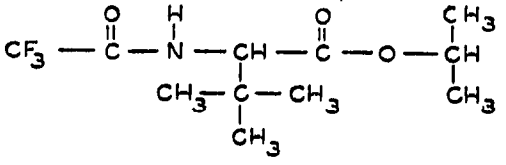
CHROM. 5821

The complete resolution of enantiomeric tertiary-leucine derivatives by gas–liquid chromatography

Optically-active-dipeptide stationary phases have been shown to separate enantiomeric pairs of amino acids[1–8]. Phases in current use are N-trifluoro-acetyl (TFA)-L-valyl-L-valine cyclohexyl ester[1–3], N-TFA-L-phenylalanyl-L-leucine cyclohexyl ester[4–7] and N-TFA-L-valyl-L-leucine cyclohexyl ester[8].

The nature of the interaction between phase and amino acid involves steric considerations as well as the formation of a hydrogen-bonded diastereoisomeric complex[6]. Particular difficulties arise in the separation of D,L-*tert.*-leucine derivatives (I) which have never been resolved by gas chromatography (GC).



N-TFA-D,L-*tert.*-leucine isopropyl ester (I)

Previous attempts at separating D,L-*tert.*-leucine enantiomers on optically active phases have encountered difficulties due to the bulky nature of its side-chain precluding proper steric orientation with the dipeptide derivative; however, the other leucine isomers, norleucine*, isoleucine and leucine itself are very well separated[9]. The influence of the alkyl group attached to the asymmetric carbon of the solute seems to be fairly complex and very dependent on the positions of branching. The substituent is primary in norleucine, secondary in isoleucine, and tertiary in *tert.*-leucine. Leucine has an isobutyl group attached to the α-carbon.

Since stationary phases with bulky groups attached to the α-carbon of the chiral solvent did not resolve D,L-*tert.*-leucine derivatives (*e.g.* N-TFA-L-valyl-L-valine cyclohexyl ester, N-TFA-L-phenylalanyl-L-leucine cyclohexyl ester), phases with less bulky groups should be able to achieve the desired separation.

We wish to discuss the behavior of two new optically active dipeptide phases in the resolution of N-TFA-D,L-*tert.*-leucine isopropyl ester by capillary-column GC and possible applications to the preparative scale separation of D- and L-*tert.*-leucine. N-TFA-L-alanyl-L-alanine cyclohexyl ester (Ala–Ala), a phase with the smallest side-chain possible attached to the asymmetric carbon, and N-TFA-L-norvalyl-L-norvaline cyclohexyl ester (Norval–Norval)**, with *n*-propyl groups, have been synthesized and used as stationary phases in the GC separation of enantiomeric amino acid derivatives.

Material and methods

Both dipeptide derivatives were obtained in good yield by coupling the N-*tert.*-butyloxycarbonyl(BOC)amino acid hydroxybenzotriazolate[10] esters with the cyclo-

* Norleucine = D,L-α-aminocaproic acid.

** Norvaline = L-α-aminovaleric acid.

hexyl esters of the appropriate amino acids in the presence of dicyclohexyl carbodiimide; removing the BOC group from the resulting dipeptide and adding the TFA group to the free amino terminus[10].

The GC experiments were carried out using a Varian Aerograph 1200-1 instrument with attachments for a capillary column and flame ionization detector. The columns employed in this investigation were 400 ft. × 0.02 in. I.D. stainless steel and were coated with 10 % solutions of the dipeptide phases in ether. Both phases were investigated at temperatures of 100 and 110° (See also Table I and Figs. 1 and 2)

