Gotlinsky, Barry

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City University of New York PH.D. 1982

SYNTHESIS OF PHOSPHONIC AND PHOSPHINIC ACID ANALOGUES OF OLIGONUCLEOTIDES AND CHOLESTEROL INHIBITORS

by

BARRY GOTLINSKY

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

1982

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

5/21/82
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Abstract

SYNTHESIS OF PHOSPHONIC AND PHOSPHINIC ACID ANALOGUES OF OLIGONUCLEOTIDES AND CHOLESTEROL INHIBITORS

by

BARRY GOTLINSKY

Adviser: Professor Robert Engel

The present thesis encompasses two separate projects. The first project described is a synthetic procedure for the preparation of a phosphinic acid analogue of a sequenced trinucleotide. The synthesis has a relatively simple starting material, D-glucose, which, through the series of reactions is developed to the oligonucleotide analogue. This analogue, having no hydrolyzable phosphate ester oxygen, is seen as being valuable for further understanding of details of protein synthesis.

The synthesis involves linking three carbohydrate ring systems via a pair of Wittig reactions, with the nucleoside base added prior to each coupling. Each ring system must also be functionalized specifically. After obtaining the trinucleotide system, the protecting groups are removed to yield the desired product.

The second project involves the synthesis of the

phosphonic acid analogue of phosphomevalonate, an important intermediate in the biosynthesis of cholesterol and its linkage to a bile acid related material. After being coupled to a bile acid derivative, the phosphomevalonate analogue is capable of being carried to the liver where it may inhibit cholesterol biosynthesis. This was confirmed by studies using a carbon-14 label in the analogue.

A series of phosphates were also synthesized and linked to the bile acid derivative for biological control experiments. The same reagents were used to accomplish the esterification as for the previously noted coupling.

ACKNOWLEDGEMENTS

I wish to thank Professor Robert Engel for his guidance, support, patience, and understanding. His confidence and encouragement made a very difficult task most pleasant, enjoyable, and rewarding.

I also wish to express my sincere gratitude to the many faculty members and graduate students whom I have had the most fortunate opportunity to meet and share this experience with.

I extend a special thanks to the thesis committee members, Professors B. Tropp, A.D. Baker, and R. Pizer, as well as Professors G. Axelrad and W.F. Berkowitz for their invaluable help.

I especially thank my family for their patience, understanding, and encouragement. They have been an extremely valuable asset to me throughout my studies.

TABLE OF CONTENTS

INTRODUCTION

Part-1

Research on nucleic acids dates back to the late nineteenth century when Freidrich Meischer investigated material termed "nuclein" (1). This material was composed mostly of long chain DNA, and was isolated from pus cells collected from bandages. Meischer later reported that he isolated "nuclein" of high molecular weight from sperm heads of Salmon. This material had a phosphorus content of 9.59%, corresponding to the phosphorus content of pure nucleic acid.

The isolation and identification of xanthine, adenine, thymine, and cytosine was also performed in the late 1800's. Kossel also began studies of the structure of nucleic acid bases.

The term "nucleic acid" was proposed by Altman in 1889 to describe the protein free acidic material which he isolated from animal tissue and yeast. The term "pyrimidine" was proposed by Pinner in 1884-5 as a result of combining the words "pyridine" and "amidine".

Emil Fischer accomplished a partial synthesis of adenine in 1897, and the total synthesis was achieved by Traube in 1904, thus proving the structure of adenine. Guanine was discovered by Magnus in 1844 and by Ungar in 1846, and was recognized as a component of nucleic acid by Kossel in 1879-83. Uracil was isolated from yeast by Ascoli in 1901, and its structure confirmed via a total synthesis by Fischer and Roeder.

In 1894 Hammersten identified the carbohydrate portion of RNA as a pentose, which was isolated by Levene and Jacobs in 1908 and identified as D-ribose. Fischer and Piloty achieved the first synthesis of L-ribose. D-ribose was synthesized by Blanksma and Ekenstein in 1913.

The role of nucleic acids as carriers of genetic information was first indicated by Avery, MacLeod, and McCarthy in 1944. In the next decade, major advances in this area were underway. In 1953, Hershey and Chase demonstrated, by using P32 labelling, that the genetic substance of bacteriophage was DNA. Watson and Crick devised the model of DNA as a complementary double helix at this time.

Since that period, major advances in nucleic acid chemistry and molecular genetics have been made. The concept of mRNA carrying information in the form of

nucleotide sequences from the gene to the site of protein biosynthesis had been established. The codons of mRNA (sequences of three adjacent nucleotides that code for an amino acid) pair sequentially with the anticodons of tRNA molecules on the ribosome. Since each tRNA is specific for a particular amino acid, the amino acid sequence may then be specified. This translation process has three basic steps: (a) initiation, (b) elongation, and (c) termination. The initiation and termination steps are unique in the synthesis of each protein, and signals for these events occur at particular sites on the mRNA molecule in the form of specific triplet codons. The elongation step is the repetitive synthesis of a peptide bond between the C-terminal of the enlarging peptide chain and the α-amino groups of the incoming amino acid. The order of addition is determined by the mRNA that moves relative to the ribosome.

The initiation of protein synthesis (fig. 1) is understood to require free ribosomal subunits, mRNA, GTP, a specific initiating aminoacyl tRNA (N-formyl-methionyl tRNA), and three protein initiating factors (IF-1, IF-2, IF-3). The initiation factors are proteins whose molecular weights are approximately 9000, 65000, and 21000, respectively. Initiation begins with the 16S ribosomal RNA (rRNA) of the 30S ribosomal subunit binding to a region of

the mRNA that precedes the first translated codon. The presence of the protein factor IF-3 is required for this binding of the mRNA to the 30S ribosomal subunit. A complex then forms when the aminoacyl-tRNA called for by the first codon interacts with GTP and the initiation factor IF-2. This complex, in the presence of the initiation factor IF-1 attaches the anticodon of the tRNA to the first codon of the message to form an initiation complex with the 30S ribosomal subunit. Upon release of the initiation factors IF-1, IF-2, and IF-3, the 50S ribosomal subunit attaches and the GTP is hydrolyzed, thus completing the formation of the 70S ribosome.

The elongation process (fig. 2) has three stages: (a) binding, (b) peptide bond formation, and (c) translocation. In the binding stage, the proper aminoacyl-tRNA is bound to the A (aminoacyl) site in the 70S ribosome. This site is free , since during initiation the aminoacyl-tRNA molecule enters at the P (peptidyl) site. Elongation factor Tu (EF-Tu, a specific cytoplasmic protein) forms a complex with GTP and the entering aminoacyl-tRNA, allowing the aminoacyl-tRNA to enter the A site with the release of EF-Tu*GDP and phosphate. EF-Tu*GDP recycles to EF-Tu*GTP with the aid of EF-Ts and GTP. The α-amino group of the new aminoacyl-tRNA in the A site carries out a nucleophilic

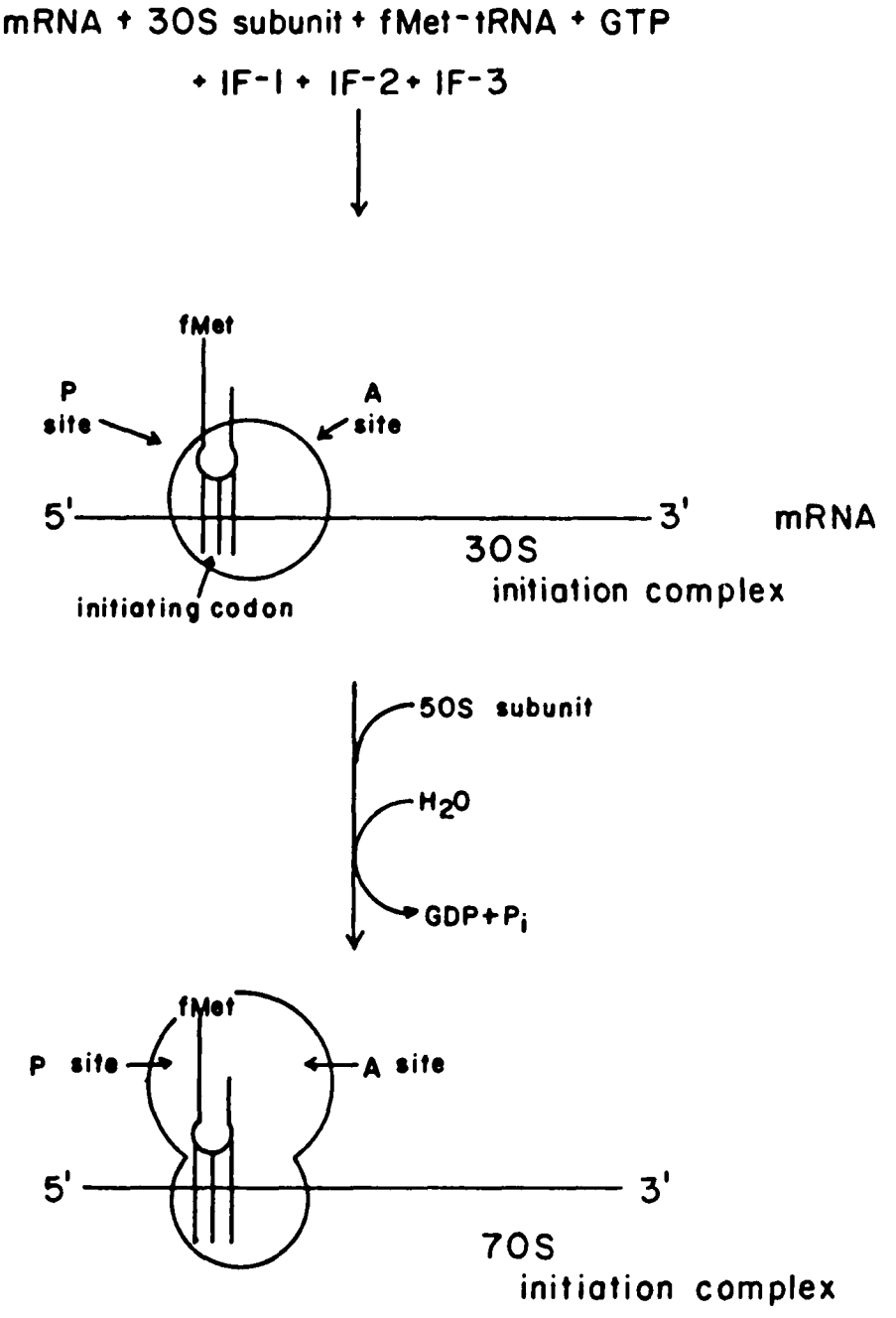

mRNA + 30S subunit + fMet-tRNA + GTP
+ IF-1 + IF-2 + IF-3
fMet
P site
A site
5'
3'
mRNA
30S
initiation complex
initiating codon
50S subunit
H_2O
GDP+P_i
fMet
P site
A site
5'
3'
70S
initiation complex

fig. 1

attack on the esterified carboxyl group of the peptidyl-tRNA occupying the P site. This reaction is catalyzed by peptidyl transferase, a protein component of the 50S ribosomal subunit. The result of this peptide bond forming stage is the attachment of the growing peptide chain to the tRNA in the A site. Translocation of the newly formed peptidyl tRNA at the A site into the vacated P site requires elongation factor G (EF-G) and GTP. The GTP is hydrolyzed to GDP and phosphate. This process frees the A site for another cycle of aminoacyl-tRNA codon recognition and elongation.

When the terminating codon of mRNA (the sequences UAA, UAG, or UGA) appears in the A site, termination will occur (fig. 3). This will take place after multiple cycles of elongation resulting in linkage of the specific amino acids into a protein molecule have occurred. There is no tRNA with an anticodon to recognize such a termination signal. Releasing factors, which are capable of recognizing that a termination signal resides in the A site, hydrolyze the bond between the peptide and the tRNA occupying the P site. This hydrolysis releases the protein and the tRNA from the P site. The 70S ribosome then dissociates in the presence of IF-3 into its 30S and 50S subunits, which are then recycled in the protein synthesis process. Ribosomes are therefore continuously recycled during protein synthesis.

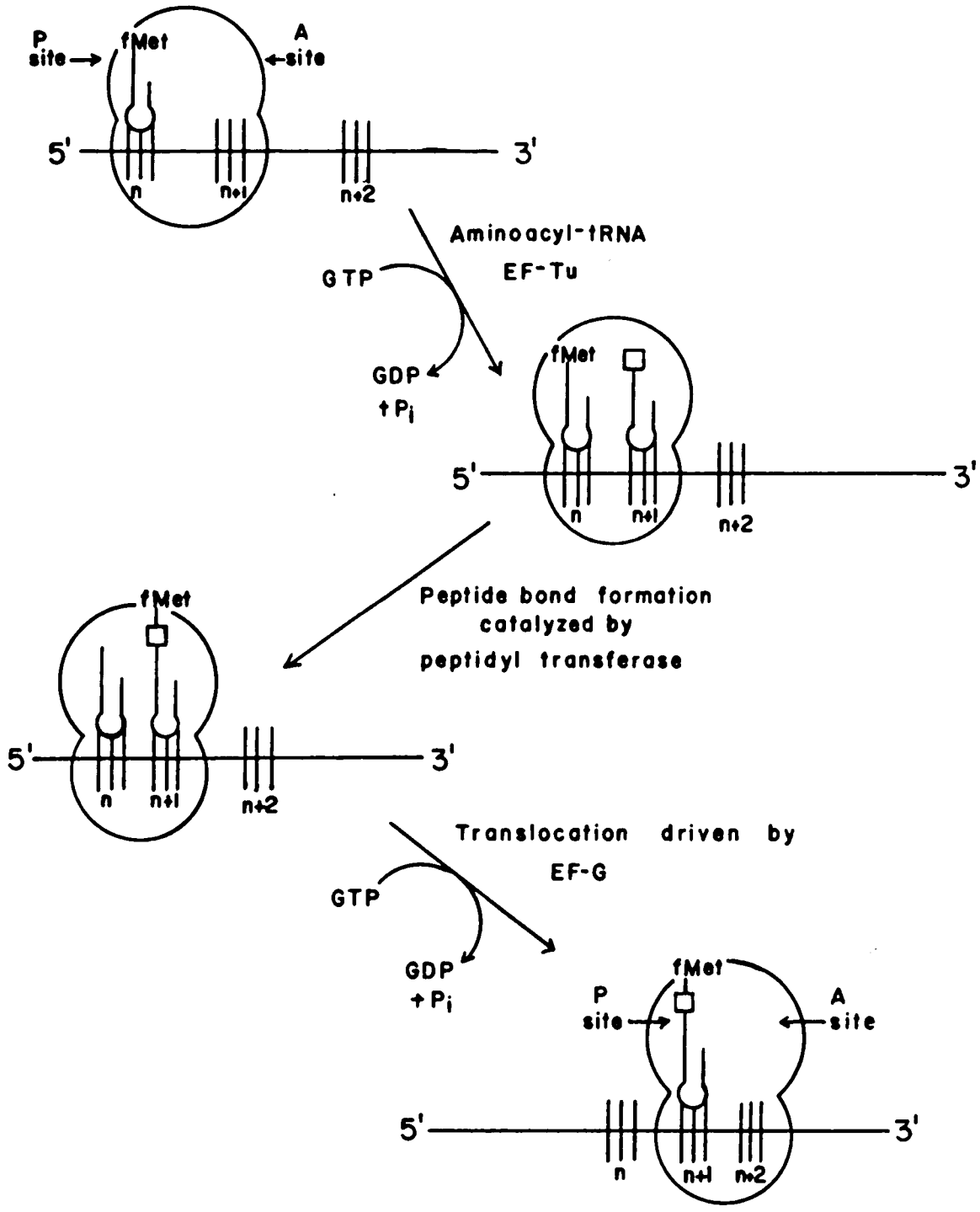

P site
fMet
A site
5'
3'
n
n+1
n+2
Aminoacyl-tRNA
EF-Tu
GTP
GDP
+Pi
Peptide bond formation
catalyzed by
peptidyl transferase
Translocation driven by
EF-G

fig. 2

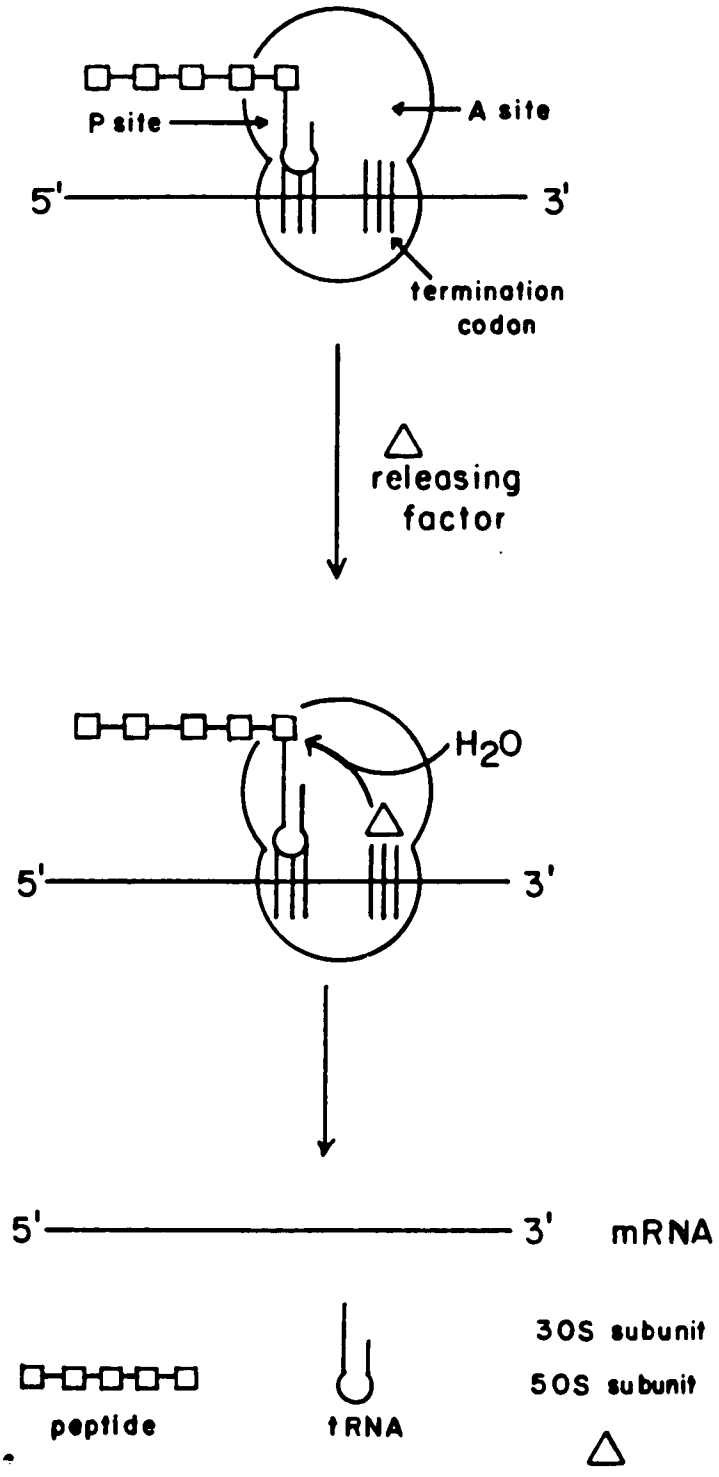
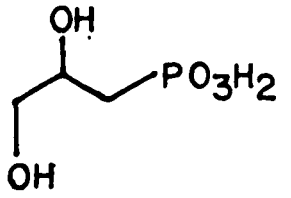

P site
A site
5'
3'
termination codon
releasing factor
H_2O
5'
3'
5'
3'
mRNA
30S subunit
50S subunit
peptide
tRNA

fig. 3

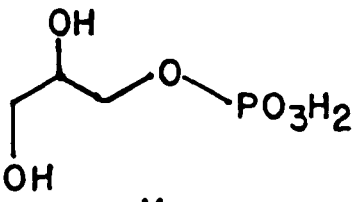
The use of drugs to treat genetic diseases at the point of protein synthesis can run into some difficulties. One of these difficulties is the result of the ability of an organism to recognize the introduction of a foreign substance, thus degrading it. This degradation would require frequent renewal of the drug. Similarly, the introduction of a corrected natural agent would result in eventual cleavage of the phosphate ester linkages after introduction.

A drug whose structure was analogous to the natural material and could undergo the normal functions of the natural material, but was immune to degradation would be quite useful. Specifically, if a substitution were made for the normal phosphate ester oxygens of a nucleic acid by a methylene linkage, the above condition would possibly be met. This substitution has been successfully used in a variety of biologically active compounds. These have included carbohydrates, products of glycolysis, phospholipids, and nucleotides (2).

Systems in which the carbon-oxygen-phosphorus linkage is replaced with a direct carbon-carbon-phosphorus linkage are of interest. The types of systems investigated have involved several structural factors, for example, the exclusion of the oxygen of the natural phosphate ester



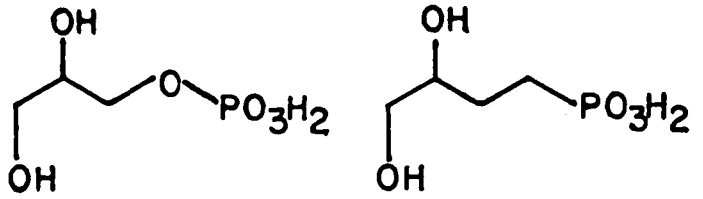
I



II

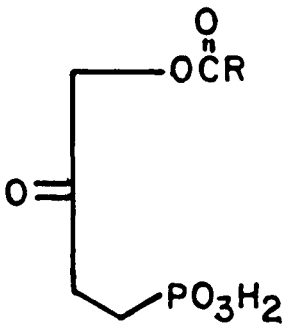
linkage, such as 2,3-dihydroxypropyl-1-phosphonic acid (I), a nonisosteric analogue of glycerol-3-phosphate (II). Such analogues have been found to possess biological activity under limited circumstances.

Analogues which are isosteric with the natural phosphates represent another structural class which is more interesting. For example, an isosteric, non-hydrolyzable analogue of glycerol-3-phosphate (II) (3,4) is 3,4-dihydroxy-butyl-1-phosphonic acid (III) (5). Although such a compound is not strictly isosteric, crystallographic data has shown that the differences between a series of phosphates and the corresponding phosphonic acids to be minimal (6-12).

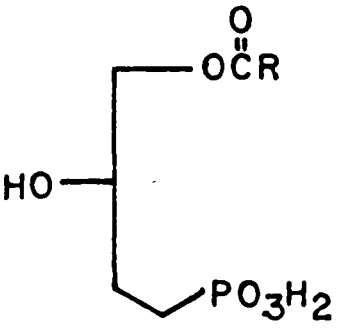


II III

With the postulate that the isosteric phosphonic acid analogue of a natural phosphate would be expected to substitute for all natural processes except for cleavage of the phosphorus, it was proposed that substitution of the analogue III for II might be able to serve as a metabolic regulator. The analogue III has indeed been found to inhibit the growth of mutant strains of E. coli (13,14). The analogue replaces glycerol-3-phosphate in the reaction catalyzed by phosphatidyl glycerol phosphate synthetase. The resultant analogue of phosphatidyl glycerol phosphate accumulates in the cell, and since cleavage of phosphorus does not take place, the natural metabolic end product is not present, and inhibition of the growth of the cell takes place.



IV

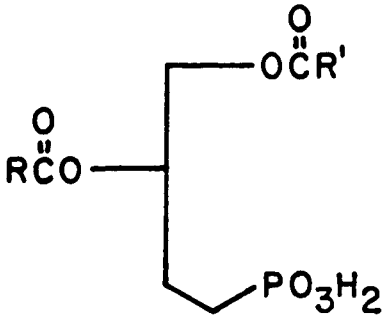


V

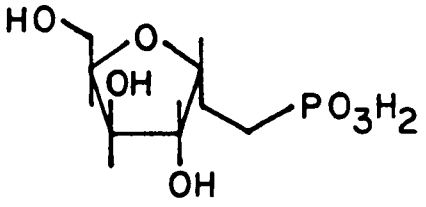
Other analogues investigated included the following phospholipid analogues: an analogue of acyl dihydroxyacetonephosphate (IV) (15), its reduced form, lysophosphatidic acid (V) (16), and the differentially substituted phosphotidic acid (VI) (16). Compound V was determined to be a substrate for lysophosphatidate acyltransferase.

Analogues of carbohydrate phosphates have also been investigated, among them the analogue of fructose-1-phosphate (VII) (17). This analogue was found to be an in vivo inhibitor of growth of E. coli.

Isosteric phosphonic acid analogues of nucleotides have been studied (2). Jones and Moffatt have investigated numerous analogues. Among them are the

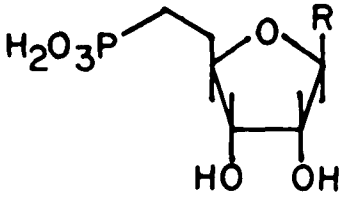


VI

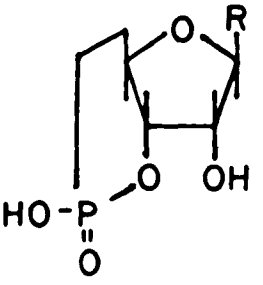


VII

5'-deoxy-5'-(dihydroxyphosphonylmethyl) nucleosides (VIII), where R is uracil or adenine (18), and its related cyclic nucleotide analogue (IX) (18), bearing a 5'-methylene substitution for the 5'-oxygen. The isomers of these compounds, X and XI, respectively,

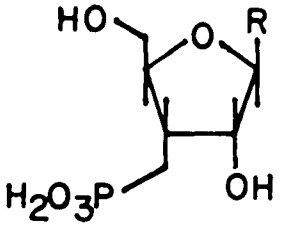


VIII

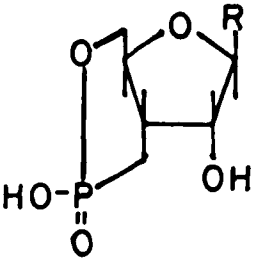


IX

where the 3'-oxygen is substituted by a 3'-methylene group, have also been reported (20,21). These compounds are reported to exhibit activity in controlling metabolic processes and in producing



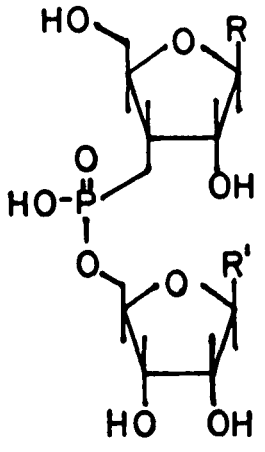
X



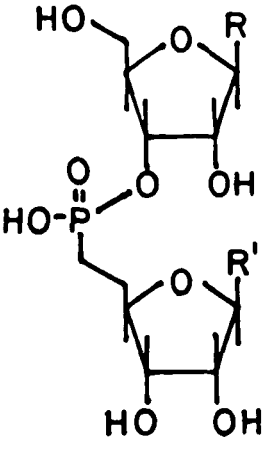
XI

metabolic deficiencies (20-22), although details have not appeared in the literature. Isosteric analogues of dinucleoside phosphates in which the 3'-phosphate oxygen is replaced by a methylene group, XII and XIII, respectively, have also been synthesized (20,21,23).

Several phosphonic acid analogues of nucleotides have been used to study the mechanism of protein biosynthesis (2). The most useful have been analogues of nucleoside pyrophosphates. In particular, the analogue of GTP (XIV),



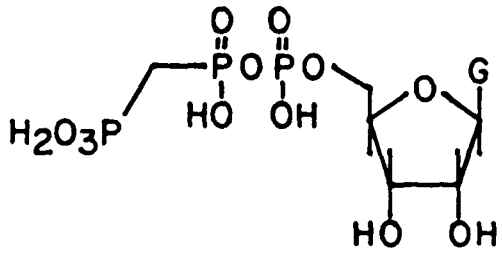
XII



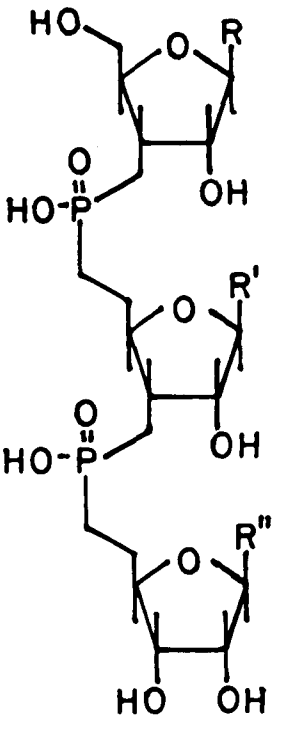
XIII

in which the β,γ-pyrophosphate oxygen is replaced by a methylene group, was found to be an inhibitor of peptide synthesis. The detailed role of GTP in the several stages of protein biosynthesis was determined using compound XIV (24-29).

Thus it might be postulated that phosphinic acid analogues of nucleotides would be quite useful for the study of the mechanism of protein synthesis. Substitution of these analogues for codon triplets might also prove to be quite interesting. Specifically, phosphinic acid analogues (XV) of trinucleotides, which are impervious to phosphorus elision, are seen as being quite valuable for further understanding of other details of protein synthesis, and have potential for uses as drugs. Incorporation of these



XIV



XV

analogues in a natural mRNA, and thus into a protein synthesizing system, would provide much information about binding sites. The absence of the esteric oxygens could affect substrate enzyme binding. This would be a direct

measure of the magnitude of the interactions involving esteric oxygens. At present, the binding requirements of phosphate esteric oxygens in oligonucleotides is unknown.

Non-hydrolyzable RNA analogues might also be useful in interferon related nucleic acids. It has also been found that synthetic homonucleosidic RNA's serve, in vivo, in mammals as interferon inducers (30). It is currently believed that interferon is significant in the control of viral infections. A problem with these drugs, though, is their inherent lability, as they are easily susceptible to cleavage (31). Non-hydrolysable phosphinic acid analogues of homonucleosidic RNA would not have this problem. If these analogues could be substituted for the natural compounds, they might function for a longer period of time, as they are not subject to phosphate cleavage, and thus could be superior in interferon generating activity.

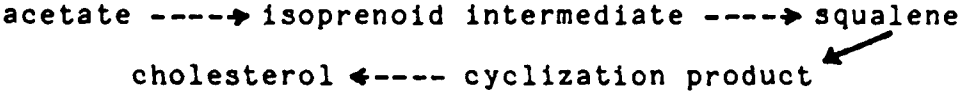
This portion of the work deals with the total synthesis of a phosphinic acid analogue of a trinucleotide. This should allow a start on the determination of biochemical utility for systems as noted above.

Part 2

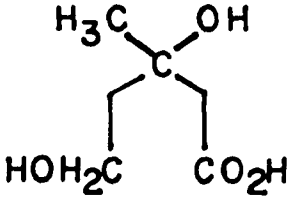
Modern research on cholesterol biosynthesis had its major beginning in 1937. Two independent groups made quite similar observations. One of these groups, Rittenberg and Schoenheimer, studied intermediary metabolism via stable isotopes (32). Incorporation of trideuterio acetate into the unsaponifiable materials of yeast was studied by the second group, Sonderhoff and Thomas (33). The conclusion of these studies was that the process of cholesterol formation involved the coupling of smaller molecules derivable from acetate. These smaller molecules were believed to be intermediates in fat and carbohydrate metabolism.

Rittenberg and Bloch believed that acetate itself was the basic building block of cholesterol biosynthesis (34-36). This was based on studies of the utilisation of labelled acetic acid for cholesterol synthesis in animal tissues. The utilisation of acetate for the biosynthesis of rubber was demonstrated by Bonner and Arreguen (37). They believed that three acetate molecules could combine to form the required isoprenoid subunit for the macromolecule via aceto acetate and methyl crotonic acid.

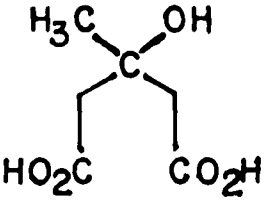
The idea that cholesterol was derived from a polyisoprenoid intermediate was now taking shape. Robinson's theory that cholesterol was formed by the cyclization of squalene (38), which is a polyisoprenoid hydrocarbon, was in accord with this idea. At this point, the biosynthesis of cholesterol was summarized as:



Wright and Folkers discovered mevalonic acid in 1956 (39). This discovery linked acetic acid to the biological isoprene unit. The intention of this investigation was to find a substitute for acetate in the nutrition of acetate



XVI



XVII

requiring strains of Lacto-bacillus acidophilus (40). Tavormina, Gibbs, and Huff noticed the structural similarity between mevalonic acid (XVI) and β-hydroxy-β-methyl-glutaric acid (XVII), that is that their carbon skeletons are identical. They therefore tested the bacterial growth factor of mevalonic acid and found it to be active as a precursor of squalene sterol (41). Via isotopic labelling, it was shown that mevalonic acid is the key intermediate in terpene and sterol biosynthesis. Labelled mevalonic acid was incorporated into squalene and cholesterol in a very high yield. Also, incubation of labelled acetate with liver slices demonstrated that acetate carbon is an immediate precursor of mevalonic acid.

The biosynthesis of cholesterol therefore is comprised of three major stages: (a) acetate conversion to mevalonic acid (the source of acetate is now known to be Acetyl-CoA), (b) mevalonic acid conversion to squalene (isoprenoid units are formed from mevalonate in this stage), and (c) squalene conversion to sterol.

In the first stage (fig. 4), mevalonic acid is formed by condensation of three molecules of acetyl-CoA. The key intermediate in this process is β-hydroxy-β-methylglutaryl-CoA. This is converted to mevalonate in a two step reduction by NADPH catalyzed by β-hydroxy-β-methylglutaryl-CoA reductase.

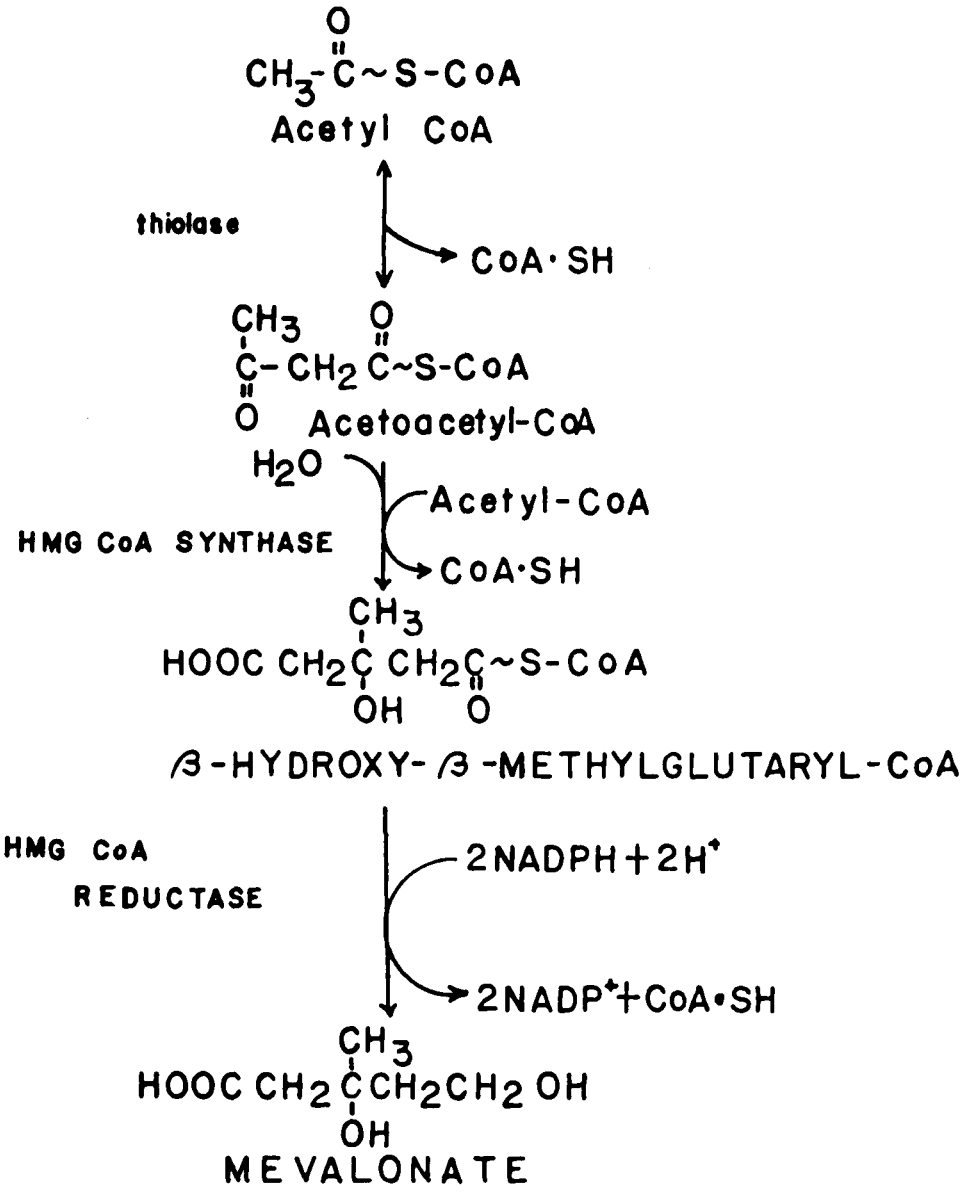

CH3-C~S-CoA
Acetyl CoA
thiolase
CoA·SH
CH3 C-CH2 C~S-CoA
Acetoacetyl-CoA
H2O
Acetyl-CoA
HMG CoA SYNTHASE
CoA·SH
HOOC CH2 C CH2 C~S-CoA
OH
β-HYDROXY-β-METHYLGLUTARYL-CoA
HMG CoA
REDUCTASE
2NADPH+2H+
2NADP++CoA·SH
HOOC CH2 C CH2CH2 OH
OH
MEVALONATE

fig. 4

In the second step (fig. 5), mevalonate is phosphorylated by ATP to the 5-monophosphate ester, and then to the 5-pyrophosphate. A third phosphorylation at carbon 3 yields an unstable intermediate which loses phosphoric acid and decarboxylates to form 3-isopentyl pyrophosphate (the active isoprenoid unit). This isomerizes to 3,3-dimethyl allyl pyrophosphate. The two isomeric isoprenyl pyrophosphates undergo condensation to yield the monoterpene geranyl pyrophosphate. Another condensation with 3-isopentenyl pyrophosphate yields the sesquiterpene farnesyl pyrophosphate. Each condensation is accompanied by the elimination of pyrophosphoric acid. Two molecules of farnesyl pyrophosphate condense to yield presqualene pyrophosphate, which is reduced by NADPH to yield squalene and pyrophosphate. These conversions are catalyzed enzymatically.

In the last stage (fig. 6), squalene undergoes attack by molecular oxygen to form squalene 2,3-epoxide. This epoxidation is catalyzed by squalene monooxygenase. The first sterol is formed when the squalene 2,3-epoxide undergoes cyclization to lanosterol. This reaction is accomplished by a series of 1,2 methyl and hydride shifts along the squalene chain resulting in closure of the four rings. These reactions occur in the microsome, and require sterol carrier protein 1 for binding.

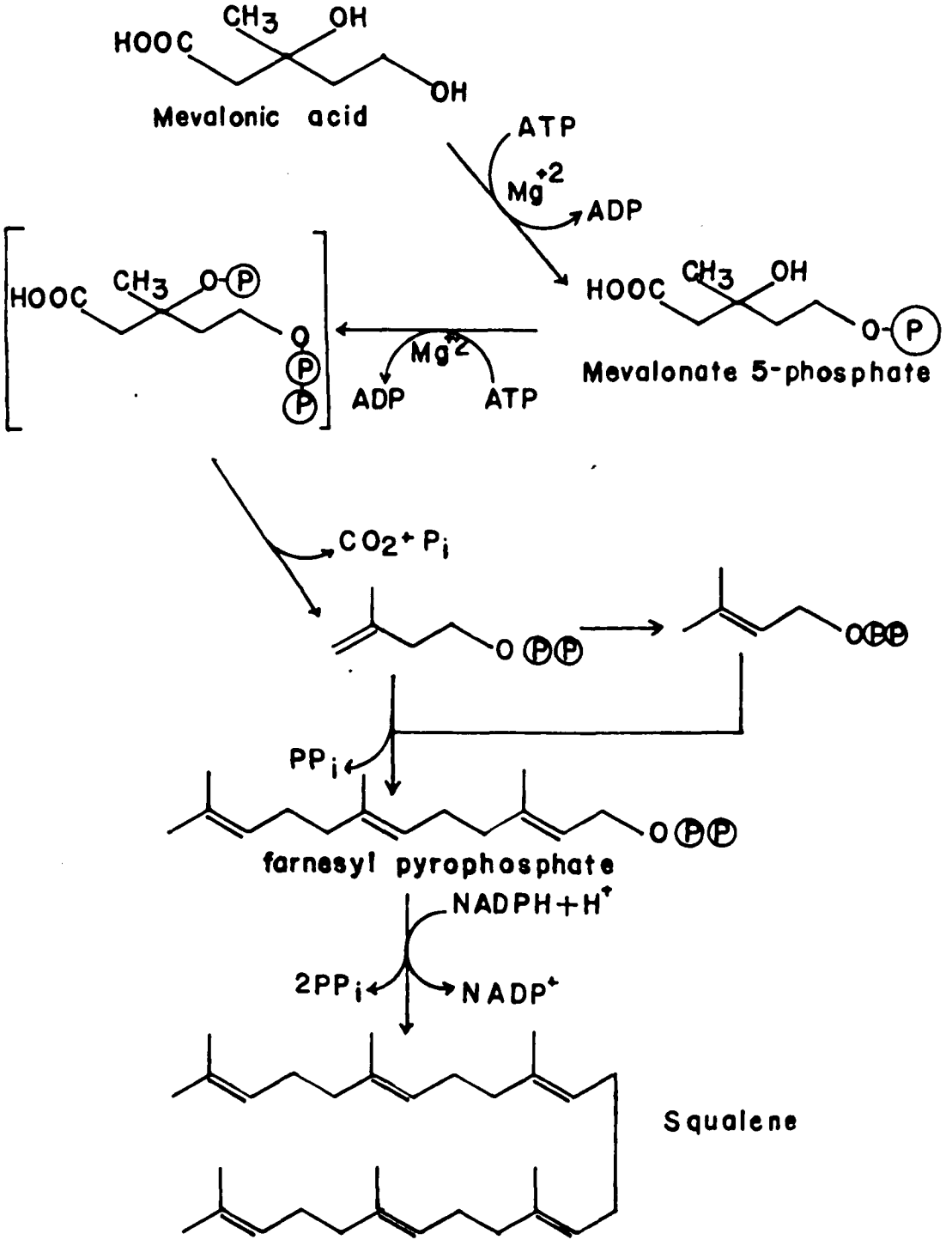

HOOC
CH3
OH
OH
Mevalonic acid
ATP
Mg+2
ADP
HOOC
CH3
OH
O-P
Mevalonate 5-phosphate
HOOC
CH3
O-P
O
P
P
Mg+2
ADP
ATP
CO2+Pi
O P P
O P P
PPi
O P P
farnesyl pyrophosphate
NADPH+H+
2PPi
NADP+
Squalene

fig. 5

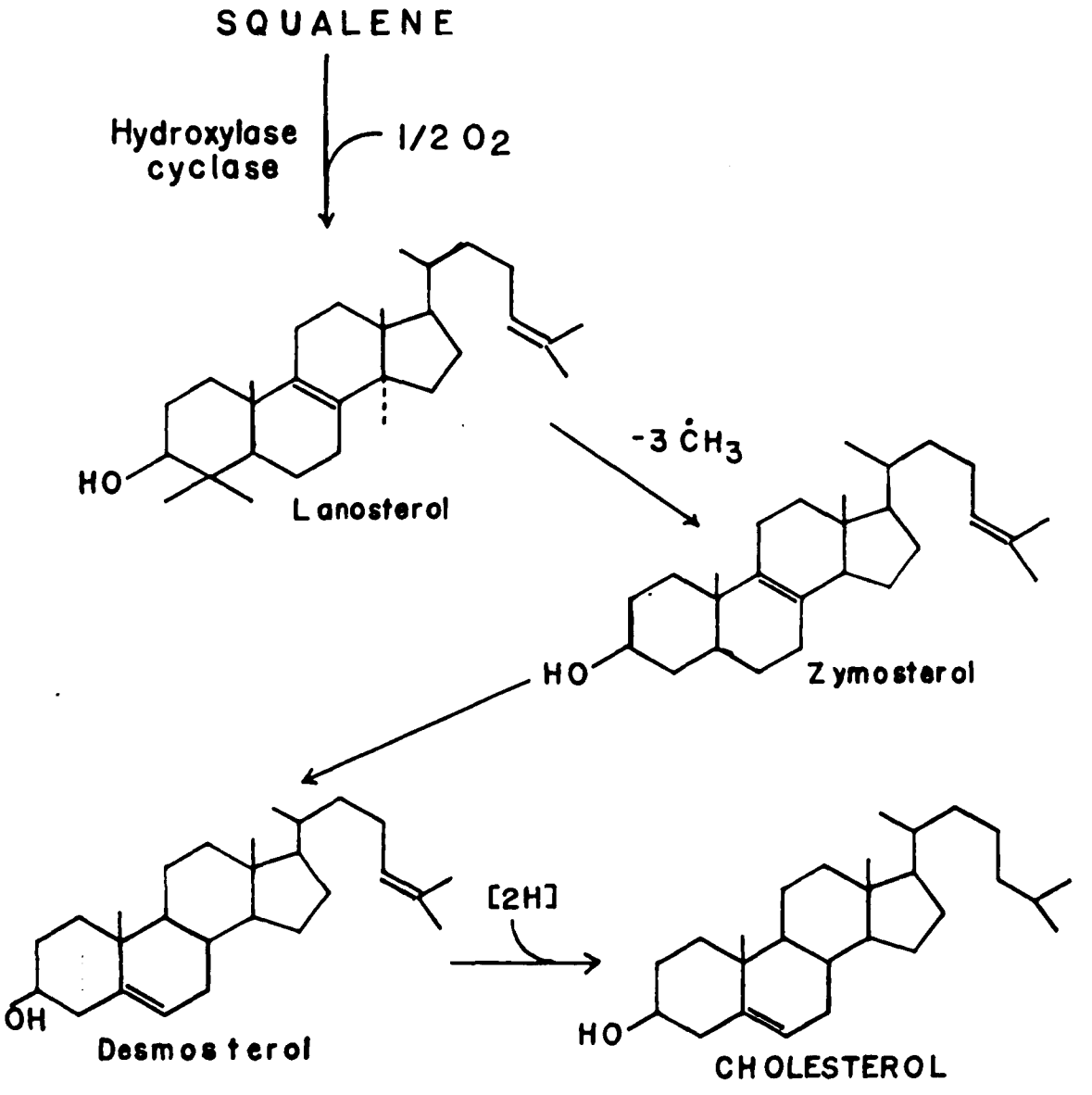

SQUALENE
Hydroxylase
cyclase
1/2 O2
HO
Lanosterol
-3 ĊH3
HO
Zymosterol
OH
Desmosterol
[2H]
HO
CHOLESTEROL

fig. 6

The conversion of lanosterol to cholesterol involves changes in the steroid nucleus and side chain. The methyl group on C14 is oxidized to carbon dioxide to form 14-desmethyl lanosterol. Two more methyl groups on C4 are then removed generating zymosterol. The C8-C9 double bond shifts to C8-C7, and then shifts further to C5-C6 to form desmosterol. Saturation of the double bond in the side chain occurs forming cholesterol.

