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The determination of steric purity of amines and amino acids gas chromatography and mass spectrometry

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THE DETERMINATION OF STERIC PURITY OF AMINES AND AMINO ACIDS BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

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1979

PREFACE

Some of the experimental work in this thesis was done in conjunction with Professor B. Halpern, Department of Chemistry, University of Wollongong.
All analytical results are those of the author.

The author wishes to thank Professor B. Halpern for his help and encouragement throughout the course of this work.

Thanks are also due to Associate Professor E. Gellert for his guidance during Professor Halpern's absence on sabbatical leave. The author acknowledges the work of Dr. A. M. Duffield and Mr. J. Korth who ran the samples described in this thesis. In particular thanks are due to my mother for the typing of this thesis.

C. Wiecek

SUMMARY

Amines and amino acids have been condensed with a mixture of N-TFA-R-prolyl chloride and N-TFA-S-(1-^{2}H)-prolyl chloride and the resulting four diastereoisomers have been separated by Gas Chromatography into two peaks. The amount of each diastereoisomer formed was then determined by Chemical Ionisation Mass Spectrometry. The steric purity of the enantiomeric mixtures was calcuated from these values and the calculated values were always found to be within 5% of the expected values.

The procedure was then repeated using a mixture of N-TFA-R-thiazolidine-4-carboxylic acid chloride and N-TFA-S-(1-$^{2}H_{2}$)-thiazolidine-4-carboxylic acid chloride. Calculated steric purity measurements were again obtained within 5% of the expected values although samples had to be kept refrigerated after preparation.

The method is not dependent on diastereoisomer formation going to completion.

CONTENTS

INTRODUCTION

The unambiguous determination of optical purity is of considerable importance. For example, since there is a large degree of configurational uniqueness of the metabolites utilised in many biochemical processes then the search for optical activity becomes an important experiment for the presence of extraterrestrial biogeny [1]. Also the retention of partial loss of optical purity in the course of a reaction can often give valuable information concerning the reaction mechanism provided the optical purities of the starting materials and the product are known[2]. Accurate knowledge of optical purities is especially important in the area of peptide synthesis as a small amount of optical impurity in the monomers can lead to a polypeptide of low biological activity[3].

Several criteria of optical purity have long enjoyed general acceptance, but as has been pointed out by Eliel[4], none is completely reliable. Specifically, resolutions have been regarded as complete when the crystalline enantiomer or precursor diastereoisomer is unchanged in melting point or rotation upon further crystallisation and/or when the two enantiomers are obtained in states of equal optical purity i.e., with equal specific rotations. While these criteria have given correct estimates of optical purity there are many instances where they have failed to yield the desired information and have given estimates which have fallen far off the mark[5], [6], as will be shown by the following example. Crawford[7] had resolved trifluoropropan-2-ol as the quinine salt of β-(1-trifluoromethylethoxy) propanoic acid and reported

$[\alpha]_D^{26}$= -2.63 (neat) for the pure enantiomer. But this value led to an incongruous result, when the stereoselective reduction of 1,1,1-trifluoroacetone by the Grignard reagent from (+)-1-chloro-2-phenylbutane was investigated by Feigl et alia[8] as their product had $[\alpha]_D^{26}$ = -3.47. On the basis of chromatography of the (-)- O-methylmandelates, the optical purity of the reduction product could subsequently be unambiguously established, and was found to be 62% (i.e. $[\alpha]_D^{26}$= -5.6 for the pure enantiomer).

Several approaches have been used for the determination of optical purity of an enantiomeric mixture. In 1940 Graff, Rittenberg and Foster[9] described a method whereby both S- and R- amino acids could be determined in biological materials by an isotope dilution method which involves adding a quantity (m grams) of labelled racemic A(SA and RA) with specific activity S_o to a test sample of unlabelled optically active A(n grams) and reisolating the racemic A. The specific activity of the reisolated racemate (S_i) is then related to the optical purity (p) of the test sample by:

$$S_i = \frac{S_o m(m+n)}{(m+n)^2 - n^2p^2}.$$

This method was later developed by Berson and Ben-Efraim[10]. The isotope dilution technique has been used to determine the optical purities of several compounds but requires considerable amounts (10-100mg) of isotopically labelled materials. Other methods involve the simultaneous analysis of the stereoisomeric components and involve techniques including polarimetry, NMR spectroscopy and differential microcalorimetry. The first of these, polarimetry,

requires mg amounts of analytically pure sample and the absolute rotation of the pure enantiomer must be known[2].

The NMR approach usually involves diastereoisomer formation (or the use of an optically active solvent [11]) and necessarily requires measurable chemical shift nonequivalence for a set of signals due to corresponding groups in the two diastereoisomers. Since no physical separation of the diastereoisomers in the mixture (SASB and RASB) is required the NMR method is applicable to the determination of optical purities of compounds which owe their chirality to deuterium substitution since the optical purities of such compounds cannot be determined by the isotope dilution method or by gas chromatographic (GC) methods because the physical properties of such enantiomers or diastereoisomers are too similar to permit separation by physical methods[2].

While the previously described methods of determining optical purity have been rather limited in their usage, extensive research has been carried out into the application of the gas chromatographic (GC) separation of diastereoisomers as the basis for a convenient method for the determination of optical purity [12],[13],[14]. The advantages of this analysis over conventional polarimetric measurements are that chemical impurities are separable during chromatography and that a steric purity measurement can be done on a mixture. In this method the enantiomers (SA and RA) are converted into a mixture of diastereoisomers (SASB and RASB)by reaction with a chiral reagent SB. Conditions are chosen so that the ratio (r) of enantiomers $^{SA}/_{RA}$ is equal to the ratio of the diastereoisomers $^{SASB}/_{RASB}$. The optical purity can then be

calculated from $^{r - 1}/_{r + 1}$. However, two conditions have to be satisfied. First the chiral reagent B must be optically pure or its optical purity must be known and second, there must be no kinetic resolution in the reaction which transforms the mixture of enantiomers into the diastereoisomers. The first requirement can be met by using a chiral reagent, preferably a natural product, whose optical purity can be determined by classical methods (i.e. biochemical transformation, isotope dilution, nuclear magnetic resonance spectroscopy). The second requirement is more difficult to establish as the system has to be tested to ensure that the reaction goes to completion and ideally the two rates of diastereoisomer formation should be determined.

In their study Halpern and Westley[13] used N- trifluoroacetyl-S-prolyl chloride as a resolving agent for the separation of neutral amino acids. They reported that as little as 0.1ug of each diastereoisomer could be detected. This represented a considerable improvement in the measurement of optical purity by polarimetry since amino acids have small specific rotations and thus at least 10ug of optically pure material is needed. Gil-Av et alia[14] demonstrated the measurement of optical purity of a series of 2-n-alkanols as determined by polarimetry and gas chromatography of the α-acetoxypropanoates. Their results agree well within the limits of experimental error. The optical purity of a compound can thus be estimated by the gas chromatographic method without knowing the specific rotation of the pure enantiomer.

Most of the recent papers concerning the determination of optical purity and the separation of diastereoisomers by GC have

dealt with the separation of derivatives of amino acids[24-24]. These have included N-trifluoroacetylamino acid esters of (-)-menthol [16],[18] and of several n-alkan-2-ols[12],[14] as well as derivatives in which an α-chloroisovaleryl [17] or α-chloropropionyl[21] residue was attached at the amino function. Dipeptide derivatives have also been studied[13],[15],[23]. In addition esters of n-alkan-2-ols[12],[14] and (-)-menthol[25] and the N-trifluroacetyl -S-prolyl derivatives of a series of amines [19] have been examined. Another GC approach uses stereo-selective sorption of enantiomers on a chiral stationary phase. Although simpler in principle, separation of enantiomers by GC on optically active substrates is less convenient in practise than prior conversion to diastereoisomers and subsequent separation on conventional achiral substrates. The method has been used with some success to separate amino acid derivatives on capillary columns coated with N-trifluoroacetyl-S-isoleucine lauryl ester[26].

The work described in this thesis was devoted to the development of a GC-Mass Spectrometric (MS) technique for the determination of optical purity which overcomes the problem of kinetic resolution during diastereoisomer formation. Such a GC/MS method has been suggested in the literature[20]. The method involves the conversion of the enantiomeric mixture (SA and RA) into diastereoisomers by reaction with a chiral reagent mixture (RB and SB^*), where SB^* contains a stable isotopic label. The diastereoisomeric mixture containing RARB, $RASB^*$, SARB, $SASB^*$ is then separated by GC into two peaks RARB, $SASB^*$ and $RASB^*$, SARB which are monitored by methane chemical ionisation MS to determine

the ratio $\frac{SASB^*}{RARB}$ and $\frac{RASB^*}{SARB}$. The enantiomeric purity of SARA can then be calculated from these ratios and they should be the same, as the rate of formation of SASB* is the same as that for the enantiomer RARB, similarly RASB* forms at the same rate as SARB. Therefore the steric purity measurement is not dependent on the reaction going to completion.

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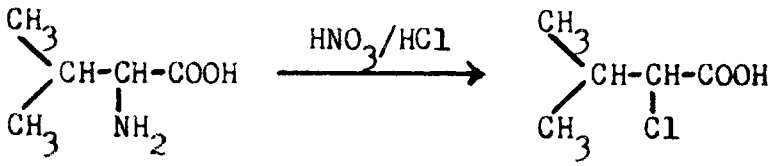
DISCUSSION AND RESULTS

Initially we chose α-chloroisovaleryl chloride (1)[1] as a resolving agent. This compound has been used as a chiral reagent for the GC resolution of amino acids[1]. R-($^2H_7$)-valine (2)[2] was used to prepare S-($^2H_7$)-α-chloroisovaleryl chloride (3) and S-valine (4) was used to prepare R-α-chloroisovaleryl chloride (1), (Scheme 1). A combination of these acid chlorides was used as a resolving agent and condensed with several amino acid methyl esters. However, we found the mixed reagent to be unsuitable for the determination of optical purity by the proposed method because the GC retention times of the S-($^2H_7$)-α-chloroisovaleryl amino acid methyl esters were substantially lower than the corresponding unlabelled compounds. For example the GC retention times of S-($^2H_7$)-α-chloroisovaleryl-S-valine methyl ester (5) and R-α-chloroisovaleryl-R-valine methyl ester (6) were 9.0 and 9.3 minutes respectively. Such partial separation of diastereoisomers is undesirable since the ratio $\underline{SASB}^*/\underline{RARB}$ is variable over the GC peak.

We then investigated the GC behaviour of the condensation products of N-TFA-prolyl chloride (7)[3] with amino acid methyl esters. This reagent has been shown to be a good chiral reagent for the GC resolution of amines and amino acids (4), (5). N-TFA-S-($^2H_7$)-prolyl chloride (8) was first synthesised from commercially available S-($^2H_7$)-proline (9) and the corresponding R-reagent (7) was synthesised from R-proline (10), (Scheme 2). Again we found the mixed reagent to be unsuitable for the purpose of determination of optical purity as

SCHEME 1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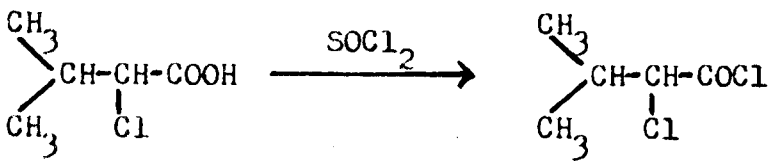
CH3 NH2
HNO3/HCl
CH3
CH-CH-COOH
CH3 Cl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SOCl2
CH3
CH-CH-COCl
CH3 Cl

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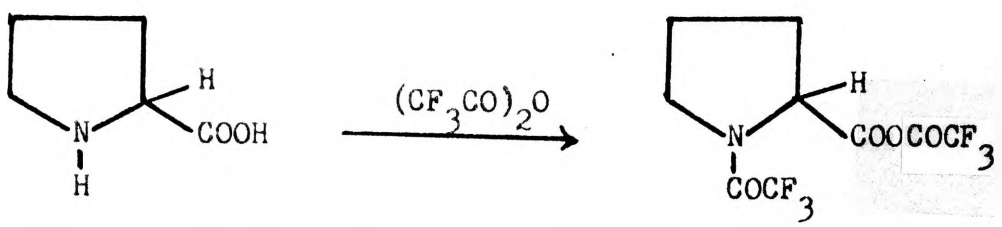
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N
H
COOH
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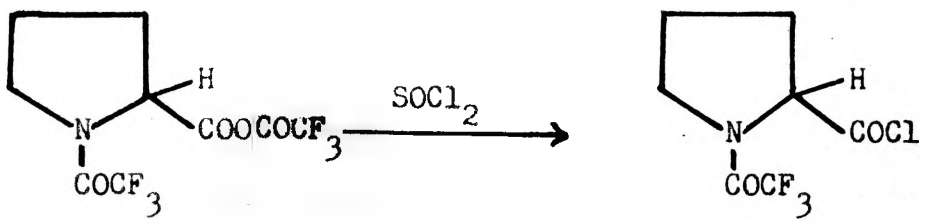
H
N
$COCF_3$
$COOCOCF_3$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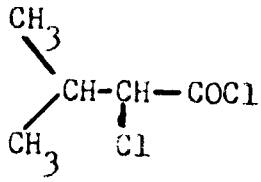
H
N
$COCF_3$
COCl



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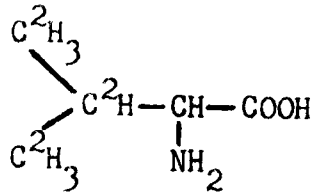

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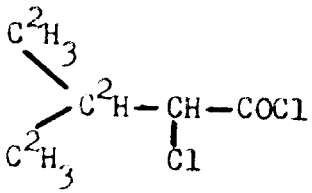

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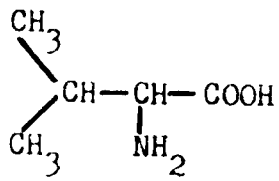

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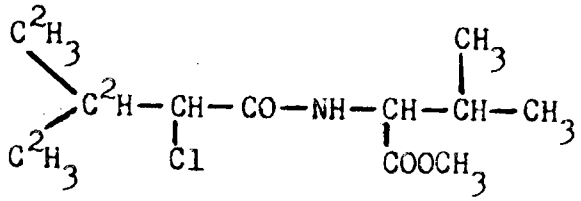

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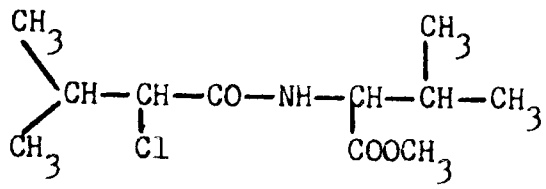
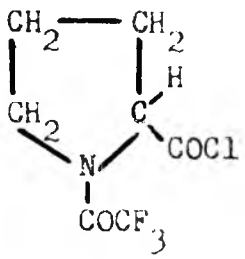


(6)

the diastereoisomeric TFA-peptide methyl esters derived from the deuterated species again had substantially lower GC retention times than the corresponding unlabelled compounds. For example the GC retention times of the labelled SASB* peptide (11) and unlabelled RASB peptide (12) derivatives of valine (4) were 7.7 and 7.9 minutes respectively. The use of other isotopically labelled derivatives of proline were then investigated. Since the chromatographic properties of ^{15}N and ^{13}C labelled analogues are essentially identical with that of the unlabelled compounds, ^{15}N or ^{13}C proline would be the best stable isotopically labelled analogues for this work. While both ^{15}N and ^{13}C proline are available commercially they were too expensive for us to use.

S-(1-^{2}H)- proline (13) was then synthesised by a stereospecific exchange reaction of the α-hydrogen atom of S-proline (10) in [Co(en)$_2$(S)-pro] I_2 (14)[6] with D_2O in the presence of Li_2CO_3. The optical purity of the S-(1-^{2}H)-proline (13) was checked by converting it to N-TFA-S-(1-^{2}H)-prolyl chloride (15) and condensing this with S-valine methyl ester. The product (16) was examined by GC/MS. Only one peak of m/e 326 was present on the GC trace indicating the optical purity of the S-(1-^{2}H)-proline (13) but the MS indicated the presence of some non-deuterated derivative (m/e 325) (12). The isotopic purity of the labelled proline (13) was determined by converting it to the N-TFA-S-(1-^{2}H)- prolyl glycine methyl ester derivative (17) and measuring the intensities of the protonated molecular



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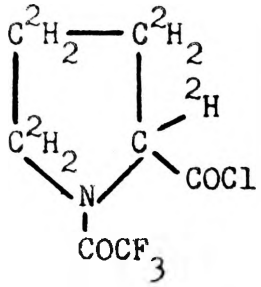

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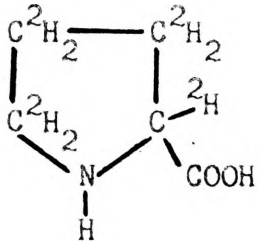

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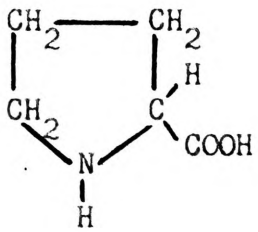

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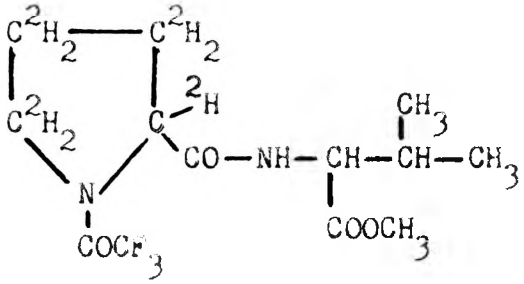

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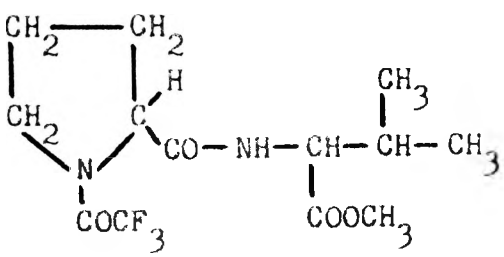

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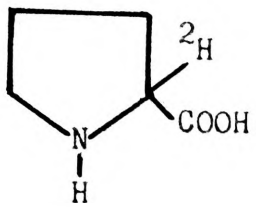

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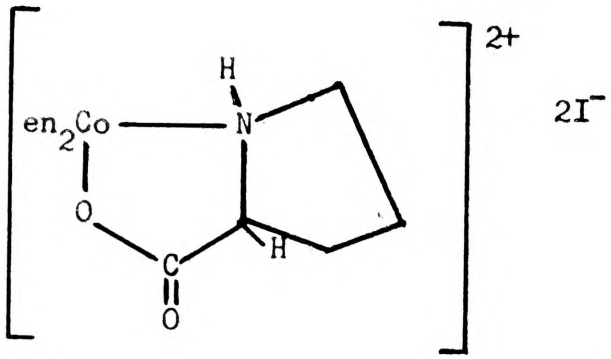

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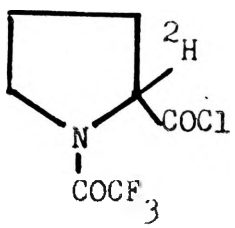

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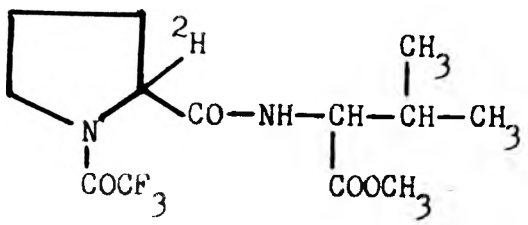

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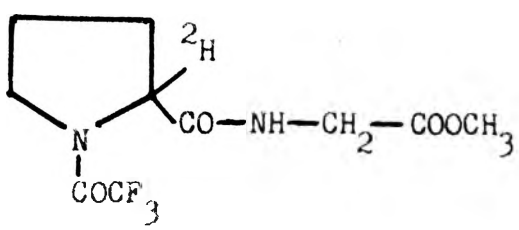

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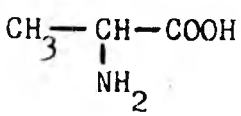

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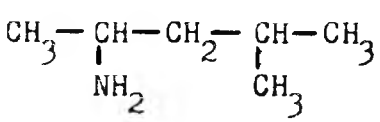


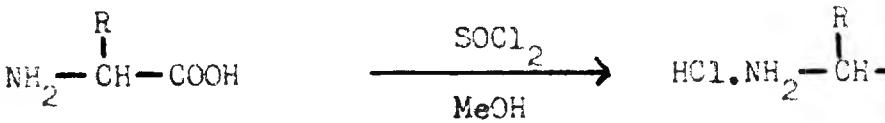
(19)

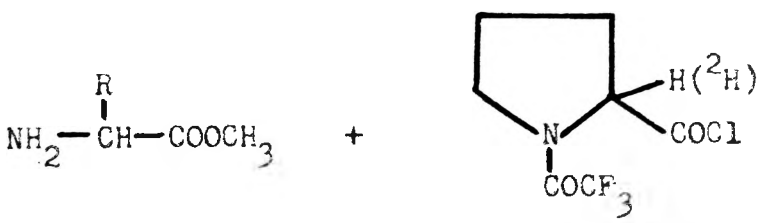
ions of the labelled (m/e 284) and unlabelled (m/e 283) compounds. The results of duplicate runs indicated that the isotopic purity of the S-(1-^{2}H)-proline (13) was 83.28%.

A reagent solution of the N-TFA-S-(1-^{2}H)-prolyl chloride (15) (SB*) and N-TFA-R-prolyl chloride (7) (RB) in CH_2Cl_2 (approximately 0.1 to 1 mg/ml) was then made up and this was used to make diastereoisomeric derivatives of amino acid methyl esters and amines.

In the condensation of the reagent (RB,SB*) with a R,S amine or amino acid (RA,SA) a diastereoisomeric mixture of RASB*, SARB, SASB* and RARB is formed (Scheme 3). This can be separated by gas chromatography into two peaks which contain RASB*, SARB and SASB*, RARB. A chemical ionisation mass spectral analysis of the intensities of the protonated molecular ions can be used to calculate the proportion of labelled to unlabelled proline in each GC peak. This ratio can then be used to determine the optical purity of the amine or amino acid. If unequal amounts of the N-TFA-S-(1-^{2}H)-prolyl chloride (15) and N-TFA-R-prolyl chloride (7) are used as the chiral reagent it is necessary to determine the ratio of the labelled to unlabelled proline derivatives from both GC peaks to obtain the optical purity of the compound. In both cases the results have to be corrected for the natural isotopic abundances and for the isotopic purity of the labelled proline reagent. For this reagent solution the $\frac{RA}{SA}$ ratio of the enantiomers is determined as follows :

SCHEME 3



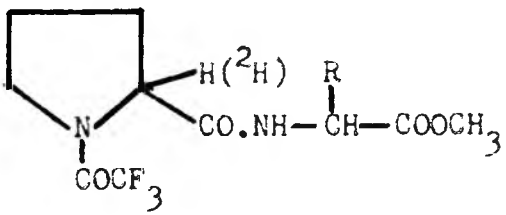


(SA,RA)

Chiral Reagent

(RB,SB*)

- $COOCH_3$



Peak 1 : RASB*, SARB

Peak 2 : SASB*, RARB

The intensity due to RA in the first GC peak =

$$(RASB - \frac{x}{100} SARB) + 0.2007\ (RASB - \frac{x}{100} SARB)$$

The intensity due to SA in the first GC peak =

$$(SARB - 0.2007(RASB - \frac{x}{100} SARB)$$

The intensity due to RA in the second GC peak =

$$(RARB - 0.2007(SASB - \frac{x}{100} RARB)$$ and

the intensity due to SA in the second GC peak =

$$(SASB - \frac{x}{100} RARB) + 0.2007\ (SASB - \frac{x}{100}\ RARB)$$

Combining the intensities due to RA in peaks 1 and 2 and dividing by the intensities due to SA in peaks 1 and 2 gives Formula 1.

Where x is the % of the natural isotopic abundances calculated from the empirical formula of the M + 1 ion of the CI MS of the TFA-peptide methyl ester and 20.07 is the percentage of unlabelled S-proline in the S-(1-^{2}H)-proline used to prepare the labelled reagent.

FORMULA 1

$$\frac{RA = (RASB^* - \frac{x}{100} SARB) + 0.2007\ (RASB^* - \frac{x}{100} SARB) + RARB - 0.2007\ (SASB^* - \frac{x}{100} RARB)}{SA = (SARB - 0.2007\ (RASB^* - \frac{x}{100} SARB) + (SASB^* - \frac{x}{100} RARB) + 0.2007\ (SASB^* - \frac{x}{100} RARB)}$$

We first applied the new procedure to the analysis of "optically pure" commercially available amines and amino acids (Table 1) [7]. The reproductibility of the method was checked by analysing an S-proline (10) solution of known optical purity ($\frac{R}{S}A$ = 1.26) six times. (The concentrations of the standard solutions were adjusted on the basis of the observed optical purity of the commercially available amino acids and amines). The mean value of $\frac{R}{S}A$ and the average mean were calculated from Formula 1 (x = 15.2%) to be 1.25 $\pm$ 0.02 .

The reaction conditions from diastereoisomer formation were checked by condensing five identical alanine (18) samples of optical purity ($\frac{R}{S}A$ = 1.32) with the resolving reagent and then working up the reaction mixture after different time intervals (0.5 min to 24 hrs.). The calculated $\frac{R}{S}A$ values were then determined by GC/MS and were found to be 1.33 $\pm$ 0.03. On the basis of these results, all other condensations were worked up within 30 minutes of adding the resolving agent.

