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Note

Improved gas chromatographic method for the quantitative determination of secondary amines as sulphonamides formed by reaction with benzenesulphonyl chloride

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It has been shown that nitrosamines can be formed *in vivo* from ingested amines and nitrite[1,2]. Moreover, the same tumours are produced in experimental animals that are fed amine plus nitrite and in those fed the corresponding nitrosamine[3,4]. Consequently, it is clear that a thorough knowledge of the occurrence of nitrosatable amines in the human environment is desirable.

Previous assays developed for these amines have involved colorimetric[5,6] and polarographic procedures[7]. Gas chromatography (GC) has more recently been used for this purpose[8,9] and has several advantages over other procedures, including high specifity and sensitivity.

We have previously reported[10] the preparation and GC separation of the sulphonamides of aliphatic secondary amines. The derivatization procedure used was based largely on the conditions developed for the derivatization of dinitrophenyl amine[11]. Satisfactory derivatization of all of the amines studied was obtained. However, during an investigation of the applicability of the method to other aliphatic and cyclic secondary amines, it was discovered that diisopropylamine was not derivatized, probably owing to steric hindrance.

We now report an improved method utilizing the same derivatization reagent (benzenesulphonyl chloride), which results in almost complete derivatization of all of the amines studied.

EXPERIMENTAL

Chemicals

All standard compounds were obtained at the highest purity available from commercial sources and used without further purification. The following amines were obtained as the hydrochloride salts from Tokyo Kasei (Tokyo, Japan): dimethylamine, diethylamine, diisopropylamine, di-*n*-propylamine, diisobutylamine and di-*n*-butylamine. The hydrochloride salts of pyrrolidine and morpholine were prepared from the free base (Wako, Osaka, Japan). Benzenesulphonyl chloride (BSC) was obtained from Tokyo Kasei. All other reagents were of reagent grade.

Procedure

Stock solutions of each amine were prepared in 0.1 *M* hydrochloric acid at a concentration of 20 μg/ml of the free base. A 1-ml volume of amine solution was pipetted into a 30-ml screw-capped test-tube containing 4 ml of 10 *M* sodium hydroxide solution, followed by 0.2 ml of BSC. The test-tube was capped and shaken vigorously for 30 sec and allowed to stand for 30 min at room temperature with occasional shaking. After adding 5 ml of 10 *M* sodium hydroxide solution, the tube was immersed in a water-bath at 80° for 30 min. The extraction and quantitation procedures were performed as described previously[10], except that diethyl ether was replaced by *n*-hexane.

Chromatography

A Shimadzu (Kyoto, Japan) Model 4BM gas chromatograph equipped with a flame photometric detector (FPD) was used. Glass columns, either 3 m or 1 m in length and of 3 mm I.D., were packed with 3% OV-1 or 3.5% SE-30, respectively, on acid-washed and DMCS-treated Chromosorb W (60–80 mesh). The chromatographic conditions for the OV-1 column were a temperature programme from 200° to 240° at 4°/min, injector temperature 250° and detector temperature 260°, and those for SE-30 column were a temperature programme from 140° to 200° at 5°/min, injector temperature 250° and detector temperature 260°. The carrier gas (nitrogen) flow-rate was 40 ml/min in both instances.

RESULTS AND DISCUSSION

The GC characteristics of the derivatives of the amines studied on 3% OV-1 and 3.5% SE-30 are shown in Table I.

Although neither stationary phase was able to separate all of the amine derivatives, those not separated on one stationary phase could be separated on the other. Thus, morpholine and diisobutylamine had identical retention times on 3%

TABLE I

CHROMATOGRAPHIC RETENTION DATA FOR SULPHONAMIDES DERIVED FROM SECONDARY AMINES

t_R = retention time (min); Rel. t_R = relative retention time (diisopropylamine = 1.0).

Amine	*Column*			
	*3.5% SE-30**		*3% OV-1***	
	t_R	*Rel.* t_R	t_R	*Rel.* t_R
Dimethylamine	2.83	0.52	2.42	0.62
Diethylamine	4.20	0.78	3.20	0.82
Diisopropylamine	5.41	1.00	3.91	1.00
Di-*n*-propylamine	6.18	1.14	4.38	1.12
Pyrrolidine	6.18	1.14	4.74	1.21
Morpholine	7.10	1.31	5.20	1.33
Diisobutylamine	7.68	1.42	5.20	1.33
Di-*n*-butylamine	9.23	1.71	6.41	1.64

* A 1 m × 3 mm I.D. glass column was used with a nitrogen flow-rate of 40 ml/min.
** A 3 m × 3 mm I.D. glass column was used with a nitrogen flow-rate of 40 ml/min.

