Substrate Analogs Based on Phosphoenolpyruvate

by

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INTRODUCTION

In order to study the mechanism of action of enzymes which utilize the enol phosphate of pyruvic acid (PEP) as a substrate, it was desired to find improved synthetic routes to the known PEP analog,[1] α-(dihydroxyphosphinylmethyl)acrylic acid (CH_2-PEP). It was hoped that these routes might be utilized for the preparation of a ^{13}C-labeled CH_2-PEP analog and stereospecifically deuterium-labeled CH_2-PEP analogs.

In recent years some elegant work[2] has revealed that the enzymes of glycolysis and gluconeogenesis catalyze reactions in a stereospecific manner, that is, in a manner whose absolute stereochemistry is determined by the optical dissymmetry of the macromolecule. It is precisely the dissymmetric nature of the macromolecule which allows it to recognize and distinguish between the faces of PEP. The optical activity of the enzymatic product, 2-phosphoglyceric acid (2-PGA), in the case of the enolase (2-phospho-D-glycerate hydrolase) reaction, is proof of this ability. While this information does not tell us whether enolase catalyzed <u>cis</u>- or <u>trans</u>-addition of water to PEP, X-ray crystallography gives us the absolute configuration of 2-PGA. Thus, two relatively simple physical measurements on enzymatically formed 2-PGA inform us that enolase catalyzed the stereospecific addition of a proton to the <u>si</u> face[4] of PEP at C-2. A more subtle and elegantly conceived physical measurement had to be made in order to prove that enolase added water with <u>trans</u>- stereospecificity.[5]

It has been shown that CH_2-PEP (the known phosphonate analog of PEP) is a substrate for the enolase-catalyzed reaction.[1,6] It would be of value to show that enolase treats CH_2-PEP with the same stereospecificity as it

PEP at C-2. A more subtle and elegantly conceived physical measurement had to be made in order to prove that enolase added water with trans- stereospecificity.

It has been shown that CH_2-PEP (the known phosphonate analog of PEP) is a substrate for the enolase-catalyzed reaction. If one could show that enolase treats CH_2-PEP with the same stereospecificity as it does PEP, they would thereby provide experimental verification of the assigned specificity of the enolase reaction. This verification is necessary in view of the fact that a large series of interlocking stereochemical assignments for glycolytic and gluconeogenic enzymes depends on the assigned stereospecificity of enolase. When the vinyl protons of CH_2-PEP can be unambiguously assigned and stereospecifically deuterium-labeled CH_2-PEP or CH_2-2-PGA analogs have been prepared, it will be possible to directly verify the enolase stereospecificity.

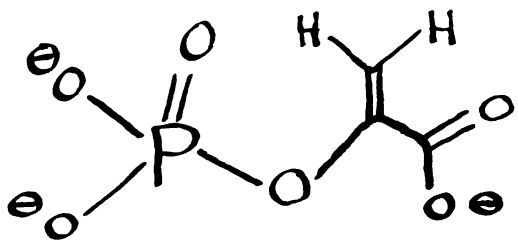
The vinyl protons of CH_2-PEP have now been assigned. Although it is not yet possible to prepare stereospecifically deuterium-labeled CH_2-PEP or CH_2-2-PGA analogs, some progress has been made towards characterizing intermediates which could eventually be used in a stereospecific synthesis. Some of these "intermediates" could prove useful in their own right as potentially interesting compounds for study in a biological system. By retaining some of the features of PEP and making small additions to the structure, some compounds were prepared which might be of interest to study

with some of the other PEP-utilizing enzymes. Some of these substituted-PEP analogs might also resemble the preferred conformation and distribution of charge of a biologically important pyrophosphate or triphosphate.

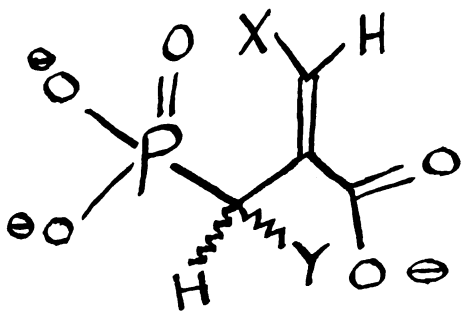
George L. Kenyon

TABLE OF CONTENTS

Below are composite drawings of some of the substituted CH_2-PEP analogs which were sought.

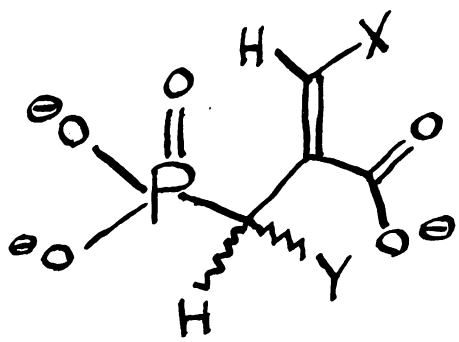


PEP



X = H
= COOH
= OH
= O–P(=O)(O⊖)(O⊖)

Y = H
= COOH
= P(=O)(O⊖)(O⊖)



X = H
= COOH
= OH

Y = H
= COOH
= P(=O)(O⊖)(O⊖)

II. HISTORICAL

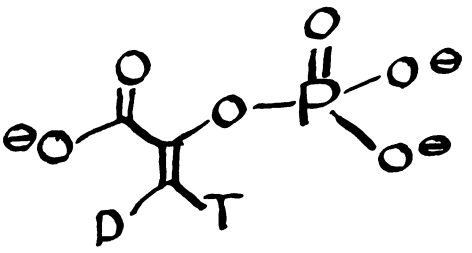
Ultimately, the assignment of the stereospecificity of each of the following enzymes of glycolysis and gluconeogenesis of the is dependent on the stereochemistry of the enolase-catalyzed reaction.

Table I

Glycolytic	Reaction Catalyzed
Pyruvate kinase[8]	$H^+ + PEP + ADP \rightleftarrows Pyr + ATP$
Malate dehydrogenase decarboxylating enzyme[8]	$NAD^+ + Malate \rightleftarrows Pyr + H^+ + CO_2 + NADH$
Gluconeogenic	
Pyruvate carboxylase[8]	$ATP + CO_2 + Pyr \overset{biotin}{\rightleftarrows} H^+ + oxalacetate + ADP + P_i$
PEP carboxykinase[9]	$PEP + CO_2 + GDP \rightleftarrows GTP + oxalacetate$
PEP carboxytransphosphophorylase	$PEP + CO_2 + P_i \rightleftarrows PP_i + oxalacetate$
PEP PEP carboxylase[9]	$PEP + CO_2 + OH^- \rightleftarrows P_i + oxalacetate$
Oxalacetate decarboxylase[10,11]*	$oxalacetate + H^+ \rightleftarrows pyr + CO_2$
3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase[12]	erythrose 4-phosphate + PEP $\rightleftarrows$ DAHP

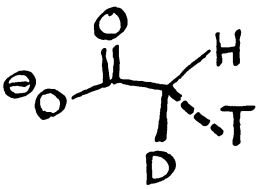
* This enzyme has been shown to be identical to pyruvate kinase. Ref.11

Specifically labeled phosphoenolpyruvates were prepared utilizing the known specificities of enolase and phosphoglucose isomerase (D-glucose-6-phosphate ketol-isomerase).[9]

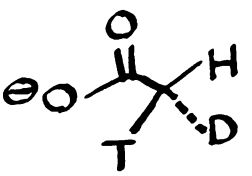


Z-isomer[13]

The malate synthetase - fumarase analytical sequence was used to distinguish isotopic enantiomers of acetate.[14,15]



(S)-2-deuterio-2-tritioacetic acid

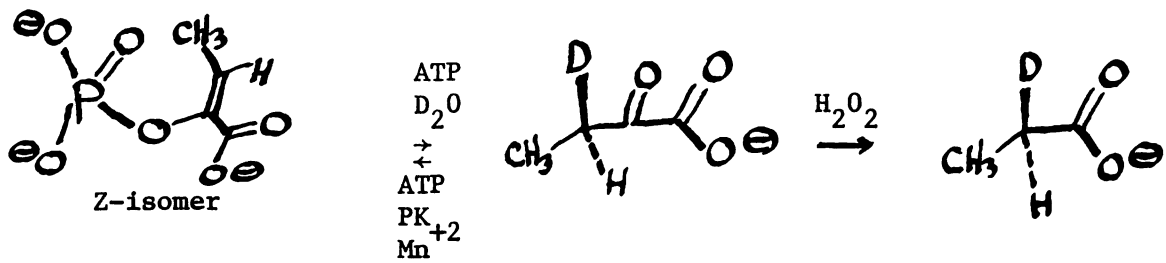


(R)-2-deuterio-2-tritioacetic acid

The assigned stereospecificities of enolase[5], phosphoglucose isomerase,[14,17] malate synthetase,[14,15] and fumarase[18] led eventually to assignments of the stereospecificities of the enzymes listed in Table I.

The ability to prepare isotopic enantiomers of pyruvate,

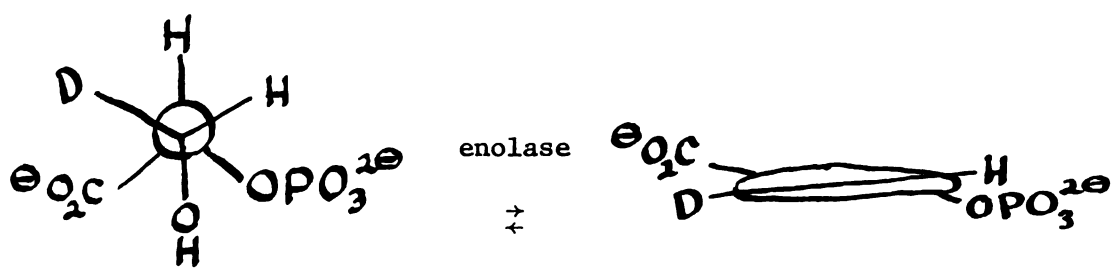
oxalacetate, and malate depends on the stereospecificity of pyruvate kinase as determined by Rose et al.[8] A specifically labeled PEP was prepared using the phosphoglucose isomerase - enolase sequence. The chiral pyruvate formed in the pyruvate kinase reaction was then analyzed by the malate synthetase -fumarase sequence. Further support for the specificity of pyruvate kinase was provided when it was found thatα-ketobutyrate is a substrate for the enzyme.[19,20]



Z-isomer

One can compare the optical rotation of the pyruvate kinase-derived α-deuteriopropionate with the rotation of α-deuteriopropionate of known absolute configuration.[21] Since it was determined that the Z-isomer of phosphoenol α-ketobutyrate is the substrate in the opposite direction, one concludes that pyruvate kinase does in fact add a proton to the 2 si, 3 re face of PEP at C-3.[22]

Cohn et al. determined the stereochemistry of the enolase reaction to be the following:

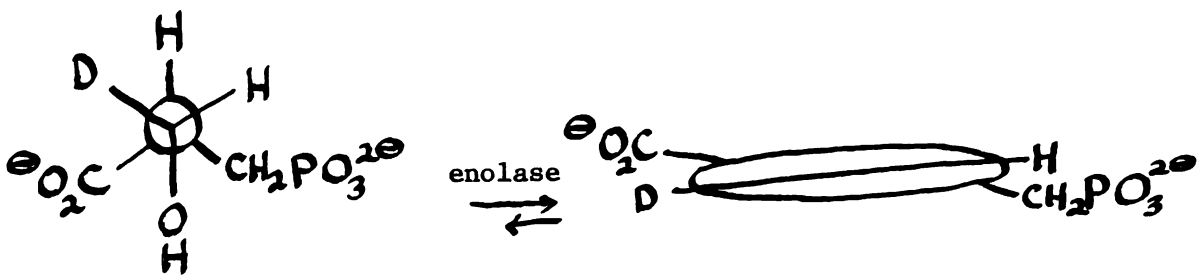


(3R)-2-PGA-3-d

PEP-3-d

The configuration at C-3 of the 2-PGA-3-d which was established in the reaction of phosphoglucose isomerase to form fructose-6-phosphate in D_2O is ultimately based[16] on the comparison of phosphoglucose-2-d derived from C-1 and C-2 of this F-6-P with specifically deuterated Li glycolate.[17] The absolute configuration of the latter was was determined by neutron diffraction. The geometry at C-3 of the PEP-3-d formed in the enolase reaction was determined on the basis of an NMR assignment* of the vinyl protons of PEP.

Direct experimental verification of the assigned stereospecificity of enolase could be provided if 1) the vinyl protons of CH_2-PEP can be assigned in the NMR spectrum, 2) specifically deuterium-labeled CH_2-PEP or CH_2-2-PGA can be prepared, and 3) it can be shown that enolase treats CH_2-PEP with the same specificity as it does PEP.**



(3R)-CH_2-2-PGA-3-d CH_2-PEP-3-d

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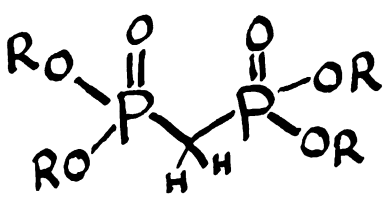
* The NMR assignment of the vinyl protons of PEP is based on the experimental observation (Ref. 23) and theoretical verification (Ref. 24) that J trans > J cis for spin-spin coupling between magnetic nuclei substituted directly to a double bond.

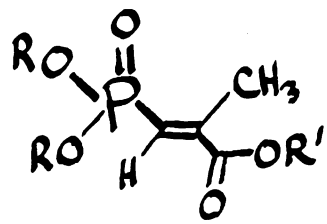
**NMR kinetic data (Refs. 1, 6) indicate that this condition obtains but one should not assume this a priori.

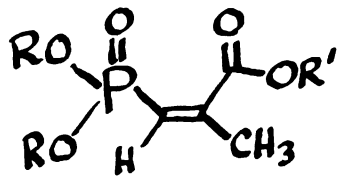
III. DISCUSSION AND RESULTS

A. Modified Wittig reactions as synthetic routes to phosphonate analogs of PEP

In order to prepare phosphonate analogs of phosphoenolpyruvate, the Wadsworth-Emmons-Horner (modified Wittig) reaction,[25,26] was explored. When the preformed α-anion of bis-(dimethoxyphosphinyl) methane (1a) was treated with ethyl pyruvate in a manner similar to the procedure of Huff, Moppett, and Sutherland,[27] a mixture of E and Z isomers of ethyl (α-methyl-β-dimethoxyphosphinyl)acrylate (2Ea) and (3Za) was isolated in good yield.



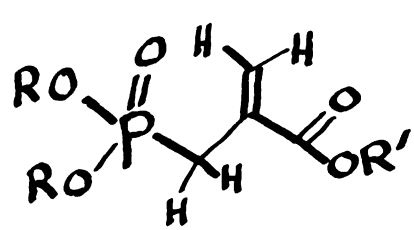




1a	R = CH_3	2Ea	R = CH_3	R' = Et	3Za	R = CH_3	R' = Et
b	R = Et	b	R = Et	R' = Et	b	R = Et	R' = Et
C	R = Isopropyl	c	R = Et	R' = H	c	R = Et	R' = H
		d	R = CH_3	R' = CH_3	d	R = CH_3	R' = CH_3
		e	R = H	R' = H	e	R = H	R' = H

When the reaction was conducted with an excess of sodium hydride, substantial amounts of the known itaconate analog, ethyl (α-dimethoxyphosphinylmethyl)acrylate[1] (4a), were formed as well as 2Ea and 3Za.

4a	R = CH_3	R' = Et
b	R = Et	R' = Et
c	R = Et	R' = H
d	R = CH_3	R' = CH_3
e	R = H	R' = H



Treatment of a mixture of 2Ea and 3Za with sodium hydride under equilibrium conditions gave varying amounts of rearrangement to 4a. The yield for this rearrangement reaction has not yet been optimized. The modified Wittig reaction and subsequent rearrangement was found to also occur when the more readily accessible bis-(diethoxyphosphinyl)-methane (1b) was treated with ethyl pyruvate. Bis-(diisopropoxy-phosphinyl)-methane (1c), however, failed to undergo the modified Wittig reaction with ethyl pyruvate, presumably due to steric hindrance.

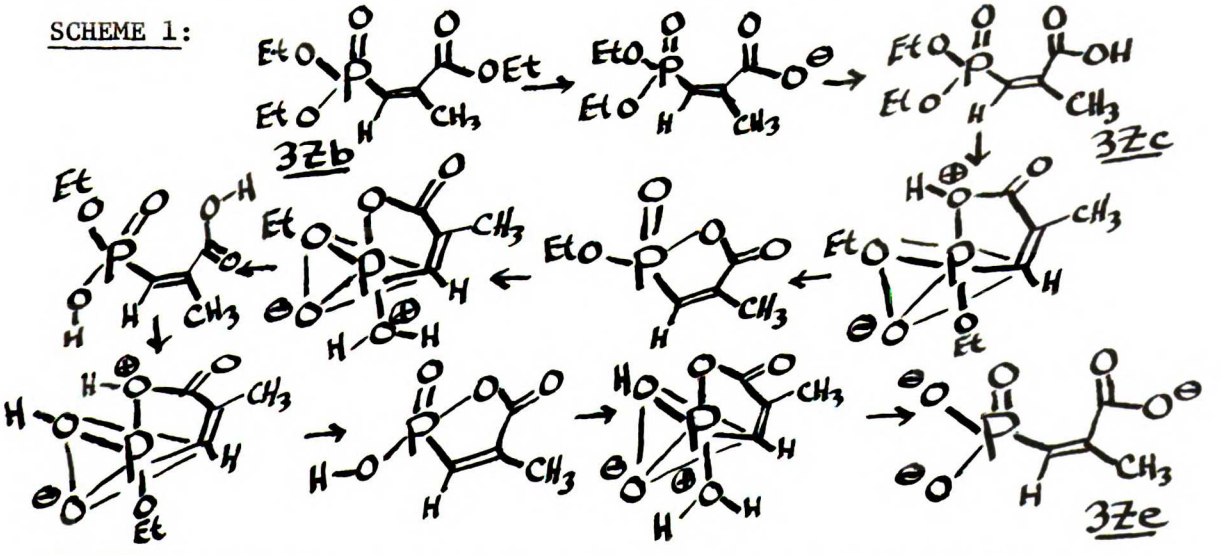
Treatment of a mixture of 2Eb and 3Zb with sodium ethoxide in ethanol under equilibrium conditions gave rearrangement of 3Zb to 4b as judged by NMR. Compound 2Eb remained unchanged. Treatment of the mixture of 2Eb and 3Zb with potassium hydride in tetrahydrofuran both at 0° and -78° resulted in polymerization of the reaction mixture. This presumption is supported by the fact that no vinyl protons were observed in the NMR spectrum of the reaction mixture.

A definitive assignment of the geometries of 2Eb and 3Zb has been made on the basis of the following observations. A mixture of 2Eb and 3Zb was dissolved in water, stirred 3 days at room temperature with one equivalent of sodium hydroxide, acidified with 12N HCl to pH 2, and thorougly extracted. The CH_2Cl_2 extract was found to contain only 2Ec in the amount consistent with its being formed from 2Eb. The aqueous layer was found to contain only 3Ze in the amount consistent with its being formed from 3Zb. Compound 2Ec was

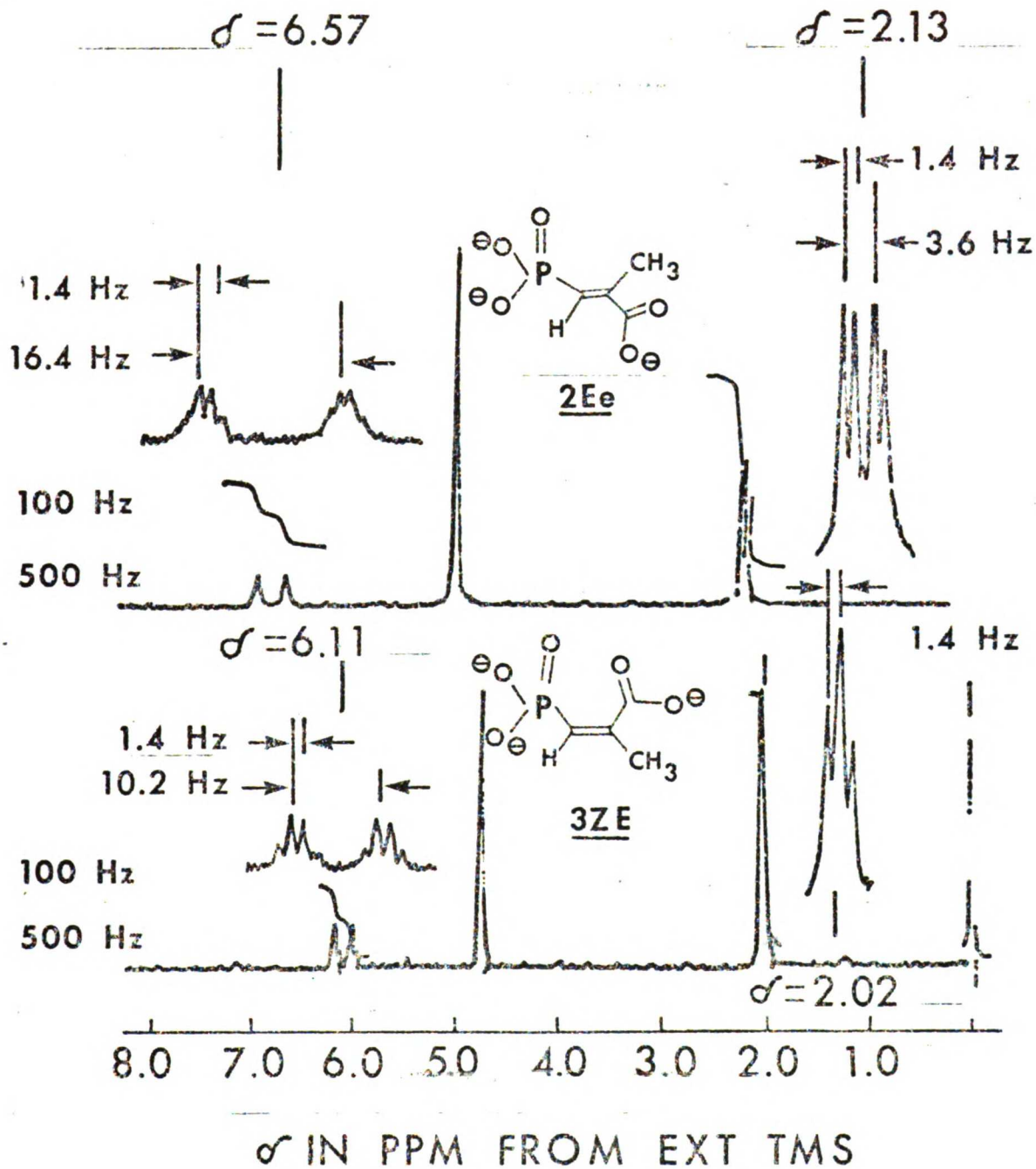
converted to 2Ee by heating at reflux for 36 hr in 6N HCl. Compound 4b, 4c, and 4e were not observed in either the organic or aqueous phases.* (See page 11 for a comparison of the NMR spectra of 2Ee and 3Ze.)

The rapid conversion of 3Zb to 3Ze at room temperature (less than 10 min at pH 2) is indicative of a reaction proceeding via intramolecular nucleophilic catalysis.[51,52] An "in-line", S_N2-type mechanism can be invoked to explain these results. Both exocyclic and endocyclic cleavages are thought to occur. An "adjacent" mechanism involving only exocyclic cleavages is probably prohibited since pseudorotation would violate one of the preference rules[41] for pentacoordination at phosphorus. In Scheme 1 below, proton transfer steps have been omitted for the sake of clarity.

SCHEME 1:



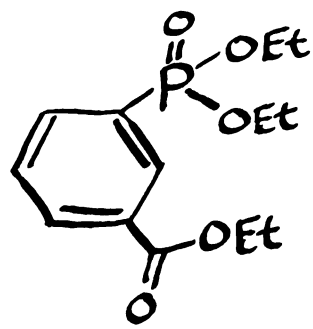
* When the reaction sequence was repeated in D_2O with 40% NaOD and 6N DCl, no evidence for base- or acid-catalyzed deuterium exchange was observed as judged by examining the NMR spectra of the products obtained, 2Ee and 3Ze.


δ =6.57
δ =2.13
1.4 Hz
3.6 Hz
1.4 Hz
16.4 Hz
2Ee
100 Hz
500 Hz
δ =6.11
1.4 Hz
1.4 Hz
10.2 Hz
3ZE
100 Hz
500 Hz
δ=2.02
8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
δ IN PPM FROM EXT TMS

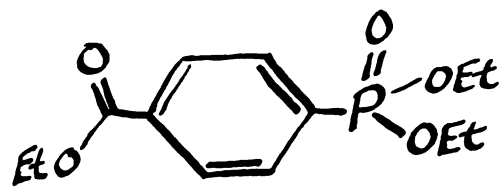
2Ec 2Ee

Strong acid and heat are required to convert 2Ec to 2Ee. Under these conditions, the conversion of 2Ec to 2Ee probably proceeds through an S_N1 mechanism wherein a carbon-oxygen bond is broken.[44] Exchange experiments with ^{18}O have shown[70,71] that in the acidic hydrolysis of trimethyl phosphate, C-O fission predominates. In addition, alkyl phosphonates of optically active alcohols are hydrolyzed with extensive racemization in acid solution.[72]

For both 2Eb and 3Zb, the initial base-catalyzed hydrolysis is thought to occur exclusively at the carboxy ester.[42,43] This assumption is substantiated by the work of Freedman and Jaffe (1954)[42] who showed that 5 and 6 below could be selectively hydrolyzed to 7 and 8 under basic conditions.



5



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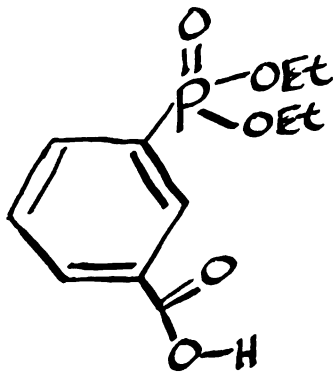

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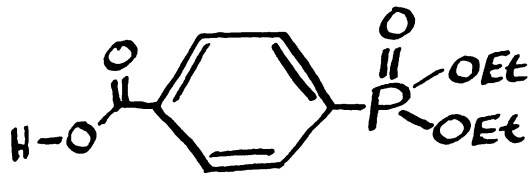
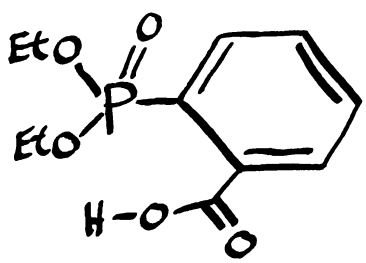


8

Precedent for intramolecular carboxyl-group participation leading to accelerated rates of hydrolysis in phosphonate esters is found in the work of Gordon, Notaro, and Griffin (1964)[40] and Blackburn and Brown (1968).[39] A vastly increased rate of hydrolysis (8 X 10^7-fold) was observed for 9 as compared to the para-substituted 10. An "in-line", S_N2-type, mechanism was proposed[39] to explain the results.



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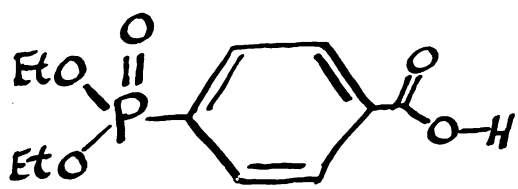


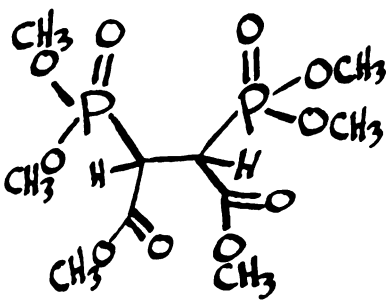
10

The structure proof and assignment of geometries to (α-methyl-β-dihydroxyphosphinyl)acrylic acid (2Eb) and 3Zb) confirms the predictions that 1) the vinyl proton of 2Eb will resonate at lower field than the vinyl proton of 3Zb due to its cis-relationship to a methoxycarbonyl group[28] and 2) the allylic methyl protons of 2Eb will resonate at lower field than those of 3Zb due to their

cis-relationship to a dimethoxyphosphinyl group. Anisotropic deshielding effects in the NMR spectrum are widely used to make geometric assignments in the absence of other data.[28]

Hydrolysis of a mixture of 2Ea, 3Za, and 4a with 48% HBr for 1.5 hr at reflux gave a mixture of triacids 2Ee, 3Ze, and 4e whose ratio of integrated NMR intensities corresponded to the ratio of the initial mixture of esters. The conditions employed for the hydrolysis were apparently not severe enough to achieve thermal equilibration of the mixture of esters or the triacids.* Fractional crystallization from water gave a 54% yield (based on 4a) of α-(dihydroxyphosphinylmethyl) acrylate (4e) which possessed an NMR spectrum identical to that previously reported.[1]

The modified Wittig reaction of hexamethyl 2,3-diphosphono-succinate[32] (10) with paraformaldehyde was found to give mixtures of 11 and 12.



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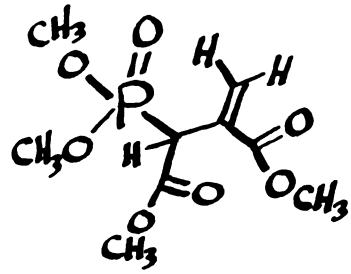
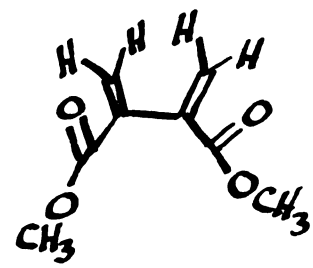


11



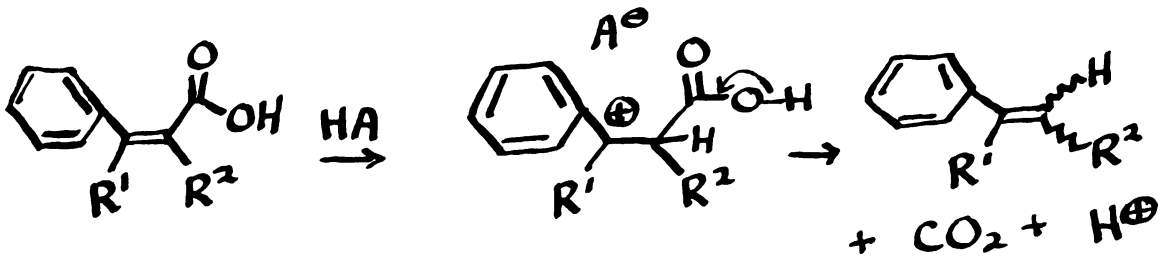
12

*Sakai[31] observed that the thermal rearrangement of citraconic to itaconic acid can be achieved in H_2O by heating to 170° for 22 hr in a bomb.

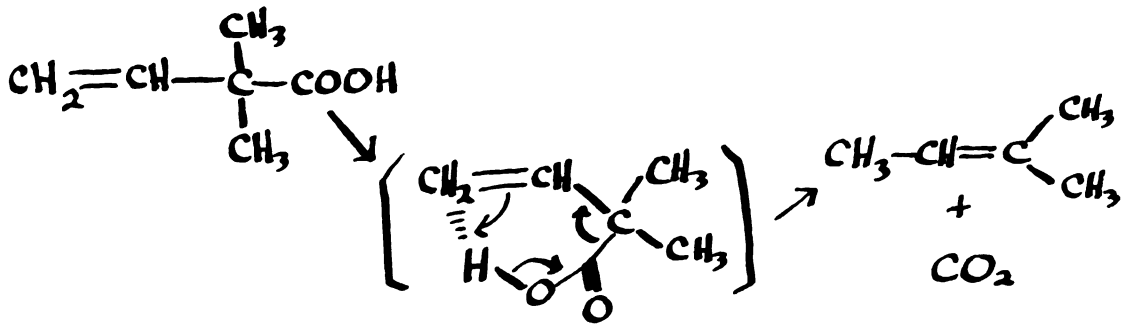
The overall yield and relative amount of each product varied considerably depending on 1) temperature, 2) reaction time, 3) sequence of addition, 4) solvent, 5) stoichiometry, and 6) nature of base employed. Compound 12 was always the major product in greater than 60% isolated yield regardless of the conditions employed. Compound 11 could be short-path distilled and on hydrolysis (1.5 hr, 48% HBr reflux) and recrystallization gave 4e whose NMR spectrum was again identical to that previously reported.[1]

The decarboxylation of 11 without double bond rearrangement was not unexpected in view of the apparently greater thermodynamic stability of 4a with respect to 2Ea and 3Za. The α-dimethoxyphosphinyl group probably stabilizes a cyclic transition state for decarboxylation. Methyl (dimethoxyphosphinyl)acetate is known to decarboxylate on thermal, acid-catalyzed hydrolysis.[33] The failure of 4e to decarboxylate under these conditions is probably due to its inability to isomerize to a β,γ-unsaturated carboxylic acid and the reluctance to form a β-substituted primary carbonium ion. The following examples lend support to this hypothesis.

Under acidic conditions, cinnamic acids are thought to decarboxylate via a β-carbonium ion mechanism.[35]

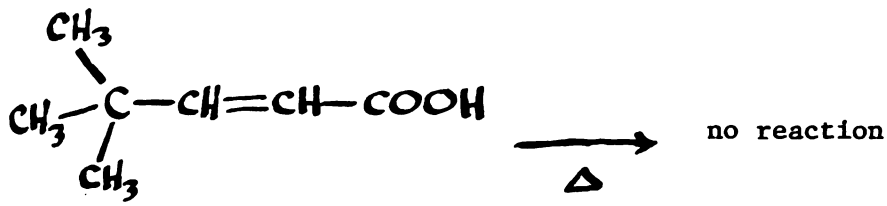


When R^1 = H and R^2 = CH_3, the slowest rates of decarboxylation were achieved. In another study, the pyrolysis of 2,2-dimethyl butenoic acid (13) has been shown to give 2-methyl-2-butene (14) as the sole olefinic product.[34]



13 14

Compound (15) 4,4-dimethyl penten-2-oic acid, however, remained unchanged on attempted pyrolysis.



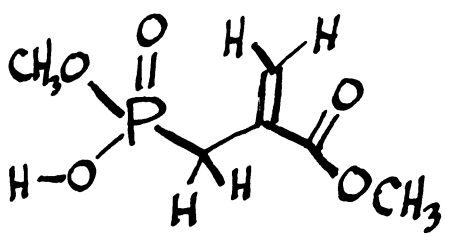
15

B. Synthesis of ^{13}C-labeled α-(dihydroxyphosphinylmethyl)acrylate

In order to assign the vinyl protons of α-(dihydroxyphosphinylmethyl)acrylate unambiquously, a suitable labeled synthesis was sought. A modification of the Stobbe condensation[45] seemed appropriate.

The normal course of the Stobbe condensation is addition to the carbonyl of a ketone followed by cyclization of the β-hydroxy alkoxide anion to a γ-lactone follwed by E_1 or E_2 elimination to give an α,β- unsaturated itaconate monoester.[65]

Martin, Gordon, and Griffin[53] observed that Stobbe condensation of diethyl β-carboethoxyethylphosphonate with a variety of ketones gives a series of β,γ-unsaturated phosphonates. No evidence was observed for subsequent base-catalyzed rearrangement to β,γ-unsaturated phosphonates. On attempting the analogous reaction with paraformaldehyde, condensation occurred α to the carbonyl as was previously observed. However, the expected mono-acid Stobbe-product, methyl-α-(hydroxymethoxyphosphinylmethyl)acrylate (16), was not observed.