In a similar manner a number of amine and amino acid solutions of known optical purity were analysed. In all cases the $\frac{R}{S}A$ values were within 5% of the expected values (Table 2)[7].

The technique was then used to monitor the progress of an optical resolution of 2-amino-4-methylpentane (19) and 2-methyl-piperidine (20) via salt formation with (+) -tartaric acid [8], [9]. The results indicated that after 3 recrystallisations (+)-2-methyl-piperdine (20) and (+)-2-amino-4-methylpentane (19) of 99.3% and 94.3% enantiomeric purity respectively were obtained.

Table 1: Optical purity of some commercially available amines and amino acids as determined from the diastereoisomeric N-trifluoroacetyl-R and S-(1-^{2}H)-prolyl derivatives of their methyl esters

Compound	Uncorrected Intensities				Natural # Isotopic Abundance(%) x	Calculated ratio $\frac{RA}{SA} = r$	Optical Purity $\frac{r-1}{r+1}$! !
	SARB	RASB*	RARB	SASB*			
R-proline	2,277	10,958	36,180	5,499	15.2	369	0.995
S-proline	11,472	2,070	2,441	14,345	15.2	0.000847	0.998
R-alanine	1,950	10,367	14,283	1,977	13.0	459	0.996
S-alanine	14,126	1,908	1,926	9,936	13.0	0.00237	0.995
R-leucine	1,147	5,803	8,390	1,452	16.4	133	0.985
S-leucine	7,712	1,305	814	4,099	16.4	0.00552	0.989
S-valine	57,988	9,472	7,189	37,454	15.3	0.00604	0.988

cont.

Table 1: Optical purity of some commercially available amines and amino acids as determined from the diastereoisomeric N-trifluoroacetyl-R and S-(1-^{2}H)-prolyl derivatives of their methyl esters

Compound	Uncorrected Intensities				Natural # Isotopic Abundance (%) x	Calculated ratio $\frac{RA}{SA} = r$	Optical Purity $\left\|\frac{r-1}{r+1}\right\|$!
	SARB	RASB*	RARB	SASB*			
R-1-phenylethylamine	1,072	5,163	24,163	4,455	15.2	29.4	0.934
S-1-phenylethylamine	9,094	1,651	743	3,308	15.2	0.0334	0.935
R-2-aminoheptane	299	1,258	8,949	1,491	16.3	0.0496	0.904
S-2-aminoheptane	13,773	1,395	2,374	2,909	16.3	113	0.983

Calculated from the empirical formula of the diastereoisomers

! The absolute value of $\frac{r-1}{r+1}$

Table 2: Optical Purity analysis of amines and amino acids as determined from the diastereoisomeric N-trifluoroacetyl-R and S-(1-^{2}H)-prolyl derivatives of their methyl esters

Compound	Uncorrected Intensities SARB	RASB*	RARB	SASB*	Natural Isotopic Abundance (%)# x	Calculated Ratio RA/SA	Expected Ratio RA/SA
proline	9,498	7,510	9,141	4,711	15.2	1.28	1.26
proline	5,359	4,198	4,488	2,243	15.2	1.26	1.26
proline	18,119	14,387	15,658	8,505	15.2	1.23	1.26
alanine	11,297	3,137	3,338	7,088	13.0	0.211	0.216
alanine	7,183	3,398	4,170	4,539	13.0	0.551	0.571
alanine	4,139	6,987	9,330	2,884	13.0	3.45	3.39
leucine	3,367	4,003	4,447	1,984	16.4	1.99	2.06
leucine	3,373	1,917	2,639	2,365	16.4	0.718	0.699
leucine	4,263	1,387	1,357	2,715	16.4	0.237	0.237
valine	53,373	38,655	29,939	35,609	15.3	0.714	0.704
valine	16,439	9,602	9,529	15,603	15.3	0.475	0.490
valine	19,763	8,867	7,120	14,466	15.3	0.330	0.326
valine	46,426	16,785	12,724	30,900	15.3	0.234	0.245

cont.

Table 2: Optical Purity analysis of amines and amino acids as determined from the diastereoisomeric N-trifluoroacetyl-R and S-(1-^{2}H)-prolyl derivatives of their methyl esters

Compound	Uncorrected Intensities SARB	RASB*	RARB	SASB*	Natural Isotopic Abundance (%)# x	Calculated Ratio RA/SA	Expected Ratio RA/SA
1-phenylethylamine	6,010	2,911	11,750	5,661	15.2	1.30	1.25
1-phenylethylamine	1,951	1,518	6,652	2,297	15.2	2.42	2.38
1-phenylethylamine	10,697	3,438	10,038	7,545	15.2	0.627	0.656
2-aminoheptane	2,372	1,213	5,462	1,317	16.3	2.34	2.32
2-aminoheptane	4,822	1,127	2,209	1,198	16.3	0.425	0.420
2-aminoheptane	2,076	643	2,080	720	16.3	0.958	0.970

Calculated from the empirical formula of the diastereoisomers.

The use of a resolving agent that could be made synthetically, and thus would be isotopically pure was then investigated. Since both enantiomers of thiazolidine-4-carboxylic acid (24) are readily available[10] (Scheme 4) and deuterium can be incorporated into position 1 with deuteroformaldehyde N-TFA-thiazolidine-4-carboxylic acid chloride (21) was investigated as a resolving agent [10], [11]. For the derivatives of this resolving agent the $\underline{RA}/\underline{SA}$ ratio of the enantiomers is given by the Formula 2 :

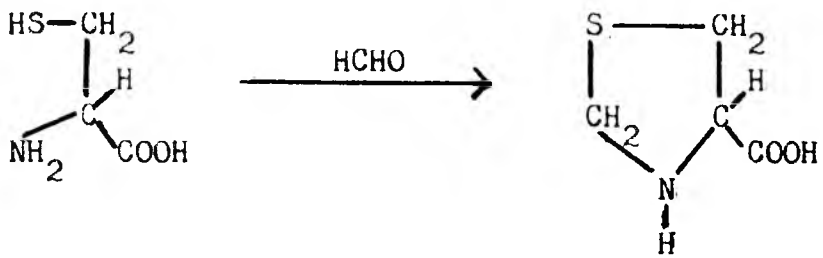
FORMULA 2

$$\frac{\underline{RA}}{\underline{SA}} = \frac{\underline{RARB} + (\underline{RASB}^* - \frac{x}{100}\,\underline{SARB})}{\underline{SARB} + (\underline{SASB}^* - \frac{x}{100}\,\underline{RARB})}$$

where x is the % of the natural isotopic abundances calculated from the empirical formula of the M + 2 ion of the CI MS of the TFA-peptide methyl ester.

S-(1-$^2H_2$)-thiazolidine-4-carboxylic acid (22) was synthesised from S-cysteine (23) and deuteroformaldehyde[10], and R-thiazolidine-4-carboxylic acid (24) was synthesised from R-cysteine (23) and formaldehyde. The N-TFA-acid chloride derivatives of these amino acids were then synthesised and used as a resolving agent. The reagent was first used to analyse solutions of known optical purity of proline (10) and then solutions containing valine (4) and alanine (18); and 2-aminoheptane (25) and 1-phenylethylamine (26)combined (Tables 3 and 4) [12]. In all cases the $\underline{RA}/\underline{SA}$ values were within 5% of

SCHEME 4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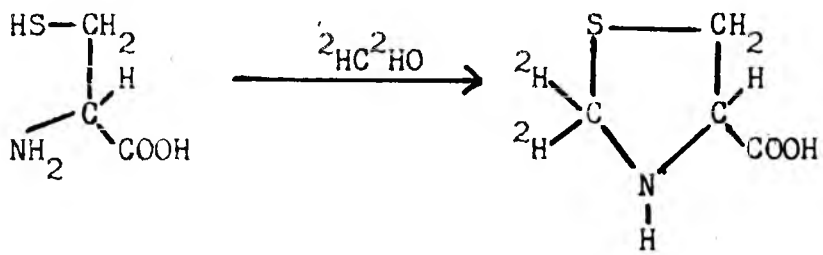
C
NH2
COOH
HCHO
S—CH2
CH2
H
C
COOH
N
H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2H
2H
C
H
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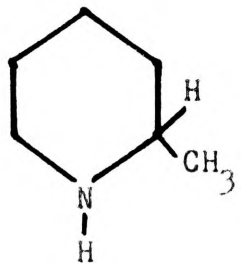

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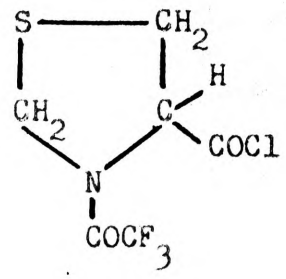

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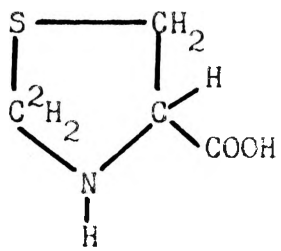

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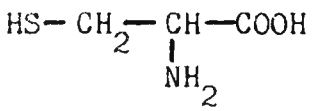

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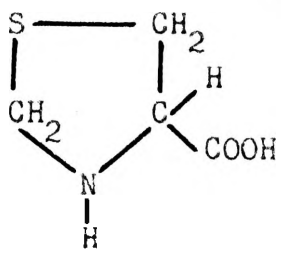

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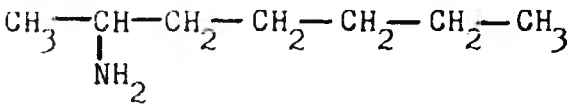

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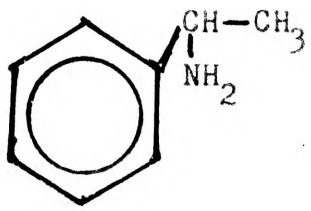

(26)

the expected values. Unfortunately, these derivatives proved particularly unstable and satisfactory analyses could not be obtained on samples that were not kept refrigerated after preparation.

Table 3: Optical Purity of some commercially available amino acids and amines as determined from their diastereoisomeric N-trifluoroacetyl-R and S (1-$^{2}H_2$) -thiazolidine-4-carbamoylamino acid methyl esters and amides

Compound	Uncorrected Intensities SARB	RASB*	RARB	SASB*	Natural Isotopic Abundance (%)# x	Calculated Ratio RA/SA = r	Optical Purity $\frac{r-1}{r+1}$!
R-proline	4.2	303.7	108.4	7.5	4.80	59.6	0.967
S-proline	53.7	6.5	6.2	223.1	4.80	0.0373	0.928
R-valine	1.4	70.8	6.8	0.7	4.80	45.6	0.957
S-valine	109.6	7.6	3.1	205.4	4.80	0.0184	0.964
R-alanine	3.1	156.8	74.5	6.2	4.80	38.4	0.949
S-alanine	22.3	3.3	3.6	60.6	4.80	0.0508	0.903
S-2-aminoheptane	133.9	11.1	26.4	264.7	4.80	0.0794	0.853
R-2-aminoheptane	3.4	85.4	98.8	7.8	4.80	27.1	0.929
S-phenylethylamine	20.6	1.7	2.6	42.5	4.80	0.0531	0.899
R-phenylethylamine	2.5	48.7	57.0	4.7	4.80	14.7	0.873

calculated from the empirical formula of the diastereoisomers

! The absolute value of $\frac{r-1}{r+1}$

Table 4: Optical Purity analysis of amino acids and amines as determined from their diastereoisomeric N-trifluoroacetyl-R- and S-(1-$^2H_2$)-thiazolidine-4-carbamoylamino acid methyl esters and amides

Compound	Uncorrected Intensities				Natural Isotopic Abundance #	Calculated Ratio RA/SA	Expected Ratio RA/SA
	SARB	RASB*	RARB	SASB*			
proline	179.9	181.9	43.3	49.5	4.80	0.955	0.983
proline	121.4	223.3	35.5	14.5	4.80	1.89	1.88
proline	113.4	73.8	15.5	49.6	4.80	0.519	0.509
alanine and	182.6	318.0	146.3	46.2	4.80	2.05	2.10
valine	147.8	67.8	26.1	39.4	4.80	0.469	0.449
alanine and	360.1	510.2	267.4	119.0	4.80	1.63	1.61
valine	56.8	15.1	4.8	21.4	4.80	0.223	0.235
1-phenylethylamine and 2-aminoheptane	53.7	37.6	138.5	49.5	4.80	1.79	1.75
	324.9	91.0	318.6	315.4	4.80	0.631	0.646
1-phenylethylamine and 2-aminoheptane	324.6	51.4	148.8	369.0	4.80	0.270	0.265
	253.2	443.1	760.9	138.5	4.80	3.33	3.27

calculated from the empirical formula of the diastereoisomers

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EXPERIMENTAL

Reagents were obtained as follows : Thionyl chloride and all S-amino acids from B.D.H. Chemicals Ltd. Poole, England, R-alanine, R-cysteine and R and S-phenylethylamine from Fluka, Buchs, Switzerland, R-proline from Calbiochem, San Diego, Cal. U.S.A., R-leucine from Cyclo, Los Angeles, Cal. U.S.A., R and S-2-aminoheptane from Norse, Newbury Park, Cal. U.S.A., and trifluoroacetic anhydride from Sigma, St. Louis, U.S.A.

All solvents used were analytical reagent grade and were supplied by Ajax Chemicals, Sydney, N.S.W., Australia. D_2O (99.7%) was obtained from the Australian Atomic Energy Commission, Lucas Heights, N.S.W., Australia.

PART 1

Preparation of R and S-($^2H_7$)-2-chloro-3-methylbutanoyl (R, S) amino acid methyl esters and N-TFA-R and S-($^2H_7$)-prolyl (R, S) amino acid methyl esters.

G.C. separations were carried out using a 5 ft. (2 mm I.D.) glass column coated with 6% Apiezon N on Chromosorb W (80 - 100 mesh). The samples were chromatographed with nitrogen as a carrier gas at a flow rate of 20 ml/minute. All samples were run isothermally at 130°. Under these conditions the retention times of the 2-chloro-3-methylbutanoyl amino acid methyl ester diastereoisomeric pairs were : valine (8.5, 9.3 min) and alanine (7.4, 8.7 min). The retention times of the ($^2H_7$)-2-chloro-3-methylbutanoyl amino acid diastereoisomeric pairs were valine (8.2, 9.0 min) and alanine (7.1, 8.4 min). The retention times of the

N-trifluoroacetyl prolyl amino acid methyl esters were : valine (7.9, 8.7 min) and alanine (7.3, 8.5 min). The retention times of the N-trifluoroacetyl-($^{2}H_{7}$)-prolyl amino acid methyl esters were : valine (6.9, 7.7 min) and alanine (6.0, 7.1 min).

Preparation of R-2-chloro-3-methylbutanoyl chloride

A mixture of concentrated hydrochloric acid (1.5 ml) and concentrated nitric acid (0.5 ml) was added to S-valine (500 mg) at 40-50°C. After 20 minutes the mixture was extracted with ether, and the ether extracts washed with water and dried over anhydrous sodium sulphate. The solvent was removed under vacuum and the residue treated with thionyl chloride (1 ml) at 60°C for 1 hour. Excess thionyl chloride was removed under vacuum and the residue distilled to yield R-2-chloro-3-methylbutanoyl chloride (360 mg. b.p. 150-152°C) which was dissolved in methylene chloride (100 ml).

Preparation of S-2-chloro-($^{2}H_{7}$)-3-methylbutanoyl chloride

This compound was prepared in identical manner to R-2-chloro-3-methylbutanoyl chloride using R-($^{2}H_{7}$)-valine [1] instead of S-valine.

Preparation of R and S-($^2H_7$)-2-chloro-3-methylbutanoyl (R, S) amino acid methyl esters

Methanol (1 ml) was cooled in a dry ice-alcohol bath and thionyl chloride (0.1 ml) added dropwise. This solution was added to the amino acid (10 μmol) and the resulting solution refluxed for 30 min. The reaction mixture was then concentrated under vacuum and dry methanol (0.5 ml) added and the reaction mixture again concentrated under vacuum. To the residue was then added a mixture of R-2-chloro-3-methylbutanoyl chloride reagent (0.55 ml) and S-2-chloro-($^2H_7$)-3-methylbutanoyl chloride reagent (0.55 ml) and the resulting solution was adjusted to pH9 with triethylamine. The reaction mixture was then washed in turn with water (2 ml)HCl (2 ml, 1 M) and $NaHCO_3$ solution (10%, 2 ml) and then dried over Na_2SO_4. The solvent was then removed and the residue dissolved in dry methanol (0.20 ml) for GC analysis.

Preparation of N-Trifluoroacetyl-S-($^2H_7$)-prolyl chloride

Trifluoroacetic anhydride (0.4 ml) dissolved in anhydrous ether was added to S-($^2H_7$)-proline (100 mg) at -10°C. After 10 minutes the flask was removed from the cooling bath and kept at room temperature for 2 hours. The ether and unreacted trifluoroacetic anhydride were removed under vacuum at room temperature. A solution of thionyl chloride (1 ml) in dry benzene (2 ml) was then added to the residue at 0°C and the reaction mixture kept at room temperature for a further 2.5 hours. The benzene and unreacted thionyl chloride were then removed under vacuum at below 40°C. The residue was dissolved in dichloromethane and the

solvent then removed under vacuum. This process was repeated twice more. The residue was then transferred quantitatively to a volumetric flask with dry dichloromethane and made up to 100 ml. Molecular sieve (Linde Type 3A) was then added and the reagent stored at $0^{o}C$.

Preparation of N-Trifluoroacetyl-R-prolyl chloride

This compound was prepared in identical manner to N-trifluoroacetyl S-($^{2}H_{7}$)-prolyl chloride using R-proline instead of S-($^{2}H_{7}$)-proline.

Preparation of N-trifluoroacetyl-R and S-($^{2}H_{7}$)-prolyl (R, S) amino acid methyl esters

Methanol (1 ml) was cooled in a dry ice-alcohol bath and thionyl chloride (0.1 ml) added dropwise. This solution was added to the amino acid (10 μmol) and the resulting solution refluxed for 30 min. The reaction mixture was then concentrated under vacuum and dry methanol (0.5 ml) added and the reaction mixture again concentrated under vacuum. To the residue was then added a mixture of N-trifluoroacetyl-R-prolyl chloride reagent (0.55 ml) and N-trifluoroacetyl-S-($^{2}H_{7}$)-prolyl chloride reagent (0.55 ml), and the resulting solution was adjusted to pH9 with triethylamine. The reaction was then washed in turn with water (2 ml) HCl (2 ml, 1 M) and $NaHCO_{3}$ solution (10%, 2 ml) and then dried over $Na_{2}SO_{4}$. The solvent was then removed and the residue dissolved in dry methanol (0.20 ml) for analysis by GC.

PART 2

Preparation of N-TFA-R and S-(1-^{2}H)-prolyl (R, S) amino acid methyl esters and amides

Reagents were obtained as previously described.

Mass spectra were recorded on a Finnigan 3200 chemical ionisation mass spectrometer interfaced to a Finnigan Model 6110 Data System. GC separations were carried out using a 5 ft (2 mm ID) glass column coated with either Tabsorb (Supelco) or 3% OV-225 on Gas Chrom.Q (100-120 mesh) (Applied Science Lab. State College Pen., U.S.A.)

The samples were chromatographed with methane as a carrier gas at a flow rate of 20 ml/minute. The following chromatographic conditions were used : alanine (Tabsorb) programmed 1 minute after injection from 160^{o} at 6^{o}/min; 2-aminoheptane (OV-225) from 140^{o} at 6^{o}/min. 2-amino-4-methylpentane (Tabsorb) from 125^{o} at 4^{o}/min; glycine (Tabsorb) from 160^{o} at 6^{o}/min; leucine (Tabsorb) from 170^{o} at 6^{o}/min; 2-methylpiperidine (OV-225) from 140^{o} at 6^{o}/min; 1-phenylethylamine (OV-225) from 150^{o} at 10^{o}/min; proline (OV-225) from 200^{o} at 6^{o}/min; and valine (Tabsorb) from 150^{o} at 6^{o}/min.

Under the above conditions the retention times of the diastereoisomeric pairs were : alanine (4.1, 4.7 min), 2-amino-heptane (7.5, 8.1 min), 2-amino-4-methylpentane (4.8, 5.3 min), leucine (4.4, 4.7 min), 2-methylpiperidine (8.8, 9.2 min), 1-phenylethylamine (7.2, 7.8 min), proline (6.2, 6.5 min) and valine (3.9, 4.6 min).

Preparation of S-(1-^{2}H)-proline

To a slurry of trans-$[Co(en)_2Br_2]Br$ (20.9g) [2] and S-proline (5.75 g) in methanol (800 ml) at 60° was added $LiOH.H_2O$ (2.09g) in methanol (200 ml). The solution was refluxed for 45 minutes, diluted with water (3 l), and sorbed on Dowex 50WX2 exchange resin (H^+ form). Elution with HCl(1M) resulted in the separation of three major bands $[Co(en)_2Br(MeOH)]^{2+}$, $[Co(en)_2$ (S)-pro$]^{2+}$, and $[Co(en)_3]^{3+}$, in order of elution. The $[Co(en)_2$ (S)-pro$]^{2+}Cl_2$ (60-70%) was reduced to dryness on a rotary evaporator and dissolved in water and the solution neutralised (pH ∿ 5) with 0.5M LiOH solution. Addition of NaI and cooling resulted in a preferential crystallisation of one of the diasteroisomers Δ-$[Co(en)_2$ (S)-pro$]I_2$. This material was crystallised from warm water, and all fractions had similar rotations: $[\alpha]_{589}$, - 279°; $[\alpha]_{546}$ - 471° in water at 25° from an 0.1% solution. Anal. Calcd for $[Co(en)_2$ (S)-pro$]I_2.H_2O$: C, 19.1; H, 4.6; N, 12.4. Found C, 19.1; H, 4.7; N, 12.1.

Δ-$[Co(en)_2$ (S)-pro$]I_2$ (11 g) was dissolved in D_2O (100 ml) and $NaDCO_3$(0.1g) was added. The D_2O was removed in vacuo and the residue was redissolved in D_2O (100 ml) and Li_2CO_3 (2 g) added. The mixture was then left for 48 hours. The mixture was then filtered to remove undissolved Li_2CO_3 and the pH adjusted to 1 by the addition of conc. HCl (13 ml). The Co^{3+} was reduced to Co^{2+} by the addition of $NaBH_4$ (1.2g) which was added slowly with vigorous stirring. The pH was kept less than 4 by the addition of conc. HCl. The reduced solution was diluted to 1l and was sorbed on AG50W-X8 cation exchange resin

(H^+ form) and the S-(1-^{2}H)-proline was eluted with ammonia solution (10%). The ammonia eluate was reduced to dryness at 40°C. The yield of S-(1-^{2}H)-proline was 1.4g.

The S-(1-^{2}H)-proline (80 mg) was converted to N-TFA-S-(1-^{2}H)- prolyl chloride and this was condensed with glycine methyl ester as described below. The product was analysed by GC/MS and the intensities of the protonated molecular ions (*m/e* 283 and 284) were recorded for duplicate runs. These were 410, 2102 and 8392, 42526 respectively. After correcting for the theoretical natural isotopic abundances of the M + 1 ion in the CI MS of the non-deuterated species (11.55%) the average isotopic purity of the S-(1-^{2}H)-proline was calculated to be 83.28%.

Preparation of N-Trifluoroacetyl-R-prolyl chloride [3]

Trifluroacetic anhydride (0.4 ml) dissolved in anhydrous ether (2 ml) was added to R-proline (100-120 mg) at -10°C. After 10 minutes the flask was removed from the cooling bath and kept at room temperature for 2 hours. The ether and unreacted trifluoroacetic anhydride were removed under vacuum at room temperature. A solution of thionyl chloride (1 ml) in dry benzene (2 ml) was then added to the residue at 0°C and the reaction mixture kept at room temperature for a further 2.5 hours. The benzene and unreacted thionyl chloride were then removed under vacuum at below 40°C. The residue was dissolved in dichloromethane (0.5 ml) and the solvent then removed under vacuum. This process was repeated twice more. The residue was then transferred quantitatively to a volumetric flask with dry dichloromethane and made up to 100 ml. Molecular sieve (Linde Type 3A) was then

added and the reagent stored at 0°C.

Preparation of N-Trifluoroacetyl-S-(1-^{2}H)-prolyl chloride

This compound was prepared in identical manner to N-trifluoroacetyl-R-prolyl chloride using S-(1-^{2}H)-proline (80-100 mg) instead of R-proline.

Preparation of N-trifluoroacetyl-R and S-(1-^{2}H)-prolyl (R, S) amino acid methyl esters

Methanol (1 ml) was cooled in a Dry Ice-alcohol bath and thionyl chloride (0.1 ml) added dropwise. This solution was added to the amino acid (10 μmoles) and the resulting solution refluxed for 30 minutes. The reaction mixture was then concentrated under vacuum and dry methanol (0.5 ml) added and the reaction mixture again concentrated under vacuum. To the residue was then added a mixture of N-trifluoroacetyl-R-prolyl chloride reagent (0.55 ml) and N-trifluoroacetyl-S-(1-^{2}H)-prolyl chloride (0.55 ml) and the resulting solution was adjusted to pH9 with triethylamine. The reaction mixture was then washed in turn with water (2 ml) HCl (2 ml, 1M) and $NaHCO_3$ solution (10%, 2 ml) and then dried over Na_2SO_4. The solvent was then removed and the residue dissolved in dry methanol (0.20 ml) for analysis by GC/MS.

