combined volume of the column, the sample injector and the detector was determined before packing the column by measuring the volume corresponding to the peak maximum of an organic solute in the dry nitrogen carrier gas and with no solvent or solid support in the column. The volume of the solvent was determined by an internal standard. The volume of the solid support was calculated from its density as determined by a specific gravity bottle. The results from the two methods differ by 2 %. The difference was mainly due to uncertainty in the effective volume of the solid support.

It is concluded that with hydrogen flame ionization detector it is now possible to determine directly, by air peak, the gas hold-up of GLC apparatus by presaturating the carrier gas with an organic solvent prior to the injection of the air sample. In the case of non-volatile solvents, the carrier gas may be saturated with any other suitable organic solvent of low volatility at the column temperature.

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Resolution of amino acids by gas chromatography*

The resolution of racemic mixtures of secondary *n*-alkanols by gas chromatography of the corresponding diastereoisomeric α-hydroxypropionates has been reported earlier[1,2]. The present communication describes a similar approach to the separation of α-amino acids.

The amino acids examined were chromatographed in the form of N-trifluoroacetyl (N-TFA) esters[3] of 2-*n*-alkanols, the latter serving to introduce an additional asymmetric center. The derivatives were prepared in nearly quantitative yields by esterification in the presence of HCl, and treating the resulting amino ester hydrochlorides in methylene chloride solution with excess trifluoroacetic anhydride at —20° with stirring. The reaction mixture was allowed to warm up to room temperature and left for 1 h.

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Gas chromatography was carried out on capillary columns of 150 ft. length and 0.01 in. I.D., provided with a hydrogen flame ionization detector. The diastereoisomers derived from alanine, valine, leucine, isoleucine, proline, phenylalanine and glutamic acid could be separated under the experimental conditions given in Table I.

TABLE I

GAS CHROMATOGRAPHIC SEPARATION OF THE DIASTEREOISOMERS OF N-TFA-AMINO ACID ESTERS

*N-TFA-Amino acid ester**		*Column***	*Temperature*§	*Relative retention time diethyl sebacate = 1*		$r_{LL/LD}$§§
Amino acid	*Alcohol*			*LL or DD*	*LD or DL*	
(±) Ala	(±) 2-Octanol	A	140	0.314	0.343	1.09
		A	160	0.421	0.440	1.05
		B	150	0.210	0.227	1.08
	± 2-Butanol	A	140	0.109	0.114	1.04
(±) Val	(±) 2-Octanol	A	140	0.433	0.466	1.08
		A	160	0.525	0.555	1.06
		B	150	0.251	0.263	1.05
	± 2-Butanol	A	140	0.143	0.147	1.03
(±) Leu	(±) 2-Octanol	A	140	0.576	0.615	1.07
		A	160	0.646	0.683	1.06
		B	150	0.385	0.401	1.04
(±) Pro	(±) 2-Octanol	A	140	1.351	1.490	1.10
		A	160	1.335	1.460	1.09
		B	150	0.890	0.955	1.08
(±) Phe	(±) 2-Octanol	A	140	3.10	3.26	1.05
		A	160	2.56	2.67	1.04
(±) Glu	± 2-Butanol	A	140	1.44	1.49	1.04

* Allocation of the peaks of the isoleucine derivatives (see text) has not yet been made; they overlap those of the N-TFA-leucine 2-octyl esters.

** A = capillary column (Perkin Elmer) FS-1265 coated with trifluoropropylmethyl polysiloxane; flow rate 1.5 ml N_2/min. B = capillary column (Perkin Elmer) coated with polypropylene glycol; flow rate 2.6 ml N_2/min.

§ The retention time of diethyl sebacate (in min) is: for 140° 12.1, for 150° 23.2, and for 160° 4.55.

§§ Ratio of the retention times of the diastereoisomers.

The degree of resolution obtained for some of the above compounds can be seen in Fig. 1. Isoleucine, which contains two asymmetric carbon atoms, can be resolved in the form of the N-TFA ester of 1-octanol, whereas the corresponding 2-*n*-octyl derivative gives four peaks as expected. Trifluoropropyl-methyl polysiloxane is preferred to polypropyleneglycol as stationary phase, because of the shorter analysis time and generally higher ratio between the retention times of the diastereoisomers. A temperature of 140° was found suitable for the separation of all derivatives studied, except for N-TFA-phenylalanine 2-octyl ester, which was better resolved at 180°.

In order to allocate the peaks, derivatives were prepared from optically active reagents. As in the case of α-alkanoyloxypropionates[1,2] the DL (or LD) compounds had

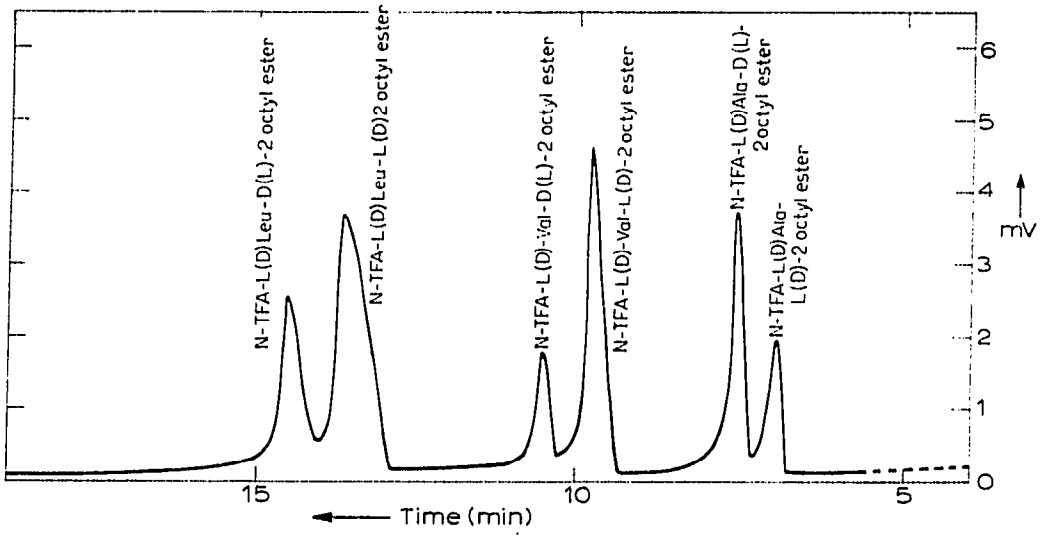

Fig. 1. Chromatogram of diastereoisomers of N-TFA-amino acid 2-octyl esters. Column, see Table I, column A; temperature 130°; flow rate 1.5 ml N_2/min.

in all cases a higher retention volume. Small amounts (1–5 %) of the second diastereoisomer found in the products of these syntheses are considered to result from incomplete steric homogeneity of the reagents.

For application of the method to amino acids, the N-TFA esters of which have relatively low retention times, 2-octanol is a convenient reagent, since its enantiomorphs are commercially available. 2-Butanol is suitable for amino acids, such as glutamic acid, which lead to compounds with high retention times. Modified types of diastereoisomeric derivatives, *e.g.*, amino acid methyl esters having an asymmetric centre in the group attached to the nitrogen atom, might be needed for extension of the resolutions to other natural amino acids.

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