5,5-Dimethyloxazolidine-2-thione Formation from Glucosinolate in *Limnanthes alba* Benth. Seed

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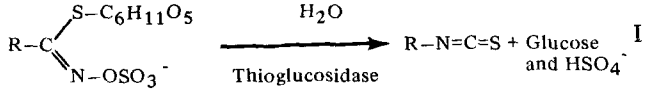
ABSTRACT

A crystalline compound, identified as 5,5-dimethyloxazolidine-2-thione was isolated from defatted *Limnanthes alba* var. *alba* (meadowfoam) seed meal by either of two procedures, described below. The precursor glucosinolate was separated and then hydrolyzed with an added thioglucosidase prepared from mustard seed. Alternatively, endogenous thioglucosidase was allowed to act in an aqueous slurry of the *Limnanthes alba* meal, and subsequently 5,5-dimethyloxazolidine-2-thione was purifed from the autholysis products. Previous workers reported 5,5-dimethyloxazolidine-2-thione formation in seed meals of only two Cruciferae genera. Among seed samples we analyzed representing the *Limnanthaceae* species, production of the rare oxazolidinethione derivative was unique to *Limnanthes alba*.

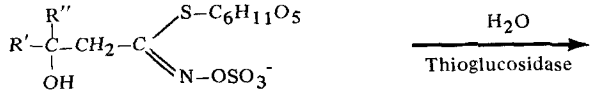
INTRODUCTION

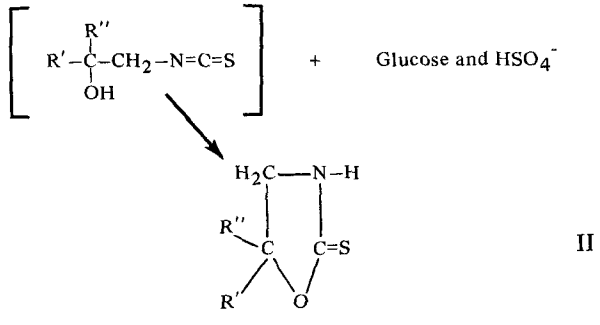
The excellent potential of the *Limnanthes* (meadowfoam) as a source of industrial oil and the botanical and agronomic characteristics of this possible new crop were reported by Gentry and Miller (1).

The glucosinolates are a class of compounds, usually associated with the Cruciferae family, that may yield isothiocyanates and oxazolidinethiones on enzymatic hydrolysis (equations I and II) (2,3). The oxazolidinethiones arise through cyclization of an initially formed hydroxyisothiocyanate:



I





II

In some instances, alternate pathways of glucosinolate breakdown to form other products from the aglucon portion also are known to occur (4).

[1]ARS, USDA.

The presence of glucosinolates in defatted *Limnanthes* seed meals could detract from their potential use as animal feeds (4). Ettlinger and Lundeen (5) found that seed from *Limnanthes douglasii* contains a glucosinolate which they characterized as the precursor of m-methoxybenzylisothiocyanate. Miller, et al., (6) reported the amounts of isothiocyanate that could be obtained from several different species of *Limnanthes* and found that one of them (*Limnanthes alba* Benth.) also produced an unidentified oxazolidinethione. We now report the isolation and identification of this oxazolidinethione as 5,5-dimethyloxazolindine-2-thione (DMOT), the product of equation II with $R' = R'' = CH_3$.

EXPERIMENTAL PROCEDURES

For preparation of DMOT from the separated glucosinolate precursor, typically, a 100 g sample defatted meal was heated dry in a steam jacketed vessel for 5-10 min, and then 1-1/2 liters boiling water was poured onto the hot meal with stirring. The steam heating was continued an additional 15 min. Most meal solids were separated by filtration of the aqueous slurry through layers of cheesecloth. The residue meal was reextracted with hot water (1 liter) for ca. 10 min and the solids again separated. The combined extracts, which included a third wash, totaled ca. 3-1/2 liters and were clarified by filtering through Celite under vacuum. The glucosinolates then were separated from most of the other solubles by anion exchange on a 4 x 33 cm column of Bio-Rad AG 1 x 2 (Cl^-) 50-100 mesh. The glucosinolates were eluted with 10% NaCl solution. A large amount of the NaCl in the eluate was removed after first distilling off most of the water under reduced pressure in a rotating evaporator followed by trituration of the residue with 90-95% aqueous ethanol (ETOH). The aqueous alcohol solution was concentrated to a syrup, dissolved in ca. 40 ml water, and applied to a 5 x 100 cm Sephadex G-10 column. The glucosinolates were separated on the Sephadex column with water as elutriant at a flow rate of 46 ml/hr, and 23 ml fractions were taken.