The following MH^+ ions were monitored for alanine (297, 298), for leucine (339, 340) for proline (323, 324) and for valine (325, 326).

Preparation of N-Trifluoroacetyl-R and S-(1-^{2}H)-prolyl amides

To a solution of the amine (10μmoles) in dichloromethane (5 ml) was added a mixture of N-trifluoroacetyl-R-prolyl chloride (0.55 ml) and N-trifluoroacetyl-S-(1-^{2}H)-prolyl chloride (0.55 ml) and the resulting solution to pH 9 with triethylamine. The reaction mixture was then washed in turn with water (2 ml), HCL (5 ml, 1M) and $NaHCO_3$ solution (10%, 5 ml) and then dried over Na_2SO_4. The solvent was then removed and the residue dissolved in dry methanol (0.20 ml) for analysis by GC/MS.

The following MH^+ ions were monitored for 2-aminoheptane (309, 310), for 2-amino-4-methylpentane (295, 296) for 2-methylpiperidine (293, 294) and for 1-phenylethylamine (315, 316).

Establishment of Reactions Conditions for formation of Diastereoisomers

Standard solutions of alanine were prepared by dissolving S-alanine (270.62 mg) in distilled water (100 ml) and R-alanine (358.29 mg) in distilled water (100 ml). 10 ml of both solutions were then mixed and five 1 ml samples of the resulting solution were freeze dried. The residues were esterified as described above and treated with the mixed resolving agent (4 ml) at pH 9 as described above. The samples were then worked up after time intervals or 0.5 minute, 30 minutes, 60 minutes, 120 minutes and 24 hours. The calculated $^{RA}/_{SA}$ ratios as determined by GC/MS were found to be 1.32, 1.36, 1.36, 1.26 and 1.36 respectively.

Preparation of Reference Solutions of Amino Acids of known Optical Purity

Standard solutions of amino acids were prepared by dissolving R-proline (51.56 mg), S-proline (41.10 mg), R-alanine (162.17 mg), S-alanine (189.85 mg), R-leucine (43.96 mg), S-leucine (62.97 mg), S-valine (144.10 mg), and R, S-valine (273.19 mg) in turn in distilled water (50 ml). From each solution a 2.0 ml sample was withdrawn and freeze dried. It was then condensed with the mixed resolving agent as described above (Table 1).

The R and S amino acid solutions were then used to prepare reference samples of known optical purity by pipetting different volumes of each of the two enantiomeric amino acid solutions. For proline three samples were prepared each containing 1.0 ml of both the R and S -proline standards, for alanine three samples were prepared containing 0.50 ml of the R and 2.0 ml of S-alanine standards, 1.0 ml of the R and 1.5 ml of S-alanine, and 2.0 ml of the R and 0.50 ml of S-alanine; for valine four samples were prepared containing in each case 0.50 ml of the R, S-valine standard with 0.20 ml, 0.50 ml, 1.0 ml and 1.5 ml of S-valine; for leucine three samples were prepared containing 1.5 ml of the R and 0.5 ml of S-leucine standard, 1.0 ml of both R and S-leucine, and 0.5 ml of R with 1.5 ml of S-leucine.

These reference samples were then freeze dried and condensed with the resolving agent (1-5 ml) as described above (Table 2).

Preparation of Reference solutions of Amines of known Optical Purity

Standard solutions of amines were prepared by dissolving R-1-phenylethylamine (121.1 mg), S-1-phenylethylamine (95.2 mg), S-2-aminoheptane (110.26 mg) and R-2-aminoheptane (100.48 mg) in turn in dichloromethane (100 ml). A 2.0 ml sample of each solution was condensed with the mixed resolving agent as described above (Table 1).

The R and S amine solutions were then used to prepare reference samples of know optical purity by pipetting different volumes of the two enantiomeric amine solutions. For 1-phenylethylamine three samples were prepared containing 2.0 ml of both R and S-1-phenylethylamine standards, 2.0 ml of the R and 1.0 ml of S-1-phenylethylamine, and 1.0 ml of the R and 2.0 ml of the S-1-phenylethylamine; for 2-aminoheptane three samples were prepared containing 5.0 ml of the R and 2.0 ml of S-2-aminoheptane standards, 2.0 ml of the R and 5.0 ml of S-2-aminoheptane, and 2.0 ml of both R and S-2-aminoheptane. These reference samples were then condensed with the resolving agent (1.5 ml) as described above (Table 2).

Preparation of the N-Trifluoroacetyl-R and S-(1-^{2}H)-prolyl derivatives of (+)-2-methylpiperidine and (+)-2-amino-4-methylpentane

In separate experiments the racemic amine (5 g) was added to a warm solution of (+)-tartaric acid (8.4 g) in methanol (20 ml) and allowed to cool. The resulting tartrate

was recrystallised three times from methanol (20 ml, 10 ml and 8 ml) [4] [5]. A sample of the (+)-tartrate salt of the amine (1 mg) was then dissolved in Na OH (10%, 1 ml) and the solution extracted with dichloromethane (2 ml). The extract was dried over anhydrous Na_2SO_4 and treated with the mixed reagent as described above. Analysis by GC/MS indicated enantiomeric purities for (+)-2-methylpiperidine and (+)-2-amino-4-methylpentane of 99.3% and 94.3% respectively.

PART 3

Preparation of N-TFA-R and S-(1-$^2H_2$) thiazolidine-4-carbamoylamino acid methyl esters and amides

Reagents were obtained as previously described.

Mass spectra were recorded on a MAT-44 digital GC/MS. system. GC separations were carried out using an open-coupled 25 m SE-S4 (Jaeggi) WCOT column, 0.3 mm I.D.

The samples were chromatographed with helium as a carrier gas at a flow rate of 2 ml/minute. The following chromatographic conditions were used : alanine, valine programmed 1 minute after injection from 150° at 6°C /min; proline programmed 1 minute after injection from 150°C at 10°C /min; 1-phenylethylamine, 2-aminoheptane programmed 1 minute after injection from 150°C at 4°C /min and programmed at 10°C /min 9 minutes after injection.

Under the above conditions the retention times of the diastereoisomer pairs were : alanine (4.7, 5.0 min), 2-aminoheptane (6.7, 7.0), 1-phenylethylamine (10.1, 10.7), proline (3.7, 4.1 min) and valine (5.9, 6.4 min).

Preparation of R-thiazolidine-4-carboxylic acid

To a solution of R-cysteine hydrochloride[6] (4 g) in water (10 ml) was added formaldehyde solution (2.5 ml, 40%) and the solution stirred for 3 hours at room temperature. The pH was then adjusted to 4 with pyridine, and ethanol (10 ml) added to yield crystals of R-thiazolidine-4-carboxylic acid (3 g, m.p. 196-197°C).

Preparation of S-(1-$^{2}H_{2}$)-thiazolidine-4-carboxlyic acid

This compound was prepared in identical manner to R-thiazolidine-4-carboxylic acid using S-cysteine hydrochoride and deuteroformaldehyde instead of R-cysteine hydrochloride and formaldehyde.

Preparation of N-trifluoroacetyl-R-thiazolidine-4-carboxylic acid chloride

Trifluoroacetic anhydride (0.4 ml) dissolved in anhydrous ether (2 ml) was added to R-thiazolidine-4-carboxylic acid (80-100 mg) at -10°C. After 10 minutes the flask was removed from the cooling bath and kept at room temperature for 2 hours. The ether and unreacted trifluoroacetic anhydride were removed under vacuum at room temperature. A solution of thionyl chloride (1 ml) in dry benzene (2 ml) was then added to the residue at 0°C and the reaction mixture kept at room temperature for a further 2.5 hours. The benzene and unreacted thionyl chloride were then removed under vacuum at below 40°C. The residue was dissolved in dichloromethane (0.5 ml) and the solvent then removed under vacuum. This process was repeated twice more. The residue was then transferred quantitatively to a volumetric flask with dry dichloromethane and made up to 100 ml. Molecular sieve (Linde Type 3A) was then added and the reagent stored at 0°C.

Preparation of N-trifluoroacetyl-S-(1-$^2H_2$)-thiazolidine-4-carboxylic acid chloride

This compound was prepared in identical manner to N-trifluoroacetyl-R-thiazolidine-4-carboxylic acid chloride using S-(1-$^2H_2$)-thiazolidine-4-carboxylic acid instead of R-thiazolidine-4-carboxylic acid.

Preparation of R and S-(1-$^2H_2$)-thiazolidine-4-carbamoylamino acid methyl esters

Methanol (1 ml) was cooled in a Dry Ice-alcohol bath and thionyl chloride (0.1 ml) added dropwise. The solution was added to the amino acid (10-20 μmoles) and the resulting solution refluxed for 30 minutes. The reaction mixture was then concentrated under vacuum and dry methanol (0.5 ml) added and the reaction mixture again concentrated under vacuum. To the residue was then added a mixture of N-trifluoroacetyl-R-thiazolidine-4-carboxylic acid chloride reagent (0.5 - 1.0 ml) and N-trifluoroacetyl-S-(1-$^2H_2$)-thiazolidine-4-carboxylic acid chloride (0.5-1.0 ml) and the resulting solution was adjusted to pH 9 with triethylamine. The reaction mixture was then washed in turn with water (2 ml) HCL (2 ml, 1M) and $NaHCO_3$ solution (10%, 2 ml) and then dried over Na_2SO_4. The solvent was then removed and the residue dissolved in dry methanol (0.10 ml) for analysis by GC/MS.

Preparation of Reference Solutions of Amino Acids of known Optical Purity

Standard solutions of S-amino acids were prepared by dissolving S-proline (189.63 mg), S-valine (250.91 mg) and

S-alanine (126.39 mg) in turn in distilled water (500 ml). Standard solutions of R-amino acids were prepared by dissolving R-proline (38.77 mg), R-valine (22.51 mg) and R-alanine (53.54 mg) in turn in distilled water (100 ml). From both proline solutions a 1.0 ml sample was withdrawn and freeze-dried. From the S-valine and S-alanine solutions 1.0 ml samples were withdrawn, combined and freeze dried. From the R-valine and R-alanine solutions 1.0 ml samples were withdrawn, combined and freeze dried. These samples were then condensed with the mixed resolving agent as described above (Table 3).

The R and S amino acid solutions were then used to prepare reference samples of known optical purity by pipetting different volumes of the enantiomeric amino acid solutions. For proline three samples were prepared containing 10.0 ml of the R and 10.0 ml of the S-proline standards, 20.0 ml of the R and 10.0 ml of the S-proline, and 10.0 ml of the R and 20.0 ml of the S-proline. For alanine and valine two samples were prepared; the first contained 10.0 ml of each alanine and valine solution, the second containing 20 ml of the S and 15 ml of the R-alanine standards and 20 ml of the S and 10 ml of the R-valine standards. 1.0 ml aliquots of these reference samples were then freeze dried and condensed with the mixed resolving agent (1.5 ml) as described above (Table 4).

Preparation of Reference Solutions of Amines of known Optical Purity

Standard solutions of amines were prepared by dissolving R-1-phenylethylamine (106.78 mg), S-1-phenylethylamine (194.59 mg), R-2-aminoheptane (159.68 mg) and S-2-aminoheptane (114.65 mg) in

turn in dichloromethane (100 ml). From each solution a 5.0 ml sample was withdrawn and condensed with the mixed resolving agent as described above (Table 3). The R and S amine solutions were then used to prepare two reference solutions of known optical purity by pipetting different volumes of the enantiomeric amine solutions. The first contained 5 ml of the R-2-aminoheptane and S-1-phenylethylamine solutions and 2 ml of the S-2-aminoheptane and R-1-phenylethylamine solutions. The second contained 2 ml of the R-2-aminoheptane and S-1-phenylethylamine solutions and 5 ml of the S-2-aminoheptane and R-1-phenylethylamine solutions. The resulting solutions were then condensed with the mixed resolving agent as described above (Table 4).

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APPENDIX

GC CI MS outputs of commercially available amino acids and amines (Table 1).

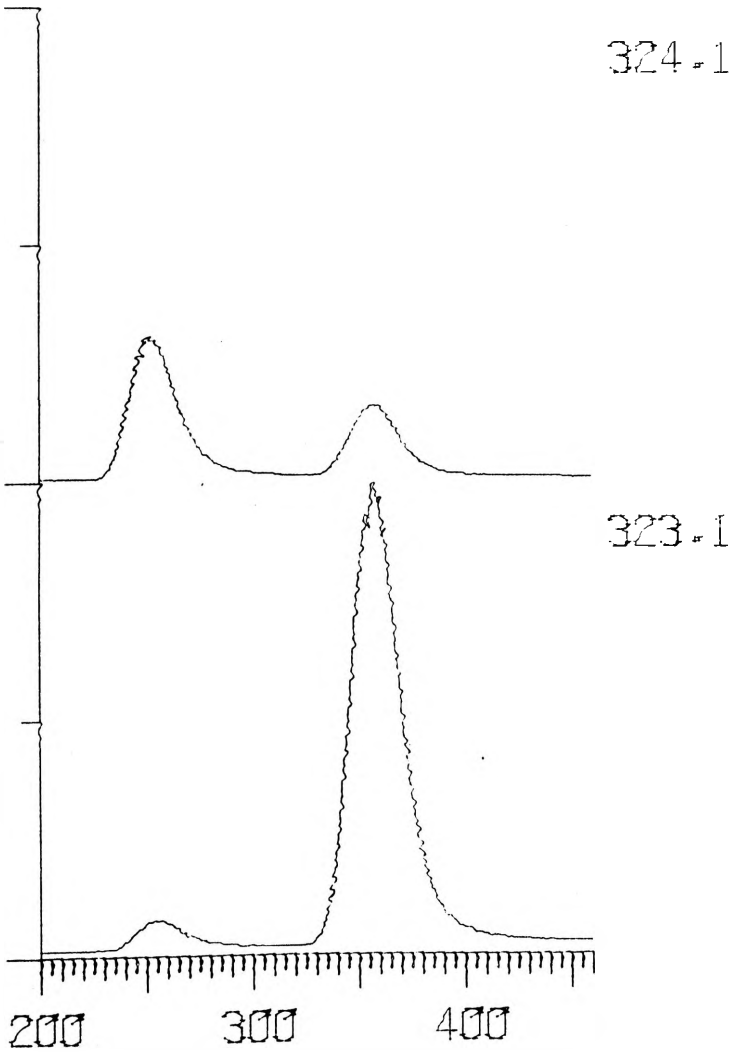
GC CI MS analyses of amino acids and amines (Table 2).

GC CI MS outputs of commercially available amino acids and amines (Table 3).

GC CI MS analyses of amino acids and amines (Table 4)

A09207

3% OV-225 200/6 CI/CH4



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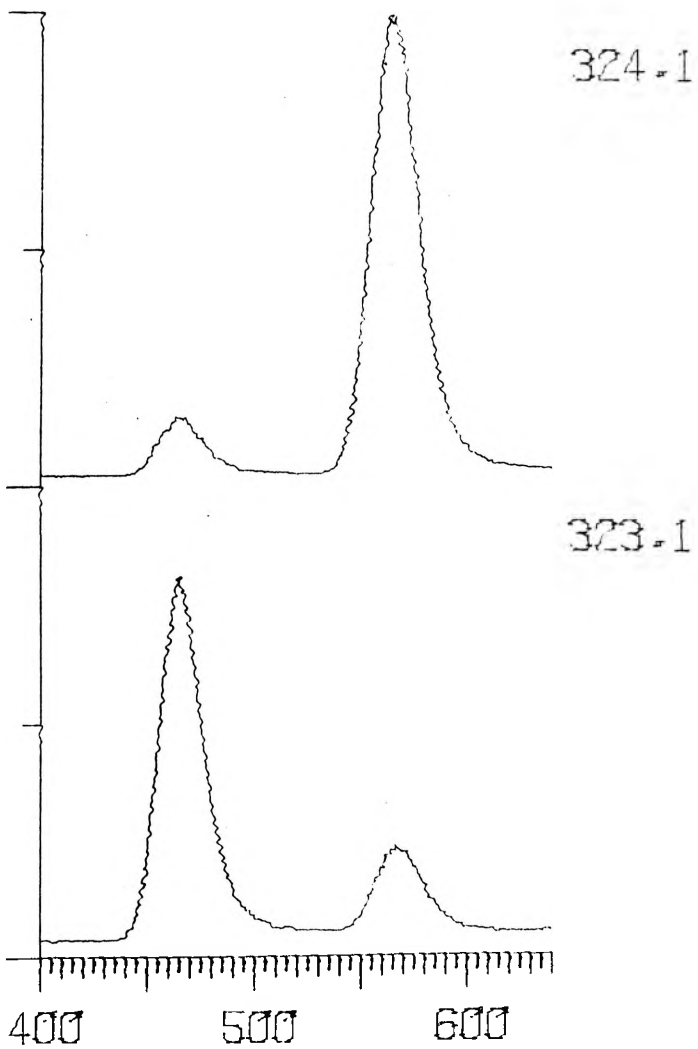

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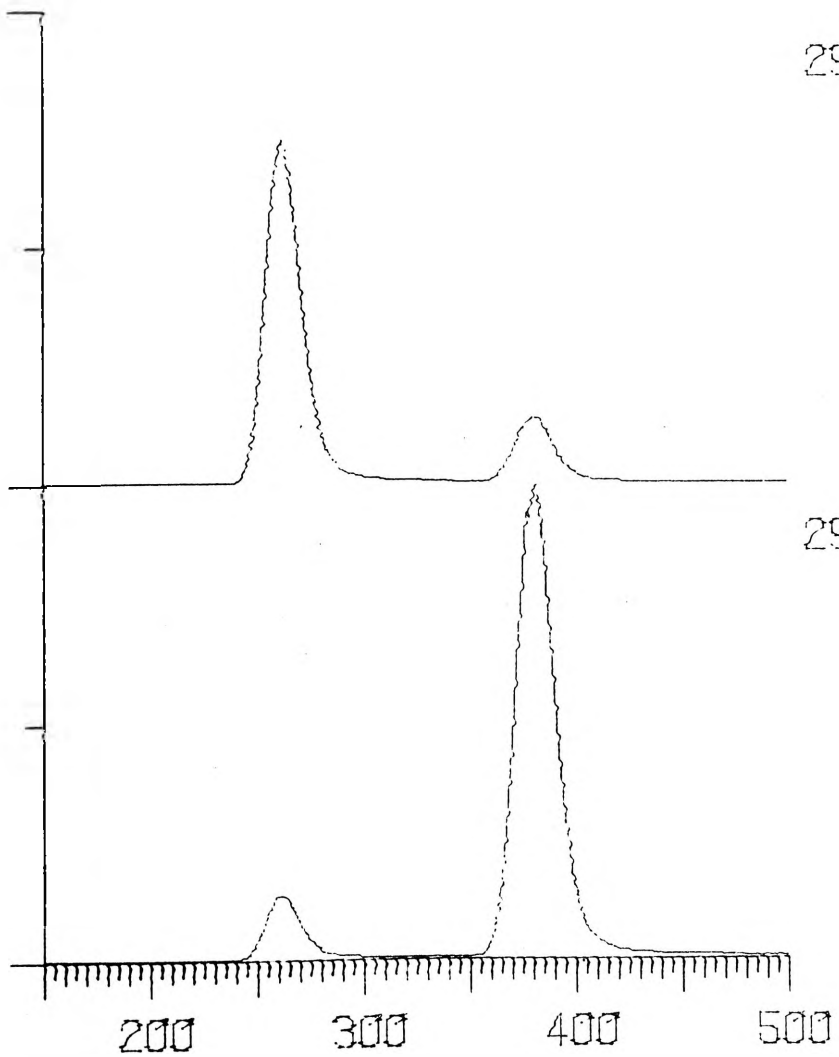

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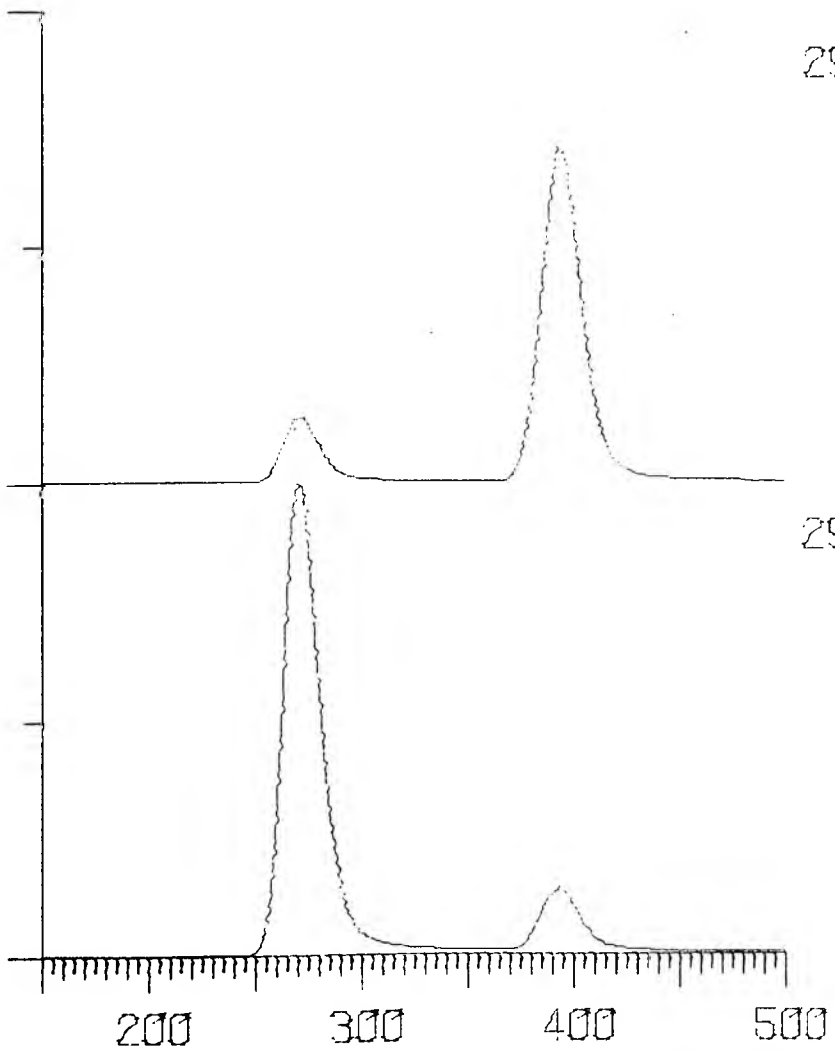

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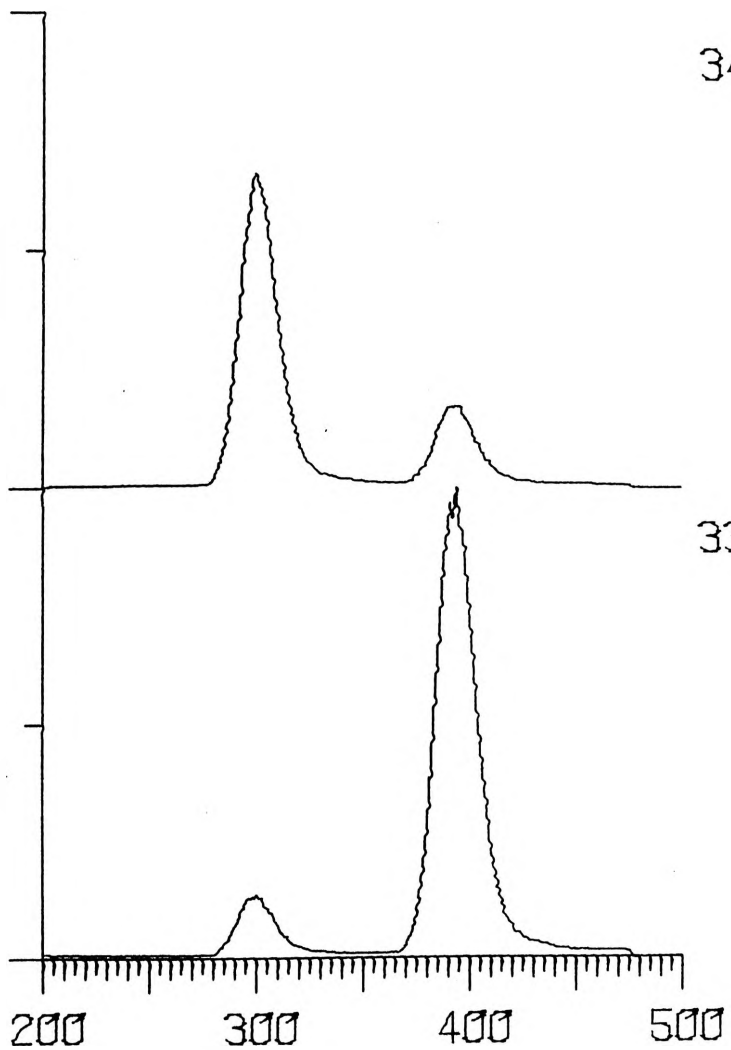

