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Screening Crucifer Seeds as Sources of Specific Intact Glucosinolates Using Ion-Pair High-Performance Liquid Chromatography Negative Ion Electrospray Mass Spectrometry

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Seeds, of either commercial crucifer crops or some wild and weed relatives, were screened for intact glucosinolates using a previously developed ion-pair LC-MS method. This method, in contrast to GC-MS techniques, ensures the accurate measurement of all classes of glucosinolates. Many crucifer seeds contained very high concentrations of glucosinolates with low concentrations of additional pigments and secondary metabolites. The other common seed metabolites were cinnamoylcholine esters, for example, sinapine. Glucosinolates derived from homologues of L-methionine were characteristic of *Brassica* and related crucifer species. In addition, significant concentrations of 4-hydroxy-3-indolylmethylglucosinolate were found in the majority of *Brassica* species. Wild and weed species often had relatively simple glucosinolate profiles: either a single glucosinolate or a predominant glucosinolate together with trace amounts of others. Species identified with seed glucosinolate profiles suitable for purification included various *Alyssum*, *Erysimum*, and *Iberis* species for 3-methythiopropyl-glucosinolate and 3-methylsulfinylpropyl-glucosinolate and various *Alyssum*, *Erysimum*, and *Lepidium* species with very high concentrations of C4−C6 aliphatic glucosinolates. Seeds of *Arabis*, *Barbarea*, *Lepidium*, *Moringa*, and *Sinapis* species were good sources of aromatic glucosinolates, and *Azima tetracantha* was a good source for *N*-methoxy-3-indolylmethyl-glucosinolate. MS data are reported for all of the intact glucosinolates detected from the screening process.

KEYWORDS: Glucosinolates; seeds; standards; functional foods; LC-MS

INTRODUCTION

Glucosinolates are secondary metabolites found in plants of the order Capparales (**Figure 1**) and are derived from protein and nonprotein L-amino acids (*1*, *2*). Glucosinolates differ from each other in the structure of their aglycon moieties; these are generally classified as alkyl, aliphatic, alkenyl, hydroxyalkenyl, aromatic, or indole (**Figure 2**). Further modification of the basic structures gives rise to a wide range of structures, and >120 individual glucosinolates have been identified. All plants containing glucosinolates also contain myrosinase (thioglucosidase, EC 3.2.3.1), a glucosinolate-hydrolyzing enzyme encoded by a multigene family (*3*). Disruption of crucifer tissues brings myrosinases and glucosinolates into contact, liberating a glucose molecule and an unstable intermediate that can degrade into a variety of compounds (**Figure 2**). The nature of the hydrolysis products is dependent on factors such as pH, temperature, metal ions, protein cofactors (epithiospecifier protein), and the properties of the side chain. Common hydrolysis products that have been identified include isothiocyanates, nitriles, cyanoepithioalkanes, and 5-substituted-oxazolidine-2-thiones (*1*, *2*, *4*).

Glucosinolate hydrolysis products are a major component of the mixture of compounds that give crucifers their characteristic smell and flavor (*1*, *5*). Isothiocyanates, often a major product of glucosinolate hydrolysis, can react with the sulfhydryl groups (forming dithiocarbamates) and amino groups (forming thioureas) of amino acids, glutathione, and proteins (*4*, *6*). Interest in these glucosinolate hydrolysis products, especially the isothiocyanates, has increased over the past two decades since it was demonstrated that these molecules affect human health, either beneficially or adversely (*7*, *8*). Sulforaphane (4-methylsulfinylbutylisothiocyanate), derived from glucoraphanin, a predominant glucosinolate in broccoli, was identified as a major inducer of anticarcinogenic enzymes (*9*). Specific isothiocyanates and indoles may have several different biological effects. These include the ability to modulate xenobiotic detoxification mechanisms by affecting proteins in key signal transduction pathways, leading to changes in the expression of phase I and phase II enzymes, resulting in beneficial anticarcinogenic properties (such as inducing apoptosis), and also affecting immune functions via modulation of cyclooxygenase-2 (COX-2) (*8*−*12*). In contrast, certain hydrolysis products have a deleterious effect on animals, for example, goitrin (5-vinyloxazolidine-2-thione) and crambene (1-cyano-2-hydroxy-3-butene) (*13*, *14*).

Glucosinolates are present in all members of the plant order Capparales, which is subdivided into 16 families (**Figure 1**),

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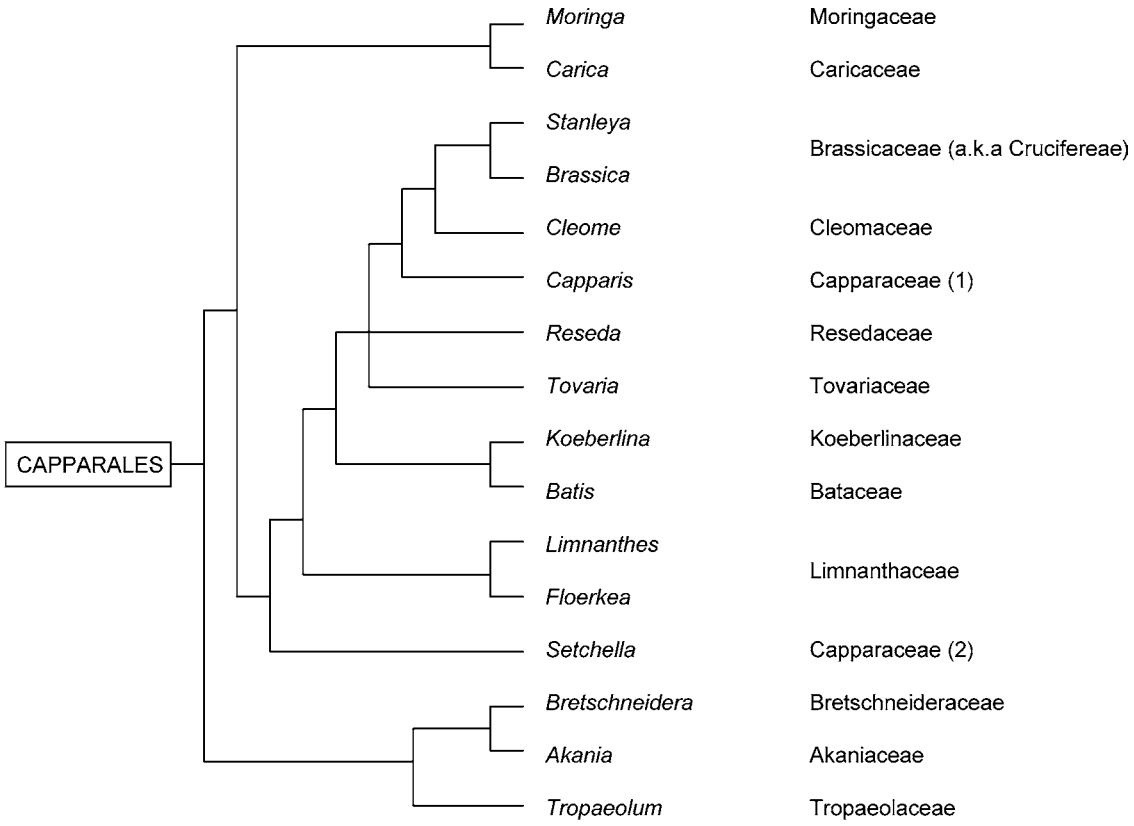

Figure 1. Taxonomy of glucosinolate-containing plants based on data from Rodman et al. (*15*).

