INTRODUCTION

Horseradish (Armoracia rusticana) is a perennial herb of the Brassicaceae family. Its rhizomes are widely used as a condiment and as a source of horseradish peroxidase. Horseradish peroxidase is a glycoprotein commonly used as a reagent for clinical diagnosis and analytical immunoassays (1). Wasabi (Wasabia japonica), called Japanese horseradish, is also a member of the Brassicaceae family of vegetables. Either in the fresh form or as a dried powder, wasabi is widely used in the Japanese cuisine to garnish traditional dishes such as sushi and sashimi.

A high consumption of vegetables and fruits has been correlated with a decreased cancer risk. High consumption of Brassica vegetables lowered colon cancer risk in men and women, but the risk was increased in women for rectal cancer (2). Consumption of yellow-green vegetables has been correlated with a decreased cancer risk. High consumption of Brassica vegetables lowered colon cancer risk in men and women, but the risk was increased in women for rectal cancer (2). Consumption of yellow-green vegetables has been correlated with a decreased cancer risk. High consumption of Brassica vegetables lowered colon cancer risk in men and women, but the risk was increased in women for rectal cancer (2).

Cyclooxygenase and human tumor cell growth inhibitory extracts of horseradish (Armoracia rusticana) and wasabi (Wasabia japonica) rhizomes upon purification yielded active compounds 1–3 from horseradish and 4 and 5 from wasabi rhizomes. Spectroscopic analyses confirmed the identities of these active compounds as plastoquinone-9 (1), 6-O-acyl-β-D-glucosyl-α-sitosterol (2), 1,2-dilinolenyl-3-galactosylglycerol (3), linolenoyloleoyl-3-β-galactosylglycerol (4), and 1,2-dipalmitoyl-3-β-galactosylglycerol (5). 3-Acyl-α-sitosterol, sinigrin, gluconasturtiin, and phosphatidylcholines isolated from horseradish and α-tocopherol and ubiquinone-10 from wasabi rhizomes isolated were inactive in our assays. At a concentration of 60 μg/mL, compounds 1 and 2 selectively inhibited COX-1 enzyme by 28 and 32%, respectively. Compounds 3, 4, and 5 gave 75, 42, and 47% inhibition of COX-1 enzyme, respectively, at a concentration of 250 μg/mL. In a dose response study, compound 3 inhibited the proliferation of colon cancer cells (HCT-116) by 21.9, 42.9, 51.2, and 68.4% and lung cancer cells (NCI–H460) by 30, 39, 44, and 71% at concentrations of 7.5, 15, 30, and 60 μg/mL, respectively. At a concentration of 60 μg/mL, compound 4 inhibited the growth of colon, lung, and stomach cancer cells by 28, 17, and 44%, respectively. This is the first report of the COX-1 enzyme and cancer cell growth inhibitory monogalactosyl diacylglycerides from wasabi and horseradish rhizomes.

KEYWORDS: Horseradish; wasabi; anticancer; galactosyl diacylglycerides; cyclooxygenase enzyme

Materials. Fresh wasabi rhizomes were purchased from Pacific Farms (Eugene, OR). Fresh horseradish rhizomes were purchased from a supermarket in East Lansing, MI. All solvents were ACS reagent grade and purchased from Spectrum Chemical Co. (Gardena, CA). Ibuprofen, Naproxen, dimethyl sulfoxide (DMSO), and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Celebrex and Vioxx were physician’s professional samples supplied by Dr. S. Gupta, Sparrow Hospital, Michigan. COX-1 enzyme was prepared in...
Memorial Institute-1640 (RPMI-1640) medium were purchased from Michigan State University. Fetal bovine serum (FBS) and Roswell Park lysate in the Department of Biochemistry and Molecular Biology at Michigan State University. Human tumor cell lines, SF-268 (central nervous system, CNS), NCI-H460 (lung), and MCF-7 (breast) were purchased from the National Cancer Institute (NCI, Bethesda, MD) and HCT-116 (colon) and AGS (stomach) from American Type Culture Collection (ATCC, Rockville, MD). All cell lines were maintained at BNPP, Michigan State University.

