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Note

Gas chromatographic determination of cysteic acid

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The development of a satisfactory gas chromatographic (GC) procedure for the analysis of cysteic acid has been hampered by the difficulty of preparing a suitable volatile derivative of this compound. The N-heptafluorobutyryl isobutyl ester (HBB)[1,2] and the trimethylsilyl (TMS)[3,4] derivatives used in previous studies appear to be inadequate for reliable quantitative work in view of the fact that the HBB derivative is thermally unstable[1] and the TMS derivative is very sensitive to moisture[4].

Recently, we developed a specific and reliable method for the determination of taurine by GC in which taurine was analysed as its N-isobutoxycarbonyl (N-isoBOC) dibutylamide derivative[5], and we demonstrated that complex biological materials such as urine and blood could be analysed accurately and precisely by the method[5,6]. In the present paper, we report the logical extension of this work to the analysis of cysteic acid.

EXPERIMENTAL

Reagents

Cysteic acid and homocysteic acid as an internal standard (I.S.) were purchased from Nakarai (Kyoto, Japan), and each was dissolved in water to make a stock solution at a concentration of 1 m*M*. Isobutyl chloroformate (isoBCF) was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Cetyldimethylbenzylammonium chloride (CDMBA-Cl)[7] obtained from Tokyo Kasei Kogyo was dissolved in methanol at the concentration of 10%. Hydrogen chloride in methanol (HCl–methanol) obtained from Tokyo Kasei Kogyo was diluted with methanol at the concentration of 0.5 *M*. Thionyl chloride and *n*-dibutylamine (DBA) were purchased from Nakarai and used after distillation. All other chemicals were of analytical grade.

Analytical derivatization procedure

An aliquot of the sample solution (containing 5–500 nmol of cysteic acid) was pipetted into a 10-ml pyrex glass tube with a PTFE-lined screw-cap. After addition of 0.25 ml of the I.S. solution (0.1 or 1.0 m*M*), 0.1 ml of 0.5 *M* sodium hydroxide was added and then the total reaction volume was made up to 1 ml with distilled water if necessary. Immediately after the addition of 0.1 ml of isoBCF, the mixture was shaken with a shaker set at 300 rpm (up and down) for 5 min at room temper-

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ature. The reaction mixture was washed twice with 3 ml of diethyl ether after adjustment to pH 1–2 with 0.5 *M* hydrochloric acid. Subsequently, 0.05 ml of 10% CDMBA-Cl was added to the aqueous layer, and the ion-pair compounds formed were extracted into 2 ml of methylene chloride by shaking for 3 min at room temperature. After centrifugation for 1 min the organic layer was transferred to another tube, and the solvent was evaporated to dryness under a stream of nitrogen. To the residue was added 0.2 ml of 0.5 *M* HCl–methanol solution, and the tube was tightly capped and heated at 80°C for 15 min. The excess HCl–methanol was removed at 80°C under a stream of nitrogen. To the residue was added 0.2 ml of thionyl chloride, and the tube was tightly capped and heated at 80°C for 15 min. The excess thionyl chloride was removed at 80°C under a stream of nitrogen. To the residue was added 0.2 ml of 2 *M* DBA in acetonitrile and the mixture was allowed to stand for 5 min at room temperature after tightly capping. The reaction mixture was acidified with 1 ml of 20% orthophosphoric acid and then extracted twice with 3 ml of *n*-pentane. After the solvent was evaporated to dryness at 60°C the residue was dissolved in 0.1 ml of ethyl acetate, and 2–4 μl of this solution were injected into the gas chromatograph.

Preparation of reference compound

A reference sample of methyl β-dibutylsulphamoyl-α-(isobutoxycarbonylamino)propionate, m.p. 55–57°C, was prepared from 50 mg of cysteic acid in essentially the same manner as the analytical derivatization procedure. The data for elemental analysis are as follows: calculated for $C_{17}H_{34}N_2O_6S$: C, 51.75; H, 8.69; N, 7.10. Found: C, 52.07; H, 8.80; N, 7.08.

Gas chromatography

GC analysis was carried out with on a Shimadzu 4CM gas chromatograph equipped with a hydrogen flame ionization detector. The column packing, 1.0% OV-17–0.2% FFAP on Uniport HP (100–120 mesh) was prepared using toluene as a coating solvent according to the solution coating technique[8], and was poured into a silanized glass column (1.0 m × 3 mm I.D.). The packed column was conditioned at 275°C for 24 h with nitrogen at a flow-rate of 30 ml/min. The operating conditions were as follows: nitrogen flow-rate, 45 ml/min; hydrogen flow-rate, 35 ml/min; air flow-rate, 0.75 l/min; injection and detector temperature, 280°C; oven temperature, 220°C; chart speed, 1.0 cm/min; sensitivity, 10^2 (× 10^6 Ω); range, 4–32 (× 0.01 V).

