of authentic glucosinolate standards. The support of the U.S. Food and Drug Administration Visiting Scientist Program for G.J.S. is gratefully acknowledged.

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Separation and Identification of Glucosinolates from *Brassica* Vegetables Using High-Performance Capillary Gas Chromatography (GC)–Positive-Ion Chemical Ionization Mass Spectrometry (PICIMS) and GC–PICIMS/MS

G. John Shaw,[1] Denis Andrzejewski,* John A. G. Roach, and James A. Sphon

A new, general-purpose analytical procedure for identification of desulfoglucosinolates from *Brassica* vegetables has been developed by combining high-performance capillary gas chromatography (HPCGC) with positive-ion chemical ionization mass spectrometry (PICIMS) and PICIMS/MS. HPCGC on a 25 m × 0.2 mm Ultra 1 WCOT fused silica column gave highly reproducible base-line separation of alkyl, alkenyl, hydroxyalkenyl, aryl, and indole desulfoglucosinolates as their per(trimethylsilyl)desulfoglucosinolate analogues. PICIMS and PICIMS/MS of these compounds using methane as the reagent gas gave conventional and daughter ion mass spectra in which the major ions were highly diagnostic of the glucosinolate structure.

Glucosinolates (1) are an important class of β-D-thioglucosides whose natural occurrence is limited to several plant families within the dicotyledonous angiosperms. Although approximately 100 glucosinolates (Kjaer and

Skrydstrup, 1987) have been identified in the Cruciferae family, only a few of these (ca. 20) appear to be associated with the economically important genus *Brassica* (Fenwick et al., 1983).

Recently, monitoring endogenous levels of these compounds in existing and new plant varieties has become of interest because of the physiological activities of the associated breakdown products (isothiocyanate, nitrile thiocyanate, and oxazolidine-2-thiones) following endogenous, enzyme-mediated hydrolysis in disrupted, un-

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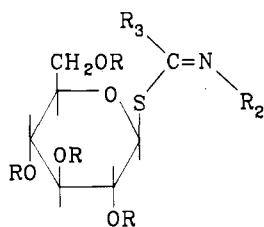
STRUCTURE	R	R_2	R_3
1	H	OSO_3^-	side chain
2	H	OH	side chain
3	TMS	OTMS	side chain

heated plant tissue and the high human and domestic animal consumption of *Brassica* crops. Numerous studies have collectively demonstrated that the breakdown products not only are responsible for the characteristic pungent volatile flavor of these crops but also are associated with food taints, cyto- and phytoplasmic toxicity, anticarcinogenic effects, and various nutritive and antinutritive effects (Fenwick et al., 1983).

Mass spectrometry (MS) has become an increasingly important technique for the qualitative and quantitative determination of glucosinolates (McGregor et al., 1983). Newer developments in sample introduction and ionization techniques have made it possible to analyze intact or desulfoglucosinolates directly by desorption electron impact (Fenwick et al., 1980), desorption chemical ionization (CI) (Fenwick et al., 1980; Eagles et al., 1981a) with methane, isobutane, and ammonia, and positive- and negative-ion fast atom bombardment (Taylor et al., 1981; Fenwick et al., 1982). Although it has been clearly demonstrated that such methods provide valuable structural information, chromatographically based techniques are still the most popular methods of analysis for these compounds.

The most widely used direct method for determining the distribution of glucosinolates from crucifers relies on desulfation of resin-bound glucosinolates using purified aryl sulfatase and then chromatography of the desulfoglucosinolate (2) mixture. Analysis of these compounds by direct high-performance liquid chromatography (HPLC) (Helboe et al., 1980; Truscott et al., 1982a,b, 1983; Goetz and Schraudolf, 1983; Sang and Truscott, 1984) and HPLC-MS (Hogge et al., 1987) has been reported. Extensive use has been made of packed-column gas chromatography (GC) (Underhill and Kirkland, 1971; Heaney and Fenwick, 1980a–c, 1981; Olsen and Sorensen, 1980) and packed-column GC-MS (Olsson et al., 1977; Eagles et al., 1981b; Christensen et al., 1982) in the analysis of the corresponding per(trimethylsilyl)desulfoglucosinolate analogues.

