High-Performance Liquid Phase Separation of Glycosides. 5. Determination of Individual Glucosinolates in Cabbage and Rapeseed by Laser-Induced Fluorescene Capillary Electrophoresis via the Enzymatically Released Isothiocyanate Aglycon

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A capillary electrophoresis (CE) method was developed for the profiling and determination of individual glucosinolates (GSs) via their isothiocyanate degradation products upon myrosinase digestion. The resulting isothiocyanates, the structures of which are reflective of the parent GS's, were then converted to their corresponding amines via base hydrolysis or reaction with 1,2-benzenedithiol. Subsequently, the amines were fluorescently labeled to allow their sensitive detection by laser-induced fluorescence (LIF). The CE method involved the use of in situ charged micelles for the separation of isothiocyanates and their corresponding fluorescently labeled amines by micellar electrokinetic capillary chromatography (MECC). The term "in situ charged micelles" refers to micelles formed by complexing the polar hydroxyl groups of glycosidic surfactants with borate. The MECC method with on-column LIF detection was applied to the determination of GSs in white cabbage, rapeseed leaves, and rapeseed roots.

Keywords: *Glucosinolates; isothiocyanates; capillary electrophoresis; laser-induced fluorescence*

INTRODUCTION

Glucosinolates (GSs) are important natural products (Figure 1) occurring mainly in plants of the Cruciferae family, for example, broccoli, cabbage, radish, and Brussels sprouts. GSs are found in all parts of the plant, that is, in the roots, stems, leaves, and seeds (Heaney and Fenwick, 1984, 1987; Shibamoto and Bjeldanes, 1993; Tookey et al., 1980; Van Etten and Tookey, 1979). GSs differ among themselves by the nature of the R group (Figure 1). This leads to a wide range of flavors and off-flavors and nutritive as well as toxic effects upon consumption of the crucifers (Van Etten and Tookey, 1983).

As a continuation to our recent contribution to the determination of total GSs in a given plant tissue by capillary electrophoresis (CE) via the glucose released upon myrosinase digestion (Figure 1) (Karcher and El Rassi, 1999), we are reporting here a CE method for the determination of individual GSs via the isothiocyanate degradation products that are obtained by the myrosinase digestion of GSs. The structures of the isothiocyanate degradation products are reflective of their parent GSs. Myrosinase is an enzyme that accompanies the GSs in the same plant tissue. When the plant tissue is crushed or masticated, the enzyme myrosinase enters into an intimate contact with the GSs and consequently hydrolyzes the GSs into glucose and their corresponding isothiocyanates (Figure 1). This natural enzymatic process was exploited in this study for the determination of the individual GSs. This was accomplished by first denaturing the endogenous myrosinase, followed by extracting the GSs from the plant of interest, and finally adding to the GS extract the exogenous myrosinase to produce the corresponding isothiocyanates. To enhance the detectability of the individual GSs via their corresponding isothiocyanates, the isothiocyanates were first converted to amine compounds and then the resulting amines were labeled with a fluorescent tag (Figure 2) for sensitive detection by laser-induced fluorescence (LIF).

The CE method described in this paper for the determination of individual GSs via their isothiocyanate degradation products was based on micellar electrokinetic capillary chromatography (MECC) with in situ charged micelles. In situ charged micelles, which were introduced from our laboratory (Smith et al., 1994; Mechref et al., 1995; Cai and El Rassi, 1992), consisted of glycosidic surfactants complexed with borate. The surface charge density of the resulting in situ charged glycosidic micelle can be conveniently varied by changing the pH and the borate concentration, thus allowing the adjustment of the migration time window to best suit a given separation problem (Cai and El Rassi, 1992; Mechref et al., 1995; Smith et al., 1994).

The rationale behind the MECC−LIF method described here for the determination of GSs via their isothiocyanate degradation products is the lack of authentic GS standards. In fact, only two GS standards are currently available from commercial sources. These are sinigrin (i.e., allyl glucosinolate) and gluconasturtiin. On the other hand, some of the isothiocyanate degradation products, the structures of which are reflective of

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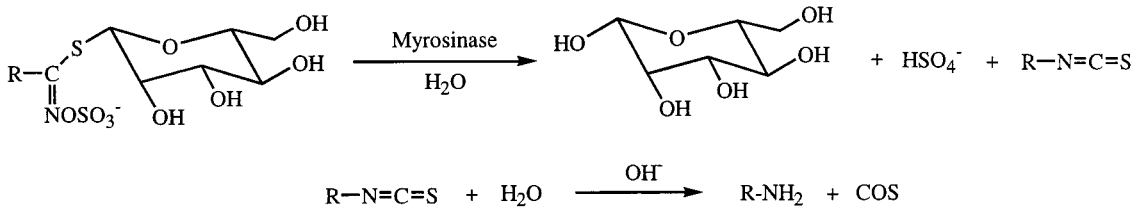


