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ARTICLE

Analysis and Anti-*Helicobacter* Activity of Sulforaphane and Related Compounds Present in Broccoli (*Brassica oleracea* L.) Sprouts

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A crude methanol extract prepared from fresh broccoli sprouts was extracted with hexane, chloroform, ethyl acetate, and butanol sequentially. Residual water fraction was obtained from the residual aqueous layer. The greatest inhibition zones (>5 cm) were noted for *Helicobacter pylori* strain by the chloroform extract, followed by the hexane extract (5.03 cm), the ethyl acetate extract (4.90 cm), the butanol extract (3.10 cm), and the crude methanol extract (2.80 cm), whereas the residual water fraction did not show any inhibition zone. Including sulforaphane, five sulforaphane-related compounds were positively identified in the chloroform extract, of which 5-methylsulfinylpentylnitrile was found in the greatest concentration (475.7 mg/kg of fresh sprouts), followed by sulforaphane (222.6 mg/kg) and 4-methylsulfinylbutylnitrile (63.0 mg/kg). Among 18 sulforaphane and related compounds synthesized (6 amines, 6 isothiocyanates, and 6 nitriles), 2 amines, 6 isothiocyanates, and 1 nitrile exhibited >5 cm inhibitory zones for *H. pylori* strain. The results indicate that broccoli sprouts can be an excellent food source for medicinal substances.

KEYWORDS: Anti-*Helicobacter pylori*; broccoli sprouts; isothiocyanates; sulforaphane

INTRODUCTION

First grown in California in the early 1900s, broccoli (*Brassica oleracea*), derived from the cabbage family Brassicaceae (formerly Cruciferae), is one of the most popular vegetables in the United States. From World War II to the present, the United States has been the world's largest broccoli producer. In 1998, the United States produced 133,000 acres of broccoli with a value of $554 million (*1*). Widely considered to be a healthful food, broccoli is high in vitamins C, K, and A and soluble fiber and contains multiple nutrients. However, potent anticancer properties found in broccoli and broccoli sprouts have received much attention recently. Epidemiological studies suggest that broccoli and broccoli sprouts reportedly induce carcinogen-detoxifying enzyme systems and decrease the occurrence of cancer (*2*, *3*). For example, an extract from broccoli sprouts protected test subjects from skin carcinogenesis induced by UV light (*4*). Sulforaphane present in broccoli sprout is the primary bioactive compound, which may prevent prostate cancer (*5*). Also, a freeze-dried aqueous extract of broccoli sprouts containing isothiocyanates inhibited bladder cancer in experimental rats (*6*).

In humans, *Helicobacter pylori* is highly associated with a number of diseases of the upper gastrointestinal tract, including gastric inflammation, chronic superficial gastritis, duodenal and gastric ulcers, gastric adenocarcinoma, and non-Hodgkin's lymphomas of the stomach (*7*, *8*). *H. pylori* infections are prevalent worldwide and common in both developed and developing countries. In developing countries, 70−90% of the population carries *H. pylori*, whereas the prevalence of infection in developed countries is lower, ranging from 25 to 50% (*7*, *9*). Most infections by *H. pylori* are acquired during childhood and persist lifelong if not eradicated properly. *H. pylori* eradication has been provided principally by the use of conventional antibacterial drugs, including potent triple therapies consisting of mixture of two antibiotics such as amoxicillin, clarithromycin, and/or metronidazole with bismuth or a proton pump inhibitor, which are still the most effective (*8*). They have a success rate of 80−90% (*10*), but serious side effects such as taste disturbances, nausea, diarrhea, dyspepsia, headache, and angioedema (*8*) as well as disturbance of human gastrointestinal microflora (*11*, *12*) have occurred. The cost of combination therapy is significant. Additionally, widespread use of antibiotics has often resulted in the development of resistance (*8*, *9*, *13*). These problems indicate the need for the development of new improved antibacterial agents and strategies for the prevention or eradication of *H. pylori*.

Recent reviews describe various biological activities of broccoli and its sprouts (*14*, *15*) containing a high level of an isothiocyanate, the so-called sulforaphane, reported to have powerful bactericidal activity against *H. pylori* infection (*16*, *17*). In addition to these activities, broccoli and its sprouts have antioxidant activities (*18*−*20*) and cholesterol-lowering and antiobesity effects (*21*).

