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Isothiocyanates, sterol and triglycerides from *Raphanus sativus*

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ABSTRACT

*Chemical investigation of the dichloromethane extract of freeze-dried Raphanus sativus roots led to the isolation of 4-methylthio-3-butenyl isothiocyanate or raphasatin (**1a**), 4-(methylthio)butyl isothiocyanate or erucin (**1b**), β-sitosterol (**2**) and unsaturated triglycerides (**3**). The structures of **1a** and **1b** were elucidated by extensive 1D and 2D NMR spectroscopy, while those of **2** and **3** were identified by comparison of their NMR data with those reported in the literature.*

Keywords: *Raphanus sativus*, Brassicaceae, 4-methylthio-3-butenyl isothiocyanate, 4-(methylthio)butyl isothiocyanate, β-sitosterol, triglycerides

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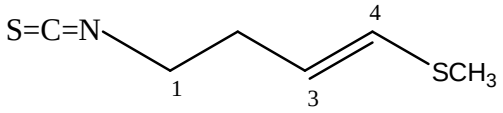
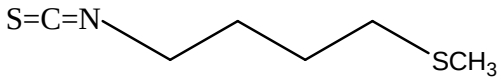
INTRODUCTION

Raphanus sativus (radish), locally known as labanos is used as vegetable and reputed to possess diverse medicinal properties. It is used as anthelmintic, antifungal, antibacterial, antiscorbutic, diuretic, laxative, tonic, carminative, antiscorbutic, stimulant, stomachic, cholagogue, lithotriptic, emmenagogue [1]. The aqueous extract of the bark of *R. Sativus* significantly decreased the weight of kidney stones and showed an increase in the 24 h urine volume of rats [2]. The fresh juice of radish exhibited gastro protective potential [3]. Another study reported that Japanese radish sprout exhibited hypoglycemic activity in both the normal and diabetic rats and partly improved lipid metabolism in the normal rats [4]. Furthermore, radish sprouts extracts exhibited antioxidant properties and significantly induced bile flow in rats [5]. The methanolic and water extracts of radish reduced the carbon tetrachloride induced hepatotoxicity in albino rats [6].The aqueous extract of radish seeds exhibited antibacterial properties attributed to the active principle, raphanin [7]. Radish sprouts and mature taproot contain glucosinolates, isothiocyanates, phenolics and anthocyanins [8]. 4-Methylthio-3-butenyl isothiocyanate was reported as a principal antimutagen in radish [9]. It induces detoxification enzymes in HepG2 human hepatoma cell line [10]. It reduces cell proliferation in a dose-dependent manner and apoptosis in colon carcinoma cell lines [11]. Another constituent of radish is 4-(methylthio)butylisothiocyanate which increases significantly the p21 protein expression and ERK1/2 phosphorylation in a dose-dependent manner to inhibit PC3 cell proliferation($P \leq 0.01$) [12]. It was also reported to selectively affect cell-cycle progression and apoptosis induction of human leukemia cells [13]. Another study reported that the major fatty acids in seed lipids of radish were erucic, oleic, linoleic, and linolenic acids,while the major fatty acids in radish family lipids were linolenic acid (52–55%), erucic acid (30–33%), and palmitic acid (20–22%) [14].

We earlier reported the isolation of β-sitosterol, unsaturated triglycerides and the essential fatty acids, linoleic acid and α-linolenic acid [15].We report herein the isolation and identification of 4-methylthio-3-butenyl isothiocyanate (**1a**), 4-(methylthio)butyl isothiocyanate (**1b**), β-sitosterol (**2**) and unsaturated triglycerides (**3**) from a local collection of *Raphanus sativus* roots.



1a



1b

MATERIALS AND METHODS

General Experimental Procedures

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layerchromatography 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.

Sample Collection

Three(3) kg of radish roots was bought from *Arranque* market, Manila, Philippines in January 2014. It was identified as *Raphanus sativus* at the Botany Division, Philippine National Museum.

Extraction and Isolation

Fresh radish roots (three kilos) were peeled and cubed in one inch dimensions before lyophilization. The resultant dried samples (294.6732 g) were ground fine and incubated with freshly grated radish and two liters of distilled water for three hours. One liter of CH_2Cl_2 was added to the mixture which was then left in a closed vessel for three days. The mixture was filtered to separate the CH_2Cl_2 extract which was then concentrated using a rotary evaporator. Subsequent drying over N_2 gas afforded 0.7602 g of crude extract.

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.

The crude extract (0.7602 g) was chromatographed by gradient elution using increasing proportions of acetone in CH_2Cl_2(10% increments) as eluents. The 10% to 20% acetone in CH_2Cl_2 fractions were combined and rechromatographed (4 ×) using 2.5% EtOAc in petroleum ether to afford **3** (4.2 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) in CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9 by volume ratio) to yield **2** (12.5 mg) after washing with petroleum ether. The 40% to 50% acetone in CH_2Cl_2 fractions were combined and rechromatographed (4 ×) in CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume ratio) to afford a mixture of **1a** and **1b** (7.0 mg) after washing with petroleum ether.

4-Methylthio-3-butenyl isothiocyanate (**1a**): colorless oil. ^1H NMR ($CDCl_3$, 600 MHz): δ 2.25 (s, Me), 3.53 (t, *J* = 6.6Hz, H_2-1), 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 ($CDCl_3$, 150 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 (**1b**): colorless oil. ^1HNMR ($CDCl_3$, 600 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, H_2-4); ^{13}C NMR ($CDCl_3$, 150 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 freeze-dried *Raphanus sativus* roots afforded a mixture of 4-methylthio-3-butenyl isothiocyanate (**1a**) and 4-(methylthio)butyl isothiocyanate (**1b**), β-sitosterol (**2**) and unsaturated triglycerides (**3**). The structures of **1a** and **1b** were elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of their ^{13}C NMR spectroscopy with those of 4-methylthio-3-butenyl isothiocyanate [16, 17] and 4-(methylthio)butyl isothiocyanate [18], respectively.

The structures of **2** and **3** were confirmed by comparison of their ^{13}C NMR data with those reported in the literature for β-sitosterol [19] and unsaturated triglycerides [20], respectively. The presence of α-linolenic acid in the triglycerides (**3**) was deduced from the methyl triplet at δ 0.96 (t, J = 7.8 Hz), the double allylic methylenes at δ 2.78 and the olefinic protons at δ 5.34 (m) [27]. The presence linoleic acid in **3** was deduced from the methyl triplet at δ 0.86 (t, J = 6.6 Hz), the double allylic methylene at δ 2.78 and the olefinic protons at δ 5.34 (m) [28]. Based on integrations of the triglyceride methyls at δ 0.96 (t, J = 7.8 Hz) and δ 0.86 (t, J = 6.6 Hz), the ratio of linolenic acid and linoleic acid in **3** is about 1:1.

4-Methylthio-3-butenyl isothiocyanate (**1a**) was reported to be the principal antimutagen of 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 (**1b**) 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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