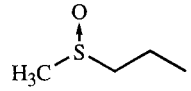
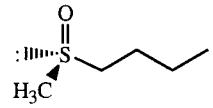
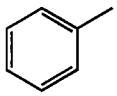
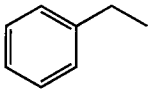
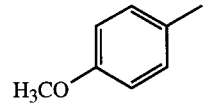
Where R =	Parent glucosinolate	Corresponding isothiocyanate	Corresponding FITC or Dns-Cl labeled amine
	Glucoiberin	3-(methylsulfinyl)-propylisothiocyanate (Iberin) (1)	3-(methylsulfinyl)-propylamine (1)
	Glucoraphanin	4-(methylsulfinyl)-butylisothiocyanate (Sulphoraphane) (2)	4-(methylsulfinyl)-butylamine (2)
	Sinigrin	Allyl-NCS (3)	Allylamine (3)
	Glucotropaeolin	Benzyl-NCS (4)	Benzylamine (4)
	Gluconasturtiin	Phenethyl-NCS (5)	Phenethylamine (5)
	Glucoaubrietin	4-Methoxyphenyl-NCS (6)	4-Methoxyphenylamine (6)

Figure 1. Degradation of GSs to isothiocyanates by the enzyme myrosinase and the subsequent transformation of the isothiocyanates to amines upon base hydrolysis. Also shown in this figure are the structures of the GSs for which standard isothiocyanates and amines were commercially available for our study.

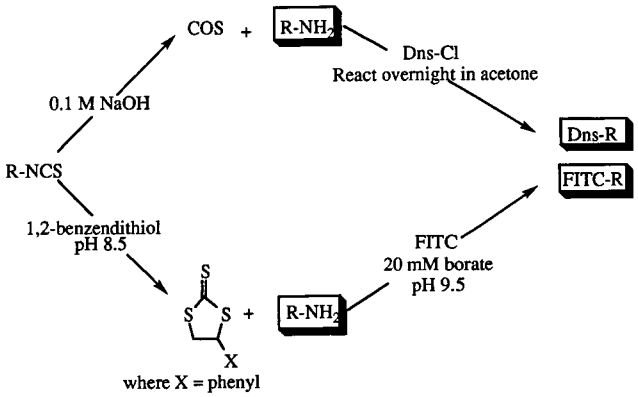


Figure 2. Reaction schemes for the conversion of isothiocyanates to amines via base hydrolysis or 1,2-benzenedithiol reaction and the fluorescent labeling of the resulting amines with either Dns-Cl or FITC.

the parent GS's, are available as pure standards or can be readily synthesized. The applicability of the MECC–LIF method to the determination of individual GSs in real samples was demonstrated with extracts from rapeseed leaves, rapeseed roots, and white cabbage.

MATERIALS AND METHODS

Reagents and Materials. The isothiocyanates (R—NCS) including benzyl-NCS, allyl-NCS, phenethyl-NCS, and 4-methoxyphenyl-NCS were purchased from Aldrich (Milwaukee, WI). 3-(Methylsulfinyl)propyl-NCS (iberin) and 4-(methylsulfinyl)-butyl-NCS (sulforaphane) were purchased from LKT Laboratories (St. Paul, MN). Structures for these aglycons produced from myrosinase hydrolysis of glucosinolates are shown in Figure 1. The myrosinase (thioglucosidase, EC 3.2.3.1) was purchased from Sigma (St. Louis, MO). 1,2-Benzenedithiol, which was used to convert the isothiocyanates to amines, was also obtained from Aldrich. All of the amines (R—NH_2) including benzylamine, allylamine, phenethylamine, and 4-methoxyphenylamine and the internal standard *N*,*N*-dimethylaniline as well as the derivatizing agent, 5-(dimethylamino)-1-naphthalenesulfonyl chloride (Dns-Cl), and fluorescein isothiocyanate (FITC) were supplied by Aldrich. The two surfactants, *n*-octyl-*β*-D-glucoside (OG) and *n*-nonyl-*β*-D-glucoside (NG) were obtained from Anatrace (Maumee, OH).

CE Instrument and Capillary Column. The instrument for CE was a Beckman P/ACE instrument (Fullerton, CA). It consisted of a Model 5510 equipped with a diode array detector and an Omnichrome (Chino, CA) Model 3056-8M He—Cd laser multimode, 8 mW at 325 nm. For the detection of the FITC derivatives, a Beckman laser pace system Model 488 was used as the excitation source. It consisted of a 3 mW, 488 nm (air-

cooled) argon ion laser. Unless otherwise stated, in all experiments, the temperature of the capillary was held constant at 25 °C. A personal computer and P/ACE station software were utilized for data handling purposes. The UV detection of the isothiocyanates and their corresponding amines was performed at 200 nm. For the LIF detection of the fluorescent derivatives, a fluorescence emission band-pass filter of 520 ± 20 nm was purchased from Corion (Holliston, MA). A 360 nm cut-on filter obtained from Corion was used to reject the laser beam of the He−Cd and a 488 nm notch filter for rejection of the laser beam of the Ar ion laser. All experiments were performed in fused-silica capillaries obtained from Polymicro Technology (Phoenix, AZ). The dimensions of the capillaries were 50 cm to the detection window and 57 cm total length with 50 μm internal diameter and 365 μm outer diameter. Samples were pressure injected at 0.034 bar (i.e., 3.5 kPa) for various lengths of time. Between runs, the capillary was rinsed with 0.1 M NaOH, distilled water, and running electrolyte for 2, 3, and 1 min, respectively.