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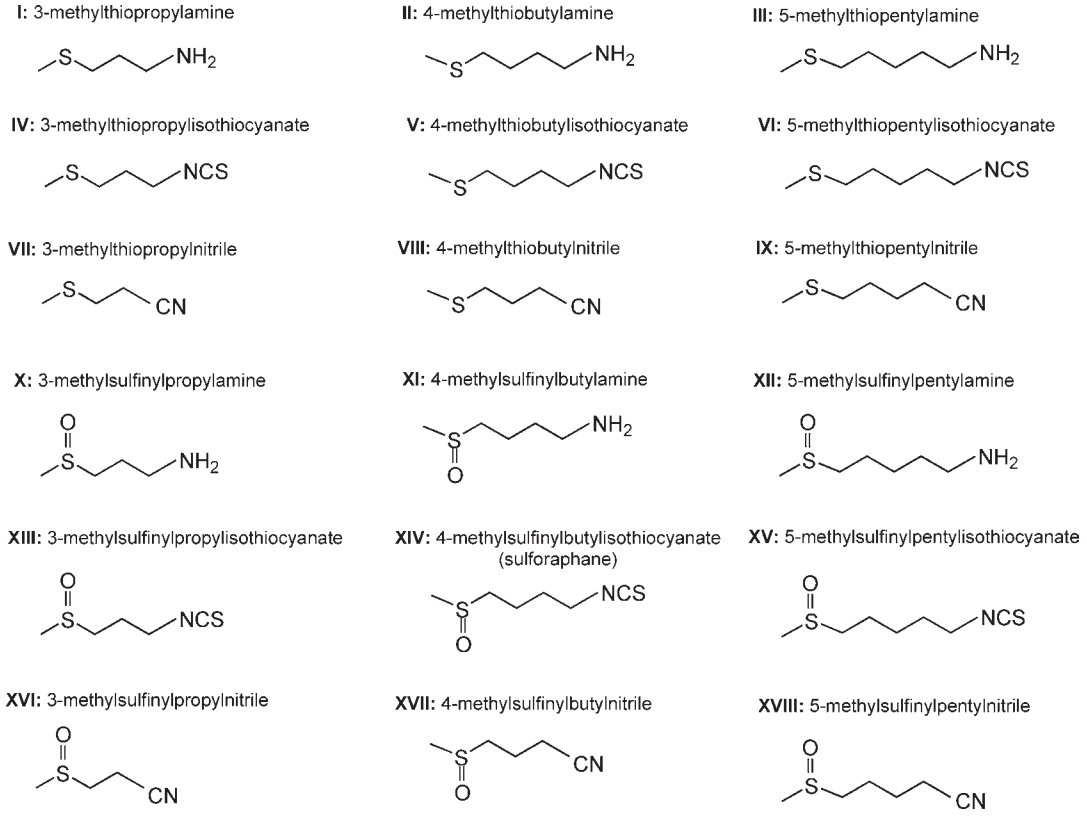

Figure 1. Sulforaphane and related compounds synthesized and their structures.

Many studies reporting on the analysis of chemicals in fresh broccoli and its sprouts have generally been conducted to test for association with biologically active components, such as antioxidants (*22*). The antioxidant activity of broccoli and its sprouts is commonly induced by one of their major components, sulforaphane, which is yielded from glucosinolates (*23*). Polyphenols with potent antioxidant activity, such as quercetin, were also reported in broccoli (*24*, *25*). A possible anticancer agent, sulforaphane has been detected in broccoli and its sprouts using either HPLC (*26*) or GC-MS (*27*). The levels of sulforaphane in four varieties of broccoli ranged from 1.9 to 3.7 mg/g (*27*).

In the present study, essences obtained from fresh broccoli sprouts were investigated for their chemical compositions and their bactericidal activity against *H. pylori*.

MATERIALS AND METHODS

Chemicals and Materials. HPLC grade methanol, acetonitrile, acetic acid, hexane, chloroform, ethyl acetate, butanol, hydrogen peroxide solution (30%), potassium hydroxide, sodium hydroxide, and sodium sulfate were bought from Fisher Scientific Co. (Rochester, NY). 3-Methylthiopropylamine (3-MTPA) was purchased from TCI Chemical (Portland, OR). 4-Bromobutyronitrile, 3-bromopropionitrile, 5-bromovaleronitrile, lithium aluminum hydride, ninhydrin, thiophosgene ($CSCl_2$), and sodium thiomethoxide were purchased from Sigma-Aldrich Corp. (St. Louis, MO).

