

OPTICAL RESOLUTION OF D,L AMINO ACIDS BY GAS

CHROMATOGRAPHY AND MASS SPECTROMETRY

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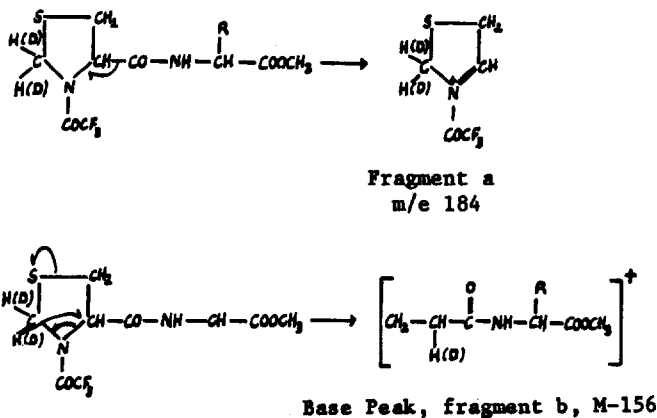
The significance of optical activity for the recognition of life and hence its utility for biochemical exploration needs no elaboration (Lederberg 1965), (Ulbricht 1962). Recently, a number of gas liquid chromatographic (G.L.C.) procedures have been developed, whereby important metabolites, like amino acids, can be scanned for optical activity, with very high sensitivity (Gil-Av., Fischer and Charles 1965), (Halpern and Westley 1965 a,b), (Pollock 1965). The same principle can be generalized for complex mixtures by ratio-detection of D- and L- input reagents when these form resolvable diastereoisomeric complexes with the target material. We now show the use of mass spectrometry for the ratio-detection, as well as to identify the optically active species.

For this purpose we prepared an artificial mixture of D and L enantiomeric resolving agents, in which the L reagent was labelled with 2 deuterium atoms (L*). After coupling with the target material, the product was gas chromatographed and the peaks collected and passed into a mass spectrometer. For each symmetrical molecule (e.g. glycine), the D and L reagents are unresolved and the label ratio will remain uniform through the peak. However, if an asymmetric molecule is encountered, which gives rise to resolvable diastereoisomers, the deuterated reagent will be concentrated in one peak, distorting the ratio. If the target molecule is racemic (D L), two peaks will also be

formed (one containing L*D plus D L; the other L*L plus D D); but the label ratio in each peak will remain constant. We chose trifluoroacetyl-thiazolidine-4-carboxylic acid chloride as the reagent, because both enantiomers are available (Ratner and Clarke 1937), and deuterium can be incorporated into position 2 with deuterioformaldehyde. Also mass spectrometric fragmentation patterns of its condensates with amino acid esters yielded characteristic peaks which could be used to identify both the reagent and the amino acid (Figure 1).

Figure 1

MASS SPECTRAL FRAGMENTATION OF TFA-THIAZOLIDINE-4-CARBOXYLIC ACID CONDENSATION PRODUCTS



In a typical assay, the amino acid sample was esterified with thionyl chloride-methanol and the excess reagent and solvent removed. An excess of the resolving agent (L* plus D) in an inert solvent was added to the residue and the suspension neutralized with triethylamine. After washing with water, the solution was injected into the gas chromatograph and the emerging components collected for introduction into the mass spectrometer. By monitoring the ratio for fragment (a) [184:186] as well as the ratio (b:b+1) for the base peak (M-156]

a fast sensitive recording for optical activity was obtained. In addition, the position of the base peak was also used to confirm the identity of the optically active amino acids present (Table), (Figure 2).

TABLE: MASS SPECTRAL MONITORING OF G.L.C. FRACTIONS
CORRECTED FOR ISOTOPIC ABUNDANCE. (*)

G.L.C. Fraction	Ratio(a:a+2) m/e 184:186	Fragment(b) m/e	Ratio (b:b+1)	Molecular Weight (b+156) and Iden- tity of Amino Acid	Optical Identity of Fraction
1	28:2.5	158	100:8.5	314-alanine	L
2	1:24	158	4.5:100	314-alanine	L
3	38:41	144	97:100	300-glycine	DL
4	55:56	172	100:96	328-aminobutyric acid	DL
5	33:32	172	100:98	328-aminobutyric acid	DL
6	2.5:33	200	8.5:100	356-leucine	D
7	23:2	200	100:8	356-leucine	D
8	100:2	184	100:5	340-proline	L
9	12:31	184	12:100	340-proline	L

