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THE SYNTHESIS OF PHOSPHONIC ACID AND PHOSPHONOLIPID ISOSTERES OF NATURAL PHOSPHATES

by

DIZA P. BRAKSMAYER

A dissertion submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1977

This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

3 October 1977
date

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Chairman of Examining Committee

October 4, 1977
date

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Supervisory Committee

The City University of New York

ABSTRACT

THE SYNTHESIS OF PHOSPHONIC ACID AND PHOSPHONOLIPID ISOSTERES OF NATURAL PHOSPHATES

by

DIZA P.BRAKSMAYER

Advisor: Professor Robert Engel

Over the years analogues of natural substrates have been used in enzyme -catalyzed reactions as probes for the elucidation of factors controlling the enzyme-substrate interactions. However, analogues can also have profound influence upon cell metabolism. Such considerations resulted in the study of analogues of natural organic phosphates with their potential to serve as metabolic regulators and chemotherapeutic agents.

The carbon-phosphorus bond is not hydrolyzed by the enzymes commonly involved in cleavage of phosphate esters. This fact suggested several approaches for metabolic regulation or modification by compounds bearing such a linkage. Ideally, the general design of the phosphate analogues would bear only one structural variation from the parent compound. The structural variation considered in the present work is the substitution of a methylene group for the esteric oxygen in a phosphate ester linkage.

The preparation of 4-hydroxy-3-oxobutyl-1-phosphonic acid, the isosteric analogue of dihydroxyacetone phosphate, was under-

taken as an alternative synthesis approaching the isosteric analogue of glycerol-3-phosphate, 3,4-dihydroxybutyl-1-phosphonic acid, allowing for tritium labelling at the C-3 atom. The biological investigations based on this radioactive analogue revealed a perturbation of the normal phospholipid production and distribution, and futhermore, its incorporation into the phosphonic acid analogue of phosphatidylglycerophosphate by cultures of Escherichia coli and Bacillus subtilis.

In view of the effect that this analogue had on bacterial phosphoglyceride metabolism, the synthesis of the following phosphonolipids was attempted and accomplished: 3,4-dipalmitoyl-oxybutylphosphonylcholine, the isosteric analogue of lecithin; 3,4-dipalmitoyloxybutylphosphonylethanolamine, the isosteric analogue of cephalin; and 3,4-dipalmitoyloxybutylphosphonyl-glycerol, the isosteric analogue of phosphatidylglycerol. Attempts were also made toward the synthesis of (1,2-dipalmitoyl)-glyceryl--4'-phosphoryloxy-3'-hydroxybutyl-1'-phosphonic acid, a new phosphonolipid derivative formed through the interaction of the radioactive isosteric analogues of glycerol-3-phosphate with enzymes involved in phosphoglyceride metabolism. The preparation of diphenyl and dibenzyl phosphonates was attempted as intermediates in a converging synthesis of more complex phosphate analogues. For greater solubility in aqueous systems for biological investigations, an attempt was made on the synthesis of 3,4-dicaproyloxybutyl-1--phosphonic acid, an isosteric analogue of phosphatidic acid.

ACKNOWLEDGEMENTS

I sincerely thank Professor Robert Engel who was my teacher in the beginning, then my advisor and now a friend and colleague. His patience, understanding and unwavering confidence in me made this thesis a reality.

I am grateful to the people whom I have met during this program of study and appreciate their help. With time many of these acquaintances became close friends. But the completion of this thesis work does not mark the end of our friendships that have since developed.

Special thanks to the City University of New York for financial support.

TABLE OF CONTENTS

Portions of this thesis have been published in:

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TO MY FATHER,

memoria in aeterna

INTRODUCTION

During the past fifteen years significant attention has been directed toward the preparation and investigation of phosphonic acids which might be regarded as analogues of naturally occurring phosphates. This interest has developed from an appreciation that phosphonic acids and their esters possess potential as metabolic regulators or modifiers when used in place of naturally occurring phosphates.

The fact that the carbon-phosphorus bond is incapable of being hydrolyzed by the enzymes commonly involved in cleavage of phosphate esters indicated several routes of practicability for metabolic regulation by compounds bearing such a linkage in place of the usual phosphate ester linkage. For example, the use of a phosphonic acid analogue, in place of a natural phosphate which acts as a metabolic regulator using enzymatic reactions at sites distant from the phosphate ester linkage, but by other extraneous routes is hydrolyzed to inorganic phosphate, might be expected to result in a net enhancement of regulatory activity. Likewise, a phosphonic acid or a phosphonate ester, substituting for a natural phosphate metabolite in which the enzymatic site is near the phosphate linkage may be capable of inhibiting or perturbing the normal metabolic pathways of an organism simply by non-participation in a normal phosphate cleavage process. (It should be noted that the presence of the carbon-phosphorus linkage does not preclude enzymatic cleavage of phosphorus--ester linkages also present.)[1]

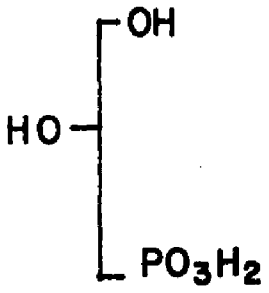
To summarize, the use of phosphonic acid analogues of natural orgainic phosphates as probes into basic metabolic problems and as chemotherapeutic agents represents a systematic approach to metabolic regulation. Ideally, in the design of an analogue of a natural metabolite, it would be desired that it would bear only one structural variation from the parent compound. The structural variation considered in the present work is the substitution of a methylene group in place of the esteric oxygen atom in a phosphate ester linkage. Isosteric analogues strictly refer to compounds of identical size and shape. It should be noted that crystallographic data obtained on the compounds 2-aminoethyl phosphate[2,3] and 2-amincethylphosphonic acid,[4] closely related species, indicated that the distances between the phosphoryl oxygen and some other position varies only by about 0.8% for a phosphate in comparison to its nominally isosteric phosphoric acid analogue. Although the systems involved in this thesis do not meet most rigorously the conditions for isosteric analogues, the bond angles and lengths involved are similar enough, in light of the afore mentioned crystallographic data, that the term "isosteric" may reasonably be applied to those compounds where a methylene group substitutes for the esteric oxygen atom of a phosphate ester linkage.

HISTORICAL

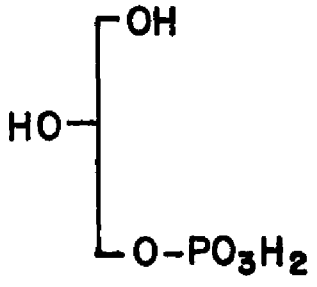
Analogues of Phosphate Products of Glycolysis

A multitude of reports has shown regard for the possibilities for use of phosphonic acids as metabolic regulators when used as substitutes for natural phosphates. Both isosteric and non-isosteric analogues for all simple phosphorus-containing products of carbohydrate degradation have been synthesized and investigated.

Rosenthal and Geyer[5] were the first to report synthetic work on this topic with the preparation of 2,3-dihydroxypropyl-1-phosphonic acid (I). This non-isosteric analogue of glycerol-3-phosphate

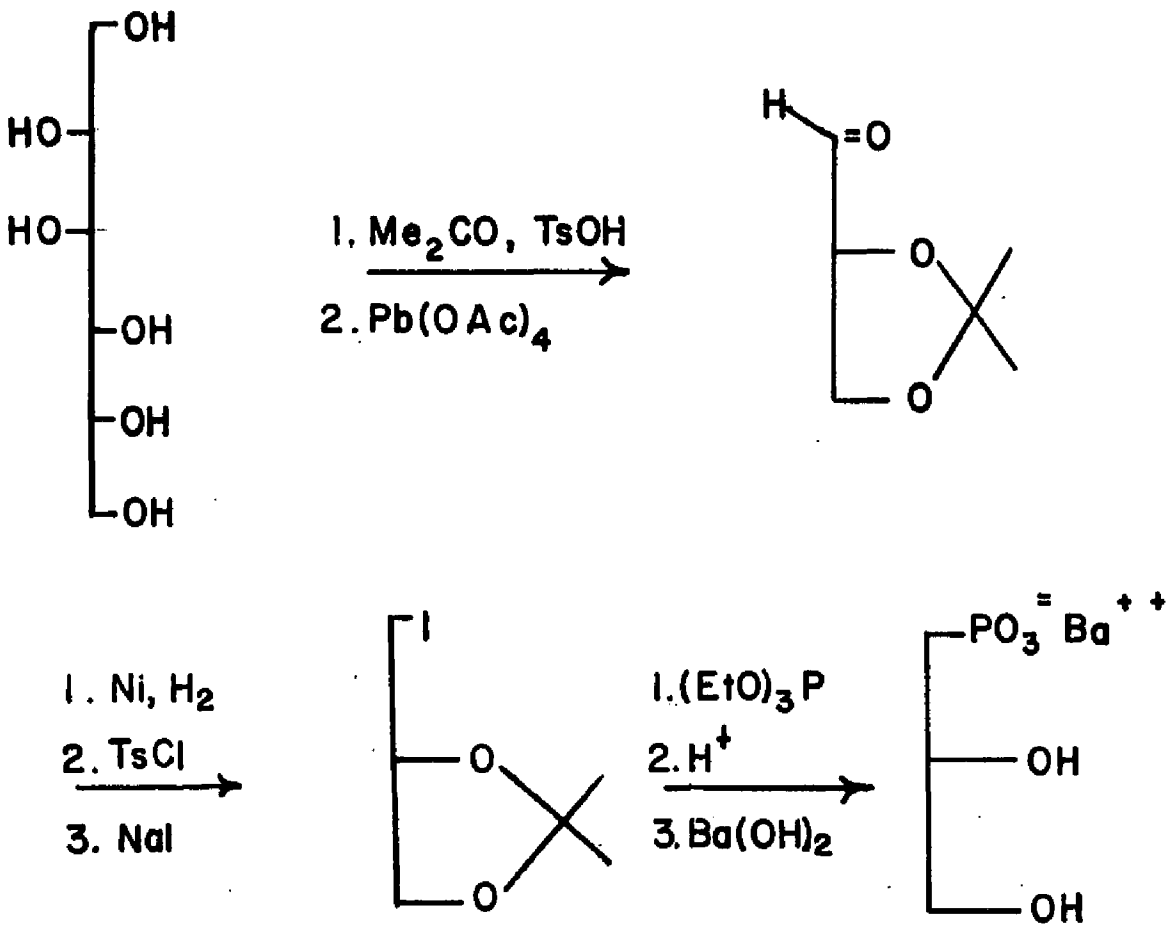


I



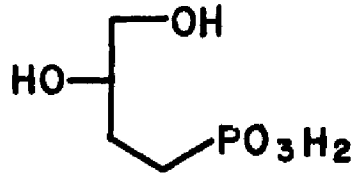
GLYCEROL - 3 - PHOSPHATE

was synthesized via an Arbuzov reaction on allyl bromide followed by hydroxylation and ester hydrolysis. The optically active form, R-(-)-2,3-dihydroxypropyl-1-phosphonic acid,[6] bearing the same absolute configuration as the natural sn-glycerol-3-phosphate about the internal hydroxyl was later reported by Baer and Basu.[7] This route began with D-mannitol diacetonide and, after cleavage, involved an Arbuzov reaction on the derived iodide. (Scheme 1).



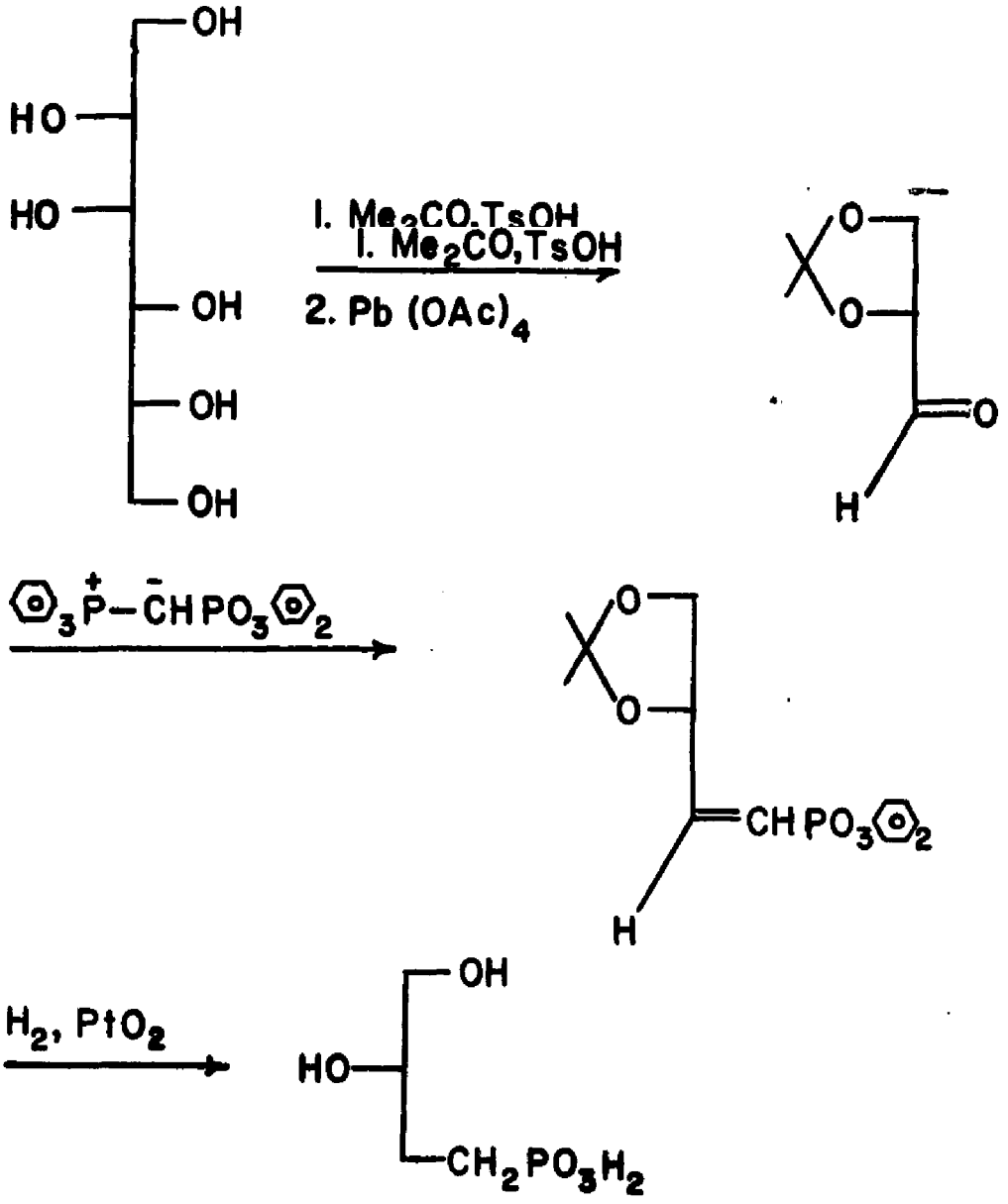
SCHEME I

The racemic isosteric analogue, 3,4-dihydroxybutyl-1-phosphonic acid (II) was then reported by Kabak, et al.[8] utilizing a route paralleling that of Rosenthal and Geyer.[5] Since this first report, synthesis of



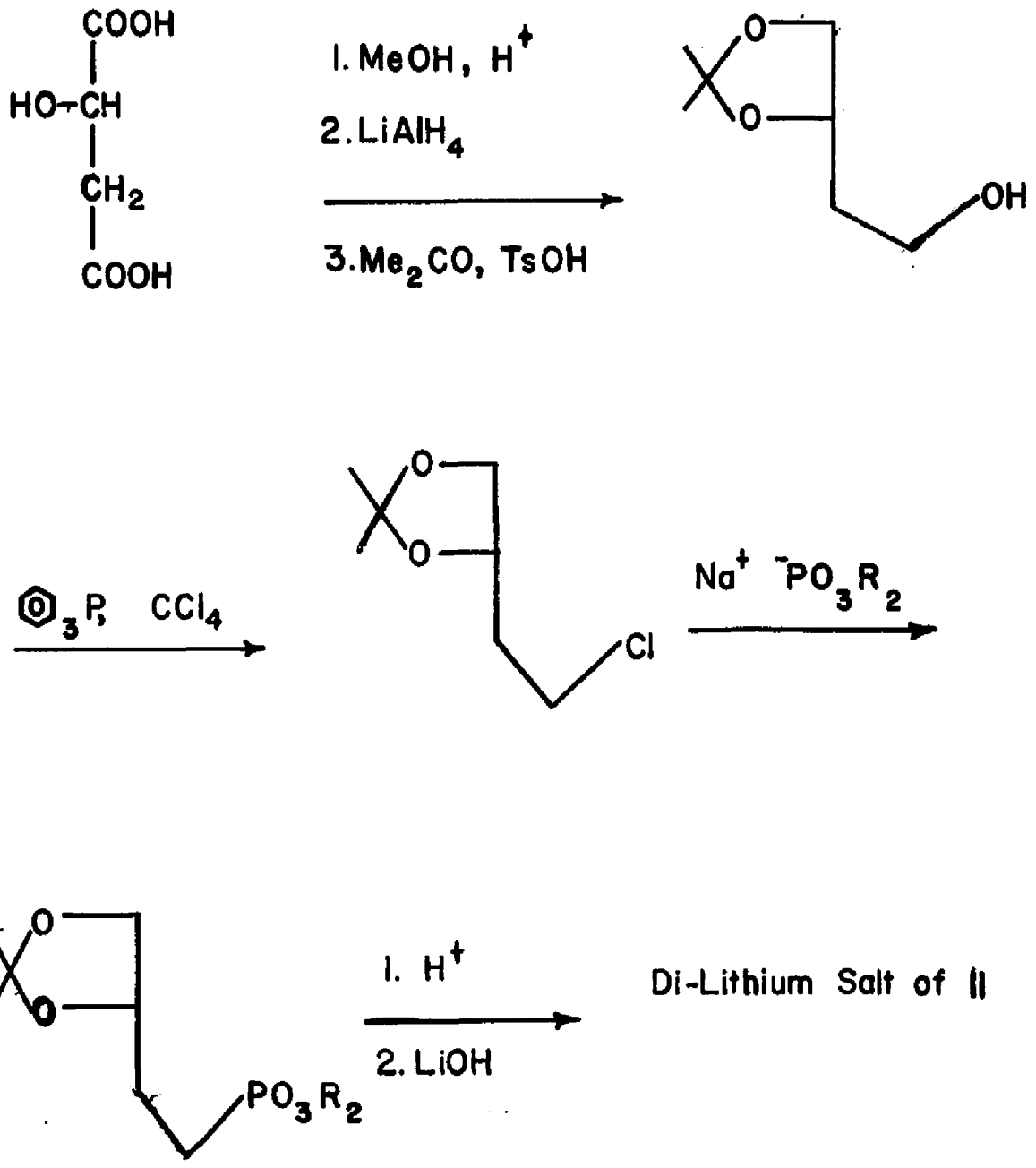
II

the optically active form, S-(+)-3,4-dihydroxybutyl-1-phosphonic acid, has been attained by several different routes. One synthesis began with D-mannitol diacetonide[6] which was cleaved to the aldehyde. This intermediate was then phosphonylated by a Wittig reaction. (Scheme 2).



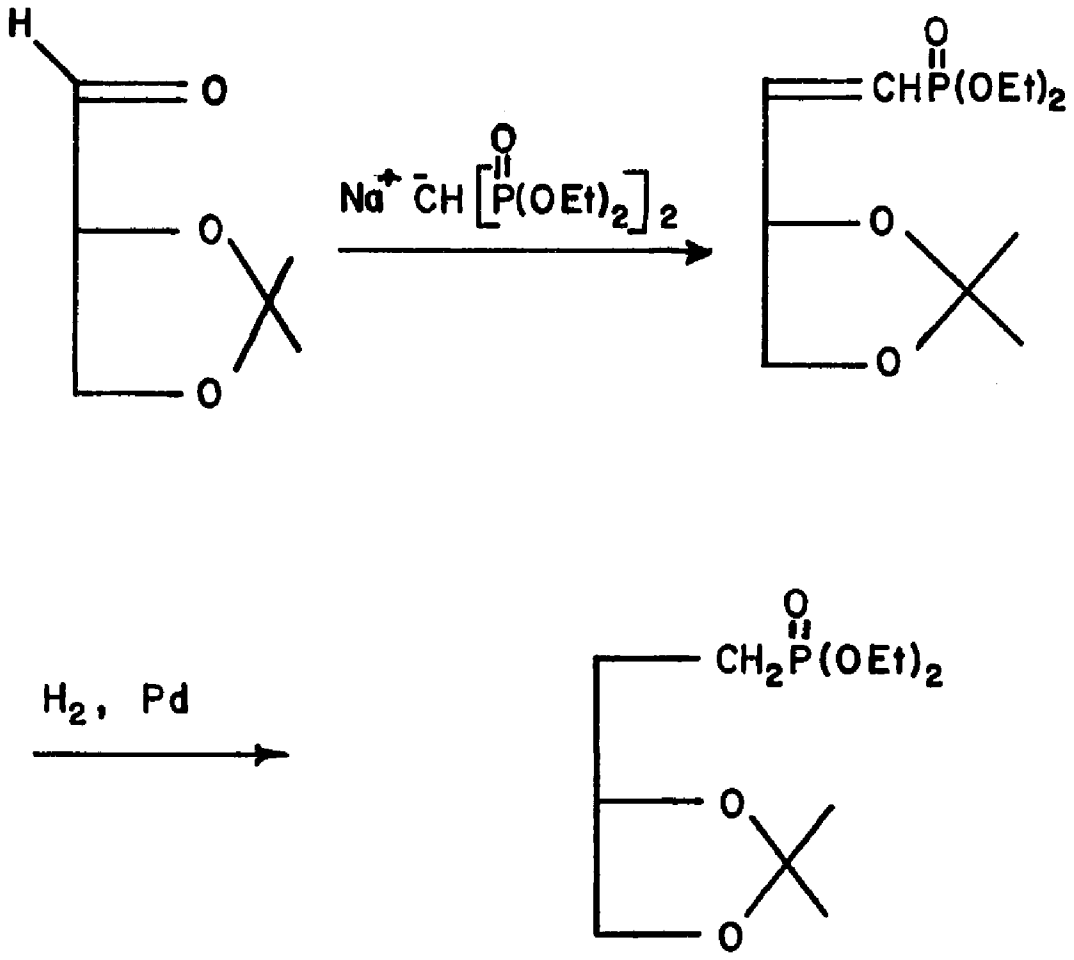
SCHEME 2

Another preparation involved reduction of L-malic acid, formation of the acetonide, conversion of the remaining hydroxyl to the chloride, and phosphonylation via a Becker reaction.[9] (Scheme 3).



