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Two New Natural Products from the Roots of *Raphanus sativus* L.

Shun-bin Wu[1, 2, a], Feng Wang[2, b], Xiao-bao Jin[1, c] , Tao Liu[1, d], Lirong Wu[1, e], Wei Wang[3, f], Dan Mei[3, g], Zhong Zheng[3, h] and Jia-yong Zhu[1, *]

[1.] Guangdong Provincial Key Laboratory of Pharmaceutical Bioactive Substances, Guangdong Pharmaceutical University, Guangzhou 510006, China;

[2.] College of Traditional Chinese Materia Medica, Guangdong Pharmaceutical University, Guangzhou 510006, China;

[3.] Xi'an AMJAN Pharmaceutical Co., Ltd., Xi'an 710400, China.

[a]richard1357@163.com, [b]wfeng1230@163.com, [c]jinxf2001@163.com, [d]tliu@foxmail.com, [e]wlirong11@gmail.com, [f]13500017819@139.com, [g]echo_md@sina.com, [h]zhengzhong@an-health.com.cn, [*]corresponding author: zhujy@gdpu.edu.cn

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Abstract: The roots of *Raphanus sativus* L., also called radish, has been used as a traditional anti-migraine drug in China for hundreds of years. However, its bioactive substances and pharmacological mechanism were still not very clear. In this study, two new natural products were isolated from the roots of *Raphanus sativus* L. by successive chromatographic procedures, such as open silica gel, Sephadex LH-20 and RP-18 column chromatograph. The structures of isolated compounds were elucidated as Cis-(1-methylazetidin-2-yl)methanol and cyclo-(4-methyl-Val-4-methyl-Val) on the basis of spectral data analysis, including MS and NMR (^{1}H-NMR, ^{13}C-NMR, NOESY, HSQC and HMBC). The new natural products would be potential candidates for early anti-migraine drug study.

Introduction

Migraine is a severe and persistent headache which can cause nausea and light sensitivity, and one in five people in the world are suffering from this disease. Some migraineurs also experience an "aura" around objects which warns them an attack is on the ways [1~3]. *Raphanus sativus* L. is a plant from Cruciferae family, which is not only a delicious food, but also a medicine used as antitumor, antimicrobial and antiviral agents [4~6]. Furthermore, according to the ancient Chinese medical books, the juice of *Raphanus sativus* L. can treat migraine through intranasal administration. However, little research was conducted on the effective ingredients and the pharmacological mechanism of *Raphanus sativus* L.. Besides, the existing anti-migraine drugs, such as sumatriptan and aspirin, were expensive or just analgesic, which couldn't heal the disease but eased the pain temporarily [7, 8]. *Raphanus sativus* L. might be a good candidate for anti-migraine drug study.

In this study, the fresh radish was juiced and concentrated to dry extracts. Then the dry extracts were partitioned successively with n-hexane, CH_2Cl_2, EtOAc, and n-BuOH. The active n-BuOH extracts, demonstrated through experimental animal models of migraine [9~11], was further isolated with silica gel, Sephadex LH-20, RP-18, and HPLC. The isolated compounds were identified by spectral data analysis.

Experimental section

General Experimental Procedures. UV spectra were determined by U-2900 spectrophotometer (Hitachi, Japan). IR spectra (KBr pellets) were determined by Nicolet 6700 FT-IR (Thermo Electron, USA). ESI-MS and HR-ESI-MS data were obtained using a Q-Tof Micro mass spectrometer (Waters, USA). NMR spectra were recorded on Avance DRX-500 MHz spectrometer (Bruker, Germany) with TMS as the internal standard. Analytical HPLC was performed on Waters e2695/2998 Series HPLC system equipped with a C_{18} column (4.6×250 mm, 5 μm, SunFire^{TM}, Waters, USA). HPLC-grade solvents were purchased from Merck (Germany). Silica gel (100-200 and 200-300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China) and Sephadex LH-20 gel (GE Healthcare, USA) were both used for column chromatography. TLC analysis was run on GF254 precoated silica gel plates (Merck, Germany), and spots were visualized at 110°C after spraying 10% H_2SO_4-EtOH. All solvents used for extraction and isolation were at least of analytical grade.

Plant Material. The *Raphanus sativus* L. (Yantai, Shandong, China) was purchased from Guangzhou Carrefour (Franch) in July 2012 and botanically identified by Prof. Honghua Cui from Guangdong Pharmaceutical University, Guangzhou, China. A voucher specimen (RS20120716) is kept at College of Traditional Chinese Materia Medica, Guangdong Pharmaceutical University, China.

