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GAS CHROMATOGRAPHIC SEPARATION OF LOWER ALIPHATIC PRIMARY AMINES AS THEIR SULPHUR-CONTAINING SCHIFF BASES USING A GLASS CAPILLARY COLUMN

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SUMMARY

Thirteen C_1–C_7 aliphatic primary amines were quantitatively converted to the corresponding sulphur-containing Schiff bases by the reaction (60°, 1 h) with 2-thiophene aldehyde. Complete separation of the derivatives of the 13 amines was achieved on a 30 m × 0.25 mm I.D. glass capillary column (125°), packed with PEG-20M.

INTRODUCTION

The trace analysis of lower aliphatic amines is important because these amines cause odours and problems in agriculture, such as the decay of foods and fish. It is particularly important to know the components of the amines, especially the primary amines, and any carbonyl compounds additionally present, because these compounds easily produce Schiff base condensates.

In general, when packed columns such as those listed in Table I are employed, the complete separation of free amines, such as ethyl-, *n*-propyl-, isopropyl-, *tert.*-butyl- and allylamines, is very difficult. Seven columns, *viz.*, THEED + TEP*[1], triethanolamine[2], Squalane + glycerine[3], Chromosorb 103[4,5], Pennwalt 223[5] and PEG 1500 and 20M[6] have been tested but all were unsatisfactory for the purpose of complete separation.

Gas chromatography (GC) using chemical reactions for the biologically important primary amines such as epinephrine, dopamine, catecholamines, phenethylamine and amphetamine has been reported. Several derivatives, such as trimethylsilyl ethers[7], Schiff bases[8–12], *p*-tosylamides[13], pentafluorobenzoylamides[5,14,15], 2,4-dinitrophenyl derivatives[16,17], isothiocyanates[18,19] and trifluoroacetates[20–22] have been studied for this purpose. Pentafluorobenzaldehyde, pentafluorobenzoyl chloride, 2,4-dinitrofluorobenzene and trifluoroacetic anhydride have been used as derivatization agents for the electron-capture detection of picogram amounts of several primary amines.

However, most of these techniques have been applied to high-molecular-weight

* THEED = N,N,N′,N′-Tetrakis (2-hydroxyethyl)ethylene diamine; TEP = tetraethylene pentamine.

compounds, with few applications to lower aliphatic primary amines[5,17,23,24]. The reactions of pentafluorobenzoyl chloride[5] with ammonia and lower aliphatic primary and secondary amines such as methyl-, dimethyl-, ethyl-, diethyl-, *n*-propyl- and di-*n*-propylamines gave white, curdy precipitates, which may be the hydrochlorides of the amines, so that the reactions were not quantitative. Day *et al.*'s procedure[17], involving partial separation into three distinct zones by thin-layer chromatography, is required as a preliminary treatment prior to GC analysis. The procedure for the derivative-formation reaction (2,4-dinitrophenylamines) is also sometimes complex.

In contrast, the reaction of Schiff base formation with the lower aliphatic primary amines using benzaldehyde and pentafluorobenzaldehyde take place easily and rapidly at room temperature. The by-product of these reactions is water, which does not undergo secondary reactions in the reaction systems involved. These methods resulted in sharper peaks, high precision and high selectivity with the lower aliphatic primary amines. While the minimum detectable amount of fluorine-containing Schiff bases[24] was about 0.02 ng with an electron-capture detector (ECD), that of non-fluorine-containing Schiff bases was about 60 ng with a flame-ionization detector (FID)[23]. However, in these methods, the separation of the derivatives of *n*-propyl-, allyl- and *tert.*-butylamines was poor.

This paper describes a complete GC separation of 13 lower aliphatic primary amines as their sulphur-containing Schiff bases using a glass capillary column, which has a high resolving power.

EXPERIMENTAL

Preparation of sulphur-containing Schiff base

The procedure for the preparation of sulphur-containing Schiff bases derived from lower aliphatic primary amines with 2-thiophene aldehyde (THA) was as follows. The amine ($1 \cdot 10^{-5}$ mole) and THA ($1–10 \cdot 10^{-5}$ mole) were added to 1 ml of ethanol and the mixture was allowed to react at temperatures from room temperature to 60° for 0.5–4 h. The rate of reaction was evaluated by measuring the disappearance of the amine or THA by GC using Tenax-GC as column packing.

