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DETECTION OF OPTICAL ACTIVITY AS A SIGN OF LIFE

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Living organisms work against the law of increasing disorder and concentrate order within themselves. One of the most fundamental aspects of this order is the optical purity of those metabolites which are built up into polymers (enzymes, nucleic acids) and as a consequence of this, biological processes usually exhibit steric (optical) discrimination.

Gas Liquid Chromatographic (G.L.C.) procedures have been developed whereby important metabolites such as amino acids can be scanned for optical activity with very high sensitivity. By using a combination of G.L.C. and mass spectrometry we have found a method of ratio-detection as well as identification of optically active compounds. More recently we have observed that within 24 hours of inoculating non-sterile soil with a racemic mixture of certain substrates, marked changes in the D:L ratio of the unused substrate can be detected.

Biogenetic macromolecules, having ordered asymmetric centers, contain 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.[1,2] This discrimination of optical isomers 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 liquid chromatography (G.L.C.) and as little as 0.1 μg of each antipode can be detected.[3,4]

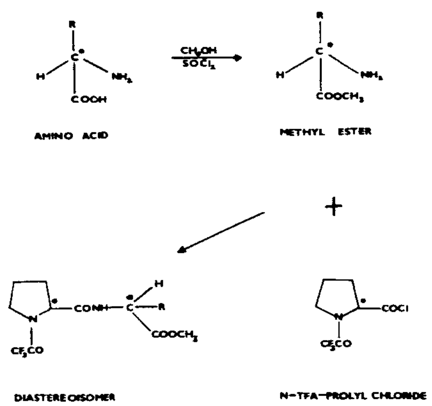


Fig. 1. Preparation of Diastereoisomers

The method of preparing diastereoisomers is summarized in Fig. 1 and a typical gas chromatographic separation of a synthetic mixture of D,L amino acids as their N-trifluoracetyl-L-prolyl peptide methyl esters is illustrated in Fig. 2.

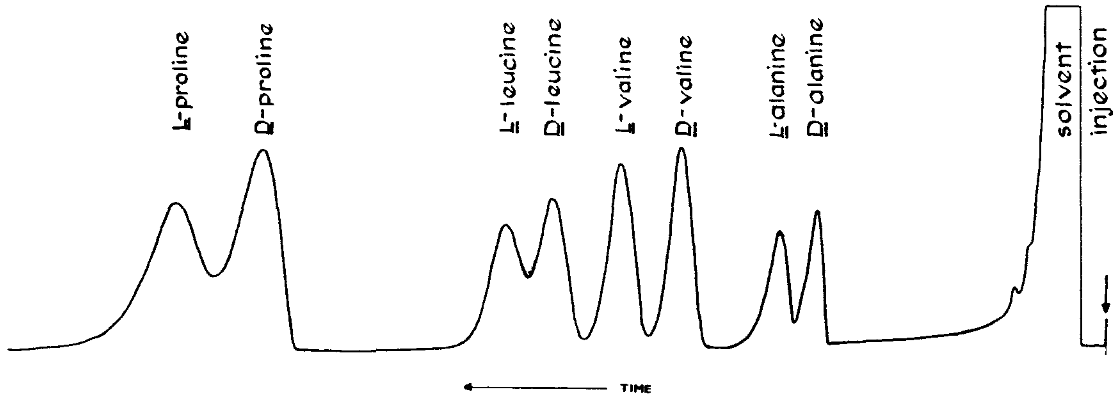
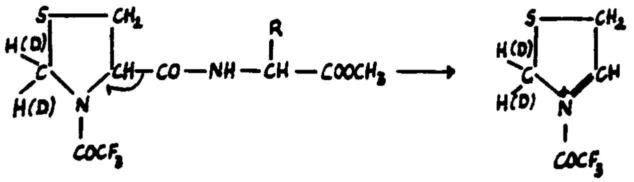


