Journal of Chromatography, 76 (1973) 464–466

CHROM. 6484

Note

Separation of O-alkylhydroxylamines by gas chromatography

O-Alkylhydroxylamines are a group of amines that possess interesting physiological properties[1]. Some of these compounds have anaesthetic, antitumour, antirheumatic, diuretic and blood-pressure depressing activity.

In the course of systematic studies of N-hydroxyamino acids[2], we synthesized a series of O-alkylhydroxylamines[3], the physical constants of which, such as boiling point, n_D value and pK_a value, were measured. Their fragmentation principles in mass spectrometric processes were also defined[4]. The latter technique, in particular, required the determination of the degree of purity of the amines obtained. Experiments involving thin-layer and paper chromatography did not give satisfactory results, although many different solvent systems were tested. Only thin-layer chromatography of the dansyl derivatives of these amines seems promising.

Gas chromatography was therefore used as a fundamental method for the above purpose, and a suitable liquid phase and optimum operating conditions were looked for. The proof of the separation of four O-alkylhydroxylamines on Carbowax 1500 by BARGIGIA AND GAMBI[5] was unsatisfactory, probably because of the similar physico-chemical properties, such as small differences in pK_a values and boiling points, of these compounds (see Table I).

TABLE I

SOME PROPERTIES OF O-ALKYLHYDROXYLAMINES AND THEIR RELATIVE RETENTIONS

Compound	*Boiling point (°C) (ref. 3)*	*pK_a (ref. 3)*	*Relative retention*
CH_3-ONH_2	50	4.51	1.00
C_2H_5-ONH_2	64–65	4.56	1.41
n-C_3H_7-ONH_2	85–86	4.60	2.45
i-C_3H_7-ONH_2	79–80	4.50	1.52
n-C_4H_9-ONH_2	115	4.64	4.16
i-C_4H_9-ONH_2	96–97	4.62	3.14
sec.-C_4H_9-ONH_2	103–104	4.51	2.87
tert.-C_4H_9-ONH_2	92–93	4.31	1.91
n-C_5H_{11}-ONH_2	146–147	4.56	7.71
i-C_5H_{11}-ONH_2	138	4.55	5.96
sec.-C_5H_{11}-ONH_2	125–126	4.62	4.60

In the present work, several representative O-alkylhydroxylamines were separated on different liquid phases, the selection of which was based on papers describing amine separations[6–7]. Finally, Triton X-305 was chosen and optimum conditions for separations on this phase were established.

Experimental

All work was carried out with a Willy Giede Model GCHF 18.3 gas chromatograph, equipped with a flame ionization detector (FID) and a 1.9-mV recorder.

The following columns were tested:

20% Squalane on 80–100 mesh Sterchamol;

20% Apiezon L on 80–100 mesh Sterchamol;

Porapak Q, 80–100 mesh;

5, 10 and 15% Versamid 900 on 60–80 mesh Chromosorb W;

5, 10 and 15% Triton X-305 on 60–80 mesh Chromosorb W.

Results and discussion

The model mixture of eleven synthesized O-alkylhydroxylamines (Table I) was injected into the columns, and the most suitable temperature and flow-rate were established so as to obtain optimum resolution. Satisfactory results for some of the O-alkylhydroxylamines were obtained on the column packed with 10% Versamid 900 on 60–80 mesh Chromosorb W, but unfortunately two pairs were not completely separated, namely O-*tert.*-butylhydroxylamine from O-*n*-propylhydroxylamine and O-*sec.*-butylhydroxylamine from O-isobutylhydroxylamine. The best resolution was given by the column with 10% Triton X-305 on 60–80 mesh Chromosorb W. Fig. 1 illustrates the separation of all eleven O-alkylhydroxylamines on this column. The low relative retention of O-*tert.*-butylhydroxylamine is probably connected with its lower basicity.

After choosing the most suitable liquid phase and determining the optimum

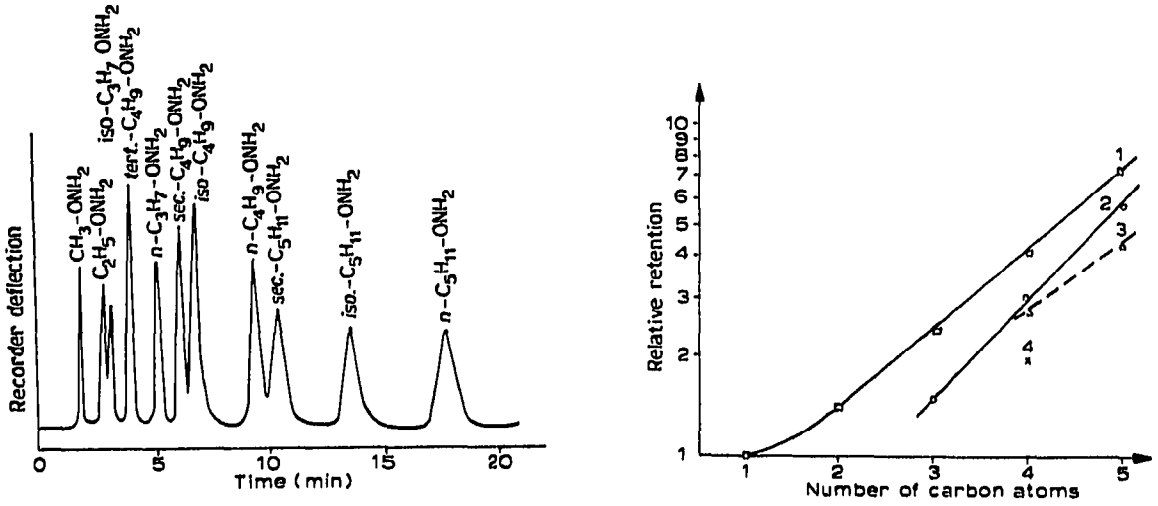

Fig. 1. Chromatogram of model mixtures of O-alkylhydroxylamines on a 3-m × 4-mm I.D. column packed with 10% Triton X-305 on 60–80 mesh Chromosorb W. Column temperature, 90°. Injector port temperature, 130°. Flow-rate of argon carrier gas, 25 ml/min; hydrogen, 40 ml/min; air 400 ml/min.

Fig. 2. Logarithm of relative retention *versus* number of carbon atoms for the individual series of O-alkylhydroxylamines. 1 = O-*n*-alkylhydroxylamines; 2 = O-isoalkylhydroxylamines; 3 = O-*sec.*-alkylhydroxylamines; 4 = O-*tert.*-alkylhydroxylamines.

GLC conditions, the pure amines were injected successively into the column, and their relative retentions were calculated (Table I).

Based on the results obtained, the logarithm of the relative retention was plotted against the number of carbon atoms in the molecule of each O-alkylhydroxylamine for each homologous series (Fig. 2).

The plots for the homologous series of O-*n*-alkylhydroxylamines (except for O-methylhydroxylamine) and O-isoalkylhydroxylamines, and probably also for the O-*sec.*-alkylhydroxylamines (only two points), were straight lines. This relation can thus be used for the identification of compounds from these groups.

The GLC conditions described above permit the determination of the degree of purity of synthetic O-alkylhydroxylamines, and these conditions can also be used in gas chromatograph–mass spectrometer systems.

The authors thank Dr. R. STASZEWSKI for helpful discussions and comments.

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Received November 10th, 1972