Sources of GSs. The GS standard was sinigrin, which was purchased from Sigma. The white cabbage was bought at a local grocery outlet. Dr. H. A. Melouk, USDA-ARS, Department of Entomology and Plant Pathology, Oklahoma State University, grew the rapeseed plants in the USDA-ARS greenhouse in Stillwater, OK. At the time of the harvesting, the rapeseed plants (Dwarf Essex) were approximately 10 in. tall and had been growing for 102 days.

Extraction of the Intact GSs and Their Isothiocyanate Degradation Products after Treatment with Myrosinase. The GSs from the cabbage and the rapeseed leaves and roots were extracted following the previously developed procedures (Karcher and El Rassi, 1998, 1999), but with the following modifications. After isolation of the intact GSs in methanol, four vials were first dried via Speed Vac and then reconstituted with 250 μL of a 50 mM sodium phosphate buffer (pH 6.2). These solutions were combined and filtered through a 0.2 μm Titan syringe filter from Scientific Resources Inc. (Eatontown, NJ). To this solution was added ~1.0 mg of solid myrosinase, and the mixture was stirred for 1 h; 500 μL of methylene chloride was then pipetted into the solution and stirred. The organic layer, which contained the isothiocyanates, was removed, and the aqueous solution was washed twice more with 500 μL portions of methylene chloride. The combined washings were dried via Speed Vac and reconstituted with acetonitrile and analyzed for isothiocyanate content.

Conversion of the Isothiocyanates to Amines by Reaction with 1,2-Benzenedithiol. The schematic of the reaction of 1,2-benzenedithiol with R−NCS is shown in Figure 2. This well-established conversion has been previously performed by numerous other research groups (Bertelli et al., 1998; Zhang et al., 1992, 1996). Briefly, 25 mM 1,2-benzenedithiol dissolved in methanol was mixed with 5.0 mM standard isothiocyanates in methanol, and finally an equal volume of 100 mM potassium phosphate (pH 8.5) was added. This solution was stirred at room temperature for at least 4 h. Thereafter, the hydro-organic solution (i.e., water−organic mixture) was dried via Speed Vac, reconstituted in 600 μL of methylene chloride (CH_2Cl_2), and stored at 0 °C.

Conversion of the Isothiocyanates to Amines by Base Hydrolysis. For this reaction, 100 μL of the R−NCS was pipetted into 300 μL of 0.1 M NaOH and stirred magnetically. This chemical conversion of the R−NCS to amine is shown in Figure 2. Following this, the solution was dried under reduced pressure, reconstituted with CH_2Cl_2, and stored at 0 °C.

Labeling of Amines with Dansyl Chloride (Dns-Cl). The reaction of Dns-Cl with primary amines (Figure 2) has been studied in detail in numerous research reports [see Geerdink (1988) as a typical reference]. Briefly, 300 μL of the amine dissolved in methylene chloride was pipetted into an amber vial and roughly 30 mg of solid sodium carbonate was added. Following this, 320 μL of an equal molar solution of Dns-Cl in acetone was added and heated at 45 °C for 45 min. Next, the volume of the solution was reduced to ~100 μL via a gentle stream of nitrogen and allowed to react overnight at 45 °C. The solution was then dried via a stream of nitrogen and reconstituted with acetonitrile and filtered through a 0.45 μm syringe filter.

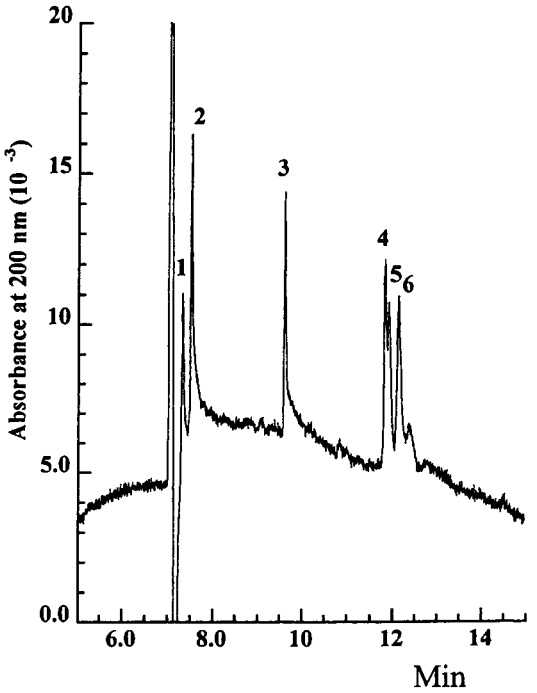


Figure 3. Separation of the standard isothiocyanates. Conditions: electrolyte, 200 mM borate (pH 9.5) containing 100 mM NG; voltage, 18 kV; thermostating the capillary, 25 °C; capillary, fused silica, $l = 50$ cm, $L = 57$ cm, 50 mm i.d.; solute 1, 3-(methylsulfinyl)propyl-NCS; solute 2, 4-(methylsulfinyl)-butyl-NCS; solute 3, allyl-NCS; solute 4, benzyl-NCS; solute 5, phenethyl-NCS; solute 6, 4-methoxyphenyl-NCS.