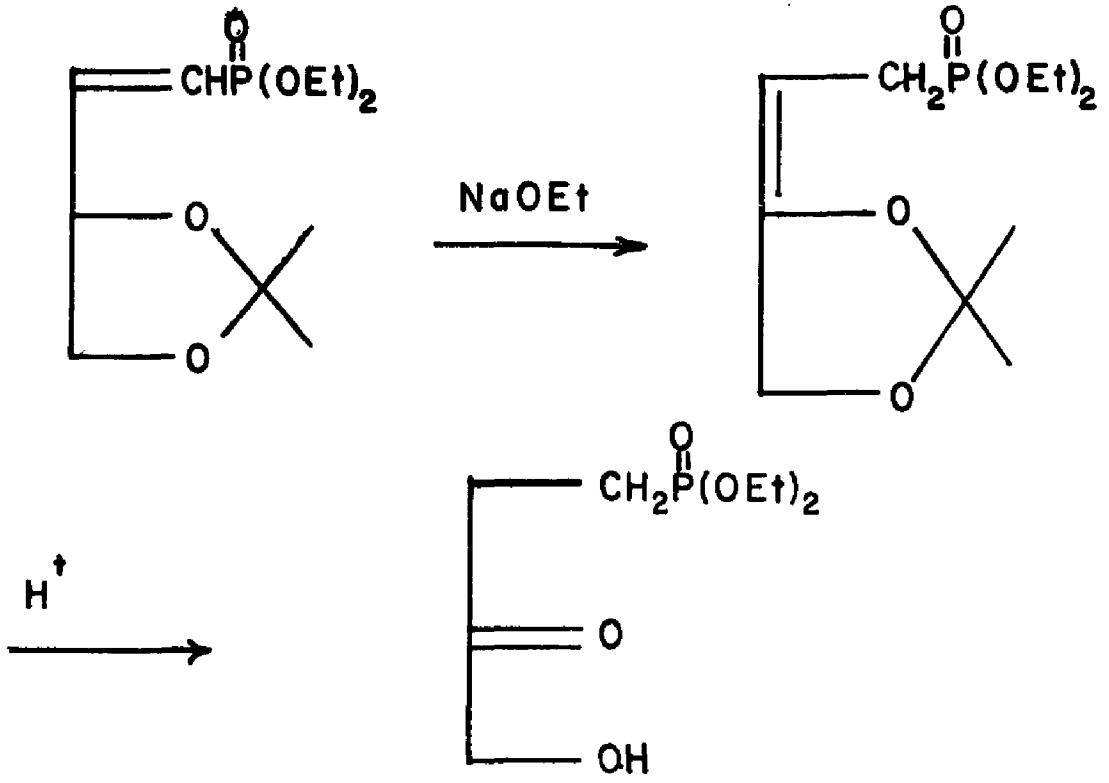
SCHEME 3

Paulsen and Bartsch reported[10] the synthesis of II by a Horner reaction on the protected glyceraldehyde followed by reduction and phosphonate ester cleavage using trimethylchlorosilane. (Scheme 4).

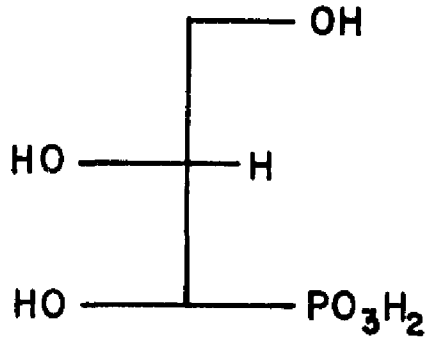


SCHEME 4

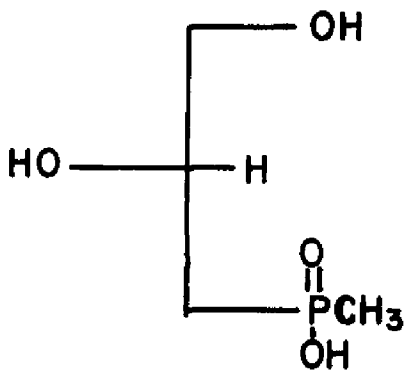
Treatment of the vinyl phosphonate intermediate with base isomerized the carbon-carbon double bond and subsequent acid cleavage of the acetonide function yielded the diester of the isosteric analogue of dihydroxyacetone phosphate (vide infra).



Two other analogues of glycerol-3-phosphate, both non-isosteric in design, have also been reported.[6] They are the 1,2,3-trihydroxypropyl-1-phosphonic acid (III) and the phosphinic acid (IV).



III

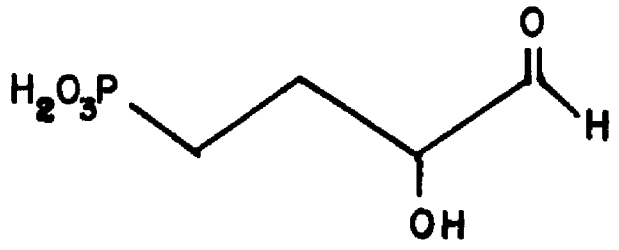


IV

Baer, et al.[11] studied the biological activity of L-glycerol-3-phosphate:NAD oxidoreductase on the non-isosteric analogue I and found that it did not interact. No affinity was exhibited towards the analogous phosphonate. Thus, they concluded that "the phosphate ester bond of L-α-glycerophosphoric acid ... is an essential structural feature for enzyme activity." However, through the efforts of Kabak, et al.,[8] and Shopsis, et al.[12-14] it was found that the isosteric analogue II was capable of inhibiting the growth of mutant strains of E.coli at low concentration.

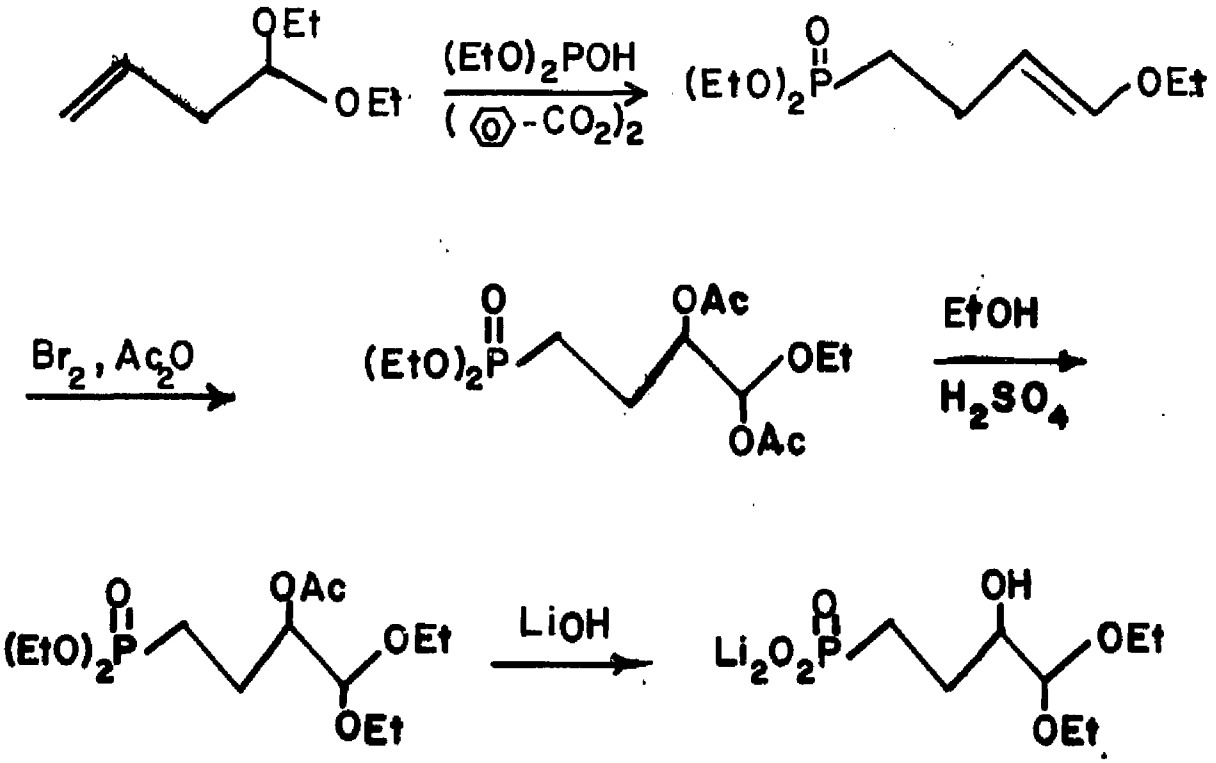
Thus it may be concluded that the ester oxygen is not necessary for activity, as was incorrectly stated by Baer, et al.[11] However, correspondence of size and shape with the natural substrate is an important factor. The ability of II to substitute for glycerol-3-phosphate in enzymatic processes was demonstrated by Cheng, et al.[89] and later confirmed by Adams, et al.[6] In their work the action of glycerol-3-phosphate dehydrogenase (rabbit muscle) on S-(+)-3,4-dihydroxybutyl-1-phosphonic acid and III was investigated. It was found that S-(+)-3,4-dihydroxybutyl-1-phosphonic acid behaved as a substrate showing Michaelis-Menten kinetics and kinetic parameters very similar to those obtained with the natural substrate, sn-glycerol-3-phosphate. The similarity in kinetic parameters suggests that the esterified C-3 oxygen of sn-glycerol-3-phosphate has no major direct interaction with the enzyme. The analogue III could not substitute likewise. It appears that the spatial relationship of C-3 to P in S-(+)-3,4-dihydroxybutyl-1-phosphonic must approximate that in the CH_2-O-P linkage for efficient binding to the enzyme.

The isosteric analogue of glyceraldehyde-3-phosphate (V) was prepared by Goldstein, et al.[15] The synthesis involved a free radical



V

addition of diethyl phosphite across the olefinic linkage of 1,1--diethoxy-3-butene to yield diethyl 1-ethoxybut-1-enyl-4-phosphonate. The enol ether was converted to the diacetate which was treated with sulfuric acid in ethanol to obtain the corresponding diethyl acetal. Cleavage of the diethyl phosphate linkages was accomplished with lithium hydroxide. The product was isolated and stored as the dilithium salt of the diethyl acetal and converted to the free aldehyde form prior to use by treatment with Dowex 50 in the H^+ form. (Scheme 5).

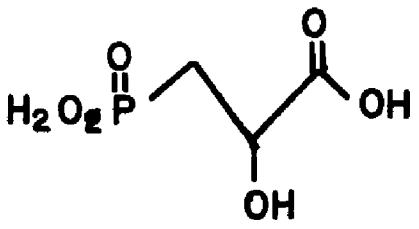


SCHEME 5

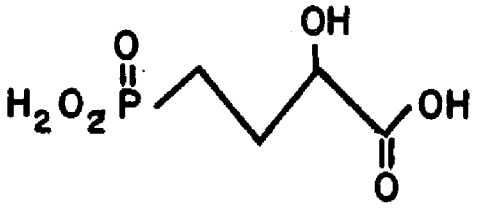
Earlier, Baer and Robinson[16] reported a multi-step synthesis of the non-isosteric analogue beginning with DL-glyceraldehyde dimer. Analogue V was found[15] to be a substrate for glyceraldehyde-3-phosphate dehydrogenase. It is transported into cells of E. coli, as is the natural phosphate, and inhibits their growth at low concentration.[17]

Recent reports have shown that the affinity of oxygen for hemoglobin can be altered by 2,3-diphosphoglyceric acid,[18,19] the major organic phosphate in the red blood cell. Interest in improving performance of oxygen transport mechanisms via the 2,3-diphosphoglyceric acid-hemoglobin interaction encouraged investigations directed at altering the hemoglobin-oxygen dissociation curve with isosteric analogues of phosphoglyceric acid.

Five syntheses of analogues of 3-phosphoglyceric acid have been reported. Pfeiffer, et al.[20] described the preparations for both the non-isosteric and isosteric analogues, VI and VII, respectively.

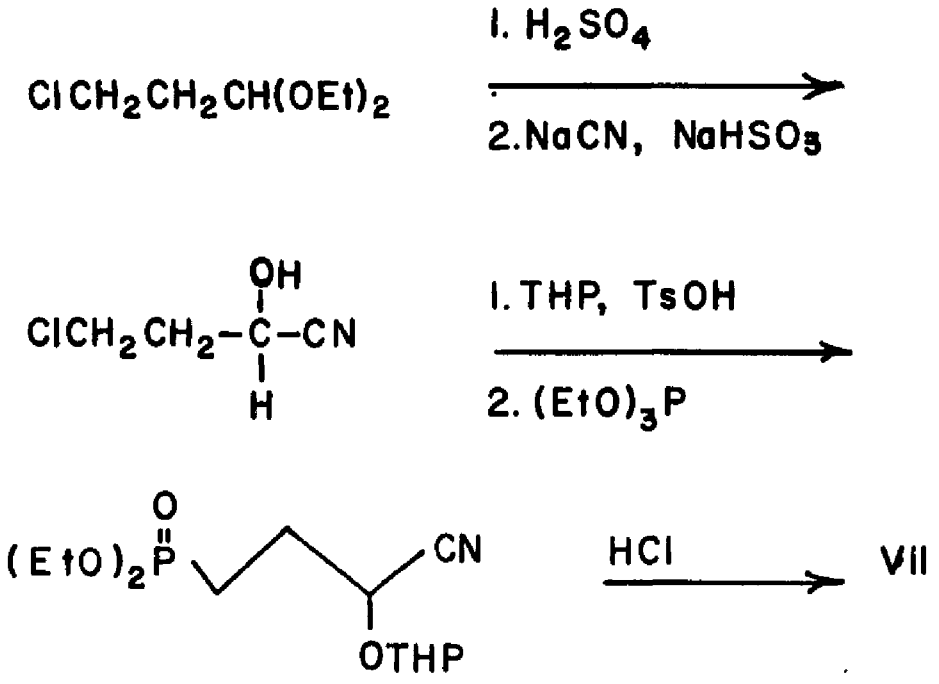


VI



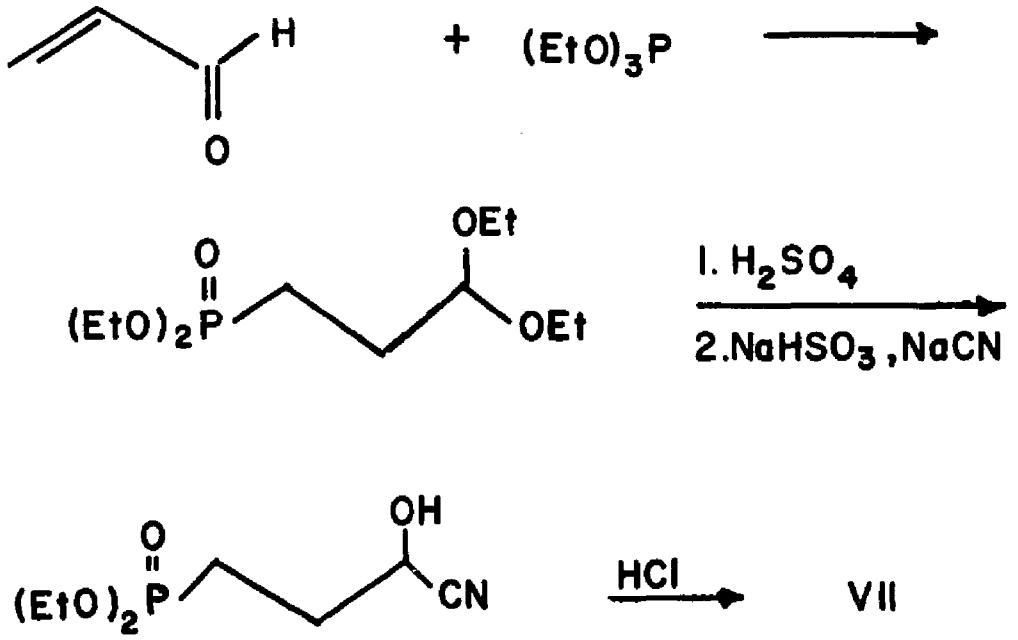
VII

Compound VII was synthesized by the route shown below. (Scheme 6).



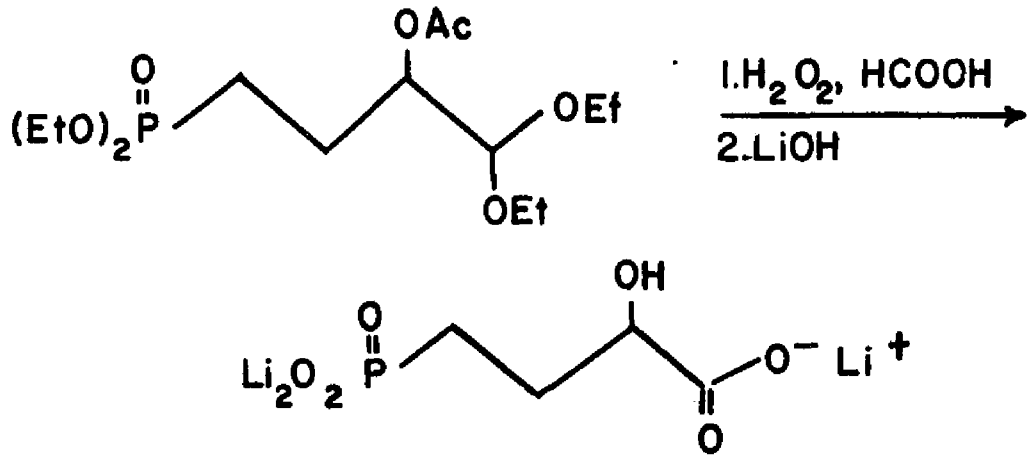
SCHEME 6

Later work by K.-C.Tang, et al.[9] involved C-P bond formation in VII by the reaction of triethyl phosphite with acrolein via a Michael--type addition yielding the diethyl acetal. After treatment with acid the resulting aldehyde is used in cyanohydrin formation followed by hydrolysis to VII. (Scheme 7).



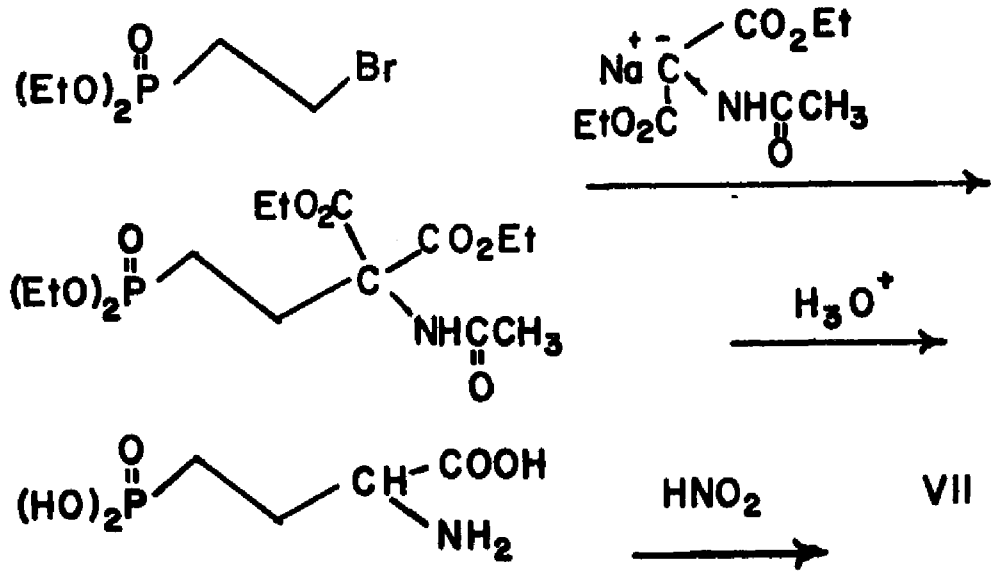
SCHEME 7

Goldstein, et al.[15] reported the preparation of the trilithium salt of VII by the oxidation of an intermediate in the preparation of the analogue of glyceraldehyde-3-phosphate. (Scheme 8).



SCHEME 8

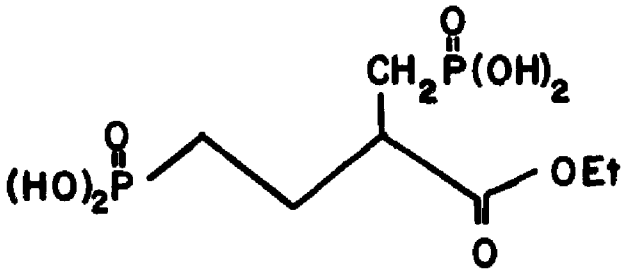
Dixon and Sparks reported[21] a route for the preparation of VII from diethyl 2-bromoethylphosphonate. (Scheme 9).



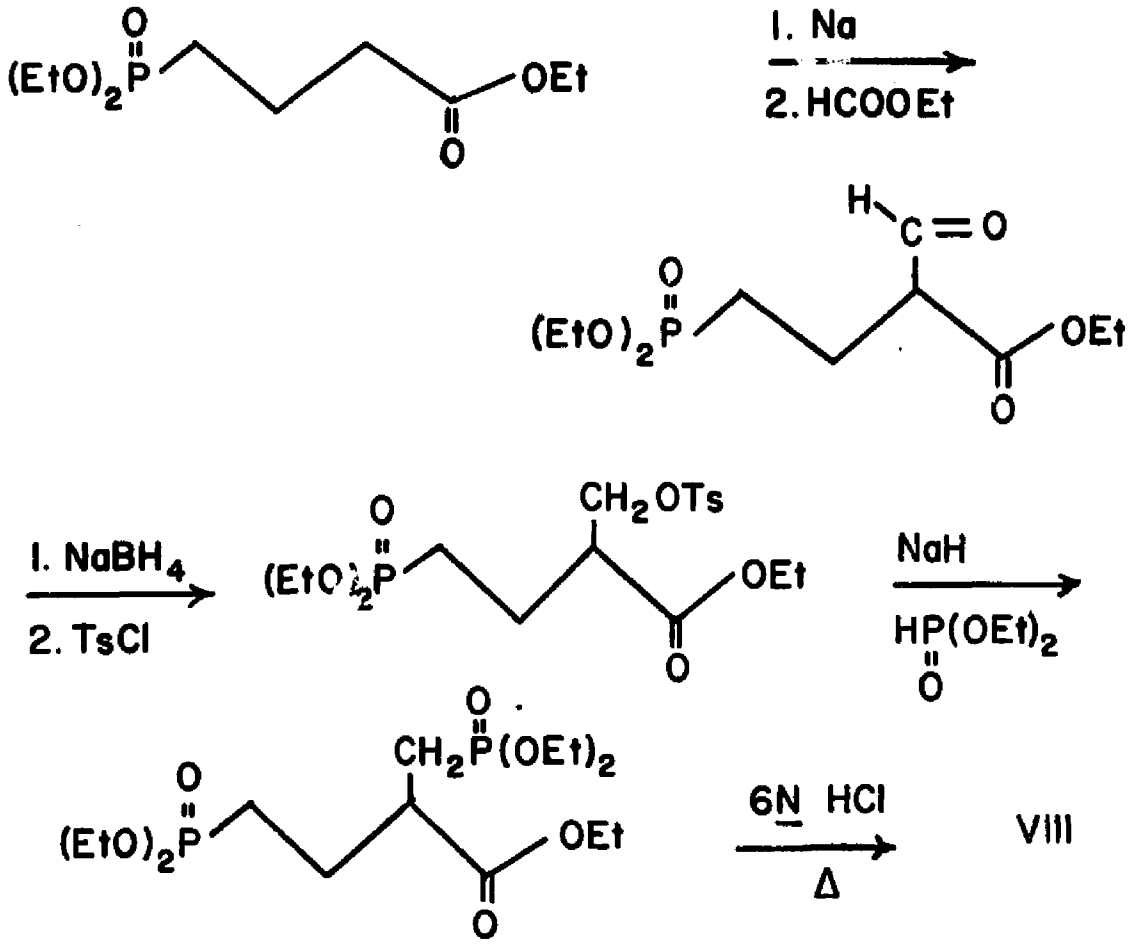
SCHEME 9

Biochemical investigations[20] of VI and VII indicated that neither one altered the hemoglobin-oxygen dissociation curve. No antibacterial activity was observed in these studies. However, it was found[21] that VII was capable of replacing the natural material in oxidation of NADH. Orr and Knowles observed[22] it to be a substrate for phosphoglycerate kinase with a similar kinetic value to that of the natural material.

