SYNTHESIS OF INHIBITORS OF CHOLESTEROL BIOSYNTHESIS

by

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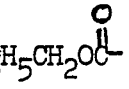
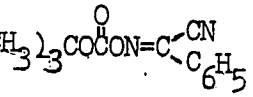
TABLE OF CONTENTS

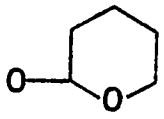
TABLE OF ABBREVIATIONS

Z..... 

Gly..... 

t-BOC-ON..... 

PPTS.....Pyridinium p-toluene sulfonate

-OTHP..... 

Abstract

SYNTHESIS OF INHIBITORS OF CHOLESTEROL BIOSYNTHESIS

by

Virender K. Sarin

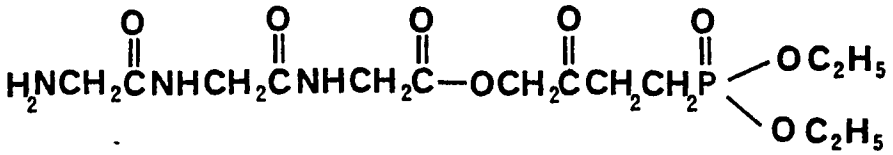
Advisor: Professor Robert Engel

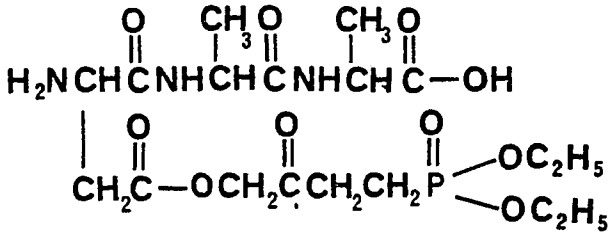
The work described here deals with the synthesis of inhibitors of cholesterol biosynthesis, in vivo and potentially in vitro. Among the different compounds synthesised here, that of greatest importance is 3-carboxy-4-hydroxy-4-methylpentyl-1-phosphonic acid, the isosteric phosphonic acid analogue of 5-phosphomevalonate. Three different approaches for its synthesis are described. All of these approaches use Diethyl 4-oxopentyl-1-phosphonate as the starting compound. The first route involves the use of a Reformatsky reaction with methyl bromoacetate followed by the hydrolysis of the carboxylic ester with a base. The second approach utilizes Lithium Naphthalene to generate the dianion of acetic acid which in turn reacts with the starting Keto compound to yield 3-carboxy-4-hydroxy-4-methylpentyl-1-phosphonic acid in the form of the diethyl phosphonate. The last route uses Lithium diisopropylamide to generate the carbanion of ethylacetate which in turn reacts

with the starting ketone to give the desired compound again in the form of the diethyl phosphonate. In all the three routes, the Phosphonate ester was hydrolysed to the free phosphonic acid by reaction with Trimethylsilyl Bromide followed by hydrolysis with water. This isosteric phosphonic acid analogue of 5-phosphomevalonate has been found to serve as a potent inhibitor of squalene synthesis using the S_{10} homogenate of rat liver. The specific point of inhibition has been determined to be phosphomevalonate phosphokinase.

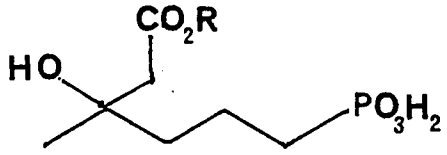
For this compound to be used as a drug, however, it must be transported inside an intact cell. Unfortunately, the simple compound appears to be incapable of this by itself. Thus an approach of "illicit transport" has been utilised where the potential drug is chemically attached to a "chemical vector" for which a mode of transport into the intact cell exists. Once the potential drug with its chemical vector has entered the cell, it is expected that the extraneous portion will be cleaved by enzymes normally present. Two types of chemical vectors have been considered here, the first of these being a tripeptide with the potential drug attached through a functional group to a carboxyl group along the tripeptide. Such a system is expected to gain entrance to the cell via an oligopeptide transport system. For this approach, two tripeptide systems have been synthesised. The first is a Diglycylglycine with the drug coupled to the terminal carboxyl group of the tripeptide leaving a free terminal amino function. The second tripeptide synthesised is L-Aspartyl-L-alanyl-L-alanine with the phosphonate analogue coupled to the β-carboxyl function of the aspartic acid. This coupled system

has both free carboxyl and a terminal amino function. The potential drug coupled to the tripeptide system as a model test system is Diethyl-4-hydroxy-3-butanone-1-phosphonate, an analogue of dihydroxy-acetone phosphate which is an important product of glycosis in lipid metabolism.





The second type of "chemical vector" is a large lipophilic function which is capable of taking part in micelle formation. A micelle once formed, could then simply be enveloped by a cell. For this approach, the potential drug chosen is the phosphonic acid analogue of 5-phospho-mevalonate. The lipophilic function is a large aliphatic unit.



Finally, reactions of differentially substituted haloketones with Triethyl phosphite and Diethylphosphite have been discussed.

Historical

The phase of continuous research on cholesterol biosynthesis began in 1937 with two independent and remarkably complementry investigations. Rittenberg and Schoenheimer from their studies on intermediary metabolism with the aid of stable isotopes, arrived at the conclusion that the process of cholesterol formation involved the coupling of smaller molecules "possibly those which have been postulated to be intermediates in the fat and carbohydrate metabolism[1]." Sonderhoff and Thomas came to the same conclusion based on their studies of the incorporation of trideuterio acetate into the unsaponifiable materials of the yeast[2].

Rittenberg and Bloch[3,4,5] predicted that a two-carbon metabolite, acetate, is the principal building block of cholesterol. This prediction was based on a study of the utilisation of labeled acetic acid for cholesterol synthesis in animal tissues. More convincing evidence for the exclusive origin of the sterol molecule from acetate came from studies done on a mutant of Neurospera crassa. A deficiency in pyruvate metabolism made the growth of this mutant dependent on exogenous acetate. Cells of the mutant strain grown on labeled acetate produced ergosterol with essentially no dilution of the isotope. This demonstrated that no other carbon source contributed significantly to the synthesis of the sterol skeleton.[6]

Bonner and Arreguen demonstrated the utilisation of acetate for the biosynthesis of rubber and speculated on the way that three acetate molecules could combine to form the requisite isoprenoid subunit for the macromolecule via acetoacetate and methyl crotonic acid.[7] fig. I.

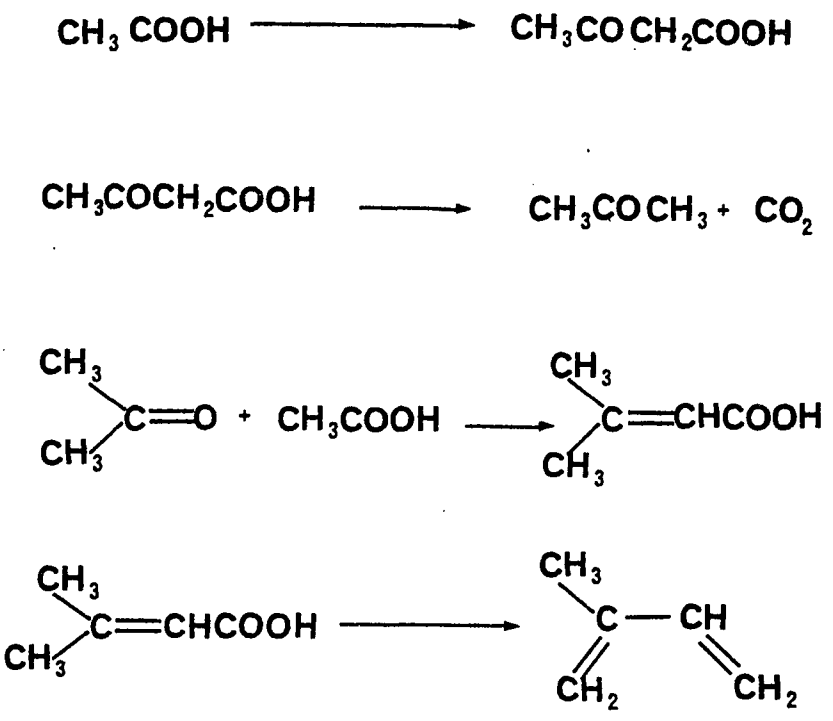
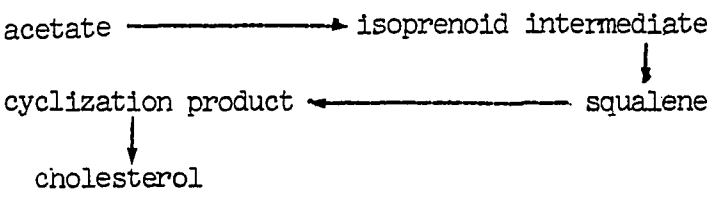


Fig 1

From this postulate evolved the thought that cholesterol, like many other natural substances, was derived from a polyisoprenoid intermediate. For this view to take hold, the ground was well prepared by Robinsons hypothesis according to which cholesterol was formed by the cyclization of squalene, a polyisoprenoid hydrocarbon.[8,9] An outline of the major stages of the overall process emerged as shown below:



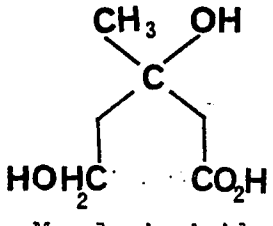
The link between acetic acid and the biological isoprene unit remained a mystery until the discovery of Mevalonic acid in 1956 by Wright, Folkers and associates at the Merk Sharp and Dohme laboratories.[10] The original purpose of these investigations was to isolate and characterize a factor which was exceptionally active as a substitute for acetate in the nutrition of acetate requiring strains of Lacto-bacillus acidophilus[11]. Noting the structural resemblance of mevalonic acid and hydroxymethyl-glutarate — their carbon skeletons are identical (fig. 2.) —— Tavormina, Gibbs and Huff tested the bacterial growth factor and found it to be remarkably active as a precursor of squalene and sterol[12]. Conversion was essentially quantitative, assuming that only one of the enantiomorphs was active. Mevalonic acid is in fact, the key intermediate in the terpene and sterol biosynthesis, as shown by the isotope labeling experiments. Isotopically labeled mevalonic acid was incubated with liver slices and found to be incorporated into squalene and cholesterol with a very high yield. Furthermore, incubation of labeled acetate with liver slices showed that acetate carbon was an immediate precursor of mevalonic acid. The following section will deal with the enzymatic mechanisms by which

(1) acetate is converted to mevalonic acid.

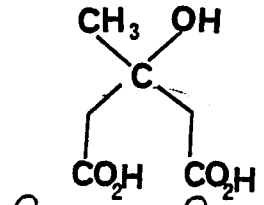
(2) mevalonic acid is converted into squalene.

(3) squalene is converted into cholesterol.

The enzymatic link between acetyl coenzyme A (Co A) and mevalonate by way of acetoacetyl Co A and hydroxymethylglutaryl Co A was established in the laboratories of Rudney[13,14] and of Lyden[15,16] (fig. 3.).



Mevalonic Acid



β-Hydroxy-β-Methyl Glutaric Acid

Fig 2

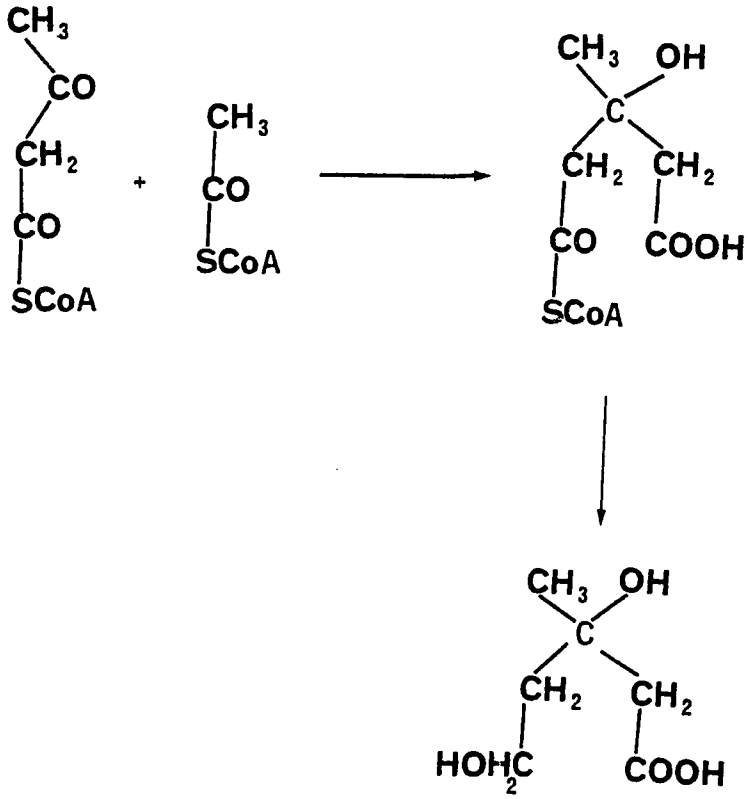


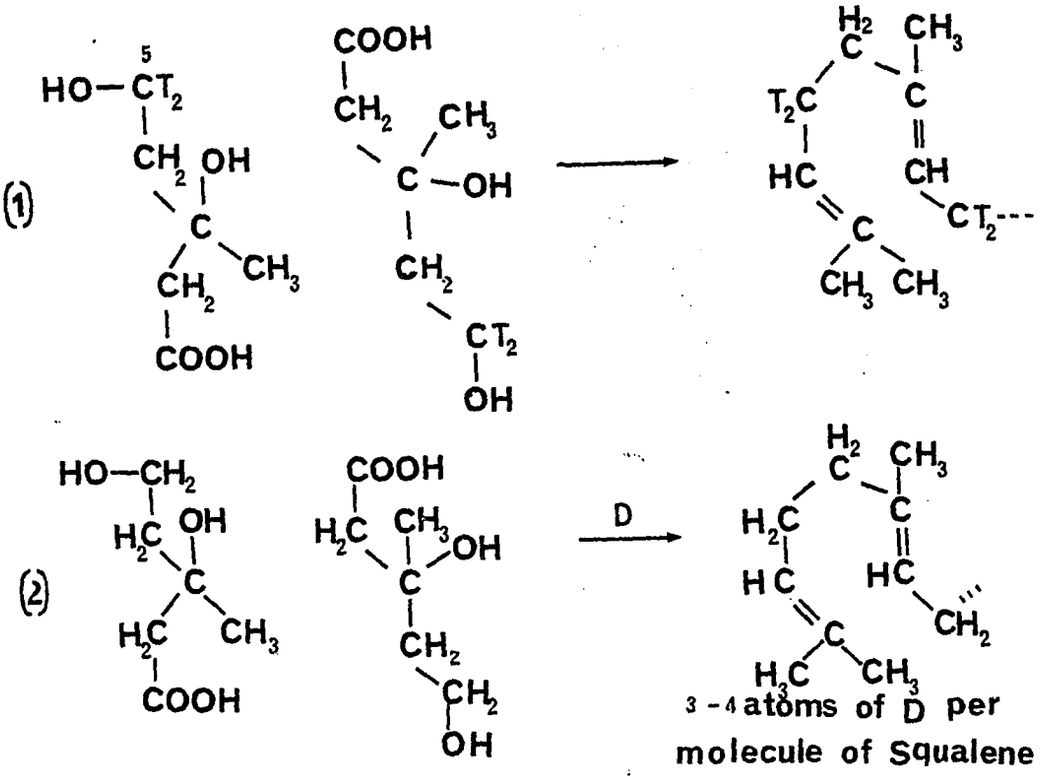
Fig 3

β-Hydroxy-β-methyl glutaryl Co A undergoes reduction of one of its carbonyl groups and loss of CoA by the action of β-hydroxy-β-methyl glutaryl CoA reductase to yield mevalonic acid.

β-hydroxy-β-methylglutaryl CoA + 2NADPH + $2H^+$ ⟶ mevalonic acid + $2NADP^+$ + CoA.

Bloch[17] studied the retention of tritium in the enzymatic conversion of 5,5-di-(^{3}H)-mevalonate to squalene and found that in this transformation only a small fraction of the 12 hydrogen atoms attached to C_5 of the six participating mevalonate molecules was removed. Thus, one of the two bond forming centers (C_5) seemed to remain in the reduced state during the process of carbon — carbon bond formation. Later experiments with D_2O and 5,5-di-(^{3}H)-mevalonate strengthened the above conclusion and allowed Bloch to make the same deduction for carbon atom 2 of mevalonate[18] (fig.4.). Therefore, in the coupling of C_5 and C_6 sub-units, bond formation had to occur without loss or reintroduction of hydrogens at the reacting centers — that is, by interaction of mevalonic acid deriyatiyes containing $-CH_2-$groups at both the C_2 and C_5 positions. From the same experiments, it could be inferred further that the removal of the tertiary hydoxyl group and the loss of the carboxyl function of mevalonic acid proceed concertedly to a C_5 compound bearing methylene group. Possible structures for the reactive condensing unit are thereby limited to isoprene itself or a derivative of isopentene[18].

$$6\ C_6H_{10}T_2O_4 \longrightarrow C_{30}H_{38}T_{11-12}$$



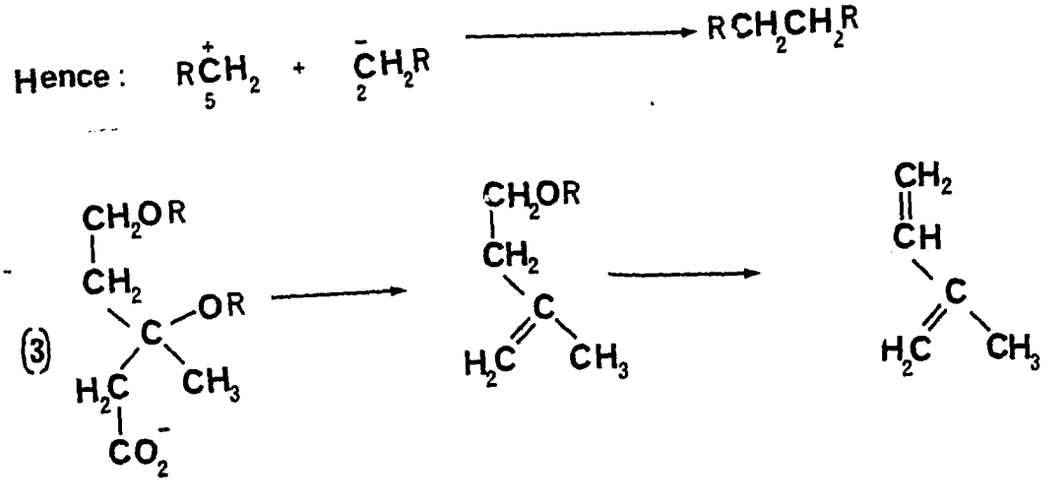
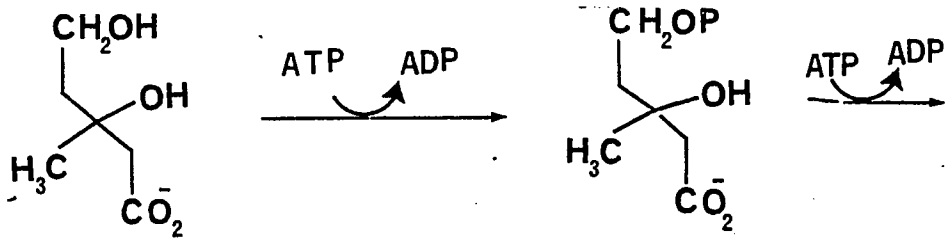
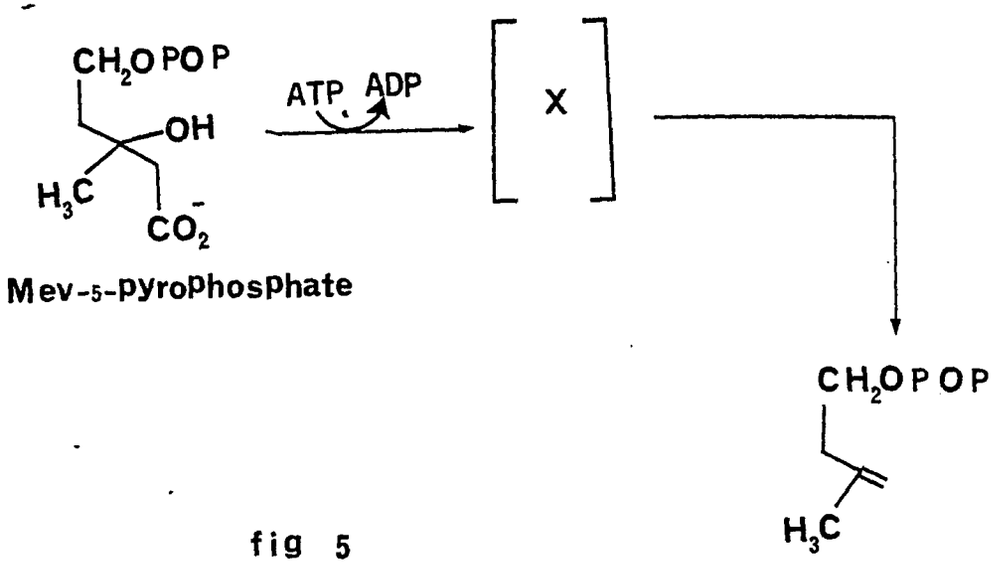


fig 4

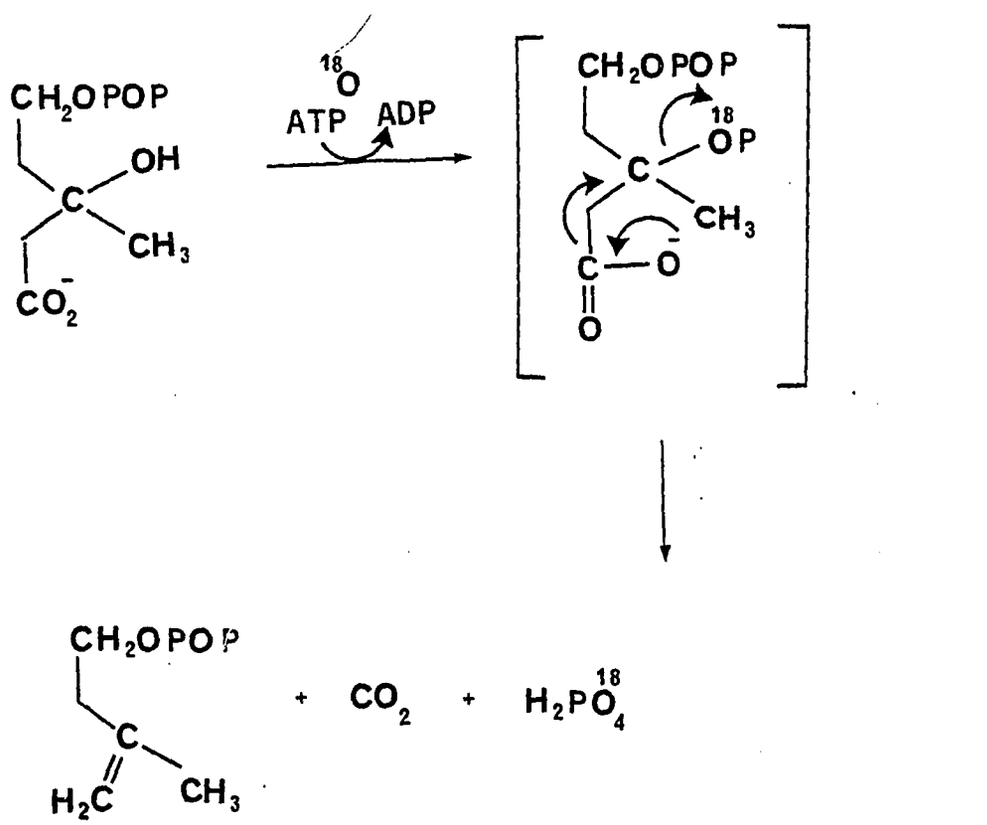
In the synthesis of squalene from mevalonic acid in yeast extract, both adenosine triphosphate (ATP) and reduced triphosphopyridine nucleotide (TPNH) had to be supplied to the enzyme system as cofactors.[17] Mevalonic acid is phosphorylated by ATP to the 5-monophosphate ester as shown by T.T. Tchen who isolated the stable monophosphate of mevalonic acid and the requisite kinase enzyme[19,20]. 5-Phosphomevalonic acid is once again phosphorylated to mevalonate-5-diphosphate by ATP (fig.5.) The next reaction in the sequence is the formation of isopentenyl-pyrophosphate by the ATP-facilitated decarboxylative β-elimination of mevalonic acid-5-pyrophosphate. The concerted nature of this reaction had been shown by deuterium studies as depicted in fig. 4. It was further demonstrated by studies using the purified "anhydrodecarboxylase" which catalysed the coordinated removal of the carboxyl group and of the tertiary hydroxy group[18]. Data obtained with ^{18}O suggest that 3-phosphomevalonic-5-pyrophosphate is a transient intermediate[21]. ATP serving as the phosphorylating agent for the tertiary hydroxyl group and thereby promoting its elimination. (fig. 6.)





Mev-5-pyroPhosPhate

fig 5



+ CO_2 + $H_2P^{18}O_4$

fig 6

In the symmetrical squalene molecule there are two terminal isopropylidene groups, and, since the biological "isoprene unit" has the isopropenyl structure, two of the six isopentenyl groups must isomerize at some stage of squalene synthesis. Lynen and his associates proposed a mechanism involving the isomerisation of free isopentenyl-pyrophosphate to dimethylallyl pyrophosphate prior to condensation[22] (fig.7.) Support and proof for this mechanism were provided by Lynen et al, when they isolated the requisite isomerase and showed that this enzyme is an essential component in the coupling system when isopentenyl pyrophosphate is the sole substrate[23].

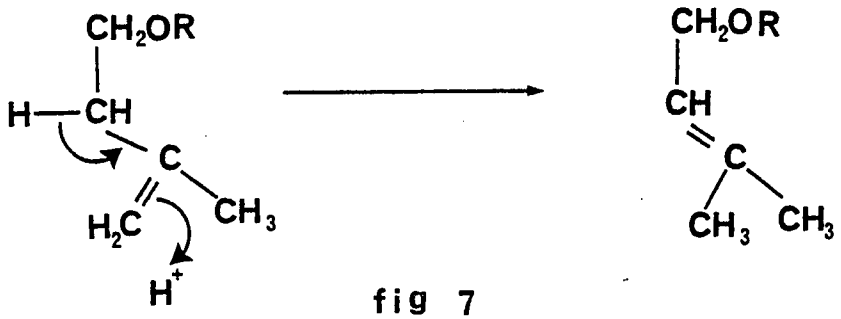
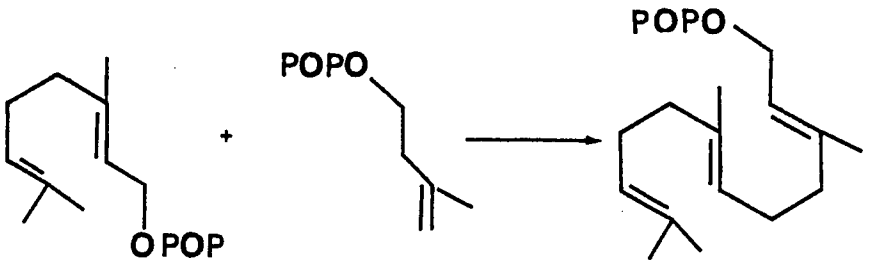


fig 7

These two isomeric isoprenyl pyrophosphates then undergo condensation with the elimination of pyrophosphate to form the monoterpene derivative trans-geranyl pyrophosphate.[24] A third isoprenyl pyrophosphate then reacts, again with elimination of pyrophosphate to yield the sesquiterpene trans-Farnesyl-pyrophosphate. (fig.8.)



Geranyl pyro— Phosphate

C_{10}

Farnesyl Pyrophosphate

C_{15}

Fig 8

One can formulate both the initial interaction of two C_5 units and subsequent C_5 additions as resulting from a nucleophilic attack by the π-electrons of the exomethylene group of isopentenyl pyrophosphate on an incipient cation formed by the pyrophosphate elimination from the allyl pyrophosphate. All these events are considered as concerted. (fig.9a.)

A covalent enzyme-substrate complex might be formed initially between the allyl pyrophosphate and the condensing enzyme with elimination of pyrophosphate. Allyl-enzyme rather than the free allyl pyrophosphate would then react with a second C_5 unit to form the new carbon-carbon bond. (fig. 9b.)

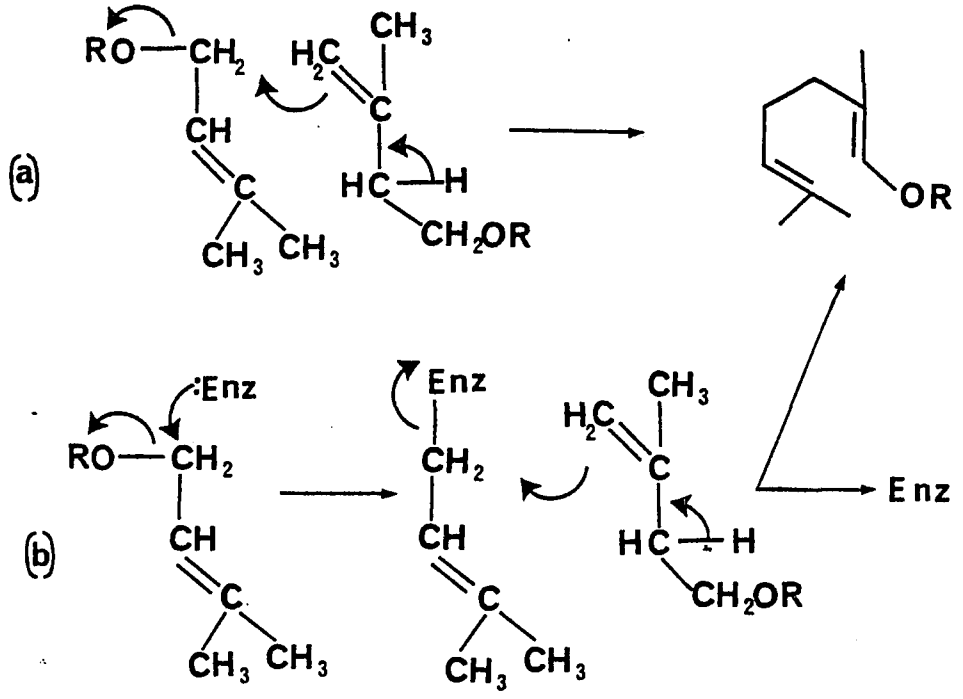


Fig 9

In the absence of any cofactor, except Mg^{2+}, two molecules of farnesyl pyrophosphate are condensed by the enzyme presqualene synthetase to presqualene pyrophosphate, accompanied by loss of one H atom from C-1 of one of the two molecules of farnesyl pyrophosphate; farnesyl coupling is reductive and proceeds by tail to tail linkage.

Rilling and Epstein[25] isolated an intermediate from TPNH-starved yeast subcellular particles and assigned the structure[26] as shown in fig.11. Altman and Rilling[27] have synthesised this intermediate presqualene pyrophosphate and confirmed its conversion to squalene by yeast subcellular particles.

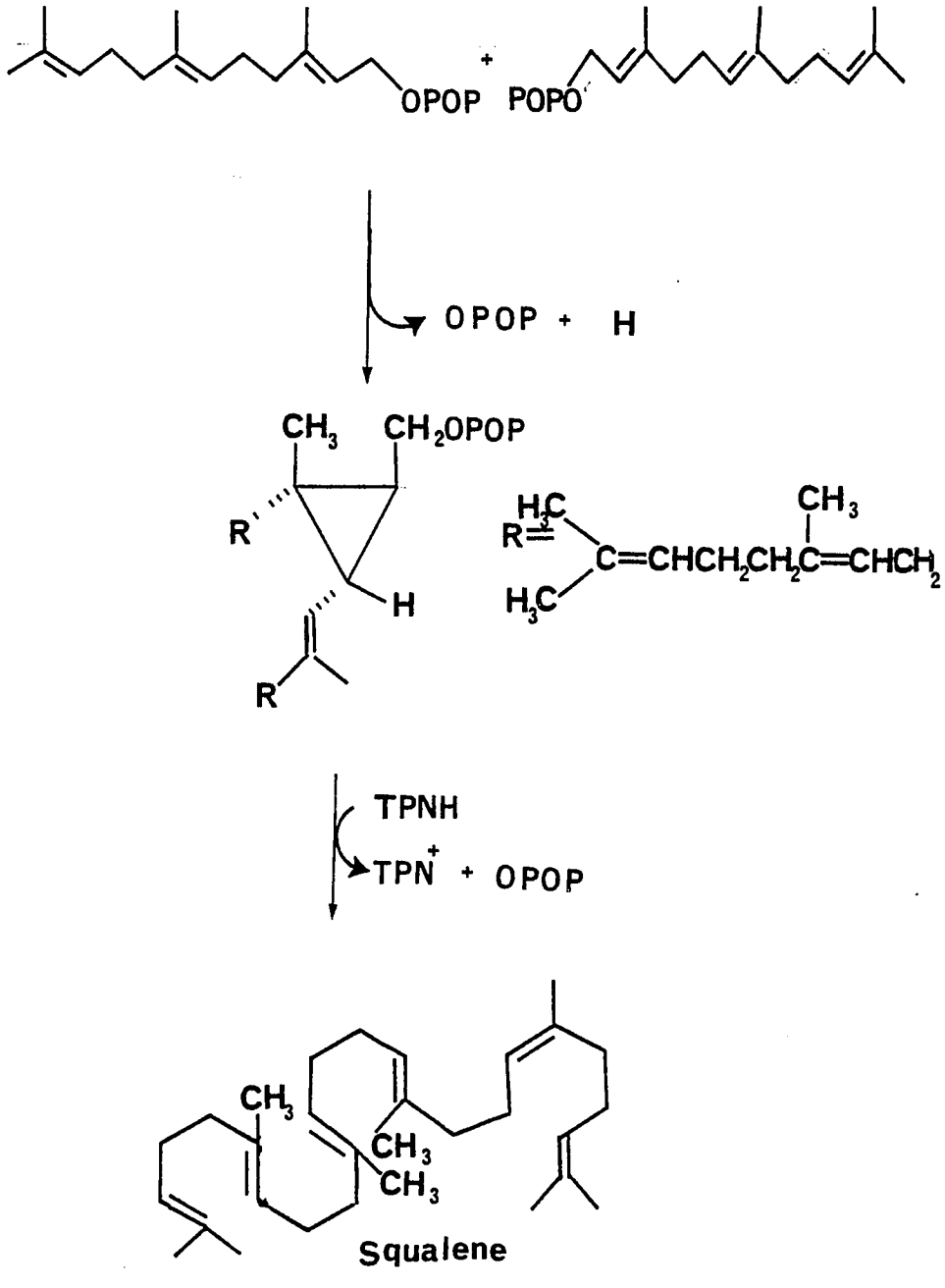

OPOP
POPO
OPOP + H
CH3
CH2OPOP
R
H
R
H2C
R
CH3
C=CHCH2CH2C=CHCH2
H3C
TPNH
TPN+ + OPOP
CH3
CH3
CH3
Squalene

Fig 10

Presqualene pyrophosphate, which contains three assymetric centers at the three carbon atoms of the cyclopropane ring, is rearranged and reduced with TPNH as co-enzyme to the symmetrical C_{30}-terpene, squalene The H atom lost in the previous reaction, is now replaced by direct transfer from TPNH. Altman and Rilling have proposed a mechanism for the biological conversion of presqualene pyrophosphate to squalene based on the well established equilibrium between cyclopropyl carbinyl, cyclobutyl, and allylcarbinyl cations generated in solvolysis and deaminations[27]. This mechanism is shown in Fig. 11 as proceeding through classical ions.

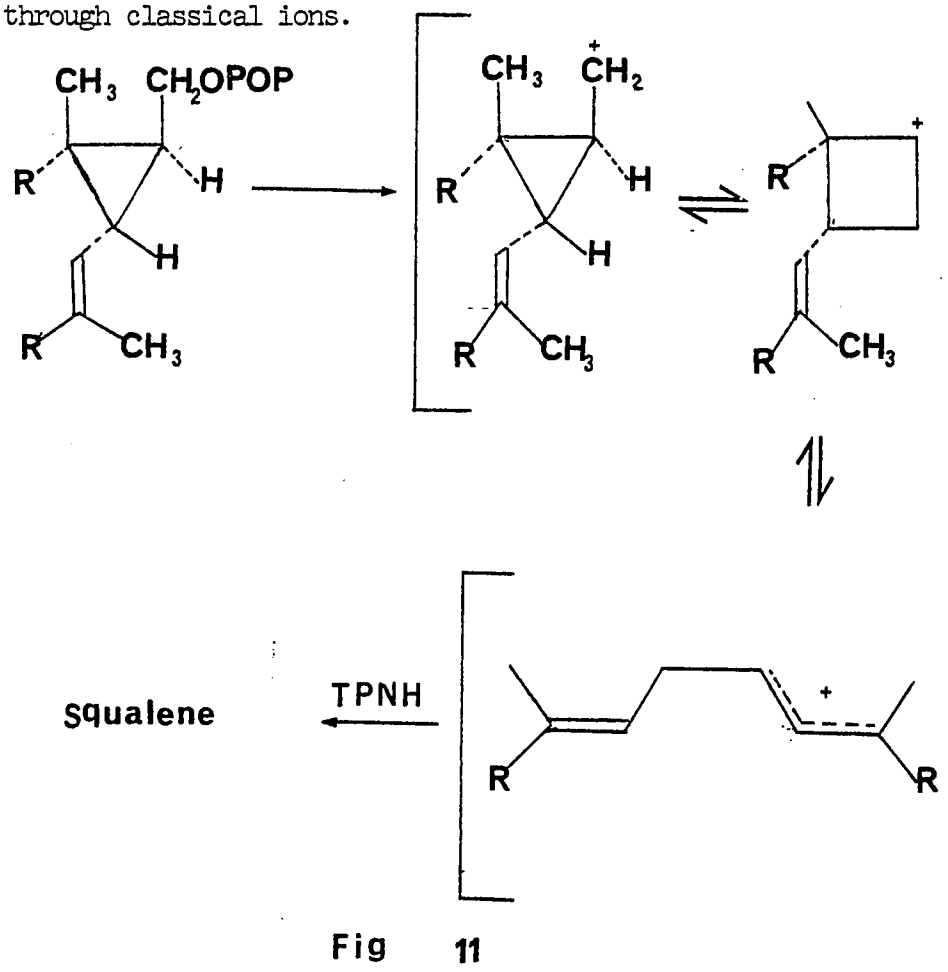
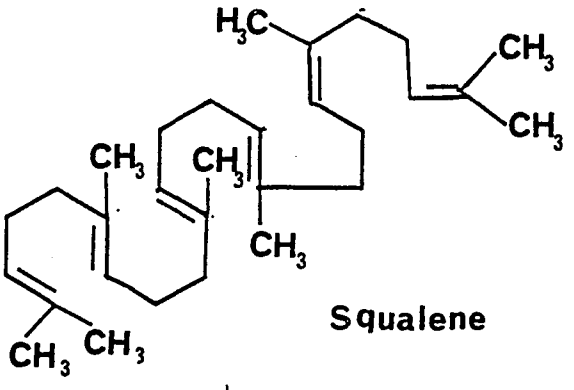


Fig 11

Squalene is transformed into the tetracyclic steroidal configuration by the pathway depicted in fig. 12. The enzyme squalene epoxidase catalyses conversion of squalene to the 2,3-oxide by molecular oxygen. T.T. Tchen showed that molecular oxygen and not water, is the source of the 3-hydroxyl group of cholesterol. Furthermore, his experiments showed that the enzymatic cyclisation of squalene to lanosterol in a D_2O medium proceeds without attachment of deuterium to carbon[28] as it would if any in the series of presumptive carbonium ions fail to stabilise. The cyclization of squalene-2,3-oxide is postulated to be initiated by attack of a proton on the oxide ring and is followed by concerted electron shifts leading to ring closures and formation of a transient carbonium ion at C_{20} (fig.12). Lanosterol is derived by a series of concerted hydride and methyl shifts and elimination of the proton from C-9.



Squalene

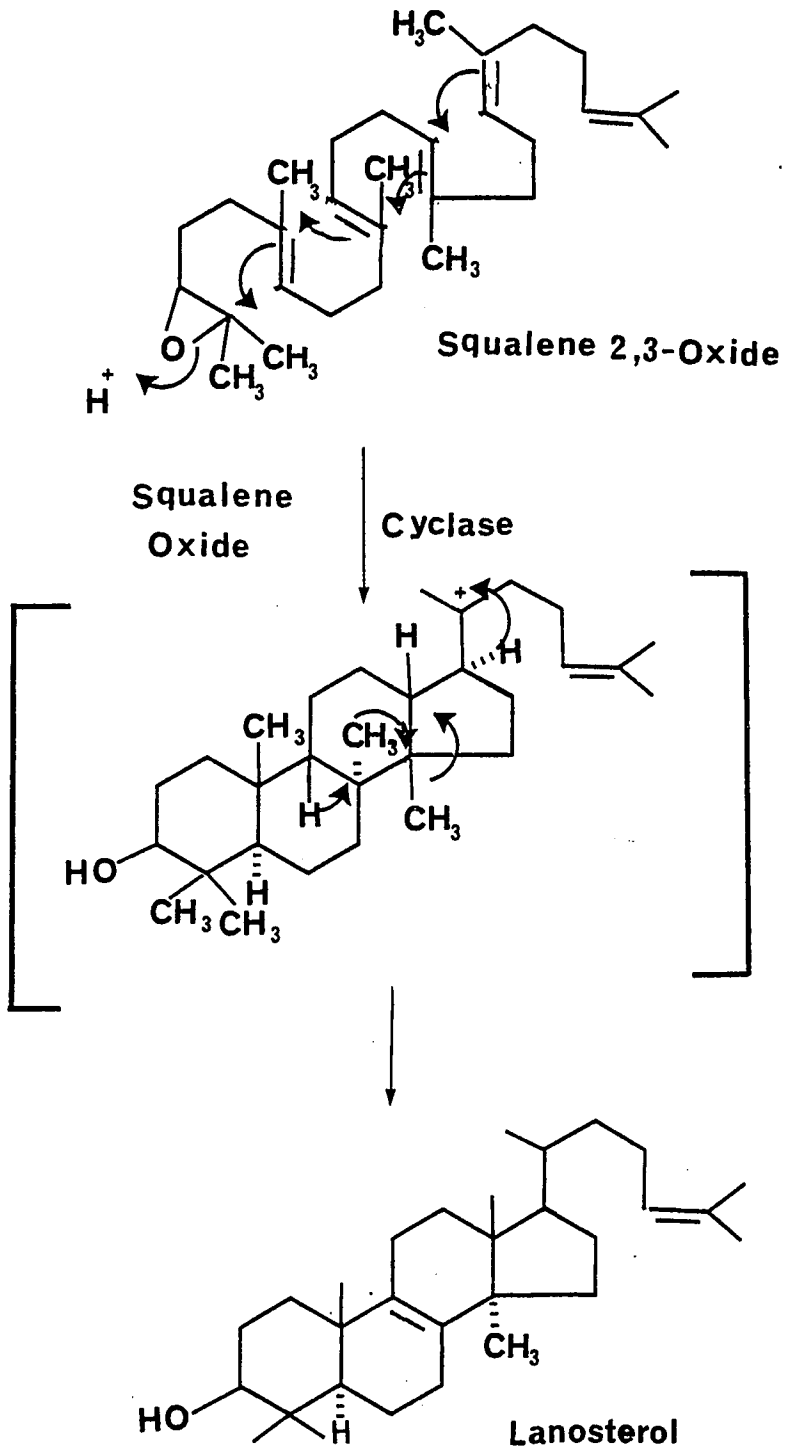
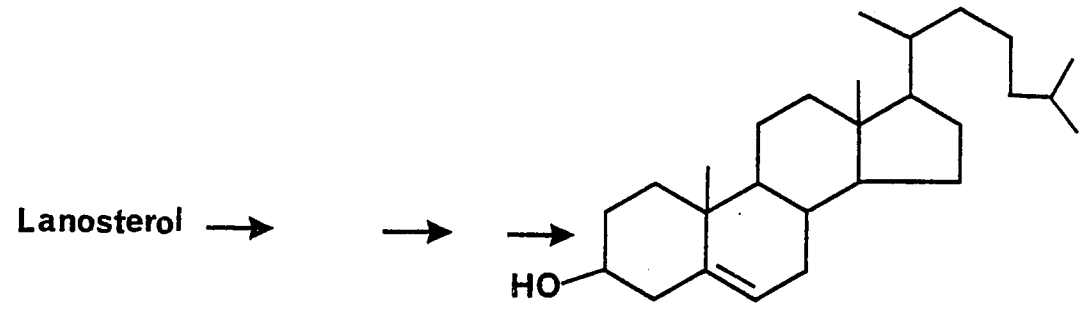

H3C
CH3
CH3
CH3
O
CH3
CH3
H+
Squalene 2,3-Oxide
Squalene
Oxide
Cyclase
H
H
CH3
CH3
H
CH3
HO
H
CH3 CH3
HO
H
CH3
Lanosterol

Fig 12

The conversion of Lanosterol to cholesterol involves the removal of three methyl groups (at C_4 and C_{14}), saturation of the double bond in the side chain, and the migration of the double bond from position 8,9 in lanosterol to position 5,6 in ring B. The removal of three methyl groups is an oxidative process since the carbon atom appears as CO_2,[29] suggesting that the methyl group in lanosterol at C_4 and C_{14} are first oxidised to the carboxyl functions and that their loss is a decarboxylation process.



Cholesterol

The inhibition of cholesterol biosynthesis has been approached from several directions. Consider first some *in vivo* studies and the conclusions from them.

The *in vivo* studies of Schoenheimer and Breusch[30] provided strong suggestive evidence that when cholesterol is added to the diet the ability of an animal to synthesise cholesterol is greatly diminished. Later isotopic experiments of Gould et al[31] and Bloch[32] confirmed the above study. They found that when animals are fed a high cholesterol diet, the ability of liver slices from such animals to synthesise cholesterol from acetate-^{14}C is markedly inhibited.

The sensitivity of this feedback system as well as the extent to which cholesterol synthesis can be suppressed by such a feedback inhibition is well illustrated by the data in table I[33].

Table I.

Cholesterol in diet (%)	Cholesterol synthesis (n moles of added acetate-2-^{14}C)
0.0	67.0
0.1	36.0
0.25	28.0
0.5	1.8
1.0	2.4
2.5	1.8

Both the chemical and subcellular sites at which the cholesterol feedback system operates in liver have been examined in a number of laboratories. Gould and Popjak[34] and subsequently Buscher et al,[35] noted that cholesterol feeding causes a more marked inhibition in the conversion of acetate-^{14}C to cholesterol than was observed when labelled mevalonate was used as a sterol precursor. On the basis of this study, both the laboratories concluded that cholesterol feeding inhibits cholesterol synthesis at a site prior to synthesis of mevalonate.

