Journal of Chromatography, 90 (1974) 157–161

CHROM. 7167

GAS CHROMATOGRAPHIC ANALYSIS OF AMINES SEPARATED AS URETHANE DERIVATIVES

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(Received October 12th, 1973)

SUMMARY

A simple method for the separation of low molecular weight amines as urethanes is described. Diethyl pyrocarbonate is used under restricted pH conditions in the reaction. Analysis of the derivatives was carried out on four columns using gas chromatography. The times of elution and substance-specific correction factors were determined.

INTRODUCTION

Diethyl pyrocarbonate is used in various biochemical and microbiological applications and has so been used for many years in our laboratory. The reaction with amines is well known and produces a carbethoxy derivative (a urethane):

$$R{-}NH_2 + C_2H_5O{-}CO{-}O{-}CO{-}OC_2H_5 \rightarrow R{-}NH{-}CO{-}OC_2H_5 + CO_2 + C_2H_5OH$$

This type of derivative has been made and analysed by Boehm and Mehta[1] and Pauli and Genth[2], but gas chromatography has been used for only a few amino products[3,4]. Special interest has been shown in herbicides. Spengler and Hamroll[5] investigated urethanes with a ring structure.

In this work low-molecular-weight amines are detected as urethanes.

MATERIALS

Compounds were obtained as follows: diethyl pyrocarbonate from Schuchardt, München, G.F.R.; ammonium chloride, propylamine, methylamine hydrochloride, 2-methyl-propylamine, 3-methyl-butylamine hydrochloride, aniline and methylaniline from E. Merck, Darmstadt, G.F.R.; ethylamine hydrochloride from Hopkin & Williams, Chadwick Heath, Great Britain; and butylamine from Eastman-Kodak, Rochester, N.Y., U.S.A. The pentylamine and 2-methylbutylamine were prepared by decarboxylation of norleucine and isoleucine[6]. Distillation into HCl was used to purify the amines for the gas chromatographic studies.

EXPERIMENTAL

Preparation of samples

The analysis was made on 0.05–0.7 mg amine in 5 ml aqueous solution treated with 10 mg diethyl pyrocarbonate for 30–40 minutes at room temperature. The pH was adjusted with NaOH to 9.5.

The excess of diethyl pyrocarbonate and the duration of treatment were enough for the complete carbethoxylation of all available amino groups (*cf.* the reaction kinetics of diethyl pyrocarbonate[7,8]). Mixtures of stock solutions were used to determine the times of elution and specific correction factors. The substance-specific correction factors, f_i, were calculated according to Kaiser[9] by

$$f_i = \frac{\text{wt. per cent}_i \times \text{area standard}}{\text{wt. per cent standard} \times \text{area}_i}$$

where wt. per cent_i = wt. per cent of the substance i in the calibration mixture and area_i = peak area for i.

The butylamine derivative was used as the standard.

Analysis

A Perkin-Elmer F11 gas chromatograph having a flame ionisation detector (FID) and all-glass column system was used in the experiments. The conditions for analysis are given in Table I. Injections were made with a 5-µl Scientific Glass (London, Great Britain) syringe. A 10-ml vial with a membrane was used for the reaction of each mixture. Before analysis the pressure was lowered with a needle through the membrane of the vial. *n*-Decane was added for comparison of elution times. Quantitative measurements were made on a Carbowax column. Aniline derivatives had to be separated on the other columns. Apiezon N and SE-30 columns were used in this part of the work. Peak areas were measured with a planimeter.

TABLE I

GAS CHROMATOGRAPHIC CONDITIONS

	Column			
	SE-30	*Apiezon N*	*Carbowax 1540*	*XF-1125*
Stationary phase	20% SE-30 + 1% KOH	10% Apiezon N	8% Carbowax 1540	1% XF-1125
Support	Gas Chrom. Q (100–120 mesh)	Chromosorb G (60–80 mesh)	Chromosorb W (80–100 mesh)	Chromosorb W (60–80 mesh)
Column dimensions	1.10 m × 2.0 mm I.D.	1.20 m × 2.0 mm I.D.	1.5 m × 3.0 mm I.D.	2.2 m × 2.0 mm I.D.
Gas (N_2) flow-rate	37 ml/min	60 ml/min	75 ml/min	20 ml/min
Sensitivity	× 100	× 100	× 100	× 100
Amount injected	1 µl	1 µl	2 µl	1 µl
Recorder speed	40 mm/min	40 mm/min	40 mm/min	40 mm/min

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of some aliphatic amines separated in the form of carbethoxy derivatives on an 8% Carbowax 1540 column. The derivative from ammonia (urethane) is eluted after butylamine. Under the conditions used on this polar column the aniline derivative could not be found.

A high percentage (20%) of SE-30 stationary phase resulted in a suitable separation of the urethanes (Fig. 2). The performance of this column was even better than that of the Apiezon N and XF-1125 columns used in the experiments.

