Journal of Chromatography, 169 (1979) 153–159

CHROM. 11,348

ENANTIOMERIC PHASES IN ANALYTICAL GAS CHROMATOGRAPHY

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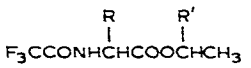
(First received April 25th, 1978; revised manuscript received July 25th, 1978)

SUMMARY

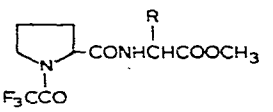
An improved three-step synthesis has been developed for the preparation of N-docosanoyl-D-valine *tert.*-butylamide. This gas chromatographic phase, and the corresponding previously described enantiomeric L-valine phase, which bring about a reversal in the elution patterns of antipodes, were found to perform with comparable accuracy and precision when used for the gas chromatographic analysis of D- and L-leucine mixtures. This reversal of the elution pattern of enantiomeric eluates offers a number of hitherto unattainable advantages in both qualitative and quantitative gas chromatographic analyses of enantiomer mixtures.

INTRODUCTION

The gas chromatographic (GC) resolution of amino acid enantiomers was first accomplished after their conversion to N-trifluoroacetyl (N-TFA) 2-butyl[1] (1a) or 2-octyl[2] (1b) ester diastereomers or to N-TFA-L-prolylamino acid methyl ester diastereomers[3] (2), and specific applications of these techniques have recently been reviewed[4,5]. Gil-Av *et al.*[6] subsequently introduced GC resolutions involving amino acid enantiomers by using optically active stationary phases such as N-TFA-L-isoleucine lauryl ester[6] (3), N-TFA-L-valyl-L-valine cyclohexyl ester[7] (4) and others[8]. More recently N-lauroyl-L-valine *tert.*-butylamide[9] (5a) and N-docosanoyl-L-valine *tert.*-butylamide[10] (5b) have been recommended as superior stationary phases for the



1a. $R' = CH_3CH_2$
1b. $R' = CH_3(CH_2)_5$



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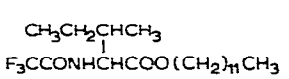

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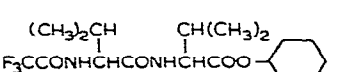

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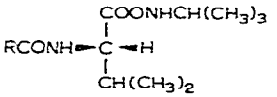
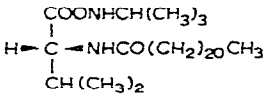
GC resolution of enantiomeric N-TFA-amino acid esters. Finally, in 1977 Frank *et al.*[11] introduced the use of a chiral diamide phase of high thermal stability, consisting of a polysiloxane backbone coupled to N-propionyl-L-valine *tert.*-butylamide. This phase effectively separated and resolved the enantiomer pairs in a 17-component synthetic mixture of N-pentafluoropropionyl-D,L-amino amino acid isopropyl esters.



5a, R = $CH_3(CH_2)_{10}$
5b, R = $CH_3(CH_2)_{20}$



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Since our recent research interests necessitated routine GC analyses for D/L ratios of a number of amino acids, we have undertaken[5] to evaluate the efficacy of GC resolutions involving the diastereomeric derivatives 1a and 2 using conventional GC phases, as well as the resolution of N-TFA-amino acid isopropyl ester enantiomers with the optically active phase 5a. Using capillary columns and an electronic digital integrator, each method showed comparable accuracy (0.03–0.7% absolute error) and precision (0.03–0.6% standard deviation) in the analyses of a series of D- and L-leucine mixtures of known enantiomeric composition[5]. The optically active phase 5a, however, proved in practice to be more convenient to use, and accordingly we have employed the optically active L-valine phases 5a and 5b in a number of subsequent studies investigating potential mechanisms for the abiotic origin of optical activity[12–17]. In certain of the latter investigations, however, the products analyzed were such that the optically active phases 5a or 5b afforded chromatograms which were somewhat ambiguous as to the identity of the GC peaks displayed or were not of a sort which permitted optimum accuracy in peak area integration. It seemed to us that such difficulties might be obviated if the enantiomeric GC phases were available for comparative studies. We have therefore undertaken to prepare, test and employ a capillary column coated with N-docosanoyl-D-valine *tert.*-butylamide (6), the enantiomer of the previously employed L-valine phase 5b. The present paper describes an improved synthesis for 6, its experimental evaluation and comparison with 5b as a GC phase, and a summary of the advantages which we feel are offered by the use of enantiomeric phases for optical resolutions by GC.

