THE UNIVERSITY OF MANITOBA

PART I: THE TOTAL SYNTHESIS OF ANISOMYCIN

PART II: SYNTHETIC APPROACHES TO DAUNOMYCIN

by

Arend Jan te Raa

B.Sc. (Hons.), Manitoba, 1967

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF

Doctor of Philosophy

in the Department

of

Chemistry

WINNIPEG, MANITOBA

May, 1972

TABLE OF CONTENTS

	PAGE
ABSTRACT	iii
ACKNOWLEDGEMENTS	v
INTRODUCTION (PART I)	2
RESULTS AND DISCUSSION (PART I)	7
EXPERIMENTAL (PART I)	25
INTRODUCTION (PART II)	41
THE STRUCTURE OF DAUNOMYCIN	43
SYNTHESIS OF DAUNOMYCIN	50
RESULTS AND DISCUSSION (PART II)	51
EXPERIMENTAL (PART II)	63
INFRARED SPECTRA	79
P.M.R. SPECTRA	113
REFERENCES	131
CURRICULUM VITAE	134

ABSTRACT

Part I of this dissertation describes the total synthesis of anisomycin.

2R, 3R, tartaric acid was successfully converted to natural anisomycin and its enantiomer, (+)-anisomycin. Key steps in the synthesis were the reaction of p-methoxybenzyl magnesium chloride with N-benzyl-2R, 3R, tartarimide, the product of which was not isolated, but immediately subjected to hydride reduction to give N-benzyldeacetyl anisomycin plus its C-2 epimer.

N-benzyldeacetyl anisomycin was selectively acetylated at the C-3 position. Debenzylation gave natural anisomycin.

The hydroxyl groups of the C-2 epimer were inverted, via the cis epoxide, to give the enantiomeric form of N-benzyldeacetyl anisomycin. Selective acetylation and debenzylation gave (+)-anisomycin.

Part II describes a synthetic approach to daunomycin. 2,5-dimethoxybenzaldehyde was condensed with acetyl acetone. The product was successively hydrogenated, alkylated with ethyl bromoacetate, hydrolyzed, and cyclized to 3-acetyl-5,8-dimethoxy-1-tetralone. Hydrogenation under acidic conditions reduced the aryl ketone to a methylene. The product was successfully condensed with phthalic acid mono-

methyl ester in trifluoroacetic anhydride. The product was hydrolyzed, then cyclized to give the linear tetracyclic skeleton of daunomycinone.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. C.M. Wong for his patient guidance and instruction during the course of the work.

He also wishes to acknowledge his colleagues in Dr. Wong's laboratory for their companionship - especially Bob Schwenk, who shared many a moment of success and failure with the author during the past four years.

The author sincerely thanks his wife, Joan, for typing the manuscript and for her encouragement, patient understanding, and assistance, especially during the preparation of this manuscript.

Finally, the financial support received from the National Research Council of Canada and from the Government of Manitoba is greatly appreciated.

To My Dear Wife

Joan

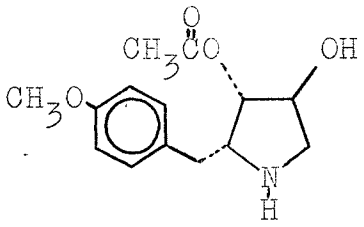
PART I

THE TOTAL SYNTHESIS OF ANISOMYCIN

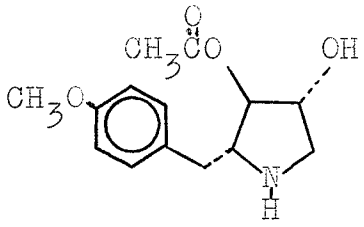
INTRODUCTION

Anisomycin was first isolated in 1954 by Sobin and Tanner[1] as a fermentation product isolable from various Streptomyces species. In vitro studies have shown that anisomycin has excellent activity against several pathogenic protozoa and on occasion it has been used for the treatment of amebic infections[2].

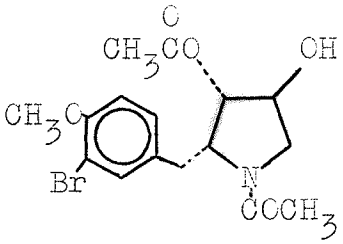
The structure of anisomycin was elucidated in 1964 by Beereboom and coworkers[3]. They assigned structure (1a) to the antibiotic, but this structure



(1)



(1a)

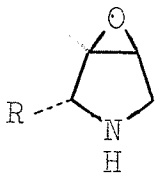


(1b)

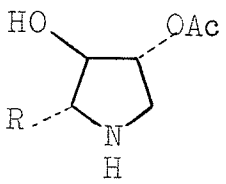
was later revised to (1) on the basis of the X-ray analysis[4] of the bromo derivative (1b) of N-acetylanisomycin.

Anisomycin is a white, crystalline compound (m.p. = 141.5 - 142°C) with a pKa value of 7.9. The molecule is a trisubstituted pyrrolidine, namely 2-(p-methoxybenzyl)-3-cis-4-trans-hydroxy-pyrrolidine. It is readily acetylated to N-acetylanisomycin (2) (scheme I, page 6) in cold acetic anhydride or to N,O-diacetylanisomycin (3) in acetic anhydride in the presence of pyridine. Hydrolysis of anisomycin in either acid or base gives deacetylanisomycin (4). Treatment of anisomycin with PCl_5 in chloroform yields a chloro compound which on treatment with ethanolic KOH gives the cis-epoxide (5). Opening of the epoxide (5) with acetic acid gives isoanisomycin (6) as the only product. Similarly, opening of (5) by amines, alkoxides, or mercaptans gives a 4-trans substituted-3-cis-hydroxy-2-(p-methoxybenzyl) pyrrolidine as the only product.

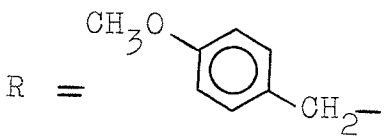
The stereospecific chemical behaviour of the epoxide had led Beereboom and coworkers[3] to assign the trans configuration (5a) to the epoxide. They argued



(5a)



(6a)



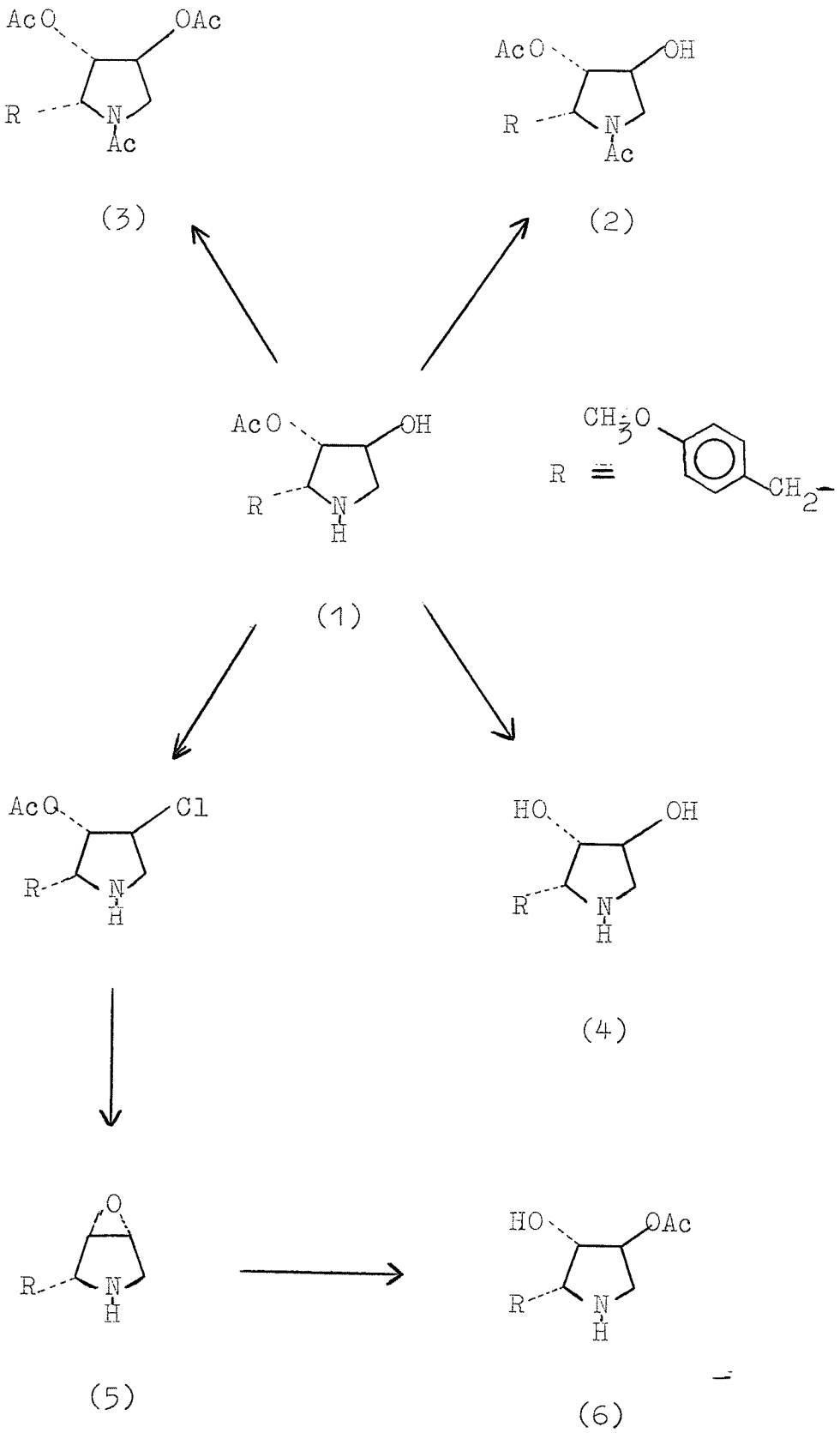
that the p-methoxybenzyl group at C-2 was sterically

inhibiting nucleophilic attack at C-3 of the pyrrolidine ring. This would be expected to occur only if the bulky group at C-2 was trans to the epoxide, thereby forcing attack at C-4 to give (6a) (i.e. the all trans compound). Correlation of anisomycin with (6a) led them to assign the all trans configuration (1a) to anisomycin. However, since only one product is obtained from the opening of (5), it appears that a factor other than steric controls the opening of the epoxide (5). It is now well known that diaxial products predominate in opening of epoxides. Many examples can be cited from the steroid field for instance[5]. It is reasonable to assume that in the epoxide (5) the bulky C-2 substituent is equatorial. Therefore, a diaxial product would have a cis C-3, trans C-4 configuration. This is experimentally observed.

A number of synthetic approaches to anisomycin have been reported. Wong[6] established the absolute configuration of anisomycin by a chemical correlation to L-tyrosine as 2R, 3S, 4S. This configuration is correctly represented in (1). Oida[7] synthesized racemic anisomycin starting from racemic tyrosine. Salmón and Walls[8] synthesized isomers of anisomycin from pyrrole. Buccini[9] used a similar approach to synthesize racemic deacetylanisomycin. More recently, Felner and Schenker[10] reported the total synthesis of anisomycin beginning with L-(+)-tartaric acid diethylester. Reference will be made to this work in connection with the work described

in this thesis. The object of the work described in this part of the thesis was to develop a synthetic scheme that would lead to the total synthesis of optically active anisomycin. The naturally occurring isomer of tartaric acid was chosen as starting material.

SCHEME I

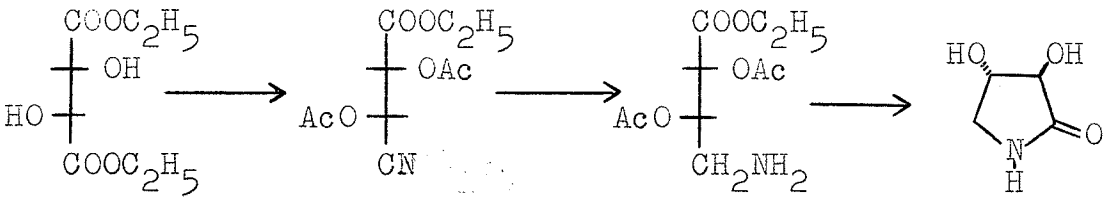

AcO
OAc
R
N
Ac
(3)
AcO
OH
R
N
Ac
(2)
AcO
OH
R
N
H
(1)
CH3O
R
CH2
AcO
Cl
R
N
H
HO
OH
R
N
H
(4)
O
R
N
H
(5)
HO
OAc
R
N
H
(6)

RESULTS AND DISCUSSION

Natural anisomycin and its enantiomorph, (+)-anisomycin, have been stereospecifically synthesized starting from L-(+)-tartaric acid[11,12].

L-(+)-tartaric acid was chosen as starting material because it is readily available and, more important, it has two optically resolved carbon atoms. Since the tartaric acid was destined to become the carbon skeleton of the pyrrolidine ring of anisomycin, the C-3 and C-4 positions would be optically resolved and also have the correct trans substituents. The problem was, then, essentially the introduction of a nitrogen atom to complete the pyrrolidine ring and the addition of the p-methoxybenzyl side chain to complete the skeleton of anisomycin.

A literature survey showed that 3R, 4S, dihydroxy pyrrolidone (9) had been synthesized by Japanese workers[13] from L-(+)-tartaric acid diethylester according to the scheme shown below. The pyrrolidone seemed an attractive



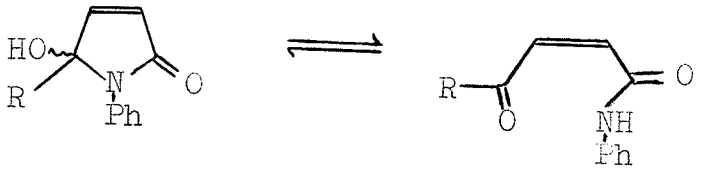
(7) (8) (9)

intermediate for the introduction of the p-methoxybenzyl side chain. However, the reduction of the nitrile (7) according to the conditions of the Japanese workers (62 atmospheres of H_2) posed a problem since a high pressure hydrogenator was not available to us at the time; also the overall yield (10%) of the conversion of the nitrile (7) to the lactam (9) is very poor. Since we could not find a known suitable alternative for the synthesis of (9) we abandoned the thought of using a pyrrolidone as an intermediate*. Instead of 3,4-dihydroxy pyrrolidone, we decided to use tartarimide (16) (scheme III, page 23) as the intermediate to introduce the p-methoxy-benzyl side chain. The N-benzyl derivative was chosen because the benzyl group is readily removed later on by hydrogenolysis. N-benzyl tartarimide[14] is readily obtained by condensing tartaric acid with benzylamine in refluxing xylene.

The next problem was the introduction of the side chain. Grignard additions to imides are well known. However, there is some question as to whether the product

*It is interesting to note that Felner and Schenker[10] used 3R, 4S, dimethoxy pyrrolidone (11) as an intermediate in their synthesis of anisomycin (scheme II, page22). They obtained (11) from the nitrile (10) in 80% yield using hydrogen at atmospheric pressure.

is a 2-hydroxy-5-pyrrolidone or a γ-keto amide. The literature is somewhat unclear on this point. Recently Queen and Reipas[15] established that hydroxy pyrrolinones

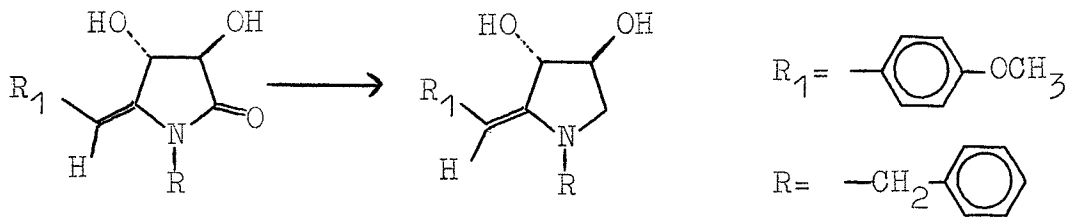


are the products obtained on addition of Grignard reagents to N-aryl maleimides. This is contrary to the published findings of Awad and coworkers[16] who proposed that the open chain γ-keto amides are the resulting products. In maleimides the presence of the double bond may favor the cyclic structure for the products, while in succinimides the open chain may be favored. However, Jocelyn and Queen[17] and also Walton[18] have shown that Grignard additions to N-alkyl succinimides also lead to the cyclic 2-hydroxy-5-pyrrolidones. This does not agree with earlier findings of Lukes and Prelog[19] who concluded that the products were open chain keto-amides. More recently, Sekiya and Terao[20] have shown that succinimide itself reacts with two equivalents of Grignard reagent to yield γ-keto amides in excellent yield. However, evidence suggests that reaction of (16) with anisyl magnesium chloride should give the pyrrolidone (17).

The Grignard reaction on N-benzyl tartarimide failed in anhydrous ether due to solubility problems.

