The S-C Cleavage of S-(*cis*-1-Propenyl)-L-cysteine Sulfoxide by OH Radicals from Irradiated Aqueous Solutions†

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Sulfoxide amino acids, such as S-alkyl-L-cysteine sulfoxides (alkyl=methyl, *n*-propyl, allyl, 1-propenyl), are found in *Allium*,²⁾ *Brassica*³⁾ and *Phaseolus*⁴⁾ species. Though sulfide amino acid (methionine) is generally known to be highly sensitive to γ -irradiation and a number of studies have been carried out on the degradation mechanism,⁵⁻⁷⁾ the γ -radiolysis of sulfoxide amino acids of biological interest has never been adequately investigated.

In connection with flavor deterioration accompanying food irradiation, the authors have studied the mechanism of formation of volatile products from sulfur-containing amino acids (precursors of onion and garlic flavors) irradiated by γ -rays in oxygen-free aqueous solutions.⁸⁻¹⁰⁾ In addition, a new *cis-trans* isomerization has recently been found during studies on the γ -radiolysis of S-(*cis*-1-propenyl)-L-cysteine sulfoxide (PeCSO) (isomers of naturally-occurring lachrymatory precursors of onion).¹⁾ As the cleavage of the S-C (alanine moiety) bond must be involved in the mechanism, it is considered important to elucidate the mechanism of formation of alanine from PeCSO irradiated in aqueous solution.

In the previous paper,⁸⁾ the authors tentatively suggested that OH radicals formed during the γ -radiolysis of water contributed to the formation of alanine. More recently, Taniguchi *et al.*¹¹⁾ proposed, on the basis of electron spin resonance (ESR) spectroscopy, that α -aminobutyric acid is produced by the reaction of methionine with OH radicals generated chemically in a Tj^{3+} - H_2O_2 system.

This paper is concerned with the identification of ninhydrin-positive products from PeCSO irradiated in oxygen-free aqueous solutions and with the determination of the rate constant for the formation of alanine from measurements of the competitive kinetics.

S-(*cis*-1-Propenyl)-L-cysteine sulfoxide (PeCSO) was

prepared by the synthetic procedure reported previously.¹⁾

Irradiation was carried out with cobalt-60 γ -rays (3 kCi) at a dose rate of 6.24×10^4 rad/hr at room temperature. Oxygen-free neutral solutions of PeCSO (20 mM) dissolved in triply-distilled water were placed in stoppered pyrex tubes and irradiated with $10^4 \sim 10^6$ rad. Irradiation in the presence of KBr (a specific scavenger for OH)¹²⁾ was also carried out to permit measurement of the rate constant for the formation of alanine.

A two-dimensional thin-layer chromatogram of the ninhydrin-positive products of irradiated PeCSO is shown in Fig. 1. Among these products, alanine (7), S-(1-propenyl)-cysteine sulfone (5), S-(1-propenyl)-cysteine (1), glycine (8), serine (9), cysteic acid (11) and cystine (13) were identified by comparing their R_f values* with those of reference compounds. The identities of alanine and S-(1-propenyl)-cysteine were further confirmed by IR and mass spectroscopy** after separation by column chromatography using Avicel-celite (3:1).

FIG. 1. TLC of ($\pm$)S-(*cis*-1-Propenyl)-L-cysteine Sulfoxide (4) Irradiated in Oxygen-free Aqueous Solution (0.02 M, 1Mrad).

From quantitative analysis data obtained with an amino acid analyzer, the G-value for the formation of alanine from irradiated PeCSO was 2.93 (total dose; 10^6 rad). The G-value for alanine from PeCSO is considerably greater than those of S-*n*-propyl-L-cysteine sulfoxide or S-allyl-L-cysteine sulfoxide, which were previously reported.⁸⁾ This evidence indicates that the S-C (alanine moiety) bond in PeCSO is easily cleaved by γ -irradiation.

* Other solvent systems, *n*-butanol-acetic acid-water (63:10:27 v/v) and phenol-acetic acid-water (7:1:2) were also used.

** The mass spectral fragmentation of S-(*cis*-1-propenyl)-L-cysteine was reported previously.¹³⁾

† Studies on γ -Radiolysis of Sulfur-containing Amino Acids. Part V, see reference 1). A part of this work was presented at the Annual Meeting of the Japanese Research Association for Food Irradiation, Tokyo, November 19, 1971.

The reaction of potassium bromide with OH radicals is given by the following equation.

If the formation of alanine by the reaction of PeCSO with OH radicals is simply expressed by the following equations,

then on the basis of the competition between potassium bromide and PeCSO as represented in eq. (1) and (2), the following relationship can be derived.

$$\frac{1}{G(\text{Ala})} = \frac{1}{G(\text{OH})} \left[1 + \frac{k_1}{k_2} \frac{(\text{Br}^-)}{(\text{PeCSO})} \right] \quad (3)$$

$G(\text{Ala})$ represents the experimentally observed alanine yield, (PeCSO) the concentration of S-(*cis*-1-propenyl)-L-cysteine sulfoxide, and k_2 the rate constant for the formation of alanine. A plot of $1/G(\text{Ala})$ vs. $(\text{Br}^-)/(\text{PeCSO})$ should be linear with a slope of $[1/G(\text{OH})][k_1/k_2]$. Experimental plots were found to give a straight line, as shown by the typical data in Fig. 2.

FIG. 2. Typical Plots of Reciprocal Alanine Yields as a Function of $[\text{KBr}]/[\text{PeCSO}]$ for $(\pm)\text{S}-(\text{cis}-1\text{-Propenyl})\text{-L-cysteine Sulfoxide}$.

Taking $k_1 = 1.35 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ as determined by

Allen¹²⁾ and $G(\text{OH}) = 2.74$, according to Bielski and Allen,¹⁴⁾ the authors obtained the value $k_2 = (4.80 \pm 0.06) \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$.

This value compares favorably with the value of $4.2 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ determined from the data of Meissner *et al.*¹⁵⁾ obtained by the pulse radiolysis technique for the rate constant of the reaction of dimethyl sulfoxide with OH radicals.

Thus, it may be concluded that the S-C bond in PeCSO is mainly cleaved by the attack of OH radicals, one of the most important species with regard to food irradiation.

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