General Experimental Procedures. 

Extraction of Wasabi and Horseradish Rhizomes. 

Extraction of horseradish rhizomes was conducted using a Silica Gel 60 plate (Merck, Darmstadt, Germany). The rhizomes were powdered to <40 μm and then extracted with acetone (3:1, v/v) as the mobile phase. Two bands were obtained, and the lower band was further purified by preparative silica TLC (250 μm) using hexane–acetone–acetic acid (7:3, v/v). The active fraction was further purified by preparative silica TLC (250 μm) using MeOH–CHCl₃ (1:6, v/v) as the mobile phase to yield compound 3 (Rf 0.8, 20 mg).

Purification of Compounds from Wasabi Rhizomes. 

The ethyl acetate extract of horseradish rhizomes was stirred with MeOH to afford a MeOH soluble fraction (610 mg). The MeOH soluble fraction (325 mg) was purified by preparative silica (1000 μm) TLC using hexane–acetone (3:1, v/v) as the mobile phase. Two bands were collected at Rf 0.1 (200 mg) and Rf 0.25 (32 mg). The higher Rf band (15 mg) was further purified by preparative silica TLC (250 μm) using hexane–acetone (3:1, v/v) as the mobile phase to afford compound 2 (Rf 0.125, 10 mg). The lower Rf fraction (180 mg) was further purified by preparative silica TLC (250 μm) using MeOH–CHCl₃ (1:6, v/v) as the mobile phase to yield compound 3 (Rf 0.8, 20 mg).

Compound 3 (Colorless Oil). 

1H NMR spectra were recorded on Varian INOVA (300 MHz) and VXR (500 MHz) instruments. 13C NMR spectra were obtained at 75 and 125 MHz. 1H NMR chemical shifts are reported in ppm relative to CDCl₃ at 77.0, DMSO at 39.50, D₂O at 4.80, and CD₃OD at 3.30 ppm, respectively. 13C NMR chemical shifts are reported in ppm relative to CDCl₃ at 77.0, DMSO at 39.50, D₂O at 4.80, and CD₃OD at 3.30 ppm, respectively. 13C NMR chemical shifts are reported in ppm relative to CDCl₃ at 77.0, DMSO at 39.50, D₂O at 4.80, and CD₃OD at 3.30 ppm, respectively. 13C NMR chemical shifts are reported in ppm relative to CDCl₃ at 77.0, DMSO at 39.50, D₂O at 4.80, and CD₃OD at 3.30 ppm, respectively. 13C NMR chemical shifts are reported in ppm relative to CDCl₃ at 77.0, DMSO at 39.50, D₂O at 4.80, and CD₃OD at 3.30 ppm, respectively. 13C NMR chemical shifts are reported in ppm relative to CDCl₃ at 77.0, DMSO at 39.50, D₂O at 4.80, and CD₃OD at 3.30 ppm, respectively. 13C NMR chemical shifts are reported in ppm relative to CDCl₃ at 77.0, DMSO at 39.50, D₂O at 4.80, and CD₃OD at 3.30 ppm, respectively. 13C NMR chemical shifts are reported in ppm relative to CDCl₃ at 77.0, DMSO at 39.50, D₂O at 4.80, and CD₃OD at 3.30 ppm, respectively. 13C NMR chemical shifts are reported in ppm relative to CDCl₃ at 77.0, DMSO at 39.50, D₂O at 4.80, and CD₃OD at 3.30 ppm, respectively. 13C NMR chemical shifts are reported in ppm relative to CDCl₃ at 77.0, DMSO at 39.50, D₂O at 4.80, and CD₃OD at 3.30 ppm, respectively.
Cyclooxygenase Enzymes Inhibitory Assay. Cyclooxygenase enzymes inhibitory activities of extracts and pure compounds were evaluated using COX-1 and COX-2 enzymes. The rate of oxygen consumption during the initial phase of the enzyme-mediated reaction with arachidonic acid as substrate was measured using a Model 5300 biological oxygen monitor (Yellow Spring Instruments, Inc., Yellow Springs, OH). The reaction mixture, consisting of 0.1 M Tris, 1.0 mM phenol, 17 μg hemoglobin, the enzyme, and 10 μL extract dissolved in DMSO at 1.5% (DMSO alone as solvent control), was held in a 600-μL micro-oxygen chamber (Instech Laboratory, Plymouth Meeting, PA) at 37 °C. After 3 min of incubation, 10 μL of arachidonic acid (1 mg/mL of Tris buffer) was added to initiate the reaction. Data were recorded using Quicklog for Windows (Strawberry Tree Inc., Sunnyvale, CA). The positive controls Ibuprofen, Aspirin, Celebrex, Vioxx, and stearic acids were also methylated using diazomethane and used as standards.