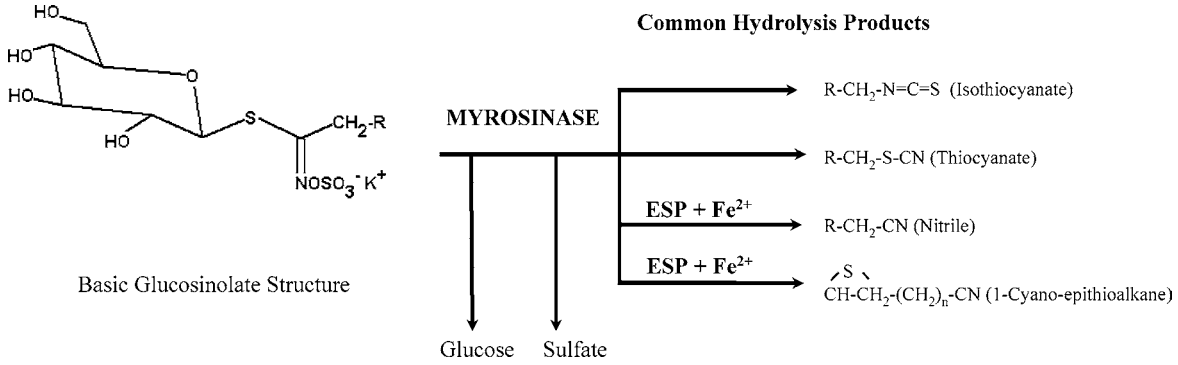

Figure 2. Basic glucosinolate structure and common myrosinase hydrolysis products.

and glucosinlolates have been found in all the tissues of these species (*15*). Structural type and individual glucosinolate concentrations differ according to various factors, for example, species, tissue type, physiological age, and plant health and nutrition (*1*, *2*, *16*). Glucosinolate concentrations in the reproductive tissues (florets/flowers and seeds) are often as much as 10−40 times higher than in the vegetative tissues. Thus, seed material can be a very good source of glucosinolates. Some crucifer seeds also contain modified glucosinolates, with the addition of sugars and phenolic residues to the glucose moiety, for example, that are not present in other tissues such as those eaten by humans (*17*). Considerable effort is currently being expended to promote sprouts (young seedlings) as functional foods for human consumption, particularly *Brassica oleracea* species that generate anticancer isothiocyanates (ITC) during digestion. Broccoli sprouts have been investigated for their ability to release higher amounts of sulforaphane, a potentially important dietary isothiocyanate with anticarcinogenic activity, as part of the increasing number of human intervention studies (*18*, *19*). Studies have also been done on the differences between isothiocyanates and their nitrile analogues (which are often formed from glucosinolates during the consumption of fresh, uncooked, crucifer vegetables; **Figure 1**) (*13*, *20*, *21*). However, robust data detailing the concentrations of bioactive compounds in these materials are often minimal. To develop functional foods, it is essential to obtain reliable data on the concentrations of bioactive components, so that directed breeding can be performed, for example, to selectively increase the concentrations of beneficial compounds such as glucosinolates and flavonoids. There is also increasing focus on developing supplements based on crucifer tissues and specific compounds, for example, sulforaphane and indole-3-carbinol.

We are conducting a systematic evaluation of seeds and seedlings of crop and noncrop *Brassica* and non-*Brassica* glucosinolate-containing species as a starting point in this process. Although there are many articles and reviews detailing

Table 1. ESI-MS Data for Glucosinolate, Abbreviation Used in Tables, and Retention Time (RT)

ref	glucosinolate semisystematic name	common name	RT (min)	$[M - H]^-$ (%)	$[SO_4H]^-$	$[SO_4]^-$	additional diagnostic ions
1	methyl	glucocapparin	3.2	332 (100)	72	20	
2	*n*-propyl		8.2	360 (100)	76	22	
3	isopropyl	glucoputranjivin	7.7	360 (100)	88	37	
4	*n*-butyl		11.4	374 (100)	83	32	
5	isobutyl		10.6	374 (68)	100	43	
6	2-methylpropyl		9.8	374 (70)	100	43	
7	*n*-pentyl		14.4	388 (53)	100	39	
8	*n*-hexyl		20.7	402 (47)	100	37	
9	2-hydroxy-2-methylpropyl	glucoconringiin	6.3	390 (88)	100	26	
10	3-benzyloxypropyl	glucomalcomiin	22.7	480 (100)	81	16	
11	3-methylthiopropyl	glucoiberverin	10.8	406 (91)	100	29	
12	4-methylthiobutyl	glucoerucin	13.8	420 (100)	97	30	
13	4-methylthio-3-butenyl	dehydoerucin	14.2	418 (100)	62	13	
14	5-methylthiopentyl	glucoberteroin	18.0	434 (99)	100	30	
15	6-methylthiohexyl	glucolesquerellin	22.5	448 (100)	100	29	
16	7-methylthioheptyl		27.5	462 (100)	27	4	
17	8-methylthiooctyl		31.3	476 (100)	69	12	
18	9-methylthiononyl		34.4	490 (100)	61	12	
19	10-methylthiodecyl						not detected
20	3-methylsulfinylpropyl	glucoiberin	5.2	422 (100)	93	38	
21	4-methylsulfinylbutyl	glucoraphanin	6.5	436 (100)	77	27	
22	4-methylsulfinyl-3-butenyl	glucoraphenin	6.7	434 (100)	50	5	
23	5-methylsulfinylpentyl	glucoalyssin	8.1	450 (100)	81	24	
24	6-methylsulfinylhexyl	glucohesperin	10.8	464 (100)	76	23	
25	7-methylsulfinylheptyl	glucosiberin	13.8	478 (100)	76	37	
26	8-methylsulfinyloctyl	glucohirsutin	17.3	492 (100)	66	39	
27	9-methylsulfinylnonyl	glucoarabin	21.3	506 (82)	100	38	
28	10-methylsulfinyldecyl	glucocamelinin	25.5	520 (100)	43	10	
29	3-methylsulfonylpropyl	glucocheirolin	5.6	438 (100)	68	23	
30	4-methylsulfonylbutyl	glucoerysolin	6.5	452 (100)	78	27	
31	5-methylsulfonylpentyl		8.5	466 (100)	59	22	
32	8-methylsulfonyloctyl		17.9	508 (100)	24	9	
33	9-methylsulfonylnonyl		22.6	522 (100)	69	21	
34	10-methylsulfonyldecyl		25.8	536 (100)	57	16	
35	2-propenyl	sinigrin	6.2	358 (100)	96	47	
36	3-butenyl	gluconapin	9.0	372 (100)	50	12	
37	4-pentenyl	glucobrassicanapin	12.3	386 (100)	47	11	
38	(*R*)-2-hydroxy-3-butenyl	progoitrin	6.0	388 (100)	50	8	
39	(*S*)-2-hydroxy-3-butenyl	epiprogoitrin	6.6	388 (52)	100	31	
40	(*R*)-2-hydroxy-4-pentenyl	gluconapoleiferin	8.0	402 (43)	100	31	
41	benzyl	glucotropaeolin	13.3	408 (100)	48	11	
42	2-hydroxybenzyl		10.4	424 (50)	100	71	
43	3-hydroxybenzyl	glucolepigramin	8.8	424 (87)	100	43	
44	4-hydroxybenzyl	glucosinalbin	8.6	424 (100)	92	37	
45	2-methoxybenzyl		16.6	438 (99)	100	37	
46	3-methoxybenzyl	glucolimnanthin	15.8	438 (100)	95	31	
47	4-methoxybenzyl	glucoaubrietin	15.2	438 (100)	92	33	
48	4-hydroxy, 3-(apiosyloxy)benzyl		9.2	572 (100)	71	6	440 (9)
49	4-hydroxy, 3-(3′,4′-dimethoxy-benzylapiosyloxy)benzyl		25.7	736 (100)	45	3	
50	2-(α-L-rhamnopyranosyloxy)benzyl		15.8	570 100)	71	17	
51	4-(α-L-rhamnopyranosyloxy)benzyl		10.3	570 (100)	59	12	
52	3-hydroxy, 4-(α-L-rhamno-pyranosyloxy)benzyl		9.2	586 (100)	68	7	
53	3,4-dihydroxybenzyl	glucomatronalin	7.2	440 (76)	100	11	
54	3,4,5-trimethoxybenzyl		16.0	482 (46)	100	27	
55	2-phenylethyl	gluconasturtiin	17.7	422 (56)	100	33	
56	(*R*)-2-hydroxy-2-phenylethyl	glucobarbarin	12.5	438 (42)	100	22	
57	(*S*)-2-hydroxy-2-phenylethyl	glucosibarin	13.2	438 (62)	100	25	
58	*p*-hydroxy-2-phenylethyl		11.6	438 (100)	84	20	
59	*p*-methoxy-2-phenylethyl		19.1	452 (100)	94	23	
60	(*R*)-*p*-hydroxy-2-hydroxy-2-phenylethyl		7.0	454 (87)	100	18	
61	(*R*)-*p*-methoxy-2-hydroxy-2-phenylethyl		13.4	468 (94)	100	16	
62	3-indolylmethyl	glucobrassicin	15.7	447 (100)	39	11	
63	4-hydroxy-3-indolylmethyl	4-hydroxyglucobrassicin	10.9	463 (100)	96	7	477 (15), 493 (8)
64	4-methoxy-3-indolylmethyl	4-methoxyglucobrassicin	19.5	477 (100)	72	28	
65	*N*-methoxy-3-indolylmethyl	neoglucobrassicin	22.8	477 (100)	42	12	
66	*N*-sulfo-3-indoylmethyl	*N*-sulfoglucobrassicin	10.4	527 (15)	19	6	447 (100)