The regulation of cholesterol biosynthesis has been approached in various ways. Extensive *in vivo* studies have been made. Schoenheimer and Breusch provided evidence that when cholesterol is added to the diet of an animal, the ability to synthesize cholesterol is decreased (42). This was confirmed by isotopic experiments by Gould (43) and Bloch (44). When animals were fed a high cholesterol diet, the ability of liver slices from these animals to synthesize cholesterol from labelled acetate is inhibited. Investigations by Gould and Popjak (45) and Buscher (46) indicated that cholesterol feeding caused a more marked inhibition in the conversion of labelled acetate to cholesterol than was observed when labelled mevalonate was used as a sterol precursor. Cholesterol feeding therefore inhibits cholesterol synthesis at a site prior to the synthesis of mevalonate.

A specific biochemical localization of this feedback reaction site was discovered as a result of work by Siperstein (47-50). It was shown that feeding of cholesterol has no effect on the following conversions:

acetate ----→ acetoacetic ester or β-hydroxy-β-butyric acid

mevalonate ----→ squalene

squalene ----→ cholesterol

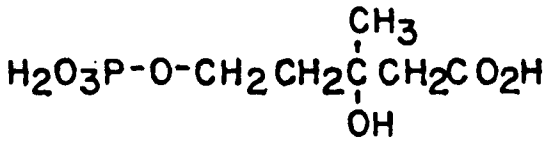
From this data, it was concluded that cholesterol inhibits its own synthesis at the point of conversion of β-hydroxy-β-methyl glutarate to mevalonate (47,51). This was proven in a study using GLC to isolate the carbon-14 labelled mevalonate in which feeding of cholesterol resulted in a marked inhibition of the synthesis of mevalonate while having no detectable effect upon the synthesis of β-hydroxy-β-methyl glutarate (48,50,52). It has also been found that fasting depresses cholesterol synthesis at the same enzymatic site.

Another approach to the inhibition of cholesterol biosynthesis is via the use of drugs. Since the biosynthesis of cholesterol involves a series of phosphate esters, a good specific inhibitor would involve the

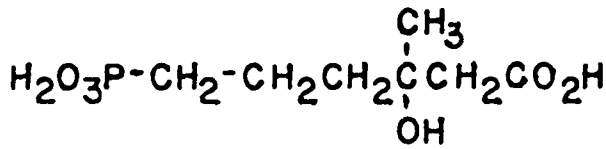
introduction of a phosphonic acid species as a substitute for the natural metabolite. As discussed in Part 1 of the Introduction, isosteric analogues of the natural phosphates in which a methylene group is substituted for the phosphate ester oxygen would be ideal for such inhibitors. As discussed previously, the non-hydrolyzable phosphonic acid would cause inhibition in the biosynthesis at the point where phosphate cleavage would occur in the natural system.

5-Phosphomevalonic acid (XVIII) represents the first stage at which a phosphonic acid analogue might be introduced which would be of use in the inhibition of squalene synthesis. The phosphonic acid analogue (XIX) related to XVIII was chosen. This analogue retains all the reactive sites necessary for the continuation of the normal reaction sequence until the beginning stages of condensation of isopentenyl units, where the phosphate ester linkage is involved (53-55).

A major difficulty in working with phosphonic acid analogues is the inability of many of these charged compounds to enter the cell. A potential solution to this problem involves linkage of the analogue to a molecule that is transported by a pre-existing transport system. Since in the liver there is a lipoprotein feedback mechanism operating to regulate sterol synthesis which involves the



XVIII



XIX

uptake of chylomicron remnants, the liver can sense the amount of cholesterol that is entering the body through the intestine. Linking the phosphonic acid analogue to a molecule which is transported into the liver cells would seem the ideal solution. Recent studies on bile acid transport into liver cells (56,57) provide a promise of viable transport for drugs. The very high binding capacity to the plasma membrane suggests that adsorption is primarily due to unspecific interaction with the lipid phase of the plasma membrane (56). It is also believed that the uptake of bile acids is mediated by more than one carrier and a particular carrier has affinity for more than one bile acid (57).

This portion of the work involved the synthesis of the phosphonic acid analogue of 5-phosphomevalonic acid, both labelled and unlabelled, and the coupling of the analogue to a bile acid related carrier. The drug carrier conjugate was then tested in rat hepatocytes for satisfactory inhibition of cholesterol biosynthesis. In addition, a number of compounds similar to XIX were coupled to the carrier as a control for measuring the effects of the carrier itself.

RESULTS AND DISCUSSION

Part-1

The synthesis of phosphonic acid analogues of oligonucleotides involves the stepwise coupling of the carbohydrate rings. Each of the rings (labelled A, B, and C in fig. 7) must be specifically functionalized, with the nucleoside base added prior to each coupling. The units were linked via Wittig reactions between the ylid of the phosphinic phosphonium salt at the 3'-position of one unit and a 5'-aldehyde function of a second unit.

The route may be briefly summarized. The starting material for this scheme, D-glucose, was converted into diacetone glucose (XX) by the method of Glen *et. al.* (58) using an excess of anhydrous acetone with anhydrous zinc chloride and phosphoric acid as catalysts (Scheme I). The diacetone glucose was oxidized with dimethylsulfoxide/acetic anhydride (59,60) to give the ketone (XXI) which was purified via repeated distillations. This ketone was then converted, via several transformations to the phosphinic phosphonium salt (XXII). The following transformations are

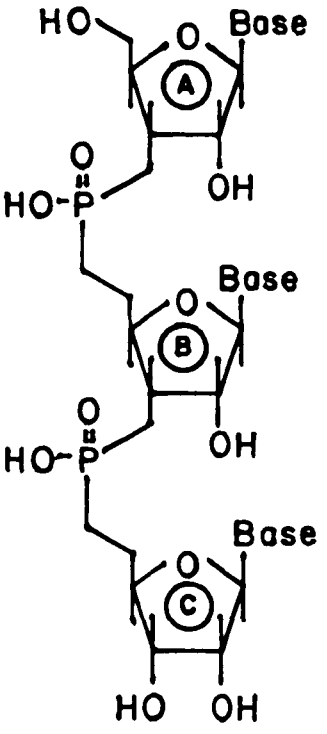
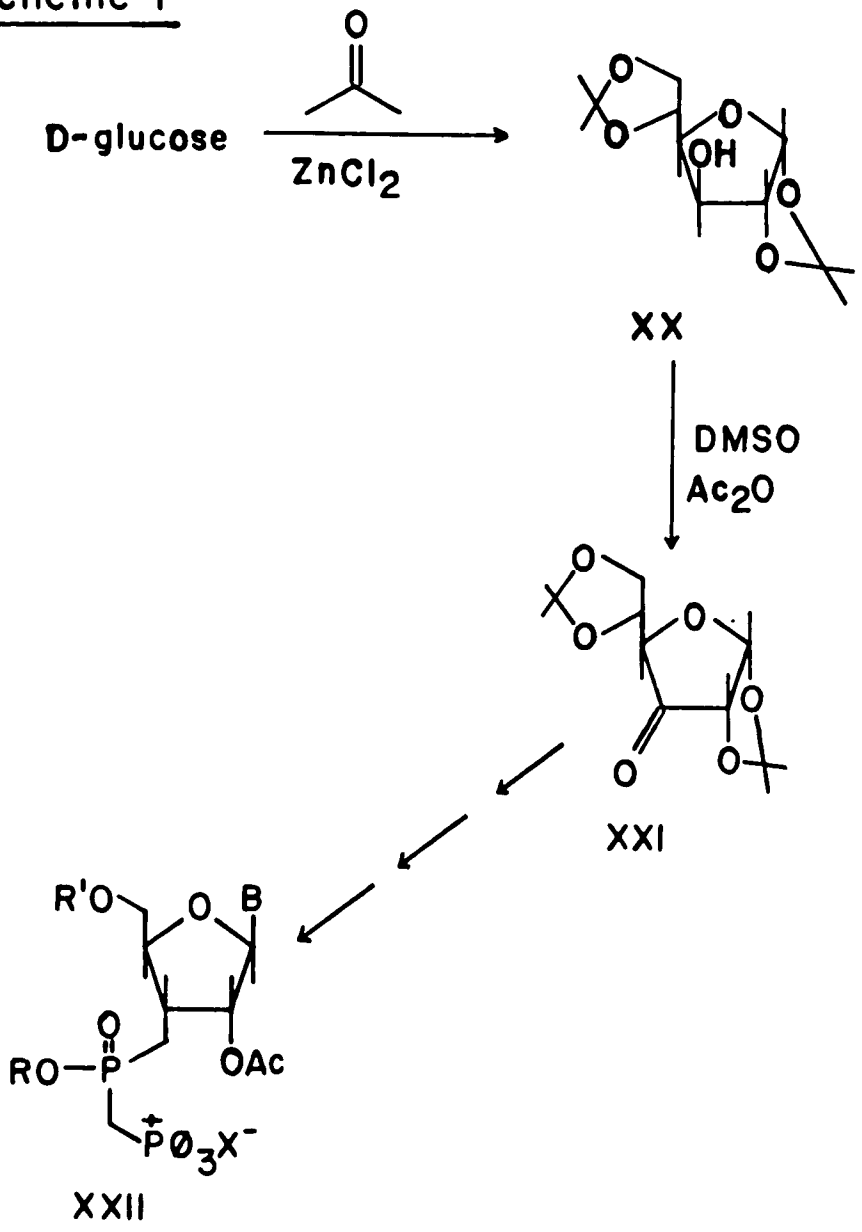


fig. 7

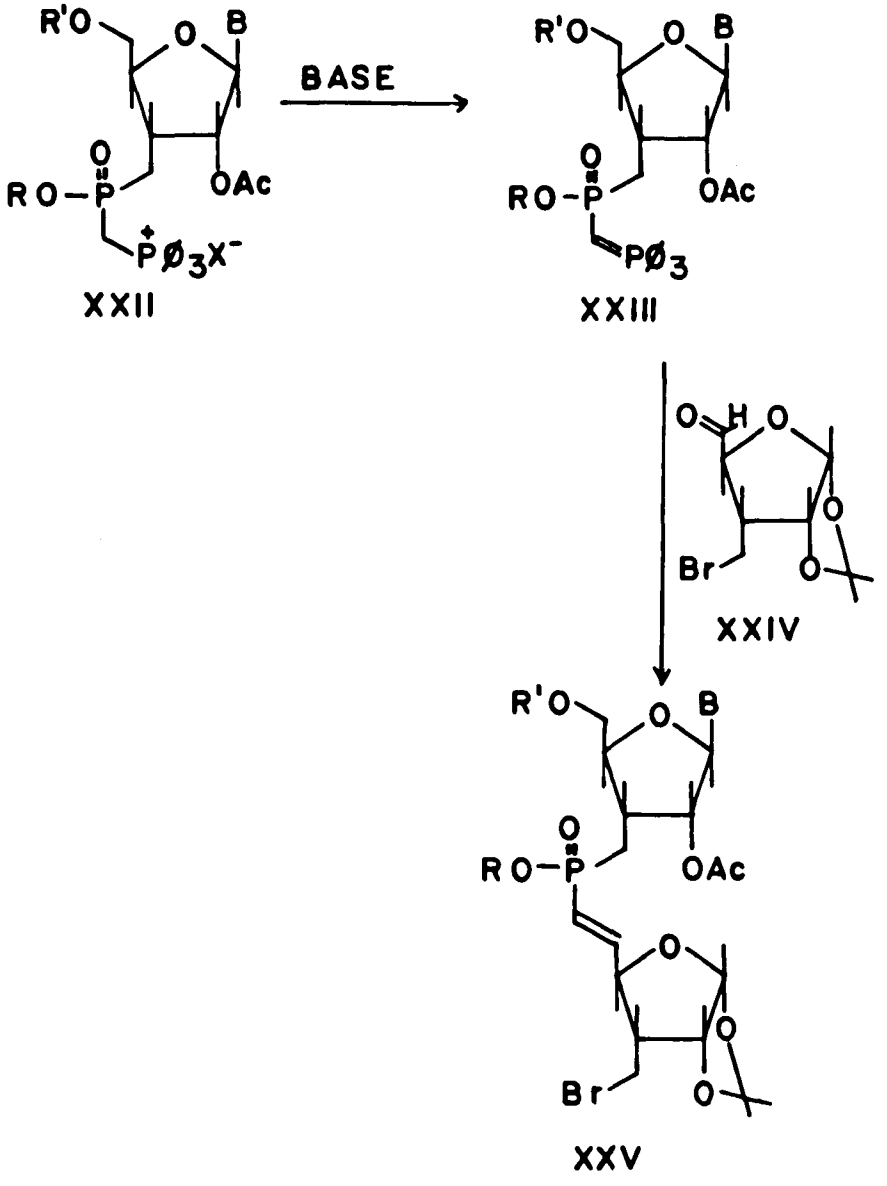
involved: (a) selective removal of the 5,6-isopropylidene function, cleavage of carbon-6 from the remainder, and protection of the 5'-oxygen function; (b) introduction of a methyl phosphonic acid at the 3'-position, which could

eventually be transformed into the required phosphonium salt; and (c) removal of the 1,2-isopropylidene group and introduction of the nucleoside base at the 1'-position.

Scheme I



Scheme II

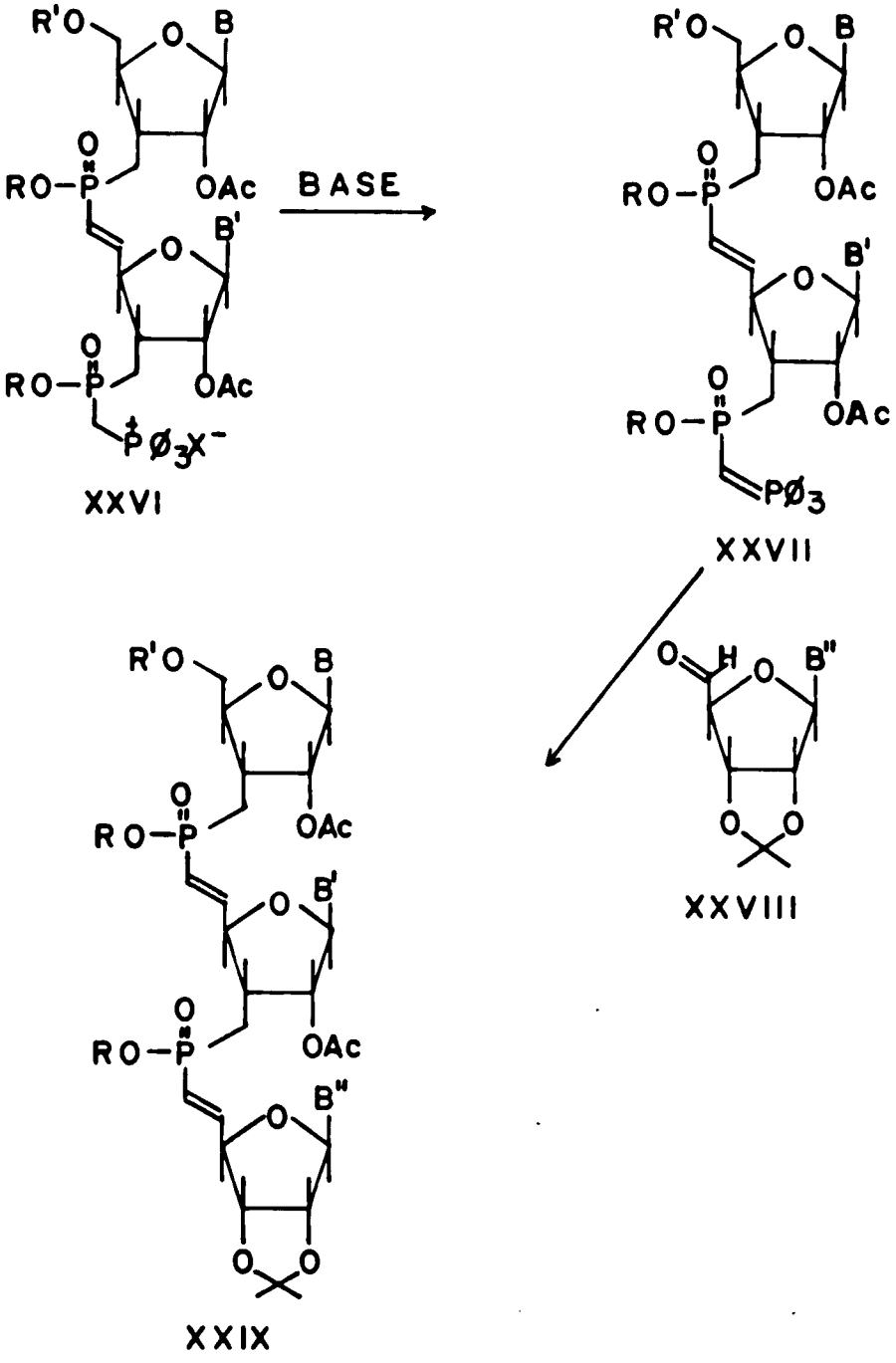


Once the phosphonium salt (XXII) was generated, it was treated with base and allowed to react with a suitably functionalized ring system having an aldehyde at the 5'-position (XXIV). This resulted in the A-B system (Scheme II).

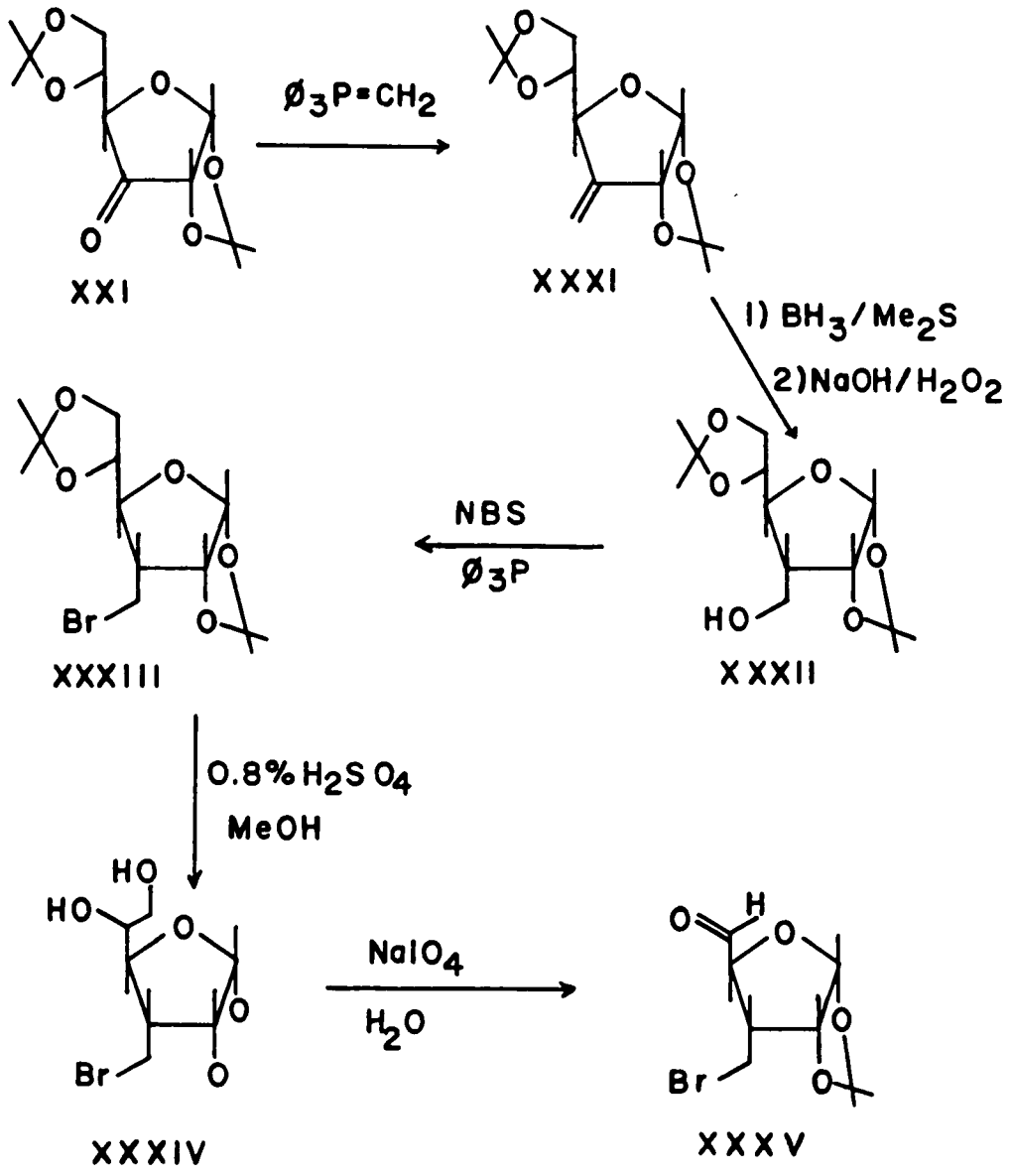
Using procedures similar to those used for the A ring system, the methyl phosphonic acid was introduced at the 3' position of the B ring in XXV, which will eventually be transformed into the required phosphonium salt, and the nucleoside base was introduced at the 1'-position. Once the phosphonium salt was generated (XXVI), it was treated with base and a suitably functionalized ring system having an aldehyde in the 5'-position (XXVIII) to yield the protected trinucleotide (XXIX) (Scheme III). After deprotection, the desired trinucleotide is obtained (XXX).

As the total synthesis is rather long (over 30 steps), it will be discussed in several stages. The first stage is conversion of the ketone (XXI), obtained in two steps from D-glucose, as already discussed, to the bromo-aldehyde XXXV. This compound is both an intermediate to the fully functionalized A ring system (XXII in Scheme II) as well as the B ring system which is added to the A system via a Wittig reaction (XXIV in Scheme II).

Scheme III



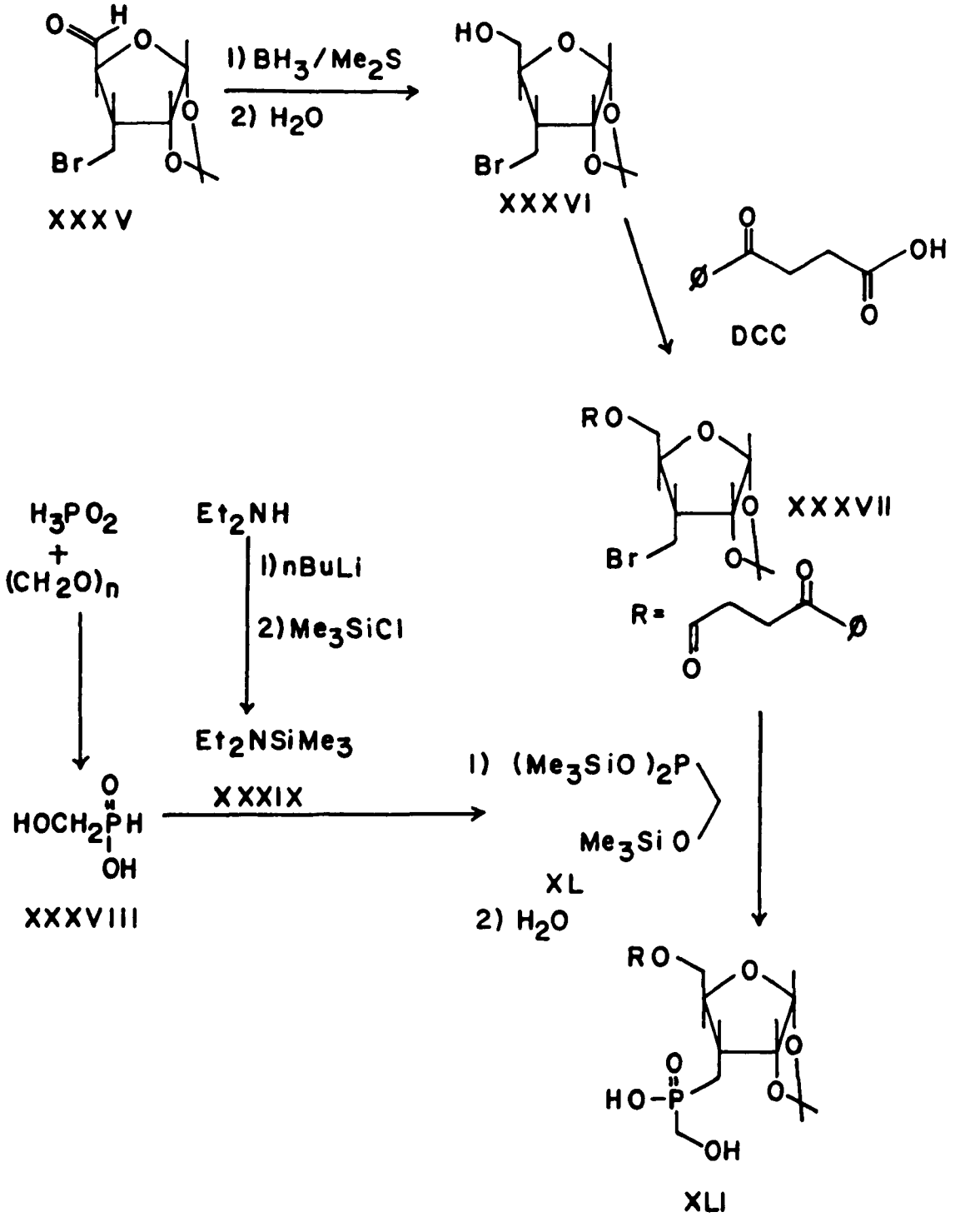
Scheme IV


Ø3P=CH2
XXI
XXXI
1) BH3/Me2S
2)NaOH/H2O2
NBS
Ø3P
Br
XXXIII
HO
XXXII
0.8% H2SO4
MeOH
HO
HO
Br
XXXIV
NaIO4
H2O
H
O
Br
XXXV

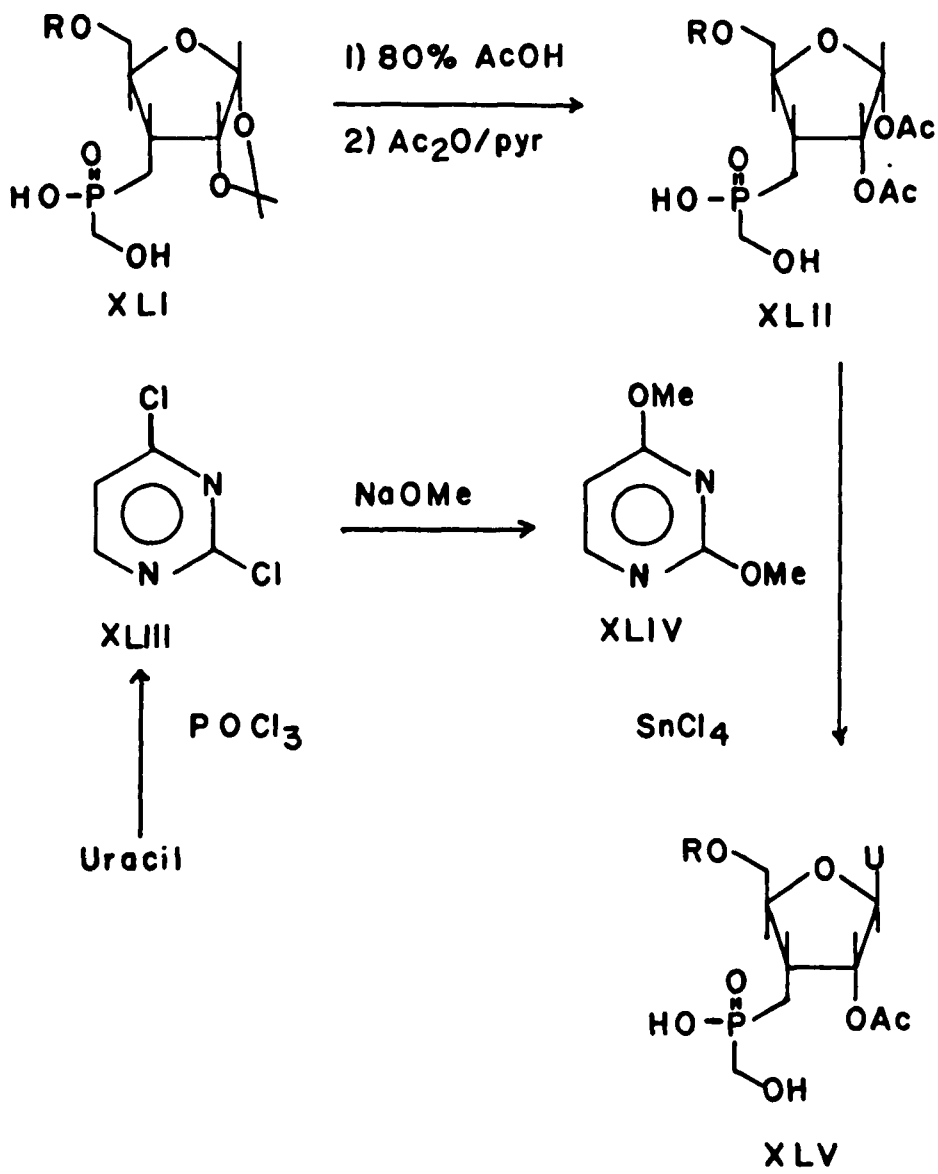
The ketone (XXI) underwent a Wittig reaction with methylene-triphenylphosphorane, generated by n-butyllithium and triphenylmethylphosphonium bromide, to yield the alkene (XXXI). In order to obtain the anti-Markovnikov alcohol from the alkene, XXXI was treated with a borane-dimethylsulfide complex, followed by oxidation with alkaline hydrogen peroxide to afford the primary alcohol (XXXII) in good yield. The primary alcohol (XXXII) was converted to the bromide (XXXIII) by using N-bromosuccinimide/triphenylphosphine (61).

Next, the 5,6-isopropylidene group was cleaved using 0.8% sulfuric acid in methanolic solution (62), yielding the 5,6-diol (XXXIV). Carbon-6 was cleaved by treating the diol (XXXIV) with 0.05M sodium metaperiodate (63). On workup with barium hydroxide, the aldehyde (XXXV) was obtained.

Scheme V


1) BH_3/Me_2S
2) H_2O
XXXV
XXXVI
DCC
XXXVII
R=
H_3PO_2
+
$(CH_2O)_n$
Et_2NH
1) nBuLi
2) Me_3SiCl
Et_2NSiMe_3
XXXIX
XXXVIII
1) $(Me_3SiO)_2P$
Me_3SiO
XL
2) H_2O
XLI

Scheme VI


RO
O
HO-P
O
OH
XLI
1) 80% AcOH
2) Ac2O/pyr
OAc
OAc
XLII
Cl
N
Cl
XLIII
NaOMe
OMe
OMe
XLIV
POCl3
Uracil
SnCl4
U
XLV

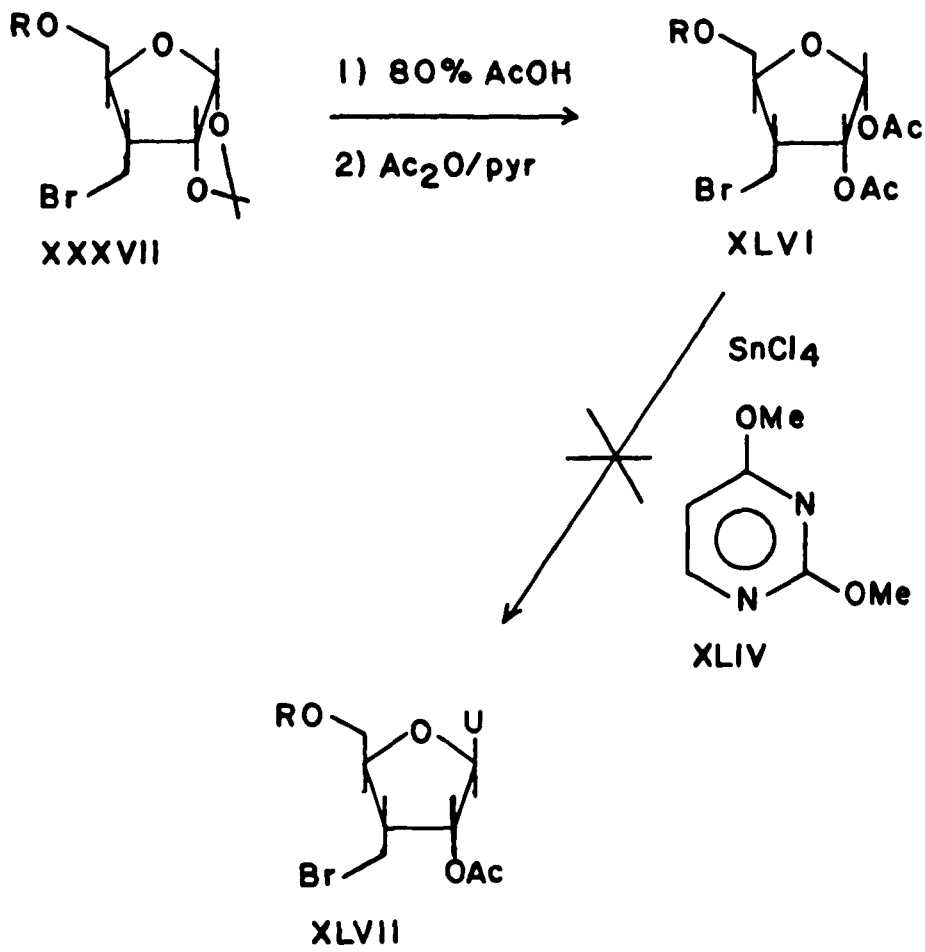
The next stage involves protection of the 5-oxygen and generation of the methyl phosphinic acid at the 3-position. The 5-oxygen was first reduced from the aldehyde (XXXV) to the alcohol (XXXVI) via the borane/dimethylsulfoxide complex, followed by hydrolysis. Borane was chosen as the reducing agent as it does not affect the 3-methyl bromide. The 5-alcohol was then protected with a 3-benzoyl propionyl group with DCC to yield XXXVII. This protecting group was chosen since it is stable to acid and can be removed under essentially neutral conditions (hydrazine hydrate in pyridine buffered with acetic acid) (64).

At this point the methyl phosphinic acid was generated at the 3-position. This was accomplished by reaction of XL with XXXVII to yield XLI after hydrolysis. The phosphonate reagent (XL) was made via the route reported by Rosenthal (65). Hypophosphorous acid was treated with paraformaldehyde to yield hydroxymethyl phosphinic acid (XXXVIII). This was then treated with trimethylsilyldiethylamine (XXXIX) to yield the phosphonate reagent XL. Trimethylsilyldiethylamine (XXXIX) was formed from the reaction of diethylamine and n-butyllithium with trimethylsilylchloride.

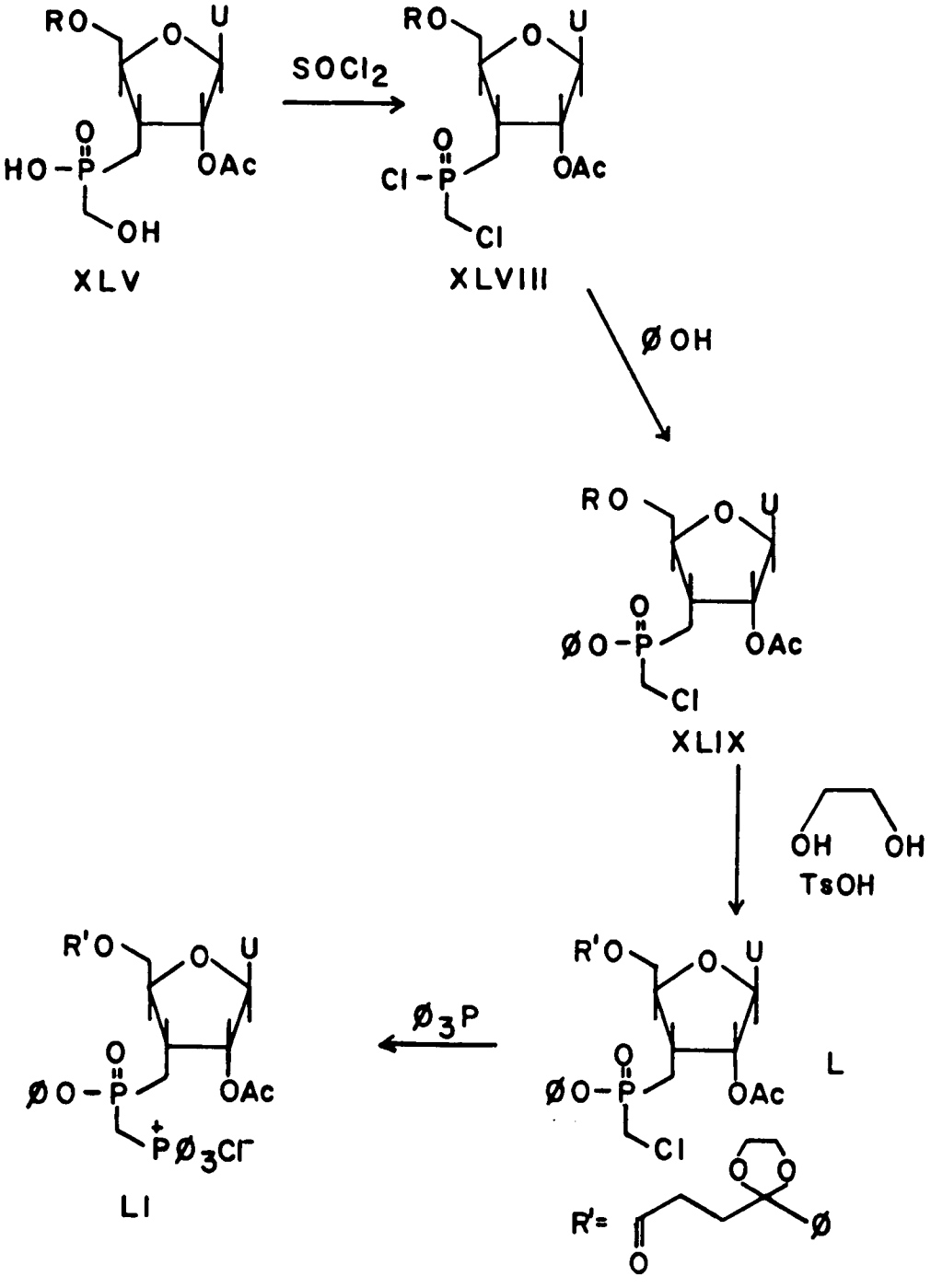
The 1,2-isopropylidene group was then cleaved using 80% acetic acid at 80 degrees C. for 24 hours. After removal of

the solvents under vacuum, the residue was treated directly with acetic anhydride and pyridine to yield XLII. The nucleoside base was then added using a modified (67) Hilbert-Johnson reaction (68). Compound XLII was treated with 2,4-dimethoxypyrimidine (XLIV) and stannic chloride to yield XLV. 2,4-Dimethoxypyrimidine (XLIV) was formed from sodium methoxide and 2,4-dichloropyrimidine (XLIII), which was formed from phosphorus oxychloride and uracil.

Scheme VII


RO
O
O
Br
O
XXXVII
1) 80% AcOH
2) Ac2O/pyr
RO
O
OAc
Br
OAc
XLVI
SnCl4
OMe
N
N
OMe
XLIV
RO
O
U
Br
OAc
XLVII

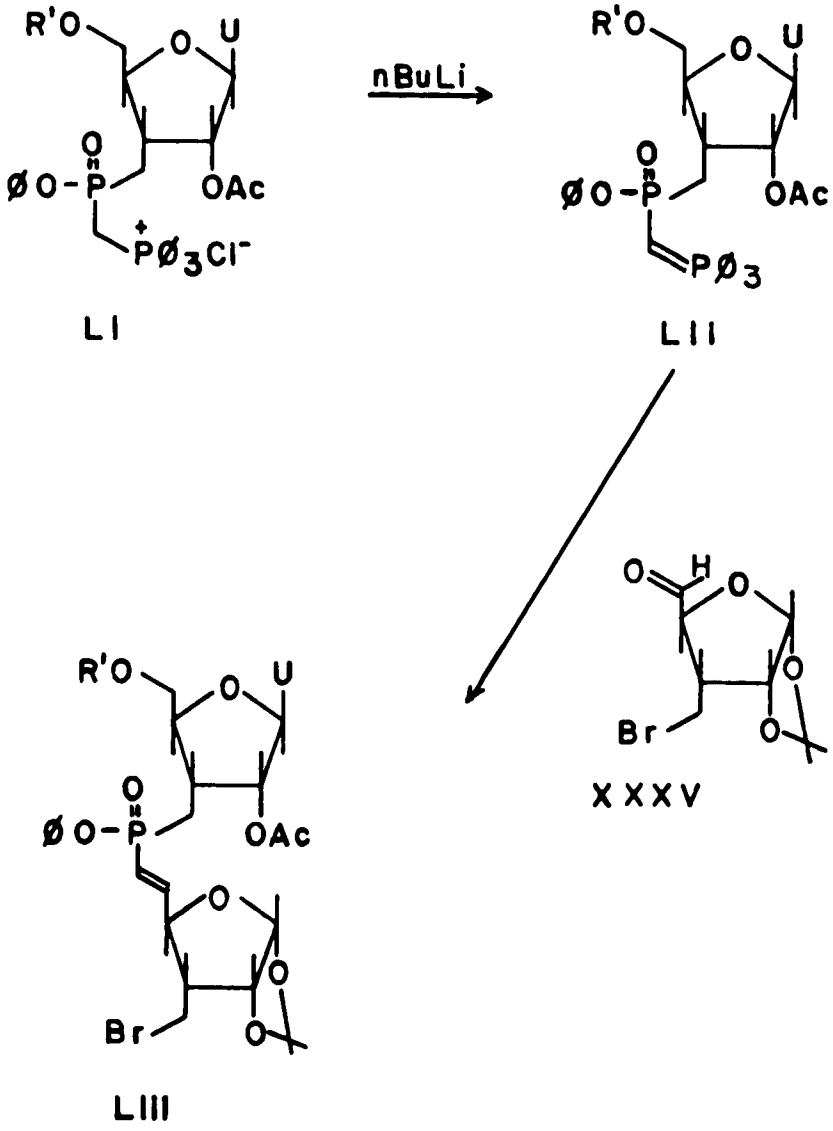
Scheme VIII


RO
U
O
HO–P
O
OAc
OH
XLV
SOCl2
Cl–P
Cl
XLVIII
ØOH
ØO–P
XLIX
OH
OH
TsOH
R'O
L
R'=
Ø
Ø3P
ØO–P
+PØ3Cl⁻
LI

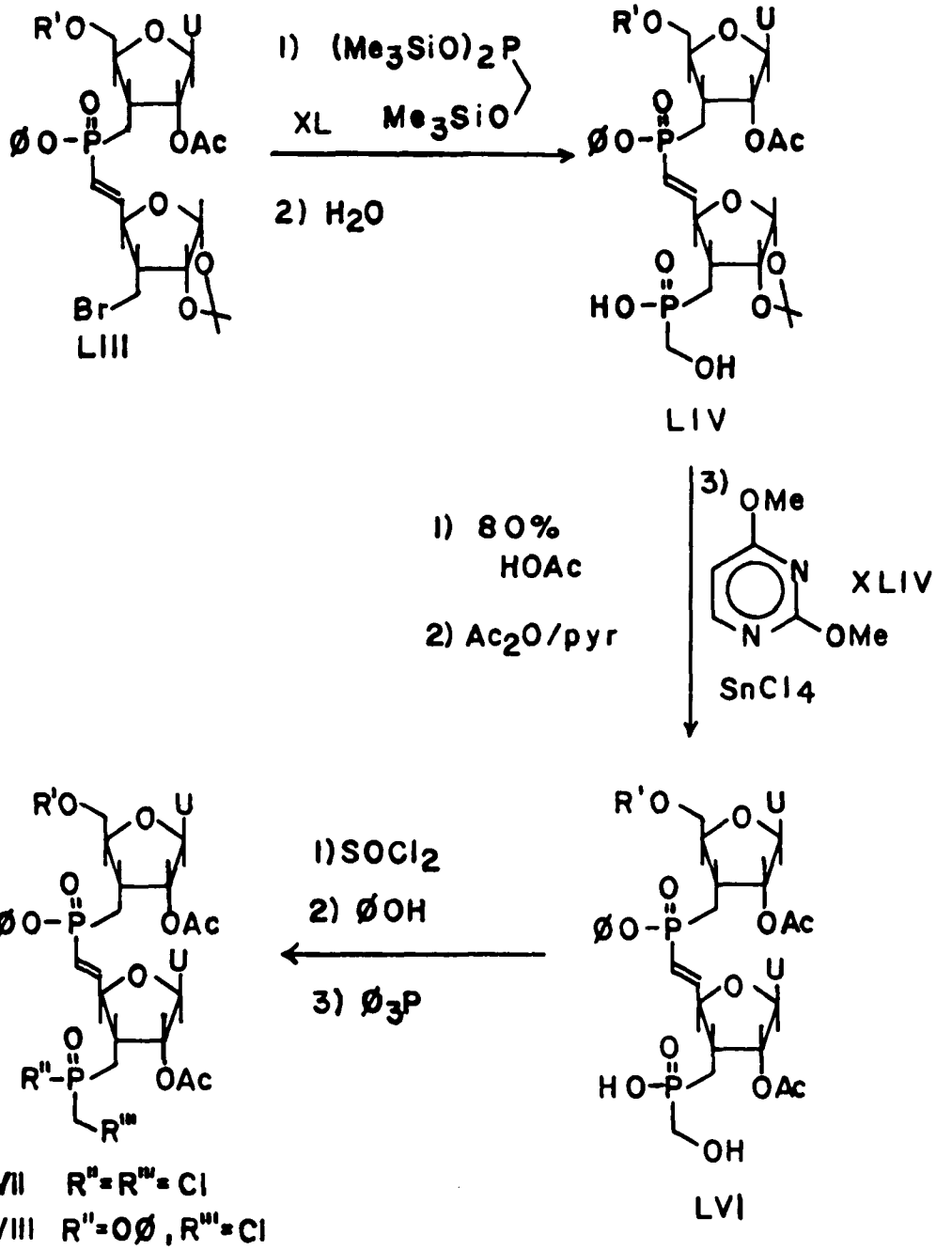
An alternative reaction sequence , in which the nucleoside base was added before the methyl phosphinic acid was generated at the 3'-position was attempted. Compound XXVII was treated first with 80% acetic acid, and then with acetic anhydride and pyridine to cleave the 1,2-isopropylidene group and then form the 1,2-diacetate (XLVI). The nucleoside base was then attempted to be added at the 1-position with 2,4-dimethoxypyrimidine (XLIV) and stannic chloride. Analysis showed that the bromide on the 3-methyl group was displaced rather than the acetate at the 1-position.

Returning to the succesful scheme, the phosphinic acid now needs to be converted to the phosphinic phosphonium salt. The phosphinic acid (XLV) was converted to the chloro-methyl phosphinyl chloride (XLVIII) with thionyl chloride (69). Treatment with phenyl gave the phenol ester (XLIX). At this point the ketone in the 3-benzoyl propionyl group must be protected because of the oncoming Wittig reaction. This was accomplished with p-toluenesulfonic acid and ethylene glycol to yield compound L. The phosphonium salt (LI) was then formed as a crystalline salt by treating compound L with triphenylphosphine.