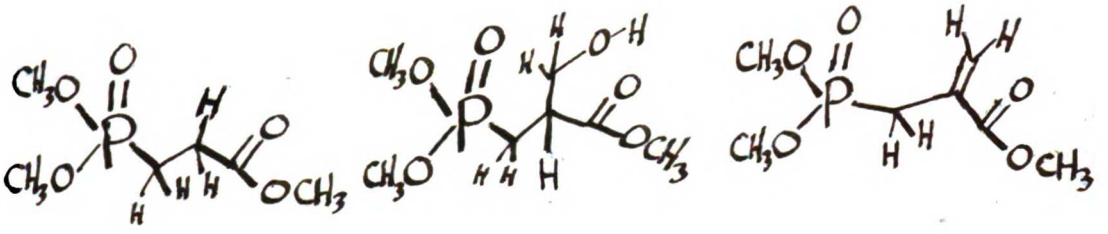


16

Treatment of dimethyl β-carbomethoxyethylphosphonate (17) with potassium hydride in tetrahydrofuran followed by paraformaldehyde gave a 40% yield based on 17 of 4d.* Elimination from an initially

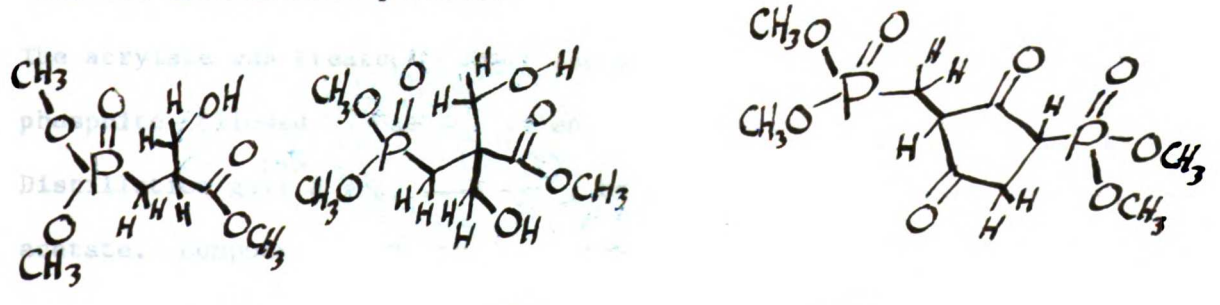
* Alternate methods for introducing methylene groups ∝ to carboxyl groups have been recently reviewed. Ref 48.

formed β-hydroxy (or β-alkoxy) intermediate 18 would explain the observed product.



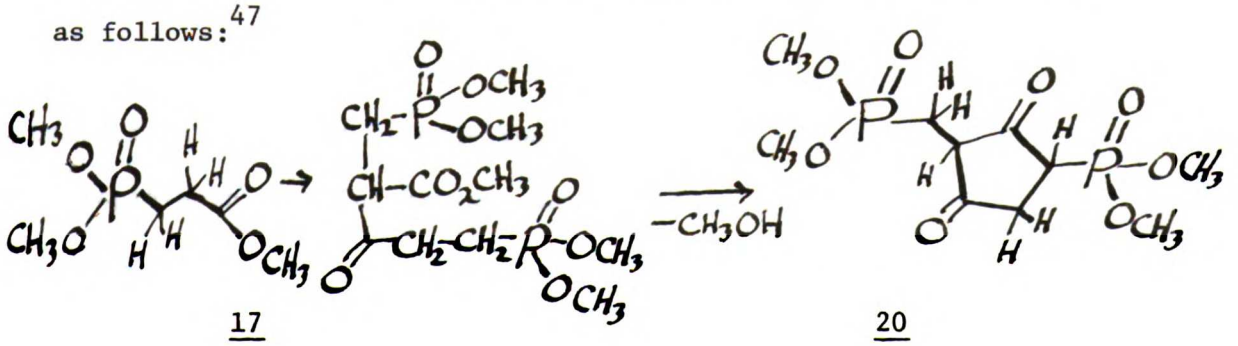
17 18 4d

The NMR spectrum of 4d was qualitatively similar to that of 4a. In addition to 4d, which comprised greater than 75% of the isolated product, unreacted 17 (10%) and at least one unidentified product (15%) were observed. Compounds 18, 19, and 20 are possible candidates for the unidentified product.



18 19 20

Compound 20, the self-condensation product of 17, could be formed as follows:[47]



17 20

Continuous extraction of the acidified reaction mixture might possibly have revealed the anticipated mono-acid Stobbe-product, 16. No evidence was observed for base-catalyzed rearrangement to α,β-unsaturated phosphonates.

Acid-catalyzed hydrolysis of partially purified 4d gave 4e after several purification steps. This synthesis was then employed to prepare a 90% ^{13}C-enriched carboxyl-labeled 4e for the purpose of assigning the vinyl protons of 4d and 4e in the NMR spectrum. Compound 17 with a 90% ^{13}C-enriched carboxyl group was prepared in approximately 60% yield *via* a three-step sequence from ethyl bromoacetate ^{13}C-labeled in the carboxylate position (Koch Isotopes). An Arbusov reaction[65] of triethyl phosphite with ethyl bromoacetate gave a 96% yield of ethyl (diethoxyphosphinyl)acetate. A modified Wittig reaction with paraformaldehyde gave ^{13}C-labeled ethyl acrylate. The acrylate was treated without isolation with an excess of dimethyl phosphite followed by one equivalent of sodium methoxide in methanol.[49] Distillation gave a 62% yield of 17 based on ethyl (diethoxyphosphinyl) acetate. Compound 17 was then treated with potassium hydride and paraformaldehyde in tetrahydrofuran as described yielding 4d. Compound 4d was hydrolyzed and isolated as the dilithium salt of 4e in 12% yield overall based on ethyl bromoacetate.

The ^{1}H NMR spectra of ^{13}C-labeled 4d and 4e are shown for comparison with that of non-labeled 4d and 4e on page 20 and 21. For spin-spin coupling between nuclei substituted directly

FIGURE LEGENDS

Figure 1 (page 20)

Top The proton NMR spectrum ($CDCl_3$) at 60 MHz showing the vinyl proton region of methyl α-(dimethoxyphosphinylmethyl)acrylate

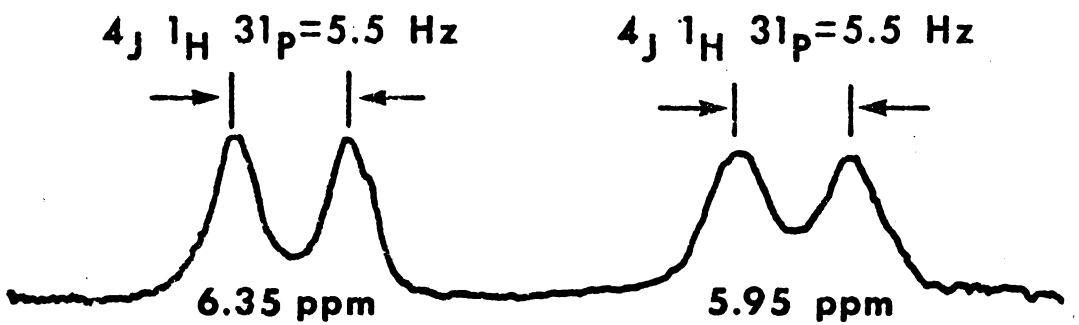
Bottom 90% ^{13}C-enriched in the carbonyl position

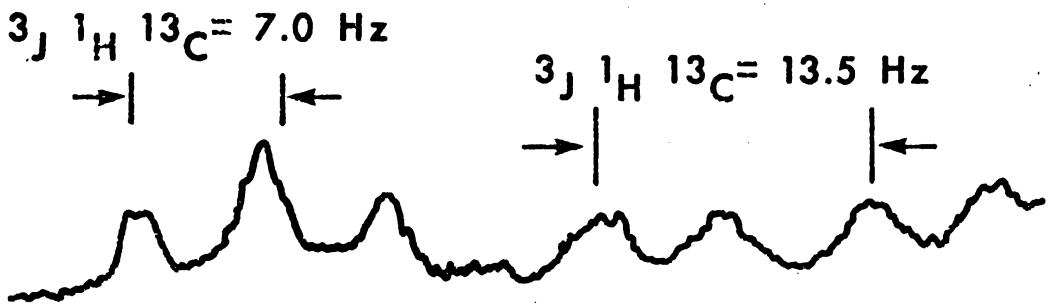
Figure 2 (page 21)

Top The proton NMR spectrum (D_2O) at 60 MHz showing the vinyl proton region of α-(dihydroxyphosphinylmethyl)acrylate dilithium salt

Bottom 90% ^{13}C-enriched in the carbonyl position

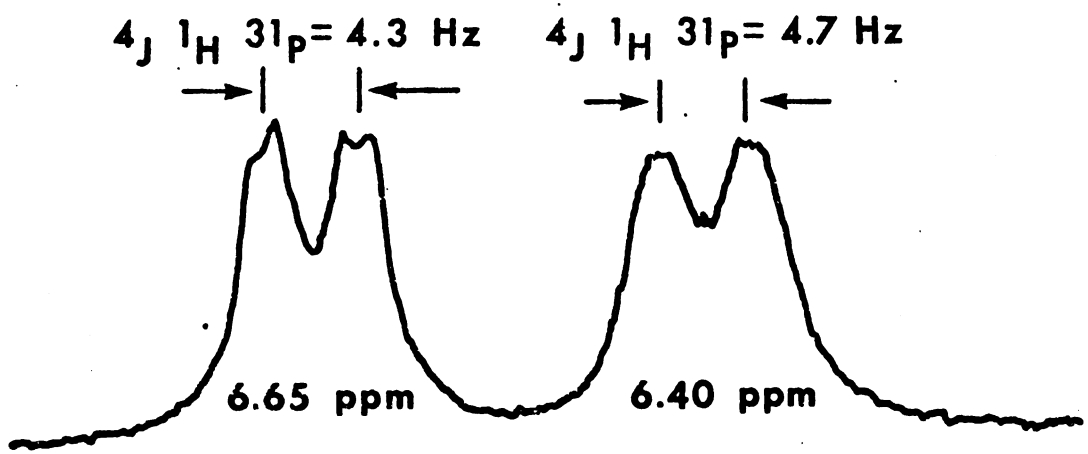
METHYL α-(dimethoxyphosphinylmethyl) ACRYLATE

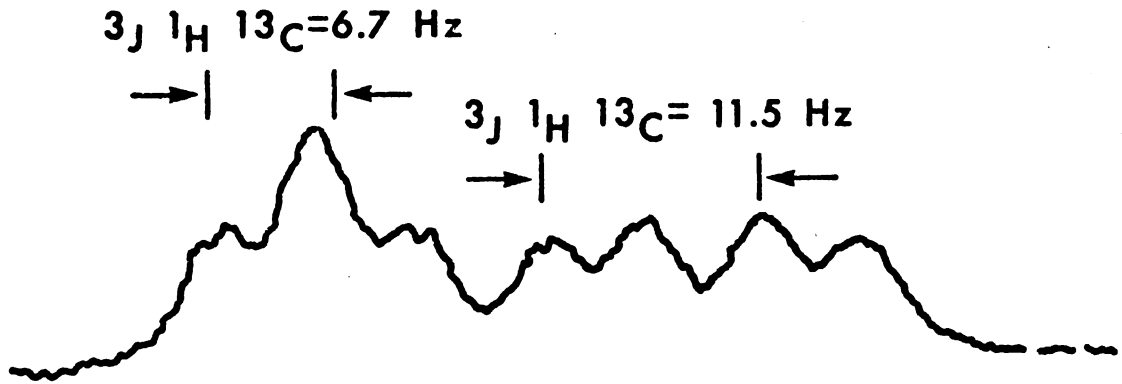




90% ^{13}C-ENRICHED IN THE CARBOXYLATE POSITION

α-(dihydroxyphosphinylmethyl)
ACRYLIC ACID DILITHIUM SALT

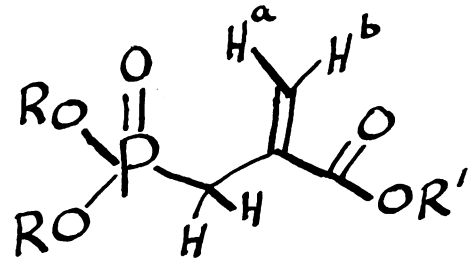




90% ^{13}C-ENRICHED IN THE CARBOXYLATE POSITION

to carbons of a double bond it has been shown without exception both experimentally[23] and theoretically[24] that J trans > J cis. In 4d we observe $^{3}J\ ^{1}H\text{-}^{13}C$ values of 7.0 Hz and 13.5 Hz for the δ = 6.35 ppm and the δ = 5.95 ppm vinyl protons, respectively. In 4e we observe $^{3}J\ ^{1}H\text{-}^{13}C$ values of 6.7 Hz and 11.5 Hz for the δ = 6.65 ppm and the δ = 6.40 ppm vinyl protons, respectively. It is clear from the data that the vinyl proton which is cis to the carboxyl group in both 4d and 4e resonates at lower field than that which is trans to the carboxyl group.

Chemical Shifts[6]
Rel. to TMS/DSS



		Ha	Hb		
4d	δ =	5.95 ppm	6.35 ppm	4d	R = CH_3 R' = CH_3
4e	δ =	6.40 ppm	6.65 ppm	4e	R = H R' = H

The tentative assignment[6] of the vinyl protons on the basis of differences in $^{4}J\ ^{1}H\text{-}^{31}P$ values was considered invalid because of two examples where ^{4}J coupling between ^{31}P and ^{1}H was shown to be J cis >J trans.[7] Similarly, the small differences in $^{4}J\ ^{1}H\text{-}^{1}H$ values were considered inadequate to constitute an unambiguous assignment.[7] A tentative assignment of the vinyl protons of 4e was made by analogy with the assignment of the vinyl protons of PEP. In PEP, the vinyl proton cis to the carboxylate group was shown to

resonate at lower field with $^{3}J\ ^{1}H\text{-}^{13}C$ cis = 3.1 Hz and $^{3}J\ ^{1}H\text{-}^{13}C$ trans = 9.2 Hz for the δ = 5.33 ppm and δ = 5.15 ppm protons, respectively.[5] Similarly, in phosphoenol α-ketobutyrate, it was shown that $^{3}J\ ^{1}H\text{-}^{13}C$ cis = 2.9 Hz and $^{3}J\ ^{1}H\text{-}^{13}C$ trans = 9.5 Hz for the δ = 6.82 ppm and δ = 6.52 ppm protons, respectively.[22]

Many examples are known where the anisotropic deshielding effect of a carbonyl group substituted to a double bond results in the vinyl proton substituted cis to be downfield from the geometric isomer with the vinyl proton substituted trans.[28] In assigning 4d and 4e, we have shown that the anisotropic deshielding of a vinyl proton by a carboxyl group substituted to a double bond is greater than that of a phosphinylmethyl group similarly substituted to a double bond in both the triester and triacid state.

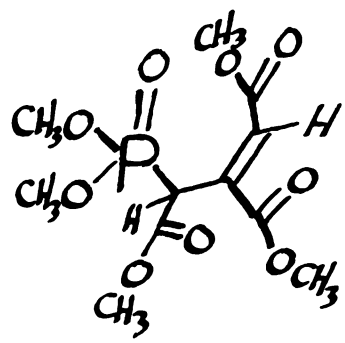
C. Michael additions to dimethyl acetylenedicarboxylate as a synthetic route to phosphonate analogs of PEP

Further evidence for the strong thermodynamic preference for double bond isomers deconjugated*[50,53] from phosphinyl groups is provided by the following results. Michael addition of the preformed

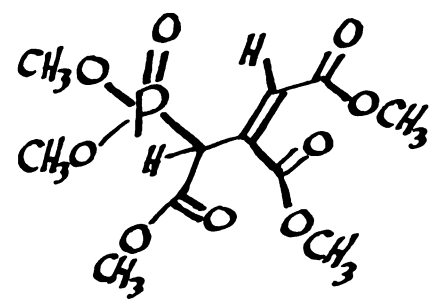
---

* Ionin and Petrov[50] have shown the existence of a base-catalyzed equilibrium between β,γ- and α,β-unsaturated (butenyl) phosphonates with the former isomer predominating.

α-anion of methyl (dimethoxyphosphinyl)acetate to dimethyl acetylene-dicarboxylate under equilibrium conditions gave a mixture of E and Z isomers of β,γ-unsaturated phosphonates 21E and 22Z in the ratio 75E:25Z.

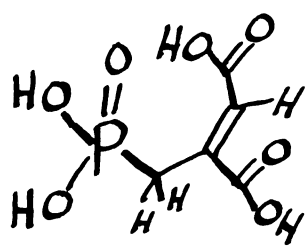


21E

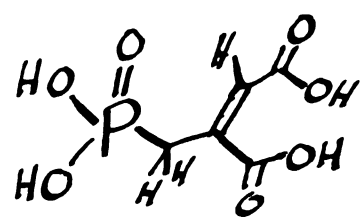


22Z

Hydrolysis of a 75E:25Z mixture of 21E and 22Z with 12N HCl during a 2 hour reflux gave a mixture of E and Z isomers of β,γ-unsaturated phosphonates 23E and 24Z in approximately the same ratio, 75E:25Z. Decarboxylation without double bond rearrangement occurred as predicted. (See page 15)



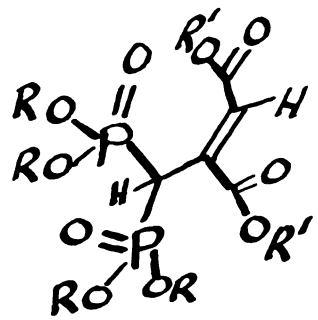
23E



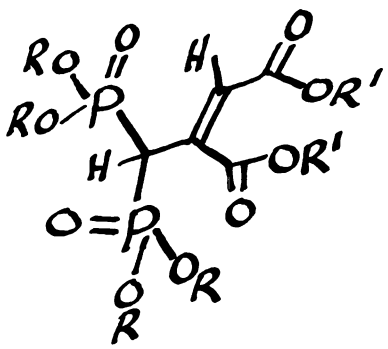
24Z

Compound 23E was separated from 24Z by fractional crystallization from water, the former isomer being slightly less soluble in water. (See page 26 for the NMR spectra of 23E and 24Z.)

Similarly, the preformed α-anion of bis-(dimethoxyphosphinyl) methane (1a)* was treated with dimethyl acetylenedicarboxylate under equilibrium conditions to give a mixture of E and Z isomers of β,γ-unsaturated phosphonates 25Za and 26Ea in the ratio of 65Z: 35E.



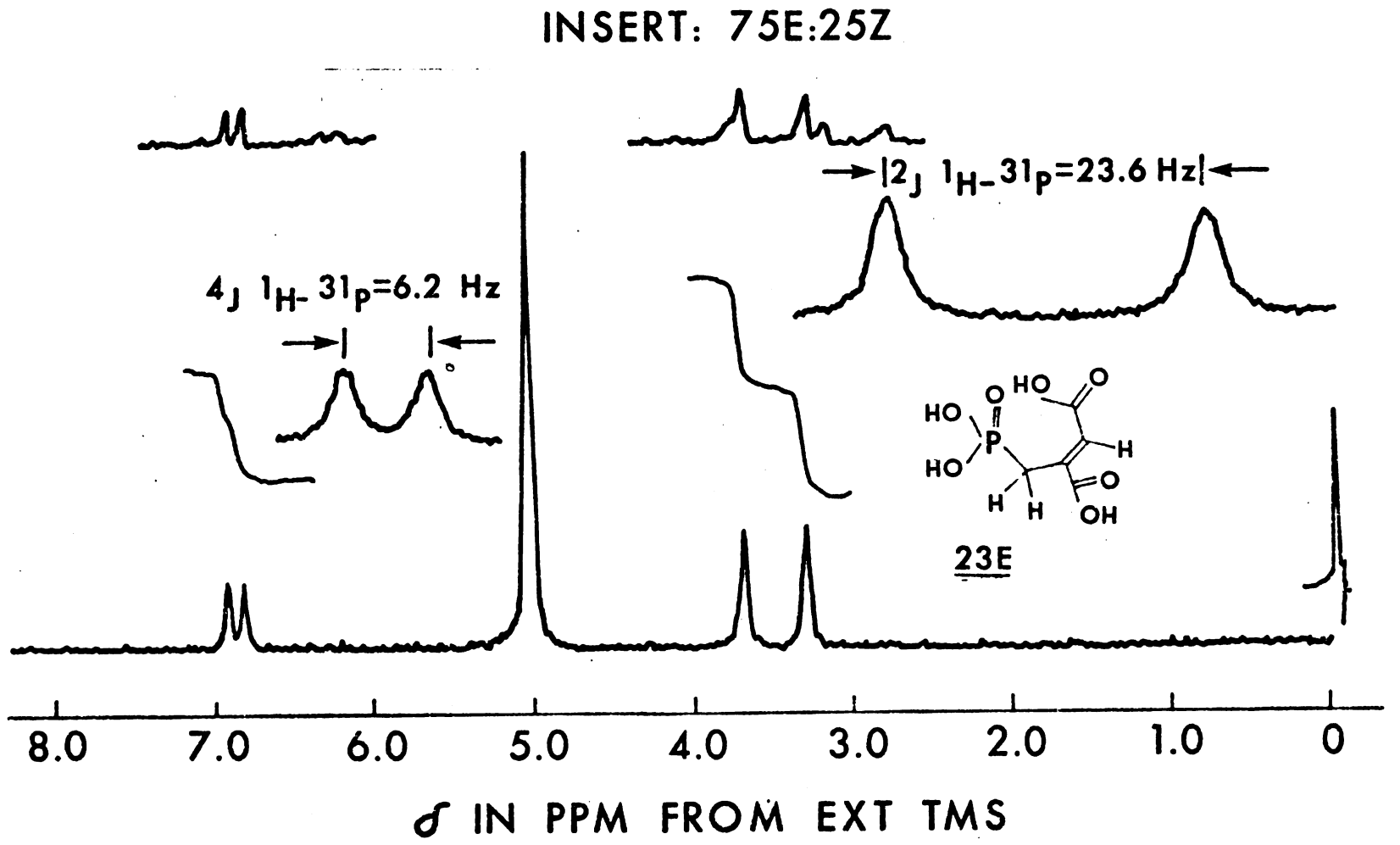
25Za R = CH_3 R' = CH_3
b R = Et R' = CH_3
c R = Si $(CH_3)_3$ R' = CH_3



26Ea R = CH_3 R' = CH_3
b R = Et R' = CH_3
c R = Si $(CH_3)_3$ R' = CH_3

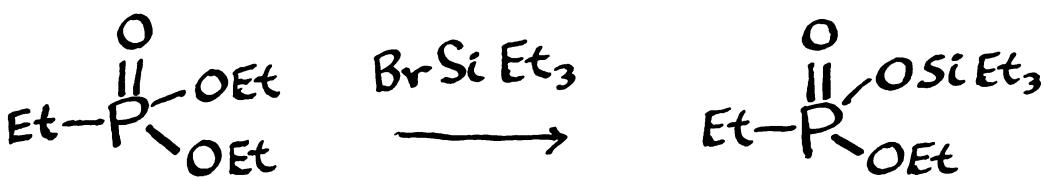
---

* The reaction was also found to occur with the more readily accessible bis-(diethoxyphosphinyl)-methane (1b).


INSERT: 75E:25Z
$^{2}J\ ^{1}H\text{-}^{31}P$=23.6 Hz
$^{4}J\ ^{1}H\text{-}^{31}P$=6.2 Hz
23E
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
δ IN PPM FROM EXT TMS

Treatment of a mixture of E and Z isomers of 25Za and 26Ea in the ratio 40Z:60E* with excess trimethylsilyl bromide, neat, at room temperature, for 15 min., gave a 40Z:60E mixture of 25Zc and 26Ec. Precedent for this mild phosphonate transalkylation reaction comes from several workers,[66,67] L. Malatesta[68] (1950) being one of the earliest.

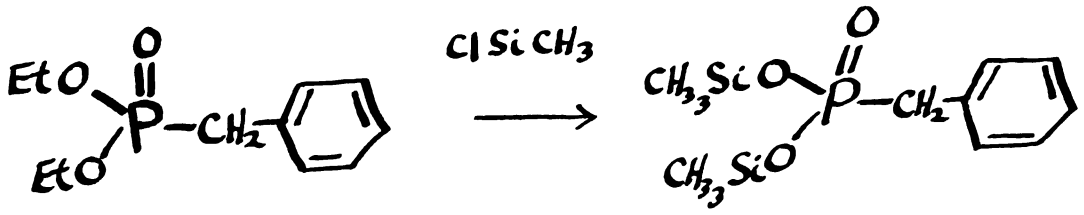
Malatesta[68] showed that treatment of diethyl ethylphosphonate (27) with triethylsilyl bromide gives ethyl triethylsilyl ethylphosphonate (28).



27 28

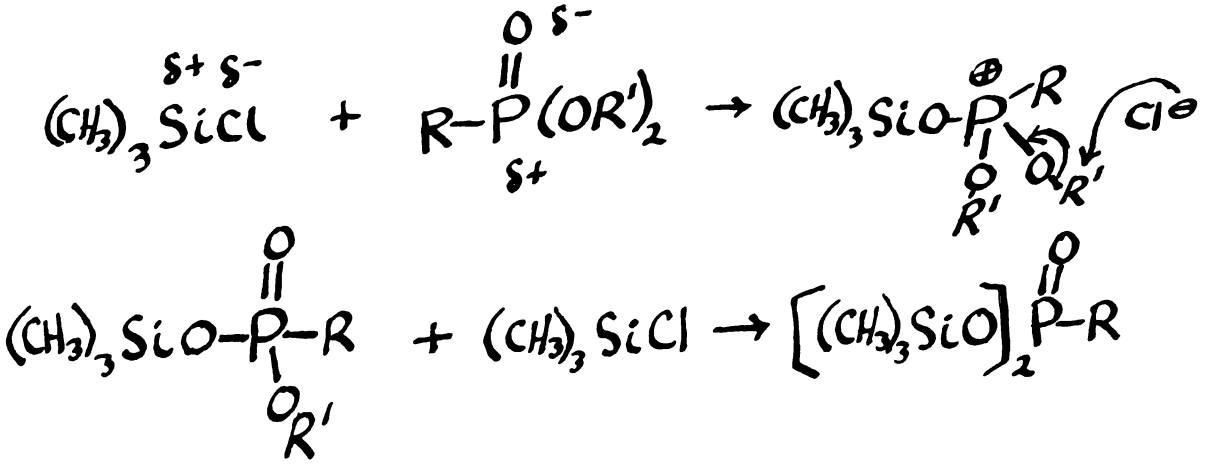
More recently, Rabinowitz[66] (1963) has shown that treating diethyl benzylphosphonate (29) with excess trimethylsilylchloride at reflux for 4 days gives a 93% yield of bis-(trimethylsilyl) benzylphosphonate (30). Rabinowitz also studied the hydrolysis

*This 40Z:60E mixture of 25Za and 26Ea is due to an enrichment of the E isomer during column chromatographic purification of the equilibrium 65Z:35E mixture.

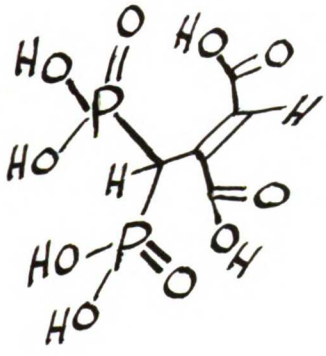


29 30

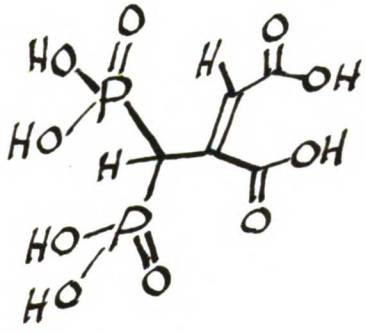
of the resultant silyl phosphonates. He points out that the conventional means of converting a phosphonic acid ester into the corresponding phosphonic acid by refluxing in concentrated aqueous acid[73] is not applicable to phosphonates containing acid or water sensitive groups. The mechanism he proposes for the transalkylation reaction is the following:



Hydrolysis of the equilibrium 40Z:60E mixture of 25Zb and 26Eb with 12N HCl during a 3 hour reflux gave a mixture of E and Z isomers of β,γ-unsaturated phosphonates 31Z and 32E in approximately the same ratio 40Z:60E. Compound 31Z was separated from 32E by fractional crystallization from water, the former isomer being slighly less soluble in water. (See page 30 for the NMR spectra of 31Z and 32E.)

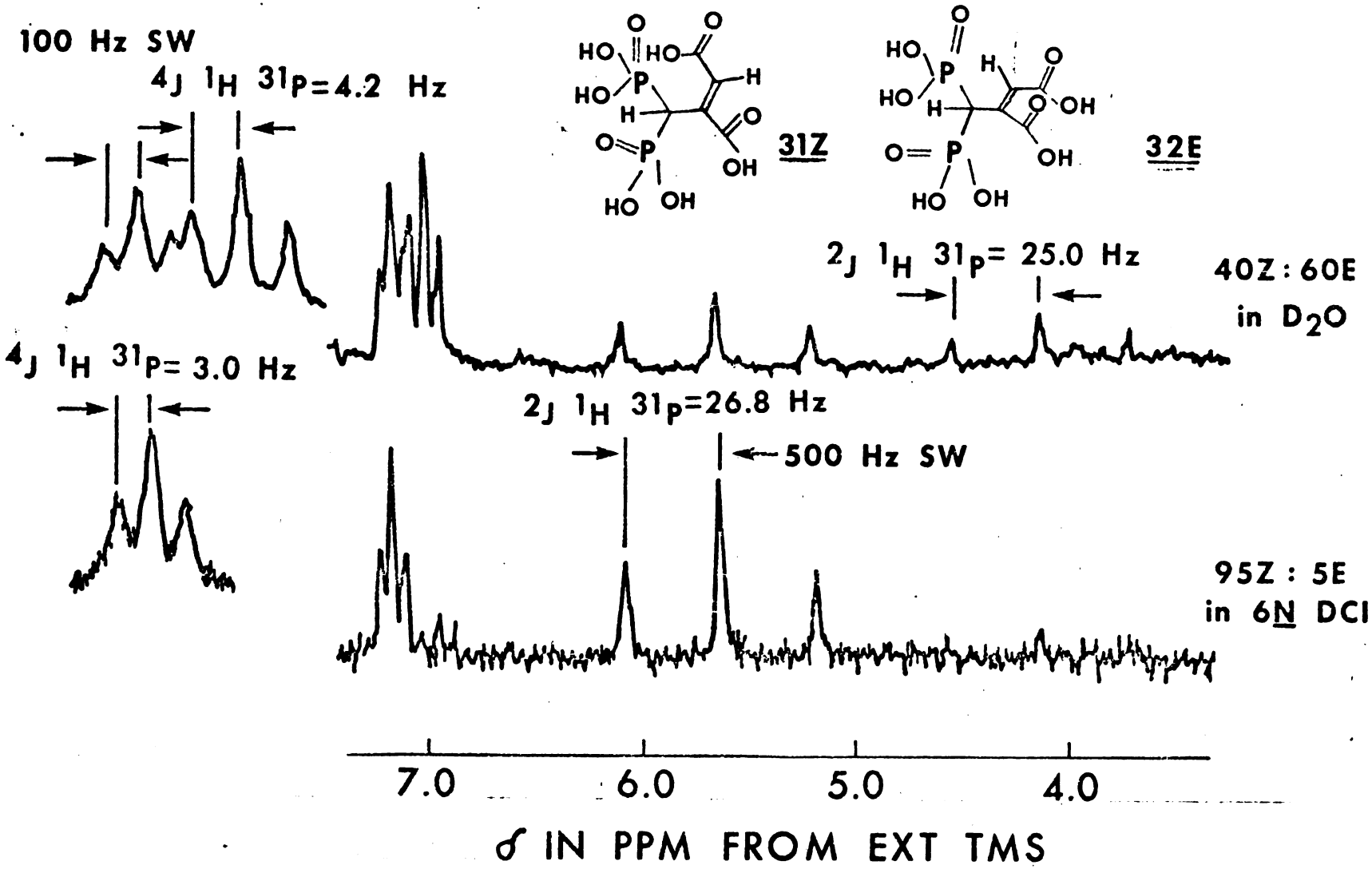


31Z



32E

Thermal equilibration of geometric isomers under the severe conditions employed for the hydrolysis of 21E, 22Z, 25Za, and 26Ea cannot be definitely ruled out. Differences of less than 5% in the NMR integration would not be discernible. The geometric assignment of 4d. (see page 22) Since it has been shown that the anisotropic deshielding effect of a carboxy group is greater than that of a phosphinylmethyl group, the vinyl protons of 21E and 22Z can be

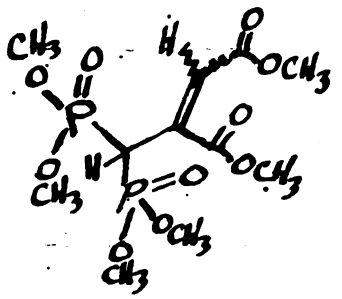

VINYL REGION
100 Hz SW
4J 1H 31P=4.2 Hz
4J 1H 31P= 3.0 Hz
31Z
32E
2J 1H 31P= 25.0 Hz
40Z : 60E
in D2O
2J 1H 31P=26.8 Hz
500 Hz SW
95Z : 5E
in 6N DCl
7.0
6.0
5.0
4.0
δ IN PPM FROM EXT TMS

assigned chemical shifts of δ= 6.91 ppm and δ = 6.76 ppm, respectively. Similarly, the vinyl protons of 25Za and 26Ea must resonate at δ =6.89 ppm and δ = 6.85 ppm, respectively. Support for the validity of these assignments is given by the prediction that the allylic proton which is cis to a carboxyl group directly substituted to the double bond should resonate at lower field relative to that which is trans-substituted.

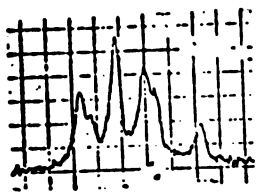
Since the geometric assignment of the vinyl protons in 4d did not change on hydrolysis to 4e (see page 22), it is a reasonable assumption that the geometric assignment of 21E and 22Z will not change on hydrolysis to 23E and 24Z. This assumption is verified by the ^{1}H NMR integration of each isomer. Although the chemical shift non-equivalence of the vinyl proton in each geometric isomer is much smaller, the same argument can be applied to 25Za and 26Ea on being hydrolyzed to 31Z and 32E.

In 25Zb and 26Eb, the assignment of the vinyl proton of each geometric isomer appears to be reversed from that of 25Za and 26Ea and the hydrolysis products 31Z and 32E. The vinyl protons of 25Zb and 26Eb can be tentatively assigned chemical shifts of δ= 6.82 ppm and δ = 6.87 ppm, respectively. This assignment is based on the ^{1}H NMR integration of each geometric isomer and the assumption that the allylic proton in each geometric isomer will experience greater deshielding if it is substituted cis to a carboxyl group on the same double bond. The assignment is further

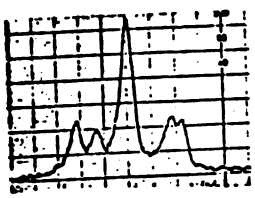
Vinyl Region

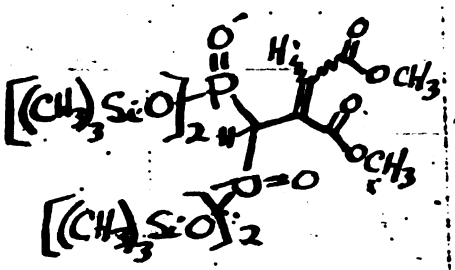


65Z:35E

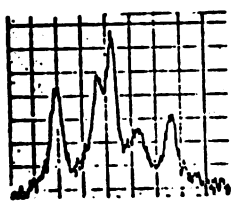


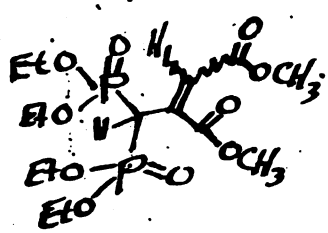
Same but 40Z:60E



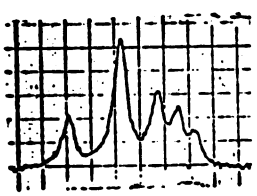


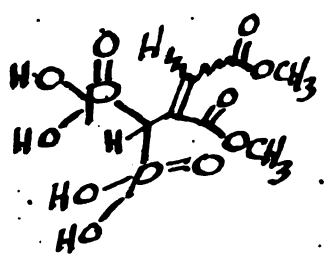
40Z:60E



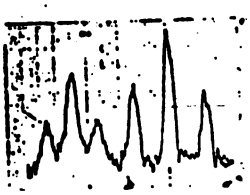


40Z:60E





40Z:60E



based on a comparison of the $^{4}J\ ^{1}H\text{-}^{31}P$ coupling constants in 25Zb and 26Eb with those observed in 25Za, 26Ea, 31Z, and 32E. (See Table V, page 79; also see page 31 for a comparison of the vinyl region at 100 Hz sweep width.)

