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HIGH SENSITIVITY OPTICAL RESOLUTION OF POLY-FUNCTIONAL AMINO ACIDS BY GAS LIQUID CHROMATOGRAPHY

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Whilst gas liquid chromatography of diastereoisomers has been used with some success for the determination of steric purity of neutral amino acids [1][2][3], no general procedure for the g.l.c. resolution of poly-functional amino acids has been described. This has rather limited the usefulness of this new analytical tool, since thirteen of the twenty essential amino acids contain a third functional group besides the characteristic α-amino and carboxyl group.

We have now found that by suitable derivatisation of the functional group, racemic hydroxy and β-mercapto-amino acids can be resolved by g.l.c. and that the choice of polyethylene glycol adipate as the column liquid phase, permits the separation of diastereoisomers derived from the acidic-α-amino acids.

In typical assays (Table) the β-hydroxy-α-amino acids (0.1mM) were esterified with thionylchloride-methanol[4] and the excess reagent and solvent removed. Hexamethyldisilazane (0.5ml) was added and the suspension refluxed till a clear solution resulted. The excess reagent was removed in vacuum and the residue condensed with a solution of N-TFA-L-prolylchloride in methylenechloride (0.1mM in 1ml)[1]. After 1 hour, part of the solution (2μl) was injected into the gas chromatograph. To avoid β-elimination of the mercapto

group during g.l.c. analysis the β-mercapto-α-amino acids were converted to the corresponding thiazolidine-4-carboxylic acids, by reaction with excess aqueous formaldehyde[5]. After several hours at room temperature the water was removed by freeze drying and the residue esterified and coupled with the TFA-L-prolyl chloride reagent as described previously for the resolution of neutral amino acids[1]. In all cases quantitative yields of the derivatised polyfunctional amino acids were obtained.

Since we have previously shown that the coupling of amino acid esters with N-TFA-L-prolylchloride proceeds without detectable racemization[1]; this new analytical technique can now be used to discriminate the antipodes of all but four of the essential amino acids. Due to the excellent g.l.c. separation of the diastereoisomers (retention time ratios of 1.08 to 1.56) this procedure is ideally suited to the quantitative steric analysis of individual amino acids. However, it is still not possible to carry out a complete steric amino acid analysis due to some overlap of retention times between different amino acids (e.g., L-methionine and D-glutamic acid). Because of the low volatility and the polar nature of the N-TFA-L-prolyl peptide esters derived from arginine, histidine, tryptophane and tyrosine, alternative derivatisation and different column phases are under investigation.

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TABLE

Gas Chromatographic Separation of Poly-functional D,L-amino Acids as their N-trifluoracetyl-L-prolyl Peptide Methyl Esters.*

D,L Amino Acids	Derivative of amino acid ester coupled with resolving agent.	Retention times (min.) LD	LL	Ratio of retention times $\frac{LL}{LD}$
Threonine	O-trimethylsilylether (O-TMS)	5.1	6.25	1.23
Allo threonine	O-TMS	5.4	6.25	1.16
Serine	O-TMS	5.6	6.85	1.22
β-hydroxyvaline	O-TMS	6.6	8.05	1.21
Homoserine	O-TMS	9.0	11.05	1.23
ɣ-hydroxy proline	O-TMS	18.3	22.1	1.21
β-hydroxy glutamic acid	O-TMS	27.0	29.7	1.10
Aspartic acid		18.0	19.4	1.08
Glutamic acid		29.3	33.7	1.15
Methionine		25.7	29.3	1.14
Cysteine	thiazolidine-4-carboxylic acid	19.5	30.5	1.56
Penicillamine	5,5-dimethyl-thiazolidine-4-carboxylic acid	16.45	19.0	1.16

*G.l.c. analyses were carried out on a Wilkens 600 C aerograph equipped with a flame ionization detector. The 5' x 1/8' steel column was packed with 0.5% polyethylene glycol adipate on chromosorb W, and during the analyses the nitrogen flow was 46ml/min. and the separation temperature was 185° C.

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