and appears to be the end product of the herbicide degradation in soil (Verloop, 1972). Leach et al. (1971) reported that 2,6-dichlorobenzamide is water soluble and is moved readily by soil water. The accumulation of this major breakdown product under eastern Ontario conditions may suggest that due to the insufficient rainfall in this area the material is not leached away from the root zone.

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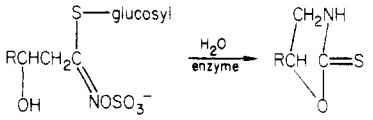
(+)-5-Allyloxazolidine-2-thione, an Enantiomer of Turnip Antithyroid Factor Isolated from *Berteroa incana* (L) D.C.

A new potential thyroid toxicant, (+)-5-allyloxazolidine-2-thione, has been isolated from *Berteroa incana* (L) D.C. seed. The new compound was characterized by comparison of its GLC, ORD, NMR, UV, and MS data to those of the previously known levo isomer from turnips and Chinese cabbage.

Oxazolidinethiones are well-known as antithyroid agents (Astwood et al., 1949; Tookey et al., 1980). They are readily formed from certain glucosinolates (GS's) in crucifer plants such as turnips, rutabagas, and Chinese cabbages. Glucosinolates that contain a β-hydroxy function (I) may hydrolyze to an intermediate isothiocyanate that



I II

cyclizes to form an oxazolidine-2-thione (OZT) (II) or may hydrolyze to form alternate aglucon products. Because glucosinolates impart flavor and can affect nutritional quality of foods and feeds, they have evoked much interest and have been extensively reviewed (Kjaer, 1960; Ettlinger and Kjaer, 1968; Tookey et al., 1980).

We wish to report a new potential toxicant, (+)-5-allyl-OZT, isolated from enzymatically hydrolyzed GS's of *Berteroa incana* (L) D.C. seed. Tapper and MacGibbon (1967) reported the corresponding levo isomer from turnips and rutabagas. The new OZT compound, and by inference a new precursor GS, was characterized by comparison with ORD and NMR data from the literature and by direct comparison of GLC, UV, and MS data with those of the authentic levorotatory isomer. We also report a convenient preparation of (+)-5-allyl-OZT that may be of use in gaining further knowledge of the toxicology of this class of thyroid poisons.

EXPERIMENTAL SECTION

Seed Preparation Analytical Procedures. Seed was collected from Wisconsin, Michigan, and Yugoslavia. Seed meal preparation, glucosinolate extraction, and subsequent analytical determinations for GS-glucose and quantitative determinations of individual aglucons were as summarized previously (Daxenbichler et al., 1980). GLC was performed with Packard 7400 series and supporting equipment as described by Daxenbichler and VanEtten (1977). GC–MS determinations were obtained as described by Spencer and Daxenbichler (1980). UV measurements were recorded with a Beckman Model DK-2A spectrophotometer. Optical rotation measurements were taken in 0.66% solution in methanol with a Perkin-Elmer No. 241 polarimeter in a 1 DM microcell and the data recorded at five wavelengths 589 (Na) and 578, 546, 436, and 365 nm (Hg). ^{1}H NMR spectra were recorded with a Varian HA-100 instrument. HPLC separations were performed with a Waters Associates instrument.

Isolation of Allyl-OZT. *Seed Preparation.* Whole seed (grown in Wisconsin) was ground in a Conco hammer mill and defatted by five separate 60-min steeps with pentane–hexane in a glass percolator. Then the meal was air-dried in a hood (yield: 77% of starting weight).

GS Extraction. Glucosinolates were extracted by inactivation of the endogenous enzyme system with boiling methanol followed by three extractions with 70% (v/v) aqueous methanol as described by Daxenbichler et al. (1980).

Thioglucosidase–Buffer Preparation. A crude thioglucosidase–buffer medium was prepared from *Sinapis alba* seed meal as follows: Whole *S. alba* seed (provided by French Co.) was flaked and defatted with pentane–hexane percolation at room temperature in a manner similar to the defatting operation of *Berteroa* seed described above.