The geometric assignment of 25Zc and 26Ec can be made by analogy with the assignment of 4d. (see page 22). The vinyl protons of 25Zc and 26Ec can be tentatively assigned chemical shifts of δ = 6.88 ppm and δ = 6.86 ppm, respectively. This assignment is based on the NMR integration of each geometric isomer and the assumption that the allylic proton in each geometric isomer will experience greater deshielding if it is substituted cis to a carboxyl group on the same double bond. The assignment is further based on a comparison of the $^{4}J\ ^{1}H\text{-}^{31}P$ coupling constants in 25Zc and 26Ec with those observed in 25Za, 26Ea, 31Z, and 32E. (See Table V, pages 79 and 80; also see page 31 for a comparison of the vinyl regions at 100 Hz sweep width.)

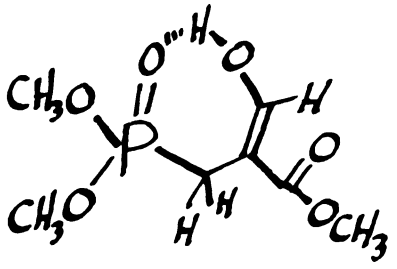
D. A crossed-Claisen condensation of methyl formate as a synthetic route to phosphonate analogs of PEP

The crossed-Claisen condensation of dimethyl β-carbomethoxyethylphosphonate (17) with methyl formate in glyme using sodium hydride proceeds exothermically at room temperature[48] and occurs α to the carboxyl group exclusively.[47] On workup, an amber-colored oil was obtained which crystallized on standing at 0° after several days. Sodium hydride in glyme is not a sufficiently strong base to preform the α-anion of 17 at room temperature. In the

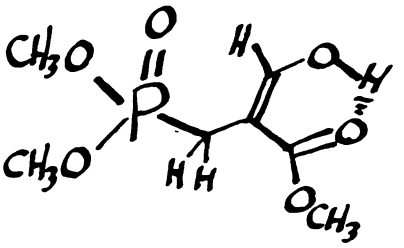
presence of methyl formate, however, the reaction with 17 in glyme is autocatalytic. An analogous reaction with dimethyl succinate in protic solvents is known to give both mono- and di-formyl derivatives depending on the reaction conditions employed.[54] Detailed structural characterizations, however, were not described.

An NMR spectrum of the crude product, methyl (dimethoxyphosphinylmethylhydroxymethylene)acetate (33E) revealed the presence of only one geometric isomer with a chelated enolic hydroxyl resonance at δ = 9-10 ppm and no aldehyde proton resonance. The $^{2}J\ ^{1}H\text{-}^{31}P$ coupling constant of 20.4 Hz observed for the allylic protons is in excellent agreement with the observation of $^{2}J\ ^{1}H\text{-}^{31}P$ = 22.0 $\pm$ 1.0 Hz for a wide variety of model benzyl and allyl phosphonates.[53] On standing overnight at room temperature in $CDCl_3$ or after recrystallization from CH_2Cl_2, the NMR spectrum ($CDCl_3$) revealed the presence of an additional geometric isomer 33Z. Again no aldehyde proton resonance was visible. A $^{2}J\ ^{1}H\text{-}^{31}P$ coupling constant of 19.2 Hz was observed for the allylic protons in 33Z. The chemical shift of the allylic protons of 33Z was upfield by $\Delta\delta$ = 0.21 ppm from that of 33E in $CDCl_3$ and $\Delta\delta$ = 0.66 ppm upfield in D_2O. The chemical shift of the vinyl proton in 33Z was upfield by $\Delta\delta$ = 0.66 ppm from that of of 33E in $CDCl_3$ and $\Delta\delta$ =2.79 ppm in D_2O. Microdistillation of a sample of 33E and 33Z produced a 80E:20Z mixture in $CDCl_3$, while in D_2O, a ratio of 65E:35Z

was observed. The geometries of 33E and 33Z can be tentatively assigned as follows:



33E

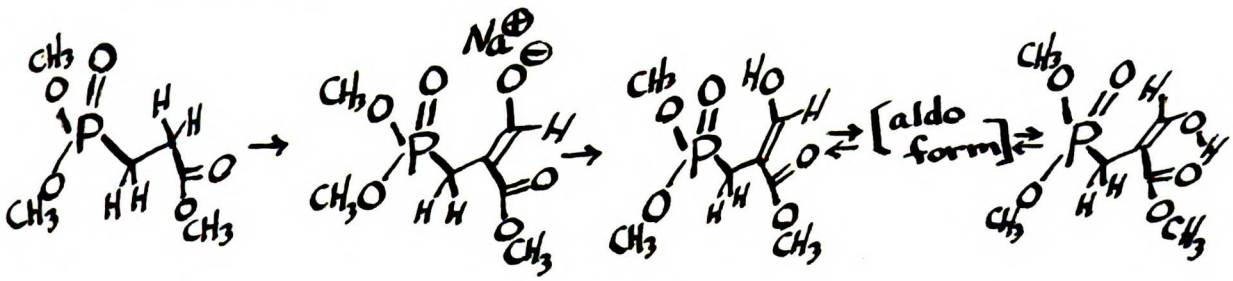


33Z

This assignment is based on the demonstration that the anisotropic deshielding effect of a carboxyl group on a vinyl proton is greater than that of a phosphinylmethyl group similarly substituted to a double bond. (see page 22) The assignment is supported by the observed shift to higher field of the allylic protons in 33Z which is predicted to result from increased distance to the enolic hydroxyl group, hence resulting in less deshielding. The effect of phosphoryl hydrogen-bonding on the chemical shifts of the vinyl and allylic protons in 33E and 33Z is difficult to predict and might invalidate the geometric assignment. The lack of resolvable $^{4}J\ ^{1}H\text{-}^{31}P$ coupling at 60 MHz to the vinyl proton in 33Z in both $CDCl_3$ and D_2O suggests a conformation of the dimethoxyphosphinyl group with respect to the plane of the vinyl group which is unfavorable for coupling.

If this tentative assignment of 33E and 33Z is valid, we observe a thermodynamic preference for 33E over 33Z in both $CDCl_3$ and D_2O

based on on the NMR integration of each isomer. The observation that initially only one isomer is present 33E which isomerizes on the order of days at room temperature in $CDCl_3$ to an equilibrium mixture suggests that the thermodynamically favored enolate anion is that of 34E.

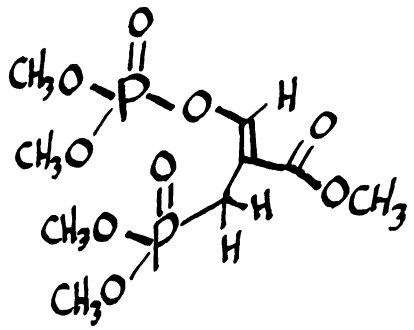


17 34E 33E 33Z

Phosphorylation of the sodium enolate anion, 34E presumably, in benzene with dimethyl phosphorochloridate led to a single enol phosphate 35E. When an equilibrium mixture of 33E and 33Z in the ratio 80E:20Z is treated with sodium hydride, a mixture of enolate anions 34E and 34Z must be formed which equilibrate during the 5 hour before the acid chloride is added. Alternatively, 34E could react faster with the acid chloride than does 34Z.

The tentative assignment of the geometry of 35E can be made by analogy with the geometric assignment of 33E since the chemical shifts of the allylic and vinyl protons are predicted not to change greatly on phosphorylation. Removal of the effect of the hydrogen-bond on the chemical shifts in 35E results in small upfield shifts of $\Delta\delta = 0.14$

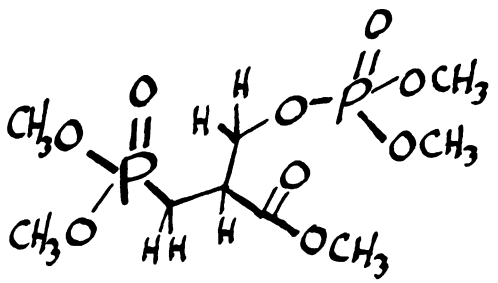
ppm and $\Delta\delta$ = 0.02 ppm for the vinyl and allylic protons, respectively,relative to 33E.



35E

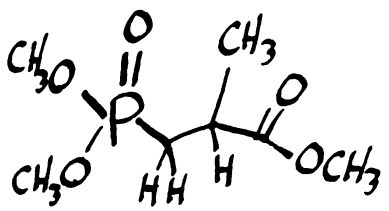
Further support for the tentative geometric assignment of 35E is given by the fact that the $^{4}J\ ^{1}H\text{-}^{31}P$ coupling constant of 5.2 Hz in 33E is maintained on phosphorylation in 35E where $^{4}J\ ^{1}H\text{-}^{31}P = {}^{3}J\ ^{1}H\text{-}^{31}P$ = 6.2 Hz.

Catalytic hydrogenation of 35E was attempted with the hope of preparing 36.

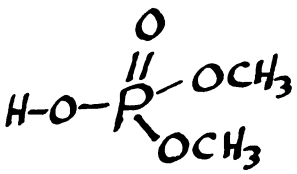


36

Instead, the products of hydrogenolysis followed by hydrogenation were observed 37 and 38.

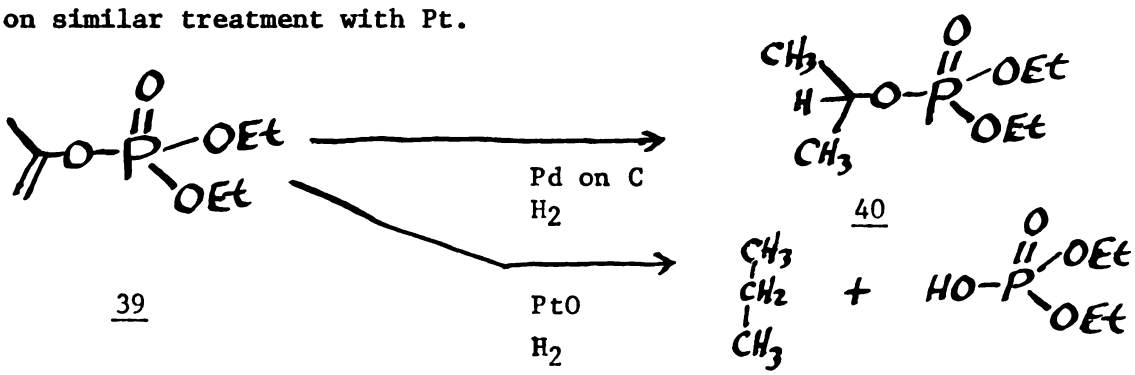


37



38

The identity of methyl (RS)-α-(dimethoxyphosphinylmethyl)propionate (37) was confirmed by an unambiguous synthesis of 37[46] and NMR spectral comparison. Compound 37 was observed in each instance when Rd, Pt, and Pd were used as catalysts in ethyl acetate at 3 atmospheres pressure of H_2. This result is at variance with the report of Jacobson et al.[56] wherein diethyl isopropenyl phosphate 39 gave diethyl isopropyl phosphate 40 on hydrogenation with Pd and gave diethyl hydrogen phosphate and propane (ie. hydrogenolysis) on similar treatment with Pt.

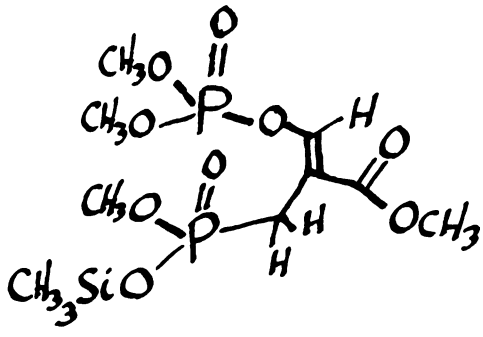


39

40

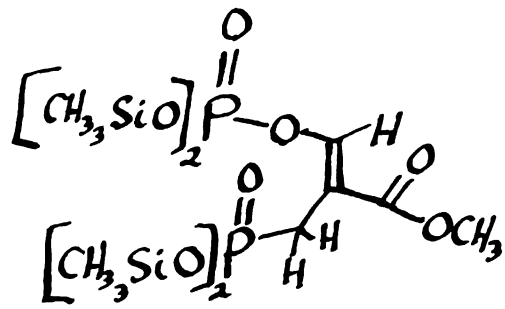
Further attempts to reduce 35E were abandoned.

Treatment of 35E with one equivalent of trimethylsilyl bromide at room temperature for 15 min (neat) gave 41E on inspection of the NMR spectrum of the crude reaction mixture.



41E

Evidence for this structure is twofold: 1) the vinyl proton is now a broadened 1:2:1 apparent triplet with approximately the same chemical shift as in 35E, and 2) the allylic protons now show geminal coupling becoming resolvable without a significant change in their chemical shifts relative to 35E. Treatment of 35E with a greater than five-fold excess of trimethylsilyl bromide at reflux in benzene for 0.5 hour gave a single product 42E on microdistillation of the reaction mixture.



42E

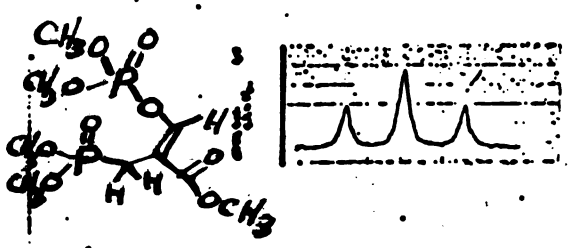
The NMR spectrum of 42E shows a doublet of doublets for the vinyl proton centered at approximately the same chemical shift as in 35E with $^{3}J\ ^{1}H\text{-}^{31}P$ and $^{4}J\ ^{1}H\text{-}^{31}P$ coupling constants very similar to the $^{3}J\ ^{1}H\text{-}^{31}P = {}^{4}J\ ^{1}H\text{-}^{31}P = 6.2$ Hz coupling constant of 35E. The allylic protons of 42E are qualitatively similar to those of 35E in both chemical shift and $^{2}J\ ^{1}H\text{-}^{31}P$ coupling constant. There is therefore little doubt that the geometry of 42E is identical to that of 35E. (See page 39 for a comparison of the vinyl and allylic regions at 100 Hz sweep width.)

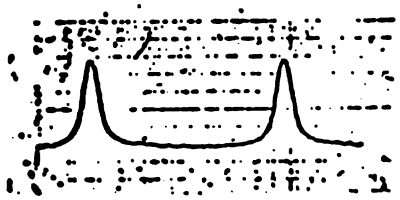
When a mixture of 33E and 33Z was allowed to stand at room temperature in D_2O, it was noticed that hydrolysis of the dimethoxyphosphinyl ester groups was occurring at a significant rate ($t_{1/2} \simeq 25$ days).* After approximately 50 days at room temperature, hydrolysis of the methoxycarbonyl ester group was less than 10% complete. On heating 33E and 33Z in D_2O at 50°, the dimethoxyphos-

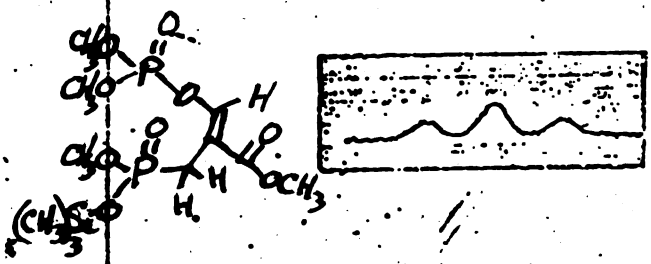
*Ester hydrolyses were followed conveniently by comparison of the NMR integration of the methoxyphosphinyl ester doublet (J = 11 Hz) with that of the methanol peak. Satisfactory mirroring curves (ref. 40) were obtained in all cases.

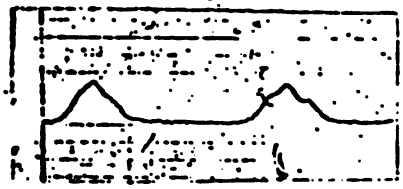
Vinyl Region

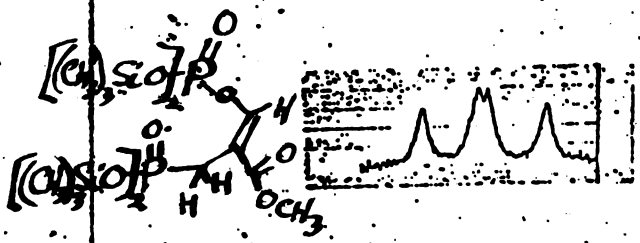
Allylic Region

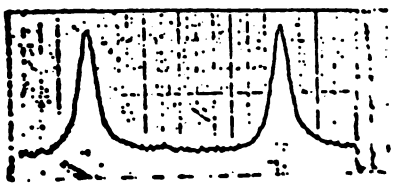


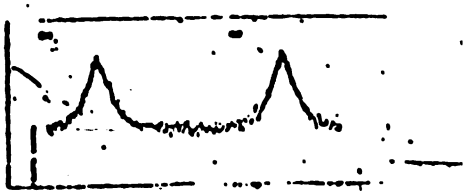




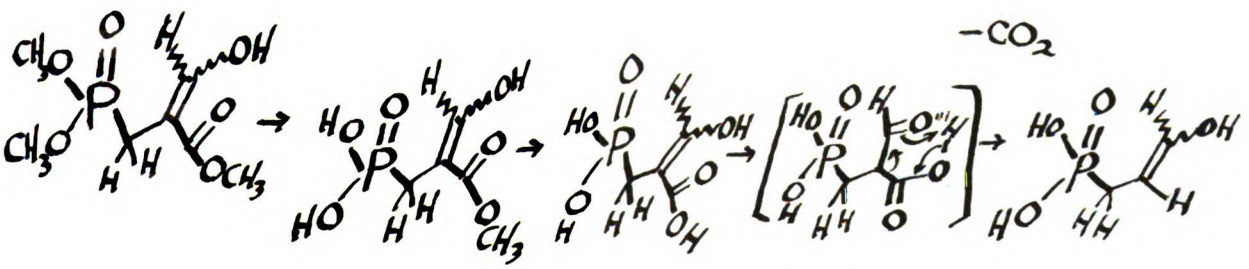








phinyl ester hydrolysis occurred at a much faster rate ($t_{\frac{1}{2}} \simeq 2$ days). At room temperature and at 50°, hydrolysis of both dimethoxyphosphinyl ester groups was observed. These observations can be explained by assuming that the reaction at room temperature proceeds via intramolecular nucleophilic catalysis involving both endocyclic and exocyclic cleavage.[51,52,63,64] When a mixture of 33E and 33Z was treated at room temperature with 6N DCl, methoxyphosphinyl ester hydrolysis was complete in approximately 2 days. Shortly thereafter, the evolution of CO_2 was observed suggesting that decarboxylation of 44 to give 45 occurred as the methoxycarbonyl ester group was hydrolyzed.



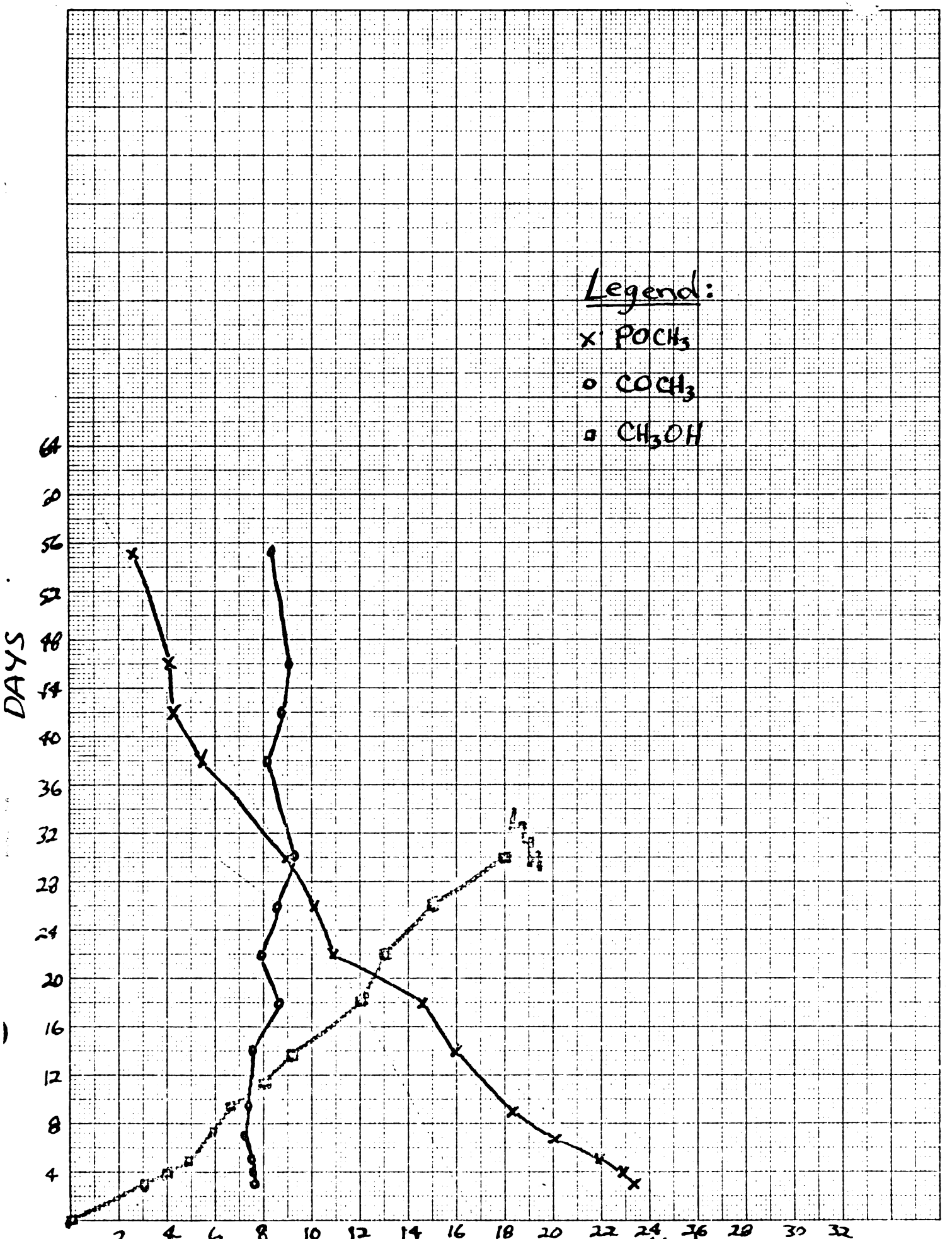
33E 43 44 45
33Z

In the hydrolysis of 33E and 33Z at room temperature pseudorotation is probably precluded by the energy required to place both oxygen and

carbon atoms of the five-membered ring into equatorial positions of a trigonal bipyramidal pentacoordinate intermediate. Several instances have been documented, however, where accelerated rates of hydrolysis have been obtained even while pseudorotation was precluded by one or more of the preference rules[41] for pentacoordination about phosphorus.[30,40,52,63,64]

In the hydrolysis of 33E and 33Z at 50°, the energy required to place the five-membered ring di-equatorial via pseudorotation might be present. All cleavages could then be exocyclic and the rate-limiting step might well be the initial general acid catalyzed cyclization. Unfavorable electrostatic interactions would be minimized by requiring only a single cyclication step in the overall hydrolysis. At 50° and at room temperature both methoxyphosphinyl ester groups are hydrolyzed.

Preliminary kinetic data suggests that at room temperature the second methoxyphosphinyl ester group is hydrolyzed approximately 2-fold more slowly than the first ester group. (See page 42 for the mirroring curves[40] obtained for 33E and 33Z.) A more detailed study of the kinetics might be unwarranted since the matter is complicated by slow but significant methoxycarbonyl ester hydrolysis. Another complicating factor in the kinetic analysis is the observation that the ratio of E to Z isomers in 33 is quite different from the ratio of E to Z isomers in 43. At room temperature, the ratio of E to Z isomers was followed as a function of time. If the tentative


Legend:
× $POCH_3$
o $COCH_3$
□ CH_3OH
DAYS
64
60
56
52
48
44
40
36
32
28
24
20
16
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4
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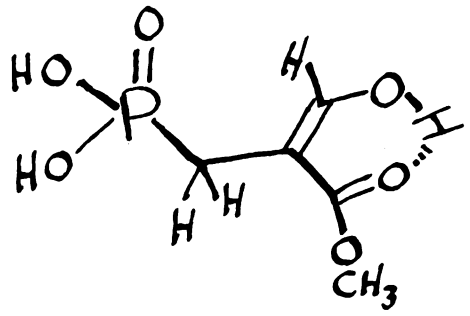
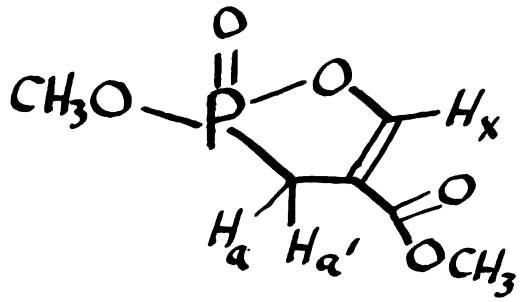
32

assignment of structures to 33E and 33Z is correct, the thermodynamically-favored hydrogen-bond in 43 is that of 43Z. Large changes in the chemical shifts of the vinyl and allylic protons are not observed on hydrolysis. The $^{4}J\ ^{1}H\text{-}^{31}P$ coupling constant in 43E is similar to that observed in 33E. No $^{4}J\ ^{1}H\text{-}^{31}P$ coupling is resolvable at 60 MHz for 43Z just as was observed for 33Z.



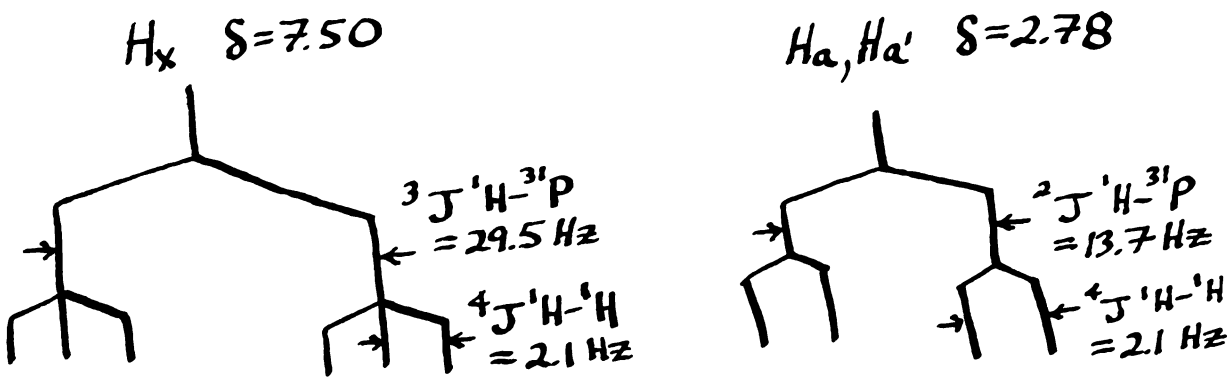
43Z

Support for the mechanism of intramolecular nucleophilic catalysis in the auto-catalyzed hydrolysis of 33E and 33Z is provided by the synthesis of a postulated intermediate in the hydrolysis. Compound 44 was prepared by heating a mixture of 33E and 33Z to reflux under vacuum for 2 hour to remove methanol as it was formed followed by short path distillation of the product. Confirmation of the structure 44 is provided by examination of the NMR spectrum.

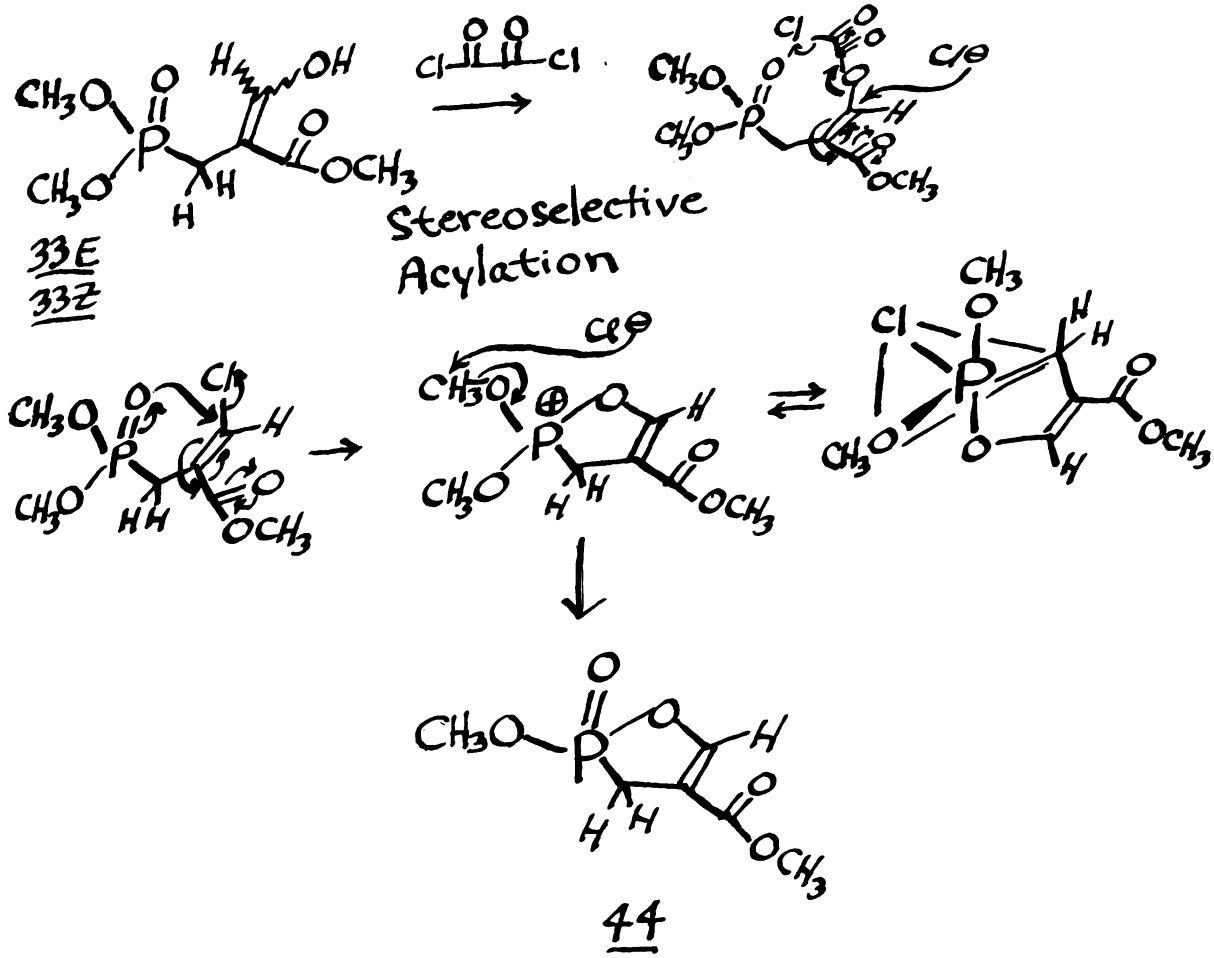


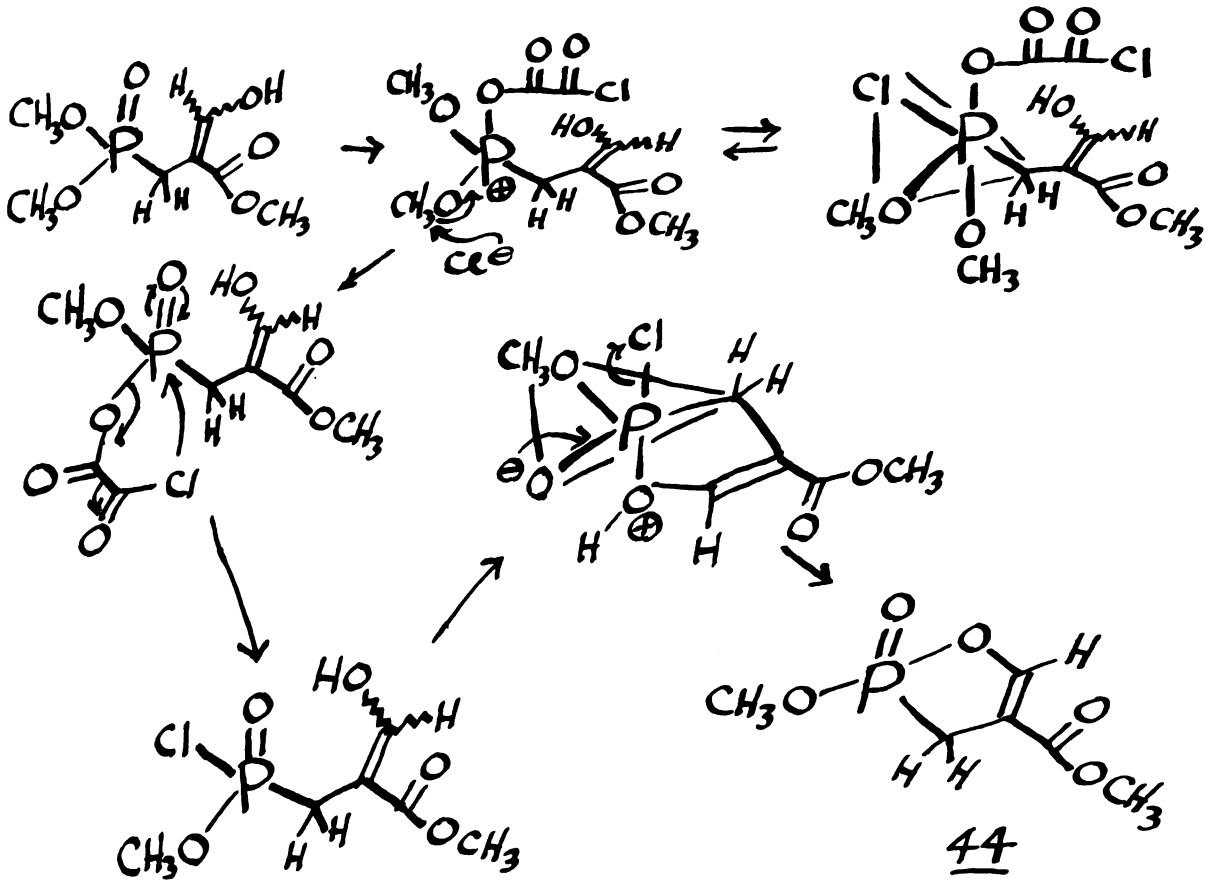
44

The large $^{3}J\ ^{1}H\text{-}^{31}P$ coupling constant of 29.5 Hz is a manifestation of the trans-orientation between the coupling nuclei which is a maximum on the Karplus curve.[61,62] In addition, the σ-mode of $^{4}J\ ^{1}H\text{-}^{31}P$ coupling could now be a maximum. The large $^{4}J\ ^{1}H\text{-}^{1}H$ coupling constant of 2.1 Hz is characteristic of many small-ring compounds with restricted conformational mobility and optimum coupling angles.[60] At 60 MHz, both H_a and H_a' are chemical shift equivalent.

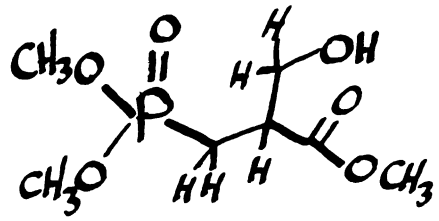


Difficulty in rigorously purifying 44 has resulted in an elemental analysis which deviates slightly from theoretical. Additional support for its structure is provided by an alternate synthesis. When a mixture of 33E and 33Z was heated with oxalyl chloride, the distillate from the reaction mixture gave an NMR spectrum which is virtually identical to that of 44. A CIMS of the distillate gave a parent M+1 peak corresponding to the mass of 44. Several mechanisms can be invoked to explain the formation of 44. Two likely mechanisms are the following:

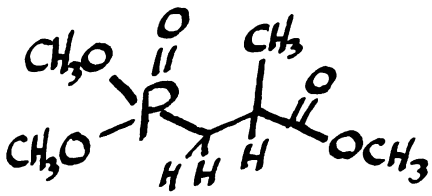




When a mixture of 33E and 33Z was treated with palladium on charcoal in methanol in a Parr hydrogenation apparatus with 3 atomspheres H_2, a slow reduction requiring longer than 24 hours to 45 was observed. The complexity of the NMR spectrum of 45 is the result of pronounced 2nd order splitting.



