



Glucosinolate hydrolysis products from various plant sources: pH effects, isolation, and purification[☆]

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Abstract

Glucosinolates are a class of organic anions that can be hydrolyzed either enzymatically with myrosinase or non-enzymatically to form primarily isothiocyanates and/or nitriles. The isolation and purification of these hydrolysis products are of particular interest both for their potential use in organic synthesis and for their biological activities. Methods were developed for the isolation and purification of gram-scale quantities of several ω -(methylthio)alkyl-, ω -(methylsulfinyl)alkyl-, ω -(methylsulfonyl)alkyl-, and substituted benzyl glucosinolate hydrolysis products; the isothiocyanates erucin [1-isothiocyanato-4-(methylthio)butane], iberin [1-isothiocyanato-3-(methylsulfinyl)propane], cheirolin [1-isothiocyanato-3-(methylsulfonyl)propane], lesquerellin [1-isothiocyanato-6-(methylthio)hexane], hesperin [1-isothiocyanato-6-(methylsulfinyl)hexane], sulforaphene [1-isothiocyanato-4-(methylsulfinyl)but-3-ene], 3-methoxybenzyl isothiocyanate, and 4-hydroxybenzyl isothiocyanate; and the nitriles iberiverin nitrile [1-cyano-3-(methylthio)propane], erucin nitrile [1-cyano-4-(methylthio)butane], and 3-methoxybenzyl nitrile using defatted seedmeal from several different genera within the crucifer family (Brassicaceae) and meadowfoam family (Limnathaceae) as the source of parent glucosinolates. The procedures use solvent extraction of autolyzed defatted seedmeals from various plant sources together with variable reaction pHs and solvent partitioning to obtain relatively pure (generally >97%) compounds without the need for chromatographic separation.

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1. Introduction

The crucifer family, Brassicaceae, is an economically important family for its many food and oilseed crops as well as containing many important ornamental plants and noxious weeds. Crucifers are characterized by the presence of a group of secondary compounds called glucosinolates. Several other plant families in the same plant order as the Brassicaceae, the Capparales (e.g., the Capparidaceae, Moringaceae, Resedaceae, Stegnospermaceae, and Tovariaceae)

[☆] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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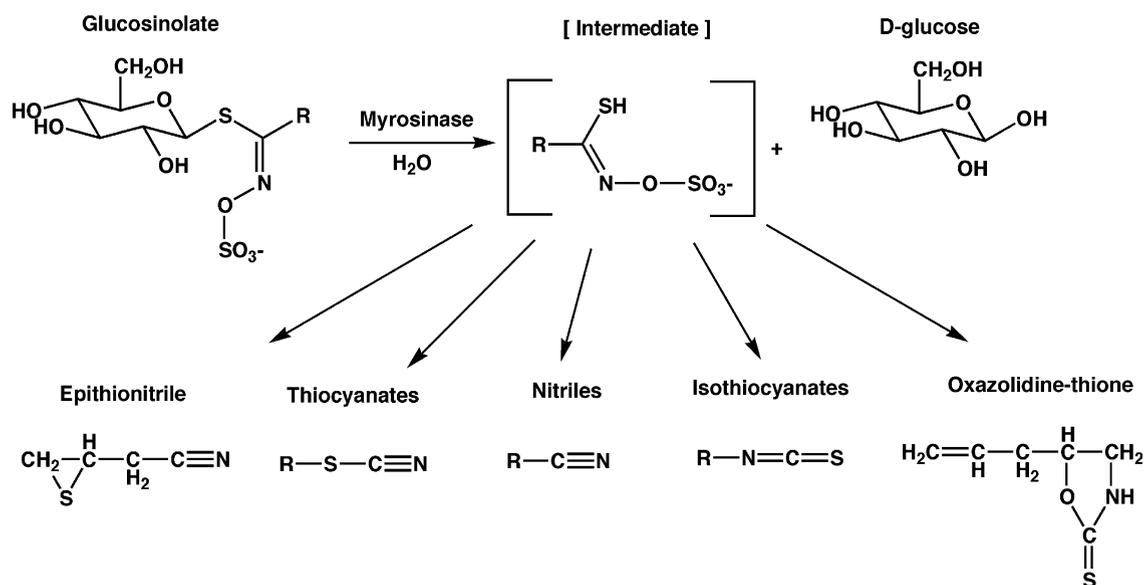


Fig. 1. The general structure of glucosinolates and their enzymatic degradation products. Adapted from Rask et al., 2000.

