Purification of the ω-(Methylsulfinyl)alkyl Glucosinolate Hydrolysis Products: 1-Isothiocyanato-3-(methylsulfinyl)propane, 1-Isothiocyanato-4-(methylsulfinyl)butane, 4-(Methylsulfinyl)butanenitrile, and 5-(Methylsulfinyl)pentanenitrile from Broccoli and *Lesquerella fendleri*†

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The purification of the ω-(methylsulfinyl)alkyl glucosinolate hydrolysis products 1-isothiocyanato-3-(methylsulfinyl)propane (IMSP), 1-isothiocyanato-4-(methylsulfinyl)butane (IMSB), 4-(methylsulfinyl)-butanenitrile (MSBN), and 5-(methylsulfinyl)pentanenitrile (MSPN) from the seeds of broccoli and *Lesquerella fendleri* (Gray) S. Watson is described. The procedure uses solvent extraction of autolyzed defatted seed meals, followed by purification of the hydrolysis products using gel filtration chromatography and reversed-phase high-performance liquid chromatography (HPLC). Purity and confirmation of the compounds were monitored and verified using gas chromatography with flame ionization detection (GC-FID), thin-layer chromatography (TLC), gas chromatography–mass spectroscopy (GC–MS), and nuclear magnetic resonance (NMR). The techniques are useful for the preparative-scale isolation of structurally related glucosinolate hydrolysis products arising from ω-(methylsulfinyl)alkyl glucosinolates and should facilitate more extensive studies into the biological effects of these naturally occurring compounds.

INTRODUCTION

Cabbage, Brussels sprouts, cauliflower, and broccoli are among the most commonly consumed cruciferous vegetables in the human diet and have been shown to be rich in glucosinolates possessing ω-(methylsulfinyl)alkyl side chains (Fenwick et al., 1983). Glucosinolates may break down to form isothiocyanates and/or nitriles in plant material during processing by the action of the endogenous enzyme thioglucoside glucohydrolase (EC 3.2.3.1) (Cole, 1976) or within the gastrointestinal tract by the action of commensal microflora (Nugon-Baudon et al., 1988).

Crucifers may have a potential role in cancer chemoprevention in addition to their high nutritional value. Several epidemiologic studies have identified a correlation between the consumption of cruciferous vegetables with glucosinolate composition very rich in ω-(methylsulfinyl)-alkyl glucosinolates and a decreased incidence of colorectal cancer (Graham et al., 1978; Haenszel et al., 1980; Lee et al., 1989; Manousos et al., 1983). In several studies with laboratory species, a chemoprotective effect against known carcinogens by diets supplemented with cruciferous plants or specific glucosinolates and their hydrolysis products has been confirmed (Stoewsand et al., 1978, 1988; Wattenberg, 1979b). The specific glucosinolate-derived compounds in crucifers responsible for these observed effects toward carcinogens are unknown, but the high relative concentrations of ω-(methylsulfinyl)alkyl glucosinolates in cabbage, Brussels sprouts, cauliflower, and broccoli make these compounds and their hydrolysis products interesting candidates.

The administration of selected glucosinolate hydrolysis products has been shown to inhibit both initiation and promotion of chemically induced neoplasia in many organs of laboratory rodents (Shertzer, 1983, 1984; Stoner et al., 1991; Wattenberg, 1977, 1979a,b, 1981; Wattenberg et al., 1985). The mechanisms by which these compounds prevent neoplasia are incompletely understood. According to Wattenberg (1992), dietary inhibitors of carcinogenesis, which include the glucosinolate hydrolysis products, may be classified according to their mechanism of action into two main categories: blocking agents and suppressing agents. Blocking agents are administered before and during exposure to carcinogens and seem to prevent carcinogens or their metabolites from reaching or reacting with critical target sites by either inhibiting reactions requiring metabolic activation, inducing the activity of enzyme systems that detoxify and/or facilitate the elimination of carcinogenic agents, or trapping of reactive carcinogenic species. Other inhibitors may act during the promotion of carcinogenesis as suppressing agents which prevent the evolution of the neoplastic process in cells previously exposed to doses of carcinogenic agents. The biochemical mechanisms of action for suppressing agents are not well-defined, but they may act to modulate the consequences of the genetic changes initiated in the early stages of the neoplastic process. Finally, some inhibitors show dual blocking and suppressing action. These inhibitors may stimulate a coordinated cell protective system that includes a detoxification component and a suppressive component.