Scheme IX



Scheme X



LVII $R'' = R''' = Cl$

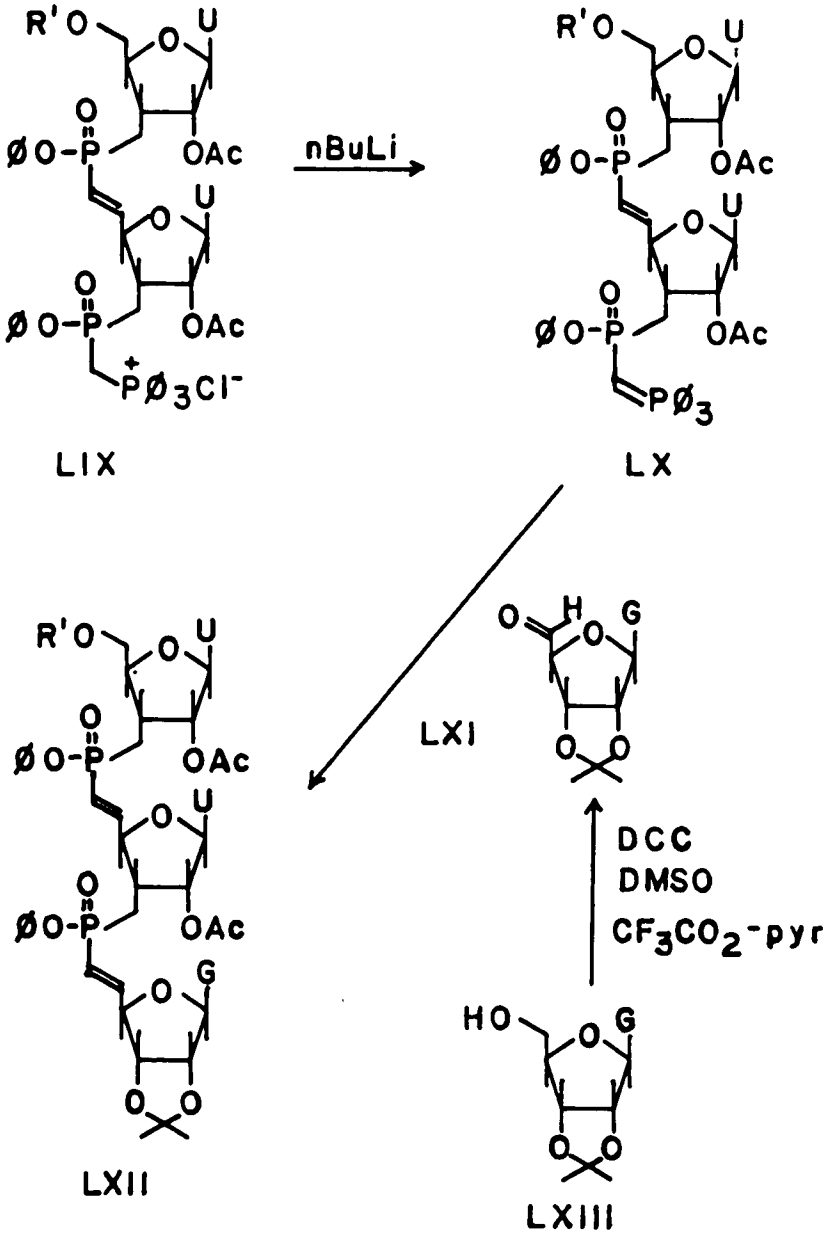
LVIII $R'' = O\emptyset$, $R''' = Cl$

LIX $R'' = O\emptyset$, $R''' = P\emptyset_3Cl$

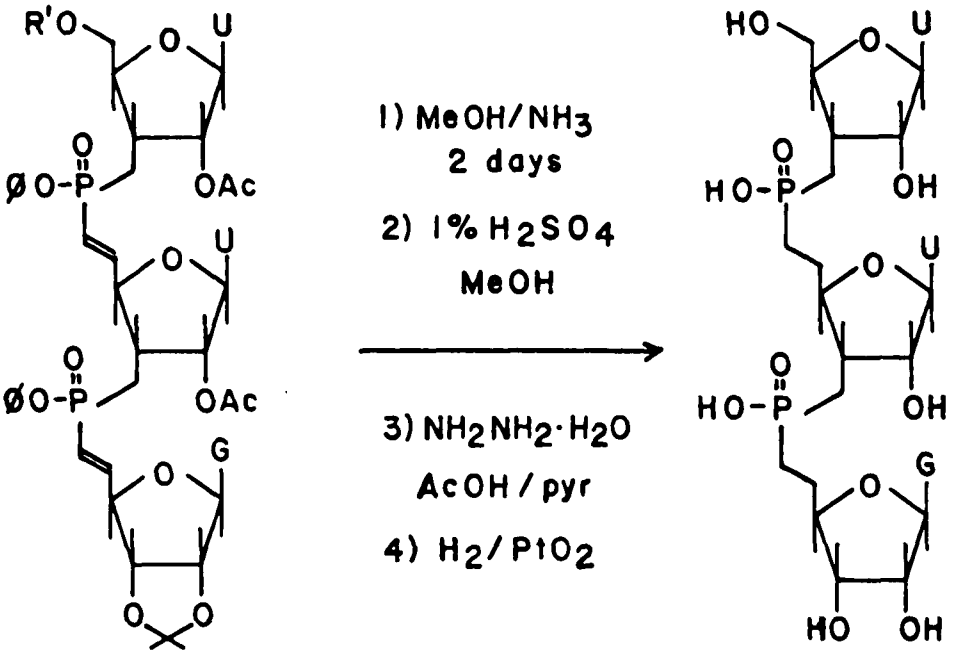
The A-B system is now formed. The phosphonium salt (LI) was treated with n-butyllithium to form the ylid (LII), which undergoes a Wittig reaction with the aldehyde XXXV, to yield compound LIII.

The functionalization of the B ring , i.e., the generation of the methyl phosphinic acid at the 3-position and the attachment of the base at the 1-position will now be discussed. The phosphinic acid function was the first to be added, by treating LIII with the phosphonate reagent XL, followed by hydrolysis, to yield LIV. The 1,2-isopropylidene group was then cleaved with 80% acetic acid, and directly converted to the 1,2-diacetate with acetic anhydride and pyridine to yield LV. The nucleoside base was attached at the 1-position using 2,4-dimethoxypyrimidine (XLIV) and stannic chloride , to yield LVI. The phosphinic acid (LVI) was then converted to the chloromethyl phosphinyl chloride (LVII) with thionyl chloride. The phenyl ester (LVIII) was obtained by reaction with phenol. The phosphonium salt (LIX) was then formed by treatment with triphenylphosphine.

Scheme XI


R'O
U
OAc
nBuLi
$P\emptyset_3Cl^-$
$P\emptyset_3$
LIX
LX
LXI
DCC
DMSO
$CF_3CO_2^-pyr$
HO
G
LXII
LXIII

Scheme XII



LXII

LXVII

Through another Wittig reaction, the C ring can now be attached. This was accomplished by treating LIX with n-butyllithium, to form the ylid, LX, and allowing it to react with a protected guanosine in which the 5'-position has been oxidized to an aldehyde (LXI) to yield the protected trinucleotide (LXII). Compound LXI was obtained by oxidizing 2',3'-isopropylidene guanosine (LXIII) with DCC, DMSO, and pyridinium trifluoroacetate.

The final four steps of the synthesis are for deprotection. Treatment of LXII with methanol saturated with ammonia on a mechanical shaker in a sealed bottle, heated by an IR lamp, cleaved the acetate groups in the 2'-positions of the A and B rings to yield LXIV. Treatment with 1% sulfuric acid in methanolic solution cleaved the 2',3'-isopropylidene group off the C ring to yield LXV. The 3-benzoyl propionyl group was removed by treatment with hydrazine hydrate in pyridine buffered by acetic acid, giving LXVI. Finally, the phenyl esters were removed and the double bonds were saturated with the use of hydrogen and platinum oxide catalyst to yield the finished trinucleotide, UUG (LXVII).

The UV data on the finished trinucleotide corresponds to the UV data of the naturally occurring nucleoside bases. Guanosine absorbs at 256 nm and uridine at 254 nm. The

absorption of the completed trinucleotide analogue is 256 nm.

The trinucleotide sequence UUG specifically codes for the amino acid leucine. Biochemical studies on the final product will potentially show the effects of the phosphinic acid linkages on the behavior of this codon.

The finished trinucleotide is extremely hygroscopic, which caused the elemental analysis to fluctuate.

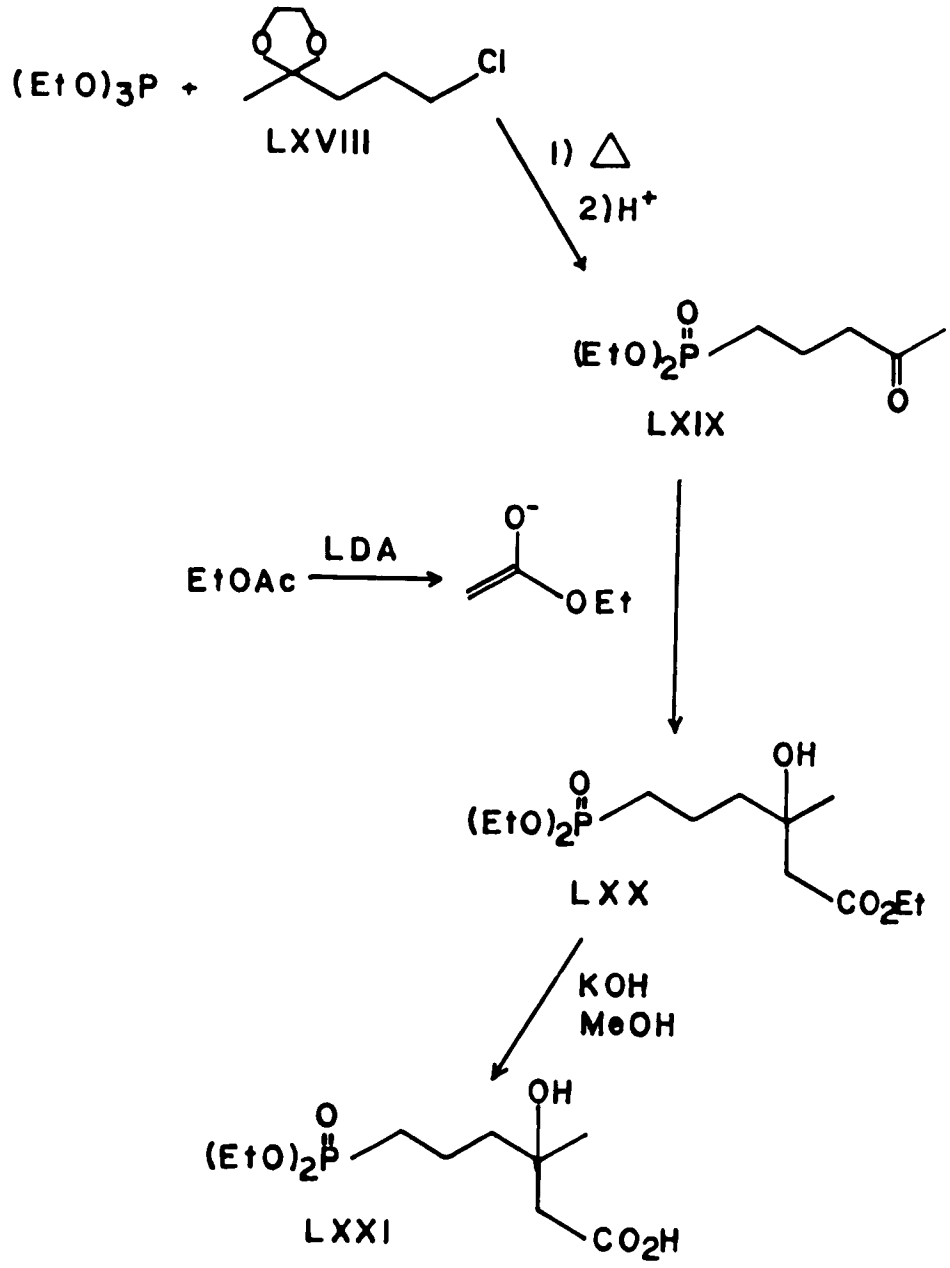
Part-2

Work on the phosphonate analogue of 5-phosphomevalonic acid was begun by Sarin, et. al. (70). The present synthesis involved treating the ketone LXIX, formed by an Arbuzov reaction (71) of triethylphosphite on the ethylene ketal of 5-chloro-2-pentanone (LXVIII), with ethylacetate. This was then treated by lithium diisopropylamine, to form LXX, which was hydrolyzed to LXXI (Scheme XIII).

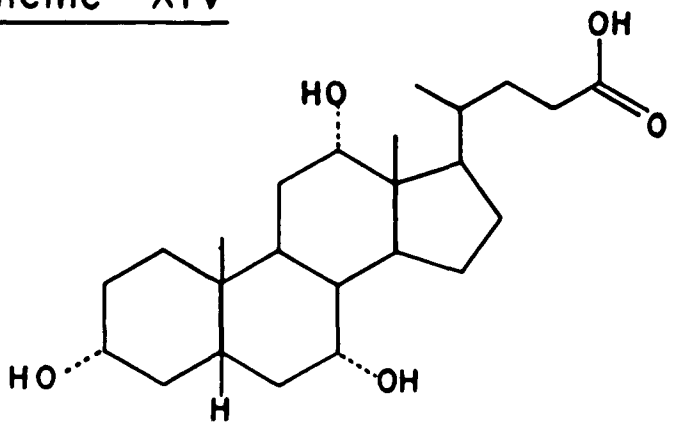
The next problem was to couple the analogue LXXI to a bile acid related material. An esterification appeared to be the most feasible route, so cholic acid (LXXII) was reduced with lithium aluminum hydride to the tetrahydroxy form (LXXIII).

Several methods were attempted to perform the esterification, including imidazole and dimethylformamide, and trichloroacetonitrile. The method that worked involved the use of triisopropylbenzenesulfonylchloride (LXXIV) in pyridine. The esterification resulted in the analogue (LXXI) coupled to LXXIII to form LXXV.

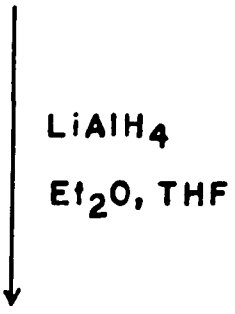
Scheme XIII

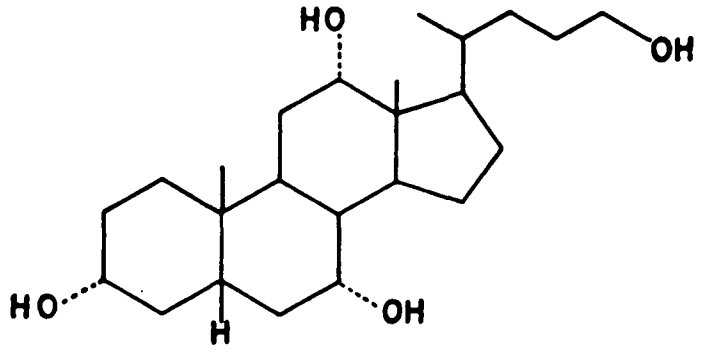

(EtO)3P +
Cl
LXVIII
1) △
2) H+
(EtO)2P
O
LXIX
EtOAc
LDA
O-
OEt
OH
LXX
CO2Et
KOH
MeOH
LXXI
CO2H

Scheme XIV



LXXII



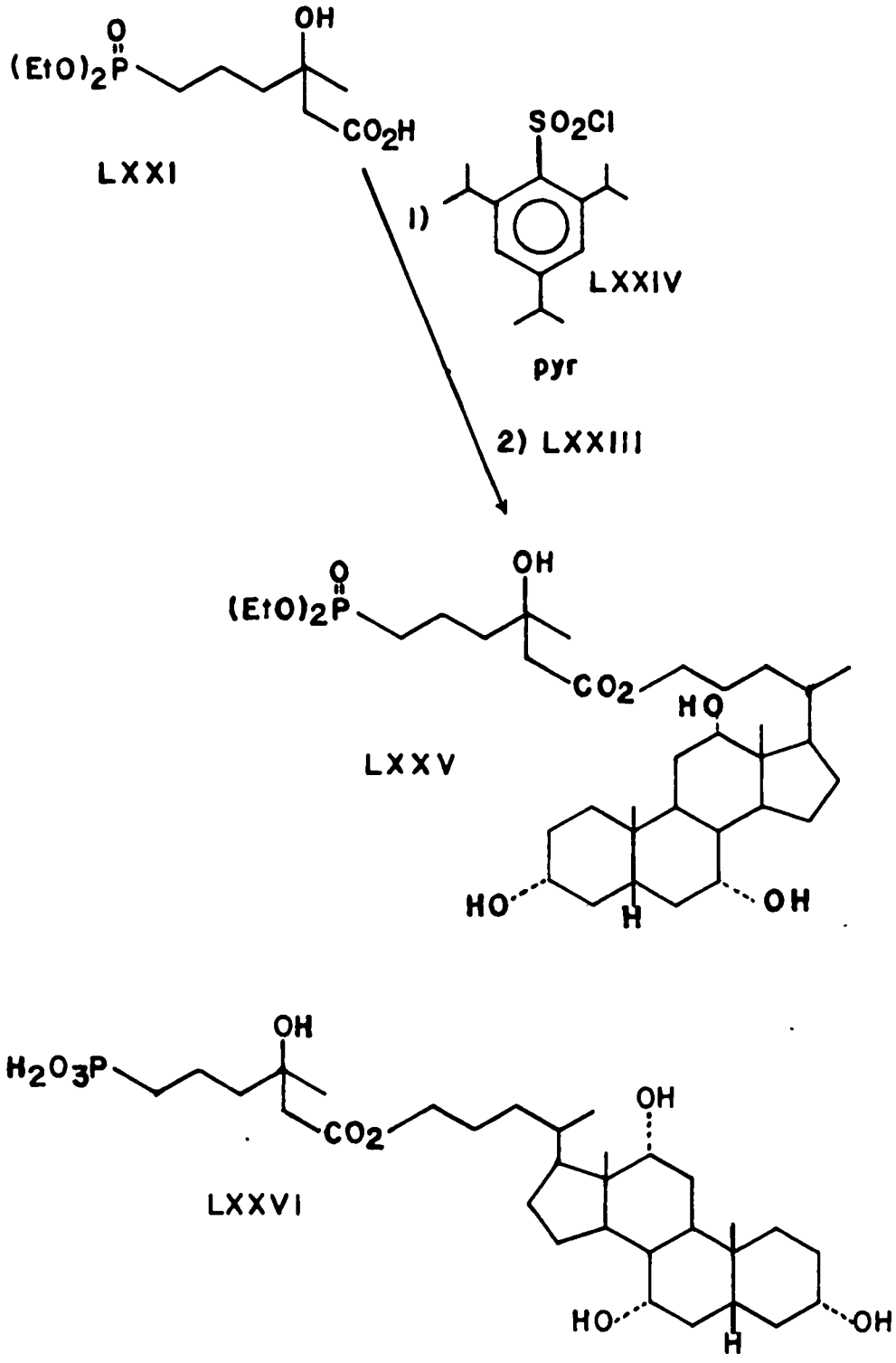


LXXIII

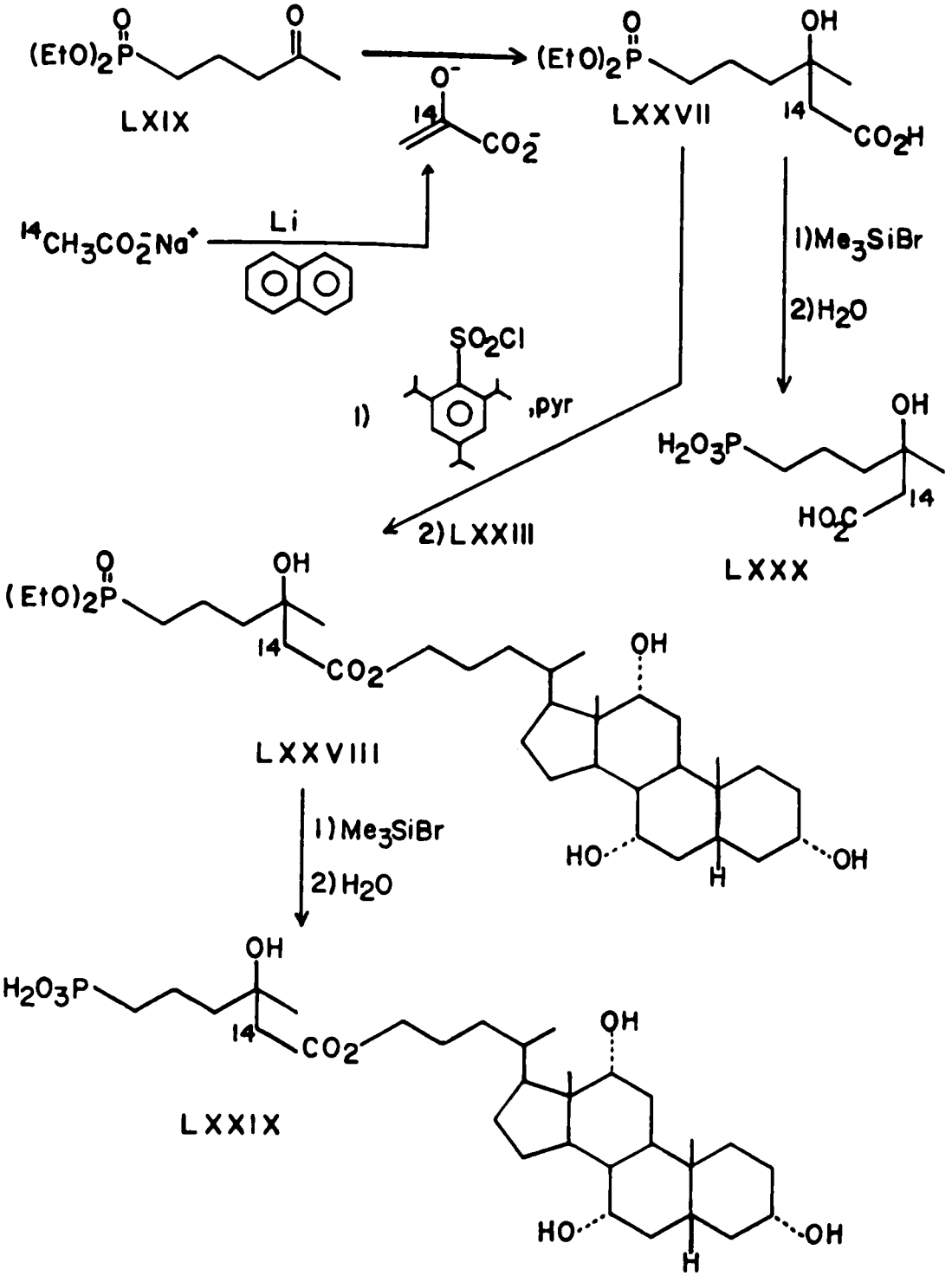
The remaining phosphonate diester was cleaved by the method of Rabinowitz (72), but by using trimethylsilylbromide (73) rather than trimethylsilylchloride. The bromide cleaved the esters overnight while the chloride took several days to cleave the esters.

The coupled analogue (LXXVI) was also synthesized with a carbon-14 label on the carboxy carbon. The synthesis started with the ketone LXIX. This was treated with the dianion of carbon-14 labelled acetic acid, formed by reaction with lithium metal and napthalene (74,75), to form the labelled analogue LXXVII. This route was chosen since labelled ethyl acetate is not available. The labelled analogue LXXVII was then coupled to LXXIII by treating with triisopropylbenzenesulfonylchloride and pyridine to yield LXXVIII. The phosphonate diester was cleaved by using trimethylsilylbromide, followed by hydrolysis to give LXXIX. The phosphonate diester on the analogue itself (LXXVII) was also cleaved by the same route yielding LXXX.

Scheme XV


(EtO)2P
O
OH
CO2H
LXXI
1)
SO2Cl
LXXIV
pyr
2) LXXIII
(EtO)2P
O
OH
CO2
HO
LXXV
HO
OH
H
H2O3P
OH
CO2
OH
LXXVI
HO
OH
H

Scheme XVI

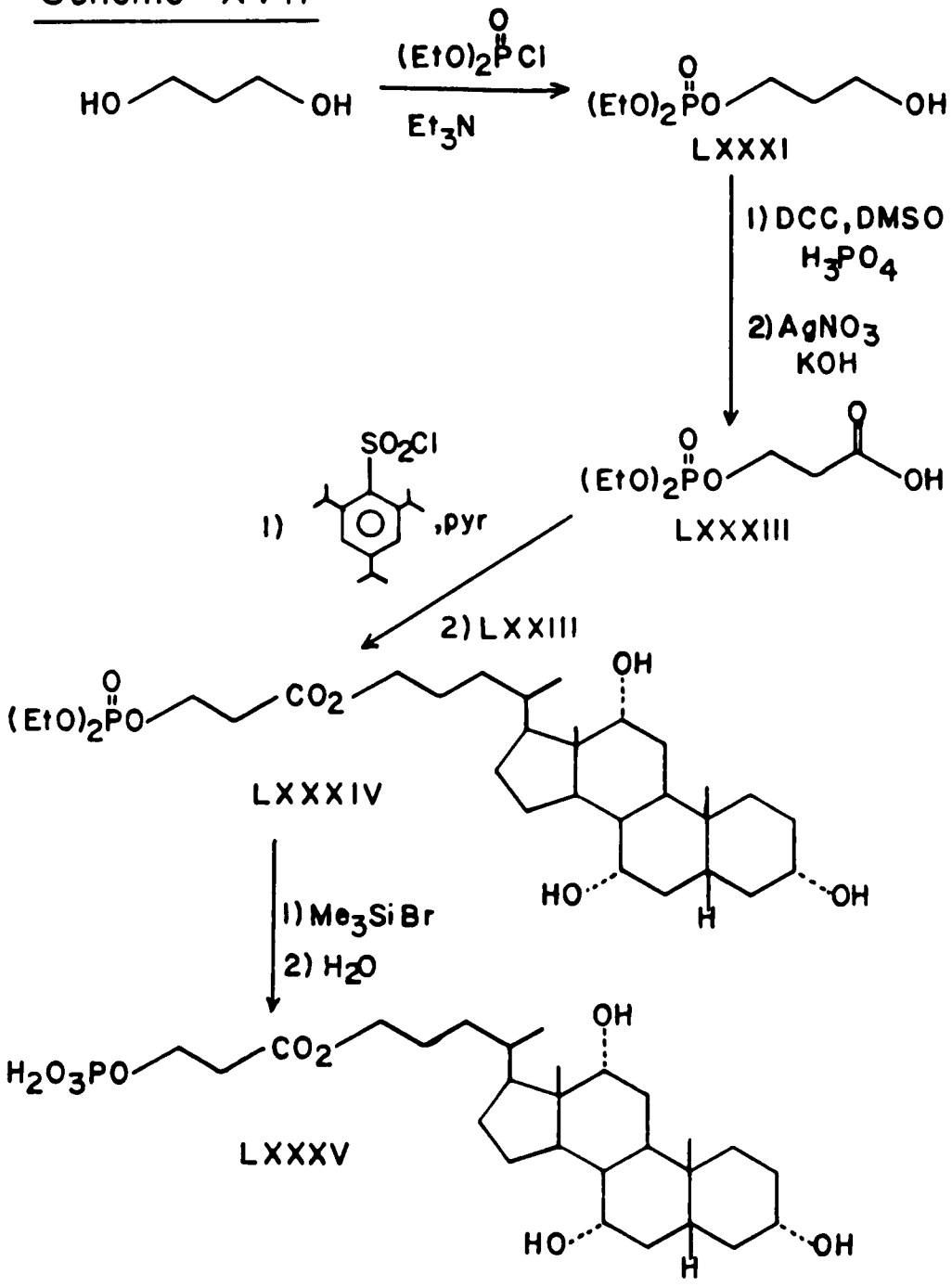

(EtO)2P
O
LXIX
14CH3CO2-Na+
Li
LXXVII
1) Me3SiBr
2) H2O
1) SO2Cl, pyr
2) LXXIII
H2O3P
HO2C
LXXX
LXXVIII
LXXIX
HO
OH
H

Compounds LXXI, LXXVI, LXXX, and LXXIX were tested biologically (76,77,78) by Professor T. Parker at Rockefeller University, using the supernatant of homogenized rat liver supplemented with cofactors and incubated under nitrogen. The activity of the agent was measured by comparison of carbon dioxide release and phosphate uptake upon addition of natural substrate to the enzyme system. Cholesterol biosynthesis was found to be inhibited. The supernatant of homogenized rat liver contains all the enzymes needed to convert mevalonate to sterols. Testing of the labelled material with rat liver hepatocytes has shown that these compounds are taken up from solution by the cells which are performing the cholesterol biosynthesis in the liver.

A series of phosphates were also linked to LXXIII for biological control experiments. The first of these compounds (LXXV) was synthesized as follows.

Diethylchlorophosphate was treated with 1,3-propanediol in the presence of triethylamine to produce LXXXI. The alcohol was oxidized to the aldehyde (LXXXII) using DCC, DMSO, and phosphoric acid. This was oxidized to the acid (LXXXIII) using silver nitrate and potassium hydroxide, after which it was coupled to LXXIII by treating with triisopropylbenzenesulfonylchloride and pyridine to produce

Scheme XVII

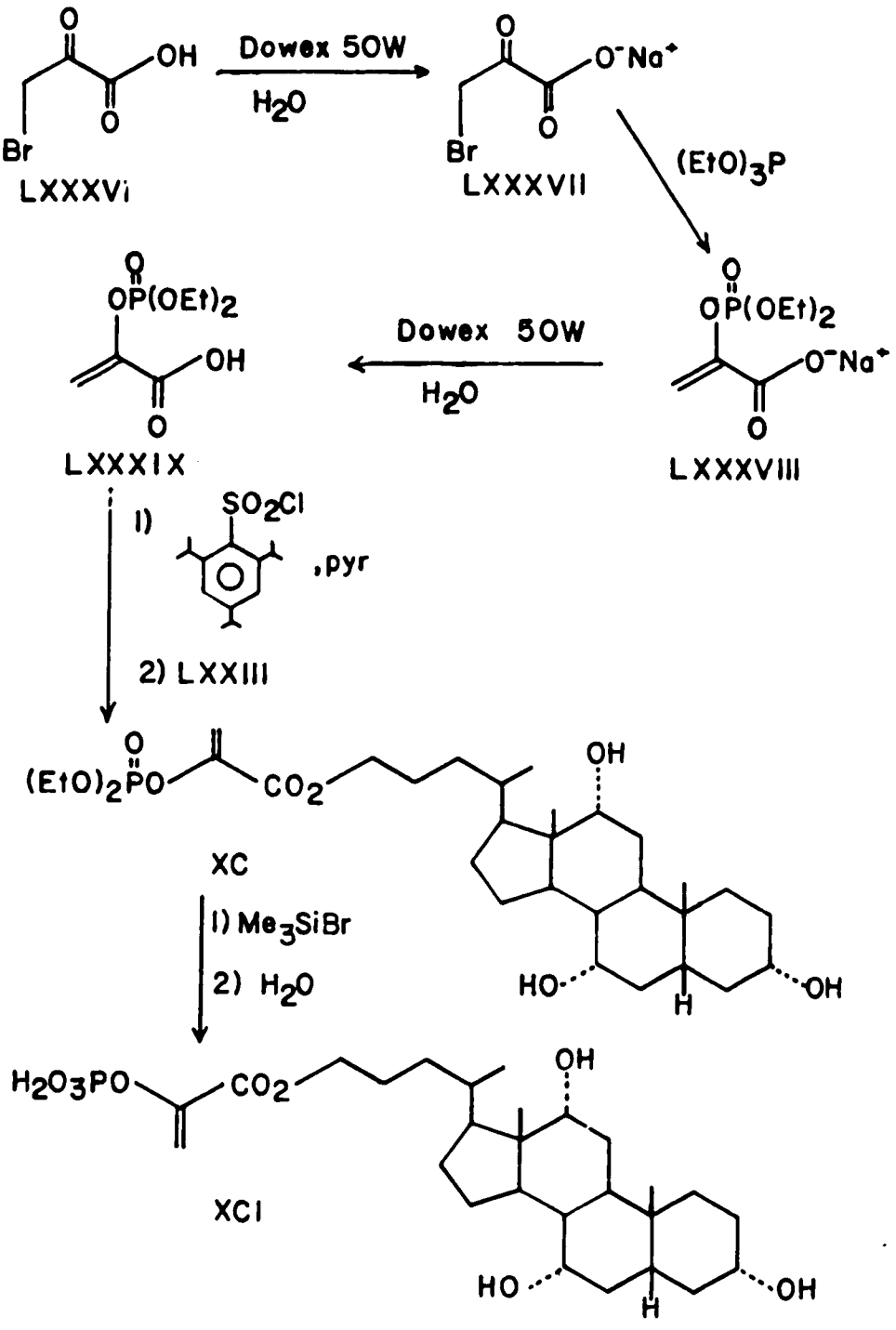

HO
OH
(EtO)2PCl
Et3N
(EtO)2PO
OH
LXXXI
1) DCC, DMSO
H3PO4
2) AgNO3
KOH
SO2Cl
1)
,pyr
(EtO)2PO
OH
LXXXIII
2) LXXIII
(EtO)2PO
CO2
OH
LXXXIV
HO
OH
H
1) Me3SiBr
2) H2O
H2O3PO
CO2
OH
LXXXV
HO
OH
H

LXXXIV. The phosphonate diester was cleaved by using trimethylsilylbromide, followed by hydrolysis to yield LXXXV.

Another phosphate linked to LXXIII was LXXXIX. The synthesis began by treating 3-bromopyruvic acid (LXXXVI) with the basic form of Dowex 50W to generate LXXXVII. This was treated with triethylphosphite to yield LXXXVIII, which was treated with the acid form of Dowex 50W to generate the acid LXXXIX. This was coupled to LXXIII by treating first with triisopropylbenzenesulfonylchloride in pyridine, and then LXXIII. The phosphonate diester was cleaved using trimethylsilylbromide, followed by hydrolysis to yield XCI.

Finally, LXXIII was tetra-acetylated with acetic anhydride and pyridine to yield XCII.

Scheme XVIII



EXPERIMENTAL

General

All chemicals were of reagent quality and used without further purification with the following exceptions: pyridine was distilled and stored over KOH pellets; hexane was dried over sodium metal; THF was distilled over lithium aluminum hydride and stored over molecular sieves; methanol was distilled over magnesium metal prior to use; dimethylsulfoxide was distilled over calcium hydride and stored over molecular sieves; thionyl chloride, stannic chloride, triethylamine, and diethylamine were distilled prior to use. Thin layer chromatography was performed using Polygram Sil-N-HR sheets (Brinkman); visualization was effected using an iodine chamber and spray reagents such as molybdate spray (for phosphorus containing compounds). Silica gel for column chromatography was purchased from Baker Chemicals (40-140 mesh). Separations were performed on Waters Associates analytical and Prep500 HPLC. Infrared spectra were measured using a Perkin-Elmer 598 spectrophotometer, ultra-violet spectra were measured with a

Perkin-Elmer Lambda 3 spectrophotometer, and nmr spectra were obtained with a Varian EM-360 instrument.

Synthesis of diacetone glucose (XX) (Ref. 58):

D-glucose (450 g, 2.5 moles) was stirred with 360 g anhydrous zinc chloride, 22.5 g 85% phosphoric acid, and 3 liters acetone for 2 days at room temperature. The mixture was then filtered and the undissolved glucose washed with acetone. The filtrate was brought to pH 8 with 50% KOH while stirring in an ice bath. The precipitated zinc oxide was filtered and washed with acetone. The combined washings and filtrate were concentrated under vacuum. The resulting material was dissolved in water and extracted with chloroform. The combined chloroform extracts were washed with water, dried over anhydrous magnesium sulfate, filtered, and the filtrate concentrated under vacuum to yield 116 g (50.2%) of pure XXI. Analytical data: nmr ($CD(Cl)_3$, δ): 1.45(d of d,12H), 2.65(d,1H), 3.9-4.65(m,6H), 6.05(d,1H).

Synthesis of 1,2:5,6-di-O-isopropylidene-α-D-ribo-hexos-3-ulose (XXI) (Ref. 58,59):

Diacetone glucose (30 g, 0.12 mole) was stirred in 350 mL dimethylsulfoxide and 230 mL acetic anhydride in a tightly stoppered round bottomed flask overnight at room temperature. The volatile materials were then removed from the reaction mixture by vacuum distillation at 32-45 C at

0.2 Torr. The product was isolated by vacuum distillation at 112-120 C at 0.006 Torr to yield 18 g (58%) of pure XXXI. Analytical data: nmr (CD(Cl)3,δ): 1.4(d,12H), 3.9-4.5(m,5H), 6.15(d,1H); ir (CH(Cl)3,cm-1): 2990, 1775, 1375, 1235, 1163, 1065, 855.

Synthesis of 1,2:5,6 di-O-isopropylidene-3-deoxy-3-methylene- α -D-hexofuranose (XXXI):

Methyltriphenylphosphonium bromide (57.3 g, 0.16 mole) was stirred in 700 mL anhydrous ether under nitrogen. To this was added dropwise 100 mL n-butyllithium (1.6 M in hexane), and the mixture was stirred for 1 hour. Compound XXI (41.4 g, 0.16 mole) in 100 mL anhydrous ether was added dropwise and the mixture stirred overnight at room temperature. The reaction mixture was then filtered, washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The product was isolated by vacuum distillation at 75-80 C at 0.004 Torr to yield 25.9 g (65%) of pure XXXII. Analytical data: nmr (CD(Cl)3,δ): 1.1-1.5(m,12H), 3.65-4.2(m,2H), 4.35-4.6(broad s,1H), 4.8(d,1H), 5.35(m,2H), 5.75(d,1H); ir (CH(Cl)3,cm-1): 2995, 1452, 1380, 1370, 1235, 1162, 1050, 1005, 930, 840.

Synthesis of 1,2:5,6 di-O-isopropylidene-3-deoxy-3-hydroxymethyl-α -D-hexofuranose (XXXII):

Compound XXXI (13.2 g, 0.051 mole) was stirred in 100 mL sodium dried hexane under a nitrogen atmosphere. To this was added 20 mL of borane-dimethylsulfide complex (10M) dropwise over a period of 30 minutes, while cooling in an ice bath. The mixture was stirred for two hours at room temperature, after which 100 mL absolute ethanol was added dropwise while cooling in an ice bath. Sodium hydroxide (64 mL, 3N) was then added, followed by the addition of 22 mL of 30% hydrogen peroxide, added dropwise over a period of 15 minutes. The ice bath was removed, and the mixture stirred overnight at room temperature. Ether (200 mL) was added, and the aqueous phase was saturated with sodium chloride. The mixture was filtered, the ether phase separated, and the aqueous phase extracted with ether. The combined ether extracts were washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum to yield 11.9 g (87.9%) of pure XXXII. Analytical data: nmr (CD(Cl)3, δ): 1.1-1.6(m,12H), 2.6(s,2H), 3.0(s,1H), 3.3-4.3(m,5H), 4.55(d,1H), 4.65(t,1H), 5.7(m,1H); ir (CH(Cl)3,cm-1): 3470, 2980, 1452, 1382, 1372, 1308, 1240, 1162, 1060, 1010, 872, 845.

Synthesis of 1,2:5,6 di-O-isopropylidene-3-deoxy-3-bromomethyl-α-D-hexofuranose (XXXIII):

Compound XXXII (31.85 g, 0.12 mole) was stirred in 450 mL dry dimethylformamide in a 1 liter round bottom flask fitted with a drying tube. Triphenylphosphine (61 g, 0.24 mole) was added, and the mixture was then cooled in an ice bath. N-bromosuccinimide (42 g, 0.24 mole) was added in portions over a period of 10 minutes. The mixture was stirred overnight at room temperature, after which 100 mL methanol was added, and the solvents were evaporated under vacuum. The residue was fractionated between ether and water, dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The residue was purified on a silica gel column eluted with ethylacetate:hexane (1:9) to yield 19.5 g (50%) of pure XXXIII (Rf=0.4 ethylacetate:hexane,1:9). Analytical data: nmr (CD(Cl)3,δ): 1.2-1.6(m,12H), 2.1-2.6(m,2H), 3.3-4.2(m,5H), 4.75(t,1H), 5.75(d,1H); ir (CH(Cl)3,cm-1): 2990, 1665, 1430,1375, 1235, 1165, 1115, 1055, 1010, 840.

Synthesis of 1,2-O-isopropylidene-3-deoxy-3-bromomethyl-α-D-hexofuranose (XXXIV):

Compound XXXIII (35.74 g, 0.11 mole) was dissolved in 150 mL methanol and mixed with 150 mL of 0.8% sulfuric acid overnight at room temperature. The solution was neutralized with sodium bicarbonate, filtered and extracted with chloroform. The combined chloroform extracts were dried and evaporated under reduced pressure to yield 33 g (97%) of pure XXXIV. Analytical data: nmr (C(Cl)4, δ): 1.2-1.7(m,8H), 2.2-2.6(m,1H), 2.95(d,1H), 3.3-4.4(m,5H), 4.75(t,1HO, 5.7(d,1H); ir (C(Cl)4,cm-1): 3410, 2990, 1670, 1435, 1375, 1230, 1165, 1120, 1065, 1010, 860.

Synthesis of 1,2-O-isopropylidene-3-deoxy-3-bromomethyl-5-oxo-α-D-ribofuranose (XXXV):

Compound XXXIV (4.2 g, 0.014 mole) was dissolved in 100 mL water. The solution was cooled in an ice bath and covered with aluminum foil to exclude light. A 0.05M solution of sodium metaperiodate (400 mL) was added dropwise. The mixture was then stirred overnight at room

temperature. There was then added 8 g of barium hydroxide, the mixture filtered, and the filtrate concentrated under vacuum to yield 2.4 g (57%) of pure XXXV. Analytical data: nmr (CD(Cl)3,δ): 1.1-1.7(m,4H), 2.75(s,1H), 2.95(d,2H), 3.3-4.2(m,4H), 4.4-5(m,1H), 5.7-6.0(m,1H); ir (CH(Cl)3,cm-1): 3000, 2880, 1710, 1670, 1385, 1260, 1095, 1030, 860.

Synthesis of 1,2-O-isopropylidene-3-deoxy-3-bromomethyl-α-D-ribofuranose (XXXVI):

Compound XXV (2.4 g, 0.008 mole) was stirred in 50 mL dry tetrahydrofuran under a nitrogen atmosphere. To this was added dropwise 2.2 mL of borane-dimethylsulfide complex (10M) . The mixture was stirred overnight. There was added 12 mL of water dropwise while cooling in an ice bath. The reaction mixture was extracted with chloroform, the combined chloroform extracts dried, and evaporated under reduced pressure to yield 1.9 g (85%) of pure XXXVI. Analytical data: nmr (CD(Cl)3,δ): 1.1-1.4(m,5H), 1.6(q,1H), 2.4(s,1H), 2.7(d,1H), 3.1-3.8(m,4H), 4.5(t,1H), 5.6(d,1H); ir (CH(Cl)3,cm-1): 3300, 2990, 1670, 1420, 1380, 1170, 1010, 875.

Synthesis of 1,2-O-isopropylidene-3-deoxy-3-bromomethyl-5-O-(3-benzoyl-propionyl)-α-D-ribofuranose (XXXVII):

Compound XXXVI (5.4 g, 0.02 mole) was stirred with 10.7 g (0.06 mole) of 3-benzoyl propionic acid and 16.5 g (0.08 mole) of DCC in 125 mL pyridine overnight at room temperature. There was added 20 mL water dropwise, and the mixture stirred for 2 hours. The reaction mixture was filtered, the precipitate was washed with chloroform, and concentrated under vacuum. The product was isolated by column chromatography using silica gel eluted with chloroform:ethylacetate (2:1) to yield 4 g (50%) of pure XXXVII (Rf=0.6 chloroform:ethylacetate,2:1). Analytical data: nmr (CD(Cl)3,δ): 1.1-2.1(m,12H), 2.6-2.9(m,2H), 3.15-3.6(m,3H), 3.8-4.6(m,4H), 5.75(d,1H),7.2-8.1(m,5H); ir (CH(Cl)3,cm-1): 3000, 2930, 1730, 1685, 1450, 1220, 1160, 1010.

Synthesis of hydroxymethyl phosphonous acid (XXXVIII) (Ref. 65):

A 50% solution of hypophosphorus acid (210 mL) was concentrated under vacuum at a temperature not exceeding 45

C until a concentration of 80% was obtained. Paraformaldehyde (58.42 g) was gradually added over a period of 1.5 hours at 40 C. The mixture was stirred for 12 hours at 50 C, and then for 30 hours at 60 C. The solution was concentrated under vacuum in the presence of excess formaldehyde to yield 16.7 g (87%) of XXXVIII.

Synthesis of trimethylsilyl-diethylamine (XXXIX) (Ref. 65):

A 100 mL solution of n-butyllithium (1.6M in hexane) was stirred in 100 mL pentane at -20 C under nitrogen. Diethylamine (17 mL, 0.16 mole) was added dropwise. Trimethylsilylchloride (22 mL, 0.17 mole) in 50 mL pentane was then added dropwise at -5 C. The mixture was stirred overnight at room temperature, after which the precipitate was filtered and the product collected by distillation at 120-126 C to yield 18 g (80%) of pure XXXIX.

Synthesis of bis-trimethylsilyl-trimethylsiloxymethyl phosphonite (XL) (Ref. 65):

To 3.1 g (0.032 mole) of XXXVIII in 15 mL acetonitrile was added dropwise 16 g (0.11 mole) of XXXIX at 0 C under nitrogen. The mixture was then refluxed for 2 hours, after which the solvent was evaporated under vacuum and the product collected by vacuum distillation at 54 C, 0.005 Torr to yield 6.3 g (66%) of pure XL.

Synthesis of 1,2-O-isopropylidene-3-deoxy-3-(hydroxy hydroxymethyl phosphinyl methyl)-5-O-(3-benzoyl-propionyl)-α-D-ribofuranose (XLI):

A mixture of 7.5 g (0.024 mole) of XL and 10.4 g (0.026 mole) of XXXVII in 20 mL tetrahydrofuran was refluxed for 4 hours under nitrogen. After cooling to room temperature, a mixture of 10 mL tetrahydrofuran, 10 mL t-butyl alcohol, and 10 mL water was added, and the reaction mixture stirred for two hours at room temperature. The mixture was concentrated under vacuum, 25 mL of water was added, and the mixture was extracted with chloroform. The combined chloroform extractions were dried, and concentrated on a rotary evaporator. The product was isolated on a C18 reverse phase column eluted with methanol:water (9:1) using a Waters Prep500 HPLC to yield 5.6 g (63%) of pure XLI (Rf=0.75

methanol:water,9:1). Analytical data: nmr (CD(Cl)3,δ): 1.1-2.1(m,18H), 2.85(t,2H), 3.5(t,2H), 3.9-4.4(m,1H), 7.3-8.2(m,5H); ir (CH(Cl)3,cm-1): 3310, 3000, 2925, 2850, 1695, 1650, 1520, 1450, 1400, 1360, 1230, 1000, 785, 690.

Analysis: calculated for C(20)H(27)O(9)P: C,54.30; H,6.15% found: C,54.60; H,6.45%

Synthesis of 1,2-di-O-acetyl-3-deoxy-3-(hydroxy hydroxymethyl phosphinyl methyl)-5-O-(3-benzoyl-propionyl)-α-D- ribofuranose (XLII):

Compound XLI (3 g, 0.008 mole) was heated with 120 mL of 80% acetic acid at 80 C overnight. The solvents were evaporated under vacuum, and the residue stirred with 30 mL acetic anhydride and 60 mL pyridine overnight at room temperature. The mixture was concentrated under vacuum, and the product isolated by column chromatography using a silica gel column eluted with chloroform:ethylacetate (1:1) to yield 2.5 g (60%) of pure XLII (Rf=0.3 chloroform:ethylacetate,1:1). Analytical data: nmr (CD(Cl)3, δ): 1.1-2.2(m,16H), 2.85(t,2H), 3.5(t,2H), 3.85-4.3(m,1H), 7.3-8.2(m,5H); ir (CH(Cl)3,cm-1): 3310, 3000, 2925, 2850, 1750, 1695, 1650, 1520, 1450, 1400, 1215, 910, 780, 730, 670.