However, excellent yields (70-80%) were obtained in anhydrous tetrahydrofuran. Careful temperature control was essential for a successful reaction. Below 30°C the formation of the p-methoxybenzyl magnesium chloride was too slow. At reflux temperatures the Grignard reagent coupled with unreacted p-methoxybenzyl chloride to form 1,2-di(p-methoxyphenyl)ethane in high yield. Optimum temperature for the formation of the Grignard reagent was 33-37°C. The product obtained after work up was a pure white solid. The infrared spectrum of this solid (figure 1) showed absorptions at 1720 cm^{-1} and also at 1630 cm^{-1}. Obviously these bands represent a ketone and an amide carbonyl stretch respectively. In other words we did not obtain the pyrrolidone (17), but instead the keto-amide (18). The presence of an amide N-H stretch at 3280 cm^{-1} in addition to the hydroxyl bands at 3450 cm^{-1} and 3175 cm^{-1} supported this conclusion. The ring opening was a disappointment, but we felt that opening had probably occurred during work up. Therefore we subjected the Grignard addition complex (19) directly to a lithium aluminum hydride reduction. The products expected were the isomeric amines (20) and (21). Experimentally, three amines were isolated : A, B, and C. The yields were 10%, 1%, and 0.5% respectively. These yields were reproducible over a large number of runs. Variations in reaction conditions and methods of

work up had little effect on the total yield. A puzzling phenomenom was the extensive decomposition that occurred during work up. In the immediate work up 90-95% of the theoretically expected material was soluble in dilute hydrochloric acid; hence presumably present as amines. The product yellowed, then blackened immediately upon isolation. After one hour the product was redissolved in chloroform, and the dark chloroform solution extracted several times with dilute hydrochloric acid. The acidic solution then gave only a 15% yield of the expected amines. From this material the three compounds A, B, and C were isolated without further decomposition in crystalline form. The major product (75-80%) had, however, decomposed. The structure of the unstable amine may have been the enamine (34) shown below.



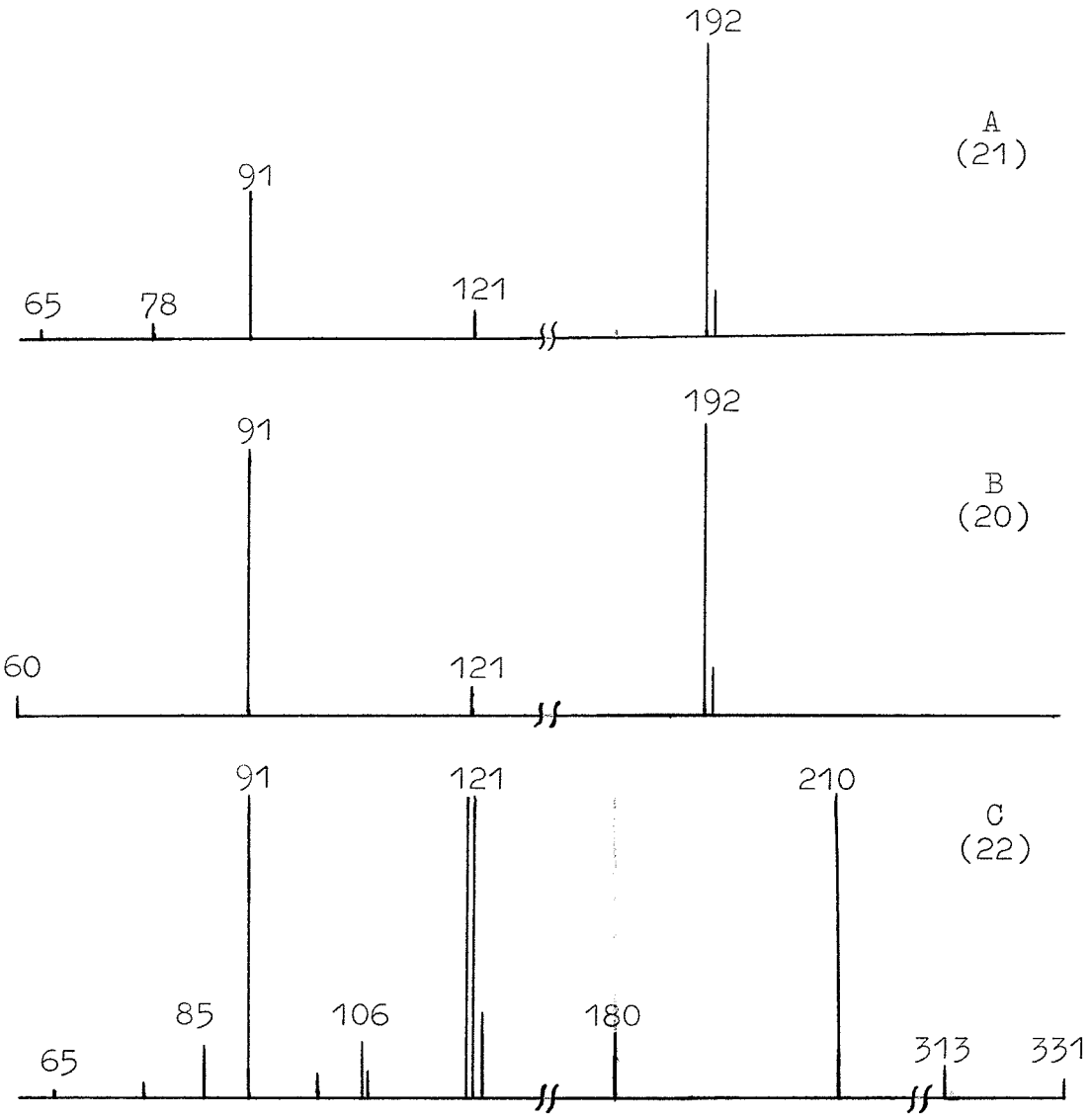
(29) (34)

Although there is no conclusive evidence, there is some circumstantial evidence for this. Later on in the research, an attempt was made to synthesize (34) from the lactam (29). The reduction of (29) by lithium aluminum hydride in anhydrous tetrahydrofuran went smoothly. An excellent yield of material soluble in

dilute hydrochloric acid was obtained. However, the product yellowed upon isolation and then blackened. This was remarkably similar to the above mentioned pattern. Furthermore, after an hour no acid soluble material could be isolated. It is reasonable to assume that the enamine (34) had been obtained. However, the enamine must be very unstable and decompose rapidly*.

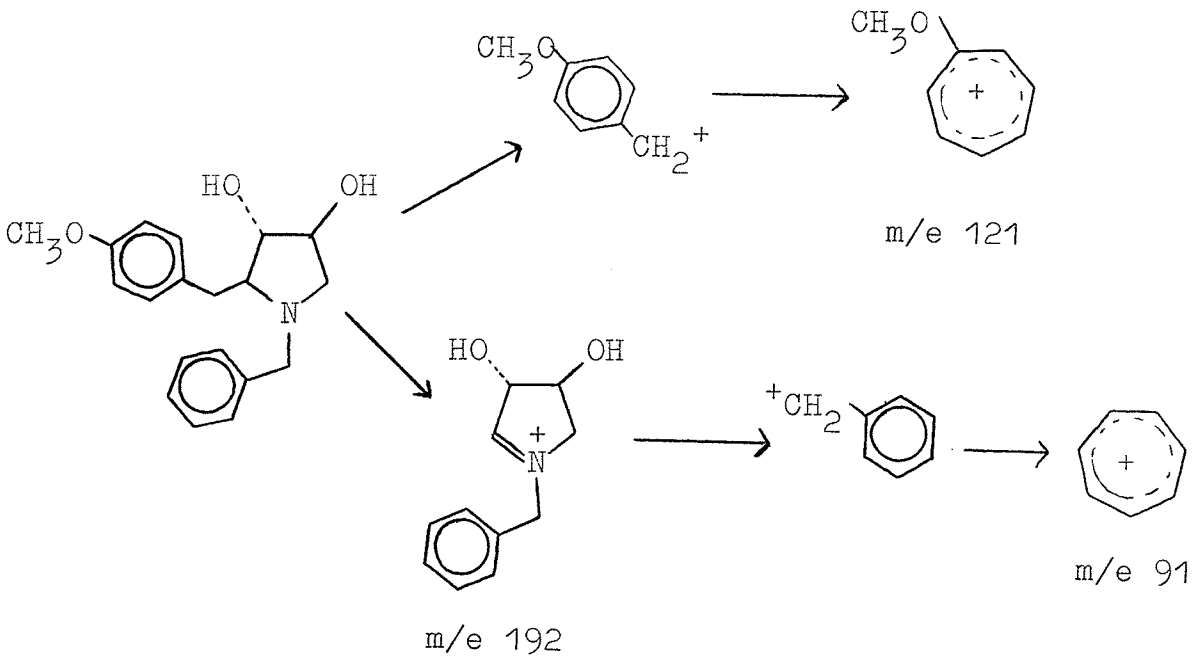
All three of A, B, and C were characterized. The infrared spectra (figures 2-4) showed hydroxyl absorptions and were lacking in carbonyl absorptions. The proton magnetic resonance (p.m.r.) spectra (figures 1 - 3) clearly indicated the presence of a mono-substituted phenyl ring (5 proton singlet), a p-substituted phenyl ring (A_2B_2 pattern), and a methoxy group. On the basis of the infrared and p.m.r. spectra any one of the three compounds A, B, and C could be the desired compound (20). The mass spectra were a little more enlightening. A and B gave nearly identical fragmentation patterns (page 13) suggesting that these two compounds were probably isomeric and most likely (20) and (21). However, the

*The work of Fellner and Schenker[10] supports this observation. They synthesized the enamine (13) (scheme II page 22) and found it to be unstable to the extent that they could not isolate it, but had to hydrogenate it immediately to the amines (14) and (15). Yields were 14% and 43% respectively.



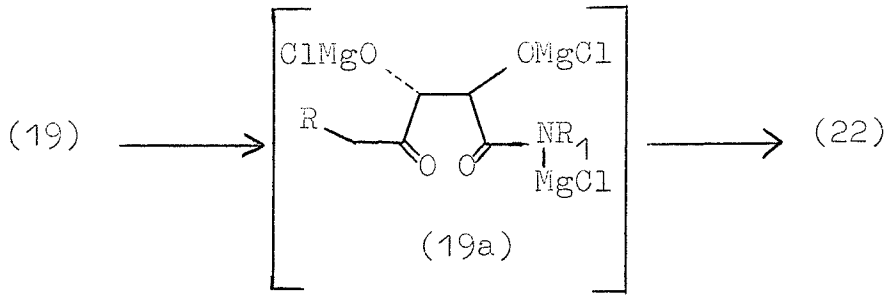
Mass spectra of A, B, and C

molecular ion (M^+ = 313) was not observed in either spectrum. A logical explanation would be the apparent facile cleavage of the C-2 side chain bond. This cleavage generates the methoxytropyllium ion (m/e = 121) or the m/e = 192 ion as shown below. The latter ion may



fragment to give the tropyllium ion (m/e = 91). The tropyllium ion could also result directly from the parent ion. The mass spectrum of C was significantly different. The 192 peak is missing; instead there is an intense peak at 210 - which is 18 mass units higher. The parent peak occurs at m/e 331. This is 18 mass units higher than the molecular weight of (20) or (21). This would indicate the presence of an additional molecule of water somewhere on the pyrrolidine fragment. The greater relative intensity of the methoxytropyllium ion indicates that the charge is more effectively carried by this ion; thus suggesting a structural difference involving the C-2 carbon atom. It occurred to us that compound C could possibily be the triol amine (22).

If the complex (19) rearranged to (19a), then subsequent

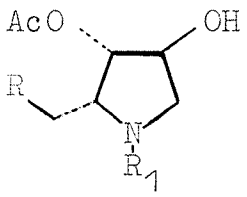


reduction should yield (22). An authentic sample of the triol amine (22) was prepared from the keto amide (18) by lithium aluminum hydride reduction. Physical and spectral data showed C to be identical with the triol amine (22).

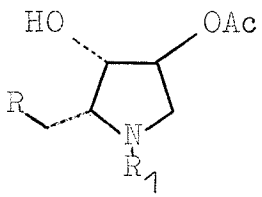
In order to establish which of the two compounds A and B was the desired N-benzyldeacetyl anisomycin (20), it was decided to synthesize (20) from natural deacetyl anisomycin (4). Reacting (4) with benzyl bromide in chloroform overnight at room temperature produced N-benzyldeacetyl anisomycin (20) in low yield. Sufficient material was obtained for spectral data. Comparison of spectral data showed compound B, obtained in 1% yield, to be identical in all respects (including optical rotation) with N-benzyldeacetyl anisomycin.

During the characterization of N-benzyldeacetyl anisomycin, it was noted that rapid esterification to a hydroxyacetate (infrared figure 6) occurred in cold acetic anhydride. Prolonged contact of the crude

hydroxyacetate with cold acetic anhydride did not produce any further changes in the infrared spectrum. Hence esterification stopped at the mono acetate stage. Repeated chromatography showed the hydroxyacetate was pure. Hence esterification was stereospecific. Two isomeric hydroxyacetates are possible: N-benzylanisomycin (23) or N-benzylisoanisomycin (23a). Hydrogenolysis of



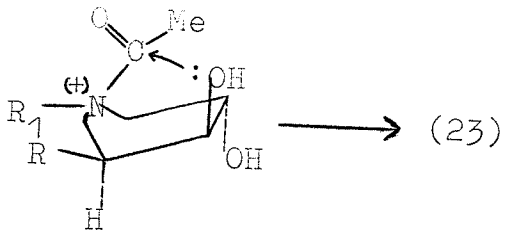
(23)



(23a)

the hydroxyacetate yielded crystalline anisomycin hydrochloride in excellent yield. Hence (23) must be the correct structure of the hydroxyacetate.

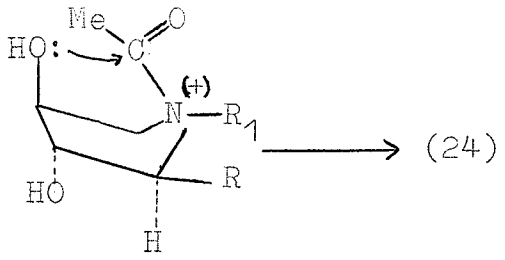
The selective esterification is really not very surprising since the nitrogen of the pyrrolidine ring acts as an internal catalyst. The preferred conformation of the pyrrolidine ring is as shown, with the bulky C-2



substituent equatorial. Molecular models show that other conformations increase steric interaction between R and R_1. These models also show why acyl transfer occurs only to the C-3 hydroxyl oxygen. The C-4 hydroxyl group is not properly oriented for acyl transfer to occur.

Synthetic anisomycin was obtained from its hydrochloride salt. The physical and spectral properties of the synthetic compound and the natural sample were compared and found to be identical. Mixed melting point determination did not cause any lowering of the melting point.

Having achieved the total synthesis of anisomycin, we decided to attempt to invert the two hydroxyl groups of the all trans diol (21) and thus synthesize the enantiomorphic form of anisomycin. A successful attempt would also prove the structure of (21). The problem would be simple if we could synthesize the cis-epoxide (25) , and the epoxide (25) could be readily made if we could synthesize the hydroxyacetate (24) - that is, a selective acetylation at the C-4 hydroxyl group of (21). This problem turned out to be very simple. Analogous to the selective acetylation of the diol (20),the all trans diol should selectively acetylate at C-4. Consider again the preferred conformation of the pyrrolidine ring as shown below. In this case the C-4 hydroxyl is cis to



the N-acyl group. Therefore, acyl transfer will occur to the C-4 hydroxyl oxygen. Experimentally only one hydroxyacetate (24) (infrared figure 10) was obtained in nearly quantitative yield. It decomposed on standing and was therefore immediately reacted with PCl_5 in chloroform to form a chloroacetate. This in turn was treated with ethanolic KOH to yield the cis-epoxide (25) in good yield. The infrared spectrum (figure 11) of (25) lacked any hydroxyl absorptions. The absorption at 865 cm^{-1} indicated that we indeed had the cis-epoxide and not the isomeric trans-epoxide. This was concluded from a comparison with a number of infrared spectra of cis and trans epoxides synthesized by my colleague, Dr. John Buccini[9], who had found that the cis-epoxide absorption always occurred at 865 cm^{-1} whereas the trans-epoxide absorption always occurred at 848 cm^{-1}.

The epoxide (25) opened readily in refluxing acetic acid to give a mixture of hydroxyacetates. These were not isolated, but directly hydrolysed in aqueous sodium hydroxide solution. Chromatography of the crude hydro-

lysate yielded the diol (26) and the all trans diol (21) in a 1:1 ratio in good yield. The infrared spectrum of (26) was superimposable on the infrared spectrum of the diol (20). The specific optical rotation of (26) was found to be $+51.4^{o}$ compared to that of -51.0^{o} for the diol (20). Thus (26) is indeed the enanthiomorphic form of N-benzyldeacetylanisomycin. The diol (26) was converted to (+)-anisomycin according to the procedure developed for the diol (20).