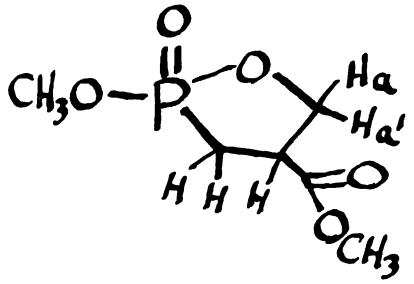
45



37

It was felt that additional evidence for the structure of 45 would be worth obtaining.

Treating a mixture of 33E and 33Z with sodium cyanoborohydride in aqueous methanol at pH 3 to 4 for 6 hr at room temperature and workup, gave an NMR spectrum of the reduction product which was identical to that obtained via the catalytic hydrogenation route. The catalytic hydrogenation route is considered less efficient since about 5% of the mixture was the hydrogenolysis product 37. Microdistillation at low vacuum (3 mm) of a sample of 45 obtained by sodium cyanoborohydride reduction gave NMR, IR, and elemental analysis results which are consistent with the phostonate 46.

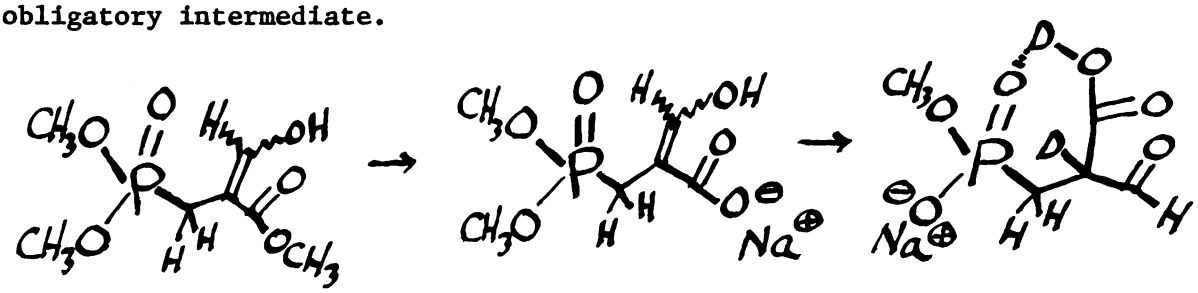


46

The cyclization which occurred on distillation was probably catalyzed by traces of acid or water. Compound 46 lacks the hydroxyl proton resonance in its NMR spectrum which was present in 45. Compound 46 also lacks the hydroxyl group stretch in the IR which was present in 45. The NMR spectrum of 46 is quite complex due to substantial 2nd order splitting. A new complex multiple in the NMR spectrum of 46 at δ = 3.9 - 4.55 ppm which was absent in 45 is undoubtedly due to the H_a and H_a' protons labelled above. These protons are now experiencing more fully the deshielding effect of the methoxyphosphinyl group and possess chemical shifts which are roughly the same as the methylene protons in diethoxyphosphinyl groups where δ = 4.2 ppm.

E. Base-catalyzed hydrolysis of methyl α-(dimethoxyphosphinylmethyl-hydroxymethylene)acetate

Treatment of 33E and 33Z with one equivalent of sodium deuteroxide in D_2O at 0° is thought to give 47. Compound 48 is thought to be an obligatory intermediate.

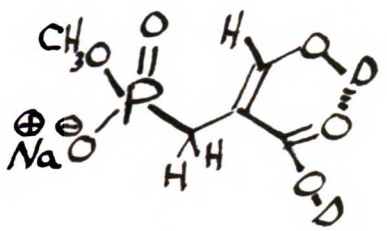


33E
33Z

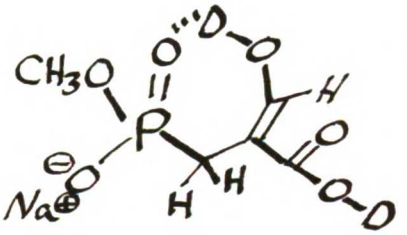
48

47

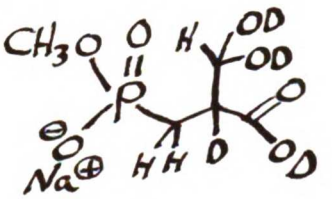
Alternate structures, however, are also compatible with the NMR spectrum obtained.



49Z



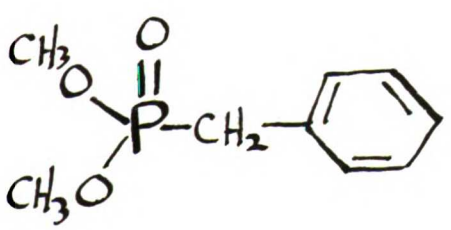
49E



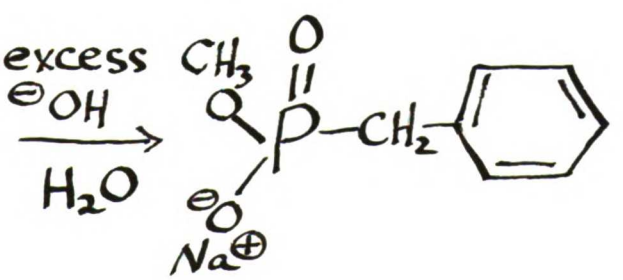
50

Treatment of 33E and 33Z with both two and three equivalents of NaOD under the same conditions gave an NMR spectrum which was virtually identical to that of 47 if one allows for small differences in chemical shift due to differences in the pD of the sample.

Hudson and Keay[44] studied the kinetics of phosphonate hydrolysis and state that only the first group is removed under alkaline conditions. Organic phosphates behave similarly; basic hydrolysis of trimethyl phosphate is said to remove only one methyl group.[74] Similarly, Rabinowitz[75] found that heating dimethyl benzylphosphonate (51) at reflux for 16 hr with excess aqueous NaOH gave a high yield of monoester 52.



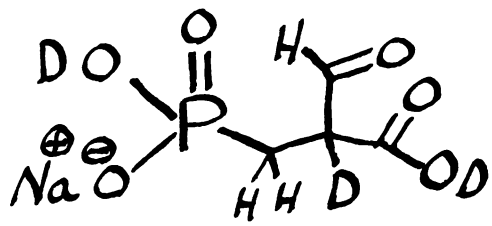
51



52

Rabinowitz pointed out that these observations are readily understandable since for further reaction to occur, the already negatively charged portion of the molecule must accept an additional negative charge.

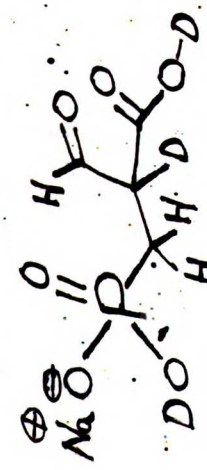
It is not surprising that when 33E and 33Z were heated to 95° for 12 hr with excess NaOD in D_2O, the second methoxyphosphinyl group remained intact. When 33E and 33Z were treated with just one equivalent of NaOD in D_2O at 0°, allowed to stir at room temperature for 3 days, and then heated to 50° for several days, the second methoxyphosphinyl group remained intact. However, when heated to 80° for 3 days, the second methoxyphosphinyl group was completely hydrolyzed to a single product, 53 presumably. (See page 50 for the NMR spectrum of 53.)



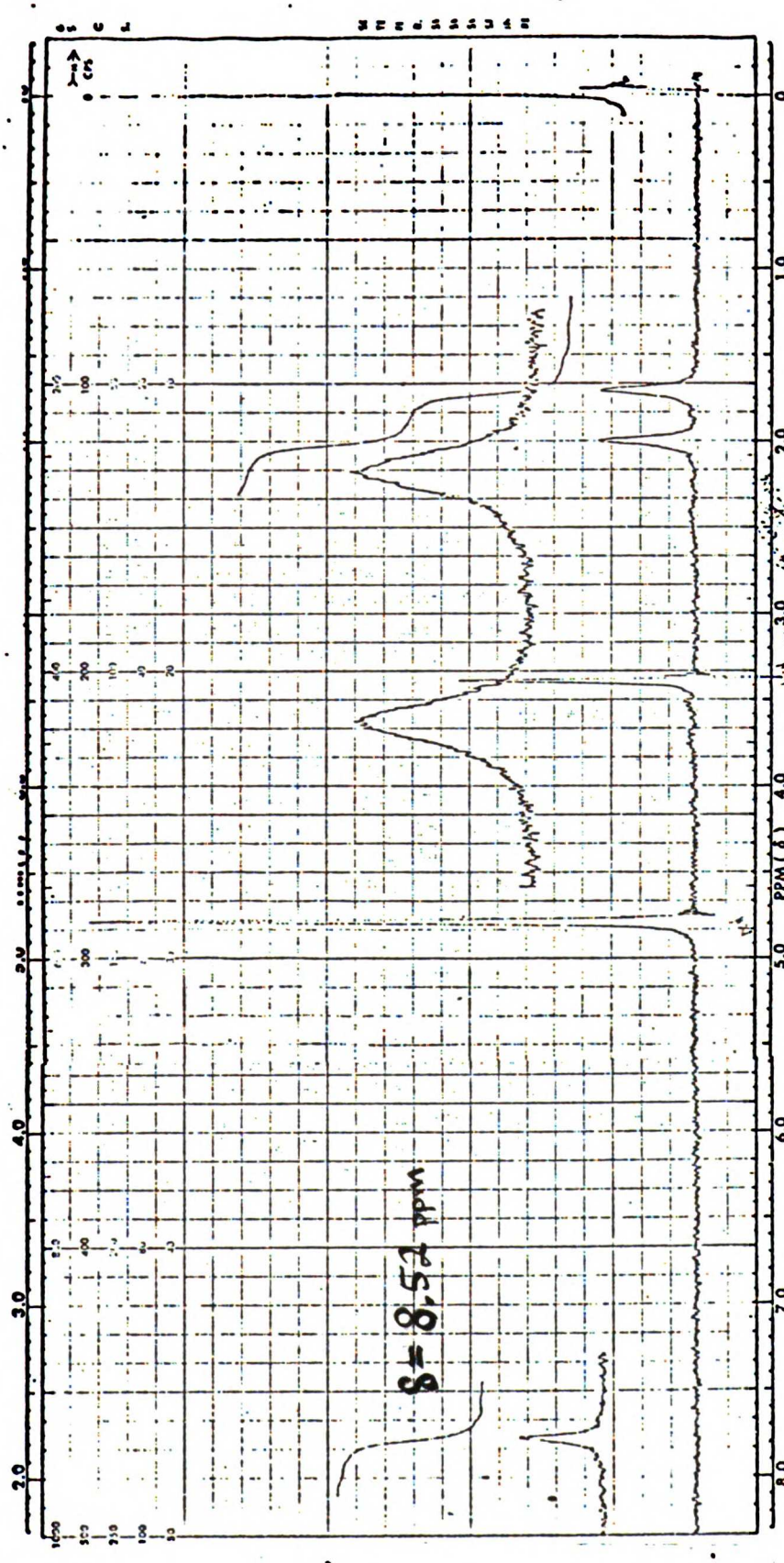
53

F. Base-catalyzed hydrolysis of a silylated enol phosphate

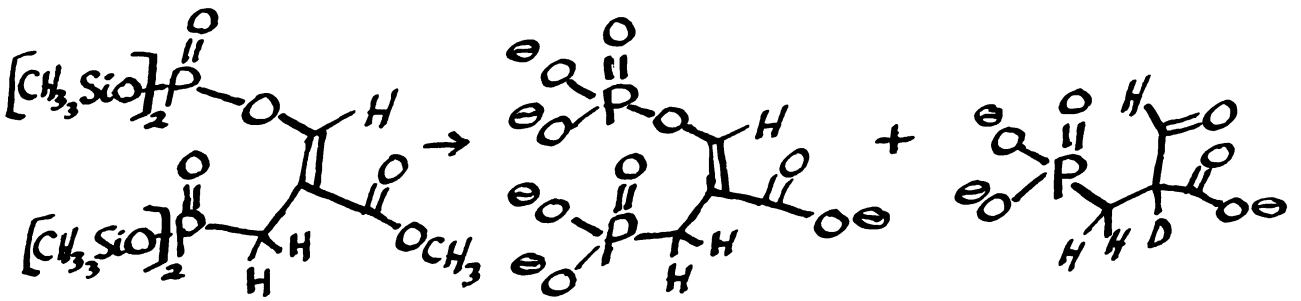
To compound 42E was added a cold solution of 10% NaOD in D_2O. A vigorous exothermic reaction ensued. After a few minutes, the initial turbidity of the solution disappeared and the solution became homogeneous. An excess of NaOD was employed. Examination of the NMR spectrum of the mixture revealed a major product presumed to be 54E and a minor product



53



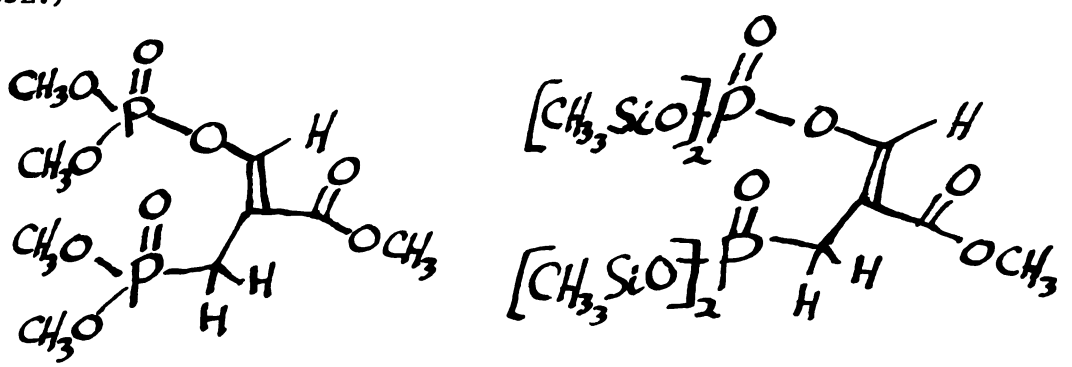
presumed to be 53 in a 95:5 ratio, respectively. (see page 52 for the NMR spectrum of 54E.)



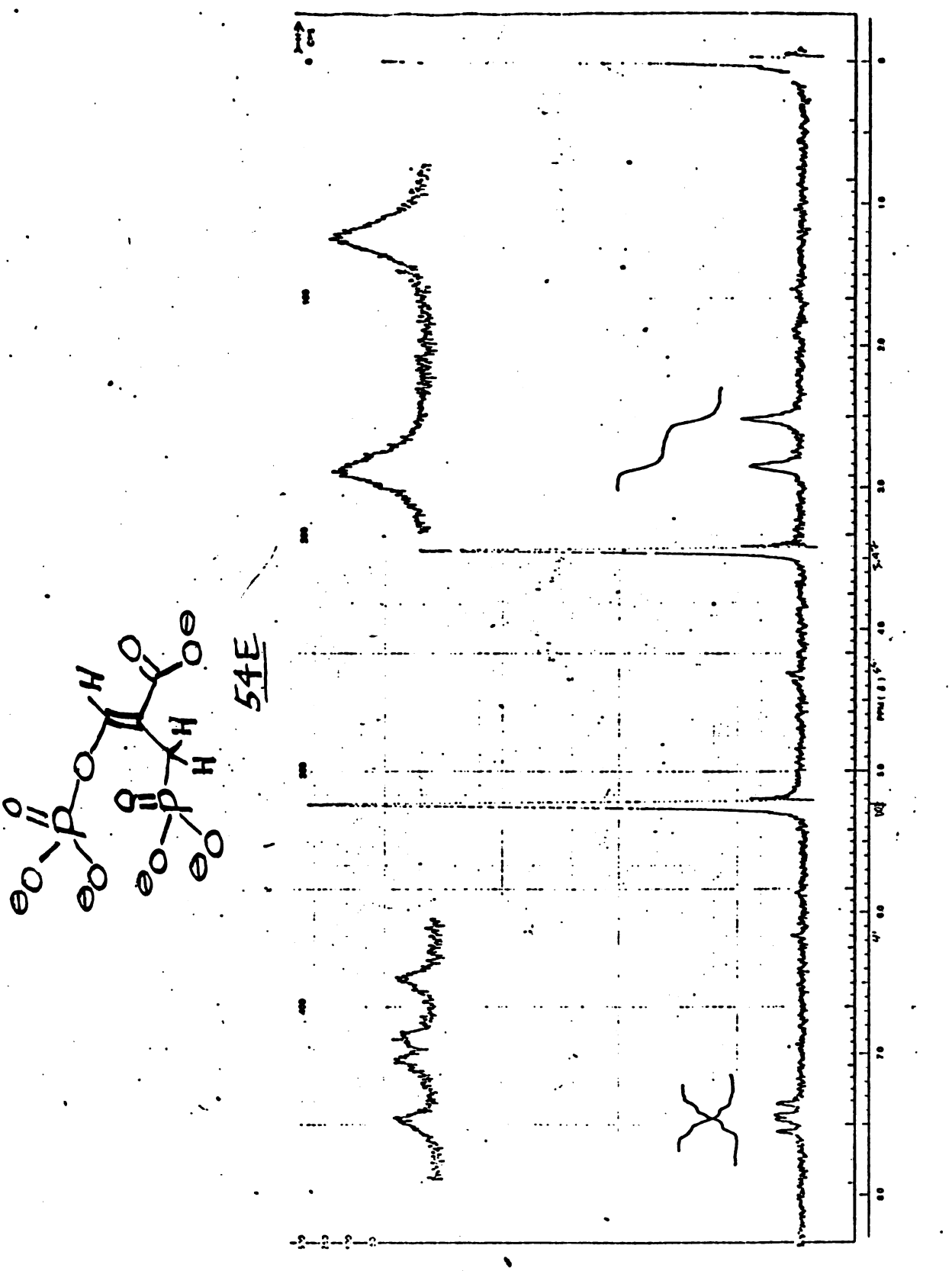
42E 54E 53

The NMR spectrum of the mixture was essentially unchanged after 4 days at 0°. After 4 days at room temperature, the proportion of 53 seemed to have increased slightly. The NMR spectrum of 53 obtained by this method is identical to that obtained via alkaline hydrolysis of 33E and 33Z.

The NMR spectrum of 54E is consistent with its having a unique geometry. The geometry is thought to be the same as that of its precursors 42E and 35E. (See page 35 for the original assignment of 35E.)



35E 42E

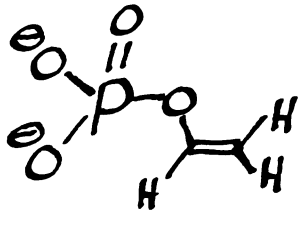


54E

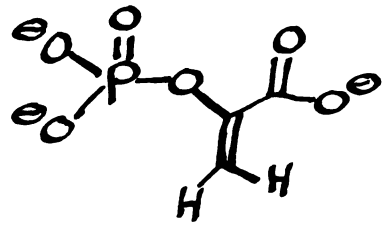
The similarity of the chemical shifts of the vinyl and allylic protons as well as the similarity of the $^{4}J\ ^{1}H\text{-}^{31}P$ and $^{3}J\ ^{1}H\text{-}^{31}P$ coupling constants is highly suggestive that all three compounds, 35E, 42E, and 54E, have the same unique geometry. (See page 39 for a comparison of the vinyl and allylic regions at 100 Hz sweep width.)

Compound 54E has the same net charge at physiologic pH as do all of the naturally-occuring nucleotide triphosphates. The pK_A value of the least acidic titratable proton of adenosine 5'-triphosphate (ATP) is 7.1, while the pK_A value of the least acidic titratable proton of 5'-adenylyl methylenediphosphonate (the α,β,βγ-methylene analog of ATP) is 8.4 - 8.5.[76,88]

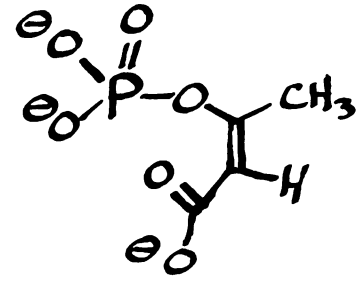
Only three vinyl dihydrogen phosphates were known as of 1961:[77] the vinyl (55), 1-carboxyvinyl (PEP), and 2-carboxy-1-methyl vinyl phosphates (56). (See Refs. 1 and 22 for other vinyl dihydrogen phosphates.)



55



PEP

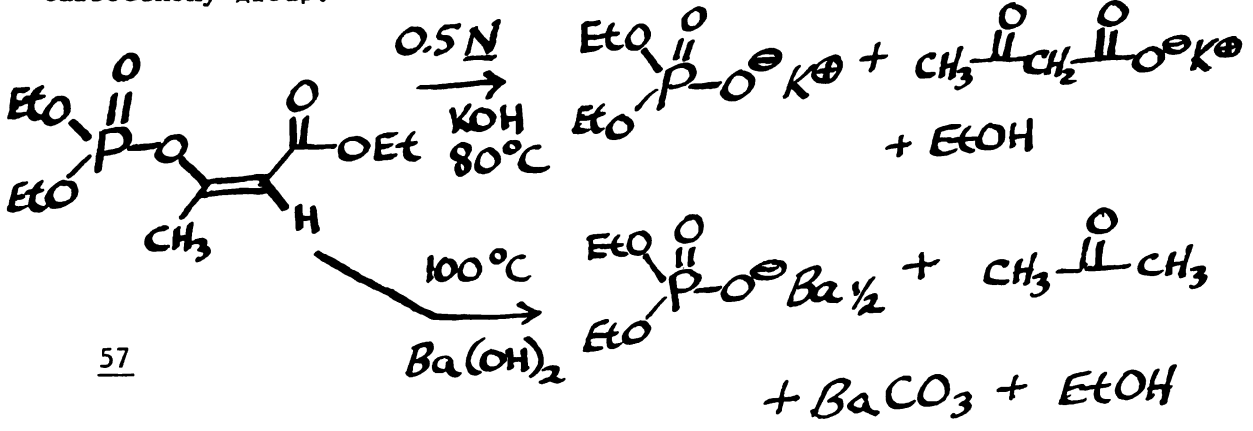


56

Compound 55 is extremely labile towards acid and the free acid could not be prepared from the salt without cleaving the enol ester linkage.[78] PEP and 56 are somewhat more stable. Compound 56 yielded acetone and carbon dioxide on heating with dilute acid.[79] The unimolecular reaction con-

stant of PEP during hydrolysis in 1N HCl at 100° is 3.5 X 10^{-3} ($t_{1/2}$ = 8.6 min) sec^{-1}.[80]

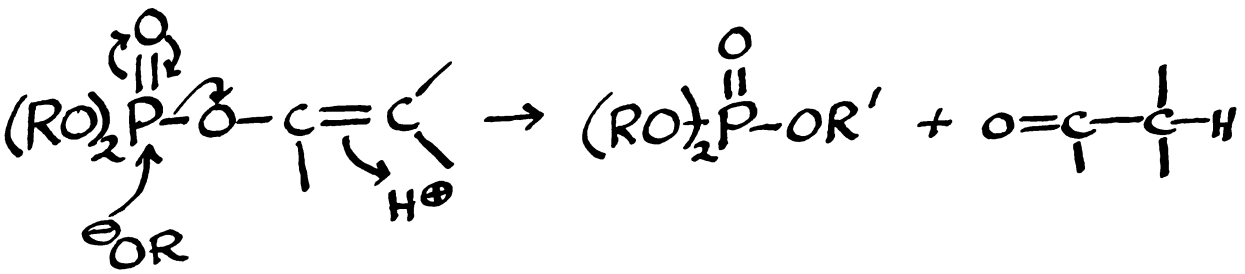
The alkaline hydrolysis of various dialkyl vinyl phosphates with 0.5N potassium hydroxide at 100° was found to cleave only the enol ester linkage. Diethyl 2-carboethoxy 1-methylvinyl phosphate (57), consumed two moles of base owing to simultaneous hydrolysis of the carboethoxy group.[81,82]



57

With barium hydroxide at 100° the corresponding barium salt of dialkyl hydrogen phosphate is formed in good yield. Compound 57, however, undergoes simultaneous decarboxylation under these conditions, yielding, in addition to barium diethyl phosphate, acetone, barium carbonate, and ethanol.[81,82]

In a thorough review article, Lichtenthaler[77] has pointed out that certain dialkyl vinyl phosphates react with cleavage of the enol ester linkage yielding mixed anhydrides: namely, acyl phosphates 58: R' = RC(O) and pyrophosphates 59: R' = $(RO)_2P(O)$.



58 R' = RC(C)

59 R' = $(RO)_2P(O)$

In this acidolysis reaction, the different enol phosphates showed similar differences in reactivity as were found for the hydrolysis in 0.1N HCl.[83] When nucleotides, such as thymidine 3'-phosphate or adenosine 3'-phosphate, were used in the acidolysis, the thymidyl and adenyl pyrophosphates, initially formed, can undergo further reaction to form oligonucleotides.[33,84]

A pertinent quotation from Lichtenthaler's review article[77] is the following:

> The reaction of phosphoenol (PEP) with phosphoric acids, although having a very important role in carbohydrate metabolism - namely, the enzymatic acidolysis by adenosine mono (di)phosphate to form adenosine di(tri)phosphate and pyruvic acid - has not yet been achieved *in vitro*. The rather slow acidic hydrolysis (ref. 86) of phosphoenol pyruvate and its subsequent classification (ref. 87) as intermediate between stable and labile organic phosphates indicate that acidolysis *in vitro* would not proceed under conditions comparable to those *in vivo*.

IV. EXPERIMENTAL

All melting and boiling points are uncorrected. Nuclear Magnetic Resonance (NMR) spectra were determined on a Varian A-60A and a Perkin-Elmer R12B spectrometer (in δ units with TMS or DSS as reference). The infrared (IR) spectra were recorded on a Beckman 4 infrared spectrometer. Microanalyses were performed by the University of California Microanalytical Laboratory, Berkeley, California.

Bis-(dimethoxyphosphinyl)-methane (1a) was prepared by the methods of Roy[36] and Nicholson et al.[37] bp 80-85° (0.02 mm) [Lit[37] bp 87-90° (0.05 mm)].

Bis-(diethoxyphosphinyl)-methane (1b) was prepared by a modification of the method of Roy.[36] bp 85-90° (0.02 mm) [Lit bp 90-94° (0.1 mm)].

Bis-(diisopropoxyphosphinyl)-methane (1c) was prepared by the method of Roy.[36] bp 125-130° (0.01 mm) [Lit bp 87-90° (0.003 mm)].

E-ethyl (α-methyl β-dimethoxyphosphinyl)acrylate (2Ea) and Z-ethyl (α-methyl β-dimethoxyphosphinyl)acrylate (3Za): Sodium hydride (3.2 g of 56%) was washed with hexane under N_2 and 100 ml of dimethoxyethane (Aldrich, distilled from sodium hydride) was added. Compound 1a (18.5 g) dissolved in 25 ml dimethoxyethane was added dropwise at 0° and allowed to stir for 2 hr at room temperature under N_2. Freshly distilled ethyl pyruvate (9.3 g, Aldrich) in 25 ml dimethoxyethane was added dropwise at 0° and the mixture was mechanically stirred for 0.5 hr at 0° after the addition was complete. Cold, saturated, aqueous KH_2PO_4 (50 ml) was added

and the reaction was then extracted thoroughly with five 100 ml portions of CH_2Cl_2. The extract was dried on Na_2SO_4 overnight, filtered, and the solvent removed in vacuo. Short path distillation at 85-90° (0.03 mm) gave 14.9 g (84% yield) of a mixture of 2Ea and 3Za in a 50E:50Z ratio. NMR ($CDCl_3$ δ rel to int TMS) 2Ea 1.33 (t, J=7 Hz, 3H), 2.25 (d of d, $^4J\ ^1H\text{-}^1H$=1.3 Hz, $^4J\ ^1H\text{-}^{31}P$=3.6 Hz, 3H), 3.73 (d, J=11 Hz, 6H), 4.25 (q, J=7 Hz, 2H), 6.6 (d of q, $^2J\ ^1H\text{-}^{31}P$=16.4 Hz, $^4J\ ^1H\text{-}^1H$=1,3 Hz, 1H) and 3Za 1.33 (t, J=7Hz, 3H), 2.11 (ap t, $^4J\ ^1H\text{-}^1H$= $^4J\ ^1H\text{-}^{31}P$=1.8 Hz, 3H), 3.75 (d, $^3J\ ^1H\text{-}^{31}P$=11 Hz, 6H), 4.25 (q, J=7 Hz, 2H), 5.8 (d of q, $^2J\ ^1H\text{-}^{31}P$=15.0 Hz, $^4J\ ^1H\text{-}^1H$=1.8 Hz, 1H).

Anal. Calcd for $C_8H_{15}O_5P$: C, 43.25; H, 6.80. Found: C, 43.21; H, 6.77.

E-ethyl (α-methyl β-diethoxyphosphinyl)acrylate (2Eb) and Z-ethyl (α-methyl β-diethoxyphosphinyl)acrylate (3Zb): These compounds were prepared by following the procedure outlined above for 2Ea and 3Za substituting 22.7 g of 1b for 1a. Short-path distillation at 100-110° (0.03 mm) gave 16.2 g (81% yield) of an analytically pure sample of 2Eb and 3Zb in a 50E:50Z ratio.

NMR ($CDCl_3$ δ rel to int TMS) 2Eb 1.33 (t, J=7 Hz, 3H), 1.35 (t, J=7 Hz, 6H), 2.25 (d of d, $^4J\ ^1H\text{-}^1H$=1.0 Hz, $^4J\ ^1H\text{-}^{31}P$=3.6 Hz, 3H), 4.15 (m, J=7 Hz, 6H), 6.58 (d of q, $^2J\ ^1H\text{-}^{31}P$=15.8 Hz, $^4J\ ^1H\text{-}^1H$=1.0 Hz, 1H) and 3Zb 1.33 (t, J=7 Hz, 3H), 1.35 (t, J=7 Hz, 6H), 2.11 (ap t, $^4J\ ^1H\text{-}^1H$= $^4J\ ^1H\text{-}^{31}P$=1.4 Hz, 3H), 4.15 (m, J=7 Hz, 6H), 5.78 (d of q, $^2J\ ^1H\text{-}^{31}P$=14.6 Hz, $^4J\ ^1H\text{-}^1H$=1.4 Hz, 1H)

Anal. Calcd for $C_{10}H_{19}O_5P$: C, 47.99; H, 7.65. Found: C, 47.68; H, 7.73.

Structure proof: rearrangement of 2Ea and 3Za to ethyl α-(dimethoxyphosphinylmethyl)acrylate (4a): Rearrangement could be achieved either 1) by conducting the reaction described above to prepare 2Ea and 3Za with a 20% molar excess of hexane-washed sodium hydride in 300 ml dimethoxyethane and allowing 6 hr mechanical stirring at room temperature or 2) by adding a 5.0 g mixture of 2Ea and 3Za dropwise to a 5% molar equivalent of hexane-washed sodium hydride in 100 ml dimethoxyethane at 0° and stirring for 6 hr at room temperature before the work-up described above. On carrying out the rearrangement with the second method , 5.0 g of a 50E:50Z mixture of 2Ea and 3Za were rearranged to 2.05 g (41% yield) of the known compound, ethyl α-(dimethoxyphosphinylmethyl)acrylate (4a).[1] The amount of rearrangement to 4a seemed to vary and only occasionally was complete rearrangement to 4a observed, along with the concurrent formation of much polymeric material. The ratio of unrearranged 2Ez to 3Za varied over a wide range. The NMR ($CDCl_3$) spectrum of the short path distilled bp 90-95° (0.03 mm) Lit[1] bp 103-105° (1 mm) rearrangement product 4a was identical to that previously reported for 4a.[1] The yield for the rearrangement to 4a is generally less than 40% with the other 30% of the material recovered being unrearranged 2Ea, 3Za, and polymer in varying proportions.

Rearrangement of 2Eb and 3Zb to ethyl α-(diethoxyphosphinylmethyl) acrylate (4b): The reaction conditions discussed above were empolyed to rearrange a 5.0 g mixture of 2Eb and 3Zb in a 40E:60Z ratio to 4b. The identity of 4b in the distilled mixture bp 100-110° (0.03 mm), 2.8 g (56% yield) is suggested by a comparison of its NMR ($CDCl_3$) spectrum

with that of 4a. As judged by the integration of the allylic region of the NMR spectrum, 4b comprised 65% of the mixture, the other 35% being unrearranged 2Eb and 3Zb.

NMR ($CDCl_3$ δ rel to int TMS) 1.33 (t, J=7 Hz, 3H), 1.35 (t, J=7 Hz, 6H), 2.94 (d, $^2J\ ^1H-^{31}P$=22 Hz, 2H), 4.15 (m, J=7 Hz, 6H), 5.81 (d, J=5 Hz, 1H), 6.21 (d, J=5 Hz, 1H).

Hydrolysis of 4a to α-(dihydroxyphosphinylmethyl)acrylic acid (4e): A mixture (5.0 g) of 4a, 2Ea, and 3Za in a 60:20:20 ratio, respectively, was heated at reflux for 1.5 hr in 48% HBr according to the procedure of Stubbe.[1] Compound 4e (1.21 g, 54% yield based on 4a) was isolated via fractional crystallization from water as white flakes, mp 168-170° (Lit[1] mp 118-120°).* The NMR spectrum of 4e in D_2O is identical to that previously reported for 4e.[1]

Stereoselective rearrangement of 3Zb to ethyl α-(diethoxyphosphinylmethyl) acrylate (4b): A mixture of 3Zb and 2Eb in the ratio 60Z:40E (1.0 g) was added dropwise to 40 ml of 0.1M sodium ethoxide in ethanol and refluxed for 0.5 hr under N_2. The reaction mixture was then cooled to 0°, 25 ml of saturated, aqueous KH_2PO_4 added, extracted thoroughly with four 100 ml portions of CH_2Cl_2, dried with $MgSO_4$, filtered, and the solvent removed in vacuo. The NMR ($CDCl_3$) spectrum of the yellow oil (0.76 g, 76% recovery) showed no 3Zb present and a 40:60 mixture of 2Eb and 4b, respectively, based on the integration of the vinyl region of the

*The mp 168-170° is considerably higher than the mp 118-120° reported for 4e. (ref 1) Samples would occasionally melt at 118-120° on recrystallization suggesting that either a crystalline polymorphism exists or thermal dehydration to a cyclic anhydride is observed. (ref 39)

spectrum. Less than 5% conjugate addition of ethanol is suspected although these addition-products were not characterized.

Attempted rearrangement of 3Zb and 2Eb to 4b with potassium hydride in tetrahydrofuran: A mixture (1.0 g) of 3Zb and 2Eb in the ratio 40E:60Z was added dropwise to 0.25 g of 25% potassium hydride in 40 ml tetrahydrofuran at 0° under N_2 and stirred for 1 hr at 0°. On workup as above an NMR ($CDCl_3$) spectrum of the yellow oil (decanted from mineral oil) showed no vinyl protons. This result was also obtained when the reaction was repeated at -78°.

Partial saponification and hydrolysis of 3Zb: A mixture (5.0 g) of 2Eb and 3Zb in the ratio 40E:60Z were dissolved in 5 ml H_2O and stirred at 0° while 2.0 g of 40% aqueous sodium hydroxide was added. The reaction mixture was stirred at room temperature for 48 hr and then acidified with 12N HCl to pH 2 and allowed to stir 3 hr before thoroughly extracting with five 25 ml portions of Ch_2Cl_2. The water layer was observed to form a white crystalline precipitate which on filtration and recrystallization from water gave 1.89 g of 3Ze or 94.8% of the theoretical yield based on 3Zb. Hygroscopic white needles were isolated mp 179-181°.

IR (nujol) 6.06, 7.6, 8.05, 8.53, 9.00, 9.86, 11.11μ

NMR (D_2O δ rel to ext TMS) 2.13 (d of d, $^4J\ ^1H-^{31}P$=3.6 Hz, $^4J\ ^1H-^1H$=1.4 Hz, 3H), 6.57 (d of q, $^4J\ ^1H-^1H$=1.4 Hz, $^2J\ ^1H-^{31}P$=16.4 Hz, 1H).

Anal. Calcd for $C_4H_7PO_5 \cdot \frac{1}{2}H_2O$: C, 27.44; H, 4.60. Found: C, 27.3; H, 4.93.

Partial saponication and hydrolysis of 2Eb: The CH_2Cl_2 layer on extraction of the above reaction mixture was evaporated in vacuo leaving 1.65 g of 2Ec which is 92.9% of the theoretical yield based on 2Eb. Compound 2Ec was converted to 2Ee by heating at reflux for 36 hr in 25 ml of 6N HCl. Compound 2Ee (1.06 g, 86% yield) was isolated on recrystallization from water as hydroscopic white plates mp 153-158°.