The isosteric analogue of 2,3-diphosphoglyceric acid VIII was synthesized[20] by the route shown below. (Scheme 10).



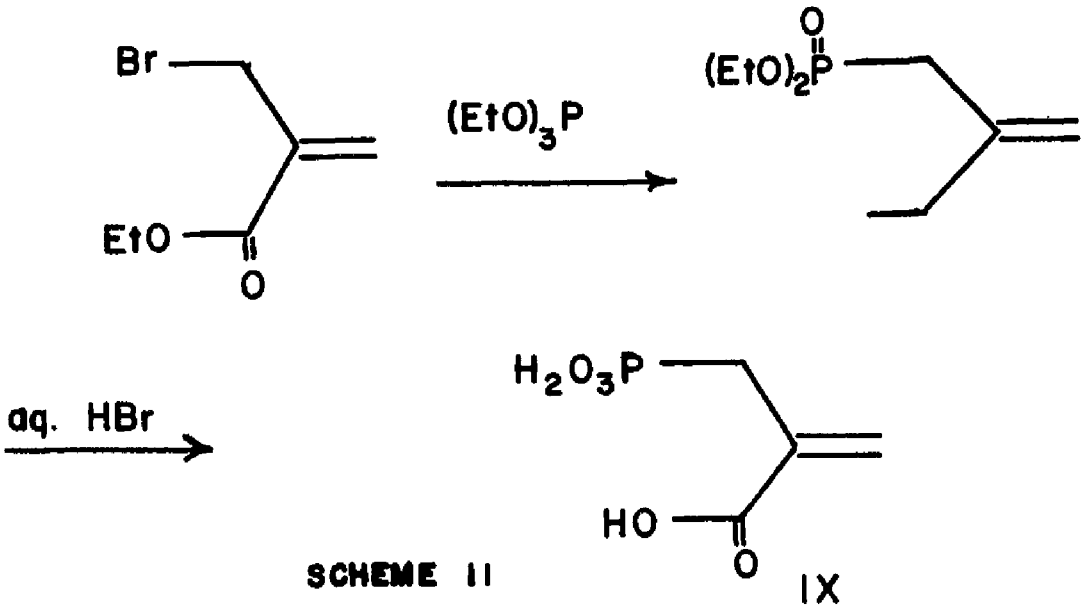
VIII



SCHEME 10

The isosteric analogue VIII again did not produce a shift in a cell-free human hemoglobin solution. Baer and Robinson[23] have cited that there is no difference in binding to hemoglobin of either D-2,3-diphosphoglyceric acid or its optical antipode.

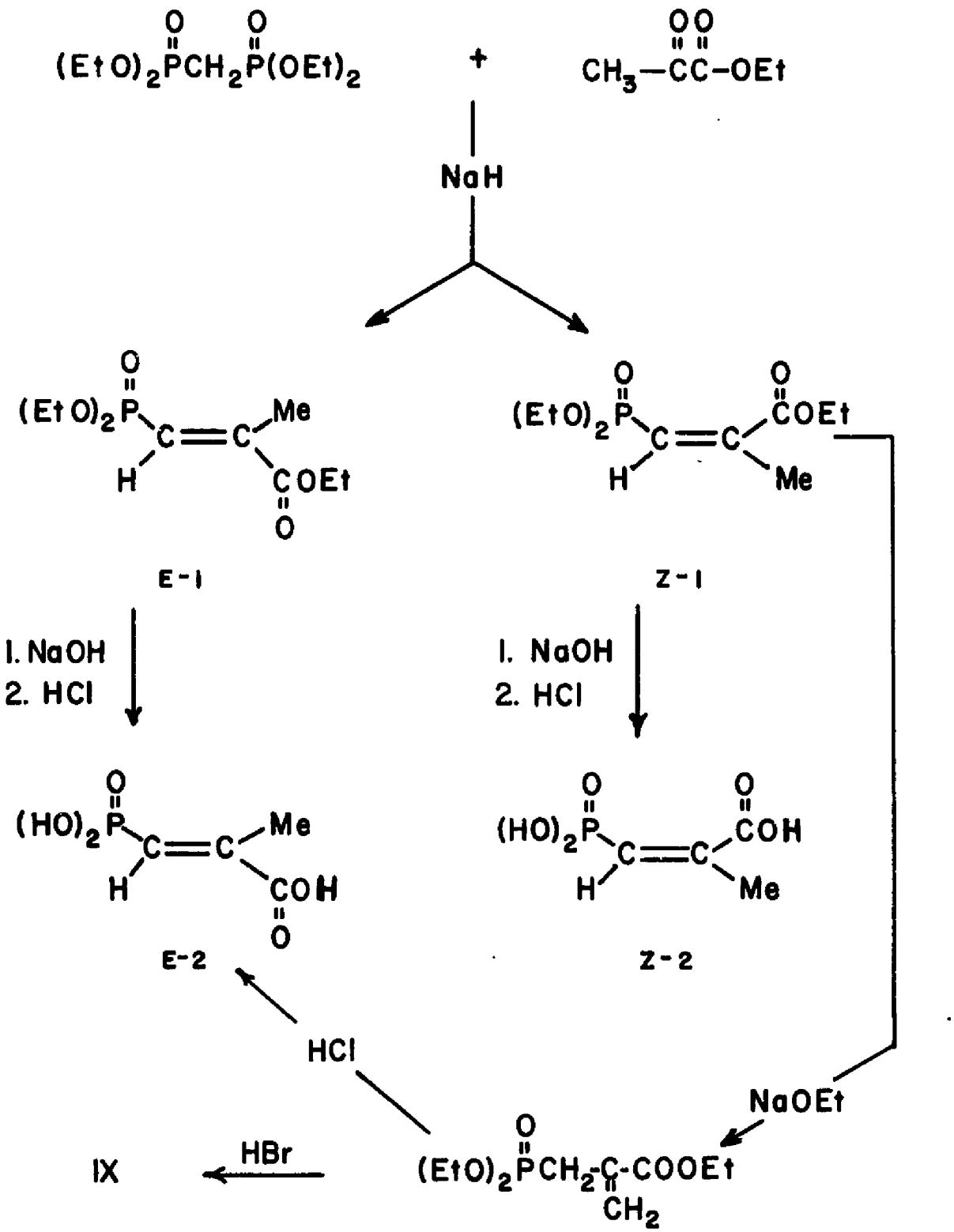
The isosteric analogue of phosphoenolpyruvate IX was prepared by Stubbe and Kenyon.[24] The route involved phosphonylation via an Arbuzov reaction. (Scheme 11).



SCHEME II

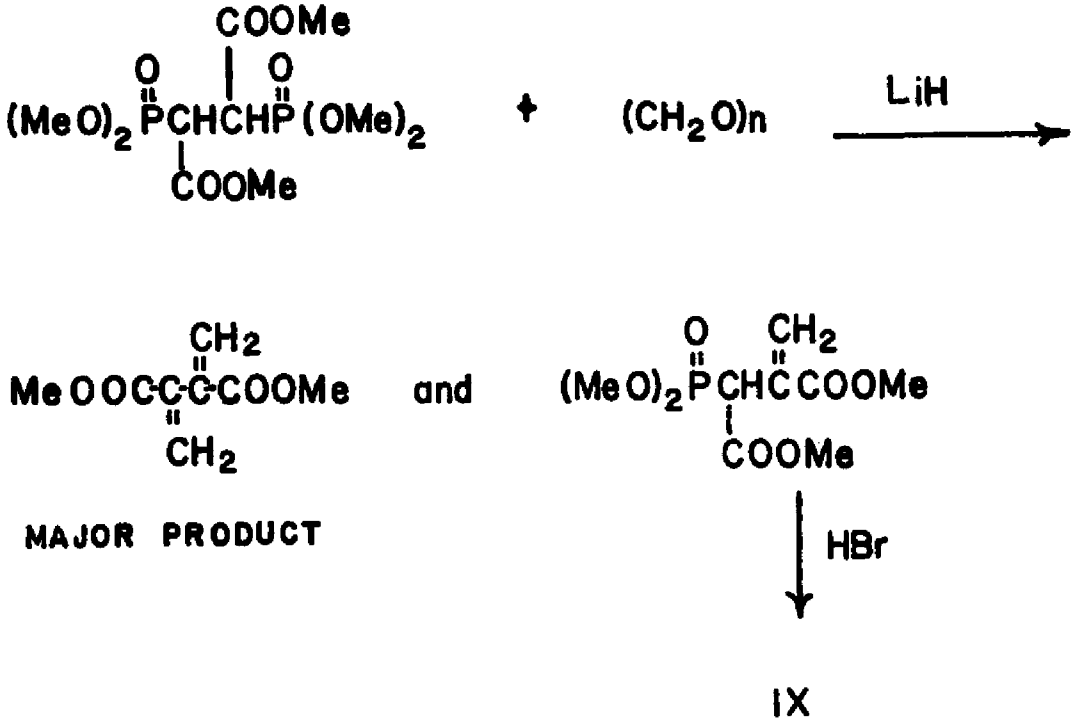
Studies[24-26] on the action of enolase on IX found it to be comparable to phosphoenolpyruvate in binding to the enzyme but much slower in overall reaction. The analogue was found to be non-inhibitory to pyruvate kinase at low ratios of analogue to substrate, but inhibitory at much higher ratios.[27]

Recently Davidson and Kenyon reported[28] three syntheses of an analogue of phosphoenolpyruvate. One approach, (scheme 12), used a Wadsworth-Emmons-Horner reaction on ethyl pyruvate to yield a 50:50 mixture of (E)- and (Z)- ethyl (α-methyl-β-diethoxyphosphinyl) acrylates, (E-1)and (Z-1) respectively. Only the (Z-1) isomer undergoes a base-catalyzed rearrangement with sodium ethoxide to ethyl α-(diethoxyphosphinylmethyl) acrylate presumably through release of unfavorable steric interaction between the cis ethoxycarbonyl and diethoxyphosphinyl substituents. In the (E-1) isomer these substituents are trans to one another. Treatment of either (E-1) or (Z-1) isomer with sodium hydroxide resulted in carboxylic ester cleavage and the free phosphonic acids, (E-2) and (Z-2) were released through acid hydrolysis.


(EtO)2PCH2P(OEt)2
CH3—CC-OEt
NaH
E-1
Z-1
1. NaOH
2. HCl
1. NaOH
2. HCl
E-2
Z-2
HCl
NaOEt
IX
HBr
(EtO)2PCH2-C-COOEt
CH2

SCHEME 12

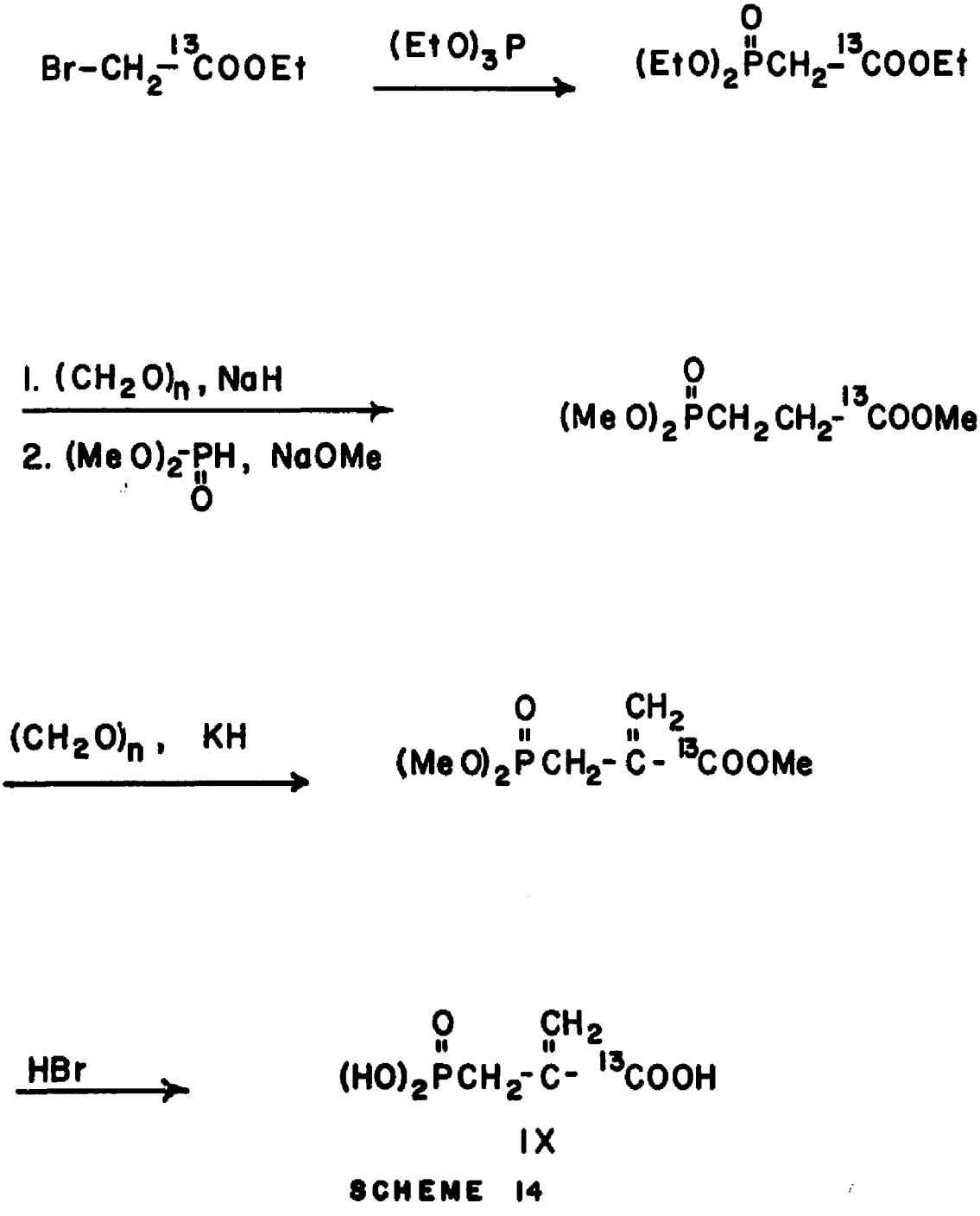
In scheme 13 is outlined another modified Wittig reaction as an approach to IX. The known compound dimethyl α,β-bis(dimethylphosphinyl) succinate was treated with formaldehyde under strongly basic conditions. A mixture of two products was generated. Upon acid hydrolysis, decarboxylation occurred without double-bond rearrangement.



SCHEME 13

Still another synthetic route is outlined in scheme 14 which allows for selective carbon-13 enrichment in the carboxyl carbon position. The starting material with C-13 labelling, which is commercially available, led to the adoption of this scheme. The approach involved an Arbuzov reaction using triethyl phosphite with ethyl bromoacetate followed by a modified Wittig procedure with formaldehyde. The labelled ethyl acrylate, not isolated, was treated with dimethyl phosphite and base to generate the labelled dimethyl β-methoxycarbonylethylphosphonate. Metal hydride treatment and condensation with formaldehyde, with subsequent acid hydrolysis of the ester functions resulted in the desired

product IX. (Scheme 14).

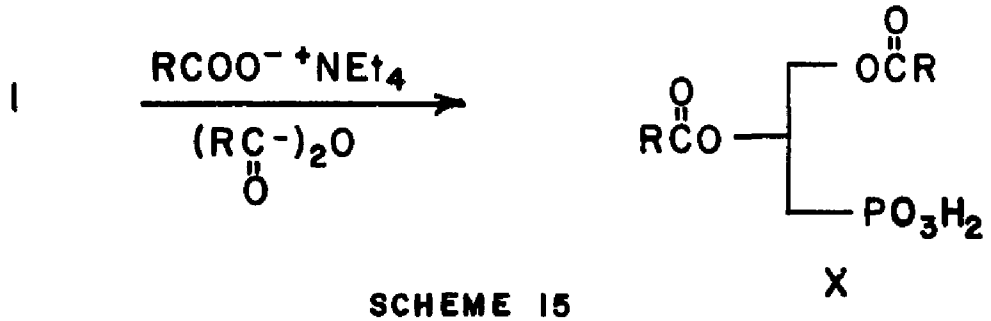


SCHEME 14

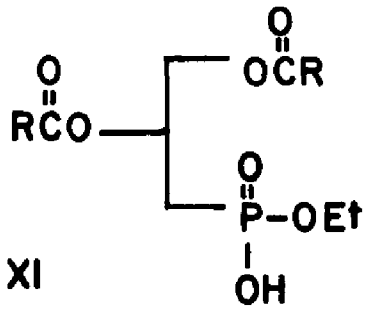
ANALOGUES OF PHOSPHOLIPIDS

Baer introduced the generic term phosphonolipids for analogues of phospholipids. One may consider here two main categories of phosphonolipids; one with structural changes in the "glycerol portion" (a C – P bond being present instead of the glycerol esteric oxygen), and the other related to aminoethylphosphonic acid (a C - P bond being present instead of the esteric oxygen of the head group). It should be noted that this latter category constitutes a "natural" system found in numerous organisms.[29-36]

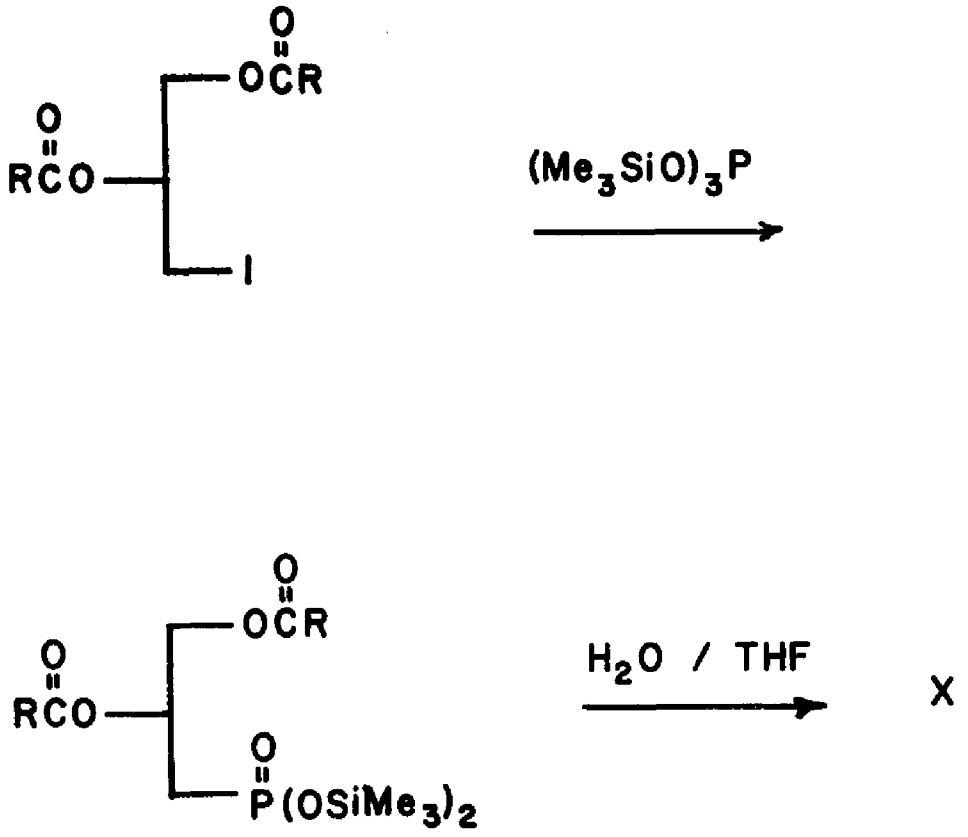
For the first category, the fundamental phosphonolipid structure is derived from analogues of phosphatidic acid, referred to as phosphotidic acids. The non-isosteric analogues of phosphatidic acid (X), bearing saturated fatty-acid ester functions were reported by Baer and Basu[37] and later by Bonsen, et al.[38] by acylation of I. The mono--ester XI related to X was also reported.[39] (Scheme 15).



SCHEME 15

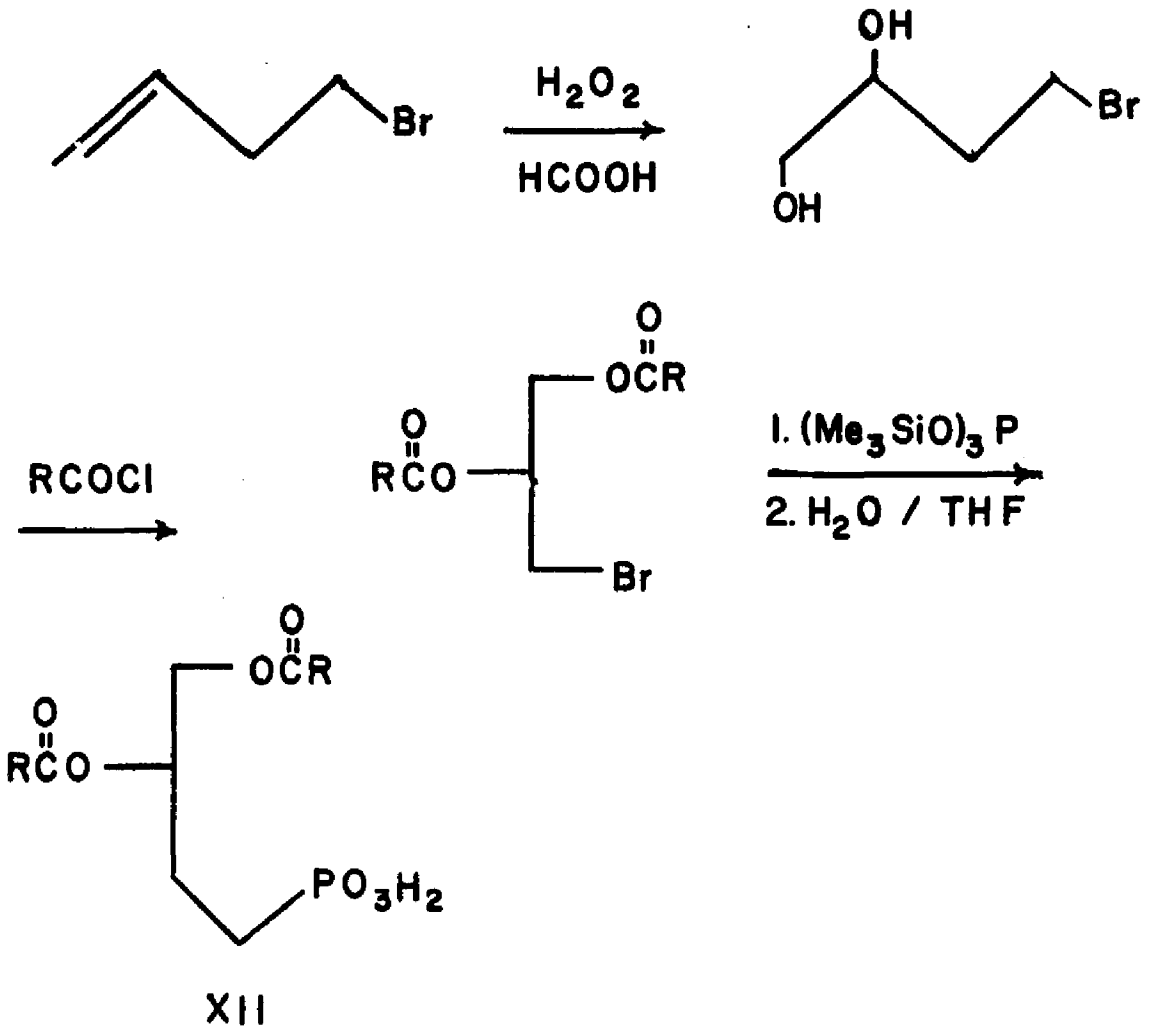


Rosenthal, et al.[40,41] reported the synthesis of non-isosteric materials X of this category of phosphonolipids using an Arbuzov reaction on 2,3-diacyl-1-iodopropanes with tris(trimethylsilyl) phosphite[42,43] followed by mild hydrolysis. (Scheme 16).