Subsequent studies by Siperstein[36,37,38,33] led to a specific biochemical localization of this feedback reaction site. They showed that feeding of cholesterol has no effect on the conversion of acetate to either acetoacetic acid or β-hydroxy-β-butyric acid, nor was the

conversion of mevalonate to squalene or of squalene to cholesterol inhibited by short-term cholesterol feeding. Since, β-hydroxy-β-methyl glutarate forms a common intermediate for the synthesis both of ketone bodies and of cholesterol[39,40], it was inferentially concluded that the specific site at which cholesterol inhibits its own synthesis is at the point of conversion of β-hydroxy-β-methyl glutarate to mevalonate[41,33] fig. 13. Using a gas-liquid chromatographic procedure, the specific effect of cholesterol feeding upon the synthesis both of β-hydroxy-β-methyl glutarate and of mevalonate, and hence the activity of β-hydroxy-β-methyl glutaryl reductase was studied[38,42]. The results of such a study are shown in table 2. It clearly demonstrates that dietary cholesterol results in a marked inhibition of the synthesis of mevalonate while having no detectable effect upon the synthesis of β-hydroxy-β-methyl glutarate[36,38]. This finding therefore, provided definitive evidence that the specific biochemical site of cholesterol feedback system is in fact localized to the point of conversion of β-hydroxy-β-methyl glutarate to mevalonate as shown in fig. 13.

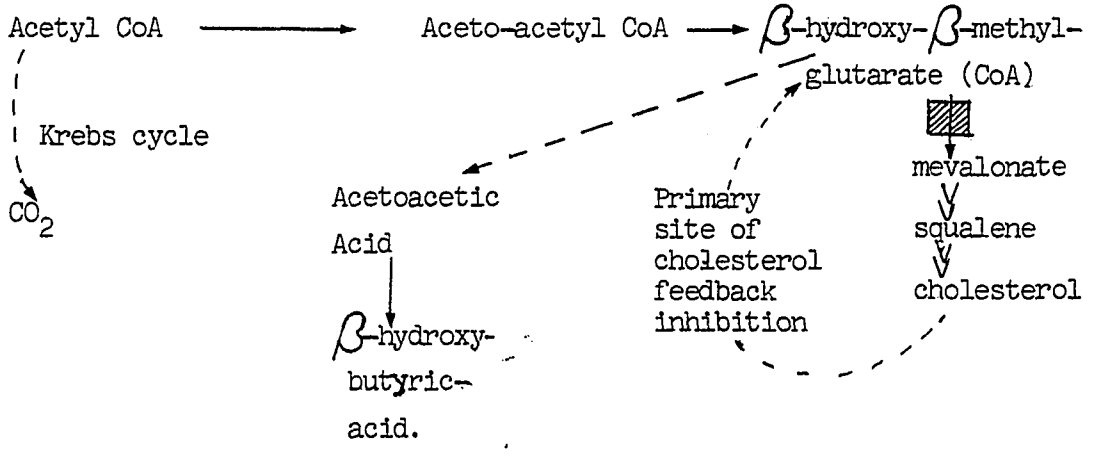


fig. 13

Further evidence for the above results has been provided by reports of Linn,[43] of Kandutsch and Saucier[44], and of Shapiro and Rodwell[45], who have demonstrated that cholesterol feeding inhibits the direct conversion of β-hydroxy-βmethylglutaryl-CoA to mevalonate in cell-free systems.

It has also been noted that fasting, or removal of cholesterol from the diet, depresses cholesterol synthesis. Back, et al[144] have suggested that this might be more sensitive than the feedback inhibition mechanism, although all others have found fasting to be significantly less effective.[145-152] It has also been found that cholesterol feedback inhibition and fasting act at the same enzymatic site.[87,88,155,156]

Table 2.

Expt. #	Cholesterol in diet %	Acetate	β-hydroxy-β-methyl-glutarate	Mevalonate	Cholesterol
I	0	3.37	0.80	16.18	28.40
	5	0.02	0.96	0.46	16.82
II	0	6.16	1.08	16.20	34.52
	5	0.09	0.96	0.46	17.36

The point to be emphasized from these results is that the feedback inhibition of cholesterol synthesis operates at the first reaction following the last quantitatively important branch point in the reactions leading from acetyl-CoA to cholesterol as shown in fig. 13. This reaction, moreover, is irreversible.[46,33]

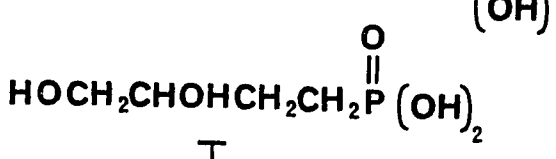
If cholesterol is fed for relatively long periods of time, i.e., 5-6 days, a modest inhibition in the conversion of mevalonate to cholesterol is superimposed upon the primary and much more striking

inhibition of mevalonate synthesis [35,33]. Gould and Swyryd[31a,b] have emphasized that with more prolonged feeding of cholesterol, a progressively greater inhibition of reactions beyond mevalonate synthesis develops, probably at a site between mevalonate and farnesyl pyrophosphate. Siperstein and Fagan[38] claimed that since mevalonate formation is clearly the rate-limiting reaction in the pathway of cholesterol synthesis, even a major reduction in the activity of the enzymes responsible for the reactions beyond the production of mevalonate could have only a modest effect upon the overall process of cholesterol synthesis. It is, moreover, likely[38,33] that this inhibition of the later reactions of cholesterol synthesis is not the result of coordinate repression, but rather represents a secondary response to the primary depression of mevalonate synthesis that follows long-term cholesterol feeding.

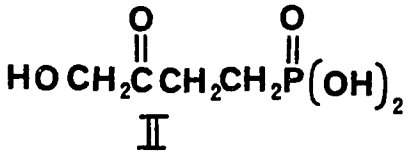
Another approach to the inhibition of cholesterol biosynthesis is by the administration of drugs. As the biosynthesis of cholesterol involves a series of phosphate esters, one approach to the development of a specific inhibitor would involve the introduction of a phosphonic acid species as a substitute for the natural metabolite. Briefly consider prior efforts of this type.

Phosphonic acids and their deriyatiyes, which can be considered to be analogues of the naturally occurring phosphates, have been given considerable attention during the last few years. This interest is generated by the recognition that phosphonic acids and their derivatives can function as analogues of naturally occurring phosphates, possess the potential to serve as metabolic regulators and drugs.

As examples, 3,4-dihydroxybutyl-1-phosphonic acid,I both in the racemic and optically active form have been prepared by Kabak et al[47,48] and 4-hydroxy-3-oxobutyl-1-phosphonic acid II,an analogue of dihydroxy acetone phosphate has also been reported by Goldstein et al[49]. It has been demonstrated by Shopsis et al[50-52], that the isosteric analogue (I) is capable of inhibiting the growth of mutant strains of E.coli at rather low concentration. The net effect is bacteriostasis which may result from a perturbation of the normal phospholipid production[53,55] resulting from an inability of the organism to cleave the phosphonate linkage which is present in place of normal phosphate. The analogue substitutes for glycerol-3-phosphate for a portion of the normal metabolic process but a point of inhibition is reached as a result of the inability to release phosphate. From this study, the authors conclude that for biological activity, there should be correspondence of size between the analogue and the natural substrate. Similar approaches have been tried with the analogue(II).

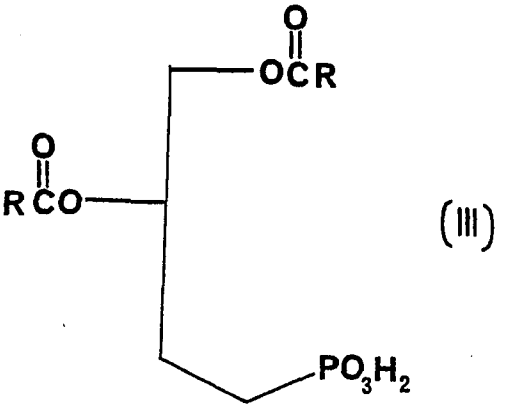


I



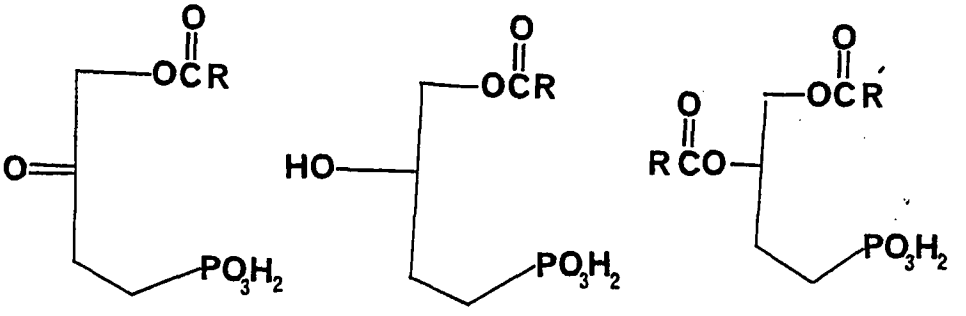
II

Some analogues of phospholipids, the isosteric phosphotidic acids (III) bearing saturated and unsaturated fatty acid ester linkages, have been prepared by Tang et al[56].



(III)

Also, a lipid derivative of the analogue of dihydroxyacetone phosphate, compound (IV), a reduced form of it, lyso phosphatidic acid prepared.[57,58] Compound (V) has been found to be a substrate for lyso-(V), and the differntially substituted compound (VI) have been prepared.[57,58] Compound (V) has been found to be a substrate for lyso-phosphatidate acyltransferase.



(IV) (V) (VI)

In the design of an analogue for a natural metabolite, it is desired that the analogue bear only one structural variation from the parent compound. The structural variation considered here is the presence of a carbon-phosphorous linkage in place of the normal phosphate ester linkage. The term "isosteric" strictly refers to compounds of identical size and shape. According to available crystallographic data for related compounds 2-aminoethyl phosphate

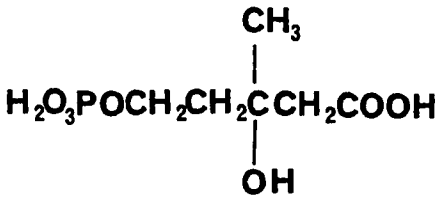
and 2-aminoethyl phosphonic acid[59-61] the distance between phosphoryl oxygen and some other position for a phosphate in comparison to its nominally isosteric phosphonic acid analogue varies by about 0.8%. Although, the compounds mentioned here do not meet this requirement of identical size and shape most rigorously, the bond length and angles involved are similar enough that the term may reasonably be applied.

Thus there is good reason to believe that phosphonic acid analogues of natural organic phosphates might serve as very useful probes for studying metabolic regulation.

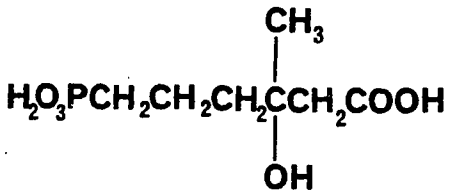
One must consider the choice of analogue which is most likely to have the desired effect, inhibition of cholesterol biosynthesis. Biosynthetic pathways are frequently regulated at either the initiation step or at branch points. The biosynthesis of cholesterol from acetate has two branch points. The first is the reductive synthesis of the crucial intermediate mevalonic acid from β-hydroxy-β-methyl-glutaryl Co A. Here the alternate fate of the substrate is degradative cleavage. The second branch on the biosynthetic route to cholesterol is the synthesis of presqualene pyrophosphate - an intermediate which Altman et al, have characterized and synthesized[27] from farnesyl pyrophosphate. Here, the alternate fate for the growing polyterpenoid molecule is continued elongation for ultimate use as the ubiquinone side chain.

5-Phosphomevalonic acid represents the first stage at which an analogue might be introduced which would be of use in the inhibition of squalene synthesis; a structural analogue which could be of value here is the phosphonate(VIII), isosteric with (VII). This might be

expected to cause inhibition of squalene biosynthesis by a feedback mechanism.

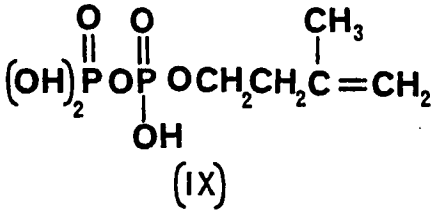


(VII)

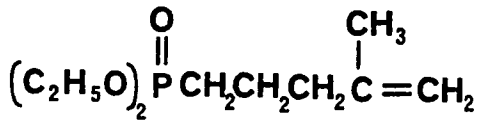


(VIII)

This structure (VIII) retains all the reactive sites necessary for continuation of the normal reaction sequence with the exception of an alkyl-phosphate esterlinkage; this ester linkage normally is not involved until the beginning stages of condensation of isopentenyl units. (24,62,63) The next step in the cholesterol biosynthesis is the enzyme facilitated decarboxylation and dephosphorylation of mevalonic acid pyrophosphate to yield isopentenyl pyrophosphate(IX). A continuation of this reasoning leads one to predict that the following structures would also represent potential significant inhibitors.

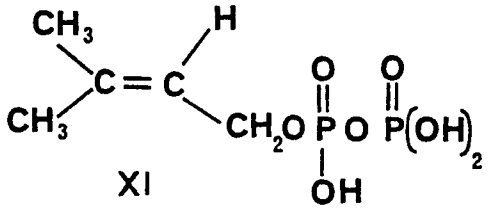


(IX)

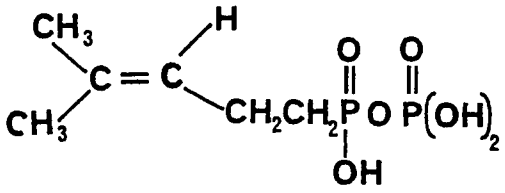


(X)

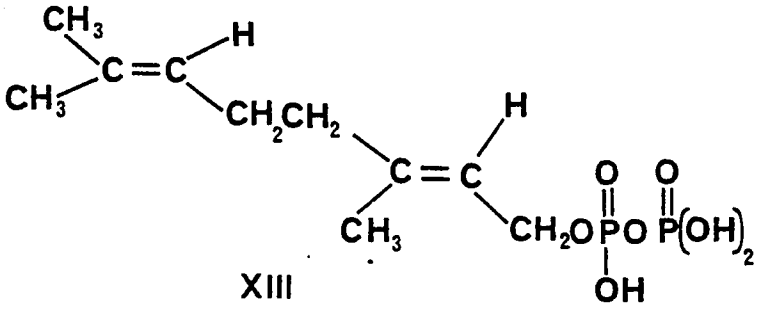
Prior to condensation, it is known that the enzymatic conversion of (IX) to dimethylallyl pyrophosphate (XI) is necessary. The phosphonic acid isostere of (XI), compound (XII), provides another site for interruption of the biosynthesis of squalene. As the normal condensation of (IX) and (XI) involves pyrophosphate cleavage from (XI) yielding geranyl pyrophosphate (XIII), such formation would be precluded by (XIII).



XI

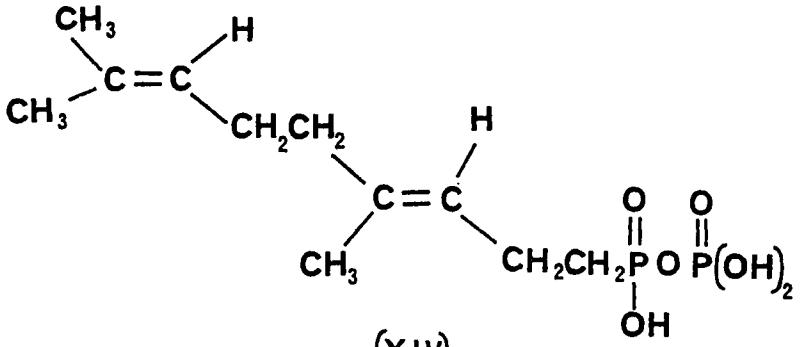


XII



XIII

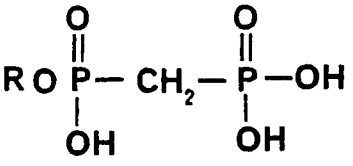
Thus, the current project was concerned with the synthesis of a series of phosphonates i.e. (VIII), (X), XII) and compound (XIV), an isostere of (XIII) and the analogue of farnesyl pyrophosphate.



(XIV)

After work on this project had begun, Corey and Volante[64] reported the synthesis of analogues of isopentenyl, γ,γ-dimethylallyl, geranyl, farnesyl and presqualene pyrophosphate. At this stage the attempt to synthesize the entire series of compounds was abandoned and concentration was placed on the phosphonic acid analogue of mevalonic acid (VIII).

A second type of phosphonic acid analogue of the natural phosphates are those isosteric with pyrophosphates in which the anhydride type oxygen has been replaced by a single methylene group as shown below:



These analogues would be of interest to define the biochemical role of the natural phosphate by looking at the interruptions or modifications of the normal process upon introduction of the phosphonic acid analogues.

Important differences are present between normal pyrophosphates and their nominally isosteric analogues. First is the loss of binding

capabilities of the β,γ-pyrophosphate oxygen in the natural compound. Thus, these isosteric analogues would be of use in mechanistic evaluation in the determination of binding requirements for enzymatic action. Also, the geometric differences between the two could result in very serious differences in binding properties of the two functions.

Along with the synthesis of the simple analogues, another chemical and biochemical problem presented itself.

A major difficulty in working with phosphonic acid analogues of natural compounds is the inability of many of these charged compounds to enter the cell. In fact, the impermeability of intact cells is a frequent problem in biochemistry. When there is no transport system for a compound, the cell membrane is a formidable barrier. For charged compounds, passive diffusion seldom produces the desired internal concentration, and the metabolism of these compounds must be studied in a cell - free extract.

Prominent among techniques used to circumvent the cell barrier is chemical modification of the compound under study in the hope that it will then be soluble in the cell membrane. One successful example is the use of N^6, 2'-O-dibutyryl cyclic AMP in the place of cyclic AMP in both prokaryotic and eukaryotic systems.[65] Another approach is to link the non-penetrating substance covalently to a molecule that is actually transported by a pre-existing transport system; an obvious advantage of using an active transport system is that high internal concentrations of the material can be reached very quickly and secondly such a method maintains to the greatest degree possible the structural integrity of the substance. In addition, the extreme measure of masking the charges

of anionic or cationic compounds need not be used, as molecules for which there exists a transport system need not be soluble in the cell membrane to reach the cytoplasm. It is known that due to special transport systems bacteria are capable of taking up nutrients even from very dilute solutions, resulting in an accumulation of solutes inside the cell. Likewise the bactericidal effect of various antibiotics can be obtained at extremely low concentration of the drug. In some cases the minimal inhibitory concentration is found to be lower *in vivo* than *in vitro*.[66] It is believed that certain antibiotics are able to 'misuse' existing transport system(s) which the bacterium needs to take up various nutrients. This raises the question of which transport system(s) can be used by which drug(s).

For several years, Gilvarg has been concerned with the utilisation of oligopeptides by *Escherichia coli* and has delineated the structural features of a peptide substrate which governs its transportability. It is a requirement of the transport system that the N-terminal α-amino group of a peptide be unsubstituted. This was shown be performing comparative studies on the growth response of *E.coli* lysine auxotroph to lysine,[67,68] oligolysine peptides and α-N-acetyl - derivatives of oligolysine. It was observed that the lysine auxotroph could use di-, tri-, and tetralysine as sources of lysine, while α-N- acetylated derivatives were inactive. This conclusion was further substantiated by the results with acetylated arginine oligopeptides. The importance of the C-terminal carboxyl group in peptide transport was also studied by Gilvarg and Payne[69,70]. They concluded that the C-terminal carboxyl group is not necessary for the uptake of oligopeptide. To this end, a series

of peptides without the free carboxyl group were synthesised. The compounds prepared were lysylcadaverine peptides. Cadaverine, which is the diamine obtained on decarboxylation of lysine, comprised only the C-terminal residue in these peptides; all other residues were lysine. All these analogues were able to enter E. coli as shown by their capacity to support the growth of lysine auxotroph. The oligopeptide system, while showing preferances for all L-forms, seems able to tolerate a degree of 'steric wobble' with respect to the third and presumably later amino acid residues relative to the N-terminus. Studies with E. coli indicate that this organism possesses an accessory permeability barrier that excludes peptides with a diffusional radius greater than a certain critical value. This feature is reflected in the inability of the higher members of homologous peptide series to enter the organism. Some of the structural specifications for oligopeptide transport in E. coli are shown in diagramatic form in fig. 14.[71A]

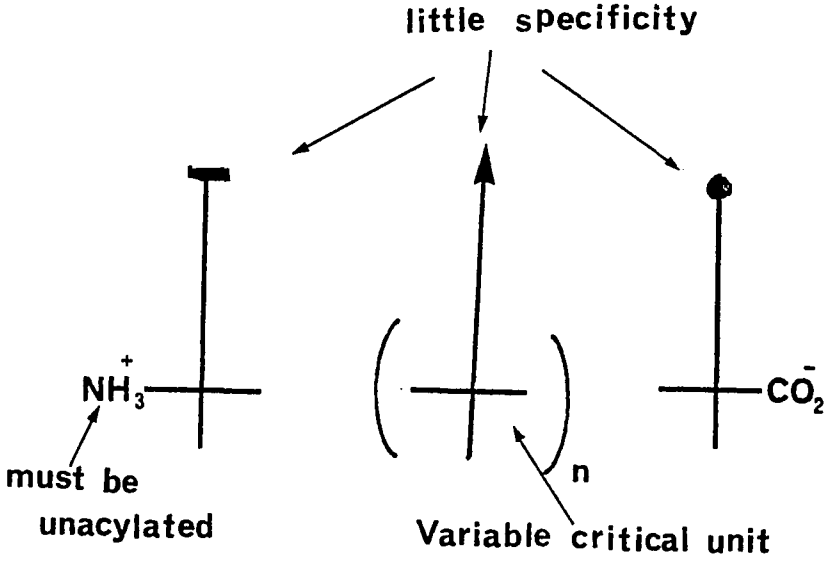


Fig 14

A striking finding was that a permease is capable of transporting peptides of widely varying amino-acid components and size.[71] The non-stringent nature of oligopeptide permease suggests that it would be an ideal candidate for carrying out the transport of normally impermeant compounds.

A particularly suitable test substance would be an amino-acid which alone could not reach the cytoplasm, but which could be linked covalently to a potentially transporting peptide. To prove that the peptide carried such an amino-aicd into the cell it would be highly desirable that the amino-acid be involved in a normal metabolic scheme so that a mutant unable to synthesize the amino-acid could be isolated and tested for its growth response to the peptide. Gilvarg and Fickel[72] showed transport of a theronine precursor, homoserine phosphate into E. coli using the oligopeptide permease as lysyl-lysyl-homoserine phosphate.

Ames et al[73], have shown the existence of oligopeptide permease in Salmonella typhimurium. They used this transport system for smuggling a histidine biosynthetic intermediate, histidinol phosphate ester into the bacteria as its glycylglycyl derivative, gly-gly-histindinol phosphate. Free histidinol phosphate ester is not transported into Salmonella.

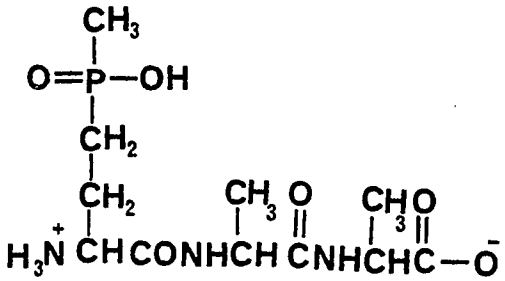
The transport of small peptides is found in mammalian systems as well. The first reports of peptide transport in mammals were those of Newey and Smyth[74,75,76,77] who demonstrated the uptake of peptides by, and hydrolysis to amino acids within, the mucosal cells of the small intestine. Subsequent studies by Craft, Sadikali and Matthews (78,79,80a,b) confirmed and extended this observation. They investi-

gated the absorption of glycine and glycylglycine in gastrointestinal disease in man. Their results indicated that a given dose of glycine was absorbed more rapidly from glycylglycine than from the free amino acid, and most rapidly when given in the form of triglycine. Similar results were obtained in rats[81a,b]. These workers indicated a resemblance between peptide transport in mammalian gut and in bacteria[82].

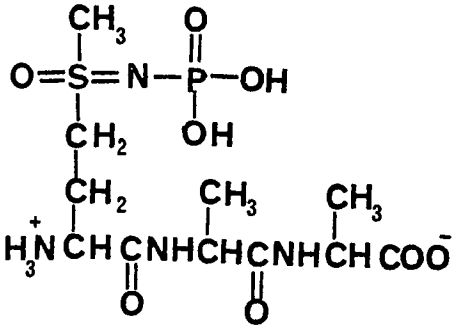
Extension of the work to the series L-methionine, L-methionyl-L-methionine and L-methionyl-L-methionyl-L-methionine gave essentially the same results as those obtained from the glycine series. Further examples of more rapid transport of neutral amino acids from peptides than from the equivalent free amino acids in mammalian gut were reported from the laboratories of Adibi and Phillips[83,84] who extended the finding to glycyl-L-leucine and L-leucylglycine. Edwards[85] reported the phenomenon with L-leucyl-L-alanine, L-leucyl-L-tyrosine, L-alanylglycine, glycyl-L-alanine and tryptophyl glycine in the rat in vivo and Rubino and Auricchio[86] found it with Glycyl-L-proline with rat small intestine in vitro.

Bayer et al,[89] have pointed out that the tripeptide antibiotic L-phosphinothricyl-alanyl-alanyl (I, fig. 15) exhibits much greater antibacterial activity against intact cells than does the constituent amino acid, phosphinothricin. In contrast, in the cell free system only the amino acid shows effective inhibition of the E. coli glutamine synthetase. It is suggested that the uptake of the antibiotic into the cell is strongly favored by the tripeptide form which is then hydrolyzed to the free phosphinothricin and alanine. This shows that the tripeptide form, but not the free inhibitor is capable of invading the cell via the oligopeptide system.[71,90]

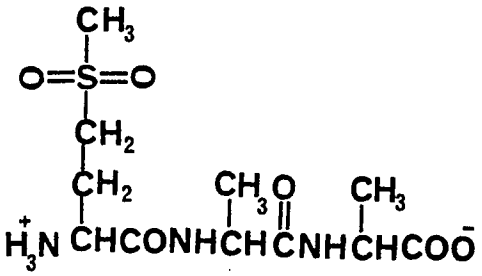
A similar transport effect has been used to explain the greater antibacterial activity of the tripeptide antibiotic L-(N^5-phosphono) methionine-5-sulfoximinyl-alanyl-alanine (II, fig. 15)[91], compared to the amino acid L-(N^5-phosphino)-methionine-5-sulfoximine, which also inhibits the glutamine synthetase of E. coli. Zahner and Jung[92] investigated the uptake of L-methionine-s-dioxide. They found that L-methionine-s-dioxide-alanyl-alanine (III, fig. 15) is taken up via the oligopeptide transport system. Inside the cell, it specifically inhibits the glutamine synthetase.



(I)



(II)

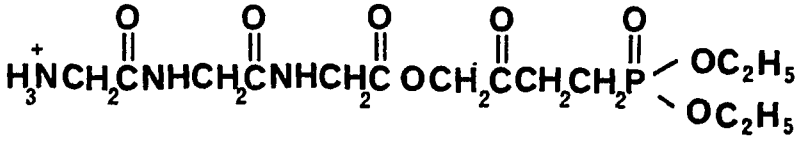


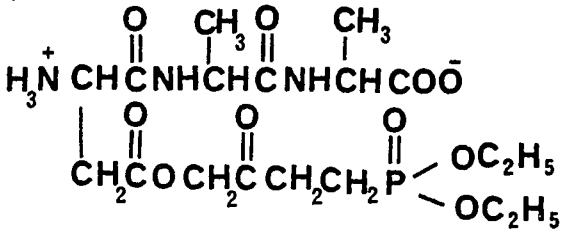
(III)

Fig 15

Thus, the oligopeptide transport system provides a basic and within certain limits, widely applicable carrier system.

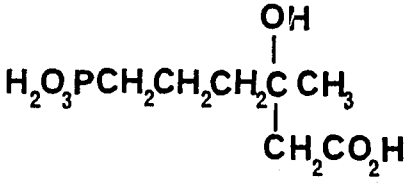
The absence of a transport system for some simple phosphonic acid analogues of natural phosphates has already been found to be a problem. The phosphonic acid analogue of dihydroxyacetone phosphate, 4-hydroxy-3-oxybutyl-1-phosphonate is reduced by the anabolic glycerol-3-phosphate dehydrogenase of E. coli[53]; unfortunately, the analogue does not have any effect on intact cells because it can not be transported. It would appear reasonable from the examples cited before that if a phosphonic acid analogue species were coupled to a tripeptide it would have a very good probability of being transported into the cell. Thus studies were initiated with 4-hydroxy-3-oxybutyl-I-phosphonate as the drug and triglycine as the peptide carrier. A tripeptide[90] linked to an analogue should have the proper hydrodynamic volume but the choice of triglycine is somewhat arbitrary. The main reason for selecting triglycine is that it appeared to present the fewest synthetic problems since there is no need to be concerned about side chain functional group or optical activity. Another tripeptide, perhaps a better transport carrier would be L-Aspartyl-alanyl-alanine with the phosphonic acid analogue coupled to the β-carboxyl group of the aspartyl unit of the tripeptide.



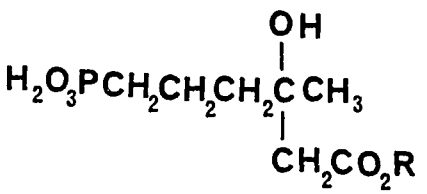


Once the analogue is transported into the cell, it is expected to be cleaved from the tripeptide by enzymes normally present. It there should be reduced by the anabolic glycerol-3-phosphate dehydrogenase into dihydroxybutyl phosphonic acid[53] (DHBP) which will then cause bacteriostasis. If 4-hydroxy-3-oxybutyl-I-phosphonate attached to a tripeptide does not inhibit cell growth, the above analogue coupled to the carboxylterminus of a tripeptide can be reduced chemically into DHBP and then tested for its inhibitory activity.

Another 'chemical vector' which can possibly transport a drug into a cell would be a large lipophilic function which is capable of taking part in micelle formation. A micelle once formed, could then simply be enveloped by a cell. This is of particular interest for the inhibition of cholesterol biosynthesis in liver. The most tractable site for attaching a lipophilic chemical vector to (VIII) is the carboxyl function. This is accomplished by preparation of an ester with a fatty alcohol as shown in (XV).



(VIII)



(XV)

Statement of Problem

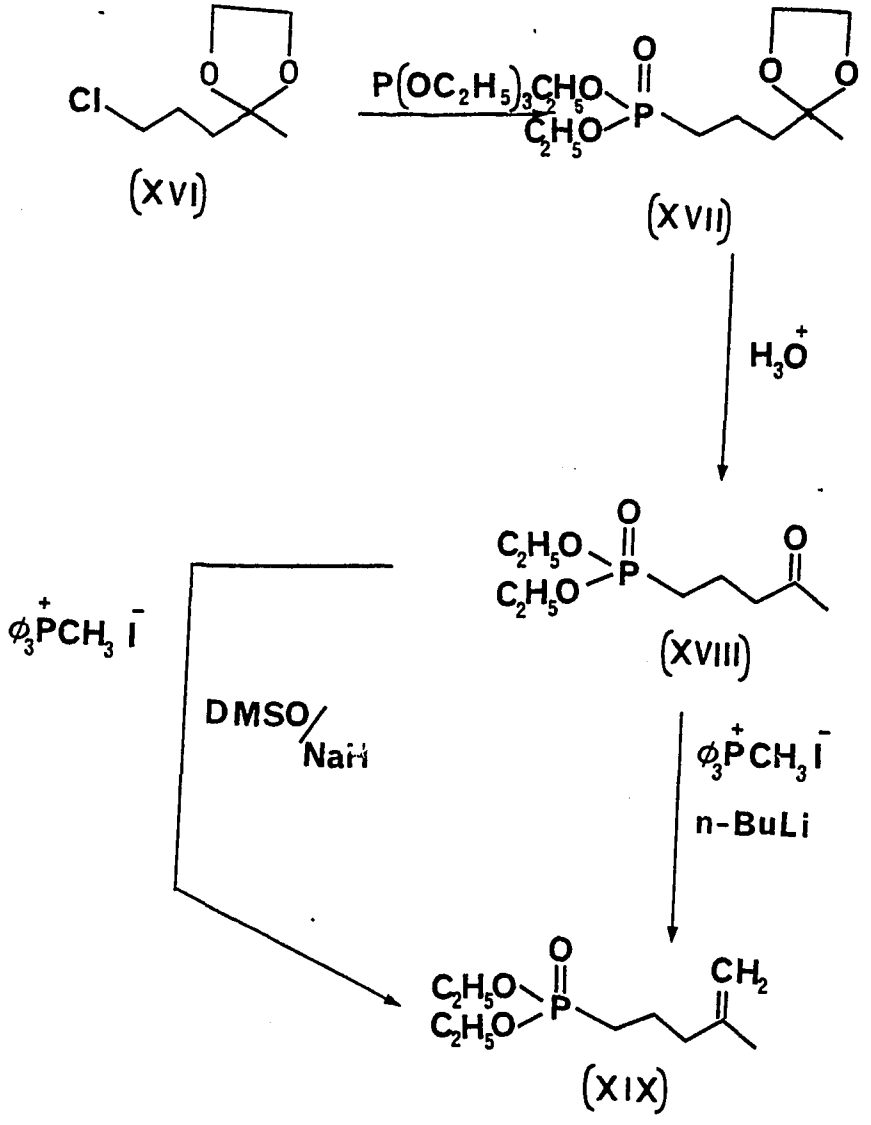
The present thesis program has had as its purpose the synthesis of a specific regulator of cholesterol biosynthesis. This was to be accomplished by the preparation of the isosteric phosphonic acid analogue of 5-phosphomevalonate, 5-carboxy-4-hydroxy-4-methylpentyl-1-phosphonic acid. In addition, analogues of the pyrophosphate bearing precursors of cholesterol were intended to be prepared where in a methylene group is substituted for the anhydride oxygen. However, once it had been found that the isostere of 5-phosphomevalonate did indeed serve as an inhibitor of cholesterol biosynthesis, but did not enter intact liver cells, this latter was replaced by the higher priority problem of designing and synthesizing the agent bearing a suitable chemical vector.

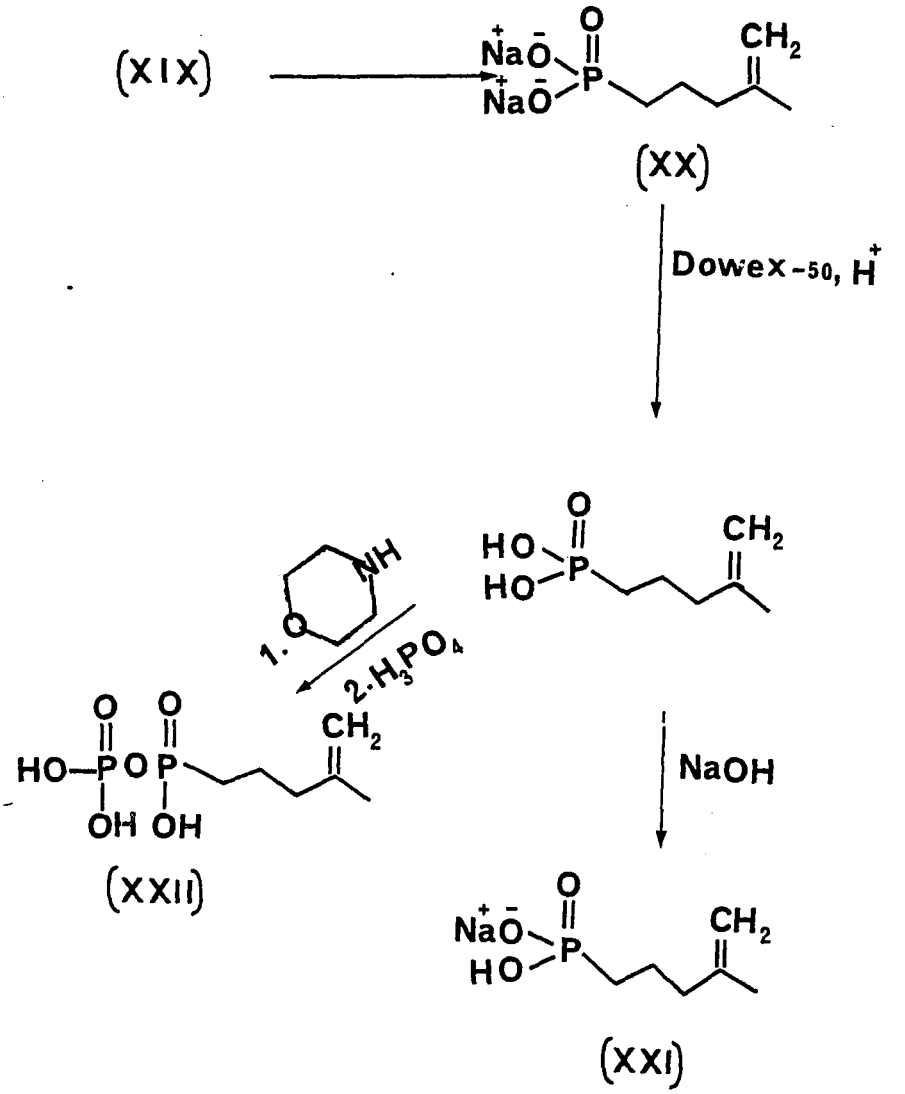
RESULT AND DISCUSSION

4-Methyl-4-pentyl-I-phosphonic acid, an analogue of isopentyl phosphonic acid has been prepared from 5-chloro-2-pentanone ethylene ketal (XVI) according to the route as shown in the scheme I. A standard Arbuzov reaction[93] was performed on (XVI) using triethyl phosphite to give the diethyl phosphonate (XVII); this is followed by mild hydrolysis to generate the free carbonyl compound (XVIII). The ketone (XVIII) is allowed to react with the methylenetriphenylphosphorane under standard Wittig[94] conditions to yield the compound (XIX) bearing the fundamental carbon structure desired. The phosphonate ester linkages are cleaved by NaI/DMF according to Moffatt's[95] procedure (see Experimental) yielding the disodium salt (XX) which is then converted to free phosphonic acid (XXI) by treatment with Dowex-50 in the hydrogen ion form. The final compound is isolated for storage and analysis as the monosodium

salt by titration with NaOH to the first inflection point of the titration curve.

Low yields of the olefin (XIX) are obtained when n-BuLi is used as the base according to standard Wittig conditions. However, yields are improved by using sodium hydride as the base in dimethyl sulfoxide according to Corey's modification.[96]





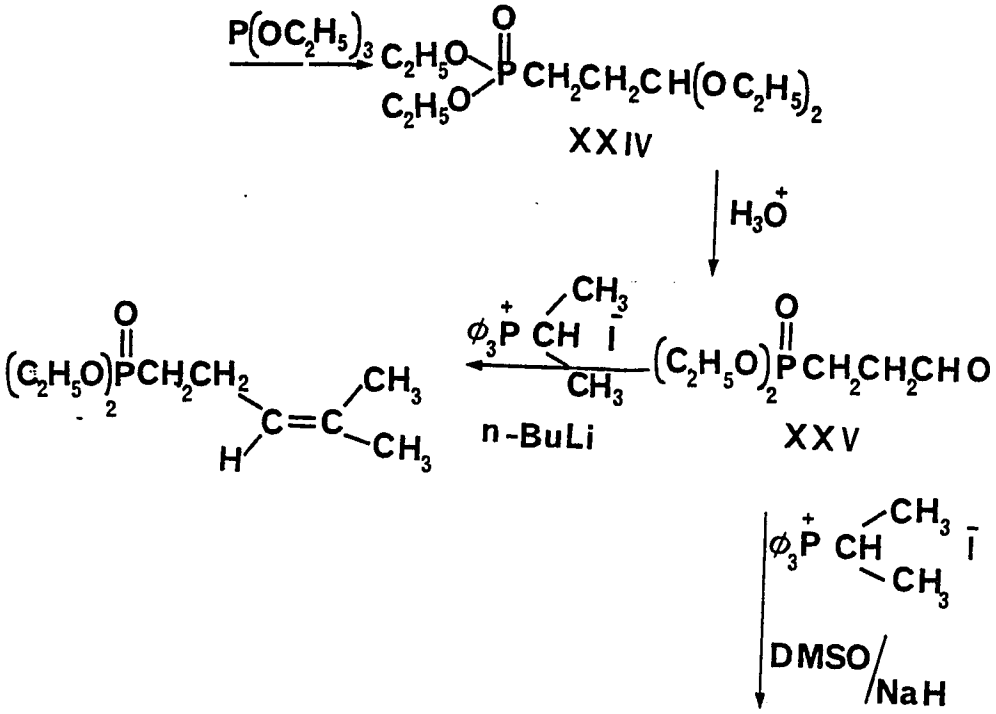
Scheme 1

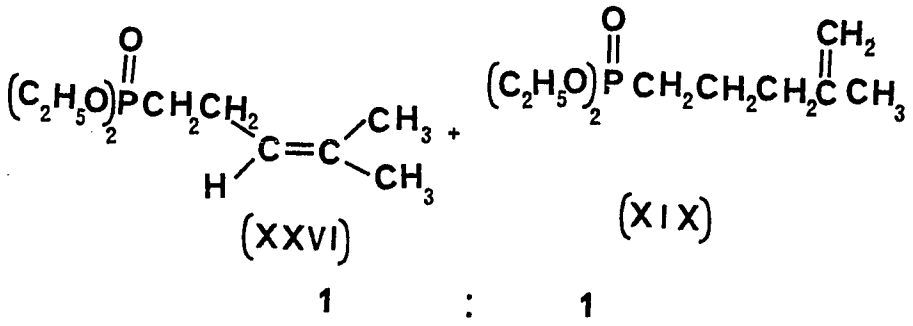
The 4-methyl-3-pentenyl-1-phosphoryl-phosphonic acid is synthesised in a manner similar to that for the previously mentioned compound (XXII). The diethyl ester of propanal-3-phosphonic acid (XXV) is allowed to react with isopropylidenetriphenyl phosphorane in a Wittig reaction using either n-BuLi[97] or the dimsyl anion derived from dimethylsulfoxide/NaH[96] as the base. The starting compound (XXV) is prepared by performing an Arbuzov reaction with triethyl phosphite on 3-chloro-1-propanal[98] (XXIII), the latter obtained by a careful reaction of acrolein with ethanol and hydrochloric acid gas[99] as shown in scheme 2.