Having synthesized (+)-anisomycin from the all trans diol (21), we then went back to try to develop an improved synthesis of N-benzyldeacetylanisomycin starting from the keto-amide (18), which we had obtained in excellent yield. A considerable amount of time was spent to find an effective procedure for ring closure. The unsaturated lactams (29) and (30) (scheme IV page 24) were finally obtained directly from (18) in 76% yield. Ring closure of (18) was effected by heating (18) in absolute ethanol at 135-140°C in a closed high pressure reaction vessel. The major isomer (29) was obtained in crystalline form. Its infrared spectrum (figure 12) showed hydroxyl bands at 3500 cm^{-1} (sharp) and at 3350 cm^{-1} (broad), a carbonyl stretch at 1730 cm^{-1}, and an intense double bond stretch at 1660 cm^{-1}. The p.m.r. spectrum (figure 5) of its diacetate showed the vinyl proton (4.09 τ, doublet), the C-3 proton (3.75 τ, quartet), the C-4 proton (4.57 τ, doublet),

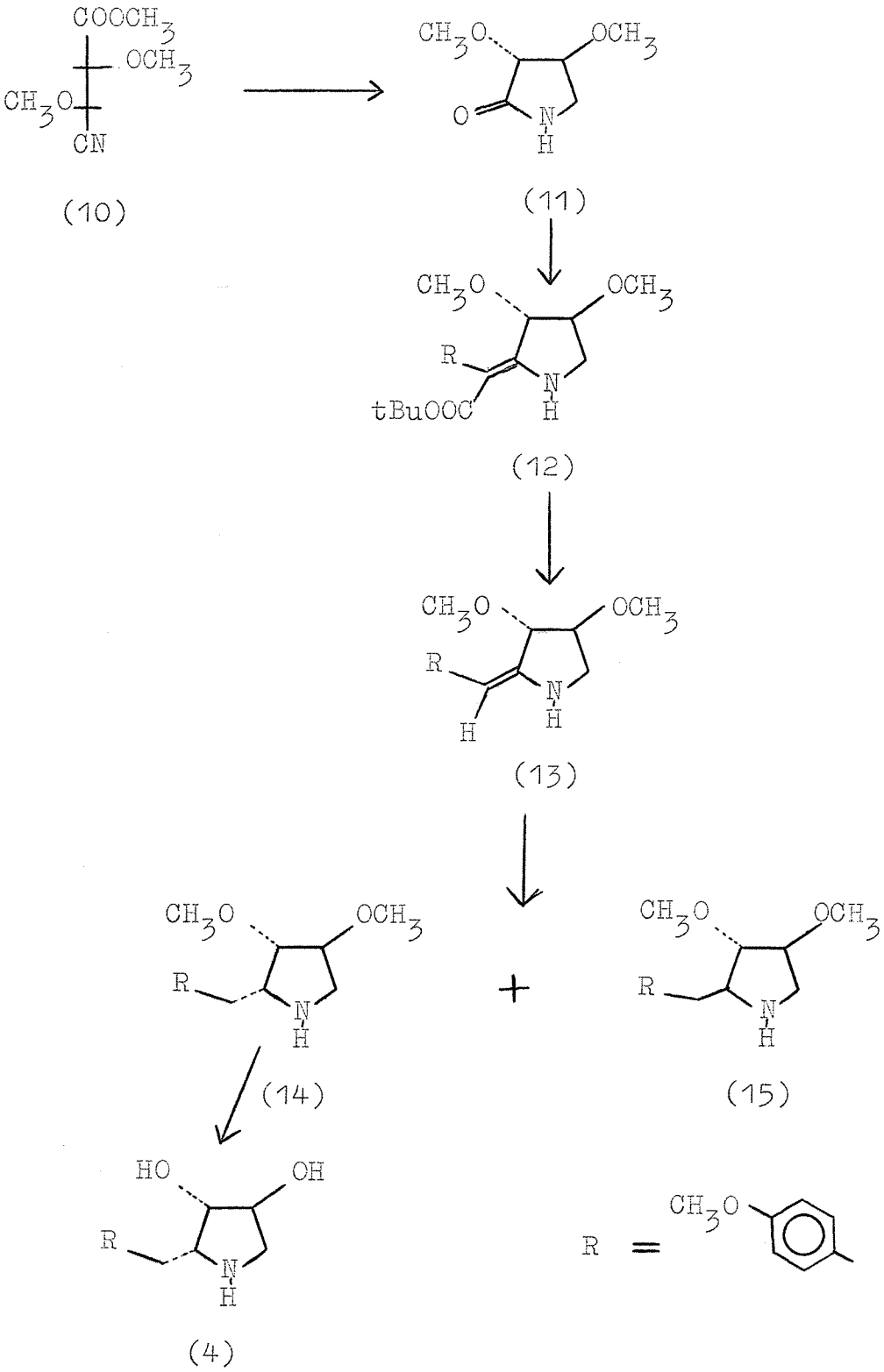
and the benzylic protons (5.15τ, broad singlet). The C-3 acetate group (8.3τ) is shielded by the aromatic ring causing an upfield shift - in contrast to the C-3 acetate group of the minor isomer (30) which has a normal chemical shift (figure 6). In fact the structural assignment of (29) and (30) was based on the p.m.r. spectra of their respective acetates. In the minor isomer the two aromatic rings are sterically interacting; this induces anisotropic effects. This accounts for the complex pattern of the aromatic protons and the AB quartet (5.05τ and 5.75τ) of the benzylic protons. The vinyl, the C-3, and the C-4 protons give rise to an ABC pattern (4.0-4.5τ).

Hydrogenation of either (29) or (30) yielded the same lactam (31) (infrared figure 14). The lactam (31) had the all <u>trans</u> configuration since lithium aluminum hydride reduction produced the all <u>trans</u> diol (21) in good yield. Attempts to obtain isomer (32) under different hydrogenation conditions failed. For instance, the pyranyl ether derivative of (29) (infrared figure 15) was prepared, in the hope that the bulky substituent might change the stereochemistry of the product. However, the only observable effect was a marked decrease in the rate of reduction. Reduction of (29) to (34) failed as previously described. However, synthesis of the all <u>trans</u> diol (21) through the lactam (29) constituted a marked improvement in total yield (43% compared to

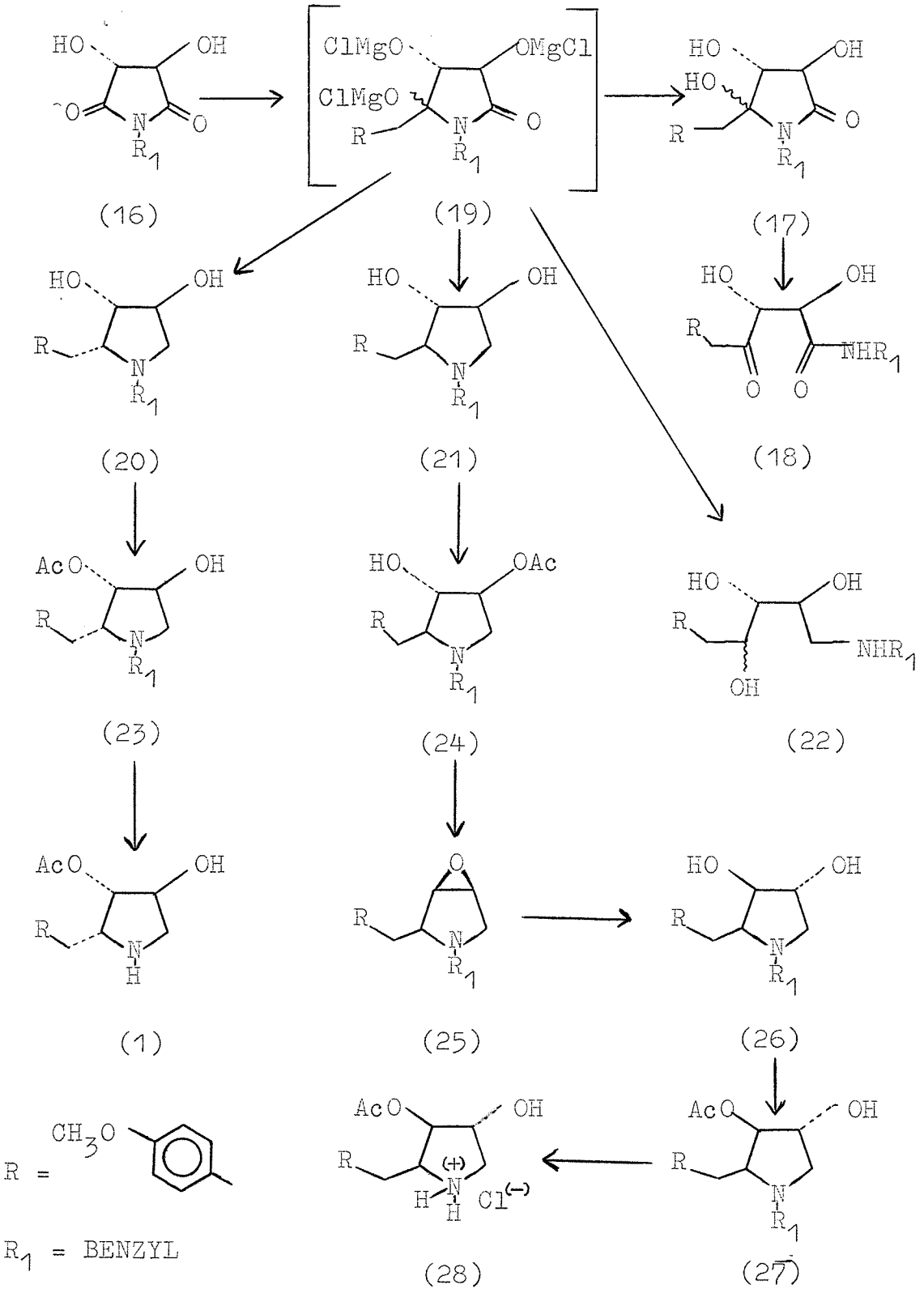
10% in the single step approach).

Conversion of (21) into (+)-anisomycin proved the structure of compound (21) as well as making (+)-anisomycin available for biological testing.

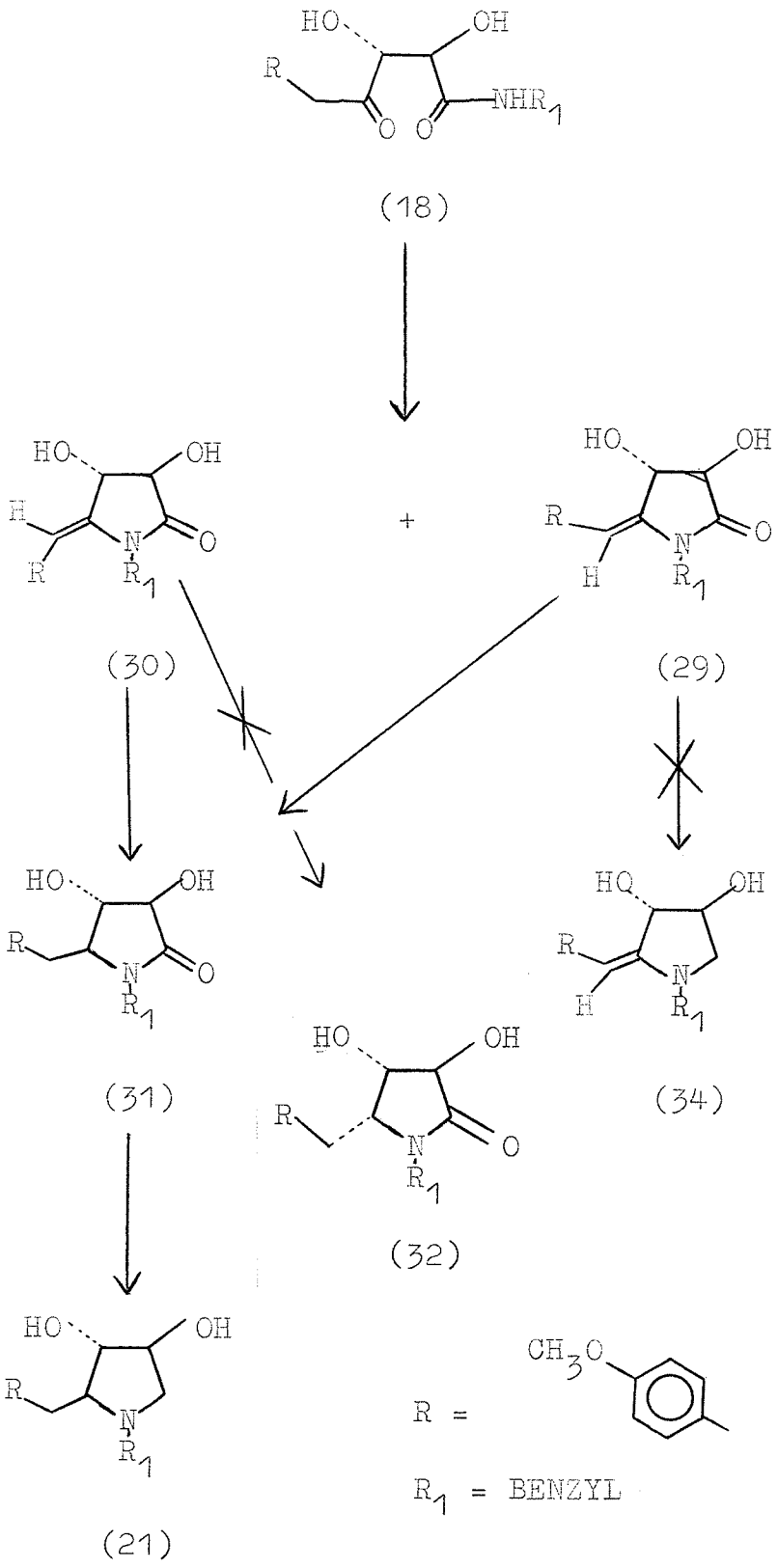
SCHEME II


COOCH3
OCH3
CH3O
CN
(10)
CH3O
OCH3
O
N
H
(11)
CH3O
OCH3
R
tBuOOC
N
H
(12)
CH3O
OCH3
R
H
N
H
(13)
CH3O
OCH3
R
N
H
+
CH3O
OCH3
R
N
H
(14)
(15)
HO
OH
R
N
H
(4)
R =
CH3O

SCHEME III



SCHEME IV


HO
OH
R
NHR1
O
O
(18)
HO
OH
H
R
N
R1
O
(30)
+
HO
OH
R
H
N
R1
(29)
HO
OH
R
N
R1
O
(31)
HO
OH
R
N
R1
O
(32)
HO
OH
R
H
N
R1
(34)
HO
OH
R
N
R1
(21)
R = CH3O
R1 = BENZYL

EXPERIMENTAL

Melting points were determined on a Fisher-Johns hot stage apparatus and are uncorrected.

Microanalyses were performed by Dr. Daesslé of Montreal.

Optical rotations were measured on a Carl Zeiss polarimeter.

Infrared spectra were recorded on Perkin-Elmer model 137 and model 700 infrared spectrometers using methylene chloride solutions or nujol mulls as stated.

Proton magnetic resonance spectra were recorded on a Varian A 56/60 A spectrometer using tetramethylsilane as internal reference.

Mass spectra were recorded on a Hitachi Perkin-Elmer RMU-6D mass spectrometer.

Preparative thin layer chromatography was performed on 1 mm. thickness silica gel G-plates, with a fluorescent indicator; visualizing with ultraviolet light.

(1) Preparation of L-(+)-N-benzyl-tartarimide (16):

A 3 liter, 3-neck, round-bottom flask was equipped with a heating mantle, an overhead stirrer, a dropping funnel, and a reflux condenser with a Dean-Stark trap. The flask was first charged with 2 liters of xylene and 75 grams (0.5 moles) of L-(+)-tartaric acid. With constant stirring the mixture was brought to reflux temperature, at which time 65 grams (0.6 moles) of benzyl amine was added dropwise over a period of 30 minutes. Refluxing was continued for 3 hours. The solution was then cooled in an ice bath and the precipitated solid was filtered off by suction, then washed twice with cold benzene. The crude product was then dissolved in 3.5 liters of boiling water and the mixture filtered hot to remove insoluble material. The filtrate yielded white crystalline needles on cooling. These crystals were collected to give 66 grams (60%) of imide, m.p. = 196-198°C

$[\alpha]_D^{25} = +126^\circ$ (CH_3OH)

Infrared spectrum (figure 5): absorptions (cm^{-1}) at 3220 (-OH); 1740 (imide carbonyl).

Mass spectrum: m^+/e = 221, 91, 71, 60.

(2) Preparation of 5-(p-methoxyphenyl)-4-keto-2,3 dihydroxy pentanoic acid N-benzylamide (18):

A dry, 3 liter, round-bottom flask was equipped with a reflux condenser, dropping funnel, thermometer, and magnetic stirrer. Provision was made for external

cooling. Approximately 5 grams of magnesium and 100 ml. of freshly distilled, dry tetrahyrofuran (thf) were placed in the flask and stirred. Five grams of anisyl chloride were added, followed by a crystal of iodine. The dark color disappeared suddenly within two minutes. Approximately 600 ml. of anhydrous thf were added, followed by 12 grams of magnesium. A dropwise addition of 64.7 grams more of anisyl chloride was then started. The temperature rose slowly and was maintained between 33 and 37°C by external cooling. When half the anisyl chloride had been added (10 minutes), an additional 500 ml. of thf was added. The external coolant was removed upon completed addition of the anisyl chloride when the temperature began to drop. Stirring continued for an additional 30 minutes. Next a warm solution of 22.1 grams (0.1 mole) of N-benzyl-tartarimide in 700 ml. of anhydrous t.h.f. was added. During this time the reaction mixture was cooled when the temperature rose above 40°C. Stirring continued for an additional 2.5 hours. The solution was then decanted into a separatory funnel and the remaining magnesium washed with 100 ml. of dry thf , then decanted. The combined solutions were washed with 500 ml. of ice cold water. This was shaken vigorously for five minutes and the liquid decanted into a large beaker. The remaining pasty mass was extracted with 500 ml. of wet thf . Anhydrous magnesium sulphate (150 grams) was placed in the combined

thf solutions and the mixture then stirred vigorously for one hour. After that the salts were allowed to settle. The liquid was then decanted and evaporated to dryness under reduced pressure. Overheating the residue was carefully avoided. The oily residue was washed successively with 200 ml. of cold benzene, 200 ml. of ice cold 3N hydrochloric acid, 200 ml. of saturated K_2CO_3 solution, and finally with water. Drying the product yielded 29 grams of crude ketone. Recrystallization from n-propanol (while avoiding prolonged heating) yielded 26.5 grams (77%) of pure (18), m.p. = 172-174°C.

Infrared spectrum (figure 1) : absorptions (cm^{-1}) at 3450 (free -OH), 3280 (-NH), 3175 (hydrogen bonded -OH), 1720 (ketone carbonyl), 1630 (amide carbonyl).

Mass spectrum: m^+/e = 343, 236, 218, 194, 165, 148, 133, 121, 108, 106, 91, 78, 77, 65.