Broccoli (*B. oleracea* L.) sprouts were provided by Cosmo Salad Co., Ltd. (Dixon, CA) as a gift. The sprouts were collected 6 days after germination.

Synthesis of Sulforaphane and Related Compounds. The structures of 18 compounds (3 methylthioalkylamines, 3 methylthioalkylisothiocyanates, 3 methylthioalkylnitriles, 3 methylsulfinylalkylamines, 3 methylsulfinylalkylisothiocyanates, and 3 methylsulfinylalkylnitriles) synthesized are shown in **Figure 1**.

Methylthioalkylnitriles. A methanol solution of sodium thiomethoxide was prepared by adding 1 g of sodium thiomethoxide to 20 mL of methanol in a 100 mL round-bottom flask. To this solution was slowly added dropwise 20 mL of a methanol solution containing 1.91 g of 3-bromopropionitrile. The solution was refluxed for 7 h using a heating nest. After the reaction solution had cooled to room temperature, methanol solvent was removed by distillation. Purified water (50 mL) was added to the residual material and extracted three times with a 100 mL portion of ethyl ether. The extracts were combined and dried over sodium sulfate overnight. After all solvent was removed by distillation, residual materials were purified using a silica gel column chromatograph with ethyl acetate/hexane (3:1).

Table 1. Amounts of Reactants Used for the Synthesis of 3-Methylthiopropylnitrile (3-MTPN), 4-Methylthiobutylnitrile (4-MTBN), and 5-Methylthiopentylnitrile (5-MTPN) and Amounts of Product and Yield

	3-MTPN	4-MTBN	5-MTPN
thiomethoxide (g)	1.00	1.63	0.90
bromoalkylnitrile (g)	1.91	2.96	2.00
product (g)	1.30	1.96	1.48
yield (%)	90	89	98

4-Methylthiobutylnitrile and 5-methylthiopentylnitrile were synthesized according to the same method as that used for 3-methylthiopropylnitrile as described above. The amounts of reactants used for each synthesis and yields are shown in **Table 1**.

Methylsulfinylalkylnitriles. Three hundred microliters of a 30% H_2O_2 solution was added to a methanol solution (3 mL) containing 300 mg of 3-methylthiobutylnitrile, 20 μL of isopropyl alcohol, and 20 μL of concentrated H_2SO_4 with stirring at room temperature for 3 h. After 5 mL of water and 5 mL of saturated NaCl solution were added, the reaction mixture was extracted four times with a 50 mL portion of $CHCl_3$. The extract was dried over anhydrous sodium sulfate overnight. After the sodium sulfate had been filtered off, the solvent was removed by evaporation. 3-Methylsulfinylpropylnitrile (320 mg) was obtained.

4-Methylsulfinylbutylnitrile and 5-methylsulfinylpentylnitrile were synthesized by the same method as that used for 3-methylsulfinylpropylnitrile. The amounts of reactants and reagents used and yields are shown in **Table 2**.

Methylthioalkylamines. A fine powder of lithium aluminum hydride ($LiAlH_4$, 1.14 g) was dissolved in 15 mL of dry ethyl ether, and the solution

Table 2. Amounts of Reactants and Reagents Used for the Synthesis of 3-Methylsulfinylpropylnitrile (3-MSPrN), 4-Methylsulfinylbutylnitrile (4-MSBN), and 5-Methylsulfinylpentylnitrile (4-MSPeN) and Amounts of Product and Yield

	3-MSPrN	4-MSBN	5-MSPeN
nitrile (mg)	300	550	250
H_2O_2 (μL)	300	600	300
methanol (mL)	3	5	3
H_2SO_4 (μL)	20	30	20
isopropyl alcohol (μL)	20	30	20
product (mg)	320	580	270
yield (%)	92	93	94

Table 3. Amounts of Reactants and Reagents Used for the Synthesis of 4-Methylthiobutylamine (4-MTBA) and 5-Methylthiopentylamine (5-MTPA) and Amounts of Product and Yield

