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SEPARATION OF ENANTIOMERS BY GAS LIQUID CHROMATOGRAPHY WITH AN OPTICALLY ACTIVE STATIONARY PHASE *

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The resolution of enantiomers by gas liquid partition chromatography on an optically active stationary phase has presented a challenge to workers in the field for many years. All efforts, however, remained fruitless, and some successes claimed in the literature (1) could not be reproduced (2). We wish now to report a series of experiments in which unambiguous and efficient resolution has been carried out on an optically active stationary phase.

The separation of optical isomers by gas liquid chromatography has been studied in the laboratory of the authors for some time. One solution of the problem consists in the conversion of mixtures of

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enantiomers into diastereoisomers, which are chromatographed on an inactive phase. By this approach methods for resolution of 2-n-alkanols, via the corresponding α-alkanoyloxypropionates (3), and of α-amino acids (4), via N-TFA esters of 2-butanol or 2-n-octanol, have been developed. Other authors have worked out similar procedures (5).

The use of optically active stationary phases for resolution was pursued concurrently. The unsuccessful attempts, mentioned in the literature (1, 2) and confirmed by our own preliminary observations, were carried out with packed columns. In order to improve efficiency of separation, it was decided to employ capillary columns instead. On the other hand, it was known that some racemic mixtures are separated by chromatographic procedures involving the use of optically active solids as the stationary phase. Thus, for instance, chromium (III) hexafluoroacetylacetonate has been resolved to some extent on dextro quartz crystals (6), and certain multifunctional amino acids have been completely separated on cellulose (7). The separation of these amino acids has been explained (8) by three-point attachment to the solid surface, as in the models assumed to represent the stereospecific binding in substrate-enzyme complexes. By analogy, it seemed that the choice of solute-solvent systems should be made preferentially in a way such that multipoint interactions could take place in the neighbourhood of the asymmetric centers.

The experiments were carried out with a Perkin Elmer Model 801 chromatograph modified for work with a capillary column and provided with a flame ionization detector. The temperatures of the injector and the column were 90 and 210°, respectively, and the nitrogen pressure 20 p.s.i. The samples were injected in ether solution (0.3-0.6 μl) with a split ratio of 100:1. The capillary columns were drawn from pyrex glass (9), and had an internal diameter of 0.25 mm and a length of 50-100 m. The capillaries were cleaned with a series of

different solvents, and then coated with a 20% ether solution of the optically active phase by the plug method. The capillaries were then dried and conditioned overnight while raising the temperature gradually to 80°.

After some unsuccessful experiments with optically active polypropylene glycol, capillaries coated with N-TFA-L-isoleucine lauryl ester were tried and other N-TFA- α-amino acid esters were taken as solutes. The possibility of hydrogen bonding and, in an intuitive way, the remote resemblance of the solute-solvent system to a peptide-enzyme complex, determined the choice of these compounds.

On a column 50 m long (about 35,000 plates with respect to 2-heptyl acetate) the peaks corresponding to the N-TFA-alanine esters of isopropanol and n-butanol showed both a slight resolution. When the experiment was repeated with a column about 100 m long (140,000 plates with respect to 2-heptyl actate; 75,000 plates with respect to N-TFA-valine ester of 2-butanol) marked separation resulted.

The following N-TFA α-amino acid derivatives have thus far been resolved on the 100 m column: N-TFA-Alanine esters of ethanol, n-propanol, isopropanol, n-butanol, 2-butanol and N-TFA-valine and leucine esters of 2-butanol. All these compounds were checked for purity by chromatography on a capillary column coated with an inactive phase (4). Derivatives with one asymmetric center gave one peak on the inactive phase and two peaks on the active phase, while those with two asymmetric carbons gave two and four peaks, respectively. Two typical chromatograms are shown in FIGS. 1 and 2. Under the experimental conditions employed some enantiomers are completely resolved, e.g. the N-TFA-valine and leucine esters of 2-butanol give four distinct peaks of equal area (FIG. 2).

Comparison of resolution of the enantiomers N-TFA-alanine esters shows the influence of the steric requirements of the alcohol chain. Thus only a single peak was observed for the derivative of methanol, while

the N-TFA esters of the C_2 to C_4 alcohols examined showed separation with partial overlap (FIGS. 1 and 2). On the other hand, complete resolution into two peaks of equal area was found for the cyclopentyl compound (FIG 1).

Compounds other than N-TFA-α-amino acid esters, such as 2-heptyl acetate and α-acetoxypropionate of 2-butanol were also chromatographed, but did not separate.

A study of the structural features influencing resolution is now under way. It is hoped that the results will permit extrapolation to other solute-solvent systems, and thus help in finding suitable stationary phases for other classes of racemic compounds.

