

soluble in alkaline solutions, which would absorb light at the wavelength at which the sulfonamide is measured.

Table VIII demonstrates the adsorption characteristics of a number of primary aromatic amine compounds which were subjected to the resin treatment as outlined in the procedure. All 13 sulfonamides follow the same adsorption and elution pattern. It is expected, then, that the methods will be effective in determining these sulfonamides.

Other compounds in the series studied behaved in different ways. Two com-

pounds, *p*-chloro- and *p*-nitroaniline, were adsorbed from the acid medium but were not eluted with the concentrated NH_4OH . Sulfanilic acid and procaine were not adsorbed by the resin but passed through in the acid effluent.

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SEED MEAL CONSTITUENTS

Oxazolidinethiones and Volatile Isothiocyanates in Enzyme-Treated Seed Meals from 65 Species of Cruciferae

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Information is unavailable concerning the amounts and types of isothiocyanate-yielding glucosides in many species of Cruciferae seeds. Because such compounds have nutritional significance, a number of unreported species were investigated. Information about the parent thioglucosides was obtained by estimation of the oxazolidinethione and steam-volatile isothiocyanate contents of enzymatic hydrolyzates of the seed meals. Significant amounts of oxazolidinethione were found in hydrolyzates from 11 species not previously known to contain such glucosidic precursors. Oxazolidinethione (calculated as vinyl oxazolidinethione) measurements ranged from 0 to 19.3 mg. per gram of pentane-hexane-extracted meal. Total volatile isothiocyanate measurements (calculated as butenyl isothiocyanate) ranged from 0 to 21.6 mg. per gram of pentane-hexane-extracted seed meal. Probable identification of the predominant volatile isothiocyanates produced in some of the hydrolyzates was obtained by paper chromatography of their thiourea derivatives.

MANY CRUCIFERAE produce potentially valuable industrial oil-seeds (78). The solvent-extracted seed meals from the species contain large amounts of protein (14 to 61%) having nutritionally favorable amino acid composition as determined by chemical analysis (79). However, Cruciferae contain thioglucosides which yield isothiocyanates with various structures (8). These isothiocyanates are liberated from their parent thioglucosides by enzymes normally present in crushed or ground moist seed. Isothiocyanates, other enzymatically formed products, or thioglucosides themselves may impart unpalatability or toxicity or both to the meals. The β -hydroxyisothiocyanates, after liberation from their thioglucosides, spontaneously cyclize to oxazolidine-

thiones (74), which are known to interfere with proper function of the thyroid (7). The literature concerning these compounds, even in very closely related species, is inadequate for predicting or assuming what types or amounts will be found in uninvestigated species.

In the present seed compositional analyses, many Cruciferae were encountered for which little or no literature information of this nature was available. Fundamental information was needed on the amount and kind of these compounds which affect the use of the seed meal as food or feed. This paper reports oxazolidinethione and volatile isothiocyanate measurements after enzyme treatment of seed meals from 65 species of Cruciferae. For comparison or because additional information was

obtained, measurements on some species cited in prior literature have been included.

Experimental

Preparation of Sample and Estimation of Volatile Isothiocyanates. Seed meals were prepared by grinding the seed in a 6-inch hammer mill, with $1/16$ -inch, round-hole screen, followed by extraction in a Butt apparatus with petroleum ether (pentane-hexane, b.p. 35° - 57° C.). Extraction by this method appeared to avoid the conversion of thioglucoside and loss of isothiocyanate encountered by Kjaer, Conti, and Larsen (10) when they extracted rapeseed with ligroin and ethanol at room temperature.

Enzyme treatment of the seed meals and estimation of the liberated oxazolidinethiones and volatile isothiocyanates were performed according to the methods described by Wetter (24, 25) except for distillation of isothiocyanates into a larger volume of ammonia without the presence of silver nitrate. [Thioglucoside conversion was allowed to proceed for 3 hours at pH 4 as described by Wetter (24). Later work in this laboratory, however, showed that when working with purified thioglucosides, optimum yield of oxazolidinethiones was obtained near pH 7.] Thiourea conversion was allowed to proceed overnight. A portion of this distillate was treated with silver nitrate to determine total volatile isothiocyanate. This slight modification of Wetter's

analytical procedure allowed paper chromatographic investigation of a portion of the distillate without significantly affecting the isothiocyanate determinations. Analysis of five species by Wetter's procedure and by the modification for comparison gave results within experimental error of each other. Total amounts of the volatile isothiocyanates formed from the seed meals were calculated as butenyl isothiocyanate. This compound, which was most widely distributed among the steam-volatile isothiocyanates, has a molecular weight near that of the average of all isothiocyanates found. Duplicate determinations varied ± 0.5 mg. of butenyl isothiocyanate per gram of meal.