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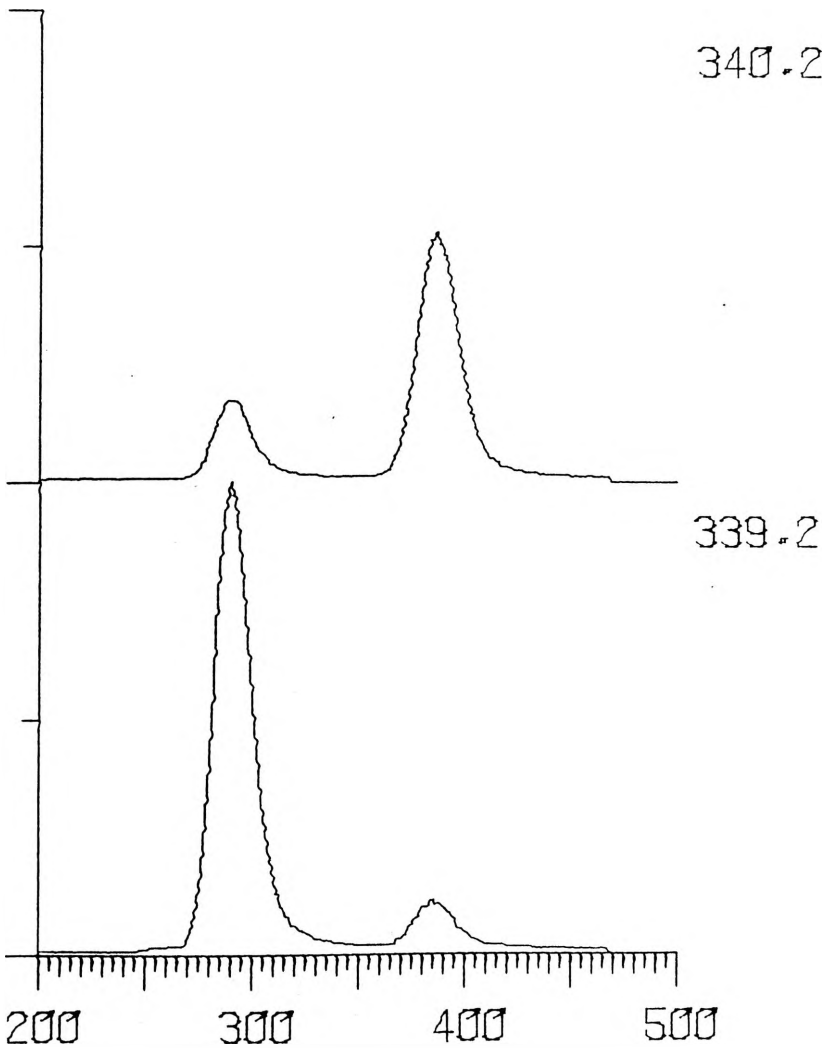

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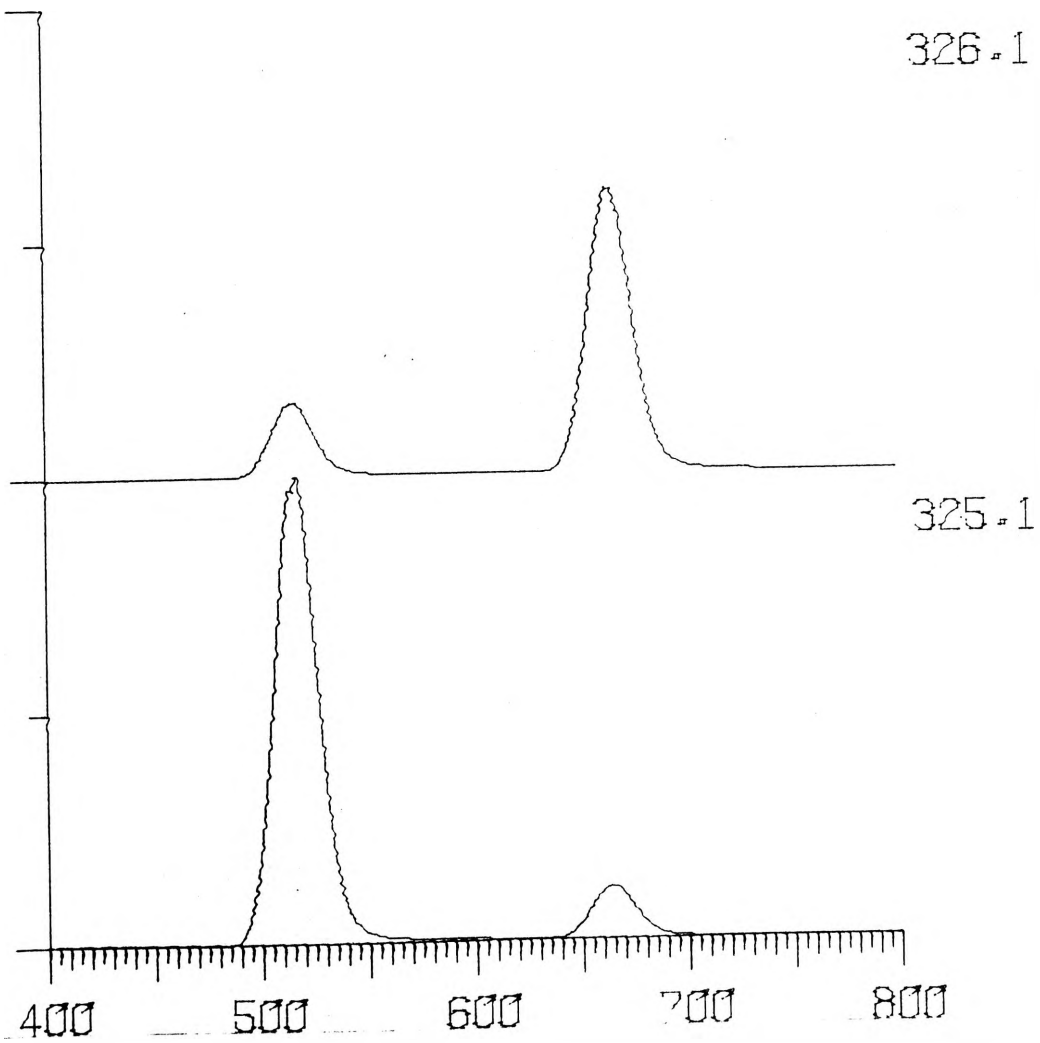

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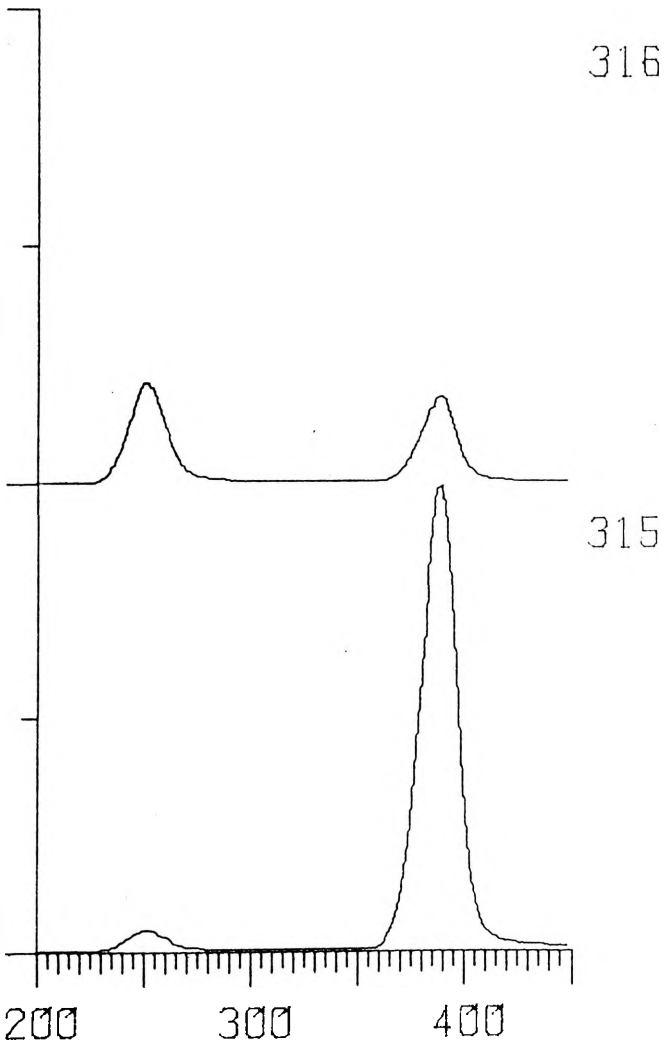
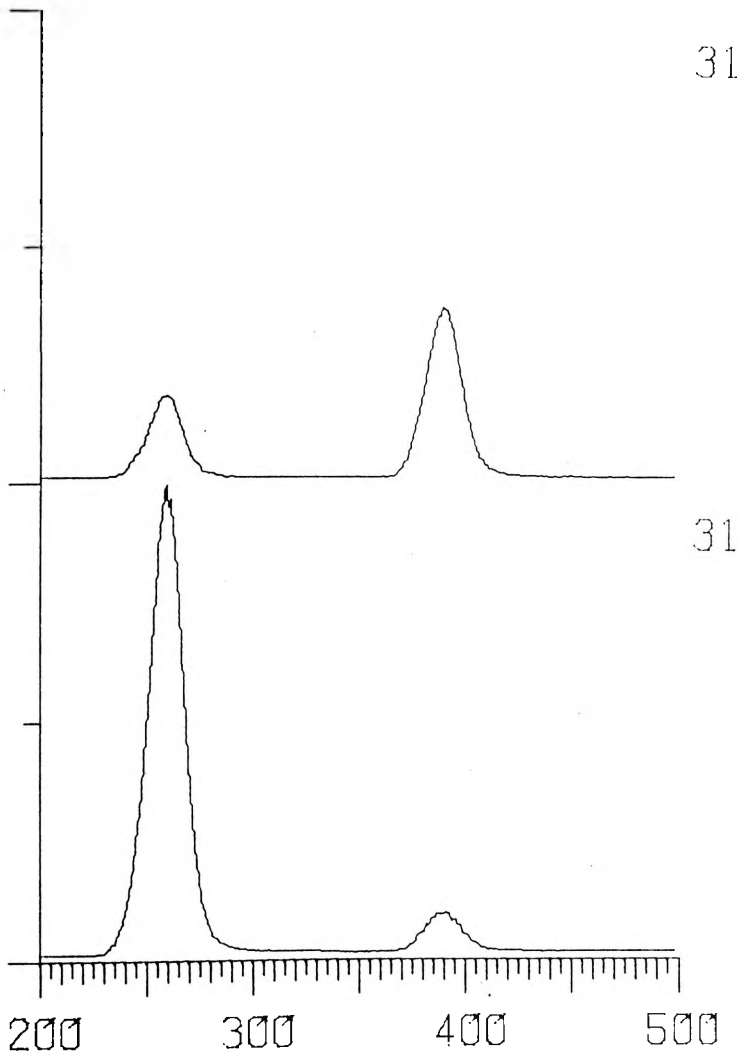


A12612 GC/MS 95 3% OV-225



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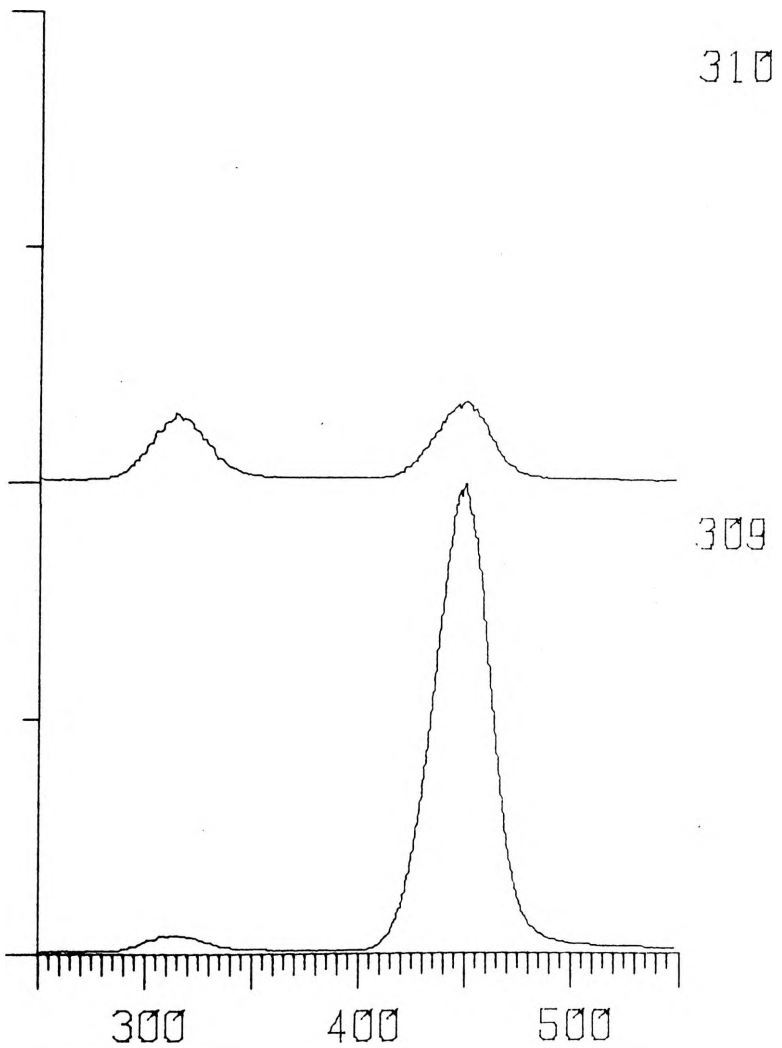
A13614



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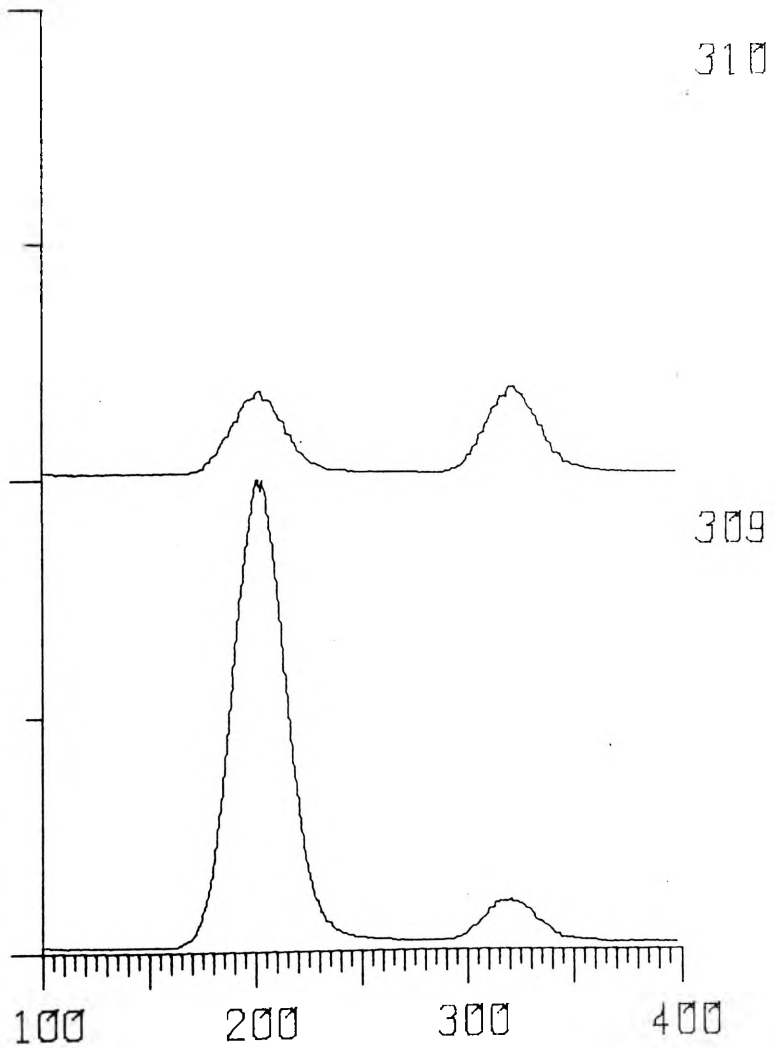
310
309
100
200
300
400

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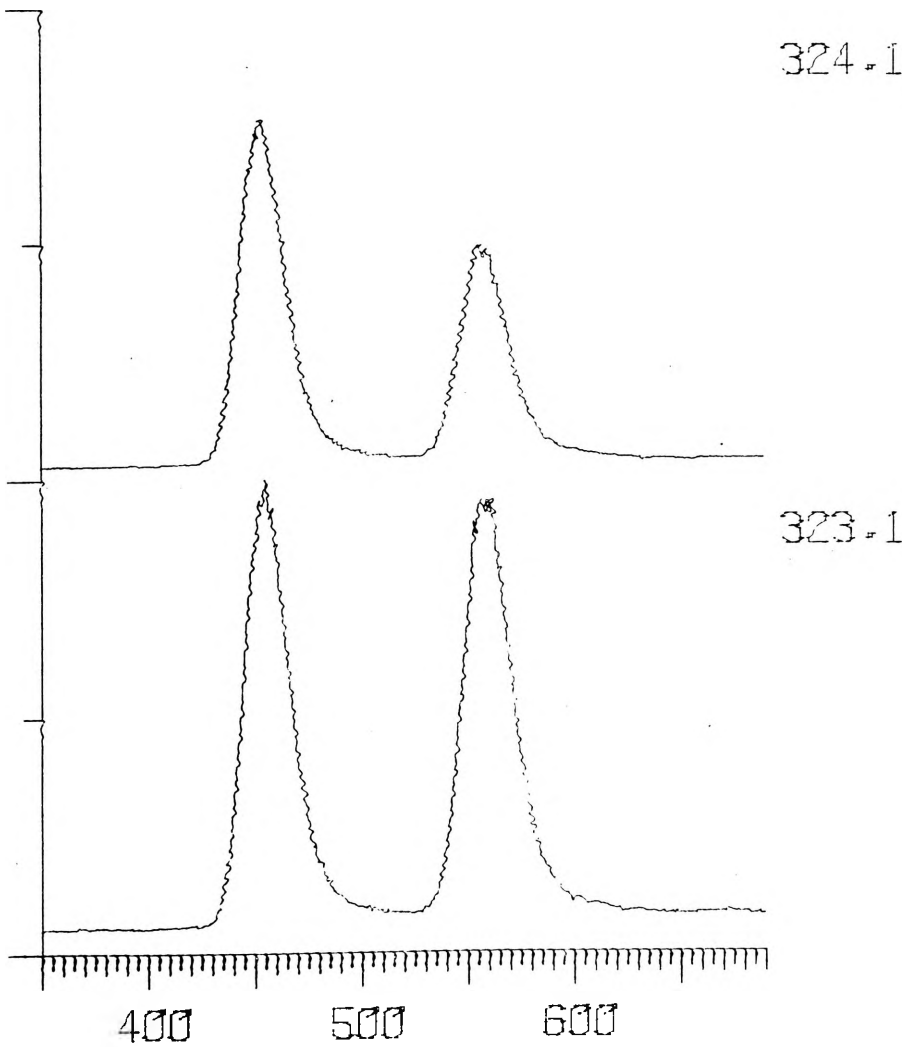

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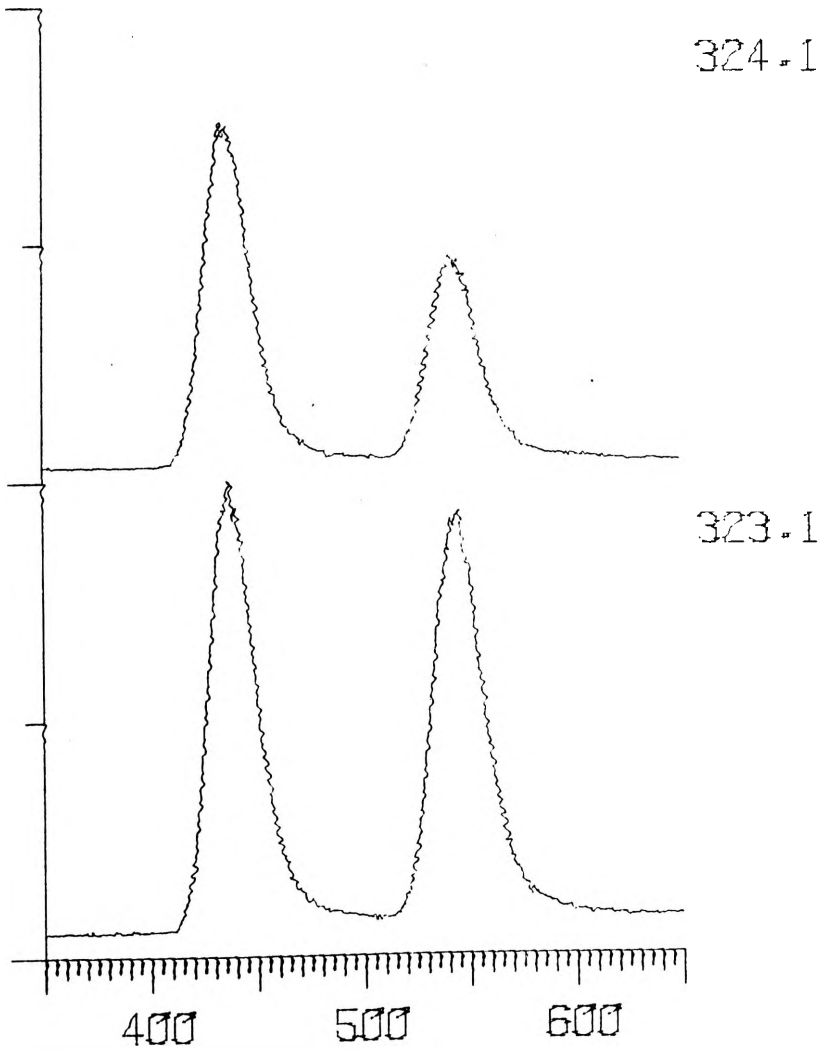

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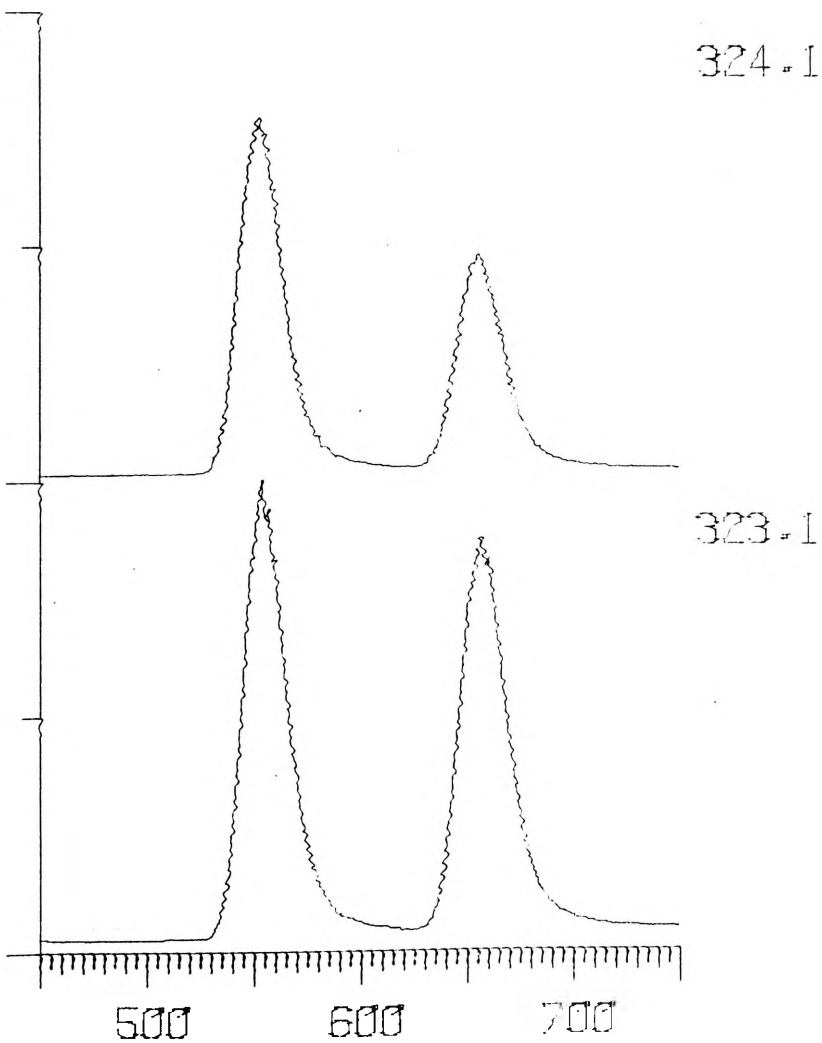

