

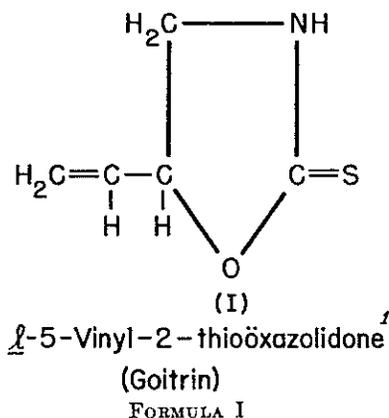
Goitrin from Fresh Cabbage*†

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Goitrin, a strongly antithyroid principle, has been found in the seeds of plant foods, of which cabbage seed is an important source. Astwood *et al.* (1) have isolated it from the roots and seeds of turnip, 0.12 to 1.0 gm. per kg., and from the seeds of cabbage, kale, and rape. They determined its structure, Formula I, and properties. It was synthesized later by Ettlinger (2).



Raciszewski *et al.* (3) have recently isolated it from rapeseed oilmeal, 2 to 4 gm. per kg.

In 1950, Greer (4), in his review, stated that cabbage has never yielded so much as a trace of this material from its leaves, even by spectrophotometric analysis. He added that the compound has been identified in small amounts in the seeds, but never in other parts of the plant.

It is of interest, therefore, that, during the course of an investigation of the non-steam-distillable isothiocyanates present in the aqueous extracts of fresh cabbage leaves (5), a few crystals of a substance with melting point and properties resembling goitrin were isolated. Furthermore, the ultraviolet absorption spectra of a number of other cabbage extracts revealed traces of a material which absorbed in the region near the maximum of goitrin, 240 mμ, (1) and gave positive paper chromatographic evidence of its presence. These observations, supported by the knowledge that goitrin is present in cabbage seeds, led to the

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† While this manuscript was in preparation, a note by A. I. Virtanen, M. Kreula, and M. Kiesvarra (*Acta Chem. Scand.*, **12**, 580 (1958)), appeared which stated, without experimental evidence, that considerable amounts of vinyl thiooxazolidone had been found in cabbage.

¹ This nomenclature conforms with previous publications. Goitrin has also been named, (-)-2-thio-5-vinyl-1:3-oxazolidone and (-)-5-vinyl-2-oxazolidinethione.

isolation and identification of goitrin from fresh cabbage leaves as described in this paper.

EXPERIMENTAL

Since it was expected that the goitrin content of cabbage varieties might vary (4, 6, 7), a 160-pound batch of seven different varieties of cabbage,² *Brassica oleracea* var. *capitata*, was processed. These included Baby Head, Golden Acre Special, Wisconsin Golden Acre, Golden Acre (Early Detroit), Small Danish, Bonanza, and Badger Market in approximately equal amounts.

Some preliminary experiments were performed to determine the most suitable conditions for isolating the goitrin with the minimal possible loss. The optimal procedure was a modification of that described by Astwood *et al.* (1). The following changes were found necessary: (a) heating the syrup above 60° was avoided because of loss of part of the vinyl thiooxazolidone, (b) a ratio of ten parts of solvent to one of syrup was needed to reduce serious emulsion formation during the extractions, and (c) extraction with chloroform (8) in addition to that with ether was required since this solvent was found to be a better extractant for goitrin than ether. The yield of goitrin in the residue from this procedure, estimated by spectrophotometric analysis, was 9.0 mg.

Additional purification was achieved by extracting the residue from the Astwood procedure repeatedly with petroleum ether. This solvent removed the remaining impurities, leaving the purified goitrin in the petroleum ether-insoluble portion.

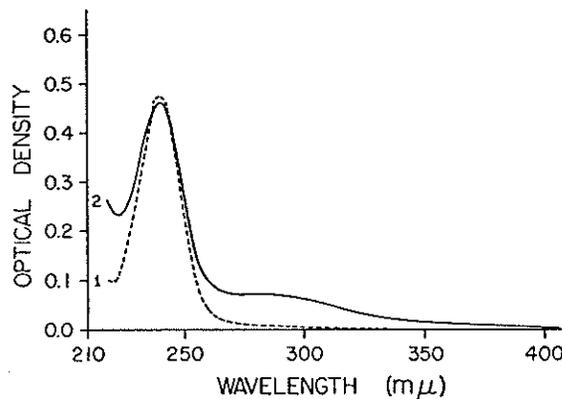


FIG. 1. Ultraviolet absorption spectrum of cabbage fraction and pure goitrin. 1 = goitrin, 2 = cabbage fraction. The concentration of each is 4×10^{-3} gm. per l. based on total solids.

² The cabbage was grown at the Waltham Experiment Station, Waltham, Massachusetts, under controlled conditions and harvested in July. It was obtained through the courtesy of Professor R. E. Young.