Several reports in particular have highlighted improvements in the capillary GC separation of persilylated derivatives of glucosinolate mixtures on different liquid phases and operating under different temperature programming conditions. Hiltunen et al. (1980) reported a method for separating alkenyl and hydroxyalkenyl desulfoglucosinolates from rapeseed using a 25-m SE30 glass WCOT capillary column temperature-programmed from 210 to 260 °C at 6 °C min⁻¹. More recently, Sosulski and Dabrowski (1984) have demonstrated the utility of using a 25-m OV1 WCOT fused silica column, temperature-programmed from 210 to 285 °C at 10 °C min⁻¹ in separating glucosinolates found in rapeseed (canola) flour. Two further reports by Truscott and co-workers have briefly referred to the separation of (4-hydroxy-3-indolyl)methyl (Truscott et al., 1982a) and (4-methoxy-3-indolyl)methyl

glucosinolate (Truscott et al., 1982b) from 3-indolylmethyl glucosinolate using a 25-m SE52 glass WCOT capillary column programmed from 200 to 280 °C at 4 °C min⁻¹.

Several studies have focused on the packed-column GC–MS analysis of persilylated desulfoglucosinolates by electron impact as well as isobutane and ammonia CI. The purpose of this study was to extend this work by investigating (1) the utility of high-performance bonded-phase fused silica capillary GC for providing more detailed information on the distribution of glucosinolates in *Brassica* vegetables than has been previously available, (2) the advantages of positive-ion chemical ionization (PICI) GC–MS using methane reagent gas to produce mass spectra in which the major ions are highly diagnostic of the glucosinolate structure, and (3) the additional information MS/MS measurements provide to conventional CI studies for both structural elucidation and increased analytical specificity.

EXPERIMENTAL SECTION

Materials. Methanol was Baker Analyzed reagent grade from J. T. Baker Chemical Co. (Phillipsburg, NJ). Single distilled, deionized water was used throughout. DEAE Sephadex A-25 and SP-Sephadex C-25 resins were obtained from Sigma (St. Louis, MO). Aryl sulfatase, Type H-1 from *Helix pomatia*, was obtained from Sigma. Pyridine (silylation grade), trimethylchlorosilane (TMCS), and *N*-methyl-*N*-(trimethylsilyl)heptafluorobutyramide (MSHFBA) were purchased from Alltech (Deerfield, IL). Silylation reagents were stored in a dry environment at 4 °C. Glucosinolate standards either were purchased from Atomergic Chemetals Corp. (Farmingdale, NY) or were gifts.

Test Sample Preparation. Rutabagas (*Brassica napobrassica* L.) and brussels sprouts (*Brassica oleracea* L.) were purchased from a local wholesale outlet. Approximately 500 g of each vegetable was coarsely chopped and rapidly frozen in liquid nitrogen before blending to a powder in a prechilled glass Waring blender. The powder was stored at −34 °C.

Extraction. A 20-g test portion of each powder was added to boiling methanol (200 mL) and extracted for 15 min (Heaney and Fenwick, 1980b). The mixture was cooled and the solvent decanted. The residue was further extracted with boiling 70% methanol (200 mL) and filtered under vacuum. The combined solutions were concentrated to ca. 20 mL on a rotary evaporator and then diluted to 50 mL with water. Extracts were stored at −12 °C.

Isolation of Glucosinolates. A 1-mL aliquot of extract was diluted with 100 µL of a 1:1 (v/v) solution of lead acetate (0.5 M) and barium acetate (0.5 M). After this extract was vortex-mixed and allowed to stand for 15 min, it was centrifuged for 3 min at 1500 rpm. Glucosinolates in the supernatant were concentrated by adsorption onto a prepacked microcolumn of DEAE Sephadex A-25 (30 mg) supported in a Pasteur pipet containing glass wool that had been preequilibrated by washing consecutively with 2 mL of 0.5 M pyridine acetate and 4 mL of water. Unbound compounds were washed from the column with 1 mL of 0.02 M pyridine acetate and discarded. Bound glucosinolates were enzymatically desulfated in situ by incubation overnight (20 °C) with aryl sulfatase (100 µL) that had been purified immediately before use (Heany and Fenwick, 1980c). Desulfoglucosinolates were eluted into a conical glass centrifuge tube with 1.5 mL of water and lyophilized in a vacuum centrifuge (Savant Industries, Hicksville, NY).

Derivatization. Desulfoglucosinolates were derivatized by suspending the residue in silylating reagent prepared