Labeling of Amines with Fluorescein Isothiocyanate (FITC). The labeling of the amine compounds with FITC was adapted from the procedure described by Li et al. (Rodriguez et al., 1996). Briefly, 1.0 mL of 1.0×10^{-4} M analyte dissolved in 20 mM borate (pH 9.5) was reacted overnight with constant stirring with 40 μL of 2.5×10^{-3} M FITC dissolved in acetone. To minimize degradation of the derivatives and initial reactants, all reactions and solutions were stored in amber vials. The schematic of this reaction is shown in Figure 2.

RESULTS AND DISCUSSION

CE of Isothiocyanates. The CE profiling of GSs in a given plant tissue via their isothiocyanate degradation products (Figure 1) must be preceded by a CE method for the separation of standard isothiocyanates. In this regard, we first performed a systematic study on the optimum conditions for the MECC of some standard isothiocyanates of interest. It is well established that MECC is the most suitable CE approach for the separation of neutral solutes such as isothiocyanates. Figure 3 shows the optimum results, which were determined through a systematic study of pH, NG concentration, and borate concentration. As can be seen in Figure 3, satisfactory separation of all the analytes was achieved for the sole purpose of qualitative analysis involving (i) the profiling of GSs via their isothiocyanate degradation products and (ii) the monitoring of the extent of the degradation of GSs to isothiocyanates by myrosinase (Figure 1). In general, isothiocyanates are reactive and degrade slowly to amines in aqueous media. Thus, the isothiocyanates liberated from the glucosinolates by the myrosinase action can only be qualitatively determined. From Figure 3, the isothiocyanates containing sulfinyl groups, for example, iberin and sulforaphane, yielded

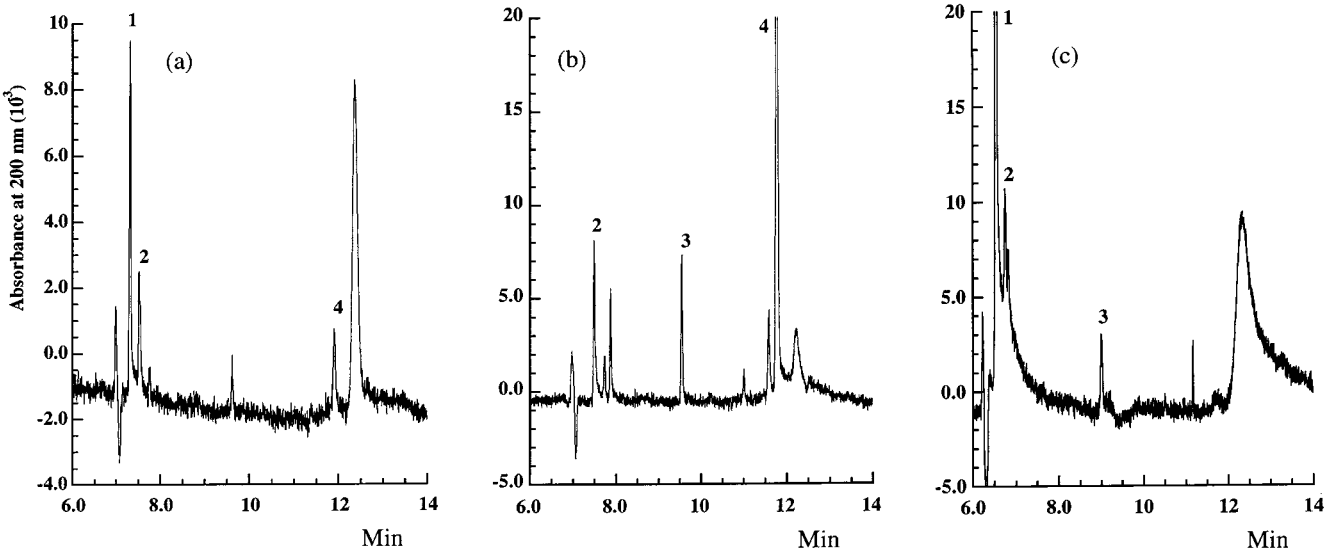


Figure 4. Profiling of the isothiocyanates in rapeseed leaves (a), in rapeseed roots (b), and in white cabbage (c) by HPCE-UV. Other conditions were as described in the legend to Figure 3.

short migration times and eluted right after t_0, which is the time of an unretained species in MECC. This can be explained by the presence of the polar sulfinyl functional groups at the end of the molecules of both isothiocyanates (Figure 1). The other four analytes were much more retarded by the micellar system due to their greater hydrophobicity. Allyl-NCS eluted before the aromatic NCS standards, which eluted in the order benzyl-NCS, phenethyl-NCS, and 4-methoxyphenyl-NCS, respectively.

In summary, with the control over the amount of charge on the NG micelle via borate complexation, the migration time window was successfully adjusted to allow for the separation of both hydrophilic and hydrophobic isothiocyanate analytes. These optimum conditions were used for the analysis and profiling of the isothiocyanates from the rapeseed and cabbage samples (see the following section).

