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Preparation and analysis of a volatile derivative of cysteic acid

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Summary. We have reported preparations and gas chromatographic analyses of volatile derivatives of sulfuric acid and taurine (Masuoka et al., 1988; 1989). By extending these studies, we have developed a method for the gas chromatographic determination of cysteic acid. Cysteic acid was converted to the N-isobutoxycarbonyl derivative by the reaction with isobutyl chloroformate in the presence of sodium hydroxide. After desalting with a cation-exchange column, the derivative was converted to the silver salt by reacting with silver oxide. The resulting silver salt was quantitatively esterified with methyl iodide in the presence of dimethyl sulfate and silver oxide. Dimethyl N-isobutoxycarbonylcysteate [methyl 2-(N-isobutoxycarbonylamino)-3-(methoxysulfonyl) propanoate] formed was analyzed by gas chromatography. The calibration curve was linear up to 5.0 μmol per ml of cysteic acid and the recovery was more than 95%.

Keywords: Amino acids – Cysteic acid analysis – Taurine analysis – Gas chromatography

Introduction

Various methods for the determination of cysteic acid (2-amino-3-sulfopropanoic acid) have been reported (Kinnier and Wilson, 1977; Kuriyama and Tanaka, 1987). However, there are only a few reports for the gas chromatographic determination of cysteic acid because of the lack of stable derivatives which are suitable for gas chromatographic analyses (Shahrokhi and Gehrke, 1968; MacKenzie and Finlayson, 1980). Recently, Kataoka et al. (1986) reported a gas chromatographic method for the analysis of a small amount of cysteic acid as the N-isobutoxycarbonyl dibutylamide methyl ester.

In order to study cysteine metabolism in the animal body, we have reported preparations and gas chromatographic analyses of volatile derivatives of sulfuric acid and taurine (Masuoka et al., 1988; 1989). By extending these studies, we have developed a method for the gas chromatographic determination of cysteic acid after its conversion to dimethyl N-isobutoxycarbonylcysteate.

Materials and methods

Reagents

L-Cysteic acid and DL-homocysteic acid (DL-2-amino-4-sulfobutanoic acid) were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. All other chemicals used were of analytical grade and were purchased from Wako Pure Chemical Ind., Ltd., Osaka, Japan.

Instruments

A Shimadzu gas chromatograph (4CM, Shimadzu Seisakusyo, Ltd., Kyoto, Japan) equipped with a hydrogen flame ionization detector was used. A column of 3% OV-101 on Chromosorb W (80–100 mesh) packed in a silanized glass tube (3 mm × 2 m) was used. The operating conditions for the determination of cysteic acid were as follows: oven temperature, 210°C; injection temperature, 240°C; flow rate of nitrogen as the carrier gas, 50 ml/min.

The identification of the derivative was performed using a gas chromatograph-mass spectrometer (GC-MS) (Shimadzu 9020-DF). Electron impact (EI) mass spectra were taken at 70 eV of ionizing voltage. Chemical ionization (CI) mass spectra were obtained with isobutane as the reacting gas. NMR spectra and infrared (IR) spectra were recorded with a Hitachi R-22 FTS FT-NMR spectrometer (Hitachi Ltd., Tokyo, Japan) and JASCO IRA-102 infrared spectrometer (Japan Spectroscopic Co., Ltd., Tokyo, Japan), respectively.

Preparation of dimethyl N-isobutoxycarbonylcysteate from L-cysteic acid

The derivative was prepared from 6.0 mmol of L-cysteic acid in essentially the same manner as the determination procedure described below. After preparation, the reaction mixture was filtered and evaporated to dryness at 30°C under a reduced pressure. The residue was applied to a silica gel column (2 × 13 cm) and eluted with 1% methanol in chloroform. The eluate was collected and evaporated to dryness at 30°C under a reduced pressure. The yield of the derivative was 68%. It was recrystallized from *n*-hexane, mp 57-58°C. Elemental analyses were as follows: Calculated for $C_{10}H_{19}N0_7S$: C, 40.40; H, 6.44; N, 4.71. Found: C, 40.16; H, 6.59; N, 4.50. Spectral data were as follows: IR(KBr): 3330(NH), 1730(COO), 1685(OCONH) cm^{-1}. NMR($CDCl_3$): δ 0.95(d, J = 7Hz, 6H, $2CH_3$), 1.9(m, 1H, CH), 3.5–3.9(m, 4H, $2CH_2$), 3.83(s, 3H, OCH_3), 3.90(s, 3H, OCH_3), 4.75(m, 1H, CH), 5.50(NH). EI: m/e (%) 283(48), 198(6), 196(15), 164(17), 152(9), 148(16), 138(100), 130(14). CI: m/e (%) 298(100, MH^+), 254(28).

Preparation of dimethyl N-isobutoxycarbonylhomocysteate, an internal standard

The derivative was prepared from 6.0 mmol of DL-homocysteic acid and purified in the same manner as that used for the cysteic acid derivative. The derivative was oil and the yield from homocysteic acid was 66%. Spectral data were as follows: IR(Neat): 3350(NH), 1730(COO, sh), 1700(OCONH) cm^{-1}. NMR($CDCl_3$): δ 0.95(d, J = 7Hz, 6H, $2CH_3$), 1.9(m, 1H, CH), 2.3(m, CH_2), 3.3(m, 2H, CH_2), 3.89(s, 3H, OCH_3), 3.9(m, 2H, CH_2), 4.01(s, 3H, OCH_3), 4.50(m, 1H, CH), 5.80(bs, 1H, NH). CI: m/e (%) 312 (100, MH^+).