	4-MTBA	5-MTPA
nitrile (g)	2.45	1.40
$LAlH_4$	1.14	0.60
product (g)	2.42	1.35
yield (%)	93	92

was placed in a 100 mL three-neck round-bottom flask, to which a dropping funnel (volume 50 mL) containing 4 mL of an ethyl ether solution of 4-methylthiobutylnitrile (2.45 g), a reflux condenser, and a nitrogen inlet were connected. The ethyl ether solution was added to the flask dropwise over 30 min while the reaction mixture was stirred with a magnetic stirrer. After all of the ethyl ether solution was added, the solution was refluxed for an additional 30 min. After the reaction mixture was cooled with an ice bath, 2 mL of water, 2 mL of a 15% NaOH solution, and 5 mL of water were added sequentially through the separatory funnel. The precipitate yielded was removed on a sintered glass filter and washed off thoroughly with fresh portions of ethyl ether into a 250 mL round-bottom flask. The pooled ethyl ether solutions were dried over KOH pellets. After removal of KOH pellets and ethyl ether, the residual material was cleaned with silica gel chromatography with dichloromethane/methanol (19:1, v/v). 4-Methylthiobutylamine (purity = 93%) was obtained (2.42 g).

5-Methylthiopentylamine was synthesized according to the same method as that used for 4-methylthiobutylamine. The amounts of reactants and reagents used and yields are shown in **Table 3**.

Methylsulfinylalkylamines. Six hundred milliliters of a 30% H_2O_2 solution was added to a methanol solution (5 mL) containing 500 mg of 3-methylthiopropylamine, 20 μL of isopropyl alcohol, and 20 μL of concentrated H_2SO_4 with stirring at room temperature for 3 h. After 5 mL of water and 5 mL of saturated NaCl solution had been added, the reaction mixture was extracted four times with a 50 mL portion of $CHCl_3$. The extract was dried over anhydrous sodium sulfate overnight. After the sodium sulfate had been filtered off, the solvent was removed by evaporation. 3-Methylsulfinylpropylamine (320 mg) was obtained.

4-Methylsulfinylbutylamine and 5-methylsulfinylpentylamine were synthesized according to the same method as that used for 3-methylsulfinylpropylamine. The amounts of reactants and reagents used and yields are shown in **Table 4**.

Methylthioalkylisothiocyanates. Three milliliters of NaOH solution (2 N) containing 200 μL of thiophosgene ($CSCl_2$) was added to a 20 mL chloroform solution of 3-methylthiopropylamine (200 mg) at room temperature. After 1 h, 20 mL of water was added to the reaction mixture. The solution was extracted with 50 mL of chloroform. After the extract had been dried over anhydrous sodium sulfate, the solvent was removed by evaporation. The residual material was cleaned by silica gel chromatography with dichloromethane. 3-Methylthiopropylisothiocyanate was obtained (145 mg).

4-Methylthiobutylisothiocyanate and 5-methylthiopentylisothiocyanate were synthesized according to the same method as that used for 3-methylthiopropylisothiocyanate. The amounts of reactants and reagents used and yields are shown in **Table 5**.

Table 4. Amounts of Reactants and Reagents Used for the Synthesis of 3-Methylsulfinylpropylamine (3-MSPrA), 4-Methylsulfinylbutylamine (4-MSBA), and 5-Methylsulfinylpentylamine (4-MSPeA) and Amounts of Product and Yield

	3-MSPrA	4-MSBA	5-MSPeA
amine (mg)	500	550	500
H_2O_2 (μL)	600	600	600
methanol (mL)	5	5	5
H_2SO_4 (μL)	30	30	30
isopropyl alcohol (μL)	30	30	30
product (mg)	350	395	340
yield (%)	61	63	62

Table 5. Amounts of Reactants and Reagents Used for the Synthesis of 3-Methylthiopropylisothiocyanate (3-MTPrITC), 4-Methylthiobutylisothiocyanate (4-MTBITC), and 5-Methylthiopentylisothiocyanate (4-MTPeITC) and Amounts of Product and Yield

	3-MTPrITC	4-MTBITC	5-MTPeITC
amine (mg)	200	200	200
$CSCl_2$ (μL)	200	210	220
product (mg)	145	195	198
yield (%)	72	73	75

