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Chemical constituents of *Raphanus sativus*

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ABSTRACT

*Chemical investigation of the dichloromethane extract of the freeze-dried roots of Raphanus sativus afforded 3-(E)-(methylthio)methylene-2-pyrrolidinethione (**1**), a mixture of 4-methylthio-3-butenyl isothiocyanate (**2**) and 4-(methylthio)butyl isothiocyanate (**3**), β-sitosterol (**4**), β-sitosteryl-3β-glucopyranoside-6'-O-palmitate (**5**), monoacylglycerols (**6**), and a mixture of α-linolenic acid (**7**)and linoleic acid (**8**). The structures of **1**-**3** were elucidated by extensive 1D and 2D NMR spectroscopy, while those of **4**-**8** were identified by comparison of their NMR data with those reported in the literature.*

Keywords: *Raphanus sativus*, 3-(*E*)-(methylthio)methylene-2-pyrrolidinethione, 4-methylthio-3-butenyl isothiocyanate, 4-(methylthio)butyl isothiocyanate, β-sitosterol, β-sitosteryl-3β-glucopyranoside-6'-O-palmitate, monoacylglycerols, α-linolenic acid, linoleic acid

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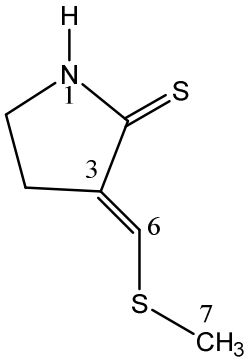
INTRODUCTION

Raphanus sativus commonly known as the radish is used as an edible vegetable and reputed to possess diverse medicinal properties. The aqueous extract of the bark of *R. sativus* has been reported to significantly decrease the weight of kidney stones and shown an increase in the urine volume of rats [1]. The fresh juice of radish exhibited gastroprotective potential [2], while the radish sprout exhibited hypoglycemic activity in rats [3] andhas also shown antioxidant properties in rats [4]. The methanolic and water extracts of the radish reduced the hepatotoxicity in albino rats [5], while the aqueous extract of radish seeds exhibited antibacterial properties [6].Another study reported that 4-methylthio-3-butenyl isothiocyanate obtained from the radish shows antimutagenic activity [7], induced detoxification enzymes in HepG2 human hepatoma cell line [8], reduced cell proliferation in a dose-dependent manner and apoptosis in colon carcinoma cell lines [9]. Furthermore, 4-methylthiobutyl isothiocyanate isolated from the radishincreased significantly the p21 protein expression and ERK1/2 phosphorylation in a dose-dependent manner to inhibit PC3 cell proliferation($P \leq 0.01$)[10] andselectively affected cell-cycle progression and apoptosis induction of human leukemia cells[11]. Glucosinolates, isothiocyanates, phenolics and anthocyanins were reported as the chemical constituents of the radish sprouts and mature taproot [12]. The major fatty acids in seed lipids of the radish were reported to be erucic, oleic, linoleic, and linolenic acids,while the major fatty acids in the radish family lipids were linolenic acid (52–55%), erucic acid (30–33%), and palmiticacid (20–22%)[13].

We earlier reported the isolation of β-sitosterol (**4**), unsaturated triglycerides and the essential fatty acids, linoleic acid (**7**)and α-linolenic acid (**8**)from *R. sativus* [14]. Recently, we reported the isolation and structure elucidation of a mixture of 4-methylthio-3-butenyl isothiocyanate (**2**)and 4-methylthiobutyl isothiocyanate (**3**), and **4** from radish roots [15]. Furthermore, β-sitosteryl-3β-glucopyranoside-6'-O-palmitate (**5**), monoacylglycerols (**6**), a mixture of **7** and **8**, and triacylglycerolswere isolated from the partially hydrolyzed radish roots [16]. In addition to compounds **2**-**8**, we report herein the isolation of 3-(*E*)-(methylthio)methylene-2-pyrrolidinethione(**1**)from the hydrolyzed radish roots.



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MATERIALS AND METHODS

General Experimental Procedures

^{1}H (500 MHz) and^{13}C (125 MHz) NMR spectra were acquired in $CDCl_3$ on a 500 MHz Agilent DD2 NMR spectrometer with referencing to solvent signals (δ 7.26 and 77.0 ppm). Column chromatography was performedwith silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} and the plates were visualizedby spraying with vanillin/H_2SO_4 solution followed by warming.

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was used for the fractionation of the crude extracts. Ten milliliter fractions were collected. Fractions with spots of the same Rf values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Sample Collection

Three (14.77) kg of radish roots was purchased from the Arranque market, Manila, Philippines inJanuary 2015. This was identified as *Raphanus sativus* at the Botany Division, Philippine National Museum.

Extraction

Fresh radish roots (14.77 kg) were peeled and cubed in one inch dimensions before lyophilization. The resultant dried samples (817.87 g) were incubated with freshly blended radish (3.53 kg) and two liters of distilled water for three hours. Two liters of CH_2Cl_2 was added to the mixture and left in a closed vessel for three days. After filtering, the residue was washed with one liter of CH_2Cl_2. The washings and supernatant were combined for concentration and eventual drying of the sample using a rotary evaporator, which afforded an 8.5 g of crude extract.