the glucosinolate composition of diverse species, it is often difficult to identify any consistent results in non-*Brassica* and noncrop glucosinolate-containing species. The common problems are caused by the lack of reliable, standard methods of glucosinolate analysis and by contradictory results arising from incorrect botanical classifications. The available analytical data for crucifer seeds, and especially wild and weed species, are not only limited but are also, in many cases, based on assay methods that do not allow all glucosinolates to be identified. The aims of this study were to screen seeds of common crop and related species that contain glucosinolates and to identify species that contained high concentrations of single glucosinolates or useful mixtures that could be used for the purification of individual glucosinolates. The first step in this process was

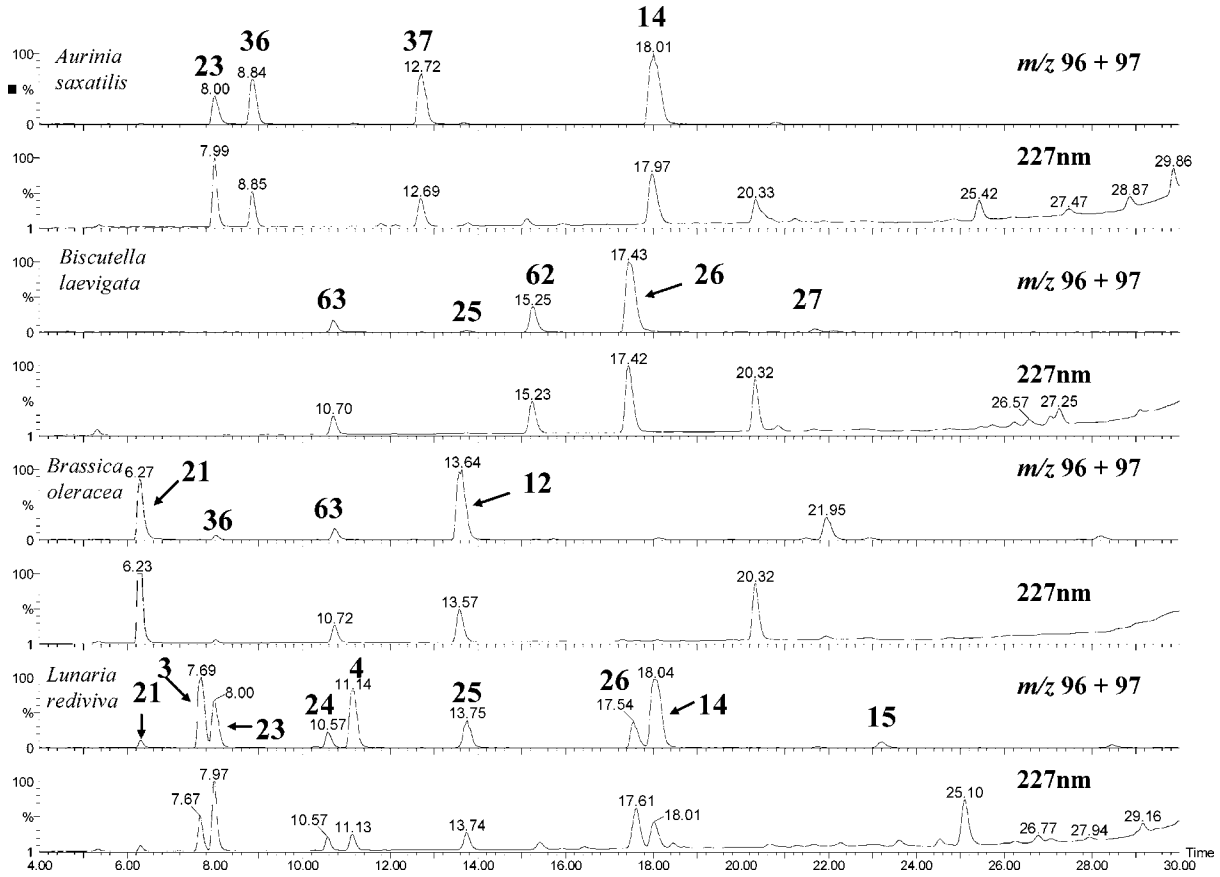

Figure 3. Examples of ion-pair LC-MS data for various seed samples; peak numbers refer to the glucosinolate listed in **Table 2**. In each sample pair *m/z* 96 + 97 (characteristic common glucosinolate MS ions) and UV trace at 227 nm are shown for *Aurinia saxatilis*, *Biscutella laevigata*, *Brassica oleracea*, and *Lunaria rediviva*, respectively. Peaks not identified in the traces are not glucosinolates; the peak at 20.3 min in *A. saxatilis*, *B. laevigata*, and *B. oleracea* is sinapine (sinapinic acid choline ester), and some of the other peaks are minor aromatic choline esters and flavonoids.

to screen seeds using the previously optimized LC-MS method (*22*). This method has been shown to be effective for all of the glucosinolates and was recently used as part of the methodology to identify the major glucosinolates in leaves of *Eruca sativa* (*5*) and various tissues of *Moringa* species (*23*).

MATERIALS AND METHODS

Seed Samples. Seeds were obtained both from commercial sources and as voucher specimens from botanical gardens (see Supporting Information). The following organizations and individuals were sources of seed material: 1 = E. W. Kings/Suffolk Herbs, Monks Farm, Kelvedon, Essex, U.K.; 2 = Dr. Nikolaus Foidl; 3 = ECHO (USA); 4 = B & T World Seeds, Paguignan, Olonzac, France; 5 = Prof. Ole Hamann, Botanic Garden, University of Copenhagen, Copenhagen, Denmark; 6 = Chiltern Seeds, Bortree Stile, Ulveston, Cumbria, U.K.; 7 = John Size, Seeds-By-Size, Hemel Hempstead, Herts, U.K.; 8 = Prof. Jutta Ludwig-Müller, Institut für Botanik, Technische Universität Dresden, Dresden, Germany; 9 = Thompson & Morgan Ltd., Ipswich, U.K.; 10 = Dr. C. Gómez-Campo, Departamento de Biologia Vegetal, Universidad Politecnica de Madrid, Spain; 11 = John Burrows, Pro-Veg Seeds Ltd., Sawston, Cambridge, U.K.; 12 = Dr. Senta Heiss (Universität Heidelberg); 13 = Ian Pearman, IACR-Rothamsted, Harpenden, Herts, U.K.; 14 = D. T. Brown & Co. Ltd., Poulton-Le-Flyde, U.K.; 15 = Unwins Seeds Ltd., Histon, Cambridge, U.K.

Chemicals. All solvents were of HPLC grade, and all water was of ultrapure grade. All chemicals were of analytical grade and were obtained from Sigma-Aldrich (Poole, U.K.).