SCHEME 16

The isosteric phosphotidic acids XII bearing saturated and unsaturated fatty-acid ester linkages have also been synthesized by the method shown.[44] (Scheme 17).



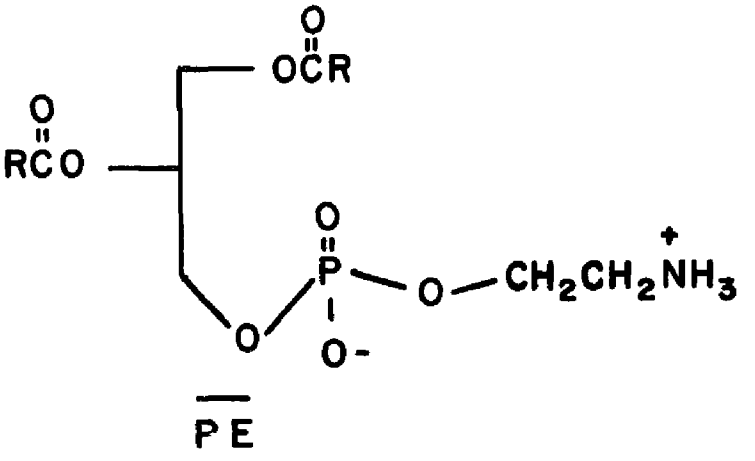
XII

SCHEME 17

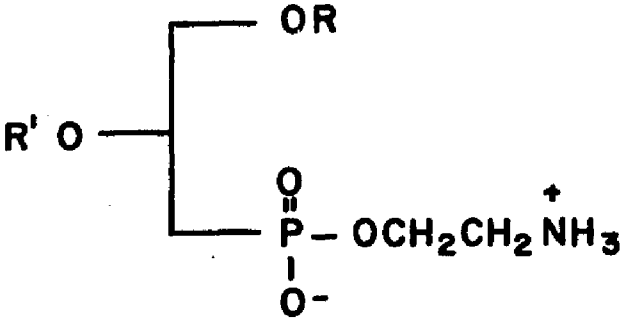
Included in the many compounds synthesized are those with ether and hydrocarbon functions [45-47] substituting for the normal fatty-acid ester functions.

The possibilities for structural variation are exhibited in a class of derivatives of phosphotidic acids, the phosphonic acids related to phosphatidylethanolamines (PE; diacylglycerylphosphoryl-ethanolamines), commonly known as cephalins. Considerable attention has been directed toward the preparation of the second category of phosphonolipids, in which the basic glycerol backbone remains, but

the oxygen has been eliminated from the ethanolamine fragment.

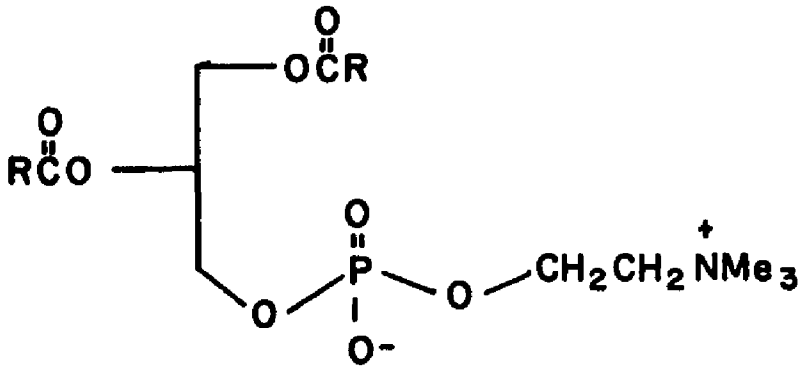


Derivatives with a carbon-phosphorus bond in the glycerol backbone, (first category of phosphonolipids) have received less attention. The non-isosteric diether derivatives XIII in this category have been prepared [45,54] with similar and differentially substituted alkoxy groups. Coupling of the diether phosphotidic acid and the protected ethanolamine was facilitated by trichloroacetonitrile.



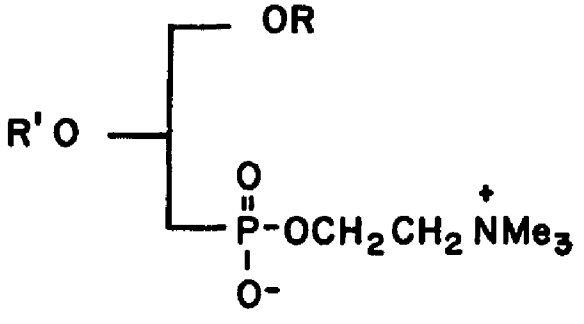
XIII

The phosphotidic acids related to phosphotidylcholines (PC; diacylglycerylphosphorylcholines), commonly known as lecithins, presented the same possible structural variations. Compounds of the

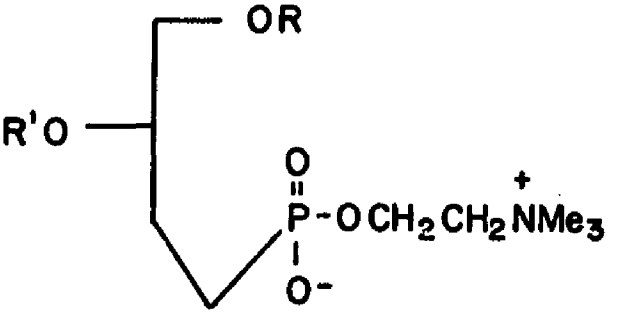


PC

types XIV and XV have been synthesized by the coupling of the corresponding phosphotidic acid with choline using trichloroacetonitrile. 55

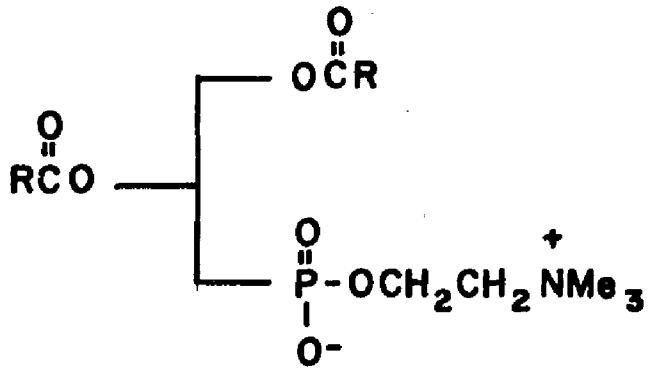


XIV



XV

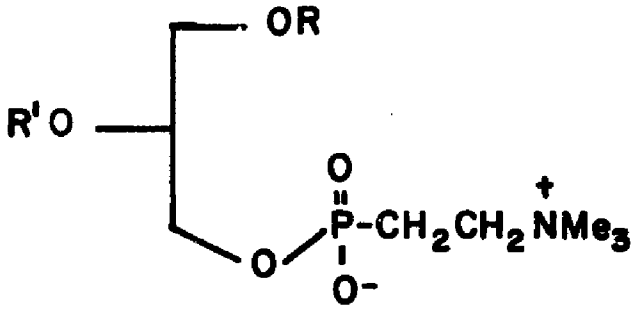
By a similar method the non-isosteric diesters XVI were prepared.[38,40,41]



XVI

Biological studies performed on the compounds in this category have been intended to determine their ability to replace natural phospholipids in various systems, being resistant to normal enzymatic cleavage processes at phosphorus, possibly serving as substitute substrates and/or inhibitors. Some interesting results developed from enzymatic studies with analogues of phosphatidylcholine. Systems lacking the ester linkage in the glycerol backbone, such as XIV, XV and XVI are inert to phospholipase C, an enzyme which hydrolyzes the bond between phosphoric acid and glycerol, and are powerful inhibitors in a dispersed state[56-58] by coating substrate micelles and forming a surface less susceptible to enzyme attack. Thus the analogue alters the electrokinetic properties of the substrate particles causing inhibition. For systems bearing an intact 3-carbon glycerol backbone but without the usual oxygen of the choline fragment,[59,60] i.e. XVII, phospholipase C facilitated hydrolysis occurs at a rate equal to that of the natural material.[61] It appears that the phosphate linkage

between the phosphoric acid and glycerol fragments has a major direct interaction with the enzyme.



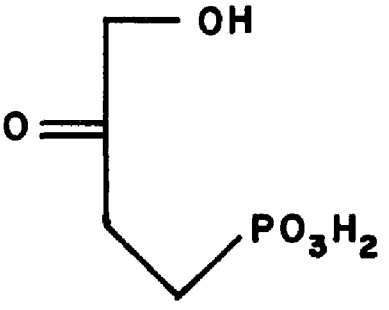
XVII

Bittman and Blau employed[62] the synthetic phosphonolipids for the preparation of liposomes and the study of their interaction with sterols. Interestingly, it was observed that the length of the glycerol backbone was significant; interaction with the sterols was exhibited only by the isosteric systems. It was also observed that the isosteric ether analogues were good substitutes for the esters indicating that the carbonyl linkage plays a negligible role, and that the spatial relationship of C-3 to P must approximate that in the CH_2-O-P linkage.

Tyhach, et al.[63-65] discovered a novel phosphonolipid to be generated by the enzymatic (in vivo and in vitro) reaction of 3,4-dihydroxybutyl-1-phosphonic acid with CDP-diglyceride. This lipid was identified as (1,2-dipalmitoyl)-glyceryl-4'-phosphoryloxy-3'-hydroxybutyl-1'-phosphonic acid.

RESULTS AND DISCUSSION

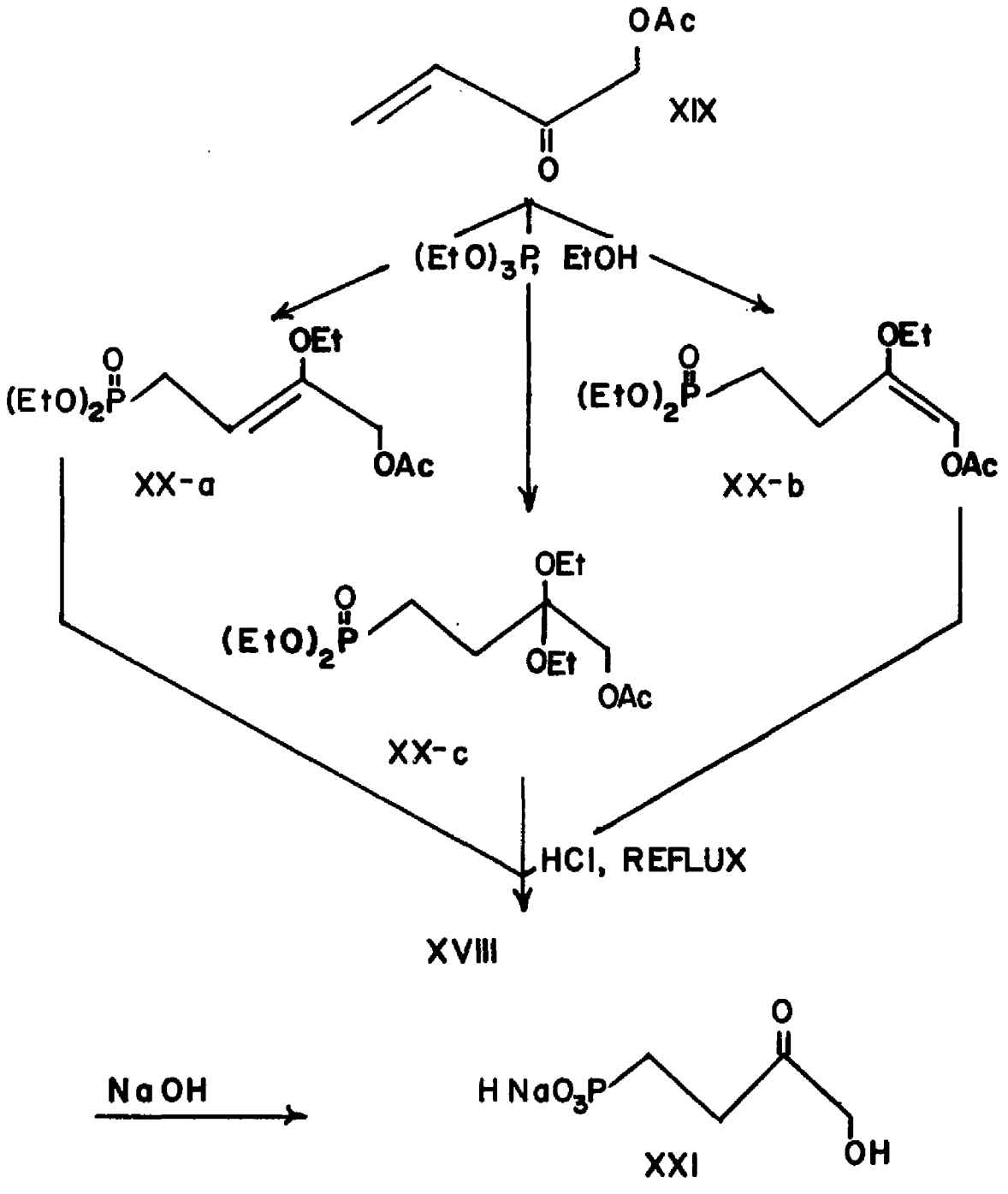
In light of the positive results of early biological investigations[12-14] using the isosteric analogue II of glycerol-3-phosphate, it became desireable to obtain the material with specific tritium incorporation at the 3-carbon. The approach taken involved preparation of the carbonyl precursor 4-hydroxy-3-oxobutyl-1-phosphonic acid XVIII, the isosteric analogue of dihydroxyacetone phosphate,[66] which was then reduced using tritium labeled sodium borohydride.



XVIII

The procedure of Hennon and Kupiecki[67] was followed to prepare the starting material, acetoxymethyl vinyl ketone (XIX). This was prepared immediately prior to use due to its tendency toward rapid polymerization. The ketone was phosphorylated with triethyl phosphite in ethanol resulting in a mixture of three compounds: XX-a, -b, -c as indicated by nmr spectra. Attempts at separation were unsuccessful but the mixture was considered suitable for the next stage in the synthesis. Acid hydrolysis resulted in cleavage of the acetate as

well as the diethyl ester linkages affording XVIII, which was titrated with sodium hydroxide and isolated as the monosodium salt XXI. (Scheme 18).



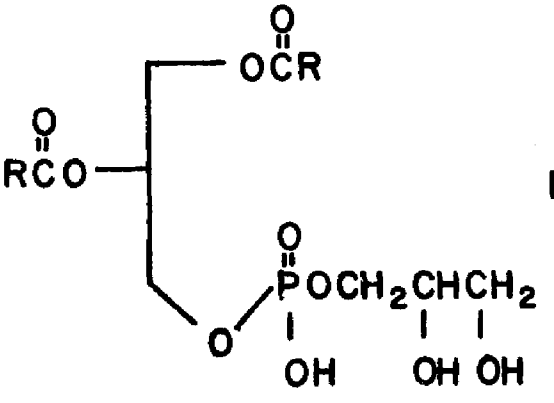
SCHEME 18

The intermediate, an isosteric analogue of dihydroxyacetone phosphate, was examined for biological activity. Whereas the analogue II was capable of penetrating the cell membranes of the organisms investigated,[12-14] the compound XVIII was not, as is the case with the natural material. It did serve as a substrate for the natural system *in vitro*, however. It was observed[89] to perform as a substrate for rabbit muscle L-glycerol-3-phosphate:NAD oxidoreductase with kinetic values similar to that of the phosphate; K_M of 182μM for the analogue compared to K_M of 133μM for the natural system. (K_M, the Michaelis-Menten constant is a dissociation constant of the enzyme-substrate complex measuring the affinity of the enzyme for the substrate.) *In vitro* studies also revealed[65] that analogue XVIII substituted for the phosphate in its action with anabolic L-glycerol-3-phosphate dehydrogenase from *E.coli*. Both enzymes reduce the carbonyl function to a hydroxyl group.

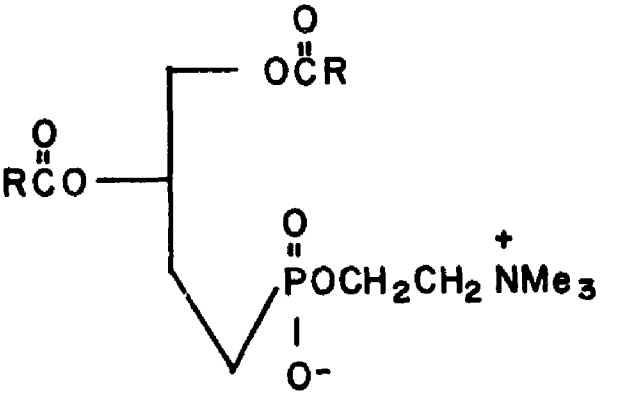
In addition to the gross inhibitory effect on the growth of mutant strains of *E.coli* observed earlier with II, this tritiated analogue now allowed definition of the effect as a perturbation of the normal phospholipid production. In treated *E.coli*, there was a rapid inhibition of the rate of phosphatidylglycerol synthesis, a slower but almost as pronounced inhibition of the rate of phosphatidylethanolamine synthesis[14] and the appearance of a new phosphoglyceride, the phosphonic acid analogue of phosphatidylglycerophosphate.[63,64] It was a substrate and an inhibitor for CDP-diglyceride:*sn*-glycerol-3-phosphate phosphatidyltransferase and *sn*-glycerol-3-phosphate:NAD oxidoreductase.[65] Analogue II was also a substrate for rabbit muscle L-glycerol-3-phosphate dehydrogenase.

In view of the effect that analogue II had on lipid synthesis (DNA and RNA syntheses were much less affected),[14] and the appearance of new phospholipid analogues derived from the interaction of II with enzymes involved in phosphoglyceride metabolism, the synthesis of phosphonolipids was suggested for structural verification.

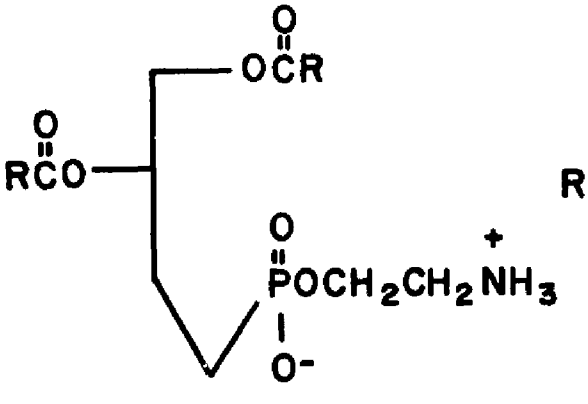
The isosteric phosphonate analogues, compounds XXII-XXIV of PC, PE and phosphatidylglycerol(PG) respectively, were synthesized providing materials in which the normal oxygen atom of the phosphate ester linkage in the glycerol backbone was substituted by a methylene group.



