Note

Isolation of N-Nitroso-2-methylthiazolidine from a Cysteamine-acetaldehyde-sodium Nitrite Model System

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Received October 25, 1978

Subsequent to the discovery that some N-nitrosamines are carcinogens,[1] many researchers have studied N-nitrosamines. There have been no reports, however, on the formation of N-nitrosamines in browning reaction systems.[2,3] Marshall and Dugan,[4] for example, reported that N-nitrosamine compounds were not found in their aldehyde-primary amine-sodium nitrite model system. Sakaguchi and Shibamoto[5] obtained some heterocyclic compounds which contain a possible source of nitrosatable nitrogen (thiazolidine, 2-methylthiazolidine, 2-formylthiazolidine, *etc.*) from their cysteamine-acetaldehyde, glyoxal or D-glucose model system. Fujimaki *et al.*[6] also isolated 2-methylthiazolidine from their cysteamine, cysteine or cystine and acetaldehyde model system. In this study, we attempted to synthesize N-nitroso compounds using a cysteamine-acetaldehyde-sodium nitrite browning model system.

A Kjeldahl flask (100 ml) containing acetaldehyde (0.3 M) and cysteamine (0.1 M), which was previously converted from cysteamine hydrochloride by the addition of 5 N sodium hydroxide solution, in 100 ml of deionized water was flame sealed and the ampule was placed in an oven at 90°C for 5 hr. Prior to flame-sealing, the ampule was cooled in ice water. The solution became dark brown in color. Following heat treatment, the reaction mixture was cooled in ice water and was adjusted to pH 2, with 6 N hydrochloric acid solution. Thirty ml of sodium nitrite solution (0.3 M) was added to the above solution, stirring with a magnetic stirrer in flask. Stirring was continued for 5 hr at room temperature. The color of the solution changed to dark red upon the addition of sodium nitrite solution, then changed gradually to orange. The formation of nitrogen peroxide gas was observed.

The reaction products were extracted with 300 ml of methylene chloride using a liquid-liquid continuous extractor for 24 hr. The methylene chloride solution was dried over anhydrous magnesium sulfate, and concentrated to yield 15.87 g of a brown oily liquid. Products were identified by comparison of the GC retention indices and mass spectra on the unknowns to those of authentic samples. The authentic sample of 2-methylthiazolidine was systhesized by the method reported previously.[6] The peak #20 (Fig. 1, area%: 31.6) was trapped in a semicapillary tube cooled with dry ice using a preparative gas chromatograph and was identified by comparison of its IR and NMR spectra

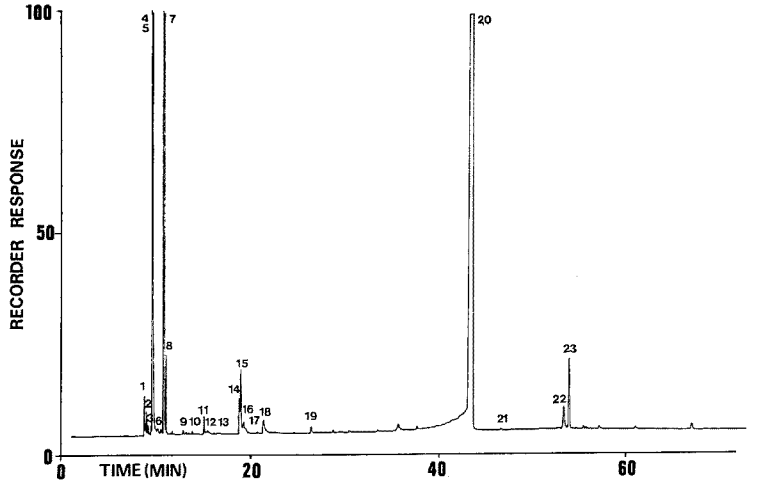

FIG. 1. Gas Chromatogram of Compounds Formed by Cysteamine-acetaldehyde-sodium Nitrite Browing Model System.

For peak identification see Table I. A Hewlett-Packard Model 5710-A equipped with a flame ionization detector, modified for capillary analyses, and a 50 m × 0.28 mm *i.d.* glass capillary column coated with Carbowax 20 M was used. The column temperature was programmed from 80～200°C at 2°/min. The nitrogen carrier gas flow was 0.6 ml/min.