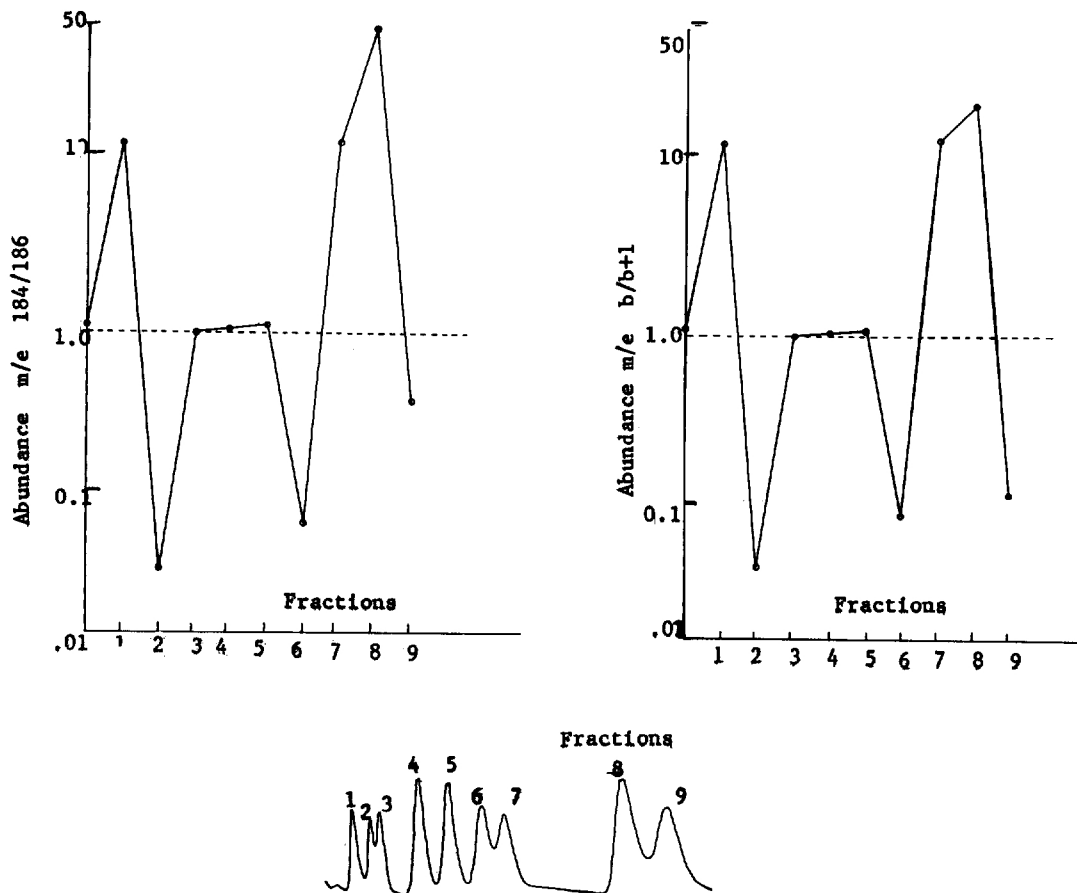
*G.L.C. analysis were carried out on a Wilkens 600C Aerograph, fitted with a micro collector and using a 5' X 1/8" S.S. column containing 5% SE 30 on chromosorb W. The separation temperature was 180°C and the N₂ flow was 28 ml/min.

Mass Spectra were determined on a Bendix-Time-of-Flight Spectrometer and the collected sample fractions introduced directly into the ion source.

The utility of mass spectrometric detection, thus substantiated, points to a general method for the speedy, facile detection and identification of minute amounts of optically active materials. Hardware for direct coupling of the gas chromatograph to the mass spectrometer (Gohlke 1959, 1962), (Ebert 1961) was not yet available to us for this study. However, the results of other

Figure 2

ANALYTICAL RESOLUTION OF LABELLED INPUT REAGENT



Gas Chromatograph*

*G.L.C. Fraction:

1. D reagent-L-alanine
2. L* reagent-L-alanine
3. D reagent-glycine and L* reagent-glycine
4. D reagent-L aminobutyricacid and L* reagent-D aminobutyricacid
5. D reagent-D aminobutyricacid and L* reagent-L-aminobutyricacid
6. L* reagent-D leucine
7. D reagent-D leucine
8. D reagent-L proline
9. L* reagent-L proline

workers suggests that the technique should thus have a sensitivity in the sub-microgram or nanogram range, rendering it useful for the monitoring of metabolic reactions as well as the identification of accumulated asymmetric metabolites.

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