have been found to possess glucosinolates. Several unrelated plant families, including the Caricaceae, Limnathaceae, and Tropaeolaceae have also been shown to contain glucosinolates. Glucosinolates are glucose and sulfur-containing organic anions (Fig. 1) whose decomposition products are produced when plant cells are ruptured, and the glucosinolates present in vacuoles are hydrolyzed by the enzyme myrosinase (β -thioglucosidase glucohydrolase; EC 3.2.3.1) (VanEtten and Tookey, 1983). These hydrolysis products, many with biological activity, include substituted isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidinethiones, which vary depending on the plant species studied, side-chain substitution, cell pH, and cell iron concentration (Cole, 1976; Daxenbichler and VanEtten, 1977; Daxenbichler et al., 1977, 1979; Gil and MacLeod, 1980a,b; Fenwick et al., 1983; Uda et al., 1986; Chew, 1988; Duncan, 1991).

Many glucosinolate degradation products are of interest because of their biological activities. Several of these hydrolysis products have biocidal activity against a wide variety of organisms, such as insects, plants, fungi, and bacteria (Vaughn, 1999). Others have human health benefits. For example, sulforaphane [1-isothiocyanato-4-(methylsulfinyl)butane], a degradation product of the glucosinolate glucoraphanin [4-(methylsulfinyl)butyl glucosinolate], is a potent inducer of phase II detoxification enzymes,

enzymes that are strongly correlated with the prevention of certain types of cancer (Zhang et al., 1992, 1994; Fahey et al., 1997; Fahey and Talalay, 1999; Brooks et al., 2001; Matusheski et al., 2001). Nutritional/health studies of these compounds require gram quantities, most of which are not commercially available. Certain other glucosinolate degradation products are utilized in organic synthesis, such as 3-methoxybenzyl isothiocyanate (limnanthin) from meadowfoam (*Limnanthes alba*) seed glucosinolates in the synthesis of substituted thioureas (Abbott et al., 2002). However, the utilization of these compounds are often limited by availability and/or their high costs. For example, 100 milligrams of technical grade iberin (97% purity) currently costs US\$ 396.90 (LKT Laboratories Inc., St. Paul, MN). Methods had been developed previously for the purification of certain glucosinolate hydrolysis products including iberin and sulforaphane (Kore et al., 1993; Bertelli et al., 1998; Matusheski et al., 2001), but in all these cases, HPLC separation was necessary for isolation of pure compounds. These compounds could also be produced by direct chemical synthesis as the preparation of synthetic sulforaphane (Schenk and Durr, 1997). Seeds of crucifers and related plants that possess relatively high concentrations of glucosinolates can be hydrolyzed to produce these degradation products (Daxenbichler et al., 1991). In this paper, we report

methods using seeds, most of which are commercially available in bulk quantities, for the gram-scale preparation, isolation, and purification of several substituted isothiocyanates and nitriles without the need for any chromatographic separation.

2. Materials and methods

2.1. Materials

White mustard (*Brassica hirta* cv. 'Martigena') seed was obtained from Dr. Rick Boydston, USDA-ARS, Prosser, WA. Arugula (*Eruca sativa*) seed was obtained from Johnny's Selected Seeds, Albion, ME. Basket of gold (*Aurinaria saxatilis*), Siberian wallflower (*Erysimum allionii*), English wallflower (*Erysimum cheiri*), Dame's rocket (*Hesperis matronalis*), sweet alyssum (*Lobularia maritima*), money plant (*Lunaria biennis*), and night-scented stock (*Matthiola longipetala* subsp. *bicornis*) seeds were obtained from Flower Art & Soul, Junction City, OR. London rocket (*Sisymbrium irio*) and shepherd's purse (*Capsella bursa-pastoris*) seeds were obtained from Valley Seed Service, Fresno, CA. Lesquerella (*Lesquerella fendleri*) seed was obtained from Dr. Terry Isbell, USDA-ARS, NCAUR, Peoria, IL. Meadowfoam (*Limnanthes alba*) seeds were obtained from Dr. Steven Knapp, Oregon State University, Corvallis, OR. Chromatographic standards for the hydrolysis products were obtained from a collection of purified glucosinolate hydrolysis products originally developed by Melvin E. Daxenbichler and Gayland F. Spencer, National Center for Agricultural Utilization Research, Peoria, IL, and maintained and supplemented to the authors. A glucoiberin standard was obtained from Carl Roth, Karlsruhe, Germany, and the sinigrin standard was obtained from Sigma, St. Louis, MO. All chemical reagents (sodium chloride and anhydrous sodium sulfate) were of analytical grade, and all solvents (hexane and dichloromethane) were of HPLC grade.

