On Sulphur-Containing Amino Acids and γ-Glutamyl Peptides in the Bulbs and Seeds of Allium Species*

by Artturi I. VIRTANEN**

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The studies in this laboratory of the organic sulphur compounds in Allium species have led to the isolation and characterization of a number of new cysteine derivatives. These findings made it possible to study the enzymatic formation of some biologically active substances and the chemical nature of these compounds. A good example of these studies is the isolation of (+)-S-(propen-1-yl)cysteine sulphoxide from the onion bulb1 and the demonstration that the lachrymatory factor is formed from this sulphoxide by an onion enzyme1,2. The lachrymatory factor was shown to be propenylsulphenic acid, the first known representative of the group of aliphatic sulphenic acids2-3. Later on the lower homologue of the propenyl derivative, S-vinylcysteine sulfoxide, was synthesized and its enzymatic splitting to vinylsulphenic acid was shown by a preparation from onion bulb4,5. Also a higher homologue of propenyl cysteine sulfoxide, S-(buten-1-yl)cysteine sulfoxide, was recently synthesized in this laboratory and the formation of butenylsulphenic acid was demonstrated6. All these sulphenic acids are so unstable that their chemical structure could be studied only mass-spectrometrically.

From the onion bulb was isolated also a cyclic sulfoxide7,8. It was shown to be 3-methyl-1,4-thiazane-5-carboxylic acid-1-oxide, the first thiazane compound found as a natural product9,10. It is present in larger amounts than any other sulphur-containing amino acid in onion. Cycloalliin was formed in dilute ammoniacal solution from S-(propen-1-yl)cysteine sulfoxide.

However, when propenyl cysteine sulfoxide labelled with 35S was injected into an onion bulb, which had been kept 6 days in moistened sand for activation of enzymes, no labelled cycloalliin was formed enzymatically. S-(propen-1-yl)cysteine sulfoxide might thus not be the precursor of cycloalliin in the plant. Studies of the mechanism of the biosynthesis of cycloalliin are being continued in this laboratory.

When 35S-sulphate was injected into an onion bulb, cycloalliin had after 12 hrs. practically as high a labelling as the spot of cystine-glutathione. After a longer time cycloalliin had the highest labelling of all the numerous sulphur compounds formed. Accordingly, the enzymatical formation of cycloalliin seems to be very rapid11.

It is interesting that Kuriyama et al.12, Oka et al.13, Takemoto14, Tominaga and Oka15 later found norcycloalliin (cycloalliin minus one CH₃-group) from brown and
red algae. When later on vinylcysteine sulphoxide was synthesized in this laboratory, the formation of norcycloalliin from the sulphoxide in ammoniacal solution could be demonstrated. The assumption that the algae may contain, in analogy with onion, a lower homologue of the lachrymatory precursor, S-vinylcysteine sulphoxide, could not be confirmed with the red alga, Chondria crassicaulis, a sample of which, preserved in ethanol, was kindly sent to me by Dr. Y. Yamada, Department of Botany, Hokkaido University.

Recently, S-crotyl- and S-buten-1-yl-cysteine sulphoxide were synthesized in this laboratory. From the butenyl derivative a lachrymatory factor, butenylsulphenic acid, was formed by the onion preparation. No cyclization of this sulphoxide to the corresponding thiazane derivative in ammoniacal solution could be demonstrated. This can depend on the proportion of cis-trans isomers in the preparation (cf. Carson and Wong) used and also on re-isomerization of butenyl derivative to the crotyl. The length of the carbon chain linked to the S-atom of the cysteine part of the molecule may also influence the cyclization.

As the free amino acid S-propenylcysteine sulphoxide is present in a relatively small amount in onion bulbs, but as the \( \gamma \)-glutamyl peptide in much higher concentration. Because the onion bulb has been shown to contain \( \gamma \)-glutamylpeptidase, the free sulphoxide can be formed from the peptide enzymatically.

\( \gamma \)-Glutamyl peptides are characteristic compounds in the bulbs and seeds of Allium species. The studies of these compounds up till 1965 have been recently reviewed by Virtanen.