EXPERIMENTAL

D-*Valine-N-carboxy anhydride (7)*

D-Valine (4.9 g; 41.8 mmoles; Aldrich, Milwaukee, Wisc., U.S.A.) was suspended in anhydrous tetrahydrofuran (75 ml; distilled from over sodium benzophenone ketyl[18]) to which 33 ml of a 3.77 *M* solution (124.4 mmoles) of phosgene in benzene had been added. The mixture was stirred at 57–64° for 4 h, then was diluted with anhydrous petroleum ether (475 ml; b.p. 35–60°; distilled from over calcium hydride), seeded with crude 7 from a previous preparation, and stored at −22°. Several crops of product totalling 5.3 g (88.5%) were recovered, m.p. 67–68°. M.p. 65° has been reported for L-valine-N-carboxy anhydride[19]. The above is an adap-

tation of the procedure of Fuller *et al.*[20]. A portion of the product was converted to N-TFA-D-valine isopropyl ester by reaction first with 2-propanol saturated with hydrogen chloride, then with trifluoroacetic anhydride[5]. GC analysis[5] on a capillary column coated with 5b showed the product 7 to be enantiomerically pure.

D-Valine tert.-butylamide (8)

A solution of the above D-valine-N-carboxy anhydride (0.643 g; 4.49 mmoles) in dichloromethane (218 ml; dried over Linde 4A molecular sieve) was added dropwise (one drop/sec) with stirring to a solution of *tert.*-butylamine (23.4 g; 320 mmoles; Aldrich) in dry dichloromethane (55 ml). The mixture was stirred for an additional 20 min, then rotary evaporated under vacuum to dryness and stored overnight at −22°. The residue was then extracted with petroleum ether (b.p. 35–60°) and the extract was rotary evaporated under vacuum to yield 0.353 g (45.7%) of crude solid D-valine-*tert.*-butylamide (8). This was purified by vacuum sublimation, m.p. 48°.

Analysis: calculated for $C_9H_{20}ON_2$: C, 62.74; H, 11.70; N, 16.26%; found: C, 62.46; H, 11.60; N, 16.14%.

A sample of the above 8 was hydrolyzed by heating at 130° (sealed tube) with 6 *N* HCl for 22 h. The recovered D-valine was converted to its N-TFA isopropyl ester[5] and analyzed by GC[5]. It proved to be enantiomerically homogeneous.

N-Docosanoyl-D-valine tert.-butylamide (6)

The above amide (8) (0.353 g; 2.05 mmoles) was dissolved in tetrahydrofuran (20 ml; dried over Linde 4A molecular sieve) and the solution was treated with docosanoic acid (0.614 g; 1.80 mmole; Sigma, St. Louis, Mo., U.S.A.) and N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (0.533 g; 2.15 mmoles; "EEDQ gold label", Aldrich). The solution was sealed in a culture tube and heated at 61° for 48 h. An additional 0.051 g of EEDQ was then added and the mixture was heated at 67° for an additional 48 h, then rotary evaporated under vacuum at 66°. The resulting solid was dissolved in petroleum ether (b.p. 35–60°) and allowed to crystallize, after which it was recrystallized twice from absolute ethanol. The final product 6 weighed 0.501 g (49.4%) and had m.p. 86–87°. Charles *et al.*[10] report m.p. 85–86° for the enantiomeric derivative 5b. The above procedure is adapted from the peptide synthesis method of Belleau and Malek[21]. The overall yield from D-valine was 20.0%.

Analysis: calculated for $C_{31}H_{62}O_2N_2$: C, 75.24; H, 12.63; N, 5.66%; found: C, 75.28; H, 12.45; N, 5.44%.

A sample of 6 was hydrolyzed by heating at 130° with 6 *N* HCl for 22 h, after which the D-valine was converted to its N-TFA isopropyl ester and analyzed by GC[5]. It proved to be 95.5% D- and 4.5% L-valine.

Gas chromatography

The enantiomeric N-docosanoylvaline *tert.*-butylamide phases 5b and 6 were coated onto 150 ft. × 0.02 in. I.D. stainless-steel capillary columns which were in turn installed in a Hewlett-Packard Model 5700A gas chromatograph coupled to a Hewlett-Packard Model 3380A digital electronic integrator-recorder. The samples described below were analyzed using the following typical operating conditions: 110° isothermal; injection port 200°; detector 250°; sample splitter ratio 11:1; helium flow-rate, 5–9 ml/min; sample size, 1 μl; sample concentration, 10^{-1}–10^{-2} *M* in dichloro-

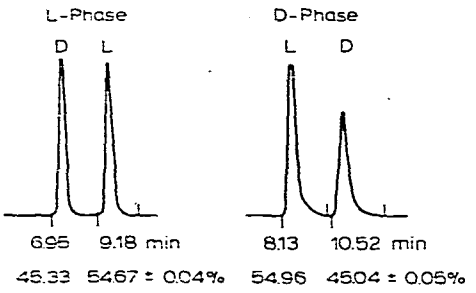
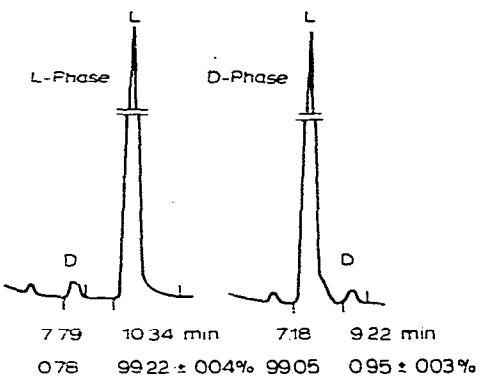


Fig. 1. Analyses of a known mixture of D- (45.38%) and L-leucine (54.62%).