2.2. Methods

2.2.1. Chromatography

Compounds present in the hexane and dichloromethane fractions were quantitated by gas chro-

matography using a flame ionization detector (GC-FID), and compounds were identified by gas chromatography-mass spectrometry (GC-MS). GC-FID analyses were performed on a Hewlett-Packard 5890 gas chromatograph equipped with a Agilent 6890 Series autosampler running HP Chemstation software. GC-MS was performed on HP 6890 GC system attached to a HP 5972A Mass Selective Detector. Columns used were fused-silica HP-5MS capillaries (0.25- μm film thickness, 30 m \times 0.25 mm i.d.). Both the GC-FID and GC-MS operating parameters were as follows: splitless injection mode, temperature programmed from 50 to 315 °C at 5 °C/min with a 2-min initial and a 10-min final temperature hold; He carrier gas flow rate at 1.1 ml/min, with the injector temperature set at 250 °C. Mass spectra were obtained by electron impact ionization (EI) over the range of 40–550 amu at a rate of 2 scans/s. The ion source temperature was 180 °C, and the electronic impact energy was 106 eV. Spectra were compared with known purified standards and by computer with the Wiley/NBS Mass Spectral Registry (McLafferty and Stauffer, 1989).

2.2.2. Isolation of glucosinolate hydrolysis products

Seedmeals were prepared by grinding aliquots of seed in a coffee grinder for 15 s. The seedmeals were subsequently defatted with hexane in a Soxhlet extractor for 24 h, after which the residual seedmeal was allowed to dry completely in a fume hood. Defatted seedmeals (10 g samples) were mixed with 25 ml (sufficient liquid to form a paste) of either: (1) 0.05 M potassium phosphate buffer, pH 7.0; (2) 0.05 M Tris buffer, pH 10.0; (3) 0.1 M HCl; or (4) 2 M HCl (the rationale behind using these solutions will be discussed in the results section). Fifty milliliters of CH_2Cl_2 was then added to each flask and the flasks were placed in an incubator shaker set at 25 °C and 200 rpm for 8 h. Following hydrolysis, 10 g of sodium chloride and 10 g of anhydrous sodium sulfate were added and mixed thoroughly. The CH_2Cl_2 was decanted and filtered through Whatman No. 1 filter paper and the residual seedmeal was extracted an additional three times with excess CH_2Cl_2 . The combined crude CH_2Cl_2 extracts were analyzed on the GC-MS for compound content. Purity of individual compounds present in the samples was determined by GC-FID. Individual crude extracts were then dried at 30 °C under vacuum in a rotoevapo-

rator. The resulting residues were partitioned between 5 ml hexane and 15 ml water and separated in separatory funnels with filtering. The hexane fraction was examined directly, whereas the water extract was further partitioned against 5 ml dichloromethane, after which the dichloromethane fraction was separated and examined by GC-FID (for discussion purposes, this fraction will be subsequently referred to as the water fraction).

2.2.3. Determination of yield of iberin and glucoiberin from *Lesquerella fendleri* seedmeal

Although, the focus of our research was to qualitatively produce glucosinolate hydrolysis products without using chromatography, it is of interest to quantify the relative conversion from the parent glucosinolates, such as in the hydrolysis of glucoiberin to iberin. For iberin quantitation using the simultaneous incubation method, defatted *L. fendleri* seedmeal (3.0 g each) replicates were incubated in sealed 250-ml Erlenmeyer flasks with 10 ml of 0.1 M HCl and 25 ml CH₂Cl₂. The flasks were placed on a controlled temperature shaker set at 25 °C and 200 rpm for 8 h. The CH₂Cl₂ extract was decanted with filtering and the residual seedmeal was extracted an additional three times with excess CH₂Cl₂. The combined extracts were dried under vacuum using a rotary evaporator. The resulting residue was dissolved in 5 ml water and washed three times with excess hexane to remove unwanted non-polar compounds. The water fraction was then placed in a separatory funnel and extracted three times with 5 ml CH₂Cl₂ aliquots. The CH₂Cl₂ supernatants were pooled and reduced to approximately 1 ml by rotary evaporation, and the supernatants were added to pre-weighed scintillation vials. The remaining CH₂Cl₂ was removed at 30 °C under a stream of nitrogen and the vials were reweighed to determine the iberin content (the purity of which was confirmed by GC-FID). It was also of interest to compare our method of simultaneously incubating the wet seedmeals with CH₂Cl₂ against incubating the seedmeals with an aqueous solution only, generally following the procedure of [Matusheski et al. \(2001\)](#). Three replicates (of 3.0 g each) were incubated with 9 ml of de-ionized distilled water (ddH₂O) in sealed 250-ml Erlenmeyer flasks at 25 °C for 8 h. Following hydrolysis, sodium chloride, wet seedmeal, and anhydrous sodium sulfate were mixed at the ratio of 1:1:0.75 (w/w/w) and mixed thoroughly. The resultant mixture was extracted three

