ACTA CHEMICA SCANDINAVICA 21 (1967) 1654–1680

Short Communications

S-(2-Carboxypropyl)-cysteine and its Sulfoxide as Precursors in the Biosynthesis of Cycloalliin

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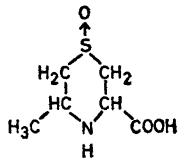
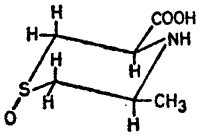
Cycloalliin or 3-methyl-1,4-thiazane-5-carboxylic acid-1-oxide (I) has been reported as the quantitatively most important sulfur compound in onion, *Allium cepa*.[1-3] It consists of a thiazane ring with the chair configuration and the sulfoxide oxygen in the axial position (II).[4] The biosynthesis of this compound has not yet been elucidated.



I



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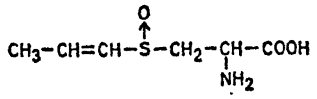
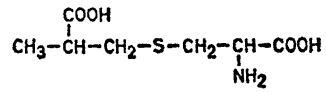


III



IV

Cycloalliin is chemically formed from S-(propen-1-yl)-L-cysteine sulfoxide (III), the lachrymatory precursor found in onion,[5,6] by ring closure in dilute ammonium hydroxide solution. However, this reaction seems not to be involved in the biosynthetic mechanism.[7] Another biosynthetic pathway will now be proposed and there will be some experimental evidence presented in its favour.

The amino acid S-(2-carboxypropyl)-cysteine (IV) may be a key compound in the biosynthesis of cycloalliin and possibly also in the biosynthesis of some S-alkylated cysteine derivatives. S-(2-Carboxypropyl)-cysteine is easily formed through reaction of cysteine with methacrylic acid. The amino acid occurs in the tripeptide S-(2-carboxypropyl)-glutathione isolated from onion,[8] but it has not earlier been found in free form in plants. However, when methacrylic acid was injected into sprouting onions, distinct spots as well of the free amino acid as of its corresponding tripeptide appeared on two-dimensional chromatograms. When radioactive cysteine was injected into onions, the spot of S-(2-carboxypropyl)-cysteine became labeled, even if the ninhydrin reaction was negative. These findings indicate that small amounts of free S-(2-carboxypropyl)-cysteine are present in onion. This may be due to a reaction between cysteine and methacrylic acid or methacrylyl-CoA, which is an intermediate in the breakdown of valine. Since in onion the amount of free cysteine is small, a quantitatively more important synthetic route may be from glutathione to S-(2-carboxypropyl)-glutathione, which is subsequently hydrolysed.

Radioactive S-(2-carboxypropyl)-cysteine was synthesized from DL-cysteine-3-^{14}C and injected into onions. The distribution of the activity in the amino acid fraction was determined after 1 day and 7 days. The amino acids were separated by two-dimensional paper chromatography,[9] the radioactivity located by radioautography and the radioactive spots ignited with Cuprox® catalyst, after which the $^{14}CO_2$ could be determined in an ionizing chamber. Table 1 shows the activity of the

Table 1. The distribution of radioactivity after injection of S-(2-carboxypropyl)-DL-cysteine-3-^{14}C into onions.

Compound	Onion 1 0 days %	Onion 2 1 day %	Onion 3 1 day %	Onion 4 7 days %
CPC	98.8	54.0	58.7	2.3
CPC-sulfoxide	1.2	0.5	0.8	1.8
Cycloalliin	—	10.9	10.2	17.5

Table 2. The distribution of radioactivity after injection of S-(2-carboxypropyl)-DL-cysteine-3-^{14}C-sulfoxide into onions.

Compound	Onion 1 0 days %	Onion 2 1 day %	Onion 3 7 days %
CPC-sulfoxide	100.0	62.4	20.7
CPC	—	1.3	1.4
Cycloalliin	—	1.4	10.5

compounds of interest in per cent of the activity recovered in the control experiment. S-(2-Carboxypropyl)-cysteine was easily metabolized (40—50 % in 1 day, 97.7 % in 7 days), the main product in the amino acid fraction being cycloalliin (10—11 % in 1 day, 17.5 % in 7 days).