Table I. Partial Collision-Induced Daughter-Ion Mass Spectra of Selected Per(trimethylsilyl)desulfoglucosinolates

glucosinolate	precursor ion	main daughter ions
isopropyl (4D)[a]	70	43 (100), 41 (36), 28 (20), 27 (26)
2-propenyl (5D)	68	
1-methylpropyl (6D)	84	57 (100), 41 (64), 29 (45), 28 (11)
3-butenyl (7D)	82	55 (100), 41 (24), 29 (14)
4-pentenyl (8D)	96	80 (12), 77 (13), 69 (21), 55 (100), 41 (53)
2-hydroxy-3-butenyl (9D)	170	74 (34), 73 (100)
2-hydroxy-4-pentenyl (10D)	184	73 (100)
2-phenylethyl (12D)	132	132 (10), 117 (23), 105 (13), 91 (100)
5-(methylthio)pentyl (13D)	144	96 (33), 81 (22), 79 (23), 69 (100), 68 (17), 55 (81), 42 (15), 41 (20)
3-indolylmethyl (15D)	229	202 (10), 130 (10), 73 (100)
(4-hydroxy-3-indolyl)methyl (16D)	317	290 (14), 277 (28), 218 (69), 217 (13), 147 (44), 74 (16), 73 (100)
(4-methoxy-3-indolyl)methyl (17D)	259	219 (52), 204 (31), 174 (17), 160 (25), 159 (21), 129 (13), 73 (100), 67 (15), 39 (12)

[a] Corresponding compound number in Figures 1 and 2.

just before use from pyridine (100 μL), MSHFBA (100 μL), and TMCS (10 μL). After sonication for 3 min, the mixture was quantitatively transferred to a 1-mL Reacti-Vial (Pierce, Rockport, IL) and heated to 120 ± 2 °C for 20 min.

High-Performance GC–MS. Qualitative GC–MS analyses were performed on either a Finnigan-MAT 4023 GC–MS or a Finnigan-MAT TSQ46 (Palo Alto, CA). The derivatized desulfoglucosinolate mixtures were separated on a fused-silica 25-m WCOT capillary column (0.2-mm i.d.) and wall-coated with a 0.33-μm film of chemically bonded methyl silicone (Ultra 1) or 5% phenyl methyl silicone (Ultra 2) (Hewlett-Packard, Avondale, PA). The injection port was configured for splitless injection at 220 °C. The column outlet was introduced directly into the ion source of the mass spectrometer through a heated transfer line (250 °C). For each analysis the column oven was held at 210 °C for 3 min after injection of the extract and then linearly temperature-programmed to 300 °C at 10 °C min⁻¹. The maximum temperature was held for 20 min. Helium carrier gas linear velocity was 27 cm s⁻¹.

PICI was performed with methane (99.9%; Matheson, Rutherford, NJ). Filament emission was 0.35 mA. Mass spectra were obtained with a nominal reagent source pressure of 0.25 Torr.

MS/MS. Collision-induced daughter-ion mass spectra were acquired by using the first quadrupole region (Q_1) of the TSQ46 to mass select a precursor ion formed under PICI (methane) conditions. The collision cell, Q_2, was configured to pass all ions formed through collisionally induced dissociation (CID). Daughter-ion mass spectra were recorded by scanning Q_3 from 20 to 420 amu in 0.8 s.

Diagnostic precursor ions (Table I) were sequentially selected by Q_1 for daughter-ion analysis in the GC–MS/MS multiple-ion experiment. Their daughter-ion spectra were acquired by repetitively scanning Q_3. The conditions of the multiple-ion experiment were selected by examining the data of prior GC–MS analyses of derivatized desulfoglucosinolates. The GC elution times of the diagnostic precursor ions were noted and used to change conditions
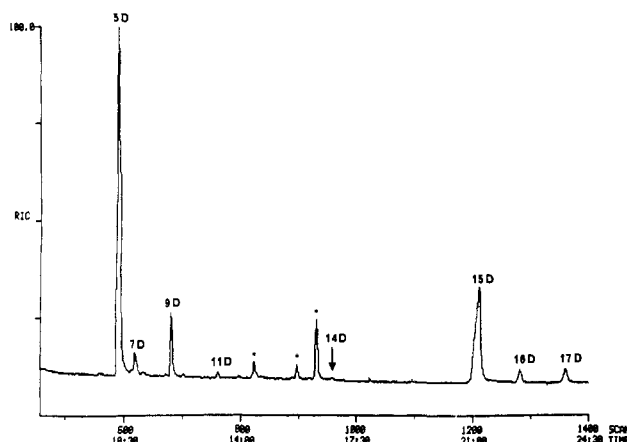

Figure 1. Capillary TIC of per(trimethylsilyl)desulfoglucosinolates from brussels sprouts; * = related to 3-(methylsulfinyl)propyl desulfoglucosinolate.

during GC–MS/MS analyses so that a daughter-ion experiment was performed for each diagnostic ion as its parent compound eluted from the GC column.

The collision cell was operated at an argon target gas pressure of (1.8–2.0) × 10⁻³ Torr, and the collision energy was –15 V. The ion source operating conditions for GC–MS/MS analyses were identical with those used for GC–MS analyses.

