cil hardness = 1 or 2), but hardness increased on further curing of the films to the values shown. Hardness and resistance to alkali were improved by increasing styrene content of the polymer.

Preliminary results of evaluation tests (11) indicate that films of a styrenated conjugated linseed polymer, when baked at either 205C for 30 min or 232C for 15 min, had excellent flexibility and resistance to reverse impact. Styrenated linseed polymers showed good compatibility with TiO_2.

Compatibility. Perhaps the most significant new property of the styrenated polymers is their compatibility with a number of commercial resins. Nonstyrenated vinyl ether polymers and copolymers were almost entirely incompatible with 20 selected commercial resins at polymer-to-resin ratios of 1:9, 1:1, and 9:1 (11). Such incompatibility greatly limits the use of vinyl ethers because they cannot be blended with other coating materials to modify their properties. A styrenated conjugated linseed vinyl ether polymer containing 30% styrene by weight is compatible at the 1:1 ratio with a number of commercial resins (Table VII). One unusual feature of this styrenated linseed polymer is that it is incompatible with other vinyl ether polymers and other styrene-containing resins, such as styrene-butadiene or styrenated alkyd resins.

Since these new products exhibit outstanding alkali resistance and compatibility with commercial resins, better methods of preparation leading to improved stability of the products should be sought.

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Isothiocyanates from Enzymatic Hydrolysis of *Lesquerella* Seed Meals[1]

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Abstract

Enzymatic hydrolyzates from seed meals of 17 species of the genus *Lesquerella*, family Cruciferae, were examined for thiooxazolidones and volatile isothiocyanates. Quantitative estimates of the total volatile isothiocyanates, calculated as butenyl, ranged from 0.6 to 13.0 mg per g of solvent-extracted meal. No thiooxazolidone was found. By means of paper chromatography, melting point, and X-ray patterns of the thiourea derivatives, the volatile isothiocyanates, 3-methylthiopropyl-, 4-methylthiobutyl-, and 6-methylthiohexyl-, were shown to be present in the hydrolyzates from three species. Evidence for identity of isothiocyanates in hydrolyzates from the remaining species was obtained by paper chromatography of the thiourea derivatives.

SEED OF THE GENUS *Lesquerella*, family Cruciferae, yields oil containing hydroxy fatty acids of potential industrial importance (7). Utilization of the protein-containing meal as feed is obviously desirable if commercialization of a species of *Lesquerella* as an oilseed occurs. The amino acid composition of the meal indicates good nutritional quality (8). However, widespread occurrence of isothiocyanate-yielding glucosides in the crucifer family is well known (11). Beta-hydroxyisothiocyanates after enzymatic liberation from the glucose spontaneously cyclize to thiooxazolidones, which adversely affect thyroid activity in animals. Volatile isothiocyanates (mustard oils) impart unpalatability to meals if present in sufficient amount and furthermore may be toxic. To our knowledge no literature information concerning the presence of these compounds in *Lesquerella* is available other than the investigation of a single species at this laboratory (1). For these reasons a study of derived isothiocyanates in seeds from *Lesquerella* species was undertaken.

Methods

Seed meals were prepared by grinding the seed in a 6-in hammer mill with $\frac{1}{16}$-in. round-hole screen followed by extraction in a Butt apparatus with petroleum ether (pentane-hexane bp 33–57C).

Hydrolysis of the meals and estimation of the liberated thiooxazolidones and volatile isothiocyanates were performed according to the methods described by Wetter (9,10) except that the volatile isothiocyanates were collected in a larger volume of ammonia without the presence of silver nitrate. An aliquot of this distillate was treated with silver nitrate to determine total volatile isothiocyanate. The remainder was used for paper chromatography of the thioureas

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TABLE I

Volatile Isothiocyanates from *Lesquerella* Seed Meals

Lesquerella species	Mg/g meal, calculated as butenyl isothiocyanate	R_{ph}±0.03[a]	Probable identity of the thiourea derivative, based on chromatographic mobility
Angustifolia	9.0	0.99	4-Methylthiobutyl-[b]
		0.00 (Trace)	.....
Argyraea[c]	2.8	0.00	.....
Densipila	5.2	1.20	6-Methylthiohexyl-
		0.13 (Trace)	.....
Engelmannii	3.5	1.20	6-Methylthiohexyl-
		0.13 (Trace)	.....
Fendleri[d]	3.9	0.77	3-Methylthiopropyl- or secondary butyl-
		0.41	Isopropyl-
Globosa	13.0	0.77	3-Methylthiopropyl-[b]
		1.20 (Minor)	6-Methylthiohexyl-
		0.00 (Trace)	.....
Gordonii	3.0	0.00	.....
Gracilis	10.6	0.77	3-Methylthiopropyl- or secondary butyl-
		0.00 (Trace)	.....
Grandiflora	6.4	1.20	6-Methylthiohexyl-[b]
Lasiocarpa	7.1	1.20	6-Methylthiohexyl-
		0.13 (Trace)	.....
Lescurii	10.2	1.20	6-Methylthiohexyl-
		0.13 (Trace)	.....
Lindheimeri	7.0	0.77	3-Methylthiopropyl- or secondary butyl-
		0.00 (Trace)	.....
Lyrata	7.1	1.20	6-Methylthiohexyl-
		0.13 (Trace)	.....
Ovalifolia	0.6	.....	.....
Perforata	7.1	1.20	6-Methylthiohexyl-
		0.13 (Trace)	.....
		0.00 (Trace)	.....
Pinetorum	4.5	0.00	.....
Stonensis	6.9	1.20	6-Methylthiohexyl-