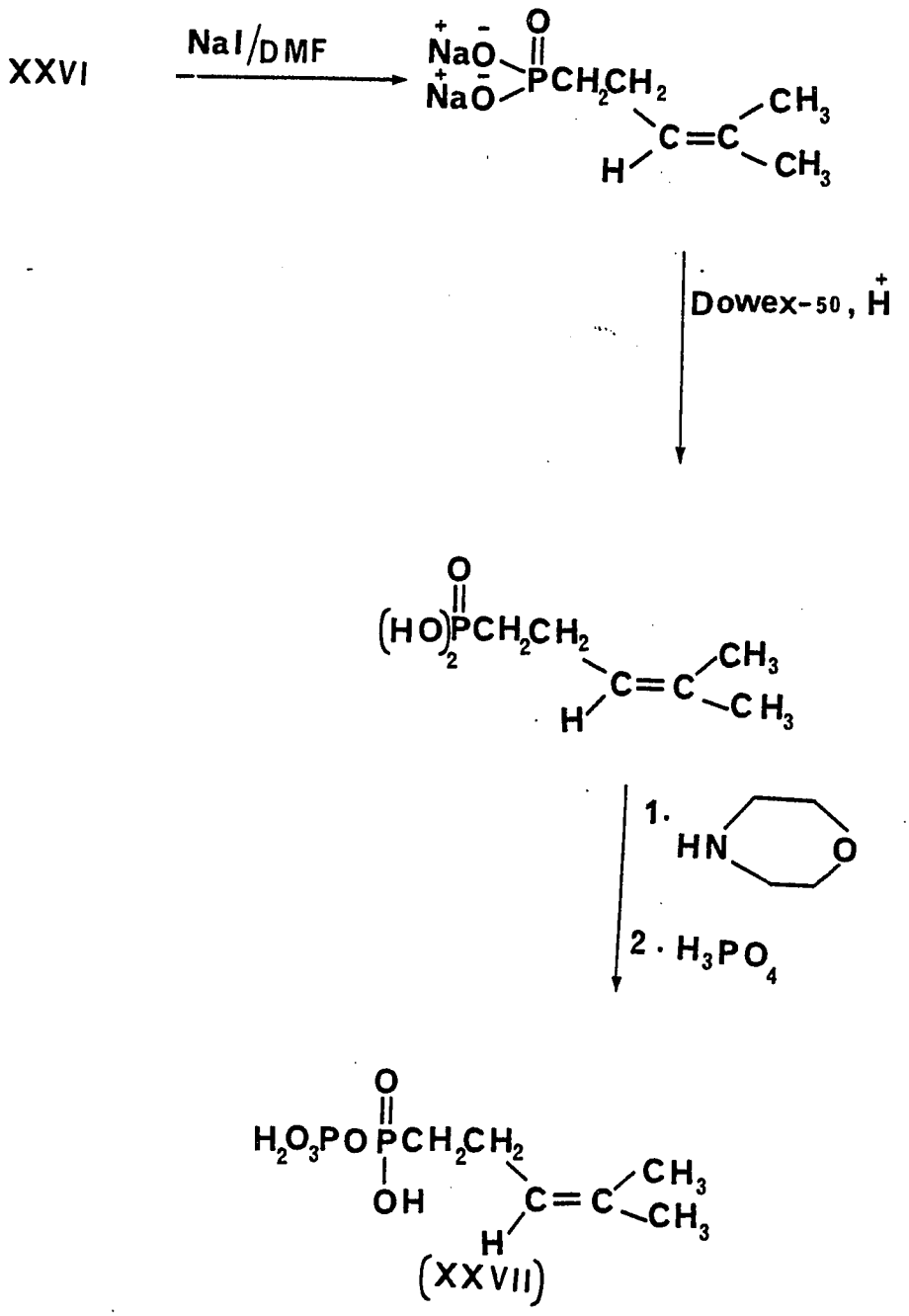
An interesting observation to be noted in this scheme is the nature of the compounds obtained in the Wittig reaction using two different bases to generate the reactive phosphorane. The exclusive formation of the compound (XXVI) is found when n-BuLi is used as the base. However reaction involving DMSO/NaH gives a 1:1 mixture of (XXVI) and (XIX). This has been confirmed by performing gas chromatographic studies as explained in the table (III). When the reaction mixture obtained from the DMSO/NaH reaction are injected together, there is observed an increase in the area of the peak corresponding to (XIX). Similarly, there is observed an increase in the area of the peak corresponding to (XXVI) when pure (XXVI) is injected with the reaction products of DMSO/NaH reaction.

$$H_2C{=}CH{-}CHO + C_2H_5OH + HCl \longrightarrow ClCH_2CH_2CH(OC_2H_5)_2$$

(XXIII)


P(OC2H5)3
C2H5O
C2H5O
O
PCH2CH2CH(OC2H5)2
XXIV
H3O+
CH3
φ3P CH I
CH3
(C2H5O)2PCH2CH2CHO
XXV
n-BuLi
(C2H5O)2PCH2CH2
CH3
C=C
H
CH3
φ3P CH
CH3
CH3
I
DMSO/NaH


(C2H5O)2PCH2CH2
CH3
C=C
H
CH3
+
(C2H5O)2PCH2CH2CH2C
CH2
CH3
(XXVI)
(XIX)
1 : 1



Scheme 2

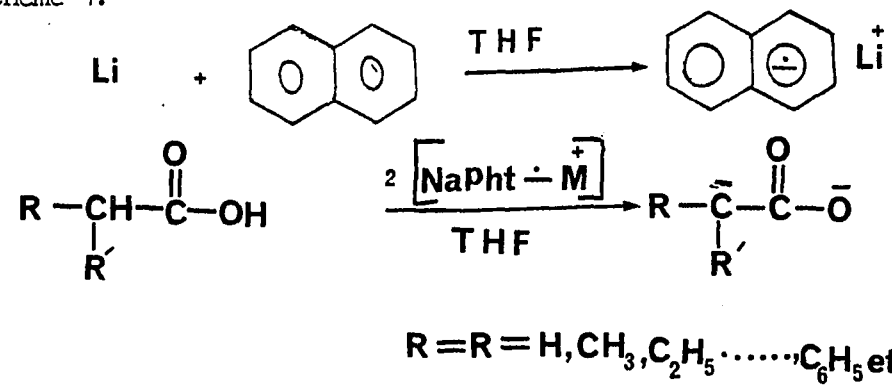
material injected	Peaks		
	(i) retention time (min)	(ii) retention time (min)	Ratio (ii) : (i)
(XIX)	8.60		
(XXVI)		9.30	
Reaction product DMSO/NaH	8.60	9.30	1.09
(XIX) + Rxn prod. (1 : 2)	8.60	9.30	0.46
(XXVI) + Rxn prod. (1 : 2)	8.60	9.30	2.50

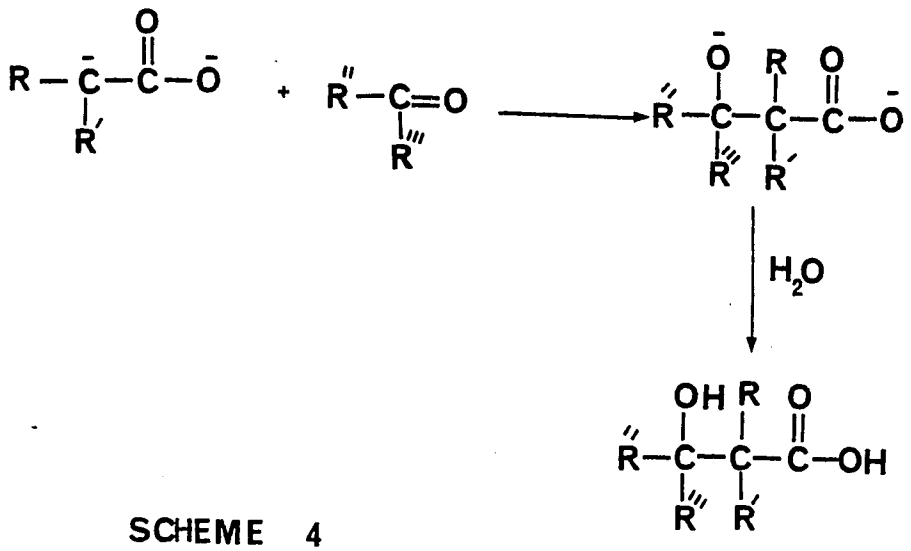
TABLE III

The synthesis of compounds (XVII) to (XXI) in Scheme I and (XXIII) to (XXVI) in Scheme 2 had been completed when the synthesis of phosphonic acid analogues of isopentenyl, γ,γ-dimethylallyl, geranyl, farnesyl and presqualene pyrophosphates were published by Corey and Volante.[64] At this stage, completion of the synthesis in this series was abandoned and attention was devoted to the analogue of mevalonic acid.

The 4-methyl-4hydroxy-5-carboxypentyl-I-phosphonic acid (XXX) is prepared from the diethyl ester of 4-oxopentyl-1-phosphonic acid (XVIII) which has been described above in scheme I. Compound (XVIII) was subjected to the Reformatsky reaction using the two-step procedure as described by Grob and Bunneisen.[100] This was followed by hydrolysis under basic conditions using sodium hydroxide in methanol[101] to yield the free carboxylic acid (XXLX). The phosphonate ester linkages were cleaved according to the Rabinowitz[102] technique using trimethylsilyl chloride followed by aqueous hydrolysis to generate the free phosphonic acid (XXX) which is isolated as the dicyclohexylammonium salt (XXXI). (Scheme 3) In spite of numerous efforts using different techniques to activate zinc for the Reformatsky reaction, the yields of the reaction could not be improved.

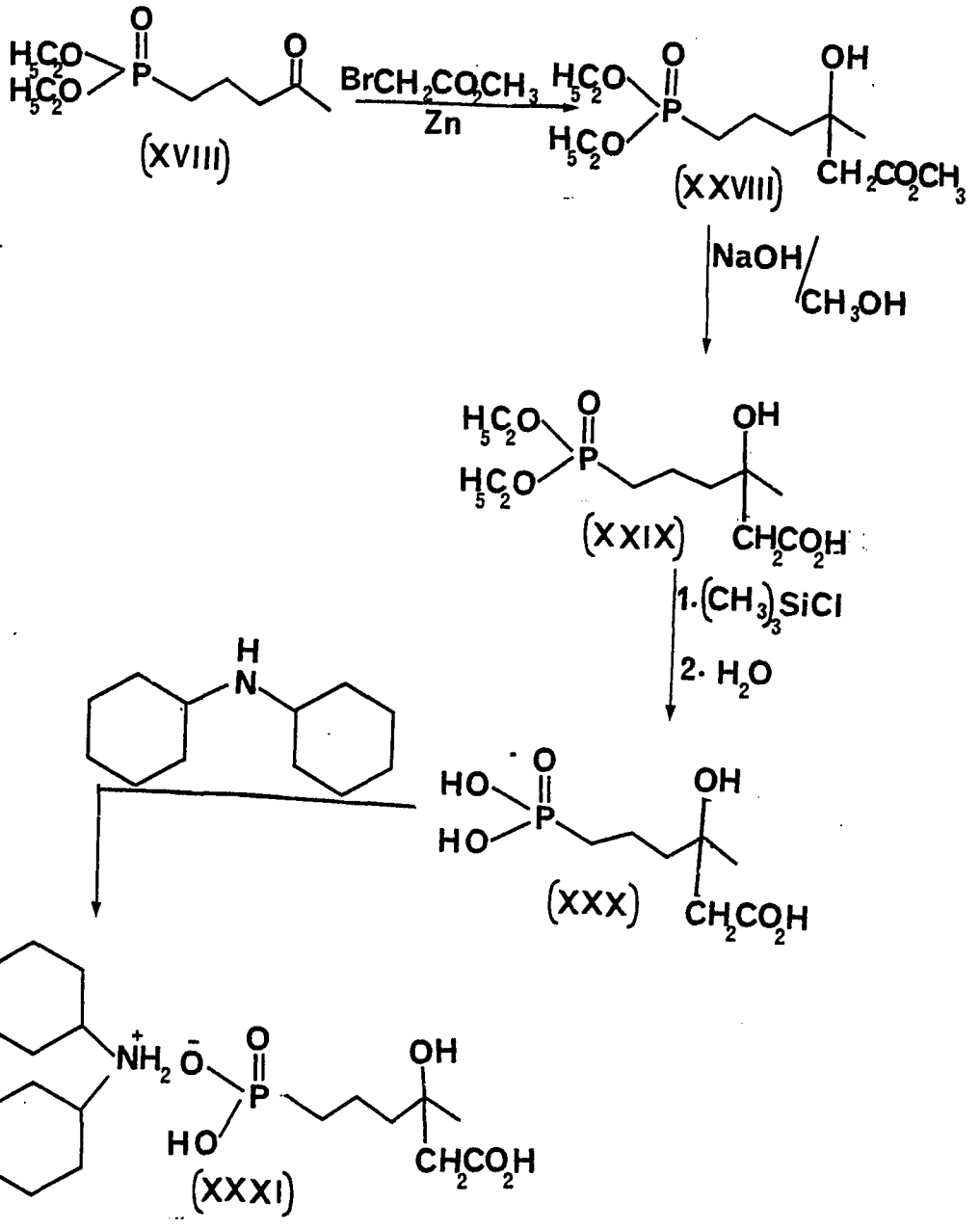
Lithium naphthalene[104,105] is a reagent used to generate a dianion from carboxylic acids. These dianions have been reported to attack carbonyl carbons generating intermediates which after hydrolysis, yield β-hydroxy acids directly. Lithium naphthalene is a radical anion which removes protons from the acid to form a dianion as shown in Scheme 4.



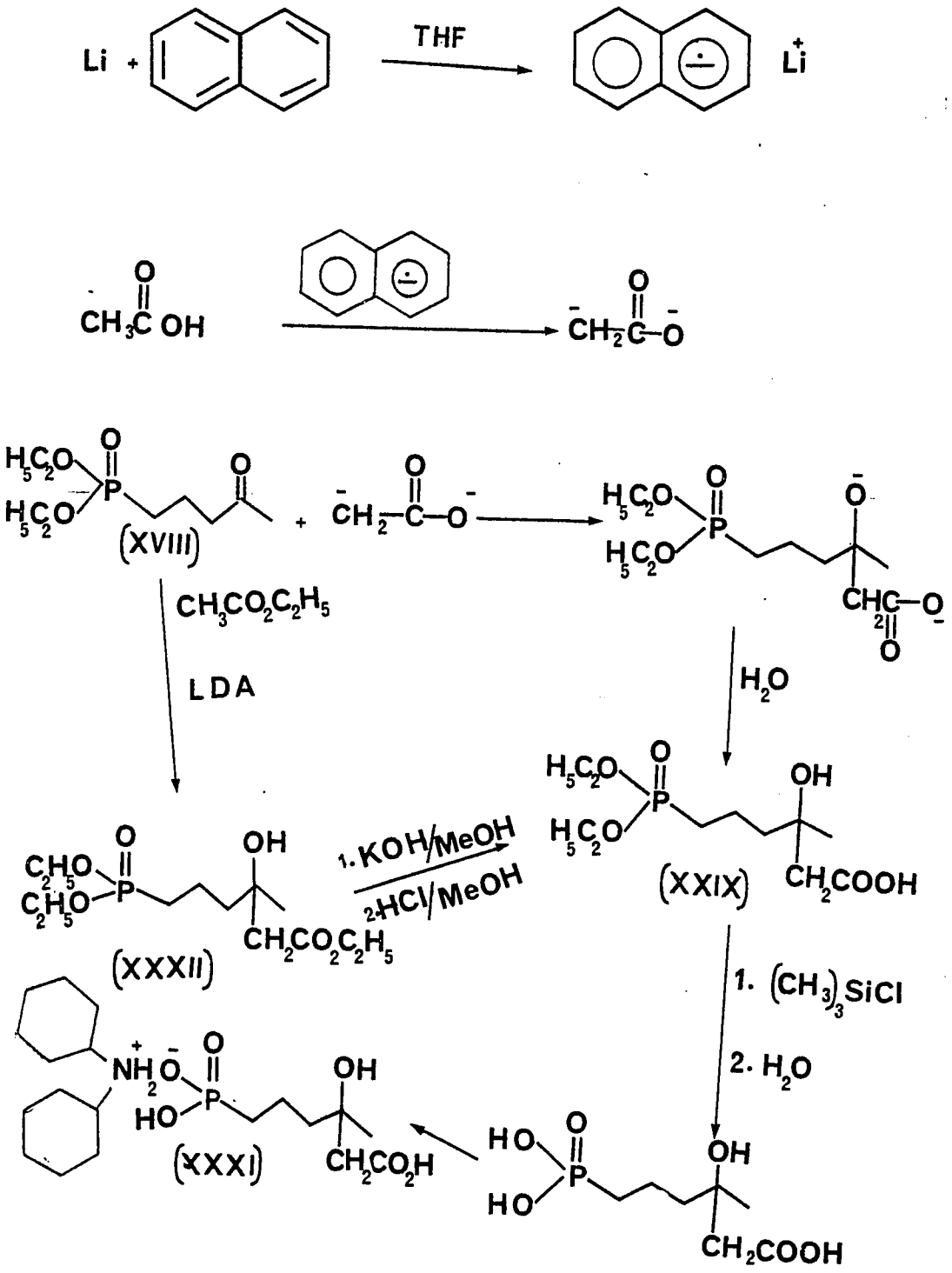


SCHEME 4

Some examples of the use of lithium naphthalene to prepare β-hydroxy acid are shown in Table (IV).[104] This approach offers several advantages over the Reformatsky reaction, although the yield of the reaction is not particularly high. Better yields of the compound (XXIX) have been obtained by allowing (XVIII) to react with the anion obtained from ethyl acetate using lithium diisopropylamide[106] as the base. The carboxylate ester is then hydrolyzed by a base (NaOH in MeOH)[107] to yield the carboxylic acid (XXIX) in good yield as shown in scheme 5.

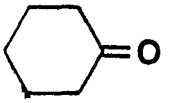
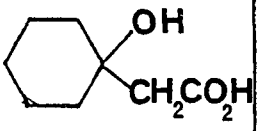
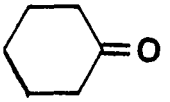
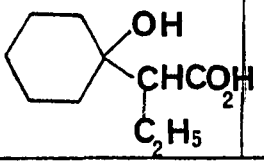

(XVIII)
BrCH2CO2CH3
Zn
(XXVIII)
NaOH/CH3OH
(XXIX)
1.(CH3)3SiCl
2. H2O
(XXX)
(XXXI)

Scheme 3

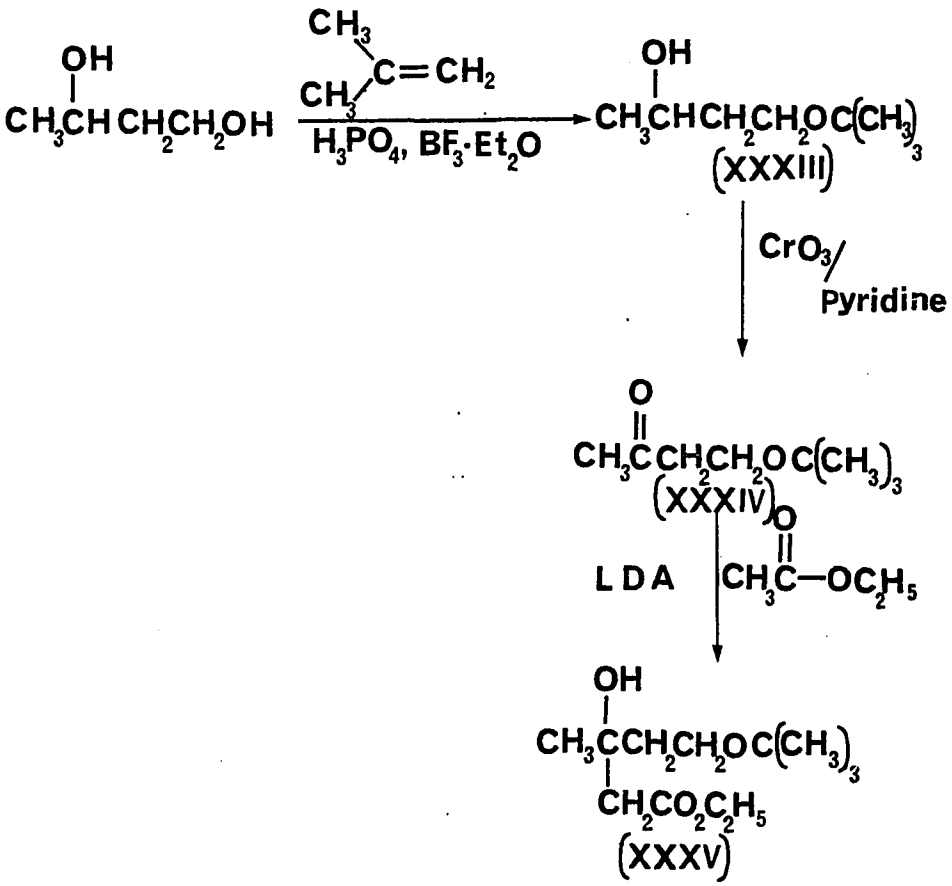

Li
THF
Li
CH3C OH
CH2C O
(XVIII)
CH3CO2C2H5
LDA
H2O
(XXIX)
CH2COOH
1. KOH/MeOH
2. HCl/MeOH
(XXXII)
CH2CO2C2H5
1. (CH3)3SiCl
2. H2O
(XXXI)
CH2CO2H
CH2COOH

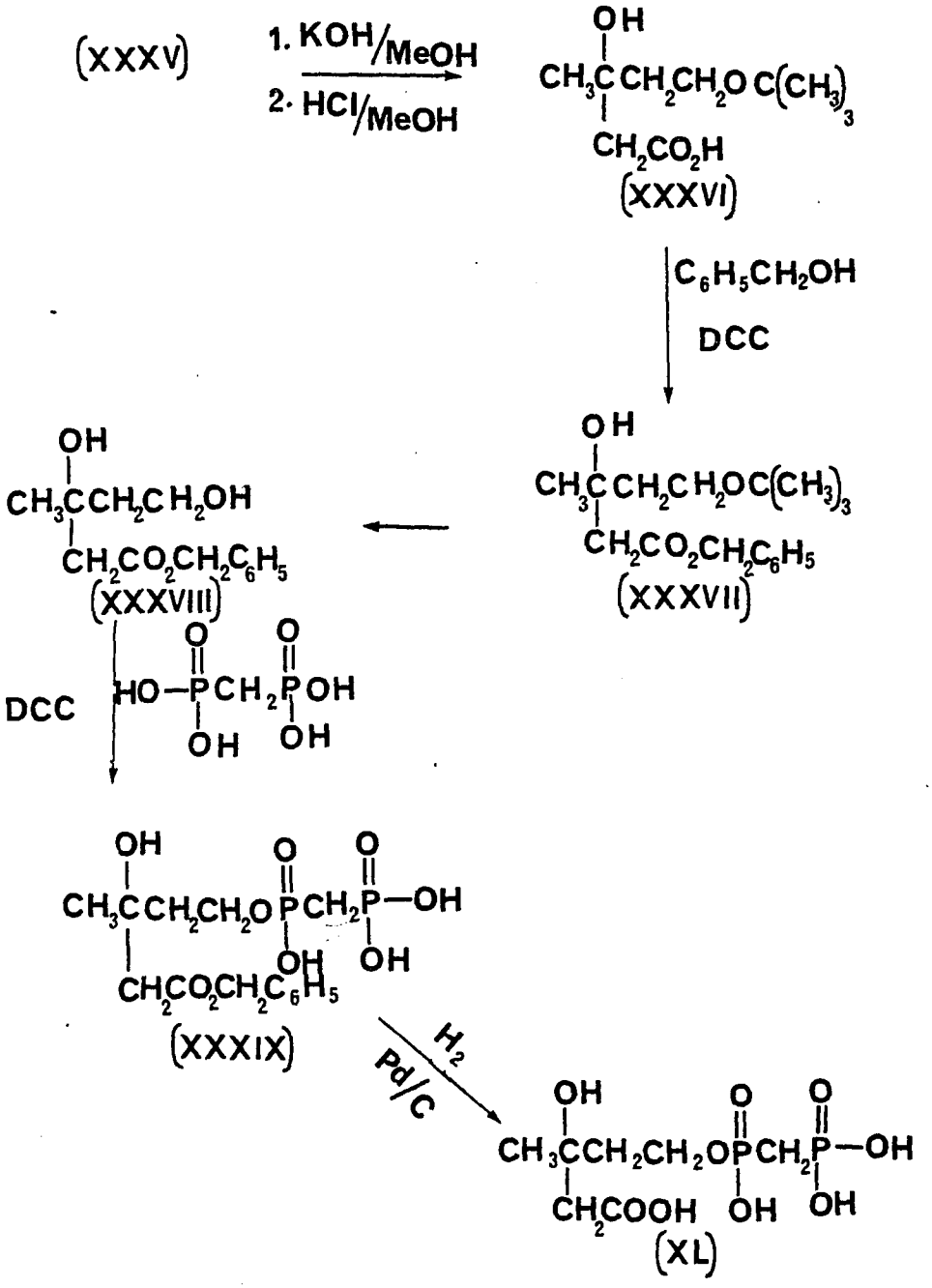
SCHEME 5

Table (IV)

ACID	Carbonyl Compound	Product	% Yield
CH_3COOH	$C_6H_5COC_6H_5$	$C_6H_5C(OH)(C_6H_5)CH_2COOH$	58
CH_3COOH			38
CH_3COOH	$CH_3CO(CH_2)_4CH_3$	$CH_3C(OH)(CH_2)_4CH_3CH_2COOH$	37·3
CH_3CH_2COOH	$pCH_3OC_6H_4COC_6H_5$	$pCH_3OC_6H_4C(HO)(CH_3)CHCO_2H$ C_6H_5	38
$CH_3CH_2CH_2CO_2H$			43

Another analogue of interest for the present work would be mevalonyl methylendiphosphonic acid in which a methylene group is present in place of the normal anhydride oxygen of the pyrophosphate function. The scheme for its synthesis would involve the preparation of mevalonic acid and its coupling to diphosphonic acid at the primary hydroxyl function of the mevalonic acid. Because of the ease with which mevalonic acid forms the lactone it is important that at no stage during its synthesis should both the primary hydroxy and carboxyl groups be allowed to be free simultaneously. Different routes for preparing the protected mevalonic acid have been tried, the first one of which is shown in scheme 6.

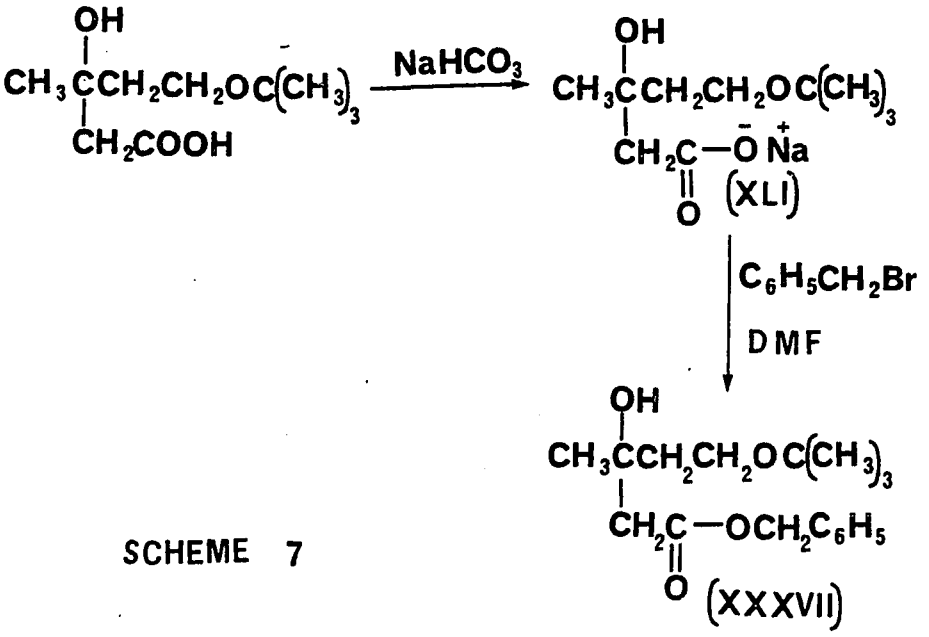



(XXXV)
1. KOH/MeOH
2. HCl/MeOH
(XXXVI)
C6H5CH2OH
DCC
(XXXVII)
(XXXVIII)
DCC
(XXXIX)
H2
Pd/C
(XL)

SCHEME 6

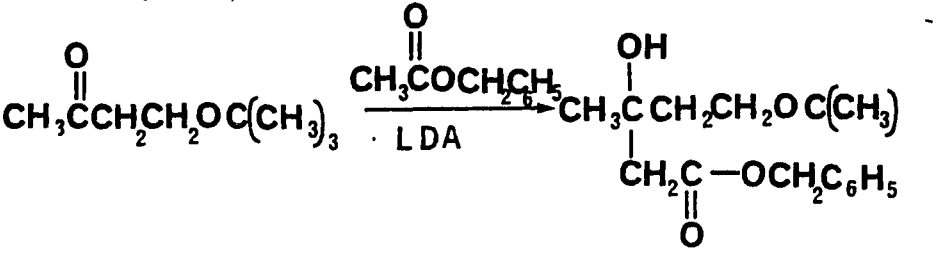
No problem was encountered in the synthesis through generation of compound (XXXVI), but the coupling of benzyl alcohol with DCC[109] could not be performed in Et_2O, $CHCl_3$ or THF at room temperature or at reflux. An alternative would be to make the sodium salt of the acid and then allow this salt to react with benzyl bromide.[110]

Scheme 7.



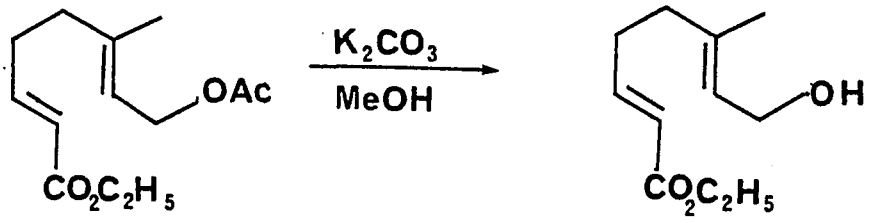
SCHEME 7

The synthesis of the benzyl ester was best achieved by performing the reaction of the carbanion obtained from benzylacetate [106] with the ketone (XXXIV) as shown below:

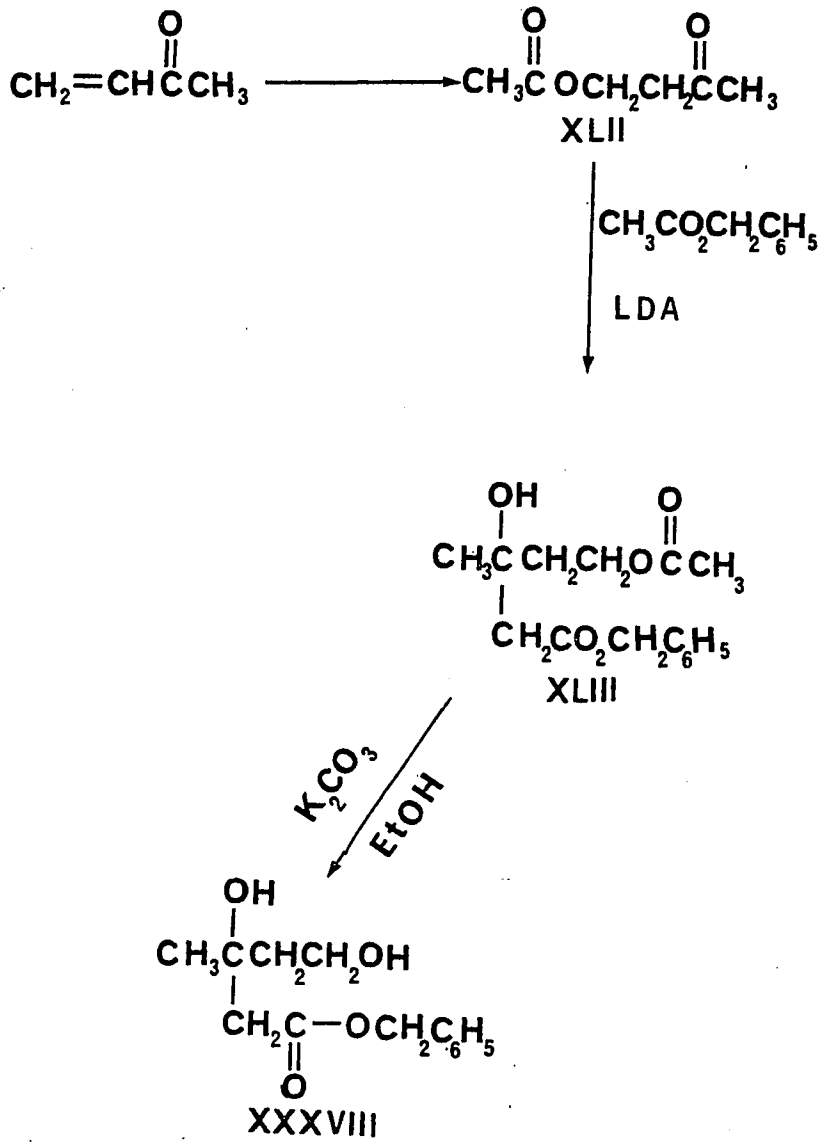


Scheme 7 had to be abandoned as the removal of the protecting t-butyl group could not be attained with an acid[124] in the presence of a tertiary hydroxyl group due to the ease of formation of a tertiary carbonium ion and the resulting extraneous reactions.

In the second scheme used for the synthesis of this compound (XL), an acetate group instead of t-butyl was used for the protection of the primary hydroxyl function.[125] At the end of the sequence, selective removal of the acetate in the presence of a benzyl ester would be tried using potassium carbonate as the base. There are examples reported in the literature where the selective removal of the acetate has been achieved in the presence of deactivated esters. Corey[126] achieved this in the synthesis of dl-sirenin using potassium carbonate in methanol as the base.

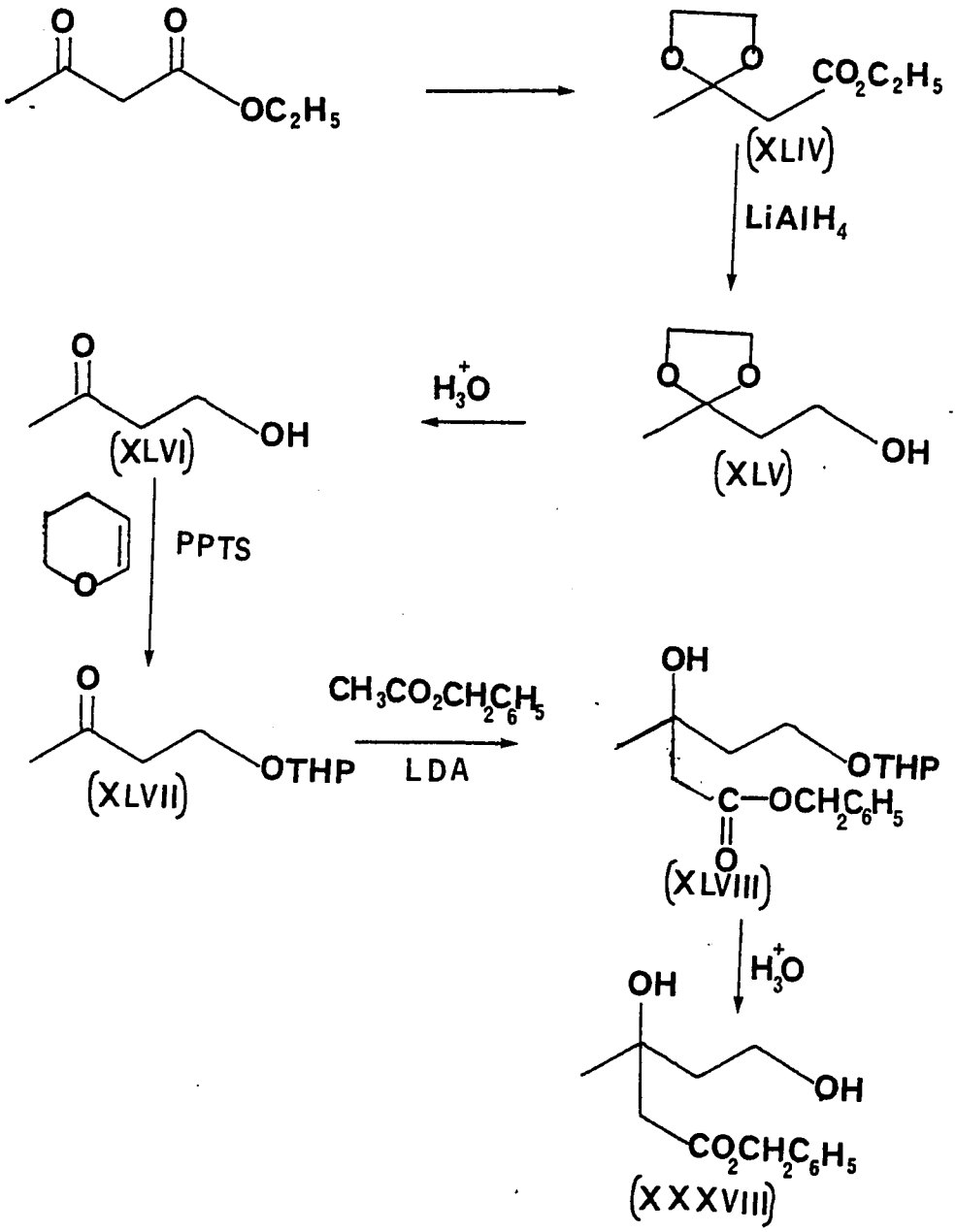


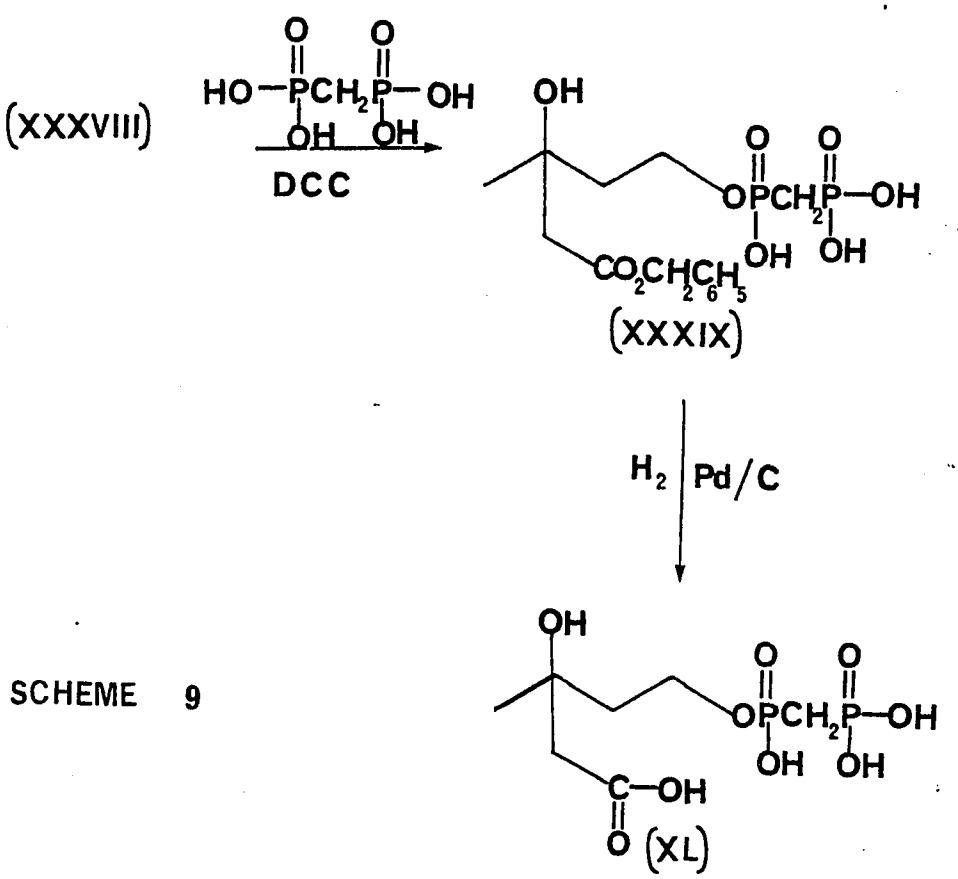
However, this selective removal could not be achieved using potassium carbonate under varying conditions of temperature and time. The Scheme 8 shown below was also abandoned after the compound (XLII) had been synthesised.


CH2=CHCCH3
O
CH3CO CH2CH2CCH3
O
O
XLII
CH3CO2CH2C6H5
LDA
OH
O
CH3C CH2CH2O CCH3
CH2CO2CH2C6H5
XLIII
K2CO3
EtOH
OH
CH3CCH2CH2OH
CH2C—OCH2C6H5
O
XXXVIII

SCHEME 8

Another possible route for its synthesis would involve the use of a tetrahydropyranyl ether as a protecting group.[127] The advantage of using this as a protecting group is that it can be removed under extremely mild acidic conditions[128] and hopefully leave the tertiary hydroxyl group untouched. (Scheme 9.)



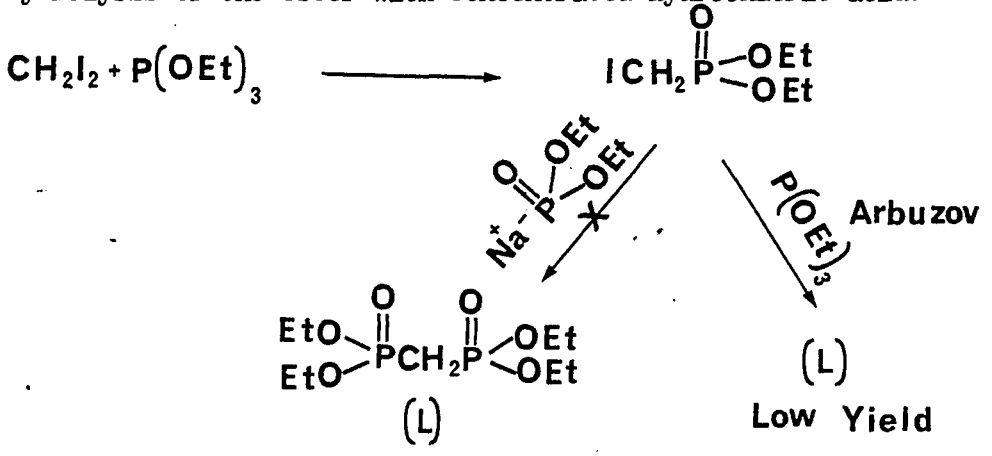


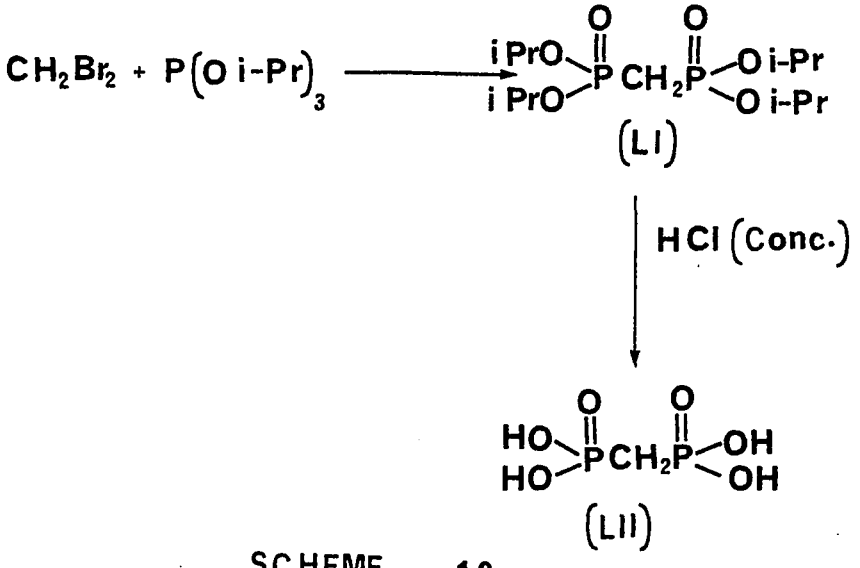
SCHEME 9

A variety of routes used to synthesize methylenediphosphonic acid all involve acidic hydrolysis of the tetraalkyl esters. The use of the Arbuzov reaction[129-132] on methylene dihalides has been reported to give the desired tetraalkyl ester in widely varying yields depending on the alkyl groups of the trialkyl phosphite and the reaction conditions used. Reaction of triethylphosphite with methylene iodide under Arbuzov conditions gave the monophosphonate (XLIX); attempts to make the desired tetraalkyl ester by performing either an Arbuzov reaction with more triethylphosphite on (XLIX) or by a Michaelis-Becker reaction on (XLIX) were not successful in our hands.

The required ester was eventually synthesised in good yield:

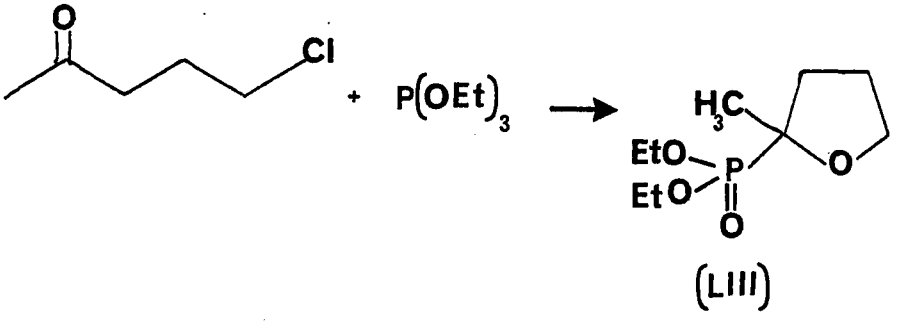
by performing a reaction between methylene bromide and triisopropyl phosphite at 140-185° using a 24 - inch fractionating column packed with glass helices.[133] Methylenediphosphonic acid was obtained by hydrolysis of the ester with concentrated hydrochloric acid.[133]



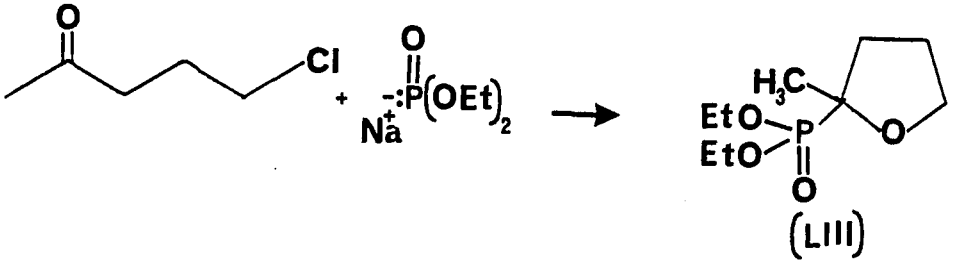


SCHEME 10

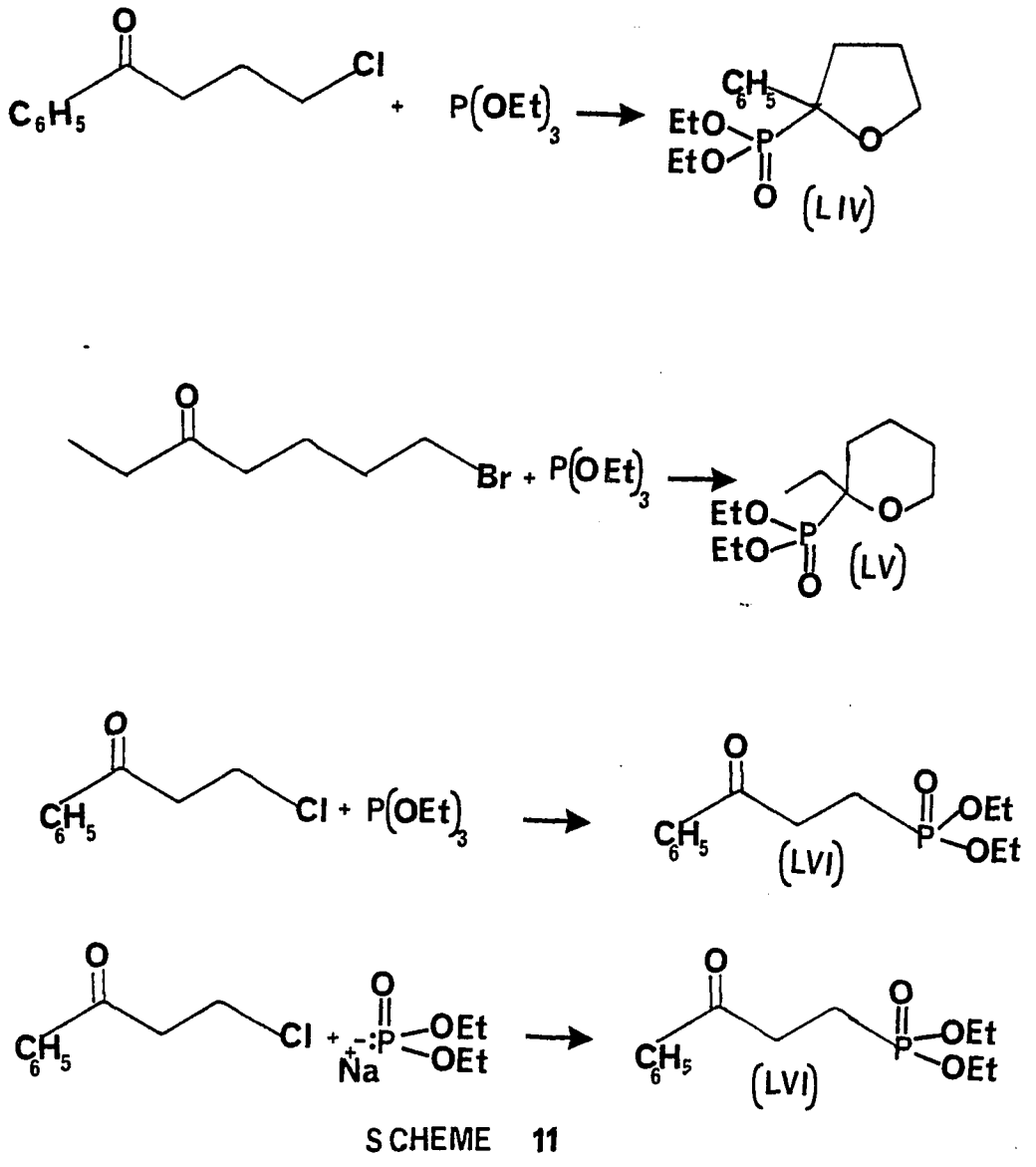
During the preparation of diethyl 4-oxopentyl-1-phosphonate (XVIII) there was performed a reaction of triethyl phosphite on 5-chloro-2-pentanone. Instead of obtaining the desired compound (XVIII), there was isolated a cyclized material. This reaction prompted the study of the reaction of different halo-ketones with Triethyl phosphite under Arbuzov conditions and with diethyl phosphite under Michaeles-Becker conditions. 1, 4-Haloketones give cyclized product with a tetrahydrofuranyl skeleton both under Arbuzov and Michaeles-Becker conditions. 1, 5-Haloketones also yield cyclized compounds with a tetrahydropyran skeleton. However, 1, 3-Haloketones give open chain compounds as shown in Scheme 11.