The acute toxicity of glucosinolates is, in general, considered to be low, in part as a result of adequate iodine in the diet. However, the consumption by animals of crucifer seed meals that are rich in glucosinolates or diets

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high in crucifers has been shown to cause a number of deleterious physiological effects (DeGroot et al., 1991). Growth depression, markedly reduced feed intake, enlargement of the liver, kidneys, and thyroid, and depletion of plasma thyroxine have all been reported in animals consuming rapeseed meal (Bell, 1984). These toxic effects are thought to be due to aglucons, such as nitriles and isothiocyanates, formed either within the meal or within the digestive tract of the animal (Paik et al., 1980). *Lesquerella fendleri* (Gray) S. Watson (*Lesquerella*, hereafter), a cruciferous weed native to western Texas, is a plant with potential economic value as a new industrial oil seed crop (U.S. Department of Agriculture, 1991). *Lesquerella* oil, a replacement for imported castor oil, is used in a number of lubricants and coatings and as an intermediate in the manufacturing of a wide range of products. The seed meal resulting after oil extraction is relatively high in lysine and has potential use as a protein supplement for beef cattle. However, the seed meal is also particularly high in 3-(methylsulfinyl)propyl glucosinolate and its hydrolysis products, 1-isothiocyanato-3-(methylsulfinyl)propane (IMSP) and 4-(methylsulfinyl)-butanenitrile (MSBN). No data are known to exist regarding the toxic effects in animals of *Lesquerella* seed meal. Limited information is available on the general acute toxic effects of ω-(methylsulfinyl)alkyl glucosinolates and their hydrolysis products in animals (Nishie and Daxenbichler, 1980). The LD_{50} of IMSP in Sprague-Dawley rats was 90 mg/kg SC (0.55 mmol/kg). Clinical signs observed in rats included excessive lacrimation, edema at the injection site, coma, and death.

Certain glucosinolates are considered important because of the nature of the side chain present. The ω-(methylsulfinyl)alkyl glucosinolates and their hydrolysis products are hypothesized to be natural pesticides in crucifers (Ames et al., 1990). Some isothiocyanates, such as 1-isothiocyanato-4-(methylsulfinyl)butane (IMSB), have been shown to have fungistatic and bacteriostatic properties (Dornberger et al., 1975), and IMSP has been shown to be a protein syntehsis inhibitor (Leblova, 1965). The chemically related isothiocyanates 1-isothiocyanato-4-(methylsulfonyl)butane and 1-isothiocyanato-3-(methylsulfonyl)-propane have been found to be cytotoxic toward He-La cells (Horáková et al., 1968). These chemical and physiologic properties of the reactive (methylsulfinyl)alkyl side chain, as well as the isothiocyanate and nitrile moieties, suggest that these compounds may have special significance in vivo.

The evaluation of the toxic or beneficial properties of ω-(methylsulfinyl)alkyl glucosinolates and their hydrolysis products in vivo requires purified compounds in relatively large amounts. Although techniques have been developed for the chemical synthesis of some glucosinolates, they have not been described for all of the ω-(methylsulfinyl)-alkyl aglucons. Many techniques have been used to identify and quantify these compounds in plant and seed material (McGregor et al., 1983). Most of these techniques have not been adapted for large-scale recovery of specific glucosinolates or their specific hydrolysis products or have been limited to the production of small quantities of pure compounds solely for structure analysis or for use as chromatographic standards.

To perform studies evaluating the biological effects of ω-(methylsulfinyl)alkyl glucosinolate hydrolysis products, it was necessary to obtain reasonably large (gram) quantities of highly purified compounds for experiments. Compounds of interest included: 1-isothiocyanato-3-(methylsulfinyl)propane (IMSP, commonly known as iberin), 1-isothiocyanato-4-(methylsulfinyl)butane (IMSB, commonly known as sulforaphane), 4-(methylsulfinyl)-butanenitrile (MSBN, commonly known as iberin nitrile), and 5-(methylsulfinyl)pentanenitrile (MSPN, commonly known as sulforaphane nitrile) (Figure 1). These compounds were not readily available; therefore, methods were developed for their separation, isolation, and purification from *L. fendleri* and broccoli seed. *Lesquerella* seed was chosen as the source for IMSP and MSBN as it has been shown to be a particularly rich source of the parent glucosinolate 3-(methylsulfinyl)propyl glucosinolate (Daxenbichler et al., 1977). Broccoli seed (*Brassica oleraceae*) was chosen as a source for IMSB and MSPN, as it has been shown to be particularly rich in the parent glucosinolate 4-(methylsulfinyl)butyl glucosinolate (Carlson et al., 1987).

