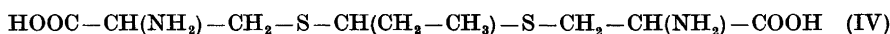
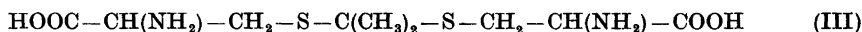
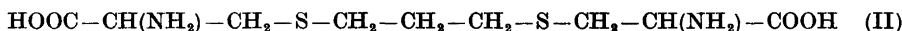
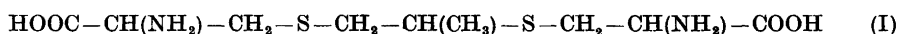


Synthesis of 3,3'-(2-Methylethylene-1,2-dithio)-dialanine, an Amino Acid Found as γ -Glutamylpeptide in the Seeds of Chive (*Allium schoenoprasum*)

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In an earlier paper¹ the isolation and the structure of a complicated γ -diglutamylpeptide (peptide R XVIII), containing two sulphur atoms, found in the seeds of chive were presented. The peptide was shown to be N,N'-bis-(γ -glutamyl)-3,3'-(2-methylethylene-1,2-dithio)-dialanine. On hydrolysis of this peptide with 1 N or 6 N hydrochloric acid or with kidney preparation 2 moles of L-glutamic acid and 1 mole of a sulphur-containing amino acid (amino acid R) were formed. When treated with Raney nickel at different temperatures only alanine could be found in the solution. In addition a gaseous product was formed which could be identified as propane mass-spectrometrically. One of the following four structures was now possible for the amino acid:



I and II were at first synthesized, as was mentioned in the former communication. Because synthetic amino acid I was identified by its IR-spectra and by paper-chromatography as amino acid R formed on hydrolysis from peptide XVIII the amino acids III and IV were not synthesized. The synthesis of amino acids I and II were performed as follows.

Synthesis of amino acid I, 3,3'-(2-methylethylene-1,2-dithio)-dialanine. 7.024 g L-cysteine·HCl·H₂O (Schwarz Laboratories Inc.) was dissolved in 85 ml 2 N NaOH and 110 ml 94 % ethanol. 2.1 ml 1,2-dibromopropane (Fluka) was added with simultaneous shaking. The solution was kept for 24 h at room temperature. A mercaptan reaction with Na-nitroprusside was still noted and 0.5 ml 1,2-dibromopropane was therefore added to the solution. After 22 h the solution, which still gave a clear mercaptan reaction, was passed through an Amberlite IR-120(H⁺ form 150 ml)-column. The column was washed with distilled water and the amino acids were eluted with 600 ml 1 N NH₃. After vacuum distillation of the eluate the residue was extracted with 400 ml isopropanol-acetic acid-water-solvent (65:10:25). The soluble part gave on a paper chromatogram a distinct spot identical with the spot of amino acid R together with faint spots of cysteine and cystine. The extract was fractionated with the above-mentioned solvent on a cellulose column. The fractions which contained amino acid I were combined and distilled to dryness *in vacuo*. The residue was dissolved in as small an amount of water as possible and the solution was treated with activated carbon to remove the colour. The amino acid was precipitated from hot aqueous solution by adding acetone gradually. The white product which resulted was recrystallized from acetone-water solution.

The insoluble portion was dissolved in 200 ml 1 N ammonia. On evaporating the solution to half its original volume, part of the dissolved substance was precipitated. On crystallization 3 g of cystine was separated which contained

as impurity amino acid I. More of the amino acid could still be separated from the solution.

The final yield of paperchromatographically pure, crystallized amino acid I was 1040 mg. (Found: C 38.23; H 6.15; N 9.75; S 22.56; O 22.65; Calc. for C₉H₁₆N₂S₂O₄: C 38.28; H 6.43; N 9.92; S 22.71; O 22.66). The substance began to decompose at about 180°C.

Synthesis of amino acid II, 3,3'-(propylene-1,3-dithio)-dialanine. 2.655 g L-cysteine (Fluka

Table 1. R_F values of γ -glutamylpeptides, synthetic amino acids I and II, and of some common amino acids.

Compounds	Solvents	
	butanol-acetic acid-water 12:3:5	phenol-water (NH ₃)
Peptide R XVIII	0.20	0.21
Amino acid R (hydrol. product of peptide R XVIII)	0.22	0.60
Synth. amino acid I	0.22	0.60
» » » II	0.16	0.57
Peptide R XIII (N,N'-bis-(γ -glutamyl)-L-cystine)	0.12	0.10
Glutamic acid	0.27	0.27
Alanine	0.32	0.57
Valine	0.50	0.74

AG, puriss.) was dissolved in 30 ml of 2 N NaOH. 40 ml of 94 % ethanol, and then 1 ml of 1,3-dibromopropane was added with mixing. After standing at room temperature in darkness for 19 h the solution no longer gave a mercaptan reaction. Thus 1,3 dibromopropane reacted with cysteine much faster than 1,2-dibromopropane. The amino acids were separated on an Amberlite column, eluting with 500 ml 1 N NH₃. The eluate was reduced *in vacuo* to a volume of 170 ml. The white precipitate thus formed was separated by filtration, and washed with a small amount of water which was combined with the filtrate. After washing with acetone the precipitate (substance b), dried *in vacuo*, weighed 2.345 g. A small amount of the substance dissolved in 1 N ammonia was chromatographed on paper both with butanol-acetic acid-water and phenol-water-NH₃ solvents. The substance proved to be amino acid II. In the former solvent it moved a little more slowly than amino acids R and I, but faster than cystine. In phenol solvent the difference in the movement was so slight that this solvent was not suitable for the separation of amino acids I and II. Amino acids R, I and II gave with ninhydrin on the paper chromatogram grayish-violet spots.

In the filtrate some of amino acid II, cystine and a substance that moved quickly on the paper chromatogram and was coloured red-violet with ninhydrin (probably a cyclic compound, formed when one of the Br atoms of dibromopropane reacted with the amino group of cysteine) were found. The solution was not treated further.

The substance b was recrystallized by dissolving it in 1 N NH₃ and then adjusting the pH of the solution to 5.0. The precipitate was washed with cold water and acetone and then dried at room temperature *in vacuo* over phosphorus pentoxide. The substance began to decompose at about 225°C. (Found: C 38.18; H 6.27; N 9.84; S 22.58; O 22.78. Calc. for C₆H₁₆N₂S₂O₄: C 38.28; H 6.43; N 9.92; S 22.71; O 22.66).

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