Studies on the Photolysis of Sulfur Containing Compounds in Foods

Part II. The Photolysis of 2-Alkyl-Thiazolidine-4-Carboxylic Acids and Mercaptals of L-Cysteine

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The aqueous solutions of 2-alkyl-thiazolidine-4-carboxylic acids and mercaptals of L-cysteine were exposed to sunlight in the presence of a small amount of riboflavin. Hydrogen sulfide, ammonia, carbon dioxide and carbonyl compounds were the photolysis products.

As for the carbonyl compounds, formaldehyde and acetaldehyde were identified from thiazolidine-4-carboxylic acid and djenkolic acid, acetaldehyde from 2-methyl-thiazolidine-4-carboxylic acid and L-cysteine-mercaptopal of acetaldehyde, and acetaldehyde and *n*-butyraldehyde from 2-*n*-propyl-thiazolidine-4-carboxylic acid and L-cysteine-mercaptopal of *n*-butyraldehyde.

As previously reported,¹⁾ S-alkyl-L-cysteines were decomposed by sunlight and evolved volatile compounds such as alkyl mercaptans, acetaldehyde, ammonia and carbon dioxide. These volatile compounds were considered as the cause of the special flavor of the foods containing these S-alkyl-L-cysteines when the foods were exposed to sunlight. In this report, the authors studied on the behaviors of 2-alkyl-thiazolidine-4-carboxylic acids and mercaptals of L-cysteine to sunlight. In the studies on the factor responsible for blackening of wall of cans containing cooked beef seasoned with soybean protein hydrolyzate, Y. Obata et al.^{2,3)} presumed the presence of heat labile sulfur containing compounds and examined for the heat instabilities of these compounds considered as the precursors of hydrogen sulfide which evolved in the course

of processing, because they found that the factor for the blackening was hydrogen sulfide in the can.⁴⁾ But it has been reported that these compounds were rather stable in boiling aqueous solutions.

The present authors observed that these compounds were easily decomposed and evolved much hydrogen sulfide when the aqueous solutions in the presence of a small amount riboflavin were exposed to sunlight even for a short time. It may be considered that the hydrogen sulfide evolved from the foods containing these sulfur compounds upon exposure to sunlight in the course of processing before they were canned caused blackening of the can-wall.

In the exposed solutions hydrogen sulfide, ammonia, carbon dioxide and carbonyl compounds were identified.

The identified carbonyl compounds were

1) Y. Obata and H. Tanaka, *This Journal*, **29**, 196 (1965).

2) J. Mizutani, Y. Obata and Y. Ishikawa, *Bull. Agr. Chem. Soc. Japan*, **24**, 382 (1960).

3) Y. Obata and J. Mizutani, *ibid.*, **24**, 562 (1960).

4) Y. Obata and S. Sakamura, Y. Ishikawa and J. Mizutani, *J. Agr. Chem. Soc. Japan*, **31**, 585 (1957).

formaldehyde and acetaldehyde from thiazolidine-4-carboxylic and djenkolic acid, acetaldehyde from 2-methyl-thiazolidine-4-carboxylic acid and L-cysteine-mercaptal of acetaldehyde, and acetaldehyde and *n*-butyraldehyde from 2-*n*-propyl-thiazolidine-4-carboxylic acid and L-cysteine-mercaptal of *n*-butyraldehyde. These carbonyl compounds were isolated as their 2,4-dinitrophenylhydrazones and identified by paper chromatographies, melting points, elemental analyses and infrared spectra.

It is also considered these volatile compounds generated upon exposure to sunlight are able to cause the representative flavor of the foods.

From these experiments, it was concluded that the photolysis of 2-alkyl-thiazolidine-4-carboxylic acids (Reaction 1) and mercaptals of L-cysteine (Reaction 2) proceeded as follows:

EXPERIMENTAL AND RESULTS

(I) Preparation of Sample Solutions of 2-Alkyl-Thiazolidine-4-Carboxylic Acids and Mercaptals of L-Cysteine.

Three kinds of 2-alkyl-thiazolidine-4-carboxylic acids and of mercaptals of L-cysteine prepared in the previous papers^{2,3)} were used. The photolysis of S-alkyl-L-cysteines has been shown to be accelerated by the addition of riboflavin,¹⁾ so this experiment was carried in the presence of a small amount of riboflavin.

2-Alkyl-Thiazolidine-4-Carboxylic Acids.

Each of 500 mg of the compounds was dis-

solved in 500 ml of water containing 25 ml of riboflavin.

Djenkolic Acid.

One hundred milligrams of djenkolic acid monohydrochloride and 50 mg of riboflavin were dissolved in 700 ml of water and adjusted to pH 6.8 with 2*N* NaOH and then the solution was made to 1,000 ml with water.

L-Cysteine Mercaptan of Acetaldehyde and of *n*-Butyraldehyde.