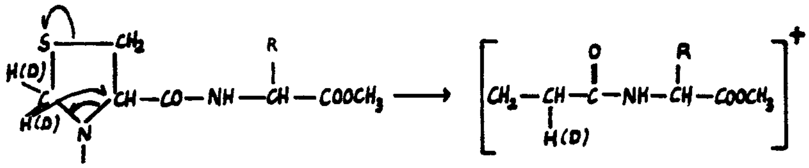
Fig. 2. GLC Resolution of Diasteroisomers

Amino acids in the soil could be identified and their optical purity determined by comparison of retention times on the gas chromatograph with a known sample. However, a peak derived from material other than amino acids could cause confusion and therefore an additional method of identification is necessary. We have now used mass spectrometry to identify the GLC peaks as well as providing a method of accurately determining the ratio of optical antipodes.[5]

For this purpose we prepared an artificial mixture of D and L enantiomeric resolving agents, in which the L reagent was labeled 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 trifluoracetyl-thiazolidine-4-carboxylic acid chloride as the reagent, because both enantiomers are available,[6] and deuterium can be incorporated into position 2 with deuteroformaldehyde. Also mass spectrometric fragmentation patterns of its condensates with amino acid esters yielded characteristic peaks which could be used to identify the reagent and the amino acid (Fig. 3).



Fragment a
m/e 184



Base Peak, fragment b, M-156

Fig. 3. 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 1), (Fig. 4).

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 was not yet available to us for this study. However, the results of other workers suggests that the technique should

Table 1

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 Identity 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' × 1/8" S.S. column containing 5% SE 30 on chromosorb W. The separation temperature was 180 °C and the N_2 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.

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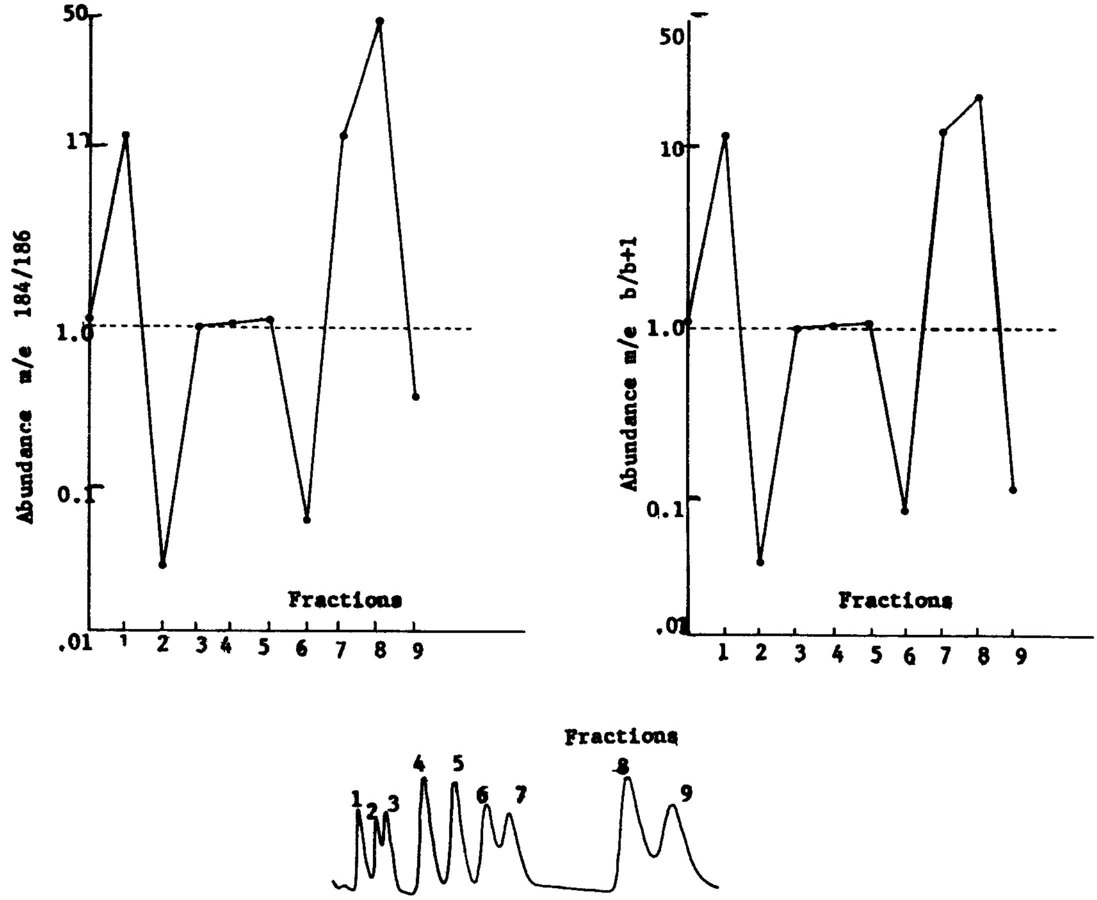
thus have a sensitivity in the submicrogram or nanogram range, rendering it useful for the monitoring of metabolic reactions as well as the identification of accumulated asymmetric metabolites.