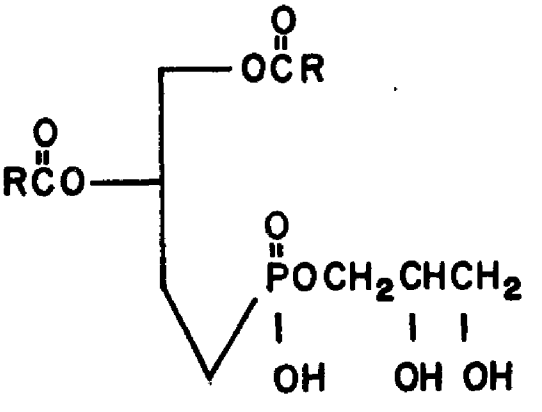
$\overline{PG}$



XXII



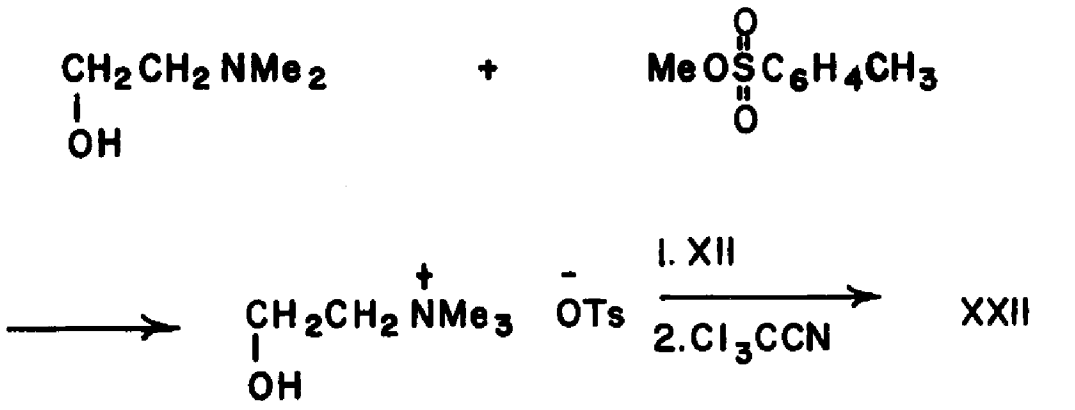
XXIII



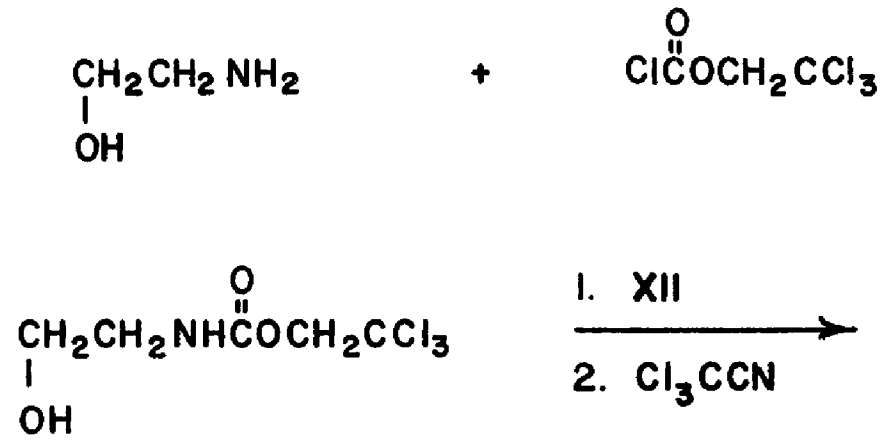
XXIV

The esterification of the previously reported 3,4-dipalmitoyloxybutyl-1-phosphonic acid,[44] with the appropriate protected hydroxyl compound was accomplished with trichloroacetonitrile using modifications of the techniques of Rosenthal,[45] and Cramer and Weimann.[68] In the preparation of XXII, choline tosylate was prepared as previously described.[45] In the synthesis of XXIII, the reported protected ethanolamine, N-(β,β,β-trichloroethoxycarbonyl)ethanolamine[69] was used with ultimate removal of the protecting function via zinc and acetic acid treatment.[69,70] For the generation of XXIV, isopropylidene glycerol was used in the coupling reaction. This was prepared by a standard method.[71]

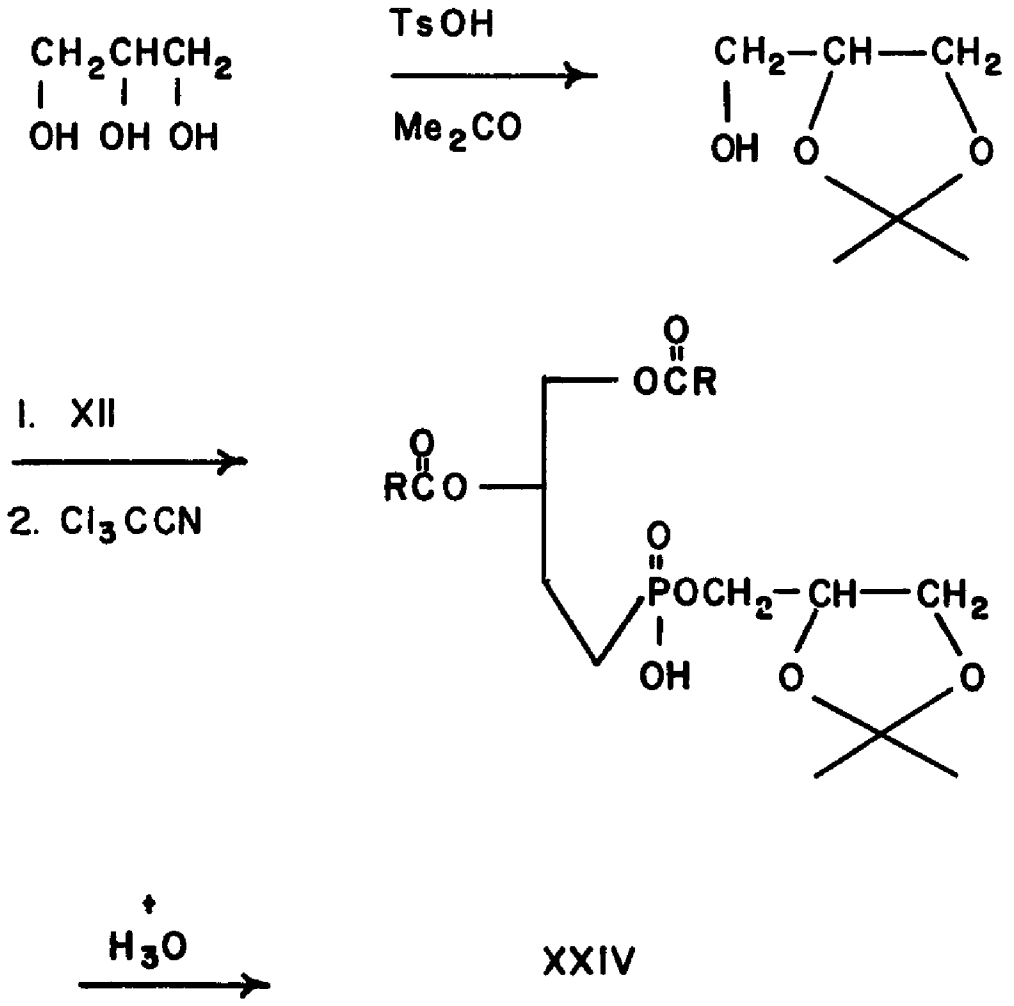
A problem common in all the three syntheses was the work-up and purification of the intermediates. The lipid property of forming emulsions between aqueous and organic layers was encountered. Treatment with saturated aqueous sodium chloride solutions and cold centrifugation of the emulsions were helpful in obtaining relatively pure intermediates. Chromatographic purification was employed involving solvent systems containing methanol which dissolved silica gel thus contaminating the eluent with the solid support. The fine particles of silica gel were removed via a Gelman Metricel Alpha-6 filter, causing some product to be lost during this stage. The esterification reaction was heat and moisture sensitive and was monitored by a thermoregulated bath, carried out under anhydrous conditions. (Scheme 19, 20 and 21).



SCHEME 19



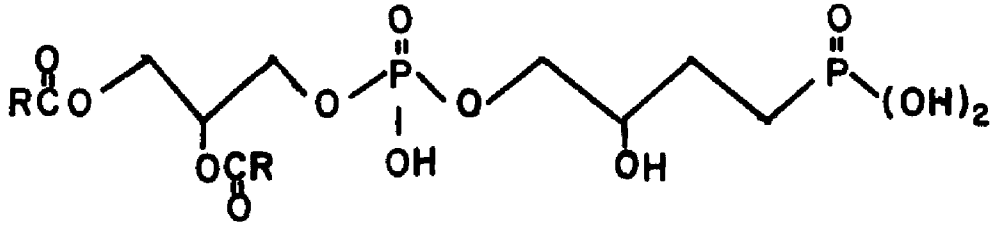
SCHEME 20



XXIV

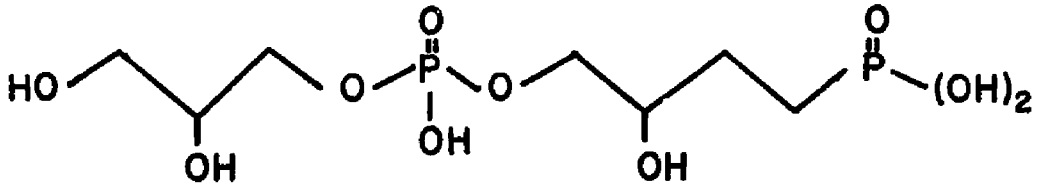
SCHEME 21

The independent non-enzymatic synthesis of (1,2-dipalmitoyl)--glyceryl-4'-phosphoryloxy-3'-hydroxybutyl-1'-phosphonic acid (XXV) was attempted. It has been determined [63-65] that XXV is the reaction



XXV

product from CDP-diglyceride and 3,4-dihydroxybutyl-1-phosphonic acid (II) mediated by CDP-diglyceride sn-glycerol-3-phosphate phosphatidyltransferase. After mild basic hydrolysis, cleaving the fatty acid linkages, XXVI remains. The approach to XXVI involved a

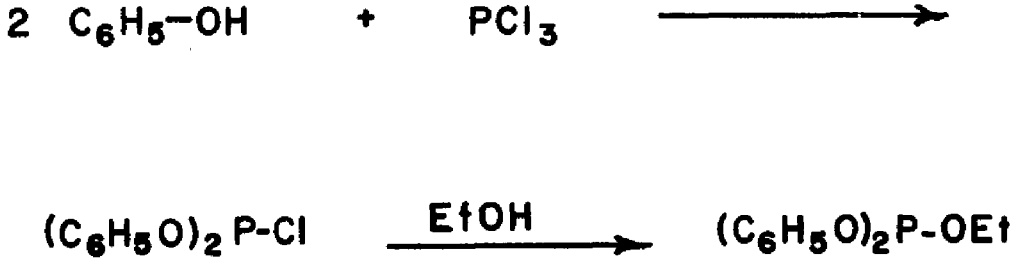


XXVI

converging synthesis of two smaller, but different compounds, II and glycerol-3-phosphate. The final stages in the scheme required the formation of a phosphate linkage joining the two protions followed by removal of the protecting groups. A carbonyl group was used as a mask for the incipient secondary alcohol of II. The free phosphonic acid was to be protected to avoid anhydride formation at this end of

the molecule during coupling. The blocking groups were chosen such that they could be introduced easily and removed easily under mild conditions. Presumably, the diphenyl and dibenzyl phosphonates could be converted to the free phosphonic acids by catalytic hydrogenolysis.[72] The diphenyl or dibenzyl phosphonates could also be employed as alternate intermediates toward the preparation of XVIII. Therefore, the synthesis appeared to require only a modification of the procedure followed for XVIII.[66]

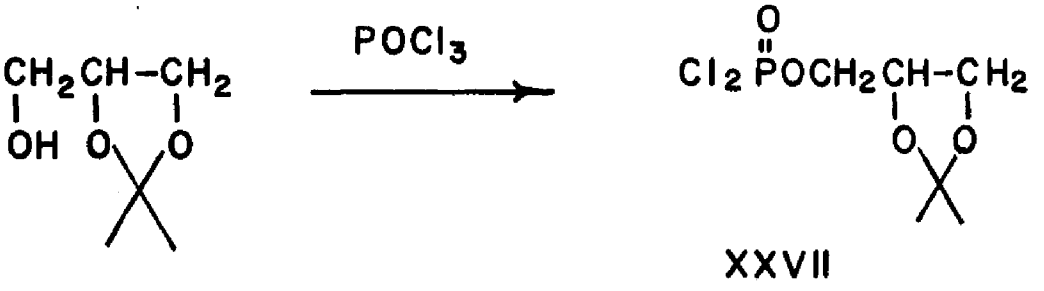
The preparation of the diphenyl and dibenzyl phosphonates presented significant problems. The synthesis of tribenzyl phosphite according to Cramer and Voges[73] was unsuccessful; only starting materials were recovered. The respective phosphorylation product could not be obtained when triphenyl phosphite was allowed to react with an alkyl halide following a procedure by Morgan and Herr.[74] Difficulties in the use of these procedures have been noted by others.[75] The use of diphenylethyl phosphite in a hydrophosphinylation reaction[76] appeared to be a good alternative. It was prepared by the reaction of diphenyl phosphorochloridite[77] with ethanol. (Scheme 22).



SCHEME 22

When scheme 18 was followed using diphenylethyl phosphite decomposition of the phosphite occurred yielding phenol and unreacted starting materials, and other adducts involving phenyl group loss as indicated by nmr. This reaction should be of interest for further mechanistic evaluation.

The hydroxyl groups of glycerol-3-phosphate were to be protected by an acetonide linkage prior to the coupling step. Baer, et al.[78] reported the synthesis of acetoneglycerylphosphorus dichloride (XXVII) by the reaction of isopropylidene glycerol[71] and phosphorus oxychloride. (Scheme 23). The reaction product was a lachrymator and difficult to

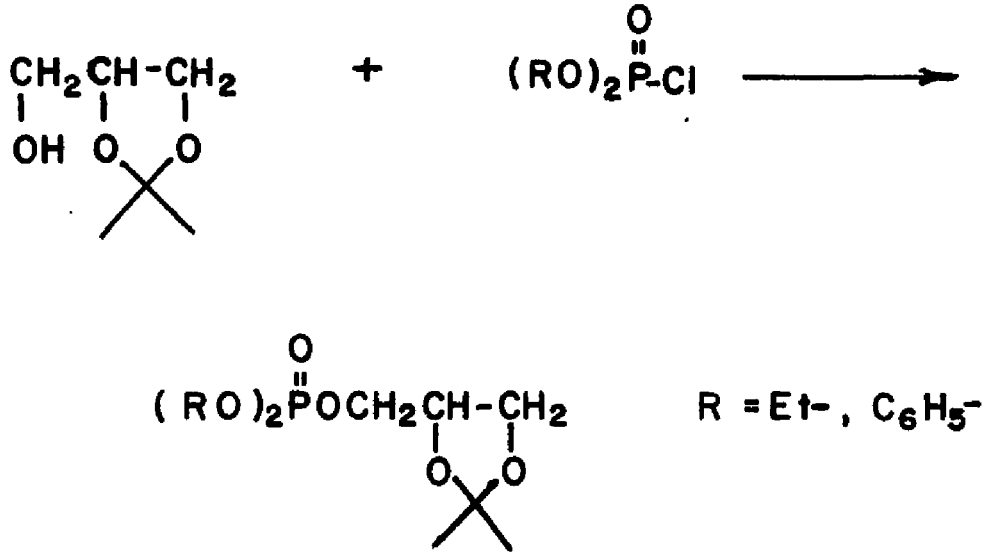


XXVII

SCHEME 23

handle and contain due to its "explosive" properties. This procedure was deemed fruitless. Based on the studies by Baer and Stancer,[79] the diphenyl and diethyl esters of glycerol-3-phosphate acetonide were prepared by the reaction of isopropylidene glycerol[71] and diphenyl phosphorochloridate and diethyl phosphorochloridate, respectively. (Scheme 24). A diethyl alkyl phosphonate was subjected to ester hydrolysis by treatment with trimethylchlorosilane and water[80] as a model for phosphate ester removal. However, no free acid was observed. In retrospect it is the belief of this author that the reaction time was not sufficient for the reaction to run to completion. (See chapter on SUGGESTIONS FOR

FUTURE RESEARCH).

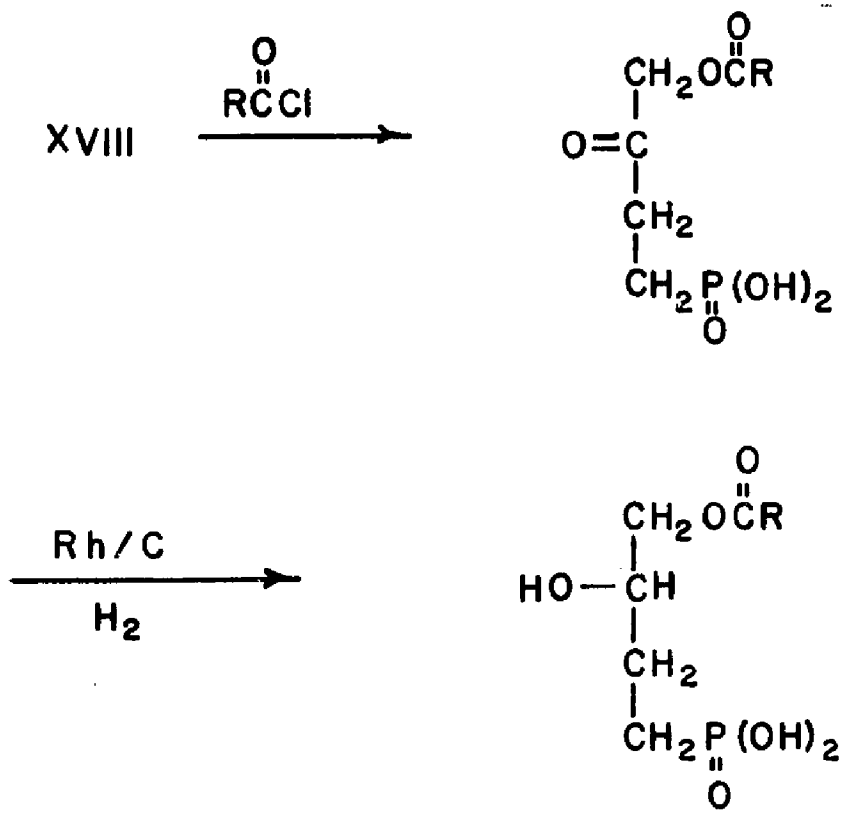


SCHEME 24

The preparation of the dicapryl derivative of phosphotidic acid was attempted by two methods inorder to obtain greater solubility in aqueous systems for biological studies. The first approach parallelled the preparation of the dipalmitoyloxybutyl-1-phosphonic acid[44] employing capryl chloride[81] in place of palmitoyl chloride. Due to the particular characteristics of this system (vide supra), including lower melting point, the 3,4-dicapryloxybutyl-1-bromide could not be isolated through crystallization or distillation. Chromatographic techniques were attempted but proved unsuccessful. It is the belief of this author that the desired product was formed, but its isolation is yet to be accomplished. A second approach involved direct acylation of II using capric anhydride through a modification of scheme 15. Again difficulties were encountered in purification.

The preparation of the capryl derivative of lysophosphotidic acid

XXVIII was attempted following a procedure reported by Tang, et al.[82] (Scheme 25). A white solid was obtained believed to correspond to



XXVIII

SCHEME 25

the carbonyl precursor. The nmr data was completely in accord with the structure of the desired compound. But elemental analysis was out of the range for experimental error.[83,84] Specially designed tlc systems indicated one component, although impurities were obviously present, probably inorganic.

EXPERIMENTAL

Reagents

Acetone, Acetonitrile, Benzyl alcohol, 4-Bromo-1-butene, 2-Butyne--1,4-diol, Capric acid, Dicyclohexylcarbodiimide, Diethyl phosphorochloridate, Dimethylaniline, Diphenyl phosphorochloridate, Hexanes, Methyl-p-toleunesulfonate, Oxalyl chloride, Phenol, Phosphorus trichloride, Trichloroacetonitrile, Trichloroethylchloroformate, Triethyl phosphite, Trimethylchlorosilane, Triphenyl phosphite, (Aldrich Chemical Co.) were used without further purification.

Palmitoyl chloride (Aldrich Chemical Co.) was distilled prior to use bp. 128° / 0.11 Torr.

Pyridine (Aldrich Chemical Co.) was dried over KOH pellets and distilled prior to use bp. 115° / 760 Torr.

Chloroform (Aldrich Chemical Co.) was distilled over phosphorus pentoxide (J.T.Baker Chemical Co.) prior to use bp. 61° / 760 Torr.

Triethylamine (Aldrich Chemical Co.) was dried over KOH pellets and distilled prior to use bp. 89° / 760 Torr.

Methanol (Aldrich Chemical Co.) was distilled prior to use bp. 65°/ 760 Torr.

Acetic acid, Acetic anhydride, 88% Formic acid, Glycerol, 30% Hydrogen peroxide, Mercuric oxide, Sodium meta-periodate, Sodium thiosulfate, Tetrahydrofuran, Trichloroethylene, p-Toluenesulfonic acid, (J.T.Baker Chemical Co.) were used without further purification.

Zinc powder (J.T.Baker Chemical Co.) was activated[70] prior to use.

Diethyl ether (J.T.Baker Chemical Co.) was stored over sodium ribbon prior to use.

Ninhydrin spray (Sigma Chemical Co.).

Phosphorus acid (K & K Laboratories, Inc.) was used without further purification.

Hydrogen bromide (Union Carbide Corp.) was used without further purification.

N,N-dimethylethanolamine (Eastman Organic Chemicals) was distilled prior to use bp. 134° / 760 Torr.

Phosphorus oxychloride (Eastman Organic Chemicals) was used without further purification.

Ethanolamine, Schiff Reagent, (Fisher Scientific Co.) were used without further purification.

MB-3 Amberlite (Mallinckrodt) was equilibrated with 9:1 solution of THF/H_2O prior to use.

Absolute ethanol (Commercial Solvents Corp., "Gold Shield") was used without further purification.

Polygram Sil N-HR sheets (Brinkmann) were used without further treatment.

Phosphospray- a molybdate visualization agent in chromatographic analyses was prepared as previously described by Dittmer and Lester.[85]

Instrumentation

NMR spectra were measured using Varian EM-360 and Varian A 60-A spectrometers. Spectra were obtained on either neat samples or in solution at a probe temperature of 37° .

Infrared spectra were measured using a Perkin Elmer model 237B. Polystyrene film was used for calibration.

Mass spectra were measured using a Varian MAT CH-7 instrument and calibrated against perflouorokerosene.

Elemental analyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, N.Y., and Galbraith Laboratories, Inc., Knoxville, Tenn.

Melting points were obtained on a Hoover Uni-Melt Capillary Melting Point apparatus.

Gelman Metricel Alpha-6 filter.

Centrifugations were performed with a Sorvall RC2-B automatic refrigerated centrifuge.

Acetoxymethyl vinyl ketone (XIX)

2-Butyne-1,4-diol (140 g, 1.63 mole) was heated with acetic anhydride (385 ml, 4 mole) on a steam cone for 2 hr. Distillation of the resulting clear orange solution afforded 243.5 g (1.52 mole, 93.4%) of the corresponding diacetate. The clear colorless liquid was collected at 88° / 0.1 Torr . Into a 1-liter 3-neck RB flask, equipped with condenser, and dropping funnel was placed 11.4 g mercuric oxide, 5 ml concentrated sulfuric acid, 114 ml glacial acetic acid. The diacetate (243.5 g, 1.52 mole) was added dropwise. The reaction temperature was maintained at 40-50° with an ice-water bath. After diacetate addition was complete, an extra 5.7 g mercuric oxide was added and the mixture was stirred 30 min. Dried sodium acetate (22.8 g) was added to neutralize the acid. The mixture was suction filtered and distilled under reduced pressure to remove acetic acid. Vacuum distillation of the remaining brown material yielded a clear colorless liquid at 59° / 0.15 Torr. 143.0 g (1.12 mole, 73.5%) XIX was collected in light-free, Dry Ice-acetone chilled flasks. This material was prepared immediately prior to use due to its tendency toward rapid polymerization.

Diethyl 1-Acetoxy-2-ethoxybut-2-enyl-4-phosphonate (XX-a)

To a cooled (0°) solution of 90 g (0.70 mole) of ketone XIX and 130 g (2.8 mole) of absolute ethanol was added dropwise 133 g (0.8 mole) triethyl phosphite.[86] The solution was stirred for 1 hr

at 0° and overnight at room temperature. Upon fractional distillation there was obtained a colorless liquid, bp 145°C / 0.3 Torr . The nmr data for this material indicated that a mixture of three compounds was present; the two isomeric enol ethers, XX-a and -b, and the diethyl ketal XX-c. Nmr (CCl_4): δ 0.9-1.40 showed a multiplet for the methyl protons in $C\underline{H}_3CH_2O$- of the diethyl esters, enol ethers and diethyl ketals. At δ 1.50-2.90 there was a multiplet corresponding to the methylene protons in $PC\underline{H}_2C\underline{H}_2$ of the diethyl ketal and enol ether XX-b and the methylene protons in $PC\underline{H}_2CH$ of the enol ether XX-c. The singlet at δ 2.05 represented the methyl protons of the acetate $O\text{-}C(O)\text{-}C\underline{H}_3$. From δ 3.20-3.60 a multiplet corresponding to the methylene protons of the enol ethers and diethyl ketal was observed. From δ 3.70-4.35 a multiplet corresponding to the methylene protons in $CH_3C\underline{H}_2O$- of the diethyl esters was shown. A singlet was observed at δ 4.60 corresponding to the methylene protons in $-C(O)\text{-}C\underline{H}_2OC(O)CH_3$ of the diethyl ketal XX-c. Further attempts at separation were unsuccessful. This mixture was considered suitable for the next stage in the synthesis, however. The yield, based on XX-a, was 140 g (0.48 mole, 68.6%).