Chromatographic examination of the thiourea solutions was carried out as

follows. The remainder of the distillate was concentrated to near dryness under reduced pressure (45° C.). The residue (usually oily) was dissolved in about 10 ml. of ethyl alcohol (95%), filtered, and reconcentrated. The thioureas were redissolved in enough ethyl alcohol (95%) to make an approximately 1% solution as calculated from the isothiocyanate measurement. From 2 to 5 μ l. of the 1% solution was spotted on paper for chromatography of the thioureas by the method of Kjaer and Rubinstein (16) in which water-saturated chloroform was the developing solvent. Thiourea derivatives were detected with Grote's reagent (5). Rate of migration of each derivative was related to that of known phenylthiourea (R_{ph}). Tenta-

Table I. Oxazolidinethiones and Volatile Isothiocy-

Genus and Species	Oxazolidinethione, Mg./Gram of Meal, Calcd. as Vinyl Oxazolidinethione	Volatile Isothiocyanate, Mg./Gram of Meal, Calcd. as Butenyl Isothiocyanate	Thiourea Derivatives of Volatile Isothiocyanates ^a		Relative size and intensity of spots
			$R_{ph} \pm 0.03$	Probable identity based on chromatographic mobility	
<i>Alyssum campestre</i> ^b L.	0	<0.5	
<i>Alyssum dasycarpum</i> ^b Steph.	0.8	<0.5	
<i>Alyssum minimum</i> ^b Willd.	0	<0.5	
<i>Alyssum saxatile</i> L.	0	8.4	0.61	3-Butenyl	P
			0.92	4-Pentenyl	T
			1.13	5-Methylthiopentyl	P
<i>Alyssum tortuosum</i> ^b Rupr.	0	9.2	1.13	5-Methylthiopentyl	
<i>Arabis alpina</i> L.	0	<0.5	
<i>Arabis glabra</i> ^b (L.) Bernh.	0	4.7	0.00	...	T
			0.13	Unidentified	T
			1.20	6-Methylthiohexyl	P
<i>Arabis laevigata</i> ^b (Muhl.) Poir.	0	7.2	0.26	Allyl	P
			0.41	Isopropyl	T
<i>Arabis virginica</i> ^b (L.) Poir.	5.1	0.9	
<i>Barbarea vulgaris</i> R. Br.	8.7	2.5	1.06	β -Phenylethyl	
<i>Boreava orientalis</i> ^b Jaub. et Sp.	0.1	11.2	0.61	3-Butenyl	
<i>Brassica campestris</i> L.	0-1.2	7.1-16.3	0.61	3-Butenyl	
<i>Brassica carinata</i> ^b A. Br.	0	11.3	
<i>Brassica juncea</i> (L.) Coss.	0	14.8	
<i>Brassica napus</i> L.	4.3-6.2	5.9-6.0	0.61	3-Butenyl	P
			0.92	4-Pentenyl	T
			1.06	β -Phenylethyl	T
<i>Brassica nigra</i> (L.) Koch.	0	21.6	0.26	Allyl	
<i>Brassica oleracea</i> var. <i>capitata</i> L.	0.1	12.4	0.26	Allyl	
<i>Brassica rapa</i> L.	1.6	6.1	
<i>Cakile edentula</i> ^b (Bigel.) Hook.	0	21.6	0.00	...	T
			0.26	Allyl	P
			0.74	<i>sec</i> -Butyl	T
<i>Camelina microcarpa</i> Andrz.	0	0.9	
<i>Camelina rupestris</i> ^b Velen.	0	<0.5	
<i>Camelina sativa</i> (L.) Crantz	0	1.3	
<i>Capsella bursapastoris</i> (L.) Medic.	0	1.4	
<i>Cheiranthus cheiri</i> L.	0	9.4 (?)	0.00	?	
<i>Conringia orientalis</i> (L.) Dumort	19.3	<0.5	
<i>Conringia planisiliqua</i> ^b Fisch and Mey.	0	9.6	0.26	Allyl	T
			0.61	3-Butenyl	P
<i>Crambe abyssinica</i> ^{b,c} Hochst. ex R. E. Fries	3.0-5.5	1.7-2.5	0.00	...	T
			0.13	Unidentified	T
			0.26	Allyl	P
			0.61	3-Butenyl	P
			0.90	4-Pentenyl or benzyl	T
			1.06	β -Phenylethyl	T
<i>Crambe orientalis</i> ^{b,c} L.	0.5	8.8	0.61	3-Butenyl	
<i>Crambe</i> ^{b,c} <i>tatarica</i> Jacq.	8.6	1.6	
<i>Descurainia millefolia</i> ^b Webb and Berth	2.6	4.8	0.26	Allyl	P
<i>Descurainia pinnata</i> subsp. <i>ochroleuca</i> ^d (Woot.) Detl.	0.5	8.5	
<i>Descurainia sophia</i> ^d (L.) Webb	0	6.3-7.4	0.26	Allyl	T
			0.61	3-Butenyl	P