Sample Processing and LC-MS Analyses. Samples were processed and extracted using the methods described previously (*22*). Essentially all seeds (1−2 g) were dried (24 h at 100 °C), and each sample was analyzed in triplicate (3 × 40 mg of dried seed) with sinigrin as an extraction standard in one replicate. Extraction was done by homogenization in 750 μL 70% v/v methanol with a small pestle and mortar that was washed with a further 750 μL 70% v/v methanol; total extraction volume was 1.5 mL. The combined homogenates were transferred to 2 mL screw-top tubes and extracted for 20 min at 70 °C with vortex mixing every 5 min, followed by centrifugation (15 min, 17500*g*, 4 °C) to pellet insoluble material. Subsamples (800 μL) of each supernatant were taken, and all solvent was removed from the subsamples using a stream of N_2 gas followed by resuspension in 800 μL of ultrapure water and filtration (0.2 μm). Samples were then divided into 8 × 100 μL fractions; one was used for LC-MS analysis, whereas the remainder were frozen at −20 °C for future use as standards. The samples were analyzed using the previously described intact glucosinolate LC-MS (identification) and LC-UV (quantification) methods (*22*). MS data are presented for every glucosinolate that was positively identified in the samples.

Generation of Additional Glucosinolate Standard by Enzymatic Treatment. *Reseda lutea* seed contains 2-(α-L-rhamnopyranosyloxy)-benzylglucosinolate. Although 2-hydroxybenzylglucosinolate occurs naturally in some *Lepidium* and related species (*24*), it was easier to obtain this glucosinolate by selective hydrolysis of the *R. lutea* glucosinolate than to obtain samples of these rare *Lepidium* species. The *R. lutea* glucosinolate was treated with naringinase; naringinase is a rhamnosidase that hydrolytically cleaves rhamnose from both high and low molecular weight rhamnosides, for example, quercetin 3-*O*-rhamnoglucoside and rhamnopolysaccharides. One hundred milligrams of naringinase, containing 511 units/mg β-rhamnosidase and 55 units/mg β-glucosidase (Sigma), was dissolved in 1 mL of ultrapure water. Two hundred microliters of the seed extract (standard 40 mg preparation) was added to either 100 μL of ultrapure water (control) or 100 μL of naringinase solution. Samples were incubated at 37 °C for 3 h; different incubation times had previously been tested, and 3 h was found

Table 2. Wild and Weed Species Containing Alkyl and Indole Glucosinolates Derived from Simple and Branched-Chain Amino Acids and L-Tryptophan, Respectively[a]

	glucosinolate												
species	**1**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	UNK	**62**	**63**	**65**
Tropaeolum majus	–	–	–	–	–	–	–	–	–	–	T	–	–
Tropaeolum pereginum	–	–	–	–	–	–	–	–	–	C	–	–	–
Azima tetracantha	–	–	–	–	–	–	–	–	–	–	A	–	C
Codonocarpus continifolius	–	–	–	–	–	–	–	–	–	B (C5)	–	–	–
Codonocarpus pyramidalis	–	–	–	–	–	–	–	–	–	C (C5)	–	–	–
Tersonia cyathifolia	–	–	–	–	–	–	–	–	–	B (C5)	–	–	–
Reseda luteola	–	–	–	–	–	–	–	–	–	–	A	–	–
Reseda odorata	–	–	–	–	–	–	–	–	–	–	B	–	–
Capparis spinosa	D	–	–	–	–	–	–	–	–	–	–	–	–
Cleome hasslerana	–	–	–	–	–	–	–	–	–	T	–	–	–
Arabis procurrens	–	–	–	–	T	–	–	B	–	–	–	–	–
Aubrietia deltoidea	–	–	–	–	–	–	–	A	–	–	–	–	–
Aubrietia hybrida	–	–	–	–	–	–	–	A	–	–	–	–	–
Biscutella laevigata	–	–	–	–	–	–	–	–	–	–	B	B	–
Cardamine hirsuta	–	–	–	–	–	–	–	–	–	–	–	A	–
Cardamine pratensis	–	A	C	A	A	A	A	A	–	A (C4)	–	–	–
Crambe cordifolia	–	–	–	–	–	–	–	–	–	–	–	A	–
Crambe maritima	–	–	–	–	–	–	–	–	–	–	–	A	–
Dentaria pinnata	–	D	A	A	–	A	–	A	–	A (C3) E (C5)	–	–	–
Draba aizoides	–	–	–	–	–	–	–	C	–	–	–	–	–
Ionopsidium acaule	–	–	–	–	–	–	–	–	–	–	–	A	–
Isatis tinctoria	–	–	–	–	–	–	–	–	–	–	–	A	–
Lunaria.annua	–	C	–	–	–	–	–	–	–	–	–	–	–
Lunaria rediviva	–	C	B	–	–	–	–	–	–	–	–	–	–
Malcomia maritima	–	–	–	–	–	–	–	–	F	–	–	–	–
Nasturtium officinalis	–	–	–	–	–	–	–	–	–	–	–	T	–
Raphanus longipinnatus	–	–	–	–	–	–	–	–	–	–	–	B	–
Raphanus sativus (1)	–	–	–	–	–	–	–	–	–	–	–	B	–
Raphanus sativus (2)	–	–	–	–	–	–	–	–	–	–	–	A	–
Sinapis alba	–	–	–	–	–	–	–	–	–	–	–	T	–
Sisymbrium officinale	–	D	–	–	A	–	–	–	–	–	–	–	–
Streptanthus insignis	–	–	–	–	–	–	–	–	–	–	–	B	–

[a] Key for all tables for the seed glucosinolate concentrations (μmol of glucosinolate/g of DW): –, not detected by LC-MS; T, trace [= <0.1 μmol (MS data collected but no quantification]; A, 0.1–10 μmol; B, 10–25 μmol; C, 25–50 μmol; D, 50–75 μmol; E, 75–100 μmol; F, 100–125 μmol; G, 125–150 μmol; H, 150–200 μmol; I, >200 μmol. Species can be cross-referenced to the table available as Supporting Information. Glucosinolate reference numbers and MS data are shown in **Table 1**. See text for a discussion on the unknown (UNK) alkyl glucosinolates. For all tables the species are listed in order of the families shown in **Figure 1** from which they are derived.

to be optimal. Samples were filtered (0.2 μm, PVDF) and analyzed using the standard ion-pair LC method. An enzyme-free 20 °C control and enzyme-only control were also analyzed.

RESULTS AND DISCUSSION

The results reported show examples of representative species selected from a large number of analyses that have been conducted in our laboratories. The seed samples were obtained from various sources and diverse geographical locations. In general, only a single example is presented for each species in the tables, unless a significant deviation from the norm was observed in the glucosinolate profiles and concentrations. However, several examples are presented for some *Brassica* species, generally those consumed as foods or that have potential as functional foods (i.e., "sprouts"), because of possible health-promoting properties. Glucosinolate distribution is described in relation to classes rather than species, because the primary aim of this study was to identify sources of glucosinolate standards. **Table 1** lists the MS data for all of the confirmed intact glucosinolates found in seeds from these analyses, and example LC-MS data are shown in **Figure 3**. **Table 1** also includes, where given, the common names used for the glucosinolates. In each of the data tables for the classes of glucosinolates the species are listed in order of the families in which they occur: Tropaeolaceae, Caricaceae, Moringaceae, Limnanthaceae, Salvadoraceae, Gyrostemonaceae, Reseadaceae, Capparidaceae, Crucifereae (aka Brassicaceae).