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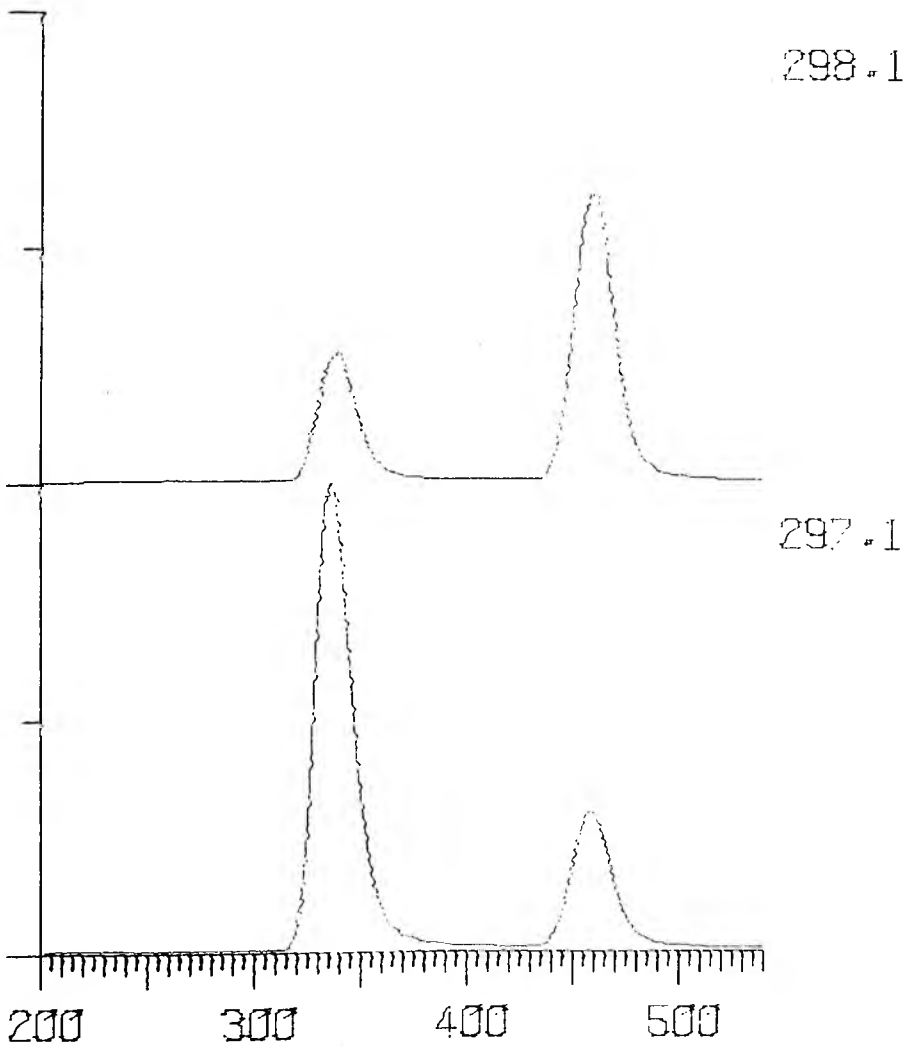

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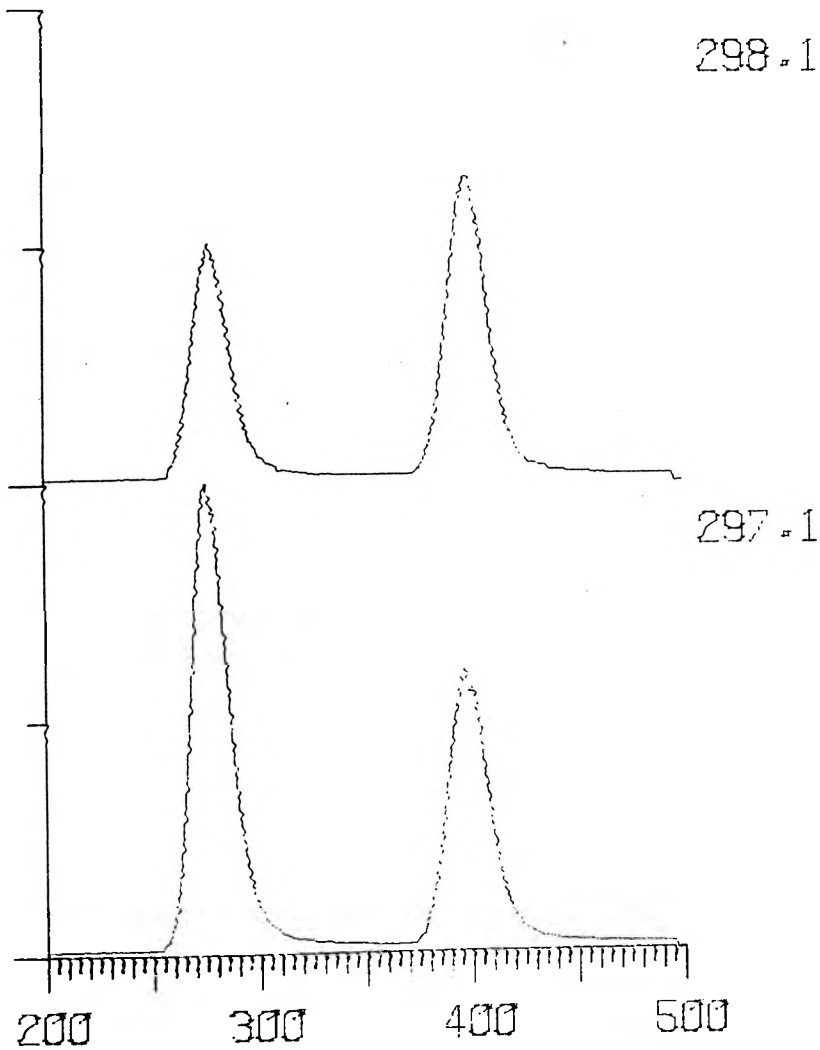

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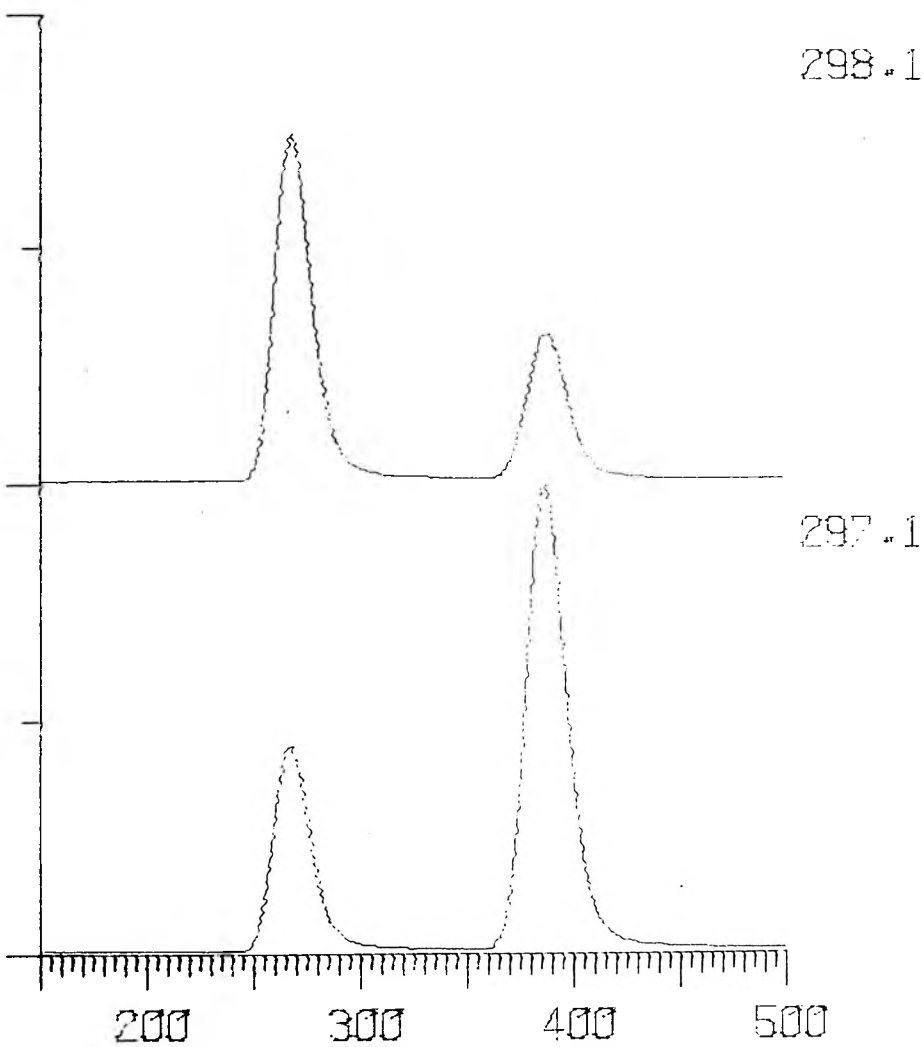

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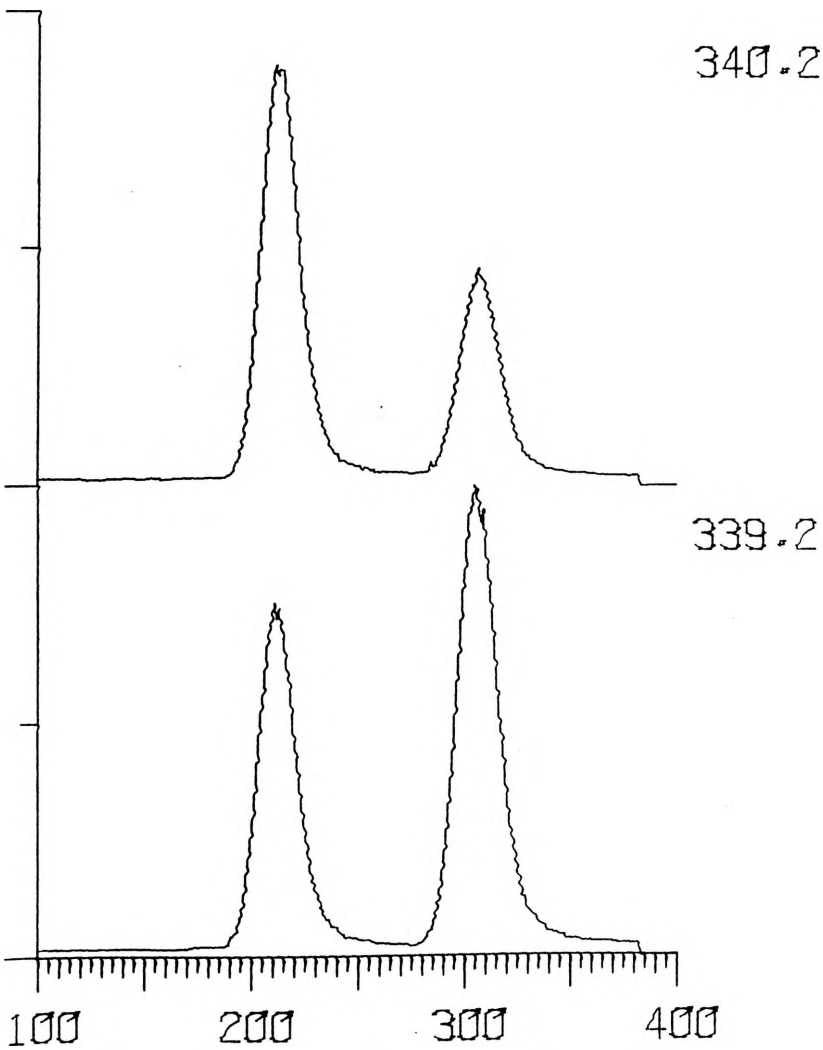
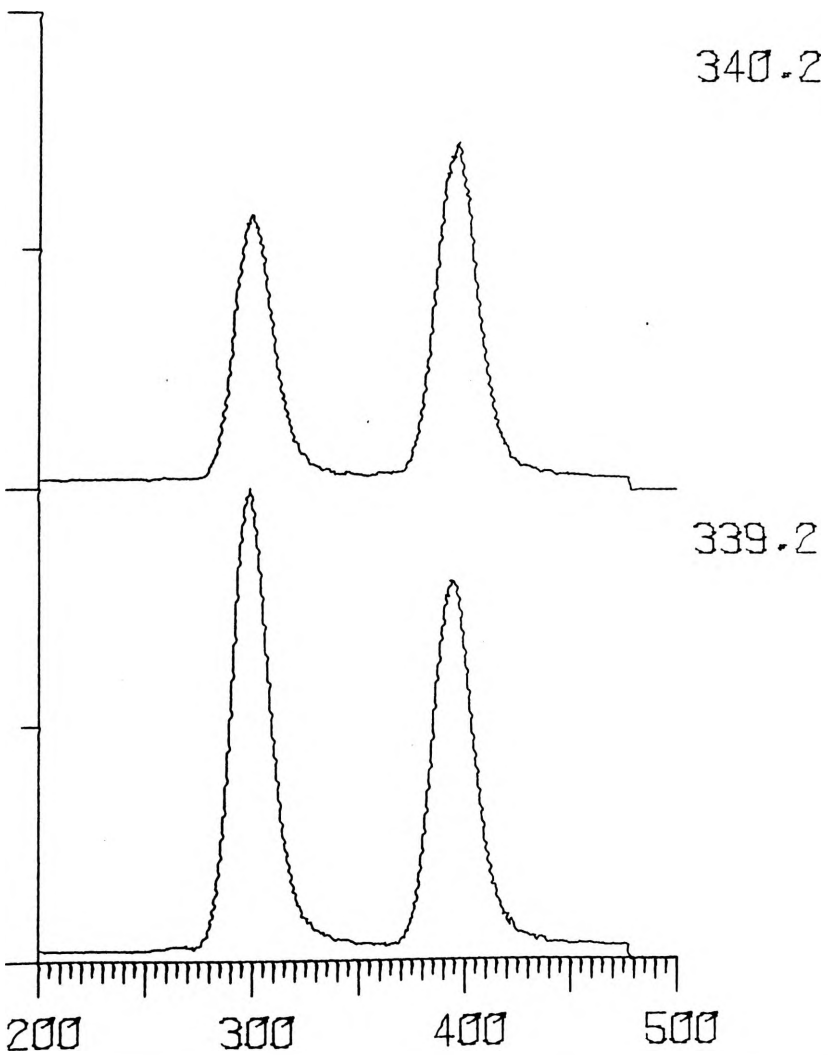
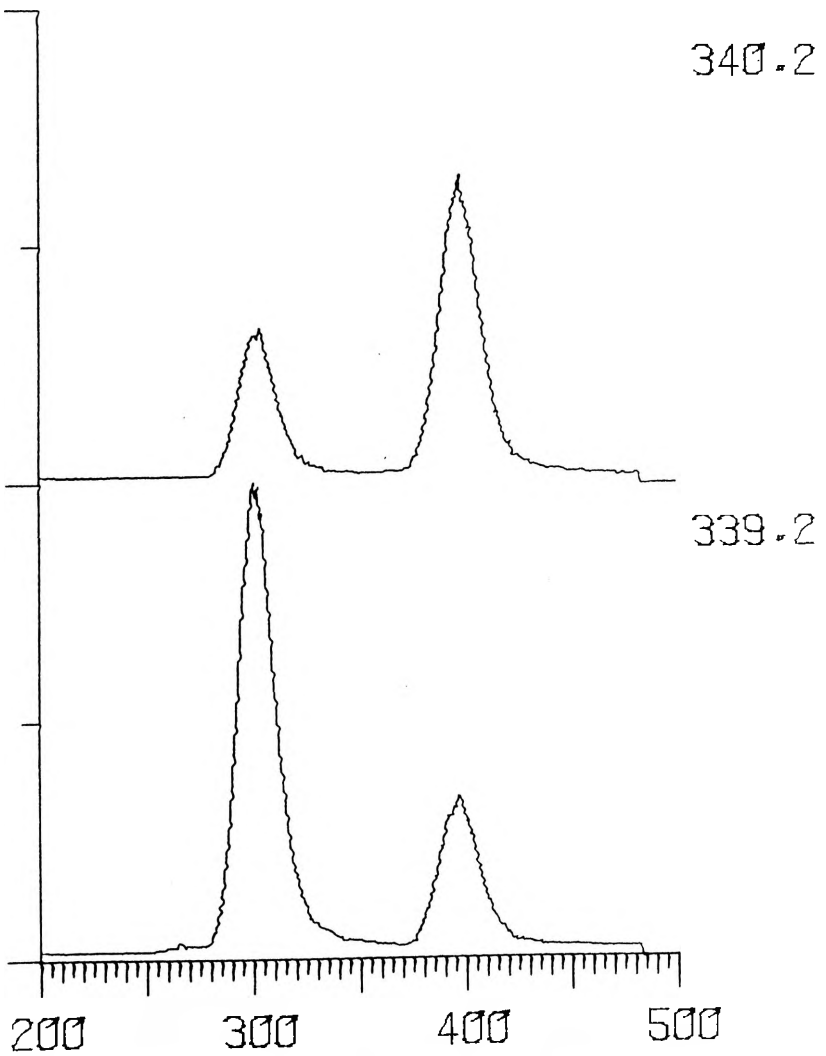


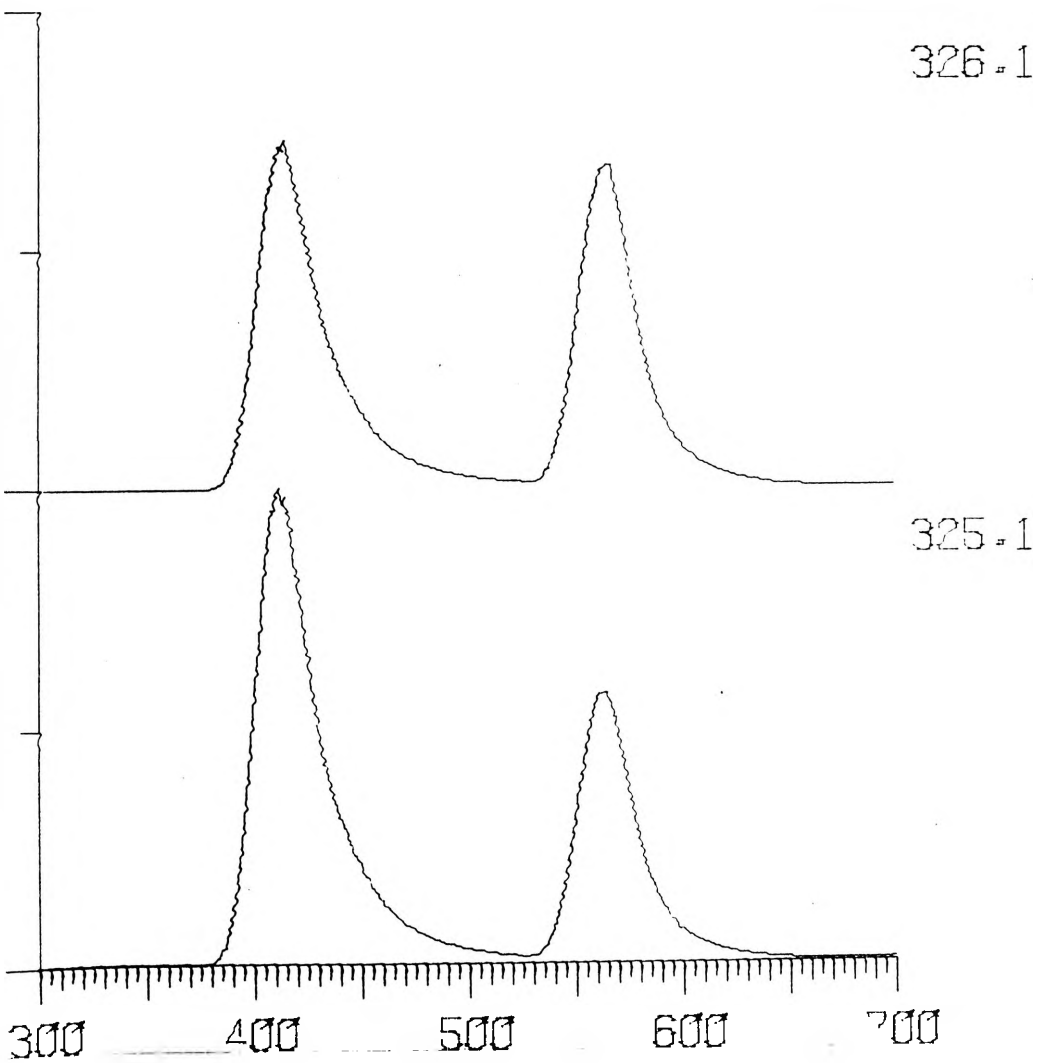
H09103 GC/MS 51 THBSORB 170/6 CI/CH4



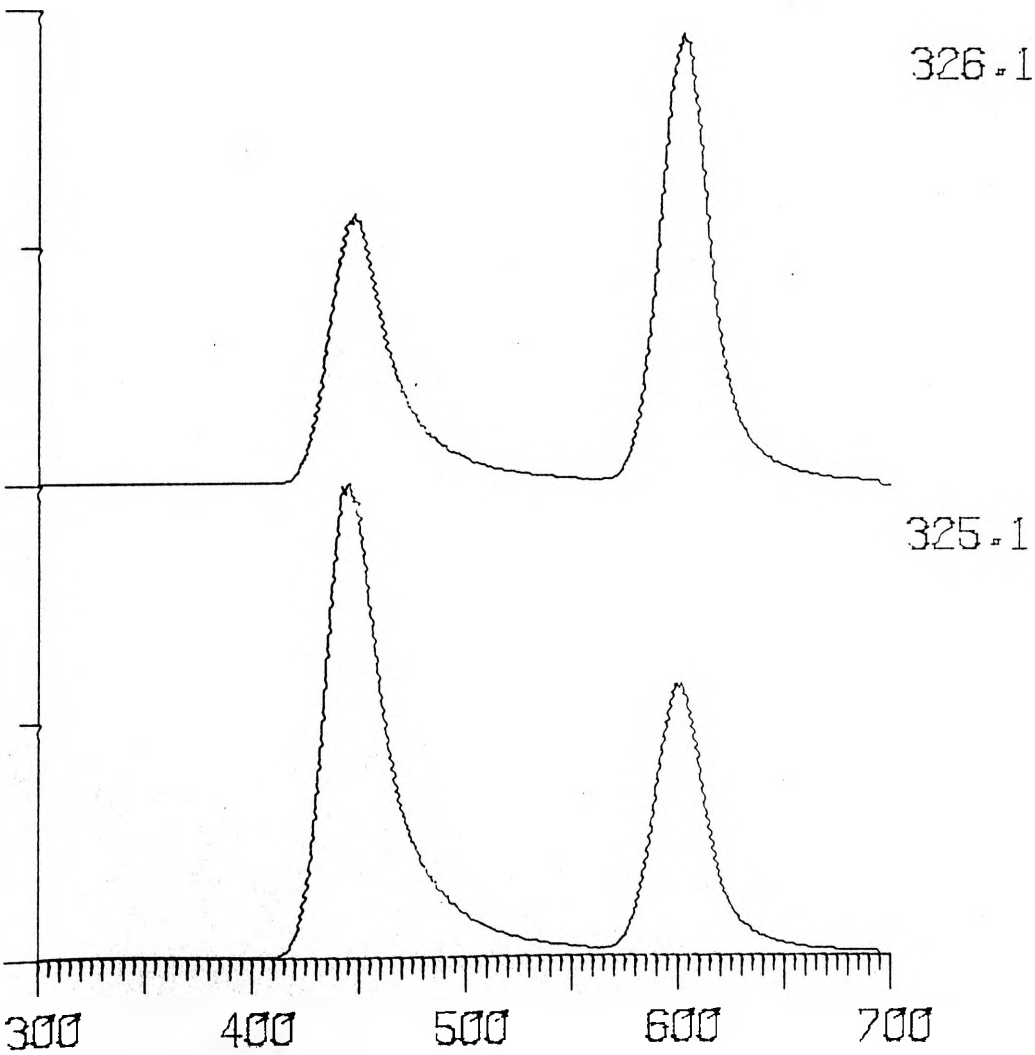
A09104 GC/MS 52 TABSORB 170/6 CI/CH4



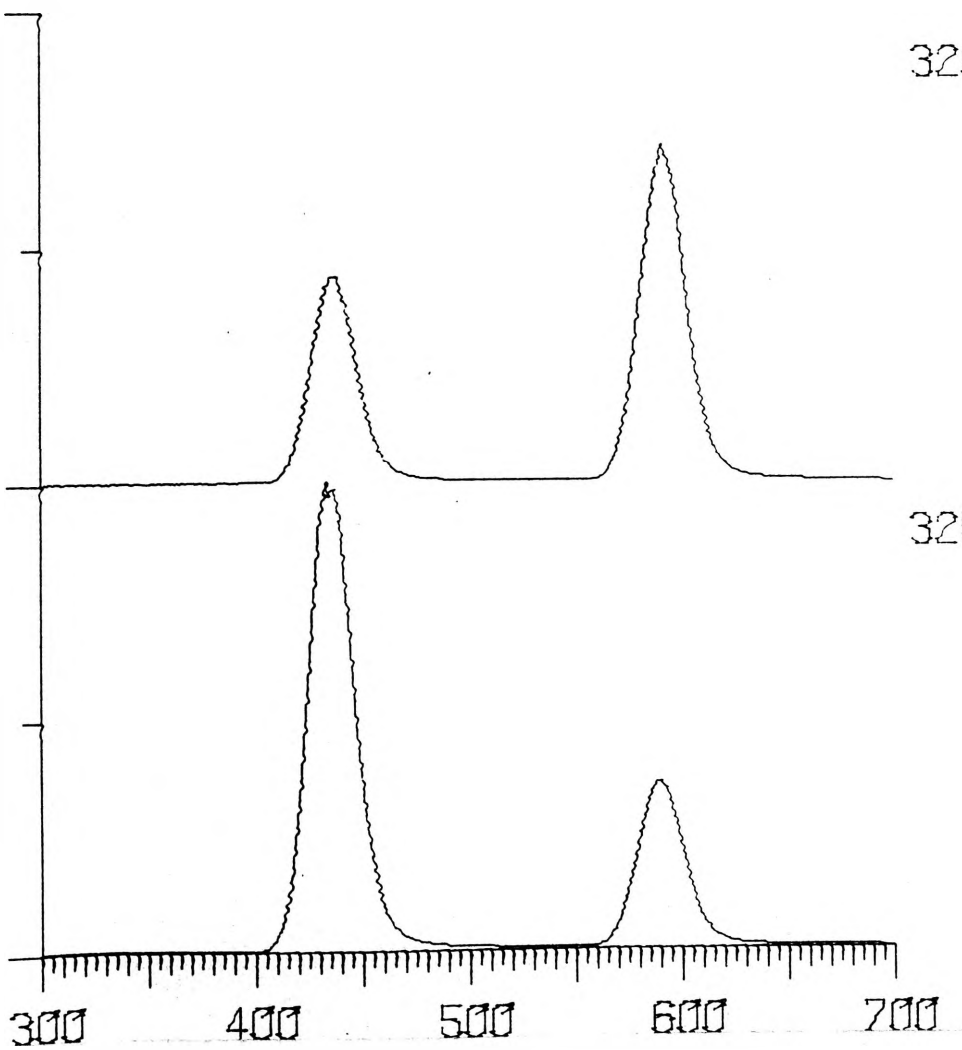
A19013 SAMPLE GC/MS 22 TABSORB 150/6 CI/CH4



