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Note

Analysis of cysteic acid and cysteine sulfinic acid by gas–liquid chromatography*

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The analysis of the protein amino acids by gas–liquid chromatography (GLC) is now routine and may be achieved using a variety of volatile derivatives[1–6]. Some of these procedures have also been applied to the analysis of both protein and non-protein amino acids in biological samples[4,7]. Nevertheless, the separation and quantitation of non-protein amino acids by GLC remains relatively unexplored and the advantages of the GLC methods have not been fully exploited. In addition, some non-protein amino acids have not yielded good chromatographic peaks because derivatives were not formed, were not volatile or were degraded on the chromatographic column.

In the course of a study of the oxidation of sulfur-containing protein amino acids, we required to separate and quantitate cysteic acid and cysteine sulfinic acid[8]. These oxidised derivatives of cysteine do not separate using conventional ion-exchange chromatography and are difficult and tedious to separate and quantitate by electrophoresis. There are very few reports of the analysis of cysteic acid by GLC. Casagrande[9] included cysteic acid in a qualitative study of a mixture of non-protein amino acids separated as their N-trifluoroacetyl (N-TFA) *n*-butyl esters and reported that cysteic acid was barely separated from ornithine. Siezen and Mague[7] chromatographed cysteic acid as its N-heptafluorobutyryl (N-HFB) isobutyl ester and observed an unexpectedly early elution and a low molar response relative to the internal standard norleucine (RMR_{NLE}). As far as we are aware, cysteine sulfinic acid has not been analysed by GLC. We, therefore, studied the GLC analysis of cysteic and cysteine sulfinic acids as their N-HFB isobutyl esters and herein report the results.

EXPERIMENTAL

Reagents

Cysteic acid and cysteine sulfinic acid were obtained from Sigma (St. Louis, Mo., U.S.A.). Heptafluorobutyric anhydride was obtained from Fluka (Buchs, Switzerland).

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* NRCC No. 17796.

Gas chromatography

All GLC was conducted using a Hewlett-Packard Model 5711 gas chromatograph equipped with a dual flame-ionization detector. Except for specific variations which are described as appropriate in the text, the chromatographic and derivatization conditions have been described elsewhere[5,6].

Mass spectra were obtained using a Finnigan Model 3300 gas chromatograph–mass spectrometer operated in either the electron impact (EI) or chemical ionization (CI) mode. Methane and ammonia were separately used as the carrier gas to obtain CI spectra. Data reduction was performed using an Incos data system.

RESULTS AND DISCUSSION

Derivatization

The conditions required for optimum formation of the N-HFB isobutyl esters of cysteic acid and cysteine sulfinic acid were examined. The RMR_{NLE} of the cysteic acid derivative was 0.58 in both 2 and 3 *M* HCl–isobutanol but slightly lower (0.55) in 5 *M* HCl–isobutanol. The RMR_{NLE} of cysteine sulfinic acid was substantially lower in 2 *M* HCl–isobutanol (0.39) than in 3 *M* HCl–isobutanol (0.56). Therefore the latter concentration was used in all subsequent experiments.

The effect of temperature on the formation of the isobutyl esters of cysteic acid and cysteine sulfinic acid is shown in Table I. The RMR_{NLE} of the derivative of cysteic acid decreased with increasing esterification temperature. The RMR_{NLE} of the cysteine sulfinic acid derivative was significantly greater at 120° than at 100° but esterification at 150° appeared to result in almost complete degradation. Reaction at 120° produced optimum esterification for the simultaneous analysis of cysteic acid and cysteine sulfinic acid. Fortuitously, the same temperature is optimal for the esterification of the protein amino acids[5].

TABLE I

EFFECT OF TEMPERATURE ON THE RMR_{NLE} OF N-HFB ISOBUTYL CYSTEIC ACID AND CYSTEINE SULFINIC ACID

Amino acid	*Esterification temperature (°C)**			*Acylation temperature (°C)***			
	100	*120*	*150*	*75*	*100*	*120*	*125*
Cysteic acid	0.61	0.58	0.53	0.56	0.58	0.68	0.54
Cysteine sulfinic acid	0.40	0.56	0.05	0.54	0.56	0.49	0.34

* Esterified for 30 min and acylated for 10 min at 100°.
** Esterified for 30 min at 120° and acylated for 10 min.

The effect of temperature on the acylation of cysteic acid and cysteine sulfinic acid isobutyl esters is shown in Table I. The RMR_{NLE} of the cysteic acid derivative was relatively unaffected by temperature in the range 75–125°. However, the RMR_{NLE} of the cysteine sulfinic acid derivative decreased when the acylation temperature was greater than 100°. Thus acylation at 100° is recommended although 150° is routinely used for the protein amino acids[6]. The RMR_{NLE} of cysteic acid in all of the above experiments was consistently greater than was observed by Siezen and Mague[7].