Each of 500 mg of the monohydrochloride compounds and 25 mg of riboflavin were dissolved in 350 ml of water, adjusted to pH 6.8 and made to 500 ml with water.

(II) Photolysis of 2-Alkyl-Thiazolidine-4-Carboxylic Acids and Mercaptals of L-Cysteine.

(1) Identification of Hydrogen Sulfide.

Aliquots of 10 ml of the above prepared solutions were placed in individual test tubes, stoppered with corks having a sheet of a

TABLE I. FORMATION OF HYDROGEN SULFIDE FROM THE COMPOUNDS

Sample	I	II	III	IV	V	VI	VII
Exposure to Sunlight (min.)							
0	—	—	—	—	—	—	—
10	+	+	+	+	+	+	—
30	+	+	+	+	+	+	—

I indicate thiazolidine-4-carboxylic acid, II 2-methyl-thiazolidine-4-carboxylic acid, III 2-*n*-propyl-thiazolidine-4-carboxylic acid, IV djenkolic acid, V L-cysteine-mercaptal of acetaldehyde, VI L-cysteine-mercaptal of *n*-butylaldehyde and VII riboflavin (5 mg per 100 ml of water).

(—) indicates the sign of negative, (+) positive and (+) more definitely recognized.

lead acetate paper respectively on each center of the cork stoppers and exposed to sunlight on a fine day.

The changing of colors on papers was checked and organoleptically the orders of hydrogen sulfide were tested periodically. The results were shown in Table I.

(2) Identification of Ammonia and Carbon Dioxide.

Aliquots of 10 ml of the solutions were placed in individual Thunberg tubes and the formed volatile compounds were detected after an hour of exposure; ammonia by the addition of Nessler's reagent into the exposed solutions and carbon dioxide by the formation of the white precipitates in an aqueous 10% Ba(OH)₂.

(3) Identification of Carbonyl Compounds.

(a) 2-Alkyl-Thiazolidine-4-Carboxylic Acids.

After an aliquot of 350 ml of each solution in individual 500-ml Erlenmeyer flask with a rubber stopper had been exposed to sunlight for three fine days, 150 ml of the 2,4-dinitrophenylhydrazine reagent¹⁾ was added to the solutions and the mixture was allowed to stand overnight at room temperature. 2,4-Dinitrophenylhydrazones formed were collected, washed with a small amount of water, dried over phosphorous pentoxide and weighed. Each 2,4-dinitrophenylhydrazone obtained was fractionated using silicic acid-celite column, recrystallized from ethanol and characterized by melting point, paper chromatographic behavior, infrared spectrum as compared with the corresponding authentic sample, and by elemental analysis. The results are summarized in Table II.

(b) Mercaptals of L-Cysteine.

An aliquot of 350 ml of each L-cysteine-

TABLE II. IDENTIFICATION OF CARBONYL COMPOUNDS FROM 2-ALKYL-THIAZOLIDINE-4-CARBOXYLIC ACIDS

Sample	Yield (mg)	M. p. (°C)	<i>R_F</i> * Value	Analysis	
				%C	%H
I	F	55.04	159~60	0.35	43.07 3.67
	Ac	"	"	"	42.86 3.57
	S	24.32	164~5	0.20	40.19 3.00
	Fo	"	"	"	40.00 2.86
II	98	159~60	0.35	43.17	3.68
III	F	56.87	121~2	0.69	47.61 5.00
	Bu	"	"	"	47.62 4.76
	S	30.98	159~60	0.35	42.85 3.76

*: D. F. Meigh, *Nature*, 170, 579 (1952).

F indicates the fast moving fraction on the column chromatogram and S slow moving fraction.

Ac indicates the authentic 2,4-dinitrophenylhydrazone of acetaldehyde, Fo that of formaldehyde and Bu that of *n*-butyraldehyde.

The calculated data were shown in the column of Analysis for the corresponding authentic samples.

TABLE III. IDENTIFICATION OF CARBONYL COMPOUNDS FROM MERCAPTALS OF L-CYSTEINE

Sample		Yield (mg)	M. p. (°C)	<i>R_F</i> Value	Species of Aldehyde Identified
IV	F	24.71	159~60	0.35	Acetaldehyde
	S	14.55	164~5	0.20	Formaldehyde
V		82.6	159~60	0.35	Acetaldehyde
VI	F	61.16	121~2	0.69	<i>n</i> -Butyraldehyde
	S	32.54	159~60	0.35	Acetaldehyde

mercaptal of acetaldehyde or *n*-butyraldehyde was placed in individual 500-ml Erlenmeyer flask and an aliquot of 700 ml of the djenkolic acid solution in 1,000-ml flask. Isolated 2,4-dinitrophenylhydrazones by the method already described were identified by mixing melting points, *R_F* values and IR spectra.

The results are summarized in Table III.

2,4-Dinitrophenylhydrazones from the solution of riboflavin (17.5 mg per 350 ml of water) were obtained in a trace amount.