Monosodium salt of 4-hydroxy-3-oxobutyl-1-phosphonic acid(XXI)

A solution of 10 g (0.034 mole based on XX-a) of the mixture XX-a, -b, -c, 30 ml concentrated hydrochloric acid, and 70 ml water was refluxed for 22 hr. The volatile components were removed under reduced pressure and the pale yellow oil was dissolved in water, treated with activated charcoal, and filtered. The water was evapor-

ated and the ir and nmr spectra of the resulting oil were in accord with the proposed structure XVIII for the phosphonic acid. The data obtained for XVIII are as follows: ir (film) 2.95, 3.60, 5.85, 7.17, 8.20, 9.45, 10.16, 10.22, 12.75, 14.13 μ; nmr (TFA) δ 2.22-3.16 (m, 4H, PCH_2CH_2), 4.62 (s, 2H, CH_2O).

This material was dissolved in water and titrated with 1N NaOH to pH 4.0 . The water was evaporated to yield a colorless semisolid to which was added 100 ml absolute methanol. The mixture was stirred until the product crystallized. The resulting white powder was isolated by suction filtration and the filtrate was concentrated to about 10 ml. The concentrate was added to 100 ml of absolute ethanol and the resulting second crop of white powder was isolated by suction filtration. The solids were combined and washed with absolute ethanol and then with anhydrous ether. The filtrate and wash solvents were combined and evaporated to give an oil which was retitrated. The powder was dried in vacuo over P_2O_5 and yielded 6.0 g (0.032 mole, 94.0%) of pure XXI: ir (Nujol) 3.00, 5.90, 6.95, 7.90, 8.17, 8.80, 9.45, 9.97, 10.20, 11.20 μ; nmr (TFA) δ 2.15-3.10 (m, 4H, PCH_2CH_2), 4.68 (s, 2H, CH_2O). Anal. Calcd for $C_4H_8O_5Na$ P: C, 25.26; H, 4.21 . Found: C, 25.05; H, 4.51 .

3,4-Dipalmitoyloxybutylphosphonylcholine (XXII)

To a mixture of 2.0 g (0.0031 mole) of 3,4-dipalmitoyloxybutyl-1-phosphonic acid,[44] (XII),[45] 5.0 g (0.018 mole) of choline tosylate, and 55 ml of pyridine, warmed to 50° , was added dropwise 15.8 g (0.11 mole) of trichloroacetonitrile. Upon addition, the colorless

solution developed a pink coloration. After stirring for 48 hr at 50° the reaction mixture had become deep red in color. At this time the solution was concentrated under reduced pressure to one-third the original volume and the crude product precipitated by the addition of 75 ml of acetonitrile. The tan colored solid was isolated by suction filtration, washed with acetonitrile, dissolved in a minimum volume of tetrahydrofuran (THF) / water (9:1) and passed through an Amberlite MB-3 column, being eluted with 100 ml of THF / water (9:1). The eluent was evaporated to dryness under reduced pressure; 2-propanol being added to assist in removal of the last traces of water. The solid residue was recrystallized first from boiling hexane and then from trichloroethylene / acetone (1:2) to yield 0.65 g (0.00089 mole, 28.7%) of the purified racemic product XXII of mp 220° (dec) which analyzed as the hemihydrate. The material chromatographed as a single spot of R_f^{85} = 0.40 (visualization with iodine or molybdate [phospho-spray]) with 65:25:4 chloroform-methanol-water and exhibited spectra in accord with the proposed structure. Further quantities of product could be isolated by evaporation of the recrystallization solvent; these exhibited minor impurities by TLC, however, and were not included in the yield as noted. Anal. calcd for $C_{41}H_{82}O_7P$ N (1/2 H_2O): C, 66.48; H, 11.22; P, 4.18; N,1.89 . Found: C, 66.42; H, 11.55; P, 3.98; N, 1.79 .

3,4-Dipalmitoyloxybutylphosphonylethanolamine (XXIII)

To a mixture of 2.0 g (0.0031 mole) of 3,4-dipalmitoyloxybutyl--1-phosphonic acid, (XII),[44] 3.5 g (0.015 mole) of N-(β,β,β-trichloroethoxycarbonyl)ethanolamine,[69] and 70 ml of pyridine, warmed to 50°, was added dropwise 14.4 g (0.10 mole) of trichloroacetonitrile. The reaction mixture was maintained at 50° for 48 hr at which time the orange solution was concentrated under reduced pressure to one-third of the original volume. The crude product was precipitated by the addition of 100 ml of acetonitrile. The tan solid was isolated by suction filtration, washed with acetonitrile and stored in a vacuum dessicator. To this adduct (2.5 g) was added sufficient glacial acetic acid for complete dissolution. There was then added 10 g of activated zinc powder[70] with 10 ml of glacial acetic acid and 20 ml of ether, and the reaction mixture stirred at rcom temperature for 16 hr. There was at this time added 200 ml of ether, the solution was filtered; the solids being washed with additional ether, and the organic solutions combined. This solution was washed first with 4 times 200 ml water, then with 200 ml of 5% sodium bicarbonate solution saturated with sodium chloride, and dried over sodium sulfate. After filtration, the solvent was removed under reduced pressure to yield an amber residue which was dissolved in a minimum of chloroform. To this was added acetonitrile until precipitation was complete. Upon suction filtration and drying under high vacuum there was isolated 0.65 g (0.00093 mole, 30.0%) of the purified racemic product XXIII as a beige solid of mp 45-47°. The material chromatographed as a

single spot of R_f = 0.75 (visualization with iodine, ninhydrin, or molybdate [phosphospray][85]) with 65:25:4 chloroform-methanol-water and exhibited spectra in accord with the proposed structure. Again, further quantities of product could be isolated by evaporation of the recrystallization solvent; those exhibited minor impurities by TLC,however, and were not included in the yield as noted. Anal. calcd for $C_{38}H_{76}O_7P$ N: C, 66.18; H, 11.03; P, 4.45; N, 2.03 . Found: C, 66.17; H, 11.21; P, 4.14; N, 1.83 .

3,4-Dipalmitoyloxybutylphosphonylglycerol (XXIV)

To a mixture of 2.0 g (0.0031 mole) of 3,4-dipalmitoyloxybutyl-1-phosphonic acid,(XII),[44] 2.0 g (0.015 mole) of isopropylidene glycerol,[71] and 50 ml of pyridine, warmed to 50° , was added dropwise 14.4 g (0.10 ml) of trichloroacetonitrile. The reaction mixture was maintained at 50° with stirring for 48 hr at which time the orange-red solution was concentrated under reduced pressure to one-third the original volume. The crude product was precipitated by the addition of 100 ml of acetonitrile. The tan solid was suction filtered, washed with acetonitrile, and dried under vacuum. The solid was taken up in 100 ml of 10% aqueous acetic acid, stirred overnight and the material precipitated by the addition of acetonitrile. This solid was suction filtered, dried, and subjected to chromatographic separation on a silica gel column (38 x 280 mm), being eluted with 65:25:4 chloroform-methanol-water. Fractions of 15 ml were taken from the column, the desired product being found in fractions 8 through 30.

These fraction were evaporated to dryness. The residue was taken up in a small volume of chloroform, filtered through a Gelman Metricel Alpha - 6 filter, and the solvent removed under vacuum. In this manner was isolated 0.15 g (0.00021 mole, 6.7%) of the purified product XXIV of mp 80° . Further amounts of product were found in later fractions of the chromatograph, but other materials were also indicated to be present by TLC. The band for XXIV is quite broad, probably due to the presence of two enantiomeric pairs, rendering the chromatographic technique less than ideal. Use of the periodate-Schiff reagent[87] in TLC visualization confirmed that rearrangement along the glycerol backbone had not occurred with the isolated product. The material chromatographed as a single spot of R_f = 0.89 (visualization with iodine, molybdate[85] or periodate-Schiff reagent[87]) with 65:25:4 chloroform-methanol-water and exhibited spectra in accord with the proposed structure. Anal.calcd for $C_{39}H_{77}O_9P$: C, 65.00; H, 10.69; P,4.29 . Found: C, 64.91; H, 10.71; P, 4.12 .

Attempted preparation of Tribenzyl phosphite

Into a 2-liter 3-neck RB flask,equipped with mechanical stirrer, condenser, dropping funnel and nitrogen flowing, added 330 g (3.0 mole) benzyl alcohol, 370 g (8.2 mole) dimethylaniline and 300 ml anhydrous ether. Set the system in an ice-water bath between 0-5° for 2 hr during which a mixture of 136 g (1.0 mole) phosphorus trichloride in 100 ml anhydrous ether was added dropwise. Allowed the reaction to stand for 30 min at room temperature. Added 200 ml ether and filtered, with suction the dimethylaniline-hydrochloride salt. The salt was washed with ether, and then placed overnight at -20° . The ethereal filtrate was saved. Liquid was decanted from the salt and combined with the ethereal filtrate saved. The solvent was removed under reduced pressure and the residue was vacuum distilled. Nmr data showed that the crude pot residue was mainly unreacted starting alcohol.

Attempted preparation of Diphenyl 3-butene-1-phosphonate

Into a 200 ml RB flask, equipped with a condenser, placed 20 g (0.15 mole) 4-bromo-1-butene and 46.5 g (0.15 mole) triphenyl phosphite. Allowed the mixture to reflux overnight, and set at room temperature to cool. The reaction flask was then chilled in the freezer for several hr. The expected [74] crystalline intermediate was never obtained, and only starting materials were observed.

Diphenyl phosphorochloridite

Into a 1-liter 3-neck RB flask, equipped with condenser, drying tube and thermometer, placed 400 g (2.92 mole) phosphorus trichloride. Gradually, 550 g (5.84 mole) phenol was added. The evolution of a gas (HCl) and a decrease in reaction temperature to -5° was noted. After the addition was complete, the solution was stirred for 1 hr and then heat was applied until the temperature attained 180° (bp.of phenol). The system was allowed to cool and was then vacuum distilled.yielding 338.5 g (1.34 mole, 45.5% yield) at 110-115° / 0.7 Torr. Mass spectra data showed a peak at 252 indicative of the desired product with a molecular weight of 252.

Diphenylethyl phosphite

Into a 1-liter 3-neck RB flask, equipped with condenser, dropping funnel, nitrogen flowing and ice bath, placed 40 g (0.5 mole) dry pyridine, 23 g (0.5 mole) absolute ethanol, and 50 ml sodium dried hexane. The flask was chilled and a solution of 108.5 g (0.43 mole) diphenyl phosphorochloridite in hexane was added dropwise. The evolution a a gas (HCl) was noted. A white precipitate formed during later stages of the reaction. The white solid was filtered with suction under nitrogen; washed with hexane. The filtrate and washings were combined and the volatile materials were removed under reduced pressure. The residue was vacuum distilled yielding 92.5 g (0.35 mole, 80.6%) at 92° / 0.025 Torr. Nmr data indicated a clean triplet at δ1.55-1.80

corresponding to the methyl protons of $\underline{CH_3}-CH_2-O$; a clean quartet at δ 4.30–4.80 representing the methylene protons of $CH_3\underline{CH_2}-O$. The phenyl protons were observed between δ 7.10–7.70 .

Attempted preparation of Diphenyl 4-acetoxy-3-oxobutyl-1-phosphonate

To a cooled (0°) solution of 71 g (0.55 mole) ketone XIX and 80 g (1.74 mole) absolute ethanol was added dropwise a solution of 92.5 g (0.35 mole) diphenylethyl phosphite in 20 g (0.43 mole) absolute ethanol. The solution was stirred for 1 hr at 0° , and for 48 hr at room temperature. Volatile components were removed under reduced pressure and the residue was vacuum distilled. Nmr data indicated phenol and other decomposed starting materials.

Attempted preparation of Acetoneglycerylphosphorus dichloride(XXVII)

Into a 2-liter 3-neck RB flask, with condenser, mechanical stirrer, dropping funnel, nitrogen flowing and ice bath, placed 95.8 g (0.625 mole) phosphorus oxychloride in anhydrous ether. The flask was chilled and a mixture of 82.5 g (0.625 mole) isopropylidene glycerol and 49.4 g (0.625 mole) dry pyridine and ether was added dropwise. After addition was complete, the system was allowed to reach room temperature (30 min). The pyridinium hydrochloride was filtered and volatile components were removed under reduced pressure. It was noted that the system created internal pressure making even distillation under vacuum hazardous. Polymers easily formed.

Diphenyl acetoneglycerylphosphate

Into a 1-liter 3-neck RB flask, equipped with condenser, dropping funnel, nitrogen flowing, mechanical stirrer and ice bath, placed 51 g (0.19 mole) diphenyl phosphorochloridate and anhydrous ether. Dropwise was added an ethereal solution of 25.1 g (0.19 mole) isopropylidene glycerol and 19.2 g (0.19 mole) freshly distilled triethylamine. After the addition was complete the system was allowed to stir 1 hr. The hydrochloride salt was filtered and washed with ether. The filtrate and washings were combined and the volatile components were removed under reduced pressure. Nmr data on the crude residue indicated that product may have formed. The observed doublet at δ 1.00-1.20 corresponded to the methyl protons in $C-(\underline{CH}_3)_2$. The multiplet at δ 3.20-4.20 represented the methylene protons in the glycerol backbone $-O-\underline{CH}_2-\underline{CH}(-O-)-\underline{CH}_2-O$. A multiplet observed at δ 6.70-7.20 was indicative of aromatic protons. Vacuum distillation of the residue resulted in decomposition to phenol and polymers.

Diethyl acetoneglycerylphosphate

Into a 1-liter 3-neck RB flask, equipped with condenser, dropping funnel, nitrogen flowing, mechanical stirrer and ice bath, placed 32.7 g (0.19 mole) diethyl phosphorochloridate in anhydrous ether. Dropwise was added an ethereal solution of 25.1 g (0.19 mole) isopropylidene glycerol and 19.2 g (0.19 mole) freshly distilled triethylamine. After the addition was complete the system was allowed to stir 1 hr. The hydro-

chloride salt was filtered and washed with ether. The filtrate and washings were combined and the volatile components were removed under reduced pressure. Nmr data on the crude residue indicated that product had formed. The observed doublet-triplet overlap at δ 1.00-1.35 represented the methyl protons of $C-(C\underline{H}_3)_2$ and $C\underline{H}_3-CH_2-O$. There was a multiplet between δ 3.30-4.20 corresponding to the methylene protons of $-C\underline{H}_2-C\underline{H}(-O-)-C\underline{H}_2-O$.

Diethyl 3-butene-1-phosphonate

Into a 250 ml RB flask, with condenser and drying tube, placed 50.2 g (0.37 mole) 4-bromo-1-butene and 83.0 g (0.50 mole) triethyl phosphite. The mixture was allowed to reflux overnight. Vacuum distillation yielded 39.5 g (0.21 mole, 56.8%) of product collected at 68-70° / 0.1 Torr. Nmr data showed a triplet at δ 0.80-1.20 indicating the methyl protons of $C\underline{H}_3CH_2-O$. At δ 1.20-2.30 there was a multiplet corresponding to the methylene protons of $-P-C\underline{H}_2-C\underline{H}_2-$. A multiplet at δ 3.50-4.00 represented the methylene protons of $CH_3C\underline{H}_2-O-$. The vinyl protons were observed as a triplet at δ 4.50-5.00 corresponding to $-CH=C\underline{H}_2$ and the $-C\underline{H}=CH_2$ as a multiplet at δ 5.30-5.90 .

Attempted preparation of 3-Butene-1-phosphonic acid

Into a 100 ml RB flask, equipped with condenser and drying tube, placed 39.5 g (0.21 mole) diethyl 3-butene-1-phosphonate. Gradually added 91.2 g (0.84 mole) trimethylchlorosilane. The system was allowed

to reflux for 48 hr. The reaction was cooled in an ice bath and 100 ml water was added. After stirring for 1 hr, chloroform was added and the layers were separated. The solvents were removed under reduced pressure. Only starting phosphonate was recovered with trimethylsilyl alcohol.

Capryl chloride

Into a 1-liter RB flask placed 68.8 g (0.40 mole) capric acid, 200 ml sodium dried benzene, and 76.0 g (0.60 mole) freshly distilled oxalyl chloride. After the solution was allowed to reflux for 3 hr, benzene and other volatile components were removed under reduced pressure. The product was vacuum distilled at 70-75° / 0.05 Torr yielding 63.0 g (0.33 mole, 82.5%).

Attempted preparation of 3,4-Dicapryloxybutyl-1-bromide

Into a 1-liter RB flask placed 20.0 g (0.12 mole) 1,2-dihydroxy--4-bromobutane[44] and sodium dried ether. The flask was chilled in an ice bath and 48.0 g (0.48 mole) freshly distilled triethylamine was added. An ethereal solution of 63.0 g (0.33 mole) capryl chloride was added dropwise. The solution was allowed to stir overnight in an ice-chilled bath. A white precipitate was suction filtered and volatiles were removed under reduced pressure. Attempted crystallization of the product as reported by Tang, et al.[44] and high pressure liquid chromatographic separations proved unsuccessful.

Capric anhydride

Into a 1-liter RB flask placed 169.0 g (0.98 mole) capric acid and 400 ml freshly distilled carbon tetrachloride. Dropwise added a solution of 100 g (0.48 mole) dicyclohexylcarbodiimide in 200 ml carbon tetrachloride. After the reaction was stirred for 48 hr, dicyclohexylurea was suction filtered and washed with carbon tetrachloride. The filtrate and washings were combined and the solvent was removed under reduced pressure. The remaining yellow liquid was chilled on ice until a solid crystallized out. This residue, 155 g (0.48 mole, 100.0%) was used as such.

Attempted preparation of 3,4-Dicapryloxybutyl-1-phosphonic acid

Into a beaker placed 6.0g(0.033 mole) dilithium salt of II[66] and dissolved it into 300 ml water. Dowex 50 in H^+ form was added and allowed to stir 0.5 hr. The resin was filtered and washed with water. Solvent was removed from the filtrate under reduced pressure, and the residue was rendered anhydrous by repeated evaporations with dry pyridine. The semi-solid was added to 155 g (0.48 mole) capric anhydride. In a closed system the reaction was allowed to stir for 40 hr at 75-80° . In order to hydrolyze any mixed anhydrides 1000 ml pyridine and 250 ml water were added to the reaction flask and allowed to stir for 48 hr at room temperature. Volatile components were removed under reduced pressure and the remaining liquid was dried under vacuum. The desired product could not be isolated.

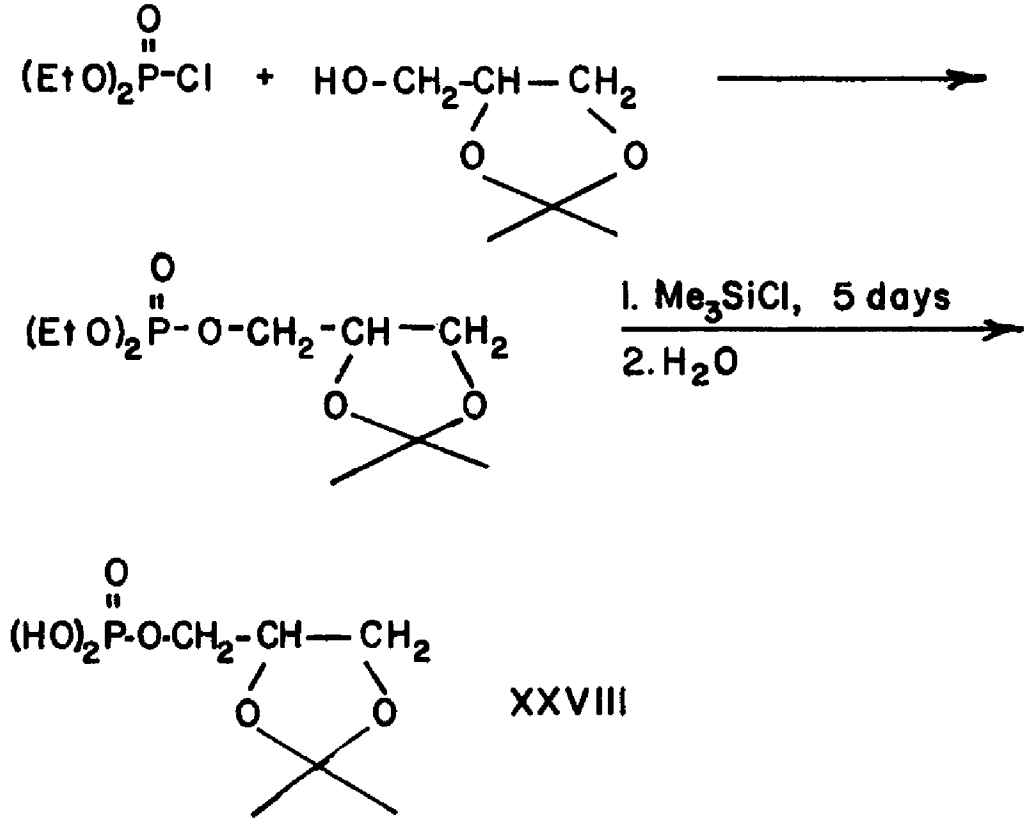
4-Capryl-3-oxobutyl-1-phosphonic acid

Into a 250 ml RB flask,equipped with stirrer, dropping funnel, drying tube and ice bath, placed 8.0 g (0.042 mole) XXI, 7.0 g (0.084 mole) dried and distilled pyridine and 40.0 ml freshly distilled chloroform. Dropwise to the chilled mixture added a solution of 19.0 g (0.10 mole) capryl chloride in chloroform. The reaction was allowed to stir 12 hr at 0°, and 50 hr at room temperature. The system was heated for 1.5 hr at 40° and then poured into 500 ml ice cold ether. The ethereal mixture was transferred into a separatory funnel and 200 ml cold 0.5 N sulfuric acid was added. The two layers were separated and the volume of each was concentrated. A solid remaining in the ether fraction was noted. A sample of this material gave a positive test indicative of a phosphorus-containing compound when treated with phosphospray on chromatographic paper. Nmr ($CDCl_3$) indicated that the compound was mostly capric acid. This yellow semi-solid was redissolved in ether and washed with an aqueous solution of sodium bicarbonate. The ether layer was now colorless and was dried over sodium sulfate. Upon filtration of the drying agent a white solid settled out. This was isolated and ir data (KBr pellet) observed was similar to that for the ir data (KBr pellet) of pure capric acid. But the appearance of a strong phosphoryl band at 1075-1250 cm^{-1} indicated possible product. When compaired to the ir data (KBr pellet) of XXI the significant changes in the band patterns clearly suggested the formation of a new compound and most likely the desired product.Nmr ($CDCl_3$) of the white solid clearly indicated the

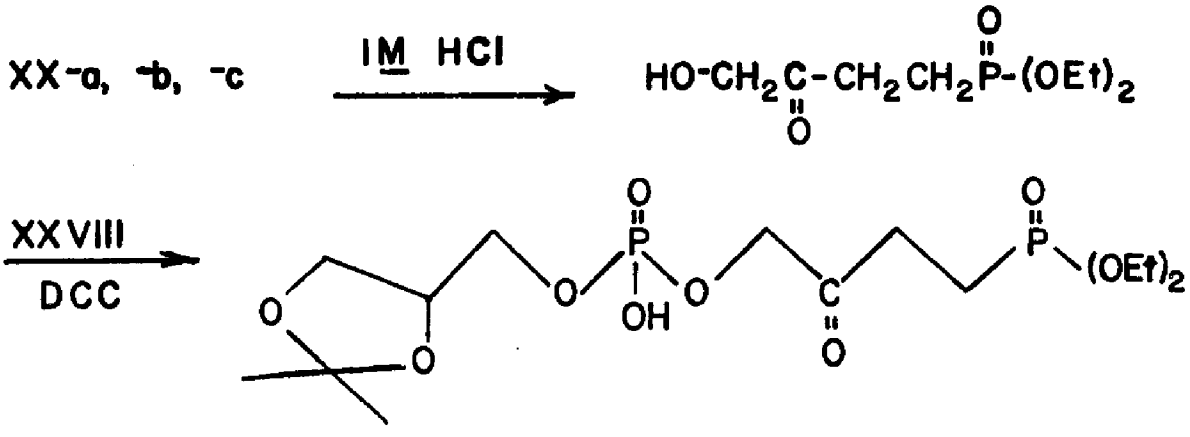
desired product. A broadened singlet at δ 0.50-0.90 represented the methyl protons of $-O-C(O)-(CH_2)_8-\underline{CH_3}$. The singlet at δ 1.20 corresponded to the methylene protons of $-O-C(O)-CH_2CH_2-(\underline{CH_2})_6-CH_3$. The methylene protons of $-P-\underline{CH_2}-\underline{CH_2}-$ are represented by a multiplet at δ 1.60-3.00 with a doublet at δ2.25-2.35 corresponding to methylene protons of $-O-C(O)-\underline{CH_2}\underline{CH_2}-(CH_2)_6CH_3$. The methylene protons of $-C(O)-\underline{CH_2}-O-C(O)-(CH_2)_8-CH_3$ are shown at δ 4.65 . A specially designed tlc system prepared by a mixture of 30.0 g Supelcosil 12A and 72.0 ml 0.01M sodium carbonate spread 0.25mm thick on glass plates and samples developed in chloroform - methanol - acetone - acetic acid - water (160:45:15:20:10) indicated only one spot at R_f 0.30 . Anal.Calcd for $C_{14}H_{27}O_6P$: C,52.17; H,8.38 . Found: C,36.97; H,5.98 . The analysis is greatly out of the range for experimental error but it should be noted that both H and C values are lower and the ratio of H:C in calcd is 0.161 and for found 0.162 . This suggested possible contamination of white solid with inorganics, possibly sodium bicarbonate during the work-up.