Defatted *S. alba* seed meal (50 g) was shaken for 30 min with 500 mL, pH 7.0, phosphate buffer (0.05 M). Some solids were separated by passage through several layers of cheesecloth (excess liquid was collected from the solids in the cheesecloth by wringing and squeezing). The liquid was also filtered through Celite supported on Whatman No. 54 paper. The Celite on the filter was washed with

Table I. Glucosinolate Composition of *B. incana* (L) D.C. Seed Meals[a]

R group of glucosinolates	% WI and MI[b]	% unknown	% Yugoslavia	% average data
4-pentenyl	0.34	0.29	0.20	0.3
2-hydroxy-4-pentenyl[c]	0.72[d]-0.82[e]	0.81[d]	0.9[d]-0.84[e]	0.8
5-(methylthio)pentyl	2.05	2.15	1.8	2.0
5-(methylsulfinyl)-pentyl	0.35	0.36	0.4	0.4
total GS (from above)	3.7	3.6	3.4	3.5
total GS calcd from glucose release method	3.5	4.0	3.8	3.8

[a] Air-dried, defatted. [b] Source of seed. [c] 5-Allyl-OZT precursor. [d] GLC. [e] UV.

about 100 mL of 0.05 M phosphate buffer. The combined filtrate and rinse extract of the *S. alba* was finally again made to 500-mL volume with 0.05 M, pH 7, phosphate buffer. This crude thioglucosidase–buffer medium was subsequently used to prepare the aglucons from the GS-containing fraction obtained from *Berteroa* seed.

Thioglucosidase Hydrolysis and Aglucon Extraction. The GS extract prepared by 70% methanol extraction of *Berteroa* (≅200 g) was concentrated to an aqueous volume of 100 mL to which was added 500 mL of the thioglucosidase–buffer medium. The mixture was covered with 600 mL of pentane–hexane and magnetically stirred for 3 h at room temperature. The pentane–hexane layer was separated, and then 600 mL of fresh solvent was added and stirred an additional 3 h and separated again. The pentane–hexane extracts were combined. Then the buffered medium was extracted 3 times with 1-L volumes of dichloromethane, and the extracts were combined and dried over anhydrous Na_2SO_4. When initial extraction with pentane–hexane was performed, a surprisingly distinctive separation of the components was obtained, distributed in the two solvents. The pentane–hexane essentially selectively extracted the 4-pentenyl isothiocyanate and 5-(methylthio)pentyl isothiocyanate, whereas the other two components (5-allyl-OZT and 5-(methylsulfinyl)pentyl isothiocyanate) were then extracted into the dichloromethane. GLC assay indicated about 100 mg of the 5-allyl-OZT was present at this stage of preparation.

Partial cleanup of the dichloromethane fraction was achieved by the use of a SiO_2 Sep-PAK cartridge. The CH_2Cl_2 solution was applied to the Sep-PAK, which was then eluted with hexane (discarded), followed by gradient elution: hexane–ether (90:10) to 100% ether. These hexane–ether fractions were combined and the components were separated by HPLC with a C_{18} semipreparative column, with acetonitrile–H_2O (1:4) as the solvent system (3 mL/min) and a UV detection system. The fraction determined to be 5-allyl-OZT (54 mg) by GLC and TLC was finally purified by chromatography on 1 × 17 cm SiO_2 (HiFlosil). The 5-allyl-OZT was eluted with hexane–ether (20:80) and was readily crystallized by removal of solvent (mp 57–59 °C). Recrystallization from ether occurred upon addition of pentane–hexane: mp 60–61 °C; lit. mp 60–61 °C.

RESULTS AND DISCUSSION

Pioneer workers in the field of glucosinolates reported that the major GS in *B. incana* seed was 5-(methylthio)-pentyl-GS and was accompanied by at least two other GS's in lesser amounts (Kjaer et al., 1955). One of the lesser GS's was identified as the oxygenated counterpart of the major component, 5-(methylsulfinyl)pentyl-GS, but the third component remained unidentified.

Table II. Optical Rotation of 5-Allyloxazolidine-2-thione Isolated from *B. incana* Seed

λ, nm	[α], deg	[Φ], deg
589 (Na)	10.0	14.3
578 (Hg)	10.0	14.3
546 (Hg)	10.6	15.2
436 (Hg)	6.05	8.65
365 (Hg)	−30.6	−43.7

Table I summarizes the GS compositional data for *B. incana* defatted seed meal that we obtained with analytical techniques developed in recent years (VanEtten and Daxenbichler, 1977; Daxenbichler and VanEtten, 1977). In addition to the two reported earlier, we obtained measurements for 4-pentenyl-GS and 2-hydroxy-4-pentenyl-GS via the aglucon products 4-pentenyl isothiocyanate and 5-allyl-OZT, respectively. The precursor GS to 5-allyl-OZT is second in quantity only to the 5-(methylthio)pentyl-GS for which this seed is the noted literature source (Kjaer et al., 1955). The good accountability of the total GS, compared to the GS-glucose released by thioglucosidase treatment in a separate measurement of the total glucosinolates present, lends assurance that the GS's present in significant quantity are noted. Initially the four GS product components were quantitated by GLC and identified by their retention times (two columns) by comparison with those of reference compounds (Daxenbichler and VanEtten, 1977). These identities were subsequently confirmed by GC–MS with the methodology we described earlier.