TABLE I. COMPOUNDS FOUND IN A CYSTEAMINE-ACETALDEHYDE-SODIUM NITRITE MODEL SYSTEM

Peak No.	Compound	Peak area %
1	Ethylene oxide	0.22
2	Acetaldehyde	0.10
3	Sulfur dioxide	0.37
4	Ethylene sulfide	1.75
5	Methylene chloride (solvent)	—
6	Croton aldehyde	0.03
7	*cis, cis*-2, 4, 6-Trimethyl-s-trioxane	62.40
8	*cis, trans*-2, 4, 6-Trimethyl-s-trioxane	0.66
9	2-Methyl-1, 3-thioxalane	[a]
10	2-Methylthiazole	[a]
11	2-Methyl-2-thiazoline	0.08
12	Unknown	[a]
13	Thiazole	[a]
14	2-Methylthiazolidine	0.22
15	2-Methyl-1, 3-dithiolane	0.42
16	Acetic acid	[a]
17	Thiazolidine	[a]
18	1, 3-Dithiolane	0.21
19	1, 4-Dithiane	0.03
20	N-Nitroso-2-methylthiazolidine	31.60
21	N-Nitrosothiazolidine (tentative)	[a]
22	3-Methyl-1, 2, 4-trithiane	0.32
23	Unknown	[a]

[a] Area percent less than 0.01.

of an authentic sample in addition to mass spectrum and GC retention index. The N-nitrosamine was confirmed by UV spectrum in addition to above method.

The products identified from the cysteamine-acetaldehyde-sodium nitrite model system are shown in Table I and their typical gas chromatogram is shown in Fig. 1. We identified the one of main peak as N-nitroso-2-methylthiazolidine. It is obvious that this nitrosamine compound was formed from the reaction of 2-methylthiazolidine with nitrite in the above amine-carbonyl browning system. Peak #21 was tentatively identified as N-nitrosothiazolidine from its mass spectrum analysis. We could not, however, obtain a sufficient amount of material for further analyses. Figure 2 shows the mass spectra of isolated samples of 2-methylthiazolidine (A) and N-nitroso-2-methylthiazolidine (B). The presence of an M^+-30 ($NO\cdot$) peak at m/e: 102 indicates a typical nitroso compound. The peaks at m/e: 88, 56, 42 are due to the 2-methylthiazolidine moiety (A, Fig. 2). The IR spectrum shows N–O stretching at 1410 cm^{-1} and N–N stretching at 1030~1150 cm^{-1}. The NMR spectrum of N-nitroso-2-methylthiazolidine indicates that this compound is a mixture of two conformers.[7]

The toxicity or carcinogenecity of N-nitroso-2-methylthiaxolidine has not been demonstrated. Thiazolidines, also, have not been found in food. Nevertheless, a large number of thiazoles and thiazolines, which are the dehydrogenated products of thiazolidines, have been found in foods.[6,8~10] Many carbonyl-amine model systems have, however, produced thiazolidines.[6,10] Some alkylthiazolidine are used as flavor ingredients.[11] The reaction system conducted in this study is not quite similar to food cooking systems because of its pH. Digestion, however, requires strongly acidic conditions, and the pH of gastric juices is less than 2. It is, therefore, possible that thiazolidines react with a nitrite to give nitrosamines during digestion.

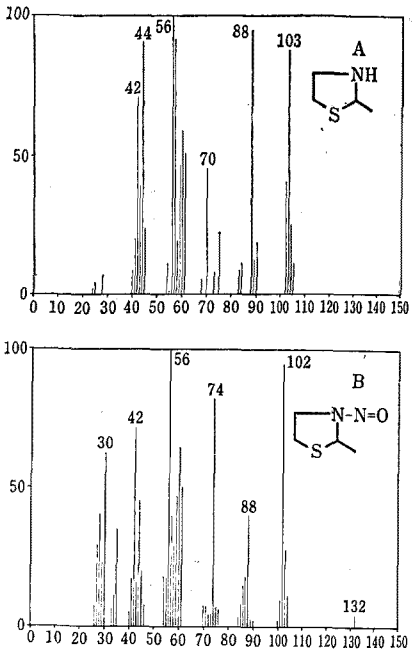

FIG. 2.
A: Mass spectrum of 2-methylthiazolidine.
B: Mass spectrum of N-nitroso-2-methylthiazolidine.

A Hitachi Model RMU-6M combination mass spectrometer-gas chromatograph (Hitachi Model M-5201) equipped with Hitachi Model M-6010 and 10 II/A data system was used.

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