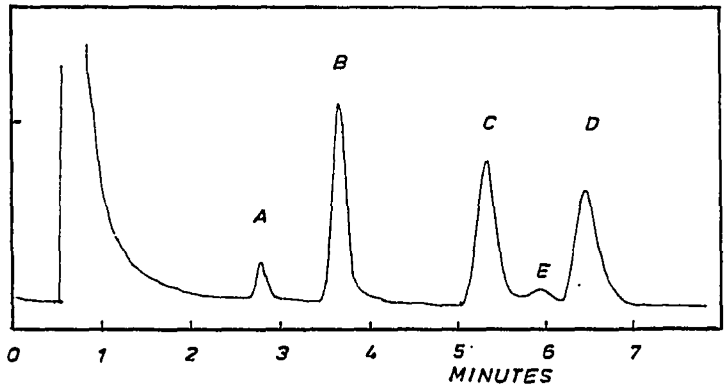
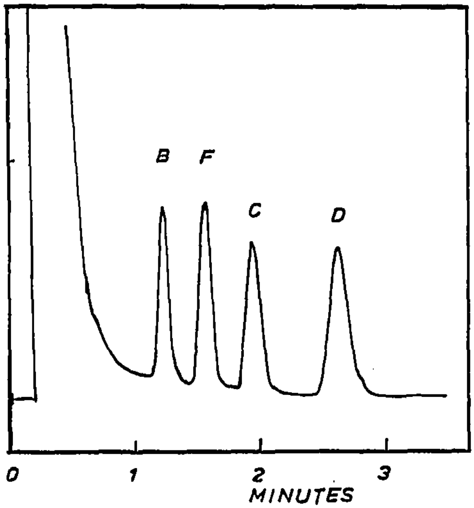


Fig. 1. Gas chromatographic separation of the urethane derivatives of methylamine (A), propylamine (B), butylamine (C), ammonia (E) and 3-methylbutylamine (D). An 8% Carbowax 1540 column was used for the analysis.



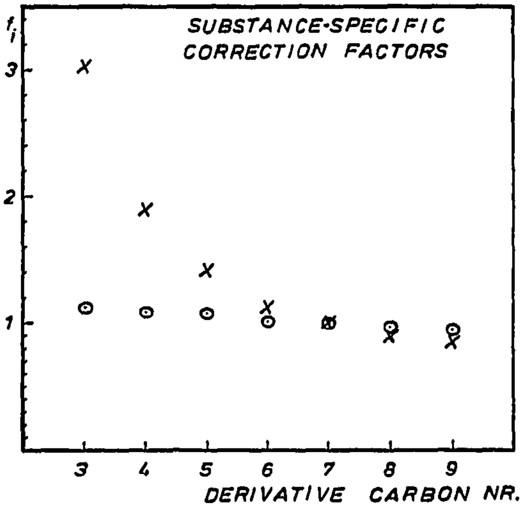


Fig. 2. Separation of the urethane derivatives of propylamine (B), 2-methylpropylamine (F), butylamine (C) and 3-methylbutylamine (D) on a 20% SE-30 column.

Fig. 3. ×, Substance-specific correction factors, f_i, as wt.% urethane produced; ⊙, f_i values as wt.% of the amine; both are plotted against the corresponding derivative carbon number.

TABLE II

ELUTION TIMES (min) OF AMINE DERIVATIVES

Compound	*Urethane derivative*	*Column*			
		SE-30	*Apiezon N*	*Carbowax 1540*	*XF-1125*
NH_3	urethane	0.725	0.70	5.78	–
CH_3NH_2	methylurethane	0.789	0.70	2.81	–
$C_2H_5NH_2$	ethylurethane	1.17	0.70	2.80	–
$C_3H_7NH_2$	propylurethane	1.55	1.30	3.68	1.16
$C_4H_9NH_2$	2-methylpropylurethane	1.98	1.65	3.89	1.24
$C_4H_9NH_2$	butylurethane	2.46	2.23	5.34	1.41
$C_5H_{11}NH_2$	3-methylbutylurethane	3.25	3.08	6.57	1.61
$C_5H_{11}NH_2$	2-methylbutylurethane	3.94	3.03	6.00	1.21
$C_5H_{11}NH_2$	pentylurethane	4.42	3.84	8.04	2.68
$C_{10}H_{22}$*		1.59	1.42	0.654	1.16

* *n*-Decane (reference)

TABLE III

ELUTION TIME (min) FOR ANILINE DERIVATIVES

Amine	*Column and temperature*		
	SE-30, 175°	*Apiezon N, 175°*	*XF-1125, 140°*
Methylaniline	2.30	2.30	2.66
Aniline	2.85	3.60	5.07

Samples injected on the XF-1125 material had tailing peaks so that some low-molecular-weight derivatives could not be observed. In Table II the times of elution at 140° on the four columns are given. For two separations, the cyclic amines, a higher temperature was needed. Table III lists the elution times for anilines.

Quantitative measurements

The derivatives have been correlated to the standard peak in two ways. Firstly, the original amount of amine was used for determination of the factors and, secondly, calculations were made for the urethanes. Fig. 3 includes both of these values.

A linear function between the carbon number and the f_i values from amines was found. One $-CH_2-$ unit alters the substance-specific correction value by 0.03 for this group. The f_i values in the urethane group are large for the low molecular weight derivatives. This is clearly due to the content of oxygen and nitrogen when analysed on an FID system.

Treatment of special samples

By use of a pH between 8.5 and 10.5 samples can be separated according to the method given. Unknown material might need extra diethyl pyrocarbonate to ensure that all the nucleophiles present react. A modification of the system to use more reagent might require the use of ethanol to give a homogeneous solution. The urethanes formed are stable for several weeks at 5° in aqueous media. If long-term storage is necessary, a suggestion would be to neutralize the sample after reaction.

In order to relate the correction factors to other systems *n*-butanol was used as standard ($f = 1.00$). A value of 3.85 ± 0.05 was obtained for urethane.

ACKNOWLEDGEMENTS

I wish to thank Dr. Siv Osterman-Golkar and Anna Gejvall, my wife, for valuable information and help. This investigation has been supported by a grant from the Swedish Atomic Research Council.

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