IR (nujol) 5.91, 7.88, 8.47, 9.71, 10.82μ

NMR (D_2O rel to ext TMS) 2.13 (d of d, $^4J\ ^1H\text{-}^{31}P$ = 3.6 Hz, $^4J\ ^1H\text{-}^1H$ = 1.4 Hz, 3H), 6.57 (d of q, $^4J\ ^1H\text{-}^1H$ = 1.4 Hz, $^2H\ ^1H\text{-}^{31}P$ = 16.4 Hz, 1H).

Partial saponication and hydrolysis of 3Zb and 2Eb in D_2O with 40% NaOD, and 6N DCl: The entire partial saponification and hydrolysis sequence of 3Zb and 2Eb (described above) was repeated in D_2O with 2.05 g 40% NaOD and 6N DCl. Neither of the products obtained, 3Ze and 2Ee, showed evidence for acid- or base-catalyzed exchange of deuterium when their NMR spectra were examined.

Hexamethyl 2,3-diphosphonosuccinate (10) was prepared according to the method of Kirillova and Kukhtin.[32] bp 150-160° (0.03 mm) [Lit[32] bp 208-210° (4 mm)].

Modified Wittig reaction of hexamethyl 2,3-diphosphonosuccinate (10): Compound 10 (3.0 g) dried in vacuo overnight over P_2O_5 was dissolved in 50 ml tetrahydrofuran. Lithium hydride (0.1 g, 50% molar excess)

was added with stirring under N_2. The reaction mixture was then heated to reflux for 15 min. After cooling to room temperature, 0.25 g of paraformaldehyde (Eastman, dried in vacuo overnight) dissolved in 20 ml tetrahydrofuran was added rapidly at room temperature. After 2 hr stirring at room temperature, the reaction mixture was filtered, and the solvent removed from the filtrate in vacuo at room temperature. An NMR ($CDCl_3$) spectrum of the micro-distilled oil, bp 65-85° (0.05 mm), 1.1 g, showed the presence of vinyl protons. These products were presumed to be 11 and 12 in the ratio 1:4, respectively, based on the integration of the vinyl region of the spectrum.

Structure proof: Hydrolysis of 11: When the modified Witting reaction was repeated on a scale ten times as large as that described above, 8.45 g of 11 and 12 in a 1:4 ratio was obtained. Short-path distillation gave two fractions, bp 45-55° and bp 100-110° (0.1 mm). The higher boiling fraction, 11 presumably (2.05 g, 9.3% yield), was then heated at reflux for 1.5 hr in 20 ml of 48% HBr (freshly distilled from $SnCl_2$). After several recrystallizations from water, 0.46 g (36% yield based on 11) of the known compound,[1] α-(dihydroxyphosphinylmethyl)acrylic acid (4e), was isolated as white flakes, mp 168-170°* (Lit[1] mp 118-120°). The NMR (D_2O spectrum of 4e was identical to that previously reported.

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*See footnote page 59

Preparation of ethyl (diethoxyphosphinyl)acetate 90% ^{13}C-enriched in the carbonyl position: Ethyl bromoacetate 90% ^{13}C-enriched in the carbonyl position (5 g, Koch Isotopes) was heated at reflux for 3 hr with 15 g of freshly distilled triethyl phosphite (Aldrich). Ethyl (diethoxyphosphinyl)acetate (6.45 g, 96% yield) was short-path distilled at bp 105-110° (1 mm) [Lit[70] bp 142-145° (9 mm)].

NMR ($CDCl_3$ δ rel to int TMS) 1.3 (t, J=7 Hz, 3H), 1.36 (t, J=7 Hz, 6H), 2.98 (d of d, $^2J\ ^1H-^{31}P$=21.7 Hz, $^2J\ ^1H-^{13}C$=7.5 Hz, 2H), 4.2 (m, J=7 Hz, 6H).

Preparation of dimethyl β-carbomethoxyethylphosphonate (17) 90% ^{13}C-enriched in the carbonyl position: A modified version of the procedure of Pudovik and Kitaev (1952) was enployed.[49] To 1.44 g of 56% sodium hydride (washed twice with hexane) in a 500 ml 4-neck flask equipped with a mechanical stirrer, thermometer, 125 ml pressure-equalized addition funnel, and N_2 bubbler, was added 100 ml dimethoxyethane (freshly distilled from sodium hydride). After cooling to 0° 6.45 g of ethyl (diethoxyphosphinyl)acetate in 50 ml dimethoxyethane was added dropwise and allowed to stir 1 hr at 0°. The reaction mixture was then cooled to -20° and 15 g paraformaldehyde (dried in vacuo overnight) was added rapidly and allowed to stir for 2 hr at 0°. The reaction mixture, while still cold, was then filtered with a nitrogen purge through a fine-fritted sintered glass filter into a 300 ml 3-neck flask and washed with 25 ml dimethoxyethane. Dimethyl phosphite (10 ml, Aldrich; freshly distilled from calcium hydride) was added to the flask

followed by the dropwise addition of 15 ml of 1M sodium methoxide over 1 hr at room temperature and then allowed to stir 1 hr at room temperature. The reaction mixture was then cooled to 0° and 25 ml saturated, aqueous KH_2PO_4 was added followed by extraction with five 100 ml portions of CH_2Cl_2. The CH_2Cl_2 extract was dried overnight over Na_2SO_4, filtered, and the solvent removed *in vacuo*. The crude product was then short-path distilled from a 15 ml pear-shaped flask at bp 95-103° (1 mm) [Lit bp 137-138° (10 mm)] yielding 3.47 g (62% yield) of 17 or 59% overall yield based on ethyl bromoacetate.

Preparation of methyl α-(dimethoxyphosphinylmethyl)acrylate (4d) 90% ^{13}C-enriched in carbonyl position: Compound 17 (3.44 g) was dissolved in 60 ml tetrahydrofuran (freshly distilled from lithium aluminum hydride) and stirred under N_2 while 4.18 g of 25% potassium hydride in mineral oil was added at room temperature and allowed to stir at room temperature for 3 hr. Paraformaldehyde (5.0 g, dried *in vacuo* overnight) was added to the mixture rapidly at room temperature and stirred vigorously for 6 hr at room temperature. Excess paraformaldehyde was filtered from the reaction mixture and washed with 25 ml tetrahydrofuran. The tetrahydrofuran was then removed *in vacuo* at room temperature leaving a yellow oil which soon separated into two layers. The upper clear layer of mineral oil was decanted leaving 1.43 g (38.8% yield based on 17) of 4d as a yellow oil. An NMR ($CDCl_3$) spectrum of crude 4d was obtained which suggested the presence of at least two products, 4d comprising better than 75% of the mixture.

NMR ($CDCl_3$ δ rel to int TMS) 2.98 (d of d, $^2J\ ^1H\text{-}^{31}P$=22 Hz, $^3J\ ^1H\text{-}^{13}C$=

4.6 Hz, 2H), 5.95 (d of d, $^{4}J\ ^{1}H-^{31}P$=5.5 Hz, $^{3}J\ ^{1}H-^{13}C$=13.5 Hz, 1H), 6.35 (ap t, $^{4}J\ ^{1}H-^{31}P$=5.5 Hz, $^{3}J\ ^{1}H-^{13}C$=7.0 Hz, 1H).

Preparation of α-(dihydroxyphosphinylmethyl)acrylate (4e) 90% ^{13}C-enriched in carbonyl position: ^{13}C-labeled 4d (1.43 g) was heated at reflux for 1.5 hr in 48% HBr (freshly distilled from $SnCl_2$). After removal of the solvent, an oil remained which was dissolved 5 ml water. Barium acetate was then added until the pH was 4.3 followed by approximately 25 ml absolute ethanol and filtration of 1.85 g precipitated crude barium salt of 4e. Crude 4e (1.85 g) as barium salt was then washed with water down a Dowex 50-W-X cation exchange resin in the lithium form and the water removed in vacuo to leave 0.95 g crude dilithium salt of 4e. Recrystallization from water and ethanol gave 0.65 g dilithium salt of 4e or 12% overall yield based on ethyl bromoacetate.

NMR (D_2O δ rel to ext TMS) 3.19 (d of d, $^{3}J\ ^{1}H-^{13}C$ = 4 Hz, $^{2}J\ ^{1}H-^{13}P$ = 20.5 Hz, 2H), 6.40 (d of d, $^{3}J\ ^{1}H-^{13}C$ = 11.5 Hz, $^{4}J\ ^{1}H-^{31}P$ = 4.7 Hz, 1H), 6.65 (ap t, $^{3}J\ ^{1}H-^{13}C$ = 6.7 Hz, $^{4}J\ ^{1}H-^{31}P$ = 4.3 Hz, 1H).

General procedure for additions of stabilized phosphonate carbanions to dimethyl acetylenedicarboxylate: The stabilized phosphonate carbanion (0.054 mole) in 10 ml benzene is added dropwise to 2.58 g of 56% sodium hydride (washed twice with hexane) under N_2 in 200 ml benzene at 0° and stirred at room temperature for 1 hr. After cooling to 0°, 7 ml of dimethyl acetylenedicarboxylate (Aldrich) in 10 ml benzene

is added dropwise and the reaction mixture is stirred for 2 hr at room temperature. The mixture is then cooled to 0° and 50 ml saturated, aqueous KH_2PO_4 is added and extracted with five 100 ml portions of ether. The ether extract is dried overnight on Na_2SO_4, filtered, and the solvent removed *in vacuo* leaving a black, viscous, oil. This oil can best be purified by short-path distillation using a salt-bath and a high vacuum. Smaller quantities can be adequately purified *via* micro-distillation. The following reactions have been carried out using this procedure:

Addition of methyl (dimethoxyphosphinyl)acetate to dimethyl acetylenedicarboxylate: A mixture of two isomeric β,γ-unsaturated phosphonates, *21E* and *22Z*, were obtained (6.5 g, 37% yield) in a *75E:25Z* ratio on short-path distillation, bp 140-145° (0.02 mm)

NMR ($CDCl_3$ δ rel to int TMS) *22Z* 3.80 (m, 15H), 4.32 (d, $^2J\ ^1H\text{-}^{31}P$ = 25.2 Hz, 1H), 6.76 (dm $^4J\ ^1H\text{-}^{31}P$ = 4.6 Hz, 1H) and *21E* 3.80 (m, 15H), 5.86 (d, $^2J\ ^1H\text{-}^{31}P$ = 28.2 Hz, 1H), 6.91 (d, $^4J\ ^1H\text{-}^{31}P$ = 3.6 Hz, 1H).

Anal. Calcd for $C_{11}H_{17}O_9P$: C, 40.75; H, 5.28. Found: C, 41.09; H, 5.36.

Addition of bis-(dimethoxyphosphinyl)-methane (1a) to dimethyl acetylenedicarboxylate: A mixture of two isomeric β,γ-unsaturated phosphonates, *25Za* and *26Ea*, were obtained (8.5 g, 45% yield) in a 65Z:35E ratio by short-path distillation, bp 155-160° (0.02 mm)

NMR ($CDCl_3$ δ rel to int TMS) *26Ea* 3.95 (t, $^2J\ ^1H\text{-}^{31}P$ = 25.3 Hz, 1H),

3.83 (m, 18H), 6.85 (t, $^{4}J\ ^{1}H\text{-}^{31}P$ =4.4 Hz, 1H), and 25Za 3.83 (m, 18H), 5.9 (t, $^{2}J\ ^{1}H\text{-}^{31}P$ = 27.0 Hz, 1H), 6.89 (t, $^{4}J\ ^{1}H\text{-}^{31}P$ = 3 Hz, 1H).

Anal. Calcd for $C_{11}H_{20}O_{10}P_2$: C, 35.30; H, 5.38. Found C, 35.00; H, 5.38.

Addition of bis-(diethoxyphosphinyl)-methane (1b) to dimethylacetylene-dicarboxylate: A mixture of two isomer β,γ-unsaturated phosphonates, 25Zb and 26Eb, were obtained (9.5 g, 44% yield) in a 40Z:60E ratio by short-path distillation, bp 165-170° (0.01 mm). NMR ($CDCl_3$ δ rel to int TMS) 26Eb 1.33 (t, J = 7 Hz, 12H), 3.8 (m, 6H), 4.2 (m, J=7 Hz, 8H), (allylic t is buried, hence unassignable), 6.87 (t, $^{4}J\ ^{1}H\text{-}^{31}P$=4.4 Hz, 1H), and 25Zb 1.33 (t, J=7 Hz, 12H), 3.8 (m, 6H), 4.2 (m, J=7 Hz, 8H), 5.77 (t, $^{2}J\ ^{1}H\text{-}^{31}P$ = 26.6 Hz, 1H), 6.82 (t, $^{4}J\ ^{1}H\text{-}^{31}P$=3.0 Hz, 1H),

Anal. Calcd for $C_{15}H_{28}O_{10}P_2$: C, 41.86; H, 6.55. Found C, 41.55; H, 6.47.

Hydrolysis of 21E and 22Z: A 75E:25Z mixture of 21E and 22Z (5.0g) was heated at reflux for 2 hr with 25 ml of 12 N HCl. On removal of the solvent, 1.75 g (55% yield of a 75E:25Z mixture of β,γ-unsaturated phosphonic acids, 23E and 24Z, was obtained on recrystallization from water.

NMR (D_2O δ rel to ext TMS) 23E 3.5 (d, $^{2}J\ ^{1}H\text{-}^{31}P$=23.6 Hz, 2H), 6.9 (d, $^{4}J\ ^{1}H\text{-}^{31}P$=6.2 Hz, 1H), and 24Z 3.01 (d, $^{2}J\ ^{1}H\text{-}^{31}P$=22.6 Hz, 2H), 6.28 (d, $^{4}J\ ^{1}H\text{-}^{31}P$=5.0 Hz, IH).

Anal. Calcd for $C_5H_7O_7P$: C, 28.59; H, 3.36. Found: C, 28.37; H, 3.39.

Compound 23E (1.05 g, 81% yield) was successfully separated from 24Z by fractional crystallization from water of 1.75 g of a 75E:25Z mixture of 23E and 24Z. Compound 23E is less soluble in water and hence is the first to fall out of solution leaving 24Z and any impurities in the

mother liquor. mp 203-205° for 23E.

IR (nujol) 5.96, 7.94, 9.43, 9.82, 10.44μ

Hydrolysis of 25Zb and 26Eb: A 40Z:60E mixture of 25Zb and 26Eb (5.0 g) was heated at reflux for 3 hr with 25 ml of 12N HCl. On removal of the solvent, 1.65 g (61% yield) of a 40Z:60E mixture of β,γ-unsaturated phosphonic acids, 31Z and 32E, was obtained after several recrystallizations from water.

NMR (6N DCl δ rel to ext TMS) 25Zb 5.63 (t, $^{2}J\ ^{1}H\text{-}^{31}P$=26.8 Hz, 1H), 7.18 (t, $^{4}J\ ^{1}H\text{-}^{31}P$=3 Hz, 1H), and 26Eb 4.15 (t, $^{2}J\ ^{1}H\text{-}^{31}P$=25 Hz, 1H), 6.98 (t, $^{4}J\ ^{1}H\text{-}^{31}P$=4.2 Hz, 1H).

Anal. Calcd for $C_5H_8O_{10}P_3 \cdot 3/4H_2O$: C, 19.78; H, 3.15. Found: C, 19.83; H, 3.34.

Several fractional recrystallizations from water gave a sample enriched in 25Zb in about a 95Z:5E ratio. 25Zb is less soluble in water than is 26Eb and hence is easier to free from water-soluble impurities.

Treatment of 25Za and 26Ea with trimethylsilyl bromide: To 0.1 g of a 40Z:60E mixture of 25Za and 26Ea was added an excess (2.5 ml) of trimethylsilyl bromide at room temperature, neat. The mixture was allowed to stir for 15 min at room temperature before removing excess trimethylsilyl bromide in vacuo. Microdistillation of the sample gave 0.16 g (88% yield) of 25Zc and 26Ec bp 150-160° (0.03 mm) in a 40Z:60E ratio.

NMR ($CDCl_3$ δ rel to int TMS) 26Ec 0.25 (m, 36H), 3.62 (t, $^{2}J\ ^{1}H\text{-}^{31}P$=25.7 Hz, 1H), 6.87 (t, $^{4}J\ ^{1}H\text{-}^{31}P$=4.8 Hz, 1H), and 25Zc 0.25 (m, 36H), 5.75 (t, $^{2}J\ ^{1}H\text{-}^{31}P$=27.9 Hz, 1H), 6.90 (t, $^{4}J\ ^{1}H\text{-}^{31}P$=3.2 Hz, 1H).

Anal. Calcd for $C_{19}H_{44}O_{10}P_2Si_4$: C, 37.60; H, 7.31. Found: C, 35.44; H, 6.88.

Preparation of methyl β-carbomethoxyethylphosphonate (17) was prepared according to the method of Pudovik and Kitaev.[49] bp 95-103° (1 mm) [Lit[49] bp 137-138° (10 mm)].

Crossed-Claisen condensation of dimethyl β-carbomethoxyethylphosphonate (17) with methyl formate: Compound 17 (15.7g) was dissolved in 150 ml of dimethoxyethane (freshly distilled from sodium hydride) and added rapidly to 4.5 g of 56% sodium hydride (washed twice with hexane) with stirring under N_2. Methyl formate (109 ml, Aldrich) was rapidly added and the mixture was allowed to stir at room temperature for 3 hr under N_2. The mixture was then cooled to 0° and 50 ml saturated, aqueous KH_2PO_4 added, followed by extraction with five 100 ml portions of CH_2Cl_2. The CH_2Cl_2 extract was dried overnight on Na_2SO_4, filtered, and the solvent removed in vacuo at room temperature to leave a viscous yellow oil which solidified on standing overnight at 0°. Recrystallization from CH_2Cl_2 gave 13.8g (77% yield) of methyl (dimethoxyphosphinylmethyl-hydroxymethylene)acetate (33E), mp 78-79° (white plates).

IR (neat 6.15, 7.23, 8.64, 9.85μ

NMR ($CDCl_3$ δ rel to int TMS) 2.92 (d, $^2J\ ^1H-^{31}P$=20.4 Hz, 2H), 3.76 (d, J=11 Hz, 6H), 3.70 (s, 3H), 7.83 (d, $^4J\ ^1H-^{31}P$=5.2 Hz, 1H), 9.18 (brd s, 1H).

Anal. Calcd for $C_7H_{13}O_6P$: C, 37.51; H, 5.85. Found: C, 37.78; H, 5.89.

Microdistillation of a sample of 33E at bp 105-110° (0.1 mm) gave an 80E:20Z mixture of 33E and 33Z in $CDCl_3$.

NMR ($CDCl_3$ δ rel to int TMS) 33E 2.92 (d, $^2J\ ^1H-^{31}P$=20.4 Hz, 2H), 3.76 (d, J = 11 Hz, 6H), 3.70 (s, 3H), 7.83 (d, $^4J\ ^1H-^{31}P$ = 5.2 Hz, 1H), 9.83 (brd s, 1H), and 33Z 2.71 (d, $^2J\ ^1H-^{31}P$=19.2 Hz, 2H), 3.76 (d, J=11 Hz, 6H), 3.70 (s, 3H), 7.17 (brd s, 1H), 9.83 (brd s, 1H).

NMR (D_2O δ rel to ext TMS) (65E:35Z) 33E 2.93 (d, $^2J\ ^1H-^{31}P$=20 Hz, 2H), 3.70 (s, 3H), 3.72 (d, J=11 Hz, 6H), 7.87 (d, $^4J\ ^1H-^{31}P$=5.2 Hz, 1H), and 33Z 2.27 (d, $^2J\ ^1H-^{31}P$=18.2 Hz, 2H), 3.73 (s, 3H), 3.72 (d, J=11 Hz, 6H), 5.08 (s, 1H).

Dimethyl phosphorochloridate was prepared according to the method of Fiszer and Michalski.[89] bp 55-58° (2 mm) [Lit[89] bp 80° (18 mm)].

Phosphorylation of sodium enolate anion of methyl dimethoxyphosphinylmethyl-hydroxymethylene)acetate 33E and 33Z: A 80E:20Z mixture of 33E and 33Z (2.0 g) was dissolved in 10 ml benzene and added dropwise with stirring under N_2 at 0° to 0.43 g of 56% sodium hydride (twice hexane-washed) in 200 ml benzene and allowed to stir for 5 hr at room temperature. Freshly distilled dimethyl phosphorochloridate (1.2 g) dissolved in 10 ml benzene was then added dropwise at 0° and allowed to stir for 3 hr at room temperature. The reaction mixture was then filtered thru a fine-fritted sintered glass filter and the solvent removed in vacuo. Short-path distillation bp 150-155° (0.01 mm) gave 2.77 g (94% yield) of the enol phosphate 35E.

IR (neat) 5.64, 6.13, 6.59, 7.76, 8.71, 9.54μ

NMR ($CDCl_3$ δ rel to int TMS) 2.94 (d, $^2J\ ^1H-^{31}P$=21.8 Hz, 2H), 3.82 (m, J=11 Hz, 15H), 7.69 (ap t, $^4J\ ^1H-^{31}P$= $^3J\ ^1H-^{31}P$=6.2 Hz, 1H).

Anal. Calcd for $C_9H_{18}O_9P_2$: C, 32.54; H, 5.46. Found: C, 32.64; H, 5.44.

Catalytic hydrogenation of enol phosphate 35E: Compound 35E (0.5 g) dissolved in 25 ml ethyl acetate and subjected to 3 atmospheres of H_2 in a Parr apparatus for 3 hr in separate runs with each of the following catalysts: 1) 100 mg platinum oxide, 2) 0.5 g of 5% palladium on charcoal, and 3) 0.5 g of 5% rhodium on aluminum. An NMR ($CDCl_3$ spectrum was obtained after filtering-off the catalyst and removal of the solvent in vacuo. In each case, the crude product showed an acidic proton at δ=11.2 ppm. The three spectra obtained were virtually superimposable suggesting identical products in each run.

NMR ($CDCl_3$ δ rel to int TMS) 1.22 (d, J=7 Hz, 3H), 1.5-3 (m, 3H), 3.65 (m, J=11 Hz, 15 H), 11.19 (s, 1H).

The identity of one of the products is confirmed by the synthesis of the presumed product, methyl (RS-α-(dimethoxyphosphinylmethyl)propionate (37) whose NMR ($CDCl_3$) spectrum is virtually identical to the spectra of the hydrogenation products.

Preparation of methyl (RS)-α-(dimethoxyphosphinylmethyl)propionate (37): Compound 37 was prepared according to the method of Pudovik and Arbusov.[46] bp 97-105° (0.02 mm) [Lit[46] bp 137-138° (10 mm)].

NMR ($CDCl_3$ δ rel to int TMS) 1.22 (d, J=7 Hz, 3H), 1.5-3 (m, 3M), 3.65 (d, J=11 Hz, 6H), 3.62 (s, 3H).

Treatment of enol phosphate 35E with excess trimethylsilyl bromide: Compound 35E (0.2 g) was dissolved in 20 ml dry benzene and 0.6 ml of trimethylsilyl bromide were added with stirring under N_2. The reaction

mixture was then heated at reflux for 45 min and after cooling, the solvent and excess trimethylsilyl bromide removed in vacuo. The crude product was microdistilled bp 135-140° (0.01 mm) to give 0.21 g (62% yield) of 42E.

NMR ($CDCl_3$ δ rel to int TMS) 0.25 (m, 36H), 2.87 (d, $^2J\ ^1H-^{31}P$=22.4 Hz, 2H), 3.73 (s, 3H), 7.68 (d of d, J=6.2 Hz, J=7.1 Hz, 1H).

Anal. Calcd for $C_{17}H_{42}O_9P_2Si_4$: C, 36.15; H, 7.49. Found: C, 35.66; H, 7.29.

Thermal cyclization of methyl (dimethoxyphosphinylmethylhydroxymethylene) acetate 33E and 33Z: Compounds 33E and 33Z (2.0 g) were heated reflux under vacuum (150° bath at 0.8 mm) for 1.5 hr in a 15 ml pear-shaped flask equipped with a magnetic stirrer and reflux condenser. After cooling, the reaction mixture was short-path distilled bp 80-85° (0.05 mm) to give 1.3 g (76% yield) of an enol phostonate 44. An NMR ($CDCl_3$) spectrum of the distillate suggested the presence of 5% of an impurity which would explain why the elemental analysis deviates slightly from theoretical.

NMR ($CDCl_3$ δ rel to int TMS) 2.78 (d of d, $^2J\ ^1H-^{31}P$=13.7 Hz, $^4J\ ^1H-^1H$= 2.1 Hz, 2H), 3.71 (s, 3H), 3.76 (d, $^3J\ ^1H-^{31}P$=11 Hz, 3H), 7.50 (d of t, $^3J\ ^1H-^{31}P$=29.5 Hz, $^4J\ ^1H-^1H$=2.1 Hz, 1H).

IR (neat) 5.78, 6.08, 6.96, 8.42, 9.67μ

Anal. Calcd for $C_6H_9O_5P$: C, 37.51; H, 4.72. Found: C, 37.29; H, 5.19.

Treatment of 33E and 33Z with oxalyl chloride: A modification of the method of Clark and Heathcock was used.[57] To 1.0 g of 33E and 33Z was added 0.56 g of oxalyl chloride with stirring, neat, under N_2, until a

homogeneous solution was achieved (10 min). The mixture was then pumped on at room temperature (0.5 mm) for 0.5 hr and then microdistilled 80-85° (0.05 mm) to give 0.06 g (7% yield) of 44 whose NMR ($CDCl_3$) spectrum is identical to 44 prepared by thermal cyclization. A chemical ionization mass spectrum of 44 prepared via the oxalyl chloride route gave the expected parent M+1 peak at 193. A 2M+1 peak at 385 was also observed.

Catalytic hydrogenation of 33E and 33Z: Compounds 33Eand 33Z (1.0 g) were dissolved in 25 ml methanol and subjected to 3 atmospheres pressure of H_2 in a Parr apparatus for 24 hr, the time necessary to achieve complete uptake of hydrogen, with 5.0 g palladium on charcoal catalyst. Filtration of the catalyst and removal of the solvent in vacuo gave 0.85 g (84% yield) of a viscous oil whose NMR ($CDCl_3$) spectrum suggested a hydrogenation product, methyl (RS)-(dimethoxyphosphinylmethyl-hydroxymethyl)acetate (45), and a hydrogenolysis product, methyl (RS)-α-(dimethoxyphosphinylmethyl)propionate (37), in a 95.5 ratio, respectively. The identity of 37 in the mixture was suggested by comparison with the NMR ($CDCl_3$) spectrum of authentic 37. The identity of 45 in the mixture was likewise confirmed by synthesis of 45 by another route and comparison of their NMR ($CDCl_3$) spectra.

Preparation of methyl (RS)-α-(dimethoxyphosphinylmethylhydroxymethyl) acetate (45) by sodium cyanoborohydride reduction of 33E and 33Z: Compounds 33E and 33Z (0.5 g) were dissolved in 25 ml methanol and 2.0 g of sodium cyanoborohydride was added with stirring. After stirring 3 hr at room temperature, a drop of methyl orange indicator was added along with 10 ml water. The pH was then lowered to 3 repeatedly with the

dropwise addition of 1N HCl until the orange color failed to disappear over a 1 hr period. The reaction mixture was then extracted thoroughly with five 75 ml portions of Ch_2Cl_2. After drying overnight on Na_2SO_4 and filtering, the methylene chloride was removed *in vacuo* leaving a white semi-crystalline solid whose NMR ($CDCl_3$) spectrum was identical to 45 obtained *via* catalytic hydrogenation. (0.36 g, 69% yield).

NMR ($CDCl_3$ δ rel to int TMS) 1.9-3.15 (complex m, 5H), 3.73 (d, ^3J ^1H-^{31}P=11 Hz, 6H), 3.73 (s, 3H), 4.52 (brd s, 1H).

IR (neat) 2.98, 5.76, 7.00, 8.21, 9.82, 12.05μ

In an attempt to purify 45 for elemental analysis, thermal cyclization to a phostonate 46 occurred.

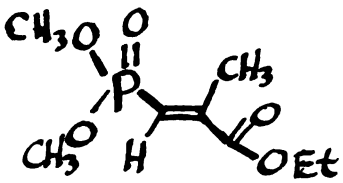
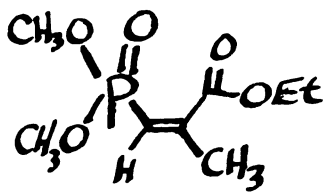
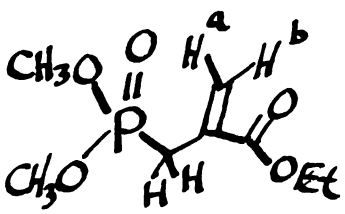
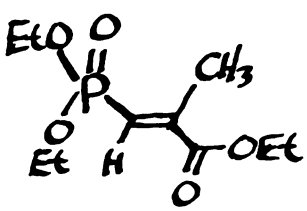
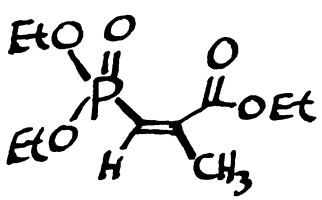
Thermal cyclization of methyl (RS)-α-(dimethoxyphosphinylmethyl-hydroxymethyl)acetate (45): Slow microdistillation at low vacuum bp 165-170° (3 mm) of 0.3 g of 45 gave 0.18 g (72% yield) of 46 as a viscous, colorless oil.

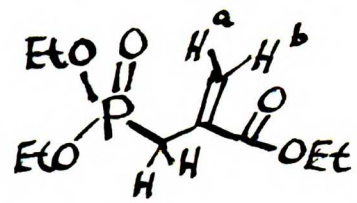
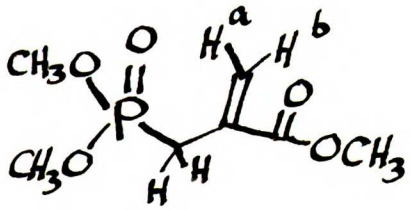
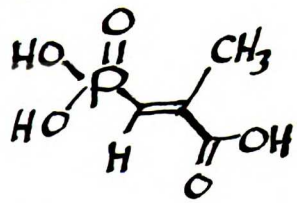
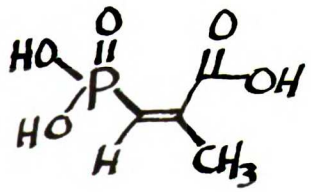
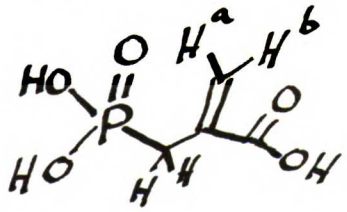
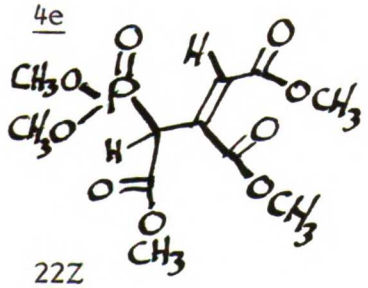
IR (neat) 5.74, 6.88, 7.48, 8.08, 9.74, 11.76 μ

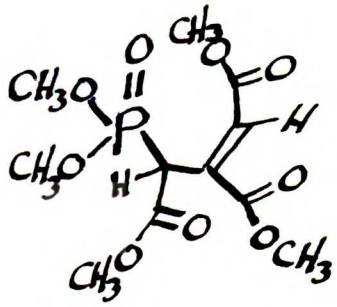
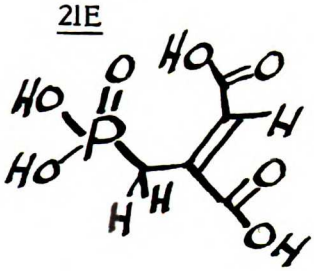
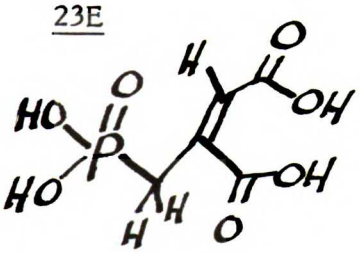
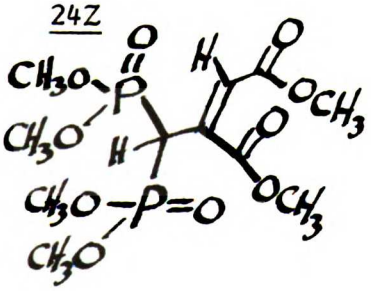
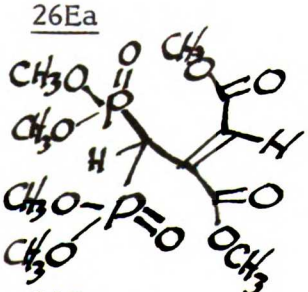
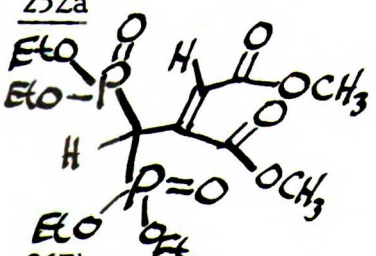
NMR ($CDCl_3$ δ rel to int TMS) 1.8-2.45 (complex m, 2H), 1.8-3.5 (complex m, 1H), 3.9-4.55 (complex m, 2H), 3.5-3.9 (complex m, 6H).

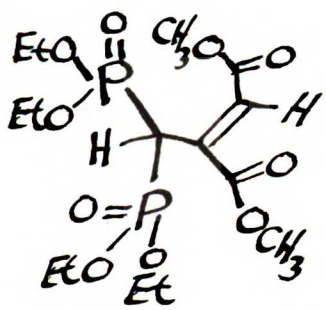
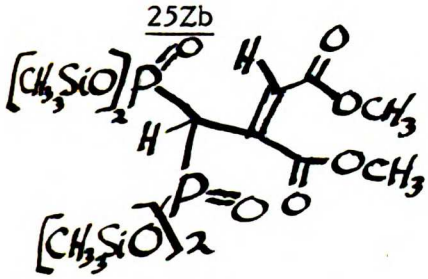
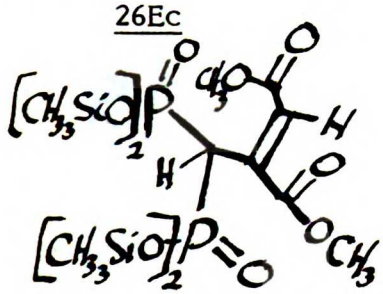
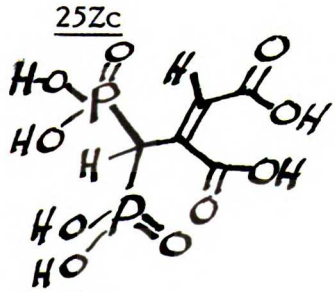
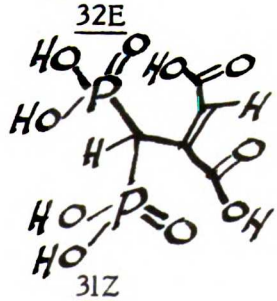
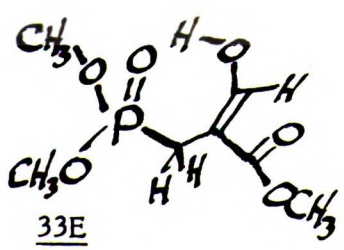
Anal. Calcd for $C_6H_{11}O_5P$: C, 37.12; H, 5.71. Found: C, 36.79; H, 5.82.