In this connection a new \( \gamma \)-glutamyl tripeptide recently isolated from the seeds of chive is especially to be mentioned. On the “map” of the \( \gamma \)-glutamyl peptides present in the seeds of chive this peptide is marked as RXI (Fig. 1).

In most of the sulphur-containing \( \gamma \)-glutamyl peptides the cysteine derivatives are in reduced form. Exceptionally, the precursor of the lachrymatory factor is present as the sulphoxide in the \( \gamma \)-glutamyl peptide of the onion bulb. In the seeds of chive the corresponding \( \gamma \)-glutamyl peptide contains the cysteine derivative in reduced form. Suzuki et al. mention also \( \gamma \)-glutamyl-S-methylcysteine sulphoxide as a component of garlic.

The new \( \gamma \)-glutamyl tripeptide RXI is structurally a unique one because it contains S-(propen-1-yl)-L-cysteine both in reduced and in oxidized form. The reduced form is connected through a \( \gamma \)-peptide bond to glutamic acid and the oxidized form through an \( \alpha \)-peptide bond to the reduced propenylcysteine. The structure of the new peptide is as follows.

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\begin{align*}
\text{CO} & \quad \text{-NH-CH-CH}_2\text{-S-CH=CH-CH}_3 \\
\text{CH}_2 & \quad \text{CO} \\
\text{CH}_2 & \quad \text{NH} \quad \text{O} \\
\text{CH-NH}_2 & \quad \text{CH-CH}_2\text{-S-CH=CH-CH}_3 \\
\text{COOH} & \quad \text{COOH}
\end{align*}
\]

The evidence to support this structure is as follows:

1. When the peptide RXI (Fig. 1) is hydrolyzed with an enzyme preparation made from cow's kidney, which hydrolyzes both \( \alpha \)- and \( \gamma \)-peptide bonds, three amino acids are formed that can be identified paper-chromatographically: glutamic acid, S-(propen-1-yl)cysteine and S-(propen-1-yl)cysteine sulphoxide.
2. Evidence that S-(propen-1-yl)cysteine sulphoxide was not a secondary product formed during enzymatic hydrolysis was obtained when the peptide RXI and the peptide RXII, which in an earlier study was proved to be γ-glutamyl-S-(propen-1-yl)cysteine, were hydrolyzed with a kidney preparation. The corresponding sulphoxide is not then formed from the latter, but S-(propen-1-yl)cysteine solely.

3. The S-propenylcysteine sulphoxide formed enzymatically from the peptide RXI was identified by the observation that an enzyme preparation from onion formed lachrymatory substance in an aqueous solution of the peptide. Another sulphur-containing amino acid, S-(propen-1-yl)cysteine, formed in the enzymatic hydrolysis of the tripeptide, was identified by oxidizing it in glacial acetic solution with hydrogen peroxide. The corresponding sulphoxide then formed gave a lachrymatory effect with onion enzyme.

4. The N-terminal amino acid was shown to be L-glutamic acid using Sanger's method as described by Waley.

5. The C-terminal amino acid was determined enzymatically with carboxypeptidase A (Sigma Chemical Company's carboxypeptidase-A DFP). This enzyme preparation hydrolyzes only α-peptide bonds. A quantitative determination showed that S-(propen-1-yl)cysteine sulphoxide is then freed and peptide RXII, γ-glutamyl-S-(propen-1-yl)cysteine, is formed at the same time. The yield of the latter was quantitative, whereas the sulphoxide began to decompose when the reaction time was lengthened.
The optical rotation of the peptide RXI was $[\alpha]_{D}^{20} = +76.0$ (in water). The glutamic acid of the peptide RXI was L-form (dextro-rotation in 6N HCl). The S-(propen-1-yl)-cysteine sulfoxide found in the peptide was also L-form, because the carboxypeptidase used hydrolyzes the peptide bond only if the amino acids are L-form.

The $R_f$-values of the peptide RXI on Whatman No. 1 paper at room temperature were: in butanol-acetic acid-water 0.54 (alanine 0.30, glutamic acid 0.24), in phenol-water (NH$_3$) 0.81 (alanine 0.60, glutamic acid 0.26).

References