times with equal volumes of CH₂Cl₂ that were combined and dried at 30 °C on a rotary evaporator. The residue was partitioned between hexane and water and treated in the same manner as the simultaneous incubation method.

For glucoiberin quantitation, a modification of a high-performance liquid chromatography (HPLC) method developed by [Betz and Fox \(1994\)](#) was used. Briefly, replicates of defatted seedmeal (5.0 g) were added to 200 ml of boiling 70% (v/v) MeOH with stirring for 15 min, and then cooled and filtered through Whatman No. 2 filter paper. The marc was washed twice with 50 ml aliquots of 70% MeOH. The resulting extract was concentrated to 5–10 ml by rotoevaporation and was diluted to 25 ml to form the working solution. The extract was analyzed on a Thermo-Finnegan Spectra System (CP4000 pump; AS3000 autoinjector, UV6000 photodiode array detector) using a C₁₈ Inertsil column (250 mm × 4.6 mm; RP C-18, ODS-3, 5 μ; Varian, Torrance, CA) running under the ChromQuest Version 4 software. The glucoiberin peak was detected by monitoring with a photodiode array at 237 nm. The initial mobile phase conditions were 12% methanol/88% aqueous 0.005 M THS at a flow rate of 1 ml/min. The binary gradient was developed to 70% methanol/30% aqueous 0.005 M THS for 20 min, and held at these conditions for an additional 15 min. Molar concentrations of glucoiberin in the samples were determined from a sinigrin-based standard curve. The relative percent yield of iberin was then calculated from the number of moles of iberin/gdw divided by the number of moles of glucoiberin/gdw.

3. Results and discussion

3.1. General

Seed sources chosen for this study had two important characteristics. First, the seed must be commercially available in kilogram quantities; secondly, the seed must contain one dominant glucosinolate whose degradation products were of interest to us. Alternately, if the seed source contained several glucosinolates, then the potential reaction products were separable by solvent partitioning alone. Most cruciferous vegetable seeds (e.g., broccoli, cabbage, kale) do

not meet this criteria as they contain several glucosinolates whose reaction products cannot be separated by simple solvent partitioning (e.g., iberin and sulforaphane, degradation products of glucoiberin and glucoraphanin, respectively, which are both found in broccoli seed). Seed which contained glucosinolates whose degradation products are low-cost and readily available—such as allyl isothiocyanate and benzyl isothiocyanate, degradation products from the glucosinolates sinigrin and glucotropaeolin, respectively—were not examined. The incubation solutions were chosen because preliminary studies indicated that these four solutions produced extremely acidic (\sim pH 0 with 2 M HCl), acidic (\sim pH 2 with 0.1 M HCl), and pH 7 and 10 with the respective buffers and formed distinct hydrolysis products.

No discernable peaks above the threshold values were found under any reaction pH for either *A. saxatile*, *L. biennis*, or *S. irio* seedmeals. All the other seedmeals contained at least one compound above the threshold levels in at least one of the reaction pHs and are discussed at greater length. Degradation products and the species from which they were found are listed in Table 1. Excellent separation of glucosinolate degradation products possessing several different R-groups was achieved without chromatography by choosing seedmeal sources which had either one predominant glucosinolate, or if containing multiple glucosinolates, had R-group substitutions that allowed for phasic separation of their degradation products. Rigorously defatting the seedmeals with hexane prior to hydrolysis allowed only those compounds produced by glucosinolate degradation to be subsequently extracted by CH_2Cl_2 . Reaction conditions were also important in determining the type of hydrolysis products formed, with acidic conditions favoring nitrile formation and higher pHs favoring isothiocyanate formation. Possibly due to the seedmeal sources used and their respective myrosinases, no thiocyanates were present in any of the preparations, although it has been shown that allyl thiocyanate is converted to allyl isothiocyanate by injector port temperatures more than 100°C (Lüthy and Benn, 1977). For the compounds isolated and identified in this paper, their prominent diagnostic mass spectral ions and relative intensities, are presented in Table 1.