Reagents

THA (b.p. 198°) and methyl- (40 wt.-% aqueous solution), ethyl- (70 wt.-% aqueous solution) and isopropylamine were obtained from Tokyo Kasei Kogyo, Tokyo, Japan. *n*-Propyl-, allyl-, *n*-butyl-, *sec.*-butyl- and *tert.*-butylamine and ethanol were obtained from Wako Pure Chemical Industries, Osaka, Japan. Isobutyl-, *n*-amyl-, isoamyl-, *n*-hexyl- and *n*-heptylamine were obtained from PolyScience Corp., Niles, Ill., U.S.A. All reagents were guaranteed or reagent-grade chemicals.

Apparatus

The gas chromatograph was a Shimadzu Model GC5AP$_5$FFp equipped with an FID and a flame-photometric detector (FPD); the FPD had an FID for monitoring. The large amounts of organic solvent present were detected with the latter FID. However, the sensitivity of this FID is lower than that of the normal FID, because it operates with a hydrogen-rich flame. The FPD and its FID accessory were operated with a separate electrometer (Shimadzu Model EM-5S). The detector signals were

recorded at 10 mV full scale simultaneously on a Shimadzu Model R-201 double-pen recorder.

The Shimadzu GC5AP$_5$FFp gas chromatograph was also equipped with a digital integrator (Shimadzu Model ITG-2A) for the determination of the rate of the sulphur-containing Schiff base formation reaction.

Chromatographic conditions

The analytical columns used were as follows: (a) a 3 m × 3 mm I.D. glass column packed with Tenax-GC (made by Enka, Arnhem, The Netherlands), 60–80 mesh; (b) a G-SCOT column with an SF-96 20 m × 0.28 mm I.D. glass capillary column (obtained from Gasukuro Kogyo, Tokyo, Japan); (c) a Hitachi Chemi-column with a PEG-20M 30 m × 0.25 mm I.D. glass capillary column (obtained from Hitachi, Ibaraki, Japan).

The GC conditions for each of the analytical columns were as follows. (a) carrier gas (nitrogen) flow-rate, 55 ml/min; air and hydrogen flow-rates, 1.0 l/min and 50 ml/min, respectively; column temperature (programmed), held for 1 min at 100°, heated at 10°/min to 250°, maintained at this temperature for 25 min, then cooled to the starting temperature; injection port and detector (FID) temperatures, 250°. (b) carrier gas (nitrogen) flow-rate, 0.97 ml/min; purge gas (nitrogen) flow-rate, 50 ml/min; air and hydrogen flow-rates, 1.0 l/min and 50 ml/min, respectively; column temperature (programmed), held for 1 min at 100°, heated at 4°/min to 200°; injection port and detector (FID) temperatures, 200°; splitting ratio, 1:140. (c) carrier gas (nitrogen) flow-rate, 0.7 ml/min; purge gas (nitrogen) flow-rate, 50 ml/min for FID and 140 ml/min for FPD; air flow-rate, 1.0 l/min for FID and 50 ml/min for FPD; hydrogen flow-rate, 50 ml/min for FID and 80 ml/min for FPD; column temperature, 125°; injection port temperature, 180°; detector temperature, 180° for FID and 205° for FPD; splitting ratio, 1:180.

RESULTS AND DISCUSSION

The complete GC separation of lower aliphatic primary amines, especially ethyl-, *n*-propyl-, isopropyl-, allyl-, isobutyl-, *sec.*-butyl- and *tert.*-butylamine, is very difficult in some packed columns. As listed in Table I, all columns tested were inadequate for the complete separation of the 13 amines of interest here.

The optimal conditions for the sulphur-containing Schiff base formation reaction were as follows: molar ratio of THA to amine, >2; reaction temperature, 60°; reaction time, 1 h. The reactions of THA with the 13 amines were quantitative under these conditions.

Fig. 1 shows a typical gas chromatogram obtained with the SF-96 glass capillary column and an FID. The derivatives of *n*-propyl- and *tert.*-butylamine were not separated, probably as a result of the low polarity of SF-96.

Fig. 2 shows a typical gas chromatogram obtained with the PEG-20M glass capillary column and an FID. The complete separation of the derivatives of the 13 amines was achieved. Unfortunately, when helium was used as the carrier gas at a flow-rate of 0.7 ml/min, the separation of the peaks of the derivatives of methyl-, *tert.*-butyl-, *n*-butyl- and allylamine was poor.