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

Fig. 4. Analytical Resolution of Labelled Input Reagent

This analytical technique can also be used to assay for living systems. An example of this is our latest experiment which has involved the use of GLC to monitor the stero-specific usage of several racemic amino acid substrates when mixed with soil. In most cases, enzymatic activity could be demonstrated after 18-24 hours. In a typical assay, soil (10g), the racemic amino acid substrate (10mg), and distilled water (10ml) were taken at room temperature. From time to time aliquots (approx. 1ml) were removed and diluted with water (10ml). The soil was centrifuged down and the supernatent solution lyophilised. The remaining amino-acid was analyzed by conversion into diastereoisomers as described in Fig. 1 and injection into the gas chromatograph. By computing the peak areas of the two diastereoisomers, a fast sensitive recording of unused D/L amino acid concentration could be obtained (Table 2 and Fig. 5).

Our results show that the L-antipodes of the substrates are preferentially attacked, but that different amino acids are used at different rates. The observation that the stereospecific action is lost after heat sterilization of the soils, confirms that a biological process is involved. Whilst the exact nature of the biological system responsible for the sterospecific attack is as yet not known, the kinetics of the experiment (Fig. 5) suggest an exponential increase of activity, which would be consistent with growth of microorganisms.

Table 2

SUSCEPTIBILITY OF DL-AMINO ACIDS TO MICROORGANISMS IN SOILS

Soil Sample	DL Amino Acid	Unused D/L Amino Acid Concentration After Period of Incubation (hrs)[*]													
		0	2	4	6	8	10	12	14	16	18	20	22	24	48
Bowers Clay[†]	Proline	1.0		1.0		1.1				1.2	1.9	3.0	6.8	9.2	
Bowers Clay Sterilized[‡]	Proline	1.0								1.0				1.0	
Bowers Clay	Glutamic Acid	1.0		1.0		1.1		1.3		1.3	1.5	2.5	2.7	5.0	
Bowers Clay Sterilized[‡]	Glutamic Acid	1.0						1.0						1.0	
Stanford Soil[‡†]	Glutamic Acid	1.0						1.8			6.7			16.0	
Stanford Soil Sterilized[‡]	Glutamic Acid	1.0						1.0						1.0	
Stanford Soil	Amino Butyric Acid	1.0				1.1								2.9	4.7

[*]GLC analyses were carried out on a Wilkens 600C Aerograph using a 5' × 1/8" column (0.5% EGA on Chromosorb W). During the analyses the nitrogen flow was 46 ml/min and the oven temperature was programmed from 140 °C to 200 °C at a rate of 4°/min. Under these conditions the retention times (min) for the N-TFA-L-prolyl derivatives were D-valine (7.6), L-valine (8.9), D-proline (13.0), L-proline (14.2), D-glutamic acid (20.2), L-glutamic acid (21.4), D-amino butyric acid (9.0), and L-amino butyric acid (10.5).

[†]Soil collected at Moffett Field, California and characterized by NASA Ames. The organic nitrogen analysis was 1,435 p.p.m. and the organic carbon was 6,380 p.p.m. The soil had a pH 6.08.

[‡]Soil samples were sterilized by heating at 135 °C for 24 hrs.

[‡†]Garden soil collected at Stanford in December, 1965.