Mass spectral data of glucocinolate hydrolysis products are presented in Table 2.

3.2. Hydrolysis products from white mustard seedmeal

White mustard seed primarily contains sinalbin (4-hydroxybenzyl glucosinolate) (Bodnaryk, 1991). Hydrolysis of the defatted seedmeal with 2 M HCl yielded low levels of several identifiable compounds, with 4-hydroxybenzyl nitrile (4-HBN; 25.1%) being the most prevalent compound. Both 0.1 N HCl and pH 7 buffer treatments produced primarily 4-hydroxybenzyl isothiocyanate (4-HBITC), with the crude pH 7 extract containing 96.3% 4-HBITC. Fractionation of the crude extract by water and hexane produced a water extract with 98.2% 4-HBITC. This compound is of particular interest as a precursor in the production of thiourea antioxidants (T. Isbell, personal communication). As white mustard seed is available commercially in large amounts, 4-HBITC production via this route would most likely be inexpensive.

3.3. Hydrolysis products from arugula seedmeal

Arugula seed contains primarily glucoerucin [4-(methylthio)butyl glucosinolate] (S.F. Vaughn and M.A. Berhow, unpublished data). Glucoerucin is normally enzymatically hydrolyzed to produce erucin [1-isothiocyanato-4-(methylthio)butane], which is also of interest to health researchers as it is an analogue of sulforaphane. Hydrolysis of the seedmeal with 2 M HCl produced nearly pure (99.6%) 1-cyano-4-(methylthio)butane (erucin nitrile) in the crude CH_2Cl_2 extract, so that further solvent partitioning is probably unnecessary for production of this compound. Hydrolysis with both 0.1 M HCl and pH 7 buffer produced primarily erucin with small amounts of erucin nitrile, which is undesirable as solvent partitioning alone does not separate these compounds. However, with the pH 10 buffer only erucin was present in the crude extract, and no solvent partitioning would be required to produce essentially pure erucin.

3.4. Hydrolysis products from English wallflower seedmeal

Glucocheirolin [3-(methylsulfonyl)propyl glucosinolate] is present in English wallflower seed (Daxenbichler et al., 1991). The crude extract of the

Table 1
Plant sources and major hydrolysis products

Species	Crude fraction	Major hydrolysis products
<i>Brassica hirta</i> (White mustard)	Tris buffer, pH 10	4-Hydroxybenzyl isothiocyanate
	Phosphate buffer, pH 7	4-Hydroxybenzyl isothiocyanate
	0.1 M HCl	4-Hydroxybenzyl isothiocyanate
	2 M HCl	4-Hydroxybenzyl nitrile
<i>Capsella bursa-pastoris</i> (Shepherd's purse)	Tris buffer, pH 10	3-Butenyl isothiocyanate
	Phosphate buffer, pH 7	3-Butenyl isothiocyanate
	0.1 M HCl	None
	2 M HCl	None
<i>Eruca sativa</i> (Arugula)	Tris buffer, pH 10	Erucin
	Phosphate buffer, pH 7	Erucin, erucin nitrile
	0.1 M HCl	Erucin, erucin nitrile
	2 M HCl	Erucin nitrile
<i>Erysimum allionii</i> (Siberian wallflower)	Tris buffer, pH 10	Erucin
	Phosphate buffer, pH 7	Erucin, cheirolin, erysolin, sulforaphane
	0.1 M HCl	Erucin, erucin nitrile, erysolin, sulforaphane, sulforaphane nitrile, cheirolin
	2 M HCl	Erucin nitrile, sulforaphane nitrile, erysolin nitrile
<i>Erysimum cheiri</i> (English wallflower)	Tris buffer, pH 10	Cheirolin
	Phosphate buffer, pH 7	Cheirolin
	0.1 M HCl	Cheirolin, hesperin
	2 M HCl	None
<i>Hesperis matronalis</i> (Dame's rocket)	Tris buffer, pH 10	Hesperin
	Phosphate buffer, pH 7	Hesperin
	0.1 M HCl	None
	2 M HCl	None
<i>Lesquerella fendleri</i> (Lesquerella)	Tris buffer, pH 10	Iberin, 3-butenyl isothiocyanate
	Phosphate buffer, pH 7	Iberin, 3-butenyl isothiocyanate
	0.1 M HCl	Iberin, 3-butenyl isothiocyanate
	2 M HCl	Iberverin nitrile
<i>Lobularia maritima</i> (Sweet alyssum)	Tris buffer, pH 10	3-Butenyl isothiocyanate, lesquerellin
	Phosphate buffer, pH 7	3-Butenyl isothiocyanate, lesquerellin
	0.1 M HCl	3-Butenyl isothiocyanate, lesquerellin
	2 M HCl	None
<i>Matthiola longipetala</i> (Night-scented stock)	Tris buffer, pH 10	Sulforaphene
	Phosphate buffer, pH 7	Sulforaphene
	0.1 M HCl	Sulforaphene
	2 M HCl	Sulforaphene nitrile