Chromatography

The derivatives of cysteic acid and cysteine sulfinic acid were well separated from the protein amino acids and, under the conditions examined, had retention times relative to norleucine (RRT_{NLE}) of 0.47 and 1.92, respectively. The cysteic acid derivative eluted before alanine with sufficient resolution to permit accurate analysis. The derivative of cysteine sulfinic acid eluted between glutamic acid and lysine and was completely resolved from these two amino acids.

Siezen and Mague[7] observed unexpectedly early elution but also a low RMR_{NLE} (0.14) for N-HFB isobutyl cysteic acid. Noticing that these authors used an injector temperature of 225° whereas for amino acid analysis we routinely use 250°, we surmised that the difference in RMR between our results and those of Siezen and Mague might be due to the difference in injector temperature. Consequently, we varied the injector temperature. At an injector temperature of 200° a very small peak was obtained for cysteic acid ($RMR_{NLE} = 0.04$). There was no significant difference in the cysteic acid RMR_{NLE} when the injector temperature raised from 250 to 300° but the peak was sharpened considerably as is illustrated in Fig. 1. Injector temperature had no significant effect on the response of the cysteine sulfinic acid derivative.

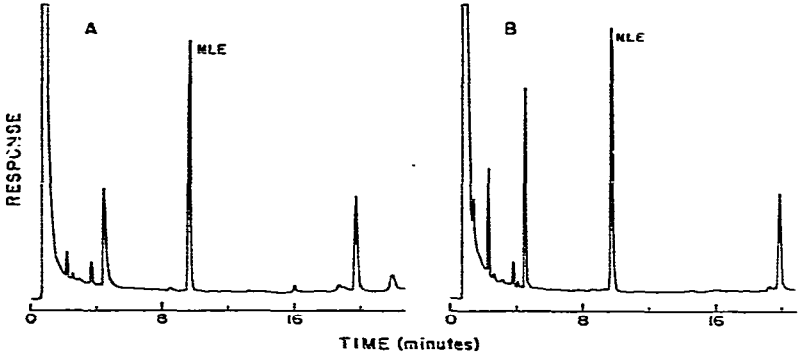


Fig. 1. Chromatograms illustrating the elution of N-HFB isobutyl cysteic acid (retention time 4.5 min) at injector temperatures of 250 (A) and 300° (B). N-HFB isobutyl cysteine sulfinic acid elutes at about 18 min.

Gas chromatography–Mass spectrometry (GC–MS)

The effect of injector temperature on the RMR_{NLE} of N-HFB isobutyl cysteic acid and the unexpectedly early elution prompted us to consider if the derivative was reacting or degrading in the injector zone of the chromatograph and to consider if the peak being observed in fact represented the derivative of cysteic acid. Thus we examined the derivative by GC–MS using a variety of techniques. In the following discussion of mass spectra, the charge designation is omitted from all ions for the sake of simplicity. For the same reason, the ions obtained in the CI spectra are described as being derived from the molecular ion M rather than the adduct ion M+1. The EI spectrum of N-HFB isobutyl cysteic acid did not contain the expected molecular ion at *m/e* 477. The largest ion observed, *m/e* 339, would correspond to the loss of $SO_3C_4H_{10}$ from the putative molecular ion. Fragments *m/e* 283, 265 and 238 corresponding to the loss of 56, 74 and 101 from the ion *m/e* 339 were also observed. The

losses are characteristic of fragmentation of a carboxylic acid isobutyl ester[10]. Evidence of acylation was also present in the spectrum at *m/e* 69 (CF_3), 119 (C_2F_5), 169 (C_3F_7) and 197 (COC_3F_7).

The CI spectrum using methane as the carrier gas is shown in Fig. 2. The ions at *m/e* 340, 368 and 380 represent the characteristic methane CI spectral feature of M+1, M+29 and M+41 indicating a molecular ion of 339. As in the EI spectrum, evidence of esterification is indicated by the ions *m/e* 284 and 266. Using ammonia as the carrier gas, only the adduct ion *m/e* 357 ($M+NH_4$) was observed. The parent ions in the EI spectra of alkyl alkane sulfonates are of low abundance (1–3%). The most abundant ions are associated with the loss of the protonated alkyl sulfonate moiety[11]. No information is avialable on the spectra of amino acid sulfonates.

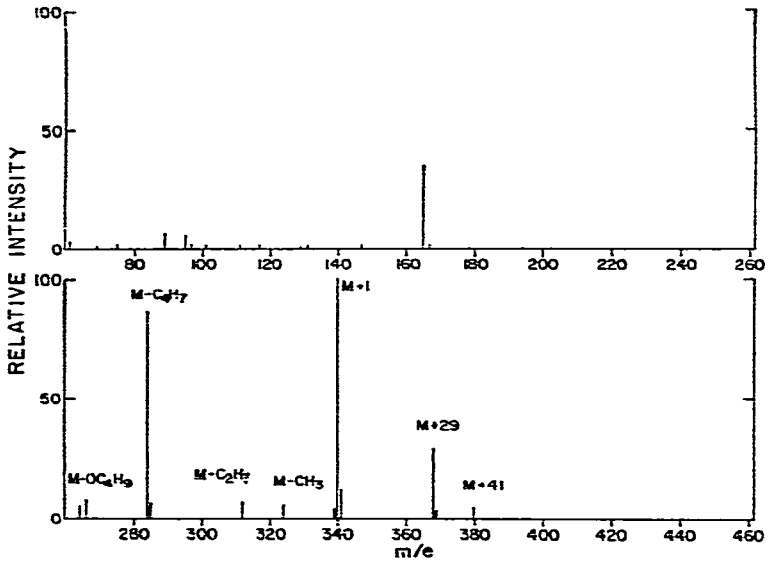
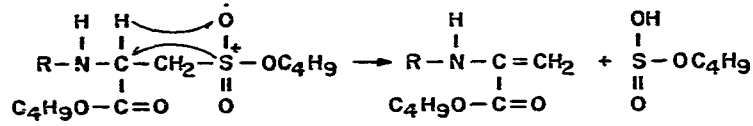