(LIII)



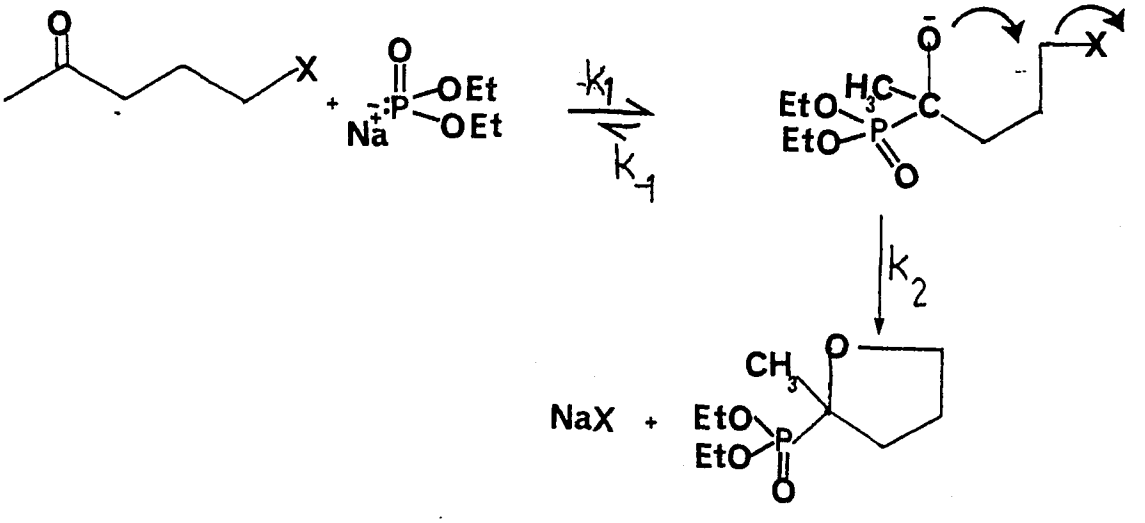
(LIII)

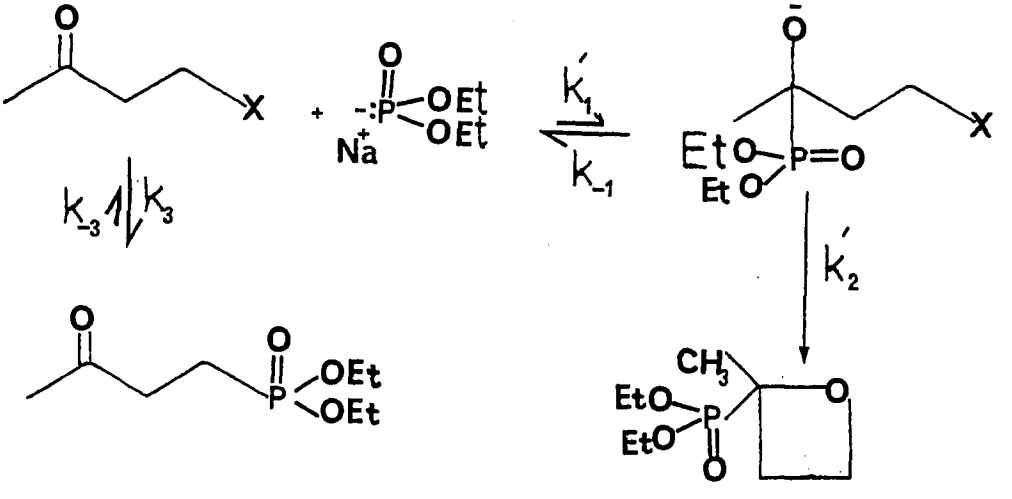


SCHEME 11

Investigations of the mechanism of this reactions led to the conclusion that for the dialkyl phosphites, reaction proceeds by attack of phosphorous at the carbonyl carbon to form an intermediate halohydrin, followed by the nucleophilic S_ni attack of oxygen on the halogen-bearing carbon. This route is sterically favorable

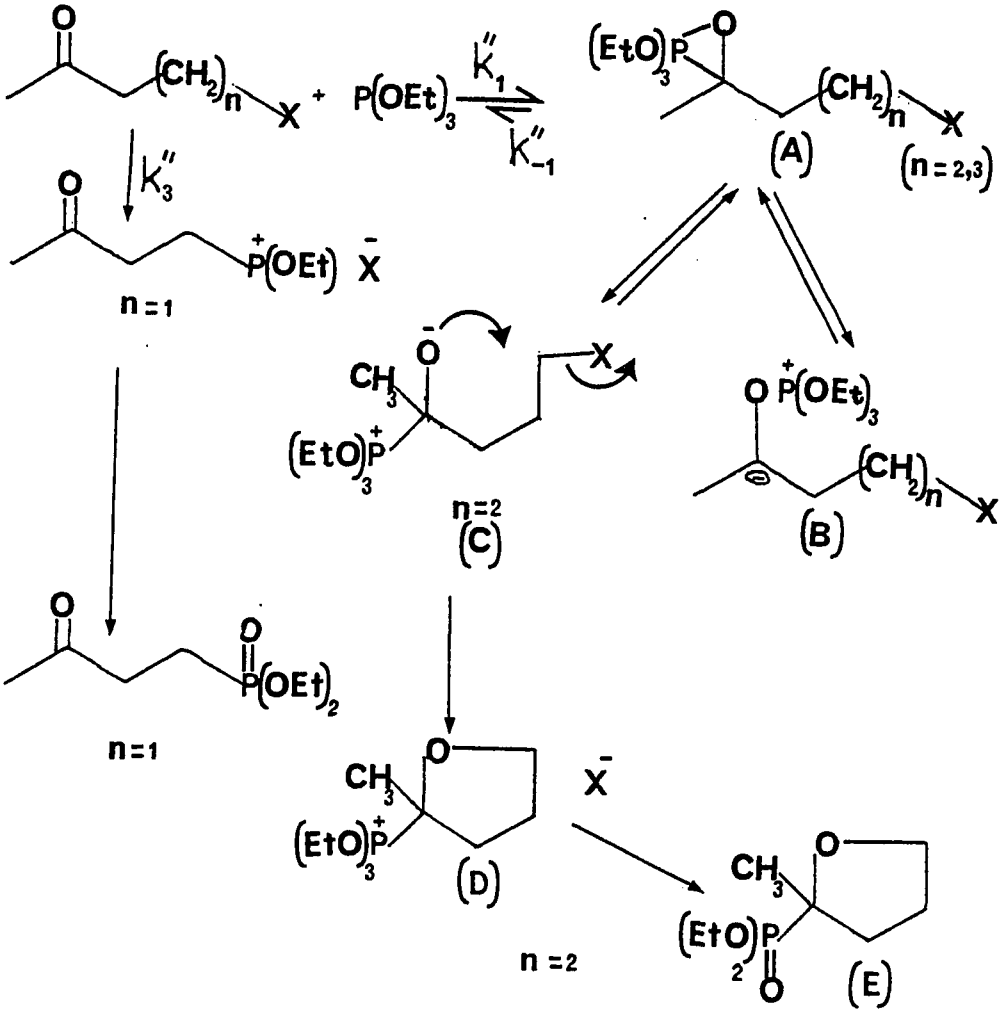
for the formation of five and six membered ring compounds. However, in the case of β-haloketones, the formation of the four membered ring is unfavorable and therefore the reaction proceeds by the attack of phosphorous at the β-carbon displacing halogen to give an open chain compound.





In the first case, k_2 is fast and therefore the reversible first step of the reaction shifts towards the right, i.e. towards the ring compound. In the second case, however k_2' is slow because of the unfavorable four-membered ring, and therefore the reversible first step of the reaction shifts towards the open chain compound.

In reaction with triethyl phosphite, phosphorous adds across the carbonyl to give a pentacovalent species as shown below. It can be considered as a tautomer of (C) and (B).



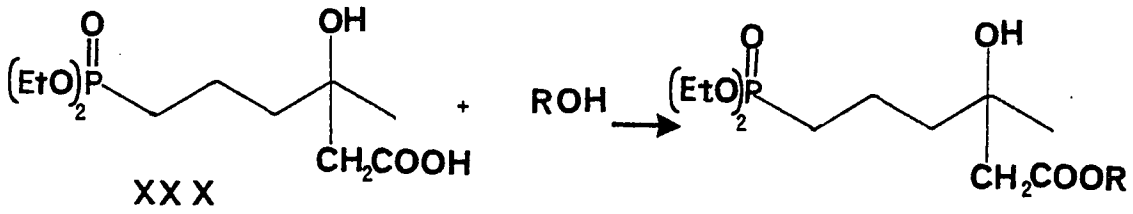
Either (A) or (C) may proceed readily to (D). This latter species may then be converted rapidly to the ring compound (E) for those systems where a five or six membered ring is formed. The last step, a displacement of an alkyl group by halide ion, similar to the second step of an Arbuzov reaction, is rapid and is not involved in the rate determining step. Phosphite addition across the carbonyl is reversible. The k''_2 is much larger than k''_{-1} for five and six membered ring compounds ($k''_2 \gg k''_{-1}$), but in the sterically unfavorable four-membered ring system k''_2 becomes small and hence k''_{-1} is significant. Therefore, phosphorous attack at the β-carbon to yield a β-ketophosphonate ($k''_{-1} \gg k''_2$) which predominates.

The phosphonic acid (XXXI) was designed as a specific inhibitor for the biosynthetic pathway leading to squalene and cholesterol. In fact, it has been found to act as a most potent inhibitor of squalene and cholesterol synthesis when used with enzyme preparations from rat liver.[108] The site of specific inhibition has been found to involve phospho-mevalonate kinase; once phosphorylated, the analogue is unable to undergo the required decarboxylation, and feedback inhibition occurs. (108). The K_i for the analogue has been found to be very similar to the K_m of the natural substrate.[108]

Unfortunately, the analogue does not appear to be transported into intact cells, a necessity for its use as a drug.[108] To overcome this problem, the potential drug is covalently attached to a "chemical vector" for which a mode of transport into the intact cell may exist. Once the potential drug with its chemical vector has entered the intact cell, it is expected that the extraneous portion will be cleaved by enzymes normally present. As described before, two types of chemical vectors

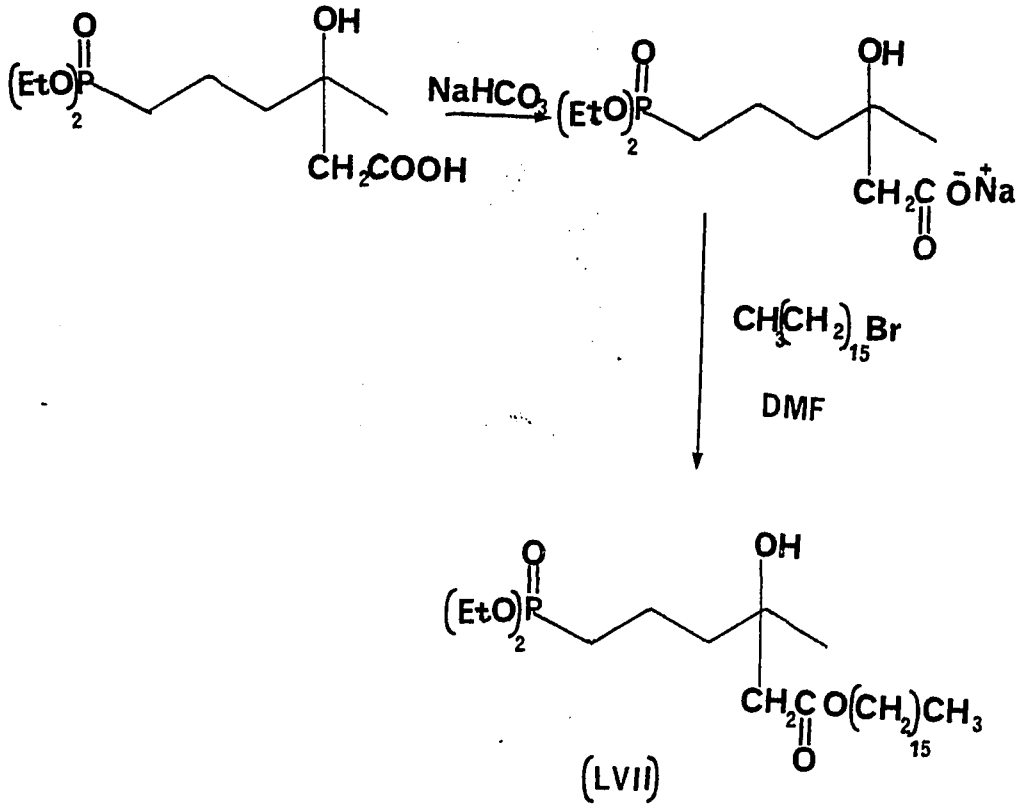
are considered here. The first of these would be a tripeptide with the potential drug attached through a functional group to a suitably reactive residue on the peptide. Such a system may then be able to gain entrance to the cell via an oligopeptide transport system. The second type of chemical vector would be a large lipophilic function which is capable of taking part in micelle formation.

The carboxyl function of the mevalonate analogue is the most suitable site for attaching a lipophilic chemical vector. It would appear reasonable that fatty alcohols could be coupled to the free carboxylic acid-phosphonate diester (XXIX) by a dicyclohexylcarbodiimide (109) mediated reaction to generate the ester (LVII) as illustrated in Scheme 12. However, dodecanol could not be coupled to the acid (XXIX) by dicyclonexylcarbodiimide using ether, chloroform or tetranydrofuran as solvent, at room temperature or at reflux temperature for time varying from 5 to 48 hours.



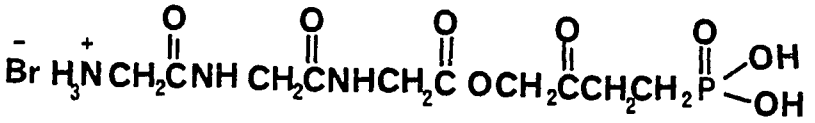
SCHEME 12

This coupling was eventually achieved by preparing a sodium salt of the carboxylic acid (XXIX) by treatment with sodium bicarbonate.[110] The sodium salt is thoroughly dried over P_2O_5 under high vacuum and is then allowed to react with cetyl bromide in DMF as the solvent. (Scheme 13)

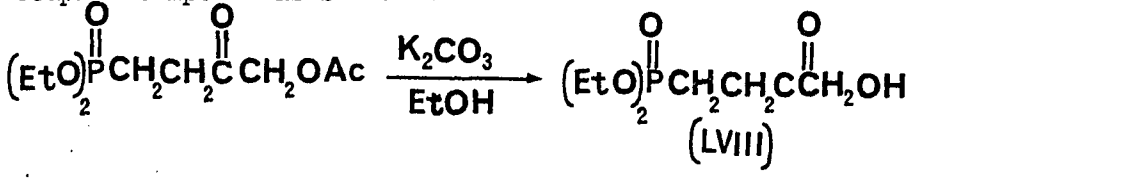


SCHEME 13

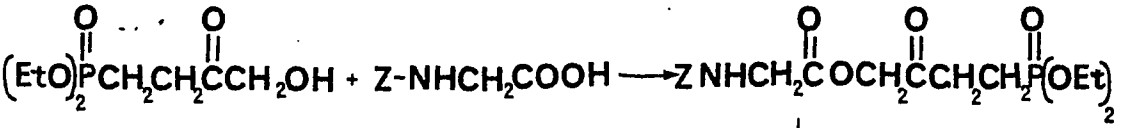
Model structures for the second type of chemical vector i.e. an oligopeptide transport system, were begun with the synthesis of triglycine as the "chemical vector" and 4-hydroxy-3-oxobutyl-I-phosphonate, the isosteric analogue of dihydroxyacetone phosphate, as the drug. The carboxyl terminus of the triglycine is coupled to the hydroxy group of the analogue.

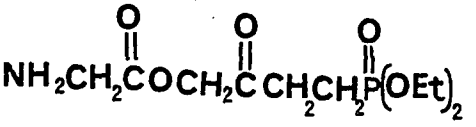


Three approaches for its synthesis were used. The first of these involves coupling the phosphonate analogue to one glycine unit and then a diglycyl unit is attached to the amino terminus of the coupled compound as shown below.



(LVIII)





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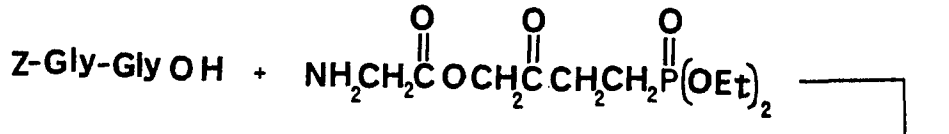
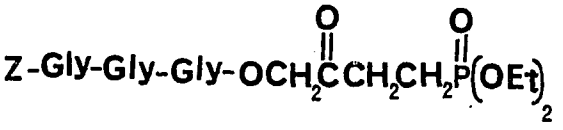
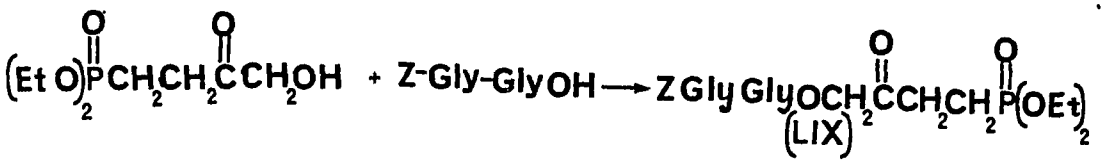
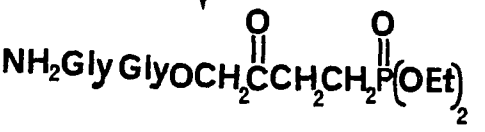




The second approach would be to couple the phosphonate analogue first to a diglycine unit and then hook up the third glycine unit.





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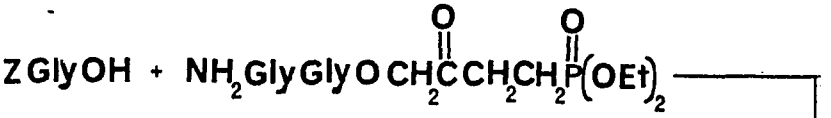
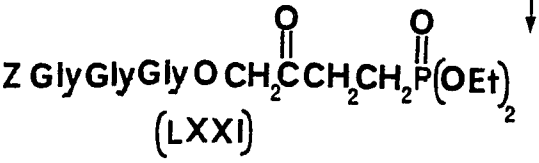
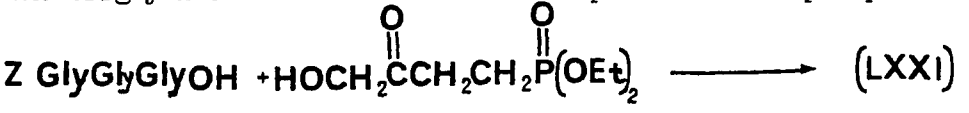


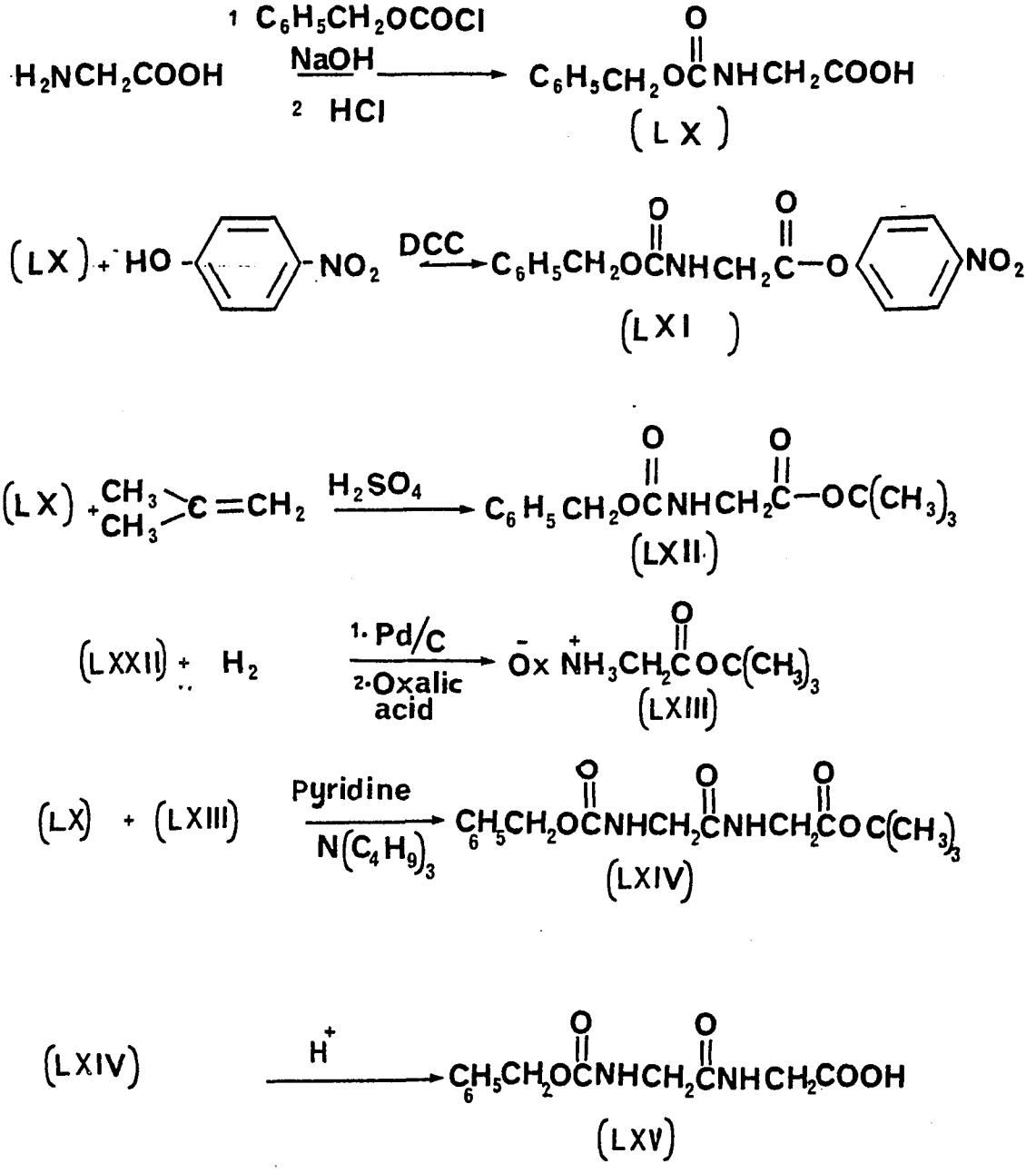


The third, and most likely the best, approach is to synthesise the triglycine unit first and then couple it to the phosphonate analogue.

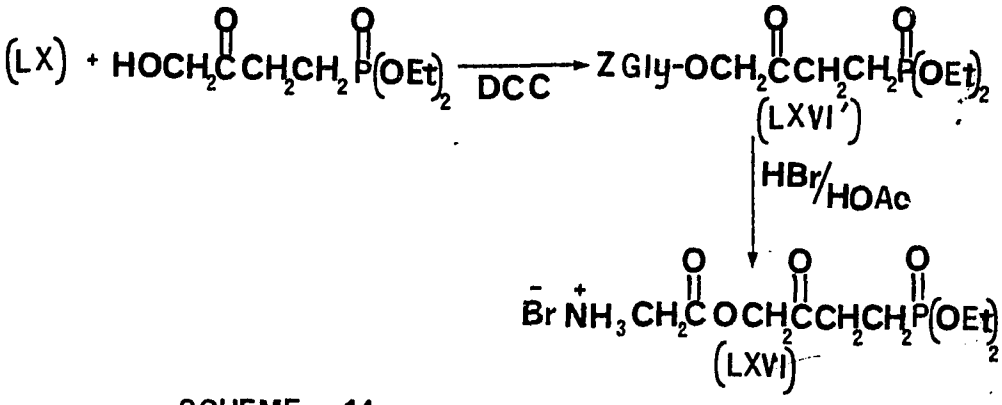


For the synthesis of the tripeptide linked to the phosphonate analogue, the carbobenzyloxy group was used for the protection of the amino group[112] whereas t-butyl esters were prepared for the protection of carboxyl group.[113] In later efforts ethyl esters[114] were

used as the carboxyl protecting group instead of the t-butyl group. For coupling glycyl units, Bodansky's[115] active ester method was used, p-nitrophenol being used as the activating group. (Scheme 14)



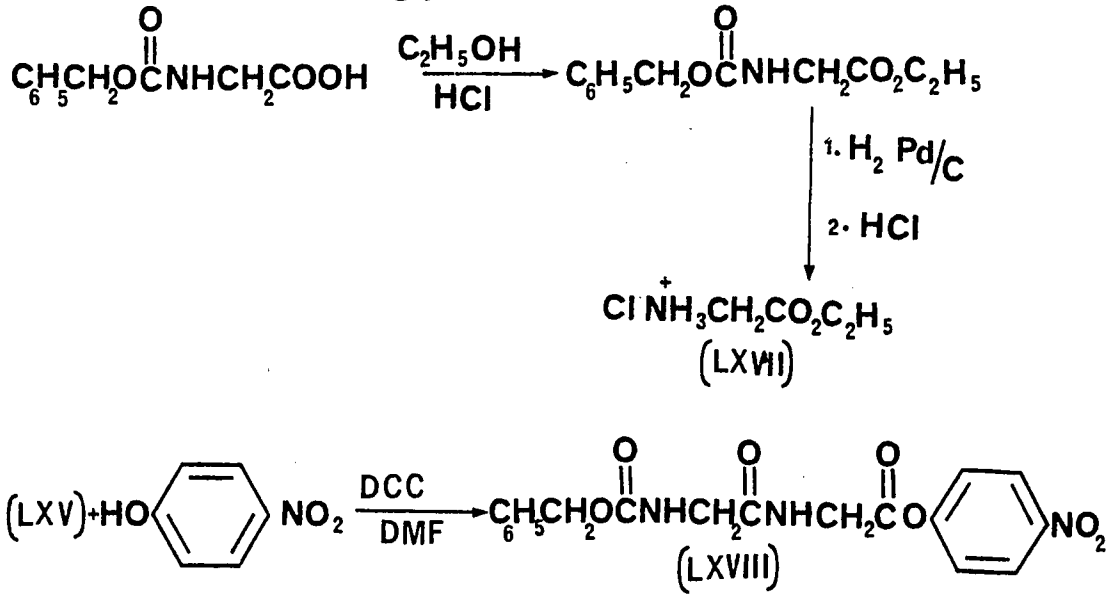
Scheme 14

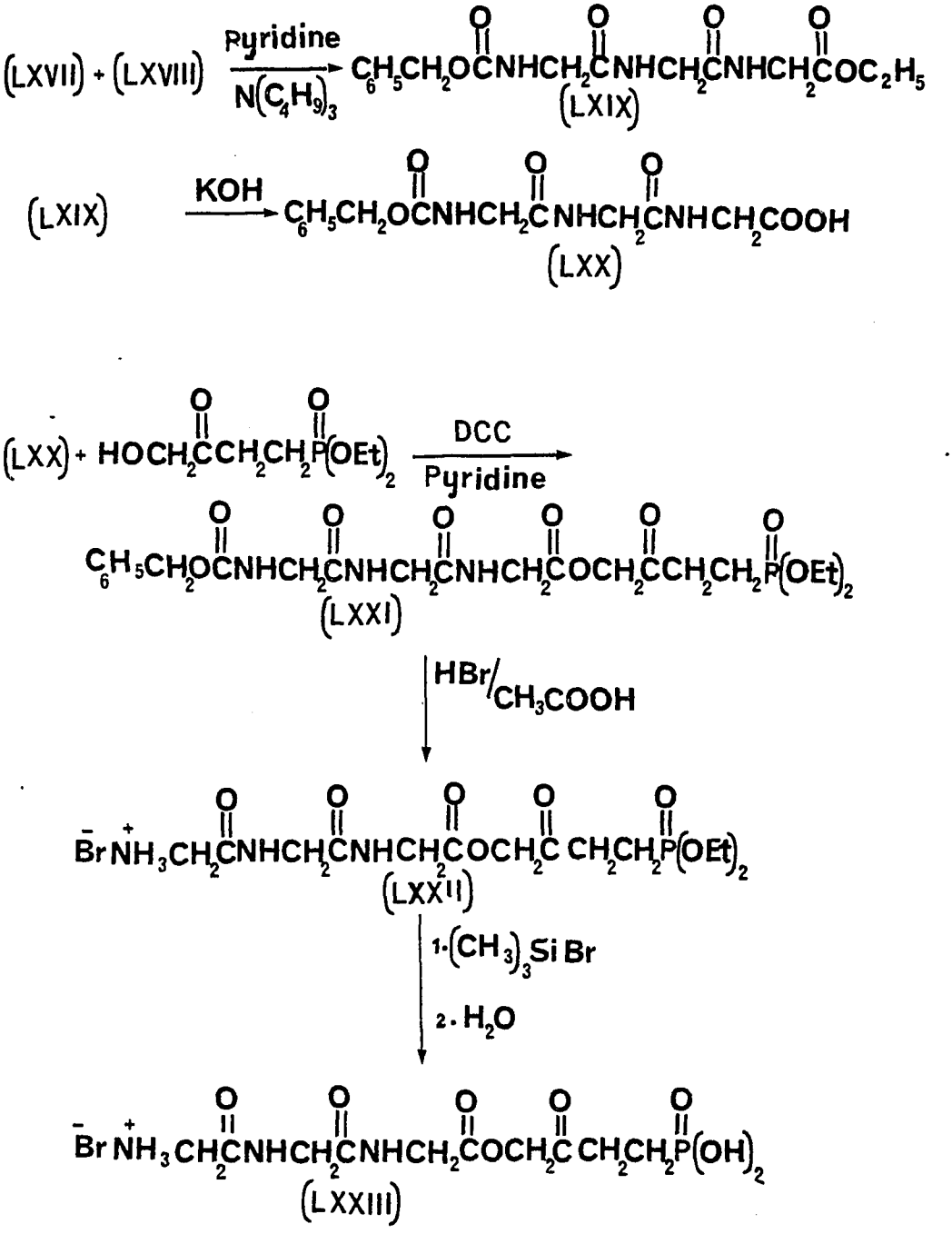


SCHEME 14

All attempts made to couple (LXV) and (LXVI) were in vain. The different methods used to couple (LXV) and (LXVI) include DCC in CH_2Cl_2, EtOAc, Et_2O as solvents, the mixed anhydride, and activated ester methods; none of these worked. Neither was success obtained with the second method, combining the phosphonate analogue with diglycine unit and then hooking up the third glycine unit.

The best results were obtained when the phosphonic acid analogue was tied to the entire triglycine unit as shown below. (Scheme 15)



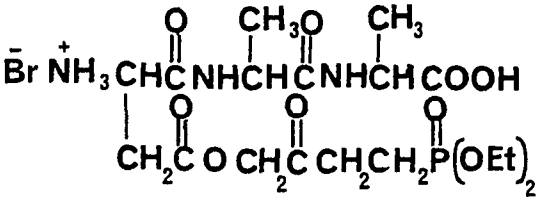

(LXVII) + (LXVIII) Pyridine / N(C4H9)3
(LXIX)
(LXIX) KOH
(LXX)
(LXX) + HOCH2CCH2CH2P(OEt)2 DCC / Pyridine
(LXXI)
HBr/CH3COOH
(LXXII)
1. (CH3)3SiBr
2. H2O
(LXXIII)

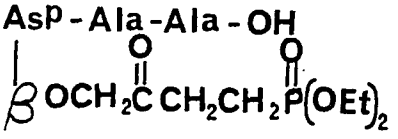
SCHEME 15

For the preparation of (LXXI) from (LXX) and the phosphonic acid analogue by DCC coupling, the only solvent which has been found to be satisfactory is anhydrous pyridine.[116] This reaction fails in ethyl acetate, methylene chloride, chloroform and ether. In the next step, removal of the carbobenzyloxy group by HBr/HOAc,[117] the ester group is left untouched as shown by NMR, IR and TLC. This is not unusual as there are examples reported in the literature[118] where this reagent has been used without cleaving the ester linkage.

Unfortunately, when the above tripeptide system was studied for its transport activity with E. coli unequivocal results were not found.

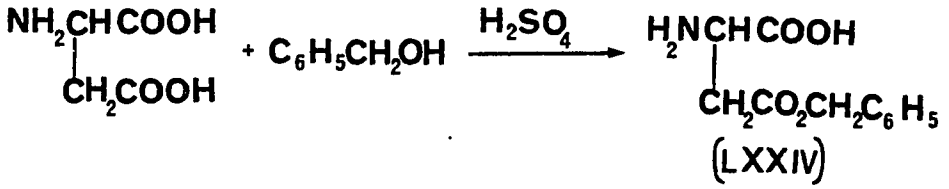
The second tripeptide, a better choice based on its hydrodynamic volume and availability of interacting functions is -



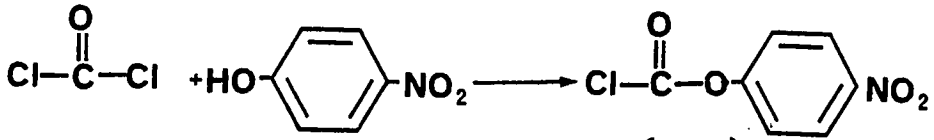


The tripeptide unit is made first and is then linked to the phosphonic acid analogue at the β-carboxyl function of the aspartic acid unit in the tripeptide. The peptide is made by combining a suitably protected aspartic acid to the alanylalanine unit. The t-butyloxycarbonyl group (t-BOC)[119] is used for the protection of the amino group in aspartic acid whereas its β-carboxyl function is

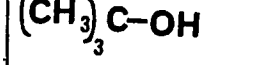
protected by a benzyl group.[120] The dipeptide is prepared as alanyl-alanine t-butyl ester[92] with a free amino group. These two units are coupled by DCC to give the required tripeptide with all the functional groups protected. The β-carboxyl function of aspartic acid is liberated by hydrogenation over palladium on carbon as catalyst. The final coupling of the analogue with the tripeptide is achieved by using DCC in anhydrous pyridine[116] as the solvent. Both the amino and the carboxyl protecting groups are cleaved using HBr in glacial acetic acid.[117] Scheme 18.

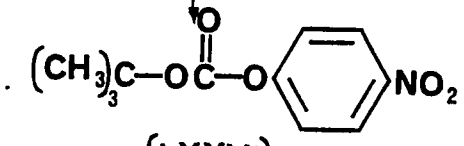


(LXXIV)

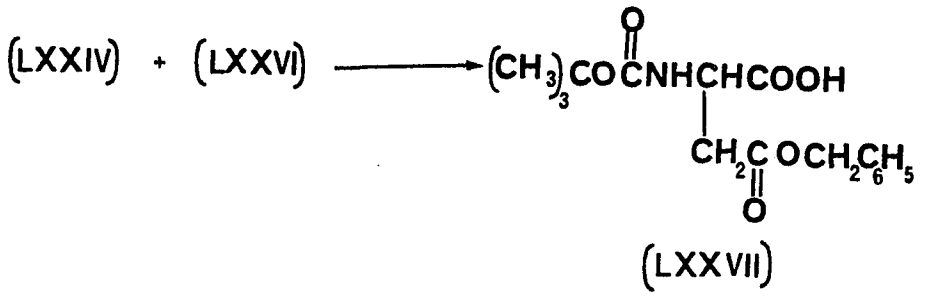


(LXXV)



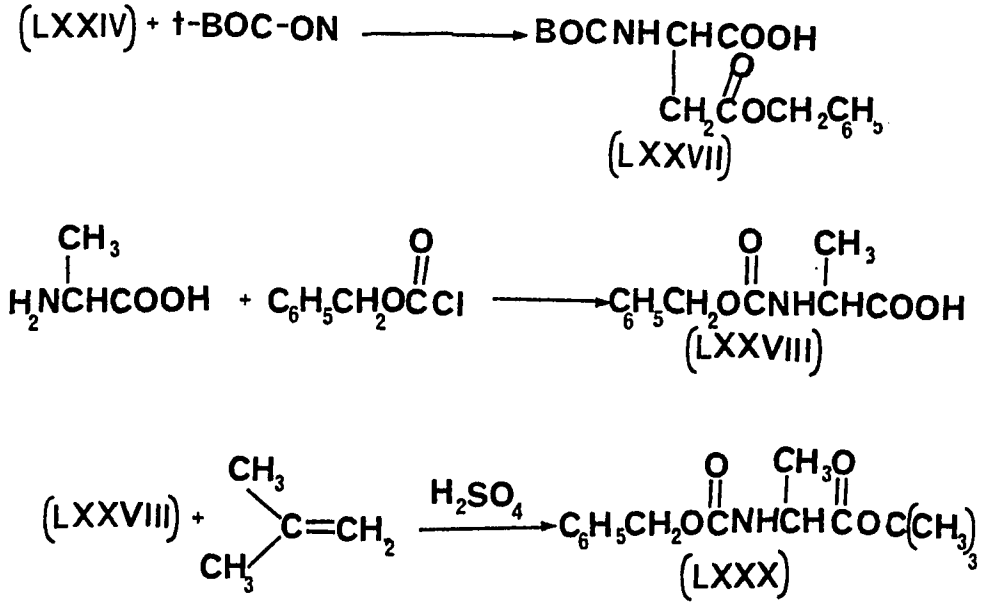


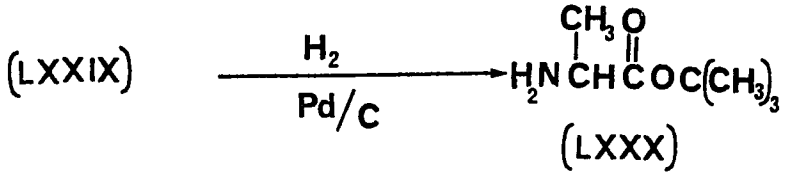
(LXXVI)



SCHEME 16

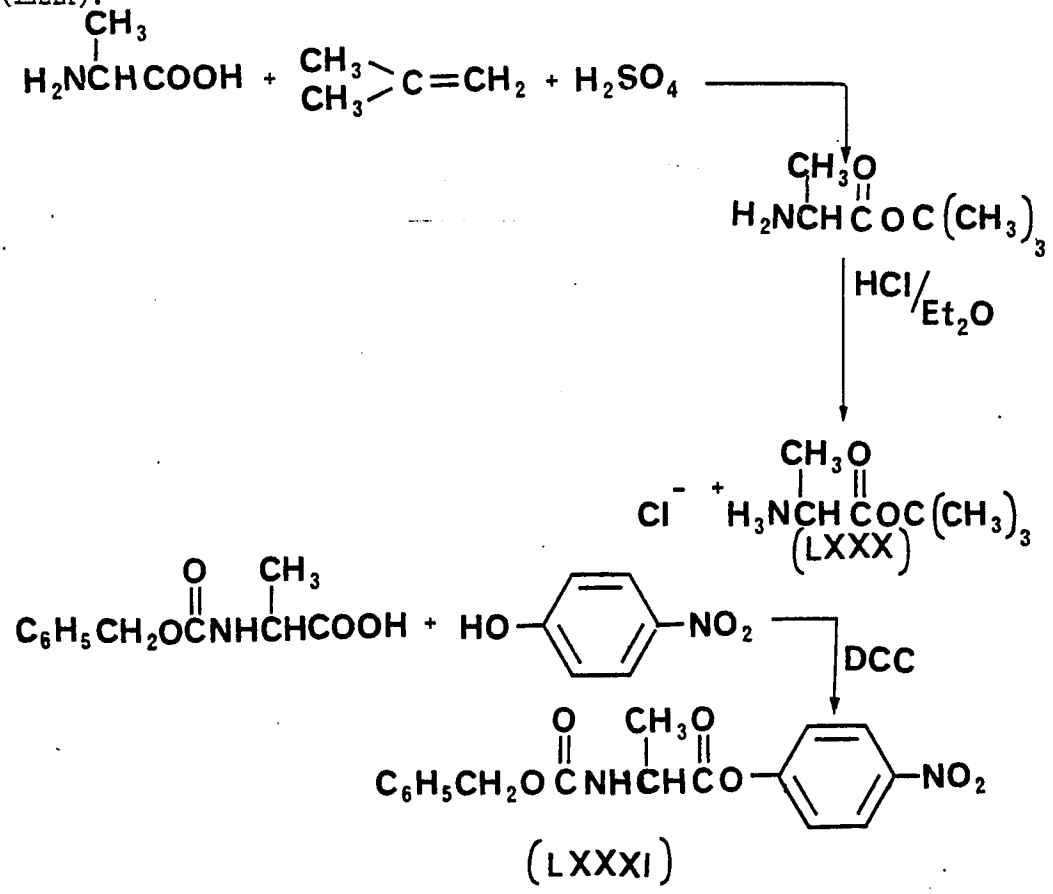
The above reaction for the introduction of the t-BOC group proceeds with only a poor yield (20-25%). More recently t-BOC-ON has been used as the reagent to introduce a t-butyloxycarbonyl group.[121] It offers the advantages of time and ease and works with a much better yield over the earlier mentioned reaction.

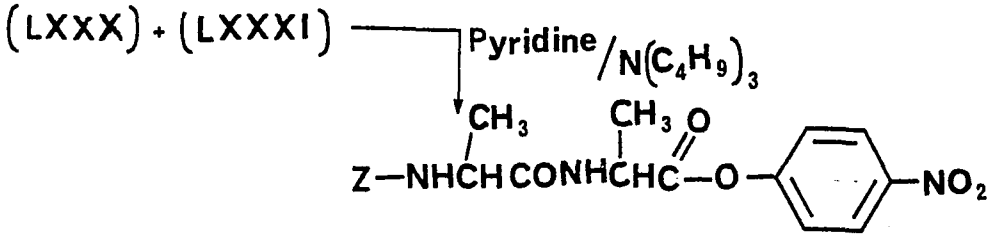




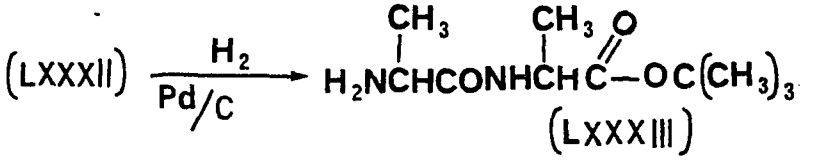
SCHEME 17

A more convenient method for making (LXXX) without protecting the amino group, is to allow alanine to react with liquified isobutylene in the presence of concentrated sulfuric acid in a pressure bottle.[122] The desired compound is isolated as the hydrochloride of (LXXX).

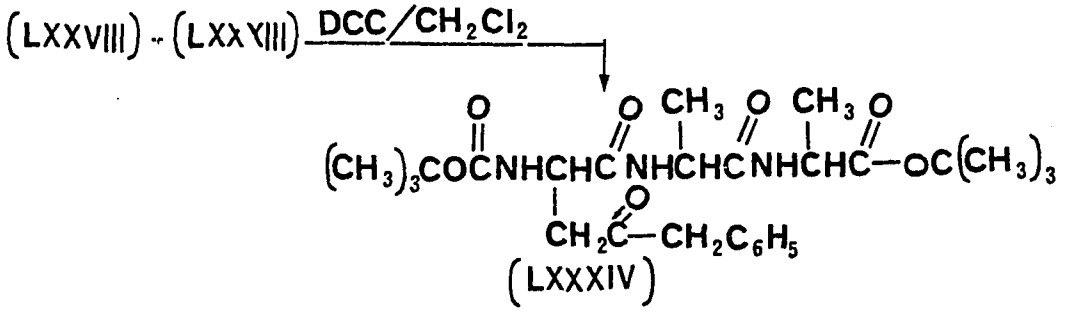




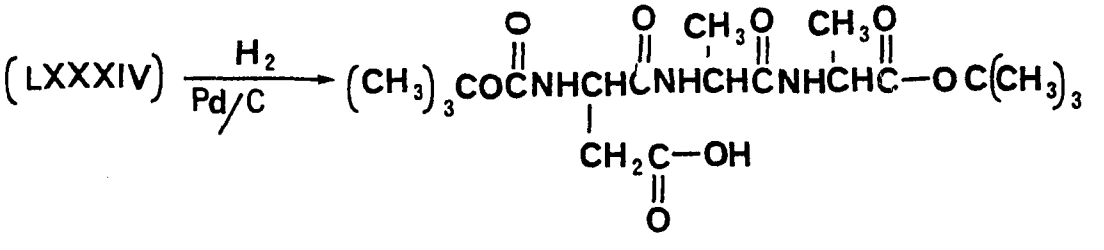
(LXXXII)



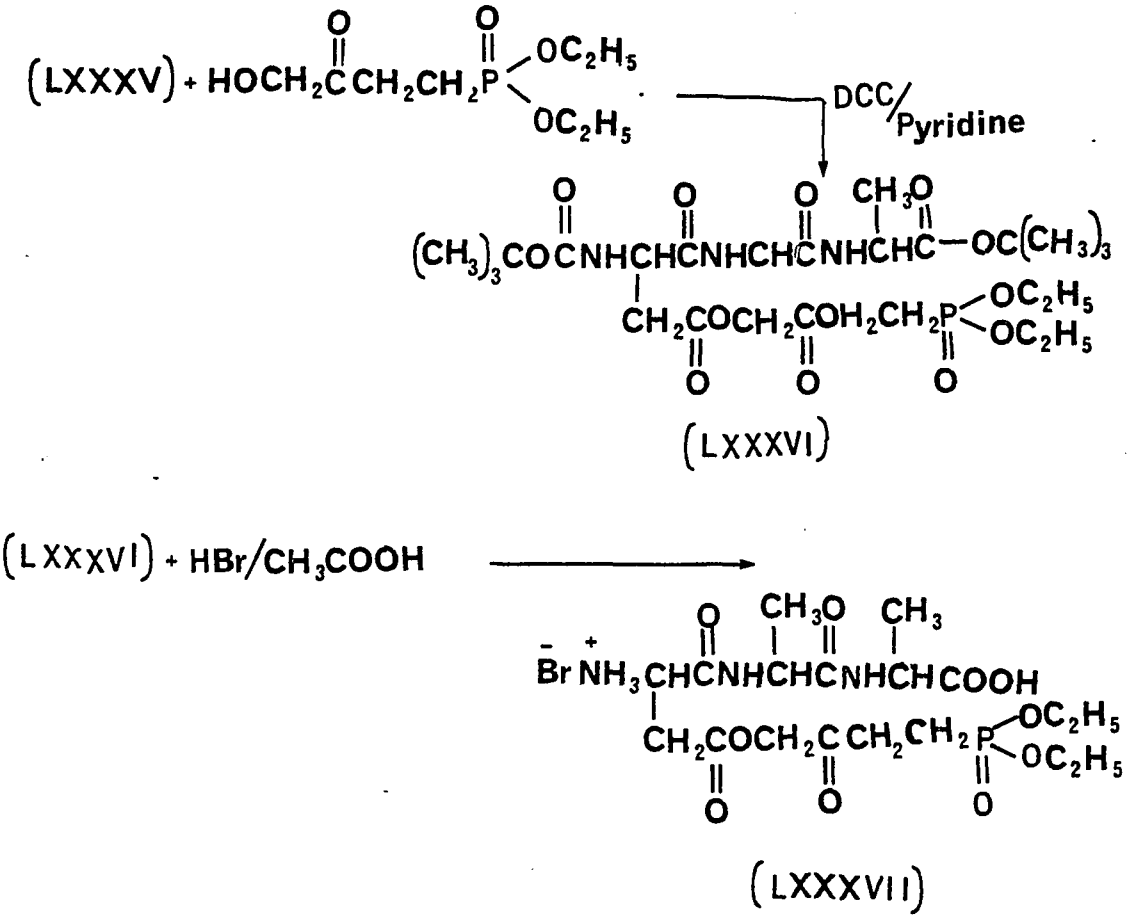
(LXXXIII)



(LXXXIV)



(LXXXV)



SCHEME 18

Experimental

General. All chemicals were of reagent quality and used without further purification with the following exceptions: benzene, pentane and hexanes were dried over sodium ribbon. DMSO and pyridine was distilled over calcium hydride. THF was distilled over lithium aluminum hydride. DMF, cetyl bromide, dioxane and methylene chloride were distilled immediately prior to use and stored over molecular sieves. Thin layer chromatography was performed using Polygram Sil N-HR silica gel sheets. Silica gel for preparative chromatography was from Baker (60-200 mesh). Infrared spectra were measured using a Perkin-Elmer 237-B spectrophotometer, and NMR spectra were measured with a Varian EM-360 or Varian A-60-A instrument. Mass spectra were measured using a Varian MAT CH-7 instrument and calibrated against perflourokerosene. Melting points were obtained on a Hoover Uni-Melt capillary melting point apparatus.