^a P, predominant spot; T, minor or trace spot; broken lines, thioureas not chromatographed. ^b Species not previously investigated.

tive identifications were based on literature R_{ph} values and comparison with available known thiourea derivatives.

Estimation of Oxazolidinethiones. An ether extract of the hydrolyzate after steam distillation was examined for absorption in the wave length range of 230 to 266 $m\mu$ with a Beckman DU spectrophotometer. Only those preparations that showed an absorption maximum at 248 $m\mu$ were considered to contain oxazolidinethione. From this absorption peak, the amount of the compound was calculated as vinyl oxazolidinethione. The six oxazolidinethiones (8, 9, 17) that have been isolated from enzymatic hydrolysis of natural products were assumed to have a maximum absorption at this wave length.

Results and Discussion

Oxazolidinethiones. Seed from 18 species formed oxazolidinethiones (Table I). Of these, only trace amounts (0.5 mg. per gram of meal or less) were found from four. The compound is formed from seed of three species of *Crambe*, two of *Descurainia*, and one each of *Alyssum*, *Arabis*, *Fibigia*, *Selenia*, *Sisymbrium*, and *Stanleyella*—none previously reported from the species examined. Of all species listed, oxazolidinethione was previously reported as formed from species of *Brassica* (7), *Barbarea vulgaris* R.Br. (13), and *Conringia orientalis* (L.) Dumort (6, 14). On the basis of the data given, thioglucosides that form oxazolidinethione are not as widespread as those that form volatile isothiocyanates.

Small amounts of absorption were obtained from 13 species, none of which had a maximum at 248 $m\mu$. Ether extracts of the hydrolyzates from *Arabis laevigata* (Muhl.) Poir., *A. glabra* (L.) Bernh., and *Malcomia maritima* R.Br. had a maximum at about 255 $m\mu$. Those of *Cheiranthus cheiri* L., *Isatis aucheri* Boiss., *Matthiola bicornis* DC., *Raphanus caudatus* L., *R. sativus* L., and *Rapistrum rugosum* (L.) All. showed a maximum at 240 to 245 $m\mu$. Ether extracts of the hydrolyzates from *Iberis amara* L., *I. umbellata* L., *Lepidium draba* L., and *Sinapis arvensis* L. did not have a maximum in the 230 to 266 $m\mu$ range but had a progressive increase in absorption as measurements were made from the longer to the shorter wave lengths. The absorption data as described for

