Gas Chromatographic Separation of Amines by Special Selectivity

By J. S. SAWARDEKER and J. L. LACH

This study deals with the separation of several aliphatic amines by the use of metal complexation in GLC.

THE USE OF complex formation in the various branches of theoretical and applied chemistry and allied fields is now generally recognized. However, the use of complex formation, although highly promising, has received little attention in gas chromatography. Bradford (1), in his gas chromatographic studies employing solid silver nitrate in glycol, achieved a selective retardation of unsaturated hydrocarbons demonstrating interaction between vapor and metal atoms. Barber (2) reported marked increase in retention times of many individual compounds using molten stearates of metals, Mn, Co, Zn, Cu, and Ni as column liquids against retention times on apiezon L. Separation of amines in general has constituted a difficult task. Recently Sze (3) achieved separation of a mixture of ten amines using tetrahydroxyethylethylenediamine and tetraethylenepentamine as partition liquids in GLC. They report that the results are promising but not satisfactory.

EXPERIMENTAL

Equipment.—F & M 500 gas chromatograph with thermal conductivity cell and equipped with Minneapolis Honeywell recorder y143x(58).

Procedure and Results.—Two columns were prepared from 1/4-in. O.D. copper tubing. In column one, the stationary phase Gas Chrom P was coated with 2% SE-30 from a solution in methylene chloride, the solvent being removed by rotary vacuum evaporator. In the second, the stationary phase (Gas Chrom P) was coated with 2% SE-30 as above and then impregnated with 4% silver nitrate. A mixture containing triethylamine (b.p. 89.5°), diethylamine (b.p. 55.5°), and *n*-butylamine (b.p. 77.8°) in the proportion 1:2:1, respectively, was investigated using the two columns. Helium was employed as the carrier gas. The chromatograms are illustrated in Figs. 1 and 2. For the column containing SE-30 only, the mixture emerged as a single peak with isothermal operation at 50°. In the second column containing SE-30 and silver nitrate temperature programming from 50 to 175° was necessary for complete separation, since isothermal operation at 50° was ineffective. Apparently no decomposition was detected on the chromatogram. Isothermal operation at higher temperature gave poor results. Interestingly enough, it was found that the amines were well retained and the order of retention for the amines was 1° > 2° > 3°. Figure 3 shows the chromatogram of a mixture of triethylamine, diethylamine, and *n*-butylamine in the proportion 1:1:2, respectively, illustrating the separation achieved which may be quantitative. However, no attempt was made to study the separation quantitatively.

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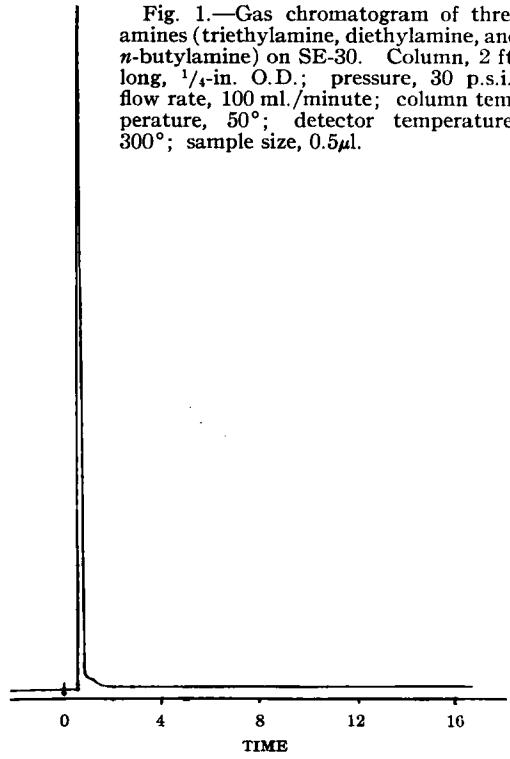

Fig. 1.—Gas chromatogram of three amines (triethylamine, diethylamine, and *n*-butylamine) on SE-30. Column, 2 ft. long, 1/4-in. O.D.; pressure, 30 p.s.i.; flow rate, 100 ml./minute; column temperature, 50°; detector temperature, 300°; sample size, 0.5 µl.

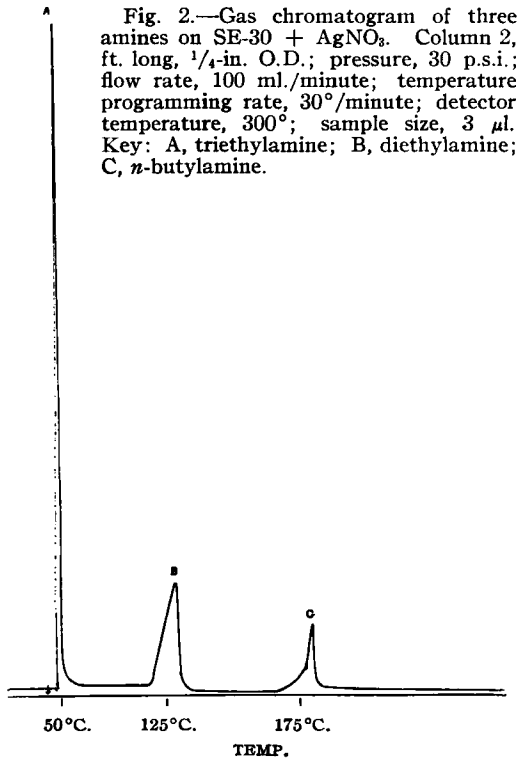

Fig. 2.—Gas chromatogram of three amines on SE-30 + $AgNO_3$. Column 2, ft. long, 1/4-in. O.D.; pressure, 30 p.s.i.; flow rate, 100 ml./minute; temperature programming rate, 30°/minute; detector temperature, 300°; sample size, 3 µl. Key: A, triethylamine; B, diethylamine; C, *n*-butylamine.