Diethyl 4-Oxopentyl-1-phosphonate (XVIII)

In a 500 mL round-bottomed flask equipped with a reflux condenser and a calcium chloride drying tube was refluxed a mixture of 50 g (0.304 moles) of 5-Chloro-2-pentanone ethylene ketal and 55.0 g (0.33 moles) of triethyl phosphite for 42-43 hours. The reaction mixture was cooled to room temperature and then stirred for 30-40 minutes with 150 mL of 0.1M HCl. The aqueous solution was extracted with 4x100 mL of benzene and the combined extracts were dried over anhydrous magnesium sulfate. After filtering the magnesium sulfate, the solvent was removed on a rotary evaporator and the residual oil distilled under reduced pressure to yield 23.1 g (36%) of the required compound as a clear liquid. bp 80-100°/.05-.025 Torr. NMR (CCl_4) δ 1.1(t,6H,OCH_2CH_3), 1.7(m,2H,CH_2), 2.1(s,3H,$COCH_3$), 2.4(m,2H,CH_2), 3.5(m,2H,CH_2CO), 4.0(m,4H,OCH_2); IR(CCl_4)cm^{-1} 3000, 1725, 1370, 1255, 1165, 1050.

Analysis: Calculated for $C_9H_{19}O_4P$ C,48.65; H,8.56
Found C,48.73; H,8.60

Diethyl 4-Methyl-4-pentenyl-1-phosphonate (XIX) Method A

sti In a 1L three-necked round-bottomed flask fitted with a mechanical stirrer, reflux condenser, a gas inlet tube, and an Erlenmeyer flask connected through Gooch tubing a gentle flow of nitrogen was maintained throughout the reaction. The flask was charged with 250 mL of anhydrous ether and (0.038 moles, 22.8 mL) of n-butyllithium (conc. about 1 mole in 600 mL of cyclohexane). The solution was stirred and 15.3 g (0.038 moles) of triphenylmethyl phosphonium iodide, dried over P_2O_5 under high vacuum, was added over a period of 5 minutes through the Gooch tubing. The solution was stirred for 4 hours at room temperature.

After 4 hours, the Gooch tubing was replaced by a dropping funnel and ketone (XVIII) (9.2 g, 0.041 moles) was added dropwise. The solution became cloudy and a white precipitate separated. The mixture was heated under the reflux overnight, allowed to cool to room temperature, and then the precipitate was removed with suction filtration . The precipitate was washed with 100 mL portions of ether, and the combined ether filtrates were washed with 100 mL portions of water until neutral. The ether extract was dried over anhydrous magnesium sulfate, the solvent was removed on a rotary evaporator, and the residual liquid was distilled under vacuum using a spinning band distilling column. bp 65-69°/0.09 Torr. Yield 2.0 g (24%). NMR (neat) δ 0.9-1.3 (t,6H,OCH_2CH_3), 1.3-1.7 (singlet superimposed on a multiplet, 7H,CH_3,CH_2CH_2), 1.8-2.1(m,2H,CH_2), 3.5-4.0 (q,4H,OCH_2), 4.45(s,2H,$=CH_2$); IR(CCl_4)cm^{-1} 1650,1450,1390,1250,1060,1030.

Analysis	Calculated for $C_{10}H_{21}O_3P$	C,54.54;	H,9.54
	Found	C,54.29;	H,9.74

Diethyl 4-Methyl-4-pentenyl-1-phosphonate (XIX) Method B

Sodium hydride (0.04 moles, 1.68 g of 57%) was washed with pentane (dried over sodium) 3-4 times in a three-necked flask. The flask was filled with nitrogen gas by alternatively applying suction and flowing in nitrogen gas. An atmosphere of nitrogen was maintained throughout the reaction. 30 mL of freshly distilled dimethylsulfoxide was injected into the flask and the flask heated at approximately 60° with the reaction mixture being stirred with a magnetic stirrer until there was no more evolution of hydrogen gas (about 40-45 minutes). At this stage, the oil bath was replaced by an ice bath.

Triphenylmethyl phosphonium iodide (0.04 moles, 15.92 g) dried over P_2O_5 under vacuum, was dissolved in 50 mL of warm DMSO in a round-bottomed flask with a minimum exposure to air. This solution was added to the three-necked flask dropwise through an addition funnel while the flask was being cooled in an ice-bath. When the addition was over, the ice-water bath was removed and the reaction mixture was stirred for 30-45 minutes at room temperature. At this stage, the reaction mixture had a dark reddish-green color. The ketone (XVIII), (8.0 g, 0.036 moles) was added through a dropping funnel. On addition of the ketone the reaction mixture became warm and there was a lightening of the color to pale yellow. The reaction mixture was stirred for 2-3 hours at room temperature and then heated at 60° for another 3-4 hours. After cooling to room temperature the reaction mixture was poured into 150 mL of distilled water. The resulting turbid solution was extracted with pentane. At this stage, a white solid precipitated which was filtered. The pentane layer was separated and the aqueous layer extracted repeatedly with 50 mL portions of pentane. The filtered solid was also washed 2-3 times with pentane. All the pentane extracts and washings were combined and dried over anhydrous $MgSO_4$. The dried solution was then passed through a colum packed with 33 g of neutral alumina. All the pentane was removed under reduced pressure and the residual liquid distilled under vacuum to yield 2.6 g (36%) of the desired compound as a colorless liquid. It was used without any further purification for the next reaction. bp 63-67°/ 0.08 Torr NMR (neat)δ 0.9-1.3(t,6H,OCH_2CH_3), 1.3-1.7(singlet superimposed upon a multiplet, 7HCH_3,CH_2CH_2), 1.8-2.1(m,2H,CH_2), 3.5-4.0(q,4H,OCH_2), 4.4(s,2H,=CH2), IR(CCl_4)cm^{-1} 1650,1450,1390,1250,1160,1050,940.

Monosodium salt of 4-Methyl-4 pentenyl-1-phosphonic acid (XXI)

A mixture of 3.3 g (0.015 moles) of diethyl 4-pentenyl-1-phosphonate (XIX) and (6.5 g, 0.042 moles) of sodium iodide in 35 mL of dimethylformamide was heated at 140-150° for 27 hours. Following this reaction period, a white solid was obtained on cooling the reaction mixture to room temperature. The mixture was evaporated to dryness at room temperature under high vacuum and the residue was triturated with 100 mL of acetone to yield a precipitate which was then washed with 500 mL of fresh acetone on a sintered glass funnel. The undissolved solid was dried and weighed (1.4 g, 45.4%). Of this disodium salt 0.3 g was dissolved in 15 mL of 50% aqueous methanol and then passed through a column of acid-form Dowex 50 to convert the above disodium salt into free phosphonic acid. The acidic eluate was then heated at 100° for one hour and evaporated to dryness to yield 0.22 g (96%) of the free phosphonic acid. It was dissolved in 50 mL of distilled water and then titrated with 0.015N sodium hydroxide to pH 5-6. The aqueous solution was evaporated under reduced pressure at 50°. The residual oil partially solidified after being kept under high vacuum overnight. To this partial solid, 15-20 mL of absolute methanol was added and then stirred for some time. Methanol was removed under reduced pressure to yield 0.26 g (90%) of a white solid which was dried under high vacuum. It gave a single spot on TLC using 10% 0.1N HCl in methanol as the developing solvent - Rf,=0.69. IR(Fluorolube)cm^{-1} 3600-3000 (broad), 1650,1465,1360,1260(s), 1200, 1110, 1030, 955,900.

Analysis: Calculated for $C_6H_{11}O_3PNa.H_2O$ C,31.85; H,5.75
Found C,31.73; H,6.12

Preparation of Dowex-50, acid form:

Dowex-50 was washed with about 150 mL of 2N sodium hydroxide and then with water till the washings were neutral. It was then washed with 250 mL of 6N hydrochloric acid on a sintered glass funnel; this was followed by water washings until they were neutral. The whole process was repeated three times and at the end Dowex-50 was allowed to stir with 250 mL of 6N hydrochloric acid for 1 hour, followed by washings with distilled water until the washings were neutral and no more color was leached from the Dowex. The Dowex-50 used had the exchange strength of 5 meq/g.

1-Chloro-3-propanyl diethyl acetal (XXIII)

A three liter three-necked flask equipped with a magnetic stirrer, a dropping funnel, a gas inlet tube, a condenser with a calcium chloride drying tube and surrounded by an ice-salt bath, was charged with 226 mL of absolute ethanol. The alcohol was saturated with hydrogen chloride at 0°. Simultaneously, 10.0 g of acrolein was weighed and kept in the addition funnel surrounded by an ice-salt mixture. When the alcohol was saturated with hydrogen chloride acrolein was added slowly to the reaction mixture with stirring, keeping the three-necked flasked immersed in an ice-salt bath at 0°. This addition required 1-2 hours taking care that the temperature of the reaction mixture did not rise above 0°. The stirring was continued for another hour and then the reaction mixture was transferred to a separatory funnel and allowed to stand until there was a separation of two layers. The lower layer of acetal was separated and treated gradually with powdered sodium bicarbonate until all the acid was neutralized. The

mixture was filtered and the filtrate washed with two 50 mL portions of ice cooled water and then dried over anhydrous potassium carbonate for 7 hours. Potassium carbonate was removed and the liquid distilled under reduced pressure using a fractionating column. bp 79-86° /19-24 Torr. Litt. bp[99] . 58-62/ 8 Torr.
Yield: 98.0 g (34%). NMR(CCl_4) δ 1.0(t,6H,OCH_2CH_3), 1.9(q,2H,CH_2CH), 3.5(m,6H,OCH_2,$ClCH_2$), 4.5(t,1H,CH).

Diethyl 3,3-diethoxy-1-phosphonate (XXIV)

Freshly distilled triethyl phosphite, 50 g (0.30 moles), and a 15% excess of the acetal (XXIII), 58 g (0.35 moles) were refluxed at 170-178° for 4 hours using a fractionating column. After 4 hours, the round-bottomed flask was cooled and a low boiling fraction was removed by distillation at 40-46°/5 Torr. The residual liquid was transferred to a 50 mL round-bottomed flask and distilled under reduced pressure using a fractionating column to yield 10.0 g (12.5%) of the required compound. bp 91-95°/0.05 Torr, Litt. bp[98] 98-100°/0.07 Torr. NMR (neat)δ 1.0(m,12 H,OCH_2CH_3), 1.3-1.9(m,4H,CH_2CH_2), 3-3.5(m,4H,OCH_2), 3.6-4.1(q,4H,OCH_2), 4.3(m,1H,CH).

Diethyl 3-oxopropyl-1-phosphonate

The above compound,(9.5 g, 0.035 moles), 53.6 mL of 3% hydrochloric acid and a small amount of hydroquinone were heated at 70-75° for 5 hours under an atmosphere of nitrogen gas. At the end of this period, water was removed using a rotary-evaporator and the residual liquid was immediately vacuum distilled to yield 5.7 g (83%) of the

required compound (XXV) bp .87-90°/ 0.13 Torr, Litt. bp[98] 92-94°/ 0.23 Torr. NMR(neat)δ 0.9-1.3(t,6H,OCH_2CH_3), 1.5-2.9(m,4H,CH_2CH_2), 3.6-4.35(q,4H,OCH_2), 9.9(s,1H,CHO). IR(CCl_4)cm^{-1} 3400,3020, 2850, 1730,1420,1390,1250,1175,1060,1040,940.

Diethyl 4-Methyl-3-pentenyl-1-phosphonate (XXVI): Method A, using n-BuLi:

In a three-necked flask equipped with a mechanical stirrer, an addition funnel, a refluxing condenser and a gas inlet tube, was placed 200 mL of anhydrous ether. n-BuLi (0.031 moles) 23 mL was injected into the flask followed by the addition of 0.031 moles (14.6 g) of triphenylisopropyl phosphonium iodide through Gooch tubing from an Erlenmeyer flask. There was a development of a dark red color after the addition of phosphonium salt. The reaction mixture was stirred for 2 days at room temperature. After 2 days, 5.8 g (0.03 moles) of the aldehyde (XXV) was added dropwise. There was the formation of a yellow precipitate and the color changed from deep red to orange to yellow as the reaction proceeded. The reaction mixture was allowed to reflux for three days. At the end of this period, the mixture was filtered and the solid washed with 200 mL of ether. The combined ethereal extracts were washed with water until the aqueous washings were no longer basic. The ether layer was dried with anhydrous magnesium sulfate. The solvent was removed on a rotary-evaporator and the residual liquid distilled under vacuum to yield 1.0 g (15%) of the required compound. bp 68-72° /0.05 Torr. NMR(neat)δ 0.9-1.3(t,6H, OCH_2CH_3), 1.4-2.5(two singlets superimposed on a multiplet, 10H,CH_2CH_2, $=C\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}$), 3.6-4.25(q,4H,OCH_2), 4.9-5.2(m,1H,HC=C). IR(CCl_4)cm^{-1} 3100,

1660, 1450, 1150, 960.

Analysis: Calculated for $C_{10}H_{21}O_3P$ C,54.55; H,9.54

Found C,54.43; H,9.60

Method B: using DMSO/NaH

Sodium hydride (0.046 moles, 2.0 g of 57%) was washed with dry pentane 4 times in a three-necked flask. An atmosphere of nitrogen gas was maintained throughout the reaction and 35.0 mL of freshly distilled dimethylsulfoxide was injected into the flask and heated at about 60° with the reaction mixture stirring until there was no more evolution of hydrogen gas (about 40-45 minutes). At this stage, the oil bath was replaced by an ice-bath. Triphenylisopropylphosphonium iodide (0.046 moles, 19.9 g), dried over P_2O_5 under vacuum, was dissolved in 50 mL of warm dimethylsulfoxide in a round-bottomed flask with a minimum exposure to air. This solution was added to the three-necked flask dropwise through an addition funnel while the flask was being cooled in ice-water bath. When the addition was over, the ice-water bath was removed and the reaction mixture stirred for 30-45 minutes at room temperature. The formation of the ylide was shown by the development of a dark red color. The aldehyde (XXV) (9.0 g, 0.046 moles) was added through a dropping funnel. The mixture was stirred for 5-6 hours and then heated at about 60-70° overnight. After cooling to room temperature, it was poured into 100-150 mL of distilled water and then extracted 5-6 times with 100 mL portions of pentane. During the extraction, a solid repeatedly precipitated; this solid was filtered and washed with 200 mL of pentane. All the pentane washings and extractions were combined and passed through a

column packed with neutral alumina. The solvent was removed under reduced pressure and the residual liquid distilled under vacuum to yield 2.9 g (29%) of a colorless liquid. bp. 60-62°/0.01 Torr. NMR (neat) δ 0.9-1.2(t,12H,OCH_2CH_3), 1.2-2.4(m,19H,CH_2CH_3,(CH_2),$=C\langle^{CH_3}_{CH_3}$), 3.5-4.15(m,8H,OCH_2), 4.5(s,2H=CH_2), 4.8-5.15(m,1H,CH=C), IR(CCl_4)cm^{-1} 3500(w), 3000,1700(w),1660,1460,1410,1260,1175,1100,975. NMR and IR indicated that the distilled material was a mixture of two compounds. This was confirmed by VPC studies. As explained in the result and discussion chapter before, these two compounds were identified as 1:1 mixture of the desired compound and diethyl 4-methyl-4-pentenyl-1-phosphonate (XIX).

Diethyl 5-Carbomethoxy-4-hydroxy-4-methylpentyl-1-phosphonate (XXVIII): Method A, Reformatsky reaction:

Washing of Zinc: About 25 g of Zinc was washed with 200 mL each of 2M hydrochloric acid, water, acetone and then with ether. At the end, it was washed with 120 mL of a 1:1 mixture of ether and benzene and then dried over P_2O_5 under high vacuum.

A 500 mL three-necked flask equipped with a mechanical stirrer, a reflux condenser, an addition funnel, and a gas inlet tube, was charged with 7.5 g (0.115 moles) of washed, dry zinc and 150 mL of a 1:1 mixture of ether and benzene as the solvent. In this was added over a period of 2 hours 19.1 g (0.125 moles) of methylbromoacetate dissolved in an equal amount of the same ether-benzene mixture. The reaction was triggered by addition of a trace of iodine and slight warming. After two-thirds of the bromoester had been added, another 5 g of zinc followed by another 4 g of zinc and at the end of the

addition (0.25 moles of zinc total). Traces of iodine were added 3 times during the course of addition of the bromoester. In the reaction mixture was generated the organo-zinc compound which appeared as a thick oil. The mixture was vigorously stirred under reflux for 4 hours. It was observed that at this stage a significant portion of the zinc was left unreacted. The reaction mixture was cooled to room temperature and 11.0 g (0.05 moles) of the ketone (XVIII) was added dropwise. It was refluxed for 3 hours under vigorous stirring and after cooling to room temperature 100 mL of glacial acetic acid was added. After the addition of ketone (XVIII), there was observed a deposition of a green precipitate at the walls of the flask which dissolved as the refluxing was continued and eventually gave a yellow solution. After 30 minutes of addition of acetic acid, the clear solution was decanted and extracted twice with 40 mL of distilled water and thrice with dilute hydrochloric acid. The combined aqueous extract was made basic by the addition of concentrated ammonia. It was saturated with sodium chloride and then extracted several times with benzene. The organic extracts were dried over anhydrous potassium carbonate and the solvent removed under reduced pressure. The residual liquid was distilled under vacuum to yield 2.6 g (17.5%) of the product. bp 55-60°/.005 Torr. Rf.(9$CHCl_3$:1 CH_3OH)=0.79. NMR(CCl_4) δ 1.2(t,9H,OCH_2CH_3, CH_3), 1.5-2.6(singlet superimposed on a multiplet, 7H,$(CH_2)_3$,CH_2CO_2), 3.5(two singlets, 4H,OCH_3,OH), 3.6-4.3(m,5H,OCH_2, CH), IR(CCl_4)cm^{-1} 3550, 3050,1735,1460,1410,1390,1250,1175,1040,950.

Analysis: Calculated for $C_{12}H_{25}O_6P$ C,48.65; H,8.45

Found C,48.89; H,8.64

Diethyl 5-Carboxy-4-hydroxy-methylpentyl-1-phosphonate (XXIX)

Method A:

The hydroxyester (XXVIII) (0.5 g, 0.0017 moles) was dissolved in 10 mL of methanol and 1.5 g of potassium hydroxide dissolved in 10 mL of water was added to it. It was refluxed for 4 hours and then brought to room temperature. The reaction mixture was acidified to congo red and then extracted three times with chloroform. The organic extracts were concentrated and then washed thoroughly with sodium bicarbonate solution. The aqueous extract was acidified to congo red and then extracted with chloroform. The organic extract was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residual liquid yielded the expected spectral analysis. The compound decomposed upon attempted distillation. It was found to be sufficiently pure on TLC and used for further reaction as such. NMR(CCl_4)δ 0.9-1.45(t,9H,CH_3,OCH_2CH_3), 1.45-1.9(m,2H,CH_2), 2.1(s,2H,CH_2CO_2), 2.3-2.7(m,2H,CH_2), 3.1-3.55(m,2H,CH_2), 3.6-4.5 (q,4H,OCH_2), 8.25(s,2H,OH,CO_2H, exchange with D_2O), IR($CHCl_3$)cm^{-1} 3300-3600(broad), 3000,1710,1450,1390,1250,1175,1050,975.

Diethyl 5-Carboxy-4-hydroxy-4-methylpentyl-1-phosphonate (XXIX)

Method B, using lithium naphthalene:

Naphthalene recrystallized (12.8 g, 0.1 moles) from 2-propanol was dissolved in 80 mL of freshly distilled tetrahydrofuran (over lithium aluminium hydride) in a three-necked flask equipped with a magnetic stirrer and a gas inlet tube. An atmosphere of argon was maintained throughout the reaction. Lithium (0.7 g, 0.1 gram atom) washed with

benzene and ether was put into the reaction flask. On stirring there developed a dark green color within 2-5 minutes. It was kept stirring for 2 hours at room temperature. At the end of this period, the flask was cooled in an ice-water bath and 3.0 g (0.05 moles) of glacial acetic acid in 30 mL of dry tetrahydrofuran was added dropwise through an addition funnel. It was stirred for 10 minutes at room temperature and then at 50-60° for the next two hours. At this stage, the reaction mixture had an orange color. The ketone (XVIII) dissolved in 30 mL of anhydrous ether was added rapidly to the reaction mixture which resulted in a rise of the temperature and decolorization of the reaction mixture to a pale yellow. It was refluxed for 2 hours at 50-60°. After cooling to room temperature, the reaction mixture was hydrolyzed with a minimum amount of water which resulted in the formation of a clear two layer mixture. The alkaline bottom layer was acidified to congo red and then extracted three times with chloroform. After drying over anhydrous sodium sulfate, the solvent was removed using a rotary evaporator leaving behind 5 g (35.5%) of an oil which exhibited spectra in accord with the proposed structure. It gave a single spot on TLC using $CHCl_3$:EtOH(9:1) mixture, Rf=0.45. NMR(CCl_4 δ 0.9-1.3 (m,16H,OCH_2CH_3,CH_3,CH_2)$_3$, CH_2CO_2), 3.2-4.4(m,5H,OCH_2,CH), 8.6(broad singlet, 2H,OH,CO_2H, exchange with D_2O). IR($CHCl_3$)cm^{-1} 3600-3300(broad), 3000, 1710, 1450,1410,1390,1250,1175,1050,975.

Analysis:	Calculated for $C_{11}H_{23}O_6P$	C,46.81;	H,8.16
	Found	C,47.30;	H,7.85

Attempts to further purify this carboxylic acid as the piperazine salt were unsuccessful. It could, however, be further purified by generation of the dicyclohexylamine salt which was prepared by carrying out the hydrolysis with trimethylchlorosilane and then treating with dicyclohexylamin.

Analysis, Calculated for $C_{19}H_{38}O_6NP$ C,56.02; H,9.34

Found C,55.73; H,9.17

Diethyl 5-carboethoxy-4-hydroxy-4-methylpentyl-1-phosphonate (XXXII) Method C, using Lithium diisopropylamide.

In a 100 mL three-necked flask equipped with two addition funnels, a gas inlet tube, a magnetic stirring bar, and a rubber septum, was placed 20 mL of anhydrous ether which was cooled to -23° under a nitrogen atmosphere. Freshly distilled diisopropylamine (2.22 g, 0.022 moles, 3.08 mL) was added via syringe followed by a solution of n-butyllithium in hexane (0.22 moles, 13.2 mL) which was added over a period of 10 minutes through an addition funnel. After stirring for 1 hour, the bath temperature was reduced to -78° and dry ethyl acetate (0.22 moles, 2.16 mL) was slowly added. The solution was stirred for 30 minutes at -78° and then 4.88 g (0.022 moles) of the ketone (XVIII) was added slowly. After 30 minutes, the reaction mixture was treated with 20% hydrochloric acid (4.0 mL) and allowed to warm to room temperature; the mixture was diluted with 4 mL of distilled water and extracted 4 times with 20 mL portions of ether. The organic extractions were combined, dried over anhydrous sodium sulfate, and the solvent was evaporated to yield 3.53 g (52%) of (XXXII) as an oil.

It gave spectral analysis in accord with the proposed structure, also showing that there was present a small amount of the unreacted starting ketone. NMR (CCl_4 δ0.9-1.8(m,16H,OCH_2CH_3,CCH_3,CH_2CH_2) 1.8-2.7(singlet superimposed on a multiplet, 4H,CH_2CO_2,PCH), 3.2-4.3(m,7H,OCH_2,OH), IR(CCl_4)cm^{-1} 3550, 3050, 1735, 1460,1410,1390,1250,1175,1060,1040, 950. It was purified in the next step by hydrolyzing the carboxylic ester to a carboxylic acid and then extracting the acid with sodium bicarbonate solution.

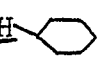
Diethyl 5-carboxy-4-hydroxy-4-methylpentyl-1-phosphonate (XXIX)

The above ester (XXXII) (3.53 g, 0.0113 moles) was dissolved in 8 mL of 1 N potassium hydroxide in methanol at -20°. After 1 hour at -20°, the reaction mixture was warmed to room temperature and allowed to stand overnight. It was neutralized with methanolic hydrochloric acid, prepared by dissolving 5.0 mL of acetyl chloride in 100 mL of methanol, to congo red. The precipitate potassium chloride was filtered and the filtrate evaporated to dryness using a rotary evaporator. The residue was dissolved in chloroform and the precipitated potassium chloride again filtered. The filtrate upon evaporation gave 2.53 g of the crude acid (79.3%). This crude acid was purified by dissolving in 10 mL of chloroform and then extracting the chloroform solution 4 times with 7 mL portions of saturated sodium bicarbonate solution. The aqueous extracts were combined and acidified aqueous solution was extracted thoroughly with chloroform. The combined extracts were dried over anhydrous sodium sulfate and the solvent removed under reduced pressure to yield 2.0 g of the purified

acid (62.7%). It gave a single spot on TLC. Rf($CHCl_3$:EtOH,9:1) = 0.45. NMR (CCl_4)δ 0.9-1.45(t,9H,CCH_3,OCH_2CH_3), 1.45-1.9(m,2H,CH_2), 2.1(s,2HCH_2CO_2), 2.2-2.7(m,2H,CH_2), 3.1-3.55(m,2H,PCH_2O, 3.6-4.5 (q,4H,OCH_2), 8.45(Broad singlet, 2H,OH,CO_2H, exchanged with D_2O) IR($CHCl_3$)cm^{-1} 3300-3600(Broad), 3000, 1710,1450,1410, 1390,1250,1175, 1050,910.

5-Carboxy-4-hydroxy-4-methylpentyl-1-phosphonic acid, dicyclohexyl-ammonium salt (XXXI).

In a 25 mL round bottomed flask, a solution of 1.67 g (0.0059 moles) of the above hydroxy acid (XXIX) and 4.56 g (0.042 moles) of freshly distilled trimethylchlorosilane was heated under reflux, under an atmosphere of nitrogen for 4 days. During this period 1 g of trimethylchlorosilane was added every day and the temperature was not allowed to exceed 85°. Excess trimethylchlorosilane was distilled off at atmospheric pressure. The residue was shaken with 15 mL of distilled water for 1 hour. At this point an oil floated on the water whereas in the original mixture the bis-trimethylsilyl derivative was in chloroform and the water layer was evaporated to dryness to yield 1.1 g of (XXX) as a thick oil (82.5%). The free phosphonic acid 0.5 g (0.0023 moles) was dissolved in 2 mL of benzene and 1.5 mL of acetone. This was added to a solution of 1.26 g (0.0069 moles) of freshly distilled dicyclohexylamine in 2 mL of benzene and 1.5 mL of acetone. On mixing,the solution immediately became turbid. The mixture was stirred for 3 hours and then the clear liquid was decanted. The thick white paste sticking to the sides of the flask was crystallized

by trituration with anhydrous ether. The solid was filtered under a nitrogen atmosphere and washed several times with anhydrous ether. The solid was recrystallized from acetone containing 4-5 drops of methanol, more acetone being added until it became turbid. The clear solution was decanted and the thick paste again crystallized as before with anhydrous ether to yield 0.72 g (76.9%) of a white solid. NMR(D_2O)δ 1.0-2.4(m,31H,5 CH_2, of ring, $PCH_2CH_2CH_2$,CCH_3,CH_2CO_2), 2.9-3.4(m,2H, ). IR(Fluorolube)3600-3200(Broad),3000,1650, 1450,1250-1050(Broad), 950.

Analysis: Calculated for $C_{19}H_{38}O_6NP$ C,56.02; H,9.34

Found C,56.33; H,9.60

Attempted preparation of diethyl 5-carbo dodecyloxy-4-hydroxy-4-methyl-pentylphosphonate (LVII'):

Diethyl-5-carboxy-4-hydroxy-4-methylpentyl-1-phosphonate (XXIX), (0.0042 moles) and 0.79 g (0.0042 moles) of dodecanol were dissolved in 10 mL of ethylacetate and then cooled to 0° in an ice-bath. After adding 0.86 g (0.0042 moles) of dicyclohexyl carbodiimide, the reaction was kept in the ice-bath for 1 hour and then at room temperature for 24 hours. The precipitated dicyclohexylurea was filtered off and to the filtrate 5-6 drops of an acetic acid-water mixture was added. It was kept at 0° overnight and the additional amount of dicyclo-hexylurea precipitated was removed. The filtrate, after washing with 1N sodium hydroxide and water, was dried over anhydrous sodium sulfate. The residue after removal of the solvent under reduced pressure showed three spots on TLC using chloroform as the developing solvent; two of

these spots corresponded to the starting acid and the alcohol, the third spot could not be accounted for. It did not give the expected spectral analysis for the desired compound. The above procedure for its synthesis was abandoned.

Diethyl 5-carbohexadecyloxy-4-hydroxy-4-methylpentylphosphonate (LVII)

Diethyl 5-carboxy-4-hydroxy-4-methylpentyl-1-phosphonate (XXIX), 2.82 g (0.01 moles) was dissolved in 15 mL of water containing 0.84 g (0.01 moles) of sodium bicarbonate. It was allowed to stir at room temperature for 30 minutes and then the water removed under reduced pressure at 40°. The residue, after drying over sodium hydroxide under high vacuum for 2 days, was dissolved in 17 mL of anhydrous dimethylformamide at 80°. To this solution, 3.05 g (0.01 moles) of cetyl bromide was added. The reaction mixture was heated with stirring at 80° for 20 hours in a stoppered round-bottomed flask. To the residue after removal of dimethylformamide under reduced pressure was added 15 mL of ether and 15 mL of water. The organic layer, after washing with saturated sodium bicarbonate and water, was dried over anhydrous sodium sulfate. The residue after removal of the solvent was purified by putting the compound on a silica gel column and eluting it first with chloroform to remove cetyl bromide and then with a mixture of 90% chloroform and 10% ethanol. It showed a single spot on TLC. Rf($9CHCl_3$:$1EtOH$)=0.72. The fraction containing the desired compound was filtered through a milli-pore filter to yield 3.0 g of the desired compound (LVII). Yield, (60%). NMR(CCl_4)δ 0.8-2.2 (m with a broad singlet, $46H, 14CH_2, OCH_2CH_3, CCH_3, CH_2CH_2CH_2$), 2.4(s,2H, CH_2CO_2), 3.5-4.4(m,7H,OCH_2,CO_2CH_2,OH), IR(CCl_4)cm^{-1} 3700-3100(broad),

3000, 1740,1450,1390,1250,1200,1060,1040,950.

Analysis Calculated for $C_{27}H_{55}O_6P$ C,63.99; H,10.94

Found C,63.73; H,11.15

N-Carbobenzyloxy glycine (LX)

To a solution of 7.5 g (0.1 moles) of glycine in 25 mL of 4N sodium hydroxide, kept at 0° for 20 minutes was added in 5 parts 17 g of benzylchloroformate and 25 mL of 4N sodium hydroxide through two separate addition funnels. This was allowed to stir for another 30 minutes. The reaction mixture, upon acidification with concentrated hydrochloric acid, yielded the desired compound as a white solid. 15.04 g (72%). mp 120-122°. Litt. mp[112] 119-121°. It was used as such without any further purification.

Diethyl 4-hydroxy-3-oxobutyl-1-phosphonate. (LVIII)

A mixture of 1.0 g of Diethyl 4-acetyloxy-3-oxobutyl-1-phosphonate and 20 mL of 1N potassium carbonate in 80% ethanolwas stirred for 15 minutes at room temperature. After 15 minutes a small piece of dry ice was added to the reaction mixture. The mixture was concentrated under reduced pressure to remove most of the ethanol. The cloudy solution was diluted with 5 mL of brine and extracted thoroughly with methylene chloride. After drying, the solvent was removed under reduced pressure to give 0.73 g (88%) of the desired compound. It was further purified on a silica gel column using 90% $CHCl_3$, 10% EtOH mixture as the eluting solvent. Rf.($9CHCl_3$:1EtOH)=0.56-NMR(CCl_4) δ0.9-1.4(t,6H,OCH_2CH_3), 1.4-2.25(m,2H,CH_2), 2.25-3.0(m,2H,CH_2), 3.8-4.3(m,6H,OCH_2,CH_2O). 4.8(broad,1H,OH), IR(CCl_4)cm^{-1} 3500,3000,1725,1450,

1250,1175,1050.

Analysis:	Calculated for $C_8H_{17}O_5P$	C,42.85;	H,7.58
	Found	C,42.66;	H,7.70

N-Carbobenzyloxy glycyl-3-oxobutyl-1-diethyl phosphonate (LXVI')

A solution of 2.30 g (0.011 moles) of N-Carbobenzyloxy glycine and 2.5 g (0.011 moles) of 4-hydroxy-3-oxobutyl-1-phosphonate in 40 mL of ethylacetate was cooled to 0° and then were added 2.27 g (0.011 moles) of dicyclohexylcarbodiimide. This mixture was kept at 0° for 4 hours and then stirred at room temperature overnight. The precipitated dicyclohexylurea was filtered and to the filtrate 4 drops of acetic acid were added and the solution kept at 0° for 6 hours. Additional dicyclohexylurea was removed and the filtrate, after drying over anhydrous sodium sulfate, was evaporated under reduced pressure to yield 4.07 g (89%) of the desired compound. This was dissolved in 10 mL of ethylacetate and then petroleum ether (30-35°C) was added until it became turbid. After cooling overnight at 0° it yielded 3.87 g (84%) of an oil which gave the correct spectral analysis and was of sufficient purity. NMR(CCl_4)δ 1.0-1.35(t,6H,OCH_2CH_3), 1.35-2.2 (m,3H,CH_2CO_2CH), 2.2-2.9(m,2H,CH_2), 3.5-4.2(m,6H,OCH_2,NCH_2), 4.55(s, 1H,OCH), 5.0(s,2H,$CH_2C_6H_5$), 6.5(broad, 1H,NH), 7.2(s,5H,C_6H_5), IR(CCl_4)cm^{-1} 3600-3200(broad), 3000,1725(broad), 1650,1500,1250,1050, 1000,975,900,695.

Attempts to couple N-Carbobenzyloxy glycyl-glycine with (LXVI)

2.10 g (0.005 moles) of (LXVI) was dissolved in a freshly

prepared saturated solution of hydrobromic acid in glacial acetic acid (15 mL), and after 90 minutes at room temperature, ether was added. After maintenance at 0° for three hours, the precipitated oil was washed with ether and reprecipitated twice form ethyl acetate/pet. ether mixture to give 1.59 g (87%) of the hydrobromide (LXVI). All attempts to couple this hydrobromide with N-Carbobenzyloxy glycyl-glycine using mixed anhydride, activated ester method or DCC coupling were unsuccessful.

t-Butyl Benzyloxycarbonyl glycinate (LXII)

Concentrated sulfuric acid (0.8 mL) was added to a solution of 15 g (0.072 moles) of carbobenzyloxy glycine in 250 mL of methylene chloride. The solution was saturated with isobutylene, causing a volume increase of about 150 mL. After 65 hours at room temperature, the solution was added to 150 mL of 5% aqueous potassium hydroxide solution. The methylene chloride layer was separated, washed with water, then concentrated under vacuum at 60° to an oil.[113] Yield, 9.56 g (50%). NMR($CDCl_3$)δ 1.5(s,9H,$C(CH_3)_3$), 3.75-3.9(d,2H,NCH_2), 5.1(s,2H, $CH_2C_6H_5$), 5.9(broad,1H,NH), 7.3(s,5H,C_6H_5).

t-Butyl glycinate oxalate salt (LXIII)

A solution of 3.95 g (0.015 moles) of t-butyl N-carbobenzyloxy glycinate (LXII) in 60 mL of methanol was hydrogenated at atmospheric pressure with 300 mg of Pd/C catalyst. The filtered solution was treated with a methanolic solution of oxalic acid and the salt precipitated with ether to yield 2.8 g (85%) of (LXIII). mp 145-146°, Litt.mp[135] 146.5-147°.

3-Oxobutyl-1-phosphonate adduct with N-carbobenzyloxyglycyl-glycine (LIX).

A solution of 0.35 g (0.0016 moles) of 4-hydroxy-3-oxobutyl-1-phosphonate and 0.40 g (0.0016 moles) of carbobenzoxy glycine in 8 mL of anhydrous pyridine was cooled to 0°. Then, 0.35 g (0.0016 moles) of DCC was added to the above solution and stirred for 4 hours at 0° and overnight at room temperature. The precipitated dicyclohexylurea was filtered and washed with 10 mL of pyridine. After removing the pyridine solvent under high vacuum at room temperature, the residue was dissolved in ethylacetate and washed with cold 1 N acetic acid and water. The organic layer, after drying over anhydrous sodium sulfate, was concentrated under vacuum to yield 0.5 g (65%) of (LIX) as a yellow oil. The crude compound, after dissolving in ethyl acetate, was decolorised using activated charcoal and then reprecipitated with petroleum ether. This purification with ethyl acetate,petroleum ether was repeated to yield 0.35 g (40%) of the compound (LIX) which exhibited spectra in accord with expected structure. Rf(4nBuOH:1HOAc:1H_2O)= 0.86. Rf(85sec.BuOH:15(10%)NH_3) = 0.81. NMR($CDCl_3$)δ1.0-1.35(t,6H,OCH_2CH_3) 1.4-2.2(m,3H,CH_2,O=C-CH), 3.6-4.3(m,8H,$NHCH_2$, OCH_2CH_3), 4.55 (s,1H,O=C-OCH), 4.95(s,2H,$CH_2C_6H_5$), 6.2(Broad, 1H,NH), 7.1-7.3 (singlet with a multiplet, 6H,NH,C_6H_5); IR ($CHCl_3$)cm^{-1} 3300, 1715,1645,1545,1410, 1275,1210,1020,955.

Analysis: Calculated for $C_{20}H_{29}O_9N_2P.\ H_2O$.

C,49.89 ; H,6-23

Found C,50.10 ; H,6.37

t-Butyl N-carbobenzoxyglycylglycylglycinate (LXIXa)

To a stirred solution of 4.57 g (0.0172 mole) of N-carbobenzoxyglycylglycine and 2.4 mL (0.0172 mole) of triethylamine in 38 mL of chloroform at -40°, was added 2.27 mL (0.0172 mole) of isobutylchloroformate at a rate which allowed the temperature to remain below -20°. The mixture was stirred for 20 minutes at -10 to -15°, cooled to -40°, and treated with 3.8 g (0.0172 mole) of t-butylglycinate oxalate salt and 4.8 mL (0.035 moles) of triethylamine. The solution was stirred for 7 hours (-10 to $+12^{\circ}$), evaporated, and the residue treated with water and ethyl acetate. The organic layer was washed with water, saturated sodium chloride solution, and dried. Addition of petroleum ether caused crystallization. The solid was recrystallized from ethyl acetate-petroleum ether to give 3.38 g (52.5%) of the desired compound (LXIXa) of mp 125-126°. Litt. mp[135] 127-128°. Rf(4n-BuOH:1HOAc:1H_2O)=0.14 NMR (dmso-d_6)δ 1.35 (s,9H,$C(CH_3)_3$), 3.45-3.7 (Distorted doublet, 6H, $NHCH_2$), 7.1-7.3 (s,6H,C_6H_5, NH), 7.85-8.3 (Distorted t, 2H,NH).

N-Carbobenzoxyglycylglycylglycine (LXX)

A suspension of 1.0 g (0.0025 moles) of t-butyl N-carbobenzoxydiglycylglycine and 0.10 g of p-toluenesulfonic acid in 20 mL of benzene was refluxed for 30 minutes. On heating, the solid dissolved but the solution then became milky white and precipitation occurred after 3-5 minutes of refluxing. After cooling, the reaction mixture was filtered and the solid washed with benzene. The crude compound (0.83 g,100%) had a mp of 165-180°. It was recrystallised from ethanol to yield 0.70 g (83.3%) of the desired compound (LXX) of

mp 193-195°. Litt. mp[136] (186-188°, dec.). Rf(4BuOH:1HOAc:1H_2O)= 0.35 NMR(dmso-d_6)δ 3.45-3.7(Broad,6H,N CH_2),4.9(s,2H,$CH_2C_6H_5$,NH), 7.7-8.1(m,2H,NH).

Ethyl glycinate hydrochloride (LXVII)

Through a suspension of 20 g of glycine in 100 mL of absolute ethanol, dry HCl gas was passed until all the material dissolved. The solvent was removed under reduced pressure and the crude product was recrystallised from alcohol-ether to yield 22.3 g (60%) of the hydrochloride. mp 143-145° Litt. mp[114] 143-145°

Benzyloxycarbonylglycylglycine p-Nitrophenyl ester (LXVIII)

To a solution of 1.62 g (0.0061 mole) of benzyloxycarbonylglycylglycine in 12 mL of dimethylformamide was added 1.02 g (0.007 mole) of p-nitrophenol. Then 1.26 g (0.0061 mole) of DCC was added to the solution at 0°. The mixture was kept at 0° for 1 hour and then overnight at room temperature. After the removal of dicyclohexylurea the filtrate was diluted with 100 mL of cold water and the precipitated solid was filtered and washed with water. On standing in air, it became a hard solid which was recrystallized from 100 mL of ethanol containing 1% acetic acid. It gave a pure product 1.80 g (76%) which melted at 163.5-164° Litt. mp[137] 163-165°.

Ethyl Benzyloxycarbonyldiglycylglycinate (LXIX)

Ethyl glycinate hydrochloride 1.25 g (0.0088 mole), benzyloxycarbonyl glycylglycine p-nitrophenyl ester 3.4 g (0.088 moles), and tri-n-butylamine (2.1 mL) were dissolved in 9 mL of pyridine and kept at room temperature for 48 hours. The mixture was triturated with 15 mL methanol and after filtration an equal amount of water was added to the filtrate. The combined precipitates were recrystallised from ethyl acetate to yield 2.3 g (75%) of the desired compound. mp 167-169° Litt. mp[138] 167-169°

Benzyloxycarbonyl diglycylglycine (LXX)

To a solution of compound (LXIX) 1.5 g (0.0042 moles) in 35 mL methanol and 5 mL water was added 2.5 mL of a solution of 2N sodium hydroxide. After 2 hours at room temperature, 1 mL of water was added. The solution was left at room temperature for 15 minutes and then another 1 mL of water was added. After a total of three hours the solution was acidified with 1N hydrochloric acid to congo-red and was kept overnight in the refrigerator. The white solid obtained was filtered, washed with methanol and then recrystallised from ethanol to yield 1.07 g(77.5%) of the product. mp 191-192° Litt. mp[136] 186-188° (dec.). R_f (4 BuOH: 1 HOAC: $1H_2O$)=0.35 NMR (DMSO-d_6) δ3.45-3.7(Broad,6H,$NHCH_2$)4.9(s,2H,$CH_2C_6H_5$), 7-7.3(Broad Singlet, 6H,C_6H_5,NH), 7.7-8.1(m,2H,NH)

Adduct of Diethyl 4-hydroxy-3-oxobutyl-1-phosphonate with benzyloxycarbonyl diglycylglycine (LXXI)

To a solution of 1.7 g (0.0053 moles) of compound (LXX) and 1.18 g (0.0053 moles) of 4-hydroxy-3-oxobutyl-1-phosphonate in 25 mL of anhydrous pyridine cooled to 0^{o} was added 1.09 g (0.0053 moles) of dicyclohexylcarbodimide. The solution was stirred at 0^{o} for 4 hours and then at room temperature for 48 hours. The solution had a dark yellow color. Pyridine was evaporated using a vacuum pump and the residue was dissolved in ethyl acetate. It was washed immediately with cold 5% acetic acid, water and cold sodium bicarbonate solution. The ethyl acetate layer, after drying over anhydrous sodium sulfate, was concentrated to 5-10 mL and then the compound precipitated with petroleum ether to give 2.36 g (84%) of the crude product. It was purified by reprecipitation with ethyl acetate and petroleum ether to yield 1.8 g (64%) of the desired product which showed one spot on Tlc using two different solvent systems. (85 Sec.BuOH:15(10%) NH_3)= 0.78. Rf(4 n-BuOH:1HOAC:1H_2O)=0.72 NMR($CDCl_3$)δ1.1-1.5(t,6H,OCH_2CH_3), 1.5-2.35(m,3H,CH_2,O=C-OCH), 2.3-3.1(m,2H,CH_2), 3.8-4.4(m,10H,$NHCH_2$, OCH_2CH_3),4.7(s,1H,O=C-OCH), 5.0(s,2H,$CH_2C_6H_5$), 6.1-6.4(m,1H,NH), 7.3(s,5H,C_6H_5) 7.3-7.5(m,2H,NH); IR($CHCl_3$)$^{cm-1}$ 3300,1715,1645 (broad)1545,1410,1275,1210,1020,955,780.