Alkyl glucosinolates were restricted to only a few of the species analyzed, but examples were found in various families (**Table 2**). They were not found in seeds of any of the *Brassica* species (**Table 3**). There did not appear to be a clear pattern to their distribution; that is, they were not taxonomic markers for any particular family. Several species contained alkyl glucosinolates that could not be fully identified because they were isomeric. For example, the C3 hydroxyalkyl glucosinolate in *Dentaria pinnata* ($[M - H]^- = 376$), on the basis of literature data, could be either 1-methyl-2-hydroxyethyl, 3-hydroxypropyl, or 2-hydroxypropyl; C4 hydroxyalkyl in *Cardamine pratensis* ($[M - H]^- = 390$), on the basis of literature data, could be either 1-hydroxymethylpropyl, 3-hydroxybutyl, or 4-hydroxybutyl; C5 hydroxyalkyl in *Codonocarpus* species ($[M - H]^- = 390$), on the basis of literature data, could be either 1-methyl-3-hydroxybutyl, 2-hydroxy-2-methylbutyl, or 2-hydroxypentyl. This is the only limitation of the current LC-MS method. Combining LC with NMR would allow these glucosinolate isomers to be distinguished. A significant concentration of isopropyl-glucosinolate was found in seeds of *Sisymbrium officinale* (**Table 2**). A high concentration of 3-benzoyloxypropylglucosinolate was found in *Malcomia maritima* (**Table 3**). Seeds of *Capparis spinosa* (caper) are a good source of methylglucosinolate (structurally the simplest glucosinolate identified in plants), as previously reported by many researchers (*25*). Apart from *C. spinosa*, none of the species containing alkyl glucosinolate are common foods. Some of these species have been reported as famine foods, but they rarely form part of a typical human diet. Methyl isothiocyanate and acetonitrile are produced by methyl glucosinolate hydrolysis on the consumption of capers.

Indole glucosinolates were also less common constituents of seeds of weed and wild crucifers (**Table 2**). There were a few

Table 3. Glucosinolates in Seeds of *Brassica* Species (C3, C4, C5, C9 = Aliphatic Glucosinolates of Chain Length Indicated)

		C3			C4					C5			C9	aromatic		indole	
Brassica species (various species)	cultivar	**11**	**20**	**35**	**12**	**21**	**36**	**38**	**39**	**23**	**37**	**40**	**33**	**44**	**55**	**62**	**63**
B. alboglabra	Green Lance	–	–	D	–	–	F	–	–	–	–	–	–	–	–	–	A
B. campestris ssp. *pekinensis*	Granat	–	–	A	–	–	B	–	–	–	A	A	–	–	–	–	A
B. campestris ssp. *rapifera*	Marion	–	B	B	–	–	A	–	–	–	A	–	–	–	–	–	A
B. japonica	Green Boy	–	–	–	–	–	C	A	–	T	A	–	–	–	–	–	A
B. juncea	Vitasso	–	–	I	–	–	–	–	–	–	–	–	–	–	–	–	A
B. kaber		–	–	–	–	–	–	–	–	–	–	–	T	H	–	–	–
B. napus	Apex	–	–	–	–	–	A	A	–	–	A	A	–	–	–	–	A
	Bienvenu	–	–	–	–	–	B	C	–	–	A	A	–	–	A	–	A
B. naous var. *napobrassica*		–	A	–	–	–	B	–	–	–	A	A	–	–	–	–	A
B. naous ssp. *pabularia*		–	–	–	–	–	A	A	–	–	–	–	–	–	–	–	A
B. nigra		–	–	D	–	–	–	–	–	–	–	–	–	–	–	–	A
B. oleracea var. *acephala* subvar. *laciniata*	Green Curled Dwarf	A	B	A	A	B	A	B	–	–	–	–	–	–	–	A	A
	Nero De Toscana	–	A	–	B	C	T	T	–	–	–	–	–	–	–	A	A
	Pentland Brig	A	A	–	A	A	–	C	–	–	–	–	–	–	–	–	A
	Red Russian	–	T	–	T	A	C	C	–	–	–	–	–	–	–	–	A
B. oleracea var. *capitata*	Hispi	–	B	B	–	–	A	–	–	–	A	–	–	–	–	–	A
	Red Flare	T	B	B	A	B	A	A	–	–	–	–	–	–	–	–	A
B. oleracea var. *capitata*	Bedford Fillbasket	–	B	C	A	B	A	C	–	–	–	–	–	–	–	–	B
	Peer Gynt	–	–	–	A	A	B	B	–	–	–	–	–	–	–	–	A
B. repandra		–	–	–	–	–	–	–	–	–	–	–	–	H	–	–	–
B. oleracea var. *botrytis* aka *B. oleracea* var. *italica* (broccoli and calabrese)																	
	Arcadia F1	–	B	–	A	B	A	–	–		–	–	–	–	–	–	A
	Brocoletto	–	–	–	–	–	C	A	–	–	B	A	–	–	–	A	A
	Corvet F1	–	H	–	C	D	C	–	–	–	–	–	–	–	–	–	B
	De Cicco	–	A	–	B	C	–	–	–	–	–	–	–	–	–	A	A
	Italian Sprouting	–	–	–	C	E	T	–	–	–	–	–	–	–	–	–	B
	Marathon F1	–	A		B	E	–	–	–	–	–	–	–	–	–	–	A
	Morses 4638	–	A	–	A	D	–	–	–	–	–	–	–	–	–	A	A
	Pabst	–	–	–	C	E	T	–	–	–	–	–	–	–	–	–	B
	Pacifica	–	C	–	B	D	–	–	–	–	–	–	–	–	–	A	A
	Purple Sprouting	–	–	–	A	B	A	–	–	–	–	–	–	–	–	T	A
	Red Arrow	B	B	A	A	B	–	–	–	–	–	–	–	–	–	T	A
	Romanesco	T	–	–	A	B	A	–	–	–	–	–	–	–	–	T	A
	Trixie F1	–	–	–	C	E	–	–	–	–	–	–	–	–	–	–	B
	White Sprouting	–	A	–	A	C	A	–	–	–	–	–	–	–	–	T	A
F1 early crop	Cape Queen	–	A	A	A	B	A	C	–	–	–	–	–	–	–	A	A
	Green Comet	–	A	A	A	A	A	C	–	–	–	–	–	–	–	A	A
	Jewel	–	A	–	B	E	–	–	–	–	–	–	–	–	–	A	A
	Mercedes	–	A	–	B	E	–	–	–	–	–	–	–	–	–	A	A
	Packman	–	A	–	B	D	–	–	–	–	–	–	–	–	–	A	A
	SG1SC	–	A	A	B	C	A	C	–	–	–	–	–	–	–	A	A
	Southern Comfort	–	A	A	B	A	A	A	–	–	–	–	–	–	–	A	A
	Stolto	–	A	A	B	D	A	C	–	–	–	–	–	–	–	A	A
F1 main crop	Dandy	–	A	–	B	E	–	–	–	–	–	–	–	–	–	A	A
	Emerald City	–	A	–	B	E	–	–	–	–	–	–	–	–	–	A	A
	Green Duke	–	A	–	B	D	–	–	–	–	–	–	–	–	–	A	A
	Pirate	–	A	–	C	E	–	–	–	–	–	–	–	–	–	A	A
	Premium Crop	–	A	A	B	E	A	C	–	–	–	–	–	–	–	A	A
F1 late crop	Ginga	–	F	–	A	B	–	–	–	–	–	–	–	–	–	A	A
	Samurai	–	F	–	A	B	–	–	–	–	–	–	–	–	–	A	A
	Shogun	–	B	A	B	E	B	C	–	–	–	–	–	–	–	A	A
	Typhoon	–	–	–	A	A	B	–	–	–	–	–	–	–	–	A	A

exceptions; for example, seeds of *Reseda odorata* contained 3-indolylmethylglucosinolate, and *Biscultella laevigata* contained significant concentrations of both 3-indolylmethylglucosinolate and 4-hydroxy-3-indolylmethyl-glucosinolate. 4-Methoxy-3-indoylmethylglucosinolate was not found in any of the seeds analyzed, although it is a common component in the leaves and roots of some crucifer species (*16*). The *Raphanus* species and the great majority of *Brassica* species contained 4-hydroxy-3-indolylmethylglucosinolate in seeds (**Tables 2** and **3**). *Azima tetracantha* had a unique glucosinolate seed profile as it contained a high concentration of *N*-methoxy-3-indolylmethylglucosinolate. As with the 4-methoxy analogue, this indole glucosinolate is more commonly found in the vegetative tissues of crucifers than in seeds. Tissues of *Azima* species have been reported as famine foods, but the glucosinolate profiles of the tissues consumed, namely, leaves and other vegetative parts, have not been reported. Indole glucosinolates can form either nitriles or carbinols (via the transient formation of unstable isothiocyanates) upon hydrolysis. Only indole-3-acetonitrile, indole-3-carbinol, and 3,3′-diindolylmethane are commercially available for bioactivity studies. Indole-3-carbinol (and other carbinols) can either form conjugates with ascorbic acid (ascorbigens) or oligomerize to form molecules such as 3,3′-diindolylmethane (*26*−*28*). Considerable evidence for the in vitro and in vivo effects of the indole glucosinolate hydrolysis products, both positive and negative, has already been established. Positive effects include the anticancer and antiestrogenic activities of indole-3-carbinol and 3,3′-diindolylmethane, as well as inhibition of DNA adduct formation and DNA methylation (*29*−*31*). Negative effects include inhibition of flavin-containing monooxygenases and induction of phase I (procarcinogenic) cytochrome P450-type enzymes that could lead to adverse food−drug interactions (*32*−*35*).