anates from Enzyme-Treated Cruciferae Seed Meals

Genus and Species	Oxazolidinethione, Mg./Gram of Meal, Calcd. as Vinyl Oxazolidinethione	Volatile Isothiocyanate, Mg./Gram of Meal, Calcd. as Butenyl Isothiocyanate	$R_{ph} \pm 0.03$	Thiourea Derivatives of Volatile Isothiocyanates ^a		Relative size and intensity of spots
				Probable identity based on chromatographic mobility		
<i>Dithyrea wislizenii</i> ^b Engelm.	0	2.6	0.13	Unidentified		T
<i>Eruca sativa</i> Mill.	0	18.2-19.9	0.99	4-Methylthiobutyl		
<i>Erucastrium strigosum</i> ^b O. E. Schulz	0	3.5 (?)	No spot	?		
<i>Erysimum perofskianum</i> Fisch and Mey.	0	6.5	0.00	...		T
			0.99	4-Methylthiobutyl		P
			1.30	Unidentified		T
<i>Fibigia clypeata</i> ^d (L.) Medik.	4.2	2.3 (?)	No spot	(?)		
<i>Hesperis matronalis</i> L.	0	2.9	0.00	...		T
			0.26	Allyl		P
			1.17	Unidentified		
<i>Iberis amara</i> L.	0	4.8 (?)	0.00	(?)		
<i>Iberis umbellata</i> L.	0	13.7	0.00	...		
			0.26	Allyl		T
			0.76	sec-Butyl or 3-methylthiopropyl		P
				3-Butenyl		T
<i>Isatis aucheri</i> ^b Boiss.	0	12.4	0.61	3-Butenyl		P
<i>Isatis tinctoria</i> L.	0	7.4	0.61	3-Butenyl		T
<i>Lepidium draba</i> ^b L.	0	3.0	0.00	...		P
			0.99	4-Methylthiobutyl		P
<i>Lepidium lasiocarpum</i> ^b Nutt.	0	1.7		
<i>Lepidium latifolium</i> L.	0	7.4	0.26	Allyl		T
			0.90	Benzyl		P
<i>Lepidium montanum</i> ^b var. <i>angustifolium</i> C. L. Hitchc.	0	10.3	0.41	Isopropyl		T
			0.74	sec-Butyl		T
			0.99	4-Methylthiobutyl		P
<i>Lepidium perfoliatum</i> ^b L.	0	11.2	0.00	...		T
			0.99	4-Methylthiobutyl		P
<i>Lepidium sativum</i> L.	0	17.1	0.90	Benzyl		
<i>Lepidium virginicum</i> L.	0	13.8	0.90	Benzyl		
<i>Lobularia maritima</i> ^d (L.) Desv.	0	8.8	0.61	3-Butenyl		P
			1.13	5-Methylthiopentyl		P
<i>Lunaria annua</i> L.	0	9.9	0.41	Isopropyl		P
<i>Malcomia maritima</i> R. Br.	0	5.4 (?)	0.00	(?)		
<i>Matthiola bicornis</i> DC.	0	2.8		
<i>Nasturtium officinale</i> R. Br.	0	10.6	1.06	β -Phenylethyl		
<i>Nerisyrenia camporum</i> ^b (Gray) Greene	0	7.9	1.13	5-Methylthiopentyl		
<i>Raphanus caudatus</i> L.	0	2.5 (?)	...	(?)		
<i>Raphanus sativus</i> L.	0	4.2 (?)	0.00	(?)		
<i>Rapistrum rugosum</i> (L.) All.	0	11.6 (?)	0.00	(?)		
<i>Selenia grandis</i> ^b Martin.	5.4	3.2	1.04	<i>p</i> -Methoxybenzyl or β -phenylethyl		
<i>Sinapis arvensis</i> L.	0	0.6		
<i>Sisymbrium garipepinum</i> ^b Burch.	6.4	0.9		
<i>Sisymbrium irio</i> ^b L.	0	5.1	0.41	Isopropyl		P
			0.74	sec-Butyl		T
<i>Stanleyella texana</i> ^b (Cory) Rollins	4.5	0.5		
<i>Thlaspi arvense</i> L.	0	11.6	0.26	Allyl		
<i>Thlaspi perfoliatum</i> L.	0	1.4		

^a Sample included pericarp. ^d Possibly investigated under another name.

these 13 species were interpreted as evidence for the presence of compounds other than oxazolidinethiones but perhaps of related structure.

Brassica campestris L. and *B. napus* L. are both commonly referred to as rapeseed. *B. napus* L. consistently formed more oxazolidinethione than *B. campestris* L., whereas the remaining species of *Brassica* examined formed only small amounts or none. Ettlinger and Thompson (4) have recently made an extensive report on the thioglucosides of *Brassica* species.

The three species of *Camelina* formed no oxazolidinethiones and little or no volatile isothiocyanates. However, Kjaer, Gmelin, and Jensen (15) isolated the nonvolatile 10-methylsulphinyldecyl isothiocyanate from *C. sativa* (L.) Crantz and found a second unidentified thioglucoside. On the basis of paper chromatography, they concluded that the same two thioglucosides are also in *C. microcarpa* Andr.

This laboratory found oxazolidinethiones were produced by *Fibigia clypeata* but not by *Isatis tinctoria*. Kjaer found evidence of ring structures from both (7).

Volatile Isothiocyanates. No information was found in the literature concerning the amounts or kinds of volatile isothiocyanates formed in 28 of the species reported (Table I). Information on four additional species under other names may have been reported by other investigators. For the most part, the present findings agree with reported literature values for the remaining species.