TABLE II

RECOVERIES OF SULPHONAMIDES (%) IN DERIVATIZATION OF SECONDARY AMINES AS A FUNCTION OF CONCENTRATION OF SODIUM HYDROXIDE SOLUTION AND REACTION TIME AT ROOM TEMPERATURE

Each value represents the average (%) of at least three determinations. The experiments were carried out using 20 μg/ml of each amine solution.

Amine	*Concentration of NaOH*											
	1 M				*5 M*				*10 M*			
	5 min	*10 min*	*20 min*	*30 min*	*5 min*	*10 min*	*20 min*	*30 min*	*5 min*	*10 min*	*20 min*	*30 min*
Dimethylamine	78.4	99.8	99.9	99.8	86.4	99.7	99.9	99.2	86.6	100.4	99.7	100.2
Diethylamine	67.9	99.5	98.5	99.9	82.3	98.9	99.2	99.8	79.3	99.9	100.2	99.8
Diisopropylamine	2.4	2.7	8.6	8.9	10.7	14.8	37.9	51.8	59.6	80.4	86.7	99.1
Di-*n*-propylamine	72.4	98.7	99.5	99.7	80.3	99.3	99.7	99.4	81.2	100.1	99.9	100.6
Pyrrolidine	80.8	99.1	98.7	100.2	77.5	99.7	100.3	100.1	76.9	99.4	98.9	99.8
Morpholine	69.7	99.4	99.5	99.7	70.8	98.9	101.0	100.4	80.2	99.9	100.8	99.9
Diisobutylamine	70.9	99.1	98.9	99.5	69.8	99.3	99.6	99.8	73.4	99.8	100.1	99.9
Di-*n*-butylamine	69.8	100.4	99.7	99.8	73.0	100.3	99.7	99.9	79.6	100.7	99.8	100.2

OV-1, but were well separated on 3.5% SE-30; pyrrolidine and di-*n*-propylamine had identical retention times on 3.5% SE-30 but they differed on 3% OV-1. In instances where the identity of a peak was ambiguous, the use of two different columns might lead to an unequivocal identification.

In order to evaluate a derivatization reaction for use in analysis, it is necessary to investigate the rate of reaction and the yield.

For optimization of the conditions for derivatization, the nature of the alkali and its concentration and the reaction time were investigated. Of the various alkalis examined, sodium hydroxide was the most suitable. Weaker alkalis such as sodium hydrogen carbonate did not effect sulphonamide formation.

Table II shows the effect of the concentration of sodium hydroxide and reaction time on the yield of sulphonamides. Comparison with synthesised and purified reference compounds showed that the derivatization of all of the amines studied, except diisopropylamine, were complete at any concentration of sodium hydroxide within 10 min. Almost complete derivatization of diisopropylamine was achieved using 10 *M* sodium hydroxide for 30 min, and this reaction time was used in subsequent routine analysis. At lower sodium hydroxide concentrations, sulphonamide formation from diisopropylamine was incomplete.

The procedure developed appeared to be the best compromise between the most rapid derivatization and optimal yield. The variability of the extent of the derivatization reaction was less than $\pm$ 3%.

The reproducibility of the chromatographic system with an FPD after derivatization was investigated by repeated analyses of each amine solution (20 μg/ml). The peak heights of each amine showed good reproducibility with a coefficient of variation of 2.8–3.4% ($n = 5$).

By use of log–log graph paper, the linearity of the peak heights as a function of the amounts of dimethylamine, diethylamine, diisopropylamine, di-*n*-propylamine, pyrrolidine, morpholine, diisobutylamine and di-*n*-butylamine were investigated over the ranges 0.5–10, 0.9–10, 1.0–15, 1.2–15, 1.0–15, 2.0–40, 1.0–15 and 1.5–20 ng, respectively. The correlation coefficients varied from 0.9947 to 0.9992.

We conclude that the proposed method is suitable for the quantitative determination of secondary amines. Further work on the identification and quantitation of secondary amines in various foods is in progress.

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