Profiling of Isothiocyanates in Fresh Rapeseed Leaves and Roots and in Fresh White Cabbage. Parts a, b, and c of Figure 4 show the electropherograms of the methanol extracts of the rapeseed leaves and roots and cabbage, respectively. From Figure 4a, it can be seen that there are at least three identifiable isothiocyanates in the rapeseed leaves. Through successive spiking of the rapeseed leaf extract with standard isothiocyanates, the three isothiocyanates were determined to be iberin, sulforaphane, and benzyl-NCS. Likewise, there are at least three identifiable isothiocyanates contained in the extract of the rapeseed roots (Figure 4b). These were determined to be sulforaphane, allyl-NCS, and benzyl-NCS. The three identifiable isothiocyanates present in the cabbage extract were determined to be iberin, sulforaphane, and allyl-NCS (Figure 4c).

In a previous paper from our laboratory (Karcher and El Rassi, 1999), we reported at least 9 minor intact GSs and 2 major GSs in the rapeseed leaves, totaling 11 intact GSs. In that same paper, we also observed that there are 5 minor intact GSs in the rapeseed roots and 1 major and 6 minor intact GSs in the white cabbage. From an inspection of Figure 4a, it can be seen that in the extract of the rapeseed leaves there are two major peaks, with one of them corresponding to iberin and four other possible minor isothiocyanates. Examination of Figure 4b shows that the rapeseed roots contain one major isothiocyanate corresponding to benzyl-NCS and possibly six other minor isothiocyanates. Furthermore, there are one major isothiocyanate corresponding to iberin and three minor possible isothiocyanates in the cabbage (Figure 4c). In all cases (i.e., Figure 4a−c), the few other peaks that may correspond to other isothiocyanates could not be identified due to the limited number of isothiocyanate standards that are commercially available at this point in time.

The observed, reduced number of isothiocyanates with respect to the number of intact GSs previously reported in the extracts from rapeseed roots and leaves and from white cabbage (Karcher and El Rassi, 1999) may be explained as follows. Most of the isothiocyanates lack strong chromophores needed for adequate UV detection, a fact that renders their detection at low levels extremely difficult. In fact, from the UV spectra of sinigrin, allyl-NCS, and allylamine, the molar absorptivities were calculated to be 8244, 3890, and 351 M^{-1} cm^{-1} at 228 nm, respectively, and 4531, 3890, and 1519 M^{-1} cm^{-1} at 204 nm, respectively. Although 228 nm is the best wavelength for maximum absorbance by GSs, 204 nm is the wavelength at which isothiocyanates and amines exhibit the maximum absorbance. These results demonstrate that intact GSs show greater detectability than their corresponding isothiocyanate and amine aglycons. Furthermore, because the molar absorptivities of the isothiocyanates are quite low, an extensive preconcentration step (i.e., solvent evaporation) is required to acquire sufficiently concentrated samples of isothiocyanates. This preconcentration step renders the sample very viscous and difficult to inject from. Therefore, there is a limit on the amount of preconcentration before analysis. On the other hand, many of the isothiocyanates are reactive in an aqueous environment degrading slowly to amines (March, 1985) during the myrosinase hydrolysis of the GS extract. The amine degradation products possess the lowest molar absorptivities with respect to glucosinolates and isothiocyanates. This may explain why no peaks corresponding to amine degradation products were detected.

Comparison of the Two Conversion Schemes of Isothiocyanates to Amines. Because the isothiocyanates degrade slowly to amines in aqueous solutions,

Table 1. Percent Conversion of Isothiocyanates to Amines via Base Hydrolysis and 1,2-Benzenedithiol

analyte	% conversion via base hydrolysis	% conversion via 1,2-benzenedithiol
phenethyl-NCS	80	64
benzyl-NCS	85	58
4-methoxyphenyl-NCS	84	68

Table 2. Percent Derivatization of the Amines with Dns-Cl and FITC

analyte	% labeling with Dns-Cl	% labeling with FITC
benzylamine	71	88
phenethylamine	77	84
4-methoxyphenylamine	78	89
3-(methylsulfinyl)propylamine	75	92
4-(methylsulfinyl)butylamine	80	94

it was imperative to convert the isothiocyanates to their corresponding amines to provide a more accurate and quantitative determination of GSs through their isothiocyanate degradation products. Although they are reactive species, the amine compounds derived from isothiocyanates are quite stable and do not degrade in aqueous solutions. In this regard, two different schemes were examined for the conversion of isothiocyanates to amines followed by the labeling of the resulting amines instead of labeling the isothiocyanates (Figure 2).

Two different procedures based on either a 1,2-benzenedithiol reaction or a base hydrolysis reaction for converting isothiocyanates to amines were studied (Figure 2). From an inspection of the data listed in Table 1, it is easily noticed that the base hydrolysis yields a higher percent conversion than the conversion via 1,2-benzenedithiol. Not only does the base hydrolysis show a higher percent conversion, but it is also much simpler experimentally.