Analysis: Calculated for $C_{22}H_{32}O_{10}N_3P$ C,49.90; H,6.05
Found: C,49.69; H,6.23

4-Hydroxy-3-oxobutyl-1-diethyl phosphonate diglycyl glycine hydrobromide (LXXII)

The compound (LXXI), 1.2 g (0.0022 moles) was dissolved in 13 mL of a freshly prepared saturated solution of hydrobromic acid in

glacial acetic acid and after standing at room temperature for 1.5 hours, 30 mL of ether was added. The oil which separated (1.0 g) was dissolved in ethyl acetate, decolorised using activated charcoal, and the purified compound was precipitated with petroleum ether to yield 0.85 g (86%) of material which exhibited spectra in accordance with the proposed structure. NMR(D_2O)δ 1.1-3.0 (t,two multiplets, 11H. OCH_2CH_3,CH_2CH_2,CO_2CH), 3.7-4.35(m,11H,NCH_2,OCH_2,CO_2CH), IR (Fluorolube)cm^{-1} 3500, 3000, 1715, 1650, 1540, 1425, 1375, 1250.

4-Hydroxy-3-oxobutyl-1-phosphonic acid diglycylglycine (LXXIII)

The compound (LXXII) 0.29 g (0.00060 moles) was placed in a nitrogen filled vial equipped with a septum as stopper. Then 0.38 g (0.0024 moles) of bromotrimethylsilane was injected. The vial was sealed and the reaction mixture stirred at 40° for 24 hours. After removing the alkyl bromide and the excess trimethylsilylbromide under vacuum, 1 mL of water was added to the vial and the reaction mixture stirred for 1 hour. It was extracted with chloroform and the aqueous layer evaporated to dryness. The residue was dissolved in ethyl acetate and then precipitated with petroleum ether to yield 0.2 g (79%) of the desired compound as an oil. NMR(D_2O)δ 1.35-2.15(m,2H,CH_2), 2.15-2.8(m,2H,CH_2), 3.5-4.2(m,8H,N-CH_2,C-CH_2,$\overset{O}{\overset{\|}{C}}-CH_2O\overset{O}{\overset{\|}{C}}$).

Benzyloxycarbonyl L-alanine (LXXVII)

To a solution of 8 g of L-alanine in 50 mL of 2N sodium hydroxide, set aside for 20 minutes at 0°, was added over 30 minutes, 17 g of benzylchloroformate and 25 mL of 4N sodium hydroxide simultaneously through two addition funnels attached to a three-necked flask. It was

allowed to stir in an ice-bath for an additional 10-15 minutes and then acidified with concentrated hydrochloric acid to congo red. The acidified solution, from which an oily compound separated, was cooled in the refrigerator for 4-5 hours. After decanting the aqueous layer, the oil was solidified by trituration with cold petroleum ether. The white solid obtained was filtered, washed with ether, dried and used without further purification. mp 82-84° Litt. mp[112] 84°. NMR($CDCl_3$)δ 1.0-1.4(d,3H,CH_3), 3.9-4.3(m,1H,CH), 4.9(s,2H,$CH_2C_6H_5$), 5.6(broad,1H,NH), 7.1(s,5H,C_6H_5), 10.1(s,1H,CO_2H), IR($CHCl_3$)cm^{-1} 3500,1720, 1510, 1455, 1350, 1075, 915, 695.

Benzyloxycarbonyl-L-alanine t-butyl ester (LXXIX)

Benzyloxycarbonyl-L-alanine 15.6 g (0.07 moles) was dissolved in 250 mL of methyl isobutyl ketone containing 0.8 mL of concentrated sulfuric acid which was saturated with isobutylene. After stirring for three days, it was washed with saturated sodium carbonate solution, water, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residual oil was dried under vacuum for 4-6 hours to yield 11.42 g (58.5%) of (LXXIX). NMR(CCl_4)δ 1.15-1.4 (two singlets,12H,$C(CH_3)_3$,CH_3), 3.85-4.3(m,1H,CH), 4.95(s,2H,$CH_2C_6H_5$), 5.8(d,1H,NH), 7.2(s,5H,C_6H_5), IR($CHCl_3$)cm^{-1} 3500, 3000, 1745, 1695, 1500, 1450, 1375, 1250, 1150, 1050, 930, 695.

Alanine t-butyl ester (LXXX)

Benzyloxycarbonyl-L-alanine t-butyl ester 11.0 g (0.039 moles) was hydrogenated using 300 mg of Pd/C as the catalyst at atmospheric

pressure. After removing the catalyst by filtration through Celite, the filtrate was concentrated over a rotary evaporator to yield 4.0 g (70%) of the compound (LXXX). It was used without further purification.[142] NMR of the hydrochloride, (D_2O)δ 1.2-1.5 (two singlets,12H, $C(CH_3)_3$,CH_3), 3.7-4.15(m,1H,CH), IR($CHCl_3$)cm^{-1} 3400(broad), 3000, 1750, 1375, 1250, 1150.

Benzyloxycarbonyl-L-alanyl-L-alanine t-butyl ester (LXXXII) Method A:

Alanine t-butyl ester (LXXX) 3.8 g (0.026 moles) was dissolved in 50 mL of pyridine and 4.3 mL of triethylamine and 8.65 g (0.025 moles) of Z-Ala-ONP[143] (LXXXI) were added. The Z-Ala-ONP was prepared by coupling benzyloxycarbonyl-L-alanine and p-nitrophenol using DCC in ethyl acetate as the solvent. The reaction mixture was kept at room temperature overnight, the solvent was evaporated under reduced pressure and the residue, taken up in chloroform, was thoroughly washed with 1N sodium hydroxide, 5% citric acid and water, dried over anhydrous sodium sulfate and evaporated to dryness. It was recrystallised twice from ether-light petroleum ether to give 7.36 g (82%) of the protected dipeptide ester (LXXXII). mp 71-75°. R_f(3N-BUOH: 1HOAC: $1H_2O$)=0.65. R_f($9CHCl_3$:$1H_2O$)=0.80. NMR($CDCl_3$)δ 1.1-1.5 (singlet with two shoulders, 15H,$C(CH_3)_3$,CH_3), 4.1-4.45(m,2H,CH), 4.9(s,2H,$CH_2C_6H_5$), 6.3(d,1H,NH), 7.1(s,5H,C_6H_5), 7.2(m,1H,NH). IR($CHCl_3$)cm^{-1} 3500, 3000, 1745, 1695, 1500, 1375, 1150, 1050, 930, 875, 695.

Alanine t-butyl ester hydrochloride (LXXX)

Concentrated sulfuric acid (10 mL, 1 Ml per g of the amino

acid) was added to a suspension of 10 g (0.112 moles) of alanine in 100 mL of dioxane in a pressure bottle. An equal volume (100 mL) of liquid isobutene was added and the mixture shaken overnight. The mixture was poured into an excess of 2N sodium hydroxide and the ester extracted thoroughly with ether. The ether extract was concentrated to about 70 mL and cooled to 0° and a dry ethereal solution of hydrogen chloride carefully added until there was no precipitation on further addition of HCl and an acidic medium was indicated by pH paper (pH 2-3). It was then cooled to -20° overnight. White crystals of alanine t-butyl ester hydrochloride were filtered and dried under vacuum to yield 8.3 g (41%). mp 164-167 (dec.), Litt. mp[122] (dec.). NMR (D_2O)δ1.2-1.5(two singlets,12H,C(CH_3)$_3$,CH_3), 3.7-4.15(m,1H,CH). IR($CHCl_3$)cm^{-1} 3400(broad), 3000, 1750, 1375, 1250, 1150.

N-Benzyloxycarbonyl-L-alanyl-L-alanine tert. butyl ester (LXXXII)

Method B:

Benzyloxycarbonyl-L-alanine 2.9 g (0.013 moles) and 2.36 g (0.013 moles) of L-alanine t-butyl ester hydrochloride were dissolved in 30 mL of dichloromethane. After cooling to 0°, N-ethylmorpholine, 1.52 g (0.013 moles), and 2.95 g (0.014 moles) of N,N-dicylohexylcarbodiimide were added and the mixture stirred for 2 hours at 0° and then overnight at room temperature. At this time, 4 drops of acetic acid were added and the reation mixture was filtered after 1 hour. The solution was washed with 4x25mL of 10% aqueous solution of citric acid, of 1M $NaHCO_3$ and of water. The organic layer was

dried over anhydrous sodium sulfate and,after removal of dichloromethane, the colorless oily residue was dissolved in dry ether (25mL) After 24 hours in the cold small amounts of additionally precipitated dicyclohexylurea were filtered and hexane added to the filtrate until it became turbid. After standing at -20° for 2 days, the crystalline product was filtered and washed with hexane. It was immediately transferred to a round-bottomed flask and dried under vacuum over P_2O_5 to yield 4.2g (92%) of (LXXXII) of mp 67-70°. Litt. mp[92] 69-71° (Hygroscopic). Rf(3 n-BuOH: 1HOAC: $1H_2O$)=0.65, R_f($9CHCl_3$: 1 EtOH)=0.80. NMR($CDCl_3$)δ 1.1-1.5(singlet with shoulder,15H,C(CH_3),CH_3), 4.1-4.4 (m,2H,CH), 4.95(s,2H,$CH_2C_6H_5$), 6.3(d,1H,NH). IR. ($CHCl_3$)cm^{-1} 3500, 3000, 1745, 1695, 1500, 1455, 1375, 1250, 1150, 1050, 930, 875, 695.

L-Alanyl-L-alanine tert. butyl ester hydrochloride (LXXXIII):

The compound (LXXXII) 4.0g (0.011 moles) dissolved in 75 mL of ethanol was hydrogenated at atmospheric pressure after addition of 150 mg. of Pd/C catalyst and two drops of acetic acid. After filtering the catalyst, the ethanol was evaporated and the residue dissolved in 25mL of dry ether. After cooling to 0°, a dry ethereal solution of HCl was added until no more precipitation occurred on further addition of HCl and a slight acidic medium (pH 2-3) was indicated by pH paper. It was kept at -20° for 24 hours. The crystalline precipitates were filtered and immediately transferred to a round-bottomed flask, and dried under vacuum over P_2O_5 to yield 2.35 g (83%) of (LXXXIII). mp 190-195° (dec.), Litt. mp[92] 195° (dec.). R_f of amine(3 n-BuOH: 1HOAC: 1 H_2O)=0.61. NMR of amine ($CDCl_3$)δ 1.4(singlet with shoulder,15H,C(CH_3)

CH_3), 3.5-3.8(m,1H,CH), 4.1-4.5(broad,2H,NH,CH), 7.9-8.2(broad,2H, NH_2). IR($CHCl_3$) cm^{-1} 3700-3000(broad), 1745, 1680, 1560, 1500, 1475, 1375, 1275, 1060, 860.

β-Benzyl L-aspartate (LXXIV)

Sulfuric acid (10 mL) was added to anhydrous ether (100 mL) followed by benzyl alcohol(100 mL) in a 500 mL. round-bottomed flask. The ether was removed under vacuum and finely ground L-aspartic acid (13.4 g) was added in several portions while the mixture was magnetically stirred. The ensuing solution was left at room temperature for 24 hours after which 200 mL of 95% ethanol was added followed by 50 mL of pyridine dropwise while the solution was stirred vigorously. The mixture was cooled overnight and the solid present was filtered and washed with ether. It was recrystallized from water containing a few drops of pyridine to yield 9.5 gm. (45%) of the desired compound (LXXIV). mp 210-212°, Litt. mp[120] 218-221°.

p-Nitrophenylchloroformate:

In a 500 mL round-bottomed flask equipped with a long refluxing condenser and an Erlenmyer flask attached by Gooch tubing was placed 77.6 mL. of a 12.5% solution of phosgene in benzene. Sodium p-nitrophenolate was dried by heating at 40-45° under high vacuum. (Dry sodium p-nitrophenolate has a red color while the hydrate is yellow). Dry sodium p-nitrophenolate was added in portions to the round-bottomed flask via the rubber tube. The reaction flask was cooled during the addition to maintain the temperature below 40°.

The mixture was then allowed to reflux gently for an hour. Excess phosgene was distilled out while heating the flask over a water bath at 50-60° for 30 minutes. Sodium chloride was removed by filtration, washed with benzene and the filtrate concentrated under vacuum on a rotary evaporator. The residue was distilled under vacuum (129-133°/ 1.5-2.0 Torr). This distilled material solidified and was recrystallized from carbon tetrachloride to yield 10.8 g. (43%) of the product. mp 80-81°; Litt. mp[134] 81-82°.

t-Butyl p-nitrophenylcarbonate (LXXVI):

p-Nitrophenylchloroformate, 12.6 g. (0.062 moles), was added slowly in portions to a stirred solution of t-butyl alcohol, 4.59 g. (0.062 moles)in 25 mL of pyridine at 0-5°. The reaction mixture was stirred at room temperature for three hours and the precipitated pyridine hydrochloride was then removed by filtration. There was then added 3.0 mL of water to the filtrate and the solution extracted 3-4 times with 25 mL portions of ether. The ether extract was washed with 1N hydrochloric acid, saturated sodium carbonate solution, and saturated sodium chloride solution. The ethereal extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in 30 mL. of absolute ethanol and the compound precipitated by the addition of water to yield 6.7 g. (46%) of the product. mp 76.5-78.5° Litt. mp[134] 78.5-79.5°. NMR($CDCl_3$)δ 1.5(s,9H,$C(\underline{CH}_3)_3$), 7.3, 8.2(symmetrical, 4H,$C_6\underline{H}_4$).

t-Butyloxycarbonyl-β-benzyl-L-aspartate (LXXVII)

A mixture of 4.14 g. (.0186 moles) of β-benzyl-L-aspartate, 6.17 g. (.028 moles) of t-butyl p-nitrophenylcarbonate, 4.93 g. (.046 moles) of sodium carbonate, 28 mL. of t-butyl alcohol, and 19 mL. of water was refluxed on a steam bath for 1 hour. The condenser was removed and the mixture concentrated by an air stream during heating to remove t-butyl alcohol. Sodium p-nitrophenolate dihydrate crystallised, was filtered after cooling, and was washed with 5 mL. of water. The filtrate was adjusted to pH5-6 by dilute hydrochloric acid and extracted with ether to remove any remaining t-butyl p-nitrophenyl-carbonate and p-nitrophenol. The aqueous portion was adjusted to pH 1 and then extracted four times with ether. After evaporation of the ether, the solid residue was recrystallized from ethyl acetate and petroleum ether to yield 3.5 g (58%) of the desired compound (LXXVII). mp 97-99° Litt. mp[140] 102-103°. R_f(3 n-BuOH:1HOAC: $1H_2O$)=0.69 NMR($CDCl_3$)δ 1.35(s,9H,$C(CH_3)_3$), 2.9(distorted doublet,2H,CH_2CO_2), 4.5(m,1H,CH), 5.0(s,2H,$CH_2C_6H_5$), 5.5(d,1H,NH), 7.15(s,5H,C_6H_5). IR($CHCl_3$)cm^{-1} 3200-3700(broad),1725(broad), 1475,1360, 1160, 925, 695.

t-Butyloxycarbonyl-β-benzyl-L-aspartate)LXXVII) Method B:

To a solution of β-benzyl-L-aspartate (2.45 g., 0.010 moles) and triethylamine (2.10 mL.,0.015 moles) in water (6 mL) was added dioxane (6 mL) and BOC-ON (2.71 g 0.011 moles) at room temperature. The mixture became homogeneous within 1 hour and stirring was continued for 2 hours. At this stage, the solution had a yellow color. After addition of water (15 mL) and ethyl acetate (20 mL) the reaction mixture was acidified with 5% citric acid solution and extracted

with ethyl acetate. The organic extract was dried over anhydrous sodium sulfate and then concentrated to about 25 mL under reduced pressure. The required compound was precipitated by the addition of light petroleum ether and cooling at -20° overnight yielding 2.14 g. - 96 %) of (LXXVII). mp 100-102° Litt. mp[140]102-104°. R_f(3 n-BUOH: 1HOAC: $1H_2O$)=0.69. NMR($CDCl_3$)δ 1.35(s,9H,$C(CH_3)_3$), 2.9(distorted d,2H,CH_2CO_2), 4.5(m,1H,CH), 5.0(s,2H,$CH_2C_6H_5$), 5.5(d,1H,NH), 7.15(s,5H,C_6H_5). IR($CHCl_3$)cm^{-1} 3200-3700(broad), 1725(broad),1475, 1360,1160,925, 695.

t-Butyloxycarbonyl-β-benzyl-L-aspartyl-L-alanyl-L-alanine tert. butyl ester (LXXXIV)

A solution of 0.00736 moles (1.59 g) of NH_2-Ala-Ala-OtBu and 2.4 g (0.0074 moles) of BOC-asparty1-β-benzyl ester in 30 mL of methylene chloride was cooled to 0°. After addition of 1.52 g. (0.0074 moles) of dicyclohexylcarbodiimide, the solution was stirred at 0° for 4 hours and then overnight at room temperature. Acetic acid (4-6 drops) was added, the solution filtered after 30 minutes, and the filtrate evaporated to dryness. The oily residue was dissolved in ethyl acetate (30 mL), washed with 5% citric acid, water, and then with 1M sodium carbonate solution. The organic layer, after drying over anhydrous sodium sulfate, was concentrated to 7-10 mL and the desired compound precipitated upon addition of light petroleum ether and cooling at -20° overnight yielded 3.08 g (80.5%) of (LXXXIV). R_f(3n-BUOH: 1HOAC: $1H_2O$)=0.76, R_f(9$CHCl_3$: 1EtOH)=0.78. NMR($CDCl_3$) δ1.2-1.5(broad s,24H,$C(CH_3)_3$,CH_3), 2.8(distorted d,2H,CH_2CO_2), 3.9-

4.8(m,3H,CH), 5.0(d,1H,NH), 7.2(s,with a multiplet,7H,C_6H_5,NH). IR($CHCl_3$)cm^{-1} 3500, 3000, 1740(broad), 1670, 1500, 1455, 1375, 1175, 930, 725, 650.

Analysis: Calculated for $C_{26}H_{39}O_8N_3$: C,59.88; H,7.48
Found: C,59.80; H,7.73

t-Butyloxycarbonyl-β-carboxyl-L-aspartyl-L-alanyl-L-alanine tert. butyl ester (LXXXV)

A solution of 2.56 g. (0.004 moles) of compound (LXXXIV) in 50 mL of ethanol was hydrogenated at atmospheric pressure using 150 mg of Pd/C as the catalyst. It was filtered through a Celite pad and then concentrated under reduced pressure to yield 1.56 g of the compound(LXXXV). It was used for the next reaction without further purification. R_f(3 n-BUOH: 1HOAC: $1H_2O$)=0.5. MNR($CDCl_3$)δ 1.55(singlet with a shoulder, 24H,$C(CH_3)_3CH_3$),2.9(broad,2H,CH_2CO_2), 4.0-4.9 (broad multiplet,3H,CH),5.9(broad,1H,NH), 7.1-7.7(broad,2H,NH), 8.8(broad,1H,CO_2H). IR($CHCl_3$)cm^{-1} 3500(broad), 3000, 1725, 1670, 1500, 1365, 1160, 920.

t-Butyloxycarbonyl-β-4-hydroxy-3-oxobutyl-1-diethyl phosphonate-L-aspartyl-L-alanyl-alanyl-tert. butyl ester. (LXXXVI)

A solution of 1.58 g (0.004 moles) of compound (LXXXV) and 0.918 g (0.004 moles) of 4-hydroxy-3-oxybutyl-1-diethylphosphonate in 10 mL of anhydrous pyridine was cooled to 0^o and to this cooled solution was added 0.86 g (0.004 moles) of DCC. The solution was stirred at 0^o for 4 hours and overnight at room temperature. The precipitated dicyclo-

hexylurea was filtered and the filtrate evaporated to a thick oil under high vacuum at room temperature. To the residue dissolved in 10 mL of ethyl acetate was added 2 drops of acetic acid. After 30 minutes the additional precipitated DCU was removed by filtration and the filtrate worked up in the usual way as described before to yield 2.1 g (82.5%) of the desired compound (LXXXVI). A small amount of it was purified for elemental analysis by precipitating it from ethyl acetate and light petroleum ether. R_f(4n-BUOH: 1HOAC: $1H_2O$)=0.69, R_f($9CHCl_3$: 1EtOH)=0.55. NMR($CDCl_3$)δ 0.9-2.1(m,32H,$C(CH_3)_3$,CH_3, $OCH_2\underline{CH}_3$), 2.3-3.2(m,4H,CH_2CO_2,$\overset{O}{\overset{\|}{C}}CH_2$), 3.6-4.8(m,9H,$\underline{CH}$,$O\underline{CH}_2CH_3$,$CO_2\underline{CH}_2$), 5.8(m,1H,NH), 6.7(m,1H,NH), 7.2(m,1H,NH). IR($CHCl_3$)cm^{-1} 3500, 3000, 1720(broad), 1675,1510, 1450, 1375, 1250, 1185,1040.

Analysis: Calculated for $C_{27}H_{48}O_{12}N_3P.1/2H_2O$. C,50.14; H,7.64
Found C,49.98; H,7.52

The protecting groups, t-butyloxycarbonyl and the t-butyl ester in the above compound (LXXXVI) were removed by using a freshly prepared saturated solution of HBr in acetic acid as described before for the triglycyl system. The diethyl phosphonate ester was hydrolyzed to the free phosphonic acid (LXXXVIII) by using trimethylsilylbromide at 35-40° for 48 hours as described earlier.

1-t. butyloxy-3-hydroxybutane (XXXIII)

1.3-Dihydroxybutane, 10.0 g (0.11 moles) was dissolved in 150 mL of methylene chloride. The solution was cooled to -20° and then to it

was added successively boron trifluoride etherate (5mL), phosphoric acid (100%, 2.1 mL). After shaking the reaction mixture in order to disperse the catalyst uniformly there was added 100 mL of liquified isobutene. The reaction mixture was shaken in a pressure bottle for 90 minutes. It was then poured into 2N aqueous ammonia with stirring and most of the excess isobutene evaporated. The two layers were separated and the aqueous layer extracted with methylene chloride. The combined extracts were washed with aqueous ammonia, water and then dried with anhydrous magnesium sulfate. Evaporation of the solvent yielded a residue which was distilled under vacuum. 79-80° /20 Torr to yield 11.0 g (68.5%) of the desired compound (XXXIII). NMR(Neat)δ 0.9-1.2(two singlets,12H $C(CH_3)_3,CH_3$), 1.2-1.65(q,2H,CH_2), 3.2-3.4(t,2H,CH_2O), 3.4-3.7(m,2H,OH,CH). IR(CCl_4) cm^{-1} 3700(broad), 3000, 1475, 1425, 1360, 1250, 1200, 1075, 1025. It was of sufficient purity and used as such for the next reaction.

Pyridinium Chlorochromate

To 92 mL of 6M HCl (0.5 moles) was added 50.0 g (0.5 moles) of chromium trioxide rapidly with stirring. After 5 minutes, the solution was cooled to 0° and 39.5 g (0.5 moles) of pyridine was added slowly over 10 minutes. Cooling to 0° gave a yellow-orange solid which was collected on a sintered glass funnel and dried under vacuum to yield 90 g (84%) of the desired compound.

1-t. butyloxy-3-butanone (XXXIV)

In a 250 mL round-bottomed flask was suspended 22.1 g (0.10 moles) of pyridinium chlorochromate in 75 mL of methylene chloride. To it

1-t-butyloxy-3-hydroxybutane (10 g, 2.068 moles) was added in one portion to the magnetically stirred solution. After stirring overnight, 100 mL of dry ether was added and the supernatant decanted from the black gum. The insoluble residue was washed several times with ether. The combined organic solution was passed through a short column of Florisil and the solvent removed under vacuum. Distillation under reduced pressure of the residue through a vigreux column gave 7.85 g (80%) of the desired compound (XXXIV). bp 60-65° /20 Torr. NMR(neat)δ 1.0(s,9H,$C(CH_3)_3$), 1.9(s,3H,CH_3CO), 2.15-2.45(t,2H,XH_2CO),3.2-3.45(t,2H,XH_2O) IR(CCl_4)cm^{-1} 3000, 1715, 1475, 1425, 1400, 1375, 1245, 1210, 1175, 1100, 1015, 900. It was used without any further purification for the next reaction.

4-carboethoxy-3-hydroxy-3-methylbutyl-1-t. butyl ether (XXXV)

Dry ether (20 mL) was cooled to -23° in a 100 mL three-necked flask under a nitrogen atmosphere. Disopropylamine (3.08 mL, 0.022 moles) was added via a syringe followed by a solution of n-butyllithium in heptane (13.2 mL, 0.022 moles) which was added over a period of 10 minutes. After stirring for 1 hour at -20°, the bath temperature was reduced to -78°and dry ethyl acetate (2.16 mL, 0.022 moles) added slowly. The solution was stirred for 20-30 minutes at -78° and then 1-t. butyloxy-3-butanone (3.2 g.,0.022 moles) added dropwise. After 15 minutes, the reaction mixture was treated with 20% aqueous HCl (4.0 mL) and then allowed to warm to room temperature. The mixture was diluted with water (4 mL) and extracted with ether. The organic layer was dried and the solvent evaporated to leave an

oil. From this oil, the unreacted ketone (0.5 g) was removed by fractional distillation under high vacuum leaving behind 2.95 g (68%) of the residue which gave spectral data in accord with the proposed product structure. NMR(neat)δ 0.9-1.1(singlet with a multiplet,15H, $C(CH_3)_3$,CH_3,OCH_2CH_3), 1.35-1.65(t,2H,CCH_2),2.2(s,2H,CH_2CO_2),3.1-3.4 (t,2H,CH_2O),3.55-4.0(t with a singlet,3H,OCH_2,OH). IR(CCl_4) cm^{-1} 3500, 3000, 1720, 1455, 1360, 1200, 1115, 1090, 925, 695.

4-Carboxy-3-hydroxy-3-methylbutyl-1-t. butyl ether (XXXVI)

To 20 mL of 1N potassium hydroxide in methanol at -20° was added 2.95 g (0.013 moles) of compound (XXXV) in 5 mL of dry ether. After 1 hour at -20°, the reaction mixture was maintained at room temperature overnight. At the end of this period the reaction mixture had a dark yellow color. It was neutralized with methanolic hydrochloric acid to congo red. The precipitated KCl was filtered and the filtrate concentrated to dryness. The residue was dissolved in chloroform and additional precipitated KCl was removed. The solvent was evaporated under reduced pressure to give 2.0 g (77%) of the desired compound. It was further purified by dissolving in chloroform and then extracting 4 times with 10 mL portions of saturated sodium bicarbonate solution. The aqueous extract was acidified to congo-red with concentrated hydrochloric acid and then extracted thoroughly with chloroform. The organic extract after drying and removal of the solvent gave 1.8 g (68%) of the pure compound. The organic extract obtained after extraction with sodium bicarbonate solution, was concentrated to give the starting ester which was again hydrolyzed

with KOH in MeOH. NMR(CCl_4)δ1.2-1.3(two singlets,12H,$C(CH_3)_3$,CH_3), 1.7-2.0(t,2H,CH_2),2.5(s,2H,CH_2CO_2), 3.5-3.7(t,2H,CH_2O), 7.7-8.0 (broad,2H,CO_2H,OH). IR(CCl_4)cm^{-1} 3700-3300, 3000, 1720, 1400, 1375, 1250, 1210, 1150, 1095, 910.

4-Carbobenzyloxy-3-hydroxy-3-methyl-1-tert. butyloxybutane (XXXVII)

Attempts to make this compound using equimolar amounts of compound (XXXVI), freshly distilled benzyl bromide and DCC in methylene chloride were unsuccessful. It was prepared by the following procedure:

The compound (XXXVI),1.16 g (0.0057 moles) was treated with 0.0478 g (0.0057 moles) of sodium bicarbonate in 7 mL of water and was stirred at room temperature for 30 minutes. Water was removed on a rotary evaporator at 40° and the residue was dried over sodium hydroxide pellets under high vacuum for 24 hours. The sodium salt thus obtained was dissolved in 8 mL of N,N-dimethylformamide at 80° and 3.92 g (0.0228 moles) of distilled benzyl bromide was added; the reaction mixture was heated with stirring at 80° C for 20 hours in a stoppered round-bottomed flask. Excess benzyl bromide and DMF were distilled off and to the residue were added 50 mL of ether and 15 mL of water. The organic layer was washed with saturated aqueous sodium bicarbonate and water and, after drying over anhydrous sodium sulfate, the solvent was removed on a rotary evaporator. The NMR of the residue indicated the desired compound to be mixed with small amounts of unreacted benzyl bromide. It was left overnight at 70-80° under 0.3 Torr pressure under a distillation column. The NMR of the residue (1.2 g.,

84%) indicated that most of benzyl bromide was removed. It was further purified by silica gel column chromatography to yield 1.0 g (60%) of the pure compound (XXXVII). NMR(CCl_4) δ 1.0-1.1(two singlets,12H, $C(CH_3)_3$,CH_3), 1.5-1.7(t,2H,CH_2),3.2-3.5(t,2H,CH_2O),3.8(s,1H,OH), 4.9(s,2H,$CH_2C_6H_5$).7.1(s,5H,C_6H_5); IR(CCl_4) cm^{-1} 3500, 3000, 1720, 1450, 1360, 1250, 1140, 1090, 915, 695.

analysis: Calculated for $C_{17}H_{26}O_4$, C,69.38; H,8.89

Found: C,69.31; H,9.02

Attempted preparation of 4-Carbobenzyloxy-3-hydroxy-3-methyl-1-butanol (XXXVIII)

The above compound (XXXVII), 2.47 g (0.0084 moles) dissolved in 100 mL of benzene (dried over sodium) was refluxed with 750 mg of p-toluenesulfonic acid for 1 hour. The reaction mixture was poured into an aqueous 5% solution of sodium hydrogen carbonate. The organic layer was worked up in the usual manner but only an intractable residue was obtained.

Attempts to remove the t-butyl ether group by using trifluoroacetic acid at 0^o were also unsuccessful.

3-Oxobutyl-1-acetate (XLII)

35 g of methyl vinyl ketone, 150 mL acetic acid, and 1 drop of water were heated under reflux overnight. After removal of acetic acid on a rotary evaporator, the residue was fractionally distilled to yield 28.6 g (44%) of the compound (XLII). bp $78-82^o$/15 Torr, Litt. bp[107] $78-84^o$/15 Torr. NMR(neat) δ 1.6(s,3H,Ch_3CO), 1.8(s,3H,$O\overset{O}{\overset{\|}{C}}CH_3$), 2.45(t,2H,$\overset{O}{\overset{\|}{C}}CH_2$), 3.95(t,2H,$OCH_2$)1 IR($CHCl_3$)$cm^{-1}$ 3500, 1725, 1360,

1250, 1175, 1050.

4-Carbobenzyloxy-3-hydroxy-3-methylbutyl-1-acetate (XLIII)

10 mL of dry ether was cooled to -23° under a nitrogen atmosphere. Diisopropylamine (1.5 mL, 10.6 mmol.) was added via a syringe followed by a solution of n-butyl-lithium in heptane (6.39 mL, 10.6 mmol.) which was added over a period of 10 minutes. After stirring for 1 hour, the bath temperature was reduced to -78° and 1.59 g (10.6 mmol.) of benzyl acetate slowly added. The solution was stirred for 30 minutes at -78° and 1-acetoxybutane-3-one (1.26 g., 10.6 mmol.) added. After 30 minutes, the reaction mixture was treated with 2 mL of 20% aqueous hydrochloric acid and then allowed to warm to room temperature. The mixture was diluted with 2-3 mL of water and then extracted with ether. The dried and evaporated organic layer yielded an oil (2.1 g) which on NMR indicated the presence of some unreacted benzylacetate. This was removed by heating the residue under a distillation column at 0.005 Torr and 80-90° bath temperature. The residue of 1.9 g (64%) gave spectral data in accord with the proposed structure. It was used for further reaction without any further purification. NMR(CCl_4) δ 1.0(s,3H,CCH_3), 1.8(singlet superimposed on a triplet,5H,$OCOH_3$,CH_2), 2.25(s,2H,CH_2CO_2), 3.5(s,1H,OH), 3.9 (t,2H,OCH_2), 4.8(s,2H,$CH_2C_6H_5$), 7.0(s,5H,C_6H_5); IR($CHCl_3$ cm^{-1} 3500, 1715, 1450, 1375, 1250, 700.

Attempted preparation of 4-Carbobenzyloxy-3-hydroxy-3-methyl-1-butanol (XXXVIII)

The above compound (XLIII) (0.48 g., 0.0017 moles) in 10 mL of

$CHCl_3$: MeOH (1:1) was stirred with 1.5 mL of 1.2N sodium hydroxide in MeOH:H_2O (1:1) at 37° for 15 minutes. At the end of this period 2.0 mL of 0.075N acetic acid was added and the reaction mixture extracted 4 times with methylene chloride. The NMR of the residue indicated that both the acetyl and the benzyl groups were removed.

Variation of temperature and time in this hydrolysis either gave the unreacted starting compound or mevalonolactone. The selective removal of acetate could not be achieved by using potassium carbonate in ethanol.

Ethyleneketal of ethylacetoacetate (XLIV)

Ethylacetoacetate (20.0 g., 0.16 moles), 14,88 g (0.24 moles) of ethylene glycol, and 100 mg of p-toluenesulfonic acid in 100 mL benzene were refluxed in a 250 mL round-bottomed flask equipped with a Dean-Stark apparatus. Refluxing was continued until no more water was collected in the Dean-Stark apparatus. After cooling the solution to room temperature, it was washed with water, saturated sodium bicarbonate, and then water again. The organic layer after drying over anhydrous sodium sulfate was concentrated under vacuum and the residue obtained distilled under vacuum to give 20 g of the compound (XLIV). bp[141] 95-100°/20 Torr. R_f($CHCl_3$+4 drops of EtOH)=0.64. NMR (neat) δ 1.4(t,3H,OCH_2CH_3), 1.6(s,3H,CCH_3), 2.75(s,2H,CH_2CO), 3.8-4.3 (singlet superimposed on a triplet,6H,OCH_2; IR($CHCl_3$)cm^{-1} 3000, 1740, 1450, 1375, 1150, 1110, 1050, 950, 875.

Analysis: Calculated for $C_8H_{14}O_4$ C, 55.17; H, 8.04
Found: C, 55.49; H, 8.13

β-Ketobutanol-ethyleneketal (XLV)

In a 250 mL three-necked round-bottomed flask was suspended 1.14 g (0.03 moles) of lithium aluminium hydride in 35 mL anhydrous ether. To this stirred suspension was added dropwise a solution of 9.5 g (0.054 moles) of ethylene ketal of ethyl acetoacetate in 30 mL anhydrous ether. The addition was maintained at such a rate that uniform refluxing occured. It was refluxed for an additional 2 hours and then cooled to room temperature. This was followed by the addition of 5 mL of 10% sodium hydroxide solution. The precipitated solids were filtered and rinsed with ether. The combined filtrates were washed with brine, dried, and evaporated. There was thus isolated 4.6 g (65%) of the desired compound (XLV). bp 100-105°/20 Torr Litt. bp[141] 85-87°/11 Torr. R_f ($CHCl_3$+4 drops of EtOH)=0.33 NMR(neat)δ 1.2(s,3H,CH_3), 1.8(t,2H,CH_2), 3.3-3.6(t,2H,OCH_2), 3.75(s,4H,OCH_2), 3.9(s,1H,OH); IR($CHCl_3$)cm^{-1} 3600, 3000, 1375, 1100, 1050, 950.

4-Hydroxy-2-butanone (XLVI)

A mixture of 10.5 g of the ketal (XLV), 7.2 mL water, and four drops of concentrated HCl was stirred for 2 hours at 60-70°. After cooling to room temperature, the aqueous solution was extracted thoroughly with ether and the ether solution washed with saturated sodium bicarbonate solution. After drying, the solvent was removed by evaporation under vacuum to yield 5 g of the residue which was distilled at11-13 Torr pressure to give 4.8 g (69%) of the desired compound (XLVI). bp 71-76°/11-13 Torr, Litt. bp[141] 70-76°/11 Torr. NMR(neat) δ 2.0(s,3H,CH_3CO), 2.45(t,2H,CH_2CO), 3.15(s,1H,OH), 3.45

(t,2H,OCH_2); IR(CCl_4)cm^{-1} 3600(broad),.3000, 1725, 1520, 1350, 1110.

4-Tetrahydropyranyl-2-butanone (XLVII)

A solution of 4-hydroxy-2-butanone (5 g, 0.057 moles) and dihydropyran (7.16 g, 0.085 moles) in 100 mL of dry methylene chloride containing 1.4 g (0.0057 moles) of pyridinium p-toluenesulfonate was stirred for 4 hours at room temperature. The solution was then diluted with ether and washed once with brine to remove the catalyst. After removal of the solvent on a rotary evaporator the residue was distilled under vacuum to yield 6.9 g (72%) of the compound (XLVII). bp 100-106°/15-18 Torr. It was used without any further purification for the next reaction. $R_f(CHCl_3)$=0.46. NMR(neat)δ 1.35(broad singlet, 6H,CH_2), 1.9(s,3H,$COCH_3$), 3.1-3.8(m,4H,OCH_2,CH_2CH_2O), 4.3(m,1H,CH); IR($CHCl_3$)cm^{-1} 3500, 3000, 1725, 1450, 1360, 1130, 1090, 1050, 925, 875.

Pyridinium p-toluensulfonate (PPTS)

In a 100 mL round-bottomed flask, p-toluenesulfonic acid monohydrate (3.7 g, 0.003 moles) was added to 12.1 mL (0.015 moles) of pyridine with stirring at room temperature. The reaction was slightly exothermic. After stirring for 20 minutes, the excess pyridine was removed with a rotary evaporator on a water bath at 55-60° to give a white solid which was recrystallised from acetone to give pure compound (6.8 g, 90%), mp 119-120°, Litt. mp[127] 120°.

4-Carbobenzyloxy-3-hydrox—3-methylbutyl-1-tetrahydropyran ether (XLVIII)

Dry ether 15 mL was cooled to -23° under a nitrogen atmosphere. Diisopropylamine (2.1 mL, 0.0147moles) was added via a syringe followed by a solution of n-butyllithium in heptane (9.2 mL, 0.015 moles) which was added over a period of 10 minutes. After stirring for 1 hour, the bath temperature was reduced to -78° and 2.25 g, (0.015 moles) of benzyl acetate slowly added. The solution was stirred for 30 minutes and 2.55 g (0.0147 moles) of tetrahydropyran-2-butanone was slowly added. After stirring for 30 minutes, the reaction mixture was treated with 3 mL of 20% aqueous HCl and then allowed to warm to room temperature. The mixture was diluted with 3 mL of water and extracted with ether. The dried and evaporated organic layer yielded 3.5 g of an oil. Unreacted benzyl acetate and starting ketone were removed by heating at $90-95^{\circ}$ at 0.01 Torr pressure in a distillation apparatus. The residue (3.3 g, 70%) exhibited spectra in accord with the proposed structure. It was further purified by column chromatography on silica gel using chloroform containing 0.75% ethanol. NMR (CCl_4) δ 1.15(s, 3H,CH_3), 1.25-2.0(m,8H,$(CH_2)_3$, CCH_2), 2.4(s,2H,CH_2CO), 3.1-4.0(m,5H, OCH_2,OH,OCH_2), 4.7(broad, 1H, CH), 5.0(s,2H, CH_2C_6), 7.1(s, 5H, C_6H_5). IR(CCl_4)cm^{-1} 3600(broad), 3000,1725,1360,1245,1050,695.

Analysis	Calculated for $C_{18}H_{26}O_5$	C,67.07;	H,8.07
	Found	C,66.75-	H,8.21

Attempted preparation of Tetraethyl methylene bisphosphonate. Kosolapoff procedure: Diiodomethane 69.0 g (0.26 moles) and 133 g (0.8 moles) of triethyl phosphite were refluxed at $160-170^{\circ}$ for 24

hours in a round-bottomed flask equipped with a reflux condenser and a Dean-Stark tube at the top of the condenser. After removal of unreacted triethyl phosphite the residue was distilled under vacuum to give 24.3 g (34%) of diethyl iodomethylphosphonate. bp 80-85°/0.05 Torr. Pure tetraethyl methylenebisphosphonate was not obtained from the residue. NMR(Neat) δ 1.2-1.5 (t,6H,OCH_2CH_3), 3.2-3.4 (d,2H,CH_2), 3.9-4.4(q,4H,OCH_2) IR(CCl_4 cm^{-1} 3000,1450,1395,1275,1175,1060,1040,975.

Becker reaction with diethyl iodomethylphosphonate (XLIX)

A solution of sodium ethoxide was prepared by dissolving 2.0 g (0.087 moles) of sodium in 36 mL ethanol. The sodium ethoxide was then added dropwise over a period of 30 minutes to a mixture of 24.3 g (0.087 moles) of diethyl iodomethylphosphonate and 12.00 g (0.87 moles) of diethyl phosphite in 7 mL of ethanol at room temperature. The reaction was very exothermic. The solution was stirred for 1 hour after which it was filtered, ethanol being removed on a rotary evaporator, and to the residue ether was added to precipitate any additional sodium iodide. The solution was filtered again and the ether evaporated. The residue did not contain the desired material.

Diethyl iodomethylphosphonate, Cade Procedure;

Freshly distilled methylene diiodide, 116 g (0.43 moles), was heated to 180° in a 500 mL three-necked round-bottomed flask equipped with an addition funnel, thermometer and a 12 inch fractionating column with a condenser at top set for distillation. Triethyl phosphite, 47.9 g (0.29 moles), was then added. There was an immediate distilla-

tion of ethyl iodide as the temperature rose to 200° in the flask. Heating was continued for another 20 minutes and the mixture distilled to give 39.5 g of unreacted methylene diiodide and 47.9 g (61%) of the desired compound. bp[132] 85-87°/0.05 Torr. The residue (4.75 g) exhibited spectrally the presence of tetraethyl methylenebisphosphonate but it could not be isolated. NMR(Neat)δ 1.2-1.5(t,6H,OCH_2CH_3), 3.2-3.4(d,2H,CH_2), 3.9-4.4(q,4H,OCH_2); IR(CCl_4)cm^{-1} 3000,1450,1395, 1060,1040,975.

Interaction of triethylphosphite and diethyl iodomethylphosphonate

Diethyl iodomethylphosphonate, 33.5 g, was heated to 220° in a three-necked round-bottomed flask equipped with an addition funnel, thermometer and a 12"fractionating column equipped with a distillation head. Triethyl phosphite, 30.2 g, was added dropwise as the ethyl iodide distilled out (66-70°). After the addition, the mixture was heated for 1 hour and then distilled. It gave 8.0 g (23%) of tetraethyl methylenebisphosphonate. bp 102-104°/0.02 Torr. Litt bp[132] 123-128°/0.5 Torr NMR(neat)δ 1.2-1.5(t,12H,OCH_2CH_3), 2.3-2.9(t, J=21 Hz,2H,CH_2), 3.9-4.5(m,8H,OCH_2); IR cm^{-1} 3000,1255,1025,925,790.

Tetraisopropyl methylenebisphosphonate (LI)

Triisopropyl phosphite (212.3 g, 1 mole) and 86.9 g (0.33 moles) of dibromomethane were combined in a 500 mL three-necked round-bottomed flask fitted with a magnetic stirrer, a thermometer and a 24 inch fractionating column for separating the isopropyl bromide as the by-product from the reaction mixture. The fractionating column was

constructed by combining two Liebig condensers which had been packed with glass helices. The temperature of the water circulating through the jackets of the Liebig condensers was maintained at 65° during the entire reaction period by passing a mixture of steam and hot water. A Dean-Stark apparatus was connected to the top of the fractionating column and to the top of the Dean-Stark apparatus was fitted a Dewar condenser cooled with Dry Ice-isopropanol mixture and protected from the atmospheric moisture by a drying tube. The Dean-Stark apparatus was heated by an electric filament. The reaction flask containing the reactants was heated until the reaction commenced at 140° and then continued for an additional 7 hours, over which time the temperature of the mixture was gradually raised until a maximum temperature of 185° was achieved. The temperature was held between 180-190° for the remaining time period (2-3 hours). The temperature must not be allowed to exceed 195°. After removal of excess of triisopropyl phosphite, the residue was distilled under vacuum to yield 89.1 g (78.5%) of the desired compound. bp 95-108°/.005-.01 Torr.

Methylenediphosphonic acid (LII)

Tetraisopropyl methylenediphosphonate 20 g (0.058 moles) was dissolved in 100 mL of concentrated HCl and the mixture refluxed for three hours. The solution was then concentrated to dryness on a flash evaporator at 40-45°. The last traces of water and hydrochloric acid was removed by adding three portions of isopropyl alcohol and reducing the volume to dryness after each addition. The white crystalline solid was filtered and washed with acetone and dried in vacuum over P_2O_5. mp 199-201°, Litt. mp[133] 203-206°. Yield:9.0 g (88%).

Diethyl 1-Methyl tetrahydrofuranyl-1-phosphonate (By Arbuzov reaction) (LIII)

A mixture of 7.2 g (0.06 moles) of 5-chloro-2-pentanone and 9.95 g (0.06 moles) of freshly distilled triethyl phosphite were refluxed for 24 hours in a 100 mL round-bottomed flask. After removing any unreacted triethyl phosphite, the residue was vacuum distilled using a small Vigreaux column to yield 8.9 g (67%) of the desired compound (LIII). bp 80-84°/.035 Torr. NMR($CHCl_3$) δ 1.1(distorted triplet, 9H,$CH_3OCH_2CH_3$), 1.3-2.3 (m, 4H,$-CH_2CH_2-$), 3.4-4.2 (m, 6H, OCH_2, OCH_2CH_3), IR(CCl_4)cm^{-1} 3000,2100,1700(w),1450,1360, 1250-1160,1025,950.