Table 6. Amounts of Reactants and Reagents Used for Synthesis of 3-Methylsulfinylpropylisothiocyanate (3-MSPrITC), 4-Methylsulfinylbutylisothiocyanate (4-MSBITC), and 5-Methylsulfinylpentylisothiocyanate (4-MSPeITC) and Amounts of Product and Yield

	3-MSPrITC	4-MSBITC	5-MSPeITC
amine (mg)	100	500	100
$CSCl_2$ (μL)	100	420	90
product (mg)	66	320	83
yield (%)	50	52	64

Methylsulfinylalkylisothiocyanates. Three milliliters of NaOH solution (2 N) containing 100 μL of thiophosgene ($CSCl_2$) was added to a 20 mL of a chloroform solution of 3-methylsulfinylpropylamine (100 mg) at room temperature. After 1 h, 20 mL of water was added to the reaction mixture. The solution was extracted with 50 mL of chloroform. After the extract had been dried over anhydrous sodium sulfate, the solvent was removed by evaporation. The residual material was cleaned by silica gel chromatography with dichloromethane/methanol (30:1−9:1, v/v) to give 3-methylsulfinylpropylisothiocyanate.

4-Methylsulfinylbutylisothiocyanate (sulforaphane) and 5-methylsulfinylpentylisothiocyanate were synthesized according to the same method as that used for 3-methylsulfinylpropylisothiocyanate. The amounts of reactants and reagents used and yields are shown in **Table 6**.

Sample Preparations for Chemical Analysis and Anti-*Helicobacter* Activity Tests from Broccoli Sprouts. Fresh broccoli sprouts (3 kg) were soaked in 10 L of methanol for 48 h. After the methanol extract had been filtered, the residual sprouts were soaked in 10 L of methanol again for 48 h. The two methanol extracts were combined and then filtered. Using a rotary flash evaporator, the methanol was removed as much as possible from the filtrate, and the residual water was removed by a freeze-dryer (FreezOne, Labconco, Kansas City, MO) to give 66.0 g of crude methanol extract. The freeze-dried sample (20 g) was dissolved in 500 mL of deionized water. The aqueous solution was sequentially extracted with 500 mL each of hexane, chloroform, ethyl acetate, and *n*-butanol using a 2 L separatory flask. Extracts and residual aqueous solution were concentrated with a rotary flash evaporator to approximately 1 mL in volume. The concentrated extracts (0.613 g of hexane, 1.294 g of chloroform, 0.969 g of ethyl acetate, and 4.83 g of butanol) and a water fraction (15.4 g) were stored at −5 °C until the assay for anti-*Helicobacter* activity. The overall sample preparation scheme is shown in **Figure 2**. The samples within frames were tested for anti-*Helicobacter* activity.

Bacterium Strain and Culture Conditions. *H. pylori* ATCC 43504 strain obtained from the College of Medicine, Dankook University,

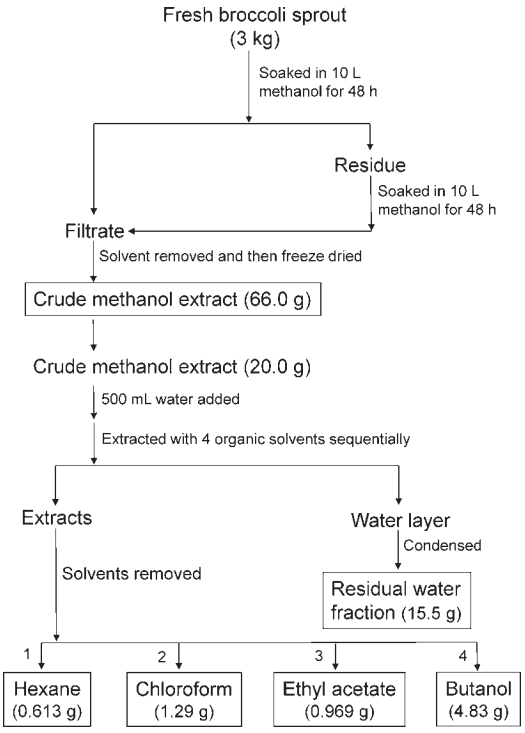

Figure 2. Overall sample preparation scheme.