Comparison of Two Labeling Procedures. As stated above, neither the isothiocyanates nor their corresponding amines are good candidates for relatively sensitive UV detection of their parent GSs in plant extracts. Therefore, it was necessary to derivatize the amine compounds derived from the isothiocyanate's degradation products of GSs before analysis by CE. In this regard, two different derivatization procedures based on either Dns-Cl (Lawrence and Frei, 1976) or FITC (Rodriguez et al., 1996) for labeling amines were examined to find the most suitable derivatives for separation and detection by MECC. Both procedures offer simple and easy experimental steps, but the FITC reaction was chosen for the quantitative analysis of the rapeseed and cabbage samples because it offers several merits not obtainable with the Dns-Cl derivatization.

Table 2 shows that the FITC labeling of amines is more quantitative than the Dns-Cl labeling. The percent yield with the FITC derivatization reaction ranged between 84 and 94% as opposed to ranging between 71 and 80% for the Dns-Cl derivatization reaction.

Figure 5a shows the electropherogram of the standard amines labeled with Dns-Cl. From this electropherogram, it can be seen that the migration order of Dns-amines is different from that of their corresponding isothiocyanates. The Dns-amine derivatives are neutral (as the isothiocyanates) at the pH of the experiment (i.e., pH 9.5). This is because the Dns-amine derivatives are charged positively only at very acidic pH (pK_a of the dimethylamino group of dansyl moiety is between 3.0 and 4.0) or negatively only at very alkaline pH (the amino group adjacent to the sulfonyl group of the dansyl moiety has a pK_a of 11.7) (Yu and El Rassi, 1993). The derivatization with Dns-Cl increases the nonpolar character of each solute to the same extent. However, when compared to isothiocyanates, the elution order, as far as peaks 4, 5 and 6 are concerned, was different, indicating that in MECC not only does the overall hydrophobicity of the solutes control retention but also polar interactions and steric effects contribute to retention. Because the Dns-amine derivatives are more hydrophobic than the parent isothiocyanates, their separation required the replacement of the NG micelle by the OG micelle, which has one methylene group fewer in its alkyl tail than in the alkyl tail of NG. As with the isothiocyanates, the separation of the Dns-amine derivatives is still difficult due to the wide differences in polarity. To separate the sulfinyl derivatives, a higher OG micellar concentration is required than what is needed for the optimum separation of the remaining derivatives. Consequently, the separation is difficult to produce.

The standard amines labeled with FITC (FITC-amines) exhibited a change in the migration order when compared to that of the Dns-amines (Figure 5b). The negative charge of the FITC function produced electrostatic repulsion between the FITC-amine derivatives and the OG-borate micelle complexes. The net result is a weak interaction with the OG micelle, and consequently the separation is also based on differences in charge-to-mass ratios among the analytes.

The Dns-Cl reaction scheme requires more experimental time than the FITC reaction (Figure 2). It also produces more side products than what is produced with the FITC labeling. These byproducts are a major problem when a complex mixture is to be analyzed, as encountered with the analysis of plant extracts. The standard amines labeled with FITC show a much cleaner electropherogram with only a few side products.

Finally, the Dns-Cl molecule has a limited solubility in an aqueous buffer. In most of the applications of this derivatization procedure (e.g., labeling of amino acids), the analytes of interest are hydrophilic and exhibit a good solubility in aqueous solutions (Lawrence and Frei, 1976). In our study, the analytes are quite nonpolar, having low solubilities in aqueous solutions, thus making their dansyl derivatives even less soluble. Therefore, low concentrations of these derivatives must be used to adjust for the limited solubility. On the other hand, the FITC derivatives are negatively charged molecules due to the presence of the carboxylic acid group in the FITC moiety. Thus, upon derivatization with FITC, the resulting derivatives show a dramatic increase in solubility in alkaline aqueous solutions (pH >7.5). Also, the negative charge introduces mobility into the analytes, a fact that explains that the separation of the FITC-amines is achieved through differences in mobility of the analytes (charge-to-mass-ratio) as well as via hydrophobic interactions with the micelle. In contrast, and solely on the basis of the hydrophobicity of the derivatives, FITC-benzylamine should have eluted before the FITC-phenethylamine and the FITC-3-(methylsulfinyl)propylamine before FITC-4-(methylsulfinyl)-butylamine.

Quantitative Analysis of the GS Content in the Rapeseed Leaves and Roots and White Cabbage. On the basis of the above results, the FITC-amine derivatives were selected to be used in the indirect