Synthesis of 2,4-dichloropyrimidine (XLIII) (Ref. 67):

A mixture of 30 g (0.27 mole) uracil and 120 mL of phosphorus oxychloride in a 250 mL round bottom flask fitted with a reflux condenser and a drying tube was heated at 110 C for 2.5 hours. After cooling to room temperature, the excess phosphorus oxychloride was distilled (30 C, 20 Torr). The remaining material was poured, very slowly, over 270 g crushed ice. There then was added 50 mL ether, and the mixture was filtered. The ether layer was separated, and the aqueous phase extracted with ether. The combined ether phases were dried, and concentrated on a rotary evaporator to yield 24.1 g (60%) of pure XLIII.

Synthesis of 2,4-dimethoxypyrimidine (XLIV) (Ref. 67):

Sodium (6.4 g) was slowly added to 100 mL of methanol and stirred for 45 minutes under a nitrogen atmosphere. To this was added 18.3 g (0.12 mole) of XLIII in 100 mL methanol dropwise. After refluxing for 45 minutes, the solution was cooled to room temperature, filtered, the precipitate washed with ether, and the filtrate concentrated under vacuum. The residue was dissolved in ether, washed

twice with 30% sodium hydroxide and then with water. The ether phase was dried and concentrated, after which the product was isolated by vacuum distillation at 54 C, 0.024 Torr to yield 13.2 g (76%) of pure XLIV. Analytical data: nmr (CD(Cl)3,δ): 3.45(d,6H), 5.82(d,1H), 7.65(d,1H).

Synthesis of 2'-O-acetyl-3'-deoxy-3'-(hydroxy hydroxymethyl phosphinyl methyl)-5'-O-(3-benzoyl propionyl)-uridine (XLV):

To 2 g (0.004 mole) of XLII in 80 mL 1,2-dichloroethane and 4 mL of 2,4-dimethoxypyrimidine was slowly added 2 mL of stannic chloride. After refluxing for 4 hours, the mixture was cooled to room temperature, 50 mL of saturated aqueous sodium bicarbonate was added, and the mixture stirred for 10 minutes. The precipitate was filtered and washed with 1,2-dichloroethane. The organic layer of the filtrate was separated, and the aqueous phase was extracted with 1,2-dichloroethane. The combined organic phases were dried and concentrated on a rotary evaporator. The product was isolated from a silica gel column eluted with chloroform:methanol (98:2) to yield 1.1 g (56%) of pure XLV (Rf=0.3 chloroform:methanol,98:2). Analytical data: nmr (CD(Cl)3,δ): 0.9-2.2(m,12H), 2.75(t,2H), 3.4-3.7(m,4H),

4.15(s,1H), 6.0(d,1H), 6.2-6.5(m,1H), 7.8-8.2(m,5H); ir (CH(Cl)3,cm-1): 3680, 3600, 3000, 2920, 2400, 1710, 1670, 1530, 1480, 1330, 1210, 1040, 1010, 935, 750, 665.

Synthesis of 1,2-di-O-acetyl-3-deoxy-3-bromomethyl-5-(3-benzoyl propionyl)-α-D-ribofuranose (XLVI):

Compound XXXVII (1.2 g, 0.003 mole) was heated with 60 mL of 80% acetic acid at 80 C overnight. The solvents were then evaporated under vacuum, and the residue was stirred with 15 mL acetic anhydride and 30 mL pyridine overnight at room temperature. The solvents were evaporated under vacuum, and the product isolated by column chromatography using a silica gel column eluted with ethylacetate:chloroform (1:1) to yield 0.77 g (60%) of pure XLVI (Rf=0.4 ethylacetate:chloroform,1:1). Analytical data: nmr (CD(Cl)3,δ): 1.1-2.1(m,14H), 2.75(t,2H), 3.3(t,2H), 3.8-4.2(m,1H), 7.15-7.9(m,5H); ir (CH(Cl)3,cm-1): 3000, 2925, 2850, 1740, 1690, 1515, 1450, 1370, 1220, 1020, 690.

Synthesis of 2'-O-acetyl-3'-deoxy-3'-(phenoxy triphenylphosphoniomethyl phosphinylmethyl)-5'-O-(3-phenyl-3- oxo[ethylene ketal]-propionyl)-uridine chloride (LI):

To 0.5 g (0.93 mole) of XLV was added 2 mL of thionyl chloride. The mixture was stirred overnight, after which the excess thionyl chloride was evaporated on a rotary evaporator to yield 0.43 g (81%) of XLVIII, which was converted to XLIX without further purification.

Compound XLVIII (0.43 g, 0.75 mmol) was heated in 8 mL of hexane and 0.5 g (5 mmol) of phenol at 70 C for 4 hours, with a gas trap to capture HCl gas evolved. The mixture was then stirred overnight at room temperature, after which the solvents were evaporated under vacuum to yield 0.31 g (68%) of XLIX, which was converted to L without further purification.

A mixture of XLIX (3 g, 4.9 mmol), p-toluenesulfonic acid monohydrate (0.5 g, 0.3 mmol), ethylene glycol (8.4 mL, 148 mmol), and 50 mL of toluene was slowly distilled, with fresh toluene added to the reaction mixture to maintain the starting volume. After 75 mL of the toluene/water azeotrope was collected the reaction mixture was cooled, washed with sodium bicarbonate solution, then with water, dried, and

concentrated on a rotary evaporator to yield 2.4 g (75%) of L, which was converted to LI without further purification.

A mixture of 3 g (4.6 mmol) of L in 100 mL toluene and 3 g (11.4 mmol) of triphenylphosphine was refluxed for 4 hours. The resulting salt was filtered, and washed with toluene and ether to yield 3.5 g (85%) of pure LI.

Analysis: calculated for C(48)H(47)ClO(10)N(2)P(2): C,63.40; H,5.21%; found: C,63.22; H,5.30%

Synthesis of 2'-O-acetyl-3'-deoxy-3'-phenoxy-[1,2-O-isopropylidene-3-deoxy-3-bromomethyl-5,6-dideoxy-5,6-didehydroallosyl]-phosphinylmethyl)-5'-O-(3-phenyl-3-oxo [ethylene ketal]-propionyl)-uridine (LIII):

Compound LI (0.1 g, 0.11 mmol) was stirred in 8 mL ether under nitrogen. n-Butyllithium (1 mL, 1.6M in hexane) was added dropwise. After stirring for 30 minutes, 0.1 g (0.3 mmol) of XXXV in 5 mL ether was added dropwise. The mixture was stirred overnight, after which it was filtered, and concentrated on a rotary evaporator to yield 43 mg (41%) of pure LIII. Analytical data: nmr (CD(Cl)3, δ): 0.8-2.8(m,20H), 3.3-3.7(m,3H), 3.9-4.2(m,2H), 4.5-4.8(m,2H), 5.7-5.9(m,2H), 7.1-7.6(m,5H); ir (CH(Cl)3,cm-1): 3010, 2980,

2400, 1710, 1690, 1510, 1470, 1420, 1260, 1210, 1050, 1025, 930, 850, 790, 720, 670, 625.

Synthesis of 2'-O-acetyl-3'-deoxy-(phenoxy-[2'-O-acetyl-3'-deoxy-3'-phenoxy triphenylphosphoniomethyl phosphinylmethyl-5',6'-didehydro uridinyl]-phosphinylmethyl)- 5'-O-(3-phenyl-3-oxo [ethylene ketal]-propionyl)-uridine chloride (LIX):

A mixture of 0.25 g (0.29 mmol) of LIII and 0.1 g (0.32 mmol) of XL was refluxed for 3 hours under nitrogen. A mixture of 1 mL tetrahydrofuran, 1 mL t-butyl alcohol, and 1 mL water was added and the mixture was stirred for 1 hour, after which it was concentrated on a rotary evaporator,diluted with water, and extracted with chloroform. The combined chloroform phases were dried and concentrated under vacuum to yield 0.16 g (65%) of LIV, which was converted directly to LV without further purification.

Compound LIV (0.16 g, 0.19 mmol) was heated in 5 mL of 80% acetic acid at 85 C overnight. The solvent was evaporated under vacuum, and the residue stirred with 2 mL acetic anhydride and 3 mL pyridine overnight at room

temperature. The solvents were then evaporated under vacuum to yield 0.12 g (60%) of LV, which was converted directly to LVI without further purification.

To 0.2 g (0.22 mmol) of LV in 8 mL 1,2-dichloroethane and 0.4 mL 2,4-dimethoxypyrimidine (XLIV) was added 0.2 mL stannic chloride. After refluxing for 3 hours, the mixture was cooled to room temperature, and 5 mL saturated aqueous sodium bicarbonate was slowly added. The mixture was stirred for 30 minutes, after which the organic layer was separated, and the aqueous phase was extracted with 1,2-dichloroethane. The combined organic phases were dried, and concentrated on a rotary evaporator to yield 0.13 g (60%) of LVI, which was converted to LVII without further purification.

Compound LVI (0.2 g, 0.21 mmol) was stirred in 4 mL thionyl chloride overnight at room temperature fitted with a gas trap. The excess thionyl chloride was evaporated under vacuum to yield 0.18 g (85%) of LVII, which was converted directly to LVIII without further purification.

Compound LVII (0.2 g, 0.18 mmol) was heated in 5 mL hexane and 0.1g (1 mmol) phenol at 70 C for 4 hours. The mixture was then stirred overnight at room temperature, after which it was concentrated on a rotary evaporator to yield 0.13 g (70%) of LVIII, which was converted directly to LIX without further purification.

Compound LVIII (0.13 g, 0.12 mmol) was refluxed in 5 mL toluene with 0.1 g (0.38 mmol) of triphenylphosphine for 4 hours. The mixture was cooled to room temperature and the crystals were filtered and washed with toluene and ether to yield 0.14 g (85%) of pure LIX.

Analysis: calculated for C(68)H(67)O(18)N(4)P(3)Cl: C,60.20; H,4.98%; found: C,59.83; H,4.76%

Synthesis of 2',3'-O-isopropylidene-5'-oxo-guanosine (LXI):

2',3'-Isopropylidene guanosine(LXIII) (1.0 g, 3.1 mmol) was stirred with 0.3 g (1.55 mmol) of pyridinium triflouroacetate, 2 g (10 mmol) of DCC, and 15 mL dimethylsulfoxide for 4 hours. The mixture was then filtered, the filtrate poured into water, washed with ether, and concentrated on a rotary evaporator to yield 0.4 g (40%) of pure LXI. Analytical data: ir (C(Cl)4,cm-1): 2990, 2900, 2810, 1740, 1685, 1425, 1060; UV (MeOH): lambda max: 256 nm; ϵ=14364.

Synthesis of 2'-O-acetyl-3'-deoxy-3'-(phenoxy-[2'-O-acetyl-3'-

deoxy-3'phenoxy-<2',3'-O-isopropylidene-5',6'-dideoxy-5',6'-didehydro guanosinyl>-phosphinylmethyl-5',6'-dideoxy-5',6'-didehydro uridinyl]-phosphinylmethyl)-5'-O-(3-phenyl-3-oxo [ethylene ketal]-propionyl)-uridine (LXII):

Compound LIX (0.1 g, 0.074 mmol) was stirred in 8 mL ether under nitrogen. n-Butyllithium (1 mL, 1.6M in hexane) was added, and the mixture stirred for 2.5 hours. Compound LXI (0.05 g, 0.16 mmol) in 5 mL ether was added dropwise, and the mixture was stirred overnight at room temperature. The mixture was then filtered, and concentrated on a rotary evaporator to yield 98 mg (45%) of pure LXII.

Analysis: calculated for C(63)H(66)N(9)O(22)P(2): C,34.97; H,4.88%; found: C,35.21; H,5.06%

Synthesis of 3'-deoxy-3'-(hydroxy-[3'-deoxy-3'-hydroxy-<5',6'- dideoxy guanosinyl>-phosphinylmethyl-5',6'-dideoxy uridinyl]-phosphinylmethyl)-uridine (LXVII):

Compound LXII (0.3 g, 0.22 mmol) was shaken in a sealed bottle with 30 mL of methanol saturated with ammonia for 2

days while being heated with an IR heat lamp. The solvent was then evaporated under vacuum to yield 0.28 g (95%) of LXIV, which was converted directly to LXV without further purification.

Compound LXIV (0.28 g,, 0.22 mmol) was stirred in 15 mL methanol and 3 mL of 0.8% sulfuric acid overnight at room temperature. The solution was then neutralized with sodium bicarbonate, and filtered. The solvents were evaporated under vacuum, the residue stirred with methanol, filtered, and the filtrate concentrated on a rotary evaporator to yield 0.27 g (98%) of LXV, which was converted directly to LXVI without further purification.

Compound LXV (0.27 g, 0.22 mmol) was stirred in 5 mL of hydrazine hydrate (85%) buffered in 8 mL pyridine/2 mL acetic acid for 3 hours at room temperature. The solvents were evaporated under vacuum, and the resulting solid washed with chloroform. The solid was then dried under vacuum to yield 0.2 g (86%) of LXVI, which was converted directly to LXVII without further purification.

Compound LXVI (0.2 g , 0.19 mmol) was dissolved in methanol (dried over magnesium). Platinum oxide was added, and the mixture was shaken under 50 p.s.i. of hydrogen. After an uptake of 2 p.s.i. hydrogen, the mixture was filtered on a celite pad, and concentrated on a rotary

evaporator to yield 0.17 g, (98%) of pure LXVII. Analytical data: UV (MeOH): lambda max: 256 nm, ϵ =13528.

Due to the extreme hygroscopic nature of this compound, the elemental analysis fluctuated. The theoretical composition is 43.29% carbon and 4.88% hydrogen. On a typical analysis a composition of 26.04% C and 7.71% H was found. This may be accounted for if the sample contained 40% water, which would yield a theoretical value of 25.97% C and 7.40% H. In addition, the extinction coefficient is unreliable due to the water in the sample.

Synthesis of diethyl 4-oxo pentyl-1-phosphonate (LXIX):

To 5-chloro-2-pentanone ethylene ketal (63 g, 0.38 mole) was added triethyl phosphite (100 g, 0.38 mole). The mixture was refluxed for 48 hours. The reaction mixture was then cooled, and stirred with 250 mL 0.1M HCl for 1 hour. The mixture was then extracted 4 times with 100 mL portions of methylene chloride. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered , and the solvent evaporated under reduced pressure. The crude product was then distilled under vacuum, the product collected at 0.004 mm Hg at 85-90 C to yield 34 g (40%) of

pure LXIX. Analytical data: nmr(C(Cl)4,δ): 1.3(t,6H), 1.7(m,2H), 2.1(s,3H), 2.4(m,2H), 4.0(m,4H); ir(C(Cl)4,cm-1): 3000, 1725, 1370, 1255, 1165, 1050.

Analysis: calculated for C(9)H(19)O(4)P: C,48.65; H,8.56% found: C,48.73; H,8.60%

Synthesis of diethyl 5-carboethoxy-4-hydroxy-4-methylpentyl-1-phosphonate (LXX):

In a 100 mL three neck flask was placed 20 mL of anhydrous ether which was cooled to -23 C under a nitrogen atmosphere. Freshly distilled diisopropylamine (2.22 g, 22 mmol) was added via a syringe followed by a solution of n-butyllithium in hexane (13.2 mL, 22 mmol) 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 C and dry ethyl acetate (2.16 mL, 220 mmol) was added dropwise. The solution was stirred for 30 minutes at -78 C and then there was added 4.88 g (22 mmol) of diethyl 4-oxopentyl-1-phosphonate (LXIX). After 30 minutes the reaction mixture was treated with 4 mL of 20% HCl and allowed to warm to room temperature. The mixture was then diluted with 4 mL of water and extracted 4 times with 20 mL

portions of ether. The organic extracts were combined, dried over anhydrous sodium sulfate, and the solvent evaporated under reduced pressure to yield 3.53 g (52%) of pure LXX. Analytical data: nmr (C(Cl)4,δ): 0.8-2.3(m,16H), 2.1(s,4H), 3.2-4.1(m,7H); ir (C(Cl)4,cm-1): 3550, 3050, 1735, 1460, 1410, 1390, 1250, 1175, 1040, 950.

Synthesis of diethyl 5-carboxy-4-hydroxy-4-methylpentyl-1-phosphonate (LXXI):

Compound LXX (3.5 g, 11 mmol) was dissolved in 20 mL ether, and was cooled to -15 C, after which 20 mL N KOH in MeOH was added. The mixture was stirred at -15 C for 1 hour, then overnight at room temperature. The mixture was neutralized with methanolic HCl, the salt filtered, and the solvent evaporated under reduced pressure to yield 3 g (95%) of pure LXXI. Analytical data: nmr (C(Cl)4, δ): 1.2-2.2(m,19H), 2.4(s,2H), 3.4-4.3(m,4H); ir (C(Cl)4,cm-1): 3300-3600(broad), 3000, 1710, 1450, 1390, 1250, 1175, 1050, 975.

Analysis: calculated for C(11)H(23)O(6)P: C,46.81; H,8.16%; found: C,47.30; H,7.85%

Synthesis of 17β-(1-methyl-4-hydroxybutyl)etiocholane- 3α,7α,12α-triol (LXXIII):

To 20 g (49 mmol) of cholic acid in 75 mL of anhydrous ether and 75 mL of tetrahydrofuran, freshly distilled over lithium aluminum hydride, was added 1.86 g (49 mmol) of lithium aluminum hydride at room temperature. The mixture was heated with stirring at 70 C for 18 hours. After cooling, the excess lithium aluminum hydride was destroyed by the addition of 25 mL water in a dropwise manner. The reaction mixture was filtered, and the solid washed with ether and tetrahydrofuran. The combined organic materials were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resultant solid was washed with water, and dried under vacuum to yield 12.5 g (65%) of pure LXXIII, m.p. 224-225 C.

Analysis: calculated for C(23)H(40)O(4): C,72.59; H,10.59%; found: C,72.51; H,10.63%.

Synthesis of diethyl 5-carbo[etiocholane-3'α,7'α,12'α-trihydroxy-17'β-(1"-methyl-4"-butyl)]oxy-4-hydroxy-4-methylpentyl-1-phosphonate (LXXV):

To 0.50 g (2.0 mmol) of LXXI in dry pyridine was added 1.25 g (4.1 mmol) of triisopropylbenzenesulfonylchloride. This mixture was stirred at 0 C for 40 minutes after which time 0.79 g (2.0 mmol) of LXXIII was added. The mixture was stirred at 0 C for 2 hours, after which it was poured into 15 mL of ice water. The resulting precipitate was filtered and purified by column chromatography on silica eluting first with 9:1 chloroform-ethanol to remove side products followed by 95% ethanol to yield 0.41 g (31%) of pure LXXV (Rf=0.1 ethanol:water,95:5).

Analysis: calculated for C(35)H(63)O(9)P: C,63.81; H,9.64%; found: C,63.88; H,9.37%

Synthesis of 5-carbo[etiocholane-3'α,7'α,12'α-trihydroxy-17'β-(1"-methyl-4"-butyl)]oxy-4-hydroxy-4-methylpentyl-1-phosphonic acid (LXXVI):

To 1 g (1.5 mmol) of LXXV in 10 mL tetrahydrofuran under a nitrogen atmosphere was added 1.8 mL (13.6 mmol) of

trimethylsilylbromide. The mixture was stirred at room temperature for 48 hours. To this was added 10 mL water, and the mixture stirred for 1.5 hours at room temperature. The mixture was concentrated under vacuum to yield 0.9 g (90%) of pure LXXVI.

Analysis: calculated for C(31)H(55)O(9)P: C,61.77; H,9.20%; found: C,61.91; H,9.12%

Synthesis of diethyl 5-C14-5-carboxy-4-hydroxy-4-methyl pentyl-1-phosphonate (LXXVII):

A mixture of 15 mL tetrahydrofuran, 0.07 g (10 mmol) of lithium, and 1.25 g (10 mmol) of napthalene was stirred for 2 hours at room temperature under an argon atmosphere. After cooling to -15 C, 0.30 mL of this mixture was transfered into a Pierce reaction vial containing carbon-14 labelled sodium acetate (8 mg, 0.1 mmol, 1mC) under an argon atmosphere. This mixture was stirred at -15 C for 10 minutes, then at room temperature for 10 minutes, then at 68-78 C for 2 hours. Compound LXIX (22 mg, 0.1 mmol) was then injected, and the mixture was stirred for 2 hours at 70 C. There was then added 0.2 mL water, and the mixture was stirred overnight at room temperature. The aqueous layer

was separated, acidified with HCl, and extracted with chloroform. The organic phase was concentrated under vacuum to yield a brown syrup.

Synthesis of diethyl 5-C14-5-carbo[etiocholane-3'α,7'α,12'α-trihydroxy-17'β-(1"-methyl-4"butyl)]oxy-4-hydroxy-4-methylpentyl-1-phosphonate (LXXVIII):

Compound LXXVII (0.1 mmol, 1 mC) was dissolved in 0.3 mL pyridine. To this was added 60 mg (0.2 mmol) of triisopropylbenzenesulfonylchloride and the mixture was stirred in an ice bath for 1.5 hours. Compound LXXIII (39 mg, 0.1 mmol) was then added, and the mixture was stirred for 2 hours in an ice bath. Water was added, and the mixture was left in a refrigerator overnight. The resulting crystals were separated from the solvents and dried under vacuum.

Synthesis of 5-C14-5-carbo[etiocholane-3'α,7'α,12'α-trihydroxy-17'β-(1"-methyl-4"-butyl)]oxy-4-hydroxy-4-methylpentyl-1-phosphonic acid (LXXIX):

Compound LXXVIII (0.1 mmol, 1 mC) was stirred with 0.1 mL tetrahydrofuran and 20uL of trimethylsilylbromide at room temperature for 2 days under nitrogen. Water (0.5 mL) was then added, and the mixture was stirred for 1.5 hours. The solvents were removed under vacuum, to yield a brown solid.

Synthesis of 5-C14-5-carboxy-4-hydroxy-4-methylpentyl-1-phosphonic acid (LXXX):

Compound LXXVII (0.8 mC) was stirred with 0.1 mL tetrahydrofuran and 20uL of trimethylsilylbromide for 2 days at room temperature under nitrogen. Water (0.5 mL) was then added, and the mixture was stirred for 1.5 hours. The solvents were evaporated under vacuum, to yield a brown liquid.

Synthesis of diethyl 3-hydroxypropyl-1-phosphate (LXXXI):

1,3-Propane diol (0.88 g, 11.6 mmol) was stirred with 1 g (5.8 mmol) of diethylchlorophosphate in 5 mL ether and 5 mL triethylamine at room temperature for 1 hour. The precipitate was filtered, and the solvents removed with a rotary evaporator to yield 1.6 g (65%) of pure LXXXI.

Analytical data: nmr (CD(Cl)3, δ): 1.2-2.2(m,8H), 2.9-3.2(m,1H), 3.6-4.7(m,6H).

Synthesis of diethyl 3-carboxypropyl-1-phosphate (LXXXIII):

Compound LXXXI (1 g, 4.7 mmol) was stirred with 4 mL (24 mmol) DMSO, 0.1 mL phosphoric acid, and 2.88 g (14 mmol) DCC overnight at room temperature. The precipitated salt was filtered, and the mixture was poured into water, and extracted with ether. The combined organic phases were dried, and concentrated under vacuum to yield 0.8 g (85%) of LXXXII, which was converted directly to LXXXIII without further purification.

Compound LXXXII (0.8 g, 4 mmol) was stirred in 15 mL absolute ethanol and 1.87 g (11 mmol) of silver nitrate in 2 mL water. To this was added 15 mL of 6% KOH , and the mixture stirred for 2 hours. The mixture was filtered, the precipitate washed with ethanol, and the filtrate acidified with HCl, then extracted with chloroform. The combined organic phases were dried and concentrated under vacuum, the product isolated by column chromatography using a silica gel column eluted with chloroform:ethanol (1:1) to remove impurities, and then 95% ethanol to yield 0.7 g (80%) of

pure LXXXIII (Rf=0.1 ethanol:water,95:5). Analytical data: nmr (CD(Cl)3, δ): 1.1-2.0(m,6H), 2.6-3.2(m,4H), 3.8-4.3(m,4H).

Synthesis of diethyl 3-carbo[etiocholane-3'α,7'α,12'α-trihydroxy-17'β-(1"-methyl-4"-butyl)]oxy-propyl-1-phosphate (LXXXIV):

Compound LXXXIII (240 mg, 1.1 mmol) was dissolved in 10 mL pyridine, and cooled in an ice bath. Triisopropylbenzenesulfonylchloride (640 mg, 2.12 mmol) was added, and the mixture was stirred for 40 minutes in an ice bath. Compound LXXIII (420 mg, 1.1 mmol) was then added, and stirred for 2.5 hours while cooling in an ice bath. The mixture was then poured into ice water and left in a refrigerator overnight. The resulting crystals were filtered and recrystallized from ethyl acetate to yield 400 mg (59%) of pure LXXXIV.

Analysis: calculated for C(31)H(51)O(8)P: C,62.19; H,8.58%; found: C,62.57; H,8.69%

Synthesis of 3-carbo[etiocholane-3'α,7'α,12'α-trihydroxy-17'β-(1"-methyl-4"-butyl)]oxy-propyl-1-phosphoric acid (LXXXV):

Compound LXXXIV (400 mg, 0.65 mmol) was stirred in 10 mL tetrahydrofuran under nitrogen. Trimethylsilylbromide (1 mL, 7.5 mmol) was added, and the mixture was stirred for 48 hours at room temperature. There was then added 5 mL water, and, after stirring for 1.5 hours at room temperature, the mixture was concentrated under vacuum to yield 350 mg (90%) of LXXXV.

Synthesis of sodium diethyl-1-carboxy-ethylene-1-phosphate (LXXXVIII):

3-Bromopyruvic acid (2 g, 12 mmol) was stirred with 10 g Dowex 50W (rinsed with sodium hydroxide, then with water to pH 7) in water for 30 minutes at room temperature. The mixture was filtered and concentrated under vacuum to yield 2.3 g (100%) of LXXXVII, which was converted directly to LXXXVIII without further purification.

To 2.3 g (12 mmol) of LXXXVII was added 4 g (24 mmol) triethylphosphite while cooling in an ice bath to maintain at room temperature. When heat was no longer evolved, the

mixture was heated at 60-70 C for 4 hours, after which it was stirred overnight at room temperature. The excess triethyl phosphite was distilled off, and the residue concentrated under vacuum to yield 2 g (67%) of pure LXXXVIII. Analytical data: nmr (C(Cl)4,δ): 1.2-1.6(m,6H), 3.5-3.8(m,2H), 3.9-4.3(m,4H).

Synthesis of diethyl 1-carboxy-ethylene-1-phosphate (LXXXIX):

Compound LXXXVIII (2 g, 8 mmol) was stirred with 10 g Dowex 50W (rinsed with HCl, then water to pH 7) in water for 1 hour at room temperature. The mixture was filtered and concentrated under vacuum to yield 1.8 g (100%) of pure LXXXIX.

Analysis: calculated for C(7)H(13)O(6)P: C,37.51; H,5.85%; found: C,36.99; H, 6.01%

Synthesis of diethyl 1-carbo[etiocholane-3'α,7'α, 12'α-trihydroxy-17'β-(1"-methyl-4"-butyl)]oxy-ethylene-1-phosphate (XC):

To compound LXXXIX (0.56 g, 2.5 mmol) in pyridine was added 1.5 g (5 mmol) triisopropylbenzenesulfonylchloride while cooling in an ice bath. The mixture was stirred for 40 minutes, after which 1 g (2.5 mmol) of LXXIII was added. After stirring for 4 hours, the mixture was poured into ice water, and left in a refrigerator overnight. The mixture was filtered, concentrated under vacuum, and recrystallised from water to yield 0.6 g (40%) of pure XC.

Analysis: calculated for C(31)H(53)O(8)P: C,63.68; H,9.14%; found: C,63.21; H,8.83%

Synthesis of 1-carbo[etiocholane-3'α,7'α,12'α-trihydroxy-17'β-(1"-methyl-4"-butyl)]oxy-ethylene -1-phosphoric acid (XCI):

Compound XC (1.2 g, 2 mmol) was stirred in 15 mL tetrahydrofuran under nitrogen. Trimethylsilylbromide (2.7 mL, 20 mmol) was added, and the mixture was stirred for 48 hours at room temperature. There was then added 10 mL water, and, after stirring for 1.5 hours at room temperature, the mixture was concentrated under vacuum to yield 1.1 g (90%) of XCI.

Synthesis of 17β-(1-methyl-4-acetoxy butyl) etiocholane-3α,7α,12α-triacetate (XCII):

Compound LXXIII (0.25 g, 0.6 mmol) was stirred in 2 mL pyridine and 5 mL acetic anhydride overnight at room temperature. The mixture was poured into water and extracted with ether. The combined ether phases were washed with saturated aqueous sodium chloride solution, then dried and concentrated under vacuum to yield 0.18 g (65%) of pure XCII.

Analysis: calculated for C(26)H(40)O(5): C,72.19; H,9.34%; found: C,72.25; H,9.23%

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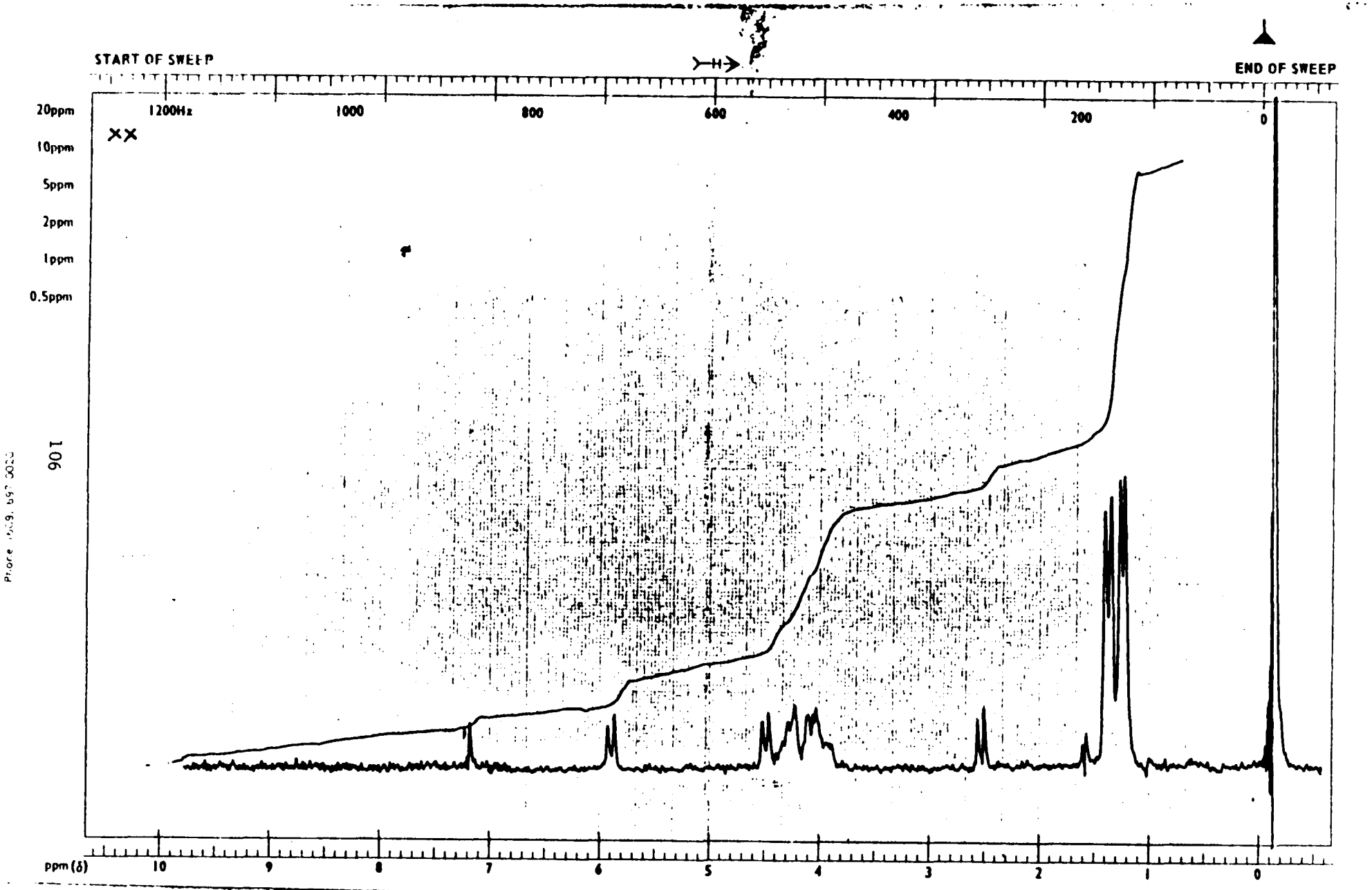
APPENDIX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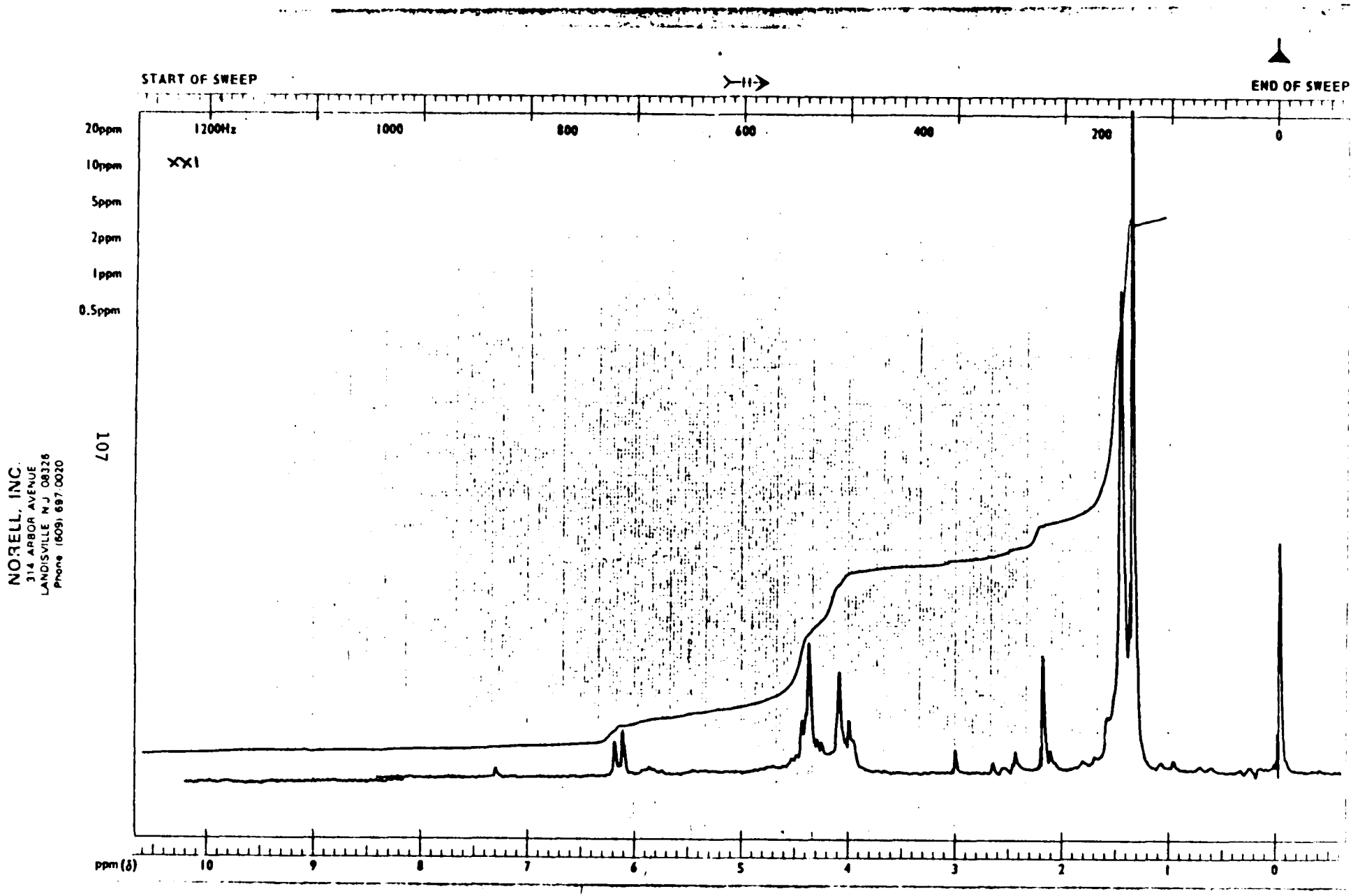
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NORELL, INC.
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LANDISVILLE N.J. 08326
Phone (609) 697-0020
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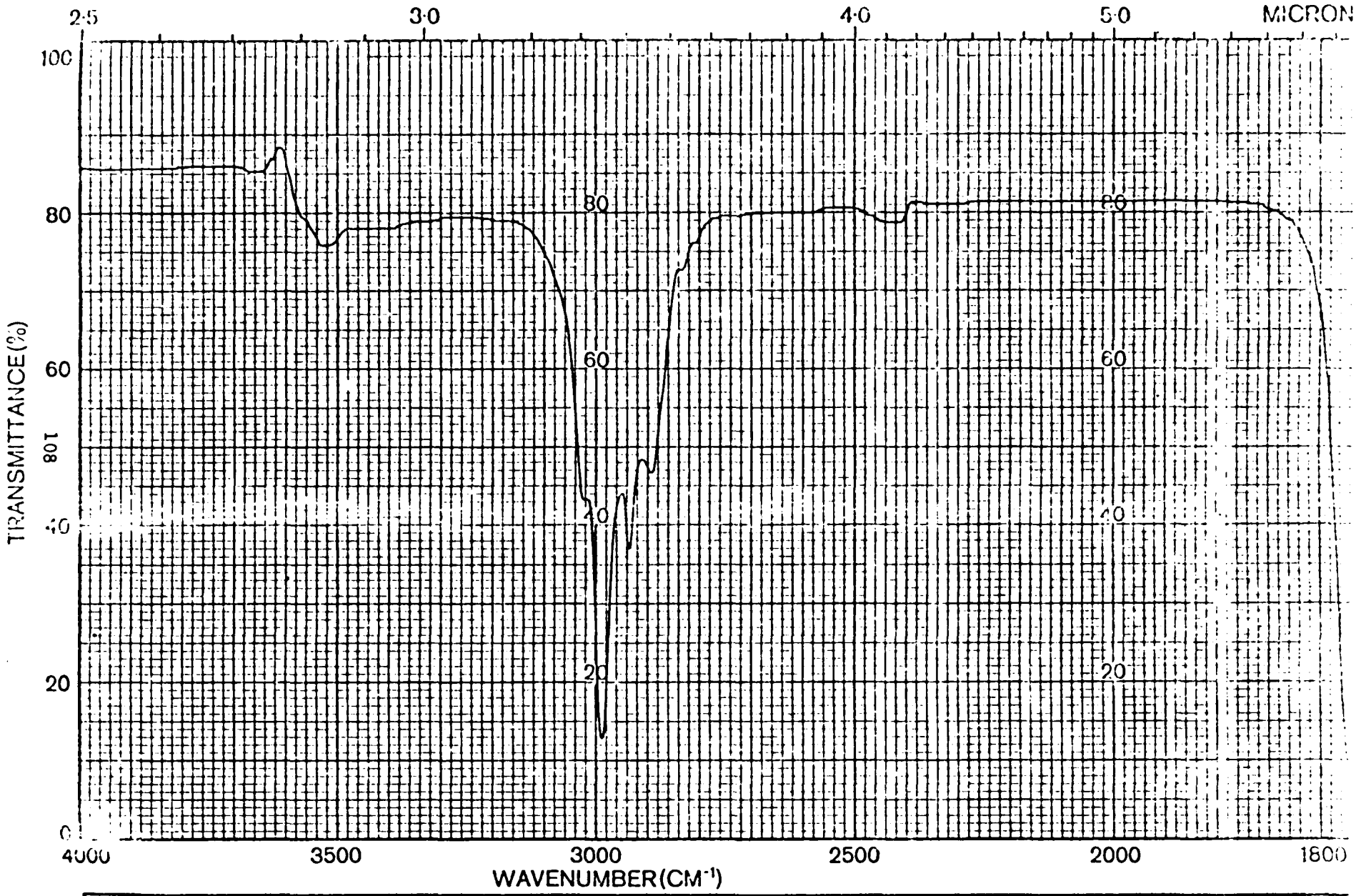
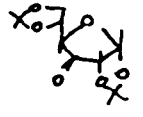
NMR of XXI