Cheonan, Korea, was used for the anti-*Helicobacter* activity test. Stock cultures of the strain were routinely stored at −80 °C on Brucella broth (Difco, Detroit, MI) containing 5% bovine calf serum (Hyclone, Logan, UT) and 20% glycerol, when required, and were subcultured on Brucella agar. After the plates had been incubated at 37 °C for 3−5 days in an atmosphere consisting of 5% oxygen, 15% carbon dioxide, and 80% nitrogen in an anaerobic chamber (Hirayama, Tokyo, Japan), the organism was grown in Brucella broth (pH 7.4) with a supplement containing 5% bovine calf serum, 10 μg/mL vancomycin, 5 μg/mL polymyxin B, 5 μg/mL trimethoprin, and 2 μg/mL amphotericin B. All cultures were examined for possible contaminations at the end of the growth cycle.

Microbiological Assay for Anti-*Helicobacter* Activity. Bacterial growth on Brucella agar was taken from the plates and resuspended in 10 mL of sterile physiological saline. The inoculum (0.1 mL) containing $1 \times 10^{7-8}$ CFU/mL was prepared by adjusting the turbidity of the suspension. A methanol solution (100 μL) containing an extract (10 mg) or a synthesized chemical (5 mg) was placed on a paper disk for bioassay (8 mm diameter and 1 mm thickness, ADVANTEC, Tokyo, Japan) using a micropippet. After the methanol had ben evaporated out, a paper disk was placed on the agar surface inoculated with *H. pylori*. The plate was incubated at 37 °C for 3−4 days in an atmosphere consisting of 5% oxygen, 15% carbon dioxide, and 80% nitrogen in an anaerobic chamber (*28*).

The assay was replicated three times for the extracts, and the results are shown in **Table 7**. The assay was replicated twice for the authentic sulforaphane and related compounds, and results are shown in **Table 8**.

Analysis of Sulforaphane and Related Compounds in Chloroform Extract of Fresh Sprouts. The chloroform extract, which exhibited the strongest anti-*Helicobacter* activity in the preliminary test, was analyzed using an Agilent model 6890 GC equipped with a 30 m × 0.25 mm i.d. (df = 0.5 μm) DB-5MS bonded-phase fused silica capillary column (Agilent, Folsom, CA) and an FID. The helium carrier gas flow rate was 1.0 mL/min at a split ratio of 20:1. The injector and detector temperatures were 250 and 280 °C, respectively. The oven temperature was programmed from 50 °C (held for 3 min) to 280 °C at 10 °C/min and then held for 20 min.

An Agilent model 6890 GC interfaced to an Agilent 5971A mass selective detector (GC-MS) was used for mass spectral identification of the GC components at an MS ionization voltage of 70 eV. GC column conditions were exactly the same as used for GC-FID.

The identification of sulforaphane and related compounds was performed by comparison with the Kovats gas chromatographic retention index (I) and by the mass spectral fragmentation pattern of each component compared with those of authentic compounds. The identification of the GC components was also confirmed with the NIST AMDIS version 2.1 software.

Table 7. Inhibitory Effects of Extracts from Fresh Broccoli Sprouts against *Helicobacter pylori* Using the Impregnated Paper Disk Bioassay

sample tested[a]	inhibitory zone[b] (cm)
crude methanol extract	2.80 ± 0.3
hexane extract	5.03 ± 0.0
chloroform extract	>5.00
ethyl acetate extract	4.90 ± 0.2
butanol extract	3.10 ± 0.1
water fraction	0.00 ± 0.00
sulforaphane	>5.00[c]

[a] Refer to **Figure 1** for samples tested. [b] Values are mean ± SD (n = 3). The dose is 10 mg/disk unless otherwise noted. [c] Dose was 5 mg/disk.

Table 8. Concentrations of Compounds Identified in the Chloroform Extract of Fresh Broccoli Sprouts[a]

no. in **Figure 1**	compound	KI[b]	concn[c] (mg/kg)
V	4-methylthiobutylisothiocyanate	1440	1.9
XIII	3-methylsulfinylpropylisothiocyanate	1612	8.8
XIV	sulforaphane	1753	222.6
XVII	4-methylsulfinylbutylnitrile	1379	63.0
XVIII	5-methylsulfinylpentylnitrile	1518	475.7

[a] Refer to **Figure 3** for gas chromatogram and **Figure 1** for structures. [b] Kovats Index on DB-5. [c] Relative to fresh broccoli sprouts.