RESULTS AND DISCUSSION

Glucosinolate Isolation and Derivatization. The method described for the isolation and derivatization of glucosinolates from brussels sprouts and rutabagas was essentially the same as that reported by Heaney and Fenwick (1980a) but with two modifications: test sample preparation in liquid nitrogen and sonication during derivatization.

Prior to the methanol extraction step, plant tissues were frozen in liquid nitrogen and then blended to a powder. Brussels sprouts could be blended whole, but it was necessary to quickly cut rutabagas into manageable-size portions before freezing. The vegetable powders were stable during long-term, low-temperature storage and permitted efficient methanol extraction of glucosinolates. The second modification arose from the observation that lyophilized desulfoglucosinolate extracts appeared to be insoluble in the silylating reagent at room temperature and that a small amount of precipitate often developed following derivatization. Sonication of this mixture for 1–2 min before derivatization at 120 ± 1 °C for 20 min either decreased or eliminated precipitate formation.

High-Performance Capillary GC (HPCGC). In this study, two 25-m apolar bonded-phase columns (Ultra 1, methyl silicone; Ultra 2, 5% phenyl methyl silicone) with 0.33-μm liquid phase film thickness were tested for their ability to separate a representative mixture of silylated desulfoglucosinolates. Columns were evaluated under different temperature-programming conditions and different carrier gas linear velocities. The best overall results were achieved with the less polar column (HP-1) and a temperature program from 210 to 300 °C at 10 °C min⁻¹. Good resolution of each component was achieved within a reasonable analysis time of 25 min with a helium carrier gas linear velocity of 27 cm s⁻¹. All subsequent separations were performed with these parameters.

Figures 1 and 2 show reconstructed total ion current (TIC) profiles for the high-performance capillary separation of glucosinolates isolated from brussels sprouts and rutabagas and analyzed as the per(trimethylsilyl)desulfo-
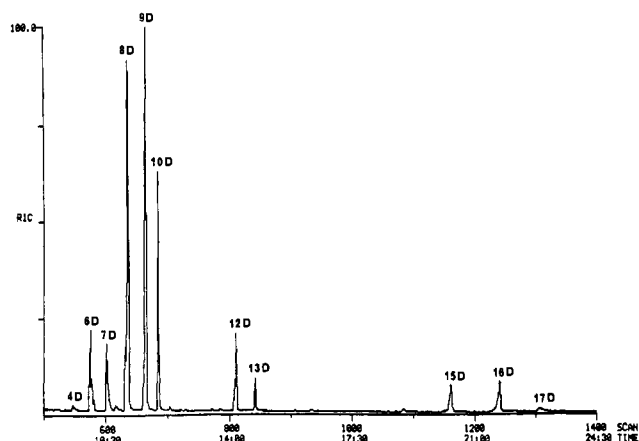
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Figure 2. Capillary TIC of per(trimethylsilyl)desulfoglucosinolates from rutabagas.

Table II. Glucosinolates Identified in Brussels Sprouts and Rutabagas

glucosinolate	trivial name	RT,[a] min:s	occurrence BS[b]	R[c]
isopropyl (4)[d]	glucoputranjivin	9:39 ± 0.02	–	+
2-propenyl (5)	sinigrin	10:05 ± 0.01	+	–
1-methylpropyl (6)	glucocochlearin	10:05 ± 0.01	–	+
3-butenyl (7)	gluconapin	10:36 ± 0.01	+	+
4-pentenyl (8)	gluco-brassicanapin	11:08 ± 0.01	–	+
2-hydroxy-3-butenyl (9)	progoitrin	11:42 ± 0.01	+	+
2-hydroxy-4-pentenyl (10)	gluconapoleiferin	12:04 ± 0.01	–	+
3-(methylthio)propyl (11)	glucoibervirin	13:05 ± 0.01	+	–
unknown (peak 1)[e]		14:02 ± 0.02	+	–
2-phenylethyl (12)	gluconasturtiin	14:16 ± 0.01	–	+
5-(methylthio)pentyl (13)	glucoberteroin	14:50 ± 0.02	–	+
unknown (peak 2)[e]		15:21 ± 0.01	+	–
unknown (peak 3)[e]		15:53 ± 0.01	+	–
3-(methylsulfonyl)-propyl (14)	glucocheirolin	16:25 ± 0.02	+	–
3-indolylmethyl (15)	glucobrassicin	20:50 ± 0.03	+	+
(4-hydroxy-3-indolyl)-methyl (16)	4-hydroxygluco-brassicin	22:12 ± 0.02	+	+
(4-methoxy-3-indolyl)-methyl (17)	4-methoxygluco-brassicin	23:45 ± 0.02	+	+