Short- to medium-chain-length aliphatic glucosinolates (C3−C6) were found mainly within members of the Crucifereae (**Table 4**). C3−C5 aliphatic glucosinolates were a common

Table 4. Wild and Weed Species Containing C3–C6 Aliphatic Glucosinolates Derived from L-Methionine Homologues

	C3				C4								C5				C6	
species	**11**	**20**	**29**	**35**	**12**	**13**	**21**	**22**	**30**	**36**	**38**	**39**	**14**	**23**	**37**	**40**	**15**	**24**
Gyrostemon ramulosus	–	–	–	–	–	–	A	–	–	C	–	–	–	–	–	–	–	–
Aethionema armenum	–	A	T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Alliaria petiolata	–	–	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Alyssoides utriculata	–	–	–	–	C	–	A	–	–	C	–	–	–	–	–	–	–	–
Alyssum alpestre	D	C	–	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–
Alyssum alyssoides	–	–	–	–	D	–	B	–	–	D	–	–	A	T	–	–	–	A
Alyssum argentum	–	–	–	–	–	–	–	–	–	–	–	–	A	D	–	–	–	–
Alyssum lobularia procumbens	–	–	–	–	–	–	–	–	–	D	–	–	A	–	A	–	D	A
Alyssum minimum	–	–	–	–	–	–	–	–	–	D	–	–	A	–	A	–	D	A
Alyssum saxatile argentums	–	–	–	–	–	–	A	–	–	–	–	–	A	E	–	–	–	–
Alyssum saxatile compactum	–	–	–	–	–	–	A	–	–	C	–	–	B	C	A	–	–	–
Alyssum wulfenianum	–	–	–	–	–	–	–	–	–	–	–	–	A	A	–	–	T	A
Aurinia saxatilis	–	–	–	–	–	–	–	–	–	B	–	–	D	B	B	–	–	–
Bunias orientalis	–	–	–	–	E	–	A	–	–	–	–	–	–	–	–	–	–	–
Cardamine bulbifera	–	–	–	–	–	–	A	–	–	E	–	–	–	–	A	–	–	–
Cardamine hirsuta	–	–	–	–	–	–	–	–	–	C	–	–	–	–	C	–	–	–
Cardaria draba	–	–	–	–	T	–	B	–	–	–	–	–	–	–	–	–	–	–
Cheiranthus allionii	–	–	B	B	B	–	B	–	–	A	–	–	–	–	–	–	–	–
Cheiranthus cheiri	–	–	C	T	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cheiranthus linfolius	T	–	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Crambe cordifolia	–	–	–	–	–	–	B	–	–	–	A	E	–	–	–	–	–	–
Crambe maritima	–	–	–	–	–	–	T	–	–	–	T	D	–	–	–	–	–	–
Dentaria bulbifera	–	–	–	–	–	–	A	–	–	E	–	–	–	–	–	–	–	–
Diplotaxis tenuifolia	–	–	–	–	E	–	A	–	–	–	–	–	–	–	–	–	–	–
Eruca sativa	–	–	–	–	D	–	A	–	–	–	–	–	–	–	–	–	–	–
Eruca vesicaria	–	–	–	–	D	–	A	–	–	–	–	–	–	–	–	–	–	–
Erysimum allionii (1)	–	A	B	–	B	–	B	–	B	–	–	–	–	–	–	–	–	–
Erysimum allionii (*2*)	–	A	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Erysimum asperum	A	A	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Erysimum concinnum	B	D		A	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Erysimum linifolium	A	A	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Erysimum perovskianum	–	A	C	–	A	–	B	–	B	–	–	–	–	–	–	–	–	–
Erysimum pumillum	F	B	T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Erysimum suffruticosum	–	A	E	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fibigia clypeata	–	–	–	–	–	–	–	–	–	–	A	F	–	–	–	–	–	–
Hesperis matronalis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	B	B
Hugueninia tanacetifolia	–	–	–	–	–	–	–	–	–	H	–	–	–	–	–	–	–	–
Iberis amara	T	E	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Iberis compacta	–	–	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Iberis crenata	–	A	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Iberis gibraltarica	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Iberis hybrida	–	E	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Iberis saxatilis	–	E	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Iberis sempervirens	D	B	–	–	E	–	B	–	–	A	–	–	–	–	–	–	–	–
Iberis umbellata	A	D	–	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ionopsidium acaule	–	–	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Isatis tinctoria	–	–	–	–	–	–	–	–	–	B	B	–	–	–	–	A	–	–
Lepidium leptopetallum	–	–	–	–	I	–	E	–	–	E	–	–	B	–	–	–	–	–
Lepidium pedicillosum	–	–	–	–	D	–	A	–	–	C	–	–	D	B	B	–	C	B
Lepidium pholidogynum	–	–	–	–	B	–	–	–	–	C	–	–	B	–	A	–	T	A
Lepidium strongylophyllum	–	–	–	–	C	–	–	–	–	B	–	–	C	B	B	–	C	B
Lobularia maritima	–	–	–	–	–	–	–	–	–	C	–	–	A	–	A	–	C	A
Lunaria annua	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	T	A
Lunaria rediviva	–	–	–	–	–	–	A	–	–	–	–	–	B	D	–	–	T	A
Malcomia maritima	–	–	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Matthiola bicornis	–	–	–	–	T	T	T	D	–	–	–	–	–	–	–	–	–	–
Moricandia arvensis	–	–	–	–	–	–	–	–	–	–	D	–	–	–	–	–	–	–
Pachyphragma macrophyllum	–	–	–	C	–	–	–	–	–	F	–	–	–	–	–	–	–	–
Physaria newberryi	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Raphanus longipinnatus	–	–	–	–	T	–	T	E	–	–	–	–	–	–	–	–	–	–
Raphanus sativus (1)	–	–	–	–	T	–	T	C	–	–	–	–	–	–	–	–	–	–
Raphanus sativus (2)	–	–	–	–	–	–	T	E	–	–	–	–	–	–	–	–	–	–
Stanleya pinnata	–	–	–	–	–	–	–	–	–	A	C	A	–	–	–	–	–	–
Streptanthus insignis	–	B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Thlaspi arvense	–	–	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–

characteristic of most *Brassica* species (**Table 2**). These short- to medium-chain-length glucosinolates are the most common L-Met-derived glucosinolates. They have been suggested as a chemotaxonomic marker for this family (*1*). Long-chain-length aliphatic glucosinolates (C7−C10) were less common and were generally restricted to a few species within the Crucifereae (**Table 5**). *Biscutella laevigata* was a particularly good source of 8-methylsulfinyloctylglucosinolate (as well as certain indole glucosinolates). Many of the *Arabis* species contained significant concentrations of these long-chain glucosinolates, either alone or in combination with modified aromatic glucosinolates. *Nasturtium* and certain wild *Lepidium* species contained C7 and C8 aliphatic glucosinolates. As chain length increases the number of species containing these glucosinolates decreases, which may reflect the increased carbon demand required for synthesis of these glucosinolates or changes in enzyme specificity at the amino acid homologue chain elongation step.