SUGGESTIONS FOR FUTURE RESEARCH

The synthesis of XXVI may be accomplished through the following approach:



XXVIII



XXVI

Diethyl phosphate esters can by converted to their free acid by allowing the ester to react with four equivalents of trimethylchlorosilane for a minimum of five days under reflux conditions. The resulting bis(trimethylsilyl) ester can then by hydrolyzed with water. The reaction by-product (trimethylsilyl alcohol) is extractable with chloroform leaving the free phosphoric acid in the aqueous layer. The solvent can then by removed leaving the desired product. This step is to be repeated in later stages of the synthesis to cleave the diethyl phosphonate ester. A recent publication by McKenna, et al.[88] reported an improved procedure using trimethylbromosilane to effect the bis(trimethylsilyl) ester. This is done by allowing a diethyl phosphate (or phosphonate) ester to react with 1-2 equivalents of the trimethylbromosilane at room temperature for 1-2 hr. The yields are in the range of 97-99% . Hydrolysis of the trimethylsilyl derivative is accomplished in neutral aqueous solution at room temperature. The extremely mild conditions employed should be especially useful for the preparation of XXVI.

One should note that there is a possibility for hydrolysis of the internal phosphate linkages of the DCC product when it is treated with trimethylbromosilane. It would be interesting to determine if preferential hydrolysis takes place favoring the external esters.

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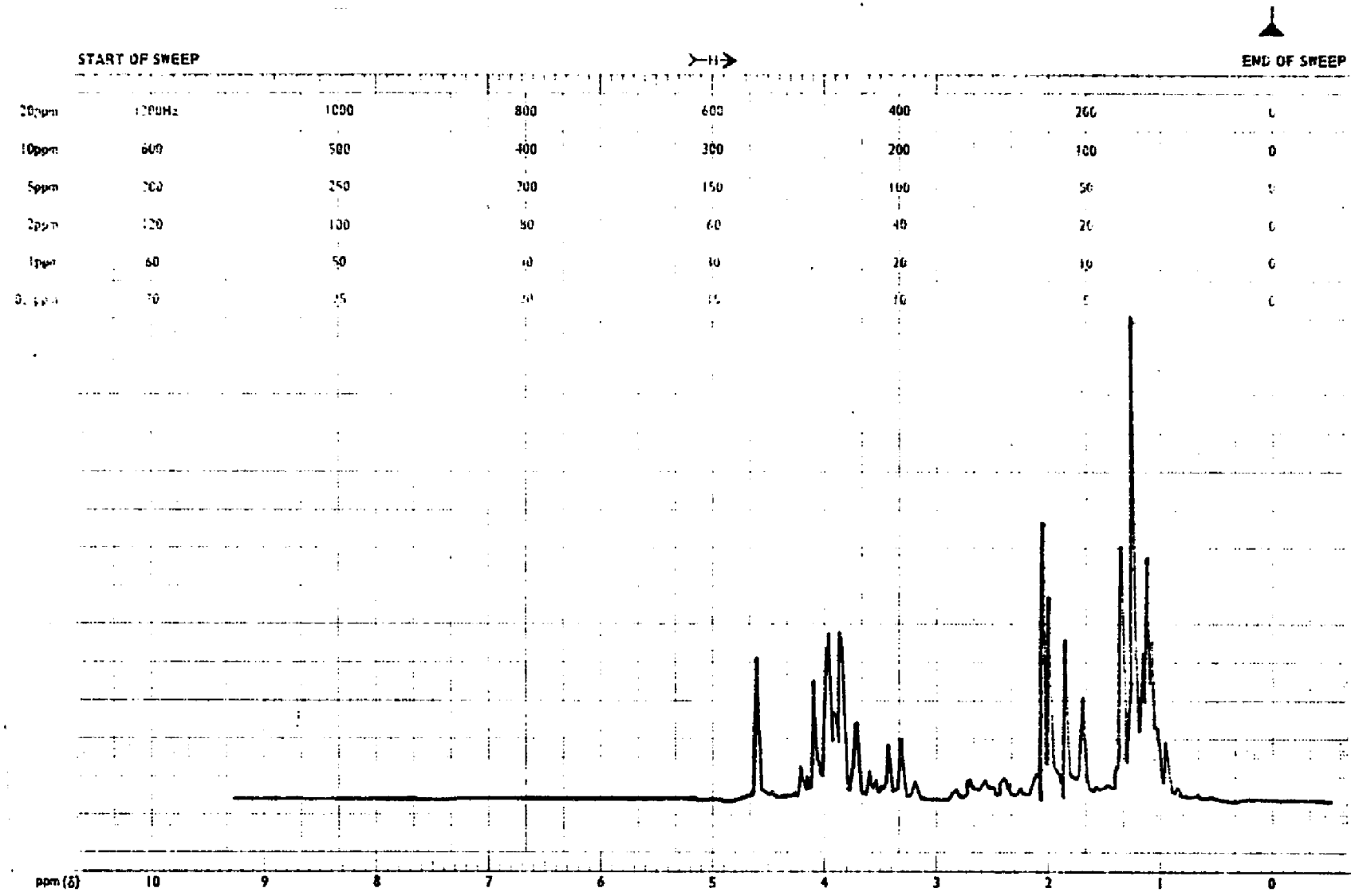
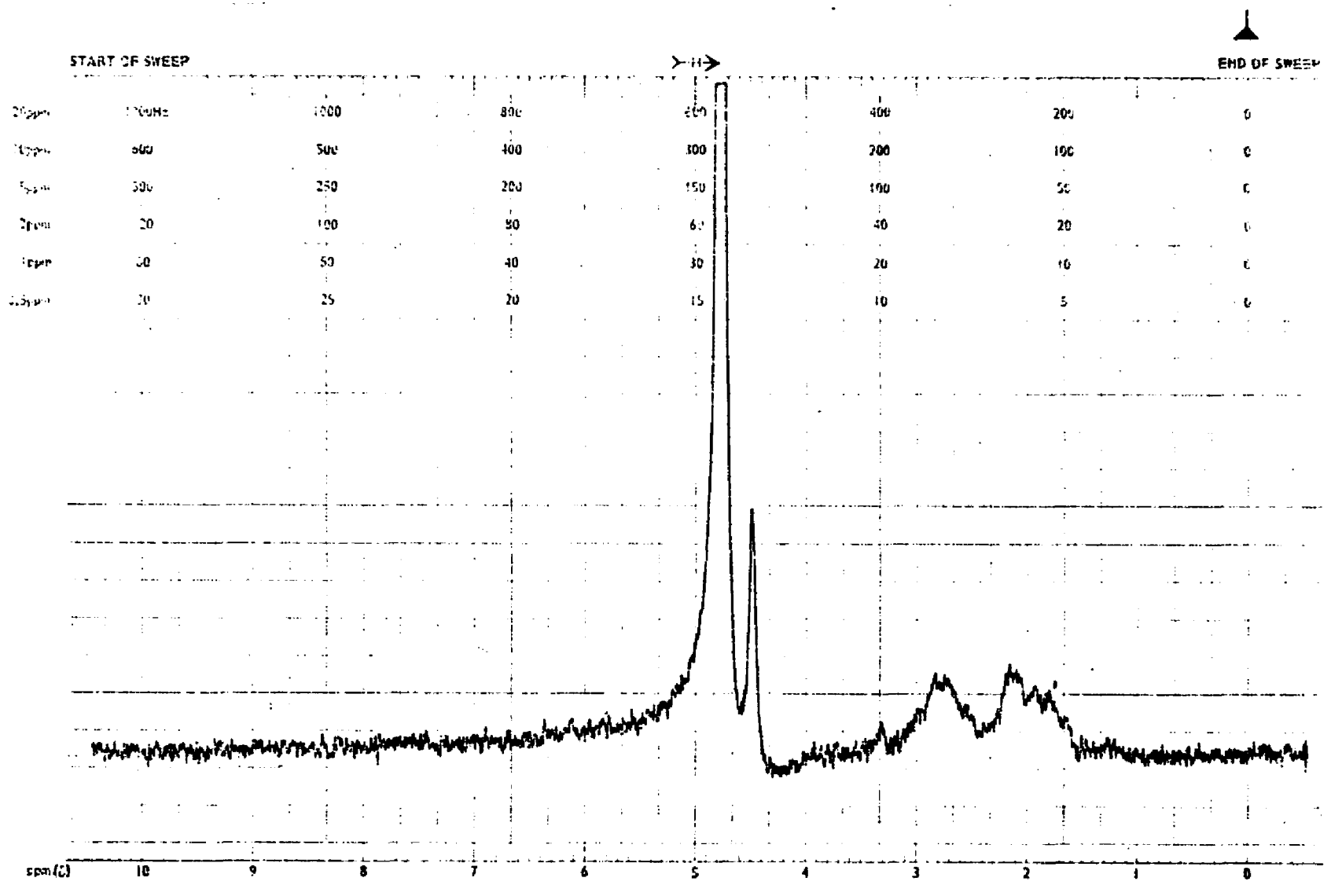
APPENDIX

