AMINO ACIDS AND PEPTIDES

II. GAS CHROMATOGRAPHY OF AMINO ALCOHOLS*

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INTRODUCTION

In recent years gas chromatography has become an important analytical tool. Although this technique is best suited to liquids, the method has been applied to the solid amino acids. To date all of the previously reported studies have been carried out on derivatives in which the amino and/or carboxyl group is usually masked or removed by a variety of chemical subterfuges. Modified systems have included methyl esters[1-3], butyl ester hydrochlorides[4], N-acetyl esters[5,6], N-trifluoroacetyl esters (N-TFA)[1,7-12], N-formyl esters[13], N-trimethylsilyl trimethylsilyl esters[14], α-hydroxy esters[15,16], α-chloro esters[17], phenylthiohydantoin and dinitrophenyl methyl ester derivatives[18-20], aldehydes[21-23], amines[24] and nitriles[25].

These various schemes have been utilized only to a very limited extent in current biochemical problems. One practical example involved the use of the dinitrophenyl methyl ester method to identify the individual amino acids formed during the hydrolysis of the polypeptide Gramicidin A[26]. In another study a variety of amino acid intermediates, used in the preparation of the hydroxyprolines, were chromatographed in the form of their acetyl and trifluoroacetyl methyl esters[27]. Recently, the N-trifluoroacetyl ester method was applied to measure the extent of racemization in peptide synthesis[28].

The N-acetyl-amino ester procedure was utilized to determine some protein and polypeptide hydrolysates some time ago[29]. Synthetic di- and polypeptides were analyzed by means of N-TFA esters[8] and O-trimethylsilyl N-TFA methyl esters[30]. Modified determinations were based on polyamino alcohols or polyamines[31] and α-chloroacids[32].

In the belief that removal of the carboxyl function in the amino acids would lead to a volatile series of compounds, the *amino alcohols* were thought to be suitable candidates for gas chromatographic analysis. These materials were previously prepared by hydride reduction of various amino acid esters and were shown to exist as either liquids or low melting solids[33-37]. To date no systematic examination of this class of compounds has been reported in the literature.

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* For the previous paper in this series, see: W. W. LEE, G. T. TONG, A. P. MARTINEZ, B. WEINSTEIN, M. G. M. SCHELSTRAETE, B. R. BAKER AND L. GOODMAN, *J. Med. Chem.*, 6 (1963) 439.

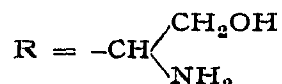
RESULTS AND DISCUSSION

The simplest amino alcohol—2-aminoethanol (*i.e.*, the glycine analog)—was systematically screened against the following stationary phases: apiezon (L), methyl silicone fluid (SF-96), cyano silicone fluid (XF-1150), fluoro silicone (FS-1265 or QF-1), methyl silicone gum (SE-30), methyl phenyl silicone gum (SE-52), cyano silicone gum (XE-60), carbowax (20M), polyglycol (UCON 50 HB-5100 and LB-1715), diethyleneglycol succinate (DEGS), ethyleneglycol succinate (EGS), didecyl phthalate (DDP), bis-2-ethylhexyl tetrachlorophthalate (EHCP), cyclohexyldimethanol succinate (CDMS), phenyldiethanolamine succinate (PDEAS), crosslinked diethyleneglycol adipate (LAC-2-R446), sucrose acetate isobutyrate (SAIB), *m*-polyphenyl ether, hallcomid (M-18), polyamide (Versamid 900), nonylphenoxypolyethylene ethanol (Igepal CO-880), tricresyl phosphate (TCP), 1,2,3-tris-(2-cyanoethoxy)-propane (TCEP) and nonylphenol.

A satisfactory peak was obtained from SF-96, XF-1150, FS-1265, SE-52, XE-60, UCON 5100, UCON 1715, DPP, Igepal and TCP. As a result, these ten substrates were treated with eight additional and different amino alcohols. FS-1265 and Igepal were soon discarded, since they did not discriminate between glycinol and alaninol, while TCP was eliminated because the retention time for glycinol was too long for practical work (11.1 min at 105°). The data for XF-1150, XE-60 and UCON 1715 were in the same category, and do not appear unusually noteworthy at this time.