Fig. 3 shows a typical gas chromatogram obtained with the PEG-20M glass capillary column and an FPD. Although the sensitivity was much higher than that

TABLE I

EFFECTIVENESS OF COLUMNS TESTED USING AN FID

Packing	*Note**	*Free amines giving overlapping peaks*
THEED + TEP	1	Ethyl-, isopropyl- and *tert.*-butylamine
Triethanolamine	2	Ethyl- and isopropylamine
Squalane + glycerine	3	Allyl- and *tert.*-butylamine
Chromosorb 103	4	Isopropyl-, isobutyl- and *tert.*-butylamine
Pennwalt 223	5	*n*-Propyl- and allylamine
PEG-1500	6	Isobutyl- and *sec.*-butylamine
PEG-20M	7	Ethyl-, *n*-propyl-, isopropyl-, allyl- and *tert.*-butylamine

* Notes:

(1) 15% THEED + 5% TEP on Chromosorb W AW DMCS, 60–80 mesh, 3 m × 3 mm I.D., 60°, N_2 flow-rate 58 ml/min.

(2) 20% triethanolamine on Celite 545, 60–80 mesh, 3 m × 3 mm I.D., 70°, N_2 flow-rate 50 ml/min.

(3) 20% Squalane + 2.5% glycerine + 2.5% KOH on Diasolid L, 60–80 mesh, 3 m × 3 mm I.D., 60°, N_2 flow-rate 50 ml/min.

(4) Chromosorb 103, 60–80 mesh, 3 m × 3 mm I.D., 130°, N_2 flow-rate 57 ml/min.

(5) 28% Pennwalt 223 + 4% KOH on Gas-Chrom R, 80–100 mesh, 3 m × 3 mm I.D., 60°, N_2 flow-rate 57 ml/min.

(6) 0.5% PEG-1500 + 0.2% KOH on Carbopack B, 60–80 mesh, 1.5 m × 3 mm I.D., 75°, N_2 flow-rate 50 ml/min.

(7) 1.3% PEG-20M + 0.3% KOH on Carbopack B, 60–80 mesh, 1.5 m × 3 mm I.D., 50°, N_2 flow-rate 50 ml/min.

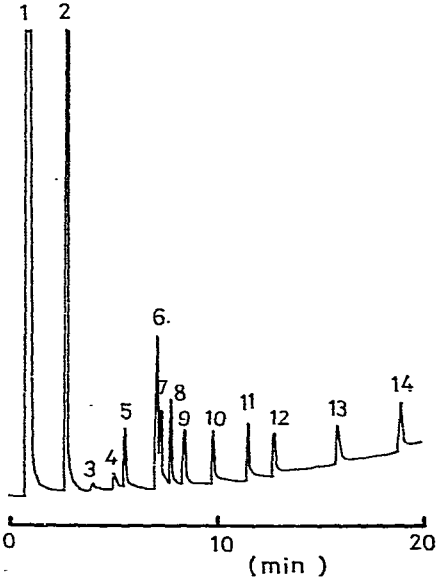

Fig. 1. Gas chromatogram of sulphur-containing Schiff bases, obtained with an SF-96 glass capillary column and an FID. GC conditions, (b). Sample concentration, $1 \cdot 10^{-5}$ mole/ml; sample injected, 1 μl. Peaks of sulphur-containing Schiff bases: 1 = ethanol (solvent); 2 = THA (excess); 3 = methylamine; 4 = ethylamine; 5 = isopropylamine; 6 = *tert.*-butylamine + *n*-propylamine; 7 = allylamine; 8 = *sec.*-butylamine; 9 = isobutylamine; 10 = *n*-butylamine; 11 = isoamylamine; 12 = *n*-amylamine; 13 = *n*-hexylamine; 14 = *n*-heptylamine.