Of the five species of *Alyssum*, three yielded only trace amounts of volatile isothiocyanates. Information obtained on *A. saxatile* L. confirmed that reported by Kjaer and Gmelin (12). Paper chromatographic results showed the formation of 3-butenyl- and 5-methylthiopentyl isothiocyanate from *Lobularia maritima* (L.) Desv. Kjaer and Gmelin reported this species as *Alyssum maritimum* Lam., from which they demonstrated the formation of the nonvolatile 5-methylsulphinylpentyl isothiocyanate (12). The formation of 6-methylthiohexyl isothiocyanate from seed of *Arabis glabra* (L.) Bernh. shows the parent glucoside to be present in one genus in addition to the *Lesquerella* (3) from which the same thiomethyl thiourea derivative was first isolated (2).

Information on *Barbarea vulgaris* R.Br. confirmed that previously reported (13). *Cakile edentula* (Bigel.) Hook. seed forms large amounts of predominantly allyl isothiocyanate. In this respect, it is similar to *Brassica juncea* (L.) Coss. and *B. nigra* (L.) Koch. Seed from *Eruca sativa* Mill. forms the single volatile 4-methylthiobutyl isothiocyanate in relatively large amounts. This isothiocyanate was isolated from *E. sativa* Mill., and characterized through the

thiourea derivative by Kjaer and Gmelin (17). *Erysimum perofskianum* Fisch. and Mey. is believed to contain five thioglucosides (22). The sulfone counterpart of 4-methylthiobutyl isothiocyanate from this species has been isolated by Schneider and Kaufmann (27). The predominant volatile isothiocyanate was found to be 4-methylthiobutyl isothiocyanate. Seed of *Hesperis matronalis* L. has been reported to contain three to five thioglucosides (8). Allyl isothiocyanate was found to be the preponderant volatile isothiocyanate from this species.

Species of *Lepidium* are usually rich in benzyl isothiocyanate (8), and the leaves of *Lepidium draba* L. have been shown to form the nonvolatile sulfoxide of 4-methylthiobutyl isothiocyanate (20). 4-Methylthiobutyl isothiocyanate was found to be the predominant volatile isothiocyanate formed from seed of three *Lepidium* species. Information obtained concerning *Nasturtium officinale* R.Br. agreed with that previously reported (10).

Neither allyl isothiocyanate nor isopropyl isothiocyanate was found in the sample of *Raphanus sativus* L. These two volatiles have been reported in small amounts for specific varieties of this species (10). The formation of allyl isothiocyanate from *Thlaspi arvense* L. was confirmed (10, 23).

The production of specific kinds of thioglucosides does not appear to reflect generic relationships. For example, *Conringia orientalis* (L.) Dumort contains large amounts of the thioglucoside which forms 5,5-dimethyl-2-oxazolidinethione (6, 14), while *Conringia planisiliqua* Fisch and Mey. contains no thioglucosides of this type. *Crambe abyssinica* Hochst. ex R. E. Fries contains both oxazolidinethione and volatile isothiocyanate-forming thioglucosides. *Crambe orientalis* L. contains nearly all volatile isothiocyanate-forming thioglucoside, and *Crambe tatarica* Jacq., nearly all thioglucosides which form oxazolidinethione. The thioglucoside from *Sisymbrium garietpinum* Burch. forms oxazolidinethione, and the thioglucosides from *S. irio* L. form volatile isothiocyanates.

Thiourea derivatives of the volatile isothiocyanates from *Cheiranthus cheiri* L., *Iberis amara* L., *Malcomia maritima* R.Br., *Raphanus sativus* L., and *Rapistrum rugosum* (L.) All. on chromatography gave only one spot which remained at the origin. According to Kjaer (7), spots remaining at the origin are probably due to sulfoxides from the corresponding 3-methylthiopropyl and 4-methylthiobutyl isothiocyanates, and the spot with R_{Fh} 0.13 is probably due to the sulfoxide from the corresponding 6-methylthiohexyl isothiocyanate. He states that these are probably artifacts formed from the corresponding thiomethyl compounds after steam distillation.

Volatiles which reacted with silver nitrate were formed from *Erucastrum strigosum* O. E. Schulz, *Fibigia clypeata* (L.) Medik, and *Raphanus caudatus* L. However, no thiourea spot could be detected by paper chromatography.

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