ANALYSIS	C	H	N
Calculated	66.46	6.15	4.08
Found	66.76	6.35	4.09

(3) Preparation of N-benzyldeacetyl anisomycin (20) and N-benzyl-2-(p-methoxybenzyl)-3-trans-4-cis-dihydroxypyrrolidine (21): The Grignard reaction was carried out as described for the synthesis of (18). Instead of adding water to the thf solution in the dropping funnel, however, the solution was added dropwise to a stirred and reflux-

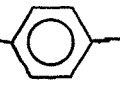
ing suspension of 15.5 grams of lithium aluminum hydride in anhydrous thf (300 ml.). Refluxing continued for 8 hours. Most of the t.h.f. was then removed by distillation, the remaining solution cooled, and ethyl acetate (1500 ml.) added carefully. This was allowed to stand with occasional shaking for 15 minutes. After that time 6N hydrochloric acid (300 ml.) was added and the mixture shaken vigorously. Next the aqueous layer was separated and washed with ethyl acetate. These ethyl acetate layers were combined. The aqueous layer was made basic and extracted with chloroform. This chloroform solution, upon evaporation, yielded only a trace of residue which was discarded. The combined ethyl acetate layers were next extracted several times with 5% hydrochloric acid. and the combined aqueous extracts made basic. This basic solution after extraction with ethyl acetate and evaporation of that solvent, then yielded a gummy oil that became increasingly darker in color.

The residue was redissolved in chloroform after 2 hours and the solution extracted with 5% hydrochloric acid. The aqueous solution was then made basic and extracted with chloroform. Evaporation of the dried chloroform solution yielded 4.8 grams of a gummy material. That residue was dissolved in a small amount of benzene and titurated with heptane. Next a crystal of (21) was added and the turbid solution allowed to stand overnight under refrigeration. Filtration then produced 3.1 grams

(10%) of the all <u>trans</u> diol (21), m.p. = 112.5-113.5°C.

$[\alpha]_D^{25} = +78^\circ$ ($CHCl_3$)

Infrared spectrum (figure2) : absorptions (cm^{-1}) at 3550 (-OH), 1620, 1590, 1510 (aromatic C=C), 1040 ($-OCH_3$).

P.m.r. spectrum (figure 1) : absorptions at 3.0τ(s, 5H's, aromatic); 3.0-3.5τ(A_2B_2, 4H's, aromatic); 6.4τ(s, 3H's, $-OCH_3$); 6.1 and 6.8τ(AB quartet, 2H's, $-N-CH_2-Ph$); 6.3τ(m, 2H's, C_3-H and C_4-H); 7-7.5τ(unresolved, 7H's, MeO-  $CH_2-CH-N-CH_2$, -OH).

Mass spectrum: m^+/e = 192, 121, 91, 78, 65.

ANALYSIS	C	H	N
Calculated	72.86	7.34	4.47
Found	72.84	7.36	4.50

The mother liquor was purified by thin-layer chromatography (t.l.c.) on silica gel : (R_f values 21 > 20 > 22, solvent 7% MeOH in $CHCl_3$).

The mother liquor yielded 310 mg. (1%) of N-benzyldeacetylanisomycin (20), and 170 mg. (0.5%) of the triol amine (22). Both (20) and (22) were identified by comparison with data of authentic samples.

<u>(4) Preparation of N-benzyldeacetylanisomycin (20)</u>:

Deacetylanisomycin (4) (450 mg.) and benzyl bromide (340 mg.) were stirred overnight at room temperature in

50 ml. of $CHCl_3$. The chloroform solution was then extracted with 5% hydrochloric acid; the aqueous solution made basic and extracted with chloroform. The chloroform solution after drying with $MgSO_4$ was evaporated under reduced pressure. The residue was subjected to t.l.c. on alumina using 3.5% MeOH in $CHCl_3$ as solvent. Elution of the fastest moving band yielded 191 mg. (30%) of (4) , m.p. = 80-82°C .

$[\alpha]_D^{25} = -51.0°$

Infrared spectrum (figure 3) : absorptions (cm^{-1}) at 3570 (-OH); 3370 (hydrogen bonded -OH); 1600, 1590, and 1500 (aromatic C=C); 1040 ($-OCH_3$).

P.m.r. spectrum (figure 2) : absorptions at 3.0τ(s, 5H's aromatic); 3.0-3.6τ(A_2B_2, 4H's, aromatic); 6.4τ(s, 3H's, $-OCH_3$), 6.0-8.1τ(unresolved multiplet, 11H's).

Mass spectrum: m^+/e = 192, 121, 91, 60, 39.

(5) Preparation of 1-p-methoxyphenyl-2,3,4-trihydroxy-5-benzylaminopentane (22):

The keto-amide (18) (500 mg.) dissolved in 20 ml. of anhydrous thf was added dropwise to a refluxing solution of lithium aluminum hydride (250 mg.) in 20 ml. of anhydrous thf . Refluxing continued for 17 hours. The solution was then distilled to a small volume and ethyl acetate (15 ml.) added carefully to

the cooled solution followed by 150 ml. of ether. The ether solution was extracted twice with 10 ml. of 6N hydrochloric acid and the acid solution made basic, then extracted with chloroform. This chloroform solution was dried ($MgSO_4$) and evaporated to dryness. The residue yielded 255 mg. (50%) of the triol amine (22), m.p. = 120-123°C.

Infrared spectrum (figure 4): absorptions (cm^{-1}) at 3450 (-OH); 3450-2650 (broad, -OH and -NH); 1620, 1590, and 1500 (aromatic C=C); 1040 ($-OCH_3$).

P.m.r. spectrum (figure 3): absorptions at 2.7τ(s, 5H's, aromatic), 2.8-3.3τ(A_2B_2, 4H's, aromatic), 6.2τ(s, 3H's, $-OCH_3$), 5.7-7.5τ(unresolved, 13H's).

Mass spectrum: m^+/e = 331, 313, 295, 210, 180, 122, 121, 120, 107, 106, 100, 91, 85, 77, 65.

(6) Preparation of N-benzylanisomycin (23):

N-benzyldeacetylanisomycin (330 mg.) was dissolved in cold acetic anhydride (25 ml.).After 30 minutes the acetic anhydride was distilled at reduced pressure and the residue subjected to t.l.c. on alumina ($CHCl_3$). Elution of the fastest spot yielded 60 mg. (14%) of diacetate. Elution of the major band yielded 256 mg. (68%) of (23). This (23) failed to crystallize and yellowed on standing or when left overnight on alumina.

Infrared spectrum (figure 6): absorptions (cm^{-1})

3600 (-OH); 1725 (ester C=O); 1610, 1590, and 1510 (aromatic C=C) .

(7) Preparation of anisomycin hydrochloride:

N-benzylanisomycin (256 mg.) was dissolved in ethanol (10 ml.); then 50 mg. of 5% Pd on charcoal was added, followed by 5 drops of 12N hydrochloric acid. This mixture was hydrogenated for 2 hours under one atmosphere of hydrogen, then the catalyst was removed by filtration. Addition of ether to the filtrate caused the hydrochloride salt to precipitate. This salt was redissolved in a small amount of methanol. Slow addition of ether yielded anisomycin hydrochloride (140 mg.) (65%) as white needles.

Infrared spectrum (figure 7): absorptions (cm^{-1}) at 3300 (free -NH, -OH), 2700 (broad, amine salt), 1750 (ester C=O). The spectrum was superimposable on the spectrum of natural anisomycin hydrochloride.

The mother liquors were combined and dissolved in water (5 ml.). This solution was made basic and exhaustively extracted with chloroform. The chloroform was dried and evaporated. T.l.c. of the residue yielded 49 mg. (30%) of deacetylanisomycin (4), m.p. = 168-170°C.

Infrared spectrum (figure 8): absorptions (cm^{-1}) at 3300 (-NH and -OH); 2700 (broad, hydrogen bonding);

1620, 1590, 1510 (aromatic C=C).

It was difficult to obtain reproducable spectra from different mulls for comparison with natural (4). For this reason synthetic (4) was converted to its hydrochloride salt. The infrared spectrum of synthetic deacetylanisomycin hydrochloride (figure 9) was superimposable on natural deacetylanisomycin hydrochloride.

(8) Preparation of N-benzyl-2-(p-methoxybenzyl)-3,4-cis-epoxypyrrolidine (25):

180 mg. of the all trans diol (21) was stirred in acetic anhydride (25 ml.) at room temperature for 20 minutes. The acetic anhydride was then evaporated under reduced pressure and the residue subjected to t.l.c. on silica gel (3% MeOH in $CHCl_3$). Elution yielded 188 mg. of the hydroxyacetate (24) (infrared figure 10). Next compound (24) was dissolved in 1.6 ml. of dry chloroform and to that solution was added 111 mg. of PCl_5. The solution was stirred for 30 minutes and then evaporated to dryness under reduced pressure. To the resulting reddish residue was added 0.6 ml. of ethanol; after dissolution of the residue 1 ml. of 20% KOH in ethanol was also added. The solution was heated to reflux, allowed to cool, then transferred to a separatory funnel and diluted with 4 ml. of water. The solution was then extracted with chloroform and the chloroform solution dried and evaporated. The residue

was subjected to t.l.c. on alumina (1:1 benzene/chloroform). Elution of the major band yielded 105 mg. (60%) of the epoxide (25). Recrystallization from ethanol/water yielded pure (25), m.p. = 113-115°C.

$[\alpha]_D^{25} = +94^\circ$ ($CHCl_3$)

Infrared spectrum (figure 11): absorptions (cm^{-1}) at 1620, 1590, and 1500 (aromatic C=C); 1040 ($-OCH_3$); 865 (epoxide ring).

P.m.r. spectrum (figure 4): absorptions at 3.0τ(s, 5H's, aromatic), 3.0-3.6τ(A_2B_2, 4H's, aromatic), 6.4τ(s, 3H's, $-OCH_3$).

Mass spectrum: m^+/e = 295, 277, 198, 186, 174, 170, 149, 121, 91, 65.

ANALYSIS	C	H	N
Calculated	77.30	7.11	4.74
Found	77.22	7.10	4.88

(9) Preparation of (+)-N-benzyl-deacetylanisomycin (26):

The cis-epoxide (25) (130 mg.) was refluxed for one hour in acetic acid. The acetic acid was then evaporated under reduced pressure and the residue refluxed one hour in 10% KOH in ethanol. The solution was then cooled and extracted with chloroform. The resulting chloroform solution was dried ($MgSO_4$) and evaporated to dryness. The residue was subjected to t.l.c. on silica gel (7% MeOH in $CHCl_3$). Elution of the faster band

yielded 61 mg. (43%) of the all trans diol (21). The slower band yielded 55 mg. (40%) of (+)-N-benzyl-deacetyl-anisomycin (26), m.p. = 79-81°C.

$[\alpha]_D^{25} = +51.4^\circ$ ($CHCl_3$).

The infrared spectrum of (26) was superimposable on the infrared spectrum of natural N-benzyl-deacetyl-anisomycin (20).

(10) Preparation of (+)-anisomycin hydrochloride (28):

The procedure followed was identical with the preparation of (-)-anisomycin hydrochloride. 55 mg. of (26) yielded 23 mg. (43%) of (+)anisomycin hydrochloride (28), m.p. = 188-189°C.

The infrared spectrum was superimposable on that of natural anisomycin hydrochloride, m.p. = 187-188°C.

(11) Preparation of lactams (29) and (30):

The glass liner of a 500 ml. Parr high pressure reaction vessel was charged with 7 grams of the keto-amide (18), 7 grams of anhydrous $MgSO_4$, and 200 ml. of anhydrous ethanol. The liner was then placed inside the high pressure reaction vessel, the vessel sealed, its atmosphere replaced by 50 p.s.i. of N_2, and the temperature rapidly raised to 130°C. The temperature was maintained between 135 and 138°C for 7 hours. The vessel was then cooled and the pressure released. The slightly colored ethanol solution was filtered and on standing for an hour

under refrigeration the unreacted (18) crystallized out (605 mg). The crystals were removed by filtration and the ethanol solution was then evaporated to dryness. Crystallization from benzene produced 3.87 grams (63%) of (29). Chromatography of the mother liquors on silica gel yielded an additional 0.5 grams of (29) for a total yield of 4.37 grams (72%) of (29), m.p. = 148-149°C.

Infrared spectrum (figure 12): absorptions (cm^{-1}) at 3500 (-OH); 3350 (hydrogen bonded -OH); 1725 (C=O); 1665 (C=C); 1610, 1580, 1510 (aromatic C=C).

P.m.r. spectrum (figure 5) of the diacetate derivative: absorptions at 2.7τ(s, 5H's, aromatic), 2.9-3.4τ(A_2B_2, 4H's, aromatic), 3.8τ(4 line m, 1H, C_3-H), 4.1τ(broad d., 1H, vinyl proton), 4.5τ(d, 1H, C_4-H), 5.1τ(broad s, 2H's, N-CH_2-Ph), 6.3τ(s, 3H's, -OCH_3), 7.8τ(s, 3H's, C_4-$OCOCH_3$), 8.3τ(s, 3H's, C_3-$OCOCH_3$).

Mass spectrum: m^+/e = 325, 321, 307, 237, 216, 188, 160, 147, 146, 108, 91, 77, 65.

ANALYSIS	C	H	N
Calculated	70.14	5.89	4.31
Found	70.28	6.08	4.52

Elution of the lower chromatographic band yielded 366 mg. (6%) of (30) as an oil.

Infrared spectrum (figure 13): absorptions (cm^{-1}) at 3300 (broad -OH); 1725 (C=O), 1680 (C=C), 1610 and

1510 (aromatic C=C).

P.m.r. spectrum of the diacetate (figure 6), absorptions at 2.7-3.5τ(m, 9H's, aromatic), 4.0-4.5τ (ABC pattern, 3H's, $-CH=C-C_3H-C_4H$), 5.1 and 5.7τ(AB, 2H's, $N-CH_2-Ph$), 6.2τ(s, 3H's, $-OCH_3$), 7.8 and 7.9τ (2s, 6H's, 2 $-OCOCH_3$).

(12) Preparation of N-benzyl-2-(p-methoxybenzyl)-3-trans-4-cis-dihydroxypyrrolidone (31):

The lactam (29) (567 mg.) was dissolved in ethanol (50ml.); 230 mg. of 5% Pd/C was added and the flask placed in a Parr hydrogenation vessel under 500 p.s.i. of hydrogen pressure. Hydrogenation was allowed to proceed for 18 hours at room temperature. The catalyst was then filtered off and the ethanol evaporated under reduced pressure. The residue was recrystallized from benzene to yield 444 mg. (85%) of (31). m.p. = 111.5-112.5°C.

Infrared spectrum (figure 14): absorptions (cm^{-1}) at 3500 and 3300 (-OH); 1690 (C=O); 1610, 1600, and 1500 (aromatic C=C).

Mass spectrum: m^+/e = 327, 206, 178, 121, 91.

ANALYSIS	C	H	N
Calculated	69.71	6.47	4.28
Found	69.93	6.26	4.49

(13) Preparation of N-benzyl-2-(p-methoxybenzyl)-3-trans-4-cis-dihydroxypyrrolidine (21) from (29):

The lactam (29) (2.2 grams) was hydrogenated in the presence of 1.1 grams of 5% Pd/C as described for the preparation of (31). The resulting crude product was dissolved in 20 ml. of anhydrous thf and then added dropwise to a warm suspension of 1.06 grams of lithium aluminum hydride in 80 ml. of thf. After the addition was completed, the mixture was refluxed for 7 hours. Then the t.h.f. solution was evaporated at reduced pressure to half the original volume, the resulting suspension cooled, and 100 ml. of ether carefully added, followed by 50 ml. of water. The mixture was transferred to a separatory funnel and shaken vigorously. The aqueous layer was then separated and extracted twice more with ether. The combined wet ether extracts were evaporated, benzene (25 ml.) added, and the benzene solution extracted with 5% hydrochloric acid. This HCl solution was made basic and extracted with chloroform. The chloroform solution was dried with $MgSO_4$ and evaporated. The residue was dissolved in a small volume of hot benzene and the benzene solution titurated with heptane. Refrigeration overnight produced 1.6 grams (74%) of (21).

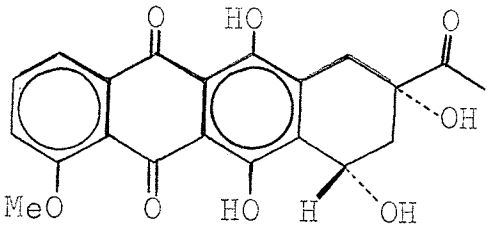
PART II

SYNTHETIC APPROACHES TO DAUNOMYCIN

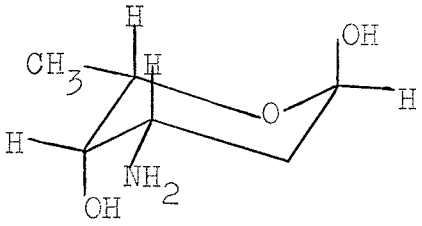
INTRODUCTION

Daunomycin, a metabolite of *Streptomyces peuceticis*, is an antibiotic which strongly inhibits the growth of a variety of experimental tumors[21]. At present it is used as an investigational anti-cancer antibiotic that appears to damage proliferative cells both by binding with DNA and by intercalating in the DNA double helix[22]. Daunomycin also has produced hematologic remissions in acute leukemia as the initial form of treatment.

Daunomycin was first isolated in 1963 by Grein *et al*[23]. It is a glycoside which on acid hydrolysis yields an aglycone, daunomycinone[24] (35), and an aminosugar, daunosamine[25] (36).



