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Note

The quantitative gas chromatographic resolution of amino ester diastereomers

MARK A. VAN DORT and WILLIAM A. BONNER*

Department of Chemistry, Stanford University, Stanford, Calif. 94305 (U.S.A.)

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The resolution of amino acid enantiomers by gas chromatography (GC) has been accomplished during the past decade by one or the other of two general techniques: (1) conversion of the amino acid enantiomers into a mixture of volatile diastereomers[1-4] [*e.g.*, N-trifluoroacetyl- (*i.e.*, N-TFA-) amino acid (+)-2-butyl esters[1]], followed by separation on capillary or packed columns loaded with conventional GC phases, and (2) conversion of the enantiomers to volatile derivatives (*e.g.*, N-TFA-amino acid isopropyl esters) which could then be resolved directly using columns loaded with optically active GC phases[5-7]. We have recently investigated[8] the application of these techniques to the quantitative analysis of mixtures of *R*- and *S*-leucine enantiomers, and found the several procedures to be of comparable precision and accuracy. Our continuing need in other studies[9-11] to analyze mixtures of amino acid enantiomers for their precise enantiomeric composition has led us to seek improvement in these techniques by a combination of the two general GC methods above, namely, the use of an optically active GC phase for the separation of N-TFA-amino acid diastereomers.

EXPERIMENTAL

RS-Leucine (±)-2-butyl ester diastereomer mixture

A mixture (0.0763 g) containing 25.9 % *R*- and 74.1 % *S*-leucine was treated with a mixture [1.33 ml; $[\alpha]_D^{24} + 2.41°$ (neat)] containing 60.8 % (+)- and 39.2 % (−)-2-butanol, which had been saturated with gaseous HCl at 0° (moles butanol/moles leucine ≈25:1). The solution was heated under reflux for 3 h (CaCl₂ tube), then was evaporated under vacuum at 50–55°. The residue was "chased" twice by vacuum evaporation of 5 ml of added dichloromethane (DCM), then was treated with 3 ml trifluoroacetic anhydride in 2 ml DCM. The solution was heated under reflux for 20 min, vacuum evaporated, and the residue was "chased" twice with 5 ml DCM as before. The dried oily residue (0.1262 g; 76.5 %) was dissolved in 4.8 ml nitromethane to give a *ca.* 0.2 *M* stock solution of a mixture of the diasteremeric esters having the "known composition" indicated in Table I.

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* To whom correspondence should be addressed.

Gas chromatographic analyses

All GC analyses were performed using a Hewlett-Packard Model 5700A gas chromatograph coupled to a Hewlett-Packard Model 3380A digital electronic integrator–recorder. Column A consisted of a 150 ft. × 0.02 in. stainless-steel capillary column coated with N-lauroyl-*S*-valyl-*tert.*-butylamide phase[7]. Column B consisted of a similar 150 ft. × 0.02 in. column coated with Ucon H 90,000 phase. Operating conditions for the GC traces in Fig. 1 were: 1A, 120° isothermal, nitrogen flow-rate *ca.* 1.6 ml/min; 1B, 125° isothermal, nitrogen flow-rate *ca.* 1 ml/min; 1C, 119° isothermal, nitrogen flow-rate *ca.* 1 ml/min. The eflux times (min) at each peak maximum are shown adjacent to each corresponding peak in Fig. 1.

RESULTS

When a mixture of the four diastereomeric N-TFA-esters prepared from a mixture of *R*- and *S*-leucine and (+)- and (−)-2-butanol was subjected to GC separation on a capillary column loaded with N-lauroyl-*S*-valyl-*tert.*-butylamide phase[7] (column A), it was immediately apparent that the optically active phase was capable of giving partial separation of all four diastereomers (Fig. 1A). This contrasts to the behavior of a typical optically inactive phase (*e.g.*, Ucon H90,000; column B) which resolves only the two enantiomeric pairs of diastereomers, *R*(+) and *S*(−), and
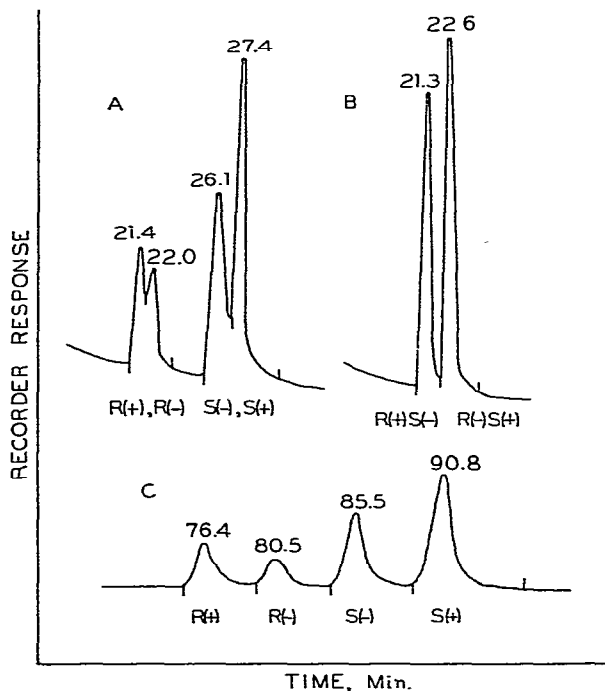

Fig. 1. Gas chromatograms of *RS*-leucine (±)-2-butyl ester diastereomer mixture with various columns. A, N-Lauroyl-*S*-valyl-*tert.*-butylamide phase; B, Ucon H 90,000 phase; C, columns A and B *in tandem.* Operational conditions: see Experimental.

