Acid hydrolysis of Vb was accomplished according to the procedure described for Va. The crude product was purified by two recrystallizations from water. The yield was 0.2 g. (54%) of white, crystalline *cis*-1,4-cyclohexanediacetic acid, m.p. 166–167°.

Anal. Calcd. for $C_{10}H_{16}O_4$: C, 59.93; H, 8.06. Found: C, 60.00; H, 8.06.

trans-1,4-Bis(2-aminoethyl)cyclohexane (VIa). *Method A.* To a well-stirred slurry of 21.3 g. (0.56 mole) of lithium aluminum hydride and 554 ml. of tetrahydrofuran was added 27.7 g. (0.17 mole) of Va in 554 ml. of tetrahydrofuran over a period of 4 hr. After addition was complete, the reaction mixture was refluxed for 2 hr. and then cooled in an ice-salt bath. Twenty-four milliliters of water, 15.7 ml. of 20% sodium hydroxide, and 68 ml. of water were added in succession to the cooled reaction mixture. The light brown precipitate which formed was separated by filtration and washed twice with 150-ml. portions of tetrahydrofuran. The combined filtrate and washings were distilled until most of the solvent had been removed. The oily residue was acidified with 200 ml. of 5% hydrochloric acid while the mixture was being cooled. The resulting acidic solution was extracted with two 100-ml. portions of ether to remove the unchanged diacetonitrile. Two hundred milliliters of 10% sodium hydroxide was added to the aqueous acidic layer and the resulting mixture was extracted with ten 80-ml. portions of benzene. After removing the solvent from the combined extracts by distillation, the crude amine (17.0 g.) was distilled *in vacuo* to yield 10.0 g. (34%) of a colorless oil, b.p. 122–126° (1 mm.).

Method B. The procedure followed for this method was adapted from that employed in the preparation of octylamine by Amundsen and Nelson[10] except that the reaction mixture was refluxed for 16 hr. From 9.3 g. (0.057 mole) of Va, there was obtained 8.25 g. (85%) of crude amine. Purification of this crude amine was accomplished in the usual manner. A yield of 5.79 g. (59%) of VIa, b.p. 122–126° (1 mm.) was realized.

cis-1,4-Bis(2-aminoethyl)cyclohexane (VIb). This isomer was prepared according to the procedure described in Method A for the preparation of VIa. Ten grams (0.062 mole) of Vb yielded 3.25 g. (31%) of crude amine. Purification of this crude amine was accomplished in the usual manner. The yield was 2.22 g. (21%) of a colorless oil, b.p. 106–110° (2 mm.).

Dibenzenesulfonamide of VIa *and* VIb. The amides were prepared by the usual method.[11] After recrystallizing the reaction product from alcohol, a 54% yield of the white, crystalline *trans*-sulfonamide, m.p. 168–169°, was obtained. Two recrystallizations of the reaction product for the *cis* isomer gave a 60% yield of the *cis*-sulfonamide, m.p. 133–134°.

Anal. Calcd. for $C_{22}H_{30}O_4N_2S_2$: N, 6.22. Found: N, 6.18 (*trans*); N, 6.29 (*cis*).

Dihydrochlorides. The dihydrochlorides were prepared by dissolving crude VIa or VIb in anhydrous ether and passing dry hydrogen chloride into the resulting solution for 10 min. The grayish white, gelatinous solid which formed was removed by filtration and washed with two 15-ml. portions of anhydrous ether. Two or three recrystallizations of this solid from ethanol gave the white crystalline dihydrochloride. The *trans* isomer melted at 319–322° dec.; yield, 34%. The *cis* isomer melted at 300–304° dec.; yield, 37%.

Anal. Calcd. for $C_{10}H_{24}N_2Cl_2$: Cl, 29.16. Found: Cl, 29.05 (*trans*); Cl, 29.72 (*cis*).

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Identification of a New, Naturally Occurring, Steam-Volatile Isothiocyanate from *Lesquerella lasiocarpa* Seed[1a]

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As part of a program evaluating the nutritional quality of seed meals from selected Cruciferae, determinations are being made of their contents of enzymatically formed steam-volatile isothiocyanates and thiooxazolidones. These compounds, formed from parent glucosides by action of enzymes normally present in the plant material, are undesirable because of the unpalatability they may confer or the effects they may have on the thyroid glands of animals ingesting them. Volatile isothiocyanates are conventionally isolated from various plant species by steam distillation and identified after conversion to corresponding thioureas by reaction with ammonia. Recent reviews in this field by Challenger[1] and by Kjaer,[2] and the structural work of Ettlinger and Lundeen,[3] provide impetus to further research in this phase of plant biochemistry.

The genus *Lesquerella* has been of special interest in our research on the chemistry of cruciferous oilseeds. Its seed oil glycerides contain major amounts of either 20-carbon or 18-carbon hydroxylated fatty acids, the structure of which will be reported in detail elsewhere. Utilization of seed oil requires profitable outlets for the coproduct protein meal. Seed meal from *Lesquerella* is therefore being investigated, and this note reports a portion of these studies.

Paper chromatography[4] of the thiourea derivatives of the volatile isothiocyanates from *lasiocarpa* seed showed one major and one very minor spot. The major spot gave an R_{ph} value (compared with phenylthiourea) different from that of any known thiourea derivative of an isothiocyanate isolated from a natural product, but was the same as that of *N*-(6-methylthiohexyl)thiourea; CH_3—S—$(CH_2)_6$—NH—CS—NH_2. This compound has been synthesized by Kjaer and Christensen.[5] Our thiourea derivative crystallized readily from alcohol on addition of water. The melting point and elementary analysis agreed with those of the synthetic compound. Infrared spectra and X-ray patterns from

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[1a] This is a contribution from a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(1) F. Challenger, *Aspects of the Organic Chemistry of Sulfur*, Butterworths Scientific Publications, London, 1959, p. 115.