SAMPLE BG-65



① XXI

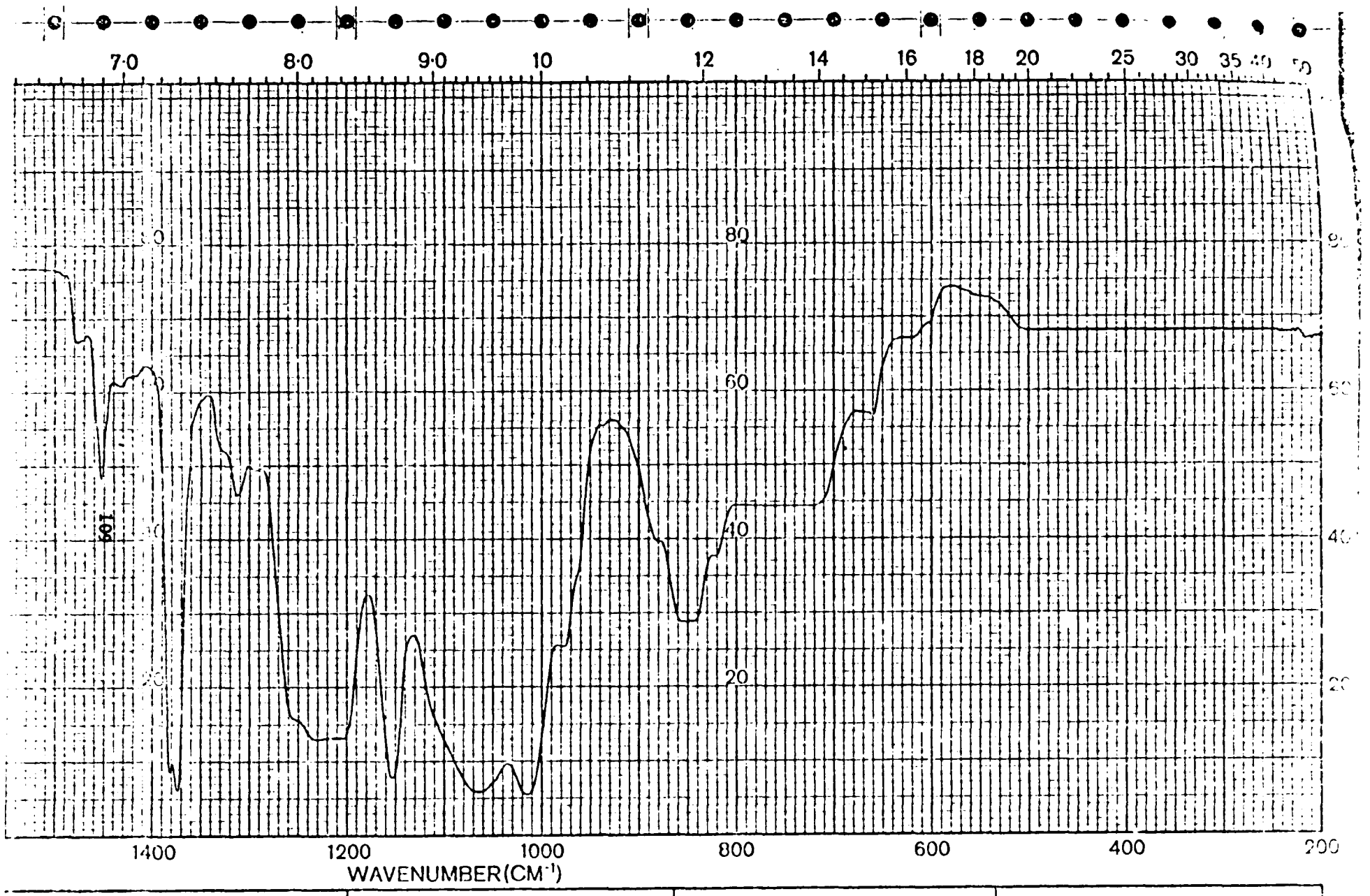
IR of XXI

SOLVENT $CHCl_3$

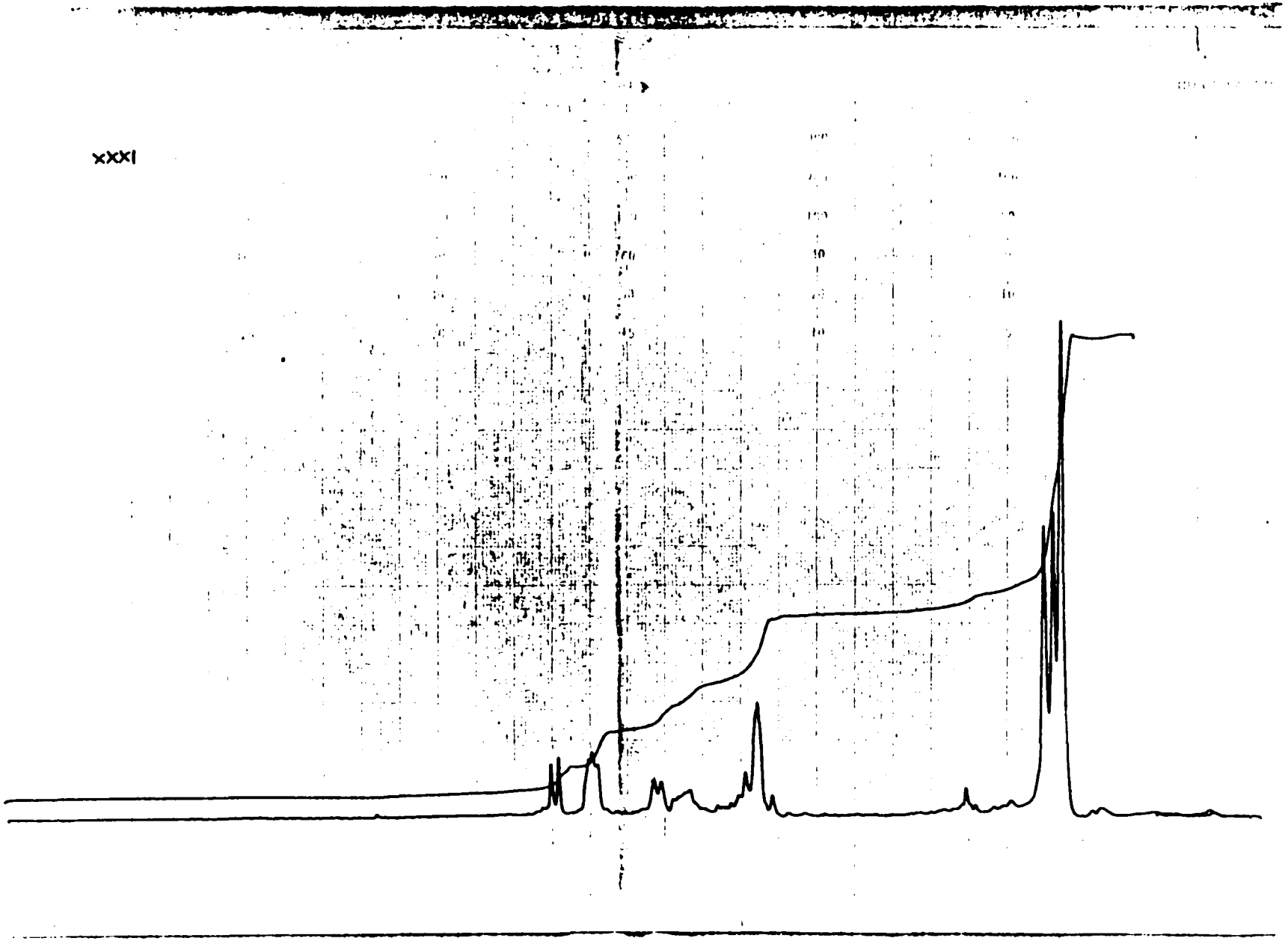
CONCENTRATION

CELL PATH

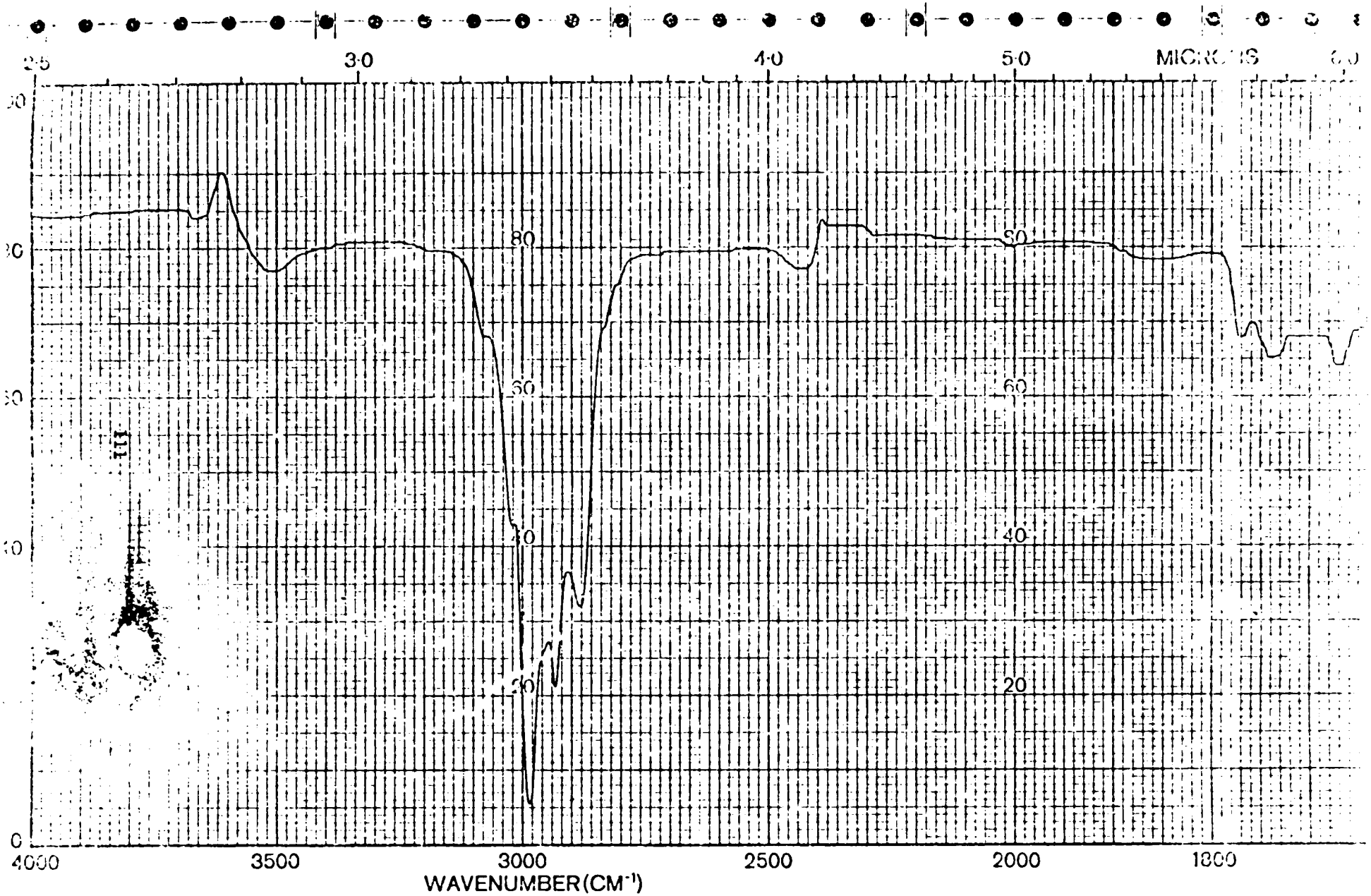
REFERENCE

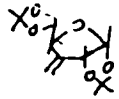


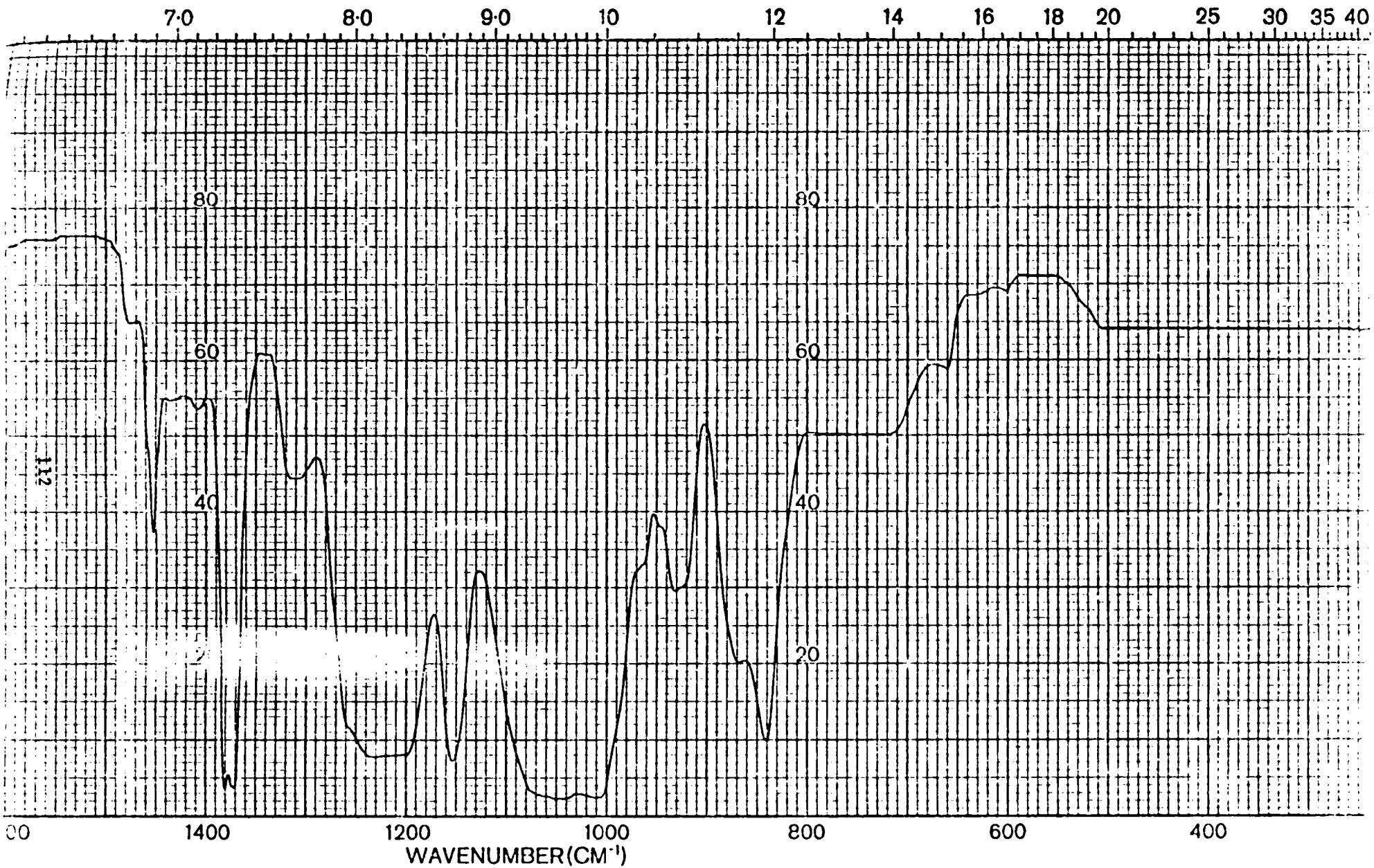
	SCAN TIME	T. SB	PERKIN ELMER
	SLIT	ORDINATE EXP.	CHART No. 5100 4367
	OPERATOR IR of XXI DATE	TIME CONSTANT	REF No.



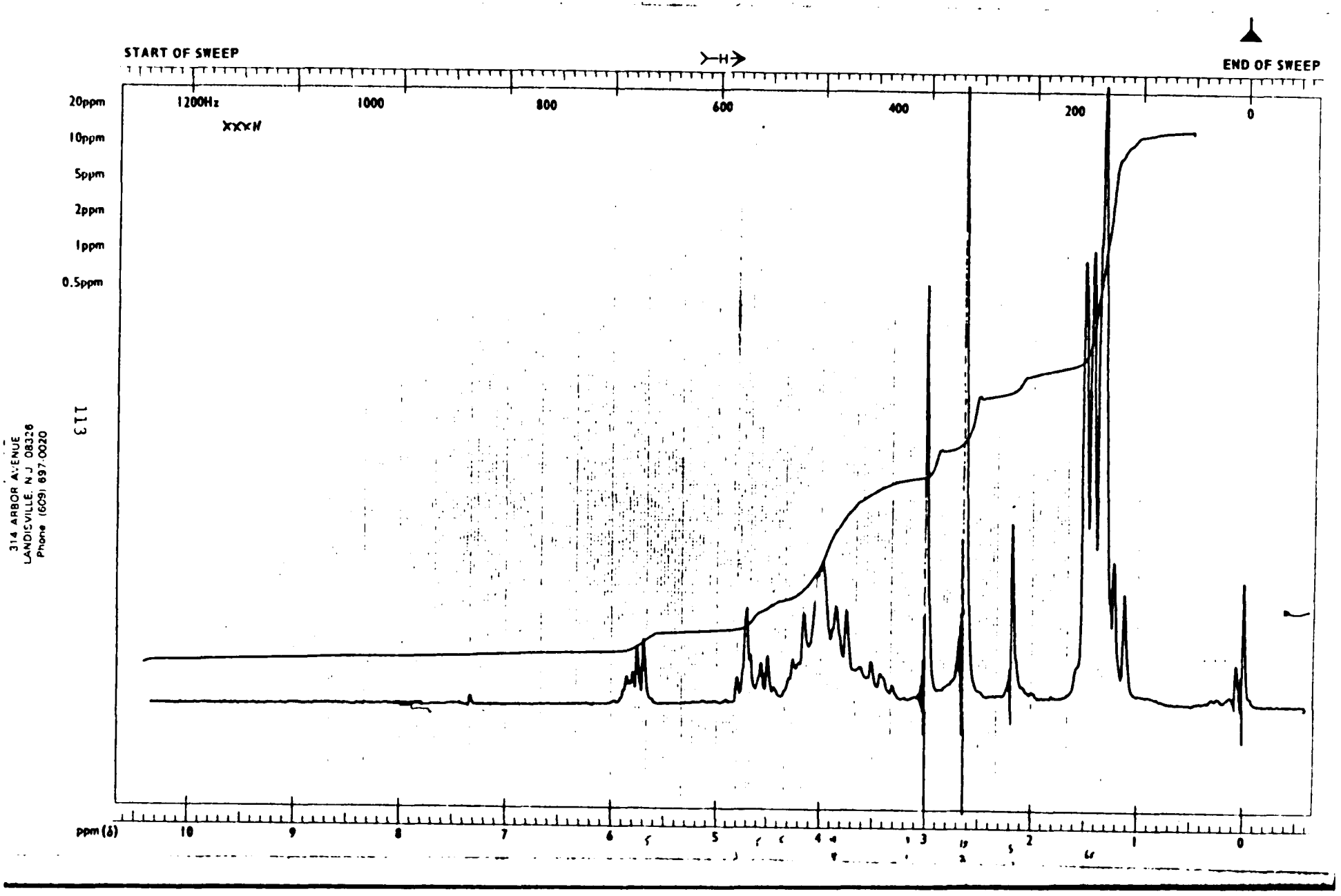
NMR of XXXI



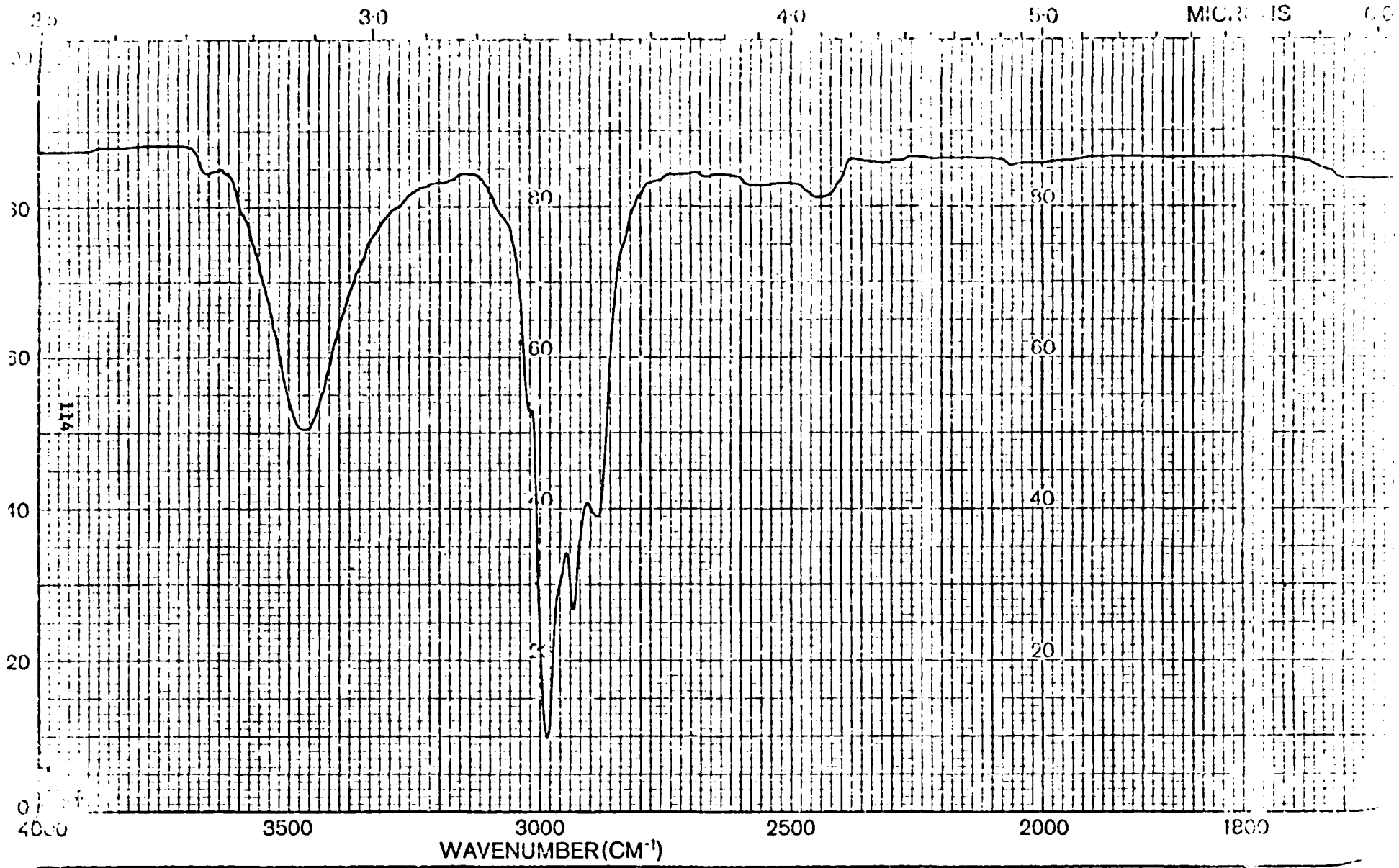
SAMPLE  (2) XXXI IR of XXXI	SOLVENT $CHCl_3$ CONCENTRATION CELL PATH REFERENCE	REMARKS

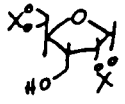


XXXI	SCAN TIME	T. SB	PERKIN ELMER
	SLIT	ORDINATE EXP.	CHART No. 5100 4367
	IR of XXXI OPERATOR DATE	TIME CONSTANT	REF No.

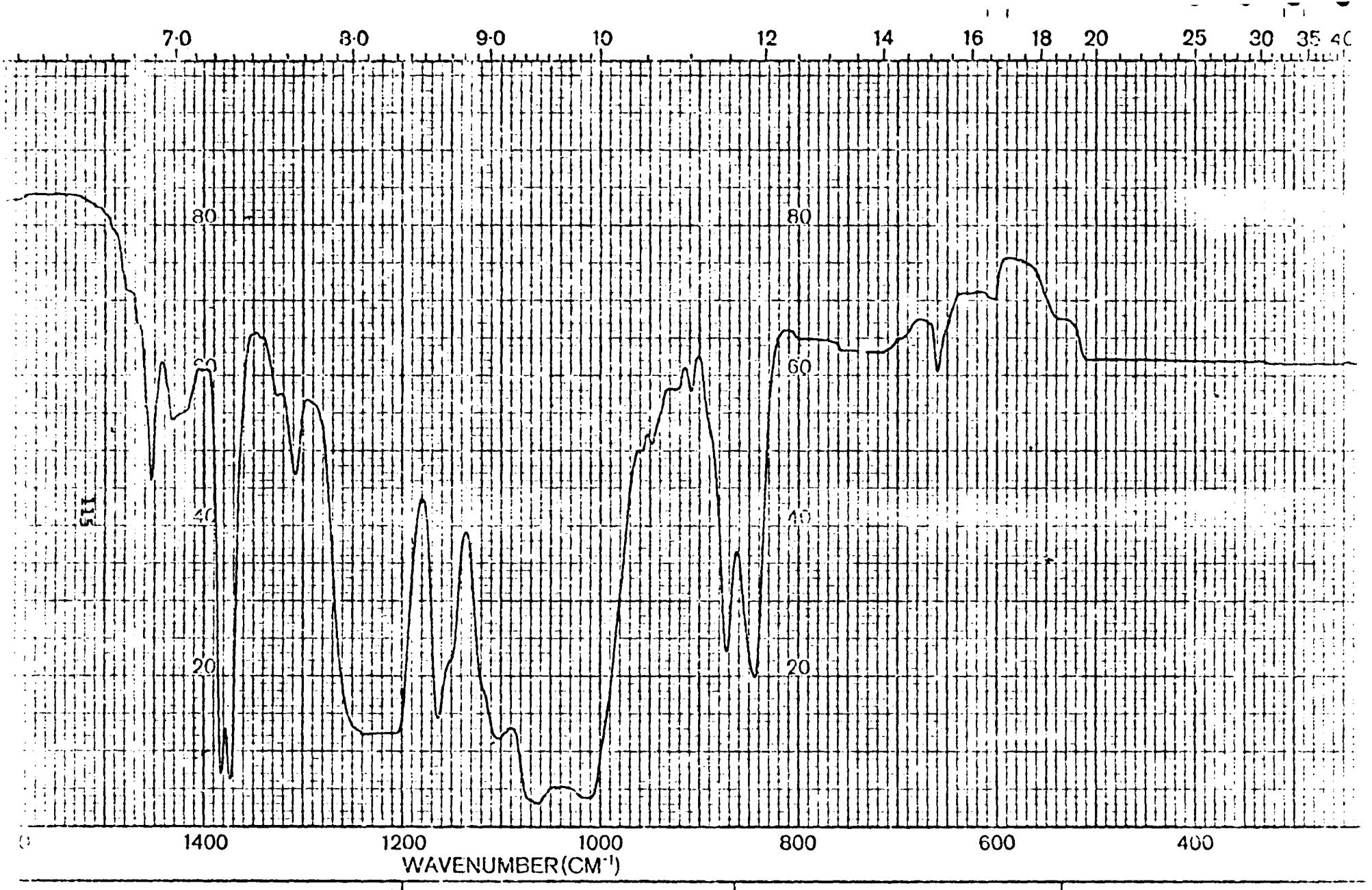

START OF SWEEP
END OF SWEEP
1200Hz
1000
800
600
400
200
0
20ppm
10ppm
5ppm
2ppm
1ppm
0.5ppm
XXXIV
113
314 ARBOR AVENUE
LANDISVILLE, N.J. 08326
Phone (609) 697-0020
ppm (δ)
10
9
8
7
6
5
4
3
2
1
0

NMR of XXXII

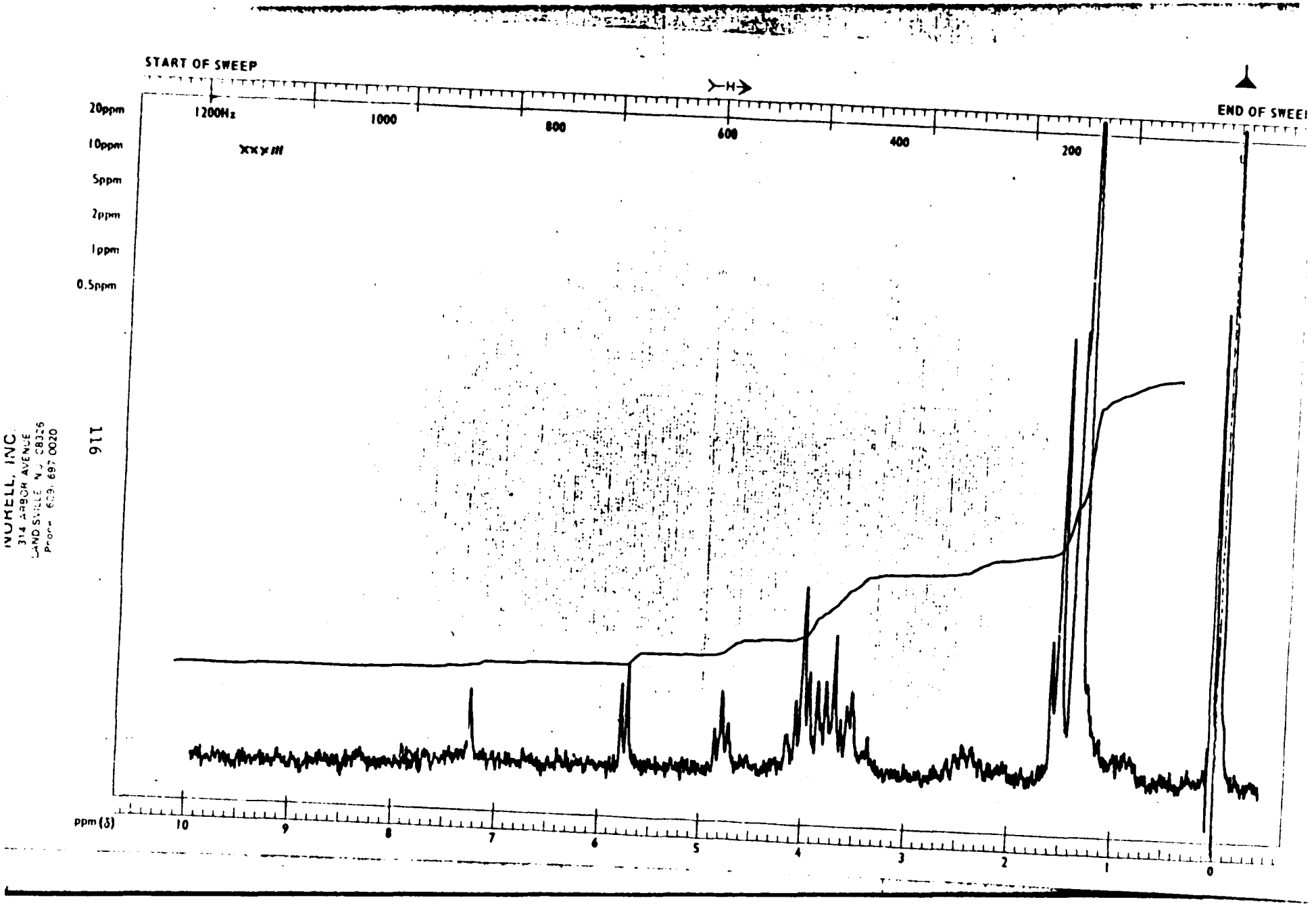


SAMPLE  XXXII	SOLVENT $CHCl_3$
IR of XXXII	CONCENTRATION
ORIGIN	CELL PATH
	REFERENCE

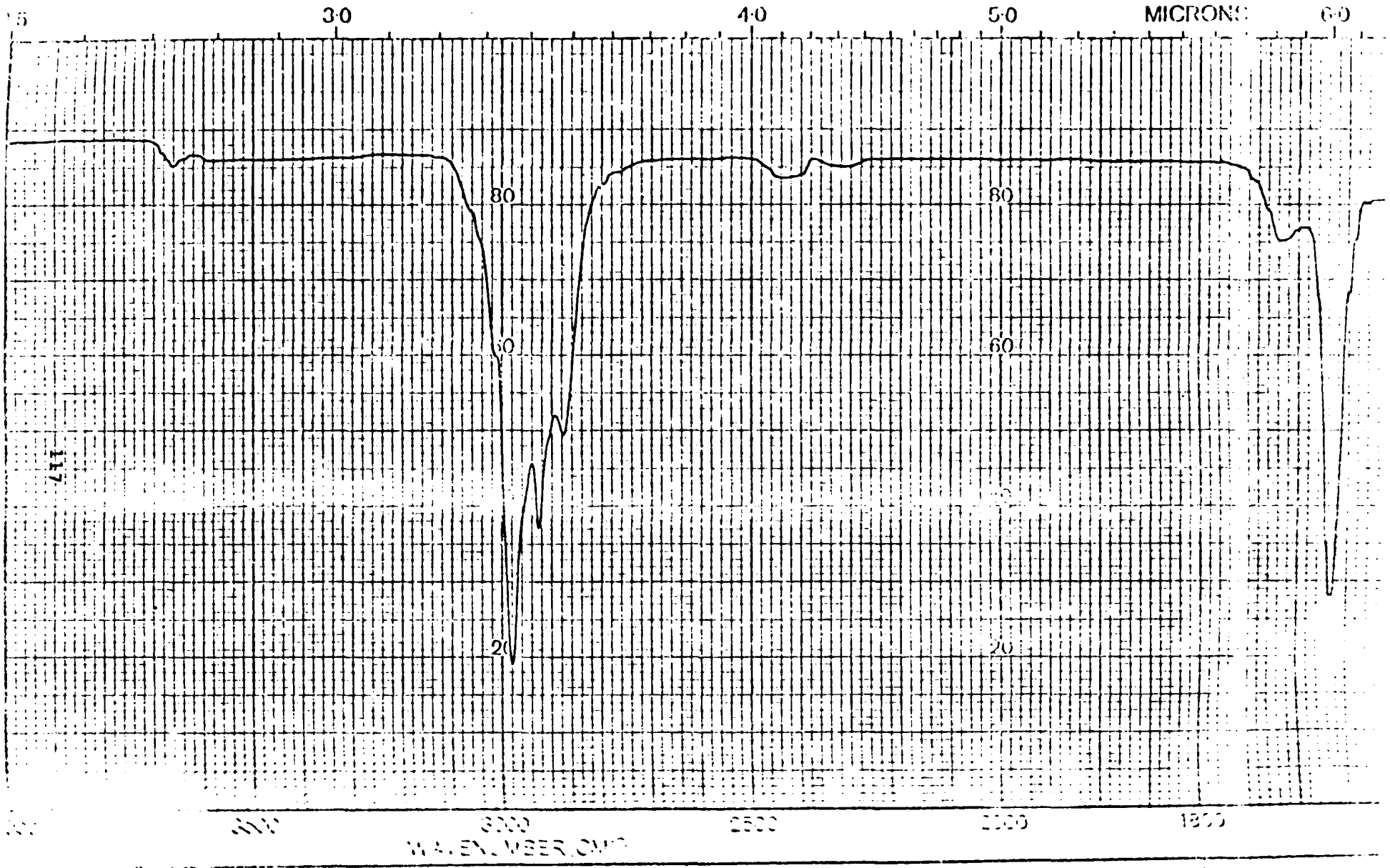
REMARKS



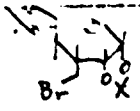
xxxii	SCAN TIME	T. SB	PERKIN ELMER
	SLIT	ORDINATE EXP.	CHART No. 5100 4367
	IR of XXXII		
	OPERATOR DATE	TIME CONSTANT	REF No.



NMR of XXXIII



SAMPLE



(1) XXXIII

IR of XXXIII

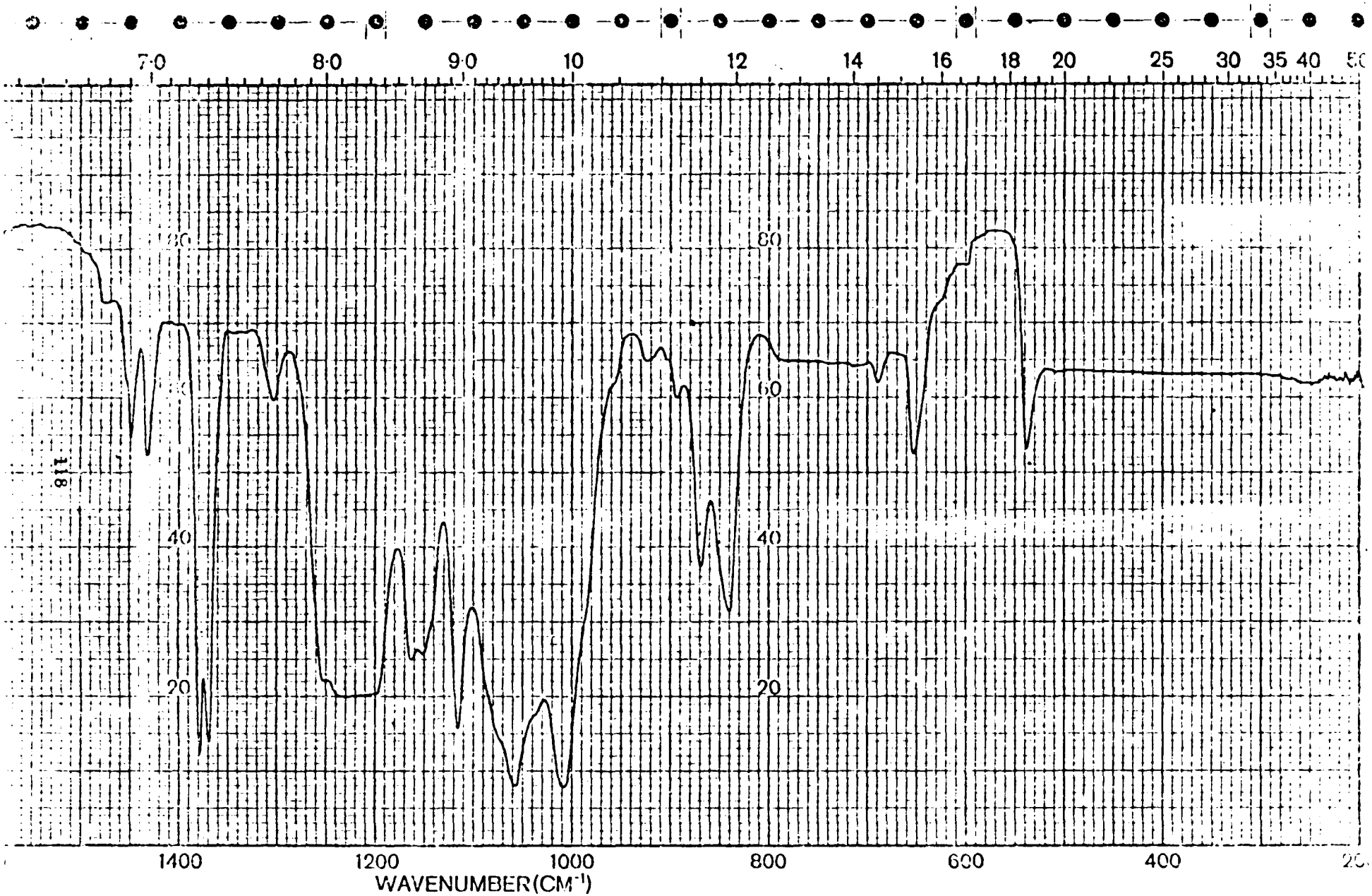
SOLVENT CCl_4

CONCENTRATION

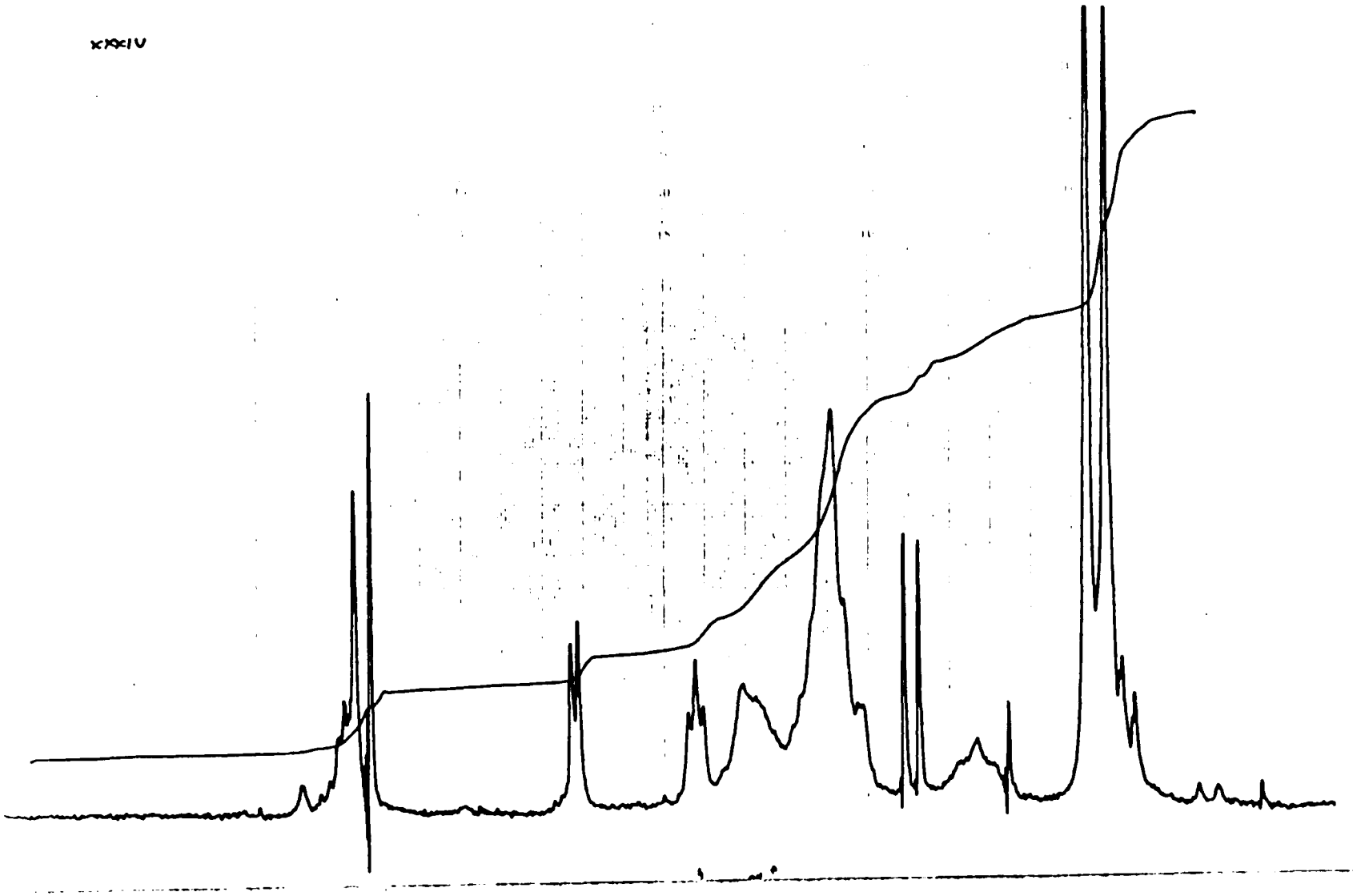
CELL PATH

REFERENCE

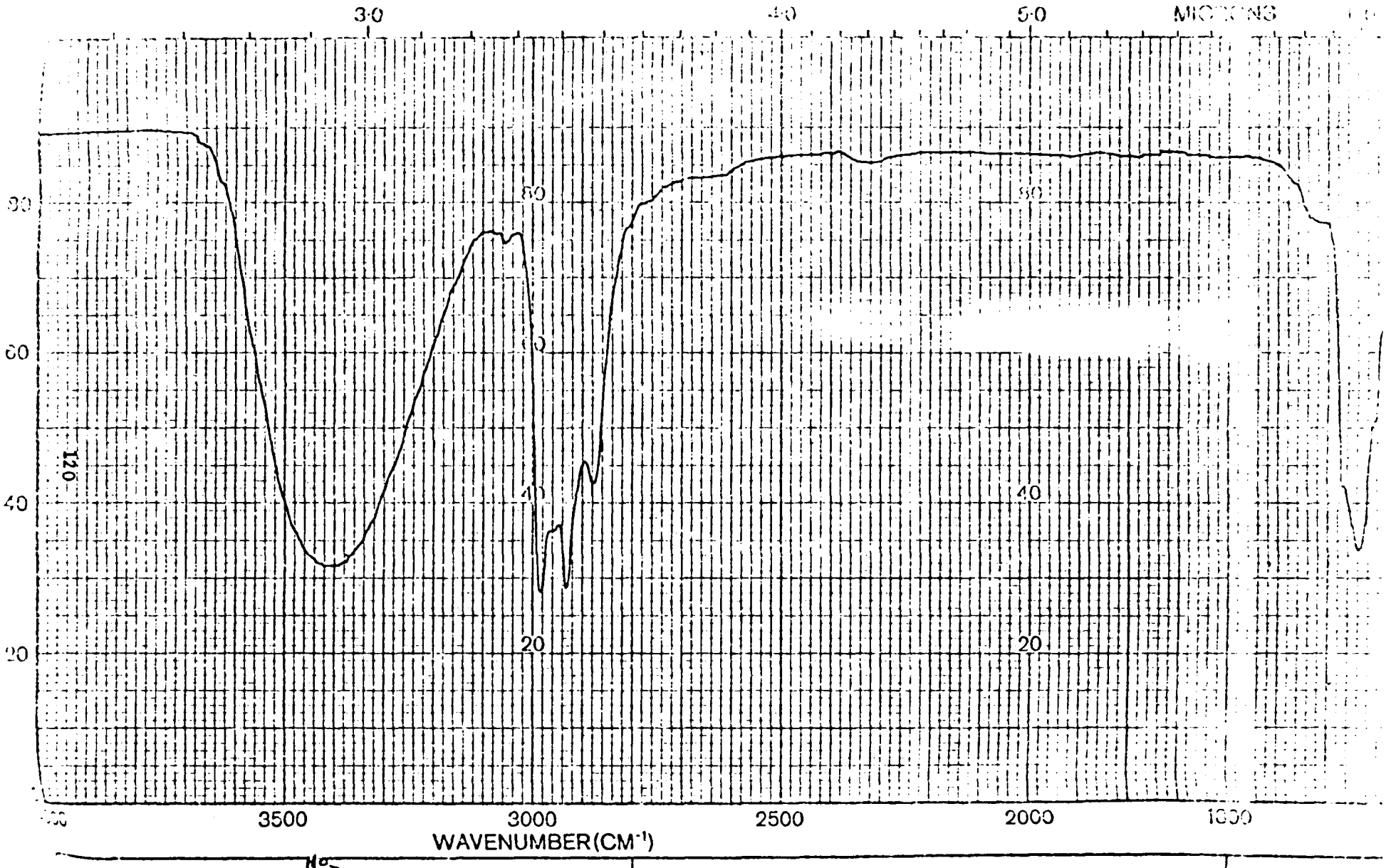
REMARKS



XXXIII	SCAN TIME	T. SB	PERKIN ELMER
	SLIT	ORDINATE EXP.	CHART No. 5100 4367
	OPERATOR IR of XXXIII DATE	TIME CONSTANT	REF No.



NMR of XXXIV

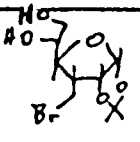


SOLVENT CCl_4

CONCENTRATION

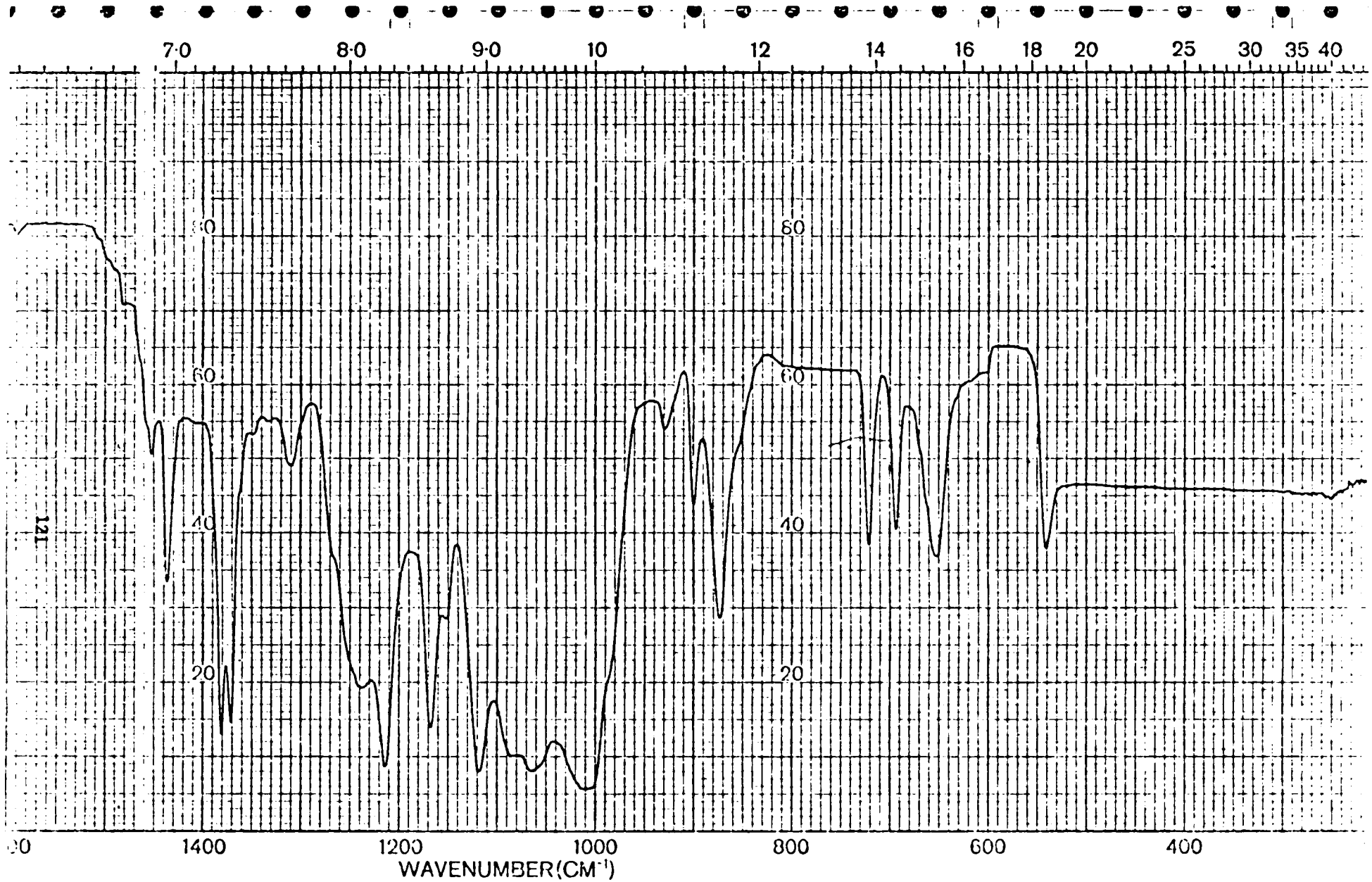
CELL PATH

REMARKS



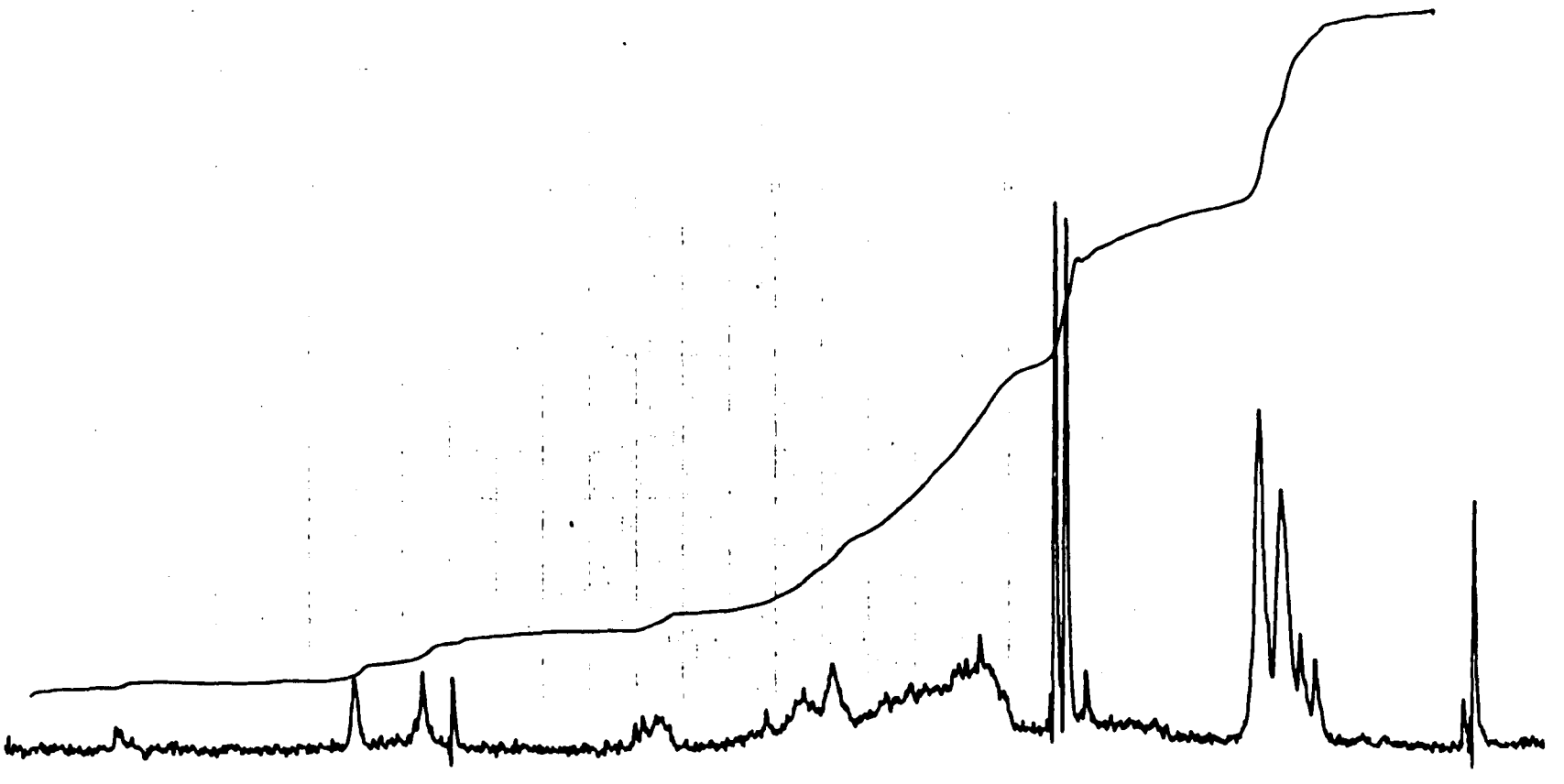
XXXIV

IR of XXXIV

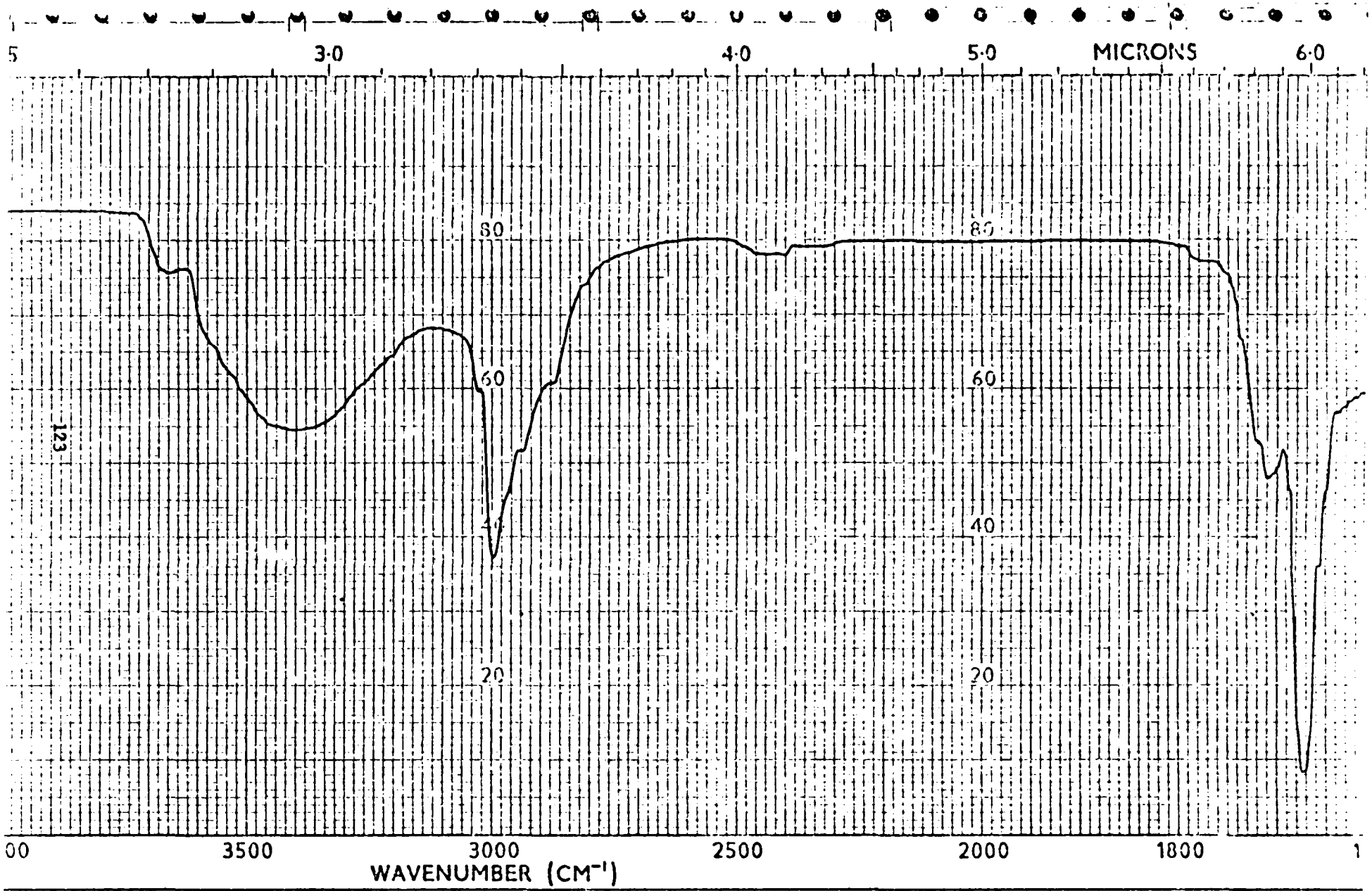


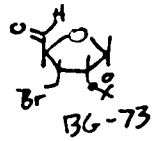
XXXIV	SCAN TIME	T. SB	PERKIN ELMER
	SLIT	ORDINATE EXP.	CHART No. 5100 4367
	OPERATOR IR of XXXIV DATE	TIME CONSTANT	REF No.