[a] RT = HPCGC retention time ±1 standard deviation; N = 5. [b] BS = brussels sprouts. [c] R = rutabagas. [d] Corresponding compound number in Figures 1 and 2. [e] 3-(Methylsulfinyl)propyl desulfoglucosinolate gave multiple peaks by GC.

glucosinolate analogues. With the exception of 3-(methylsulfinyl)propyl glucosinolate, individual glucosinolates identified are listed in Table II together with their trivial names, GC retention times, and occurrence in each vegetable extract. In the case of rutabagas, the glucosinolates identified confirm results in other reports (Gmelin and Virtanen, 1962; Mullin and Sahasrabudhe, 1977; Cole and Phelps, 1979; Cole, 1980; Mullin, 1980; Mullin et al., 1980; Carlson et al., 1981). Of the glucosinolates found in brussels sprouts, 3-(methylthio)propyl (11), 3-(methylsulfonyl)propyl (14), 4-hydroxy-3-indolylmethyl (16), and 4-methoxy-3-indolylmethyl glucosinolate (17) have not been reported previously (Gmelin and Virtanen, 1960; Lichtenstein et al., 1964; Daxenbichler et al., 1977; MacLeod and Pikk, 1978; Heaney and Fenwick, 1980b).

The chromatographic data (Table II) showed that, despite the structural similarity of some side-chain moieties and the general wide range of polarities, the major glucosinolates in each extract were completely separated and GC peak broadening of the indole glucosinolates was minimized. The bonded-phase HP-1 column showed excellent thermostability, gave a consistently stable base line, and maintained excellent chromatographic resolution.

These factors resulted in highly reproducible retention time data for each glucosinolate.

Five replicate injections were made with each extract. Retention times were measured for each glucosinolate and the mean retention time and standard deviations calculated (Table II). The average of the standard deviations for retention time and area was ±0.02. For routine chromatographic profiling of glucosinolates from *Brassica* extracts, good chromatographic reproducibility is an important prerequisite. These data suggest that, in the absence of on-line MS capability, individual glucosinolates could be identified with a high degree of confidence by GC, particularly when extracts are analyzed in conjunction with authentic standards.

While MSHFBA has proved to be a suitable derivatizing reagent for most glucosinolates reported in *Brassica* vegetables, determination of 3-(methylsulfinyl)propyl and 1-methoxy-3-indolylmethyl glucosinolate continues to be troublesome. Both compounds are unstable under the analytical operating conditions that have been used (Heaney and Fenwick, 1982). In our laboratory, the overall process, including desulfation and silylation of 3-(methylsulfinyl)propyl glucosinolate, gave three well-resolved capillary GC peaks (Figure 1). MS analysis of these derivatization products revealed none of the anticipated diagnostic ions for the persilylated desulfonated analogue. The highly reproducible GC pattern of the authentic standard suggests that, at least for qualitative analysis, demonstrating its presence or absence in plant extracts presents no difficulty.

A GC and MS comparative study using authentic standards of 3-indolylmethyl glucosinolate (15) and 1-methoxy-3-indolylmethyl glucosinolate (neoglucobrassicin) showed that these two compounds are indistinguishable under the conditions described. During derivatization, 1-methoxy-3-indolylmethyl glucosinolate gave rise to hexasilyl-3-indolylmethyl desulfoglucosinolate (Heaney and Fenwick, 1982). A recent study describing the capillary GC separation and identification of 1-methoxy-3-indolyl glucosinolate in canola extracts (Sosulski and Dabrowski, 1984) was in error on this point (Sosulski, 1987). Analysis of the same canola extracts (gift from Dr. Sosulski) in this laboratory confirms the original report of Heaney and Fenwick (1982).

**Methane PICI HPCGC–MS and HPCGC–MS/MS.** The main diagnostic ions in the methane PICI mass spectra for the acyclic, indole, and substituted indole, pertrimethylsilylated desulfated analogues (**4D–16D**) of compounds (**4–16**) are summarized in Table III. Data for alkyl (**4D, 6D**), alkenyl (**5D, 7D, 8D**), (methylthio)alkyl (**11D, 13D**), (methylsulfonyl)propyl (**14D**), and aryl (**12D**) compounds showed sufficiently dissimilar mass spectral behavior to permit the unequivocal identification of each glucosinolate. The mass spectrum of **8D** in Figure 3 is typical of this group of compounds. With the exception of **13D**, the major fragmentation pathway is attributed to a thioglycosidic cleavage at the S–C (oxime) bond and loss of the oxime trimethylsilyl (TMS) group accompanied by hydrogen migration to give the side-chain ion, $[R_3C≡NH]^+$. The corresponding 144^+ ion for **13D** is the third strongest in the mass spectrum after m/z 73 ($[(CH_3)_3Si]^+$) and m/z 75 ($[(CH_3)_2Si=OH]^+$).