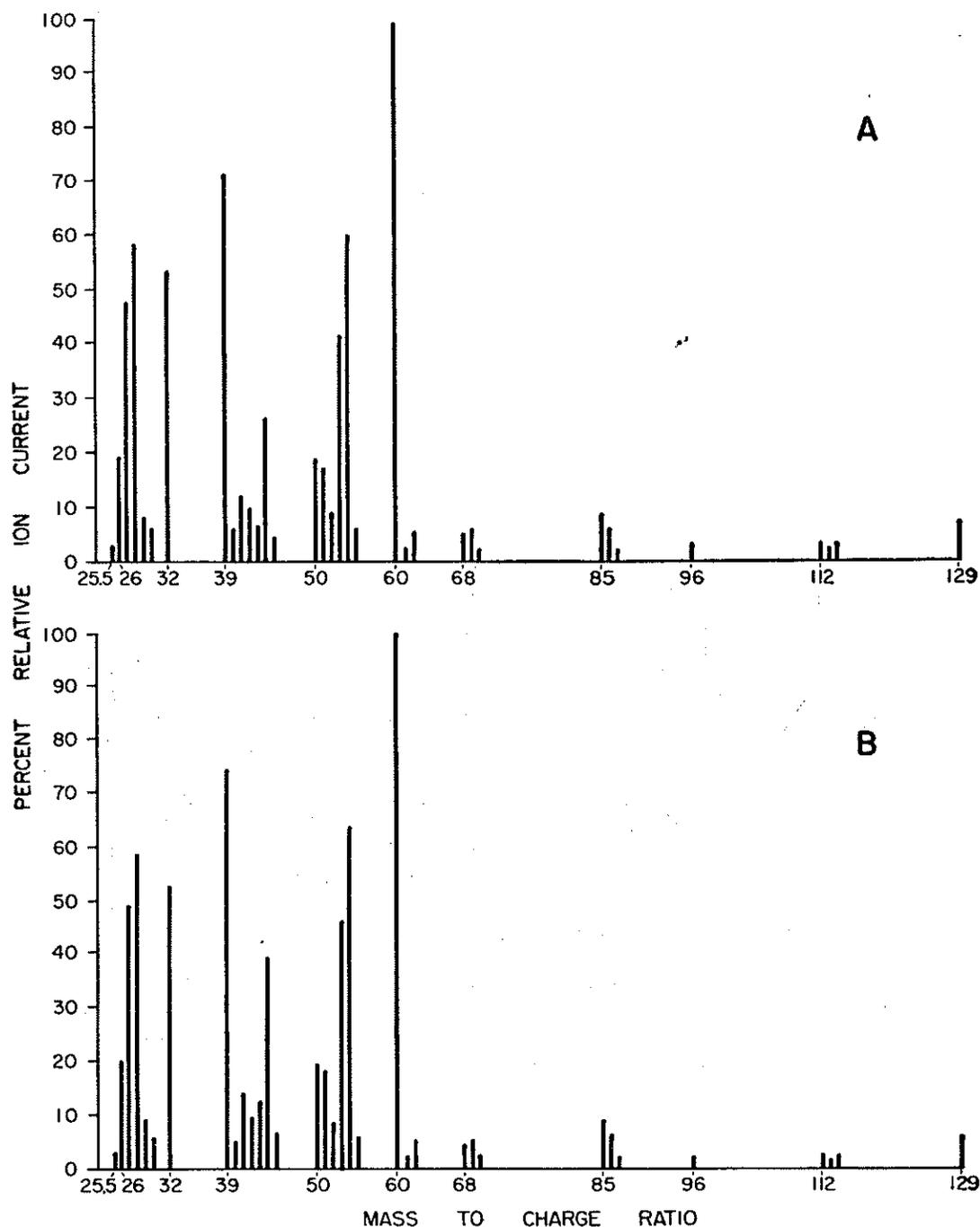


FIG. 2. Mass spectra of *l*-5-vinyl-2-thiooxazolidone and cabbage fraction. *A* = authentic goitrin. *B* = cabbage fraction.

Instrumentation—The melting points were determined with a Köfeler micro hot stage with magnification, 6 ×. For the ultraviolet absorption spectra, a Cary recording spectrophotometer model 11MS was used.

The authors are indebted to Dr. C. Merritt, Jr., and Mr. M. Bazinet of our Mass Spectroscopy Laboratory for suggesting the use of mass spectrophotometric analysis and for providing and interpreting the mass spectral data. An analytical mass spectrometer, model 21-103C, manufactured by Consolidated Electro-dynamics Corporation of Pasadena, California, with a high temperature inlet system (9), operating at 200°, was used.

The samples were admitted to the spectrometer under identical conditions through a molten indium inlet valve.