Analysis	Calculated for $C_9H_{19}O_4P$	C,48.64;	H,8.62
	Found	C,48.75;	H,8.81

Diethyl 1-Methyl tetrahydrofuranyl-1-phosphonate (LIII)

A solution of sodium ethoxide was prepared by dissolving 1.37 g (0.06 moles) of sodium in 25 mL of absolute ethanol. Sodium ethoxide prepared above was added dropwise over a period of 20-25 minutes to a mixture of 7.23 g (0.06 moles) of 5-chloro-2-pentanone and 8.28 g (0.06 moles) of diethyl phosphonate in 5 mL of absolute ethanol at room temperature. The reaction was very exothermic. After refluxing for 1 hour the solution was cooled to room temperature and then filtered. Ethanol was removed on a rotary evaporator. To the residue 25 mL of ether was added and the precipitated solid removed by filtration The ether was evaporated at reduced pressure and the residue distilled at reduced pressure using a Short-path vigreaux column to yield 9.28 g

(70%) of the compound (LIII) which gave the right spectral analysis and corresponded to the earlier prepared compound both in spectral and VPC measurements. NMR (neat). δ 1.1(distorted triplet,9H,CH_3, OCH_2CH_3), 1.3-2.4(m,4H,$-CH_2CH_2-$), 3.2-4.2(m,6H,OCH_2,OCH_2CH_3) IR(CCl_4), 3000,2100,1700(w),1450,1360-1240,1160,1020,950.

Diethyl 1-phenyl tetrahydrofuranyl-1-phosphonate (LIV) Arbuzov reaction.

A mixture of 25.0 g (0.14 moles) of γ-chlorobutyrophenone and 23.3 g (0.14 moles) of freshly distilled triethylphosphite were refluxed overnight. After removing the unreacted triethyl phosphite, the residue was distilled at reduced pressure to yield 23.5 g (60%) of the desired compound (LIV). bp 130-142°/.15-.20 Torr. NMR(CCl_4) δ1.1(two overlapping triplets, 6H, OCH_2CH_3), 1.4-2.9(m,4H,$-CH_2CH_2-$), 3.3-4.2(m,6H,OCH_2,OCH_2CH_3), 7.2(m,5H,O_6H_5) IR(CCl_4 cm^{-1} 3000,1700(w), 1600,1450,1390,1250,1150,1050,950,700.

Analysis	Calculated for $C_{14}H_{21}O_4P$	C,59.15;	H,7.39
	Found	C,59.15;	H,7.58

Diethyl 1-Ethyltetrahydropyranyl-1-phosphonate (LV) Arbuzov reaction

A mixture of 10.0 g (0.052 moles) of 1-Bromo-5-heptanone[155] and 10.79 g (0.065 moles) of triethylphosphite were refluxed at 180-185° for 16 hours in a 100 mL round-bottomed flask. The unreacted triethylphosphite was removed and the residue vacuum distilled to yield 7.7 g, 61.6% of the desired compound (LV) bp 109-112°/0.06 Torr. NMR (neat) δ.9-1.6(three overlapping triplets, 9H, OCH_2CH_3, CH_3), 1.6-2.8(m, 8H, $(CH_2)_3$, CH_2) 3.4-3.85(t,2H,OCH_2), 3.9-4.5(m,4H,OCH_2CH_3)

Analysis	Calculated for $C_{11}H_{23}O_4P$	C,52.80;	H,9.20
	Found	C,51.90;	H,9.46

Diethyl 3-phenyl-3-oxopropyl-1-phosphonate (LVI)

A mixture of 5.0 g (0.029 moles) of β-chloropropiophenone and 4.98 g (0.03 moles) of freshly distilled triethylphosphite were refluxed overnight. Excess triethylphosphite was removed and the residue distilled under reduced pressure to give 4.05 g (51.7%) of the desired compound (LVI). NMR(CCl_4) δ 1.0(t,6H,OCH_2CH_3), 1.4-2.15(m, 2H,CH_2P), 2.6-3.25(m,2H,CCH_2), 3.4-4.1(m,4H,OCH_2CH_3), 7.2-7.6(two multiplets, 5H,C_6H_5); IR(CCl_4)cm^{-1} 3000,1700(s),1600,1450,1250, 1060,1030,960,850,750.

Analysis	Calculated for $C_{13}H_{19}O_4P$	C,57.70,	H,7.03
	Found	C,56.74	H,7.08

Diethyl 3-phenyl-3-oxopropyl-1-phosphonate (LVI)

A solution of sodium ethoxide was prepared by dissolving 0.42 g (0.018 moles) of sodium in 7.5 mL of absolute ethanol. This solution of sodium ethoxide was added dropwise over a period of 15-20 minutes to a mixture of 3.0 g (0.018 moles) of β-chloropropiophenone and 2.48 g (0.018 moles) of diethyl phosphonate in 2 mL of absolute ethanol at room temperature. The reaction is exothermic. After refluxing for one hour, the reaction mixture was cooled to room temperature and then filtered. Ethanol was removed with a rotary evaporator and to the residue 20-25 mL of ether added. Any precipitated solid was filtered and then the ether was removed by evaporation at reduced pressure. The spectral data of the residue corresponded to the same compound prepared by Arbuzov reaction as described before. NMR(neat) δ 9(t,6H,OCH_2CH_3), 1.3-2.5(m,2H,PCH_2), 2.5-3.3(m,2H,CCH_2), 3.4-4.0(m,4H,OCH_2), 7.0-7.5(two m, 5H,C_6H_5). IR(neat)cm^{-1} 3000,1690,1450,1250,1150,1020,950,800,750,690.

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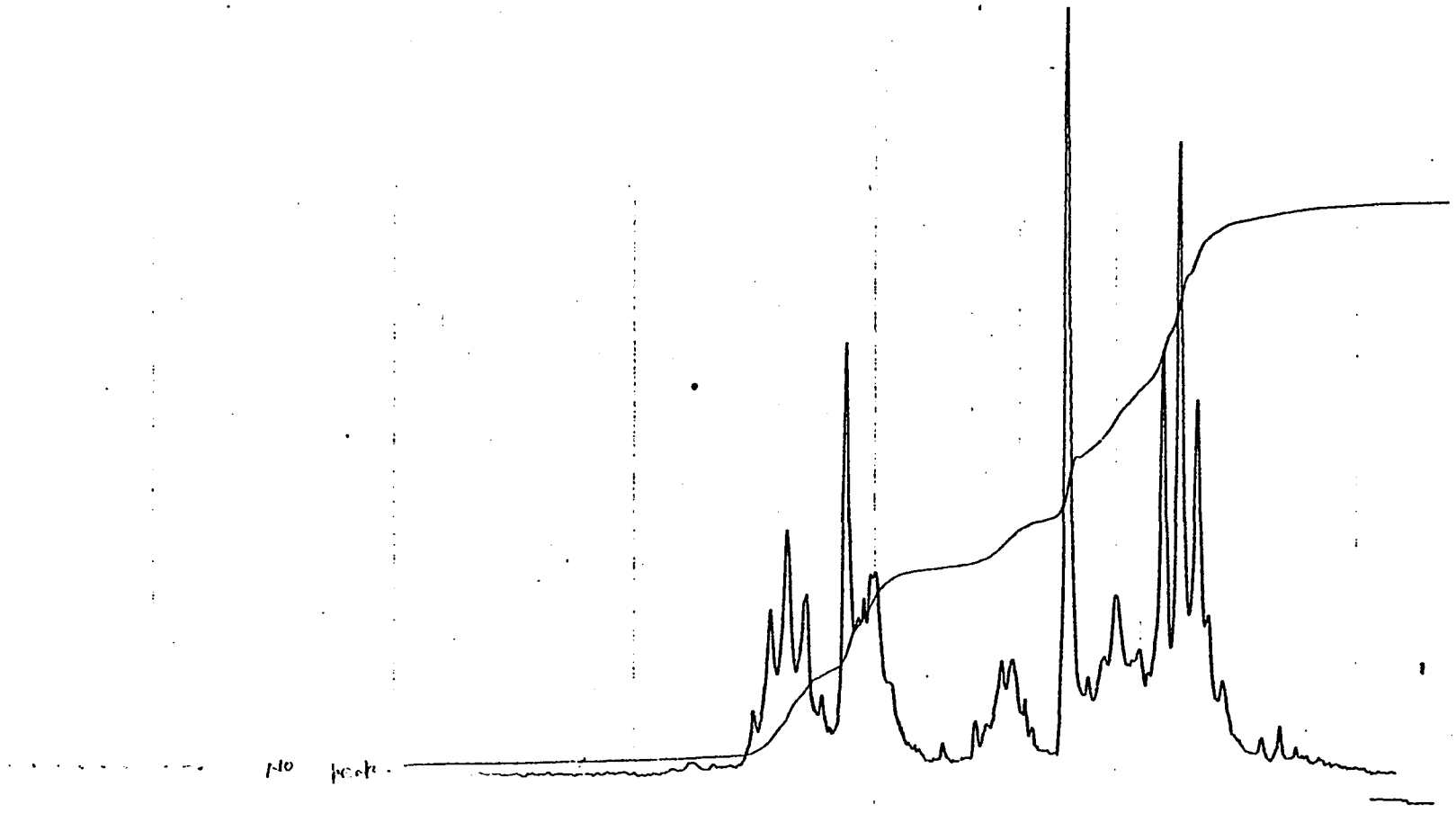
Suggestions for future research:

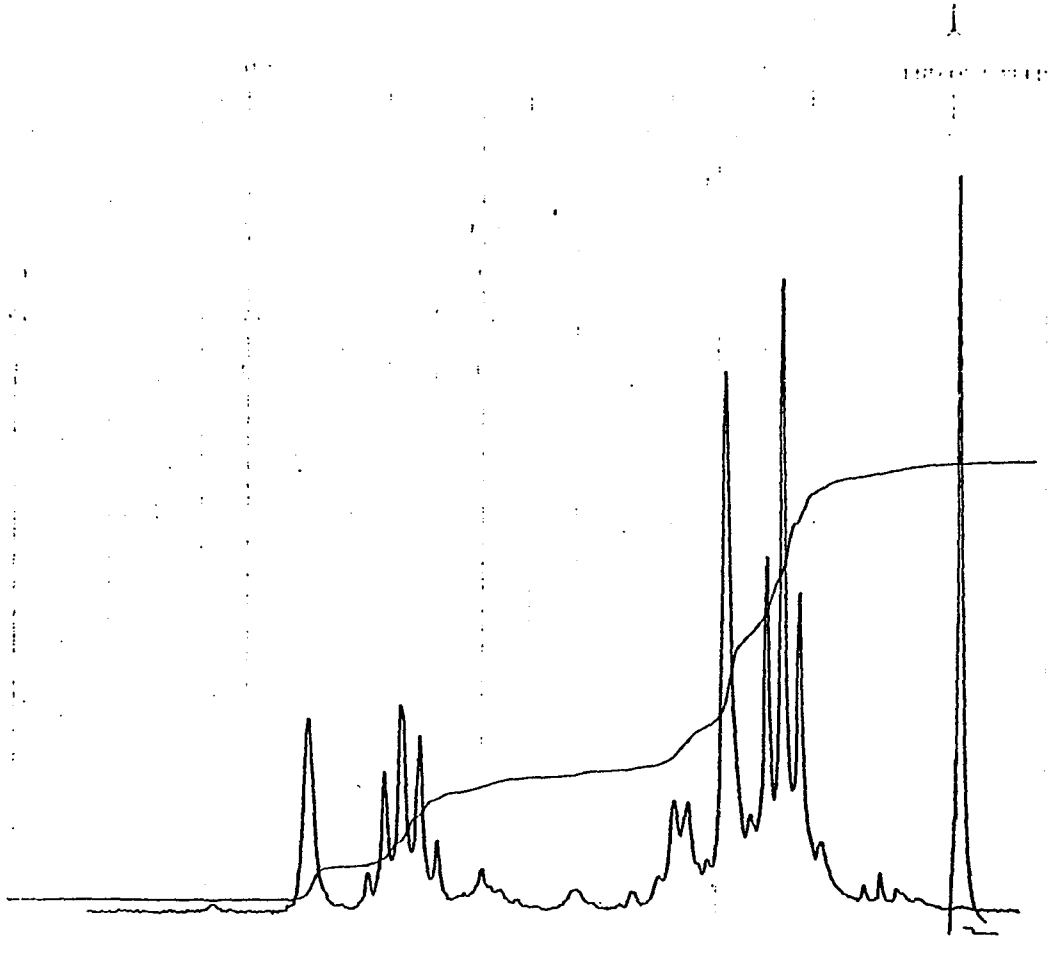
In general, the uptake of substances into a cell involves diffusion of the molecules from the bulk phase of the blood to the aqueous-membrane interface followed by translocation into the cytosolic compartments either by diffusion through the cell membrane or by interaction with a finite number of recognition sites on the cell surface prior to uptake. Dietschy et al [155] measured the increase in the mass of cholesterol esters in the liver to estimate hepatic net uptake rates of cholesterol from various serum and intestinal lipoproteins fractions. They report that liver is capable of taking up cholesterol from chylomicron remnants and at significantly lower rates, low density serum lipoproteins. One of the lipoprotein feedback mechanism operating to regulate sterol synthesis in the liver involves the uptake of chylomicron remnants and allows the liver to "sense" the amount of cholesterol that is entering the body through the intestine. This transport process apparently is present only in the liver. Therefore it seems reasonable to couple the mevalonic acid analogue to a bile acid in order to get the analogue transported inside a cell. Bile acid of choice would be tetrahydroxy-cholane.

APPENDIX

ir, ^{13}C and nmr spectra



nmr of (XVIII) in CCl_4



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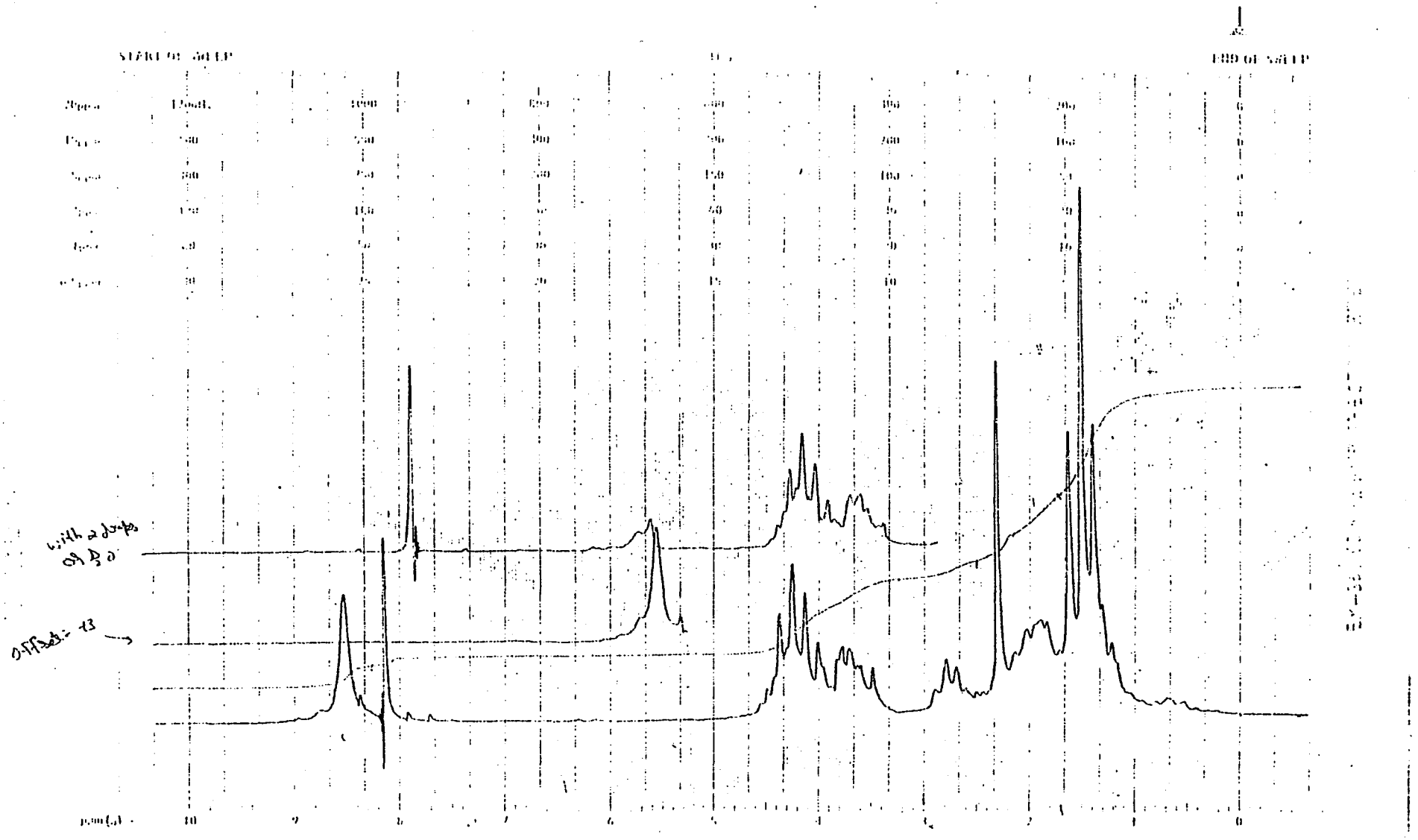

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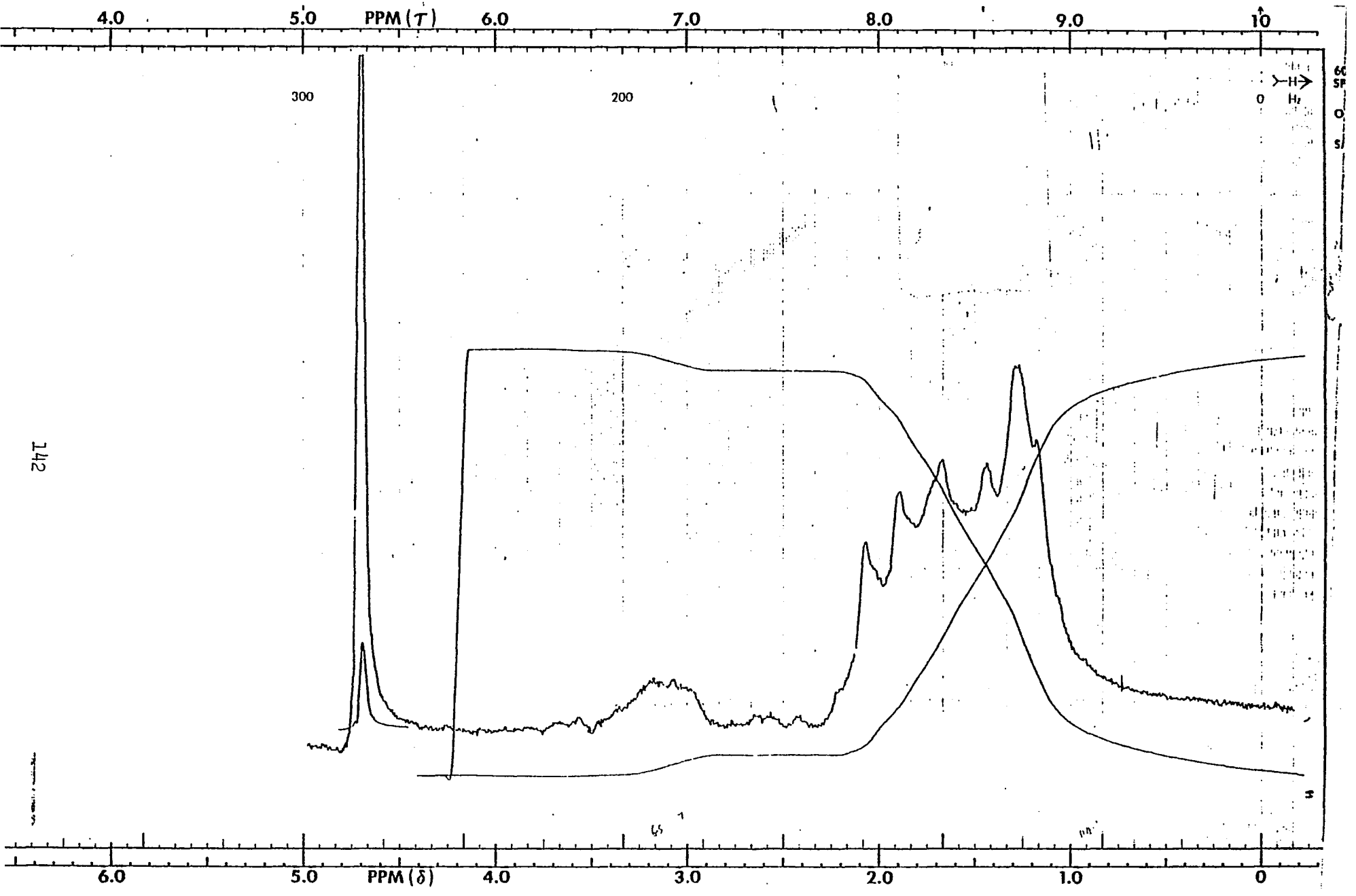
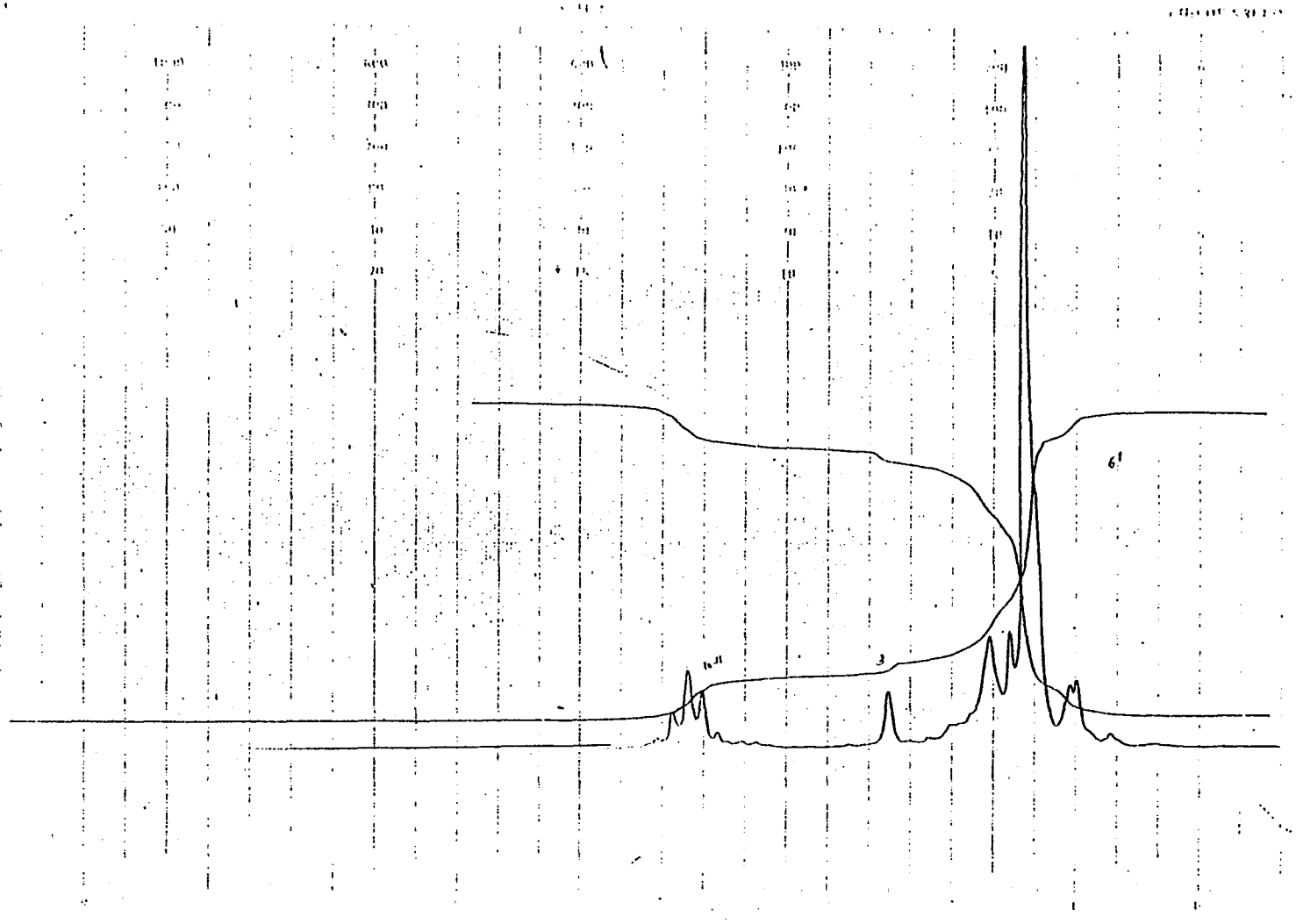


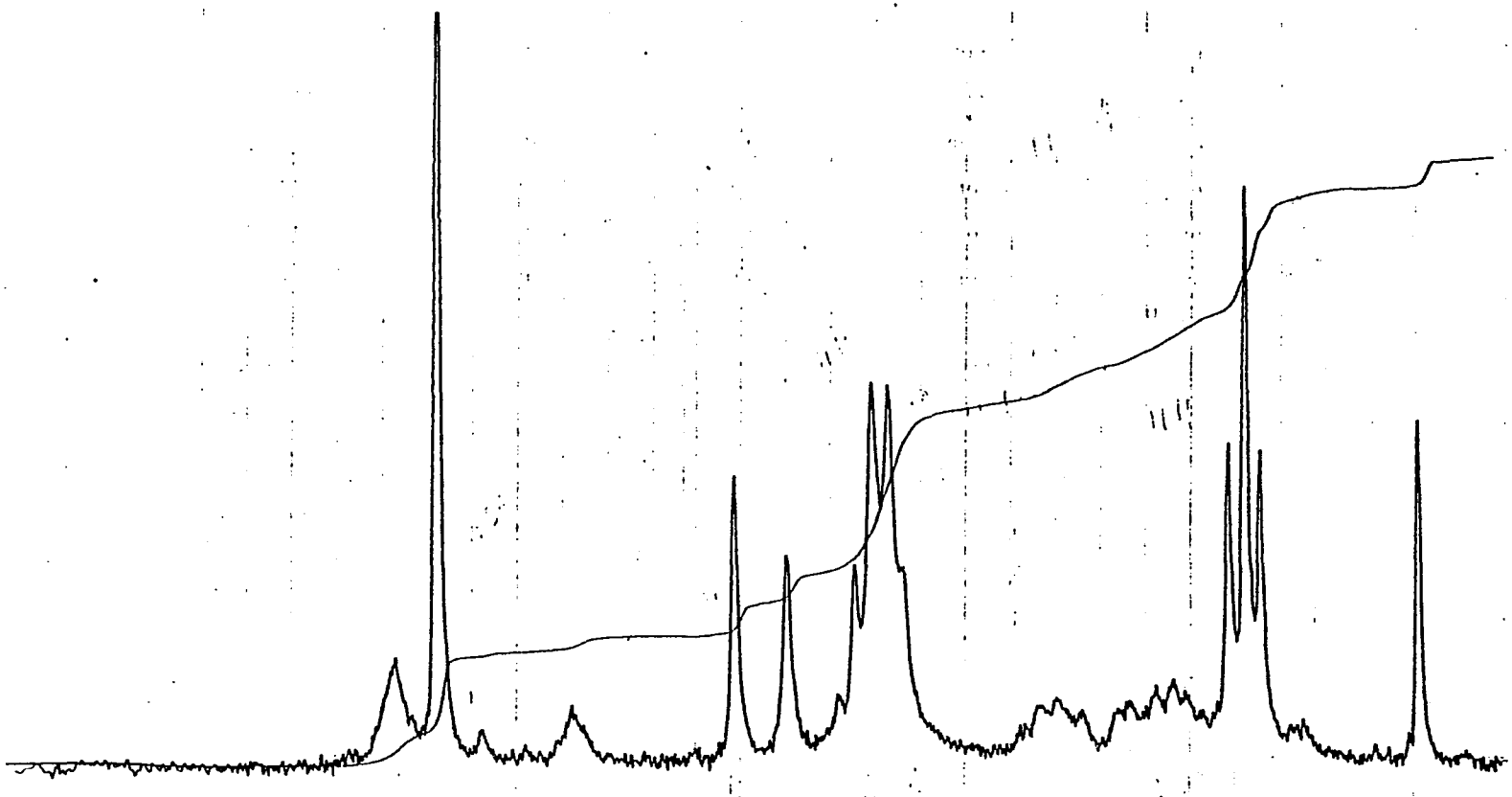
142

nmr of (XXXI) in D_2O

nmr of (XLVIII) in $CDCl_3$



nmr of (LVII) in CCl_4



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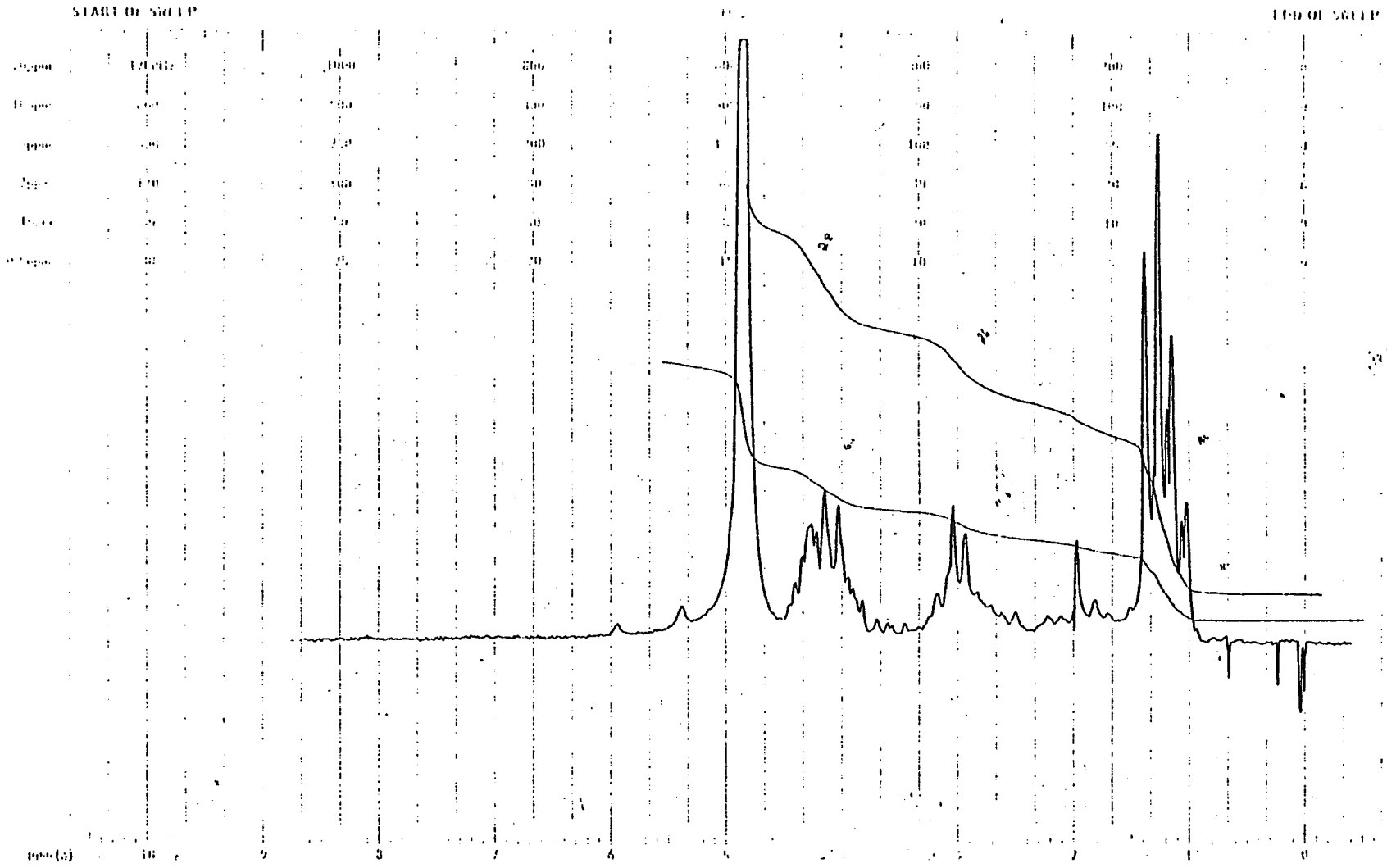

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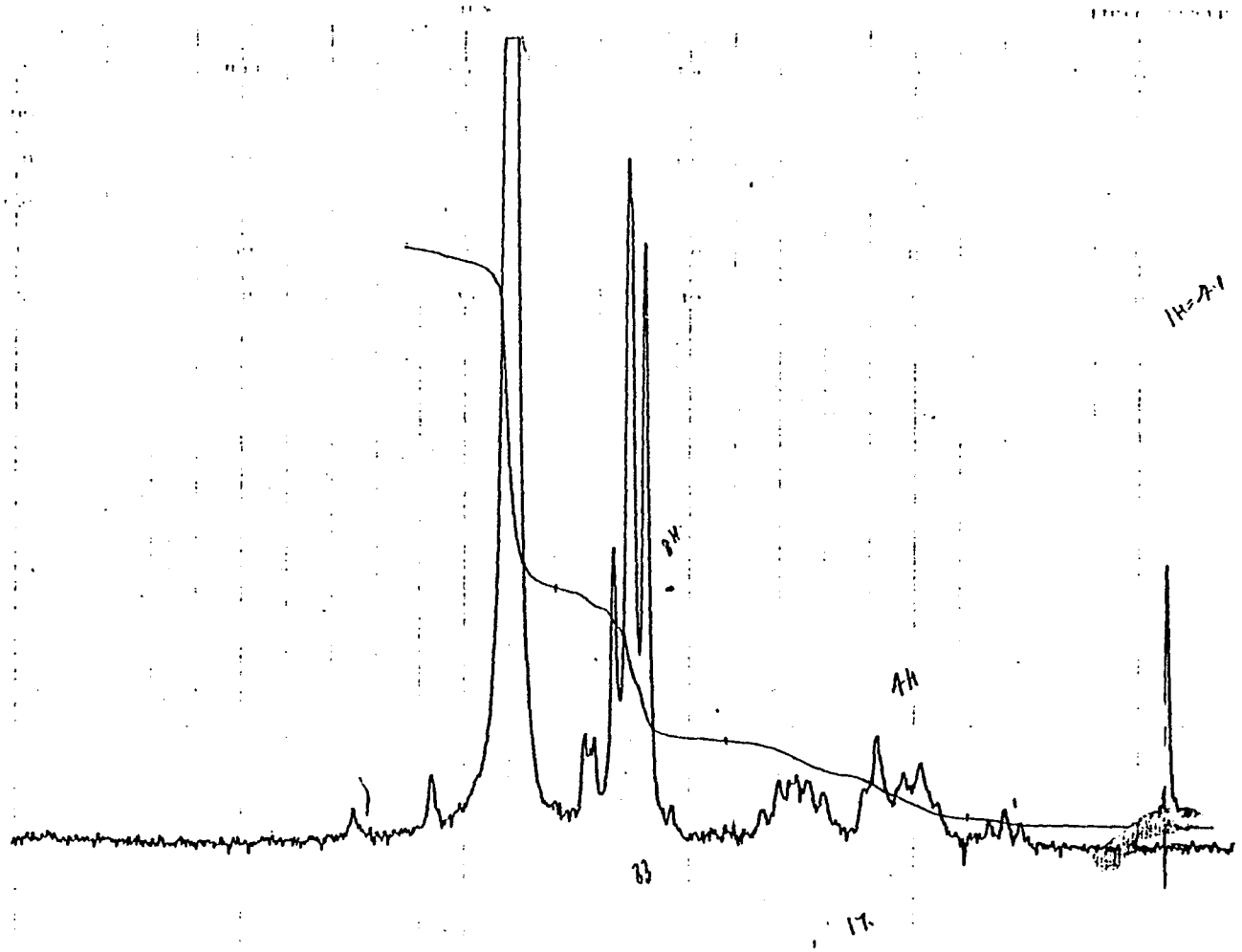

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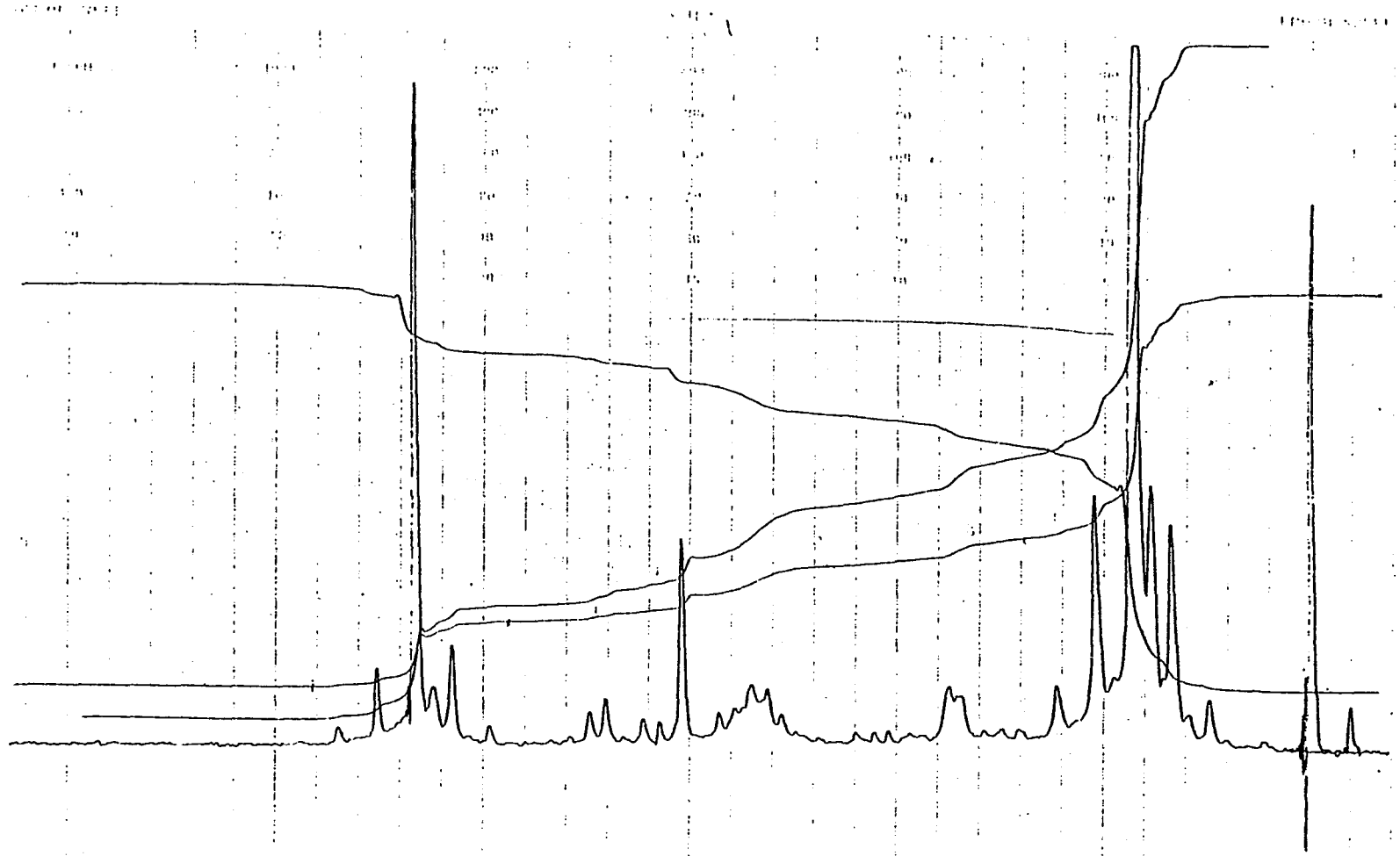

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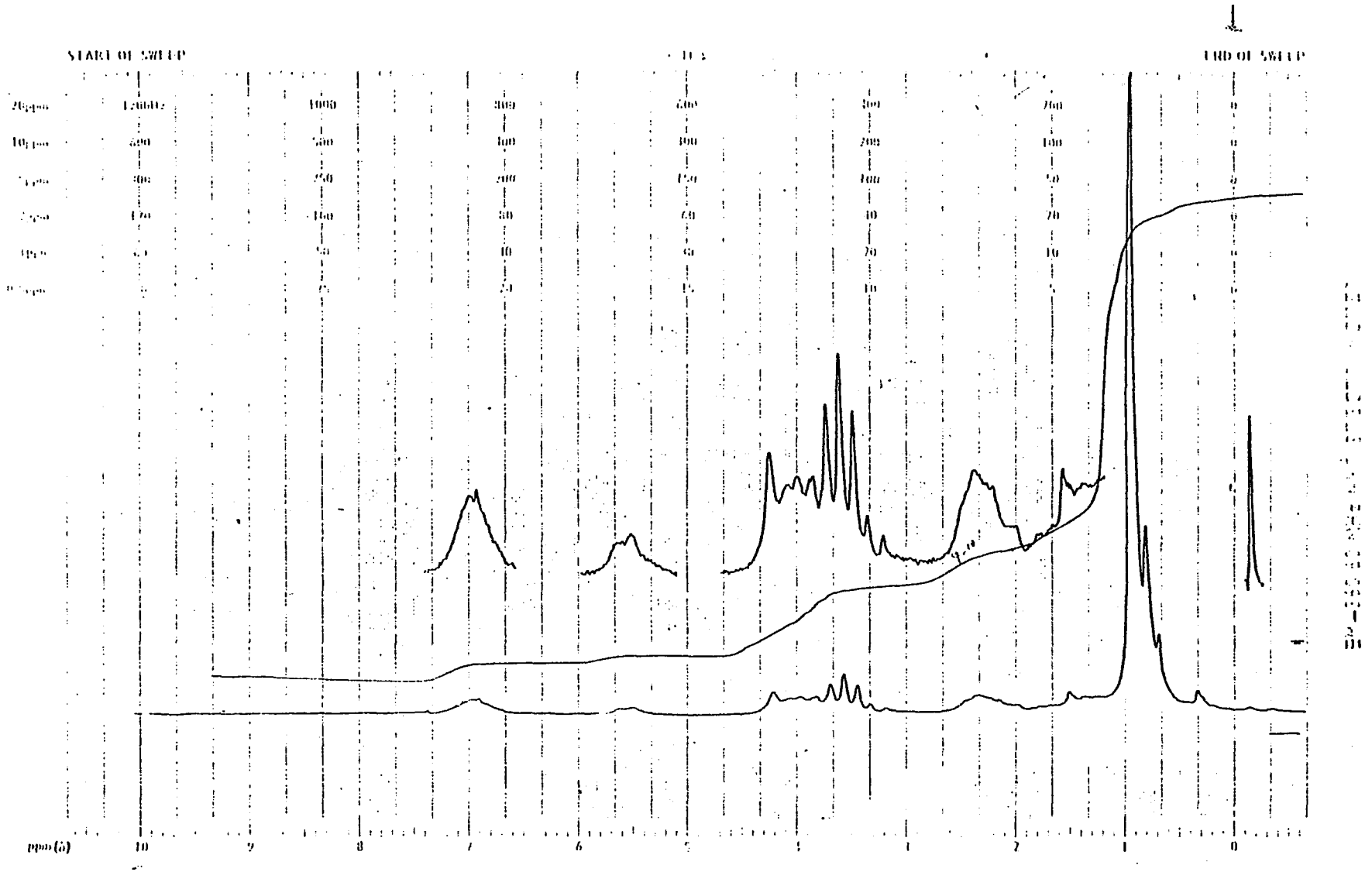