S-(2-Carboxypropyl)-cysteine is apparently spontaneously oxidized to the corresponding sulfoxide. To check the possibility that the latter compound could possibly be an intermediate in the formation of cycloalliin, radioactive S-(2-carboxypropyl)-cysteine sulfoxide was synthesized by oxidation of the thioether with hydrogen peroxide and injected into onions. The sulfoxide was reduced only to a small degree to the thioether. Thus the interconversion does not proceed easily in either direction. As can be seen from Table 2, the sulfoxide is also converted into cycloalliin, but the reaction is slow (cycloalliin formed after 1 day, 1.4 %, after 7 days 10.5 %; 62.4 % of the sulfoxide injected remaining after 1 day, 20.7 % after 7 days), although it is considered that the oxidation with hydrogen peroxide gives a mixture of the (+) and (−) sulfoxide isomers. It seems thus probable that the sulfoxide is metabolized only after a reduction to the thioether.

The results of the present as well as earlier studies [7,10] seem to justify the following conclusions: Since the sulfoxide cysteine derivatives so far tested, including cycloalliin itself, have been metabolically rather inert, the sulfoxides are not active members of the main metabolic chains, but rather end products, storage products or waste products. Thus they occur in large amounts in onion and are easily detected and isolated. The corresponding reduced forms, which have not been found in free form in onion probably because they occur in very small amounts, seem to be reactive intermediates in the metabolic chains. Work is in progress to test the validity of this general rule.

For the above reasons we propose the pathway described in Fig. 1 for the biosynthesis of cycloalliin from cysteine. The S-propen-1-yl-cysteine has probably the

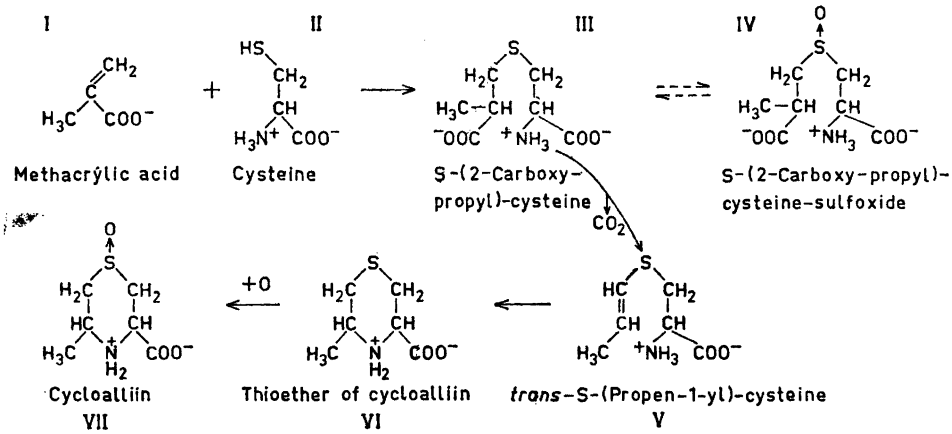


Fig. 1. Proposed pathway in the biosynthesis of cycloalliin from cysteine.

trans-configuration. Experimental evidence is still lacking for some of the intermediate steps, but work is in progress to elucidate this reaction sequence further.

This research has been financed in part by a grant made by the *United States Department of Agriculture, Agricultural Research Service.*

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Received April 27, 1967.

A Simple Laboratory Method for the Preparation of (Dimeric) Ketone Peroxides

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Peroxides from the reaction between simple cyclic ketones and hydrogen peroxide have been isolated and studied by many workers, among others by Milas,[1] Criegee,[2] Cooper,[3] Hawkins,[4] Kharasch,[5] Stoll and Scherrer,[6] and Halbig.[7] In the case of cycloheptanone and cyclooctanone the reaction has been reported to result in mixtures, from which no single well-defined peroxide could be isolated.[8]

The present author has prepared different known and some new ketone peroxides by a simple method which will be described below. The method consists of treating the ketones with 34 % hydrogen peroxide under conditions which allow a facile removal of the water from the aqueous hydrogen peroxide as well as that formed during the reaction. Reaction mixtures are spread in a thin layer on a glass or other suitable surface, and subjected to a stream of dry air. Most simply the layer is left over night in a fume hood. By spraying the reaction mixture over a