disodium salt was not analyzed, but an infrared absorption spectrum confirmed the expected structure.

A solution of PAR in water is orange and showed an absorption maximum at 410 mμ. In the pH range 6—10 PAR and lead(II) form a red complex with an absorption maximum at 515 mμ. Curves of continuous variations were plotted at pH 10 (ammonia-ammonium chloride buffer) for solutions of PAR and lead(II), and a distinct maximum was obtained at the mole ratio 1:1. The curves of continuous variations were then utilized for the calculation of the stability constant, which was found to be $K = 3.0 \times 10^6$. Ionic strength of the solutions was about 0.01.

1. Wehber, P. *Z. anal. Chem.* **158** (1957) 10.
2. Pollard, F. H., Hanson, P. and Geary, W. J. *Anal. Chim. Acta* **20** (1959) 26.
3. Langmyhr, F. J. and Kristiansen, H. *Anal. Chim. Acta* **20** (1959) 542.
4. Chichibabin, A. E. *Zhur. Russ. Fiz.-Khim. Obshchestva* **50** (1920) 512.

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A New Type of Enzymatic Cleavage of Mustard Oil Glucosides. Formation of Allylthiocyanate in *Thlaspi arvense* L. and Benzylthiocyanate in *Lepidium ruderale* L. and *Lepidium sativum* L.

ROLF GMELIN and ARTTURI I. VIRTANEN

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

The cleavage of mustard oil glucosides to *iso*thiocyanate, sulphate, and glucose, which is effected by myrosinase, is considered to be a specific enzymatic process responsible for the characteristic pungent odour or taste of many representatives of the *Cruciferae, Tropaeolaceae, Capparidaceae, Resedaceae,* and some other plant families. After having worked out a revised structural formula for the mustard oil glucosides, Ettlinger and Lundeen [1] proposed that the enzymatic formation of *iso*thiocyanates takes place through Lossen-rearrangement (reaction 1). In some other representatives of the *Cruciferae* family an unpleasant garlic-like odour is characteristic. In the literature there is no real information on the chemical nature and the way of formation of these compounds. Some plant species even derive their names from the garliclike odour (*Thlaspi alliaceum* L., *Alliaria officinalis* Andrz., *Peltaria alliacea* Jacg.).

We have now investigated the chemical nature and formation of the substances that are responsible for this strong garlic-like odour, which has led to the use of the term "garlic oils" ("Lauchöle") [2] in the literature.

With fresh plants and seeds of *Thlaspi arvense* L., *Lepidium ruderale* L., and to a somewhat smaller extent of *Lepidium sativum* L., there appears an unpleasant odour and taste when the plant material is crushed with some water. Our investigations especially with seeds of *Thlaspi arvense, Lepidium ruderale,* and *Lepidium sativum* led to the following results:

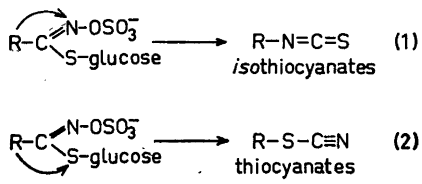
1. An enzymatic process is responsible for the formation of the unpleasant smelling substances.

2. Sinigrin in *Thlaspi arvense* and glucotropaeolin in *Lepidium ruderale* and *L. sativum* are the precursors of the smelling substances.

3. In addition to glucose and sulphate allylthiocyanate in *Thlaspi arvense*, benzylthiocyanate in *Lepidium ruderale*, and a mixture of benzyl*iso*thiocyanate and benzylthiocyanate in *Lepidium sativum* are formed as fission products. Synthetic allyl- and benzylthiocyanates have the same odour and taste as is formed in *Thlaspi arvense* and *Lepidium ruderale*. The substances with similar, unpleasant odours, characteristic of many other *Cruciferae* plants when chewed, are accordingly alkylthiocyanates — a new class of natural S-containing compounds. Reactions 1 and 2 illustrate the formation of *iso*thiocyanates and normal thiocyanates.

Experimental evidence for the conclusions:

These smelling substances are not formed when the seedpowder is treated with boiling water or boiling alcohol. In methanolic extracts from seeds of *Thlaspi arvense*



sinigrin can be detected by paper chromatography, while from seeds of *Lepidium ruderale* and *Lepidium sativum* the glucoside detected is glucotropaeolin. Both glucosides could be isolated (sinigrin in 1.4 % yield from *Thlaspi arvense* seeds, and glucotropaeolin in 1.5 % yield from *Lepidium ruderale*). They proved to be identical with authentic preparations from *Brassica nigra* Koch and *Tropaeolum majus* L. No other glucosides could be found by paper chromatography in *Thlaspi arvense* and in the two *Lepidium* species.

When the finely ground seeds are treated with cold water, after half an hour sinigrin and glucotropaeolin are no longer detectable. On the other hand the content of free glucose and sulphate increases and corresponds to the amount of cleaved glucosides. The formation of allyl*iso*thiocyanate (in *Thl. arvense*) and benzyl*iso*thiocyanate (in *Lep. ruderale*) which normally would be expected is lacking, however. In regard to the typical odour the S-containing compounds which are formed besides glucose and sulphate, are identical with synthetic allyl- and benzylthiocyanate. The identity of the two thiocyanates with allylthiocyanate and benzylthiocyanate was confirmed by the following reactions:

1. Cleavage by nascent hydrogen, by H_2S or by cysteine led to allylmercaptan and benzylmercaptan, respectively, which were isolated and analyzed as Hg-mercaptides.

2. By cleavage with NaSH thiocyanate ions were formed together with allylmercaptan and benzylmercaptan. The colorimetrically determined thiocyanate ions were in good agreement with the amounts of sinigrin or glucotropaeolin originally present.

3. In model experiments the synthetic preparations of allyl- and benzylthiocyanates behaved qualitatively and quantitatively analogously in the formation of mercaptans, mercaptides, and thiocyanate ions.

4. A small amount of benzylthiocyanate could be isolated in crystalline form from moistened, crushed seeds of *Lepidium ruderale* by steam distillation. Melting points and IR-spectra of the natural and synthetic products were identical.

The factors which are responsible for the formation of *iso*thiocyanates in most cases and of thiocyanates in some cases, are not known. Attempts to separate a thiocyanate forming enzyme led in every case to enzyme preparations with myrosinase activity which in the normal way split mustard oil glucosides to *iso*thiocyanate, glucose, and sulphate. If a certain factor which regulates or directs the migration of the alkyl residue to the S-atom instead of to the N-atom exists in the plants where thiocyanates are formed, could not yet be decided. There are, however, indications for this conclusion. It is possible that in the seeds of *Lepidium sativum*, in which benzylthiocyanate and benzyl*iso*thiocyanate are formed simultaneously by the enzymatic process, the factor is present in a smaller quantity than in *Lepidium ruderale*.

We have evidence that this new type of cleavage of the mustard oil glucosides into thiocyanates is rather common in the *Cruciferae* family. The new findings will be reported in a later communication.

Because of their reactivity with thiol-groups and because of their ability to form thiocyanate ions, normal alkylthiocyanates are by no means physiologically harmless substances. Since many important vegetable and fodder plants belong to the *Cruciferae* family, the question of the formation and physiological activity of the thiocyanates seems to be an important problem.

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1. Ettlinger, M. G. and Lundeen, A. J. *J. Am. Chem. Soc.* **78** (1956) 4172.
2. Czapek, Fr. *Biochemie der Pflanzen*, 2. Aufl., G. Fischer, Jena 1921, Vol. 3, p. 183.

Received September 12, 1959.