Benzyl and related glucosinolates (derived from L-Phe or L-Tyr) were present in species from several families. Their presence in *Brassica* species was restricted to *B. kaber* and *B. repandra*, and these appear to be anomalous. Seeds of the *Tropaeolum* species primarily contained benzyl glucosinolate (including *T. majus* species) or structurally related derivatives as found in *T. pereginum* (aka *T. canariense*) (**Table 6**). This

Table 5. Wild and Weed Species Containing C7–C10 Aliphatic Glucosinolates Derived from L-Methionine Homologues

	C7		C8			C9			C10	
species	**16**	**25**	**17**	**26**	**32**	**18**	**27**	**33**	**28**	**34**
Arabis alpina	–	–	–	–	–	T	C	–	A	–
Arabis arendsii	–	–	–	–	–	–	C	T	A	–
Arabis blepharophylla	T	–	T	D	–	T	C	–	–	–
Arabis caucasica	–	–	T	–	–	T	C	T	A	–
Arabis hirsuta	–	–	–	A	–	–	–	–	–	–
Arabis procurrens	T	A	T	B	–	–	T	–	–	–
Arabis pumila	–	A	T	B	–	–	–	–	–	–
Arabis scopoliana	–	–	T	B	–	–	–	–	–	–
Arabis soyerii	–	–	T	C	–	–	–	–	–	–
Arabis stelleri	–	–	A	C	–	–	A	–	–	–
Arabis turrita	–	–	–	A	T	–	–	–	–	A
Biscutella laevigata	–	T	–	F	–	–	A	–	–	–
Camelina sativa	–	–	–	–	–	–	B	–	C	–
Lepidium pholidogynum	–	B	–	B	–	–	–	–	–	–
Lunaria rediviva	–	B	–	C	–	–	–	–	–	–
Nastutium officinalis	–	A	–	A	–	–	–	–	–	–
Sinapis arvensis	–	–	–	–	B	–	–	C	–	–

was also found for *Carica* species (*36*). *Hesperis matronalis*, *Moringa stenopetala*, *Moringa oleifera*, and *Reseda lutea* contained unusual sugar-substituted hydroxy-aromatic glucosinolates (*2*, *17*, *23*). These unique glucosinolates were detected in only these species. Although other species were identified with high concentrations of hydroxy-aromatic glucosinolates (*Aubretia*, *Sinapis*, and *Tropaeolum*), the common modification, if any, was 4-methylation rather than derivatization with a monosaccharide (**Table 6**).

2-Phenylethyl and related glucosinolates (derived from L-homophenylalanine) were far more restricted with respect to their seed distribution (**Table 6**). These were found in members of the Resedaceae and Crucifereae but were absent from seeds from the other families. Larger homologues (3-phenylpropyl and 4-phenylbutyl glucosinolate) have been reported in *Armoracia lapathifolia* (horseradish), but this has not been confirmed (*37*). Three new glucosinolates were identified in certain *Arabis* species, namely, *p*-hydroxy-2-phenethylglucosinolate, *p*-methoxy-2-phenethylglucosinolate, and (*R*)-*p*-hydroxy-2-hydroxy-2-phenylethylglucosinolate (**Table 6**). These are either derivatives of intermediates, or actual intermediates, of the previously identified (*R*)-*p*-methoxy-2-hydroxy-2-phenylethylglucosinolate. *Brassica* root vegetables (kohlrabi, swede, and turnip) and leafy salad crucifers such as watercress (*Nasturtium officinalis*) and landcress (*Barbarea praecox*) are the common dietary sources of 2-phenylethylglucosinolate for humans. Horseradish sauce is a common source for both 2-phenylethyl ITC and allyl ITC (from 2-phenylethyl and allyl glucosinolates, respectively). There are a considerable number of reports on the biological activities of 2-phenethylisothiocyanate, including studies on its absorption, metabolism, and anticarcinogenic properties (*38*–*45*).

By far the most commonly consumed crucifers in the human diet are the *Brassica* species (**Table 3**). These include broccoli/calabrese (*B. oleracea* var. *botrytis* subvar. *cymosa* and subvar. *italica*), cabbage (*B. oleracea* var. *capitata*), Brussels sprouts (*B. oleracea* var. *gemmifera*), cauliflower (*B. oleracea* var. *cauliflora*), brown and black mustard (*B. juncea* and *B. nigra*, respectively), Chinese cabbage (*B. campestris*), kale (*B. oleracea* var. *acephala*), Chinese kale (*B. alboglabra*), swede (*B. napus* var. *napobrassica*), and turnip (*B. campestris* ssp. *rapifera*). High concentrations of 2-propenylglucosinolate and 4-hydroxy-3-indolylmethylglucosinolate were found in all *B. juncea* cultivars analyzed; 4-hydroxybenzylglucosinolate was not detected in *B. juncea* seeds, in contrast to a previous report (*46*). There has been a significant focus on the glucosinolates, and their hydrolysis products, in *Brassica* species such as broccoli and Brussels sprouts. There are numerous papers on the absorption, metabolism, cellular effects, and positive health effects of sulforaphane (4-methylsulfinylbutylisothiocyanate, derived from glucoraphanin) and allyl isothiocyanate (2-propenylisothiocyanate, derived from sinigrin), two of the predominant hydrolysis products from these two species. However, there are also other bioactive hydrolysis products such as goitrin (derived from progoitrin in Brussels sprouts) and the potentially

Table 6. Wild and Weed Species Containing Aromatic Glucosinolates Derived from L-Phenylalanine and L-Tyrosine and Their Homologues

	benzyl series												PE series						
species	**41**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**
Tropaeolum pereginum	A	–	F	–	–	C	–	–	–	–	–	–	–	–	–	–	–	–	–
Tropaeolum majus	E	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Carica papaya	E	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Carica pentagona	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Moringa oleifera	–	–	T	–	–	–	–	–	–	I	–	–	–	–	–	–	–	–	–
Limnanthes douglasii	–	–	–	–	E	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Caylusea abyssinica	–	–	–	–	–	–	–	–	–	–	–	–	A	T	B	–	–	–	–
Reseda lutea	–	–	–	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	–
Reseda luteola	–	–	–	–	–	–	–	–	–	–	–	–	–	A	C	–	–	–	–
Reseda odorata	–	–	–	–	–	–	–	–	–	–	–	–	–	T	A	–	–	–	–
Aarabis hirsuta	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	C	C	B	D
Arabis procurrens	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	T	D
Arabis scopoliana	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	C	–	C
Arabis soyerii	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	C	B	D
Arabis stelleri	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	C
Aubretia deltoidea	–	–	C	–	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–
Aubretia hybrida	–	–	C	–	–	D	–	–	–	–	–	–	–	–	–	–	–	–	–
Barbarea praecox	–	–	–	–	–	–	–	–	–	–	–	–	G	–	–	–	–	–	–
Barbarea verna	–	–	–	–	–	–	–	–	–	–	–	–	H	–	–	–	–	–	–
Barbarea vulgaris	–	–	–	–	–	–	–	–	–	–	–	–	A	A	D	–	–	–	–
Cardamine hirsuta	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cardaria draba	–	–	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Coronopus.squamatus	–	–	–	–	–	–	–	–	–	–	–	D	–	–	–	–	–	–	–
Hesperis matronalis	–	–	–	–	–	–	G	F	–	–	B	–	–	–	–	–	–	–	–
Hutchinsia alpina	C	A	A	A	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lepidium campestre	–	–	H	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lepidium sativum	H	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nasturtium officinalis	–	–	–	–	–	–	–	–	–	–	–	–	F	–	–	–	–	–	–
Sinapis alba	–	–	I	–	–	–	–	–	–	–	–	–	B	–	–	–	–	–	–
Sinapis arvensis	–	–	H	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 7. Summary of Species Identified as Good Seed Sources of Common Human Dietary Glucosinolates