XXXV



NMR of XXXV



SAMPLE  ⑥ XXXV

BG-73

ORIGIN IR of XXXV

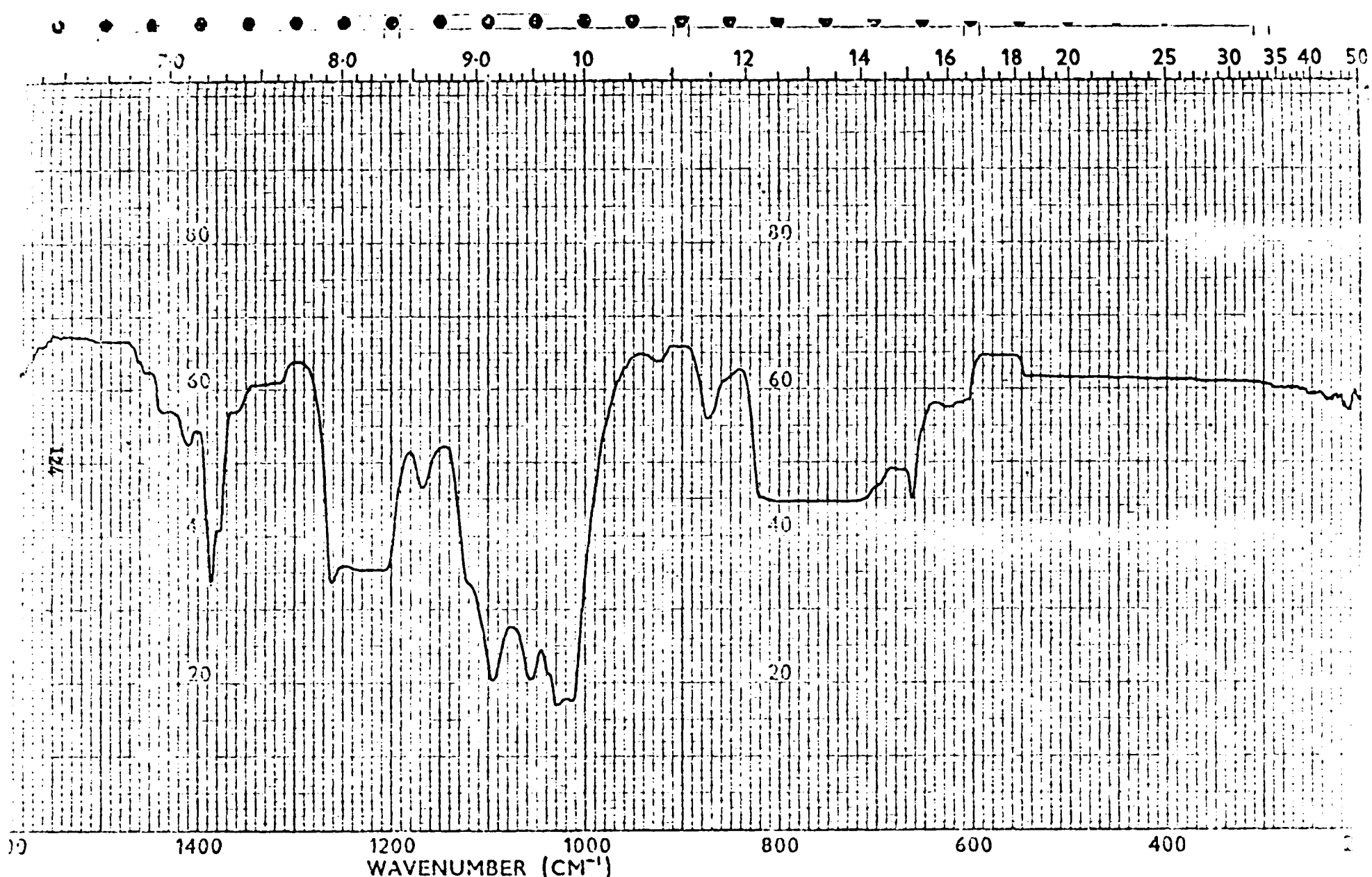
SOLVENT CH_2Cl_2

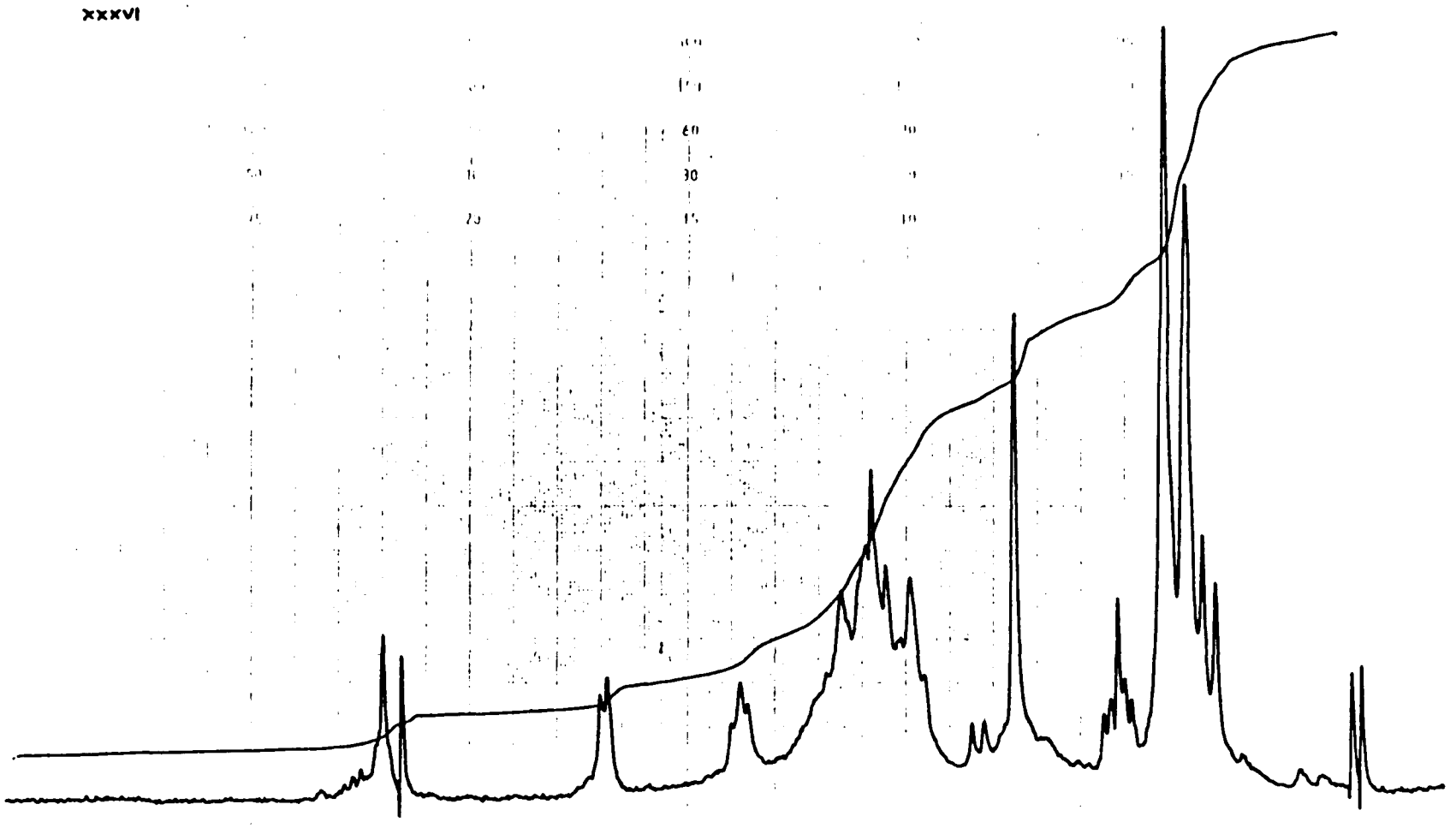
CONCENTRATION

CELL PATH

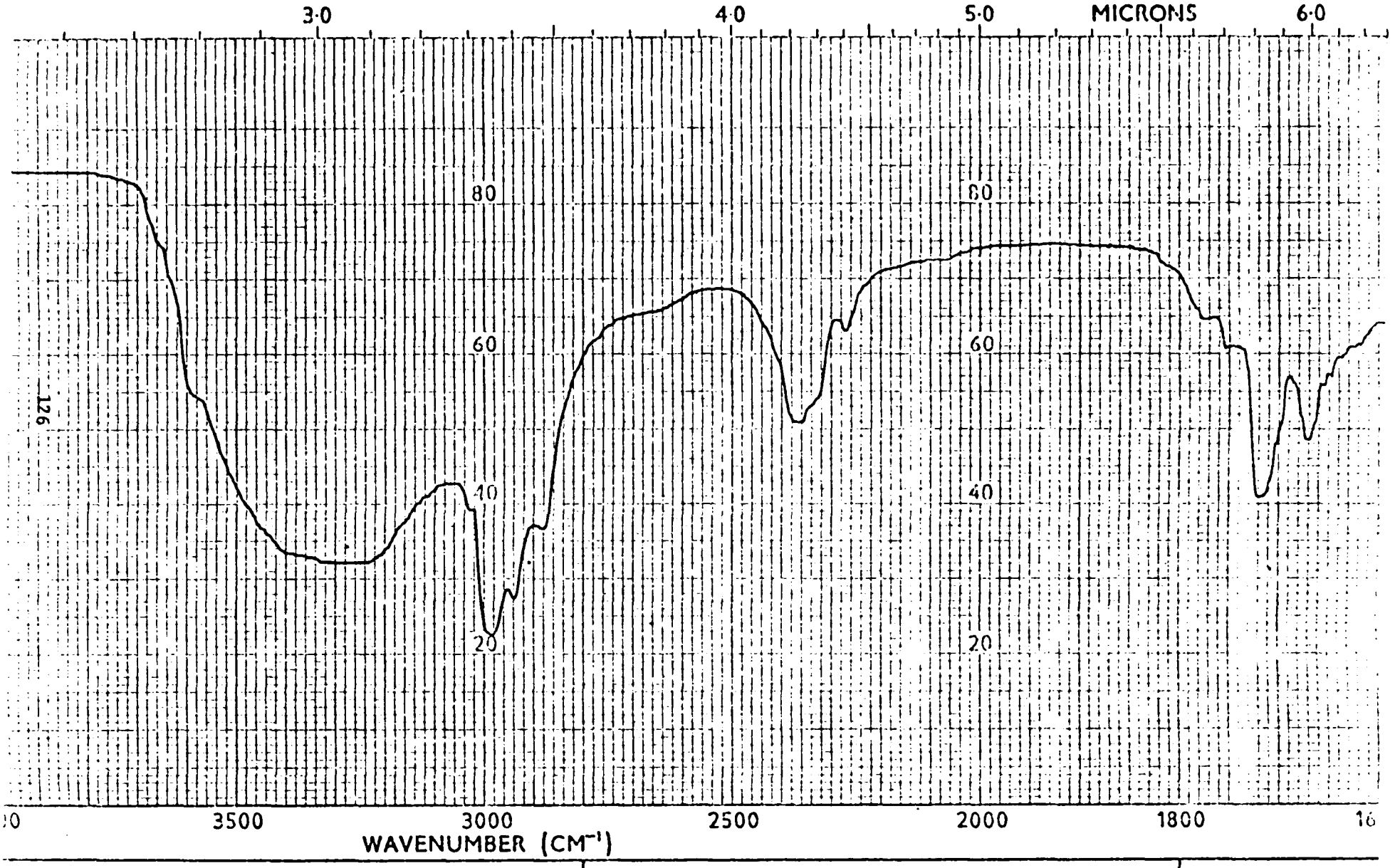
REFERENCE

REMARKS

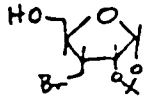

7·0
8·0
9·0
10
12
14
16
18
20
25
30
35
40
50
100
80
60
40
20
%T
1400
1200
1000
800
600
400
WAVENUMBER (CM⁻¹)
XXXV
SCAN TIME
SLIT
OPERATOR
DATE
IR of XXXV
T
SB
ORDINATE EXP.
TIME CONSTANT
PERKIN ELMER
CHART No. 5100-4367
REF. No.



NMR of XXXVI



SAMPLE



(7)

XXXVI

IR of XXXVI

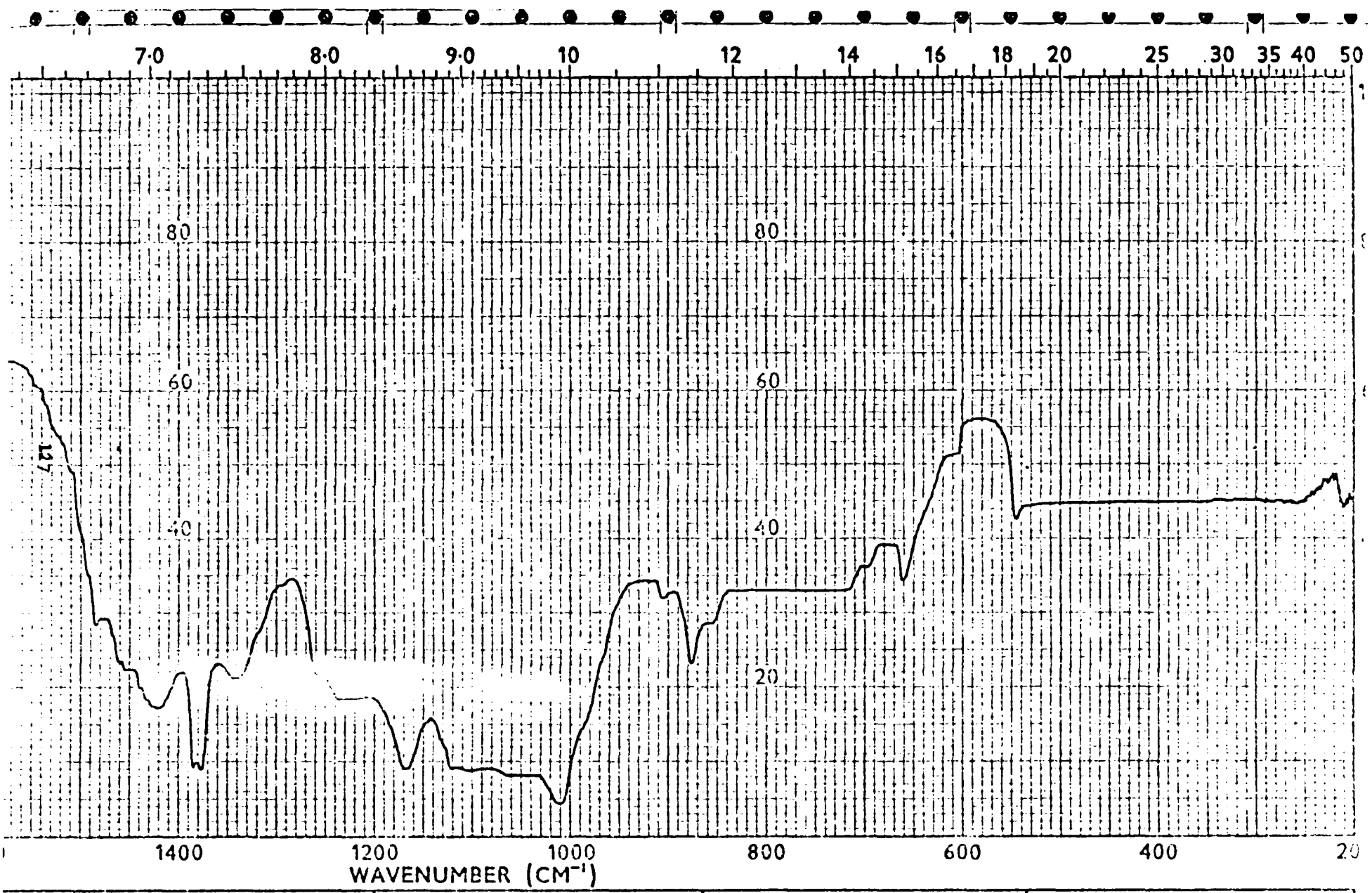
SOLVENT $CHCl_3$

CONCENTRATION

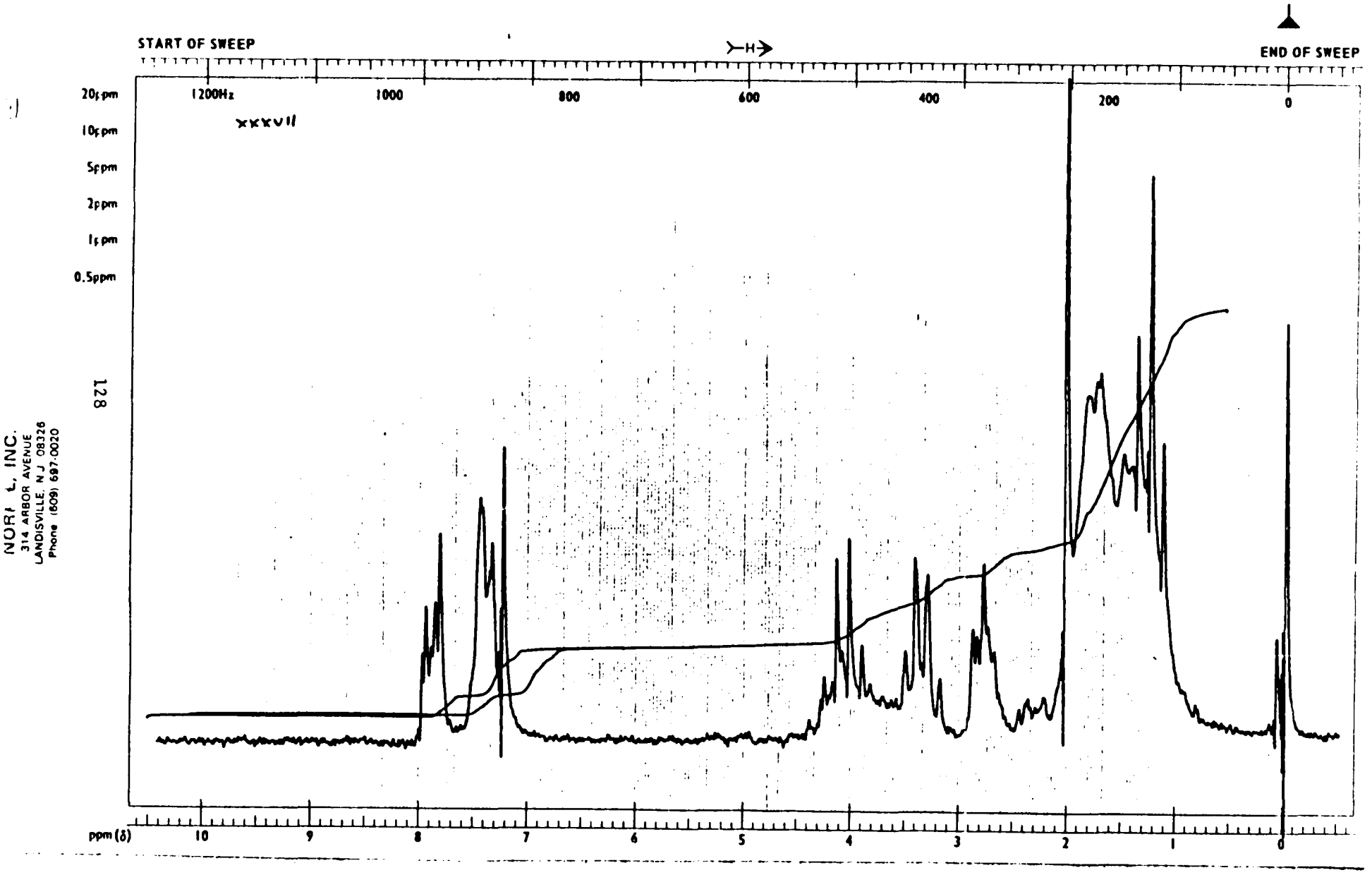
CELL PATH

REFERENCE

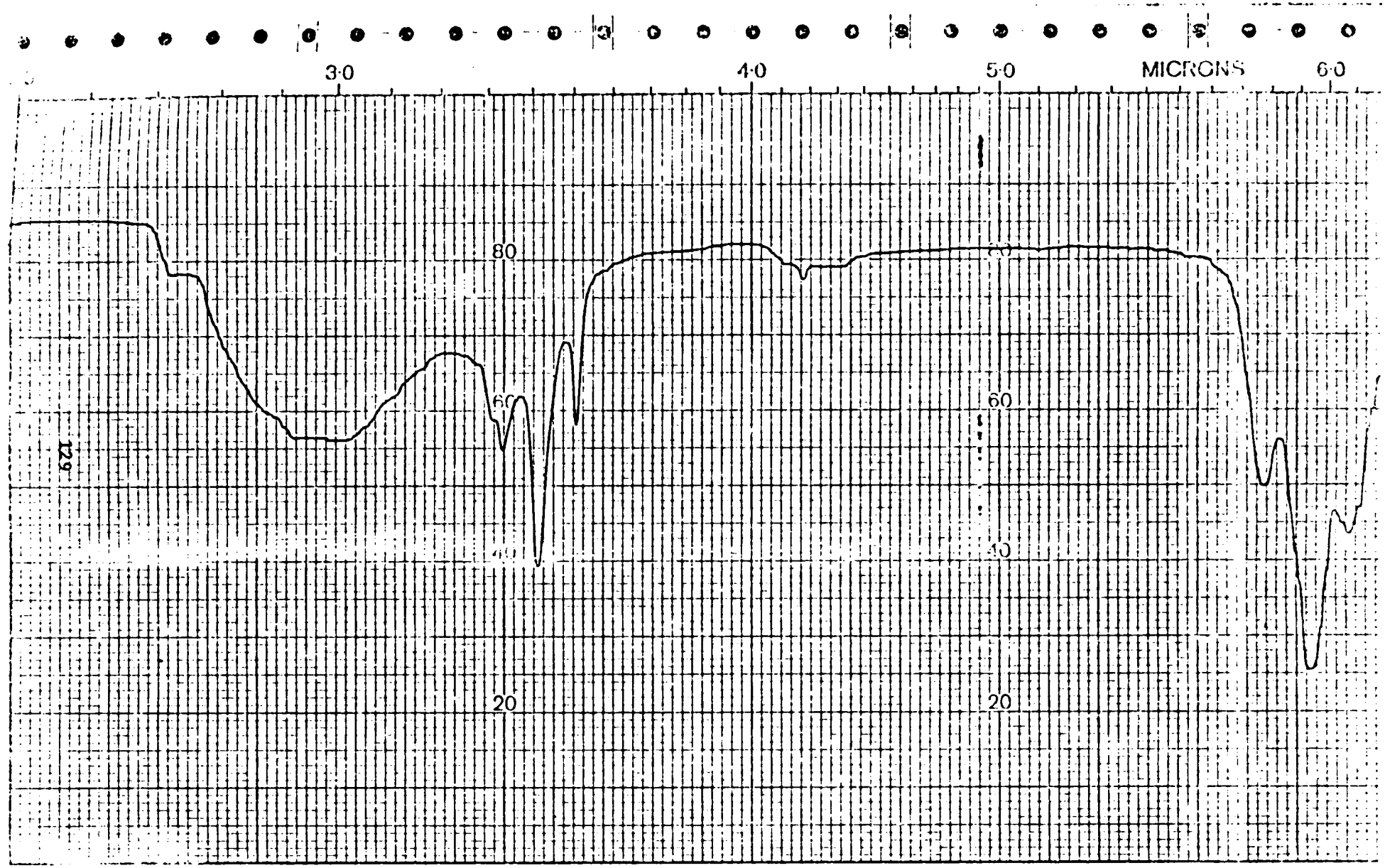
REMARKS

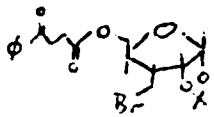


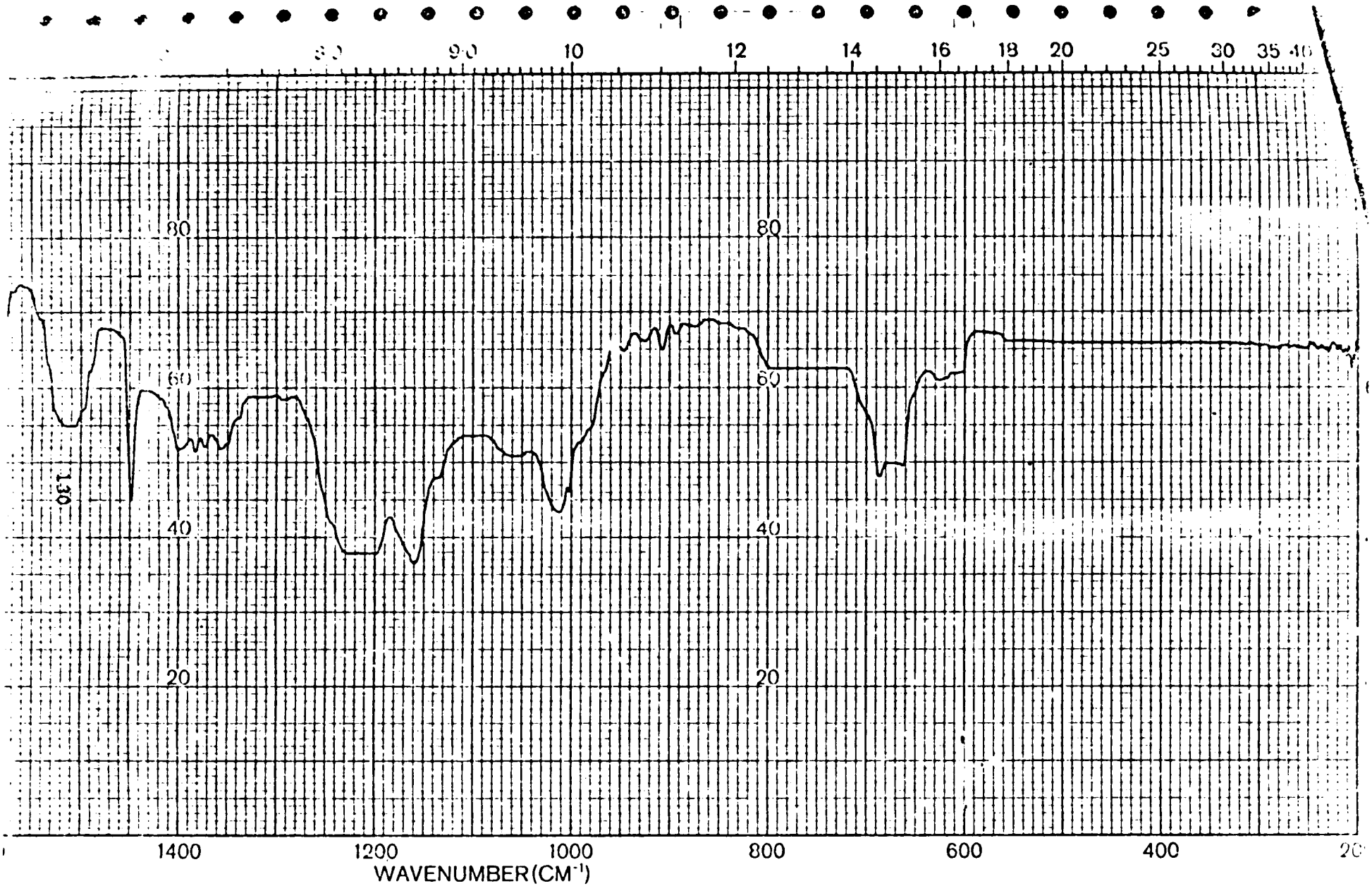
XXXVI	SCAN TIME	T SB	PERKIN ELMER
	SLIT	ORDINATE EXP.	CHART No. 5100-1367
	IR of XXXVI		
	OPERATOR DATE	TIME CONSTANT	REF. No.



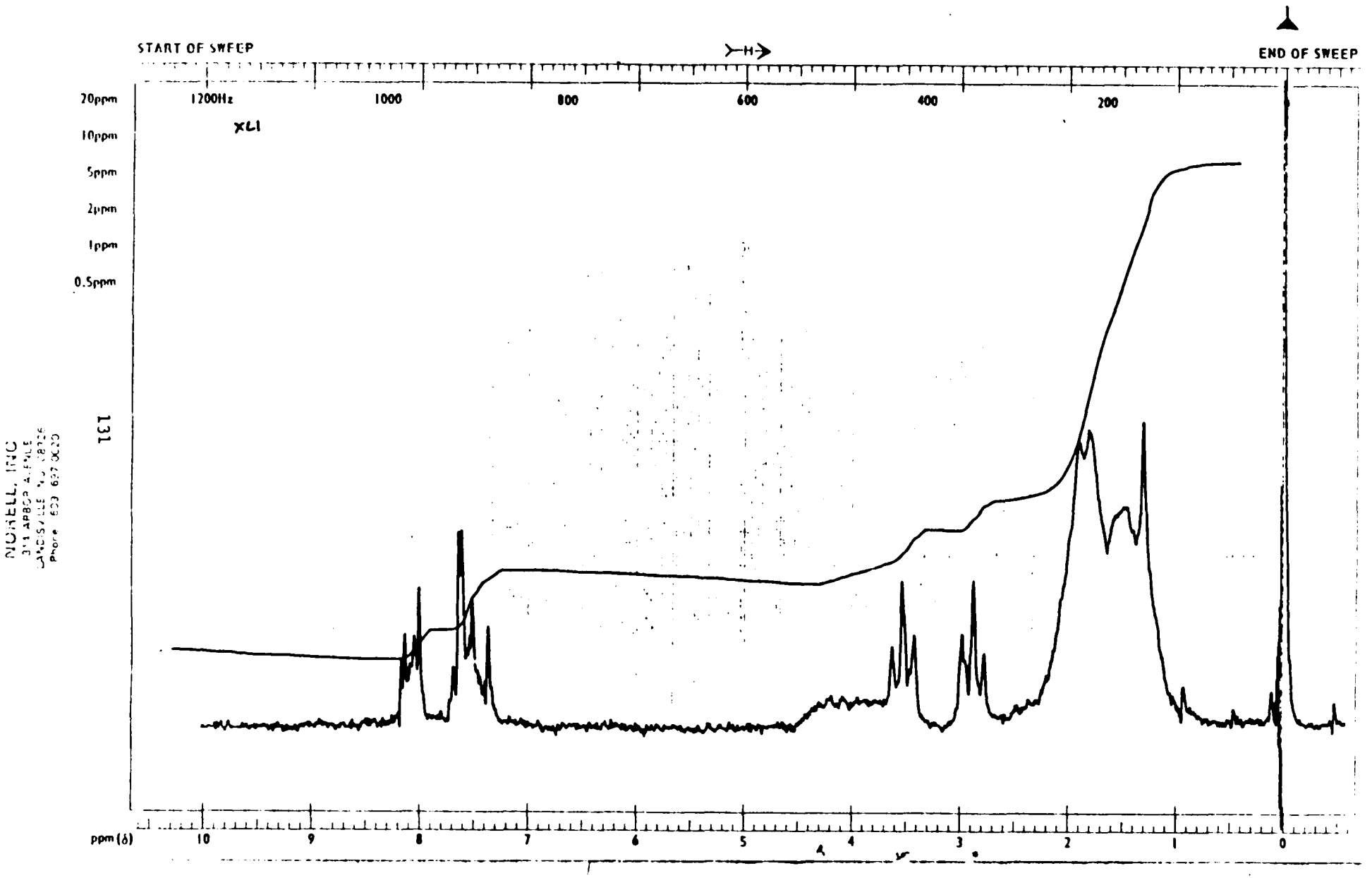
NMR of XXXVII



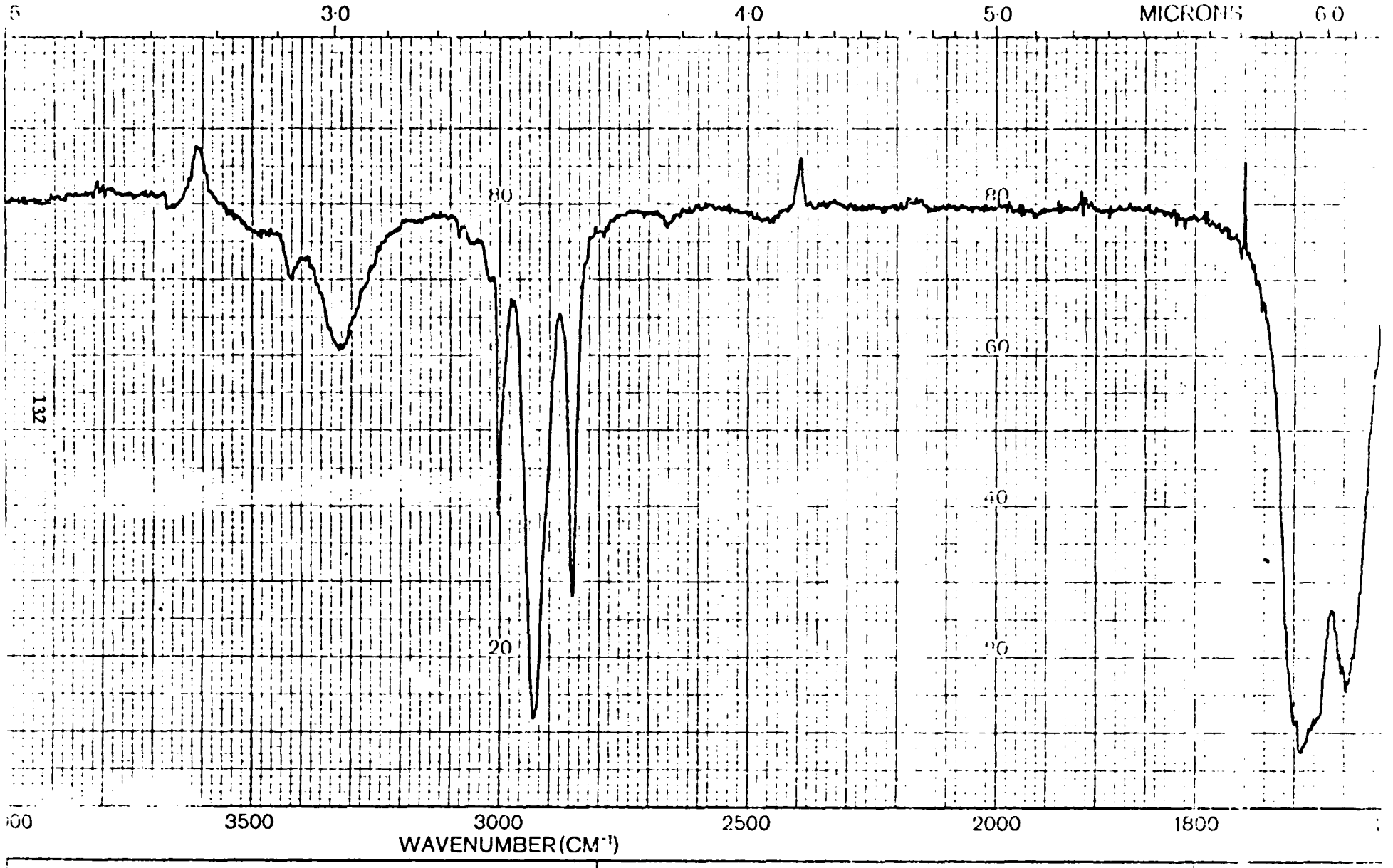
SAMPLE  (8) XXXVII IR of XXXVII	SOLVENT CH_2Cl_2 CONCENTRATION CELL PATH REFERENCE	REMARKS



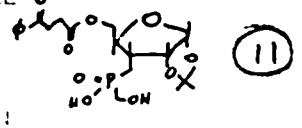
XXXVII	SCAN TIME	T. SB	PERKIN ELMER
	SLIT	ORDINATE EXP.	CHART No. 5100 4367
	OPERATOR IR of XXXVII DATE	TIME CONSTANT	REF No.


START OF SWEEP
END OF SWEEP
1200Hz
1000
800
600
400
200
20ppm
10ppm
5ppm
2ppm
1ppm
0.5ppm
XLI
131
ppm (δ)
10
9
8
7
6
5
4
3
2
1
0

NMR of XLI



SAMPLE



(11)

XLI

IR of XLI

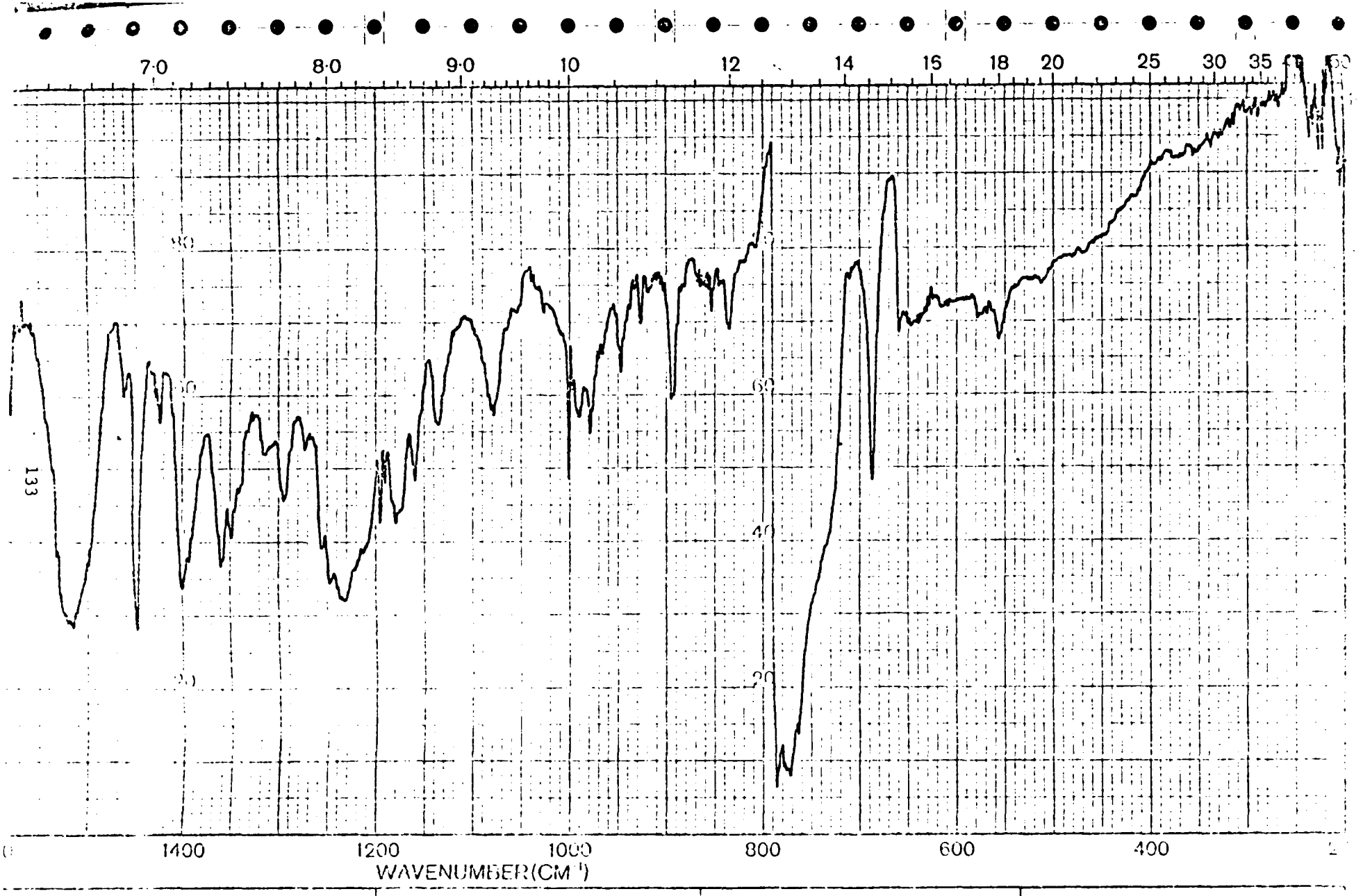
SOLVENT $CHCl_3$

CONCENTRATION

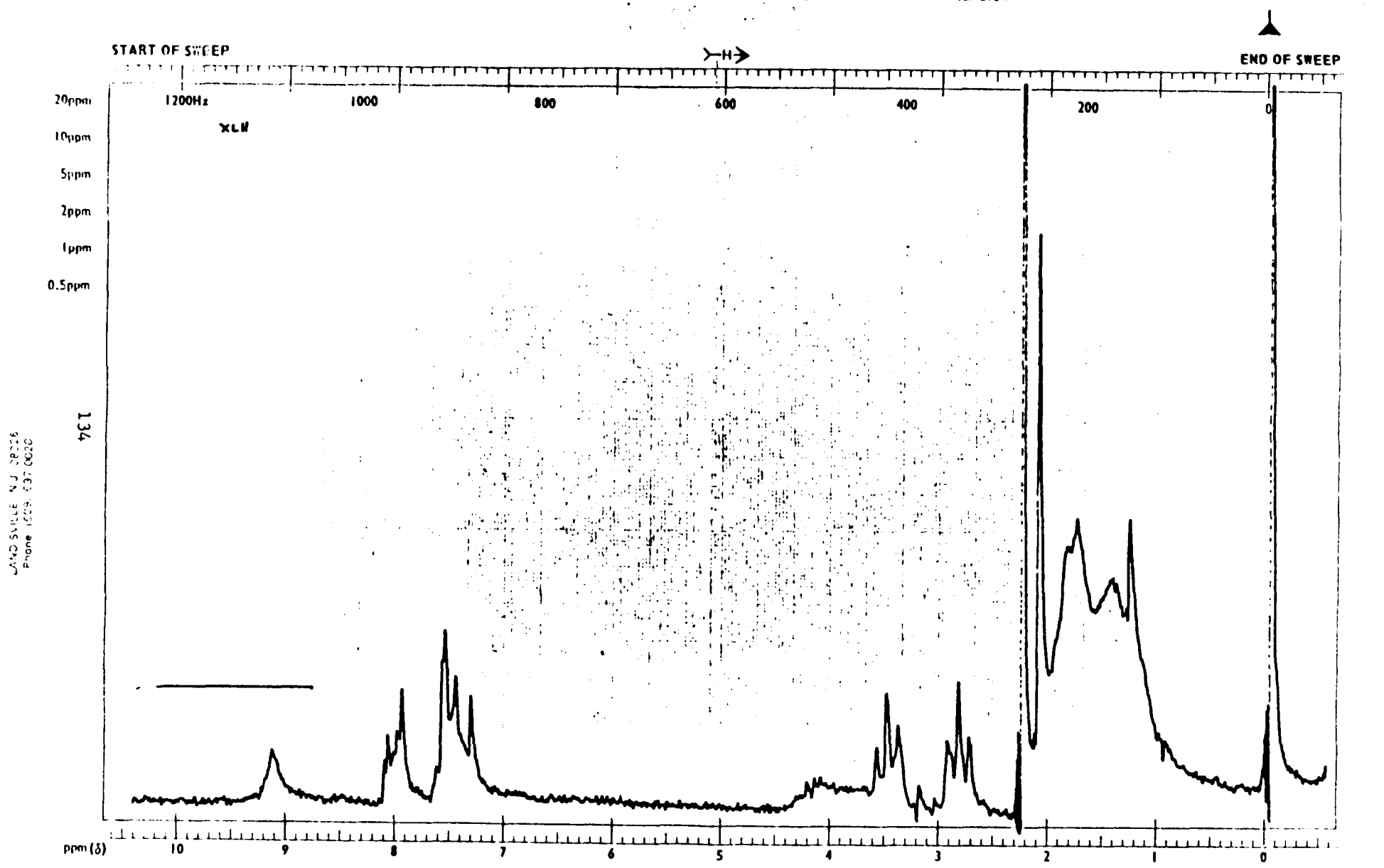
CELL PATH

REFERENCE

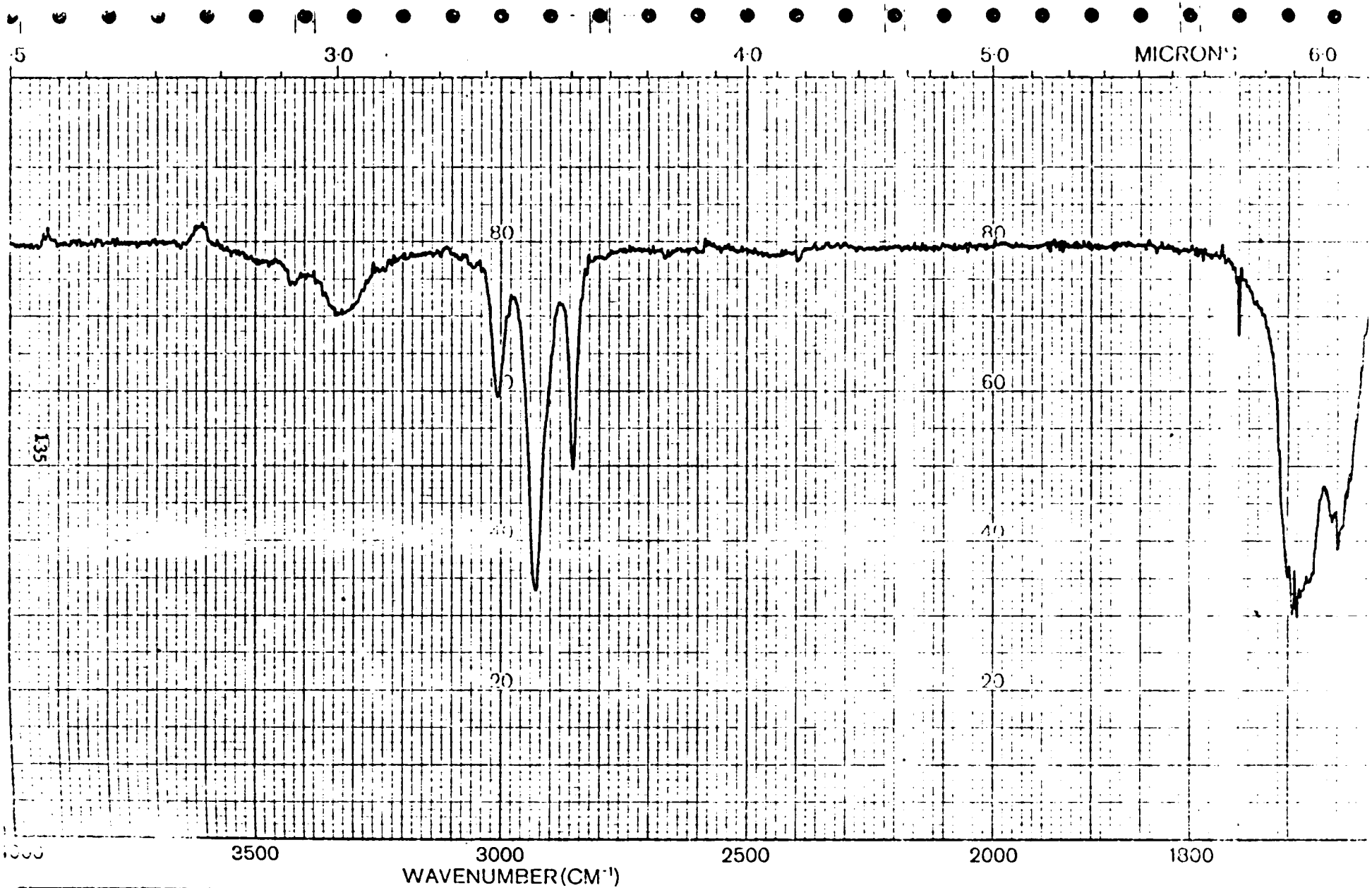
REMARKS



XLI	SCAN TIME	T. SB	PERKIN ELMER
	SLIT	ORDINATE EXP.	CHART No. 5100-436-7
	IR of XLI		
		TIME CONSTANT	REF No.