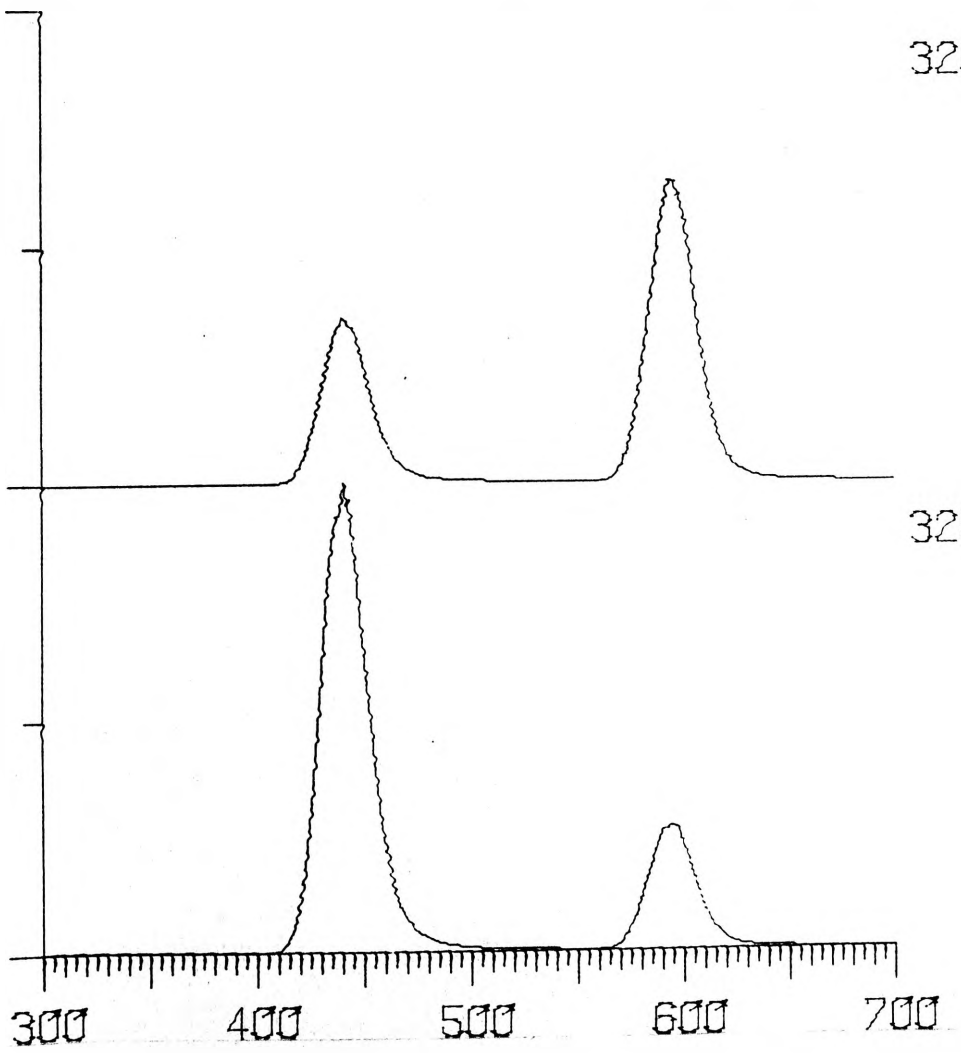
A19020 SAMPLE GC/MS 23 TABSORB 150/6 CI/CH4



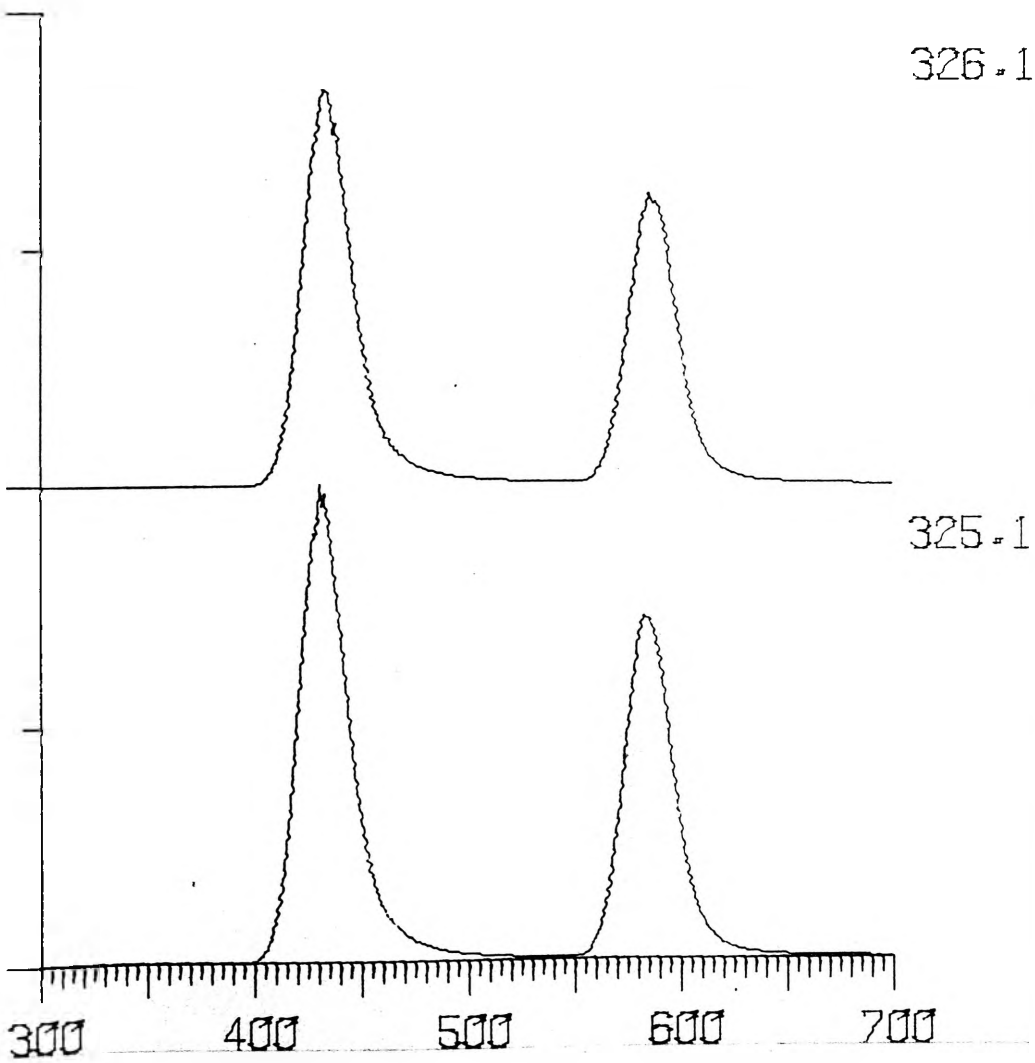
A19021 SAMPLE GC/MS 24 TABSORB 150/6 CI/CH4



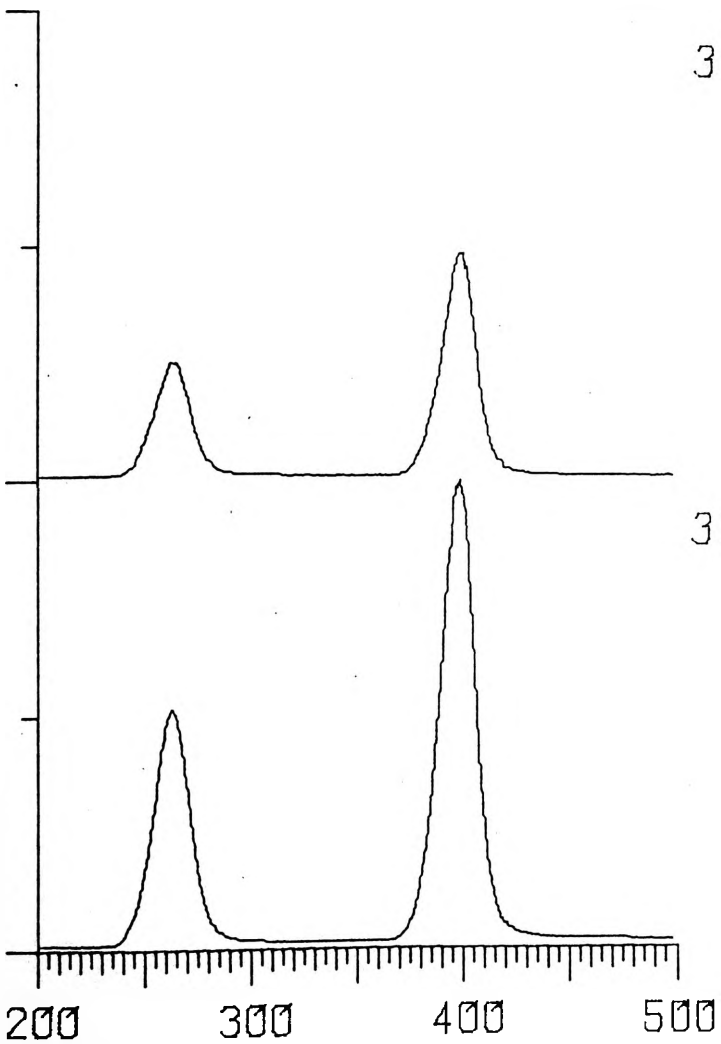
A19022 SAMPLE GC/MS 25 TABSORB 150/6 CI/CH4



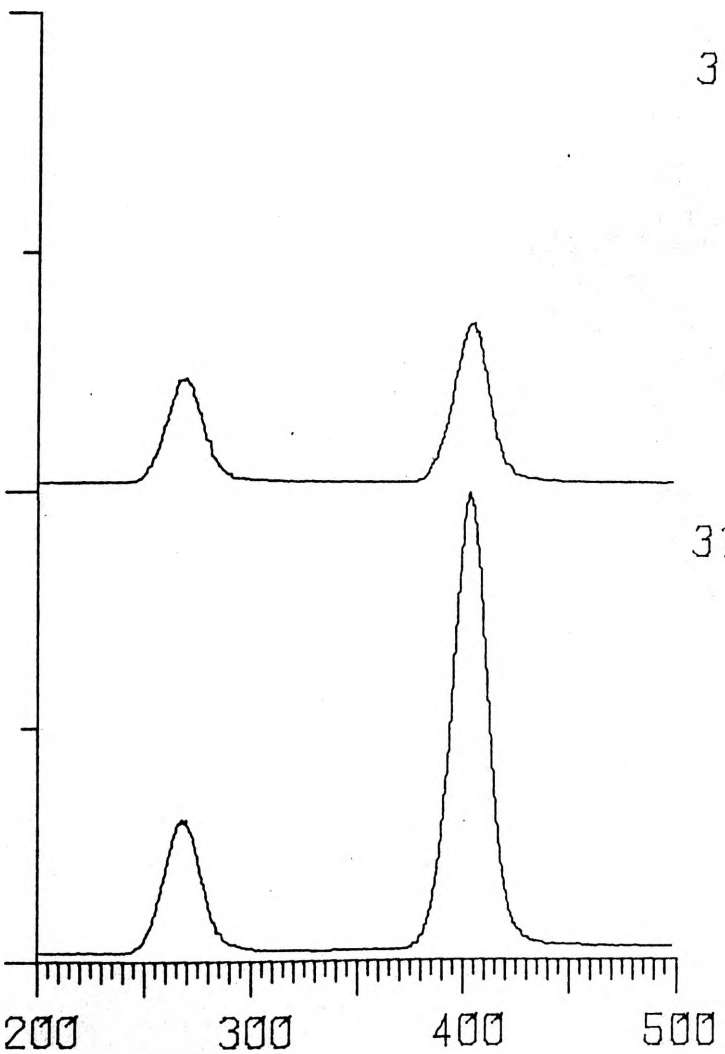
A19023 SAMPLE GC/MS 26 TABSORB 150/6 CI/CH4