Fig. 2. CI mass spectrum of the N-HFB isobutyl derivative of cysteic acid obtained using methane as the GC carrier gas.

The unexpectedly low apparent molecular weight and the apparent adduct ions could result from the addition of H, C_2H_5 and C_3H_5 to a neutral fragment following loss of $SO_3C_4H_{10}$ from N-HFB isobutyl cysteic acid. Alternatively, the apparent molecular weight may represent the true molecular weight of a compound derived from the cysteic acid derivative. The difference in retention times between the derivatives of cysteic acid and cysteine sulfinic acid appears too great to be based purely on the difference of a single oxygen atom. Thus considering the unexpectedly early elution and the dependence of RMR_{NLE} on the injector temperature, it seems probable that the peak observed represents a product derived from the cysteic acid derivative following degradation in the chromatographic injector. However, a mechanism somewhat similar to mass spectral cleavage might be expected to be operative in purely thermal degradation.



Thus the expected compounds would be the N-HFB isobutyl ester of dehydroalanine (2-amino acrylic acid) and isobutane sulfonic acid. The mass spectra obtained from the peak derived from the cysteic acid derivative are entirely consistent with the structure of N-HFB isobutyl dehydroalanine.

The CI (Fig. 3) and EI (not shown) mass spectra of the derivative of cysteine sulfinic acid were consistent with the structure of N-HFB isobutyl cysteine sulfinic acid indicating that this compound was not degraded on the chromatographic column.

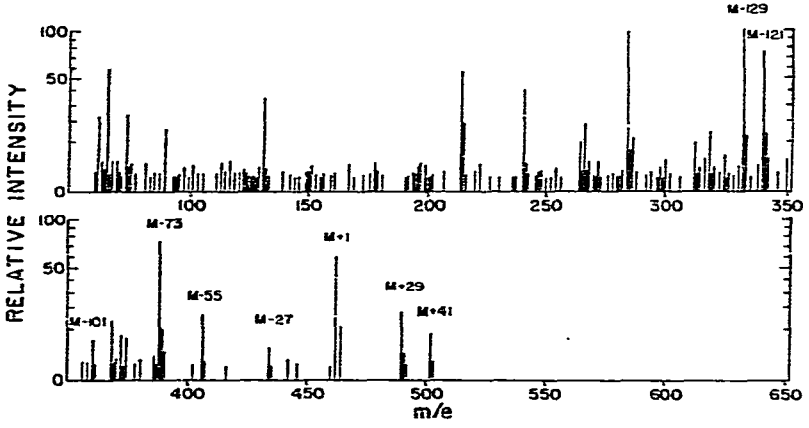


Fig. 3. CI mass spectrum of N-HFB cysteine sulfinic acid obtained using methane as the GC carrier gas.

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REFERENCES

1 F. E. Kaiser, C. W. Gehrke, R. W. Zumwalt and K. C. Kuo, *J. Chromatogr.*, 94 (1974) 113–133.
2 C. W. Moss, M. A. Lambert and F. J. Diaz, *J. Chromatogr.*, 60 (1971) 134–136.
3 J. P. Zanetta and G. Vincendon, *J. Chromatogr.*, 76 (1973) 91–99.
4 R. F. Adams, *J. Chromatogr.*, 95 (1974) 189–212.
5 S. L. MacKenzie and D. Tenaschuk, *J. Chromatogr.*, 171 (1979) 195–208.
6 S. L. MacKenzie and D. Tenaschuk, *J. Chromatogr.*, 173 (1979) 53–63.
7 R. J. Siezen and T. H. Mague, *J. Chromatogr.*, 130 (1977) 151–160.
8 A. J. Finlayson, S. L. MacKenzie and J. W. Finley, *Can. J. Chem.*, 57 (1979) 2073–2077.
9 D. J. Casagrande, *J. Chromatogr.*, 49 (1970) 537–540.
10 S. L. MacKenzie and L. R. Hogge, *J. Chromatogr.*, 132 (1977) 485–493.
11 H. Budzikiewicz, C. Djerassi and D. H. Williams, *Mass Spectrometry of Organic Compounds*, Holden-Day, San Francisco, 1967, pp. 552–565.