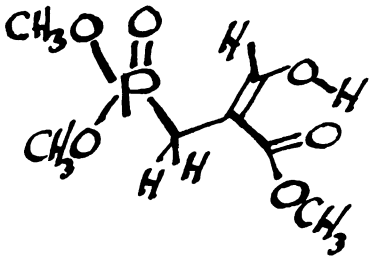
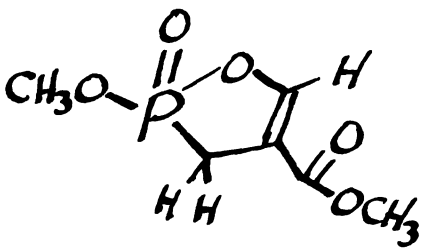
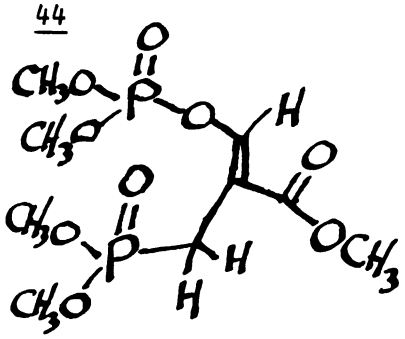
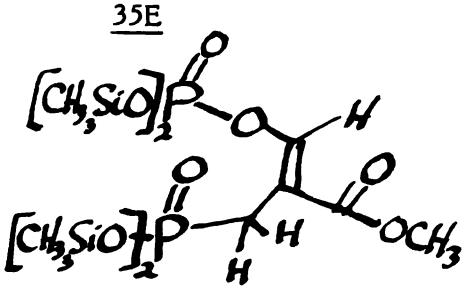
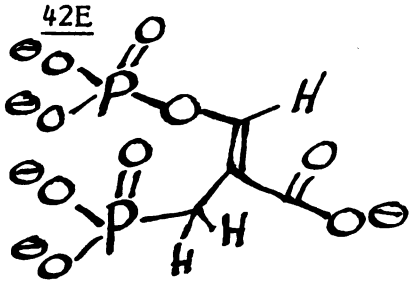
V. TABLE OF $^{4}J\ ^{1}H\text{-}^{31}P$ AND $^{2}J\ ^{1}H\text{-}^{31}P$ COUPLING CONSTANTS

Compound		$^{4}J\ ^{1}H\text{-}^{31}P$ (in Hz)	$^{2}J\ ^{1}H\text{-}^{31}P$ (in Hz)
 2 Ea	$CDCl_3$	3.6	16.4
 3Za	$CDCl_3$	1.8	15.0
 4a	$CDCl_3$	H_a=6 H_b=6	22
 2Eb	$CDCl_3$	3.6	15.8
 3Zb	$CDCl_3$	1.4	14.6

		$^4J\ ^1H\text{-}^{31}P$ (in Hz)	$^2J\ ^1H\text{-}^{31}P$ (in Hz)
	$CDCl_3$	H_a=7 H_b=5	22
4b			
	$CDCl_3$	H_a=5.5 H_b=5.5	22
4d			
	D_2O	3.6	16.4
2Ee			
	D_2O	1.4	10.2
3Ze			
	D_2O	H_a=4.7 H_b=4.3	20.5
4e			
	$CDCl_3$	4.6	25.2
22Z			

		$^4J\ ^1H\text{-}^{31}P$ (in Hz)	$^2J\ ^1H\text{-}^{31}P$ (in Hz)
	$CDCl_3$	3.6	28.2
21E 	D_20	6.2	23.6
23E 	D_20	5.0	22.6
24Z 	$CDCl_3$	4.4	25.3
26Ea 	$CDCl_3$	3.0	27.0
25Za  26Eb	$CDCl_3$	4.4	---

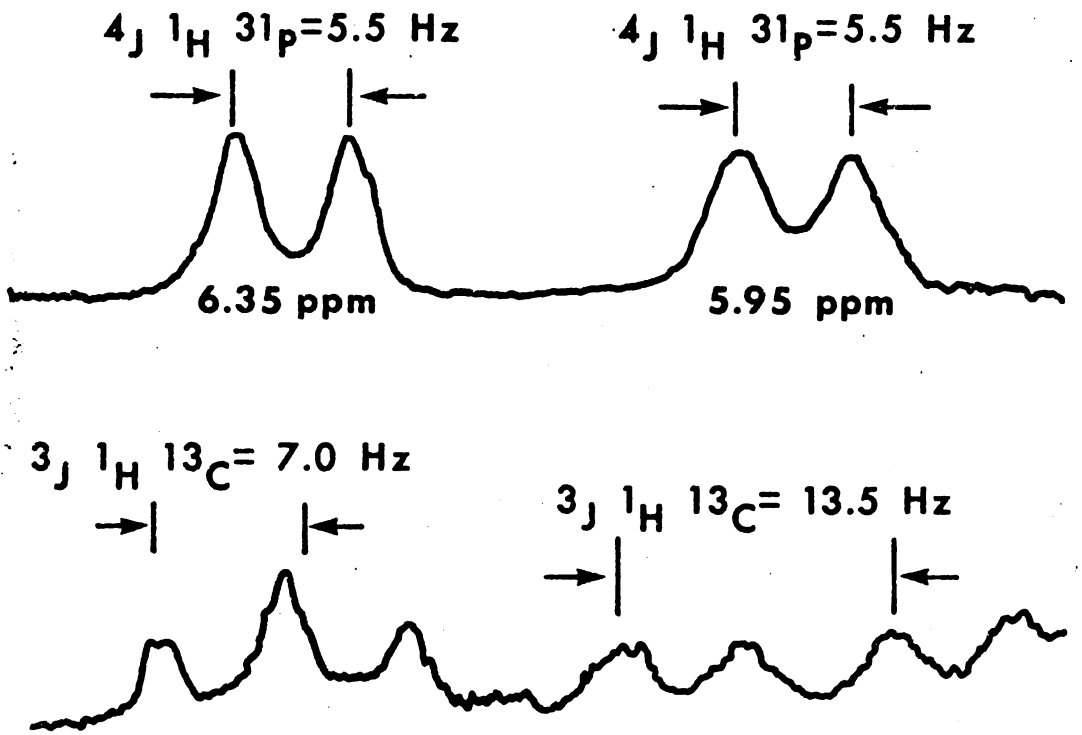
		$^4J\ ^1H\text{-}^{31}P$ (in Hz)	$^2J\ ^1H\text{-}^{31}P$ (in Hz)
	$CDCl_3$	3.0	26.6
25Zb 	$CDCl_3$	4.8	25.7
26Ec 	$CDCl_3$	3.2	27.9
25Zc 	D_2O	4.2	25
32E 	D_2O	3.0	26.8
31Z 	$CDCl_3$ D_2O	5.2 5.2	20.4 20.0
33E			

		$^4J\ ^1H\text{-}^{31}P$ (in Hz)	$^2J\ ^1H\text{-}^{31}P$ (in Hz)
	$CDCl_3$	---	19.2
	D_2O	---	18.2
33Z			
	$CDCl_3$	---	13.7
44			
	$CDCl_3$	6.2	21.8
35E			
	$CDCl_3$	6.2 or 7.1	22.4
42E			
	D_2O	5.2 or 7.0	19.6
54E			

VI. CATALOGUE OF NMR SPECTRA

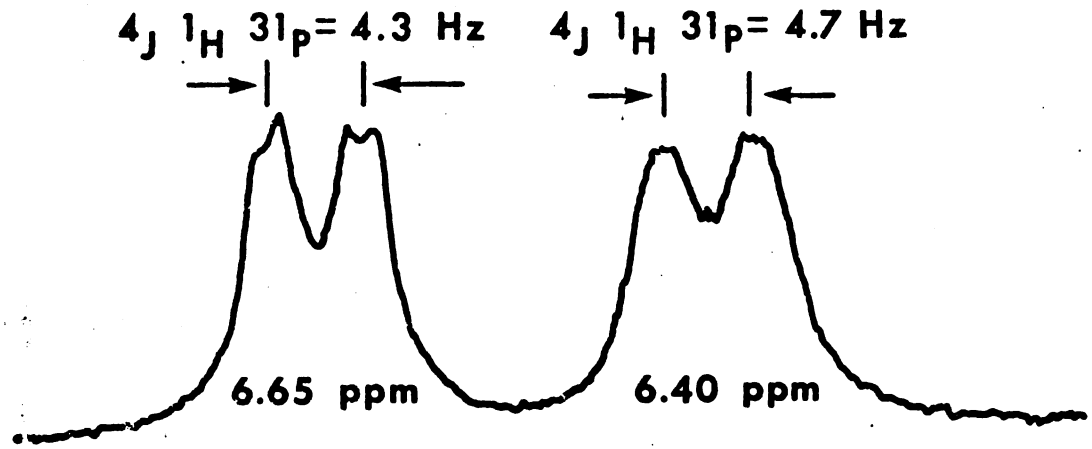
101	54E
102	Autocatalyzed hydrolysis of 33E and 33Z in D_2O (early in the hydrolysis)
103	Autocatalyzed hydrolysis of 33E and 33Z in D_2O (late in the hydrolysis)
104	47 via two equivalents of NaOD
105	47 via three equivalents of NaOD
106	53 via one equivalent of NaOD
107	44 via thermal cyclization of 33E and 33Z
108	45 via catalytic hydrogenation
109	45 via sodium cyanoborohydride reduction
110	46 via thermal cycliation of 45

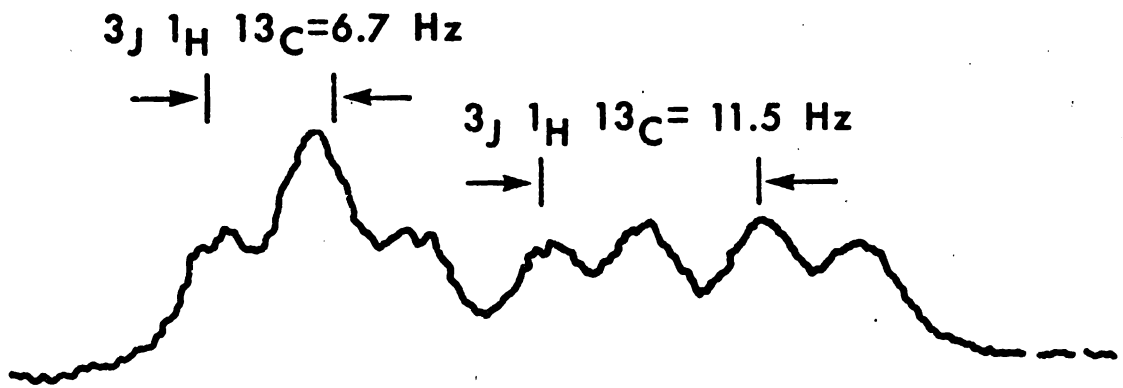
METHYL α-(dimethoxyphosphinylmethyl) ACRYLATE



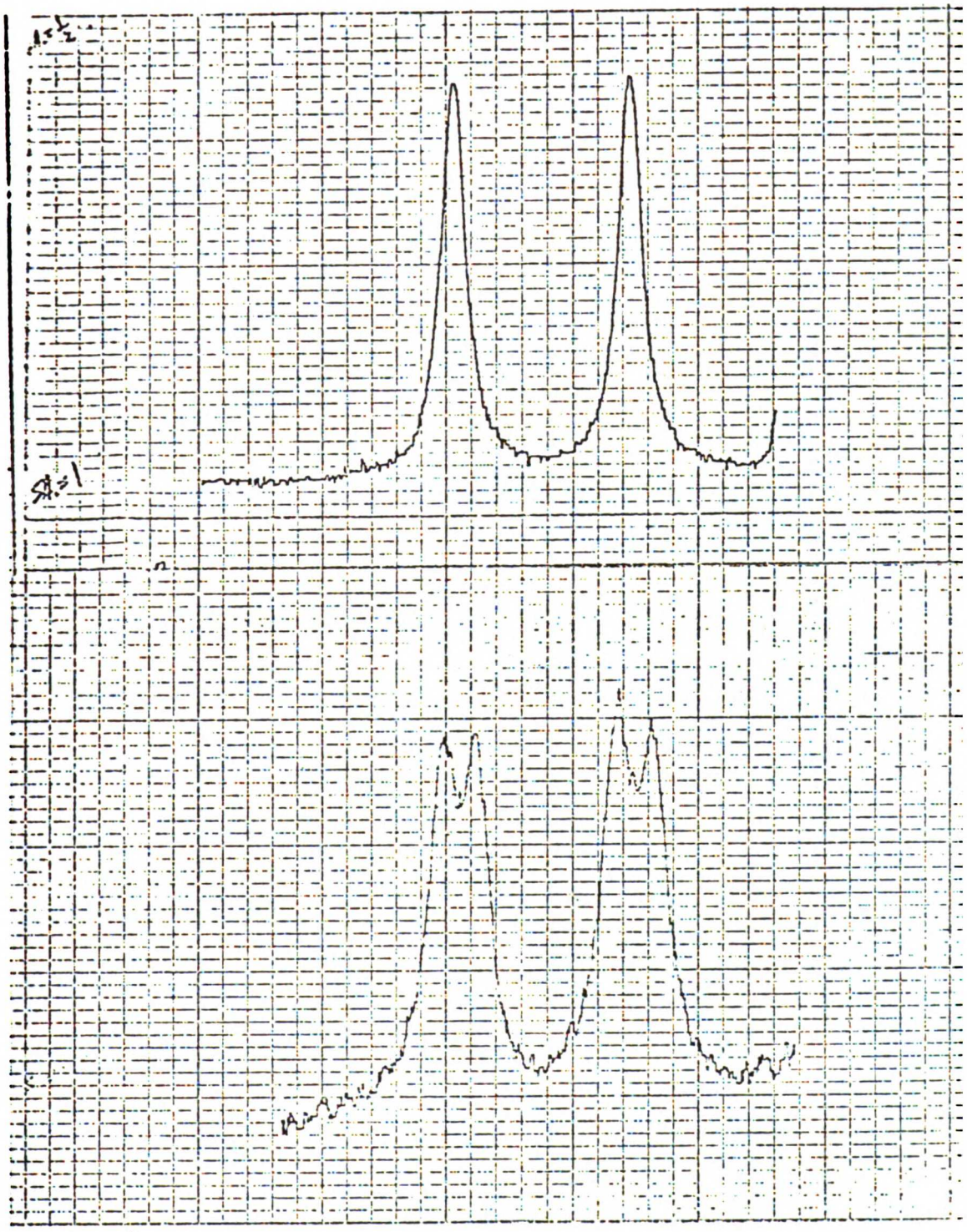
90% ^{13}C-ENRICHED IN THE CARBOXYLATE POSITION

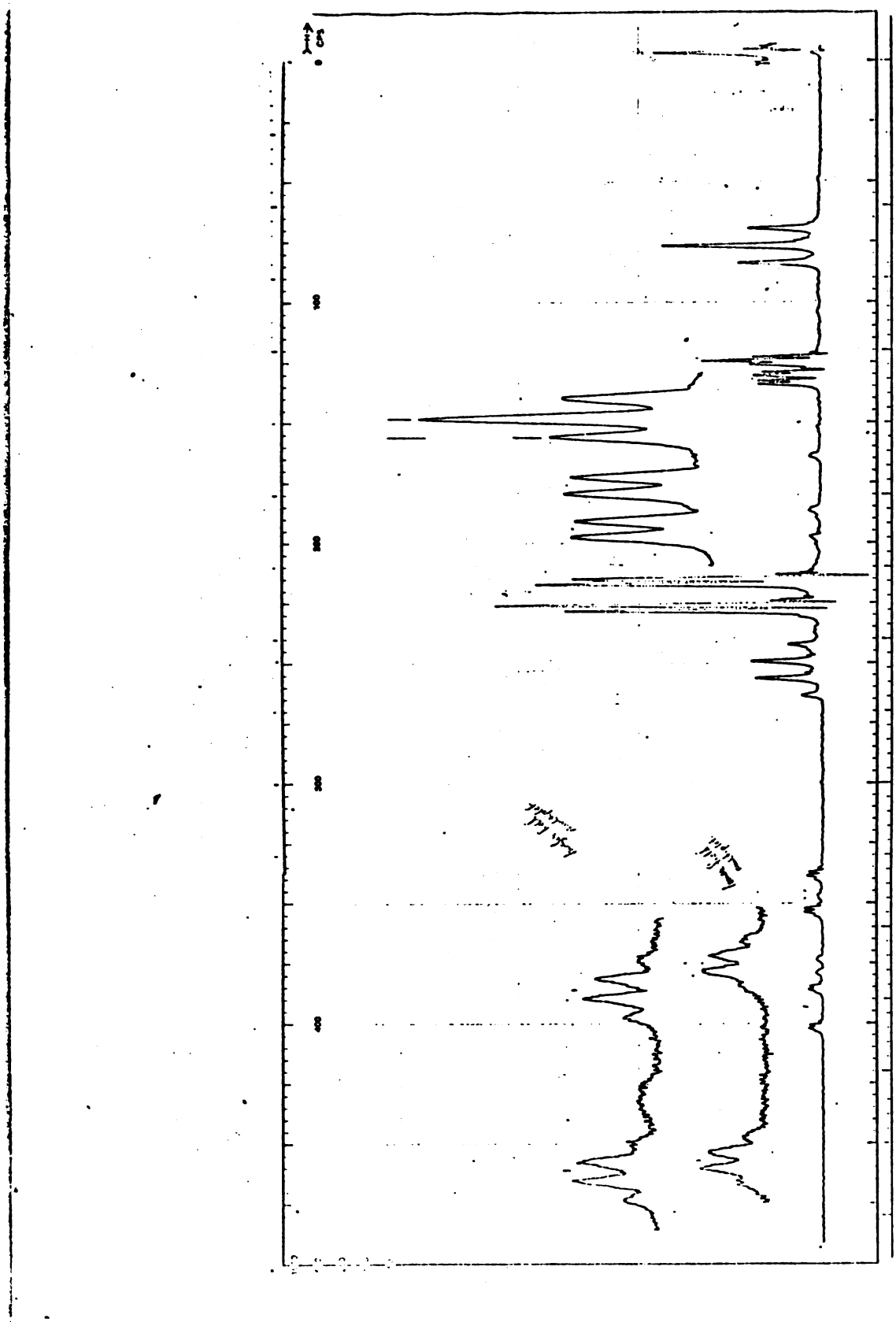
α-(dihydroxyphosphinylmethyl)
ACRYLIC ACID DILITHIUM SALT

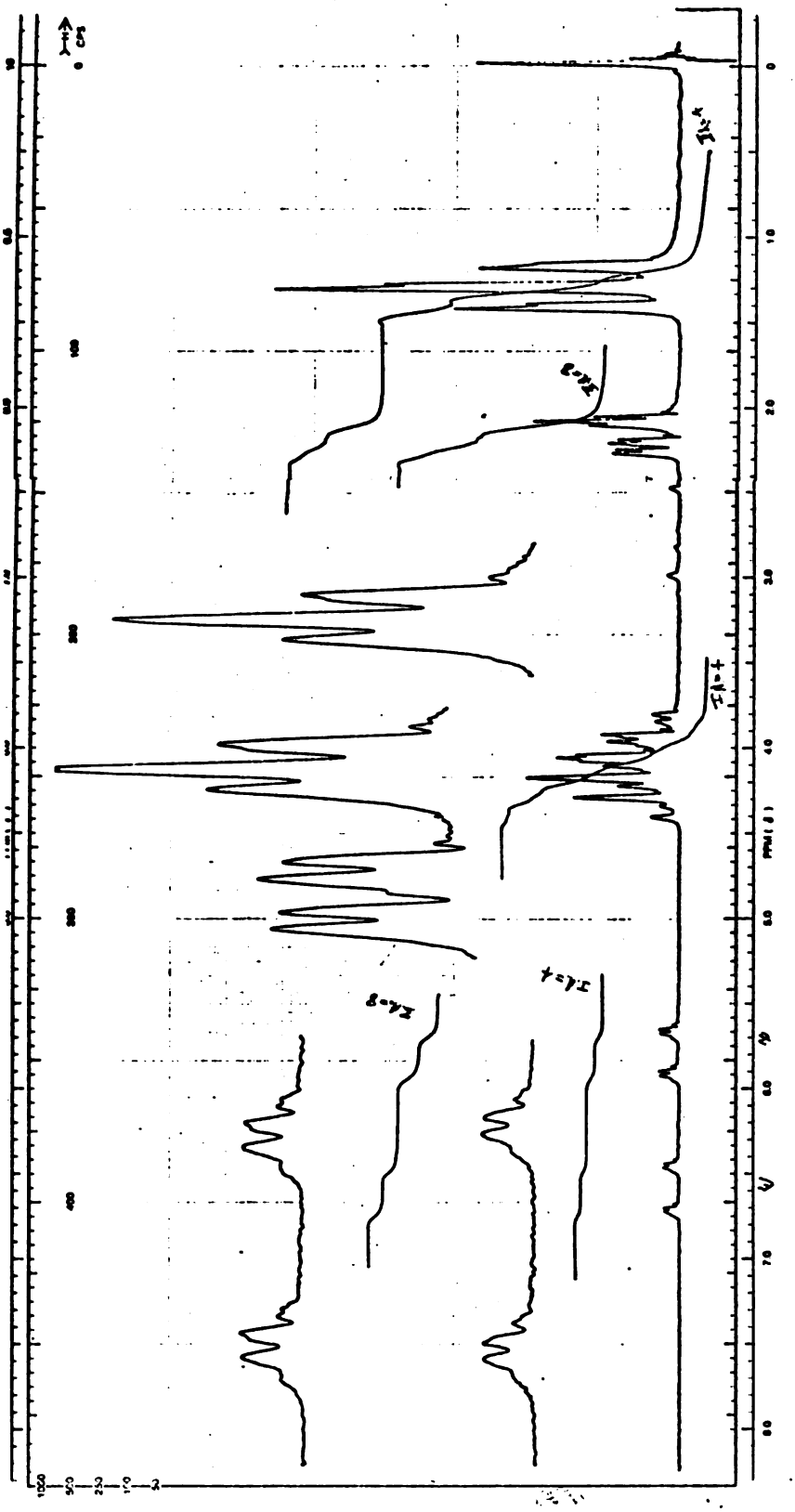


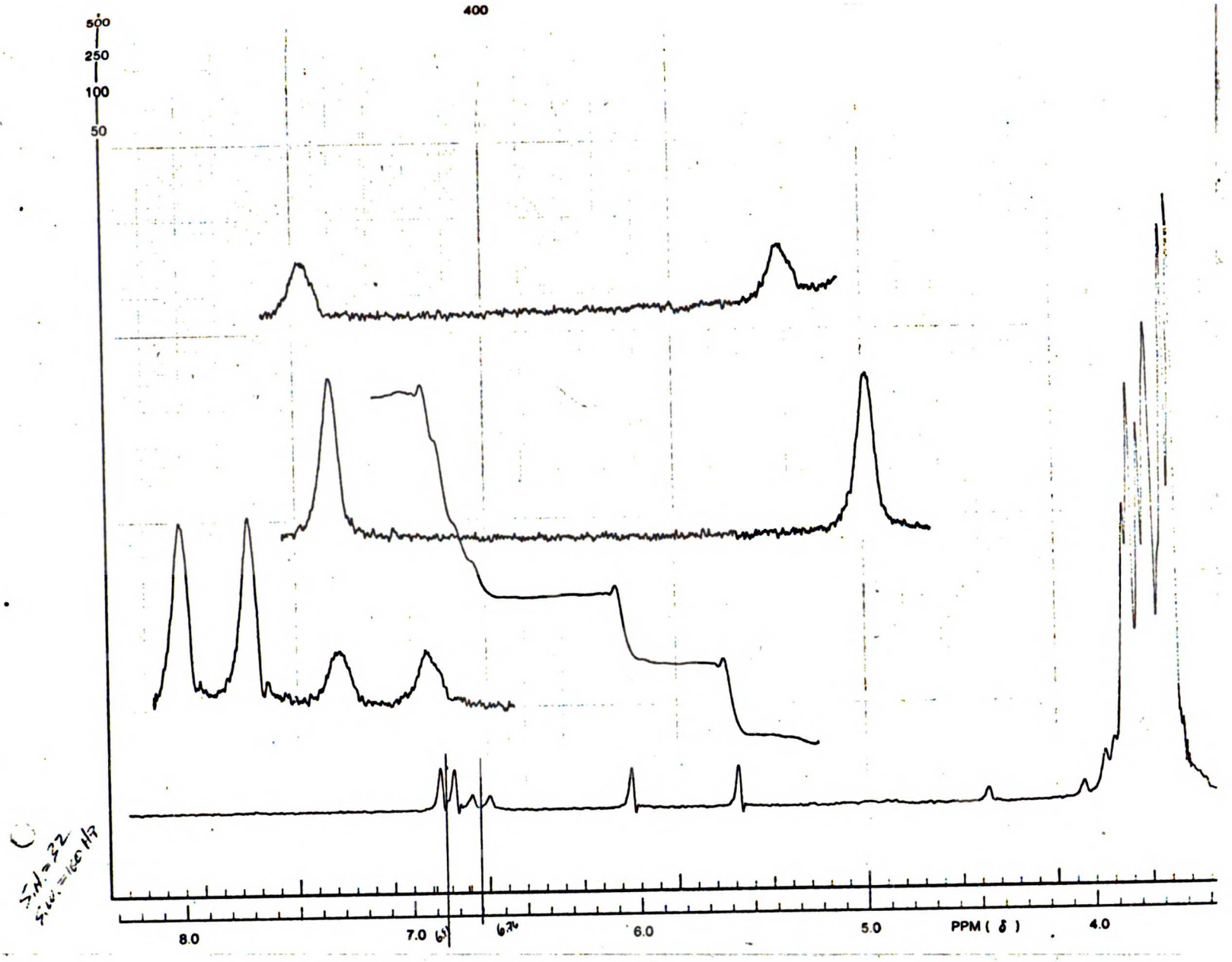


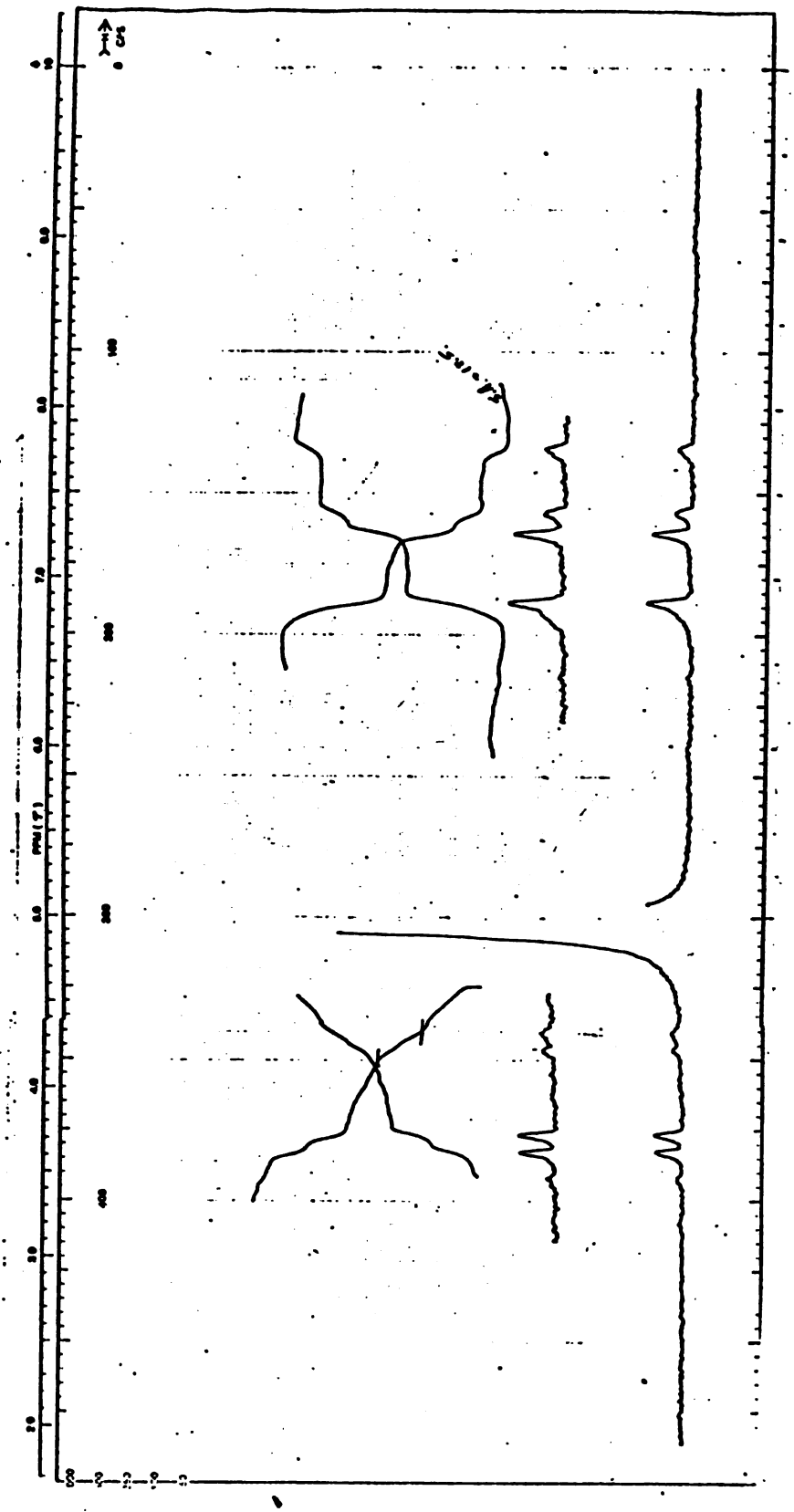
90% ^{13}C-ENRICHED IN THE CARBOXYLATE POSITION

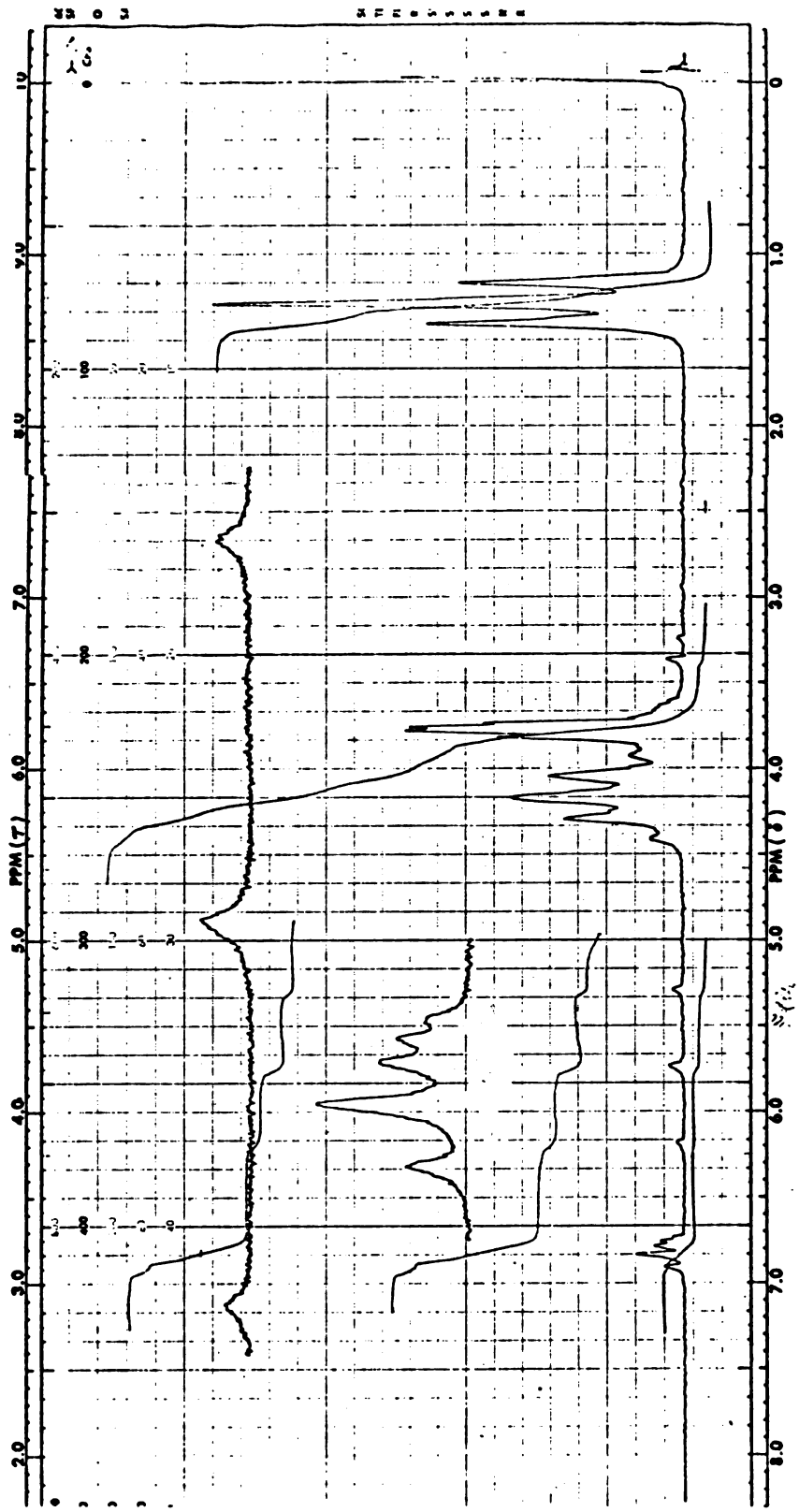


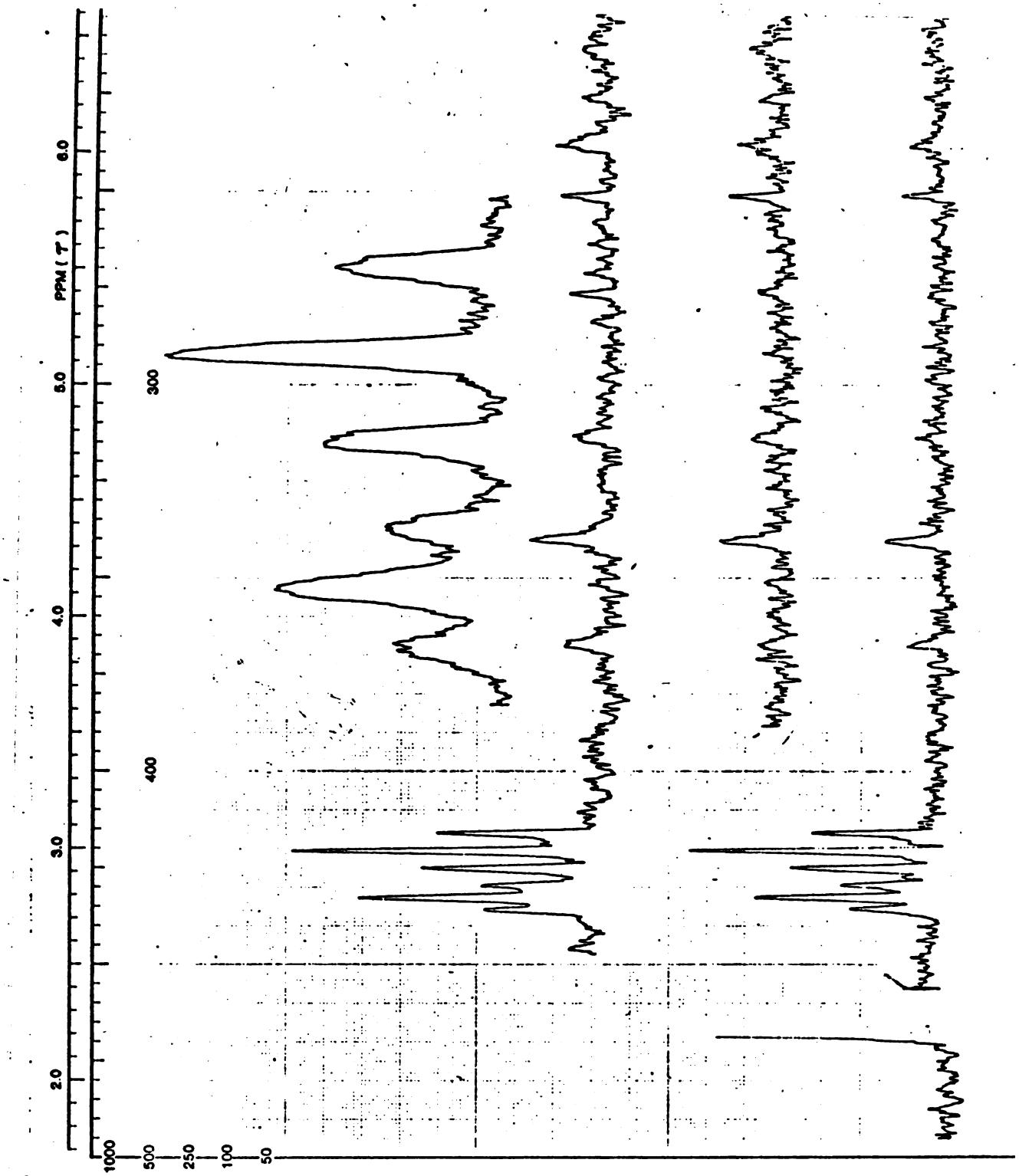


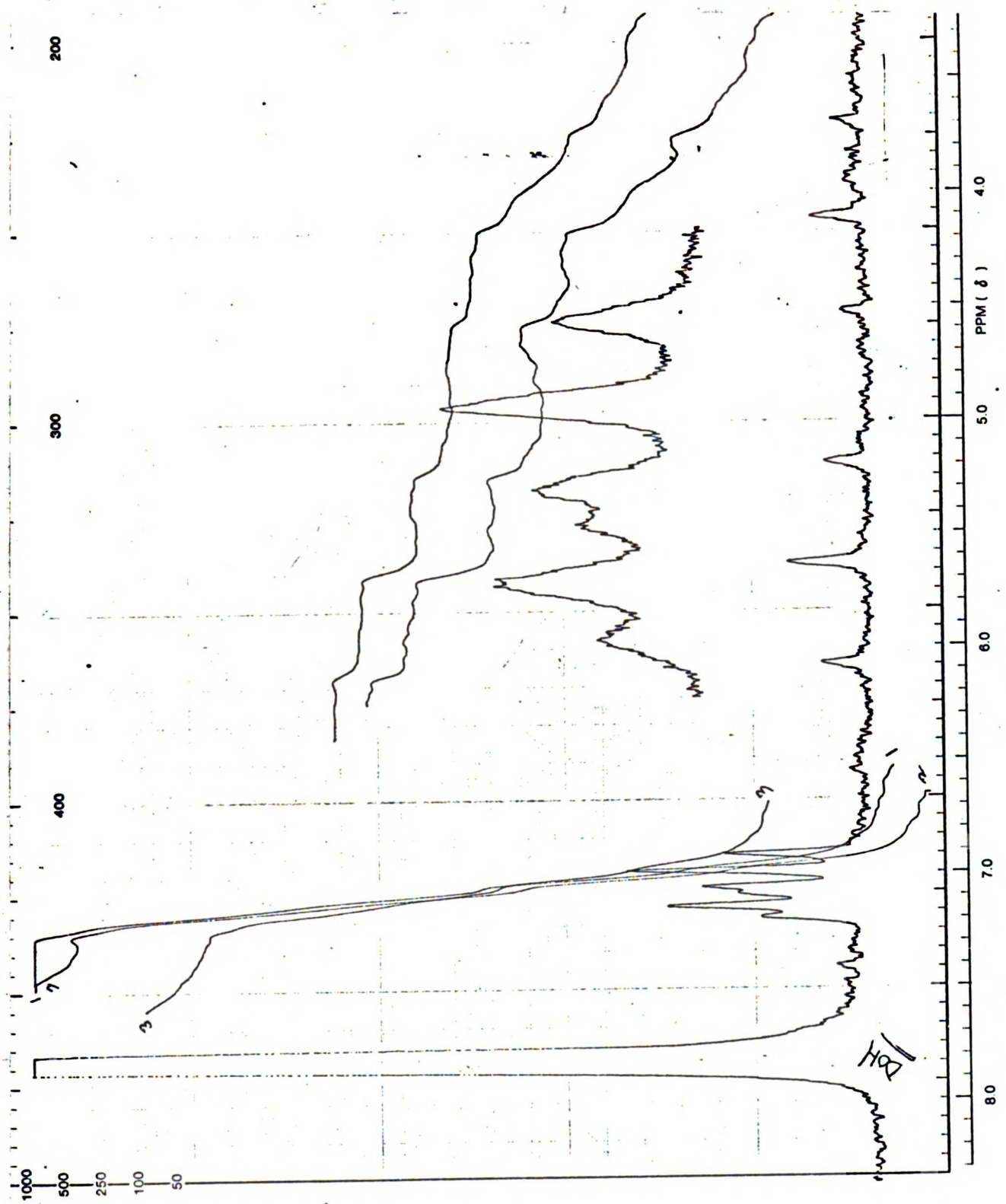

PPM(δ)