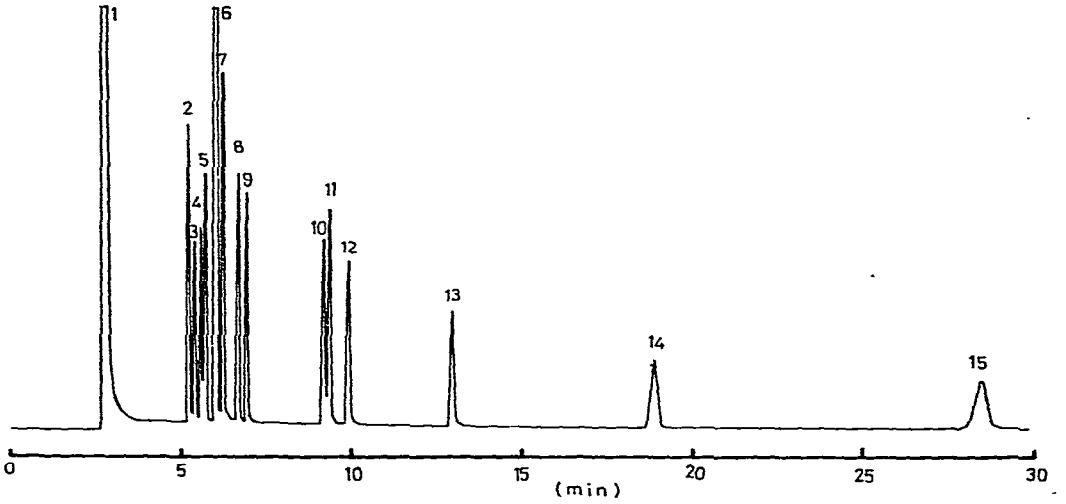

Fig. 2. Gas chromatogram of sulphur-containing Schiff bases obtained with a PEG-20M glass capillary column and an FID. GC conditions, (c). Sample concentration and volume injected as in Fig. 1. Peaks of sulphur-containing Schiff bases: 1 = ethanol (solvent); 2 = isopropylamine; 3 = methylamine; 4 = ethylamine; 5 = *tert.*-butylamine; 6 = THA (excess); 7 = *sec.*-butylamine; 8 = *n*-propylamine; 9 = isobutylamine; 10 = *n*-butylamine; 11 = allylamine; 12 = isoamylamine; 13 = *n*-amylamine; 14 = *n*-hexylamine; 15 = *n*-heptylamine.

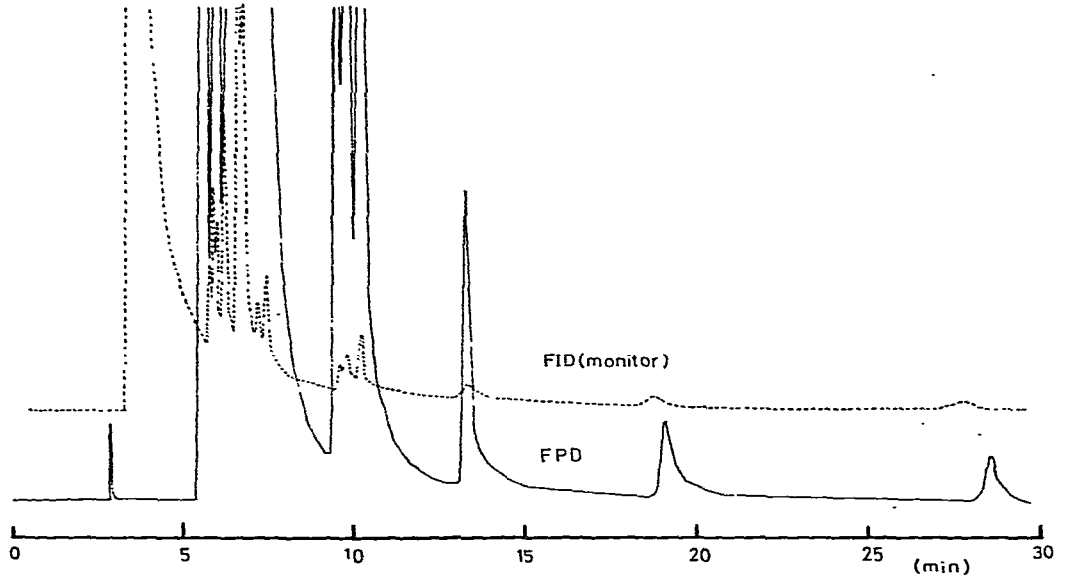

Fig. 3. Gas chromatogram of sulphur-containing Schiff bases obtained with a PEG-20M glass capillary column and an FPD and FID (monitor). GC conditions, (c). Sample concentration and volume injected as in Fig. 1.

of the FID for the derivatives of the C_1–C_4 amines, the sensitivities for the derivatives of the C_5–C_7 amines were similar. The first peak eluted from the PEG-20M column was the derivative of isopropylamine; the derivative of *n*-heptylamine was eluted within about 30 min.

The optimal flow-rates of the purge gas, hydrogen and air or oxygen in FPDs when using glass capillary columns have been described recently[25–28]. In this study of these optimal conditions the derivative of *n*-butylamine was used. At flow-rates of the purge gas from 50 to 110 ml/min, peaks with considerable tailing were obtained. At flow-rates greater than 200 ml/min the sensitivity was lower, but the tailing was only slight. Therefore, the optimal flow-rate of the purge gas was about 150 ml/min. At flow-rates of hydrogen from 40 to 70 ml/min a lower sensitivity of peak detection occurred, and the optimal flow-rate was about 80 ml/min. The flow-rate of air was maintained constant at 50 ml/min.

In the presence of 100 molar equivalents of ammonia and dimethyl-, trimethyl-, diethyl- and triethylamine, no evidence of interference was found in the gas chromatogram.

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