(35)

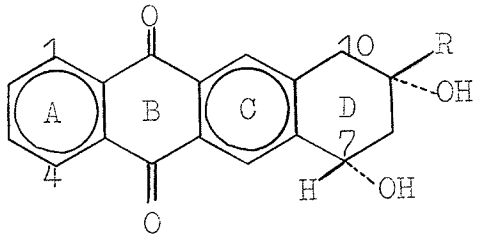


(36)

Dubost et al[26] in 1963 described an antibiotic, rubidomycin, whose properties appear to correspond with those of daunomycin.

Daunomycin is closely related to the anthracyclines[27], a group of antibiotics that contain a

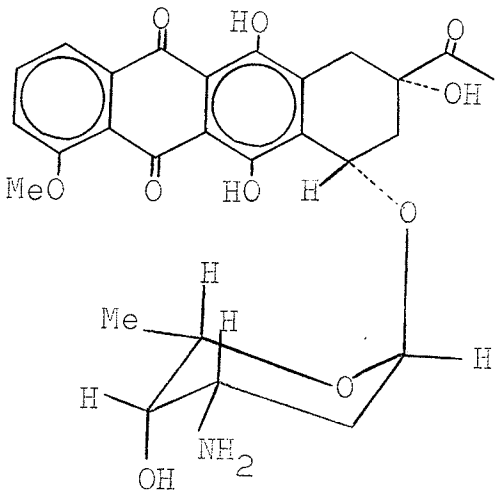
tetrahydrotetracenequinone chromophore linked to a sugar. In the anthracyclines, (R = $-CH_2CH_3$), the



number and the position of the phenolic hydroxyl groups vary. In addition, the C-10 carbon atom may carry either a hydroxyl group or a carbomethoxy group. In daunomycinone, (R = $-COCH_3$), the C-10 position is not substituted. Furthermore, the C-4 hydroxyl group is methylated.

The Structure of Daunomycin

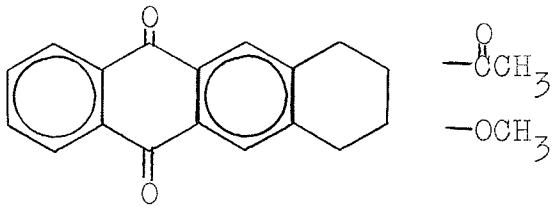
Daunomycin (37) ($C_{27}H_{29}NO_{10}$) is a red, crystalline, optically active substance. Its ultraviolet and visible



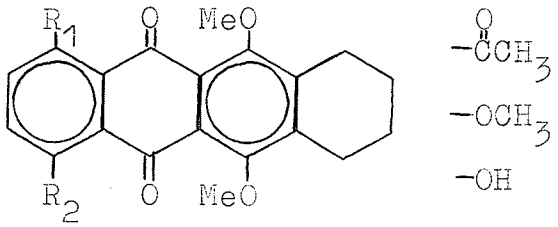
(37)

spectrum closely resembles that of 1,4,5-trihydroxy-anthraquinone[24]. The presence of the quinoid chromophore is also shown by the fact that readily reversible reduction occurs on treatment with mild reducing agents. Mild acid hydrolysis of daunomycin affords a red aglycone, daunomycinone (35) ($C_{21}H_{18}O_8$), and an amino-sugar, daunosamine (36) ($C_6H_{13}NO_3$). The ultraviolet spectrum of daunomycinone is similar to that of daunomycin. Zinc dust distillation of daunomycinone yields tetracene, identified by its ultraviolet spectrum; thus suggesting a linear tetracyclic skeleton. The infrared spectrum shows an absorption at 1718 cm^{-1},

suggesting the presence of an aliphatic ketone. The ready formation of a 2,4-dinitrophenylhydrazone derivative substantiates the presence of a ketone. The p.m.r. spectrum of daunomycinone shows absorptions for one methoxy group (s, δ=4.04) and one methylketone (s, δ = 2.69). The data given to this point accounts for the complete carbon skeleton of daunomycinone:



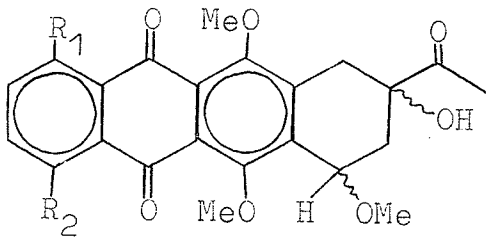
Methylation of daunomycinone with dimethylsulfate yields a trimethylether derivative, ($C_{24}H_{24}O_8$). The infrared spectrum still shows a hydroxyl band at 3350 cm^{-1}. The p.m.r. spectrum shows four sharp singlet absorptions at δ=4.00 (6H, two $-OCH_3$), 3.89 (3H, $-OCH_3$), 3.56 (3H, $-OCH_3$), and 2.40 (3H, $-COCH_3$). Further, the three proton multiplet (7-8, ABC pattern) indicates that the aromatic protons are adjacent on the same ring. Hence the following is the partial structure for the ether derivative:



where either R_1 or R_2 is a methoxy group. The absorption

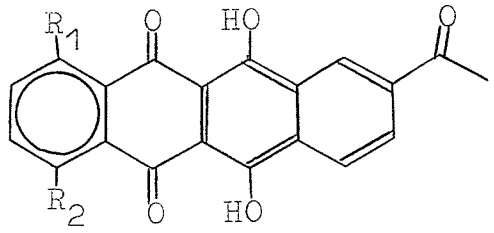
at δ=4.92 (1H, 4 lines) is the X part of an ABX spectrum. The chemical shift indicates that this proton is on an oxygen bearing carbon atom as well as being in a benzylic position. The AB part consists of two pairs of symmetrical doublets centered at approximately δ=1.87 (1H) and 2.42(1H).

The structure is now apparently:

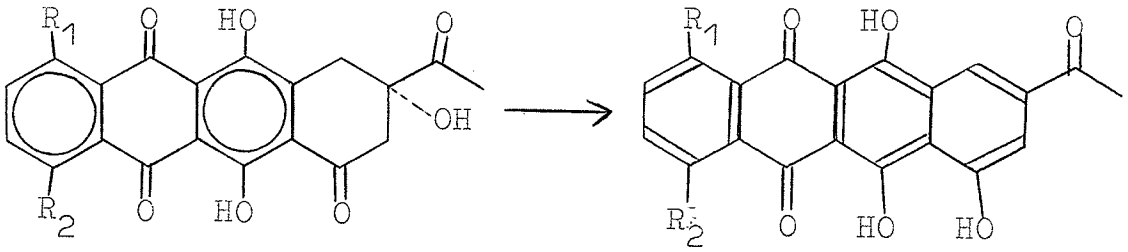


The methylketone and hydroxyl group must both be on the C-9 carbon atom, since no further proton coupling to the AB part is indicated. This leaves two benzylic geminal protons to be accounted for and these can easily be identified from the p.m.r. spectrum as two doublets at δ=3.02 and δ=3.22 (AB pattern). The fact that the remaining hydroxyl group is tertiary explains why it does not methylate.

The next problem was to establish which of the hydroxyl groups is methylated in daunomycinone. Treatment of daunomycinone with either base or acid yields bisanhydrodaunomycinone, which on oxidative

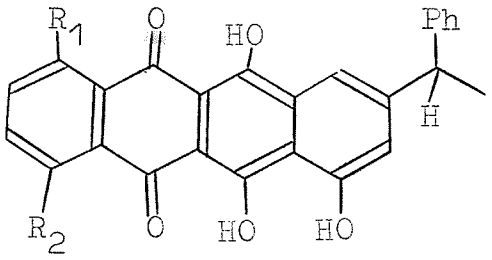


fission with permanganate yields 1,2,4-benzene tricarboxylic acid and 3-methoxy phthalic acid. Therefore, the methoxy group is located on the A ring. The only remaining question is which of R_1 or R_2 is the methoxy group. No rigorous chemical proof has yet been published. However, the position of the methoxy group may be deduced on the basis of spectroscopic evidence[29]. Daunomycinone on treatment with chromic anhydride in acetic acid yields (38), which when



(38) (39)

treated with 2N NaOH gives (39). Treatment of (39) with aluminum chloride in benzene yields (40) where



(40)

R_1 or R_2 is hydroxyl. The visible spectrum of (40) in different solvents is identical with that of 1,6,10,11-tetrahydroxy-5,12-naphthacene dione. On the basis of this evidence, it was concluded that R_2= methoxy.

The position of the glycosidic linkage may be deduced by catalytic hydrogenolysis of daunomycin. Hydrogenation yields 7-deoxydaunomycinone and daunosamine, thus confirming the attachment of the amino-sugar at C-7.

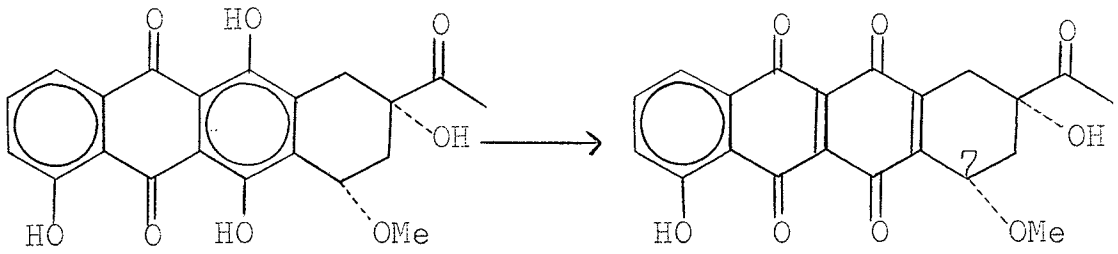
The relative cis stereochemistry of the two hydroxyl groups at C-7 and C-9 may be established by the successful conversion of daunomycinone to the 7,9 acetonide[30]. Hydrolysis of the acetonide regenerates daunomycinone, thus confirming the cis stereochemistry of the hydroxyl groups.

The structure of daunosamine was established as follows[25]. Daunosamine is a reducing sugar. It also gives a positive iodoform test, indicating a 6-deoxy sugar. This is further confirmed by the isolation of acetaldehyde on periodate oxidation. Oxidation of N-benzoyldaunosamine yields N-benzoyl-L-(+)-aspartic acid. Thus the absolute configuration at either C-2 or C-3 is (S). Since the p.m.r. spectrum shows a triplet at δ 4.75 for the C-1 proton, there are 2H's on the C-2 atom. Therefore, the C-3 carbon atom must carry the nitrogen atom. Hence the absolute config-

uration at C-3 is (S). Comparison of the molecular rotations of the eight stereoisomeric 2,6-dideoxy-hexoses with daunosamine suggests that daunosamine (36) has the L-lyxo configuration. This has been confirmed by an unambiguous, stereospecific synthesis of daunosamine[31] and the D-enantiomer[32]. A detailed study of the p.m.r. spectrum of daunomycin[30] in pyridine shows that the anomeric proton, H_1^1, is equatorially oriented and that the sugar therefore exists in the -L- form. The benzylic proton at C-7 of the aglycone exhibits spin coupling to the C-8 ax. and C-8 eq. protons of 5.0 and 1.5 Hz respectively. The magnitude of the coupling constants indicates that the proton at C-7 is pseudoequatorially oriented. Varying the solvent from pyridine -d_5 to acetone-d_6 produces a change in the spin coupling between C-8 ax. and C-7 from 5.0 to 3.0 Hz. Furthermore, after slight warming, the acetone-d_6 spectrum exhibits two sets of resonances for the amino sugar as well as the methoxyl protons. This is further evidence that the methoxyl group is situated at C-4. If the methoxyl group were located at C-1, changes in the chemical shift due to conformational changes in the sugar moiety would not be observed.

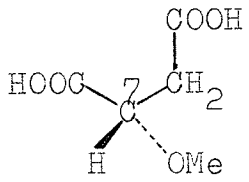
The total absolute configuration of daunomycin was established by Arcamone et al[33]. 7-O- methyl-

desmethyldaunomycinone (41) was oxidized with lead tetra-acetate to the diquinone (42). The diquinone



(41) (42)

was oxidized further with a periodate/permanganate mixture to give, after purification, S(-)-methoxy-succinic acid (43). This establishes the absolute



(43)

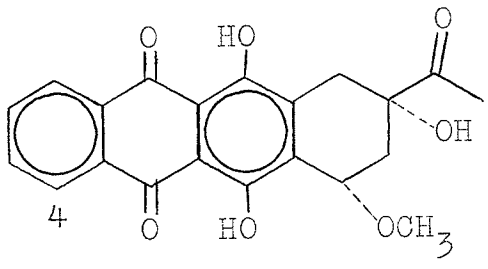
configuration of C-7. Since C-9 is sterically related to C-7, the total absolute configuration of daunomycin (37) is 7(S), 9(S), 1'(R), 3'(S), 4'(S), 5'(S).

Synthesis of Daunomycin

Although a number of the anthracyclines have been known for over two decades, it is surprising that no chemical synthesis has been reported to date on any of the antibiotics.

March[28] and coworkers reported the synthesis of compounds structurally related to daunomycin. Their objective was to determine structure-activity relationships.

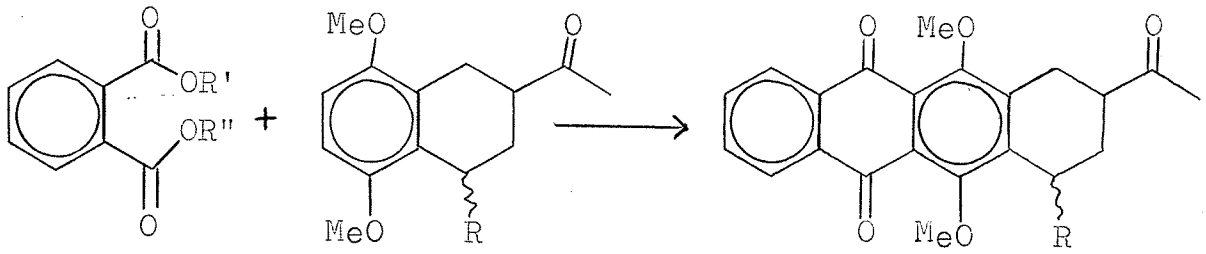
In our approach to the synthesis of daunomycin, it was decided to first synthesize racemic 7-O-methyl-demethoxydaunomycinone[34]. The synthesis of this molecule in conjunction with B. Schwenk and D. Popien constitutes the second part of this thesis. Discussion will be limited to the work carried out by the author himself.



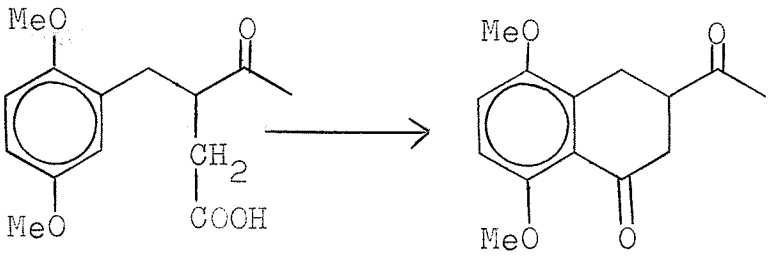
7-O-methyl-4-demethoxydaunomycinone

RESULTS AND DISCUSSION

A key step in our planned synthetic approach was the condensation of a phthalic acid derivative with a suitable bicyclic compound to give the linear tetracyclic skeleton. Since R was destined to become



a hydroxyl function, the diketone (45) was considered a useful intermediate to synthesize. The bicyclic diketone (45) would be readily available from the



(44) (45)

keto-acid (44). Initially it was decided to synthesize compound (44) by two successive alkylations of aceto-

acetic ester; followed by hydrolysis and a ketonic cleavage of the β-keto-acid (scheme V, page 60).

Diethyl acetylsuccinate was condensed with 2,5-dimethoxybenzyl chloride using sodium ethoxide as base in absolute ethanol. Upon vacuum distillation two clear liquid products were obtained. The major fraction was identified from its p.m.r. spectrum as 2,5-dimethoxybenzyl ethyl ether. The spectral data of the higher boiling fraction were consistent with the desired product (46) . Hydrolysis of (46) in cold, dilute aqueous sodium hydroxide gave the diacid (47) as the major product. Its infrared spectrum showed a broad hydroxyl absorption from 3300 to 2300 cm^{-1} and a broad carbonyl absorption at 1710 cm^{-1}. To confirm that the product was indeed a diacid, it was refluxed for 5 minutes in acetic anhydride. The infrared spectrum of the crude product lacked any hydroxyl absorptions and the carbonyl peak had shifted from 1710 cm^{-1} to 1790 cm^{-1} with an additional medium intensity band at 1870 cm^{-1}. The ready formation of the anhydride (48) confirmed the structure of (47). Warming of the anhydride in polyphosphoric acid gave compound (49) in excellent yield. Its infrared spectrum showed a broad hydroxyl band centered at 3150 cm^{-1}, a carbonyl band (acid) at 1725 cm^{-1}, and a carbonyl band (aromatic ketone) at 1660 cm^{-1}. Compound (49) was fully characterized by a colleague,

Bob Schwenk, who synthesized (49) by a different approach and successfully converted the carboxyl group to a methyl ketone.

After the diacid (47) had been removed from the reaction product, the mother liquors were concentrated. The p.m.r. spectrum showed a weak absorption attributable to a methyl ketone. The presence of the keto-acid (44) was assumed and the mixture was warmed in polyphosphoric acid at 50°C. From the neutral fraction of the product the diketone (45) was isolated by chromatography. The infrared spectrum lacked any hydroxyl absorptions and had carbonyl absorptions at 1720 cm^{-1} (aliphatic ketone) and at 1680 cm^{-1} (aromatic ketone). The p.m.r. spectrum had absorptions at δ= 2.2 (3H, $COCH_3$), δ =3.8 (6H, OCH_3), δ=6.8 (2H, AB quartet, aromatic H's), and a 5 proton multiplet in the region δ=2.5-3.5. The mass spectrum gave the correct molecular ion (m^+/e = 248).