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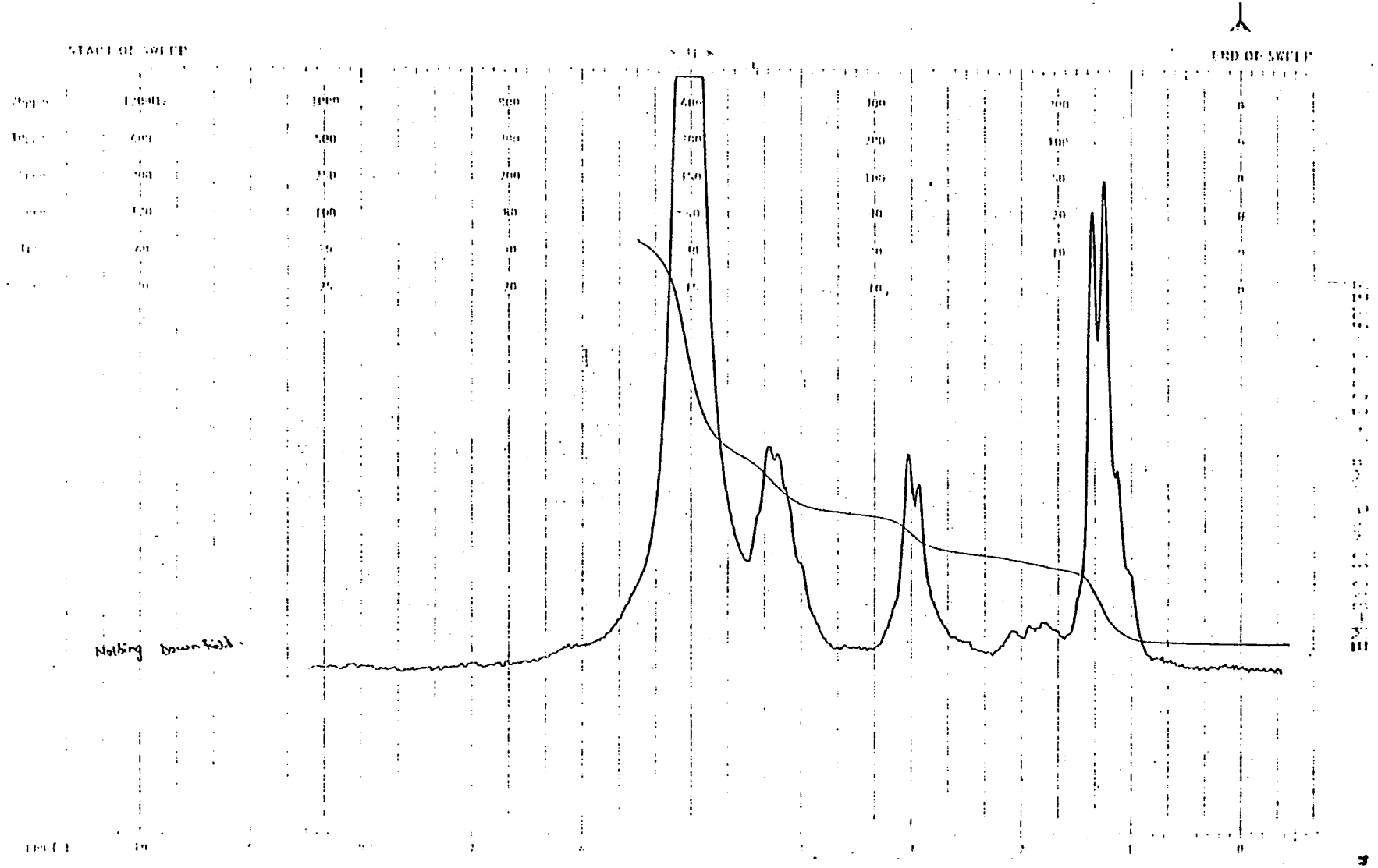
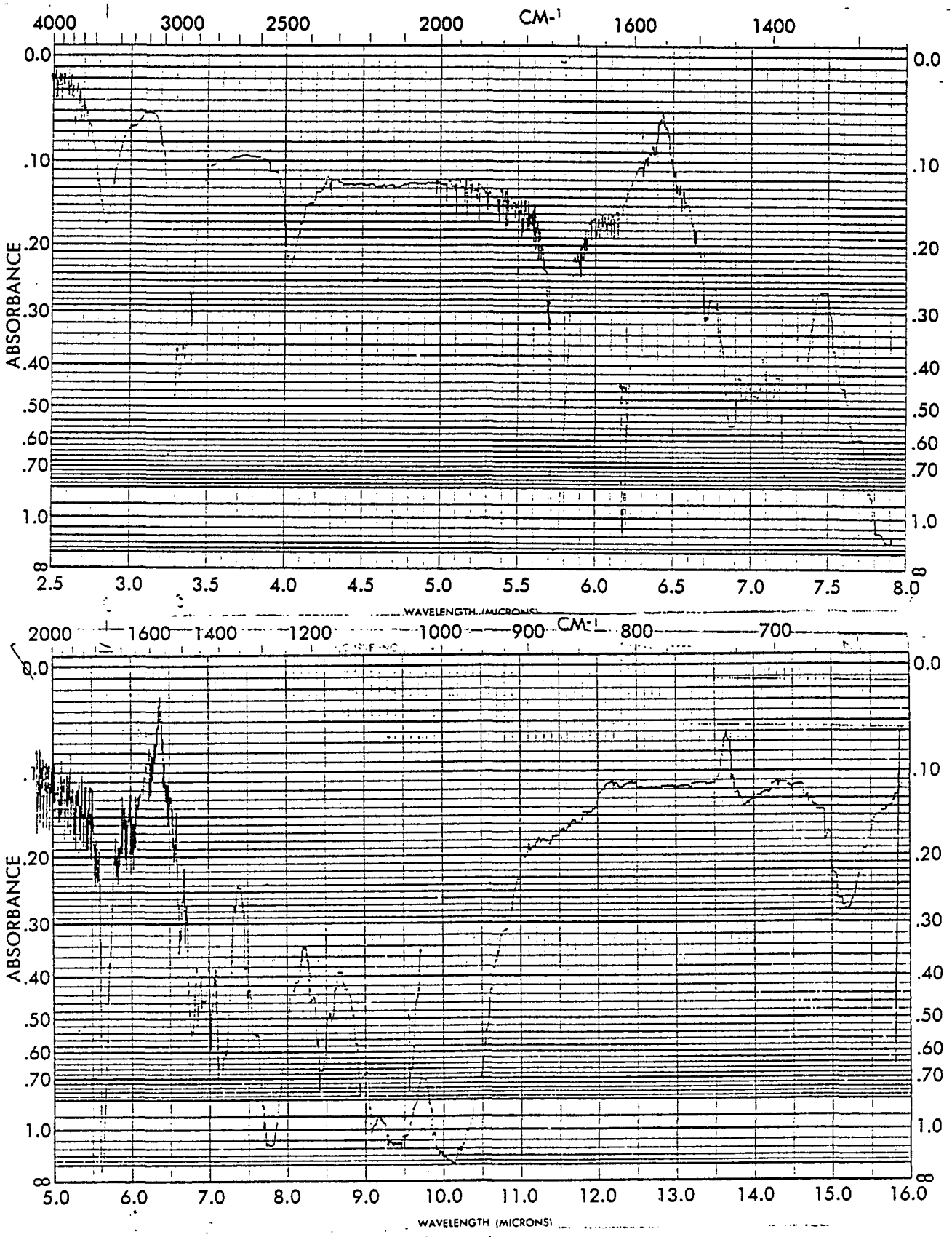
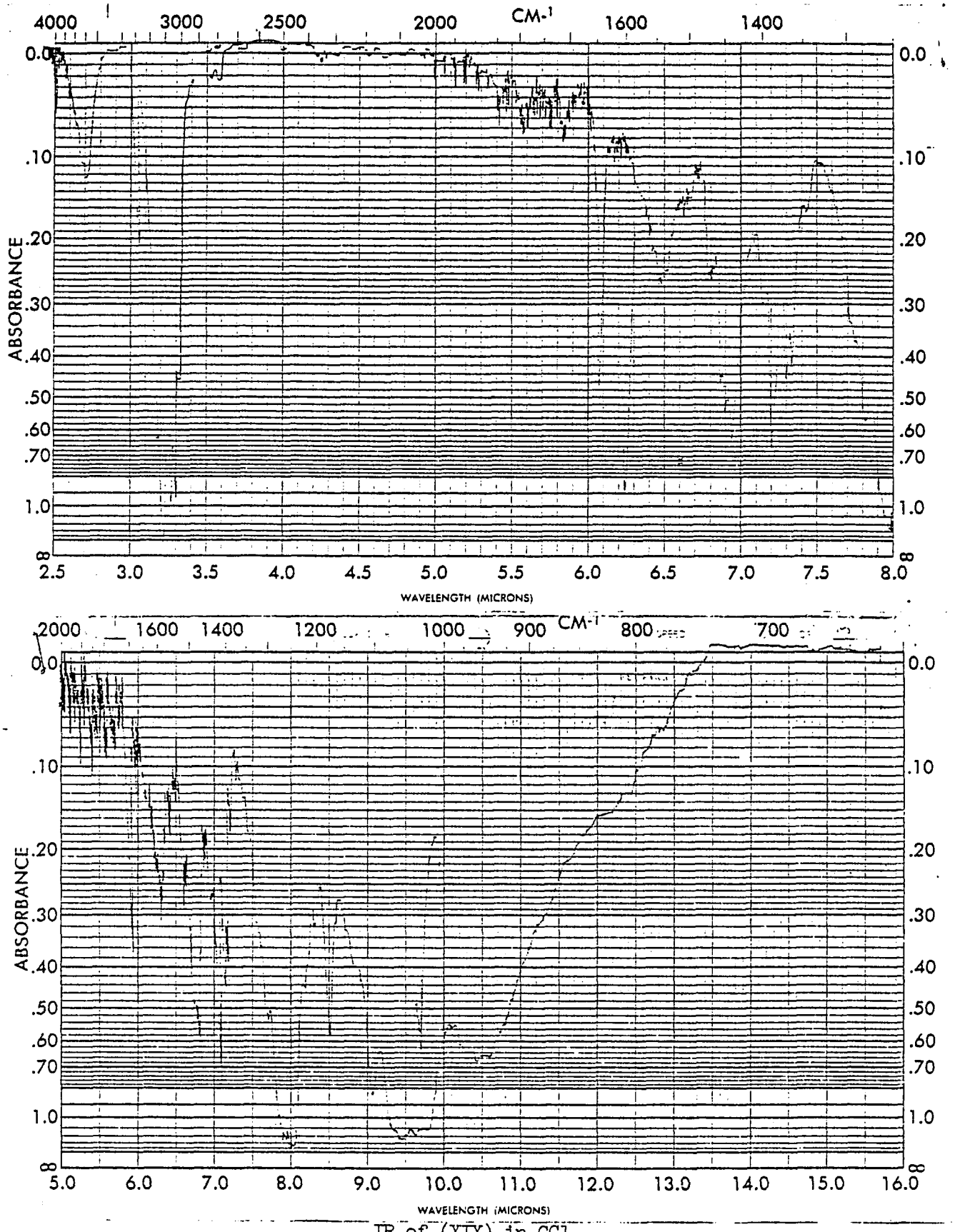


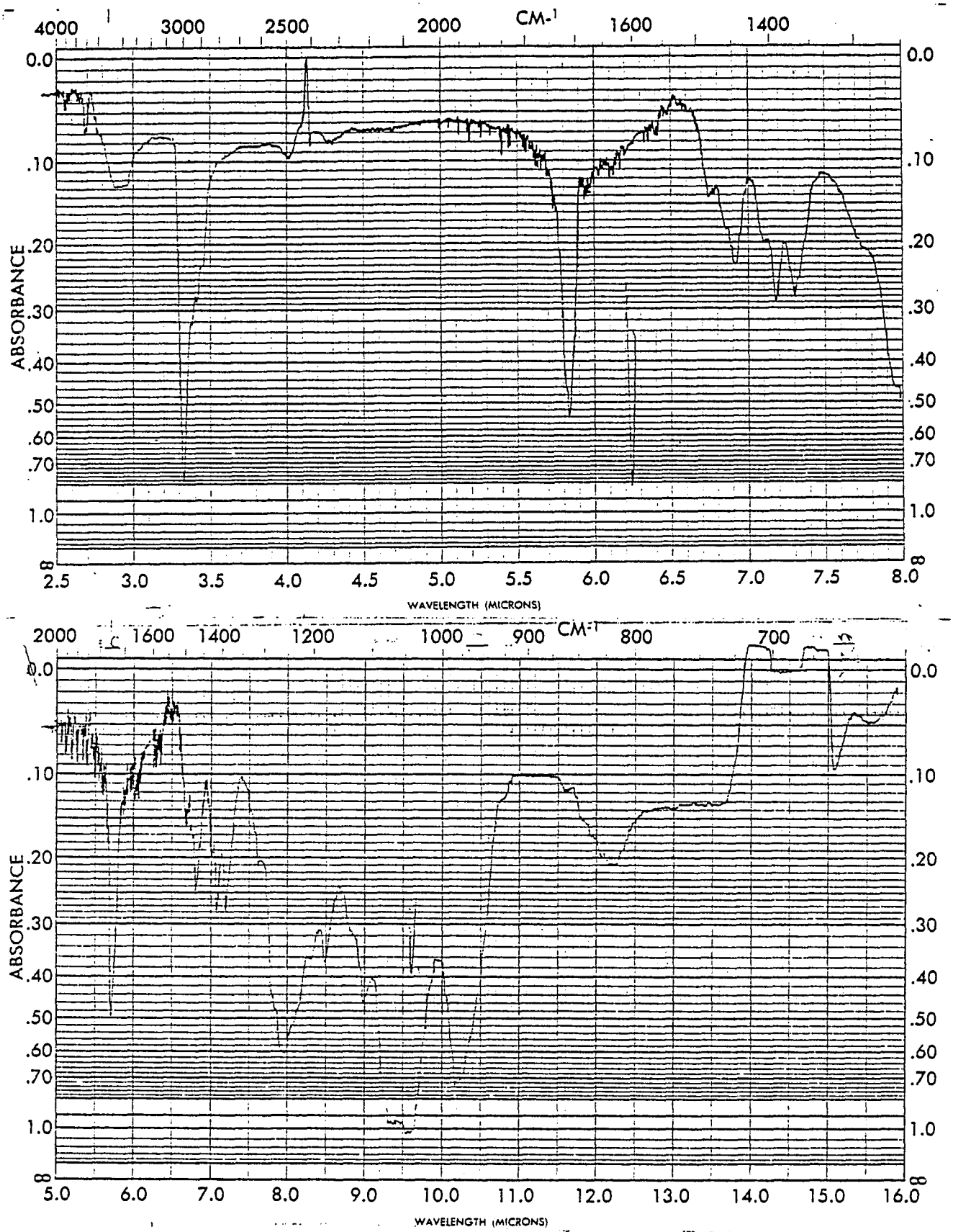
nmr of (LXXXVIII) in D_2O



IR of (XVIII) in CCl_4



IR of (XIX) in CCl_4



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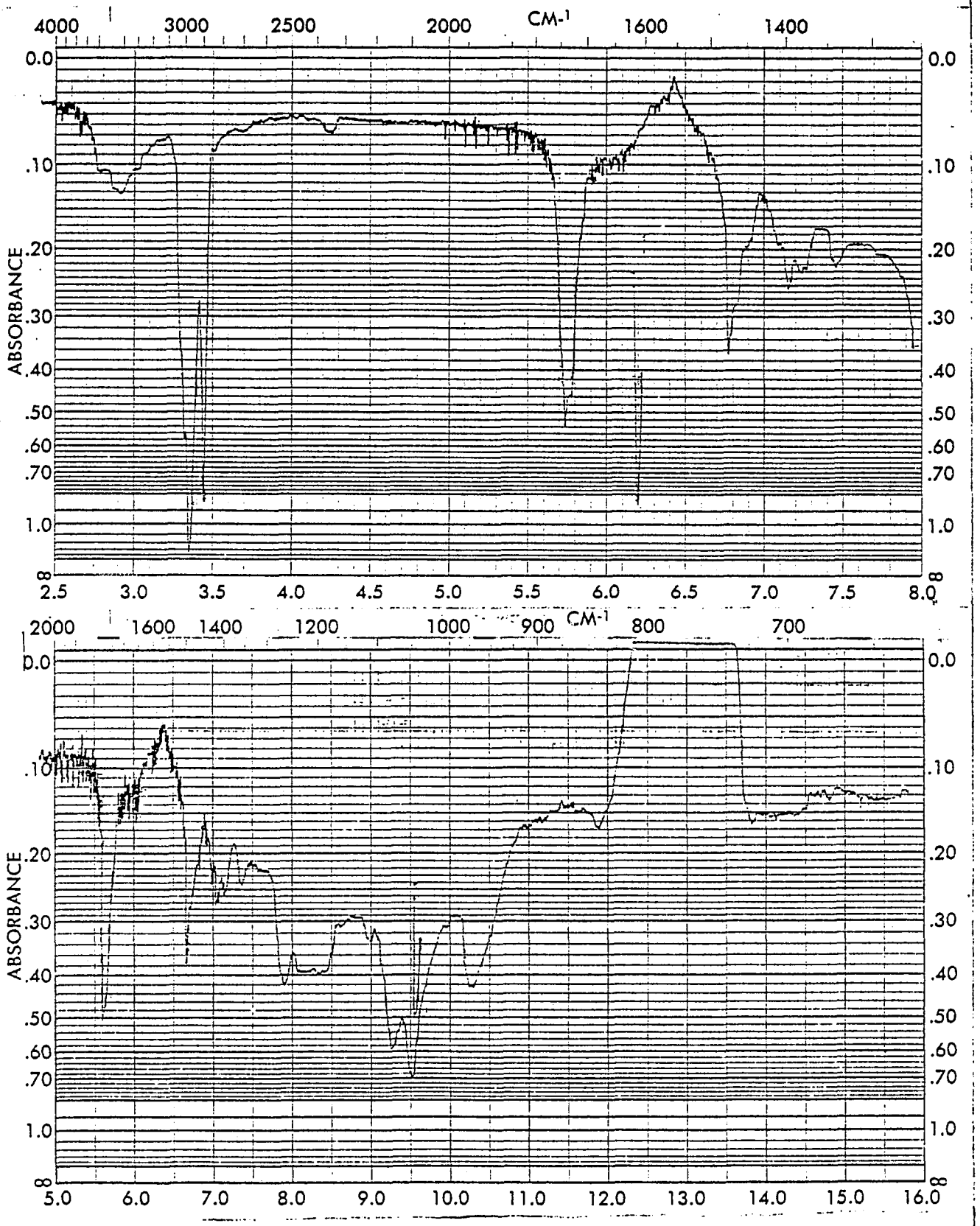
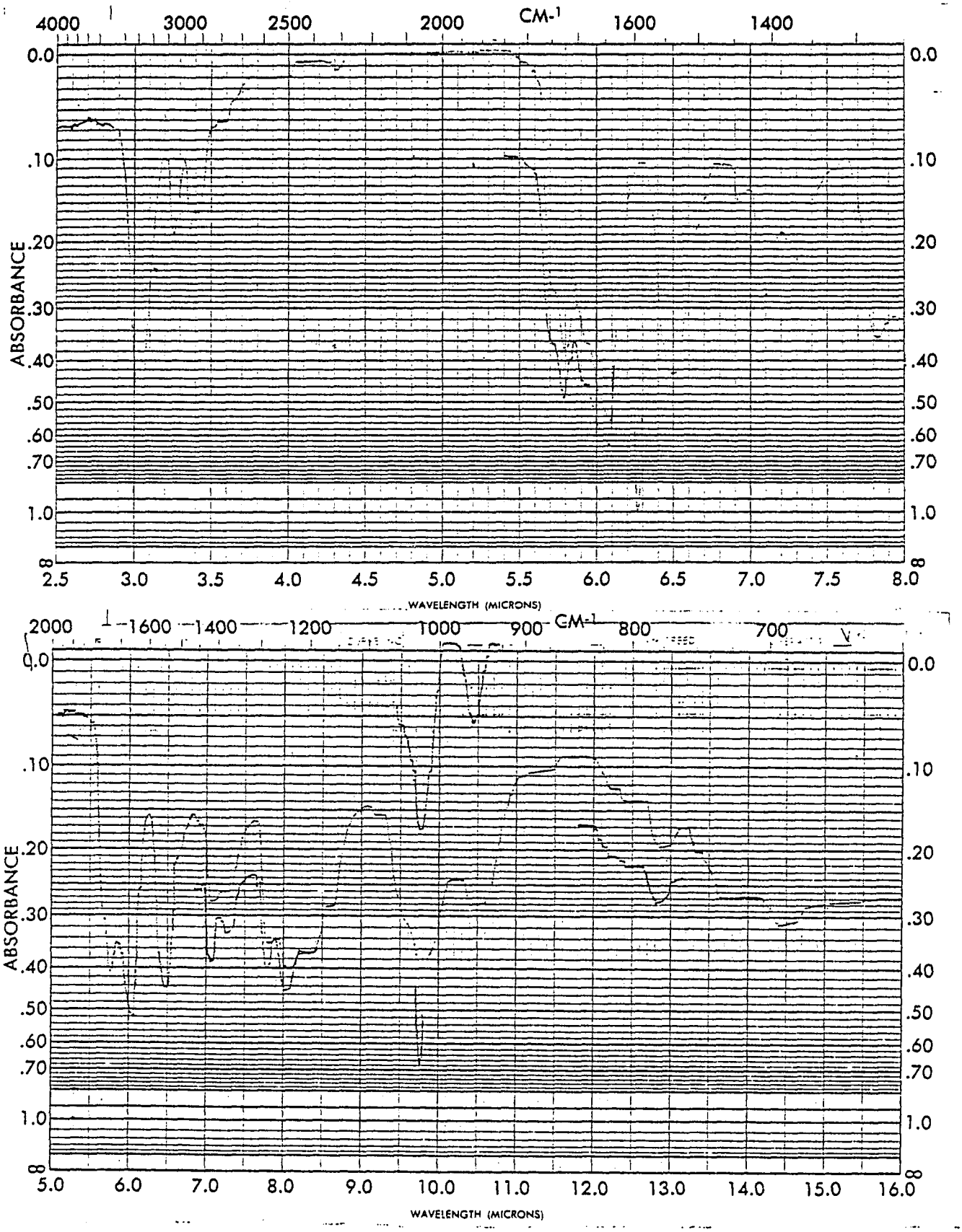
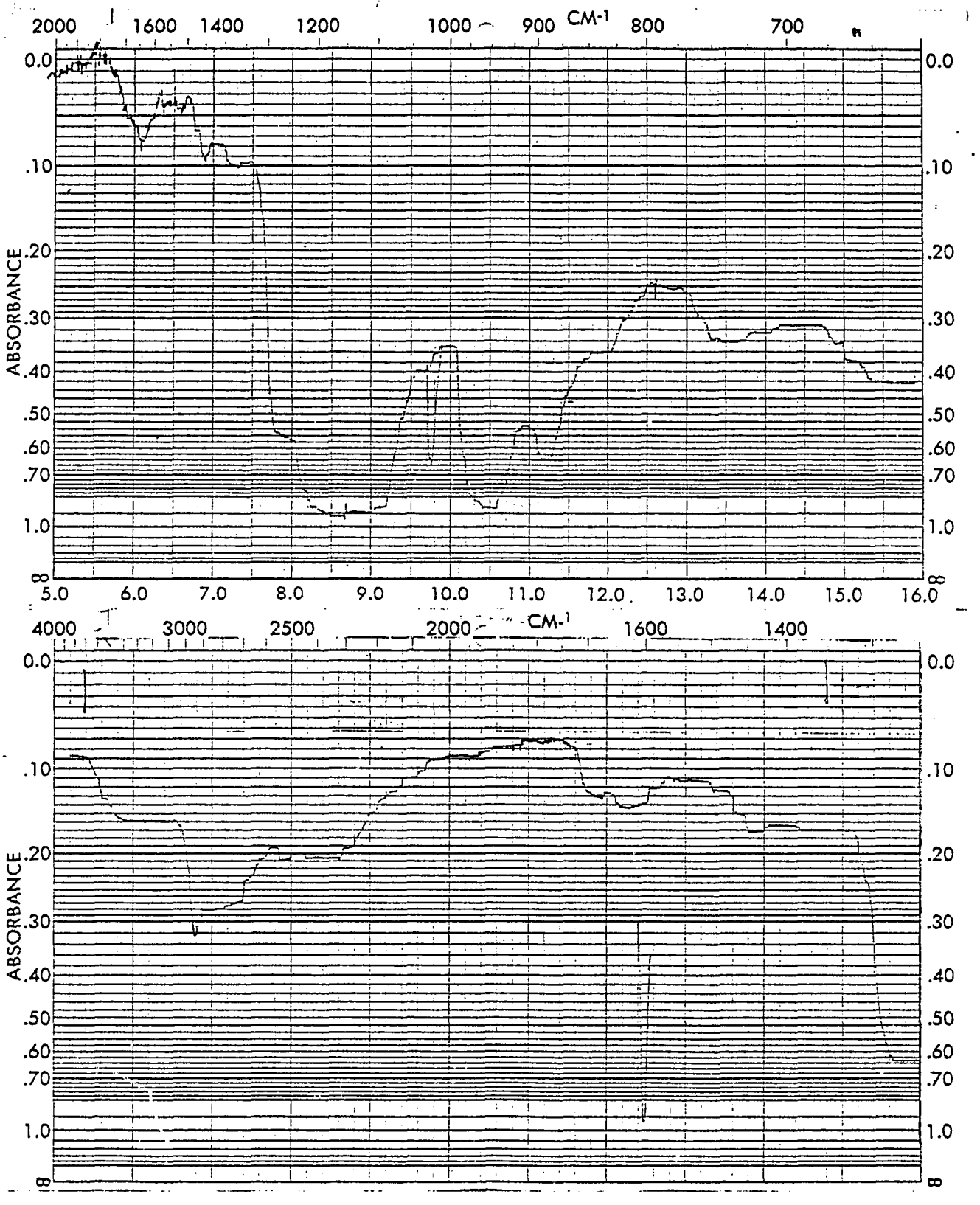


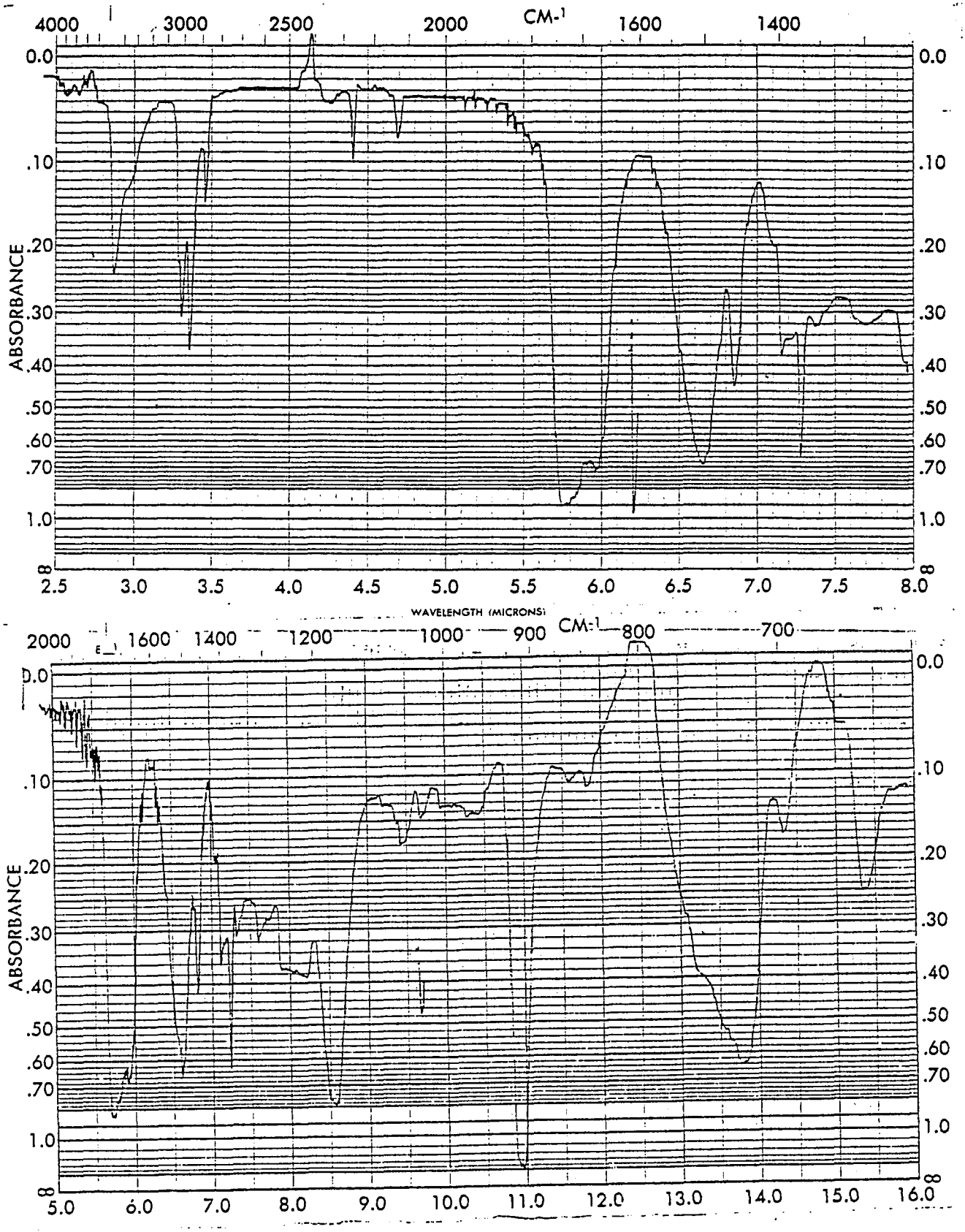
IR of (LVII) in CCl_4



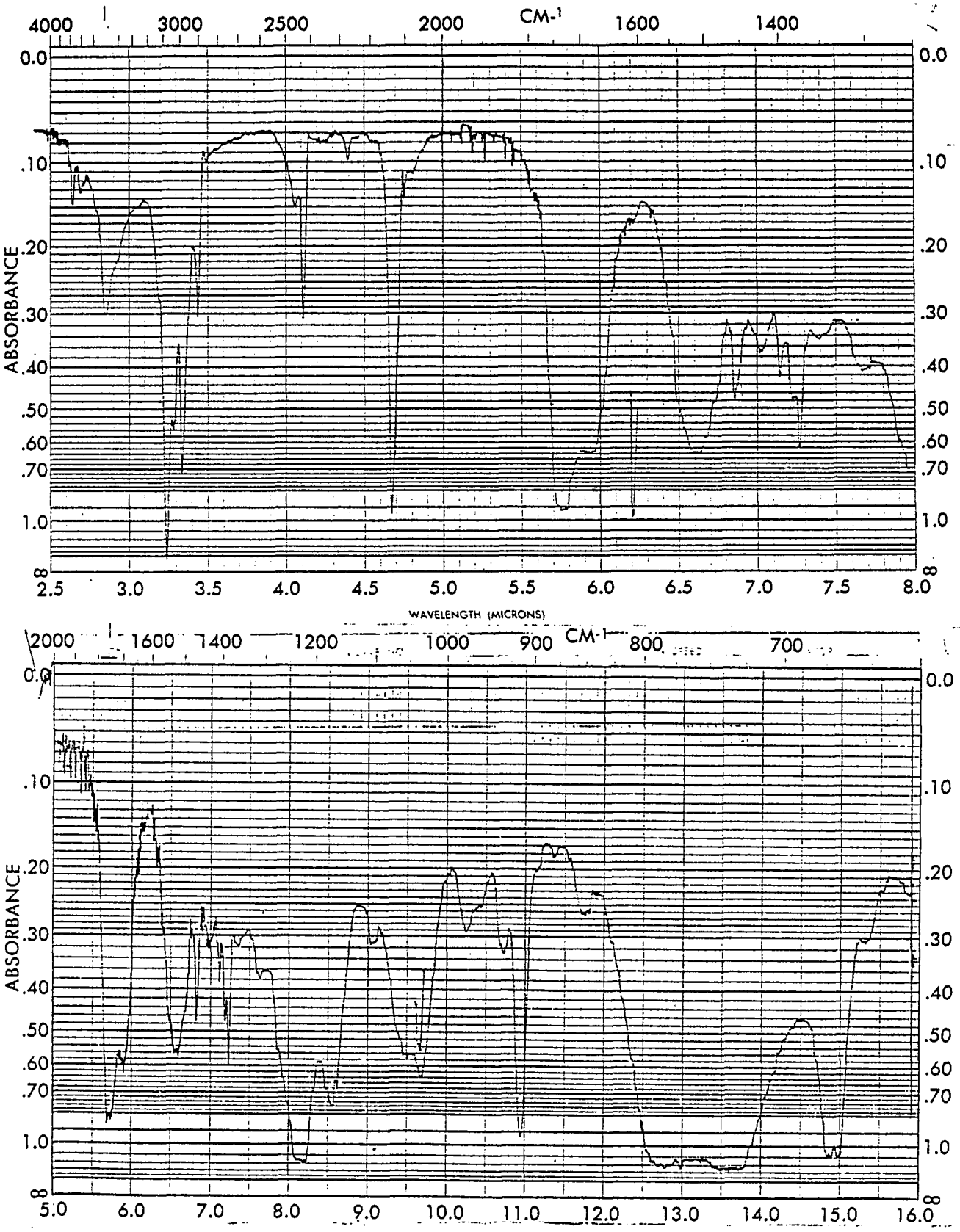
IR of (LXXI) in $CDCl_3$



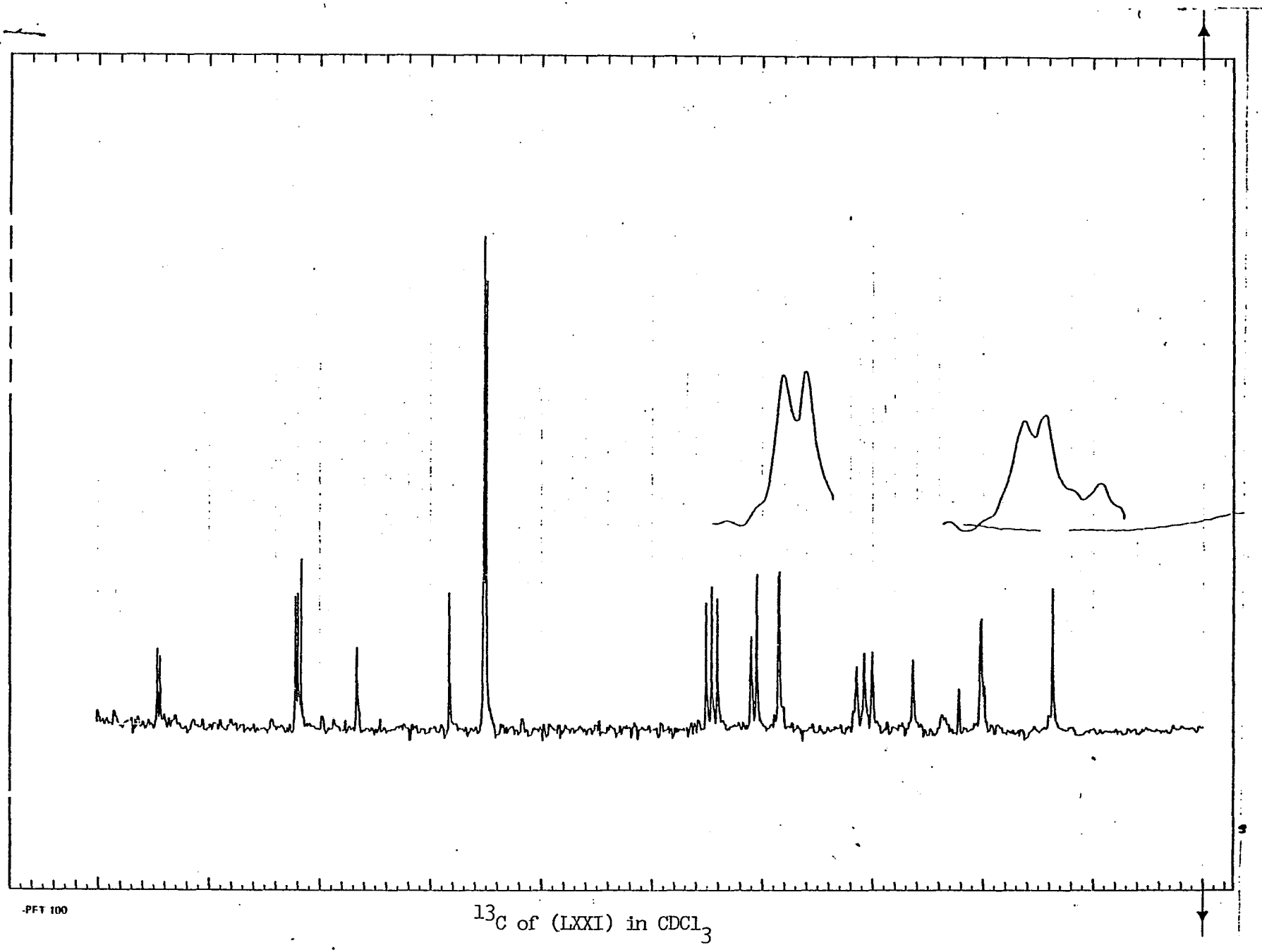
IR of (XXXI) in Fluorolube.



IR of (LXXXIV) in $CDCl_3$



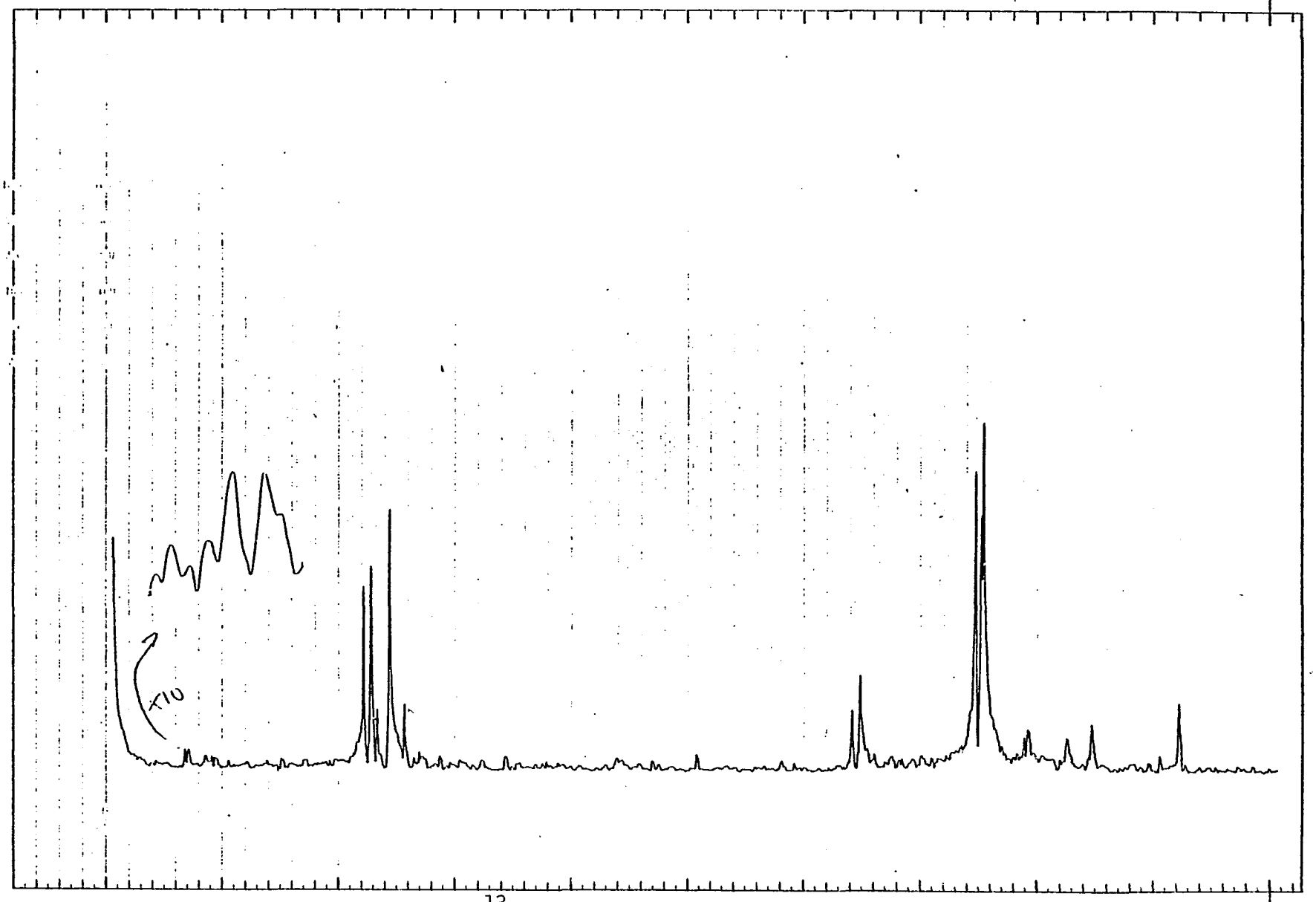
IR of (LXXXVI) in $CDCl_3$



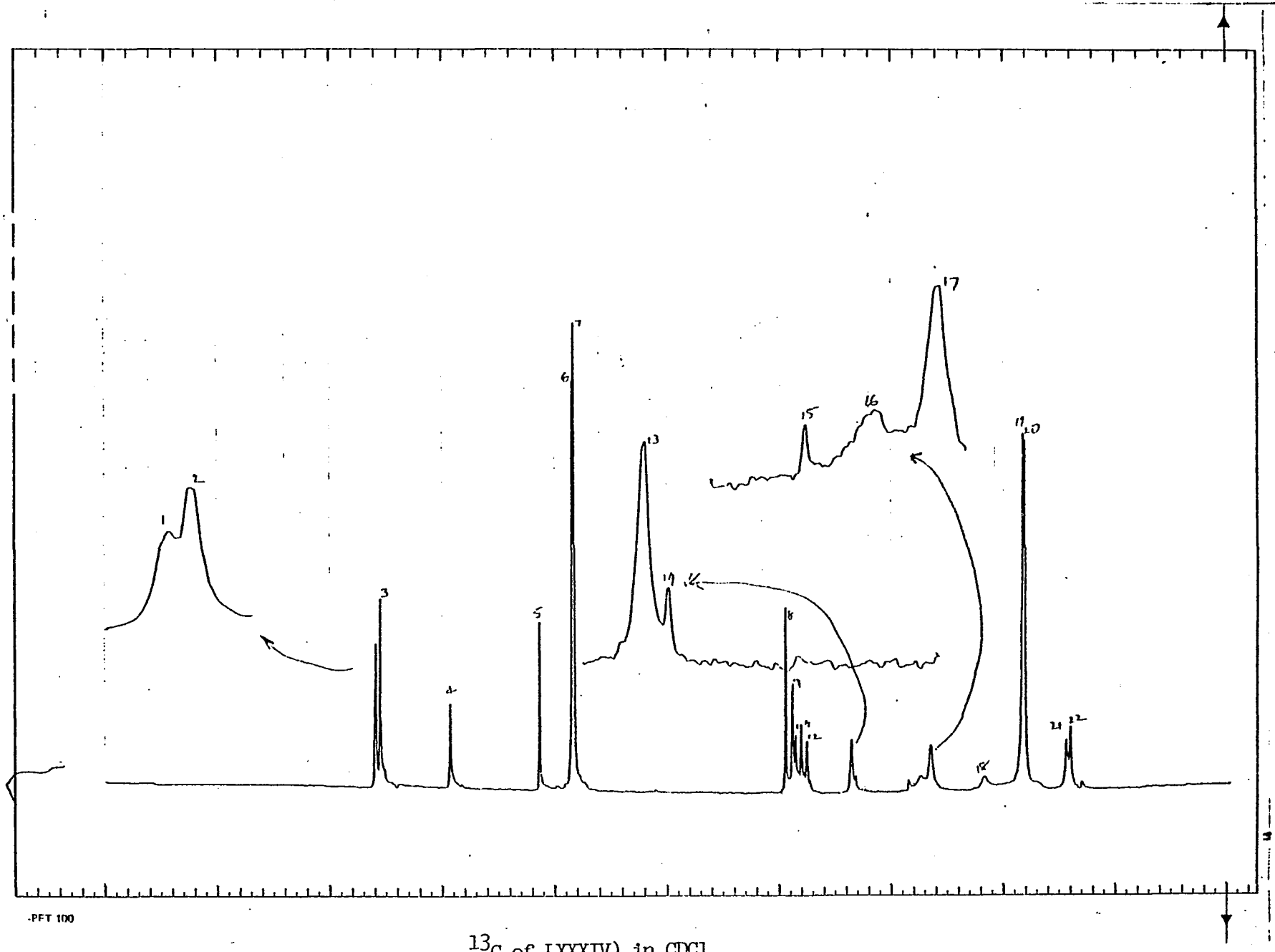
^{13}C of (LXXI) in $CDCl_3$

Chemical Shifts of (LXXI):

201.906, 201.349, 170.566, 170.020, 169.288, 156.974, 136.310, 128.444, 127.956, 68.216, 66.936, 62.120, 61.876, 44.502, 42.734, 40.905, 31.699, 21.458, 16.454, 16.215.



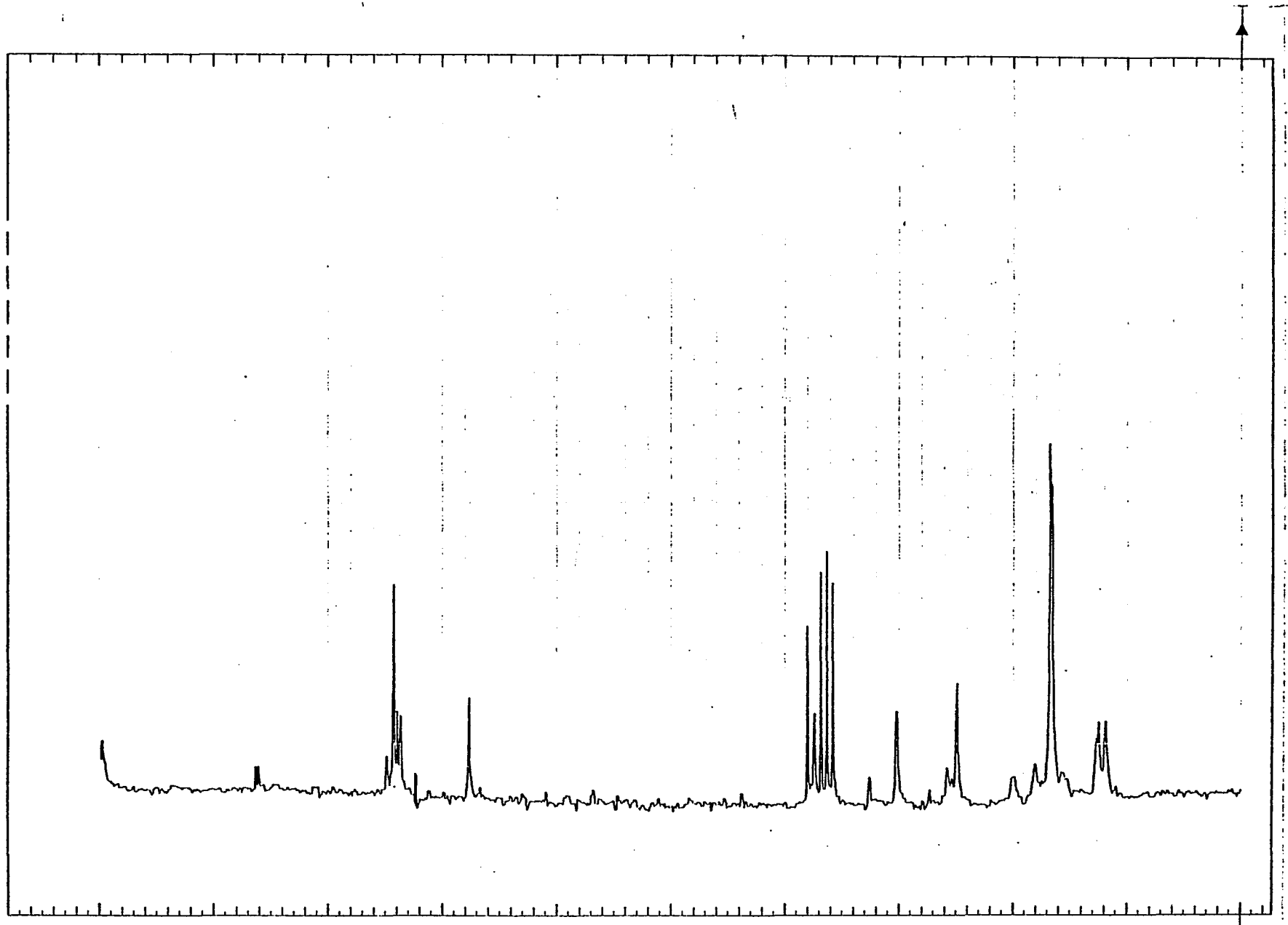
^{13}C of (LXXIII) in D_2O



^{13}C of LXXXIV) in $CDCl_3$

Chemical Shifts of (LXXXIV):

171.112, 170.937, 169.962, 154.599, 134.847, 127.532, 127.166, 80.347, 78.823, 78.153, 76.872, 75.592, 65.656, 64.802, 52.914, 50.415, 47.916, 36.333, 27.432, 27.067, 17.861, 17.008.



^{13}C of (LXXXVI) of $CDCl_3$

Chemical Shifts in (LXXXVI):

201.691, 201.076, 171.654, 171.025, 170.137, 169.922, 155.116, 81.381, 80.162, 67.728, 61.722, 61.478, 60.047, 50.749, 48.708, 48.403, 36.028, 27.981, 27.646, 17.983, 17.770, 16.246, 16.002, 15.667, 13.899, 13.777.