Gas chromatography–mass spectrometry (GC–MS)

A Shimadzu-LKB 9000 gas chromatograph–mass spectrometer with the same type of column as used for GC analysis was employed under the following conditions: trap current, 60 μA; ionizing voltage, 70 eV; accelerating voltage, 3.5 kV; ion-source temperature, 270°C; separator temperature, 260°C; helium flow-rate, 40 ml/min.

RESULTS AND DISCUSSION

Cysteic acid (α-amino-β-sulphopropionic acid) could be successfully converted into its stable volatile derivative, methyl β-dibutylsulphamoyl-α-(isobutoxycarbonylamino)propionate by essentially the same procedure as that used in the derivatiza-

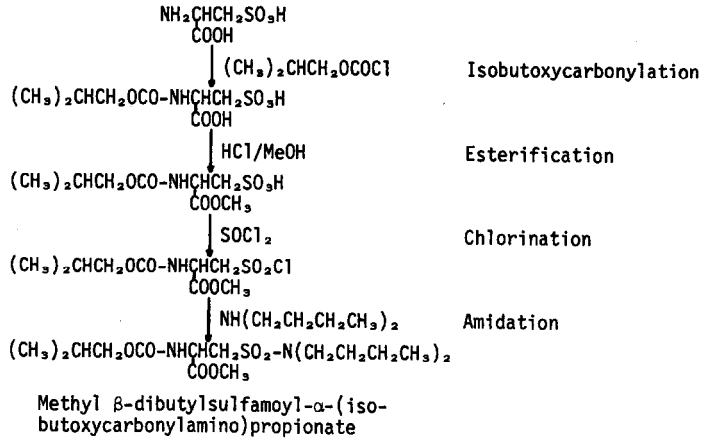

Fig. 1. Derivatization of cysteic acid.

tion of taurine[5] except for an additional step, methylation of carboxyl group, which was easily carried out with HCl–methanol before chlorination of the sulphonic acid group. The best reaction conditions for the methylation were determined to be at 80°C for 15 min (for discussion on the reaction conditions for other steps, see ref. 5). The derivatization process is outlined in Fig. 1. It should be noted that ion-pair extraction technique using CDMBA as a counter-ion results in rapid and quantitative extraction into organic layer of N-isoBOC cysteic acid dissolved in the aqueous medium. The mean derivatization yield throughout the procedure was determined to be 97% by comparison with the synthetic reference compound. The derivative preparation took *ca.* 60 min, but several samples could be treated simultaneously.

The structure of the derivative was confirmed both by GC–MS and by elemental analysis. Although the molecular ion peak (*m/e*, 394) was not observed, the prominent fragment ion peaks [$M^+ - CH_2CH(CH_3)_2$, $M^+ - N(C_4H_9)_2$, $M^+ - CH_2CH(CH_3)_2$ and $N(C_4H_9)_2$] were useful for structure elucidation. The values of

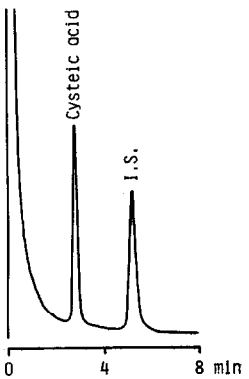

Fig. 2. Gas chromatogram obtained from a standard solution containing 250 nmol each of cysteic acid and I.S. (homocysteic acid). Column, 1.0% OV-17–0.2% FFAP on 100–120 mesh Uniport HP, 1.0 m × 3 mm I.D., glass; temperature, 220°C (isothermal). Each peak represents *ca.* 5 nmol.

elemental analysis agreed with the theoretical values calculated for the structure expected.

The derivative was very stable to moisture and, therefore, no precaution to exclude moisture was necessary in the handling and storage of it.

The derivative of cysteic acid gave a single clean peak on GC with a OV-17–FFAP column (Fig. 2); no extraneous peaks due to thermal decomposition were observed. A chromatographic run was completed within 8 min.

The calibration curve for cysteic acid prepared from peak height ratios relative to the I.S. was found to be linear in the range 5–500 nmol, corresponding to 0.1–10 nmol of cysteic acid in the portion injected, and the reproducibility of the calibration curve was found to be satisfactory.

These experiments have conclusively demonstrated that the proposed method is suitable for quantitative determination of cysteic acid. Further investigations on the application of this method to biological samples are in progress.

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