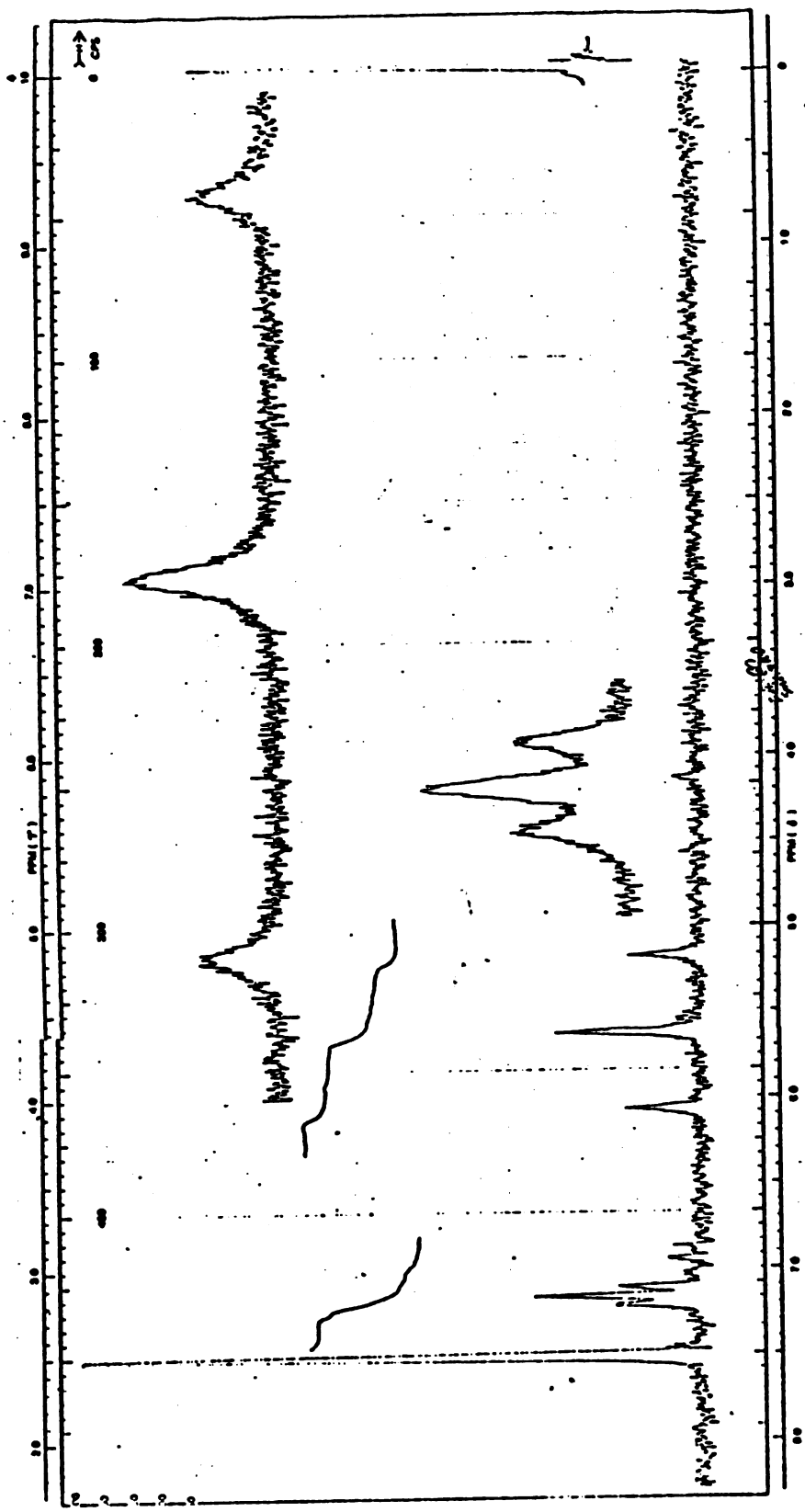

400
500
250
100
50
8.0
7.0
6.0
5.0
PPM (δ)
4.0

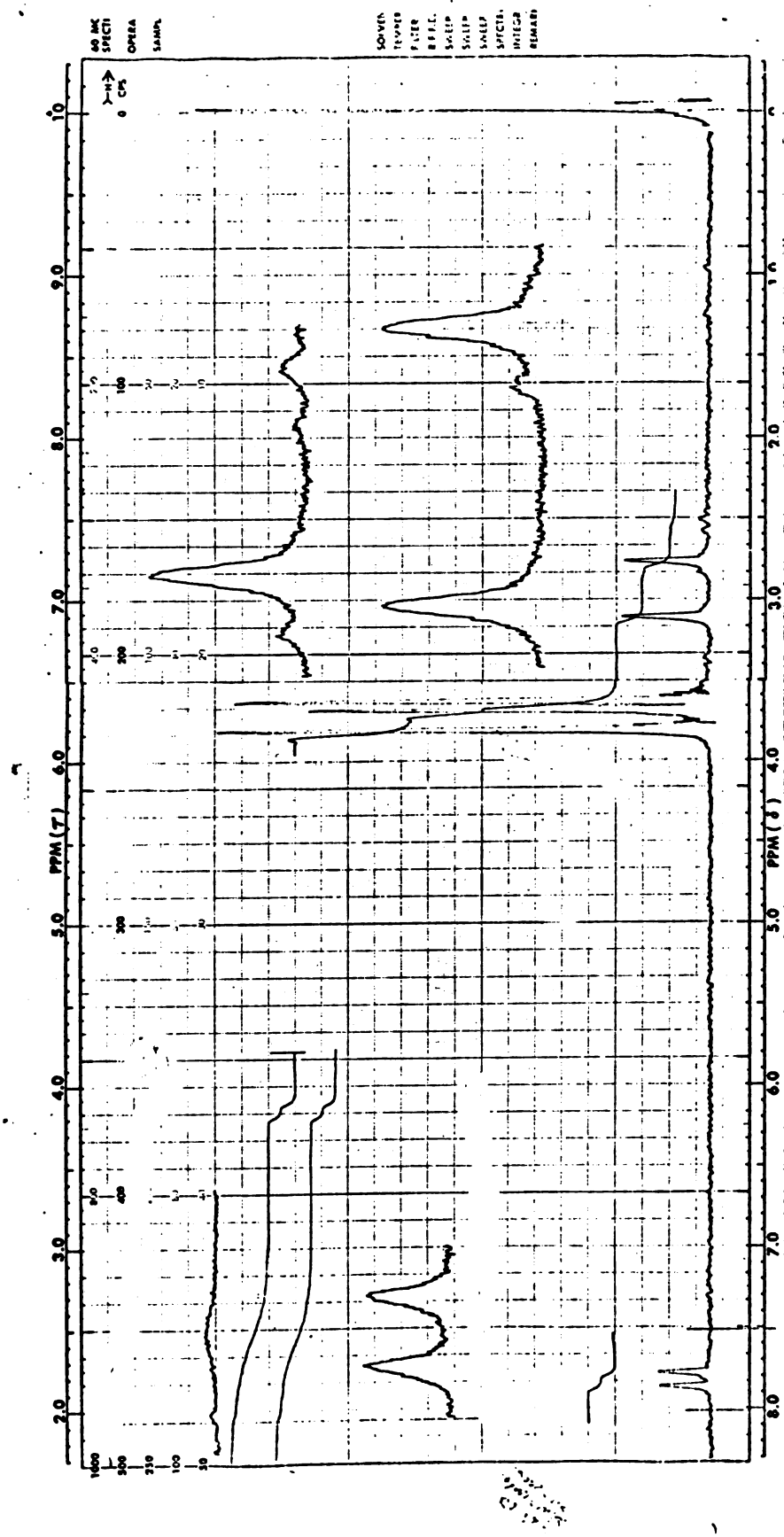


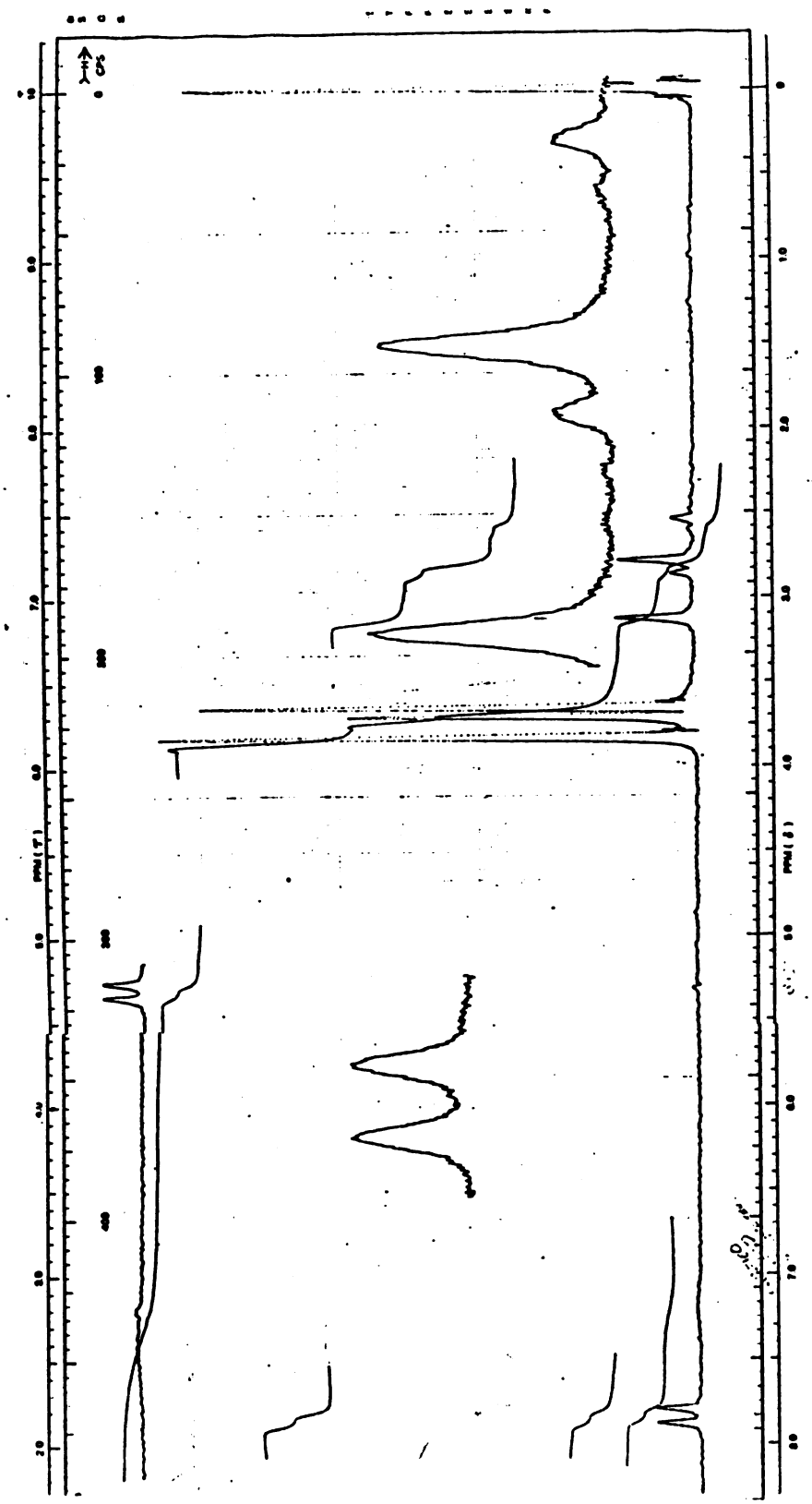

PPM (τ)
PPM (δ)
10
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0

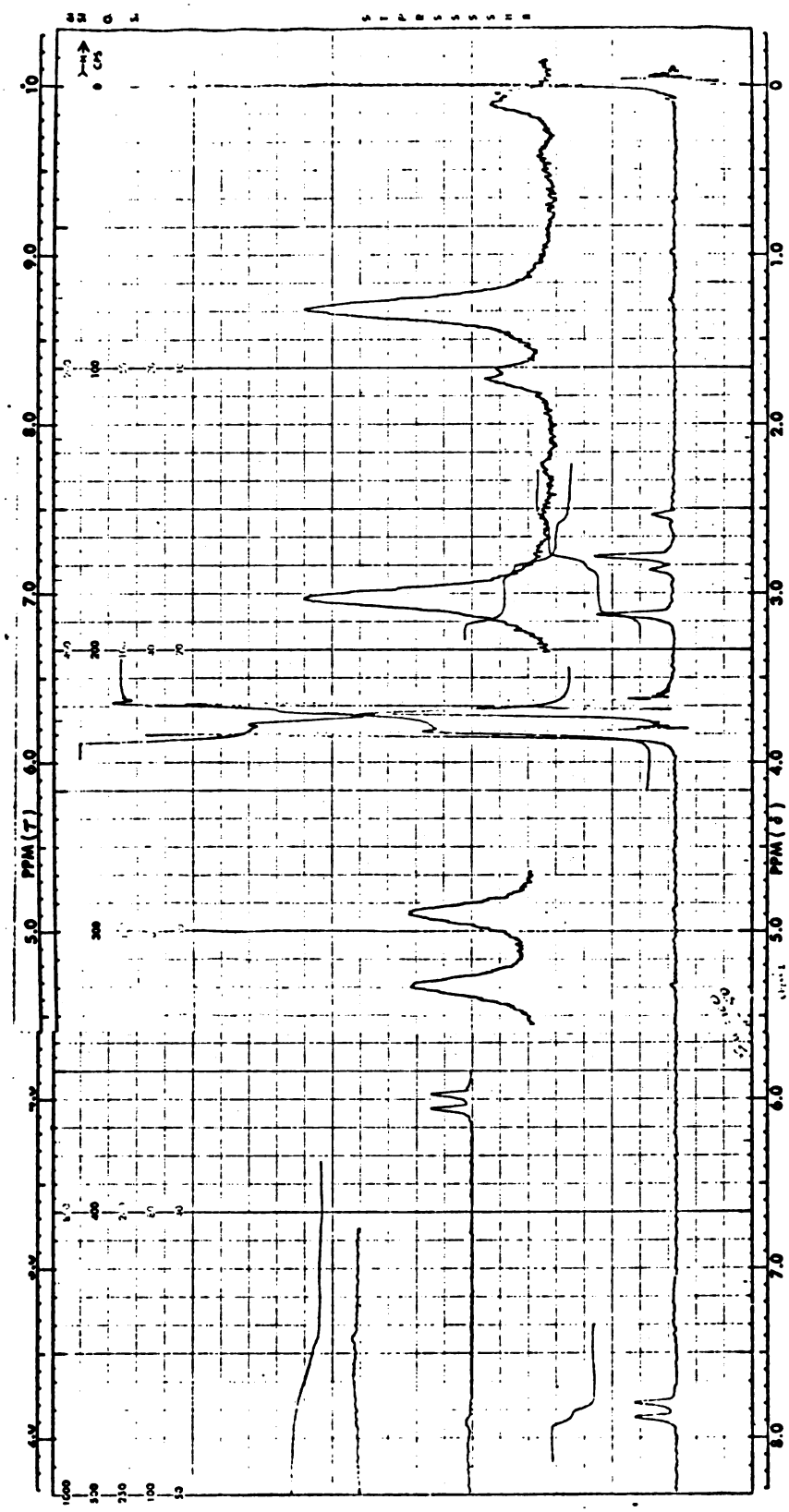

PPM (τ)
8.0
5.0
4.0
3.0
2.0
300
400
1000
500
250
100
50

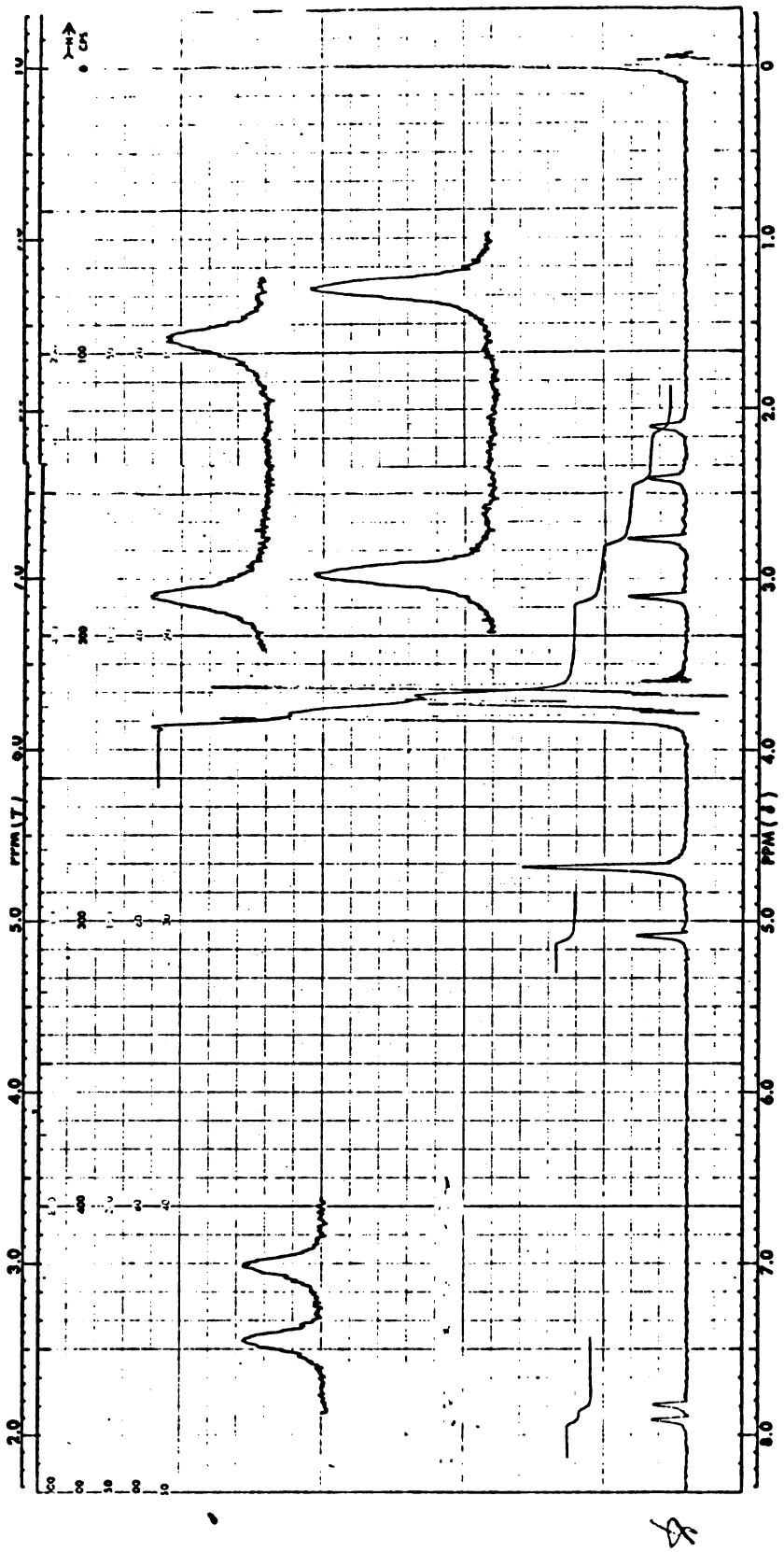

1000
500
250
100
50
200
300
400
PPM (δ)
5.0
6.0
7.0
8.0
4.0
DBH

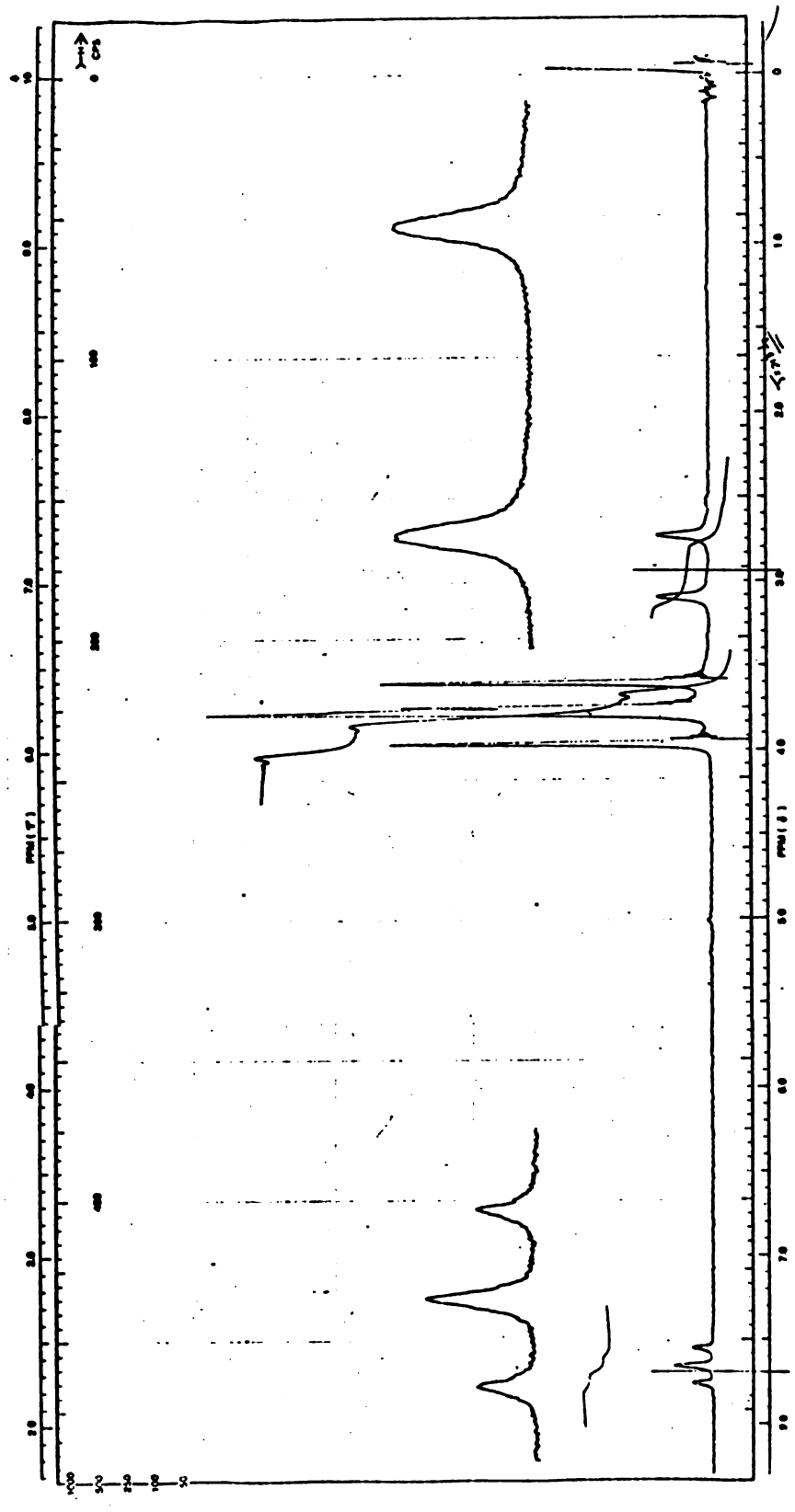


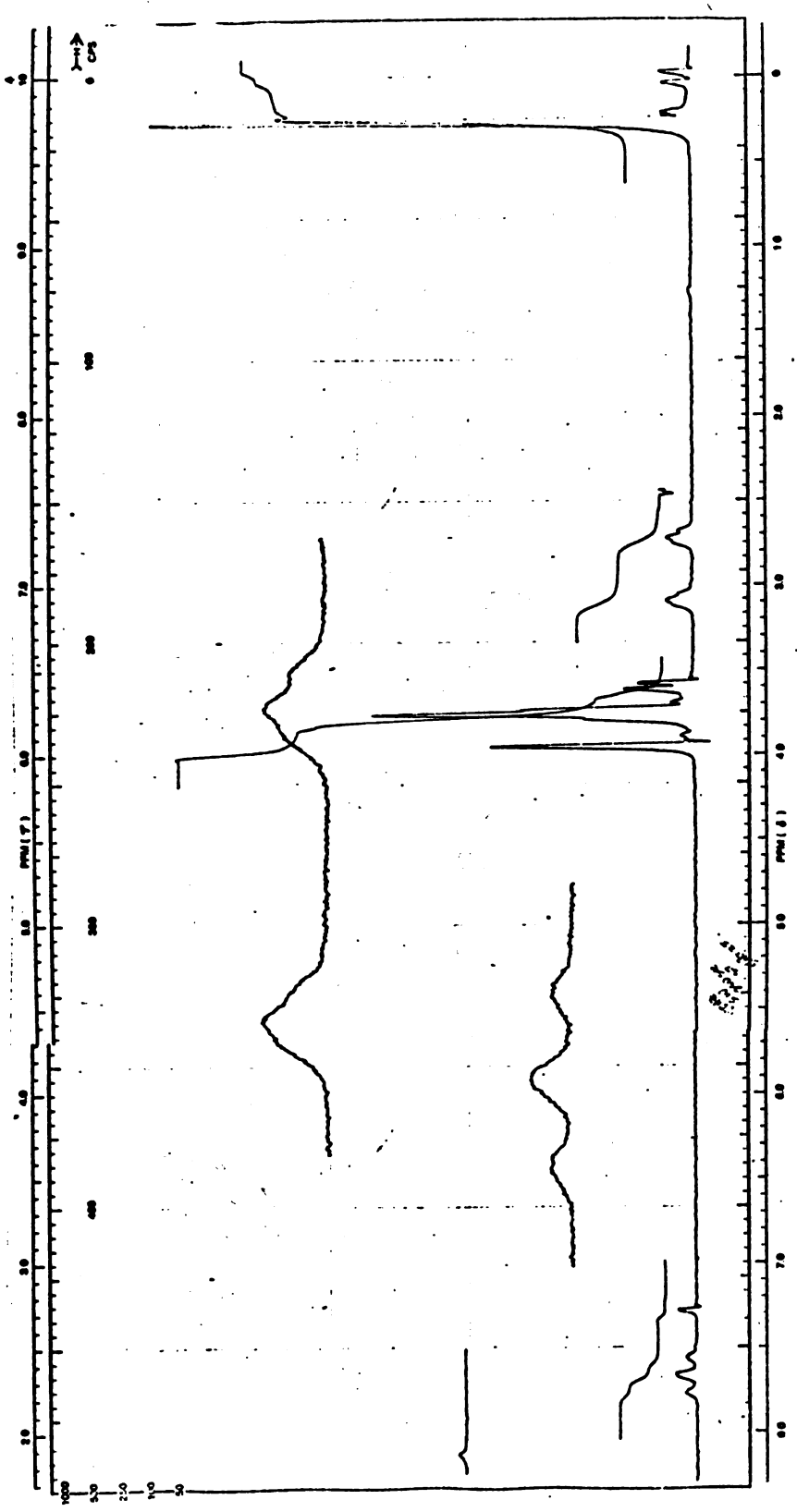

PPM (τ)
PPM (δ)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
CPS

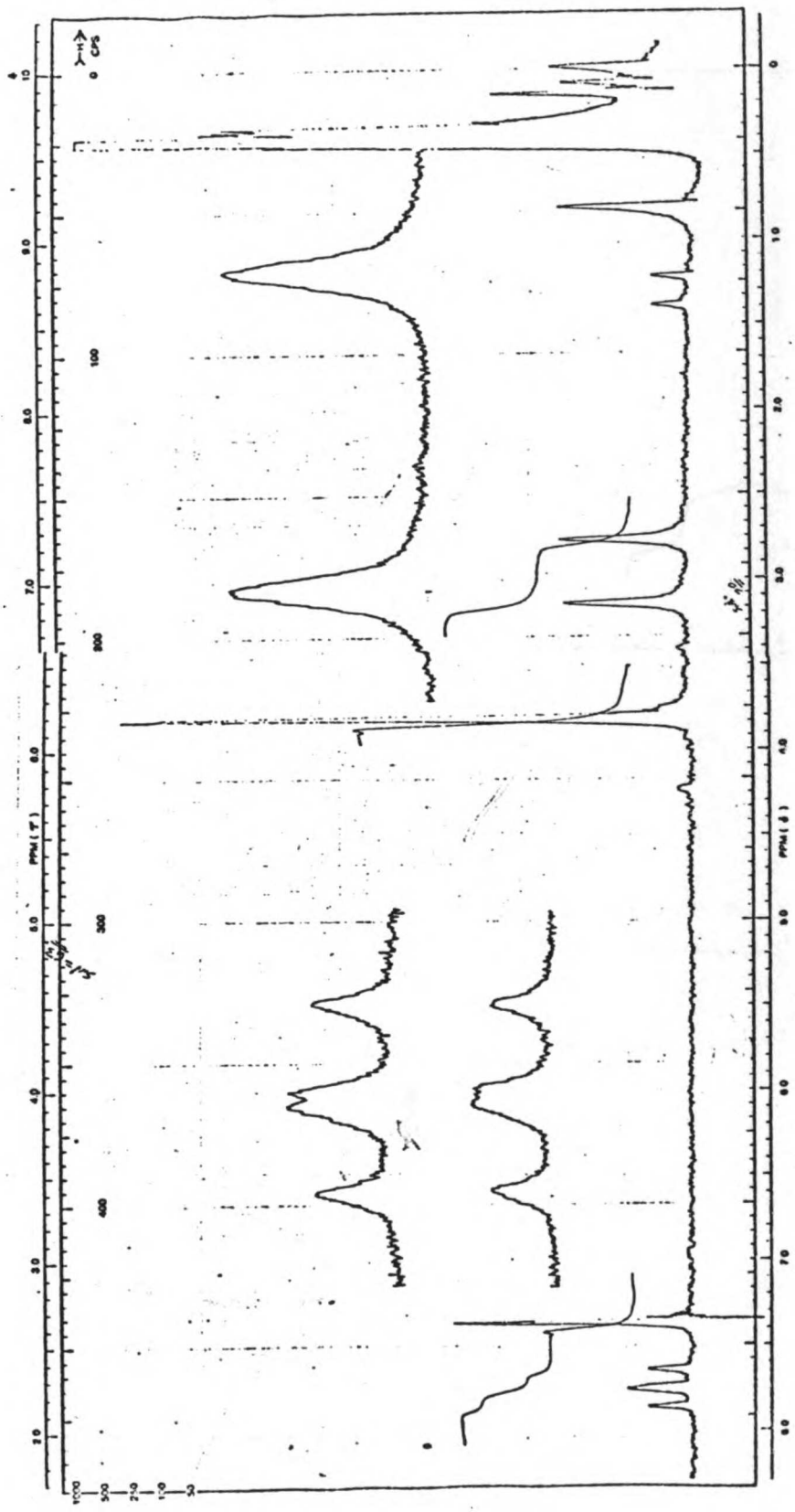


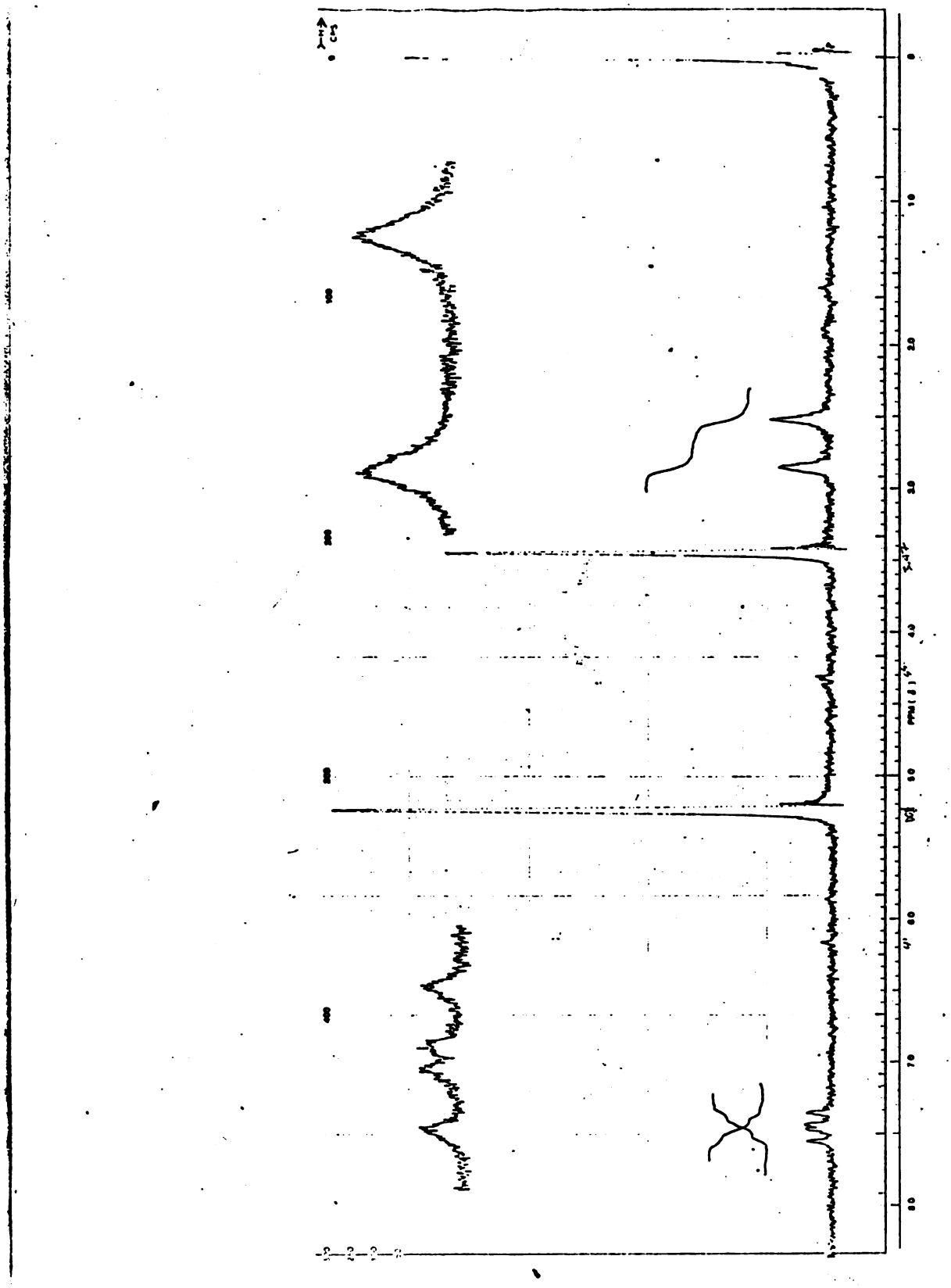

PPM(τ)
PPM(δ)