2 M HCl hydrolysate produced only an extremely small amount of CH_2Cl_2 -soluble compounds, none of which were identified. The 0.1 M HCl crude extract contained primarily cheirolin [1-isothiocyanato-3-(methylsulfonyl)propane] (84.4%) with a smaller amount of hesperin [1-isothiocyanato-6-(methylsulfinyl)hexane] (13.1%). Both these compounds have an affinity for water, so that water-hexane partitioning is

ineffective in separating these compounds. Both the pH 7 and 10 buffer crude extracts contained higher purity cheirolin (97.2 and 98.3%); partitioning between water and hexane produced water fractions that were nearly 100% cheirolin. We cannot explain the absence of hesperin at the higher pHs except that perhaps the degradation of the parent glucosinolate is suppressed at these pH levels.

Table 2
Mass spectral data of glucosinolate hydrolysis products

Hydrolysis product	MS spectral data m/z (relative intensities)
Arabin	247 (M^+ , <1), 230 (86), 83 (42), 69 (68), 55 (100)
Berteroin	175 (M^+ , 58), 160 (16), 142 (22), 129 (56), 101 (38), 72 (52), 61 (100)
3-BITC	113 (M^+ , 55), 85 (9), 72 (100), 55 (23)
Camelinin	261 (M^+ , <1), 244 (60), 198 (27), 115 (66), 81 (53), 55 (100)
Cheirolin	179 (M^+ , 100), 121 (25), 99 (90), 72 (94), 41 (41)
Cheirolin nitrile	147 (M^+ , <1), 119 (7), 94 (4), 81 (26), 68 (100)
Erucin	161 (M^+ , 13), 115 (78), 85 (36), 72 (45), 61 (100)
Erucin nitrile	129 (M^+ , 80), 114 (9), 82 (48), 61 (100)
Erysolin	193 (M^+ , 55), 135 (31), 114 (18), 86 (100), 72 (95), 55 (58)
Erysolin nitrile	161 (M^+ , <1), 98 (13), 82 (100), 80 (15), 55 (79), 41 (18)
Hesperin	188 (M^+ , 38), 142 (40), 126 (16), 72 (58), 55 (100)
4-HBITC	165 (M^+ , 4), 107 (100), 77 (20), 51 (9)
4-HBN	133 (M^+ , 100), 132 (51), 106 (31), 78 (43), 77 (29), 51 (16)
Iberin	163 (M^+ , 3), 130 (11), 116 (40), 100 (34), 86 (15), 72 (100), 41 (34)
Iberverin	147 (M^+ , 11), 101 (100), 72 (29), 61 (20), 41 (23)
Iberverin nitrile	115 (M^+ , 86), 74 (11), 68 (12), 61 (100)
Lesquerellin	189 (M^+ , 83), 156 (58), 142 (35), 128 (19), 72 (56), 61 (100)
Limnanthin	179 (M^+ , 50), 121 (100), 91 (29), 78 (14), 65 (6)
Limnanthin nitrile	147 (M^+ , 100), 132 (22), 116 (19), 104 (14), 90 (11), 77 (30)
Sulforaphane	177 (M^+ , 1), 160 (85), 114 (10), 72 (100), 64 (13), 55 (26)
Sulforaphane nitrile	145 (M^+ , 19), 128 (9), 82 (42), 64 (54), 55 (100)
Sulforaphene	175 (M^+ , 26), 112 (30), 103 (20), 87 (20), 78 (13), 72 (100)
Sulforaphene nitrile	143 (M^+ , 87), 114 (95), 87 (59), 64 (49), 53 (100), 45 (43)