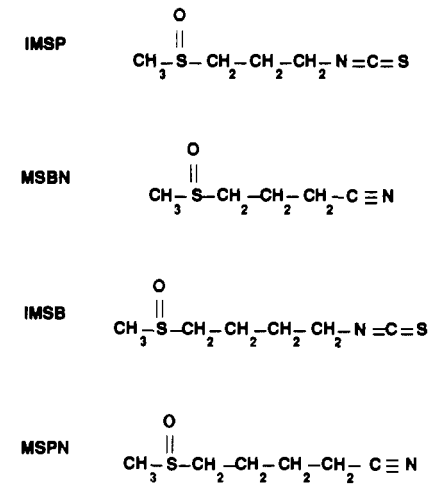

Figure 1. Chemical structures of 1-isothiocyanato-3-(methylsulfinyl)propane (IMSP; iberin) and 4-(methylsulfinyl)butanenitrile (MSBN; iberin nitrile) from 3-(methylsulfinyl)propyl glucosinolate and 1-isothiocyanato-4-(methylsulfinyl)butane (IMSB; sulforaphane) and 5-(methylsulfinyl)pentanenitrile (MSPN; sulforaphane nitrile) from 4-(methylsulfinyl)butyl glucosinolate.

MATERIALS AND METHODS

Materials and Reagents. *Lesquerella* seed (*L. fendleri*, 10 kg of 1987 crop) was kindly provided by Kenneth D. Carlson, National Center for Agricultural Utilization Research, Peoria, IL. Broccoli seed (*B. oleraceae* L. var. Botyris subvar. Cymosa Lam., Green Sprouting Variety, 10 kg of 1987 crop) was obtained from Dorsing Seeds, Inc., Nyssa, OR. Chromatographic standards for IMSP, IMSB, MNSB, and MNSP were kindly provided by Melvin E. Daxenbichler, National Center for Agricultural Utilization Research, Peoria, IL. Sephadex G-10 gel filtration medium (40–120 μm bead size) was purchased from Sigma Chemical Co., St. Louis, MO. Silica gel for flash chromatography (40-μm average particle diameter) was purchased from J. T. Baker, Inc., Phillipsburg, NJ. Whatman K6 silica gel thin-layer chromatography plates (60-Å average pore diameter) were purchased from Whatman, Inc., Clifton, NJ. All chemical reagents (sodium chloride and sodium sulfate) were of analytical grade, and all solvents (hexane, methylene chloride, methanol, acetone, and chloroform) were of HPLC grade.

Isolation of Glucosinolate Hydrolysis Product Extracts. Seed meal was prepared by grinding 500-g aliquots of either *Lesquerella* or broccoli seed in a stainless steel blender. The seed meal was defatted with 4 L of hexane at room temperature with air percolation, filtered, and allowed to air dry. The defatted seed meal was left to autolyze at room temperature for 24 h, using approximately 600 mL of water to wet the meal and using concentrated hydrochloric acid to adjust the pH of the meal to 3. Following autolysis, the meal was lyophilized and the dried meal was extracted with 8 L of methylene chloride at room

temperature. The crude methylene chloride extract was concentrated in vacuo to a dark brown oil on a rotary evaporator (40 °C). The concentrate was washed twice with 150 mL of water. The aqueous layers were pooled, saturated with sodium choride, and re-extracted in 4 L of fresh methylene chloride. The methylene chloride layer was dried over anhydrous sodium sulfate and concentrated in vacuo using a rotary evaporator. The final golden brown extract concentrates from five individual seed preparations were pooled according to seed type prior to proceeding with further purification procedures.

Sephadex Column Separation of ω-(Methylsulfinyl)alkyl Glucosinolate Hydrolysis Products. The pooled extract from either broccoli or *Lesquerella* seeds was loaded onto an open preparatory column (60 cm × 3 cm i.d.) containing Sephadex G-10 gel filtration medium (bed volume of 590 mL) and eluted by using 5% methanol in water at a flow rate of 2–5 mL/min. After the void volume (approximately 220 mL) was discarded, 100 10-mL fractions of eluate were collected. Individual fractions were extracted with methylene chloride and evaluated for the presence of IMSP, IMSB, MSBN, and MSPN using the GC-FID technique described below. Fractions derived from *Lesquerella* containing pure IMSP and pure MSBN, as determined by GC-FID, were combined separately. Fractions derived from broccoli containing isothiocyanates (IMSP and IMSB) and nitriles (MSBN and MSPN), as determined by GC-FID, were individually pooled. The aqueous portion of each of the pooled fractions was saturated with sodium chloride and extracted with methylene chloride. These methylene chloride fractions were dried over anhydrous sodium sulfate, concentrated in vacuo on a rotary evaporator, and dried under nitrogen to give semicrystalline light brown substances. Purified fraction concentrates from four Sephadex column runs for each seed type were pooled, representing the yield of glucosinolate hydrolysis products derived from 10 kg of either *Lesquerella* or broccoli seed.

The purities of the pooled fractions from *Lesquerella* (two fractions) and broccoli (two fractions) were examined using thin-layer chromatography as described below. The *Lesquerella* fraction containing IMSP was further purified using silica gel chromatography as described below. The remaining three fractions (MSBN from *Lesquerella*, IMSP and IMSB from broccoli, and MSBN and MSPN from broccoli) and semipurified fractions from the IMSP silica gel columns were further purified using the preparative HPLC technique described below.