In addition to monitoring the column eluate with a recording refractometer, the fractionation was followed by thin layer chromatography (TLC) and UV measurements of dichloromethane extracts from myrosinase treated and buffered aliquots from the fractions. The release of glucose simultaneously with the formation of the DMOT in these samples was demonstrated by use of Tes-Tape, a glucose oxidase impregnated commercial paper tape for testing for glucose in urine samples. TLC was on air-dried Silica Gel G plates with ether:hexane (3:1) as the solvent system and iodine vapor for detection. For UV measurements, the dichloromethane was evaporated and the sample dissolved in ETOH and measured at λ_{max} 243 nm with a Beckman model DK-2A recording spectrophotometer. The oxazolidinethione precursor was located, along with the remaining NaCl, in tubes 41-51, and the m-methoxybenzylisothiocyanate precursor was found in tubes 53-67. The contents of tubes 41-51 were combined and concentrated under reduced pressure to ca. 5 ml, buffered with ca. 30 ml pH 7 citric acid-phosphate buffer containing a crude enzyme preparation from white mustard (thioglucoside glucohydro-

lase EC 3.2.3.1) (7). After standing 24 hr at room temperature, the liberated DMOT was extracted into dichloromethane. After the solvent was evaporated and the residue taken up in a small volume of water, DMOT readily crystallized.

As an alternate procedure, the defatted seed meal was autolyzed at room temperature and the DMOT isolated from the hydrolyzate. A 100 g sample of defatted seed meal was blended with 500 ml water at room temperature and autolyzed for at least 10 min. The mixture was extracted by shaking vigorously with 3 liter dichloromethane. The separated CH_2Cl_2 extract was dried over Na_2SO_4, filtered, and concentrated to 2-3 ml volume. The concentrate was applied to a 2 x 34 cm column (60 g) prepared from a slurry of Adsorbosil in hexane. After the sample was applied, the components were eluted from the column with 500 ml CH_2Cl_2 followed by gradually increasing amounts of CH_3OH in CH_2Cl_2. Tubes containing 20 ml were collected, and the separation was monitored by spotting 10-15 μliters for TLC with the system described in the preceding paragraph. The m-methoxybenzylisothiocyanate, R_f 0.8, was eluted almost immediately from the column (tubes 2-6), and the DMOT, R_f 0.2, was in tubes 14-34. On concentration to an oil and taking up in a small volume of water, the DMOT readily crystallized. For recrystallization, the compound was dissolved in a minimum of warm ethyl acetate, and, as the solution approached room temperature, a few drops of hexane were added which caused immediate and copious crystallization.

Identification of the oxazolidinethione as DMOT and, therefore, its precursor glucosinolate as glucoconringiin (8), was based upon elemental analyses: analysis calculated for C_5H_9NOS (131.2): carbon, 45.77; hydrogen, 6.91; nitrogen, 10.67; and sulfur, 24.44. Found: carbon, 46.00; hydrogen, 7.05; nitrogen, 10.62; and sulfur, 24.66.

Melting point was 105 C (literature, 105 and 107 C [8]); UV max (EtOH) 243 nm (log ϵ 4.25); mass spectral data appropriate for the compound; and an NMR spectrum identical to that reproduced in an article by Kjaer and Thomsen (9).

Screening measurements for the mustard oils and oxazolidinethione compounds that could be obtained from various *Limnanthes* seed were performed essentially as described by Appelqvist and Josefsson (10) for related compounds in Cruciferae seed meals. In their procedure, the liberate isothiocyanates are extracted preferentially into isooctane and, after conversion with ammonia to thioureas, are measured by UV absorption. The oxazolidinethiones were extracted into ether and also measured by UV absorption. We slightly modified the procedure by extracting the mustard oils with isooctane and the DMOT with dichloromethane on separate aliquots. The dichloromethane was evaporated, and the DMOT was measured by UV absorption in ethanol.

RESULTS AND DISCUSSION

Previous workers gave the name gluconringiin to the glucosinolate precursor of DMOT which was first isolated from the crucifer known as hare's ear mustard or *Conringia orientalis* (8). To our knowledge, the *Conringia* and *Cochlearia* genera of the Cruciferae family are the only previously known sources.

Measurements for mustard oil and oxazolidinethione for all the known species of *Limnanthes* showed considerable variation. When calculated as glucosinolate, the total of the two types ranged from ca. 1-7% of the defatted seed meal. The amounts of m-methoxybenzylisothiocyanate found were generally as previously reported (6). Also in agreement with the previous article, the oxazolidinethione was found only in *L. alba*. The amounts of DMOT obtainable from *L. alba* Benth. var. *alba* were typically larger than those obtained from *L. alba* var. *versicolor* (Green) C.T. Mason. Indeed, from several *L. alba* var. *versicolor* accessions, little, or none, of the component was found.

For the isolation work in this article, an accession of *L. alba* Benth. var. *alba* was used. The typical amounts of m-methoxybenzylisothiocyanate isolated was 14 mg/g, and the average DMOT isolated was 3 mg/g.

Gentry and Miller (1), in discussing the habitat of *L. alba* var. *alba* point out that this ecotype, "appears to represent a contemporary case of rapid evolution." Benson (11) has pointed out: "The presence of a particular compound in one taxon and its absence from a related one are likely to be connected with the evolutionary processes which brought about the original segregation of the groups of organisms. Thus, this difference may be fundamental to classification."

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