HIGH SENSITIVITY OPTICAL RESOLUTION OF D,L AMINO ACIDS BY GAS CHROMATOGRAPHY

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Biogenetic macromolecules, having ordered asymmetric centers, have the necessary information to discriminate among the optical isomers of monomeric substrates. This one intrinsic property of "life-associated" molecules, which may be unique, will allow almost positive identification of the existence or at least previous existence of extraterrestrial life. This discrimination of optical isomers suggests that net optical asymmetry is virtually equivalent to biogeny. Paradoxically, the direct measurement of optical activity by polarimetry is an inherently insensitive method. Most biologically interesting compounds, such as the amino acids and sugars, have small specific rotations and thus at least 10 µg of optically pure material is needed.

We have now found a sensitive technique for demonstrating the asymmetry of D,L amino acids. Using N-trifluoracetyl-L-prolyl chloride as the resolving agent, the diastereoisomers of neutral amino acids can be separated by gas chromatography and as little as 0.1 µg of each antipode can be detected. The advantages of G.L.C. for the separation of diastereoisomeric trifluoracetyl-dipeptide esters have already been demonstrated by Weygand (1963) in his study of racemization in peptide syntheses. We chose N-trifluoracetyl-L-prolyl chloride because proline does not racemize during acylation or peptide syntheses (oxazolone formation is not possible), it is stable in inert organic solvents, and the rigid conformation of prolyl peptide bonds was expected to enhance differences in physical properties of its diastereoisomers.

In a typical assay, the amino acid mixture was esterified with

thionyl chloride-methanol (Brenner and Huber, 1953) and the excess reagent and solvent removed. An excess of N-TFA-L-prolyl chloride (Weygand et al, 1957) in an inert solvent was added to the residue and the suspension cooled and neutralized with triethylamine. After washing with water and drying with sodium sulphate, the solution was injected into the gas chromatograph. The Table shows the amino acids that have so far been separated as their N-TFA-L-prolyl peptide methyl esters, together with the experimental conditions used. The chromatogram in Figure 1 is a typical example of the results one obtains from a synthetic mixture of D,L amino acids.

TABLE

Gas chromatographic separation of D,L amino acids as their N-trifluoracetyl-L-prolyl peptide methyl esters.

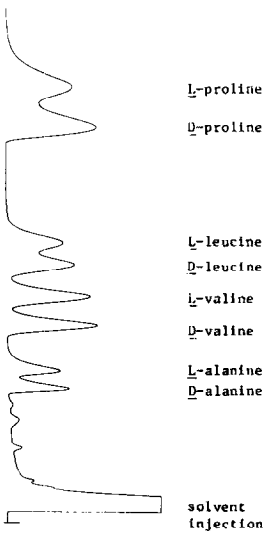
Amino Acid	Separation Temperature	Retention Time (min.)	
		D isomer	L isomer
Alanine	176°	4.4	5.0
Valine	176°	6.6	7.6
Leucine	176°	8.7	9.45
Proline	176°	13.3	14.8
Methionine	176-220°*	12.0	12.3
Phenylalanine	176-220°*	14.5	14.9

* Column programmed at 3°/min.

G.L.C. analyses were carried out on a Wilkens 600-C Aerograph, equipped with a flame ionization detector. The 5 foot column was packed with 5% S.E.-30 coated on Chromosorb W and during analyses the N_2 flow was 28 ml per min.

FIGURE 1

Gas chromatographic separation of a synthetic mixture of D,L amino acids as their N-trifluoracetyl-L-prolyl peptide methyl esters.



Finally a sample of the antibiotic gramicidin* (Hotchkiss and Dubos, 1941), which is known to contain both D and L amino acids (Ishii and Witkop, 1963), was hydrolyzed and the hydrolysate esterified and condensed with N-TFA-L-prolyl chloride. The G.L.C. analysis showed the presence of L-alanine, D and L valine, and D-leucine, and the absence of L-leucine.

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*Gramicidin N.F. from Calbiochem, Los Angeles

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