Silica Gel Purification of IMSP from Seed Extract of *Lesquerella*. The semipurified IMSP fraction from *Lesquerella* seed was loaded onto an open preparatory chromatographic column (60 cm × 3 cm i.d.) containing silica gel for flash chromatography. The column was eluted using 95:5 chloroform/methanol, and 10-mL fractions of eluate were collected and evaluated for the presence of IMSP by GC-FID and TLC. The fractions containing IMSP were pooled and concentrated into three subfractions: a fraction containing IMSP and relatively polar contaminants, a pure fraction (yielding one spot on TLC), and a fraction containing IMSP and relatively nonpolar contaminants. The polar fraction was purified further by passing it over another silica gel column with 100% acetone as the eluent. Fractions (10 mL) of eluate from the acetone column were collected and evaluated for purity by TLC. The IMSP fractions showing no evidence of contaminants from the acetone column were pooled with the purified fraction from the chloroform/methanol column, giving a final yield of 11.5 g IMSP.

HPLC Purification of ω-(Methylsulfinyl)alkyl Glucosinolate Hydrolysis Products. IMSP and IMSB were separated using a 250 × 22.5 mm Econosil C_{18} column (Alltech Associates, Deerfield, IL) with a mobile phase of 20% acetone in water pumped at 25 mL/min by a Waters Model 590-EF pump (Waters Associates, Milford, MA). Samples from Sephadex columns or silica gel purification were diluted about 3-fold with acetone and injected into a 1-mL loop in a Valco Model C6U valve (Valco Instruments, Houston, TX) in about 0.8-mL increments. Peaks were detected by differential refractometry (Waters Model 401). The eluates indicated by peaks were collected, concentrated by rotary evaporation, and finally lyophilized to viscous products. Under these conditions, MSBN and MSPN were eluted with the solvent fronts. These frontal materials were collected, concentrated, and rechromatographed with the above chromatographic system using a mobile phase of 20% methanol in water at a flow rate of 7 mL/min. In this way, MSBN and MSPN were resolved and collected.

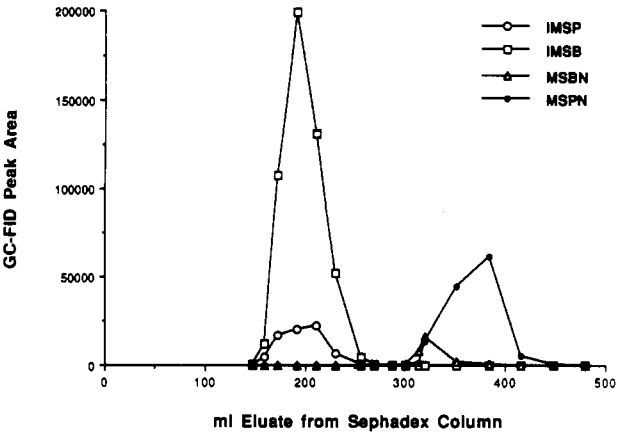

Figure 2. Sephadex column elution profile for broccoli preparations.

Following HPLC, IMSP and IMSB were purified further by chromatography on an open silica column eluted with chloroform followed by chloroform/acetone (9:1 and 4:1).

TLC Analysis of ω-(Methylsulfinyl)alkyl Glucosinolate Hydrolysis Products. Qualitative purity of the glucosinolate hydrolysis products from *Lesquerella* and broccoli seed was determined by TLC on silica gel plates using either 95:5 chloroform/methanol or 100% acetone as the mobile phase, followed by development of the plates in iodine vapor. Sample size was 1 μL of the concentrates and standards.

GC-FID Analysis of ω-(Methylsulfinyl)alkyl Glucosinolate Hydrolysis Products. The GC-FID analysis of the aglucons was conducted on a Hewlett-Packard 5840A gas chromatograph with a flame ionization detector. The column was 3% OV-17 packed on 100/120 Supelcoport (Supelco, Inc. Bellefonte, PA) (2 m × 2 mm i.d.). Operating conditions were as follows: injection volume, 1.0 μL; injector temperature, 280 °C; helium flow rate, 20 mL/min; 40 °C for 2.5 min, ramped column temperature increases at 15 °C/min to a temperature of 275 °C; column held at 275 °C for 7.5 min. The detector was kept at 275 °C with a hydrogen flow rate of 60 mL/min and an air flow rate of 240 mL/min. Retention times and peak areas of sample components were compared to those of known concentrations of highly purified standards.