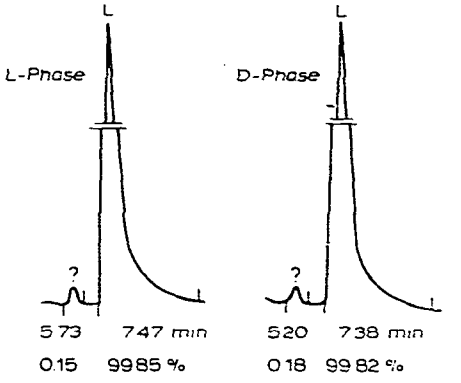


Fig. 2. Analyses of the hydrolysis product from L-leucine decapeptide.

Fig. 3. Analyses to check the optical purity of L-leucine.

methane. All samples were run in replicate (5 sample injections). Their averages, standard deviations, and typical peak eflux times are shown in Figs. 1–3.

Samples analyzed

To evaluate the above columns, D- and L-leucine mixtures having the composition or origin indicated below were converted to their N-TFA isopropyl ester derivatives as previously described[5], then were dissolved in dichloromethane for injection onto the columns. The sample analyzed in Fig. 1 was a synthetic mixture made up of 45.38 wt.% D-leucine (Sigma) and 54.62 wt.% L-leucine (Calbiochem, Los Angeles, Calif., U.S.A.). The sample in Fig. 2 was obtained by hydrolyzing L-leucine decapeptide (Vega Fox Biochemicals, Tucson, Ariz., U.S.A.) with 2-propanol–6 *N* aqueous HCl (3:1) in a sealed tube at 130° for 22 h, recovering the leucine monomer by vacuum evaporation and derivatizing as usual. The sample in Fig. 3 was derived from the above L-leucine, whose optical homogeneity we desired to establish by GC. The drawings in Figs. 1, 2 and 3 are traced from one of the actual chromatograms in each group of analyses.

RESULTS AND DISCUSSION

Fig. 1 shows that the enantiomeric GC phases 5b and 6 are each capable of resolving mixtures of N-TFA isopropyl esters of D- and L-leucine with essentially the

accuracy and precision previously noted[5] and that furthermore, as expected, the order of elution of the D- and L-leucine esters is reversed when the chirality of the phase is reversed. The sequential use of enantiomeric phases in GC analysis, originally suggested by Gil-Av[22] in 1973, and the ability of such phases to invert the elution pattern of enantiomeric eluates leads to a number of hitherto unattainable practical advantages enumerated below.

Peak identification

The identity of two peaks (of unequal size) in a chromatogram as belonging to enantiomers as opposed to optically inactive components can be established by reversing the chirality of the GC phase, since the positions of the enantiomer peaks will reverse while the relative positions of the other peaks will remain unaltered. This is illustrated in Fig. 2, where column reversal identifies the second and third peaks as belonging to the leucine enantiomers and the first peak as due to an optically inactive impurity. Similarly, in a more complex mixture the relative positions of all pairs of enantiomer peaks in the chromatogram should reverse on GC phase chirality reversal, while those of all optically inactive components should remain unaltered, again permitting distinction between the two classes of peaks.

A related advantage is the ability of phase chirality reversal to distinguish an optically active component in a mixture from an optically inactive one. Here phase reversal changes the relative positions of the peaks, but does not reverse them. This is illustrated in Fig. 3, where we undertook to establish the optical purity of our L-leucine. Using the L-phase 5b, we at first suspected the small initial peak to represent a D-leucine impurity, as in the hydrolysis product in Fig. 2. Re-examination using the D-phase 6, however, failed to reverse the positions of the peaks, thus establishing that the L-leucine was optically pure but that the derivative analyzed contained a small amount of an optically inactive contaminant.

