which may not change the N—H vibration frequency much.

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1. Yoneda, H. *Bull. Chem. Soc. Japan* **28** (1955) 125.
2. Fronæus, S. and Larsson, R. *Acta Chem. Scand.* **16** (1962) 1433.
3. Anderson, J. S., Briscoe, H. V. A. and Spoor, N. L. *J. Chem. Soc.* **1943** 361.
4. Block, H. and Gold, V. *J. Chem. Soc.* **1959** 966.
5. Hill, D. H. and Rosenberg, A. F. *J. Chem. Phys.* **24** (1956) 1219.
6. Bellamy, L. J. *The Infrared Spectra of Complex Molecules*, Meuthen & Co, Ltd, London 1958.
7. Pimentel, G. C. and McClellan, A. L. *The Hydrogen Bond*, W. H. Freeman and Co., San Fransisco and London 1960.
8. Cannon, C. G. *Spectrochim. Acta* **10** (1958) 341.

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A New γ-Glutamylpeptide, γ-L-Glutamyl-S-(prop-1-enyl)-L-cysteine, in the Seeds of Chives (*Allium schoenoprasum*)

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The precursor of the lachrymatory factor was isolated in this laboratory two years ago from onion bulbs (*Allium cepa*) and was characterized chemically as (+)-S-(prop-1-enyl)-L-cysteine sulphoxide [1]. In larger amounts this sulphoxide is present in onion bound with the γ-carboxyl group of L-glutamic acid [2] (γ-L-glutamyl-S-(prop-1-enyl)-L-cysteine sulphoxide). We have now isolated the corresponding peptide with a reduced S-atom from the seeds of chives. The isolation of the pure peptide met with difficulties because of its instability. The isolation was finally performed as follows.

1 kg of ground seeds were thoroughly extracted with 70 % ethanol, the amino acids and peptides were separated on an Amberlite IR-120 column, and 0.5 N acetic acid was added to the evaporated ammonia eluate. 200 mg of Johns-Manville filter gel was added to the turbid solution, and the mixture filtered. The fractionation of the solution was performed using a 4.9 × 59 cm column filled with Dowex 1 × 8 resin in acetate form. The eluant was at first 0.5 N acetic acid, after 170 fractions 1 N acetic acid, after 516 fractions 2 N acetic acid, and after 801 fractions 1 N HCl. 1025 fractions of 20.3 ml each were taken in all. Several unknown spots occurred on the paper chromatograms in the fractions separated.

Peptide 12 (the peptide in question) emerged from the column in fractions 594—633. The fractions were evaporated to dryness by lyophilization. The residue was dissolved in 70 % v/v isopropanol and was fractionated with this solvent on a 6.4 × 36.5 cm cellulose powder column. Peptide 12 emerged from the column in fractions 59—81, the volume of the fractions being 18.4 ml. The fractions were evaporated to dryness *in vacuo*, and the peptide was crystallized from an acetone-water mixture. The yield was 1.02 g of white needle-shaped crystals. Since the peptide is to some degree unstable, losses were probably considerable. Several faint spots which travelled slower than the peptide occurred on the paper chromatogram developed with butanol-acetic acid-water as solvent. When the peptide was separated by cutting off the corresponding zone and running a new chromatogram with the same solvent system, the same faint spots appeared again. The peptide accordingly decomposed to some extent during the preparation of the chromatogram.

Structure elucidation of peptide 12. The γ-glutamyl bond in the peptide was established by determining the free α-amino and carboxyl groups according to Linko [3]. 79.8 % of ammonia and 96.3 % of carbon dioxide were split off by the effect of ninhydrin. According to the method of Buchanan [4], 90 % of the theoretical amount of NH_3 was split off.

An enzyme preparation of calf kidney hydrolyzed the peptide in a phosphate buffer at pH 7.4. Glutamic acid and a sulphur-containing amino acid which travelled at much the same speed as S-propyl-cysteines were established by paper chro-

matography as hydrolysis products. The sulphur-containing amino acid was isolated in crystalline form by fractionation on a cellulosepowder column using butanol-acetic acid-water as solvent. It travelled as S-isopropyl-cysteine on the paper chromatogram (butanol-acetic acid-water) but the colour formed with ninhydrin distinguished it from this cysteine derivative. S-propyl-cysteine travelled somewhat faster, and S-allyl-cysteine somewhat slower than the S-containing amino acid of peptide 12.

When the precursor of the lachrymatory substance was reduced with sodium bisulfite [5], a crystalline substance was obtained. On the basis of both paper chromatography and the IR spectrum it was identical with the sulphur-containing amino acid formed from peptide 12 on enzymic hydrolysis. The unknown sulphur-containing amino acid was thus S-(prop-1-enyl)-cysteine, and hence peptide 12 was γ-glutamyl-S-(prop-1-enyl)-cysteine. In order still to corroborate the result, peptide 12 was oxidized with hydrogen peroxide in glacial acetic acid. It was then established by paper chromatography that peptide 4, γ-glutamyl-S-(prop-1-enyl)-cysteine sulphoxide, isolated from onion, was formed. Both peptides were hydrolyzed by the kidney preparation, and an enzyme preparation of onion was added to the phosphate buffer solutions of the evaporated hydrolysates. An easily recognized lachrymatory substance was then formed from both.

The configuration of the glutamic acid and the cysteine contained in peptide 12 was determined by hydrolyzing 300 mg of the peptide by adding 3 g of dry Amberlite IR-120 resin in H^+-form, and 10 ml of 70 % ethanol. The mixture was placed in a test tube from which air was removed by nitrogen gas, and the sealed tube was kept for 4 h in a water bath. The amino acids were separated in the usual way and fractionated on a Dowex 1 × 8 column. Glutamic acid, cystine, and in addition an unknown compound and very small amounts of unknown decomposition products were formed on hydrolysis. It is interesting to note that by this method of hydrolysis S-(prop-1-enyl)-cysteine remains largely undecomposed although it decomposes completely on ordinary acid hydrolysis.

The optical rotation of the glutamic acid isolated: $[\alpha]_D^{23} = +35.2$ in 6 N HCl, and of the cystine separated on a cellulose powder column: $[\alpha]_D^{23} = -179$ in 1 N HCl were found.

Both glutamic acid and cysteine are thus L-forms, and the new peptide 12 is accordingly γ-L-glutamyl-(prop-1-enyl)-L-cysteine.

The R_F-values for peptide 12 and different alkyl and alkylene cysteines are given in Table 1.

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Table 1.

Compounds	Solvents	
	butanol-acetic acid-water (63:10:27)	phenol-water (NH_3)
Alanine	0.21	0.57
γ-L-Glutamyl-S-(prop-1-enyl)-L-cysteine	0.42	0.21
S-(Prop-1-enyl)-cysteine	0.55	0.81
S-Propyl-cysteine	0.58	0.81
S-Allyl-cysteine	0.50	0.81
S-Isopropyl-cysteine	0.56	0.83

1. Virtanen, A. I. and Spåre, C.-G. *Suomen Kemistilehti B* **34** (1961) 72; Spåre, C.-G. and Virtanen, A. I. *Acta Chem. Scand.* **17** (1963) *In press.*
2. Virtanen, A. I. and Matikkala, E. J. *Suomen Kemistilehti B* **34** (1961) 84; 114.
3. Linko, P. *Suomen Kemistilehti B* **28** (1955) 96.
4. Buchanan, D. L., Haley, E. E. and Markiw, R. T. *Biochemistry* **1** (1962) 612.
5. Micheel, F. and Schmitz, H. *Ber.* **72** (1939) 992.

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