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51. **Tomoji Suzuki, Michiyasu Sugii, and Toshio Kakimoto :** Metabolic Incorporation of L-Valine-[^{14}C] into S-(2-Carboxypropyl)glutathione and S-(2-Carboxypropyl)cysteine in Garlic.

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It was previously reported that S-(2-carboxypropyl)glutathione[1] was isolated from onion and garlic. It is known that valine is metabolized via methacrylyl coenzyme A in the animal.[2] From this fact, it is probable that the origin of 2-carboxypropyl group is assumed to be an intermediate metabolite of valine, such as methacrylic acid or the coenzyme A derivative. Therefore, the present experiment has been designed to obtain a prec ise evidence for the origin of 2-carboxypropyl group of this peptide by using uniformly labeled-L-valine-[^{14}C]. The ^{14}C-labeled valine was fed to excised root of garlic under sterile conditions and localization of radioactivity of the isolated amino compounds was examined.

Methods and Results

Tissue Culture of Excised Roots——Peeled bulbs of garlic were washed with soap and 70% EtOH. After washing with sterile water, they were soaked for 5 min. in a 0.1% $HgCl_2$ solution and then washed with sterile water. The sterilized bulbs were placed in a petri dish, its bottom covered with moist filter paper. Temperature was kept at 10~15°. After 10 days, the grown roots were cut into pieces about 2 cm. long. They were collected and immediately submerged into sterile solution

Experiment A was made with 1 g. of excised root and 5 ml. of sterile White's solution[*2] in which 25 μc (3.66 mg.) of L-valine-[^{14}C] (from the Nuclear-Chicago Corp.) was added. Experiment B was carried out with 1 g. of excised root and 5 ml. of sterile White's culture solution in which 25 μc of L-valine-[^{14}C] and 2 mg. of glutathione were added.

Separation of Fractions——After 48 hr.'s cultivation at 10~15°, the roots were immediately boiled with water for 10 min. After cool, the roots were homogenized with the same water as used for boiling and the soluble fraction was obtained by centrifugation. Insoluble fraction (crude protein fraction) was rapidly dried at 80~90°. Water-soluble fraction was put through a column of Amberlite IR-120 (H form), the adsorbed amino acids were eluted with 4% NH_4OH solution (amino acids fraction). The unadsorbed effluent from the column of Amberlite IR-120 was designated as organic acid and sugar fraction.

Determination of Radioactivity——Radioactivity assays were carried out with a Geiger-Müller tube with a thin mica window. Samples were counted at an infinite thinness. If necessary, samples were converted to $BaCO_3$ by wet combustion and self-absorption was corrected.

Radioactivity of Each Fraction——The distribution of total radioactivity in each fraction is shown in Table I. There was no marked difference between A and B.

Identification and Separation of Metabolites——Composition of the amino acid fraction was examined by two-dimensional paper chromatography (solvent system: PhOH-0.08% NH_4OH=4:1, BuOH-AcOH-H_2O=5:1:4) and radioautography. The radioautogram is given in Fig. 1 and the radioactivity of the spots on paper chromatogram is shown in Table II. No significant difference could be found between A and B. The radioactive spots were identified by co-chromatography with the authentic samples. To isolate the radioactive metabolites, both amino acid fractions were combined and submitted to paper chromatography with the solvent system of PhOH-NH_4OH, followed by rechromatography with BuOH-AcOH-H_2O. Each zone was eluted with water and evaporated to dryness.

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*2 Composition : $MgSO_4$ 360 mg., $Ca(NO_3)_2$ 200 mg., Na_2SO_4 200 mg., KNO_3 80 mg., KCl 65 mg., NaH_2PO_4 16.5 mg., $Fe_2(SO_4)_3$ 2.5 mg., $MnSO_4$ 4.5 mg., $ZnSO_4$ 1.5 mg., H_3BO_3 1.5 mg., KI 0.75 mg., sucrose 20 g., glycine 3 mg., nicotinic acid 0.5 mg., pyridoxine 0.1 mg., thiamine 0.1 mg. in 1 L. of solution (L. Reinert, P. R. White : Physiol. Plant., **9**, 177 (1956)).

1) A. I. Virtanen, E. J. Matikkala : Z. physiol. Chem., Hoppe-Seyler's, **322**, 9 (1960); T. Suzuki, M. Sugii, T. Kakimoto : This Bulletin, **9**, 77 (1961).

2) M. J. Coon, W. G. Robinson, B. K. Bachhawat : Symposium on amino acid metabolism, Baltimore, p. 438 (1955).