RESULTS AND DISCUSSION

The bioassay-guided fractionation and purification of the extracts of fresh wasabi and horseradish rhizomes resulted in the isolation of active compounds 1–3 and 4–5 (Figure 1), respectively, along with 3-acyl-sitosterols, sinigrin, glucosturitum, phosphatidyicholines, α-tocopherol, and ubiquinone-10 using preparative thin-layer (TLC), medium pressure (MPLC), and high-performance (HPLC) liquid chromatographic methods. The structure of compound 1 was established as plastoquinone-9, 2,3-dimethyl-5-(2E,6E,10E,14E,18E,22E,26E,30E)-3,7,11,15,19,23,27,31,35-nonamethyl-2,6,10,14,18,22,26,30,34-hexatriacontanonaenyl-2,5-cyclohexadiene-1,4-dione by NMR spectroscopic analyses. The spectral data for compound 1 was in agreement with that reported for plastoquinone-9 (16).

The identity of compound 2 as 6-O-acyl-β-D-glucosyl-β-sitosterol was determined by NMR spectroscopic analyses. The GC-MS analysis of the hydrolysis products from compound 2 indicated the presence of palmitic, oleic, and linolenic acids at a ratio of 1:1:1. Therefore, compound 2 was characterized as an inseparable mixture of 6-O-acyl-β-D-glucosyl-β-sitosterol (17) with palmitic, oleic, and linolenic acid moieties as the acyl groups. Mixtures of 6-O-acyl-β-D-glucosyl-β-sitosterols have been previously reported as cytotoxic against several cancer cell lines including the MCF-7 breast cancer cells (18).

Compounds 3, 4, and 5 showed similar NMR spectral data and suggested that they belonged to the monogalactosyl diacylglycerol class. These compounds were hydrolyzed and the resulting products analyzed by GC-MS after methylation with diazomethane. The structure of compound 3 was determined as 1,2-dilinolenoyl-3-β-D-galactosylglycerol by spectroscopic methods and its data were in agreement with that published for 1,2-dilinolenoyl-3-β-D-galactosylglycerol (19). The two acyl groups in compound 4 were identified as oleic and linolenic acid moieties in a 1:1 ratio. It is possible that compound 4 consisted of a mixture of positional isomers of oleic and linolenic acids in its glycerol backbone. Analysis of compound 5 revealed that palmitic acid was the only acyl moiety in the molecule. The identity of compound 5 was established as 1,2-dipalmitoyl-3-β-D-galactosylglycerol.

The crude extracts from wasabi and horseradish exhibited both COX-1 and -2 enzyme inhibitory activities. The inhibitions were about 65 and 50% at 250 μg/mL for COX-1 and COX-2 enzymes, respectively. In the COX enzyme inhibitory assays with purified compounds, compounds 1 and 2 inhibited only COX-1 enzyme at 60 μg/mL (Figure 2). Similarly, compound 3 inhibited COX-1 enzyme by 75% at 250 μg/mL while showing a marginal COX-2 enzyme inhibition. The COX-1 enzyme inhibition for compounds 4 and 5 was about 45% at 250 μg/mL. The pure compounds did not account for the high COX-1 and -2 enzyme inhibitory activities exhibited by the crude extracts. This was probably due to the high content of fatty acids present in the extracts in addition to components that were not
isolated. Fatty acids are known inhibitors of COX enzymes as reported from our previous studies (20) and therefore we did not pursue the isolation of fatty acids from these extracts. 1,2-Dilinolenoyl-3-\(\beta\)-galactosylglycerol, isolated from \textit{Euphorbia cyparissias} L., was reported to exert topical anti-inflammatory activity in a mouse edema model (21). However, this is the first report of monogalactosyl diacylglycerides isolated from horseradish and wasabi rhizomes with selective COX-1 enzyme inhibitory activity.