Determination of cysteic acid in aqueous solution

To one ml of a solution containing 0.5–5.0 μmol of cysteic acid placed in a test tube (13 × 100 mm) with a Teflon-lined screw cap, 0.1 ml of 0.5 M sodium hydroxide and 0.1 ml of isobutyl chloroformate were added, and the tube was shaken with a mechanical shaker at 100 strokes per min at room temperature for 5 min. Then, the reaction mixture was extracted twice with 2 ml each of diethyl ether. The aqueous layer was applied to a Dowex 50W × 8 column (H^+ form, 0.7 × 8 cm) and eluted with water. Ten ml of the eluate was collected. To 5 ml of the eluate, 0.2 g of silver oxide was added and the mixture was shaken as above for 90 min. After centrifugation of the reaction mixture at 2,000 rpm for 5 min, 3.5 ml of the

supernatant was placed in a test tube, and evaporated to dryness using a centrifugal evaporator at 40°C under a reduced pressure. Five-tenths ml of methyl iodide, 20 μl of dimethyl sulfate and 10 mg of silver oxide were added to the test tube. The reaction mixture was shaken vigorously for 1 min and heated in a water bath at 55°C for 1 h. After cooling, 0.5 ml of 5 mM dimethyl N-isobutoxycarbonylhomocysteate in chloroform (internal standard) was added. Two μl of the mixture was analyzed by gas chromatography under conditions described above.

Results and discussion

In the present study, dimethyl N-isobutoxycarbonylcysteate, the volatile derivative of cysteic acid, was prepared in good yields. Previously, we tried to prepare this derivative by applying the derivatization procedure of taurine (Masuoka et al., 1989) as follows. Cysteic acid was converted to the silver salt of the N-isobutoxycarbonyl derivative by the reaction with isobutyl chloroformate after the addition of silver oxide. The silver salt of the derivative was esterified with methyl iodide in the presence of dimethyl sulfate and silver oxide. However, the recovery of the derivative was less than 37%, when the concentration of cysteic acid was lower than 5.0 μmol/ml. It was found that N-isobutoxycarbonylation of the silver salt of cysteic acid did not proceed efficiently. To overcome this problem, the reaction of cysteate with isobutyl chloroformate was performed with sodium hydroxide which was used in the method of Kataoka et al. (1986), and the derivatization procedure was modified as follows. Cysteic acid was converted to the N-isobutoxycarbonyl derivative by the reaction with isobutyl chloroformate in the presence of sodium hydroxide. The derivative was desalted with a cation-exchange column, and was converted to the silver salt. The silver salt was esterified as described above. Thus, the preparation of dimethyl N-isobutoxycarbonylcysteate can be done by a four-step procedure, which is simpler than the procedure preparing N-isobutoxycarbonyl dibutylamide methyl ester derivative of cysteic acid (Kataoka et al., 1986).

Fig. 1 shows a gas chromatogram of cysteic acid derivative analyzed by the determination procedure. Retention times of the cysteic acid derivative and the

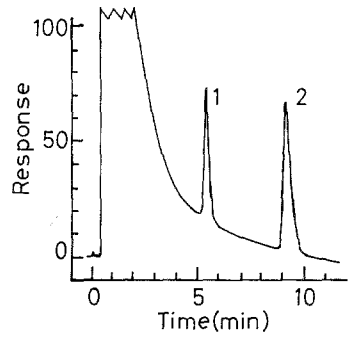

Fig. 1. Gas chromatogram of a mixture of dimethyl N-isobutoxycarbonylcysteate (derivative of cysteic acid and dimethyl N-isobutoxycarbonylhomocysteate (internal standard, a derivative of homocysteic acid). The chromatogram was obtained with a solution containing 5 μmol/ml of cysteic acid. Experimental details are described under Materials and methods. *1* dimethyl N-isobutoxycarbonylcysteate; *2* the internal standard

Table 1. Yield of dimethyl N-isobutoxycarbonylcysteate from the aqueous solution of cysteic acid[a]

Cysteic acid added (μmol/ml)	Cysteic acid determined[b] (μmol/ml)	yield[b] (%)
0.50	0.47 ± 0.03	94.7 ± 5.8
1.25	1.25 ± 0.05	100.0 ± 3.7
2.50	2.47 ± 0.04	98.9 ± 1.4
5.00	5.11 ± 0.20	102.2 ± 4.0

[a] Cysteic acid was determined by gas chromatography after the conversion to dimethyl N-isobutoxycarbonylcysteate.
[b] Values are mean ± standard deviation of 3 determinations.

internal standard were 5.4 and 9.2 min, respectively. The identification of each peak was confirmed by GC-MS analysis. Determination of cysteic acid was done by calculating the area of the peak in the chromatogram. The calibration curve was linear between 0.5 and 5.0 μmol/ml of cysteic acid and the recovery of the derivative was more than 95 % (Table 1). The present method can be applied to the exact determination of cysteic acid.

When taurine was derivatized by the present method, N-isobutoxycarbonyltaurine methyl ester was formed. The recovery of the taurine derivative were 96.5 ± 3.0 %, when 2.5 μmol/ml of taurine was analyzed. When gas chromatographic analyses were carried out at an oven temperature of 190°C, retention times of the taurine derivative, 3-(N-isobutoxycarbonylamino)propanesulfonate (internal standard) and cysteic acid derivative were 4.9, 8.8 and 10.7 min, respectively, and the peaks were separated well from each other. Therefore, the present method is useful to determine both taurine and cysteic acid at the same time.

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