Improving analytical precision

In the quantitative analysis of enantiomers using an optically active GC phase, the less prevalent enantiomer (*e.g.* L) may show up as a shoulder on the tail of the more prevalent enantiomer (D) if the latter peak precedes the former and if baseline resolution is not achieved (Fig. 4A). This situation invariably leads to an inaccurate analysis for the minor enantiomer, since the tail of the initial large peak is included in the integrated area of the second small peak. This difficulty is remedied by reversing the chirality of the GC phase, which inverts the elution pattern of the enantiomers and

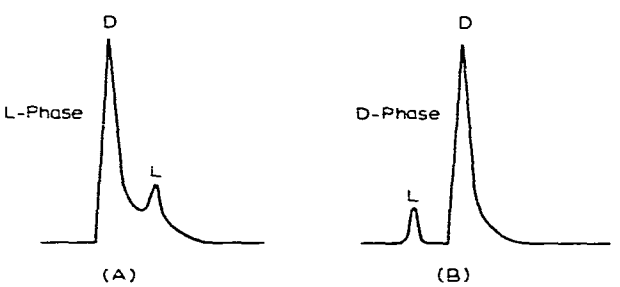


Fig. 4. Improvement in the resolution of enantiomers poorly resolved on one optically active phase.

permits accurate integration of the peak of the (now first eluted) minor L-enantiomer (Fig. 4B). Again we might emphasize that if the second peak in the hypothetical L-phase chromatogram of Fig. 4A is not due to the L-enantiomer but rather to an optically inactive impurity, peak reversal will not be achieved by using the D-phase.

Another pitfall in the quantitative analysis of enantiomers which may be recognized when GC phase chirality is reversed arises when one enantiomer peak is spuriously inflated due to co-elution with an optically inactive impurity. Column phase reversal will now lead to the artificial enhancement of the peak of the other enantiomer, giving a different D/L ratio and indicating that an optically inactive impurity is co-eluting. From the magnitude of the discrepancies in the D/L ratios the amount of the optically inactive co-eluting contaminant can be calculated and corrected for.

Elimination of systematic errors

The use of enantiomeric GC phases permits a useful "symmetry check" which can help to minimize several types of systematic errors which may be inherent in certain GC analyses of enantiomers. Consider the case of two enantiomers which are adequately resolved, but appear on the tail of a predominant solvent peak (Fig. 5A). In this hypothetical example the peak of the D-enantiomer could be erroneously enhanced over that of the L-enantiomer by inclusion of more of the solvent peak area in its integral. Phase chirality reversal should precisely reverse this situation (Fig. 5B) permitting one to average out this source of error.

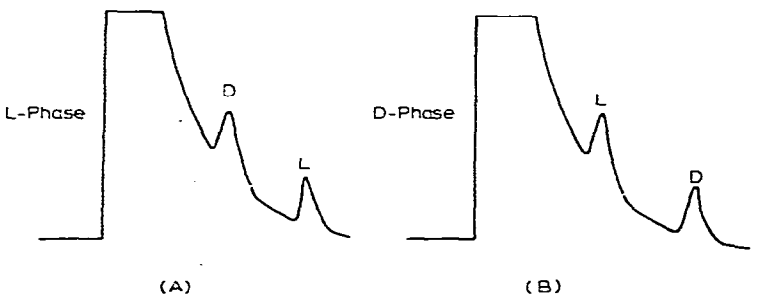


Fig. 5. Partial elimination of systematic errors due to solvent tailing.

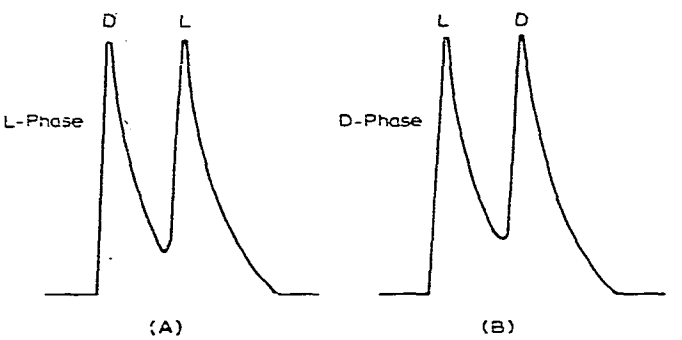


Fig. 6. Partial elimination of systematic errors due to incomplete resolution.

Finally, consider the case of two enantiomers which are not resolved to the baseline by a given chiral GC phase. In Fig. 6A the hypothetical L-phase allows the D-enantiomer to tail into the L-enantiomer, causing a spuriously high value for the integral of the second peak. This shortcoming may be compensated by reversing the column phase chirality, thus reversing the elution pattern (Fig. 6B) and permitting recognition and correction of the original error.

ACKNOWLEDGEMENTS

We are indebted to Dr. E. Gil-Av of the Weizmann Institute of Science, Rehovot, Israel for supplying us with the N-docosanoyl-L-valine *tert.*-butylamide phase used in the above studies, and to the National Aeronautics and Space Administration for a research grant (NGR-05-020-582) which supported this investigation.

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