3.5. Hydrolysis products from sweet albyssum seedmeal

Sweet albyssum seed has been reported to contain high levels of 6-(methylthio)hexyl- and 6-(methylsulfinyl)hexyl glucosinolates, both of which have hydrolysis products of interest, 1-isothiocyanato-6-(methylthio)hexane (lesquerellin) and hesperin (Daxenbichler et al., 1991). The crude extract of the 2M HCl hydrolysate did not contain large peaks, but the other three hydrolysates produced several compounds of interest, including lesquerellin. However, each of the hydrolysates contained 3-butenyl isothiocyanate in the largest amounts, which is not easily chemically separated from lesquerellin by solvent partitioning (both compounds partition into hexane). Because 3-butenyl isothiocyanate is much more volatile than lesquerellin when the pH 7 crude extract was rotoevaporated at a higher temperature (50 °C), most of the 3-butenyl isothiocyanate evaporated, primarily leaving lesquerellin behind. The remaining residue was partitioned between water and hexane, with majority

of the remaining compounds (including sulforaphane and hesperin in small amounts) present in the water fraction. The hexane fraction was found to contain lesquerellin (96.3%) with only 1-isothiocyanato-5-(methylthio)pentane (berteroin; 1.6%) as a significant contaminant.

3.6. Hydrolysis products from dame's rocket seedmeal

Dame's rocket (also known as sweet rocket) seed has 5-(methylsulfinyl)pentyl- and 6-(methylsulfinyl)hexyl glucosinolates with trace amounts of 4-(methylthio)butyl- and 5-(methylthio)pentyl glucosinolates (Daxenbichler et al., 1991). All the four crude extracts contained only very small peaks on GC-FID and only in the pH 7 and 10 buffer extracts, there was a significant peak which was identified as hesperin. However, the yields were very poor and it is unlikely that this seedmeal would be a good candidate for hesperin production, unless production of the parent glucosinolate is higher in other seed lots of this plant.

3.7. Hydrolysis products from meadowfoam seedmeal

Meadowfoam seed contains 3-methoxybenzyl glucosinolate (glucolimnanthin) in high levels (Daxenbichler and VanEtten, 1974). The crude 2 M HCl hydrolysate had one major peak, which was identified as 3-methoxybenzyl nitrile (limnanthin nitrile), but it also exhibited several other small peaks that we did not identify. Fractionation between water and hexane, with the hexane fraction washed an additional three times with water produced a fraction that had essentially pure limnanthin nitrile. The 0.1 M HCl crude extract possessed 3-methoxybenzyl isothiocyanate (limnanthin) in the greatest amount, with limnanthin nitrile (6.3%) also present. Both pH 7 and 10 buffer crude hydrolysates displayed nearly pure limnanthin (95.9 and 96.6%, respectively), and could be further purified by rotoevaporating the residue at 50 °C and redissolving the residue in hexane, followed by filtering over anhydrous sodium sulfate. This method produced limnanthin of nearly 100% purity on the GC-FID.

3.8. Hydrolysis products from night-scented stock seedmeal

Night-scented stock seed has primarily 4-(methylsulfinyl)but-3-enyl glucosinolate (glucoraphenin) (Daxenbichler et al., 1991). The crude 2 M HCl extract exhibited only a small amount of 1-cyano-4-(methylsulfinyl)but-3-ene (sulforaphene nitrile) that was not separable from several other unidentified compounds upon solvent fractionation. All the other crude extracts produced large 1-isothiocyanato-4-(methylsulfinyl)but-3-ene (sulforaphene) peaks, with the pH 7 extract being nearly pure (no other peak was above threshold levels). Although probably not necessary, the water fraction from solvent partitioning of the pH 7 extract had sulfuraphene that was essentially 100% pure.

3.9. Hydrolysis products from Siberian wallflower seedmeal

Siberian wallflower seed has several glucosinolates whose degradation products are of interest, including glucoraphanin and glucoerysolin [4-(methylsulfonyl)butyl glucosinolate] (Daxenbichler et al., 1991). Erucin nitrile (76.2%) was the principal compound