TABLE I

RETENTION TIMES OF VARIOUS AMINO ALCOHOLS



Amino alcohol	*Column*	*SF-96*		*SE-52*		*UCON*	*DDP*
	Flow rate helium, ml/min	*60*	*60*	*60*	*60*	*60*	*75*
	Oven temp., °C	*170*	*200*	*155*	*200*	*178*	*120*
Glycinol	RH	1.0	A	1.6	A	2.6	3.0
Alaninol	RCH_3	1.2	A	1.9	A	2.9	4.0
Valinol	$RCH(CH_3)_2$	2.9	A	4.9	A	4.8	9.4
Leucinol	$RCH(CH_3)CH_2CH_3$	3.7	A	6.6	3.4	11.6	F
Serinol	RCH_2OH	3.9	2.6	8.2	3.3	C	G
Isoleucinol	$RCH_2CH(CH_3)_2$	5.1	2.8	9.8	4.5	5.7	H
Methioninol	$RCH_2CH_2SCH_3$	16.2	7.4	26.1	12.1	39.3	I
Phenyl-alaninol	$RCH_2C_6H_5$	22.6	9.0	37.1	17.2	D	I
Trypto-phanol	$RCH_2C_8H_6N$	A	B	A	B	E	A

A = Compound not injected upon the column.
B = Nothing detected during a run of 150 min.
C = Nothing detected during a run of 30 min.
D = Broad peak centered at 55.3 min.
E = Nothing detected during a run of 70 min.
F = Time of peak maximum varied considerably; usually about 23.2 min.
G = Nothing detected during a run of 45 min.
H = Time of peak maximum varied considerably; usually about 22.1 min.
I = Nothing detected during a run of 90 min.

DDP and UCON 5100 were useful, but were rejected since these columns were operated at temperatures where bleeding of the liquid phase material was a definite problem. The two most outstanding phases were SF-96 and SE-52. The value of these two phases for the various amino alcohols was attributed to a combination of their low polarity and thermal stability properties.

Retention time data in minutes for these last four packings are given in Table I. Tryptophanol was not successfully chromatographed on any of the liquid phases. The separations can no doubt be improved by a temperature programming sequence and moving the analysis to a higher temperature. It is quite possible that chromatography over a lightly loaded column (*i.e.*, 2 % substrate) would produce even better results. We intend to investigate these factors at a later date and also will attempt the mass spectroscopy analysis of the amino alcohols. From a practical viewpoint these early results are encouraging to us, but at this time they do not replace the demonstrated utility of the N-TFA ester systems.

EXPERIMENTAL

Apparatus and procedure

A Wilkens Aerograph Model A-90-P2 with a thermal conductivity detection system was used in this work. Each phase was deposited as a 20 % by weight coating over Chromosorb W, 60–80 mesh; the solid support was previously deactivated with hexamethyldisilazane. The column was 2.0 m long by 6.35 mm outside diameter. The retention times given in Table I were measured for each compound from the moment of injection to the center of each sample peak. Each value represented the average of at least three determinations. Sample size was of the order of 0.2 μl.

Materials

Alaninol, isoleucinol, leucinol, methionol, phenylalaninol, serinol, tryptophanol and *valinol* were purchased from the Research Organic Chemicals Co., 3101 Floye Drive, Los Angeles 46, Calif. *Glycinol* was a Matheson Coleman and Bell product.

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SUMMARY

Amino acids may be reduced by lithium aluminium hydride to afford the corresponding amino alcohols. The amino alcohols are generally high boiling liquids or low melting solids. These compounds may be gas chromatographed over a variety of substrates. Two liquid phases, SF-96 and SE-52, separated in a short time period seven different amino alcohols. This method is an alternative to other analytical schemes for amino acids previously described in the literature.

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