Table I. Radioactivity of Each Fraction

		Total radioactivity (μc)	Recovery (%)
1) Residual culture solution	A	5.51	22.1
	B	5.10	21.0
2) Crude protein fraction	A	3.01	12.0
	B	4.25	17.0
3) Water-soluble fraction	A	13.56	54.3
	B	11.88	47.5
Amino acid fraction	A	12.00	43.3
	B	10.80	48.8
Organic acid and sugar fraction	A	0.55	2.2
	B	0.87	3.5
Total recovery (1+2+3)	A	22.08	88.4
	B	21.23	85.5

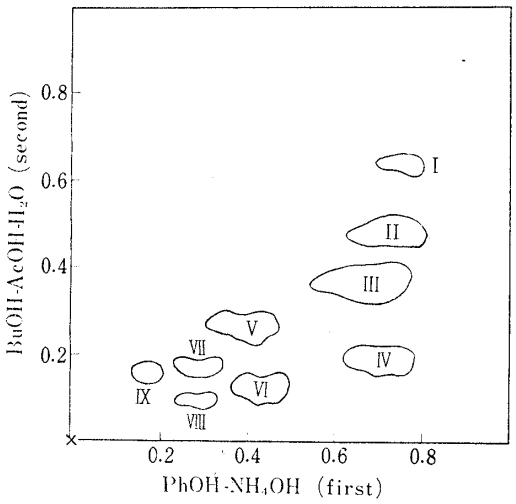

Fig. 1. Radioautogram of Amino Acid Fractions

Table II. Radioactivity of the Spots on Paper Chromatogram

Spot No.	A Total activity (88,080 cpm)		B Total activity (84,456 cpm)		Compound
	cpm	%	cpm	%	
I	635	0.8	200	0.2	——
II	3,273	3.8	2,840	3.3	Leucine
III	57,100	64.8	55,280	65.6	Valine
IV	741	0.8	892	1.1	——
V	2,606	3.1	3,906	4.5	S-(2-Carboxypropyl) cysteine
VI	947	1.1	1,588	1.9	——
VII	421	0.5	528	0.6	S-(2-Carboxypropyl) glutathione
VIII	252	0.3	254	0.4	——
IX	225	0.3	212	0.2	——
	66,200	75.5	65,700	77.8	

Degradation of S-(2-Carboxypropyl)cysteine and S-(Carboxypropyl)glutathione——To radioactive S-(2-carboxypropyl)cysteine, 1 mg, of unlabeled compound was added as a carrier and the mixture was treated with Raney Ni by the method of Fonken and Mozingo.[4] The desulfurization product was separated by treatment with Amberlite IR-120 and the total activity of the separated fraction is shown in Table III (1). It was clearly shown by paper chromatography that the activity of effluent fraction (organic acid fraction) from Amberlite IR-120 was due to isobutyric acid. The fraction (amino acid fraction) adsorbed on Amberlite IR-120 showed lower activity than the organic acid fraction and no radioactivity was found in alanine on paper chromatogram.

4) G. S. Fonken, R. Mozingo : J. Am. Chem. Soc., **69**, 1212 (1947).

To radioactive S-(2-carboxypropyl)glutathione (6,130 cpm) 2 mg. of unlabeled compound was added as a carrier and the mixture was hydrolyzed with 6*N* HCl. The hydrolyzate showed the presence of radioactive S-(2-carboxypropyl)cysteine and inactive glutamic acid and glycine on paper chromatogram. Radioactive S-(2-carboxypropyl)cysteine was collected and treated with Raney Ni as described above. The total activity of the separated fraction is shown in Table III (2) and radioactive isobutyric acid and inactive alanine were obtained on the paper chromatogram.

Table III. Total Radioactivity of Fractions in Desulfurization Experiment with S-(2-Carboxypropyl)cysteine

	S-(2-Carboxypropyl)cysteine (cpm)	Organic acid fraction (cpm)	Amino acid fraction (cpm)
(1)	29,700	27,500	300
(2)	4,050	3,950	40

Degradation of Leucine——After dilution with 20 mg. of unlabeled L-leucine, radioactive leucine was degraded by the method of McManus.[5] Carbon-1 was obtained as CO_2 by reaction with Ninhydrin. Isovaleryl aldehyde from the Ninhydrin oxidation was oxidized to isovaleric acid with alkaline permanganate. This was followed by the Schmidt degradation to yield carbon-2 as CO_2. No radioactivity was found in carbon 1 and 2. The total activity of the products at each step is shown under the formula in Chart 1.

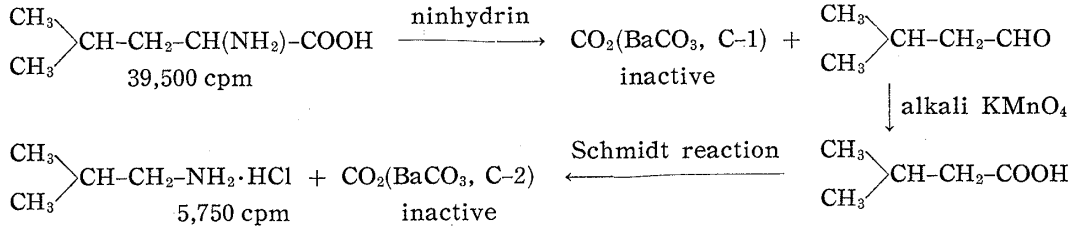

Chart 1.

Discussion

After 48 hours' cultivation of excised root of garlic bulbs in labeled valine, radioactive S-(2-carboxypropyl)glutathione, S-(2-carboxypropyl)cysteine, leucine, andsome unidentified compounds were found in the root.