An appreciable amount of additional fragmentation (Figures 3 and 4) was also observed and appeared to be generated by fragmentation of the pertrimethylsilyl β-D-glucose moiety. Other minor but nevertheless structurally relevant ions such as $[R_3C=NOTMS]^+$ and $[R_3C(S)=NOTMS]^+$ were also evident.
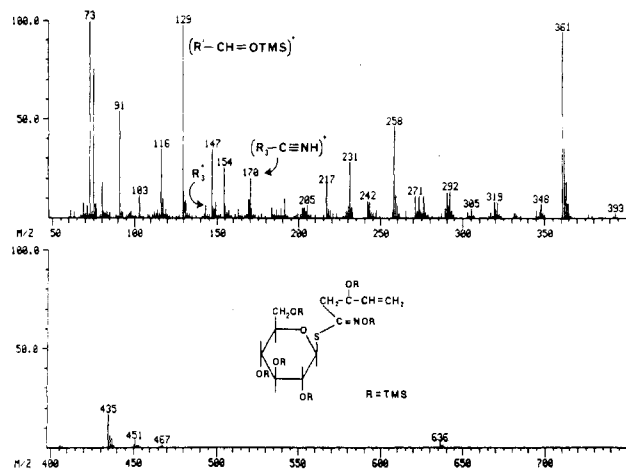
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Table III. Major Diagnostic Ions in the Methane PICI Mass Spectra of Per(trimethylsilyl)desulfoglucosinolates

glucosinolate	mol wt	$[R_3C{=}NH]^+$	$[R_3]^+$	$[R'CH{=}OTMS]^+$
isopropyl (4D)[a]	641	70 (100)	nd[b]	
2-propenyl (5D)	639	68 (100)	nd	
1-methylpropyl (6D)	655	84 (100)	nd	
3-butenyl (7D)	653	82 (100)	nd	
4-pentenyl (8D)	667	96 (100)	69 (31)	
2-hydroxy-3-butenyl (9D)	741	170 (20)	143 (8)	129 (100)
2-hydroxy-4-pentenyl (10D)	755	184 (60)	157 (7)	143 (100)
3-(methylthio)propyl (11D)	687	116 (100)	89 (12)	
2-phenylethyl (12D)	703	132 (100)	105 (9)	
5-(methylthio)pentyl (13D)	715	144 (85)	117 (10)	
3-(methylsulfonyl)-propyl (14D)	719	148 (100)	121 (10)	
3-indolylmethyl (15D)	800	229 (10)	(10)	
(4-hydroxy-3-indolyl)-methyl (16D)	880	317 (17)	290 (98)	
(4-methoxy-3-indolyl)-methyl (17D)	830	259 (10)	232 (100)	

[a] Corresponding compound number in Figures 1 and 2. [b] nd = not determined because of interference from methane reagent ion signals.
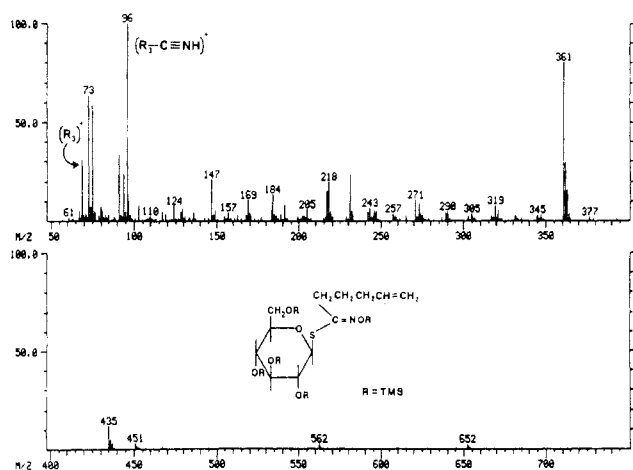
Figure 3. PICI mass spectrum of per(trimethylsilyl)-4-pentenyldesulfoglucosinolate (8D); m/z 50–700 range. Data background subtracted.

Figure 4. PICI mass spectrum of 2-hydroxy-4-pentenyl per(trimethylsilyl)desulfoglucosinolate (9D); m/z 50–700 range. Data background subtracted.

