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Secondary Metabolites from *Raphanus sativus.*

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ABSTRACT

Chemical investigation of the dichloromethane extract of the freeze-dried roots of *Raphanus sativus* led to the isolation of β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**), α-(7Z,10Z,13Z)-hexadecatrienoic acidmonoglyceride (**2**), β-sitosterol (**3**), triacylglycerols (**4**), α-linolenic acid (**5a**), and linoleic acid (**5b**). The structures of **1**-**5b** were identified by comparison of their NMR data with literature data.

Keywords: *Raphanus sativus,* Brassicaceae, β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate, α-(7Z,10Z,13Z)-hexadecatrienoic acid monoglyceride

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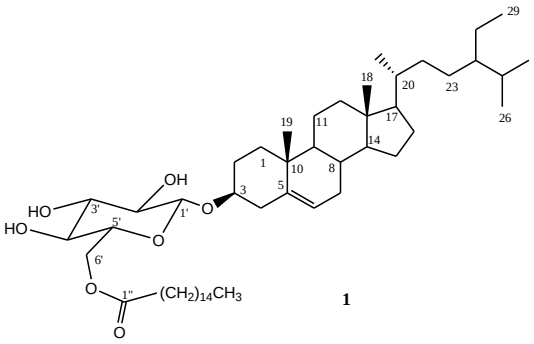
INTRODUCTION

Raphanussativus commonly known as radish is used as vegetable and reputed to possess diverse medicinal properties. A study reported that 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 [1], whilethe fresh juice of radish exhibited gastroprotective potential [2]. The radish sprout exhibited hypoglycemic activity in both the normal and diabetic rats [3], showed antioxidant properties and significantly induced bile flow in rats [4]. The methanolic and water extracts of radish reduced the carbon tetrachloride induced hepatotoxicity in albino rats [5], while the aqueous extract of radish seeds exhibited antibacterial properties attributed to raphanin [6].A principal antimutagen in radish,4-methylthio-3-butenyl isothiocyanate was reported [7]. 4-Methylthio-3-butenyl isothiocyanate induces detoxification enzymes in HepG2 human hepatoma cell line [8], reduces cell proliferation in a dose-dependent manner and apoptosis in colon carcinoma cell lines [9]. Another isothiocyanate, 4-methylthiobutyl isothiocyanate isolated from radishincreases 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 affect cell-cycle progression and apoptosis induction of human leukemia cells[11]. Glucosinolates, isothiocyanates, phenolics and anthocyanins were reported as the chemical constituents of radish sprouts and mature taproot [12]. The major fatty acids in seed lipids of radish were reported to be 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 palmiticacid (20–22%)[13].

We earlier reported the isolation of β-sitosterol, unsaturated triglycerides and the essential fatty acids, linoleic acid and α-linolenic acid from *Raphanus sativus* [14]. Recently, we reported the isolation and structure elucidation of 4-methylthio-3-butenyl isothiocyanate and 4-methylthiobutyl isothiocyanate from radish roots [15]. We report herein the isolation of β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**), α-(7Z,10Z,13Z)-hexadecatrienoic acidmonoglyceride (**2**), β-sitosterol (**3**), triacylglycerols (**4**), α-linolenic acid (**5a**), and linoleic acid (**5b**) from the partially hydrolyzed radish roots. To the best of our knowledge this is the first report on the isolation of **1** from *R. sativus.*



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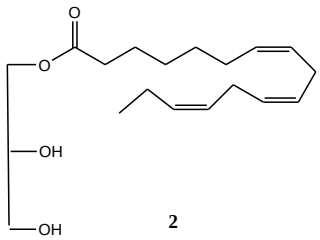

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MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR spectra. Column chromatography was performed with 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 visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

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Plant Material

Radish roots was bought from Arranque market, Manila, Philippines. It was identified as *Raphanus sativus* at the Botany Division, Philippine National Museum.

Extraction and Isolation

Fresh radish roots (13.44 kg) were peeled and cubed in one inch dimensions before lyophilization. The resultant dried samples (811.09 g) were incubated with freshly blended radish (2.05 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 a 69.63 g of crude extract.

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was used for the chromatography of the crude extracts. Twenty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f 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 of smaller fractions from the first column. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Extraction and Isolation

The crude extract (69.63 g) was chromatographed by gradient elution using increasing proportions of acetone in CH_2Cl_2 (10% increments) as elements. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using 7.5% EtOAc in petroleum ether to afford **4**(5 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 **3** (12.5 mg) after washing with petroleum ether. The 40% acetone in CH_2Cl_2 fraction from the chromatography of the crude radish roots extract was rechromatographed (4 ×) in acetonitrile:Et_2O:CH_2Cl_2 (0.5:0.5:9 by volume) afford a mixture of **5a** and **5b** (3 mg). The 50% acetone in CH_2Cl_2 fraction was rechromatographed (3×) using Et_2O:CH_3CN:CH_2Cl_2 (1:1:8 by volume) to afford **2** (1 mg). The 60% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6 by volume ratio) to afford **1** (2 mg) after trituration with petroleum ether.