Isolation

The crude extract (8.5 g) was chromatographed by gradient elution using increasing proportions of acetone in CH_2Cl_2(10% increments) as eluents. The CH_2Cl_2 fraction wasrechromatographed (3 ×) using 5% EtOAc in petroleum ether to afford a mixture of **2** and **3** (9 mg) after washing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether, followed by 20% EtOAc in petroleum ether. The fractions eluted with 15% EtOAc in petroleum ether were combined and rechromatographed

(2 ×) using less 15% EtOAc in petroleum ether to afford **4** (25 mg) after washing with petroleum ether. The fractions eluted with 20% EtOAc in petroleum etherwere combined and rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume ratio). The less polar fractions were combined and rechromatographed (2 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume ratio) to yield **1** (12 mg) after washing with petroleum ether. The more polar fractions were combined and rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume ratio) to yield a mixture of **7** and **8** **(**8 mg). The 60% acetone in $CH_2Cl_2$was rechromatographed (2 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume ratio) to afford **6** (15 mg). The 70% acetone in CH_2Cl_2fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2.5:2.5:5 by volume ratio) to afford **5** (7 mg) after trituration with petroleum ether.

3-(E)-(Methylthio)methylene-2-pyrrolidinethione (**1**): ^{1}HNMR ($CDCl_3$, 500 MHz): δ 2.79 (dt, *J* = 2.5, 7.0 Hz, H_2-4), 3.67 (t, *J* = 7.5 Hz, H_2-5), 7.58 (t, *J* = 2.5 Hz, H-6),2.51 (s, Me-7), 7.62 (br s, NH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 194.71 (C-2), 133.39 (C-3), 26.46 (C-4), 45.72 (C-5), 139.20 (C-6), 17.50 (C-7).

4-Methylthio-3-butenyl isothiocyanate (**2**): colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.25 (s, Me), 3.53 (t, *J* = 6.6Hz, H2-1), 2.50 (dt, *J* = 7.2, 6.6 Hz, H_2-2), 5.32 (dt, *J* =15.0, 7.2 Hz, H-3), and 6.18 (d, *J* =15.0 Hz, H-4); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.73 (Me), 45.13 (C-1), 33.88 (C-2), 120.04 (C-3), 129.15 (C-4), 131.39 (SCN).

4-(Methylthio)butyl isothiocyanate (**3**): colorless oil. ^{1}HNMR ($CDCl_3$, 500 MHz): δ 2.09 (s, Me), 3.56 (t, *J* = 6.6 Hz, H_2-1), 2.52 (t, *J* = 7.2 Hz, H_2-2), 1.72 (m, H_2-3), 1.80 (m, H2-4); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 15.43 (Me), 44.71 (C-1), 33.29 (C-2), 25.82 (C-3), 28.84 (C-4), 130.28 (SCN).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *R. sativus* roots afforded 3-(*E*)-(methylthio)methylene-2-pyrrolidinethione (**1**) [17], a mixture of 4-methylthio-3-butenyl isothiocyanate (**2**) [15] and 4-(methylthio)butyl isothiocyanate (**3**) [15], β-sitosterol (**4**) [18], β-sitosteryl-3β-glucopyranoside-6'-O-palmitate (**5**) [19], monoacylglycerols (**6**) [20], and a mixture of α-linolenic acid (**7**)[16]and linoleic acid (**8**) [16]. The structures of **1**-**3** were elucidated by extensive 1D and 2D NMR spectroscopy, while those of **4**-**8** were identified by comparison of their NMR data with those reported in the literature.

The pyrrolidine alkaloid, 3-(*E*)-(methylthio)methylene-2-pyrrolidinethione (**1**) previously isolated from radish seedlings was reported to inhibit hypocotyl growth in the etiolated cress seedlings at concentrations >30 mg/litre [17].4-Methylthio-3-butenyl isothiocyanate (**2**) was reported to be the principal antimutagen of the radish [21], exhibited chemopreventive effects against pancreatic carcinogenesis in hamster [22], and showed inhibition of genotoxicity in *in vivo* and *in vitro* assay systems [21, 23]. It was also reported to possess antimicrobial activity [24], exert free radical scavenging effects [25, 26], inhibit cell proliferation [23, 27, 28] and induce apoptosis in human cancer cells [24, 29]. 4-(Methylthio)butyl isothiocyanate(**3**) exhibited in vitro antineoplastic activity and selectivity toward leukemia cells [30], increased in a dose-dependent manner p21 protein expression and ERK1/2 phosphorylation to inhibit prostate adenocarcinoma cells (PC3) cell proliferation [31], demonstrated anti-cancer effects (32-35], selectively affected cancer cell growth [36], and showed potential anti proliferative activity in several cultured cancer cell lines [32, 36-38].

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