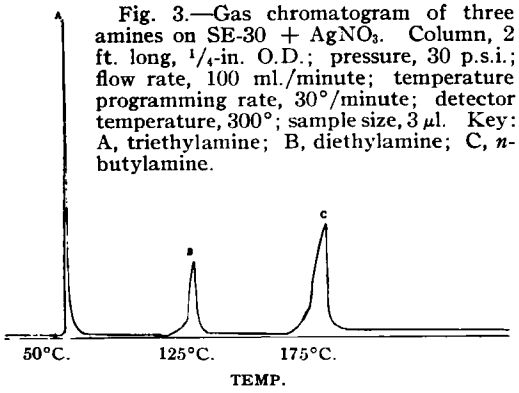

Fig. 3.—Gas chromatogram of three amines on SE-30 + $AgNO_3$. Column, 2 ft. long, 1/4-in. O.D.; pressure, 30 p.s.i.; flow rate, 100 ml./minute; temperature programming rate, 30°/minute; detector temperature, 300°; sample size, 3 μl. Key: A, triethylamine; B, diethylamine; C, *n*-butylamine.

DISCUSSION

It is evident from the chromatograms that the three amines pass through the various types of chromatographic columns at substantially different rates which are not governed by their boiling points. Silver nitrate seems to exhibit dramatic selectivity for these amines. It is difficult to state the exact nature of forces involved in the separation. However, in view of the fact that silver ion is capable of forming chelates, the strong retention behavior observed is indicative of chelate formation between the metals and the amines. It is of interest that a significant rise in temperature had to be employed to elute the 2° and 1° amines. This is consistent with the tendency for coordination increases with decreasing substitution $RNH_2 > R_2NH > R_3N$. As further evidence supporting complex formation, we observed that ethylenediamine, which theoretically is capable of forming a stronger chelate with silver, is so strongly retained that it could not be eluted even at 200°. Elution at higher temperatures was not attempted because of possible thermal decomposition of the silver nitrate. However, the use of silver bromide in place of the silver nitrate did permit use of higher temperature. In this silver bromide column ethylenediamine did emerge as a peak at a temperature of 250°.

It is of interest that the column length for this study was only 2 ft. Increasing the column length was not advantageous, and shortening it did not give good results. The method appears to be highly promising for separation of related compounds. Further studies are in progress; a complete report will be published at a later date.

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Synthesis of 5,5-Dimethyl-2,4-oxazolidinedione-2-C^{14} (DMO-2-C^{14})

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DMO-2-C^{14} was synthesized for use in measurements of the pH of intracellular water. It was prepared by condensing urea-C^{14} with n-butyl 2-methyllactate in the presence of sodium butylate in butanol solution. The yield of DMO-2-C^{14} from 1 mmole of urea-C^{14} was 58 per cent, and the product was radiochemically homogeneous as demonstrated by paper chromatography. The ionization exponent was determined by a system of solvent partitioning. A method is described for determination of DMO-2-C^{14} in biological samples.

THE MEASUREMENT of the pH of the water inside living cells is a difficult problem that has been approached in many ways (1). Of the various methods used, that based on the measurement of the intracellular and extracellular concentrations of a weak organic acid or base is probably most widely applicable and least subject to theoretical objections. Until recently, nearly all of the work based on this principle has employed carbon dioxide as the indicator compound. In 1959, Waddell and Butler (2) suggested that the weak acid, 5,5-dimethyl-2,4-oxazolidinedione (DMO), has the attributes desirable in a compound for the measurement of intracellular pH and should have advantages over carbon dioxide for that purpose. Using ultraviolet spectrophotometric methods for the analytical determinations of DMO in plasma and tissue, they employed the distribution of DMO for the calculation of the intracellular pH of dog muscle. Subsequently, DMO has been used by other workers in a number of different investigations of intracellular pH.

For an *in vitro* study of the intracellular pH of tumor cells, it became evident that the relatively large amount of DMO required in a sample for spectrophotometric measurement would impose undesirable restrictions on the design and scope of the experiments. A more sensitive method of measurement, as would be provided by the use of radioactive DMO, would avoid these restrictions. The synthesis of DMO-2-C^{14} was accordingly carried out. In the course of the subsequent study of tumor cells, experience with the use of DMO-2-C^{14} (in conjunction with inulin-carboxyl-C^{14} for measurement of extracellular water) has confirmed the expected advantages. Very small samples suffice, and the concentration of DMO added can be made low enough to cause no significant derangement of cellular function. Measurements of radioactivity

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