START OF SWEEP
END OF SWEEP
1200Hz
1000
800
600
400
200
0
20ppm
10ppm
5ppm
2ppm
1ppm
0.5ppm
XLII
ppm (δ)
10
9
8
7
6
5
4
3
2
1
0

NMR of XLII



(12)

XLII

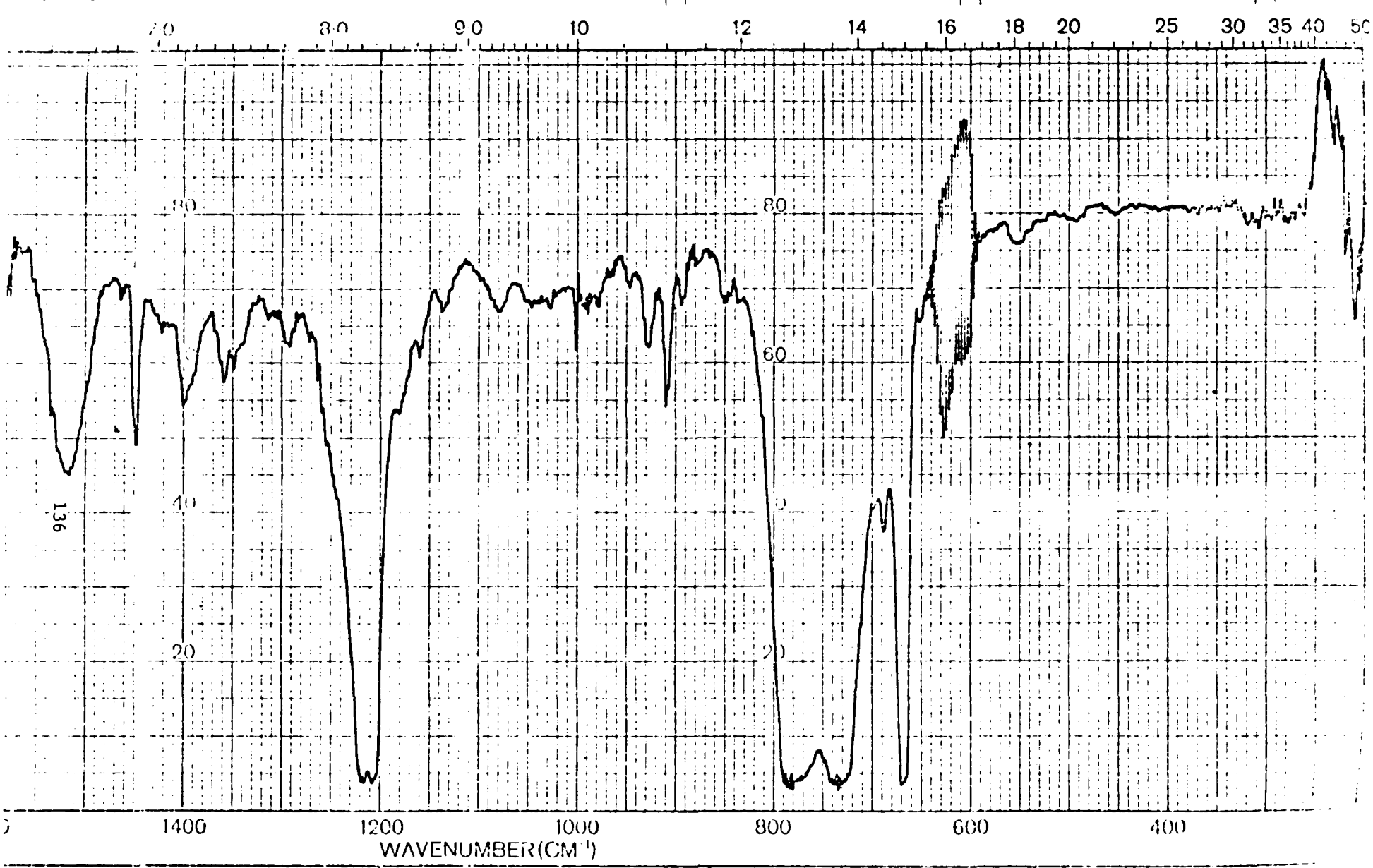
IR of XLII

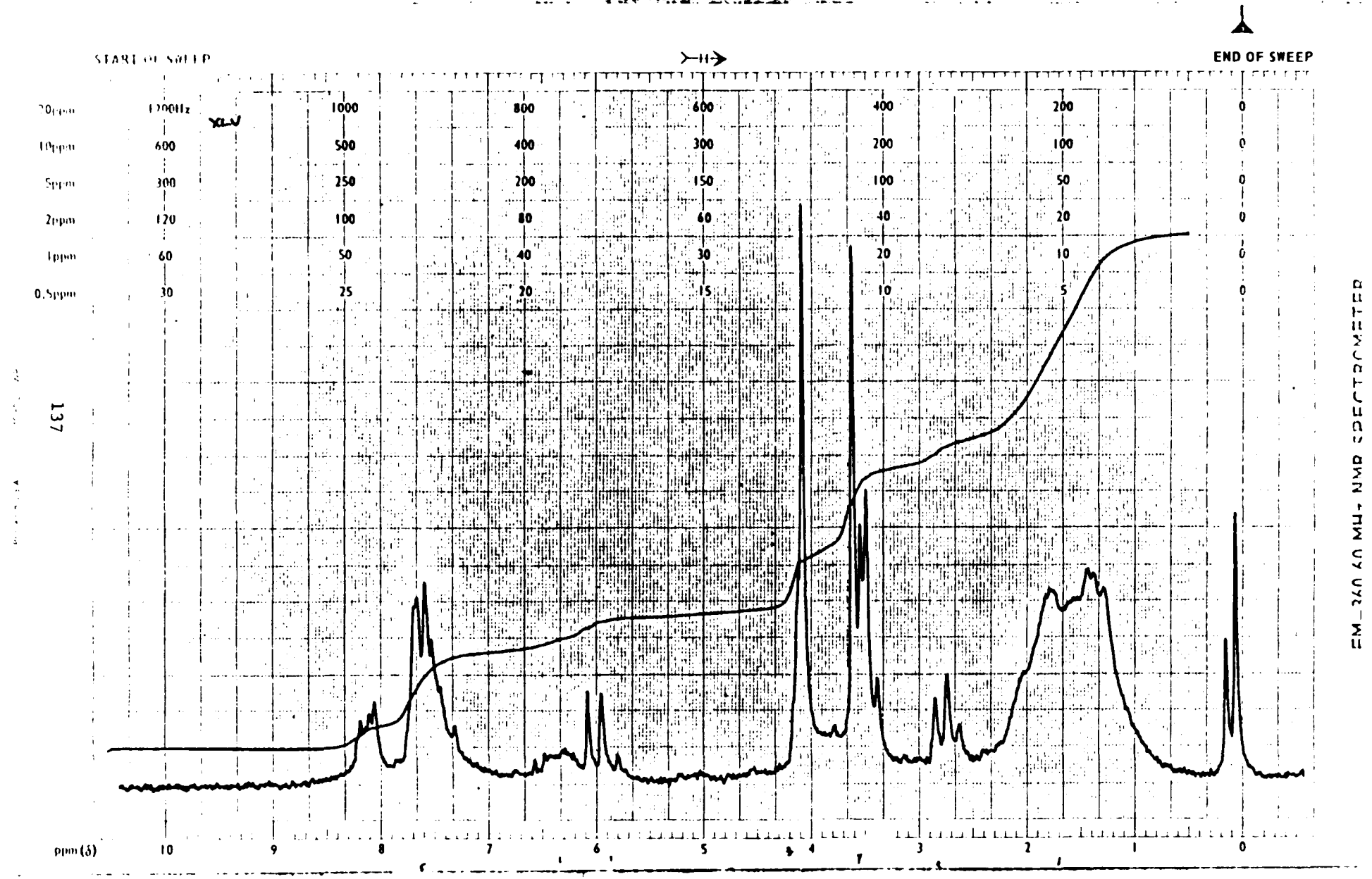
SOLVENT $CHCl_3$

CONCENTRATION

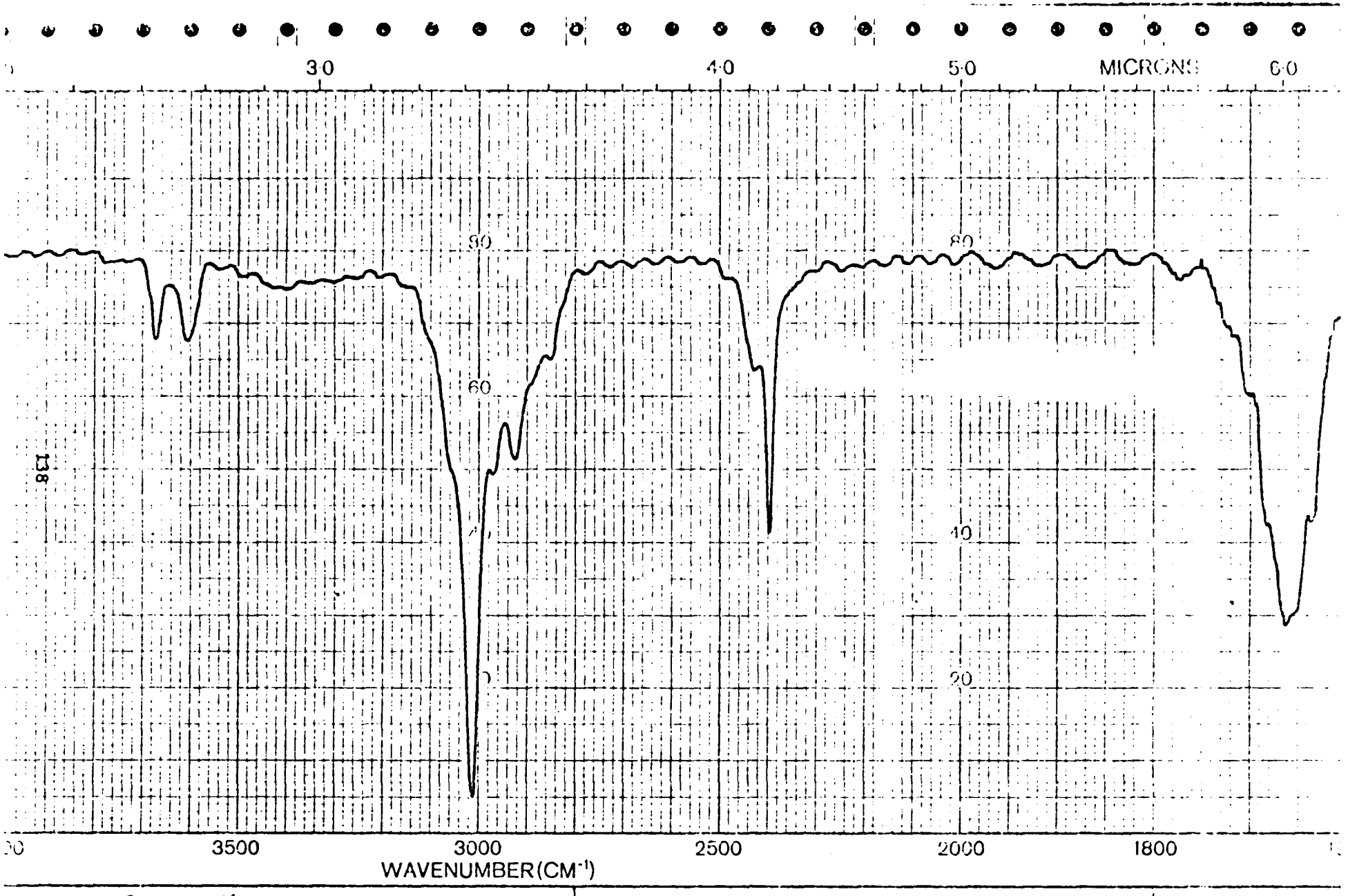
CELL PATH

REMARKS

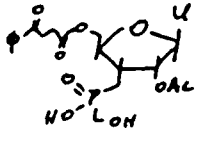

7.0
8.0
9.0
10
12
14
16
18
20
25
30
35
40
50
100
80
60
40
20
136
1400
1200
1000
800
600
400
WAVENUMBER(CM⁻¹)
XLII
SCAN TIME
SLIT
ORDINATE EXP.
CHART NO.
IR of XLII
RATE
DATE


START OF SWEEP
END OF SWEEP
XLV
20ppm 12000Hz 1000 800 600 400 200 0
10ppm 600 500 400 300 200 100 0
5ppm 300 250 200 150 100 50 0
2ppm 120 100 80 60 40 20 0
1ppm 60 50 40 30 20 10 0
0.5ppm 30 25 20 15 10 5 0
ppm(δ) 10 9 8 7 6 5 4 3 2 1 0
137
EM-360 60 MHz NMR SPECTROMETER

NMR of XLV



SAMPLE



(13) XLV

IR of XLV

ORIGIN

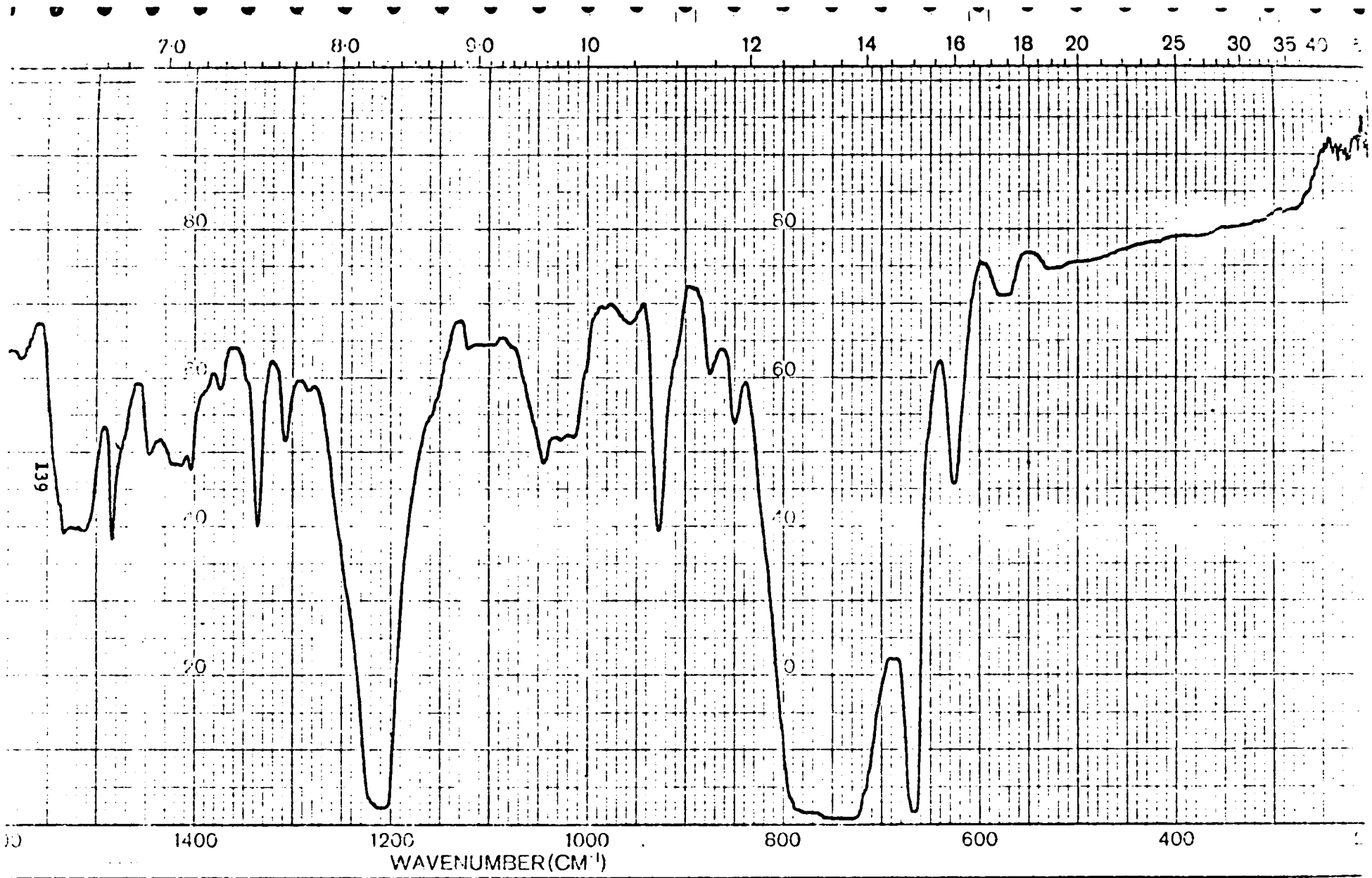
SOLVENT $CHCl_3$

CONCENTRATION

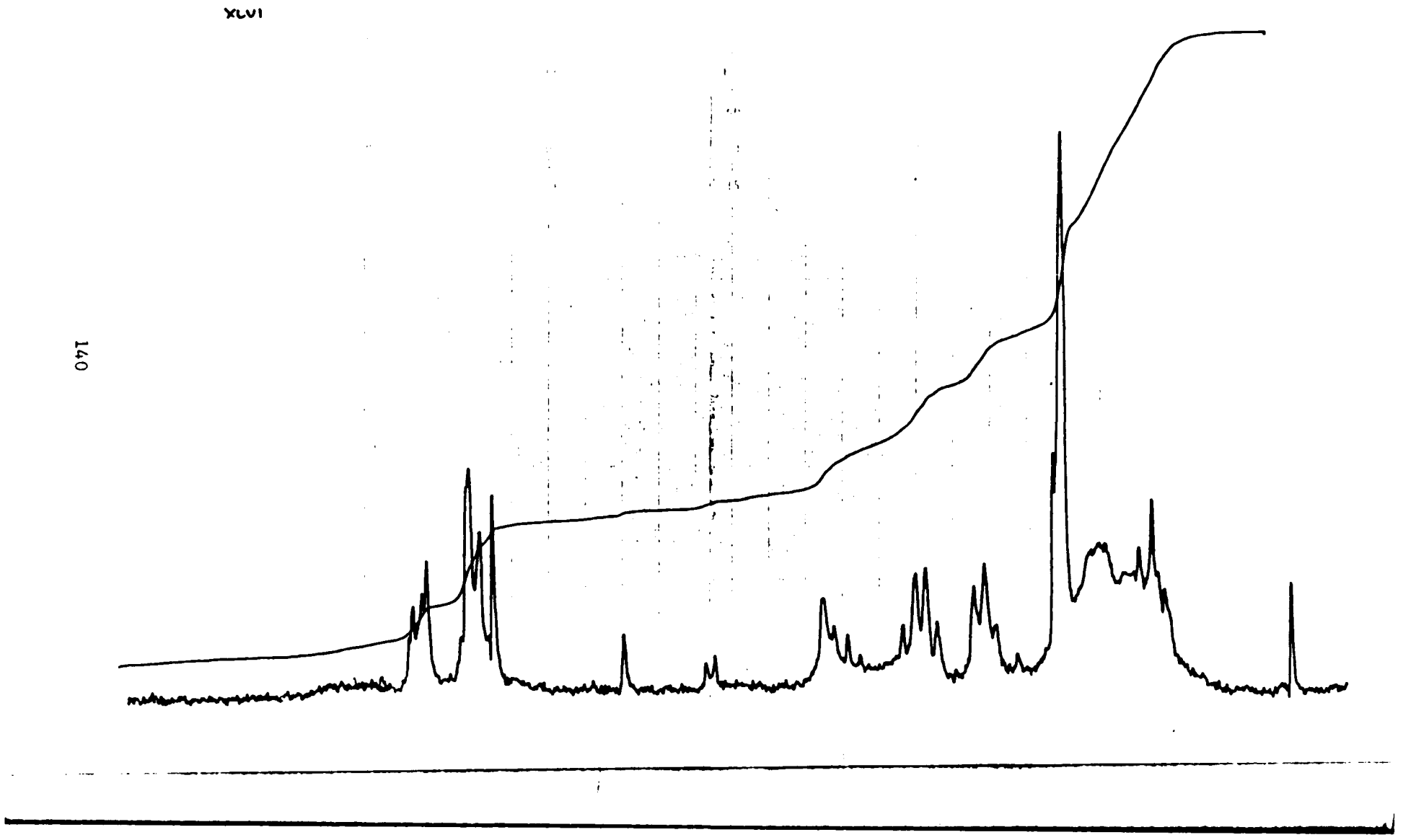
CELL PATH

REFERENCE

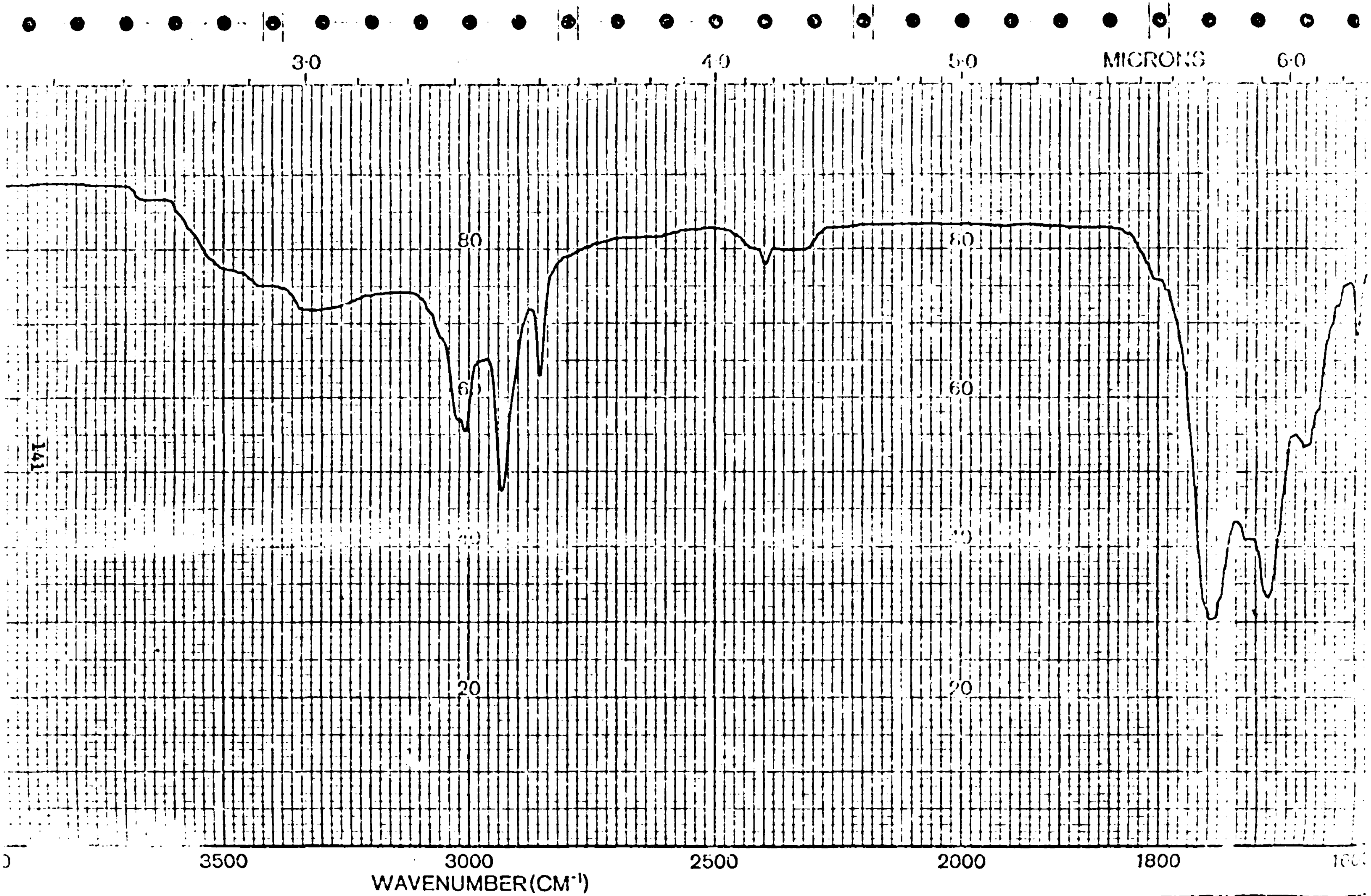
REMARKS



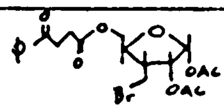
XLV	SCAN TIME	T. SB
	SLIT	ORDINATE EXP.
IR of XLV		
OPERATOR DATE	TIME CONSTANT	PERKIN ELMER CHART No 5100 4367 RUN No



NMR of XLVI



SAMPLE



(9) / [illegible]

BG-76

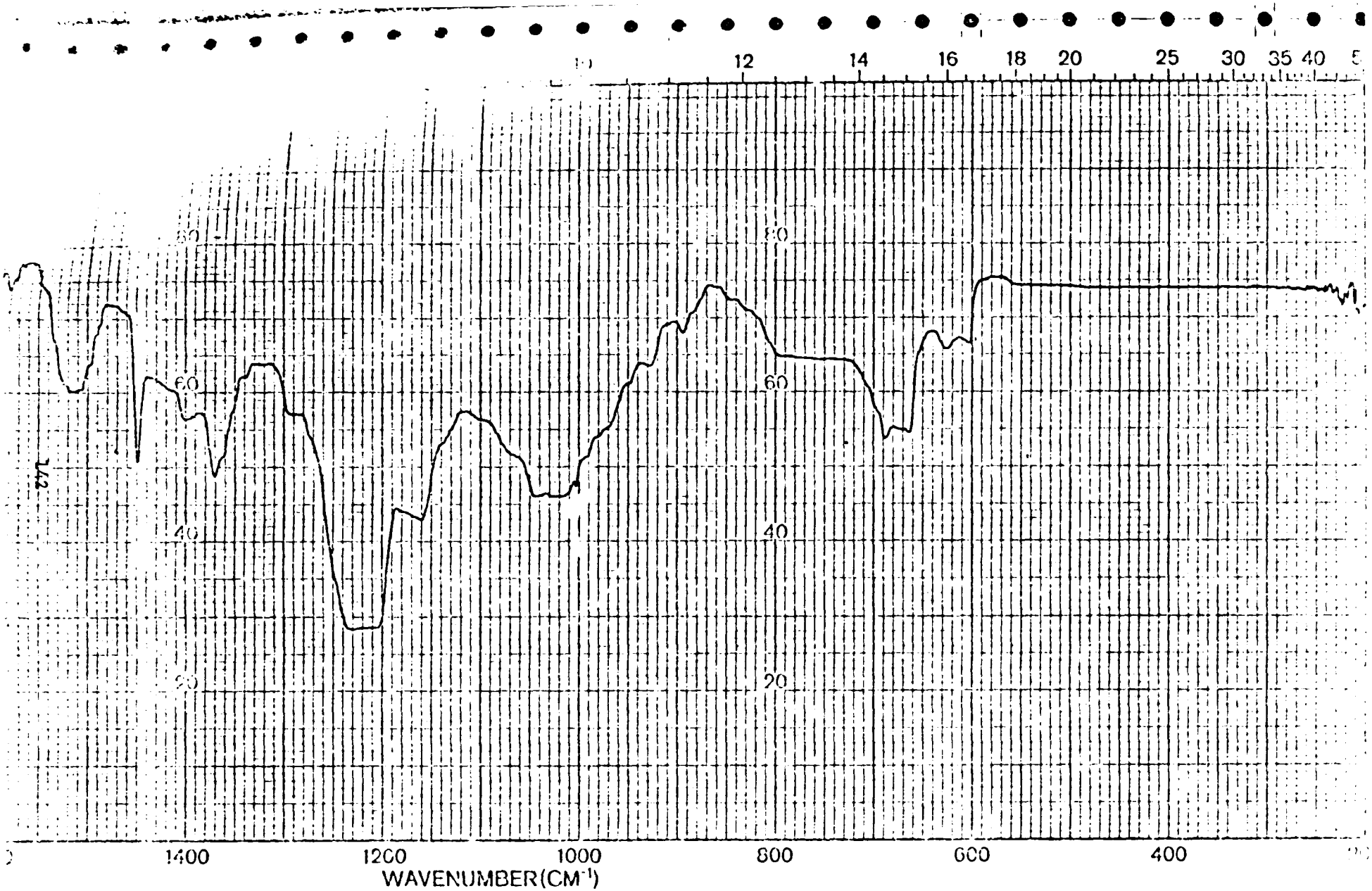
XLVI

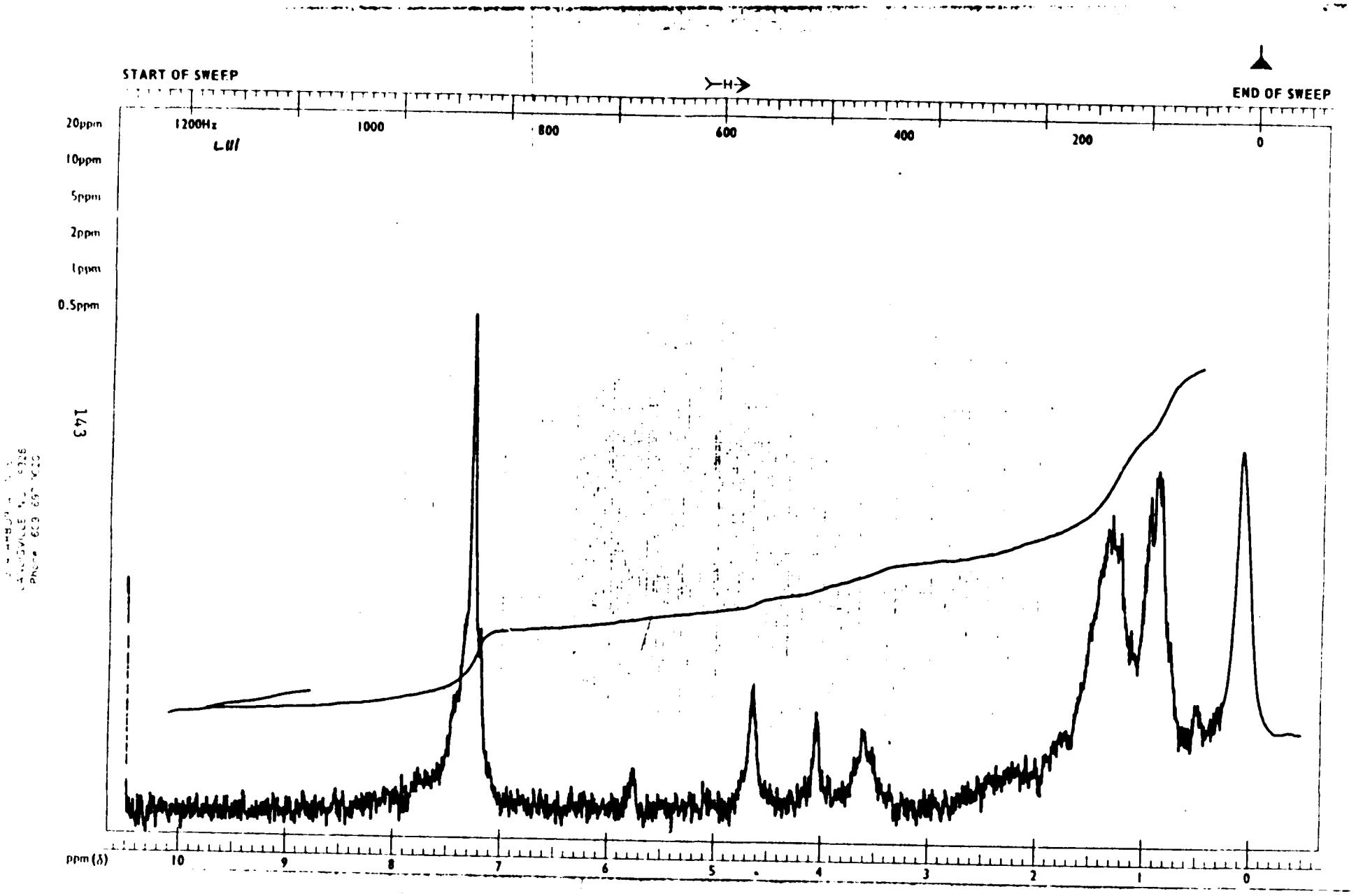
SOLVENT $CHCl_3$

CONCENTRATION

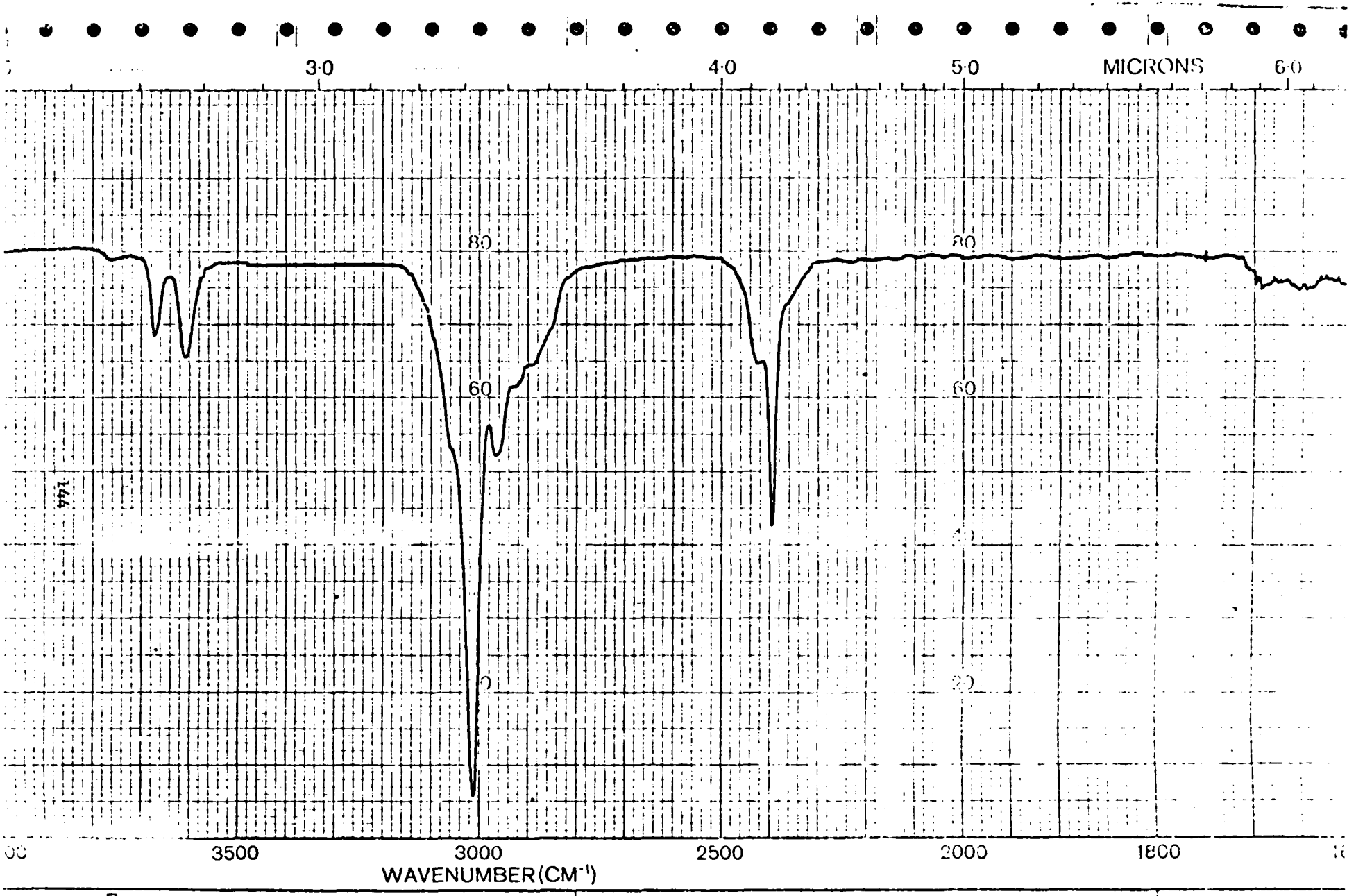
CELL PATH

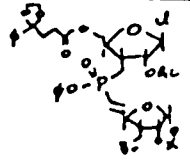
REFERENCE IR of XLVI


12
14
16
18
20
25
30
35
40
80
60
40
20
1400
1200
1000
800
600
400
WAVENUMBER(CM-1)
XLVI
SCAN TIME
SLIT
T
SB
ORDINATE EXP.
PERKIN ELMER
CHART No.
IR of XLVI
OPERATOR
DATE
TIME CONSTANT
REF No.