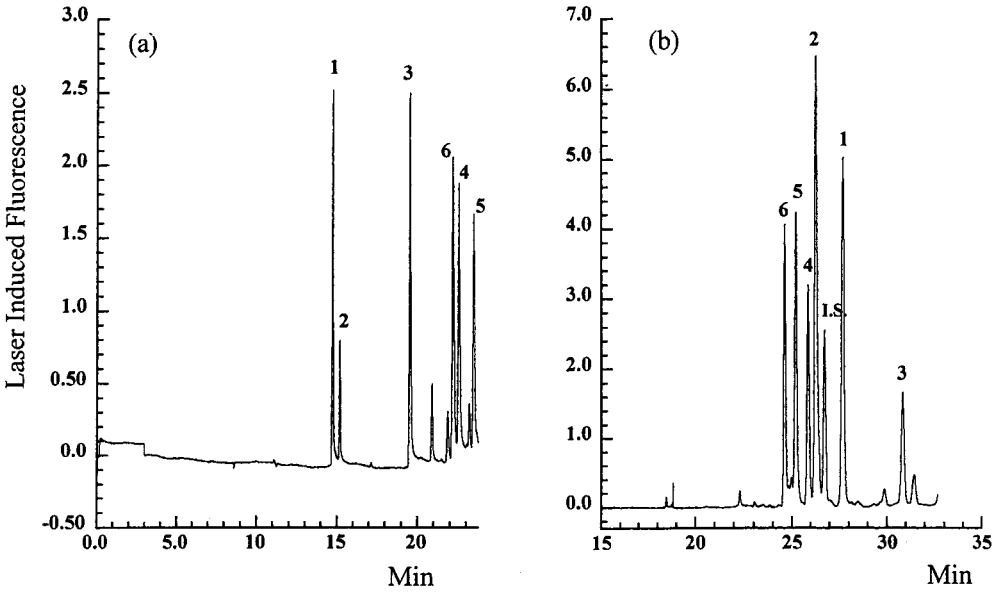


Figure 5. Separation of the standard amines labeled with Dns-Cl (a) or with FITC (b). Conditions: electrolyte, 400 mM borate (pH 9.5) containing 50 mM OG in (a) and 650 mM sodium borate (pH 9.05) containing 30 mM OG in (b); other conditions were as described in the legend to Figure 3. Solutes: I.S., internal standard; Dns-Cl (a) or FITC (b) derivatives of the amines corresponding to the isothiocyanates listed in Figure 1.

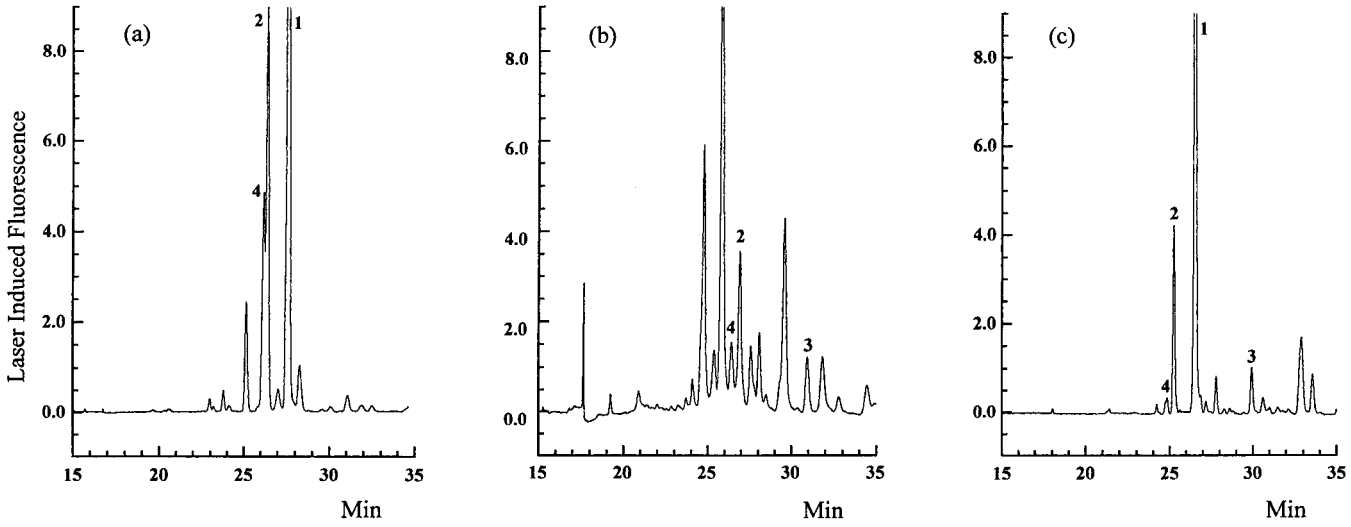


Figure 6. Profiling of the FITC labeled amines from the glucosinolates in rapeseed leaves (a), in rapeseed roots (b), and in white cabbage (c). Other conditions were as described in the legend to Figure 5.

qualitative and quantitative determination of the individual GSs. As shown in Figure 5b, the optimum conditions for the MECC separation of the FITC-amine standards were reached when 30 mM OG and 650 mM borate (pH 9.05) were used. With this high ionic strength, it was necessary to cool the capillary cartridge to 17.5 °C to reduce the amount of current and to dissipate the joule heat. A four-point calibration curve was generated for each of the six FITC-amines. In the generation of the calibration curves, all data points were an average of at least three trials. From the linear fit of these curves, the *R* values were 0.996, 0.996, 0.992, 0.997, 0.996, and 0.999 for FITC-*p*-methoxyphenylamine, FITC-phenethylamine, FITC-benzylamine, FITC-allylamine, FITC-3-(methylsulfinyl)propylamine, and FITC-4-(methylsulfinyl)butylamine, respectively.