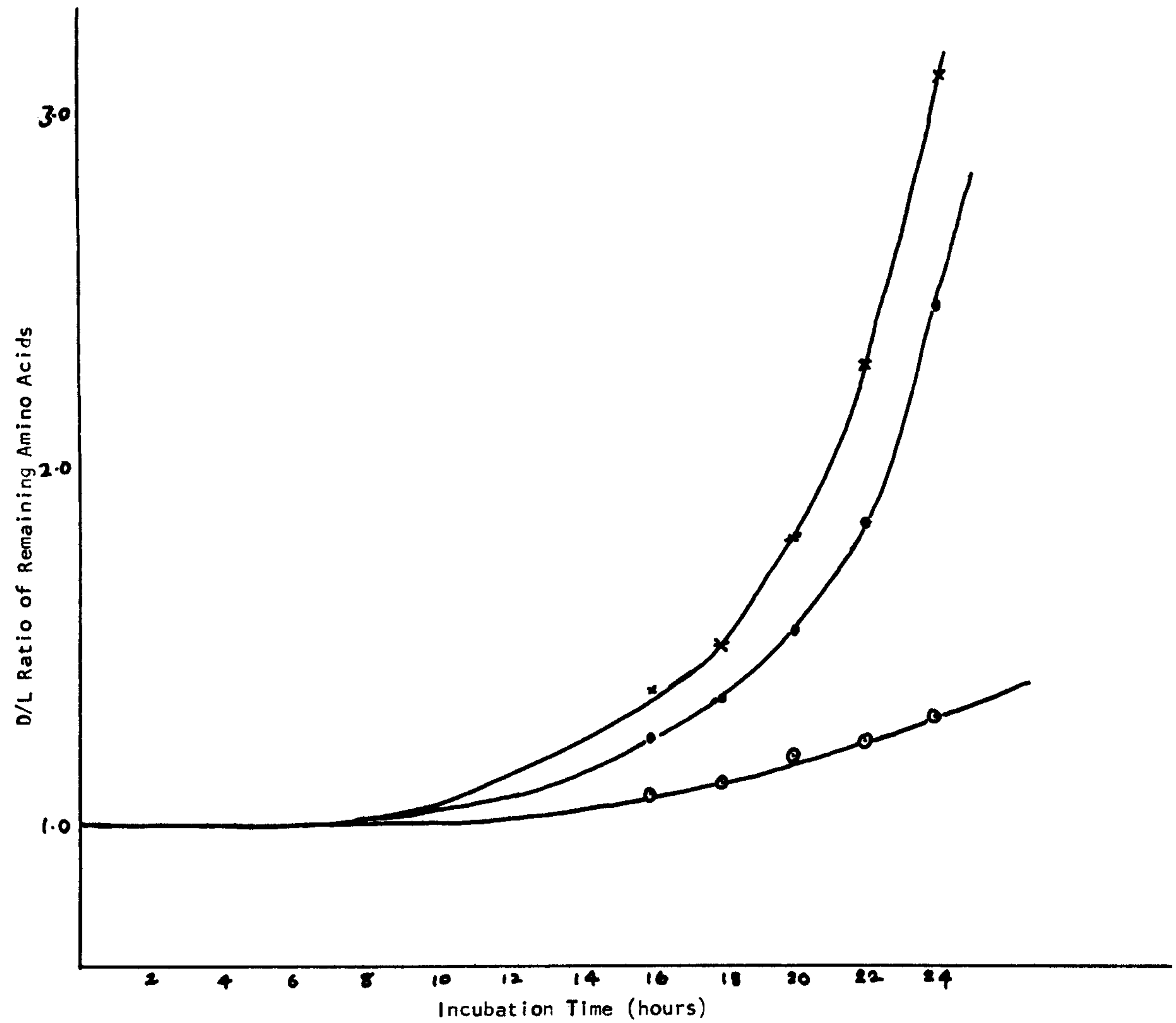


Fig. 5. Susceptibility of a Mixed D,L-Amino Acid Substrate to Microorganisms in Bowers Clay
-·- D,L-proline; -x- D,L-Glutamic Acid: -⊙- D,L-valine

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