GC–MS Confirmation of ω-(Methylsulfinyl)alkyl Glucosinolate Hydrolysis Products. A Varian Model 3400 gas chromatograph equipped with a Finnegan MAT Model INCOS 500 mass spectrometer was used for the detection, mass spectral display, and confirmation of the ω-methylsulfinyl)alkyl glucosinolate hydrolysis products. A J&W Scientific DB-5, 30 m × 0.25 mm i.d., capillary column delivered compounds to the mass spectrometer.

Operating conditions of the gas chromatograph were as follows: injection volume, 1.0 μL; injector temperature, 250 °C; helium linear velocity, 39 cm/s; oven temperature, 50 °C (held for 5 min), ramped column temperature increase to 250 °C at 10 °C/min; final column temperature held at 250 °C for 5 min.

Mass spectra were obtained by electron impact ionization (EI) over the range 35–400 atomic mass units at a rate of 0.5 scans/s. The ion source temperature was 180 °C, and the electronic impact energy was 70 eV.

RESULTS

Sephadex Column Separation. The Sephadex gel filtration column yielded good results in separating isothiocyanate aglucons from nitrile products. The nitriles eluted between 140 and 270 mL, and the isothiocyanates eluted between 300 and 450 mL (Figure 2). In the broccoli preparations, however, the isothiocyanate products (IMSP and IMSB) and nitrile products (MSBN and MSPN) could not be adequately separated from each other.

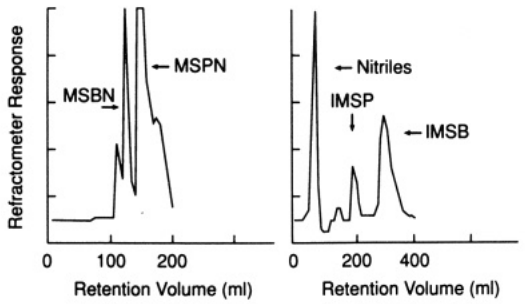

Figure 3. HPLC column elution profiles of nitriles (left) and isothiocyanates (right).

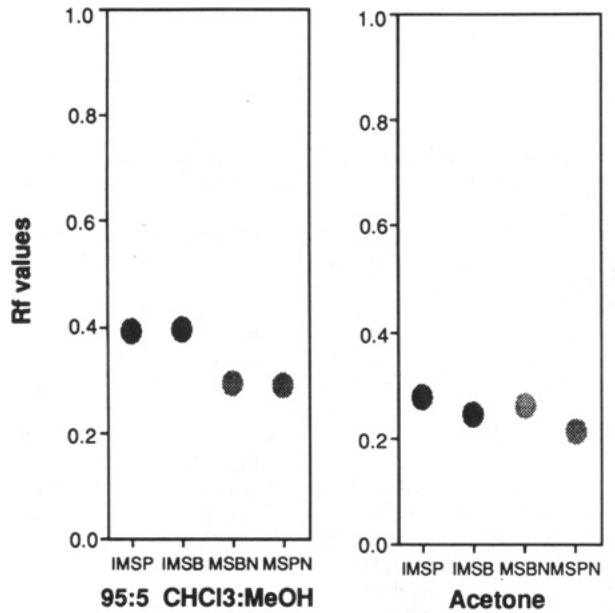

Figure 4. TLC separation of IMSP, IMSB, MSBN, and MSPN.

Silica Gel Column Separation. The silica gel column conditions were chosen to simulate the separation of IMSP from contaminating pigments and other unidentified organic molecules previously achieved with TLC using 95:5 chloroform/methanol. Acetone was used to separate IMSP from subfractions containing polar contaminants. Good separation of IMSP from impurities was achieved using the two silica gel columns to yield highly purified IMSP. Silica gel column separation of the four ω-(methylsulfinyl)alkyl glucosinolate hydrolysis products from each other was not attempted.

HPLC. IMSP was eluted between 190 and 215 mL by reversed-phase HPLC (20% acetone in water), and IMSB was eluted from 230 to 270 mL. Regarding the nitriles (20% methanol in water), MSBN was eluted from 120 to 130 mL and MSPN from 135 to 160 mL (Figure 3). Silica column chromatography was needed to adequately purify the isothiocyanates.

TLC. The chloroform/methanol solvent system was the most effective for separating the compounds of interest from contaminating pigments, although this solvent system was not useful for separating the compounds from each other (R_f values for IMSP, IMSB, MSBN, and MSPN were 0.402, 0.405, 0.303, and 0.303, respectively). Acetone yielded the best separation of the compounds from each other with R_f values for IMSP, IMSB, MSBN, and MSPN of 0.286, 0.256, 0.272, and 0.224, respectively (Figure 4). Iodine vapor was chosen in favor of more specific visualization techniques for isothiocyanates and nitriles because it is nondenaturing and reversibly bound by a wide range of organic compounds. The final purified compounds each yielded one spot on TLC using the 100% acetone and the 95:5 chloroform/methanol solvent systems.

