in this paper (Hp and the DMCP's in Fig. 1, cyclopentane–hexane, and methylcyclohexane–1-*cis*-2-DMCP) and in the literature[6–8], this phenomenon occurred with pairs of compounds consisting of either acyclic and cyclic structures or different ring structures.

Consequently, for solutes that elute close together, column temperature may often be a factor in determining order of elution. The order of elution established experimentally at one temperature may not hold true at a different column temperature, particularly for multicomponent mixtures. In addition, in programmed-temperature gas chromatography, the following situations may arise. If the column temperature is programmed during the run, a separation that would have been achieved at the lower temperature may be partly or wholly canceled out by operating at a higher temperature where a reversal in the order of elution occurs. For compounds eluting close together, the order of elution may depend on the choice of initial and final column temperatures or on the rate of heating of the temperature program.

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The influence of esterifying and acetylating groups on the retention times of amino acid derivatives in gas chromatography

A number of publications have appeared recently reporting studies on the analysis of amino acid mixtures by gas phase chromatographic separation of their more volatile derivatives. In particular the esters[1,2], N-trifluoroacetylated esters[3–8], and N-acetylated esters[9,10] have attracted considerable attention. Each of these reports have in

the main been confined to only one or two derivatives. The present communication reports data showing the relative influence of the various introduced groupings on the gas chromatographic behaviour of a number of the more suitable amino acid derivatives as an aid in the design of systems for the analysis of the amino acids.

The data presented here have been confined to two representative amino acids, *viz.* leucine, and phenylalanine. (The relative retention times for the methyl esters of leucine, phenylalanine and arginine are 0.07, 1.0 and 2.0 respectively. The relative retention times for the N-trifluoroacetyl methyl esters of leucine, phenylalanine, arginine and tryptophan are 0.1, 1.0, 9.0 and 27 respectively.) The amino acids were converted to their ester hydrochlorides by treatment with anhydrous HCl gas in the appropriate alcohol, and then recrystallized from this alcohol. The ester hydrochlorides were in turn converted either into the N-trifluoroacetyl derivative by treatment with trifluoroacetic anhydride[11] or into the acetyl derivative by treatment with acetic anhydride[10].

TABLE I

RETENTION TIMES OF AMINO ACID ESTERS

6 ft. 3/16 in. I.D. glass column packed with 2 % neopentyl glycol succinate on Fluoropak 80. Sample size 0.2 μmole.

Ester	*Leucine 135° (min)*	*Phenylalanine 190° (min)*
Methyl	2.2	4.0
Ethyl	2.7	4.6
Propyl	3.8	5.8
Butyl	6.4	8.0
Amyl	10.0	11.1

Two types of column packing were employed, 2 % neopentyl glycol succinate on Fluoropak 80 (The Fluorocarbon Co.) which was found most suitable for the amino acid esters and 2 % neopentyl glycol succinate on Chromosorb W (acid washed, 80–100 mesh) which was used for the acetylated esters. Nitrogen was the carrier gas flowing at 60 c.c./min and analysis of the effluent gas was by means of a hydrogen flame detector. The injection port and detector were at the same temperature as the column.

TABLE II

RETENTION TIMES OF N-ACETYLATED ESTERS OF LEUCINE

2 ft. 3/16 in. I.D. stainless steel column packed with 2 % neopentyl glycol succinate on Chromosorb W (acid washed, 80–100 mesh). Sample size 0.2 μmole.

Ester	*N-Trifluoroacetylated leucine*		*N-Acetylated leucine*	
	142° (min)	*181° (min)*	*142° (min)*	*181° (min)*
Methyl	1.9	0.5	11.0	1.6
Ethyl	2.1			
Propyl	3.0			
Butyl	4.4	1.0	25.7	3.1
Amyl	6.5			

The influence of ester group on retention times. The retention times obtained for the various esters of leucine and phenylalanine in the form of their free bases[12] are shown in Table I, while Table II shows the corresponding values for the N-acetylated and N-trifluoroacetylated derivatives of leucine esters.

The influence of N-acetylation on retention times. Table III gives the retention times obtained on the same column for the methyl esters and N-acetylated methyl esters of leucine and phenylalanine.

TABLE III

RETENTION TIMES OF THE METHYL ESTERS OF LEUCINE AND PHENYLALANINE AND THEIR N-ACETYLATED DERIVATIVES

6 ft. 3/16 in. I.D. glass column packed with 2% neopentyl glycol succinate on Fluoropak 80. Sample size 0.2 μmole.

	Leucine methyl ester 150° (min)	*Phenylalanine methyl ester 180° (min)*
Free base	1.4	4.0
N-Trifluoroacetylated	4.4	7.2
N-Acetylated	20.5	29.0

All other factors being equal, it is advantageous in gas phase analysis to synthesize derivatives of amino acids with low retention times. The results of our comparative studies of the amino acids have led us to favor the methoxyl radical for esterification of the carboxyl group and the trifluoroacetyl radical for acylation of the amino group.

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