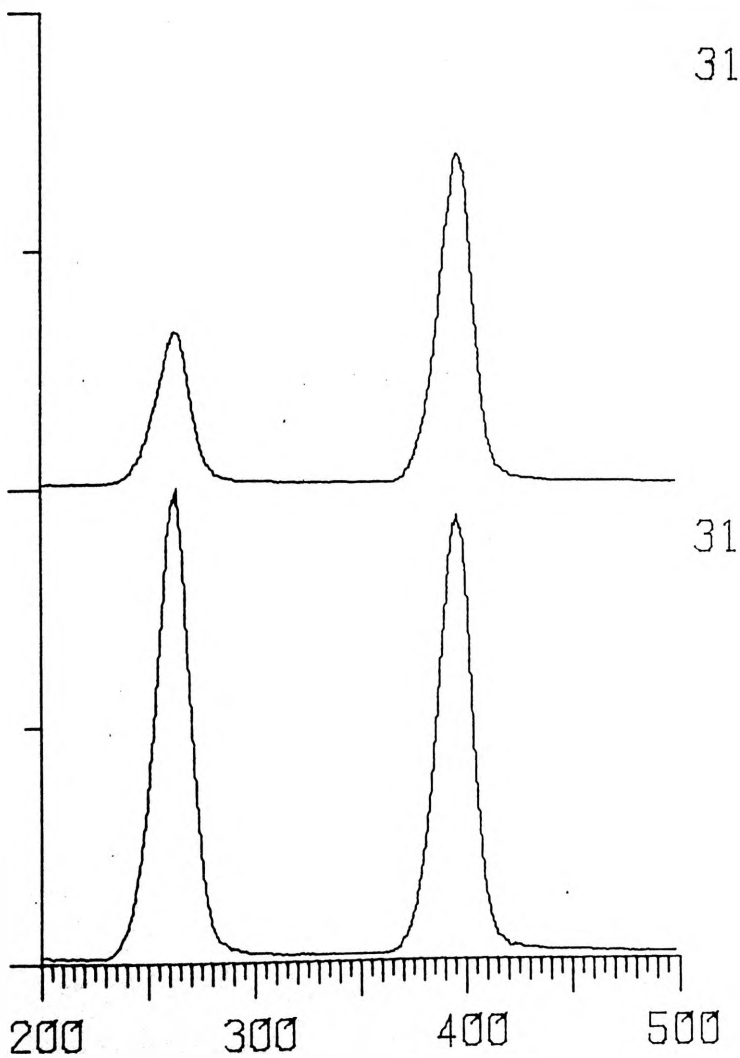
A12606 GC/MS 92 3% OV-225 150/10



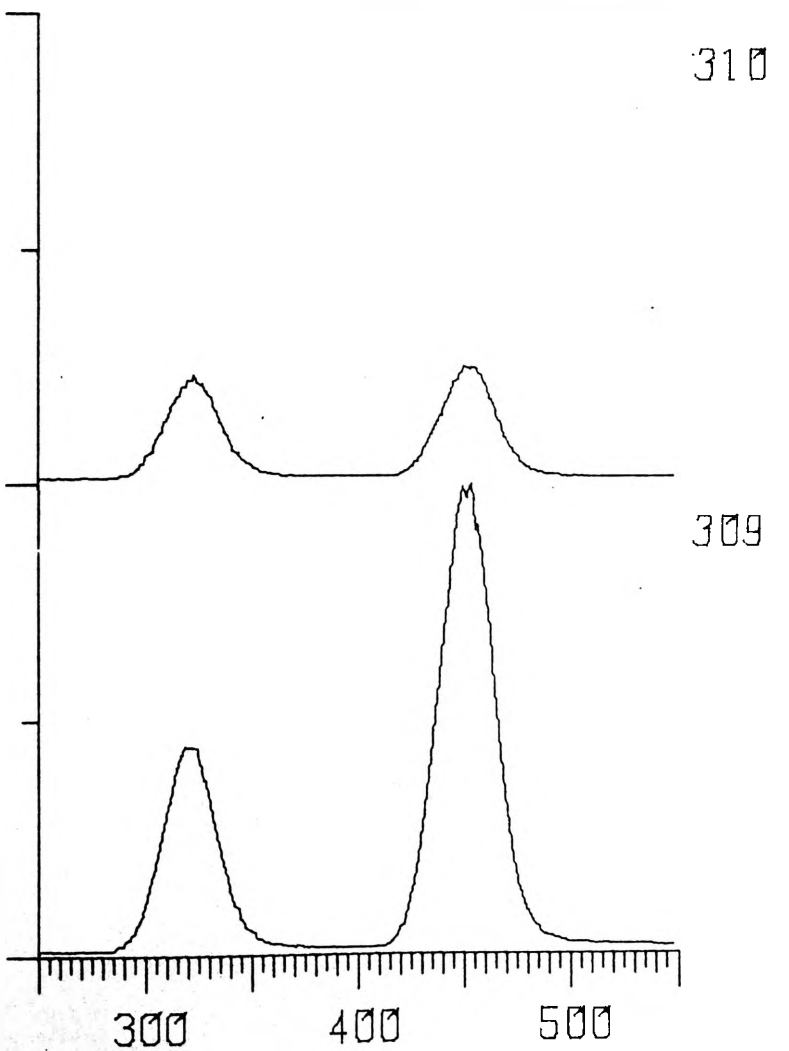
A12607 GC/MS 93 3% OV-225 150/10



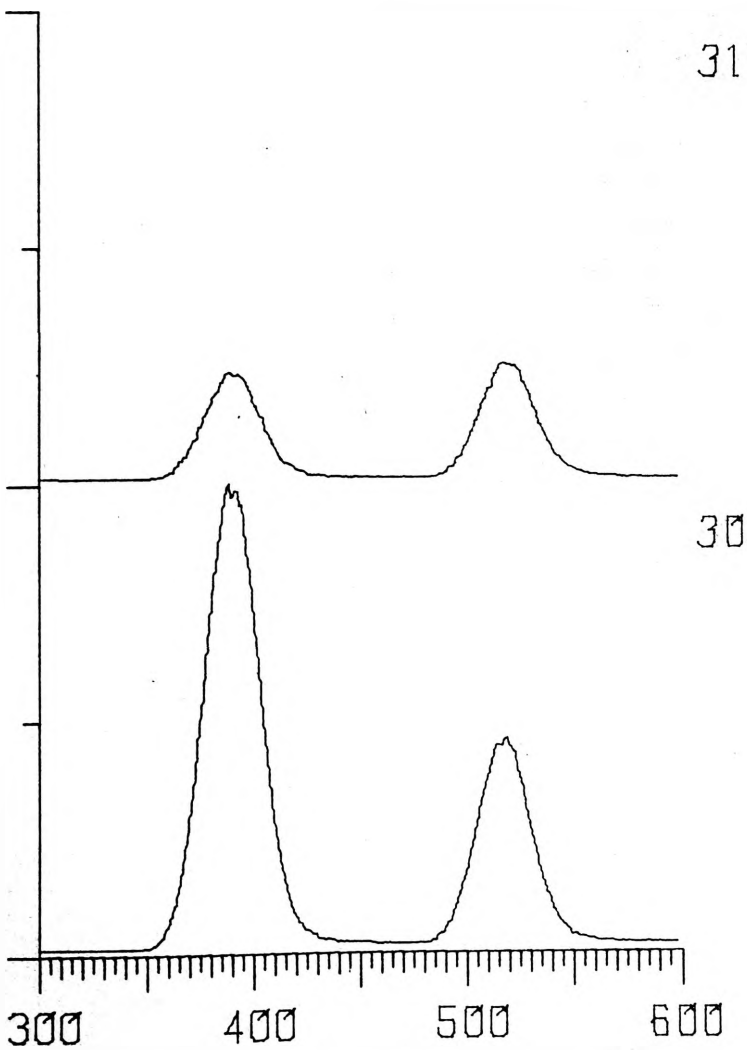
A12609 GC/MS 94 3% OV-225 150/10



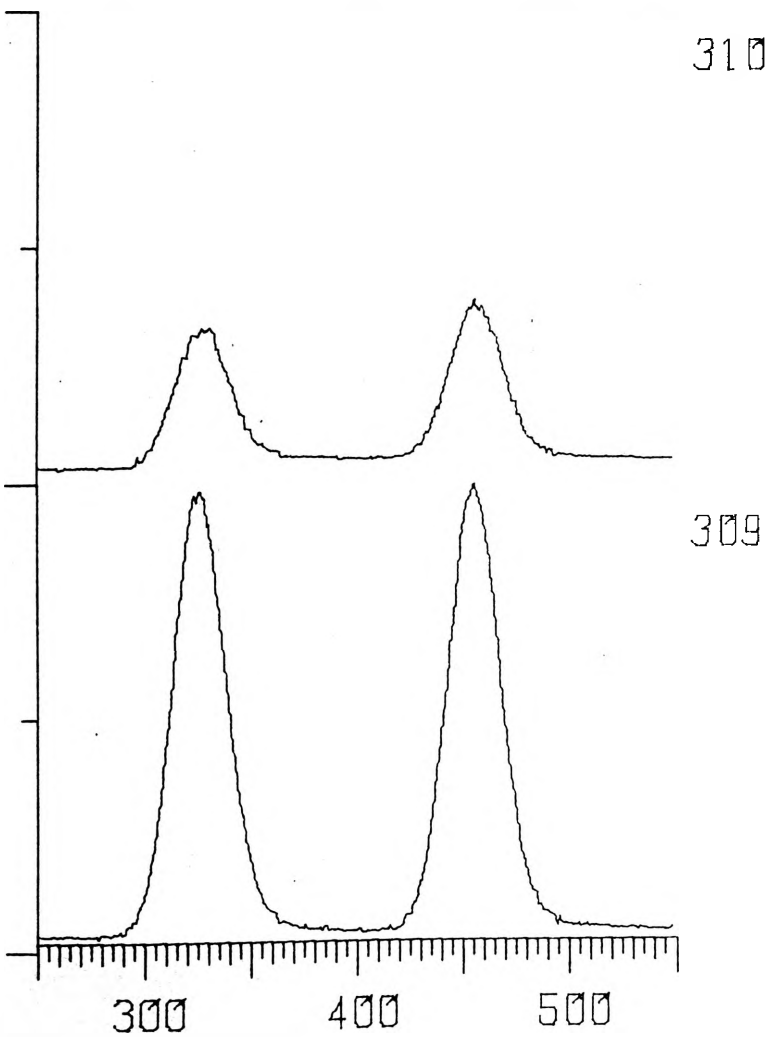
A13608 GC/MS 102 3% OV-225 130/6



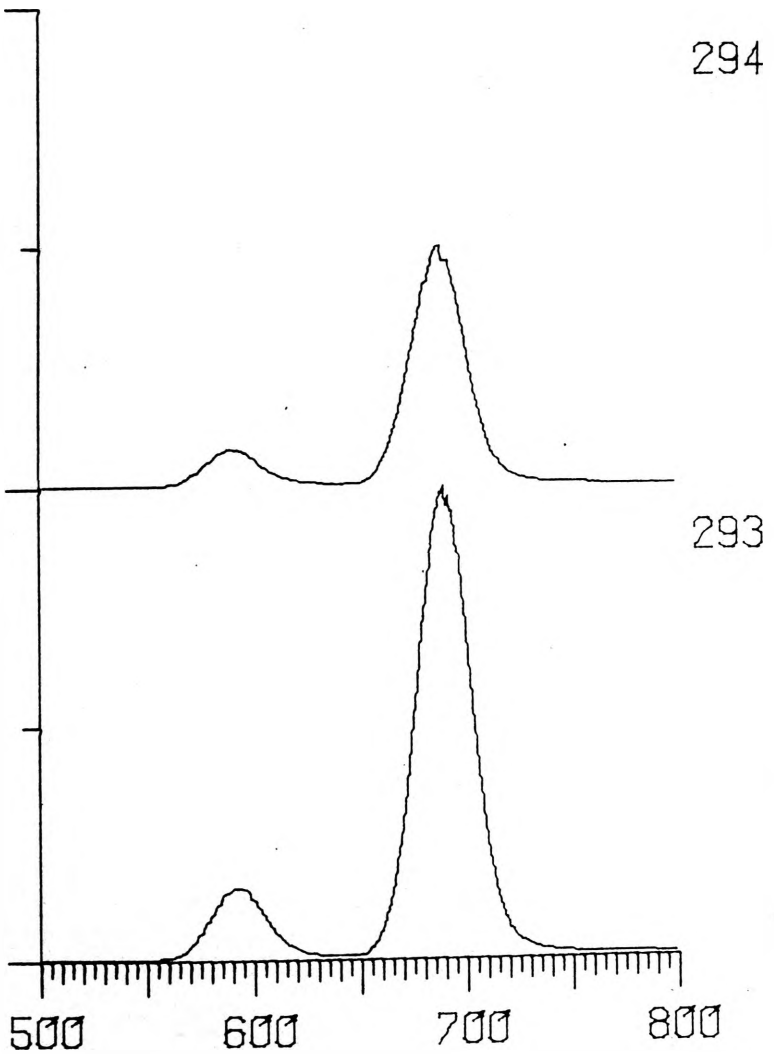
A13609 GC/MS 103 3% OV-225 130/6



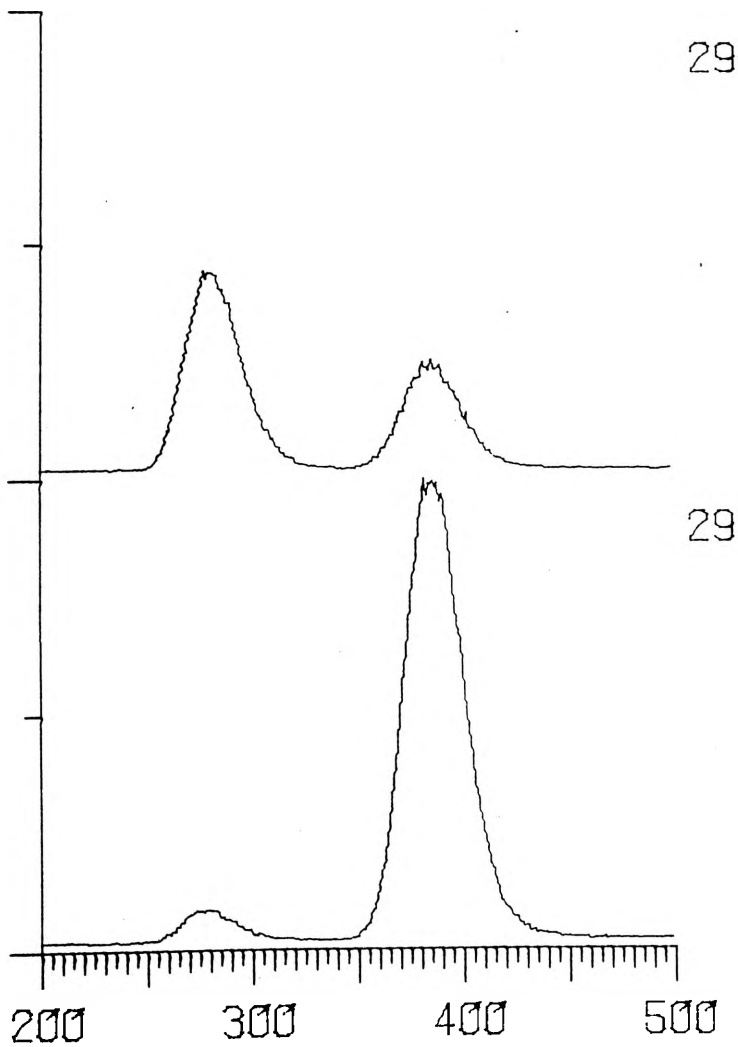
A13611 GC/MS 104 3% OV-225 130/6



A14624 GC/MS 90 3% OV-225 140/6

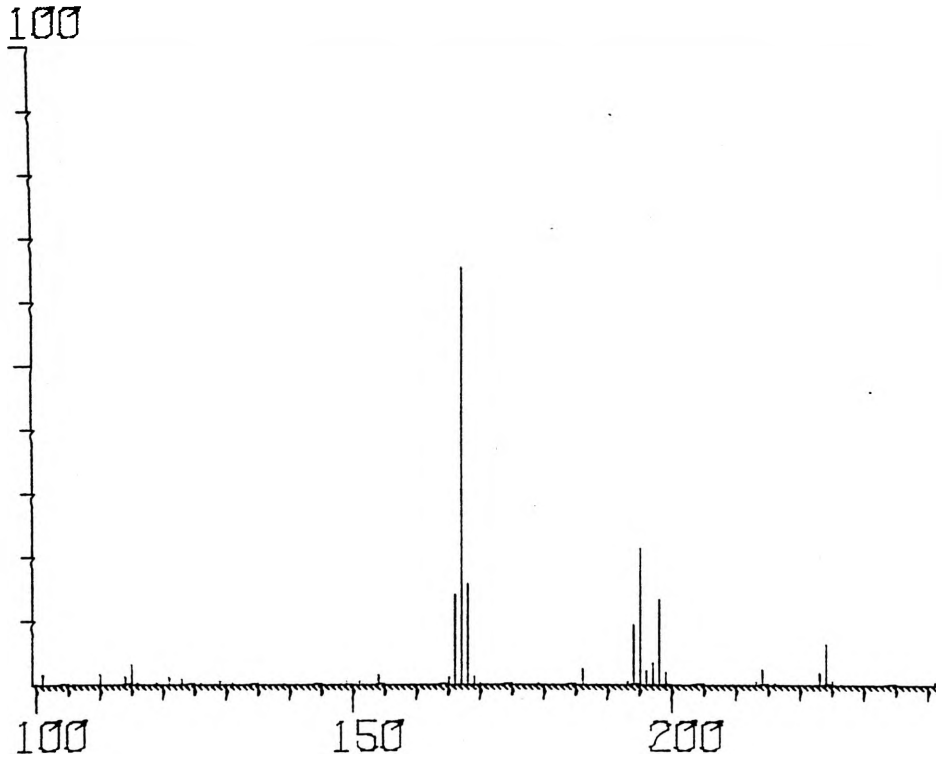


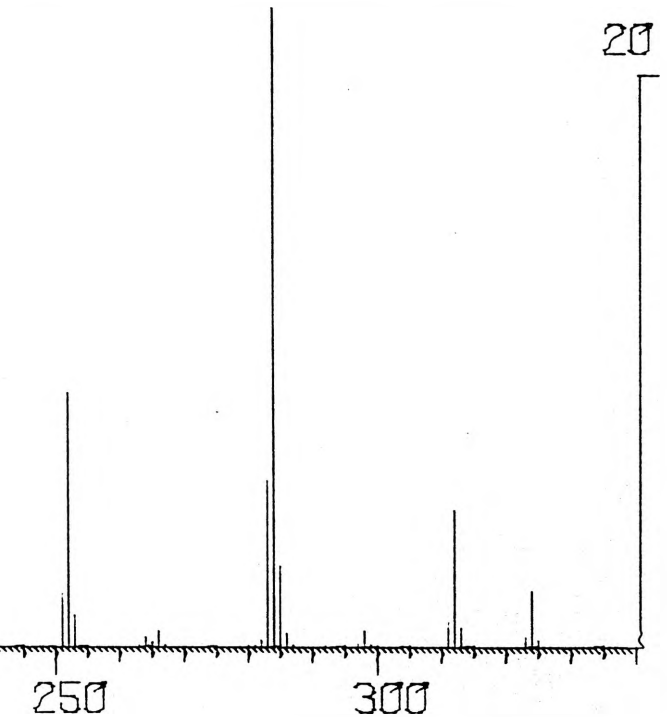
A01612 GC/MS 88 RUN #1



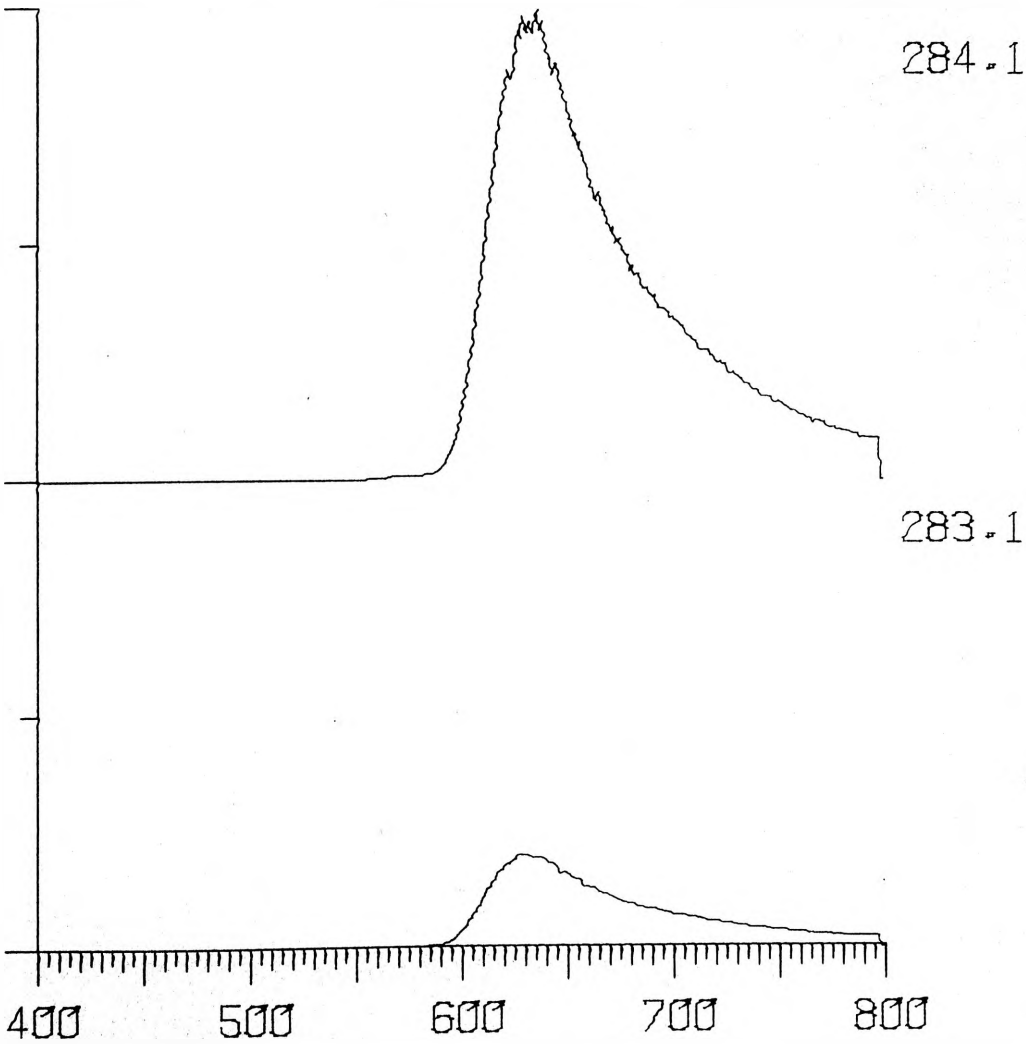
A09107 GC/MS 64 TABSORB 120/6 CI/CH4

235 -230

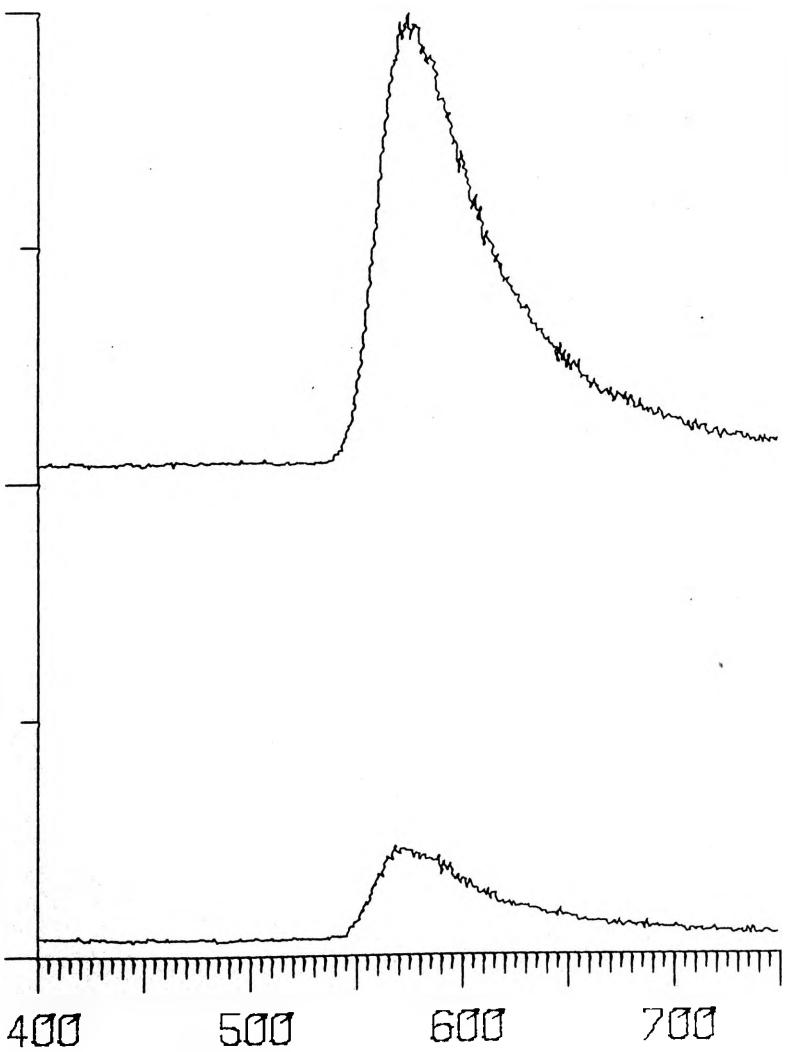



20
250
300

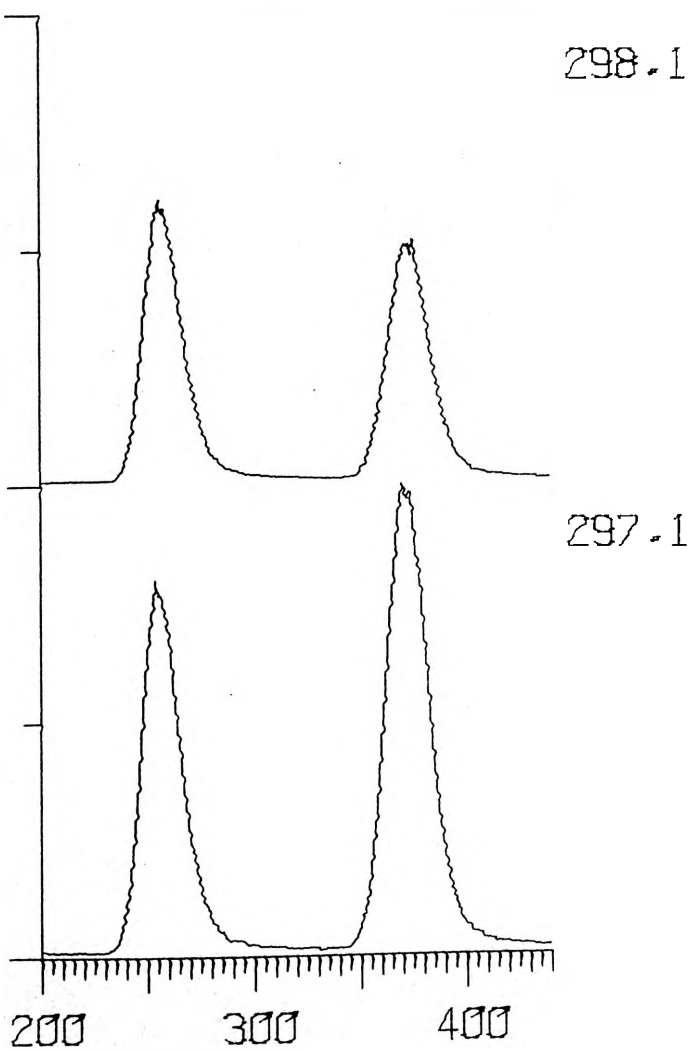
A09109 GC/MS 64 TABSORB 160/6 CI/CH4

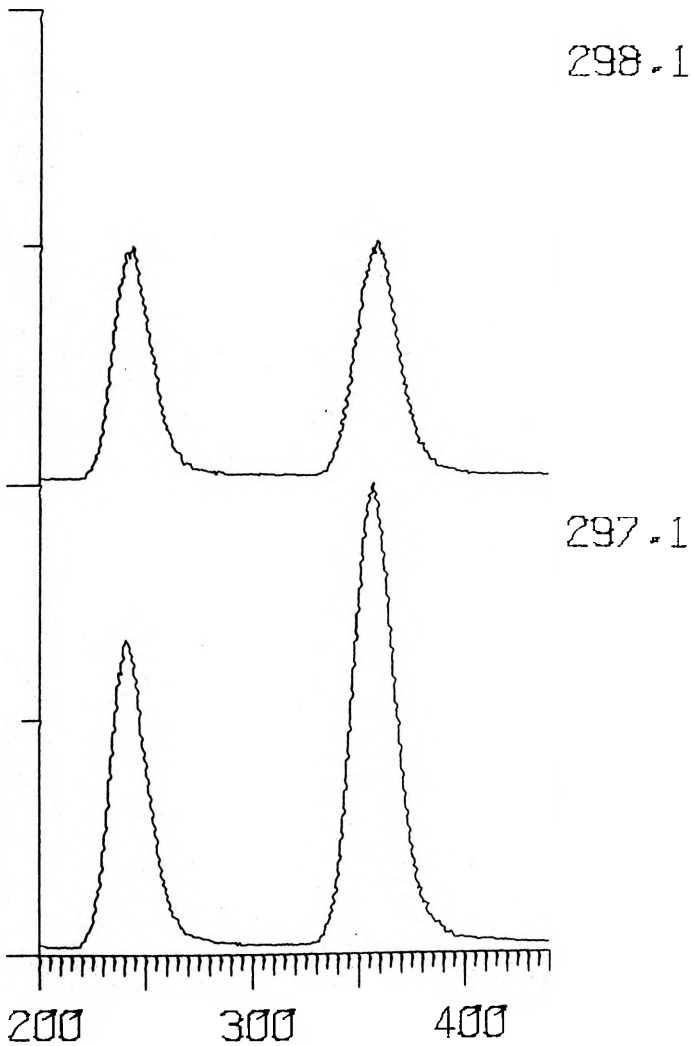


A09109 GC/MS 64 TABSORB 160/6 CI/CH4

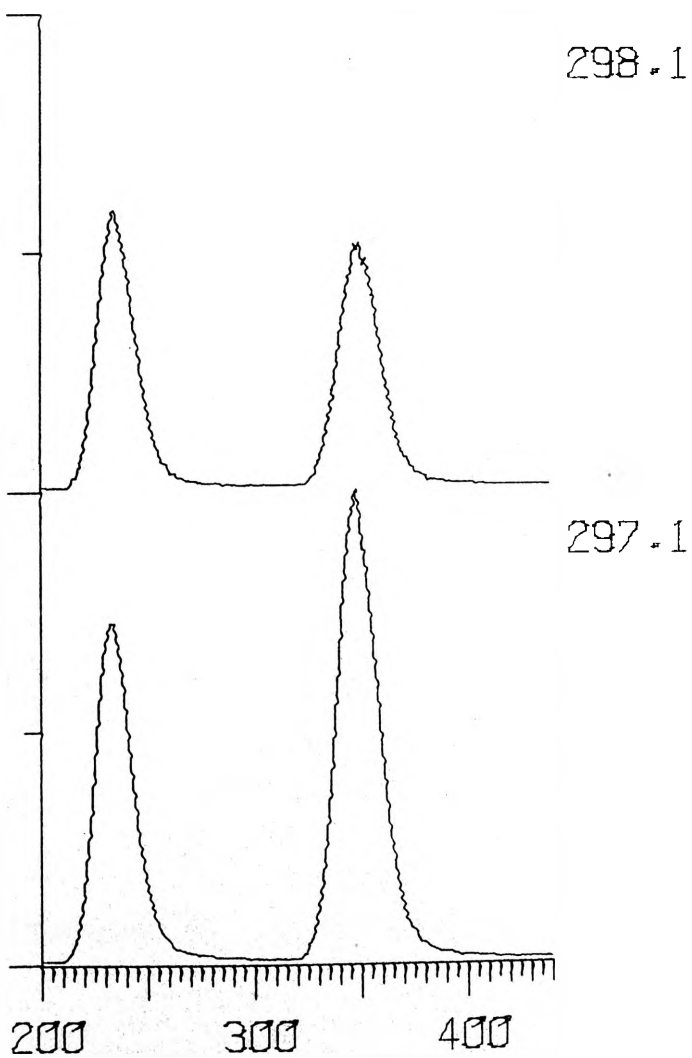


A09110 GC/MS 59 TABSORB 160/6 CI/CH4

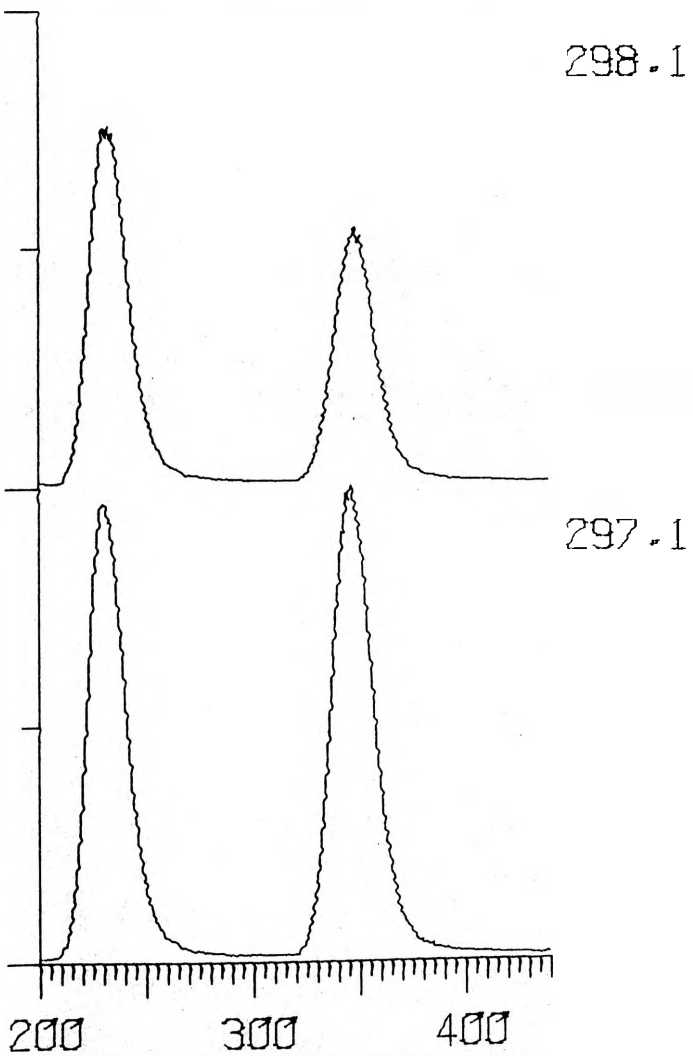



298.1
297.1
200
300
400

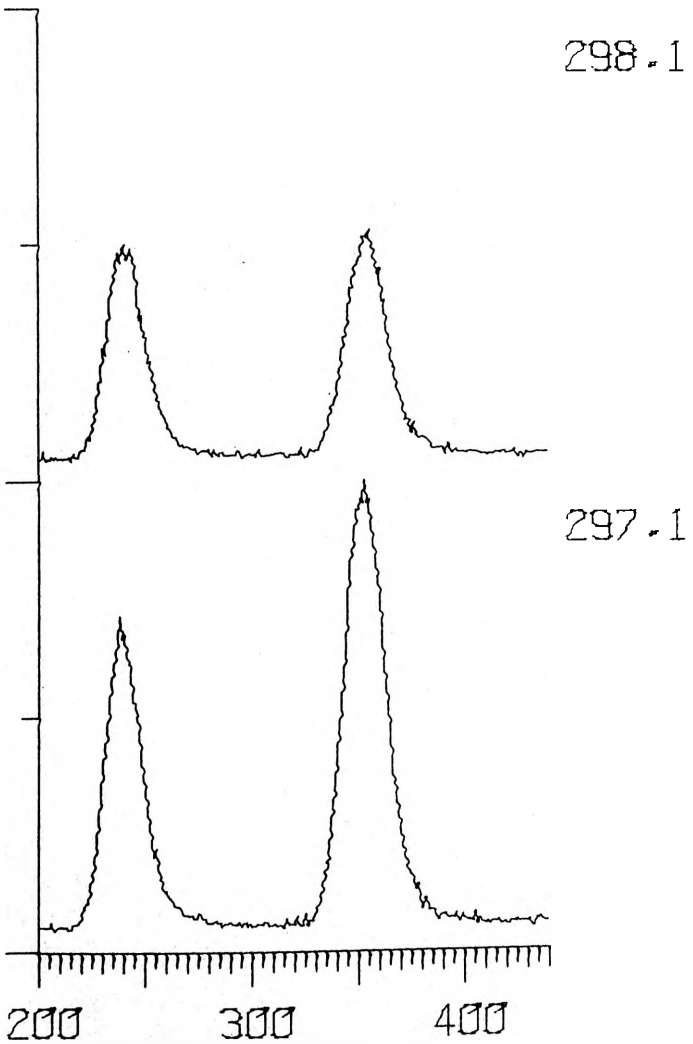
A09113 GC/MS 61 TABSORB 160/6 CI/CH4



A09114 GC/MS 62 TABSORB 160/6 CI/CH4



A09115 GC/MS 63 TABSORB 160/6 CI/CH4



PCIB=	450	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	225	°C
VELE=	162	V	TGCS=	250	°C
CEMI=	0.20	mA	TGCL=	255	°C
VSEM=	1930	V	TSOU=	195	°C

#	MASS	TIME	INTENSITY	
1	341.3	3.2	0.0	0.0
	341.3	3.7	4.2	100.0
	341.3	4.1	108.4	100.0
2	343.3	3.2	0.0	0.0
	343.3	3.7	302.7	2468.8
	343.3	4.1	7.5	6.9

1

GC

PCIB=	420	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	229	°C
VELE=	162	V	TGCS=	250	°C
CEMI=	0.20	mA	TGCL=	256	°C
VSEM=	1930	V	TSOU=	196	°C