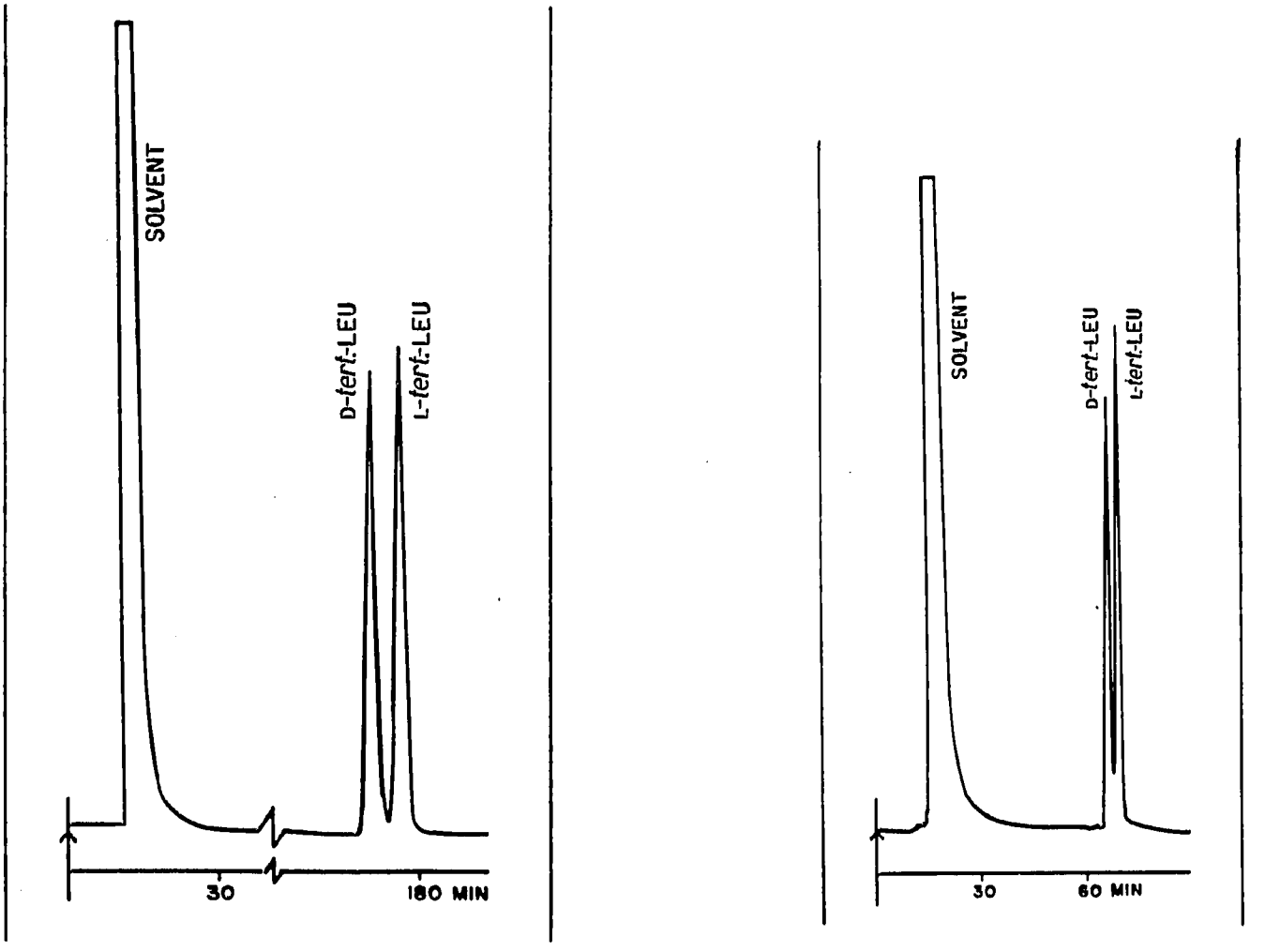


Fig. 1. Chromatogram of N-TFA-amino acid isopropyl ester with N-TFA-L-norvalyl-L-norvaline cyclohexyl ester (Norval–Norval) as the stationary phase. Chromatographic conditions: 400 ft × 0.02 in. capillary column; 100° isothermal; injector temperature 180°; detector temperature 280°; carrier gas He at 12 p.s.i.

Fig. 2. Chromatogram of N-TFA-amino acid isopropyl ester with N-TFA-L-alanyl-L-alanine (Ala–Ala) cyclohexyl ester as the stationary phase. Chromatographic conditions: 400 ft. × 0.02 in capillary column; 110° isothermal; injector temperature 180°, detector temperature 280°; carrier gas He at 12 p.s.i.

Results and discussion

The resolution capacity of the synthesized phases was of singular interest when a heretofore unresolved N-TFA-D,L-*tert.*-leucine isopropyl ester was examined. The Norval–Norval phase showed practically complete resolution of the D,L-*tert.*-leucine at 100°, whereas the Ala–Ala phase did not exhibit as high a degree of separation (Figs. 1 and 2).

From the separation factors ($t_R(L)/t_R(D)$), the quality of the separation is not evident, but the differences in retention times for the enantiomers ($t_R(L)—t_R(D)$) clearly demonstrate the preferability of Norval–Norval for this separation (Table I). Furthermore, the resolution factors $2(t_R(L)—t_R(D))/(w_D + w_L)$* show a definite improvement from Ala–Ala to Norval–Norval.

TABLE I

SEPARATION AND RESOLUTION FACTORS FOR N-TFA-D,L-*tert.*-LEUCINE ISOPROPYL ESTER ON N-TFA-DIPEPTIDE ESTER PHASES

Chromatographic conditions: 400 ft. × 0.02 in. capillary columns; temperature of colums 100° and 110° respectively; injector temperature 180°, detector temperature 280°; carrier gas He at 12 p.s.i. kept constant during the experiments.

Phase	*Temperature* (°C)	*tert.-Leucine*, D/L	t_R[a]	$t_R(L)/t_R(D)$[b]	$t_R(L) - t_R(D)$	$\frac{2(t_R(L) - t_R(D))}{w_D + w_L}$[c]
Ala–Ala	100	D L	17.0	1.000[d]	—	—
	110	D L	49.9 52.4	1.050	2.5	1.21
Norval–Norval	100	D L	155.5 164.4	1.044	6.9	1.60
	110	D L	100.9 104.9	1.040	4.0	1.29

[a] Retention time in minutes relative to chloroform.
[b] Separation factor.
[c] Resolution factor.
[d] The Ala–Ala phase could not be operated at 100° because the phase is not liquid at this temperature.

The resolution factor of 1.60 (see Table I) for Norval–Norval at 100° should allow separation of D,L-*tert.*-leucine on a preparative scale by using the phase as a coating for packed columns. A preparative scale separation of the *tert.*-leucine isomers is desirable since the regular techniques—conversion into diastereoisomers and their separation and on the other hand the enzymatic separation[11]—encounter some difficulties. The application of the Norval–Norval phase for packed columns is presently being investigated.

* $w_{L,D}$ = width of triangulated peak from L and D enantiomers at base line.

This work has been supported by the National Science Foundation (Grant GP 26019).

Department of Chemistry,
University of Houston,
Houston, Texas 77004 (U.S.A.)

W. PARR
P. HOWARD

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Received October 25th, 1971