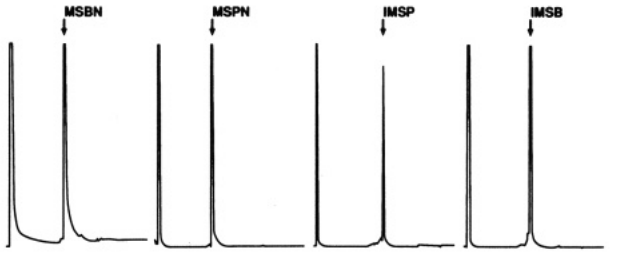

Figure 5. GC-FID profiles of MSBN, MSPN, IMSP, and IMSB. Relative retention times for the four compounds are 8.03, 8.10, 9.83, and 9.98 min, respectively.

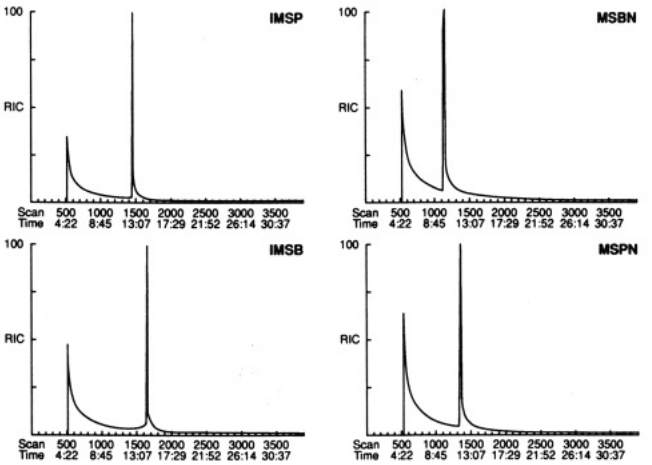

Figure 6. GC–MS reconstructed ionization chromatographs (RIC) for IMSP, IMSB, MSBN, and MSPN.

GC-FID. The order of elution of mixtures of the four ω-(methylsulfinyl)alkyl aglucons was MSBN, MSPN, IMSP, and IMSB. Confirmation of each of the peaks within the mixture was achieved by spiking samples of the mixture with pure standards of individual compounds of interest. Baseline resolution of each peak within mixtures of the hydrolysis products was not achieved using the column and conditions described. GC-FID evaluation of each of the hydrolysis products of interest gave a single peak for each purified compound. The detection limit for each compound using this system was approximately 0.5 μg. Figure 5 shows GC-FID profiles of the four aglucons in their order of elution.

GC–MS. Confirmation of the structure of the individual ω-(methylsulfinyl)alkyl glucosinolate hydrolysis products was achieved with mass spectroscopy (Figures 6 and 7). The m/e values obtained for IMSP, MSBN, IMSB, and MSPN were in agreement with previously published mass spectra (Spencer and Daxenbichler, 1980): IMSP [39 (30), 41 (80); 56 (11), 61 (16); 63 (23), 72 (100); 78 (7), 86 (12); 100 (22), 102 (5); 104 (1), 116 (25); 118 (1), 130 (6); 132 (1); 146 (1), 148 (1); M = 163 (1), 164 (1)]; MSBN [39 (25), 41 (100); 49 (9), 61 (9); 64 (74), 68 (34); 78 (5), 84 (4); 91 (1), 103 (1); 104 (1), 115 (1); M = 131 (17); 132 (1), 133 (1)]; IMSB [39 (16), 45 (13); 55 (42), 60 (6); 64 (13), 72 (100); 85 (5), 86 (5); 98 (1), 101 (1); 112 (4), 114 (9); 119 (4), 120 (1); 144 (1), 146 (1); 160 (64), 162 (6); M = 177 (1)]; MSPN [39 (24), 41 (33); 54 (23), 55 (100); 63 (18), 64 (47); 78 (7), 82 (27); 96 (1), 97 (1); 105 (1), 114 (1); 128 (4), 129 (1); M = 145 (8); 146 (1), 147 (1)].

NMR. NMR spectra were obtained to confirm the aglucon structure (Figure 8). Spectra were run at 300 MHz using $CDCl_3$ as the solvent. IMSP: δ 3.70 (m, 2 H, CH_2-NCS), 2.75 (m, 2 H, CH_2SO), 2.58 (s, 3 H, CH_3SO), and