The newly found 5-allyl-OZT from *B. incana* melts at 60–61 °C in agreement with that of the previously known levorotatory compound (Tapper and MacGibbon, 1967). GLC retention on two columns was identical with that of the known isomer, as was the UV spectra of 210–310 nm [λ_{max} 244 nm (EtOH)]. The NMR spectra agreed with the previous report for the levo isomer (Tapper and MacGibbon, 1967), as did the mass spectra described for that isomer from turnip (Spencer and Daxenbichler, 1980) and Chinese cabbage (Daxenbichler et al., 1980; Carlson et al., 1981) and as found for the reference compound supplied by Tapper.

These properties allowed structural assignments to be made. It remained, however, to establish the optical form of the 5-allyl-OZT from *B. incana*. The optical rotatory dispersion curve that could be plotted from Table II was similar in magnitude but opposite in sign to that reported from turnips and rutabagas (Tapper and MacGibbon, 1967). This literature did not include a value at the sodium D wavelength; we obtained a value $[\alpha]_D$ +10.0°. Again the cotton effect was observed but in the opposite direction to that of the turnip isomer.

Both forms of the vinyl-OZT compounds have shown pronounced goitrogenic properties. Recent studies by Nishie and Daxenbichler (1982) have also demonstrated toxicology to the liver. Studies of this type are also suggested for the allylic OZT compounds.

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Investigations on Trypsin-Hydrolyzed Peptides for Protein Identification

A chromatographic method was developed for the identification of protein species through their peptide patterns. Protein isolates of beef, pork, chicken, and soy were heated, enzymatically hydrolyzed at optimal conditions, and subsequently analyzed by sequential application of thin-layer chromatography and high-performance liquid chromatography. The chromatographic patterns of the tryptic peptides were then statistically analyzed by discriminant analysis. A classification rule was derived to identify the proteins. Results showed that beef, pork, chicken, and soy proteins were significantly ($p < 0.05$) identified. Preliminary studies indicated that an all-beef frankfurter can be discriminated from a standard frankfurter containing 35% pork protein. Further studies are required to detect and measure the presence of "nonmeat" proteins as an adulterant or additive in processed meat products.

There is a continuing demand for development of a simple and rapid analytical method to detect, quantitate, and identify nonmeat proteins in processed meat products. Currently available methods have not been generally accepted. Recent reviews by Eldridge (1981), Eldridge and Wolf (1980), and Olsman and Krol (1978) describe the principles, advantages, and disadvantages of existing analytical techniques. Olsman (1979) categorized these techniques into two broad groups: (1) identification and analysis of substances accompanying nonmeat proteins by chemical analysis or histological and microscopic studies and (2) characterization of proteins through the analysis of their hydrolysis products (amino acids or peptides) and their physicochemical properties. Immunochemical techniques employed to detect the presence of protein additives were also cited in the preceding review papers.

The detection of nonmeat protein through its peptide hydrolysis products merits further investigation since it is simple and rapid. Furthermore, the peptide hydrolysates can be subjected to various analytical techniques. Bailey and co-workers (Bailey, 1976; Llewellyn et al., 1978; Bailey et al., 1978) measured the presence of soy protein in laboratory-fabricated soy–meat products by ion-exchange chromatographic analysis of trypsin hydrolysates of heat-denatured proteins. We have developed a modification of this method and investigated the identification of beef, pork, chicken, and soy proteins.

This paper presents a preliminary report on the sequential application of thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) to peptide hydrolysates of selected proteins (beef, chicken, pork, and soy) previously heated and enzymatically hydrolyzed at optimal conditions. The chromatographic patterns of these peptides were statistically analyzed, and a classification rule was derived to identify the proteins.

EXPERIMENTAL SECTION

Preparation of Protein Isolates. Isolates of selected animal proteins were prepared by solvent extraction using acetone and ether (Morton, 1955). Fresh samples of beef, chicken, and pork (100 g each) were homogenized by blending with 200 mL of phosphate buffer (0.01 M, pH 6.5). The proteins were precipitated by adding 750 mL of cold acetone. The precipitate was allowed to settle for 30 min at 4 °C and the clear acetone layer was decanted. The precipitate was separated by centrifugation at 4080*g*, blended in 300 mL of 1-butanol, and then washed 2 times with 300 mL of petroleum ether to extract lipids. The acetone-precipitated proteins were dried under a stream of nitrogen and pulverized with a blender. The commercial soy isolate contained 92.5% protein and was utilized without further purification.

Preparation of Hydrolysates. The "superdenaturation" and hydrolysis of the proteins developed by Bailey (1976) were refined. Simultaneous optimization studies were employed on beef isolates to select the precise heating and hydrolysis conditions that would generate a fairly large number of resolvable fractions. The optimal heating temperature and time selected should be higher than those used in the processing of meat products.