Extraction and Isolation. The juice (82 kg), from the roots of fresh *Raphanus sativus* L. (100 kg), was concentrated under reduced pressure to give dry extracts (2424 g) at 60℃. This crude extracts were then suspended in H_2O (2 L) and partitioned successively with n-hexane (3 batch ×2 L), CH_2Cl_2 (3×2 L), EtOAc (3×2 L), and n-BuOH (3×6 L). The n-BuOH fraction (98.0 g), which exhibited strong anti-migraine activity, was chromatographed over a silica gel column (9 × 40 cm; 200-300 mesh) and eluted with gradient mixtures of CH_2Cl_2-MeOH (100:1, 100:2, 100:3, 100:5, 100:7, 100:15, 100:20, 100:30, 100:50, 100:100, 0:100, H_2O, each 11 L) to yield 19 pooled fractions (D-1, D-2,⋯, D-19). D-8 (1.817 g) was chromatographed over a silica gel column to give nine fractions (from D-8-1 to D-8-9). Fraction D-8-8 was chromatographed over a Sephadex LH-20 column (10 × 150 mm) using MeOH as the eluting solvent to give five subfractions (form D-8-8-1 to D-8-8-5). D-8-8-2 was further purified by Pre-HPLC using 30% MeOH in water with the detector of 254 nm to yield compound **1** (6 mg). D-7 (3.174 g) was chromatographed over a silica gel column to give ten fractions (from D-7-1 to D-7-10). Fraction D-7-7 was chromatographed over a Sephadex LH-20 column (10 × 150 mm) using MeOH as the eluting solvent to give three subfractions (from D-7-7-1 to D-7-7-3). D-7-7-2 was further purified by recrystallization to yield compound **2** (22 mg).

Results and Discussion

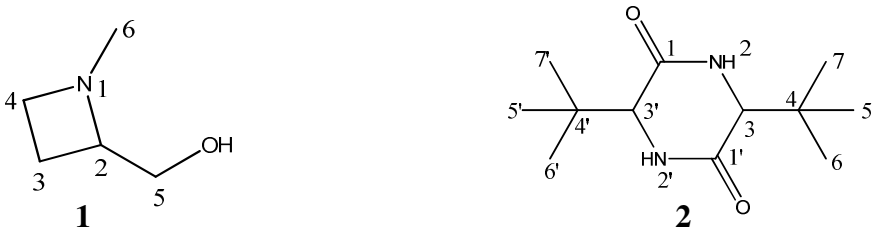
Two new natural products were isolated from the n-BuOH extracts of *Raphanus sativus* L.. The structures of the compounds were determined by MS, 1D-NMR and 2D-NMR data analysis.

Compound **1** was obtained as colorless needle crystals; its molecular formula was determined as $C_5H_{11}NO$ from the HR-ESI-MS data (*m/z* 102.1288, calcd for $C_5H_{12}NO$, 102.0919). The structural formula of **1** was shown in Fig. 1. In the ^{1}H NMR spectrum, the signals of ten H which linked to C were given and δ2.71 (3H, s, H-6) was assigned to methyl group. In the ^{13}C NMR spectrum, five C signals were given. Therefore, it was forecasted that there is one methyl, three methylenes of which one was linked by hydroxy for higher chemical shift and one methine in the molecule. The ^{1}H and ^{13}C NMR data were assigned by the HSQC and HMBC spectra (Table 1). A four-membered ring,

which was formed by two methylenes, one methine and N, was found to exist in the molecule on the basis of the fact that there is one degree of unsaturation but lack unsaturated bond. The conclusion was supported by the correlation between δ3.81 (1H, m, H-2) with C-3 (δ27.3), C-4 (δ 43.8) and C-5 (δ59.2) in the HMBC spectrum. Furthermore, the correlations between δ2.71 (3H, s, H-6) and C-2 (δ40.0), C-4 (δ43.8) revealed that methyl group was lined to N. So, CH_2OH (δ59.2, C-5) was unambiguously located at C-2.

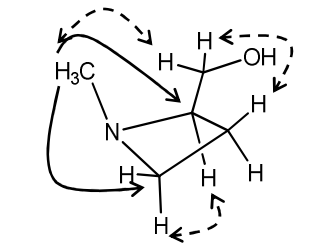
The relative configuration of **1** was established from NOESY experiments (Fig. 2). Two substituent groups, a methyl and a hydroxymethyl, both located at one side of the four-membered ring according to the NOESY cross-peaks between CH_3 and δ3.02H-5a and between CH_3 and H-5b and significant absence of the correlation between H-2α and CH_3. The configuration of remaining four H assigned to two methylenes was indicated by the correlations in NOESY spectrum between H-2α and H-4α and between H-5b and H-3β. Thus, the structure of compound **1** was determined to be Cis-(1-methylazetidin-2-yl)methanol.