in the 2 M HCl extract, with smaller amounts of sulfuraphane nitrile (7.3%) and 1-cyano-4-(methylsulfonyl)butane (erysolin nitrile; 6.3%). The chief compound in the 0.1 M HCl extract was erucin (65.3%), with smaller amounts of erucin nitrile (10.4%), 1-isothiocyanato-4-(methylsulfonyl)butane (erysolin; 8.2%), sulfuraphane (3.6%), 1-cyano-4-(methylsulfinyl)butane (sulforaphane nitrile; 2.7%), 1-cyano-3-(methylsulfonyl)propane (cheirolin nitrile; 2.4%) and cheirolin (2.5%). In the pH 7 and 10 extracts, erucin was also the most prevalent peak (73.0 and 78.3%, respectively) with smaller amounts of cheirolin, erysolin, and sulfuraphane. Fractionation of the pH 7 extract produced a hexane extract with nearly all erucin (98.7%) with the remainder erucin nitrile. The pH 10 hexane extract was pure erucin without containing any erucin nitrile. The water fraction from the pH 7 extract consisted of erysolin (38.7%), sulfuraphane (31.9%), and cheirolin (18.6%). The water fraction from the pH 10 extract also had these three compounds, but in different percentages: erysolin (64.4%), sulfuraphane (15.8%), and cheirolin (19.8%).

3.10. Hydrolysis products from shepherd's-purse seedmeal

Shepherd's purse seed has several unusual methylsulfinylalkyl glucosinolates, including 9-methylsulfinylnonyl (glucoarabin) and 10-methylsulfinyldecyl (glucocamelinin) (Daxenbichler et al., 1991). Both the 2 M and 0.1 M HCl extracts did not show any significant peaks on GC-FID. Both the pH 7 and 10 buffer extracts had one significant peak, which was identified as 3-butenyl isothiocyanate. However, no other peaks were above the threshold levels. It was possible to detect small amounts of two compounds that were tentatively identified as 1-isothiocyanato-9-(methylsulfinyl)nonane (arabin) and 1-isothiocyanato-10-(methylsulfinyl)decane (camelinin) on the GC-MS, but these peaks were below threshold levels on the GC-FID, suggesting that this seed would not be a usable source for production of these compounds.

3.11. Hydrolysis products from lesquerella seedmeal

The principal glucosinolate in lesquerella seed is glucoiberin (Daxenbichler et al., 1991). The 2 M HCl

extract exhibited several peaks, of which the largest was identified as 1-cyano-3-(methylthio)propane (iberiverin nitrile). Partitioning into water and hexane produced a water fraction that was principally iberiverin nitrile (89.2%), but several other small peaks were present and could not be removed by repeated hexane washes. Each of the other three extracts contained iberin as a major peak, with 3-butenyl isothiocyanate also present in each. The subsequent water fraction of each of the extracts contained nearly pure iberin, with the 0.1 M HCl extract being essentially pure. 1-Cyano-3-(methylsulfinyl)propane (iberin nitrile) was not detected in any of the samples. Most likely, the iberiverin nitrile found in the 2 M HCl extract was a degradation product of glucoiberin as intact glucosinolate analysis of the *lesquerella* seedmeal found no glucoiberin.

3.12. Yields of glucoiberin and iberin from *lesquerella* seedmeal

The average yield of glucoiberin as determined by HPLC was 65.0 ± 0.8 mg glucoiberin/gdw of defatted seedmeal. The yield of iberin, when incubated simultaneously with CH_2Cl_2 and determined by GC-FID, was 22.6 ± 1.1 mg iberin/gdw. The yield of iberin by the post-incubation extraction method was 12.2 ± 1.0 mg iberin/gdw. These are equivalent to 153.7 ± 1.9 μmol glucoiberin/gdw and 138.3 ± 6.7 μmol iberin/gdw. Therefore, the percentage yield of iberin was ($\# \text{mol}$ iberin/ $\# \text{mol}$ glucoiberin)100% = $(138.3/153.7)100\%$ = 90.0% yield from glucoiberin by this method. For the post-incubation extraction method, this was equivalent to 74.7 ± 6.1 μmol iberin/gdw. Therefore, the molar yield of iberin was 48.6% from glucoiberin by this method.

4. Conclusions

The methods we have described yield high purity hydrolysis products without using chromatography when certain conditions are met: (1) selection of seed with high concentrations of the glucosinolate(s) whose hydrolysis products are desired, and if more than one glucosinolate is present, then the hydrolysis products can be partitioned into hydrophobic and hydrophilic components; (2) stringently defatting the

seedmeal; (3) controlling reaction pHs. Other degradation products of interest may be produced by this method if seeds containing suitable glucosinolates are identified. Our interest in utilizing these hydrolysis products as precursors in industrial synthesis in addition to their use in health studies led us to explore ways to produce relatively pure, gram amounts of these compounds without necessitating chromatography of any kind. As we previously mentioned, if these compounds are commercially available, then their high costs currently make them prohibitive for industrial uses. Our method offers a procedure to produce relatively large amounts of these compounds at low costs when an appropriate seed source is identified.

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