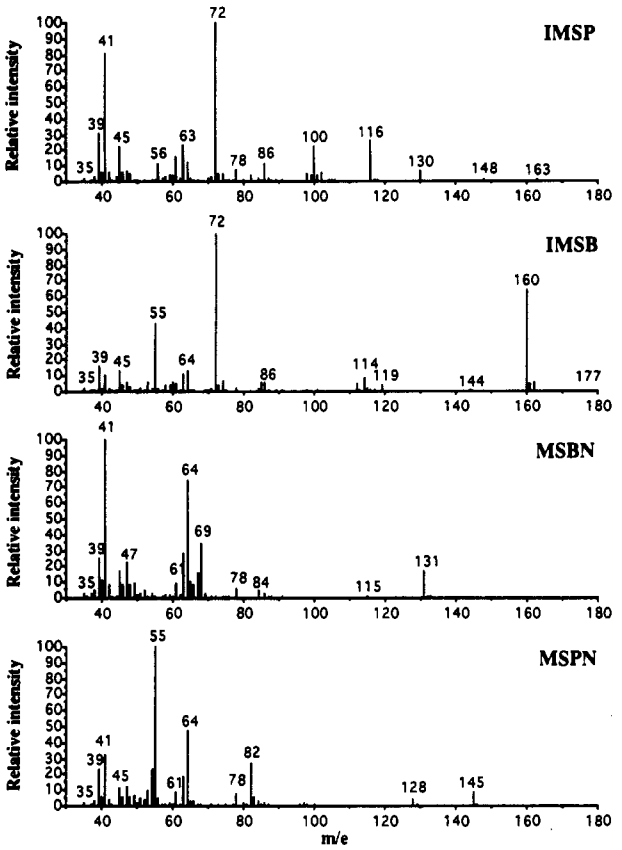

Figure 7. Mass spectra of IMSP, IMSB, MSBN, and MSPN.

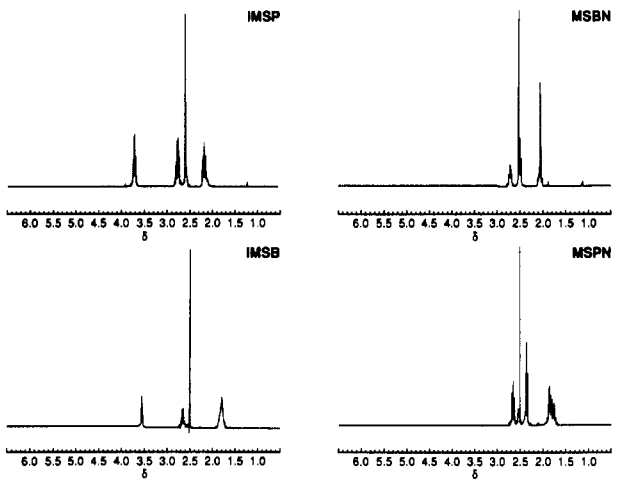

Figure 8. NMR profiles of IMSP, MSBN, IMSB, and MSPN.

2.15 (m, 2 H, CH_2). MSBN: δ 2.72 (m, 2 H, CH_2SO), 2.51 (s, 3 H, CH_3SO) 2.47 (t, 2 H, CH_2CN), 2.05 (m, 2 H, CH_2). IMSB: δ 3.60 (t, 2 H, CH_2NCS), 2.70 (m, 2 H, CH_2SO), 2.57 (s, 3 H, CH_3SO), and 1.88 (m, 4 H, CH_2CH_2). MSPN: δ 2.64 (m, 2 H, CH_2SO), 2.50 (s, 3 H, CH_3SO), 2.34 (t, 2 H, CH_2CH), 1.80 (m, 4 H, CH_2CH_2).

Yield of ω-(Methylsulfinyl)alkyl Glucosinolate Hydrolysis Products. The final yields of ω-(methylsulfinyl)alkyl glucosinolate hydrolysis products from 10 kg each of *Lesquerella* and broccoli seed were as follows: IMSP, 12.9 g; IMSB, 4.9 g; MSBN, 1.8 g; and MSPN, 6.1 g. The efficiency of the isolation method used in the present study was not monitored, since the goal was to obtain highly purified compounds rather than to determine the concentrations of the glucosinolate hydrolysis products in the starting plant material. Although the autolysis reaction conditions within the seed meals were chosen to yield a high proportion of nitrile hydrolysis products as compared to the isothiocyanates, the actual reaction in *Lesquerella* yielded almost exclusively IMSP (95%). The same autolysis conditions in defatted broccoli seed meal yielded approximately equal proportions of isothiocyanate and nitrile aglucons.