RESULTS AND DISCUSSION

The minute amount of crystals obtained as a by-product of our cabbage flavor research (5) as a fortuitous discovery had a melting point of 49–50°³ (uncorrected) (46–48° (3), 50° (1), and 50–50.5° (2) are melting points reported for *l*-5-vinyl-2-thiooxazolidone) and a mixed melting point with pure goitrin which

³ *d*-5-Vinyl-2-thiooxazolidone has been reported to have a m.p. 50–51°; the *dl*-compound melts at 64–65° (2).

showed no depression. These crystals gave positive tests for a thione, $>C=S$, group with Grote's (10) and sodium azide-iodine (11) reagents, and showed birefringence upon microscopic examination.

The residual oil, left after exhaustive petroleum ether extraction, gave a well defined ultraviolet absorption spectrum with a single absorption maximum at 240 $m\mu$ and a minimum at 222 $m\mu$. This spectrum is compared with that for pure goitrin in Fig. 1.

Additional evidence for the presence of goitrin was found by paper chromatography. Since Grote's reagent proved to be unsatisfactory because of the instability of the spot and its low sensitivity (30 μg . per spot), starch (12) and sodium azide-iodine⁴ (13) sprays, applied successively, were used. The latter yielded spots with a high degree of stability and a sensitivity of 1 μg . per spot. Utilizing this technique and prewashed chromatographic paper, a distinct white area against a pale blue background was obtained with a purified cabbage extract in a chloroform-water system (14). The R_{F} value⁵ of this spot was 1.20,⁶ identical with that for pure *dl*-5-vinyl-2-thiooxazolidone.⁷ In a second chromatographic ascending system, with benzene-ethanol-water in the ratio of 5:1:2,⁸ another cabbage fraction gave a clearly defined white spot. The R_{F} values of the fraction, 1.24, and the known goitrogen, *l*-5-vinyl-2-thiooxazolidone,⁹ were identical.

The compound has been identified unequivocally by comparison of the mass spectrum of the purest fraction isolated from the cabbage leaves with the mass spectrum of *l*-5-vinyl-2-thiooxazolidone. No previous mass spectrometric examination of this compound has been found in the literature. The spectra are shown in Fig. 2, Curves A and B, respectively. Slight discrepancies in peak intensity may be noted at mass numbers 43 and 44. The increased intensity of these peaks in the spectrum of the cabbage fraction can be attributed to impurities. However, several peaks are seen which are unique in the spectrum of the vinyl thiooxazolidone. Among these are the mass numbers 26, 27, 39, and 96, and the groupings of peaks from 50 to 55, 60 to 62, 68 to 70,

85 to 87, and 112 to 114. The molecular ion peak, mass number 129, and the doubly charged ion peak at 25.5 are particularly significant.

Although the fraction examined was known to be impure, from spectrophotometric evidence, the mass spectrum shown in Fig. 2 is practically free of interference from peaks due to impurities. This is a fortuitous circumstance, a result, undoubtedly, of the fact that the impurities in the sample which accompany the thiooxazolidone are not volatile at the temperature used in the inlet system. The remarkable correspondence of the spectrum of the unknown with that of the known compound leaves no doubt as to the identity of *l*-5-vinyl-2-thiooxazolidone in the fraction derived from fresh cabbage leaves.

The amount of goitrin present in the edible portions of the plants investigated was estimated to be one part in eight million, a quantity which is probably negligible in practical nutrition.

SUMMARY

Goitrin, a strongly antithyroid compound, previously found in seeds, has now been obtained from fresh cabbage leaves in very small amounts and has been identified by the following properties.

1. The melting point of a micro amount of crystals agreed with that of *l*-5-vinyl-2-thiooxazolidone, and a mixed melting point with authentic goitrin gave no depression.
2. The ultraviolet absorption spectrum revealed a single absorption maximum at 240 $m\mu$ and a minimum at 222 $m\mu$ consistent with those given by pure goitrin.
3. The flow rates obtained from two different solvent systems in paper chromatography agreed with those of authentic goitrin.
4. Goitrin derived from fresh cabbage has been identified unequivocally by comparison of its mass spectrum with that of *l*-5-vinyl-2-thiooxazolidone.

Acknowledgments—The authors are indebted to Professor M. G. Ettliger of The Rice Institute, Houston, Texas, for helpful advice; and to our colleagues at the Pioneering Research Division of this Command for their interest and collaboration.

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⁴ The spray reagent was diluted 1:1 with water to prevent discoloration by excess iodine.

⁵ R_{F} is defined as the ratio between the distances traveled by the individual compound and phenylthiourea.

⁶ The R_{F} value of 1.20 was obtained with chromatographic paper which had been successively washed with redistilled water, 95 per cent ethanol and chloroform to remove soluble impurities. This compares with the R_{F} value of 1.10, resulting from the use of unwashed paper.

⁷ A sample of this substance was kindly furnished by Dr. E. B. Astwood.

⁸ By private communication from Professor M. G. Ettliger.

⁹ The *l*-5-vinyl-2-thiooxazolidone was obtained from enzymatic hydrolysis of progoitrin kindly furnished by Dr. Monte A. Greer.