CID measurements of the $[R_3C{=}NH]^+$ ion at m/z 70 (4D), m/z 82 (7D), m/z 84 (6D), and m/z 132 (12D) each gave an array of relatively intense and structurally informative daughter ions (Table I), including the alkyl cation $[R_3]^+$ resulting from the loss of HCN. For 5D, no daughter mass spectrum was obtained for the m/z 68 probably because of the relatively high stability of this ion under the conditions described. Although the CI spectrum for 13D revealed an ion at m/z 117 due to $[R_3]^+$ (suggesting loss of HCN from m/z 144), the CID spectrum showed no evidence of this loss. However, data were consistent with the formation of an ion at m/z 96 via loss of CH_3SH from m/z 144. For 11D, the ion at m/z 68 arises from m/z 116 in the same manner. For 8D and 12D, this α-cleavage of $[R_3C{=}NH]^+$, leading to ions at m/z 69 and 105, is less favored. Instead, the most abundant fragment ions at m/z 55 and 91, respectively, are probably due to a cleavage β to the proposed charge site on the quaternary nitrogen. For 8D, facile loss of 41 u (C_2H_3N) gave the butenyl cation, and for 12D, the resonance-stabilized tropylium ion at m/z 91 in the appropriate CID spectra. This proposed fragmentation pathway also parallels that found in the CID

spectrum for 7D. In contrast, 6D gave an intense ion at m/z 29 due to loss of 55 u from $[R_3C{=}NH]^+$. This pattern highlights the methyl branch at C-1 of the propyl side chain. There was no evidence for loss of 41 u from $[R_3C{=}NH]^+$ in the mass spectrum of 4D, 5D, or 13D. For 4D, this indicates that the side chain was an isopropyl rather than an n-propyl moiety. No CID data were collected for 14D due to lack of material.

The hydroxyalkenyl glucosinolates, represented in this study by 2-hydroxy-3-butenyl glucosinolate (9D, see Figure 4) and 2-hydroxy-4-pentenyl glucosinolate (10D), give diagnostic ions corresponding to $[R_3C{=}NH]^+$ at m/z 170 and 184, respectively. The main fragment ions occur at m/z 129 and 143 and may arise via α-cleavage of the side chain to give $[CH_2CHCH{=}OTMS]^+$ and $[CH_2CHCH_2CH{=}OTMS]^+$. The data in Table I show that the $[R_3C{=}NH]^+$ ion for compounds 9D and 10D fragments in an identical way under CID conditions to give predominantly m/z 73 ($[(CH_3)_3Si]^+$).

The CI mass spectra for the indole glucosinolates, 15D, 16D, and 17D, were again dominated by the diagnostic ions for $[RC{=}NH]^+$ and $[R_3]^+$. In contrast to the relative stabilities of these ions found in the acyclic glucosinolates, in this group of glucosinolates the $[R_3]^+$ ions at m/z 202 (15D), m/z 290 (16D), and m/z 232 (17D) dominated each spectrum. The most striking feature in the CID spectrum of the corresponding $[RC{=}NH]^+$ ions was the daughter ion found at m/z 73. Nevertheless, each spectrum contained diagnostic information that could be correlated with the structure of the indole or substituted indole side chain. For 15D, three transitions were dominant, namely, $229^+ \rightarrow 202^+$ (loss of HCN), $229^+ \rightarrow 130^+$ (loss of HCN and the N1-TMS with H migration), and $229^+ \rightarrow 189^+$ (loss of C-3 substituent of indole with concomitant H migration to give the N-silylindole cation). The same transitions were also observed for 16D and 17D with mass shifts for each daughter commensurate with the different aromatic substitution patterns.

In summary, this study demonstrated that GC–PICIMS and GC–PICIMS/MS are valuable tools in the characterization of silylated desulfoglucosinolate mixtures from Brassica vegetables. Capillary GC on high-performance fused silica bonded-phase provided retention time data for individual glucosinolates that are reliable and reproducible. Methane PICIMS provided mass spectra in which the major fragment ions were diagnostic of the glucosinolate structure. The complimentary nature of HPCGC retention time, MS, and MS/MS data leads to unequivocal identi-

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fication of these compounds.

The value of HPCGC-PICIMS using methane was further extended in establishing the presence of coeluting compounds. We have shown, for example, that, under the GC conditions described, **5D** and **6D** were not resolved but instead emerged from the capillary column as a single symmetrical GC peak. Both compounds were readily differentiated by visual inspection of the resulting mixed mass spectra or from the reconstructed chromatograms of ions m/z 68 and 84 because these diagnostic ions accounted for a significant portion of the TIC.

Analysis of 3-(methylsulfinyl)propyl glucosinolate was complicated by multiple GC peaks resulting from the apparent instability of this compound under the assay conditions described. Nevertheless, results showed that both the GC profile and mass spectra of these peaks were reproducible. To our knowledge, no GC or GC-MS methods are currently available to distinguish neoglucobrassicin from glucobrassicin. The presence of neoglucobrassicin requires identification and quantitation by HPLC. Work is now proceeding on this problem.