The achievement of resolution of optical isomers is one of the most striking demonstrations of the efficiency of gas liquid partition chromatography. The columns described should have interesting, analytical applications and might also serve as a tool for the study of stereospecific interactions.

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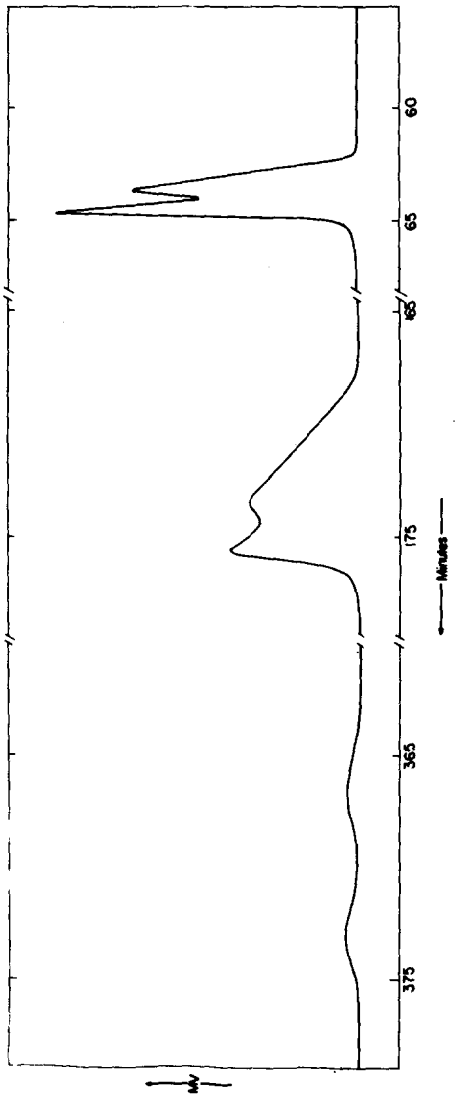

FIG. 1.

Chromatogram of enantiomers of N-TFA-alanine of isopropanol, n-butanol and cyclopentanol; retention times: 63.7, 64.7 min; 173, 175.2 min and 366:8, 373.2 min; respectively.

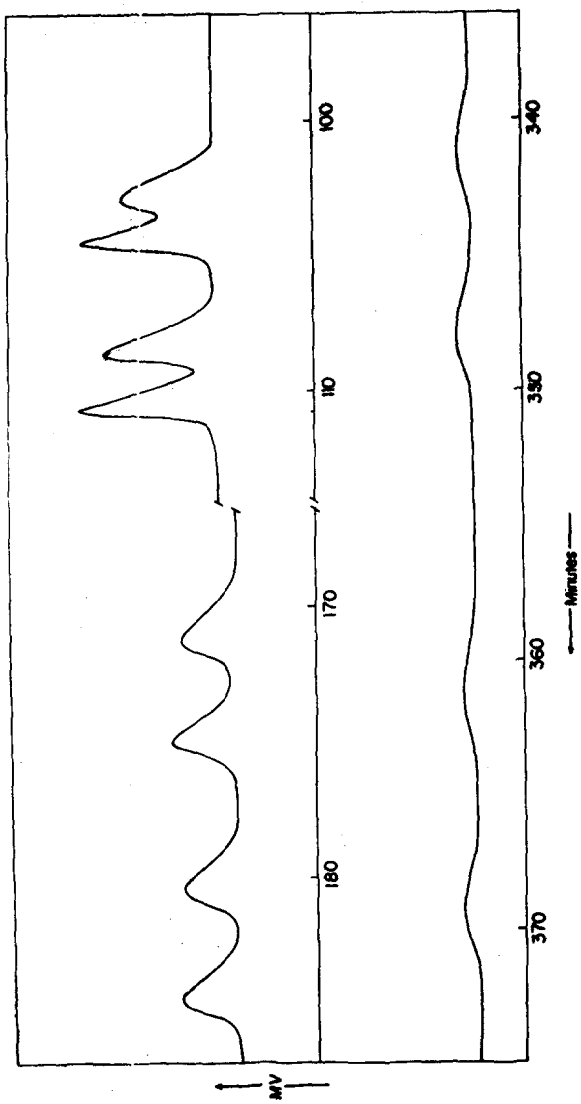

FIG. 2.

Chromatogram of the enantiomers of the 2-butanol esters of N-TFA-alanine,-valine and-leucine; retention times: 102.9, 104.5, 108.6, 110.7 min; 171.5, 175.0, 180.5, 184.7 min and 341.2, 348.0, 360.8, 369.5 min, respectively.

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