β-Sitosteryl-3β-glucopyranoside-6'-O-palmitate (**1**):^{13}C NMR (150 MHz; $CDCl_3$): δ 37.24 (C-1), 29.71(C-2), 79.54 (C-3), 38.88 (C-4), 140.26 (C-5), 122.18 (C-6), 31.92 (C-7), 31.92 (C-8), 50.15 (C-9), 36.72 (C-10), 21.06 (C-11), 39.74 (C-12), 42.31 (C-13), 56.74 (C-14), 24.28 (C-15), 28.23 (C-16), 56.06 (C-17), 11.84 (C-18), 19.34 (C-19), 36.13 (C-20), 18.77 (C-21), 33.93 (C-22), 26.06 (C-23), 45.82 (C-24), 29.13 (C-25), 19.01 (C-26), 19.81 (C-27), 23.05 (C-28), 11.97 (C-29), 101.18 (C-1'), 73.58 (C-2'), 75.89 (C-3'), 70.00 (C-4'), 73.97 (C-5'), 75.89 (C-6'), 174.77 (C-1"), 34.21 (C-2"), 24.94 (C-3"), 29.31 (C-4"), 29.52 (C-5"), 29.71 (C-6"), 29.71, 29.66, 29.60 (C-7"-C-12"), 29.36 (C-13"), 31.92 (C-14"), 22.69 (C-15"), 14.12 (C-16"). $[M+Na]^+$ = 837.71

α-(7Z,10Z,13Z)-Hexadecatrienoic acid monoglyceride (**2**): ^{13}C NMR (CD_3OD, 150 MHz): δ 175.49 (O-C=O), 132.73, 130.94, 129.21, 129.19, 129.05, 128.25 (6 CH=), 71.16 (CHOH), 66.48 (glyceryl CH_2O), 64.07 (glyceryl CH_2OH), 33.58, 30.42, 30.20, 28.12, 26.52, 26.41, 25.99, 21.49 (8 CH_2), 14.65 (CH_3).

β-Sitosterol (**3**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.23 (C-1), 31.63 (C-2), 71.81 (C-3), 42.31 (C-4), 140.73 (C-5), 121.71 (C-6), 31.90, 31.89 (C-7, C-8), 50.11 (C-9), 36.13 (C-10), 21.07 (C-11), 39.76 (C-12), 42.31 (C-13), 56.75 (C-14), 24.29 (C-15), 28.24 (C-16), 56.04 (C-17), 11.84 (C-18), 19.38 (C-19), 36.49 (C-20), 19.02 (C-21), 33.93 (C-22), 29.13 (C-23), 45.82 (C-24), 26.05 (C-25), 18.76 (C-26), 19.81 (C-27), 23.05 (C-28), 11.97 (C-29).

Triacylglycerols(**4**):^{13}C NMR (150 MHz, $CDCl_3$): δ 62.07 (2×, glyceryl CH_2); 68.85 (glyceryl CH), 173.22 (2×,C=Oα); 172.81 (C=O β); 34.16, 34.02, 33.99 (C-2); 22.55, 22.656, 22.664, 24.81, 24.84, 25.60, 27.16, 27.17, 27.19, 27.22, 29.02, 29.06, 29.09, 29.11, 29.15, 29.17, 29.25, 29.30, 29.32, 29.34, 29.45, 29.50, 29.58, 29.60, 29.63,

29.68, 29.74, 31.50, 31.90 (CH_2); 130.19, 129.97, 129.95, 128.05, 128.03, 127.86, 127.85 (CH=CH); 14.05, 14.09 (terminal CH_3).

*α-**Linolenic acid*** (**5a**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.35 (m, =CH), 2.79, 2.33 (t, *J* = 7.2 Hz, α-CH_2), 1.99-2.07 (m, allylic CH_2), 1.59-1.64 (m, β-CH_2), 1.23-1.34 (CH_2), 0.96 (t, *J* = 7.8 Hz),.

Linoleic acid (**5b**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.35 (m, =CH), 2.77, 2.33 (t, *J* = 7.2 Hz, α-CH_2), 1.99-2.07 (m, allylic CH_2), 1.60 (m, β-CH_2), 1.23-1.34 (CH_2), 0.86 (t, *J* = 6.6 Hz).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the roots of *R. sativus* yielded **1**-**5b.** Compounds **1**-**5b** were identified by comparison of their NMR data with those reported in the literature for β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**) [16], α-(7Z,10Z,13Z)-hexadecatrienoic acidmonoglyceride (**2**) [17], β-sitosterol (**3**) [18], triacylglycerols (**4**) [19], α-linolenic acid (**5a**) [20], and linoleic acid (**5b**) [21]. The presence of α-linolenic acid in the triglycerides (**4**) was deduced from the methyl triplet at δ 0.96 (t, *J* = 7.8 Hz), the double allylic methylenes at δ 2.79 and the olefinic protons at δ 5.34 (m) [20]. The presence linoleic acid in **4**was deduced from the methyl triplet at δ 0.86 (t, *J* = 6.6 Hz), the double allylic methylene at δ 2.77 and the olefinic protons at δ 5.34 (m) [21].

β-Sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**)was reported to exhibit cytotoxicity against Bowes (melanoma) and MCF7 (breast) cancer cell lines with IC_{50} values of 152 mM and 113 mM, respectively [22]. Furthermore, **1** exhibited cytotoxicity against human stomach adenocarcinoma (AGS) cell line with 60.28% growth inhibition [23]. In search of substances that inhibit the hemolytic activity of human serum against erythrocytes, **1** was evaluated on its anti-complement activity. Compound **1** was found to exhibit potent anti-complement activity (IC_{50} = 1.0 ± 0.1 μM) on the classical pathway of the complement, as compared to the positive control, tiliroside (IC_{50} = 76.5 ± 1.1 μM) [24]. On the other hand,α-(7Z,10Z,13Z)-hexadecatrienoic acid monoglyceride (**2**) was reported as an anti-bolting compound in radish plants that is responsible for the formation of the leaf rosette [17].

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