RESULTS AND DISCUSSION

Table 7 shows the results of anti-*Helicobacter* assay toward the samples prepared from fresh broccoli sprout (refer to **Figure 2** for details of each sample). Authentic sulforaphane exhibited a > 5 cm inhibition zone at the level of 5 mg/disk, indicating that the assay is valid. The chloroform extract was found to have the highest antimicrobial activity among the samples tested. The greatest inhibition zones (> 5 cm) were noted for *H. pylori* strain by the chloroform extract, followed by the hexane extract (5.03 cm), the ethyl acetate extract (4.90 cm), the butanol extract (3.10 cm), and the crude methanol extract (2.80 cm), whereas the water fraction did not show any inhibition zone, suggesting that the anti-*Helicobacter* active components are present in the organic solvent extracts. The antibacterial activities obtained in the present study were consistent with previously reported results in broccoli (*14*, *29*).

In the present study, to pinpoint the chemicals with anti-*H. pylori* activity in broccoli sprouts, the chloroform extract, which possessed the greatest activity, was analyzed by GC-MS. The typical gas chromatogram of a chloroform extract and the compounds identified are shown in **Figure 3**. The concentrations of chemicals identified are shown in **Table 8**. Three isothiocyanates (including sulforaphane) and two nitriles were positively identified in the chloroform extract. 5-Methylsulfinylpentylnitrile had the greatest concentration (475.7 mg/kg of fresh sprouts), followed by sulforaphane (222.6 mg/kg) and 4-methylsulfinylbutylnitrile (63.0 mg/kg). There are several papers on the analysis of sulforaphane and related compounds in broccoli and its sprouts. A methanol extract of lyophilized broccoli spouts contained 1153 mg/100 g of dry sample (*30*). The sulforaphane content in this study seems higher than that of the present study. However, the concentration in the dry sprouts must be much higher than that in the fresh sprouts. Therefore, the results from the present study can be considered comparable to the previous results.

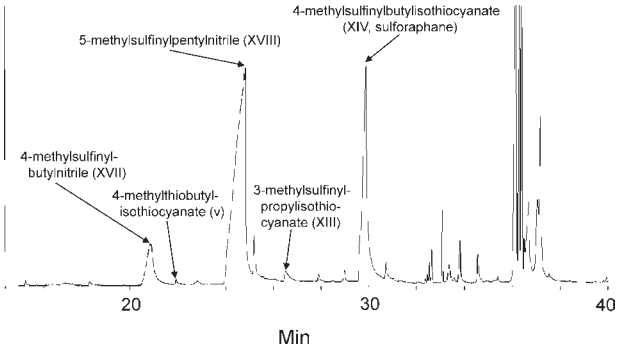

Figure 3. Typical gas chromatogram of a chloroform extract and the compounds identified (refer to **Table 8** for concentration).

Table 9. Inhibitory Effect of Sulforaphane and Related Compounds against *Helicobacter pylori* Using the Impregnated Paper Disk Bioassay

chemical	inhibitory zone[a] (cm)
I: 3-methylthiopropylamine	
II: 4-methylthiobutylamine	3.05
III: 5-methylthiopentylamine	>5.00
IV: 3-methylthiopropylisothiocyanate	>5.00
V: 4-methylthiobutylisothiocyanate	>5.00
VI: 5-methylthiopentylisothiocyanate	>5.00
VII: 3-methylthiopropylnitrile	1.60
VIII: 4-methylthiobutylnitrile	
IX: 5-methylthiopentylnitrile	3.95
X: 3-methylsulfinylpropylamine	5.10
XI: 4-methyllsulfinylbutylamine	0.95
XII: 5-methylsulfinylpentylamine	2.70
XIII: 3-methylsulfinylpropylisothiocyanate	>5.00
XIV: 4-methylsulfinylbutylisothiocyanate (sulforaphane)	>5.00
XV: 5-methylsulfinylpentylisothiocyanate	>5.00
XVI: 3-methylsulfinylpropylnitrile	>5.00
XVII: 4-methylsulfinylbutylnitrile	
XVIII: 5-methylsulfinylpentylnitrile	4.05

[a] Values are average of two duplicate experiments. The dose is 5 mg/disk.