TABLE I

GAS CHROMATOGRAPHIC ANALYSES OF *RS*-LEUCINE (±)-2-BUTYL ESTER DIASTEREOMERS

Column	Percent of diastereomer in mixture						Figure
	R(+)	*R(−)*	*S(−)*	*S(+)*	*R(+)S(−)*	*R(−)S(+)*	
Known composition	15.7	10.2	29.0	45.1	44.7	55.3	
A	12.8	11.9	26.6	48.7	39.4	60.6	1A
B	—	—	—	—	44.9	55.1	1B
A + B ± st. dev.	15.6* ± 0.3	9.3* ± 0.3	29.5* ± 0.4	45.5* ± 0.4	45.1 ± 0.5	54.8 ± 0.5	1C
B (error)**	—	—	—	—	−0.2	0.2	
A + B (error)**	0.1	0.9	−0.5	−0.4	−0.4	0.5	

* Average of closest 5 out of 6 replicate analyses.
** Percent known − percent observed.

R(−) and *S*(+) (Fig. 1B, where *R* and *S* refer to the leucine enantiomer moieties and (+) and (−) to the 2-butanol enantiomer moieties of each diastereomeric ester). In order to determine which of the four maxima in a partial separation, such as that in Fig. 1A, corresponded to which of the four diastereomeric N-TFA-esters, a known mixture of the four esters having the composition shown in Table I was prepared and subjected to quantitative analysis on the same optically active GC column A. Even though the electronic integration of the partially separated peaks in Fig. 1A gave analytical results (Table I) agreeing only to within *ca.* 1.7–3.6 % of the known percentage of each diastereomer in the mixture, the composition of the mixture was such that the analysis left no doubt that the sequence of elution of the diastereomers with increasing time was in the order *R*(+), *R*(−), *S*(−) and *S*(+).

Fig. 1A shows that the optically active phase in column A completely resolved the two *R*-leucine diastereomeric esters from the two *S*-leucine esters. However, it only partially resolved each set of *R*- and *S*-pairs. The optically inactive phase in column B, on the other hand, permitted baseline separation of the *R*(+) and *R*(−) diastereomeric esters, and likewise the *S*(−) and *S*(+) esters (Fig. 1B). Thus it seemed likely that a complete resolution (and accurate quantitative analysis) of all four diastereomers might be possible by utilizing the optically active column A and the optically inactive column B *in tandem*. This combination led to the GC trace in Fig. 1C and the final analytical figures in Table I. Here we see that true baseline separation of all four diastereomers has been achieved and, despite the inordinately long elution times and broad peak widths involved, the analytical figures obtained are in quite satisfactory agreement with the known composition of the mixture. Moreover, the sums in Table I of the *R*(+) and *S*(−) peaks (45.1 %) and the *R*(−) and *S*(+) peaks (54.8 %) in the two-column (A + B) analysis are also in good agreement with the analytical values observed for the *R*(+)*S*(−) (44.9 %) and the *R*(−)*S*(+) (55.1 %) diastereomer pair peaks observed in Fig. 1B using the optically inactive column B.

In summary, Fig. 1B shows that the optically inactive column B separates the diastereomers on the basis of either the amino acid component [*e.g.*, *R*(+) *versus* *S*(+)] or the alcohol component of the diastereomers [*e.g.*, *R*(+) *versus* *R*(−)], while Fig. 1A shows that the optically active column A separates the diastereomers on the basis of both components. In their pioneering study using optically active GC phases Gil-Av *et al.*[5] have previously separated (±)-2-butyl esters of N-TFA-*RS*-

alanine, -valine and -leucine into four diastereomeric components, while more recently Koenig[12] has reported complete resolution of the four N-PFP-*RS*-leucine ($\pm$)-3-methyl-2-butyl ester diastereomers on an optically active N-TFA-dipeptide ester GC phase.

Finally it should be emphasized that the composition of the known diastereomer mixture in Table I was calculated from the known enantiomeric composition of the reagents used in its preparation, assuming that the extent of any preferential esterification would be negligible. The correctness of this assumption, in the present case at least, is attested by the excellent agreement between the calculated analyses in Table I for the $R(+)S(-)$ and $R(-)S(+)$ diastereomer pairs and those found by analysis with column B.

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