glucosinolate	common dietary source	best seed source for purification of glucosinolate	glucosinolate content (mg of K^+ salt g^{-1} of DW of seed)
alkyl			
methyl	*Capparis spinosa*	*Capparis spinosa*	24 ± 0.1
C3 aliphatic			
3-methylthiopropyl	*Brassica* species	*Erysimum pumillum*	53 ± 0.1
3-methylsulfinylpropyl	*Brassica* species	*Iberis amara*	37 ± 0.1
2-propenyl	*Brassica* species	*Brassica juncea* cv. Vitasso	111 ± 2
C4 aliphatic			
4-methylthiobutyl	*Brassica* species	*Lepidium leptopetalum*	108 ± 6
4-methylthio-3-butenyl	*Raphanus* species	*Raphanus sativus*	3 ± 0.1
4-methyklsulfinylbutyl	*Brassica* species	*Brassica oleracea*	56 ± 0.5
4-methylsulfinyl-3-butenyl	*Raphanus* species	*Raphanus longipinnatus*	39 ± 1
3-butenyl	*Brassica* species	*Brassica alboglabra*	44 ± 2
(*R*)-2-hydroxy-3-butenyl	*Brassica* species	*Brassica napus cv. Bienvenu*	12 ± 0.1
(*S*)-2-hydroxy-3-butenyl	seakale (*Crambe maritima*)	*Fibigia clypeata*	50 ± 2
C5 aliphatic			
5-methylthiopentyl	herb (*Hesperis matronalis*)	*Lepidium pedicillosum*	33 ± 6
5-methylsulfinylpentyl	herb (*Hesperis matronalis*)	*Alyssum argentum*	29 ± 0.1
4-pentenyl	*Brassica* species	*Cardamine hirsuta*	13 ± 0.1
(*R*)-2-hydroxy-4-pentenyl	*Brassica* species	*Brassica campestris*	2 ± 0.1
C6–C8 aliphatic			
6-methylthiohexyl	herb (*Hesperis matronalis*)	*Alyssum minimum*	31 ± 0.4
6-methylsulfinylhexyl	herb (*Hesperis matronalis*)	*Lepidium pedicillosum*	8 ± 0.1
7-methylthioheptyl	watercress (*Nasturtium officinalis*)	no seed source identified	–
7-methylsulfinylheptyl	watercress	*Lepidium pholidogynum*	12 ± 2
8-methylthiooctyl	watercress	*Arabis stelleri*	3 ± 0.5
8-methylsulfinyloctyl	watercress	*Biscutella laevigata*	52 ± 0.1
aromatic			
benzyl	cress (*Lepidium sativum*) Indian mustard (*Tropaelum majus*)	*Lepidium sativum*	84 ± 4
4-hydroxybenzyl	white mustard (*Sinapis alba*)	*Sinapis alba*	136 ± 3
2-phenylethyl	watercress	*Barbarea verna*	75 ± 2
indole			
3-indolylmethyl	*Brassica* species	*Biscutella laevigata*	5 ± 1
4-hydroxy-3-indolylmethyl	*Brassica* species	*Raphanus longipinnatus*	11 ± 3
4-methoxy-3-indolylmethyl	*Brassica* species	no seed source identified	–
N-methoxy-3-indolylmethyl	*Brassica* species	Azima tetracantha	24 ± 0.1

complex mixture of indole hydrolysis products (derived from indole glucosinolates found in both species) that also need to be considered (*7*, *8*, *13*, *26*−*35*).

A large number of previously published seed glucosinolate analyses have been performed by GC-MS of volatiles released by autolysis or by exogenous myrosinase treatment (*24*). Although this technique is rapid, it is not always accurate or reliable because hydrolysis products from certain aliphatic glucosinolates, all indole glucosinolates, and many hydroxyaromatic glucosinolates are unstable and cannot be identified by GC-MS. Reverse-phase C_{18} HPLC methods for either intact glucosinolates (*22*) or desulfoglucosinolates (*16*) are preferable and are generally more accurate for determining glucosinolate content. These methods are especially robust, powerful, and selective when they form, as in the work reported here, a component of an optimized negative ion mass spectrometry LC-MS method (*22*).

In taxonomic terms the Crucifereae (aka Brassicaceae) has been a convenient group in which to place many species that have certain structural and reproductive similarities. However, in the light of results reported here, this classification may require re-evaluation and further taxonomic subdivision in the Capparales. Evidence of possible discrepancies can be seen when glucosinolate profiles are compared. A much greater diversity in glucosinolate structures was observed between the (apparently) related crucifer species compared to the more defined structural types in other families. It is clear from seed glucosinolate profiles that the proposed chemotaxonomic marker of L-Met-derived glucosinolates is not applicable to all of the species placed in the Crucifereae. However, it may be a good general marker for *Brassica* and more closely related species. We suggest that the presence of 4-hydroxy-3-indolylmethyl glucosinolate may also be a useful taxonomic marker. On the basis of glucosinolate content, there are certain "*Brassica*" species that appear to be more closely related to *Sinapis*, for example, *B. kaber* and *B. repandra*, which contain 4-hydroxybenzylglucosinolate as shown by this and earlier work (*47*). There has been a significant focus on the glucosinolates, and their hydrolysis products, in *Brassica* species such as broccoli and Brussels sprouts. There are numerous papers on the absorption, metabolism, and cellular effects of sulforaphane (4-methylsulfinylbutyl-isothiocyanate) and allyl isothiocyanate (see the Introduction).

Although many crucifers contain aliphatic glucosinolates, there are clear subdivisions based on glucosinolate content: (i) only short- to medium-chain-length aliphatic (C3, or C3 and C4 with traces of C5); (ii) only long-chain-length aliphatic; (iii) only simple aromatic; and (iv) highly substituted aromatic. The seeds of *Arabis* species characteristically contain C8−C10 aliphatic glucosinolates either with or without highly modified aromatic glucosinolates. Other crucifers such as *Alyssum* and *Aurinia* (predominance of C5 and C6 aliphatic glucosinolates) and *Biscutella* (indole and long-chain aliphatic glucosinolates) have unique profiles.

The major purpose of this work was to identify species that had specific seed glucosinolates that could be used for the rapid purification of glucosinolate standards. Examples of species that contained high concentrations of a single or a small number of glucosinolates were found and can be considered to be useful sources of large quantities of glucosinolate standards. **Table 7** summarizes the data on common dietary glucosinolates and the amount (milligrams of K^+ salt of glucosinolate per gram of dry weight of seed) in the best species identified, so far, that is those that can be used for the purification of individual glucosinolates. The main advantage of using crucifer seeds as a source of glucosinolates is their high concentration relative both to other

seed secondary metabolites and to other crucifer tissues. Other major secondary metabolites commonly found in crucifer seeds are the choline esters of phenolic acids, for example, sinapine (sinapinic acid choline ester). In addition, many of the sources identified for isolating glucosinolate standards are weed species that generally produce seed far more quickly and in much higher amounts than many of the crop species, therefore making seed production for isolating the standards more efficient.

The importance of glucosinolates, and more specifically their hydrolysis products, in human health has been demonstrated by many researchers (*7*−*12*, *18*−*21*, *27*−*35*, *38*−*45*, *48*). It is therefore important to obtain pure standards, first, for accurate quantification of glucosinolates in crucifer vegetables and related products and, second, and perhaps more importantly, for cellular and human intervention studies evaluating the effect of glucosinolate, and hydrolysis product, structures on absorption, disposition, metabolism, and excretion. Currently a limited number of glucosinolates, and their corresponding desulfoglucosinolates, are available: C3 (glucoiberin, glucocheirolin, and sinigrin), C4 (glucoerucin, glucoraphanin, gluconapin, progoitrin, epiprogoitrin, and glucoraphenin), and aromatic (glucotropaeolin, gluconasturtiin, glucobarbarin, sinalbin, and glucosibarin). None of the indole glucosinolates are available, and a limited number of hydrolysis products are commercially available. There is interest in nutraceuticals, and high glucosinolate seeds offer a covenient source for product extraction. This study provides information that can be used for the selection of species for glucosinolate purification for the subsequent evaluation of glucosinolate hydrolysis product structure−activity relationships.

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Supporting Information Available: Organizations and individuals from whom seed material was obtained. This material is available free of charge via the Internet at http://pubs.acs.org.

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