START OF SWEEP
END OF SWEEP
20ppm
10ppm
5ppm
2ppm
1ppm
0.5ppm
1200Hz
1000
800
600
400
200
0
143
ppm (δ)
10
9
8
7
6
5
4
3
2
1
0

NMR of LIII



SAMPLE 

(19) L III

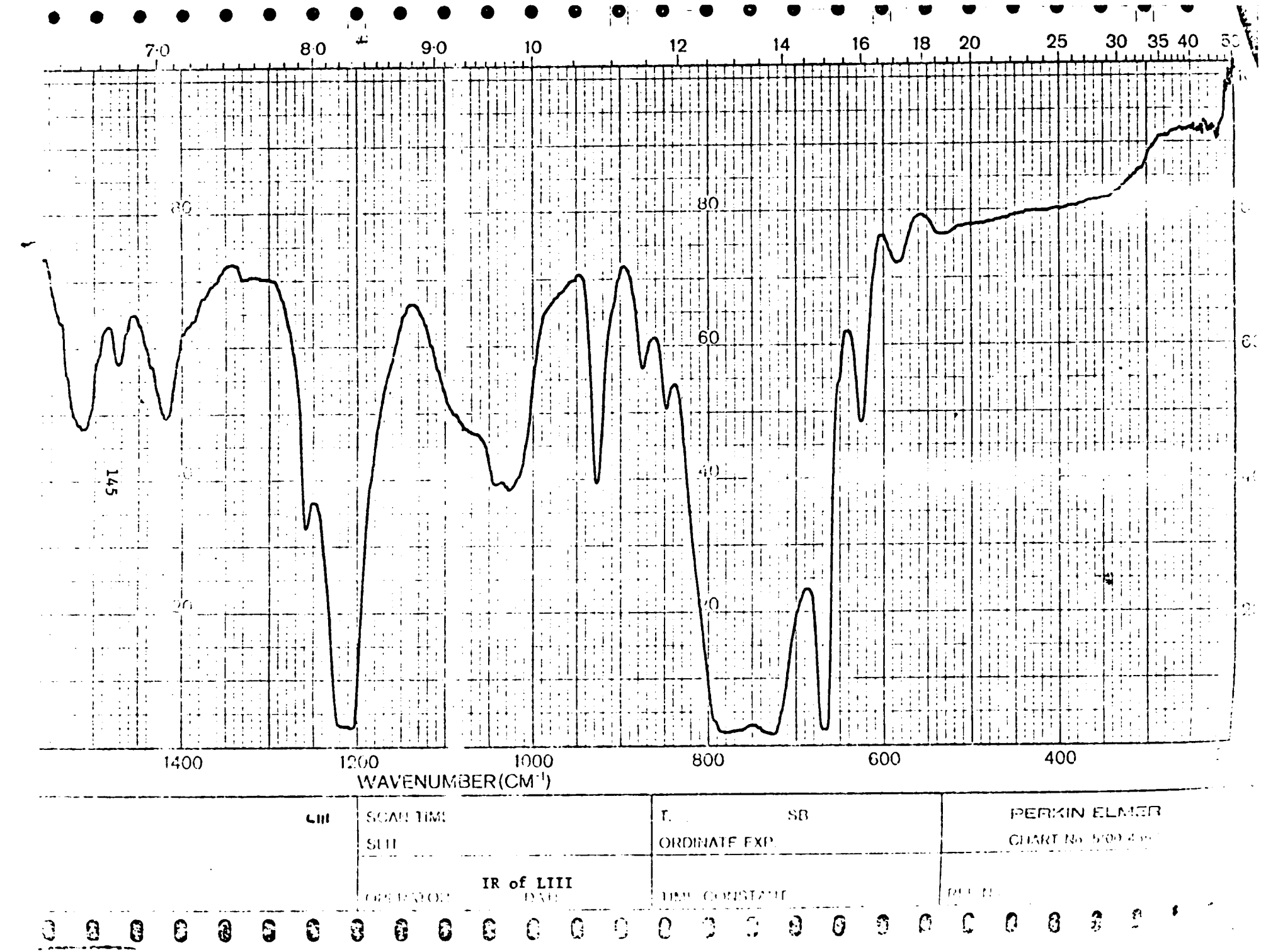
IR of LIII

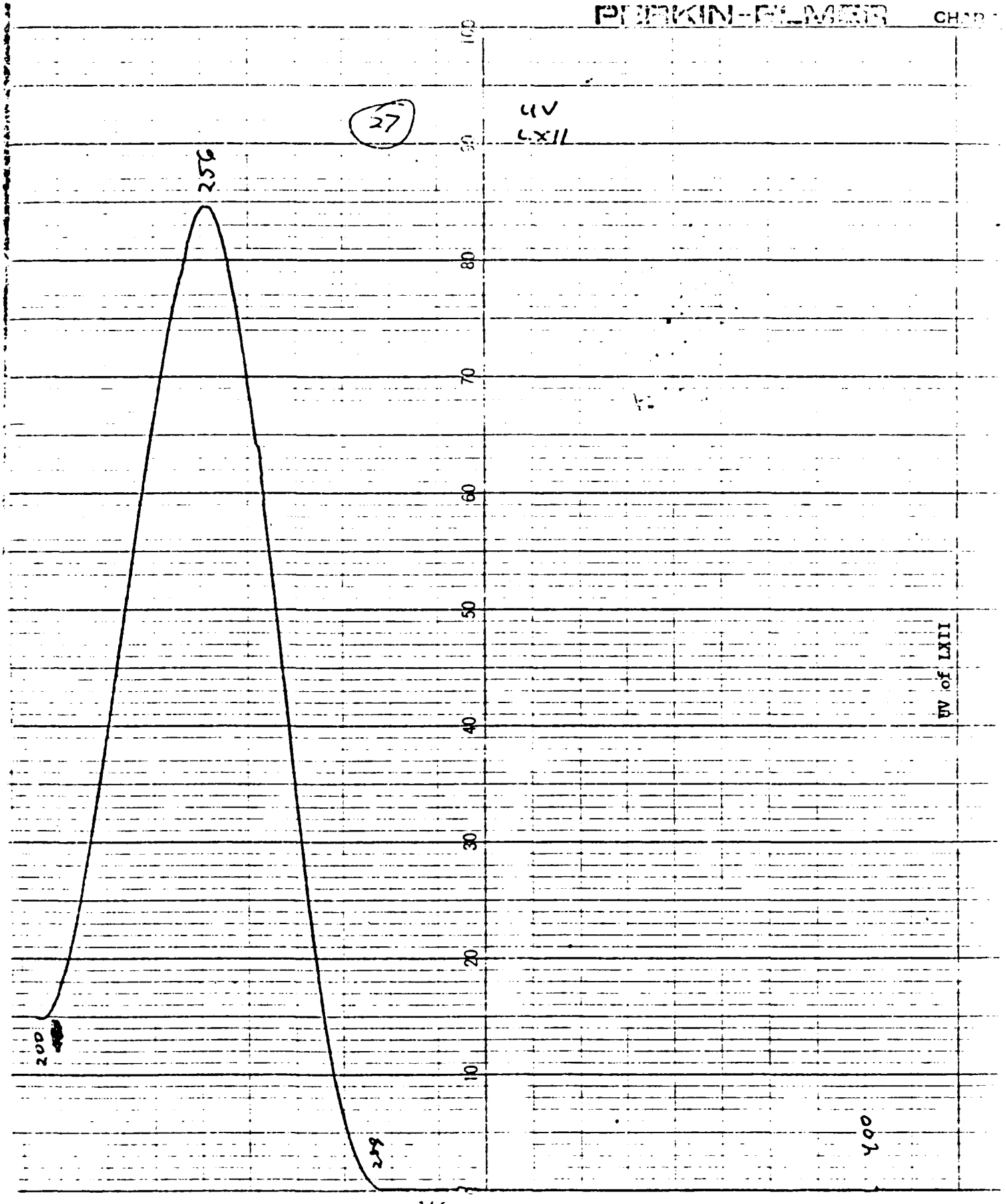
SOLVENT CH_2Cl_2

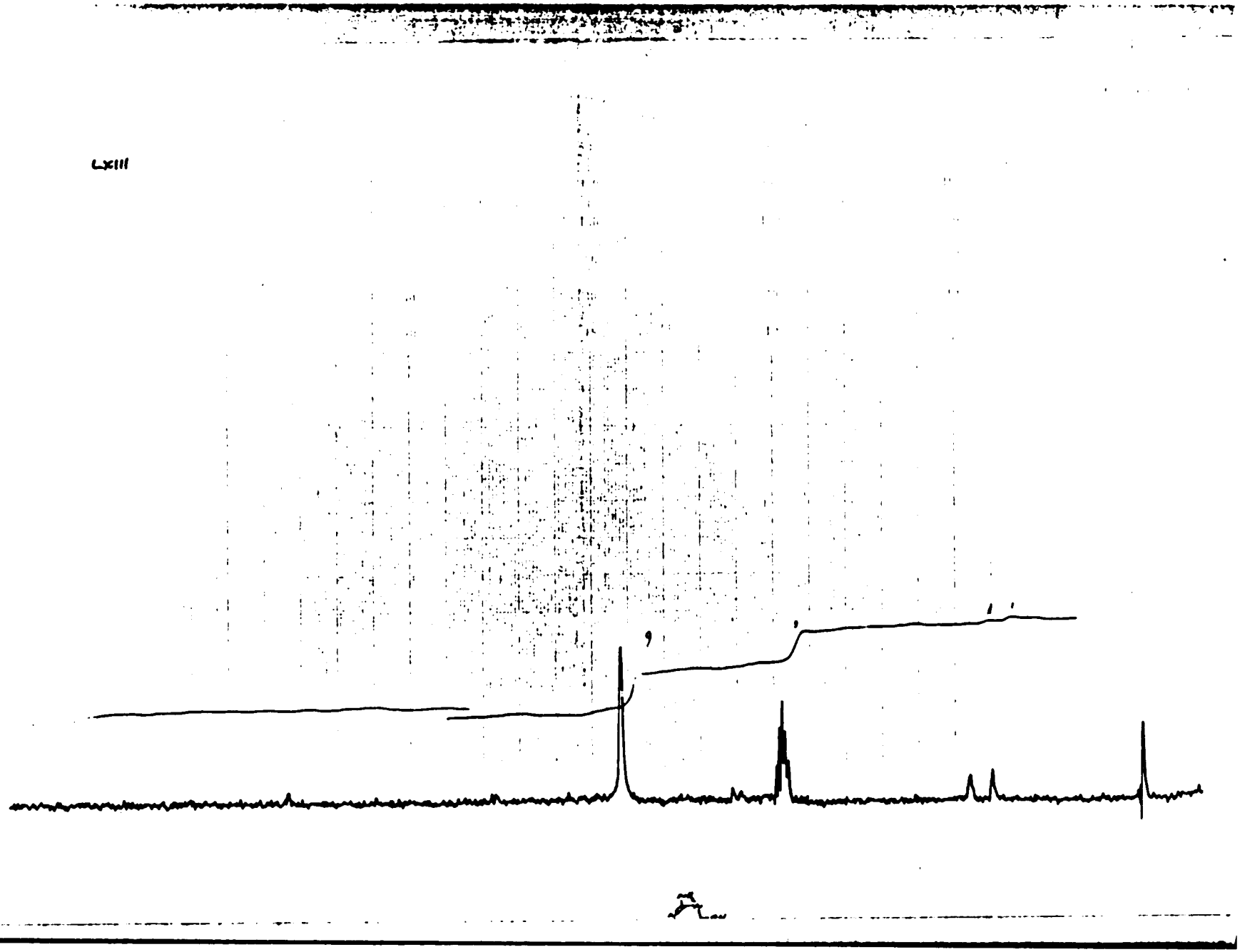
CONCENTRATION

CELL PATH

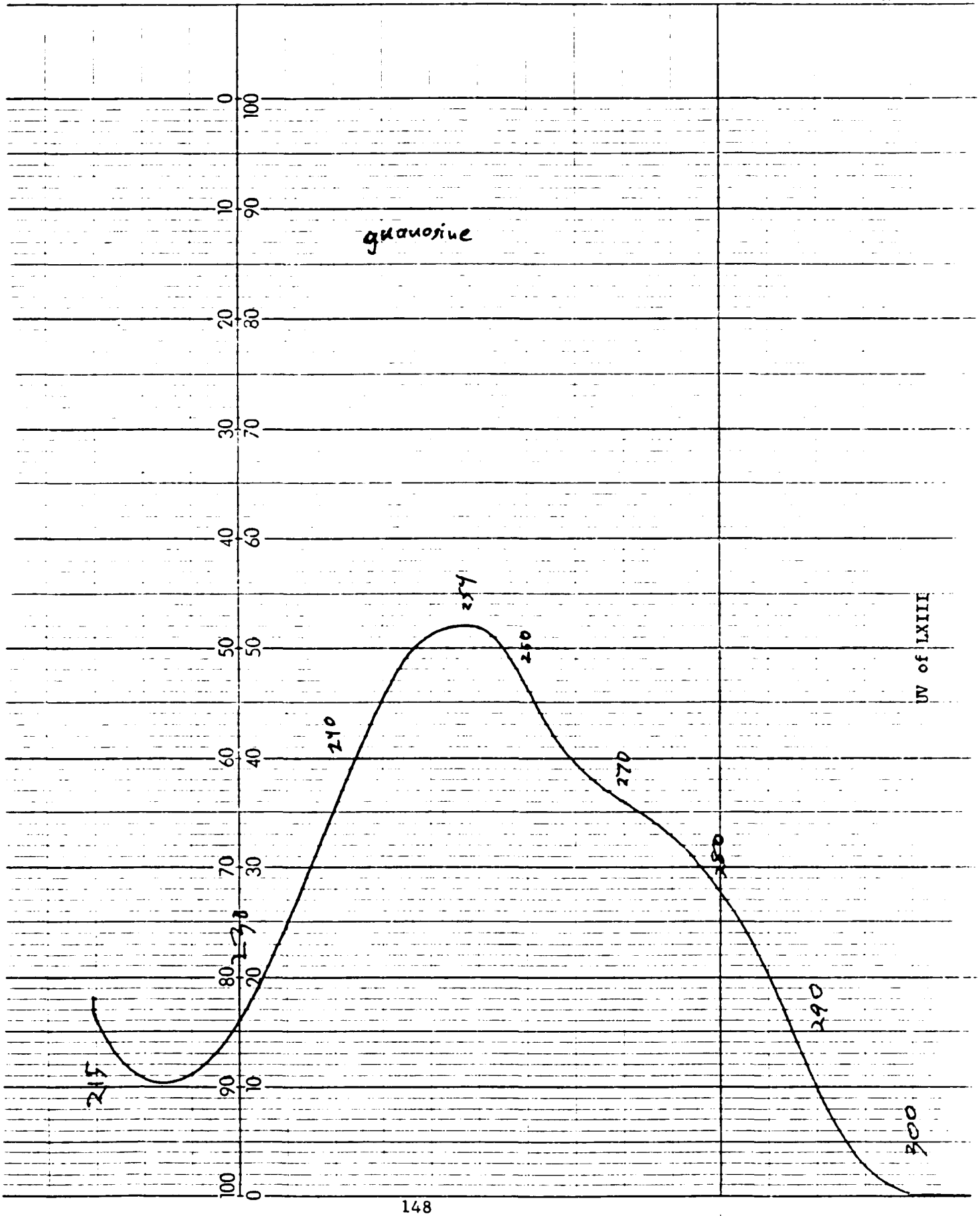
REMARKS

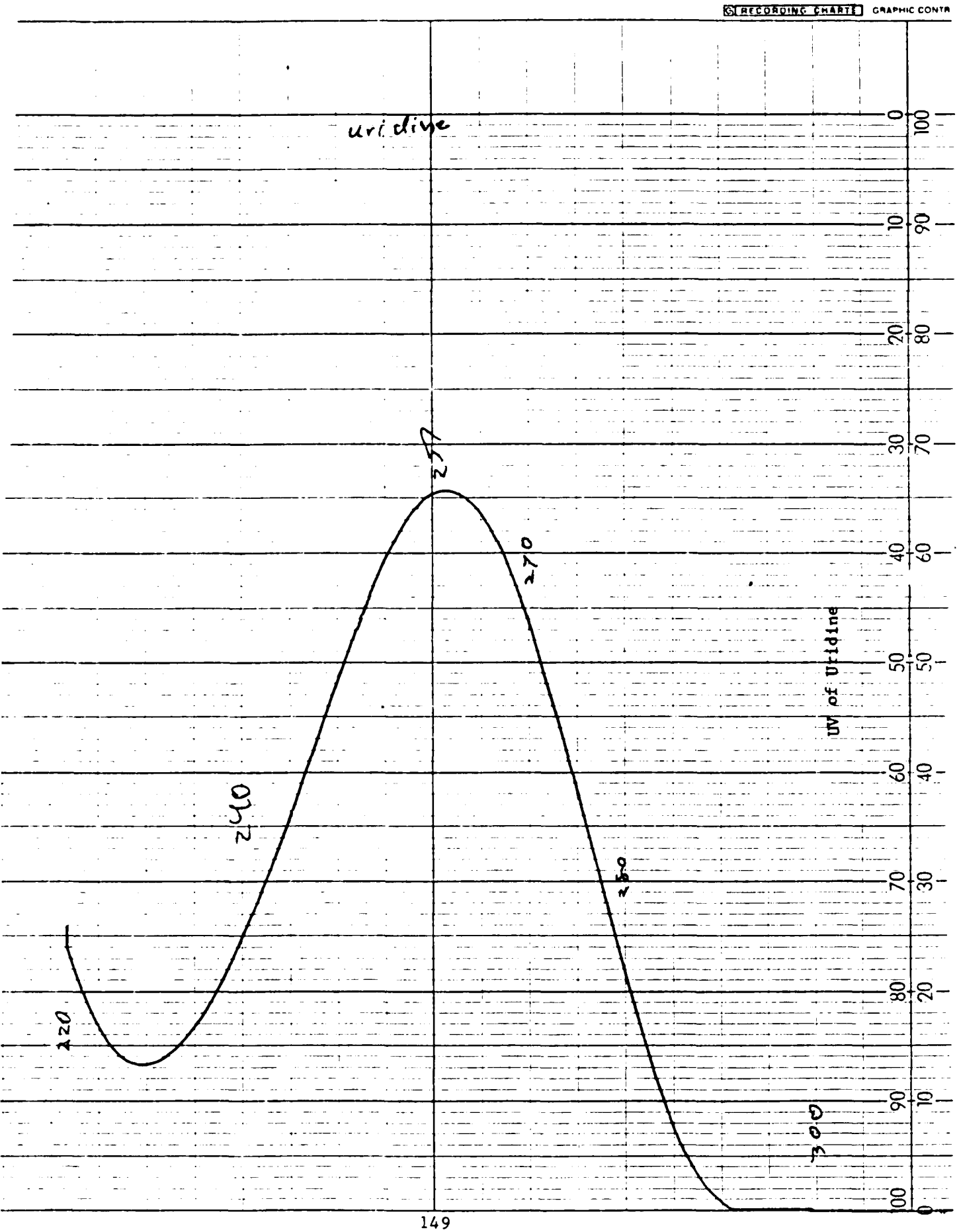

7·0
8·0
9·0
10
12
14
16
18
20
25
30
35
40
80
60
40
20
145
1400
1200
1000
800
600
400
WAVENUMBER(CM⁻¹)
SCAN TIME
SLIT
ORDINATE EXP
TIME CONSTANT
PERKIN ELMER
IR of LIII

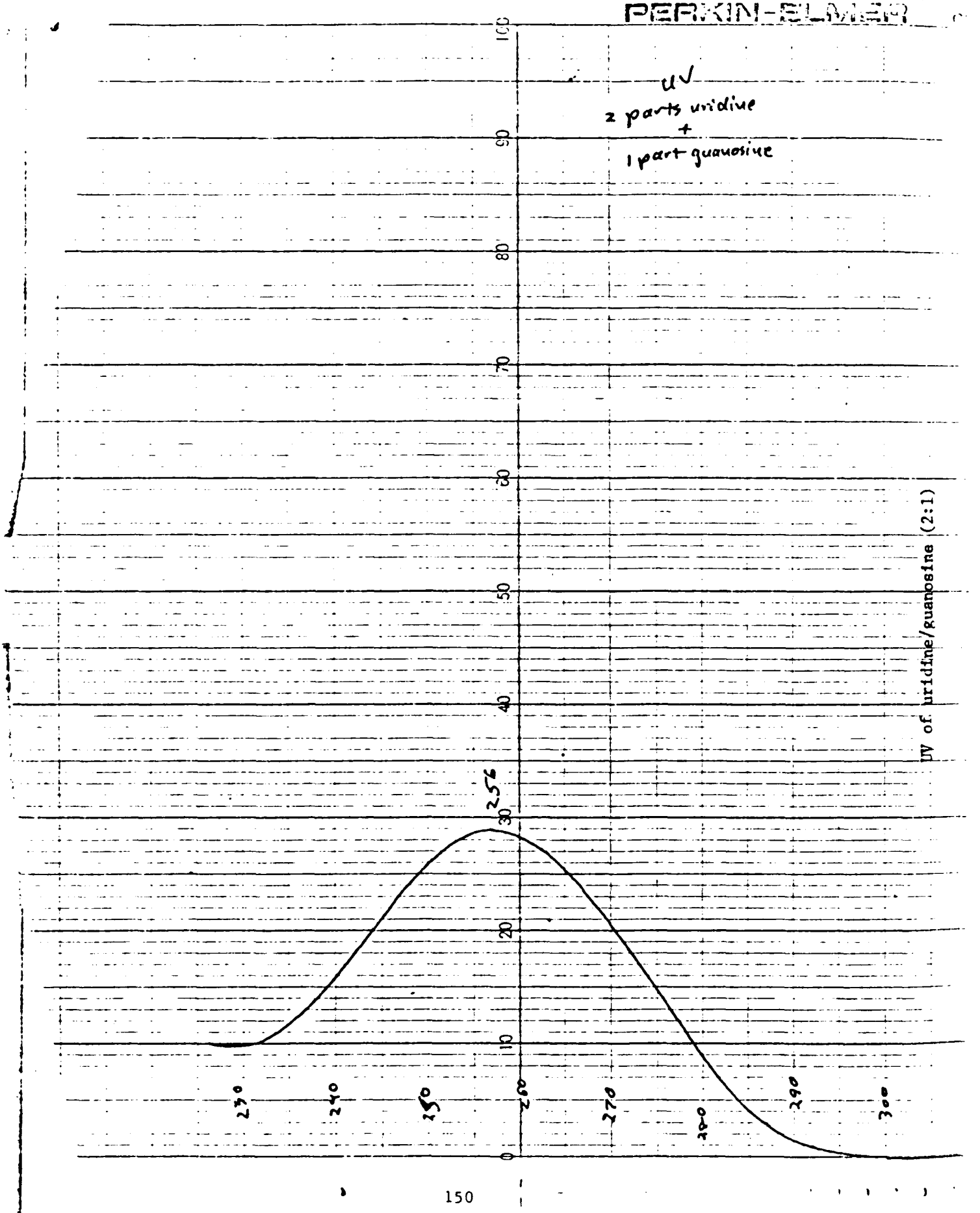

PERKIN-ELMER
27
UV
LXII
256
100
90
80
70
60
50
40
30
20
10
200
UV of LXII

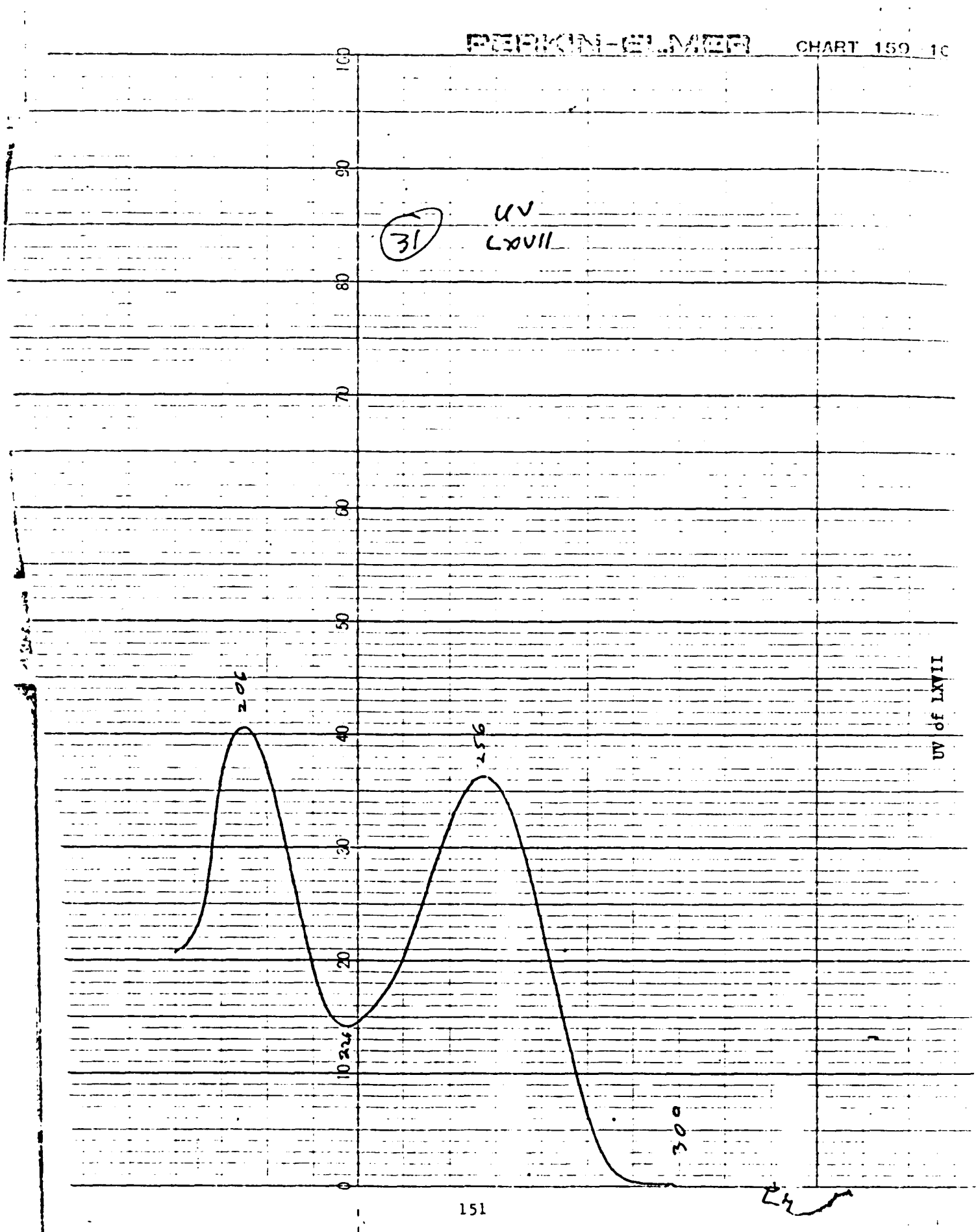


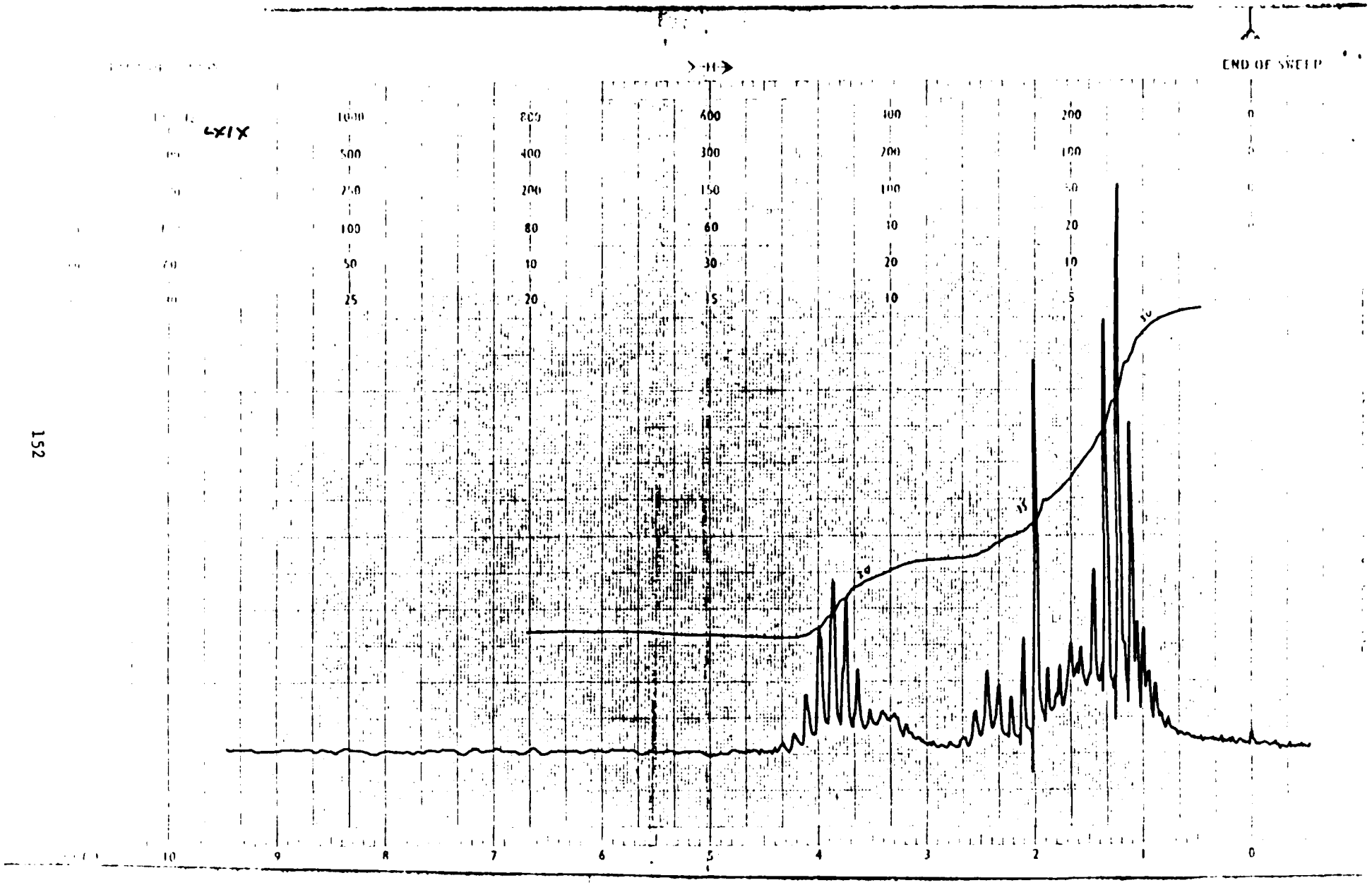
NMR of LXIII


guanosine
254
260
240
270
280
290
300
215
UV of LXIII

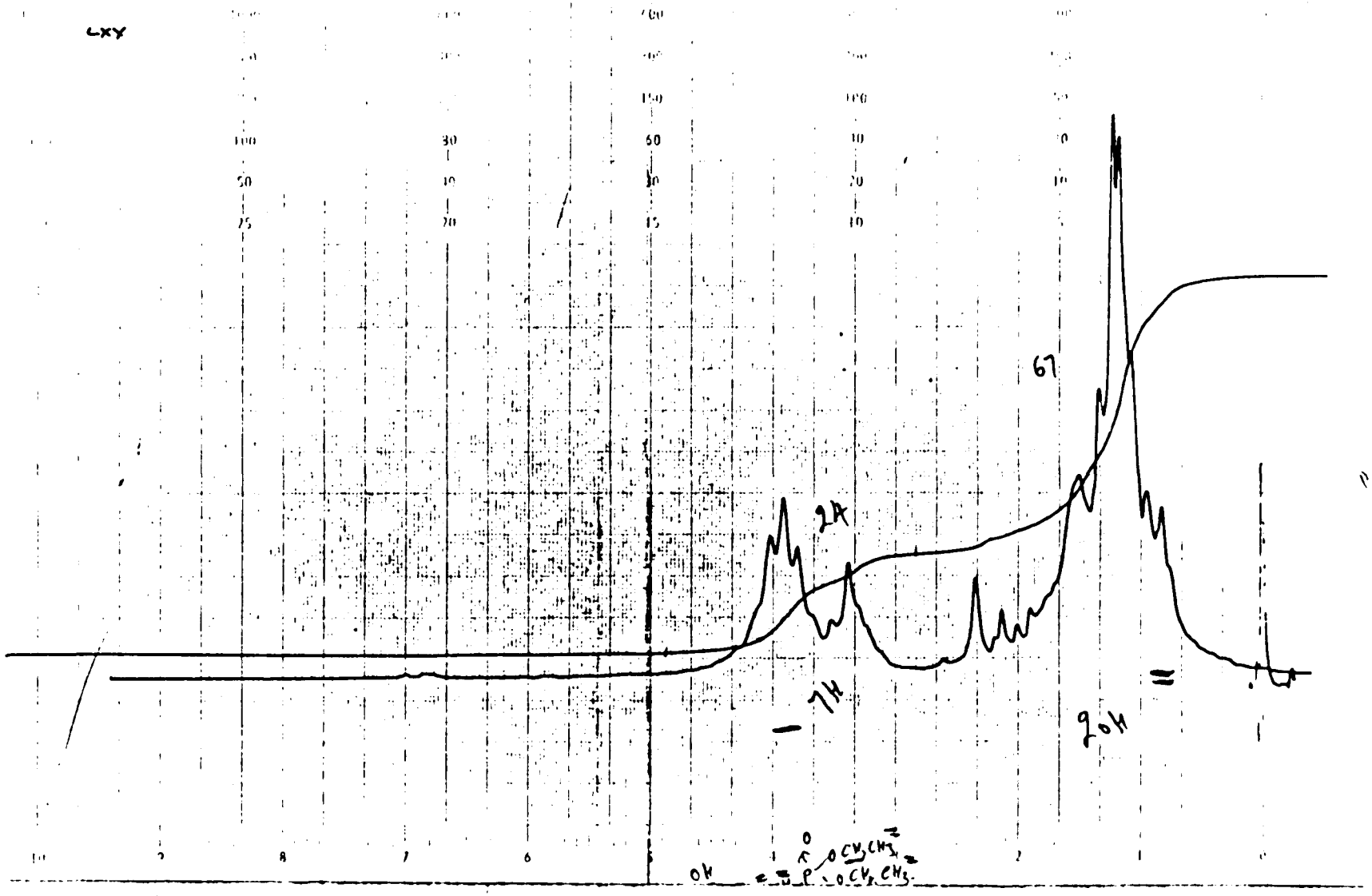

RECORDING CHARTS GRAPHIC CONTR
uridine
260
270
240
220
250
300
UV of Uridine
0
10
20
30
40
50
60
70
80
90
100


PERKIN-ELMER
UV
2 parts uridine
+
1 part guanosine
256
100
90
80
70
60
50
40
30
20
10
0
230
240
250
260
270
280
290
300
UV of uridine/guanosine (2:1)

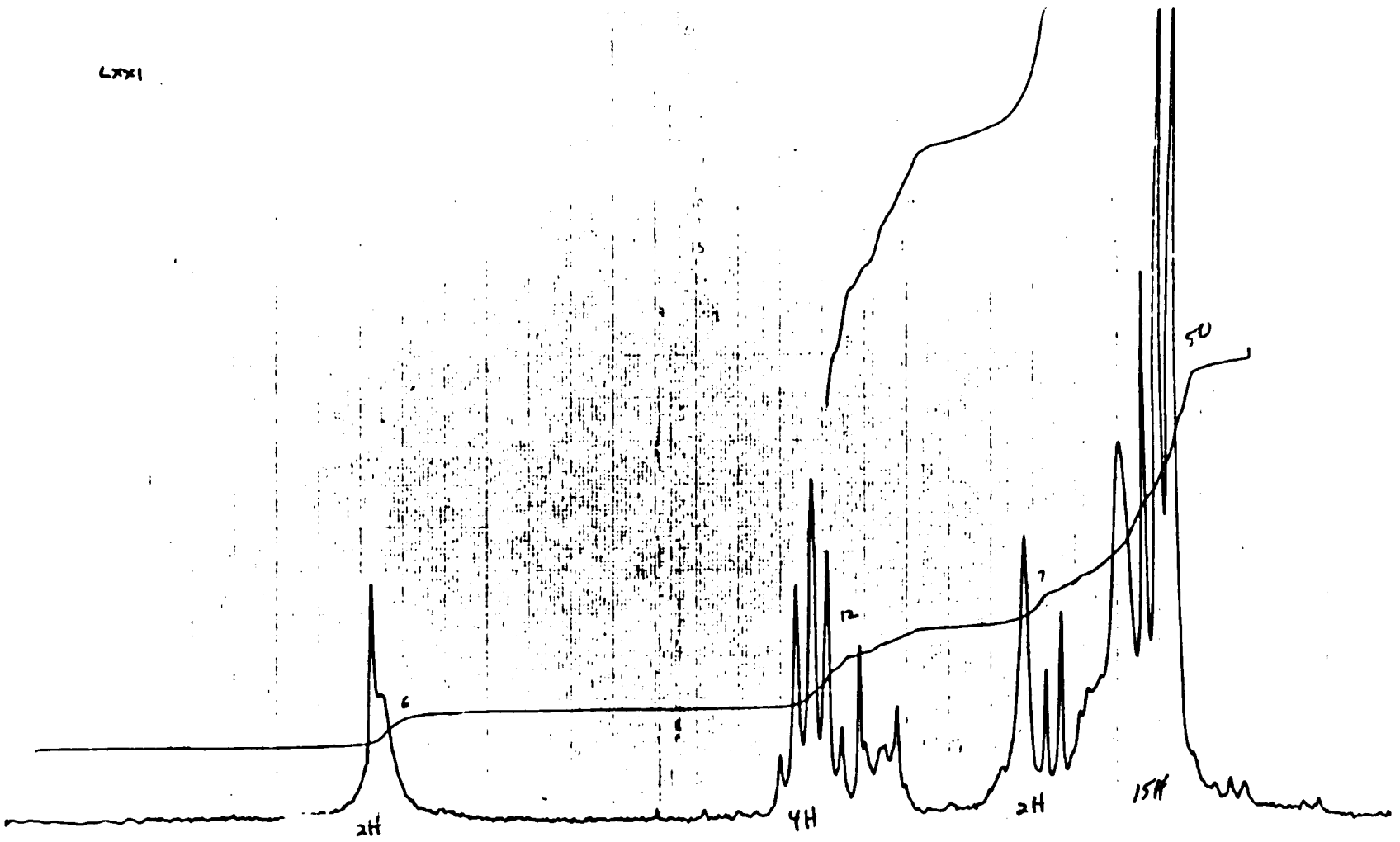

PERKIN-ELMER
CHART 159
100
90
80
70
60
50
40
30
20
10
0
31
UV
LXVII
206
256
300
UV of LXVII


LXIX
END OF SWEEP
1000
500
250
100
50
25
800
400
200
80
40
20
400
300
150
60
30
15
100
200
100
50
20
10
5
200
100
50
20
10
5
0
10
9
8
7
6
5
4
3
2
1
0

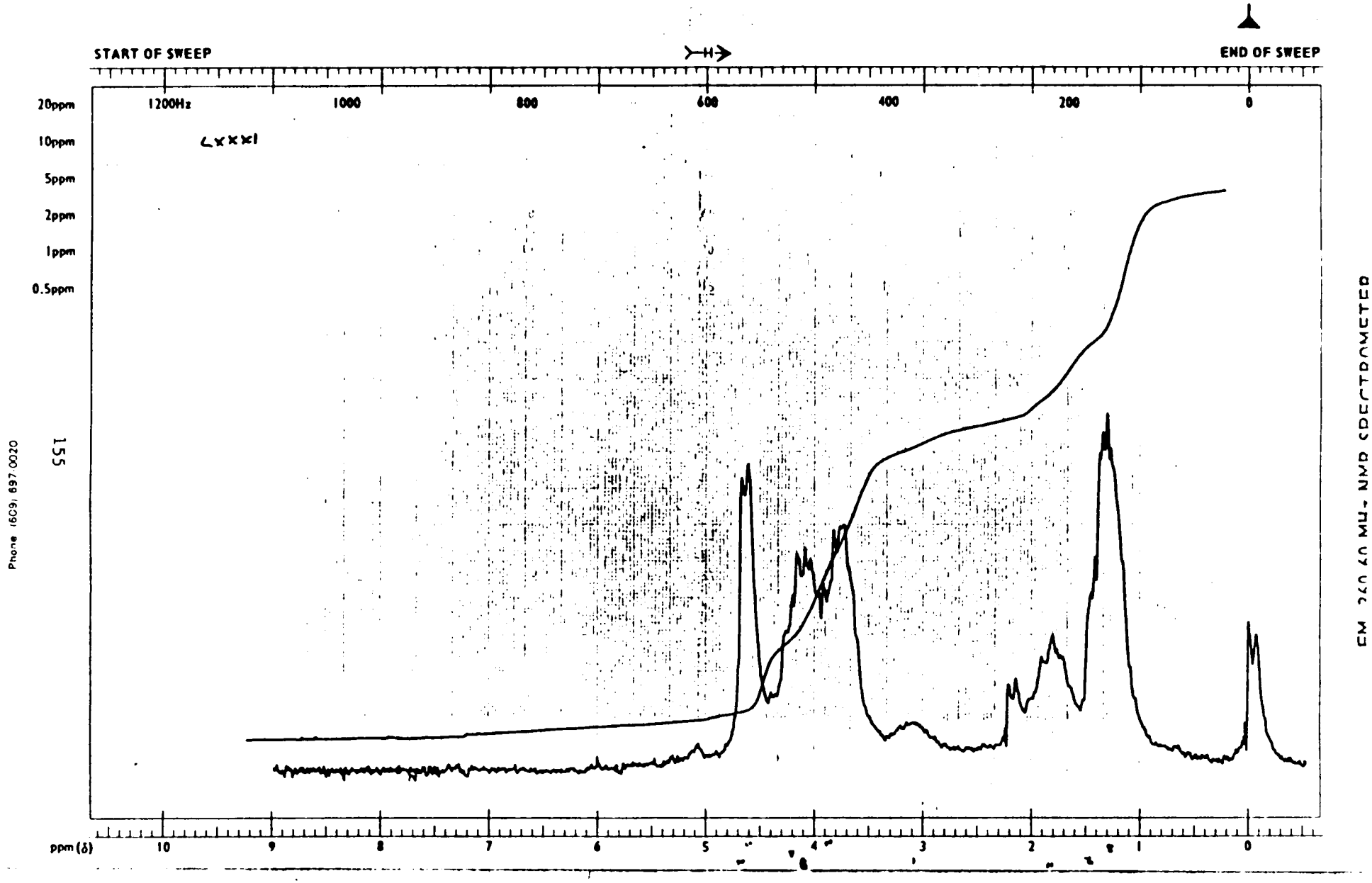
NMR of LXIX



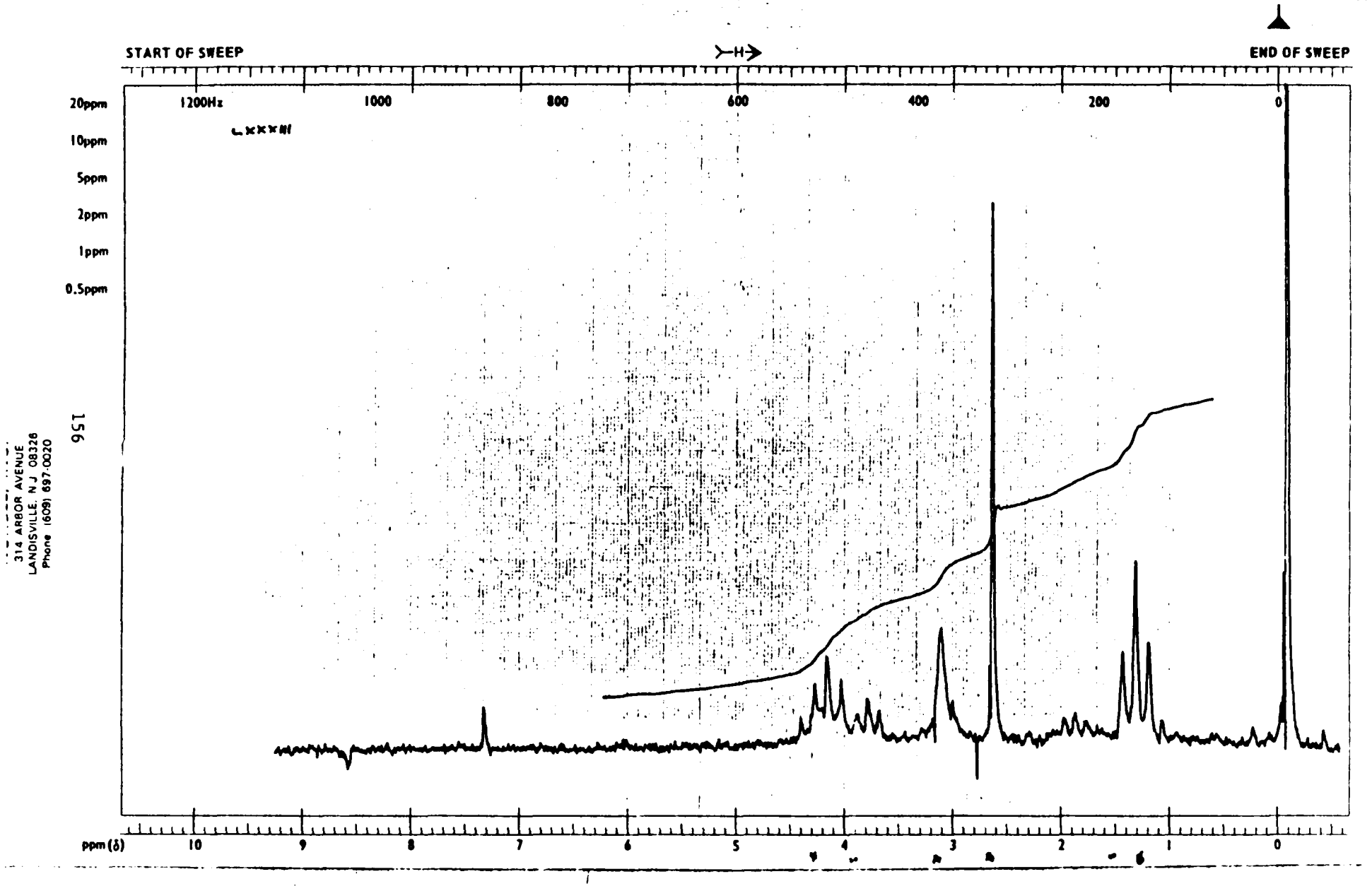
NMR of LXX



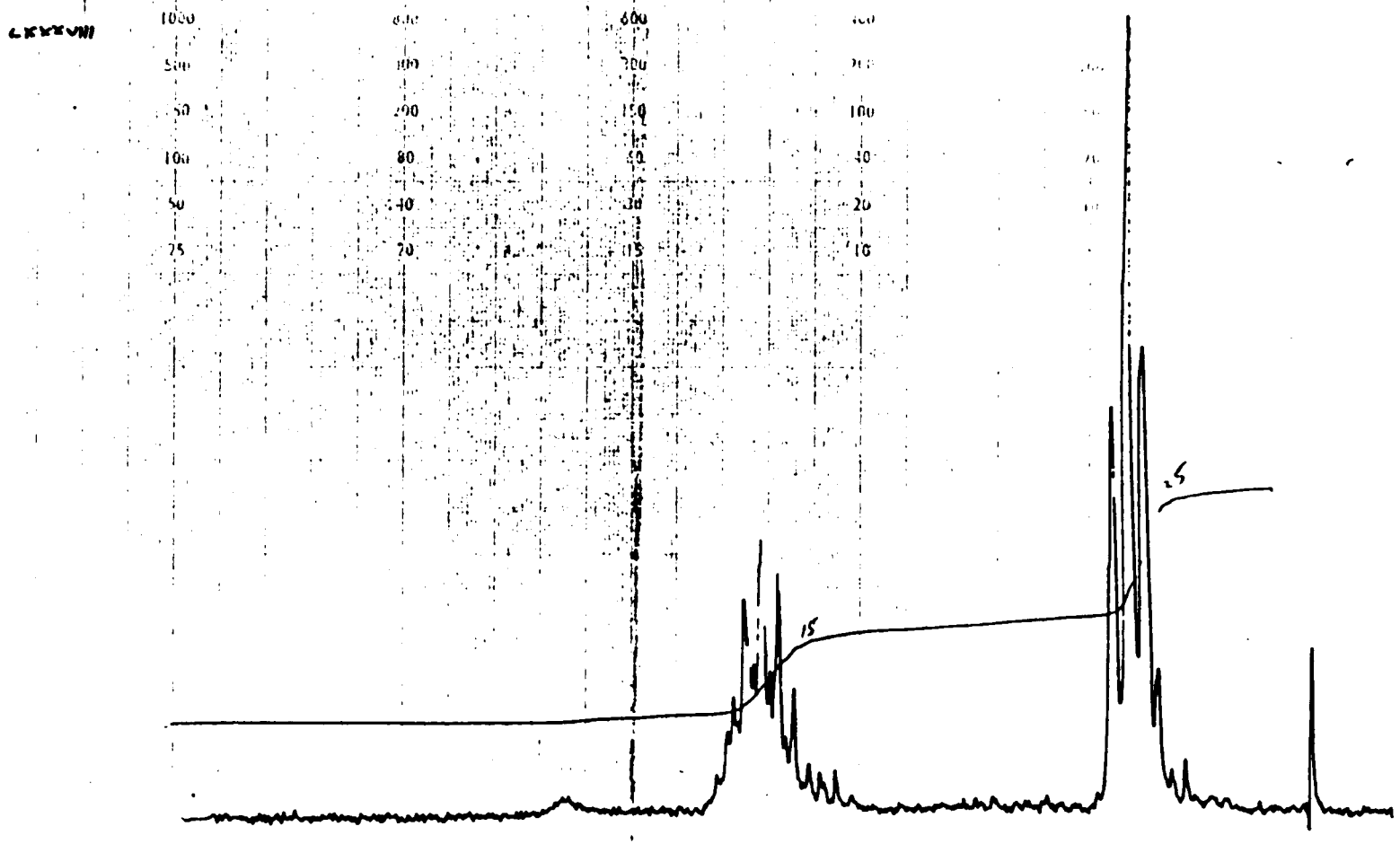
NMR of LXXI


START OF SWEEP
END OF SWEEP
1200Hz
1000
800
600
400
200
0
20ppm
10ppm
5ppm
2ppm
1ppm
0.5ppm
LXXXI
ppm (δ)
10
9
8
7
6
5
4
3
2
1
0

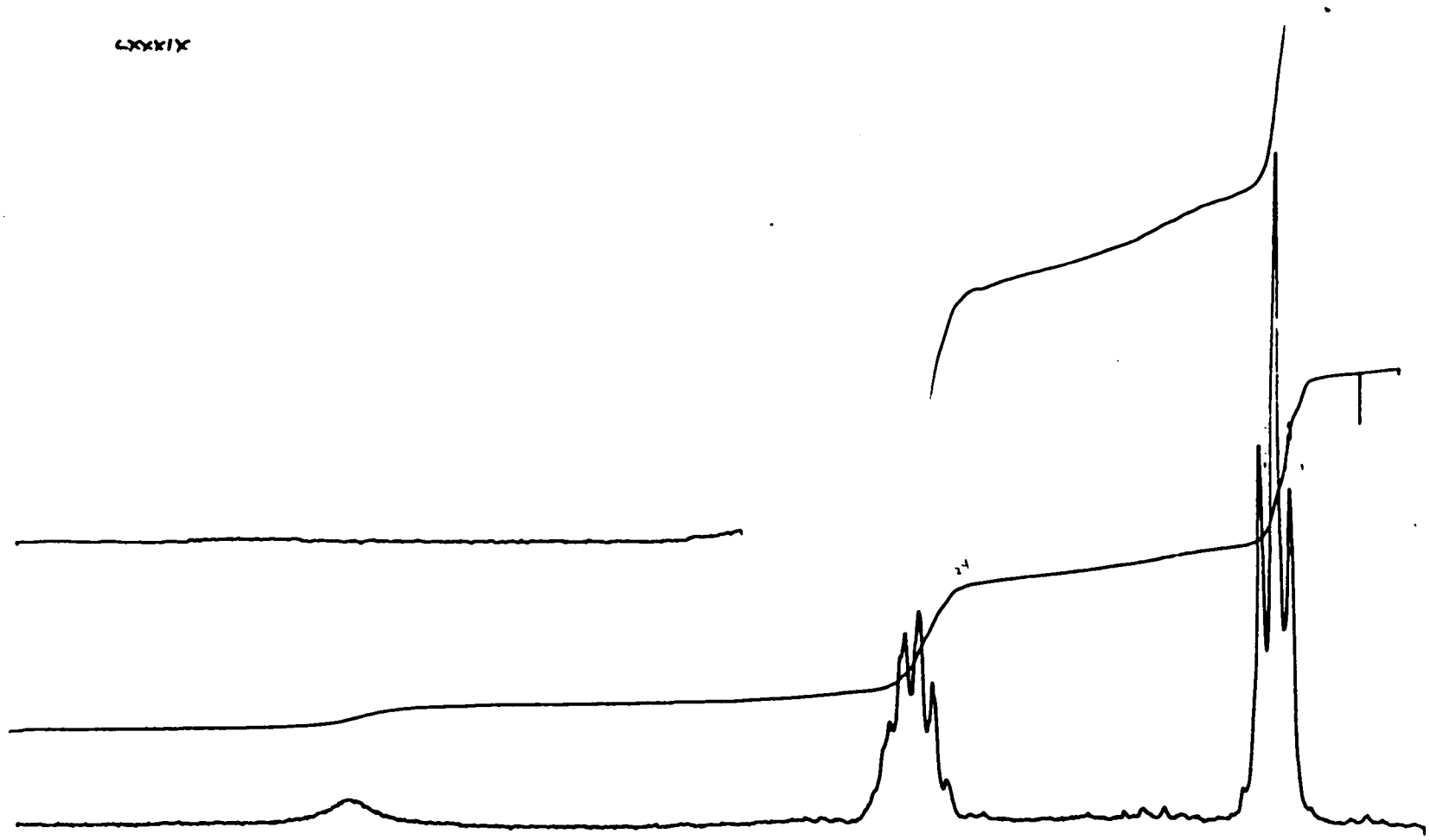
NMR of LXXXI



NMR of LXXXIII



NMR of LXXXVIII



NMR of LXXXIX