[a] $R_{ph} = \frac{\text{migration of unknown}}{\text{migration of phenylthiourea}}$

[b] Established by isolation of crystalline derivative.

[c] Heavily loaded paper chromatograms also gave trace amounts with R_{ph} values of 1.20, 0.77, and 0.13.

[d] Heavily loaded paper chromatograms also gave trace amounts with R_{ph} values of 1.20 and 0.13.

by the method of Kjaer and Rubinstein (6) in which water-saturated chloroform was the developing solvent. The thiourea derivatives were detected with Grote's reagent (2).

For the preparation of crystalline thioureas the glucosides were enzymatically hydrolyzed from appropriate samples of meal, and the resulting volatile isothiocyanates were steam distilled into ammonia in the same manner as described in the analytical procedure. The ammoniacal solution was taken to near drying under reduced pressure in a rotary evaporator at 40C, taken up in ethanol, filtered, concentrated to near dryness, and taken up in either water or aqueous ethanol from which the thiourea crystallized.

Results and Discussion

Total volatile isothiocyanate, calculated as butenyl, ranged from 0.6 to 13.0 mg per g of air-dry, extracted, seed meal (Table I). This range of total volatile isothiocyanate formed was similar to that reported for species from a number of genera of the crucifer family (3). No thiooxazolidones were found as hydrolytic products in any of the seed meals.

The yield of crystalline 3-methylthiopropylthiourea derived from 6 g of meal from *L. globosa* was 19 mg, mp 63–65C; second crop, 8 mg, 65–67C. Authentic 3-methylthiopropylthiourea melts at 66–67C (5).

Yield of crystalline 4-methylthiobutylthiourea derived from 6 g of meal from *L. angustifolia* was 15 mg, mp 49–50C. A sample of 4-methylthiobutylthiourea was also prepared from *Eruca sativa* seed meal, a known source of the glucoside from which it was derived (4). Yield from 5 g of meal was 60 mg, mp 49–50C. Reported melting point for 4-methylthiobutylthiourea is 53C (4). X-ray patterns of the two thioureas were identical. There was no depression of mixed melting point.

Yield of crystalline 6-methylthiohexylthiourea derived from 3 g of meal from *L. grandiflora* was 5 mg, mp 73–74C. This sample gave an X-ray pattern identical with that of 6-methylthiohexylthiourea whether synthesized or isolated from *L. lasiocarpa* (mp 72–73C) as previously reported (1).

Paper chromatography of the thiourea derivatives showed at least six thioureas were obtained from the genus (Table I). Comparison of migration distances with that of phenylthiourea gave R_{ph} values for four of the thioureas which agree with those reported in the literature (3–6) for the three methylthio containing thioureas and isopropylthiourea. Additional evidence for the identity of the 6- and 4-methylthio-compounds was their migration identical with the two compounds isolated from known sources, described above, when run on the same paper strip chromatogram. Secondary butylthiourea, mp 134C, migrates in the solvent system at about the same rate as 3-methylthiopropylthiourea (3). For this reason the spots with R_{ph} values of 0.77 may be due to either or both of these two compounds in the two species from which the crystalline thiourea was not isolated.

The R_{ph} value of 0.41 for the second thiourea derivative from *L. fendleri* was the same as that of isopropylthiourea (6). Seed from eight species provided thiourea derivatives for the most part present in trace amounts, which showed no chromatographic mobility. Under the same conditions methyl thiourea showed slight mobility similar to that previously reported (6). For this reason the nature of these nonmobile compounds was considered unknown. Trace amounts of material from six species gave an R_{ph} value of 0.13, which is slightly lower than the R_{ph} of 0.15 for synthetic ethylthiourea. Because of this difference in R_{ph} value the identity of these chromatographic spots was considered inconclusive.

Seed from the *Lesquerella* genus apparently contains a class of thioglucosides that yield predominantly terminal methylthioisothiocyanates on enzymatic hydrolysis. This conclusion is based on our isolation of the thiourea derivatives of isothiocyanates obtained from three selected species and on tentative identification of thioureas obtained from other species by paper chromatography. More should be known about the physiological effects of these compounds if seed meal from species containing them is fed to animals. The absence of thiooxazolidone-forming glucosides should simplify processing of the meal for feed purposes.

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