(2) A. Kjaer, *Fortschritte der Chemie Organischer Naturstoffe*, L. Techmeister, ed., Springer Vienna, 1960, p. 122.

(3) M. G. Ettlinger and A. J. Lundeen, *J. Am. Chem. Soc.*, **78**, 4172 (1956).

(4) A. Kjaer and K. Rubinstein, *Acta Chem. Scand.*, **7**, 528 (1953).

(5) A. Kjaer and B. Christensen, *Acta Chem. Scand.*, **11**, 1298 (1957).

the isolated and synthetic material were identical.

By means of paper chromatography and a specific color test[6] glucose was shown to be formed on treatment of the seed meal extract with myrosinase. This information is interpreted as showing the compound is derived from a glucoside.

The identification of this enzymatically formed isothiocyanate from *L. lasiocarpa* seed adds the homologous 8-carbon compound to the previously identified 5-, 6-, and 7-carbon compounds of similar structure elaborated in nature by Cruciferae. The lower homologs have been identified by Kjaer and co-workers[7–9] as enzymic hydrolysis products of glucosides present in the seeds of *Iberis sempervirens*, *Eruca sativa*, and *Berteroa incana*, respectively. In accordance with the method of naming the parent glucosides followed by these workers, the name glucolesquerellin is suggested for the postulated parent glucoside.

EXPERIMENTAL

Isolation and characterization of N-(6-methylthiohexyl)-thiourea. Four 5-g. samples of finely ground, petroleum ether-extracted seed meal from *Lesquerella lasiocarpa* were treated with myrosinase (for preparation see Wetter[10] and Wrede[11]) in order to hydrolyze the glucoside. To each sample was added 100 ml. of citrate buffer, *p*H 4 (0.20*M* citric acid adjusted to *p*H 4 with saturated sodium hydroxide) and 6 ml. of myrosinase solution in a 500-ml. flask that could be attached to a steam distillation apparatus. The stoppered flask was shaken for 3 hr. at room temperature. Prior to steam distillation 15 ml. of a mixture of ethanol-butanol (1:1) was added. Rapid steam distillation of the volatile isothiocyanate was continued until about 200 ml. was collected in 25 ml. of ice-cooled concd. ammonium hydroxide. The four ammonium hydroxide solutions were combined after standing overnight, concentrated to about 100 ml. on a rotary evaporator at 40°, and filtered. The filtrate was further concentrated to 25 ml. After cooling in the refrigerator for at least 2 hr., the crystalline material was collected on a micro Büchner funnel, dissolved with 4 ml. of absolute ethanol, and refiltered. On adding 8 ml. of water, recrystallization occurred. After cooling again in the refrigerator the material was filtered, air-dried and then dried *in vacuo* at room temperature to constant weight. Yield, 122 mg. Melting point on a micro hot stage, 72–73°.

Anal. Calcd. for $C_8H_{18}N_2S_2$: C, 46.6; H, 8.9; N, 13.6; S, 31.1. Found: C, 46.8; H, 8.8; N, 13.5; S, 31.0.

Comparison with authentic *N*-(6-methylthiohexyl)thiourea gave no depression of mixed melting point. Both compounds gave the same R_{ph} value of 1.19 by ascending chromatography using water-saturated chloroform as the mobile phase. Their X-ray patterns and infrared absorption spectra (2 to 15 μ) were identical. Two crude preparations of the thiourea (m.p. 70–72°) from different accessions gave yields of 99 and 132 mg. from 10 g. of each seed meal.

Evidence for the presence of the parent glucoside. A hot acetone-water (3:1) extract (200 ml.) obtained from 3 g. of oil-free meal was concentrated under vacuum to 10 ml. A portion of the solution was hydrolyzed with the myrosinase-citrate solution as used in Wetter's[10] analytical procedure. The hydrolyzed solution and appropriate controls, including the myrosinase-citrate solution, were examined by paper chromatography using two different solvent systems: (1) Ethyl acetate:pyridine:water (3:1.25:1)[12] and (2) 1-butanol:water:acetic acid (4:3:1). Two different detection reagents were used: (1) Aniline–diphenylamine–phosphoric acid in acetone[12] and (2) glucose oxidase reagent.[6]

Only trace amounts of glucose were detected when the unhydrolyzed solution was chromatogrammed. A significant amount of glucose was detected when a hydrolyzed solution equal to about one fifth of the unhydrolyzed material was chromatogrammed.

Analysis of seed meals. Composition of the seed meal for total volatile isothiocyanate determined by the method of Wetter[10] and calculated as 6-methylthiohexyl isothiocyanate was 1.2%. Seed meals from two different accessions of *L. lasiocarpa* contained about the same amounts of steam-volatile isothiocyanate. No thiooxazolidone was found.

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Transformation of Squill Bufadienolides to Pyridone Counterparts

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The bufadienolides are a group of naturally occurring α-pyrones in which the pyrone ring is linked at the 5-position to the 17β-position of a substituted steroid nucleus.[1] One of the reactions characteristic of simpler α- and γ-pyrones embodies their conversion with ammonia, or with amines, to pyridone derivatives.[2] Although the transformation has been considered general, experimental conditions required—at least those employed—have varied widely.[3]

In a study of the behavior of white squill bufa-

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