Compound **2** was obtained as a white powder with the molecular formula of $C_{12}H_{22}N_2O_2$. The structural formula of **2** was shown in Fig. 1. In the ^{1}H NMR spectrum (Table 1), the methine signals δ 3.67 (1H, s, H-3) and three overlapping methyls signals δ 2.56 (3H, s, Me-4) suggested the existence of a t-Bu. The interpretation of four carbon signals, given by ^{13}C NMR, indicated CH_3 (δ 29.9), C (δ49.1), CH (δ52.3) and C (δ176.3), further supported by HSQC. A preliminary summary could be draw there was a amide structure fragment and a symmetrical structure. The ^{1}H and ^{13}C NMR spectral data was shown in Table 1. The correlation between H-3′ (δ3.67, 1H, s, H-3) and C-1′ (δ 176.3) were performed by HMBC. In conclusion, the structure of compound **2**, was determined to be a cyclo-dipeptide, cyclo(4-methyl-Val-4-methyl-Val).



1 **2**

Fig. 1 Structures of compound **1** and **2**



HMBC

NOESY

Fig. 2 Selected HMBC and NOESY correlations of compound **1**

Table 1 Spectral data of compounds **1** and **2** (CD_3OD)

position	Compound **1**		Compound **2**	
	δ_H	δ_C	δ_H	δ_C
1	–	–	–	176.3
2	3.81 (1H, m)	40.0	–	–
3	2.46 (1H, m, α) 2.01 (1H, m, β)	27.3	3.67 (1H, s)	52.3
4	3.42 (1H, m, α) 3.57 (1H, m, β)	43.8	–	49.1
5	3.02 (1H, dd, *J* = 13.5, 5.0 Hz) 3.17 (1H, dd, *J* = 13.5, 9.5 Hz)	59.2	2.56 (3H, s)	29.9
6	2.71 (3H, s)	38.8	2.56 (3H, s)	29.9
7			2.56 (3H, s)	29.9
1′			–	176.3
2′			–	–
3′			3.67 (1H, s)	52.3
4′			–	49.1
5′			2.56 (3H, s)	29.9
6′			2.56 (3H, s)	29.9
7′			2.56 (3H, s)	29.9

Conclusions

The preliminary procedure for extraction and isolation of components from *Raphanus sativus* L. was established. Two new natural products, from *Raphanus sativus* L. were identified as Cis-(1-methylazetidin-2-yl)methanol and cyclo(4-methyl-Val-4-methyl-Val), and their full spectral data were given for the first time. The compounds would be helpful for further research about their potential anti-migraine, anti-tumor activity and other pharmacological activities.

References

[1] Emma Stanton and Claire Lemer. Hope of Migraine Sufferers, Int J Health Care Qual Assur, Volume 24, Issue 2. (2011)

[2] Natoli JL, Manack A, Dean B, et al. Global Prevalence of Chronic Migraine: A Systematic Review. Cephalalgia, 30(5):599-609. (2010)

[3] Lyngherg AC, Jensen R, Rasmussen BK, et al. Incidence of Primary Headache: A Danish Epidemiologic Follow-up Study. Am J Epidemiol, 161(11): 1066. (2005),

[4] Paul R. Hanlon and David M. Barnes. Phytochemical Composition and Biological Activity of 8 Varieties of Radish (*Raphanus sativus* L.). Sprouts and Mature Taproots. J Food Sci, 76(1): C185. (2011)

[5] Satoru Tamura, Kouji Tsuji, Piao Yongzhen, et al. Six New Acylated Anthocyanins from Red Radish *(Raphanus sativus)*. Chem. Pharm. Bull, 58(9): 1259. (2010)

[6] Yoshiaki T, Yoshihito K, Tadashi F, et al. Antioxidant Constituents of radish Sprout (Kaiware-daikon), *Raphanus sativus* L. J. Agric. Food Chem, 51(27): 8061. (2003)

[7] Hirotaka K, Yoshiyuki M, Masnobu O, et al. Chemistry and Antioxidative Activity of Hot Water Extract of Japanese radish (Daikon). BioFactors, 21(2): 211. (2004)

[8] Phil-Dong Moon and Hyung-Min Kim. Anti-inflammatory Effect of Phenethyl Isothiocyanate, an Active Ingredient of *Raphanus sativus* L. Food Chemistry, 131(4): 1332. (2012)

[9] M.G. Buzzi1, C. Tassorelli. Experimental Models of Migraine. *Handbook of Clinical Neurology* (Chapter 8), Volume 97, Pages 109–123. (2010)

[10] S Akerman, PR Holland and J Hoffmann. Pearls and Pitfalls in Experimental in Vivo Models of Migraine: Dural Trigeminovascular Nociception. Cephalalgia, 33(8): 577-592. (2013)

[11] Amol Bhandare, Neeraj Vyawahare, Ajay Kshirsagar, et al. In vivo and in Vitro Screening of Antimigraine Drug: Studies in Animal Model of Migraine. Int J of Pharma Res Dev, 2(9): 11-21. (2010)