Having obtained compound (45), we looked for means to improve its yield. Acid hydrolysis of (46) at room temperature or at reflux temperature gave tarry products. The failure to obtain satisfactory yields of compound (44) from acetoacetic ester derivatives led us to try derivatives of acetyl acetone by a similar approach. Since the β-keto ester underwent facile carbon-carbon cleavage, a β-diketone would be expected

to behave similarly.

The sodium salt of acetyl acetone was condensed with ethyl bromoacetate in absolute ethanol to give compound (50) (scheme VI, page 61). Condensation of 2,5-dimethoxy-benzyl chloride with compound (50) in ethanol also gave 2,5-dimethoxybenzyl ethyl ether as the major product and only a small yield of the desired compound (51). To circumvent this problem, the condensation was carried out in anhydrous thf using sodium hydride as base. The reaction proceeded very slowly, requiring 4 days at reflux temperature. Compound (51) was not isolated, but the reaction product was warmed overnight in 1N NaOH solution. Work up of the reaction mixture yielded the keto acid (44) in 41% yield.

A further improvement in the synthesis of compound (51) was made by condensing 2,5-dimethoxy-benzaldehyde with acetylacetone to give compound (52) in nearly quantitative yield. The infrared spectrum showed carbonyl absorptions at 1700 cm^{-1} and 1660 cm^{-1}. A medium intensity band at 1630 cm^{-1} can be attributed to the double bond. The p.m.r. spectrum showed absorptions at δ=2.2 (3H, $COCH_3$), δ=2.4 (3H, $COCH_3$), and δ=7.7 (1H, vinyl). Compound (52) was hydrogenated in quantiative yield to compound (53) and compound (53) crystallized in the enol form. The infrared spectrum showed broad carbonyl absorption at 1600 cm^{-1}.

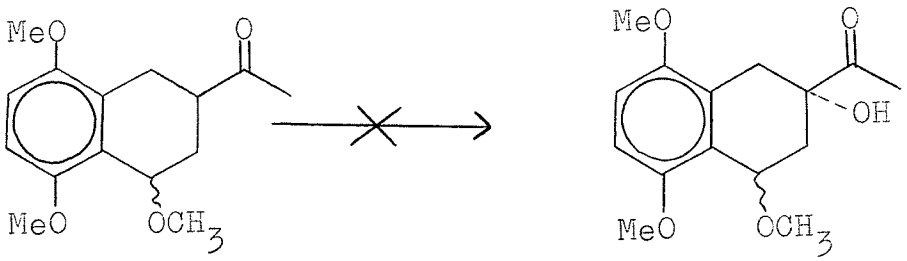
The p.m.r. spectrum in carbon tetrachloride showed a sharp peak at δ=16.6 for the enolic hydroxyl proton and a broad singlet for the methylene protons at δ=3.45. The sodium salt of (53) was condensed with ethyl bromoacetate in thf to give an excellent yield of compound (51). The infrared spectrum showed carbonyl absorptions at 1730 cm^{-1} and 1700 cm^{-1}. The p.m.r. spectrum showed the presence of 2 methyl ketones (δ=2.2), 2 methylene groups (δ=2.8 (2 H's) and δ=3.4 (2H's)) and an ethyl ester group. Compound (51), upon hydrolysis in aqueous sodium hydroxide, gave the previously obtained keto acid (44) in excellent yield. For preparative purposes compound (44) was synthesized from 2,5-dimethoxybenzaldehyde without isolating any of the intermediates. Yields of 85-90% were consistently obtained on molar scales.

Cyclisation of the keto acid (44) in polyphosphoric acid gave compound (45) in poor yield. Similar results were obtained in concentrated sulfuric acid. In anhydrous hydrogen fluoride at 20°C, however, good yields were obtained. In addition a by-product (54) was obtained in low yield. When the reaction was carried out at -20°C, compound (54) was obtained exclusively.

The problem of selective reduction of the aromatic ketone to an alcohol in compound (45) proved to have a simple solution. Ketalisation of the aliphatic ketone

in compound (45) with ethylene glycol occurred quickly with only a small percentage of diketal being formed. The diketal was selectively hydrolysed to the desired monoketal by adding a few drops of acetic acid in 95% ethanol. The monoketal (55) was obtained in high yield. The infrared spectrum lacked a carbonyl absorption at 1720 cm^{-1}, but retained the carbonyl absorption at 1680 cm^{-1}, thus showing that the aliphatic ketone was ketalised. Furthermore, the methylketone peak in the p.m.r. spectrum shifted from δ=2.2 to δ=1.4, and an additional absorption for the ketal bridge was observed at δ=4.0.

Compound (55) was reduced with sodium borohydride in methanol to give the benzylic alcohol (56). The infrared spectrum had a strong band at 3600 cm^{-1} for hydroxyl absorption. The spectrum lacked carbonyl absorptions. When compound (55) was reduced with sodium borohydride in methanol and the solution was then acidified with 6N HCl and allowed to stand for 2 hours, compound (57) was obtained in nearly quantitative yield in both epimeric forms. This was indicated by the presence of 2 aliphatic methoxy peaks in the p.m.r. spectrum at δ=3.4 and δ=3.5. The infrared spectrum lacked hydroxyl absorption bands. The aliphatic ketone absorption occurred at 1710 cm^{-1}. Numerous attempts were made to introduce the tertiary hydroxyl group α to the carbonyl function. However, none



proved successful.

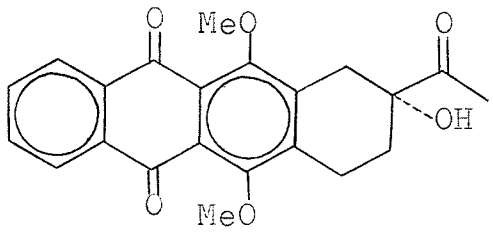
Attempts to condense phthalic acid derivatives with either compound (45) or compound (57) by various methods also failed. It became obvious that the functional group in the benzylic position was a real liability to further synthetic progress. Hence it was decided to remove the oxygen function completely. Catalytic reduction of compound (45) in acidified ethanol gave an excellent yield of the monoketone (58). A colleague, D. Popien[35], successfully converted the monoketone (58) back to the methoxyketone (57). This solved the problem of reintroducing the benzylic oxygen function.

Attempts to form the enol ether or enol acetate of compound (58) failed. However, the cyanohydrin (59) formed readily. The cyanohydrin was not purified, but converted directly to (60) by treatment with phosphorous oxychloride in dry pyridine at room

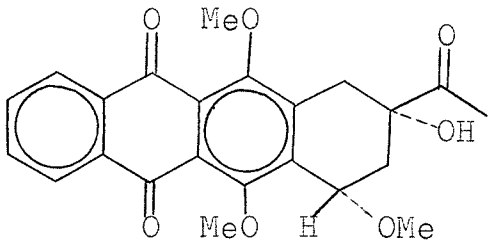
temperature. The infrared spectrum showed an absorption band at 2250 cm^{-1} for the α,β unsaturated nitrile and a band at 1650 cm^{-1} for the double bond. The p.m.r. spectrum lacked any vinyl protons, indicating that the double bond is tetrasubstituted. Oxidation of compound (60) with potassium permanganate in acetone in the presence of magnesium sulfate gave a fair yield of the desired hydroxyketone (61). The infrared spectrum showed a hydroxyl absorption at 3500 cm^{-1} and a carbonyl band at 1700 cm^{-1}. A better procedure for the synthesis of compound (61) was developed by D. Popien[35] when he successfully introduced the tertiary hydroxyl function by oxidizing compound (58) with molecular oxygen in the presence of potassium tert.-butoxide.

The monoketone (58) was successfully condensed with the monomethylester of phthalic acid in trifluoroacetic anhydride at 50°C to give two isomeric compounds (62). No attempt was made to separate the isomers. Basic hydrolysis of compound (62) yielded the isomeric acids (63). Compound (63) was dissolved in anhydrous hydrogen fluoride and allowed to stand for 2 hours at room temperature. Work up yielded the desired tetracyclic quinone (64). The p.m.r. spectrum showed it to be identical with the quinone obtained by D. Popien[35]. In his method he fused phthalic anhydride with compound (58) to give the phenol analogue

of compound (64). Methylation with dimethyl sulfate gave compound (64). The trifluoroacetic anhydride method is much milder. B. Schwenk[36] used this method successfully with the bicyclic hydroxy ketone (61) to give the following compound:

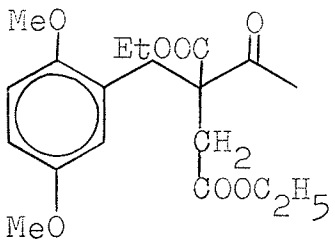


The benzylic oxygen function was introduced using D. Popien's[35] method to give:

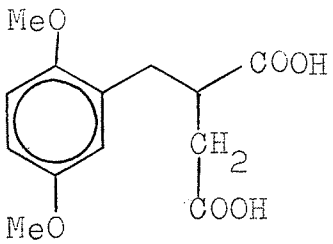


Demethylation with aluminum chloride gave the desired 7-O-methyl-4-demethoxydaunomycinone.

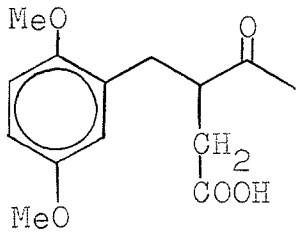
SCHEME V



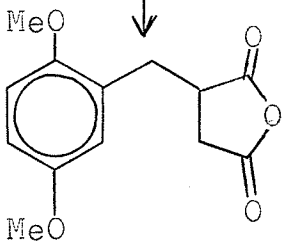
(46)



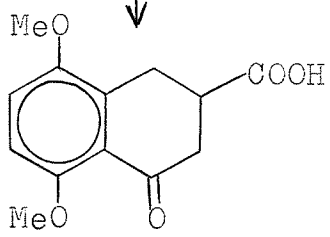
+



(47)

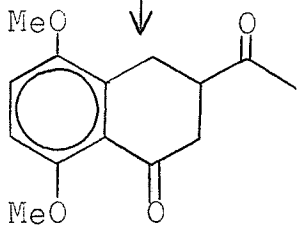


(48)



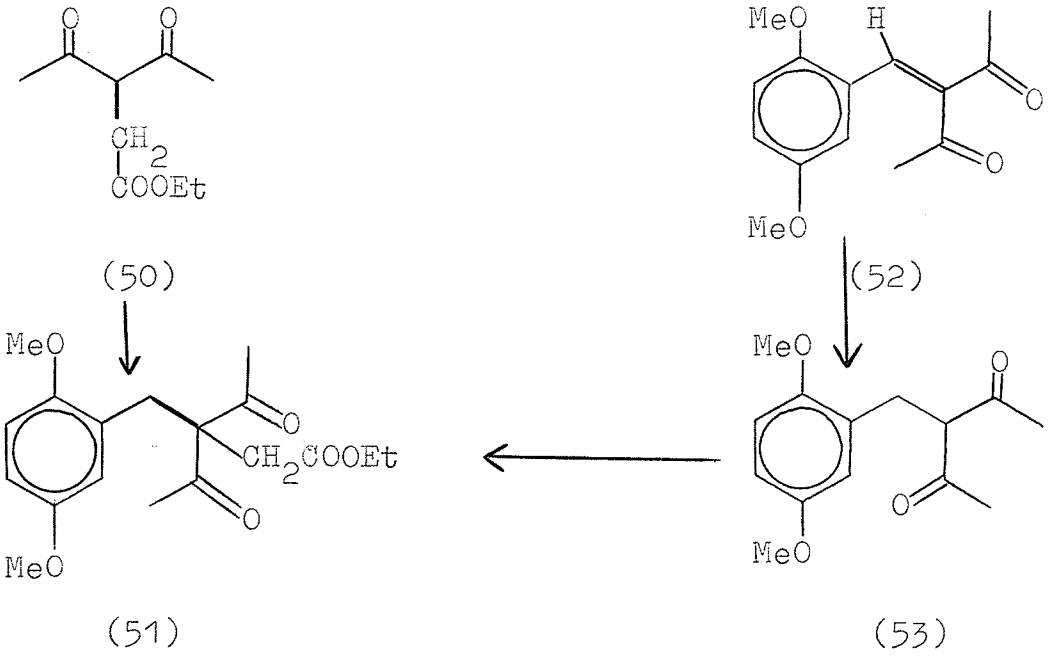
(49)

(44)



(45)

SCHEME VI

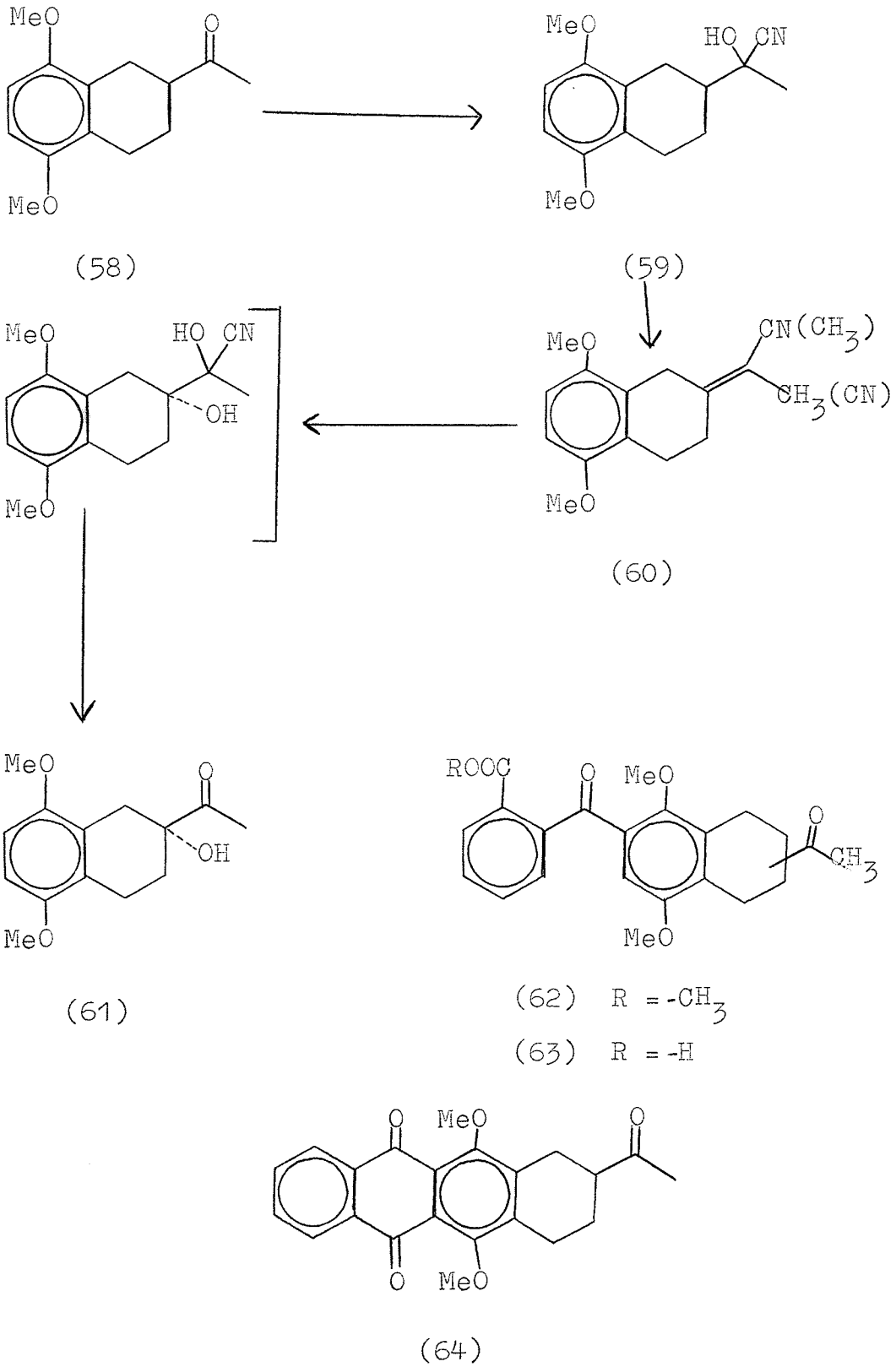


(51) (53)

(54) (55)

(57) (56)

SCHEME VII


MeO
O
MeO
(58)
MeO
HO CN
MeO
(59)
CN(CH3)
CH3(CN)
MeO
MeO
(60)
HO CN
OH
MeO
MeO
MeO
O
OH
MeO
(61)
ROOC
O
MeO
O
CH3
MeO
(62) R = -CH3
(63) R = -H
O
MeO
O
O
MeO
(64)

EXPERIMENTAL

See page 25 for related information.

(1) Preparation of ketoester (46):

A 1 liter, 3-neck, round-bottom flask was equipped with a mechanical stirrer, reflux condenser, and dropping funnel. Moisture was carefully excluded from the system. To the flask was added 50 ml. of absolute ethanol, followed by 2.3 grams (0.1 mole) of sodium. As soon as the sodium had dissolved, 21.6 grams (0.1 mole) of diethyl acetylsuccinate was added in one portion. The solution was then brought to reflux and 18.7 grams (0.1 mole) of 2,5-dimethoxybenzyl chloride in 300 ml. of absolute ethanol added over a 15 minute period. Refluxing was continued for an additional 4 hours. The mixture was then cooled and filtered and the ethanol removed under reduced pressure. The remaining mixture was again cooled and then filtered into a small distillation flask. Vacuum distillation yielded 11.2 grams (56%) of 2,5-dimethoxybenzyl ethyl ether, b.p. = 68-70°C at 0.5 mm. and 15 grams (41%) of the ketoester (46), b.p. = 180-190°C at 0.2 mm. .