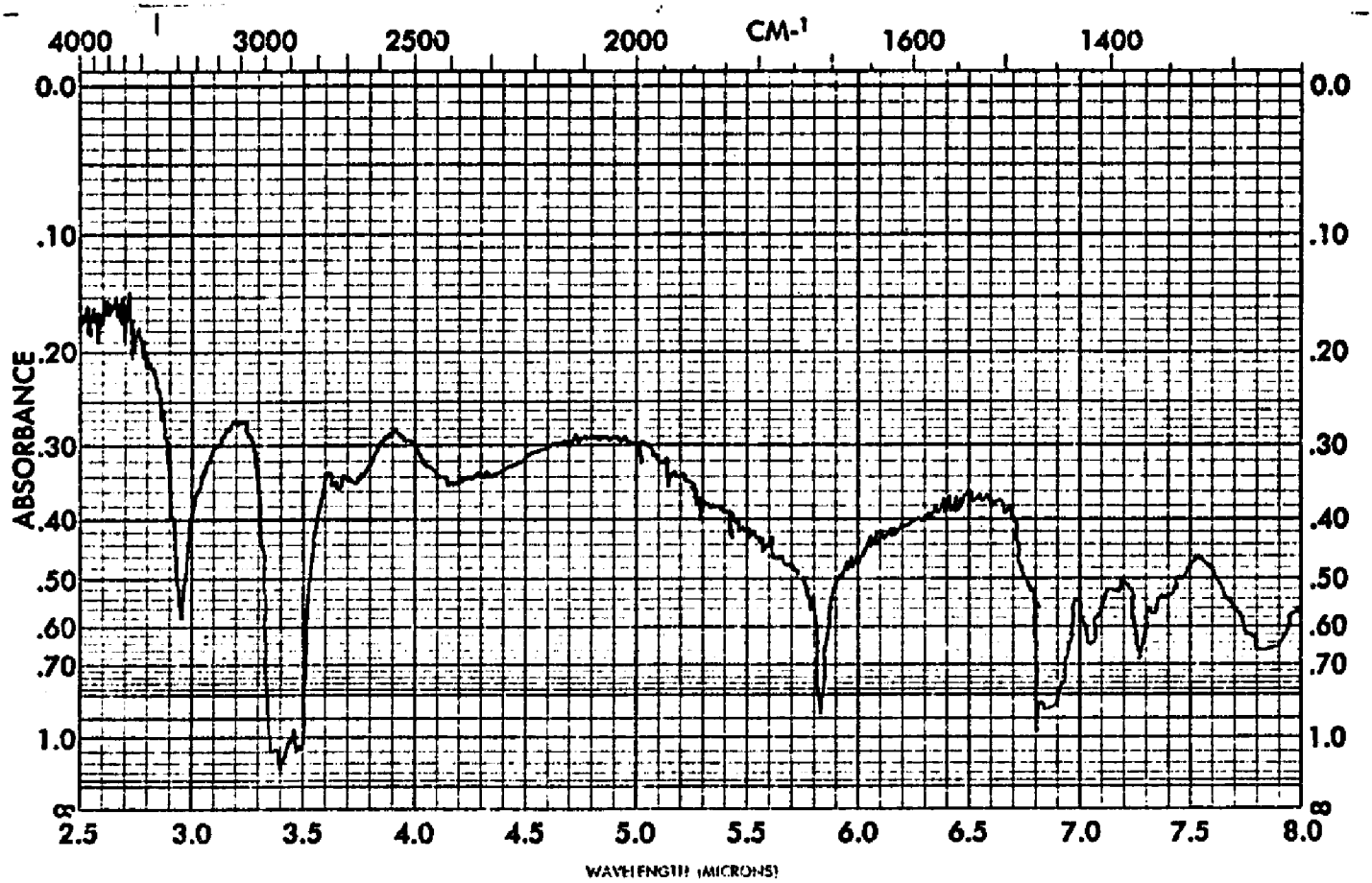
ir and nmr spectra

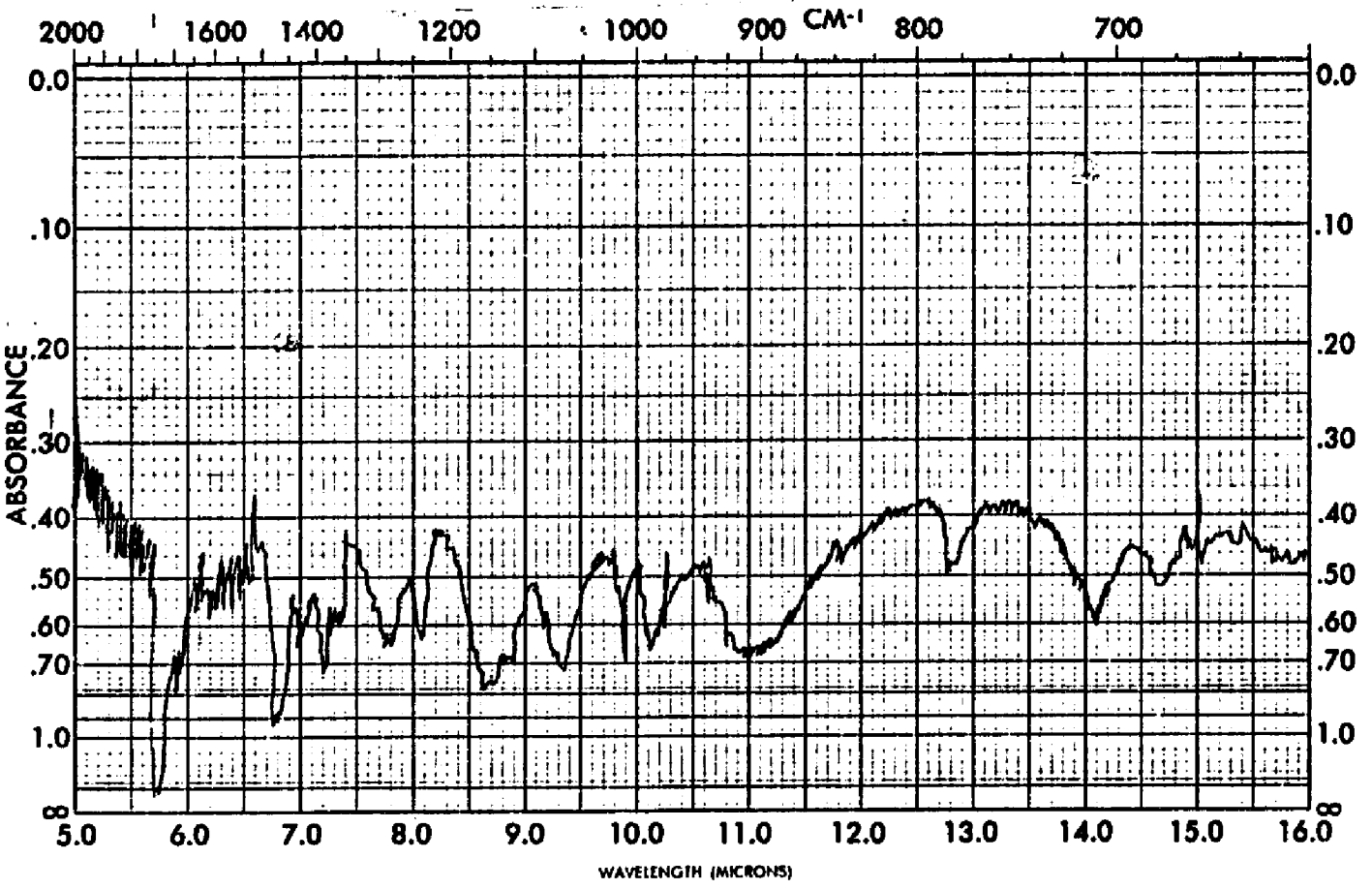


nmr of mixture XX-a, -b, -c
solvent: CCl_4

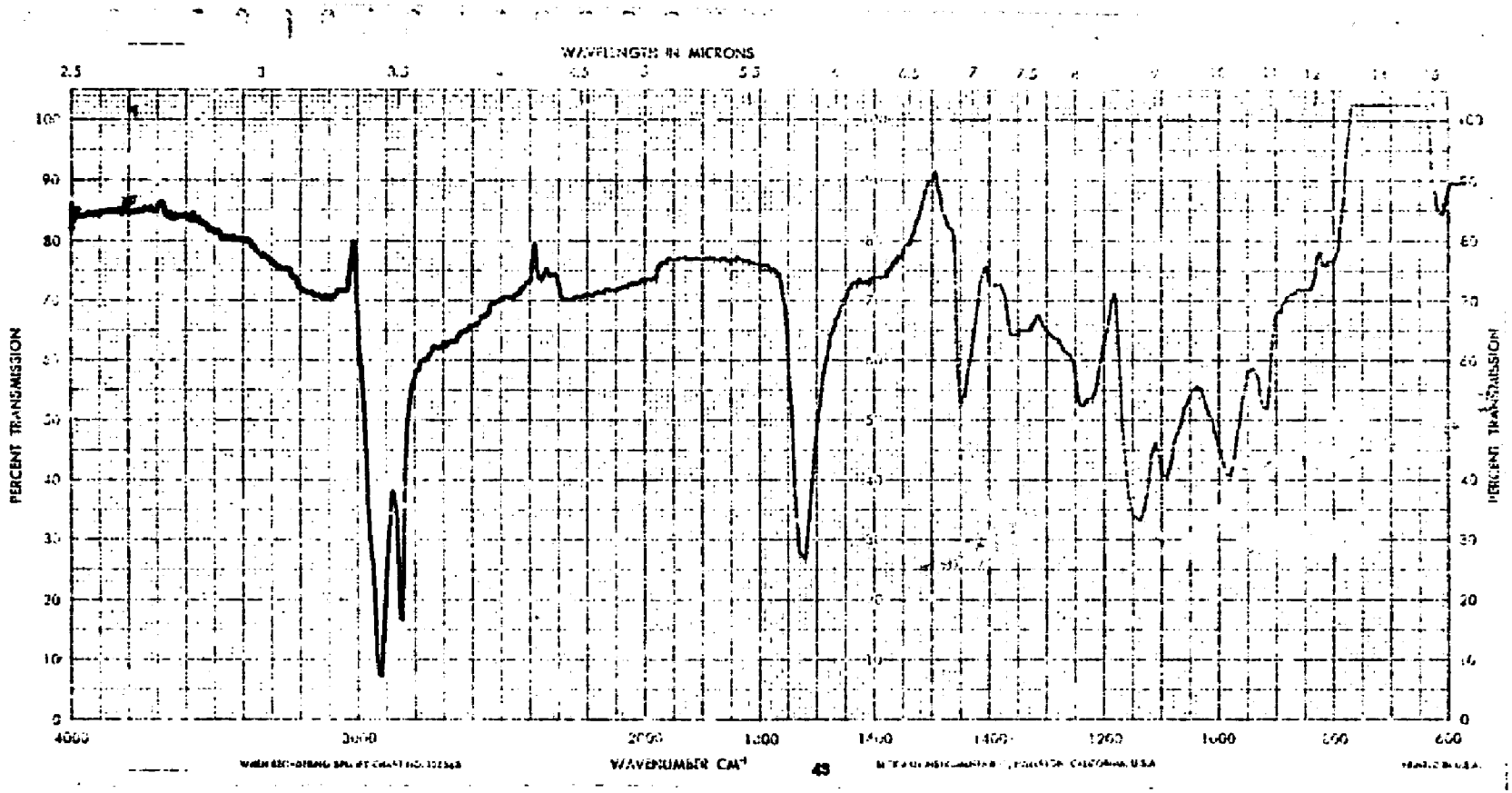


nmr (D_2O, CD_3COOD) of XXI

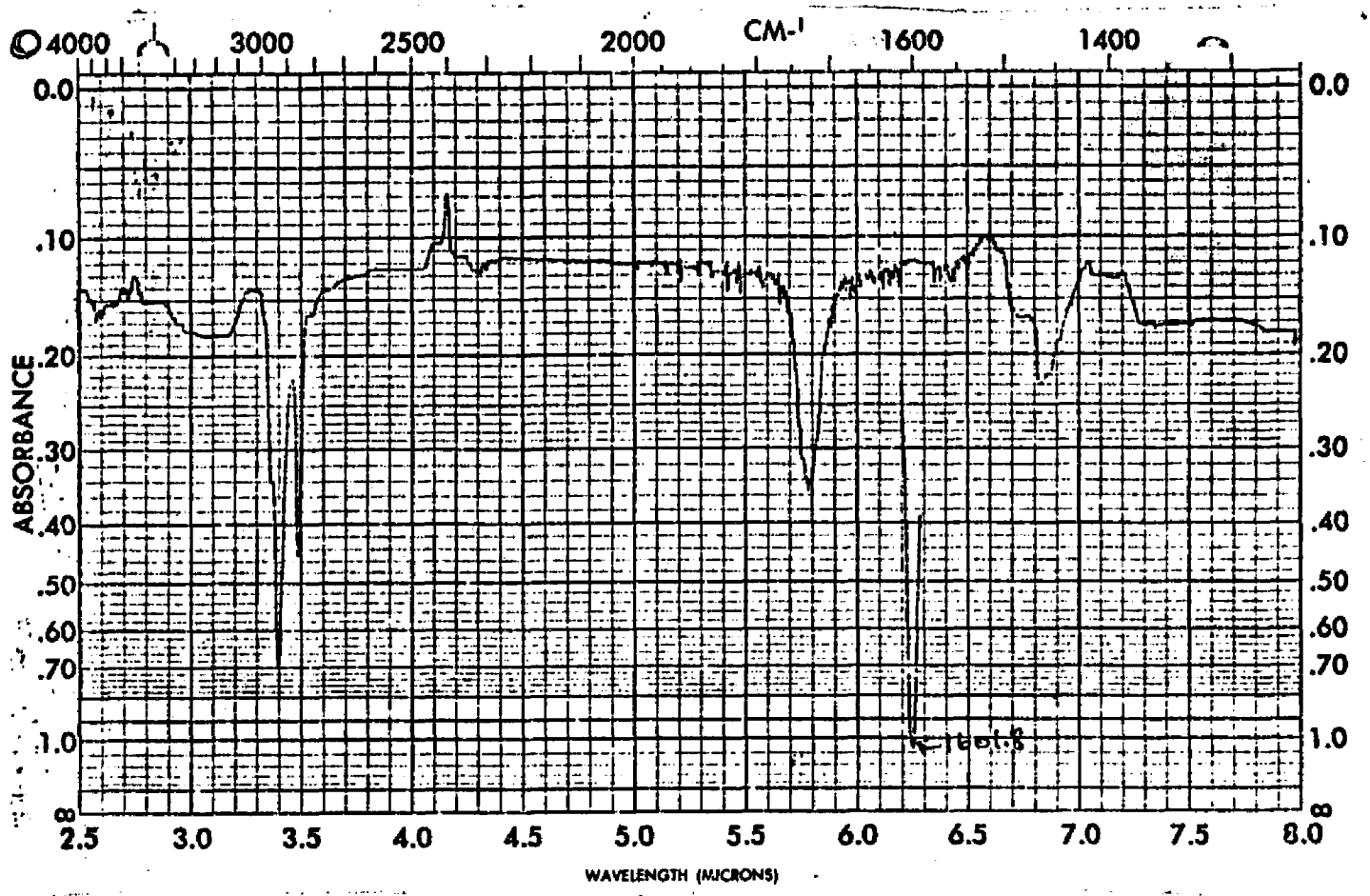


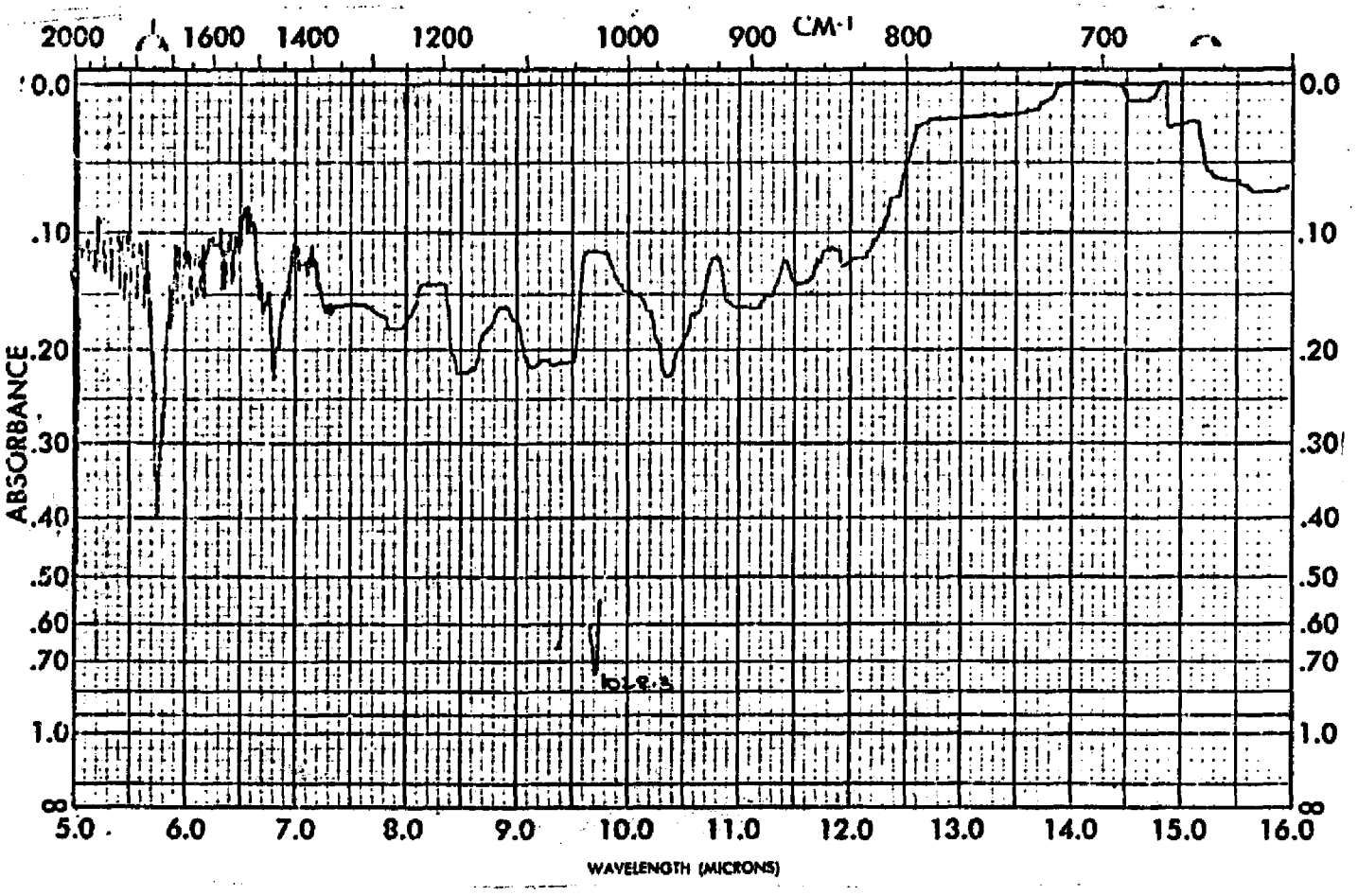


ir (mineral oil) of XXI

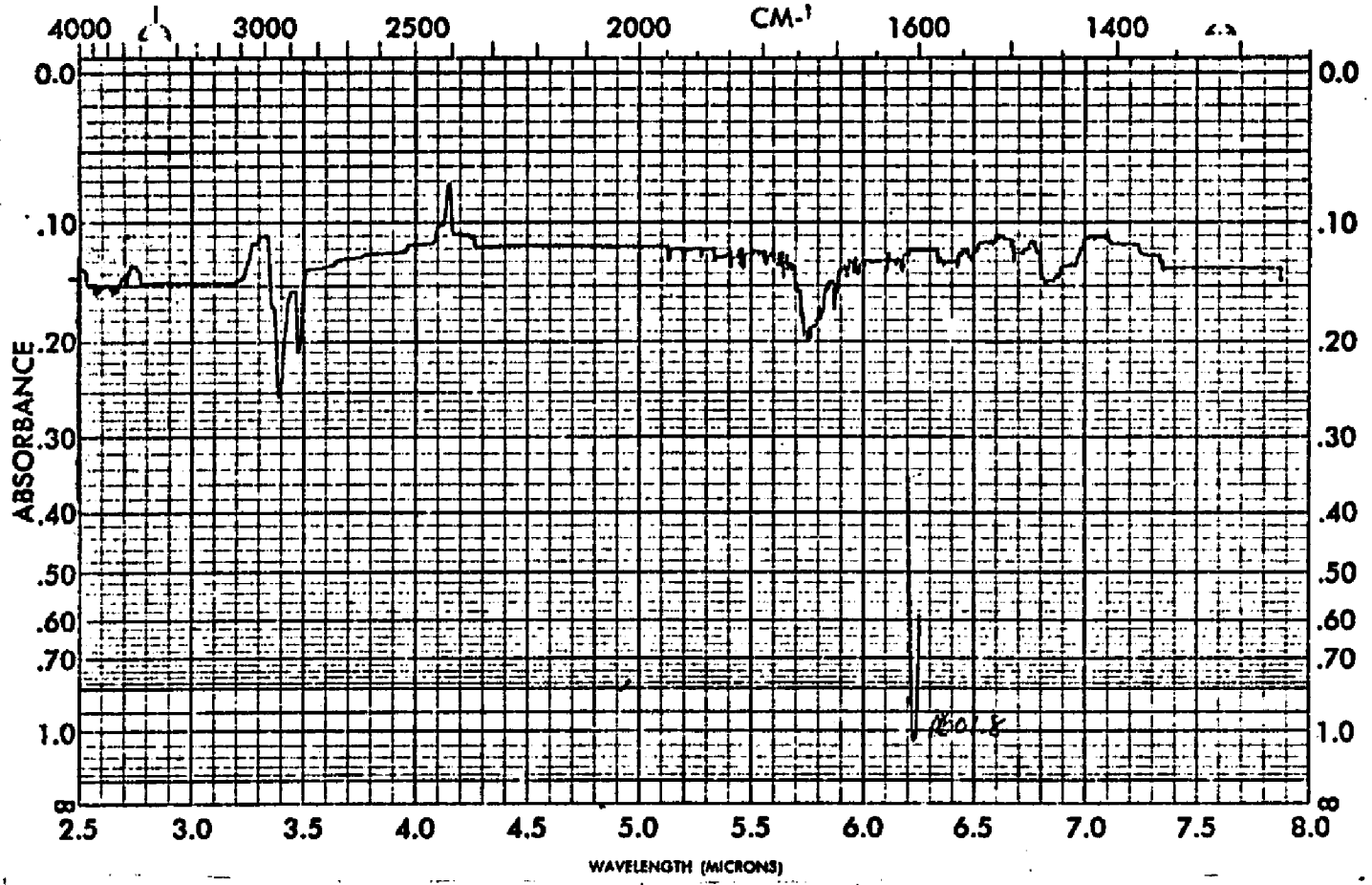


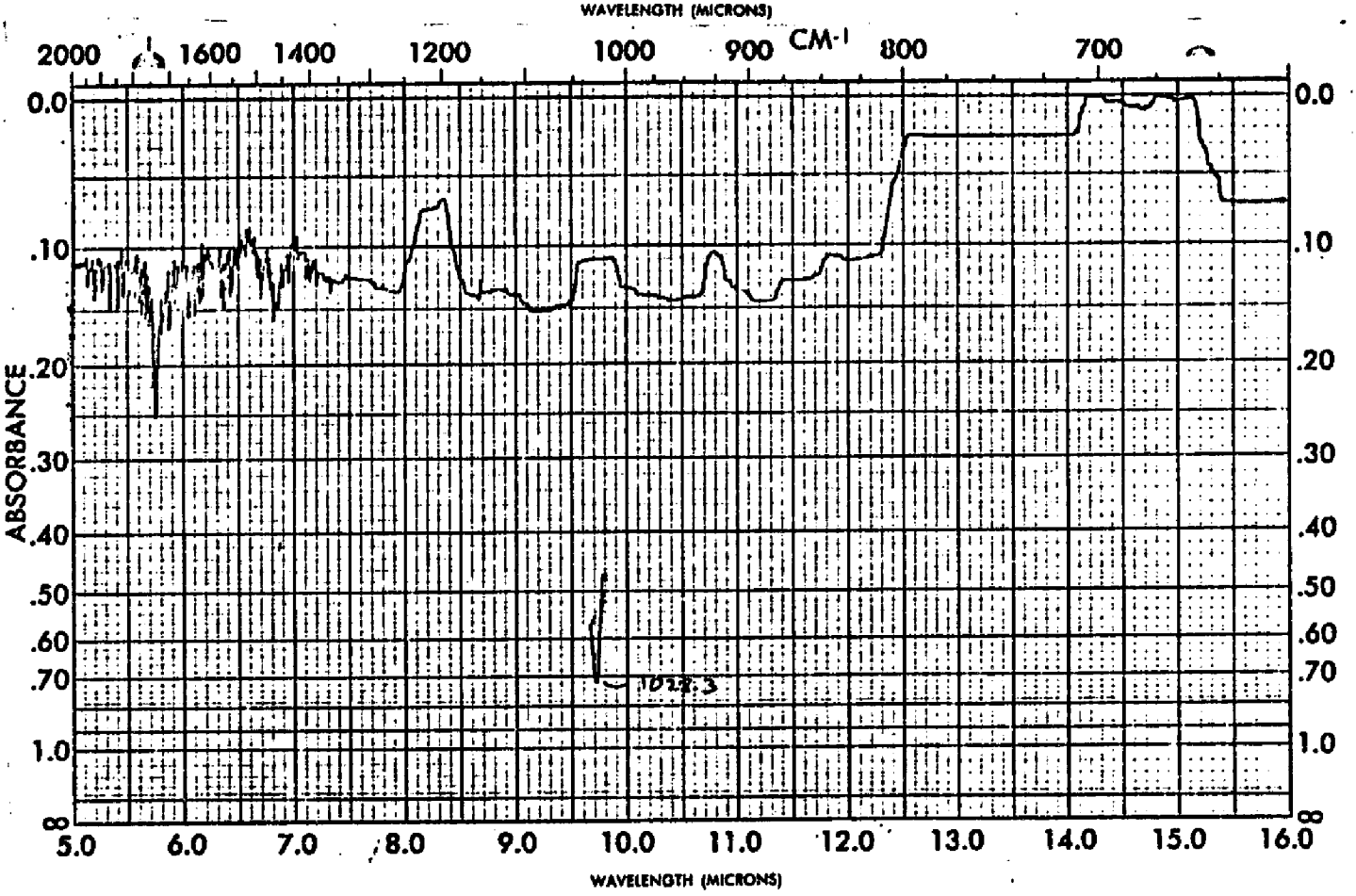
ir (mineral oil) of XII





ir ($CHCl_3$) of XXII





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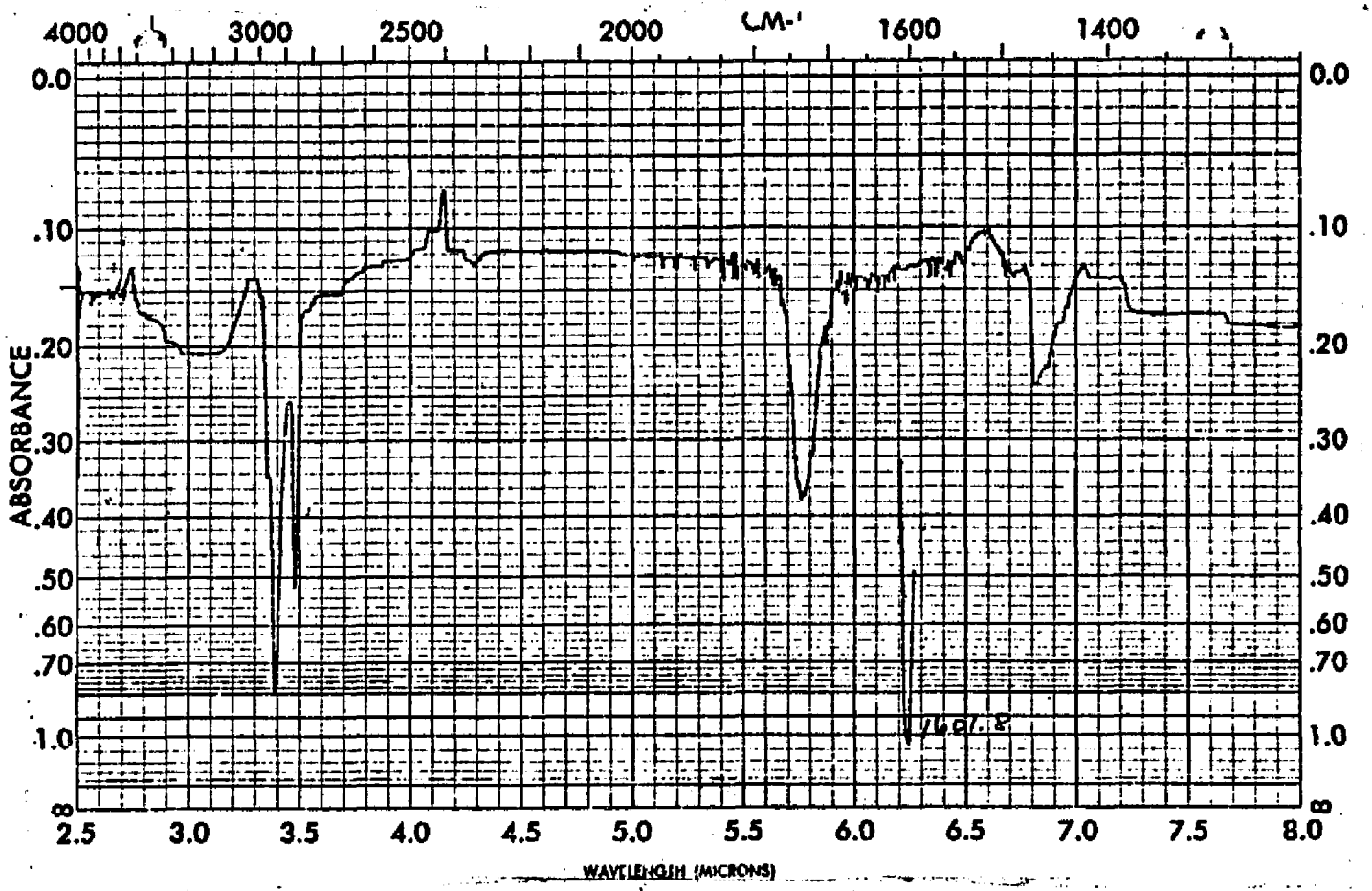

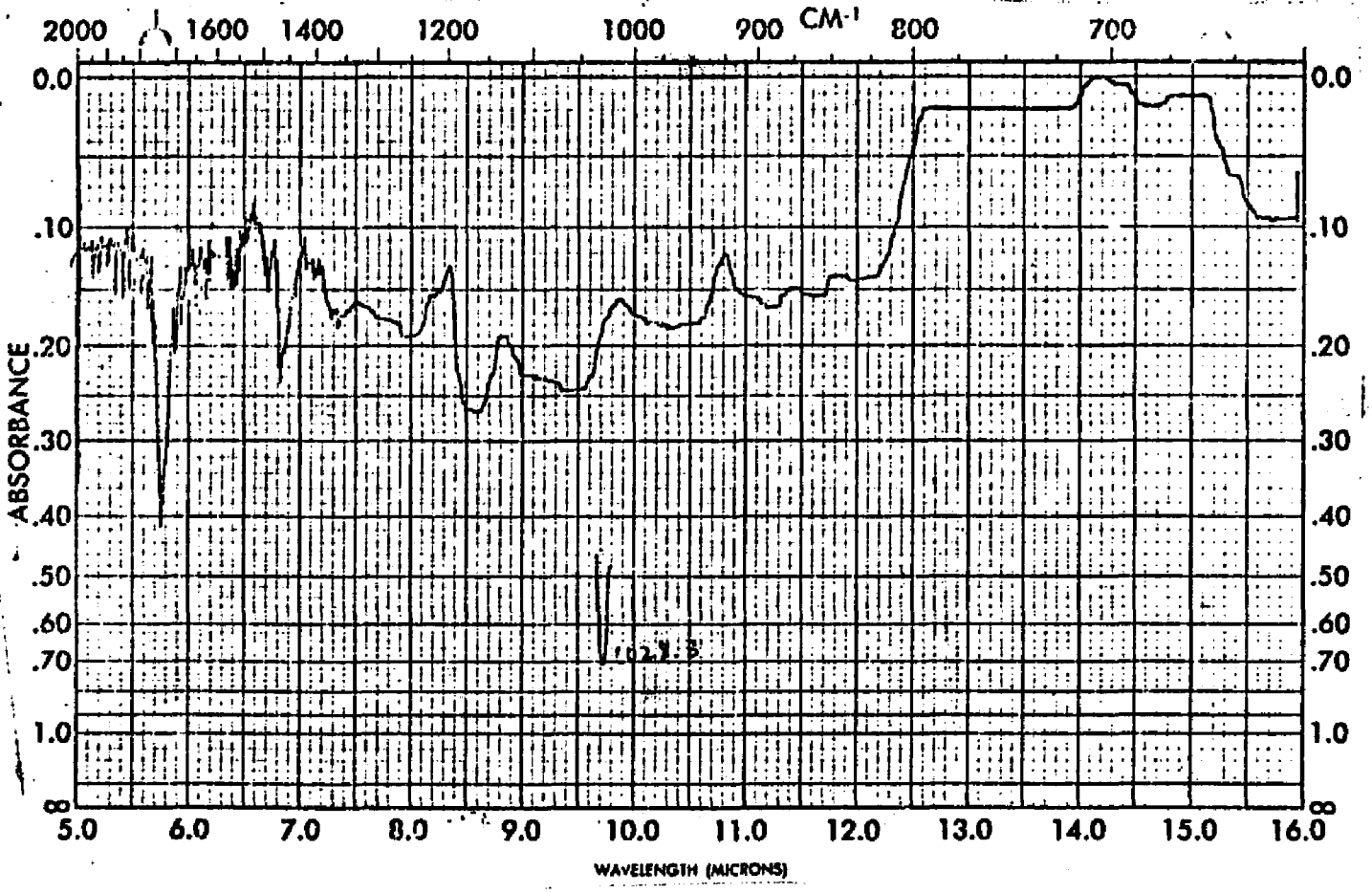

ir ($CHCl_3$) of XXIV