Recently, S-(2-carboxypropyl)cysteine was detected by Mizuhara and Omori[6] from human urine, while it was separated from the urine of rabbit as urinary metabolite of S-(2-carboxypropyl)glutathione.[7] However, the presence of this compound in the plant kingdom has not been reported previously.

Degradation of radioactive S-(2-carboxypropyl)-glutathione and S-(2-carboxypropyl)-cysteine showed that the radioactivity was located in their carboxypropyl group, proving that the previous assumption is quite probable. It may safely be said that glutathione condenses with methacrylic acid or the coenzyme A derivative and S-(2-carboxypropyl)-cysteine is formed either by the hydrolysis of S-(2-carboxypropyl)glutathione or by the cysteine conjugation with methacrylic acid or the coenzyme A derivative. Radioactive leucine was also degradated. From the distribution of isotopic carbon, it was supposed that the biosynthetic pathway to leucine from valine was the same as reported in various microörganisms.[8] However, this observation in higher plants has not been found before.

Above experimental results show that the most probable biosynthetic pathway of these compounds from valine in garlic is shown in Chart 2.

5) I. R. McManus: J. Biol. Chem., **208**, 639 (1954).
6) S. Mizuhara, S. Omori: Arch. Biochem. Biophys., **92**, 53 (1961).
7) T. Suzuki, M. Sugii, T. Kakimoto: This Bulletin, **10**, 328 (1962).
8) T. Suzuki: "Seikagaku Koza," **6**, 249 (1959), Kyoritsu Shuppan Co., Ltd., Tokyo.

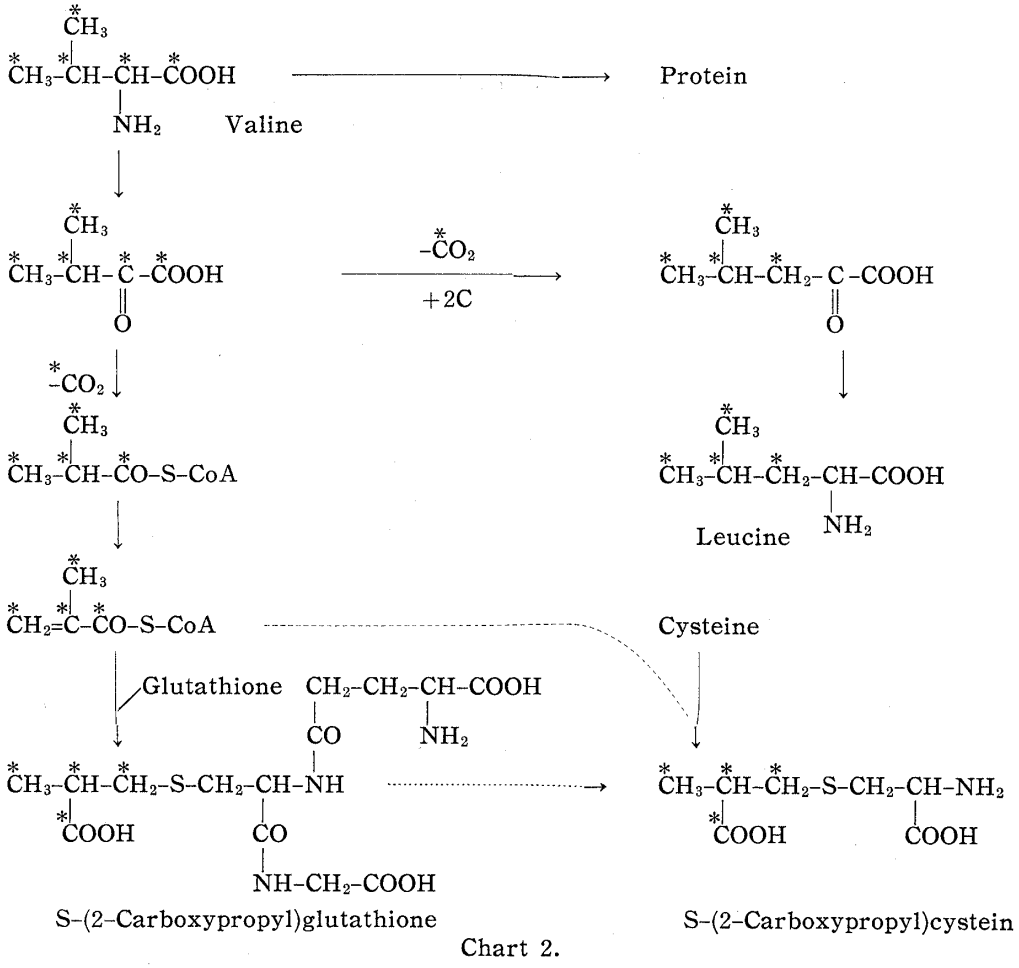

Chart 2.

Summary

It was proved that uniformly labeled L-valine-[^{14}C] is incorporated into 2-carboxypropyl group of S-(2-carboxypropyl)glutathione and S-(2-carboxypropyl)cysteine in excised root of garlic. It was also found that leucine is formed from valine in a similar fashion as reported in various microörganisms. The biosynthetic pathway of these compounds from valine in garlic is discussed.

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