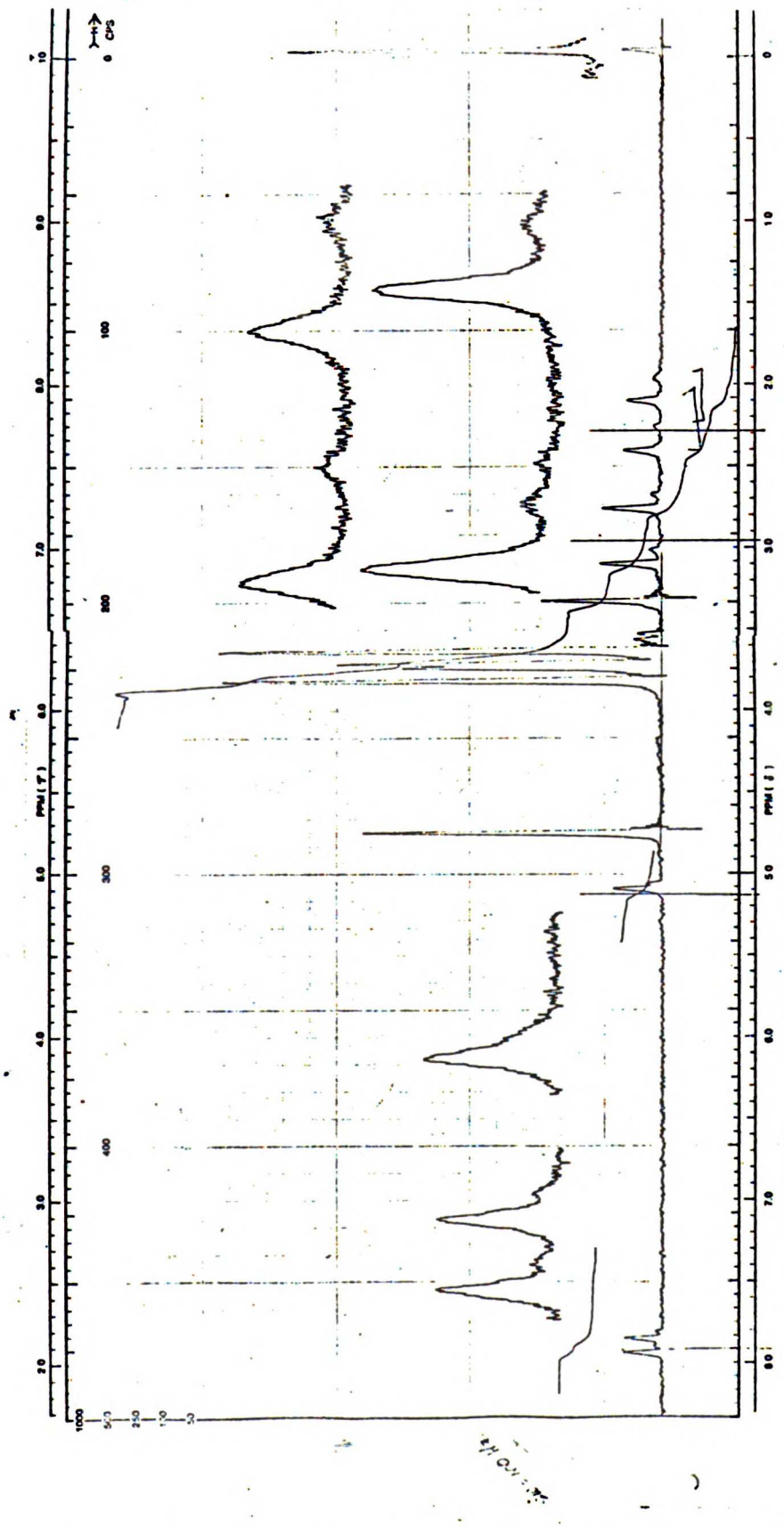

PPM(τ)
PPM(δ)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0

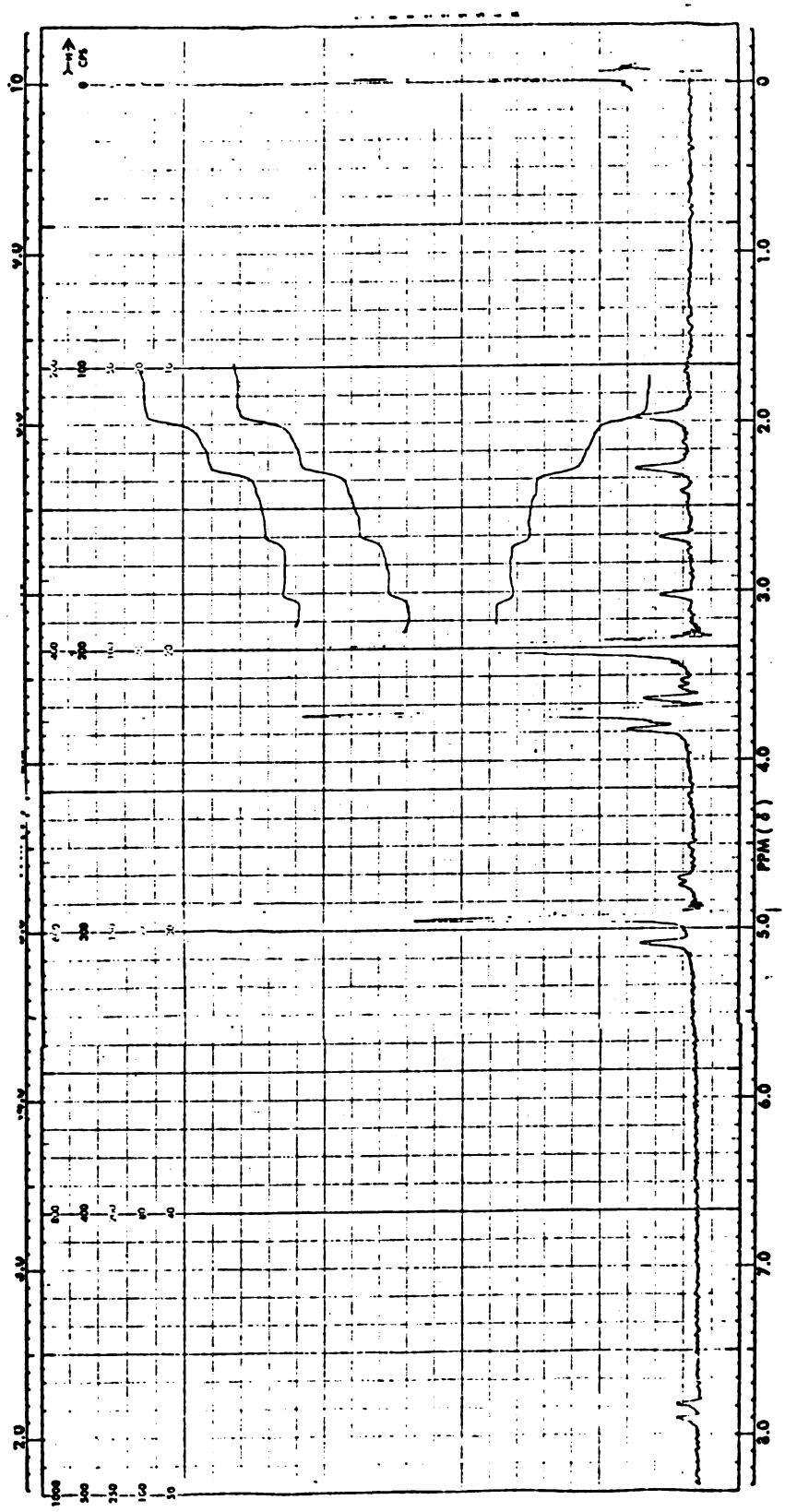


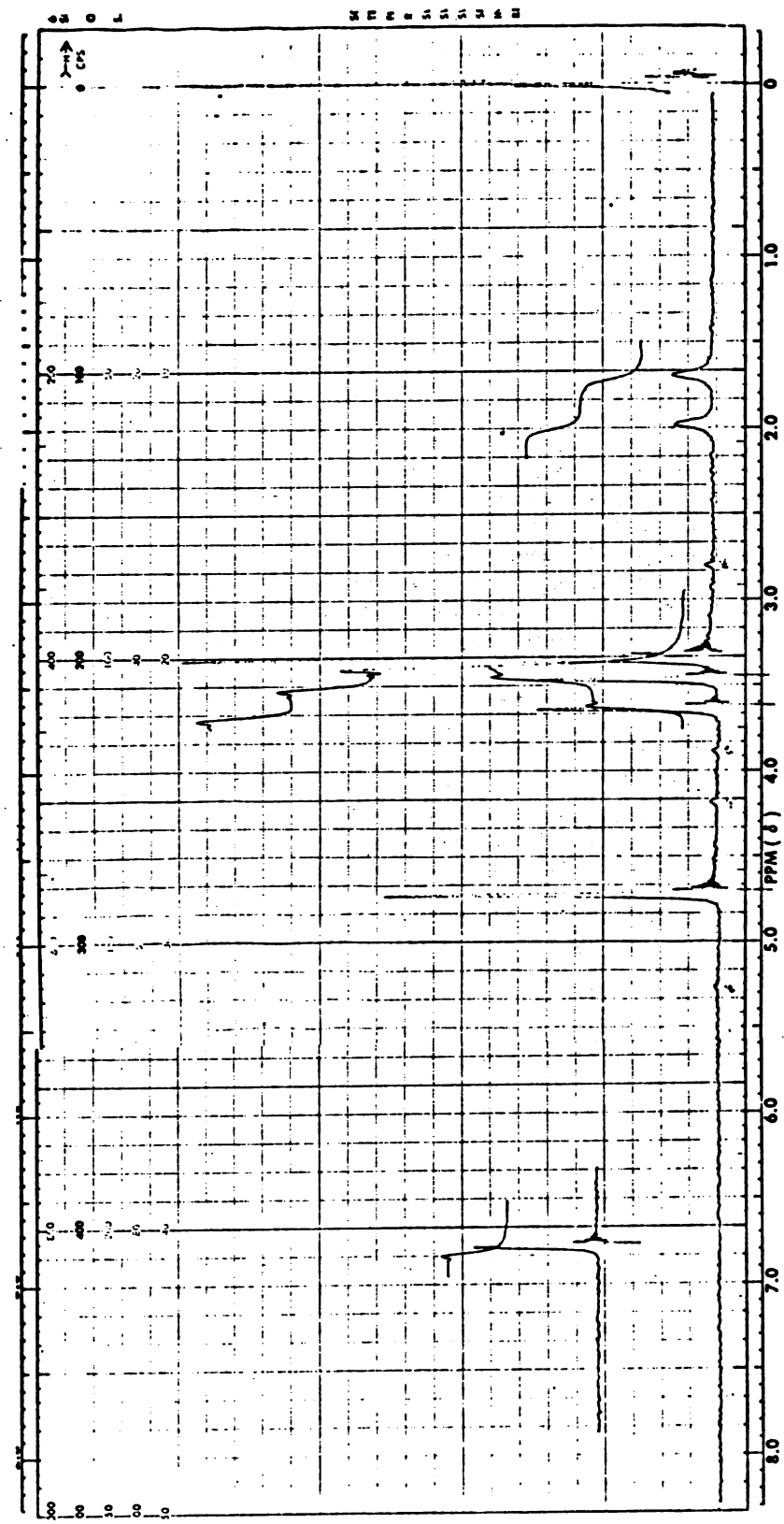


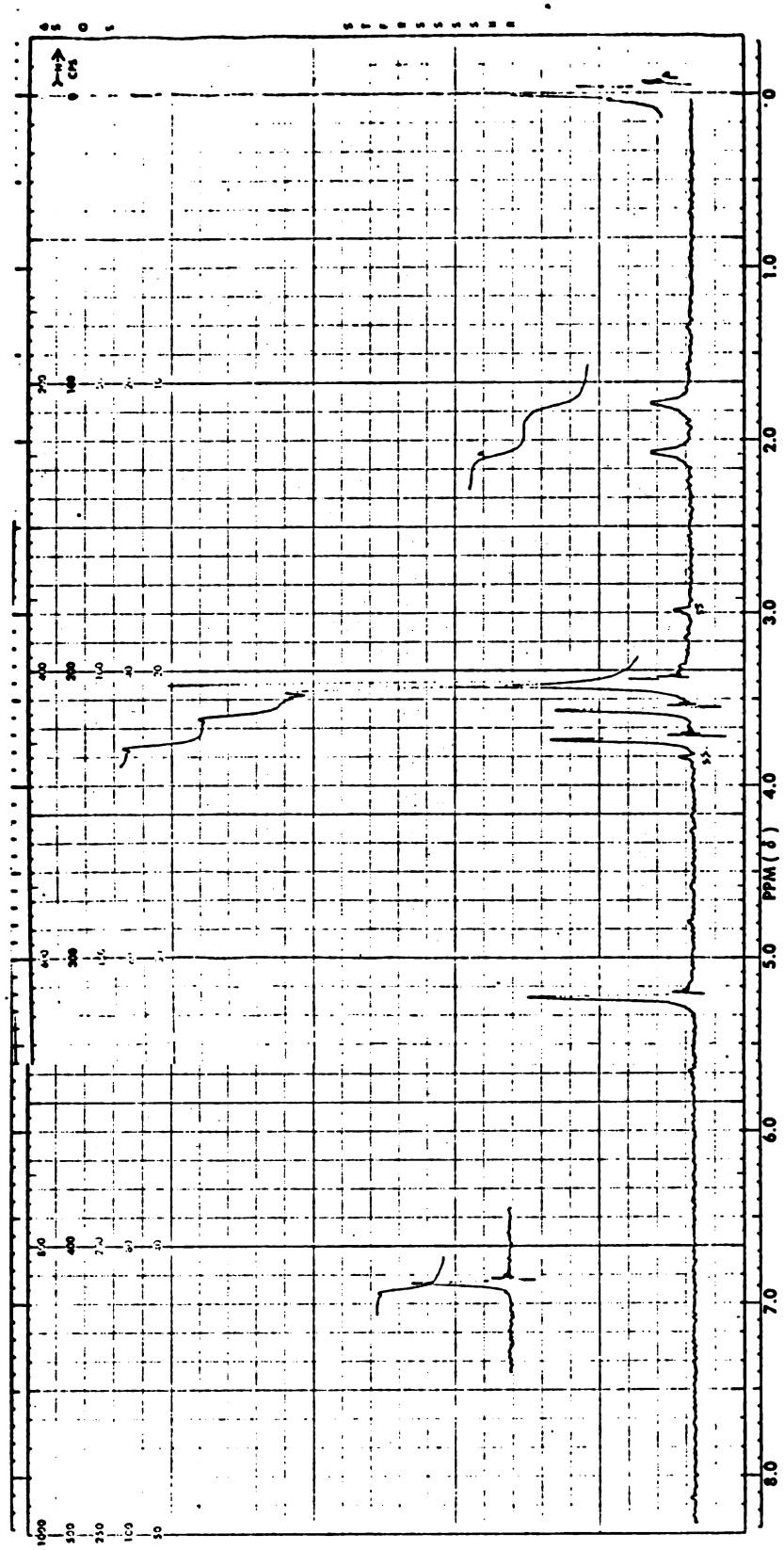


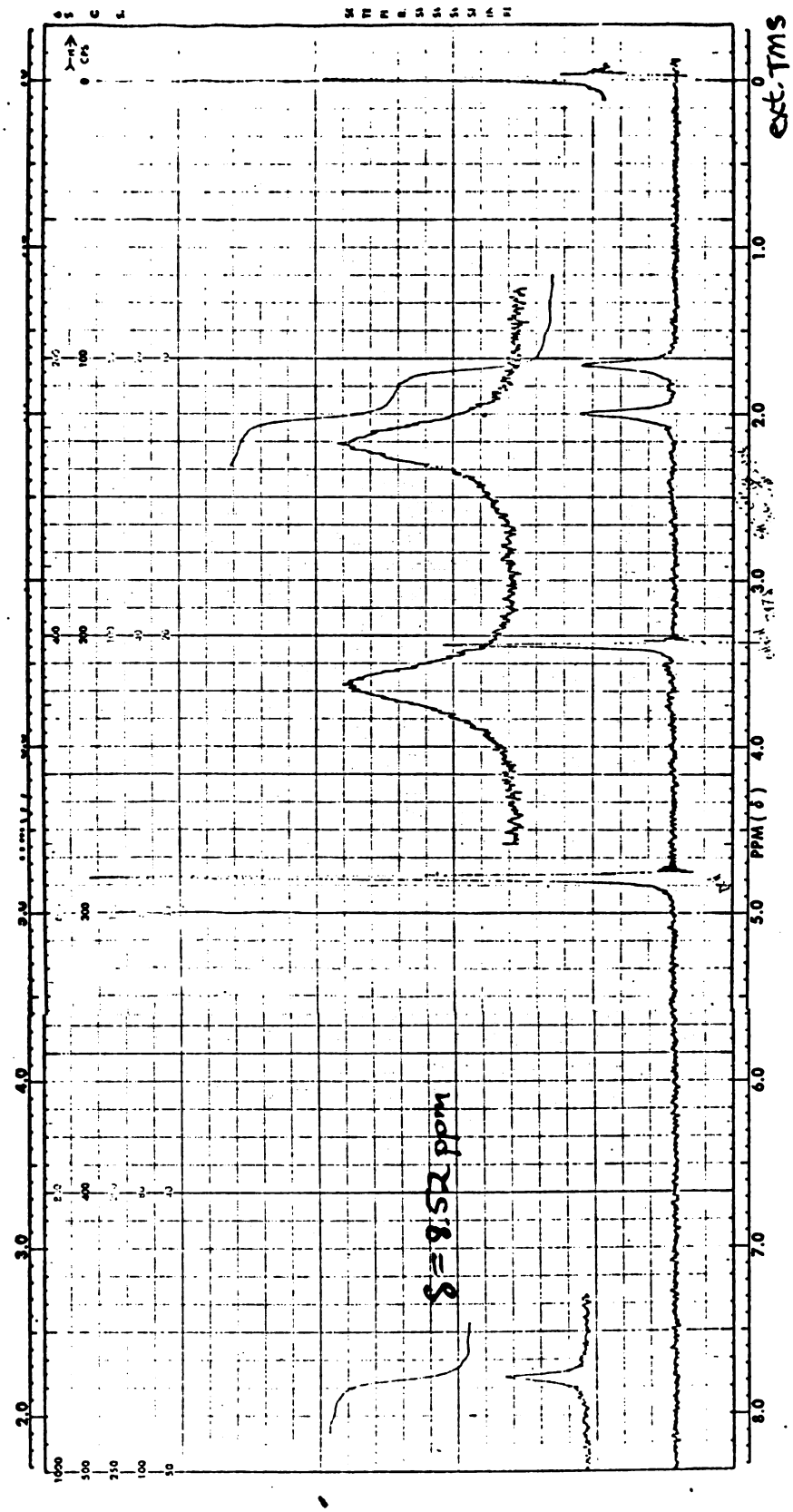


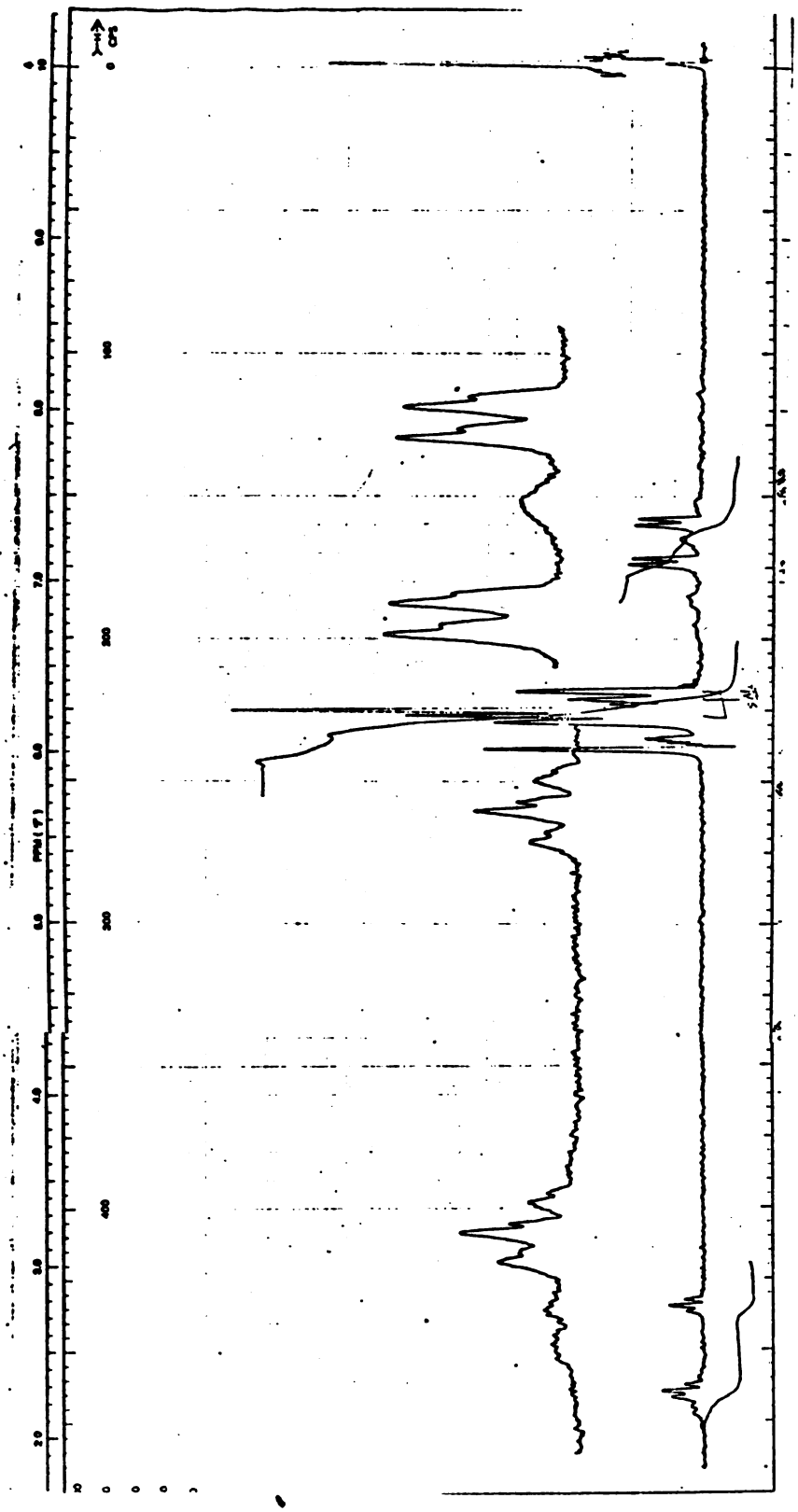


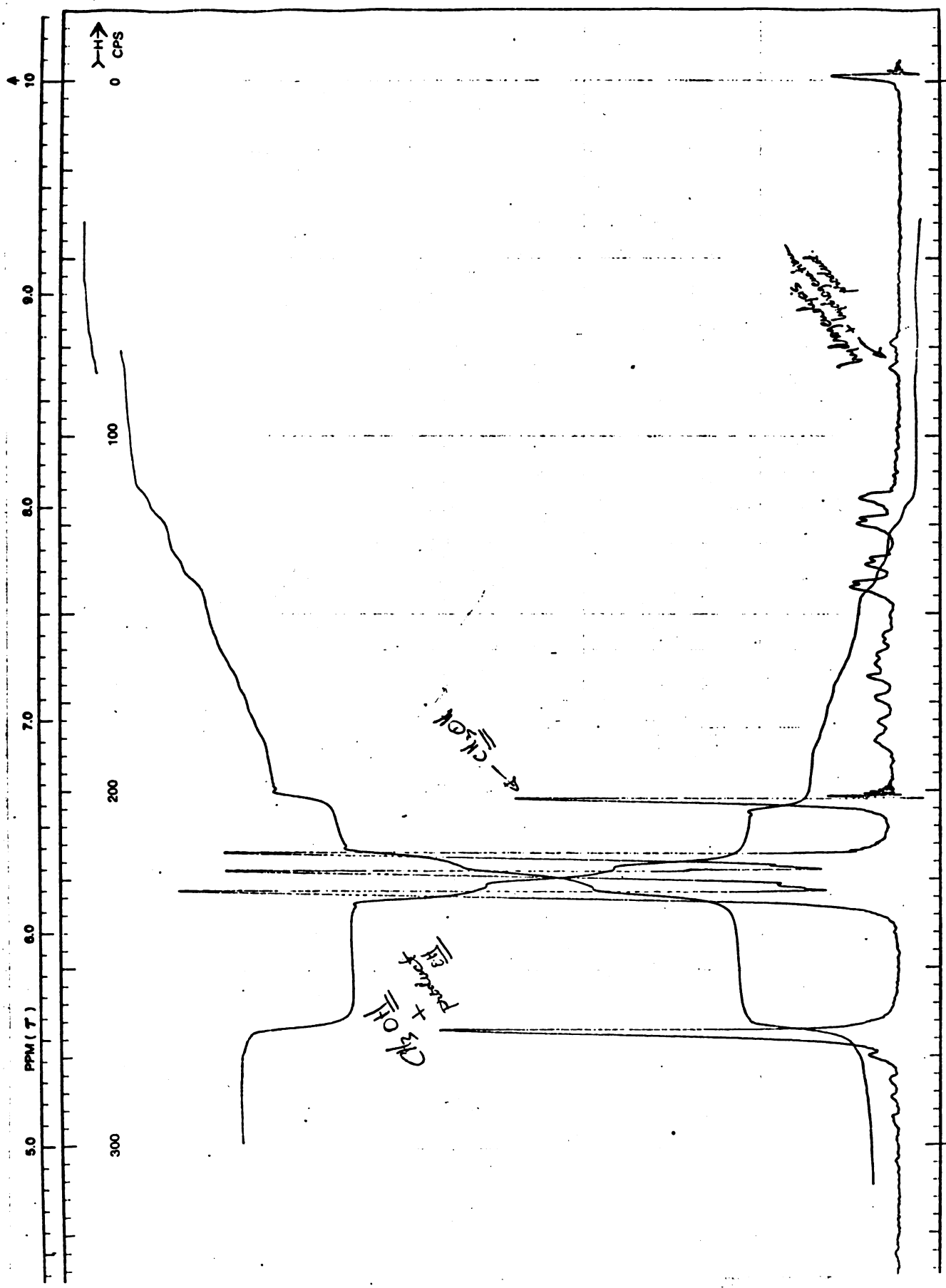

TMS
PPM (δ)

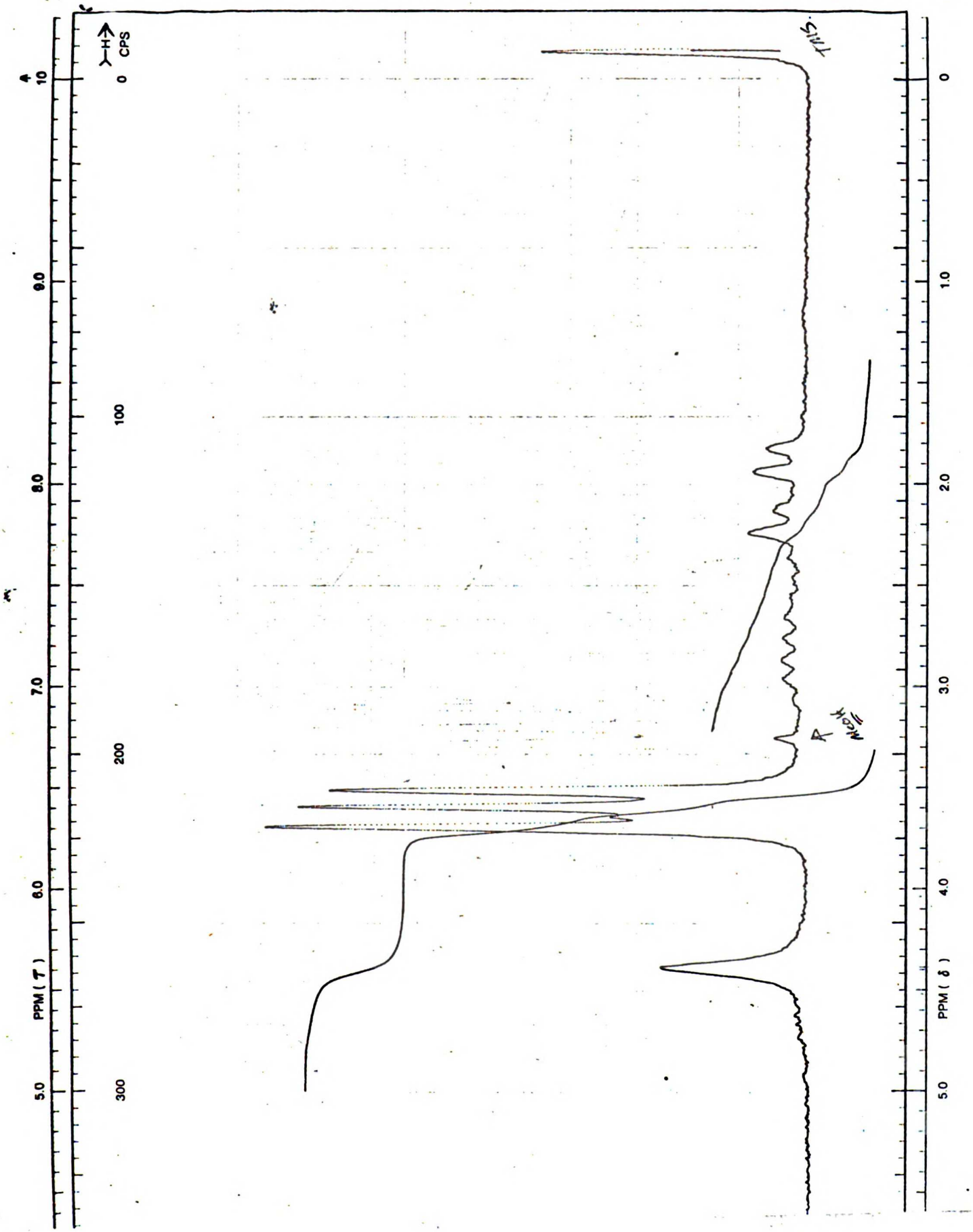

0
1.0
2.0
3.0
4.0
PPM(δ)
5.0
6.0
7.0
8.0

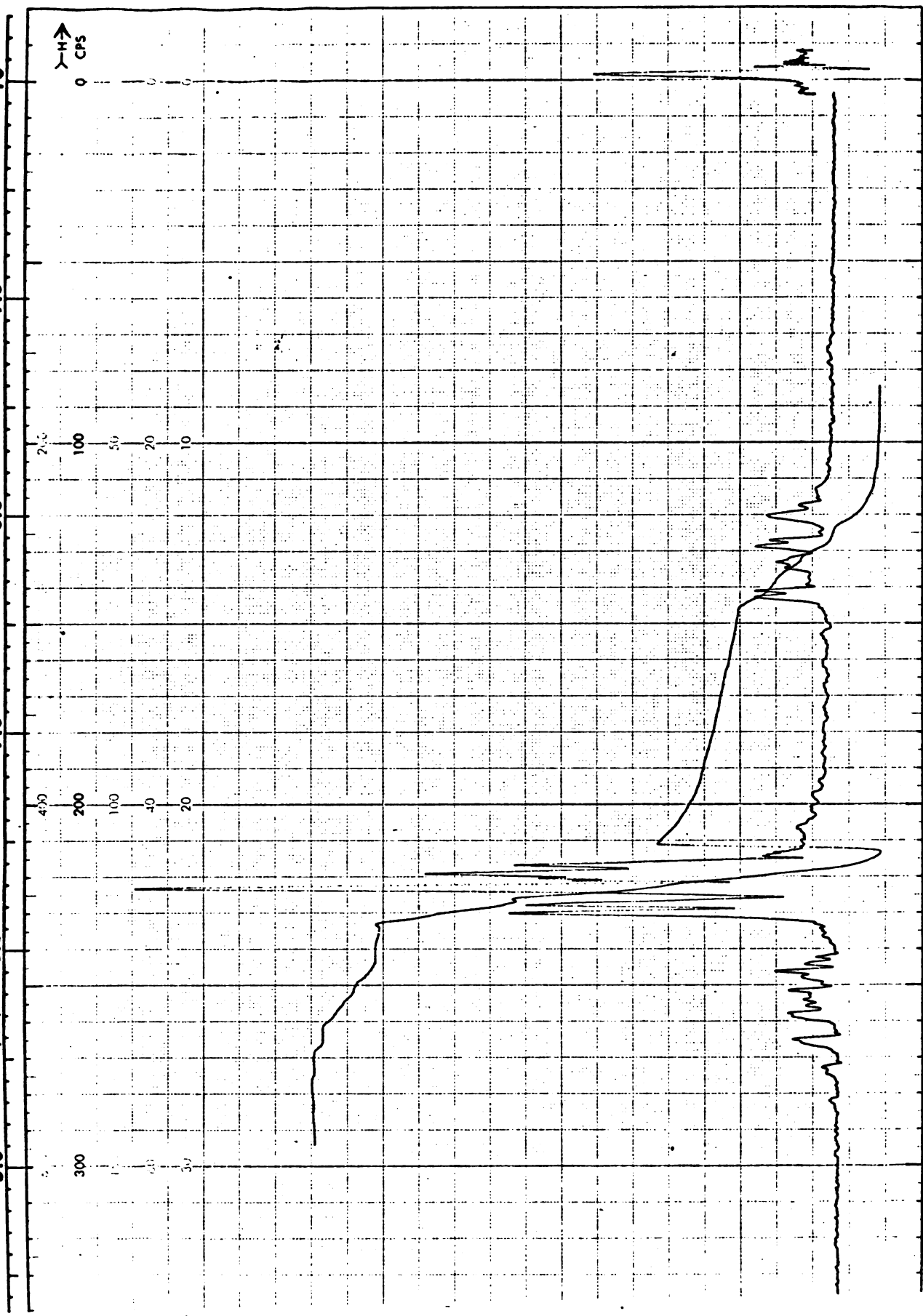

PPM(δ)
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0


ext. TMS
PPM (δ)
δ = 8.52 ppm




H
0 CPS
PPM (τ)
10
9.0
8.0
7.0
6.0
5.0
100
200
300
CH3OH
CH3OH + Benzene


HZ
0 CPS
100
200
300
10
9.0
8.0
7.0
6.0
5.0
PPM (τ)
0
1.0
2.0
3.0
4.0
5.0
PPM (δ)
Silly
R
MeOH


H
CPS
0
10
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ)
100
50
20
10
200
100
40
20
300

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