The profiling of the FITC-amines derived from rapeseed leaves is shown in Figure 6a. Through spiking, the FITC-amines identified corresponded to the parent isothiocyanate aglycons found in the rapeseed leaves shown in Figure 4a. As can be seen in Table 3, the amounts of GSs as derived from the calibration curves of their FITC-amine derivatives, the structures of which are reflective of the individual GSs, were found to be

Table 3. Amounts of Some Individual GSs in Extracts from White Cabbage, Rapeseed Roots, and Rapeseed Leaves Determined via Their Corresponding Amine Compounds

sample type	structure of R side chain	corresponding GS	amount of GS (μg/g)
white cabbage	$C_6H_5CH_2$	glucotropaeolin	1.39
	$H_3CSO(CH_2)_3$	glucoiberin	189
	$H_3CSO(CH_2)_4$	glucoraphanin	27.8
	$H_2C{=}CHCH_2$	sinigrin	25.9
rapeseed roots	$C_6H_5CH_2$	glucotropaeolin	6226
	$H_3CSO(CH_2)_4$	glucoraphanin	373
	$H_2C{=}CHCH_2$	sinigrin	2726
rapeseed leaves	$C_6H_5CH_2$	glucotropaeolin	464
	$H_3CSO(CH_2)_3$	glucoiberin	19377
	$H_3CSO(CH_2)_4$	glucoraphanin	3221

464, 19377, and 3221 μg/g of dry weight of leaves for glucotropaeolin (from FITC-benzylamine), glucoiberin [from FITC-3-(methylsulfinyl)propylamine], and glucoraphanin [from FITC-4-(methylsulfinyl)butylamine], respectively. In our previous paper dealing with total GS quantitation, we reported almost 39000 μg of total GSs/g of rapeseed leaves (Karcher and El Rassi, 1999). Thus, the three identified glucosinolates contribute over half of the total amount of GSs present in the rapeseed leaves. Also, this quantitative data closely parallels the profiling of the free isothiocyanates (Figure 4a). In Figure 4a, it is noticeable that the major constituent present in the leaves is glucoiberin. Of course, the peak height ratios in Figure 6a should not match exactly the quantitative data obtained from the standard calibration curves. This can be explained by the different fluorescent properties of the individual analytes. Not all of the analytes will exhibit a maximum emission at 520 nm. Blue or red shifts can be observed when the analytes are labeled with a fluorescent tag. Also, from Figure 4a, the peak heights of the benzyl-NCS and sulforaphane are close in height, but the reported concentrations are far apart. Again, this can be explained in terms of molar absorptivities. Benzyl-NCS has a much higher molar absorptivity when compared to that of sulforaphane.

The separation of the FITC-labeled amines in the rapeseed roots is shown in Figure 6b. Again, the identifiable FITC-amines agree with those profiled from the free isothiocyanates. Table 3 lists the individual GS contents as 6226, 373, and 2726 μg/g of dry weight of roots for glucotropaeolin (from FITC-benzylamine), glucoiberin [from FITC-3-(methylsulfinyl)propylamine], and sinigrin (from FITC-allylamine), respectively. From these data, the difference in the GS content of the roots and the leaves is quite noticeable. In the roots, the amount of glucoiberin is drastically lower than in the leaves. Also, sinigrin is found in the roots, but in the case of the leaves, sinigrin might exist but only in extremely minute amounts. The identifiable GSs correspond to over half of the total amount of GSs found in the roots (~10900 mg/g), which we reported previously (Karcher and El Rassi, 1999).

The electropherogram of the FITC-labeled amines in white cabbage is shown in Figure 6c. The amounts found for the individual GSs were 1.39, 189, 27.8, and 25.9 μg/g of dry weight of cabbage for glucotropaeolin, glucoiberin, glucoraphanin, and sinigrin, respectively. The fluorescent detection of the labeled amines allowed for the identification of glucotropaeolin. The aglycon (benzyl-NCS) was not detected by UV in the profiling of the free isothiocyanates (Figure 4c). Thus, the LIF detection allows for the trace detection of glucosinolates not detected in the UV. As with the GSs found in the rapeseed leaves and roots, the ones found in the cabbage account for a majority of the total amount (822 μg/g). In almost all previously reported data for white cabbage, glucoiberin is the most abundant GS, followed by lesser concentrations of sinigrin and glucoraphanin and minute amounts of glucotropaeolin (Van Etten and Tookey, 1979). Any quantitative comparisons with previously reported data are not very relevant because a host of factors have been reported to alter the GS content in cabbage. For instance, the size of the cabbage head, the growing conditions, the amount of pesticide used, and the length of storage are among many other factors that all affect GS content. These factors have a strong influence on precise reporting of quantitative data (Fenwick et al., 1983). Furthermore, in a previous paper by others (VanEtten et al., 1976, 1980), extremely high standard deviations among individual GSs were reported which may be due to additional parameters such as the methods used.

Conclusions. We have shown that CE is a useful tool for the profiling and determination of individual GSs via their isothiocyanate degradation products upon myrosinase digestion. This method, which involved the separation and detection of the isothiocyanates by CE−LIF, was successfully applied to the determination of GSs in white cabbage, rapeseed leaves, and rapeseed roots.

ABBREVIATIONS USED

CE, capillary electrophoresis; Dns-Cl, dansyl chloride; FITC, fluorescein isothiocyanate; GS, glucosinolate; MECC, micellar electrokinetic capillary chromatography; NG, nonyl-β-D-glucopyranoside; OG, octyl-β-D-glucopyranoside.

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