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THE TERMINATION OF AN OPTICAL PURITY BY V.P.C.

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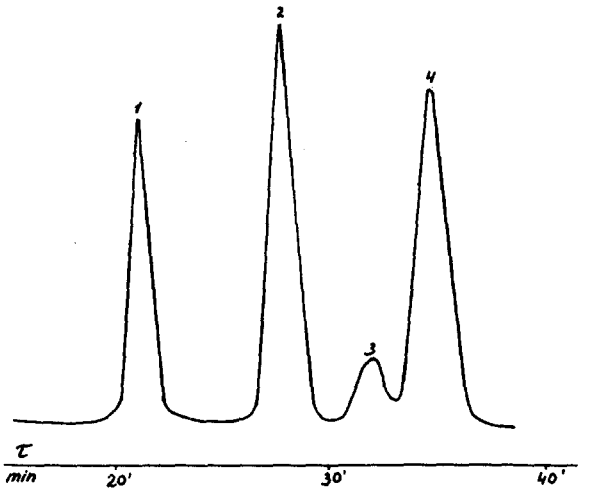
It is often important in biochemical investigations to establish the absence of racemization. Furthermore the determination of absolute configurations can be important, especially when the stereospecifity of the reactions being studied in unknown. Stereospecifity may be shown within relatively rough limits by polarimetry, but this method can not be used for complex mixtures, and is relatively insensitive to small amounts of one isomer when the other predominates.

We have found it possible to resolve diastereoisomeric derivatives of amino acids by V.P.C. on packed or capillary columns. The second asymmetric centre may belong to the amino acid itself or be introduced while synthezising derivatives [1,2]. Other work /3/ has also demonstrated the possibility of resolving stereoisomers by V.P.C. This suggested the possibility of synthezising

Fig.1.

Separation of L-menthyl N-trifluoracetyl D and L-amino acid esters ("Shandon")



1- L-Valine, 2-L-Alanine, 3-D-Alanine (12.9% in a mixture of D and L-Alanine), 4-L-Leucine.

Column: 1- 4 m, 0.4 cm i.d. stainless steel packed with 5% polyethyleneglycoladipate on 60 to 80 mesh Chromosorb W. P inlet 1.65 atm, Column temp.165°.

diastereoisomeric pairs using optically active substances, e.g. alcohols; the resolution of these pairs by V.P.C. must resolve the initial substances into optical antipodes. This supposition has been confirmed. While chromatographing amino acids as O-L-methyl esters of N-trifluoracetyl derivatives we have observed that alanine, valine and leucine may form two peaks each, the areas of which are proportional to the concentrations of D and L-forms in the initial samples/table 1/.

Table 1.

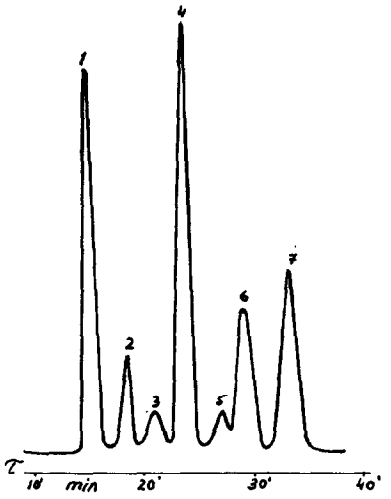
Recoveries for D and L. amino acids*

Amino acid	Mixture	Found
L - Valine	82.4; 75,1	82.4; 76.5
D - Valine	17.6; 24.9	15.8; 23.5
L - Alanine	86.77;50.00	84.77;50.0
D - Alanine	13.23;50.00	15.23;50.00
L - Leucine	83.20;84.2	82.10;82.8
D - Leucine	16.80;15.8	17.90;17.2

* Chromatographic conditions were as described for Figure 1.

Fig.2.

Separation of L-menthyl N-trifluoracetyl D and L-amino acid esters*



1-Diphenylmethan(Standart), 2-1-L-Valine, 3-D-Valine, 4-L-Alanine, 5-D-Alanine, 6-L-Leucine, 7-D-Leucine.

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* Conditions of chromatographie were as described for Figure 1.

The L-form is characterised by a smaller retention time than D-form. A number of experiments has shown that no racemization occurs in synthezising the derivatives and that the reaction between L-menthol and the amino acids is practically quantitative. Esterification of the amino acids was carried out in toluene solution of menthol (10 fold excess of menthol, 100°C in the stream of HCl gas for 1 hour). The solvent was removed in vacuo in the stream of nitrogen and the residue was treated with a large excess of trifluoracetic anhydride (room temperature, 1 hour). After evaporating in a N_2 stream the residue was dissolved in toluene and chromatographed. V.P.C. has been carried out on 4 meter column packed with polyethyleneglycoladipate (PEGA) (5% on cromosorb 60 - 80 mesh), 165°C inlet, excess pressure 1.8 atm; detection ß-ionisation(macroargon detector of "Shandon") used at a temperature of 220°C.

The relative retention times Fig.2 are shown in table 2.

This method of determining the concentration of optical antipod., seems to be the only one useful for investigation of complex mixtures of optically active substances, as well as for defindings optical purity where one of optical isomers predominates.

Table 2.

Relative retention times of L-Menthyl N-Trifluoroacetyl amino acid esters*

Diphenylmethane (standart)	1.000
L- Valine	1.330
D- Valine	1.471
L- Alanine	1.692
D- Alanine	1.954
L- Leucine	2.152
D- Leucine	2.510

References.

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3. E.Gil-Av, R.Charles, G.Fisher,J.Chromatog.17, 408(1965)

* Conditions of Chromatography were as described for Figure 1.