DISCUSSION

Many methods have been used for identification of glucosinolate hydrolysis products in plant materials, foods, and biological fluids (McGregor et al., 1983; Olsen and Sørensen, 1981), but relatively few techniques have been described for the high-yield isolation of specific compounds in highly purified form. Two main approaches to the isolation of glucosinolate hydrolysis products have been described. The first involves the isolation of the parent glucosinolate, followed by exogenous addition of thioglucoside glucohydrolase to obtain the desired glucosinolate hydrolysis products. The conditions of the enzymatic reaction can be adjusted to favor the production of either the isothiocyanate (pH 7) or nitrile product (pH 3) (Fenwick et al., 1983). This procedure was used for the first isolation of IMSP by Schultz and Gmelin in 1954, and by Daxenbichler et al. in 1977 for the isolation of MSBN. The second approach involves the autolysis of seed and/or plant meal using endogenous thioglucoside glucohydrolase, followed by purification procedures to obtain the hydrolysis products of interest. Procházka et al. (Procházka, 1959; Procházka et al., 1959, 1960) were the first to describe the purification of IMSP and IMSB using extracts of autolyzed cabbage, Brussels sprouts, and hoary cress. The purification of parent glucosinolates and their respective hydrolysis products from each other has been described using HPLC (Hogge et al., 1988), flash chromatography (Peterka and Fenwick, 1988), Sephadex gel filtration (Hanley et al., 1983), paper chromatography (Rutkowski et al., 1970), and other techniques.

Difficulties in the purification of aglucons from plant material arise because of the complex mixtures of parent glucosinolates with similar structural moieties found in botanical sources (Fenwick et al., 1989). Sources of plant materials for the isolation of glucosinolate hydrolysis products are chosen such that the starting material is known to be high in a particular parent glucosinolate. For this reason, seed meal was chosen as the most concentrated plant source of glucosinolates and for ease of purification of the compounds of interest from extraneous vegatative matter. Furthermore, *Lesquerella* and broccoli strains were specifically chosen for their known high concentrations of 3-(methylsulfinyl)propyl glucosinolate and 4-(methylsulfinyl)butyl glucosinolate, respectively. Nevertheless, difficulties were still encountered in the purification of IMSB and MSPN from the broccoli preparations as this seed also contained 3-(methylsulfinyl)propyl glucosinolate in appreciable quantities. The ratio of 4-(methylsulfinyl)butyl glucosinolate to 3-(methylsulfinyl)propyl glucosinolate in the source broccoli seed was 5:1 (Gayland F. Spencer, personal correspondence). Although crucifer seeds contain approximately 10 times the total glucosinolate concentration found in the edible portion of the vegetable, a strong correlation between the patterns of 3-(methylsulfinyl)propyl glucosinolate and 4-(methylsulfinyl)butyl glucosinolate expression found in the seeds and the patterns found in the edible portion of the vegetable has been determined for both broccoli and cabbage (Carlson et al., 1987; Tookey et al., 1980). Thus, seed meals provide a convenient and concentrated source of ω-(methylsulfinyl)alkyl glucosinolate hydrolysis products and reflect the glucosinolate patterns found in the edible portions of cruciferous vegetables.

An alternative approach to the isolation of glucosinolates and their hydrolysis products from plant materials is direct chemical synthesis of the compounds of interest. The chemical synthesis of ω-(methylsulfinyl)alkanenitriles has not been specifically described. These syntheses are theoretically possible with precursor 1,*n*-halo(methylsulfinyl)alkanes produced according to the method of Anklam (1989) and the technique of Harrison and Hodge (1980) using polymer-supported cyanide to prepare the nitriles. The chemical synthesis of IMSB has been described by Karrer et al. (1950), and the synthesis of IMSP has been described by Schmid and Karrer (1948). These chemical syntheses result in a racemic mixture of product, whereas the product isolated from plant extracts is usually the *R* form (Cheung et al., 1965).

The methods described in this paper separated the structurally closely related ω-(methylsulfinyl)alkyl aglucons from broccoli and *Lesquerella* seed meals from each other and yielded them in pure form and in sufficient quantities to enable further toxicologic evaluations of IMSP, IMSB, MSBN, and MSPN in animals. The availability of a procedure to purify these compounds should aid in the evaluation of the specific biological effects of the ω-(methylsulfinyl)alkyl glucosinolate hydrolysis products associated with consumption of cruciferous vegetables.

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Registry Numbers supplied by author: IMSP [CAS name, 1-isothiocyanato-3-(methylsulfinyl)propane; phytochemical name, 3-(methylsulfinyl)propyl isothiocyanate (3-MSPI); common name, iberin], 505-44-2; IMSB [CAS name, 1-isothiocyanato-4-(methylsulfinyl)butane; phytochemical name, 4-(methylsulfinyl)butyl isothiocyanate (4-MSBI); common name, sulforaphane], 4478-93-7; MSBN [CAS name, 4-(methylsulfinyl)butanenitrile; phytochemical name, 1-cyano-3-(methylsulfinyl)propane (CMSP); common name, iberin nitrile], 61121-65-1; MSPN [CAS name, 5-(methylsulfinyl)pentanenitrile; phytochemical name, 1-cyano-4-(methylsulfinyl)butane (CMSB); common name, sulforaphane nitrile], not registered.

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