Infrared spectrum (figure 16): absorptions (cm^{-1}) at 1720 (broad) (carbonyl bands), and at 1610 and 1590 (aromatic double bonds).

(2) Preparation of diacid (47):

Compound (46) (8.8 grams, 0.024 mole) was stirred vigorously with 100 ml. of 5% sodium hydroxide solution for 16 hours at room temperature. The basic solution was washed twice with ether and then acidified with concentrated hydrochloric acid. The precipitate was filtered off and dried. The crude product was then washed with chloroform to yield 4.6 grams (72%) of the diacid (47), m.p. = 155-156°C.

Infrared spectrum (figure 17): absorptions (cm^{-1}) at 3400-2200 (broad) (acid hydroxyl), 1705 (carbonyl stretch) , 1600 (aromatic).

Mass spectrum: m^+/e = 268, 250, 208, 151, 121, 91, 78, 77.

(3) Preparation of the bicyclic ketoacid (49) via the anhydride (48):

The diacid (47) (2.4 grams) was refluxed in 20 ml. of acetic anhydride for 5 minutes. The solvent was then distilled under reduced pressure.

Infrared spectrum (figure 18) of the residue: absorptions (cm^{-1}) at 1870 and 1790 (anhydride carbonyl).

Polyphosphoric acid (30 grams) was added to the residue and the mixture was warmed to 50°C, then stirred until homogeneous. The temperature was maintained at 50°C overnight. The resulting dark colored solution was then cooled and diluted with water; then the

cloudy aqueous solution was extracted with chloroform, dried with $MgSO_4$, and evaporated. The solid residue was recrystallized from acetone to yield 1.43 grams (64%) of the bicyclic ketoacid (49), m.p. = 203-204°C.

Infrared spectrum (figure 19): absorptions (cm^{-1}) at 3150 (broad) (hydroxyl stretch), 1730 (acid carbonyl), 1670 (aryl ketone), 1590 (aromatic).

(4) Preparation of 3-carbethoxymethyl-2,4-pentane dione (50):

A 500 ml. , 3-neck, round-bottom flask was equipped with a mechanical stirrer, reflux condenser, and dropping funnel. The flask was charged with 200 ml. of absolute ethanol and 9.2 grams of sodium. As soon as the sodium had dissolved, acetyl acetone (40 grams) was added in one portion and the solution brought to reflux. Ethyl bromoacetate (66.8 grams) was then added dropwise from the dropping funnel and refluxing was continued overnight. The ethanol was then evaporated under reduced pressure and the residue filtered. The filtrate was distilled under reduced pressure using a water aspirator and the fraction boiling between 119°C and 136°C was collected to yield 29 grams (39%) of product.

Infrared spectrum (figure 20): absorption band at 1730 cm^{-1} (broad) (carbonyl band).

(5) Preparation of compound (44) from compound (50):

The β-diketone (50) (8.5 grams) was dissolved in 150 ml. of anhydrous t.h.f. in a 300 ml. round-bottom flask. Sodium hydride (2.2 grams of a 50% suspension in mineral oil) was added, followed by 9.0 grams of 2,5-dimethoxybenzyl chloride. The solution was refluxed for 4 days. Then the solution was concentrated to a small volume and 150 ml. of a 15% sodium hydroxide solution added. This mixture was refluxed overnight, then cooled and washed twice with benzene. The aqueous fraction was acidified with concentrated hydrochloric acid and a solid precipitate was collected, dried, and recrystallized from methanol to yield 5.0 grams (41%) of the keto acid (44).

(6) Preparation of compound (52):

A 500 ml. , round-bottom flask was charged with 49.8 grams (0.3 mole) of 2,5-dimethoxybenzaldehyde, 40 grams (0.4 mole) of acetyl acetone, and 300 ml. of benzene. The mixture was stirred until homogeneous, then 2 ml. of piperidine added followed by 6 ml. of acetic acid. The flask was then equipped with a Dean-Stark trap and a reflux condenser. The solution was refluxed until no more water separated (approximately 1.5 hours) and then the benzene was distilled under reduced pressure. The residue solidified on standing.

Crystallization from methanol yielded 65 grams (88%) of condensed product, m.p. = 65-66°C.

Infrared spectrum (figure 21): absorptions (cm^{-1}) at 1700 and 1660 (carbonyl bands), 1630 (double bond , α,β unsaturated ketone), 1590 (aromatic) .

P.m.r. spectrum (figure 7): absorptions at 2.3 τ(s, 1H, vinyl proton), 3.2 τ(m, 3H's, aromatic), 6.2 τ(s, 3H's, $-OCH_3$), 6.3 τ(s, 3H's, $-OCH_3$), 7.6 τ (s, 3H's, methyl ketone), 7.8 τ(s, 3H's, methyl ketone).

Mass spectrum: m^+/e = 248, 217, 191, 176, 161, 148, 119, 105, 103, 91, 77, 43.

ANALYSIS	C	H
Calculated	67.73	6.49
Found	68.06	6.35

(7) Preparation of the β-diketone (53) from 2,5-dimethoxybenzaldehyde:

The aldehyde and acetyl acetone were condensed as described above. The crude residue, after removal of benzene, was dissolved in 1300 ml. of ethanol in a 2 liter flask and 5% Pd on charcoal (7 grams) was added. This mixture was shaken under one atmosphere of hydrogen until the uptake of hydrogen ceased (1.5 hours). The catalyst was then filtered off and the ethanol removed under reduced pressure. The residue was distilled under vacuum and the fraction boiling at 139-142°C at 0.15 mm. was collected to yield 64.6 grams (86%)

of product. This liquid product crystallized on standing.

Infrared spectrum of solid product (figure 22): absorptions (cm^{-1}) at 1600 (broad) (β-diketone enol form).

P.m.r. spectrum (figure 8): absorptions at -6.5 τ(s, 1H, enolic -OH), 3.4 τ(m, 3H's, aromatic), 6.2 τ(s, 3H'S, $-OCH_3$), 6.3 τ(s, 3H's, $-OCH_3$), 6.5 τ (s, 2H's, benzylic methylene protons), 8.0 τ(s, 6H'S, methyl ketones (enolic)).

Mass spectrum: m^+/e = 250, 207, 189, 138, 43.

(8) Preparation of compound (51) from compound (53):

The β-diketone (53) (5 grams) was dissolved in 100 ml. of anhydrous thf and then sodium hydride (1.0 grams of a 50% suspension in oil) was added. To the resulting amber colored solution was added 3.5 grams of ethyl bromoacetate and the solution refluxed for 1 hour to complete the reaction. The mixture was then concentrated to half its original volume, cooled, and 100 ml. of water added. Next the mixture was extracted with benzene three times and the benzene extracts washed with water and dried ($MgSO_4$). The benzene was evaporated at reduced pressure. The oily residue separated into 2 layers, the upper layer being the paraffin oil. The bottom layer crystallized on standing overnight under refrigeration. The oil was decanted to leave a nearly pure product. Crystall-

ization from methanol yielded 5.1 grams (76%) of compound (51), m.p. = 66-67°C.

Infrared spectrum (figure 23): absorptions (cm^{-1}) at 1730 and 1700 (carbonyl stretch), 1620 (aromatic).

P.m.r. spectrum (figure9): absorptions at 3.4 τ(m, 3H's, aromatic), 5.9 τ(q, 2H's, $-OCH_2Me$), 6.3 τ(s, 3H's, $-OCH_3$), 6.4 τ(s, 3H's, $-OCH_3$), 6.6 τ (s, 2H's, $-CH_2$ (benzylic)), 7.2 τ(s, 2H's, $-CH_2COOEt$), 7.8 τ(s, 6H's, CH_3CO-), 8.8 τ(t, 3H's, $-OCH_2CH_3$).

Mass spectrum: m^+/e = 336, 247, 151, 121, 91, 77, 43.

ANALYSIS	C	H
Calculated	64.27	7.19
Found	64.11	6.99

(9) Preparation of keto acid (44) from (53):

A 1 liter, 3-neck flask equipped with a mechanical stirrer, reflux condenser, and dropping funnel was charged with 600 ml. of anhydrous thf and 30 grams (0.12 mole) of compound (53). Sodium hydride (6.0 grams (0.125 mole) of a 50% suspecsion in oil) was added in small protions to the vigorously stirred solution. After the addition was completed, 21 grams (0.125 mole) of ethyl bromoacetate was added over a 20 minute period. The mixture was refluxed for one hour. After that, approximately 500 ml. of thf

was removed by distillation. The residue was allowed to cool and then 250 ml. of an 8% sodium hydroxide solution added. This mixture was stirred in an oil bath at 65°C for 3 hours, then allowed to cool for 2 hours. After transfer to a separatory funnel, the mixture was extracted three times with benzene and the benzene extracts discarded. The aqueous solution was transferred to a 2 liter beaker containing crushed ice and then carefully acidified with concentrated hydrochloric acid. The precipitated acid was filtered off and allowed to dry overnight. Crystallization from methanol yielded 27.1 grams (85%) of pure keto acid (44), m.p. = 106-107°C.

Infrared spectrum (figure 24): absorptions (cm^{-1}) at 3400-2400 (carboxylic acid -OH stretch), 1710 (carbonyl stretch), 1600 (aromatic).

P.m.r. spectrum (figure 10): absorptions at -0.9 τ(broad s, 1H, -COOH), 3.4 τ(m, 3H's, aromatic), 6.35 τ(s, 3H's,-OCH_3), 6.4 τ(s, 3H's, -OCH_3), 6.5-7.7 τ(m, 5H's), 7.8 τ(s, 3H's, -$COCH_3$).

Mass spectrum: m^+/e = 266, 177, 175, 151, 121, 91, 78, 77, 43.

ANALYSIS	C	H
Calculated	63.15	6.81
Found	63.30	6.58

(10) Preparation of the bicyclic diketone (45):

A 250 ml. polyethylene bottle was placed securely in a water bath at 15°C in a well ventilated hood. Anhydrous hydrogen fluoride (50 ml.) was added and the liquid stirred and allowed to warm to near the boiling point (19°C). Then 5.3 grams of the keto acid (44) was added in small portions. Stirring was continued for 3 hours, then the water bath temperature raised to 40°C. As soon as the evolution of hydrogen fluoride ceased, the residual liquid was poured into 100 ml. of ice water and the aqueous mixture extracted three times with chloroform. The chloroform solution was washed with saturated sodium bicarbonate, dried with $MgSO_4$, and evaporated. The oily residue was dissolved in hot methanol. The product crystallized on cooling to yield 3.3 grams (67%) of pure (45), m.p. = 123-125°C.

Infrared spectrum (figure 25): absorptions (cm^{-1}) at 1710 (methyl ketone), 1670 (aryl ketone), 1590 (aromatic).

P.m.r. spectrum (figure 11): absorptions at 3.2 τ(q, 2H's, aromatic), 6.2 τ(s, 6H's, $-OCH_3$), 6.5-7.5 τ(m, 5H's), 7.8 τ(s, 3H's, $-COCH_3$).

Mass spectrum: m^+/e = 248, 205, 190, 175, 91, 43.

ANALYSIS	C	H
Calculated	67.73	6.50
Found	67.74	6.78

(11) Preparation of the ketal (55):

The bicyclic ketone (45) (2.8 grams) was dissolved in 50 ml. of benzene, then 1 ml. of ethylene glycol added, followed by 100 mg. of p-toluene sulfonic acid monohydrate. The solution was slowly distilled for 1.5 hours with occasional addition of benzene to maintain volume. The benzene was then distilled off under reduced pressure and 50 ml. of ethanol containing 0.5 ml. of acetic acid added to the residue. The solution was stirred and the monoketal began to precipitate almost immediately from the solution. Stirring was continued for 2 hours. Filtration yielded 2.5 grams (76%) of pure white ketal. The filtrate was diluted with water and extracted with benzene. The benzene solution was washed with bicarbonate solution, dried with $MgSO_4$, and evaporated. Crystallization of the residue from a small amount of ethanol yielded an additional 400 mg. of product. The total yield was 2.9 grams (88%), m.p. = 139-139.5°C.

Infrared spectrum (figure 26): absorptions (cm^{-1}) at 1680 (aryl ketone), 1590 (aromatic).

P.m.r. spectrum (figure 12): absorptions at 3.2 τ(q, 2H'S, aromatic), 6.05 τ(s, 4H's, ketal bridge), 6.15 τ(s, 3H's, $-OCH_3$), 6.2 τ(s, 3H's, $-OCH_3$), 6.5-7.9 τ(m, 5H's), 8.6 τ(s, 3H's, $-\overset{\overset{O}{\|}}{C}-CH_3$).

Mass spectrum: m^+/e = 292, 205, 91, 88, 87, 43.

(12) Preparation of the methoxy ketone (57):

The ketal (55) (2.0 grams) was stirred in 50 ml. of methanol and then sodium borohydride in pellet form (550 mg.) was added to the mixture. Dissolution of the mixture occurred gradually. Stirring was continued for 30 minutes.

[Work up yielded the hydroxy ketal (56) - infrared spectrum (figure 27): hydroxy band at 3600 cm^{-1}; no carbonyl absorption.]

Hydrochloric acid (6 ml. , 6N) was added and the cloudy mixture was stirred for 2 hours at room temperature, then diluted with 200 ml. of water and extracted with ether. The ether solution was dried with $MgSO_4$ and evaporated. The oily residue slowly solidified to yield 1.78 grams (99%) of product.

Infrared spectrum (figure 28): absorptions (cm^{-1}) at 1710 (aliphatic ketone), 1610 (aromatic).

P.m.r. spectrum (figure 13): absorptions at 3.3 τ(s, 2H's, aromatic), 5.3 τ(t, 1H, -$\overset{|}{C}$HOMe), 6.1 τ(s, 3H'S, $-OCH_3$), 6.15 τ(s, 3H's, $-OCH_3$), 6.5 and 6.65 τ(s, 3H's, aliphatic $-OCH_3$), 6.5-7.6 τ (m, 4H's), 7.65 and 7.75 τ(s, 3H's, $-COCH_3$), 8.5 τ (m, 1H).

(13) Preparation of the bicyclic monoketone (58):

The diketone (45) (5grams) was dissolved in

250 ml. of ethanol and 5% Pd on charcoal (0.5 grams) was added. The mixture was shaken under one atmosphere of hydrogen for one hour, then the flask was flushed with nitrogen and 20 ml. of concentrated hydrochloric acid added. The mixture was then shaken under one atmosphere of hydrogen for an additional 45 minutes. After that the catalyst was removed by filtration. The filtrate was concentrated under reduced pressure to one third of the original volume and 150 ml. of water added. This mixture was extracted with benzene and the extract washed successively with water and sodium bicarbonate. It was then dried with $MgSO_4$ and evaporated. The residue soon crystallized and was used without further purification to yield 4.5 grams (96%) of product, m.p. = 82.5-83.5°C.

Infrared spectrum (figure 29): absorption (cm^{-1}) at 1710 (aliphatic ketone), 1610 (aromatic).

P.m.r. spectrum (figure 14): absorptions at 3.5 τ(s, 2H's, aromatic), 6.3 τ(s, 6H's, $-OCH_3$), 6.7- 8.8 τ(m, 7H's), 7.8 τ(s, 3H's, $-OCH_3$).

(14) Preparation of the bicyclic α,β unsaturated nitrile (60):

To a vigorously stirred solution of 3 grams of sodium cyanide in 50 ml. of 50% ethanol in water was added 1 gram of compound (58). The mixture was stirred for 10 minutes, then 5 ml. of acetic acid

was added and stirring was continued for one hour. The solution was then diluted with 100 ml. of water and extracted three times with ether. The ether solution was dried with $MgSO_4$ and evaporated to leave a gummy residue of the cyanohydrin (59) plus starting material.

Infrared spectrum (figure 30): hydroxyl band at 3620 cm^{-1}, weak carbonyl band (starting material) at 1710 cm^{-1}.

The residue was dissolved in 20 ml. of dry pyridine. Phosphorous oxychloride (1ml.) was added and the solution stirred at room temperature for 16 hours. The solution was then diluted with 100 ml. of water and extracted 3 times with ether. The ether solution was washed with water, dried with $MgSO_4$, and evaporated. The residue was chromatographed on silica gel to yield 490 mg. (47%) of product.

Infrared spectrum (figure 31): absorptions (cm^{-1}) at 2250 (α,β unsaturated nitrile), 1650 (double bond), 1610 (aromatic).

P.m.r. spectrum (figure 15): absorptions at 3.3 τ(s, 2H's, aromatic), 6.2 τ(broad singlet, 2 $-OCH_3$ groups plus additional protons), 8.0 τ(broad singlet, $=\overset{|}{C}-CH_3$).

(15) Preparation of α-hydroxy ketone (61):

Distilled acetone was passed through column grade silica gel and collected (5 ml.) under anhydrous

conditions. Anhydrous magnesium sulfate (500 mg.) was then added, followed by 240 mg. of the α,β unsaturated nitrile (60). The suspension was stirred vigorously and potassium permanganate (160 mg.) added. This mixture was stirred for 20 minutes. Dilute hydrochloric acid (20 ml. of 5% solution) was added and the mixture shaken vigorously, then extracted with ether. The ether solution was dried with $MgSO_4$ and evaporated. The residue was chromatographed to yield 96 mg. (38%) of product, m.p. = 102-103°C.

Infrared spectrum (figure 32): absorptions (cm^{-1}) at 3500 (hydroxyl stretch), 1700 (ketone stretch), 1605 (aromatic).