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High-Resolution Two-Dimensional Electrophoresis of Bovine Caseins[1]

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This work describes a high-resolution two-dimensional electrophoretic method for identification of bovine milk caseins. The isoelectric focusing separation was carried out in 100-µL capillary tubes using high field strengths to achieve enhanced resolution. Subsequent sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a minigel format, and the result of the combined techniques gave enhanced protein resolution while requiring substantially less time than conventional two-dimensional methods. The procedure readily distinguished various isoforms of caseins, as well as phosphorylated forms of the proteins. The method was also used to demonstrate the effect of a proteinase derived from a *Pseudomonas* species on milk proteins.

High-resolution two-dimensional electrophoresis is a phrase that has been rather loosely applied to separations involving isoelectric focusing followed by SDS-PAGE and was originally coined by O'Farrell (1975). More recently it has been shown that a significant increase in resolution of isoelectric focusing can be achieved through the use of very thin gels in combination with high field strengths (e.g., 500 V/cm) (Radola, 1980, 1984). The application of these technical improvements to two-dimensional electrophoresis holds promise for increased resolution with the bonus of greatly reduced time. For example, a typical 10 000 V-h separation at 500 V would require 20 h. In contrast, at 4000 V, that same separation could be accomplished in 2.5 h and would theoretically result in greater resolution. It is therefore desirable for purposes of clarity that the phrase "high resolution" be further defined or other terminology such as "high performance" be adopted. In this work isoelectric focusing separations were carried out with 300–400 V/cm field strengths in small-diameter tubes (approximately 1.0 mm). In addition, SDS-PAGE separations were performed in appropriately reduced size gels. The results presented here are in contrast to previous work with milk caseins employing much lower field strengths (e.g., 50 V/cm) (Trieu-Cuot and Gripon, 1981, 1982; Anderson et al., 1982; Marshall and Williams, 1988; Holt and Zeece, 1988) and yielding less resolution.

Bovine milk proteins represent a unique biological system with many functional and nutritional qualities. The principal proteins (α-, β- and κ-caseins) of bovine milk are predominantly found in a micelle complex that is responsible for the stability of the suspension and some of milk's unique functional properties (Kinsella 1984). The other major group of proteins (approximately 20% of total bovine milk protein) is the whey proteins. This class of

proteins is composed of β-lactoglobulin and α-lactalbumin (Kinsella, 1984). A significant amount of heterogeneity exists among milk proteins (especially caseins), and the electrophoretic system presented here provides excellent resolution of individual proteins while reducing time required to perform the analyses.

EXPERIMENTAL SECTION

Apparatus, Chemicals, and Reagents. Isoelectric focusing was performed using a modified Buchler disc gel electrophoresis apparatus with jacketed lower tank. The rubber grommets for holding 5-mm disc gel tubes were replaced with clear acrylic sections glued in place. Column tubing nipples (Catalog No. 90 035, Pharmacia Inc., Piscataway, NJ) were then fitted into threaded holes in the acrylic discs. These adapters held the isoelectric focusing gels, which were polymerized in 100-µL capillary tubes (Accufill 90 Micropet, volume accurate to ±0.5%; Clay-Adams Co., Parsippany, NJ) and allowed for easy placement and removal. These capillary tubes resulted in gels of 9.0 cm in length when filled to 100 µL. Isoelectric focusing was carried out on an ISCO (Lincoln, NE) Model 595 power supply capable of delivering 4000 V, 200 mA and limited to a maximum of 200 W total power output. The unit was programmed to change volts, amps, and watts in three steps with automatic crossover points (as well as end point) regulated by volt-hour values. Protection for the operator from the high voltages used was provided by the power supply's ground fault interrupt circuit and use of 5000-V rated cable for electrophoresis leads. The second-dimension separation (SDS-PAGE) was carried out in the minigel format (80 × 70 × 1.0 mm; Biorad Laboratories, Richmond CA) in constant-voltage mode.

Image analysis was performed with use of the Visage 110 (Bio Image, a Kodak Co., Ann Arbor, MI) machine vision image analyzer. The images were acquired by a solid-state CCD array 512 × 512, 8-bit camera. This camera provided 254 shades of gray and was equalized against a step wedge with 21 increments from 0 to 3.5 OD. The step wedge also allowed for pixel size determination and ranged from 0.177 mm/pixel for a 100-mm square image to 0.554 mm/pixel for a 254-mm square image. Analysis was performed with