#	MASS	TIME	INTENSITY	
1	341.3	3.2	0.0	0.0
	341.3	3.7	53.7	100.0
	341.3	4.1	6.2	100.0
2	343.3	3.2	0.0	0.0
	343.3	3.7	6.5	12.1
	343.3	4.1	223.1	555.2

PCIB=	452	µb	TGCI=	250	°C
PSOU=	0.0	nb	TGCO=	191	°C
VELE=	0.06	V	TGCS=	250	°C
CEMI=	0.00	mA	TGCL=	255	°C
VSEM=	2190	V	TSOU=	200	°C

#	MASS	TIME	INTENSITY	
1	315.3	4.7	3.1	100.0
	315.3	5.1	74.5	100.0
	343.3	5.9	0.4	100.0
	343.3	6.4	1.0	100.0
2	317.3	4.7	156.8	5580.8
	317.3	5.1	6.2	8.3
	345.3	5.9	5.0	1250.0
	345.3	6.4	0.3	30.0

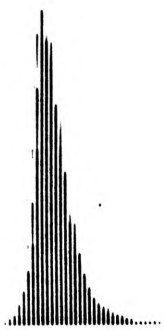
1

GC

PCIB=	450	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	183	°C
VELE=	0.06	V	TGCS=	249	°C
CEMI=	0.00	mA	TGCL=	256	°C
VSEM=	2030	V	TSOU=	197	°C

5.4

317.3

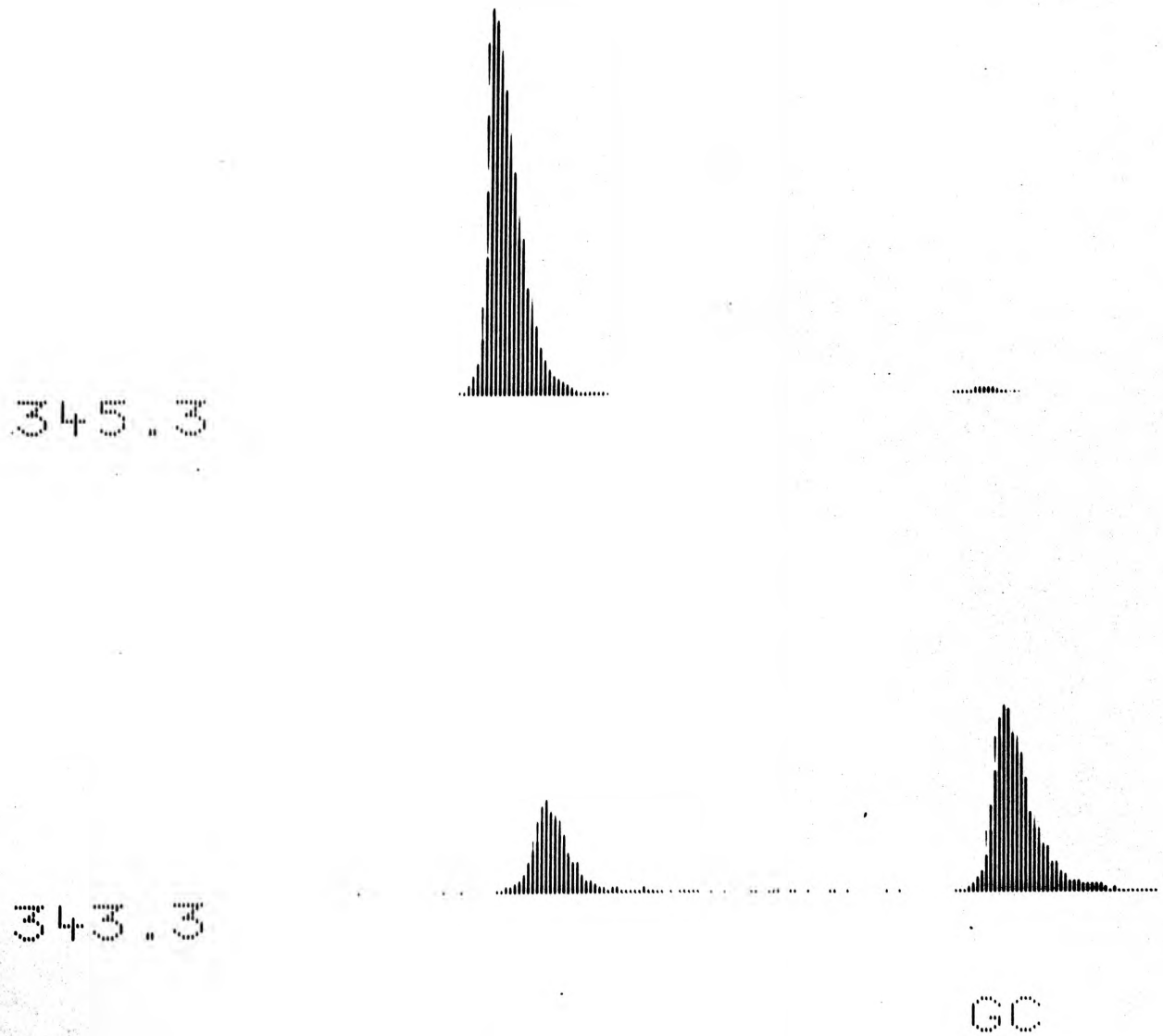


315.3

GC

PCIB=	463 µb	TGCI=	250 °C
PSOU=	0.0 mb	TGCO=	190 °C
VELE=	162 V	TGCS=	250 °C
CEMI=	0.20 mA	TGCL=	256 °C
VSEM=	2130 V	TSOU=	197 °C

6.6



PCIB=	441	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	191	°C
VELE=	0.06	V	TGCS=	250	°C
CEMI=	0.00	mA	TGCL=	255	°C
VSEM=	2030	V	TSOU=	197	°C

#	MASS	TIME	INTENSITY	
1	315.3	4.7	22.3	100.0
	315.3	5.0	3.6	100.0
	343.3	5.9	143.1	100.0
	343.3	6.4	3.8	100.0
2	317.3	4.7	3.3	14.7
	317.3	5.0	60.6	1683.3
	345.3	5.9	10.0	6.9
	345.3	6.4	235.1	4894.4

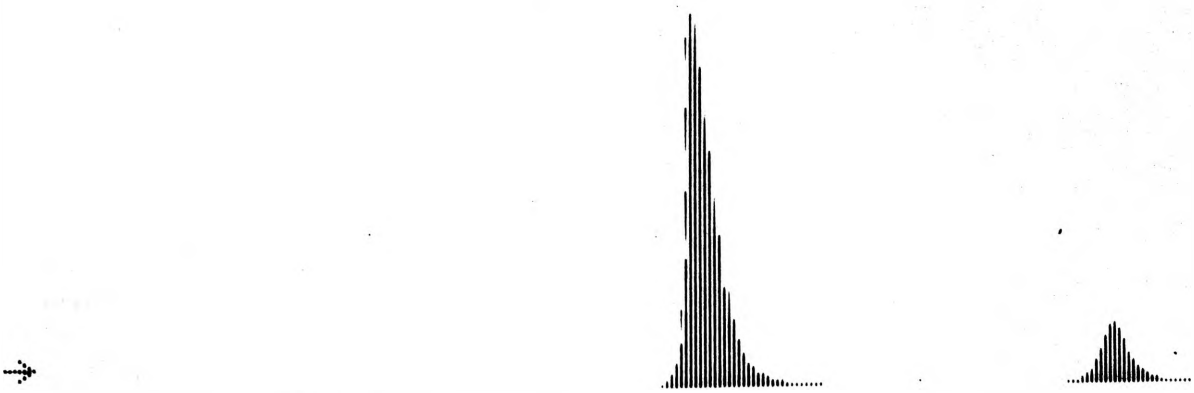
PCIB=	443	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	191	°C
VELE=	0.06	V	TGCS=	250	°C
CEMI=	0.00	mA	TGCL=	256	°C
VSEM=	2030	V	TSOU=	200	°C

#	MASS	TIME	INTENSITY	
1	315.3	4.7	15.6	100.0
	315.3	5.1	2.9	100.0
	343.3	6.0	109.6	100.0
	343.3	6.4	3.1	100.0
2	317.3	4.7	2.0	12.8
	317.3	5.1	51.7	1782.7
	345.3	6.0	7.6	6.9
	345.3	6.4	205.4	4476.8

PCIB=	439	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	182	°C
VELE=	0.06	V	TGCS=	250	°C
CEMI=	0.00	mA	TGCL=	256	°C
VSEM=	1980	V	TSOU=	193	°C

5.3

317.3



315.3

GC

PCIB=	441	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	190	°C
VELE=	0.06	V	TGCS=	249	°C
CEMI=	0.00	mA	TGCL=	256	°C
VSEM=	2030	V	TSOU=	196	°C

6.7

345.3

343.3

GC

PCIB=	452	µb	TGCI=	250	°C
PSOU=	0.0	nb	TGCO=	208	°C
VELE=	162	V	TGCS=	250	°C
CEMI=	0.20	mA	TGCL=	256	°C
VSEM=	2030	V	TSOU=	199	°C

#	MASS	TIME	INTENSITY	
1	327.4	6.7	133.9	100.0
	327.4	7.0	26.4	100.0
	333.3	10.1	2.5	100.0
	333.3	10.7	57.0	100.0
2	329.4	6.7	11.1	8.2
	329.4	7.0	264.7	1002.6
	335.3	10.1	48.7	1948.0
	335.3	10.7	4.7	8.2

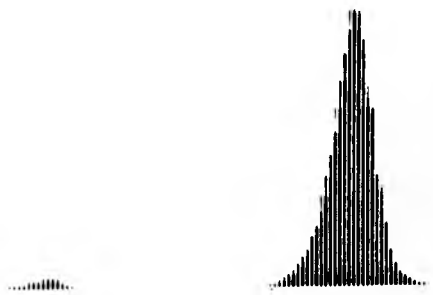
GC

1

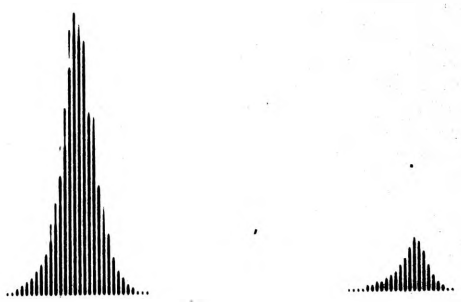
TIMH=0

PCIB=	454	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	179	°C
VELE=	162	V	TGCS=	250	°C
CEMI=	0.20	mA	TGCL=	256	°C
VSEM=	2030	V	TSOU=	197	°C

7.2



329.4

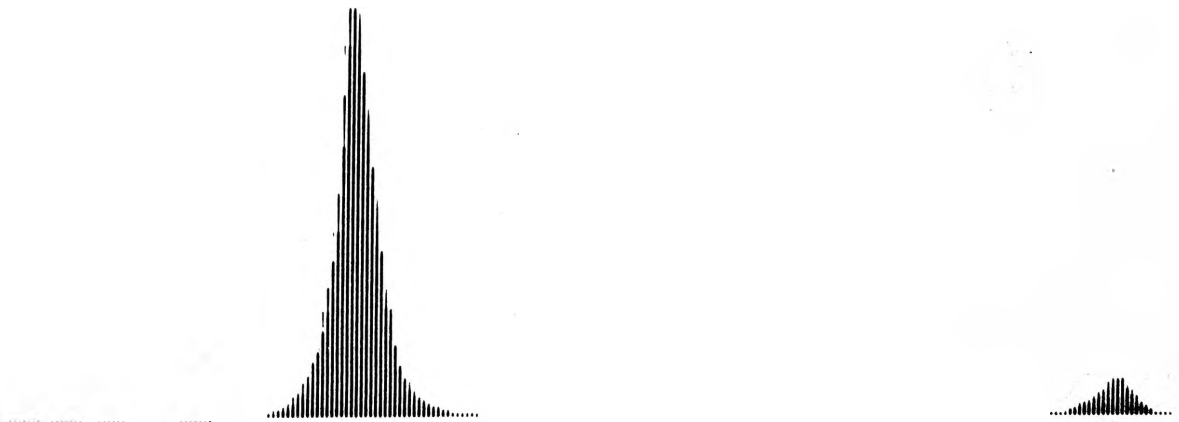


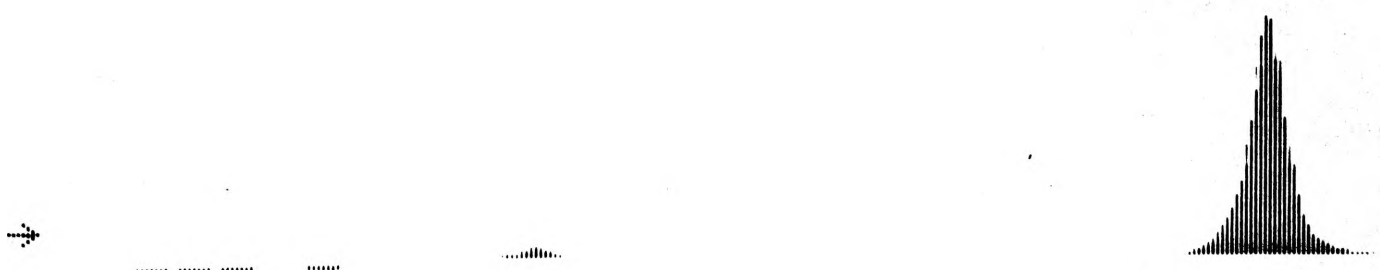
327.4

GC

PCIB=	453	mb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	205	°C
VELE=	162	V	TGCS=	250	°C
CEMI=	0.20	mA	TGCL=	255	°C
VSEM=	2030	V	TSOU=	199	°C

10.9





GC

1	327.4	6.7	3.4	100.0
	327.4	7.0	98.8	100.0
	333.3	10.1	20.6	100.0
	333.3	10.8	2.6	100.0
2	329.4	6.6	85.4	2511.7
	329.4	7.0	7.8	7.8
	335.3	10.1	1.7	8.2
	335.3	10.7	42.5	1634.6

1

GC

```
PCIB=    452  µb      TGCI=    250  °C
PSOU=    0.0  mb      TGCO=    208  °C
VELE=    162   V      TGCS=    250  °C
CEMI=  0.20  mA       TGCL=    256  °C
VSEM=  2030   V       TSOU=    200  °C
#     MASS      TIME      INTENSITY
```

PCIB=	384	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	228	°C
VELE=	162	V	TGCS=	250	°C
CEMI=	0.20	mA	TGCL=	255	°C
VSEM=	1880	V	TSOU=	196	°C

#	MASS	TIME	INTENSITY	
1	341.3	3.2	0.0	0.0
	341.3	3.8	179.9	100.0
	341.3	4.1	43.3	100.0
2	343.3	3.2	0.0	0.0
	343.3	3.8	181.9	101.1
	343.3	4.1	49.5	114.3

PCIB=	424	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	229	°C
VELE=	162	V	TGCS=	249	°C
CEMI=	0.20	mA	TGCL=	256	°C
VSEM=	1930	V	TSOU=	196	°C

#	MASS	TIME	INTENSITY	
1	341.3	3.2	0.0	0.0
	341.3	3.7	121.4	100.0
	341.3	4.1	35.5	100.0
2	343.3	3.2	0.0	0.0
	343.3	3.7	223.3	183.9
	343.3	4.1	14.5	40.8

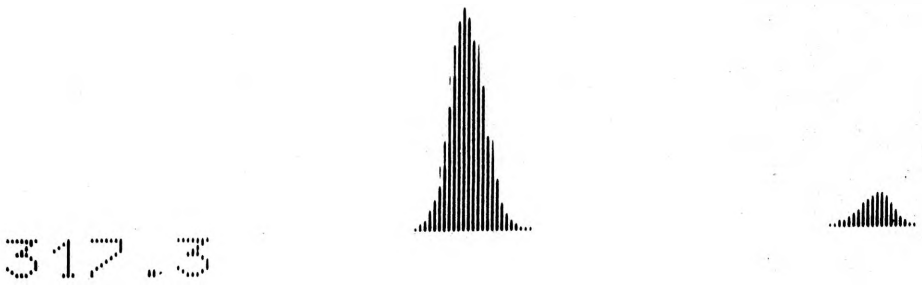
PCIB=	377	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	229	°C
VELE=	162	V	TGCS=	250	°C
CEMI=	0.20	mA	TGCL=	255	°C
VSEM=	1980	V	TSOU=	195	°C

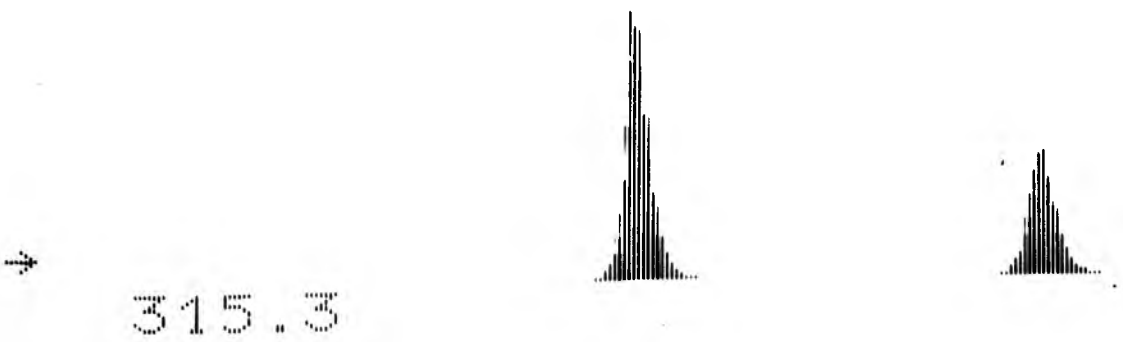
#	MASS	TIME	INTENSITY	
1	341.3	3.2	0.0	0.0
	341.3	3.7	113.4	100.0
	341.3	4.1	15.5	100.0
2	343.3	3.2	0.0	0.0
	343.3	3.7	73.8	65.0
	343.3	4.1	49.9	321.9

PCIB=	442	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	191	°C
VELE=	0.06	V	TGCS=	249	°C
CEMI=	0.00	mA	TGCL=	256	°C
VSEM=	2080	V	TSOU=	201	°C

#	MASS	TIME	INTENSITY	
1	315.3	4.7	129.0	100.0
	315.3	5.1	59.2	100.0
	343.3	5.9	56.8	100.0
	343.3	6.4	4.8	100.0
2	317.3	4.7	208.2	161.3
	317.3	5.1	32.4	54.7
	345.3	5.9	15.1	26.5
	345.3	6.4	21.4	45.8

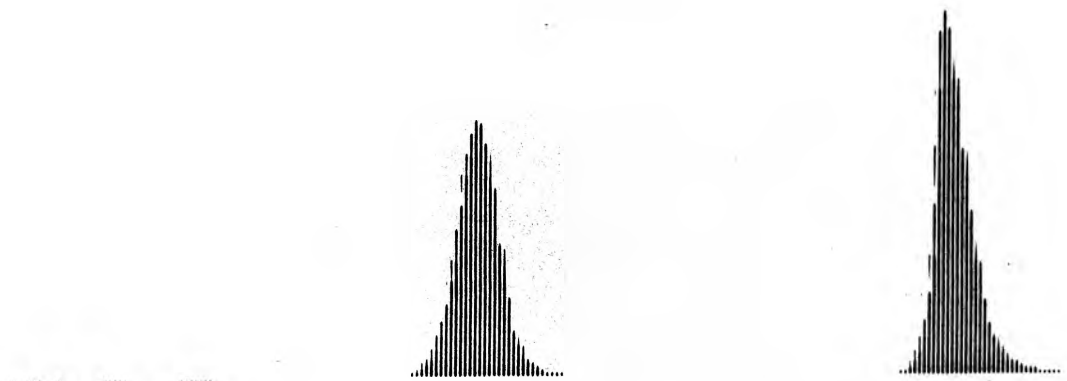
PCIB=	441	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	183	°C
VELE=	0.06	V	TGCS=	250	°C
CEMI=	0.00	mA	TGCL=	255	°C
VSEM=	2030	V	TSOU=	198	°C
	5.4				



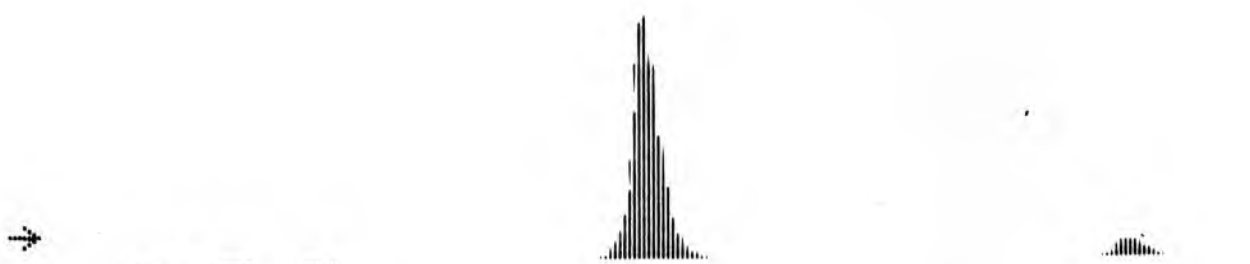


GC

PCIB=	442	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	190	°C
VELE=	0.06	V	TGCS=	250	°C
CEMI=	0.00	mA	TGCL=	255	°C
VSEM=	2070	V	TSOU=	201	°C
	6.6				



345.3



343.3

GC

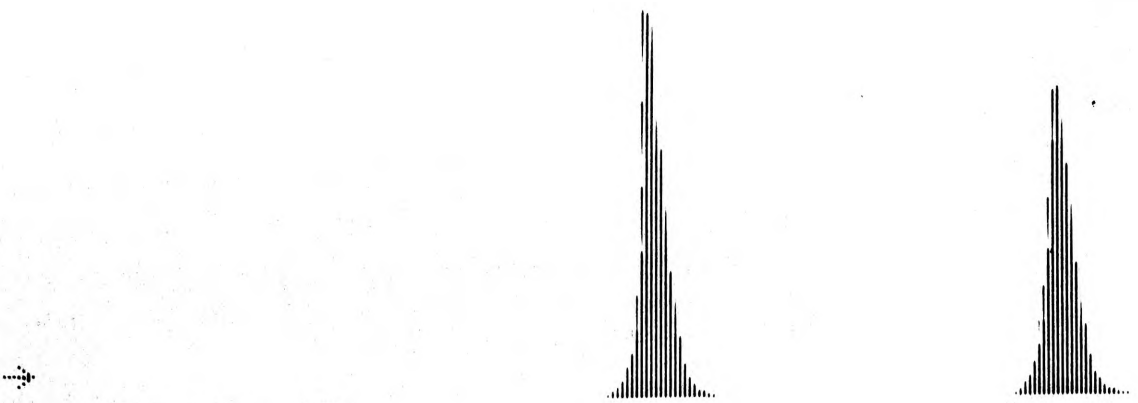
PCIB = 444 µb TGCI = 250 °C
PSOU = 0.0 nb TGCO = 191 °C
VELE = 0.06 V TGCS = 250 °C
CEMI = 0.00 mA TGCL = 256 °C
VSEM = 2080 V TSOU = 201 °C

#	MASS	TIME	INTENSITY	
1	315.3	4.7	182.6	100.0
	315.3	5.1	146.3	100.0
	343.3	6.0	147.8	100.0
	343.3	6.4	26.1	100.0
2	317.3	4.7	318.0	174.1
	317.3	5.1	46.2	31.5
	345.3	5.9	67.8	45.8
	345.3	6.4	39.4	150.9

PCIB=	444	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	182	°C
VELE=	0.06	V	TGCS=	250	°C
CEMI=	0.00	mA	TGCL=	256	°C
VSEM=	2030	V	TSOU=	199	°C

5.4

317.3

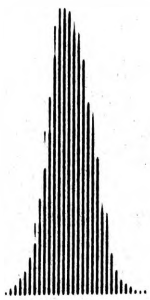


315.3

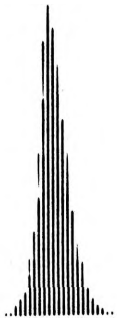
GC

PCIB=	444	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	189	°C
VELE=	0.06	V	TGCS=	250	°C
CEMI=	0.00	mA	TGCL=	255	°C
VSEM=	2090	V	TSOU=	201	°C

6.5



345.3



343.3

GC

PCIB=	448	µb	TGCI=	250	°C
PSOU=	0.0	nb	TGCO=	208	°C
VELE=	0.06	V	TGCS=	250	°C
CEMI=	0.00	mA	TGCL=	256	°C
VSEM=	2030	V	TSOU=	199	°C

#	MASS	TIME	INTENSITY	
1	327.4	6.7	324.9	100.0
	327.4	7.0	318.6	100.0
	333.3	10.1	74.9	100.0
	333.3	10.8	166.8	100.0
2	329.4	6.7	91.0	28.0
	329.4	7.0	315.4	98.9
	335.3	10.1	54.2	72.5
	335.3	10.7	54.3	32.5

PCIB=	440	µb	TGCI=	250	°C
PSOU=	0.0	mb	TGCO=	203	°C
VELE=	162	V	TGCS=	250	°C
CEMI=	0.04	mA	TGCL=	256	°C
VSEM=	2030	V	TSOU=	199	°C

#	MASS	TIME	INTENSITY	
1	327.4	6.7	253.2	100.0
	327.4	7.0	260.9	100.0
	333.3	10.1	324.6	100.0
	333.3	10.8	148.8	100.0
2	329.4	6.7	443.1	175.0
	329.4	7.0	138.5	18.2
	335.3	10.1	51.4	15.8
	335.3	10.8	369.0	247.9