{13}$C-NMR and HSQC revealed 16 carbon signals including two $sp^2$ methines ($\delta_C$ 104.8, C-2', 6'), four $sp^2$ quaternary carbons ($\delta_C$ 149.2, C-3', 5'; 136.4, C-4'; 133.1, C-1'), six $sp^3$ methines ($\delta_C$ 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 ($\delta_C$ 62.8, C-6; 36.3, C-7) and two methyl carbons ($\delta_C$ 56.8, 3', 5'-$OCH_3$). Glucose fragment was further confirmed by HSQC and HMBC anaylsis and the data was listed in Table 1. Moreover, in $^{13}$C-NMR spectrum, the anomeric carbon signal of the glucose fragment was at $\delta$76.9, which indicated that the anomeric carbon was connected with a sulfur atom, similar to 1-thio-$\beta$-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 $\delta_H$ 2.76 (H-7e) and the anomeric carbon ($\delta_C$ 76.9), that between $\delta_H$ 4.60 (H-8) and $\delta_C$ 85.5 (C-2), 133.1 (C-1'), and that between $\delta_H$ 6.70 (H-2', 6') and $\delta_C$ 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 $\beta$ based on the NOESY spectrum. Thus the structure of **1** was deduced to be as shown in Figure 1, and named raphanuside.

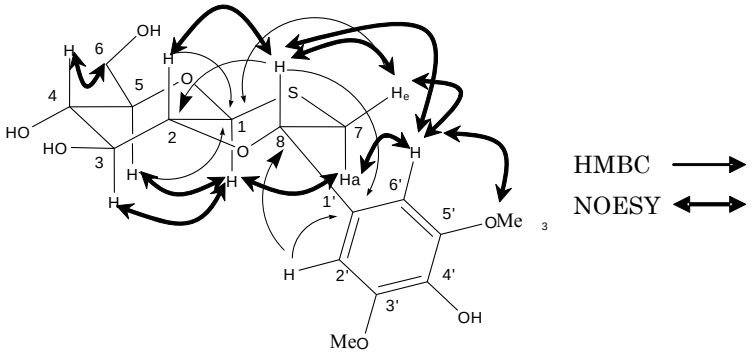


Figure 1: HMBC and NOESY correlations for raphanuside (**1**)

## 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 $CD_3OD$, $\delta$ 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 $GF_{254}$ 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 $H_2O$ 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 $CHCl_3$: 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 (10:1) was separated by HPLC (ODS column, 8μM, 250×10mm, flow rate 3.0ml/min, UV 330 nm) eluting with $H_2O$:MeCN (3:7) to afford **1** (7 mg).

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## REFERENCES

1. A. H. Gilani and M. N. Ghayur, *J. Ethnopharmacol*., 2004, **95**, 169.
2. R. Vargas, R. M.Perez, S. Perez, M. A. Zavala, and C. Perez, *J. Ethnopharmacol.*, 1999, **68**, 335.
3. M. N. Ghayur and A. H. Gilani, *Fundam*. *Clin*. *Pharmacol*., 2006, **20**, 57.
4. A. Lugasi, E. Dworschak, A. Blazovics, and A. Kery, *Phytother. Res*., 1998, **12**, 502.
5. L. Duan, B. Feng, F. Chen, W. Wang, F. Chen, G. Cai, Y. Pei, and Y. Wang, *Helv. Chim. Acta*, 2006, **89**, 2953.
6. K. Olsson, O. Theand, and P. Aman, *Carbohydr*. *Res*., 1977, **58**, 1.