Water, methanol, ethyl acetate, and hexane extracts of fresh wasabi and horseradish were tested in cancer cell proliferation inhibitory assay. The water extract of fresh wasabi inhibited 52.3, 40.7, 64.9, 46.2, and 13.2% on AGS, HCT-116, MCF-7, NCI-H460, and SF-268 cell growth at 250 \(\mu\)g/mL, respectively (Figure 3A). The methanol extract of wasabi showed 82.4, 62.7, 56.6, 53.3, and 38.4% of inhibition on AGS, HCT-116, MCF-7, NCI-H460, and SF-268 cell growth at 250 \(\mu\)g/mL, respectively (Figure 3B). Similarly, the ethyl acetate extract of wasabi demonstrated inhibitory activity on AGS, HCT-116, MCF-7, NCI-H460, and SF-268 cancer cells with 84.3, 65.6, 72.1, 73.7, and 49.4% growth inhibition at 250 \(\mu\)g/mL, respectively (Figure 3C). However, the water, methanol, and ethyl acetate extracts of horseradish and the hexane extract of both wasabi and horseradish did not exhibit significant growth inhibitory activity on the cancer cell lines tested at 250 \(\mu\)g/mL.

In tumor cell growth inhibitory assays, compounds 1 and 2 showed little or no growth inhibition of tumor cell lines tested at 30 \(\mu\)g/mL. Compound 3 inhibited the growth of colon (HCT-116) and lung (NCI-H460) cancer cell lines as determined by the MTT assay. Growth inhibitory activity was not observed for breast (MCF-7) and CNS (central nervous system, SF-268) cancer cell lines. B: Effect of linolenoyloleoyl-3-\(\beta\)-galactosylglycerol (compound 4) on the proliferation of human stomach (AGS), colon (HCT-116), and lung (NCI-H460) cancer cell lines as determined by the MTT assay. Growth inhibitory activity was not observed for breast (MCF-7) and CNS (central nervous system) (SF-268) cancer cell lines. The optical density was measured to determine the amount of formazan blue formed by viable cells and compared to the control. The data represents the mean \(\pm\) SD of three parallel experiments conducted in triplicate.

The monogalactosyl diacylglycerides, together with other glycosylated diacylglycerides, belong to the major lipids present in chloroplasts and membranes. The monogalactosyl diacylglycerides have been reported as inhibitors of 12-O-tetradecanoylphorbol-13 acetate (TPA)-induced tumor promotion in vitro and in mouse models (21–24). They are inducers of apoptosis and selective inhibitors of mammalian polymerases (25). Upon ingestion, it is expected that monogalactosyl diacylglycerides enzymatically cleave at position 1 by pancreatic lipase enzyme. This may affect their ability to inhibit cancer cell proliferation. In a recent study, monogalactosyl diacylglycerides were hydrolyzed with pancreatic lipase and the resulting monogalactosyl monoacylglycerides (MGMGs) were compared to their monogalactosyl diacylglycerides parents in cancer cell inhibitory assays. The cancer cell growth was inhibited (LD\(_{50}\)) by both classes of compounds at 40 \(\mu\)g/mL (25).
We had reported the cell proliferating effect of desulfosinigrin in wasabi and horseradish (7). Both wasabi and horseradish are rich in glucosinolates and their water extracts primarily contain beneficial and detrimental health effects. Wasabi and horseradish are probably examples of such foods with a balance of compounds that could contribute both beneficial and detrimental health effects.

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LITERATURE CITED


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