There are only a few papers on the analysis of sulforaphane and related compounds in broccoli sprouts compared to those in broccoli. For example, four varieties of broccoli contained sulforaphane ranging from 1885 to 3703 mg/kg and 5-methylsulfinylpentyl nitrile (sulforaphane nitrile) ranging from 167 to 208 mg/kg (*31*). Among them, sulforaphane, which possesses a strong antimicrobial activity, has been the most frequently reported isothiocyanate (*32*). In particular, sulforaphane has received much attention as an isothiocyanate, found in cruciferous vegetables such as broccoli, which possesses strong anti-*Helicobacter* activity (*17*, *28*, *33*). Therefore, the present study was focused on sulforaphane and related compounds for their anti-*Helicobacter* activity, and 18 sulforaphane and related compounds were synthesized and tested for anti-*Helicobacter* assay, the results of which are shown in **Table 9**.

Among the chemicals tested, nine chemicals [two amines (III, X), six isothiocyanates (IV, V, VI, XIII, XIV, XV), and one nitrile (XVI)] exhibited >5 cm inhibitory zones. As mentioned above, isothiocyanates showed strong anti-*Helicobacter* activity, which is consistent with a previous paper (*33*). Sulforaphane isomers (DL-, D-, and L-) reportedly exhibited similar and high anti-*H. pylori* activities with overall minimal inhibitory concentrations (MICs) ranging from 0.06 to 8 μg/mL (*33*). 5-Methylsulfinylpentylnitrile (XVIII), which is found in broccoli sprouts in the greatest concentration (475.7 mg/kg), exhibited an appreciable inhibition zone (4.05 cm), whereas another nitrile, 3-methylsulfinylpropylnitrile (XVI), showed a >5 cm inhibition zone but was not identified in the chloroform extract of broccoli sprouts. On the other hand, the other nitrile, 4-methylsulfinylbutylnitrile (XVII), which was identified in the chloroform extract (63.0 mg/kg), did not show any activity. Low levels of 4-methylthiobutylisothiocyanate (V, 1.9 mg/kg) and 3-methylsulfinylpropylisothiocyanate (XIII, 8.8 mg/kg) were identified. However, both isothiocyanates exhibited strong anti-*Helicobacter* activity at the level of 5 mg/disk.

Sulforaphane and 5-methylsulfinylpentylnitrile must contribute significant anti-*Helicobacter* activity to broccoli sprouts. It is hypothesized that the other sulforaphane-related compounds, which may be present in broccoli sprouts, also play a role in their anti-*Helicobacter* activity. Medicinal and chemical compositional properties found in broccoli have been also found in its sprouts. In addition to sulforaphane, some sulforaphane-related compounds, which possess anti-*Helicobacter* activities were identified in broccoli sprouts in the present study. Recent studies have indicated that broccoli sprouts possess strong antioxidant activity, which may be due to the presence of polyphenols (*34*, *35*). However, sulforaphane isolated from broccoli has been also known as an antioxidant that indirectly prevents carcinogenesis (*36*, *37*).

The stability of sulphoraphane has been reported in a several papers. For example, stability tests conducted under benchtop conditions at ambient temperature for 8 h and in long-term storage at −80 °C for 1 month showed remaining sulphoraphane ranging from 92 to 100% (*38*). It has been noted that sulforaphane has a relatively short half-life under gastric conditions (*39*). However, various studies reported its potent anti-*Helicobactor* activities as mentioned above, suggesting that sulforaphane is stable enough to reveal its activity under gastric conditions (*17*, *28*, *33*). Recently, encapsulated sulforaphane was developed to enhance its therapeutic effect and anticancer activity (*40*). It is proposed that the other sulforaphane-related compounds might have similar stabilities as sulforaphane because of their structures.

The present study discovered the presence of potent anti-*Helicobactor* active compounds in the essences prepared from broccoli sprouts in significant amounts. The major active components found in broccoli sprouts were sulforaphane and related compounds. The flavor and taste of broccoli sprouts are quite similar to those of broccoli. The chemical composition of broccoli and its sprouts are also similar. These results suggest that broccoli sprouts can be consumed in the same way as broccoli for medicinal benefit. Moreover, sprouts are quite easy to grow compared with corresponding vegetables. They require only UV light, water, and <1 week between germination and harvest. Therefore, broccoli sprouts can be an excellent food source for medicinal substances.

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