P.m.r. spectrum (figure 16): absorptions at 3.4 τ(s, 2H's, aromatic), 6.25 τ(s, 3H's, $-OCH_3$), 6.3 τ(s, 3H's, $-OCH_3$), 6.5 τ(s, 1H, -OH), 6.8-7.6 τ(m, 4H's), 7.7 τ(s, 3H's, $-COCH_3$), 8.0-8.3 τ (m, 2H's).

(16) Preparation of the tetracyclic quinone (64):

Ketone (58) (115 mg.) and phthalic acid monomethyl ester (135 mg.) were placed in a 10 ml. flask and trifluoroacetic anhydride (1 ml.) was added. The solution was warmed in an oil bath at 60°C for 12 hours, then the trifluoroacetic anhydride was evaporated. The residue was dissolved in ether

and washed with sodium bicarbonate solution. The ether solution was dried and evaporated. Chromatography of the residue on silica gel yielded 176 mg. (90%) of the isomeric compounds (62).

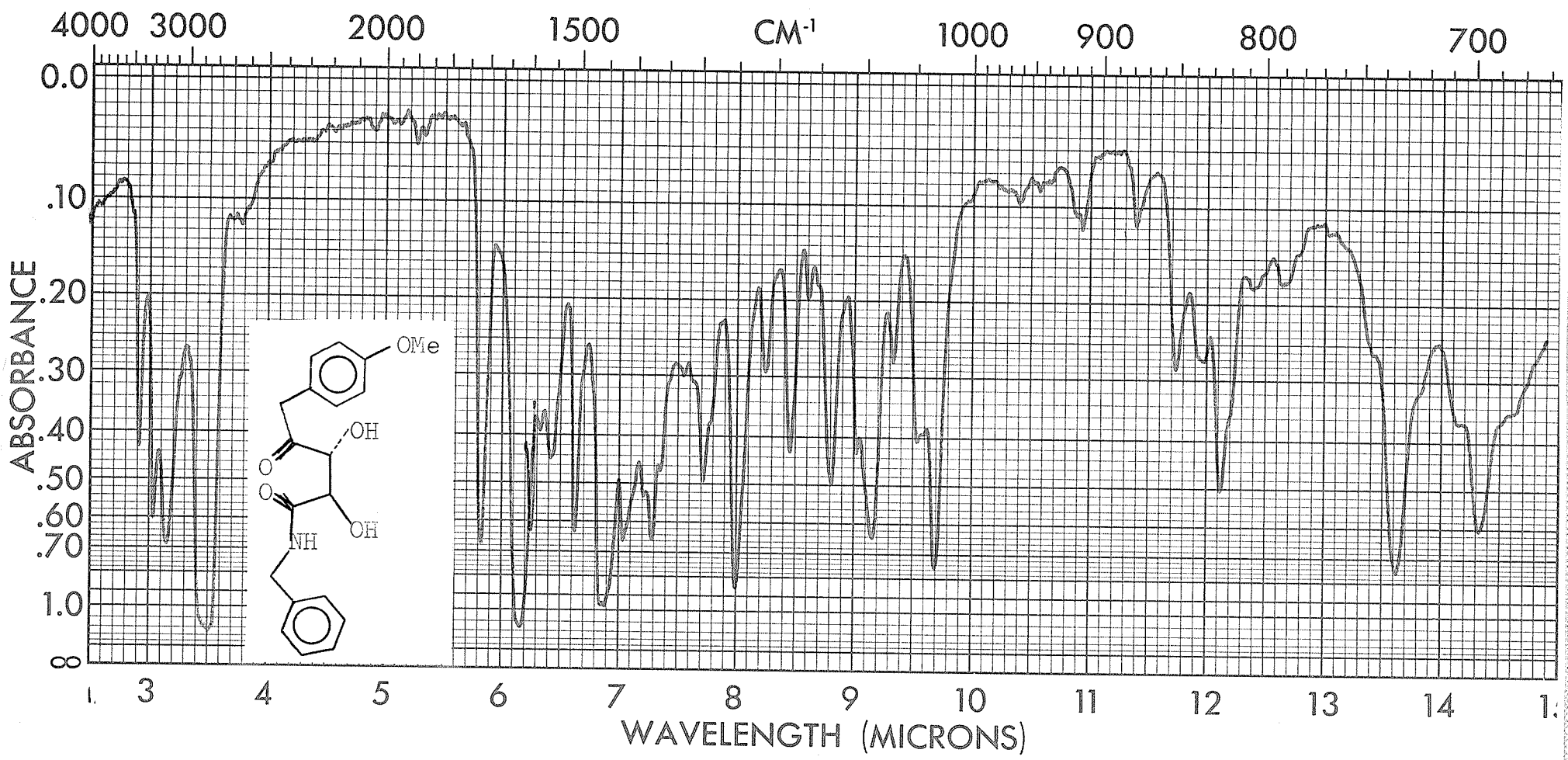
Infrared spectrum (figure 33): ketone and ester stretches at 1725 cm^{-1}, aryl ketone stretch at 1660 cm^{-1}, aromatic stretch at 1600 cm^{-1}.

P.m.r. spectrum (figure 17): absorptions at 1.9-2.9 τ(m, 5H's, aromatic), 6.1 τ(s, 3H's, $-OCH_3$), 6.2 τ(s, 3H's, $-OCH_3$), 6.6 τ(s, 3H's, $-OCH_3$ (ester)), 7.0-8.5 τ(m, 7H's), 7.7 τ(d, 3H's, $-COCH_3$).

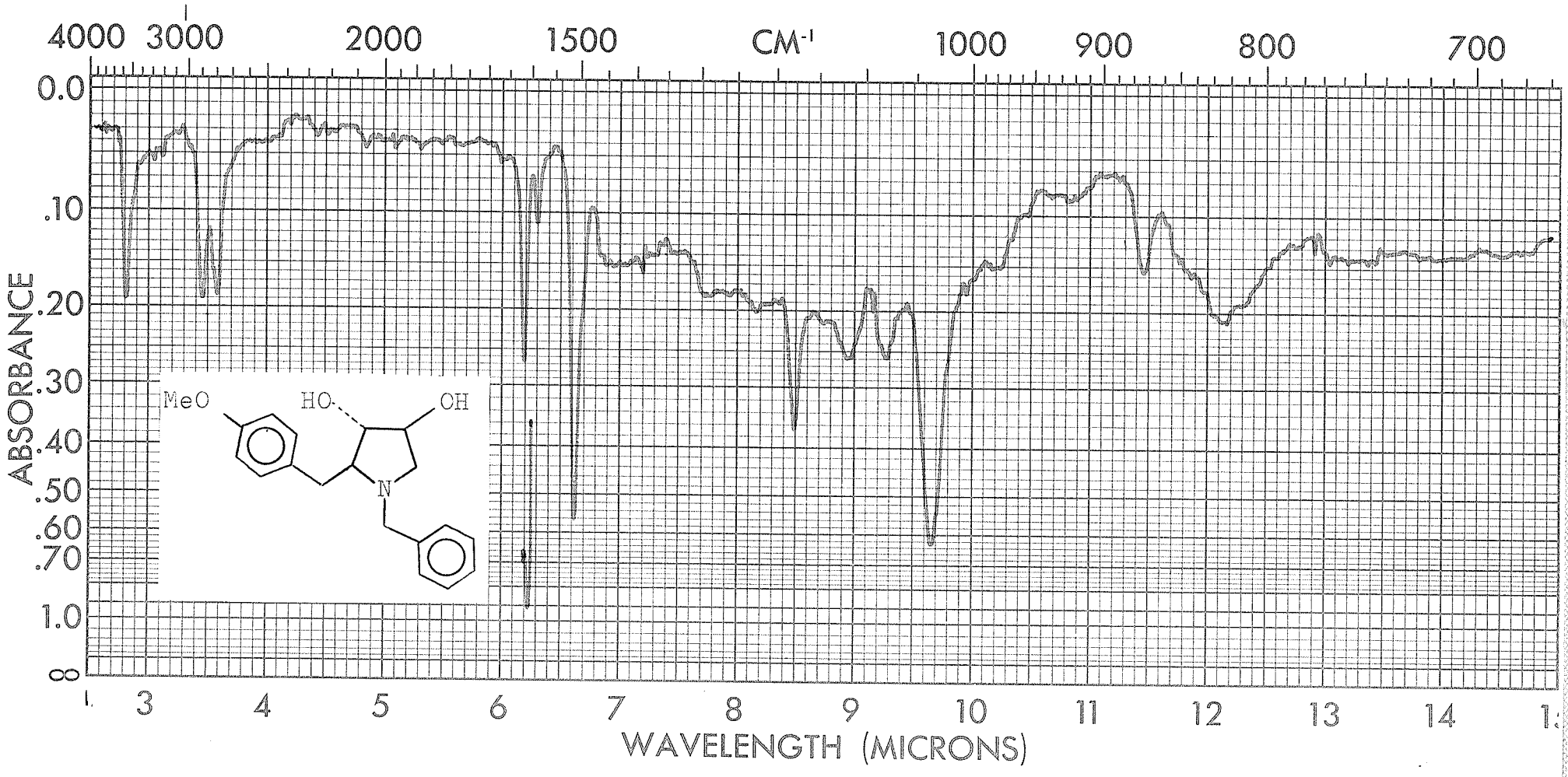
Compound (62) (176 mg.) was stirred in 5% NaOH for 3 hours at 60°C. The basic solution was cooled, washed with ether, acidified, and extracted with ether. The ether solution was dried with $MgSO_4$ and evaporated to leave 136 mg. (80%) of crude acid (63) - infrared spectrum (figure 34). The crude acid was dissolved in anhydrous hydrogen fluoride at 15°C and allowed to stand for 3 hours. Then the hydrogen fluoride was evaporated using a warm water bath. The residue was cooled and diluted with 30 ml. of ice cold water. This mixture was extracted with chloroform and the chloroform washed with bicarbonate solution, dried with $MgSO_4$, and evaporated. The crude yield was 111 mg. (67% from (62)) of the quinone (64).

P.m.r. spectrum (figure 18): absorptions at 1.8-2.5 τ(m, 4H's, aromatic), 6.15 τ(s, 3H's, $-OCH_3$), 6.2 τ(s, 3H's, $-OCH_3$), 7.8 τ(s, 3H's, $-COCH_3$).

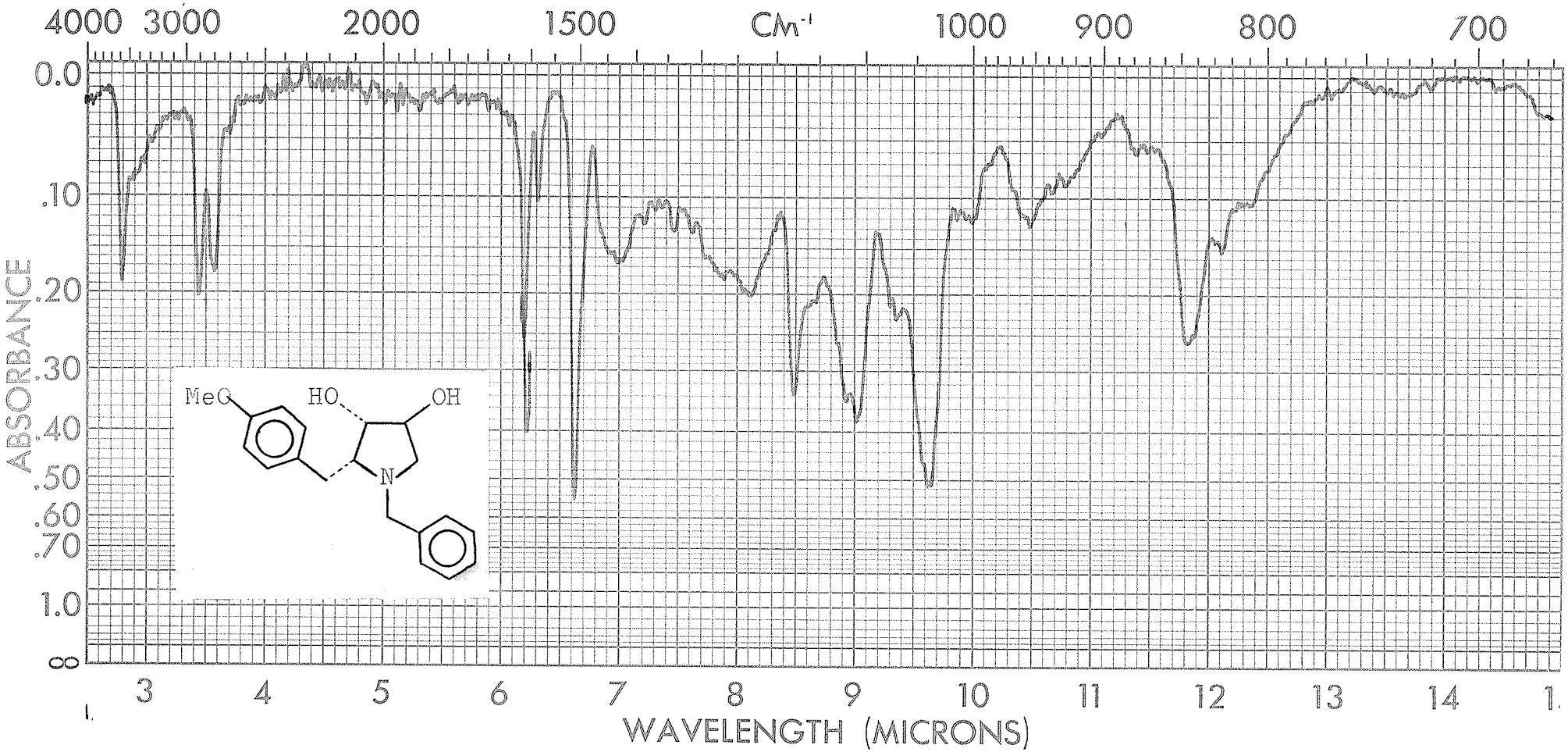
Comparison of spectra showed compound (64) to be identical with that obtained by D. Popien[35].



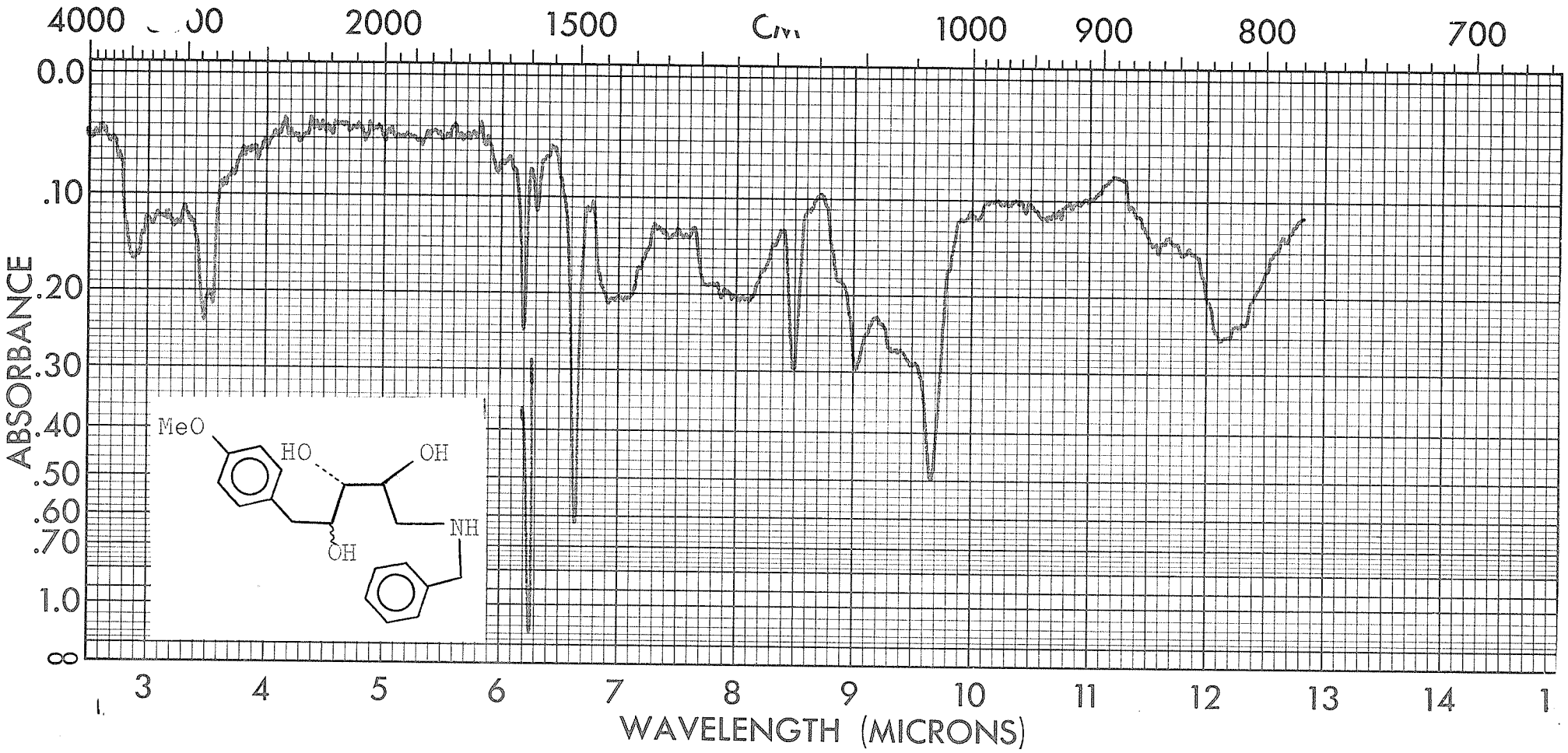
Infrared spectrum figure 1. Compound 18 (Nujol mull)



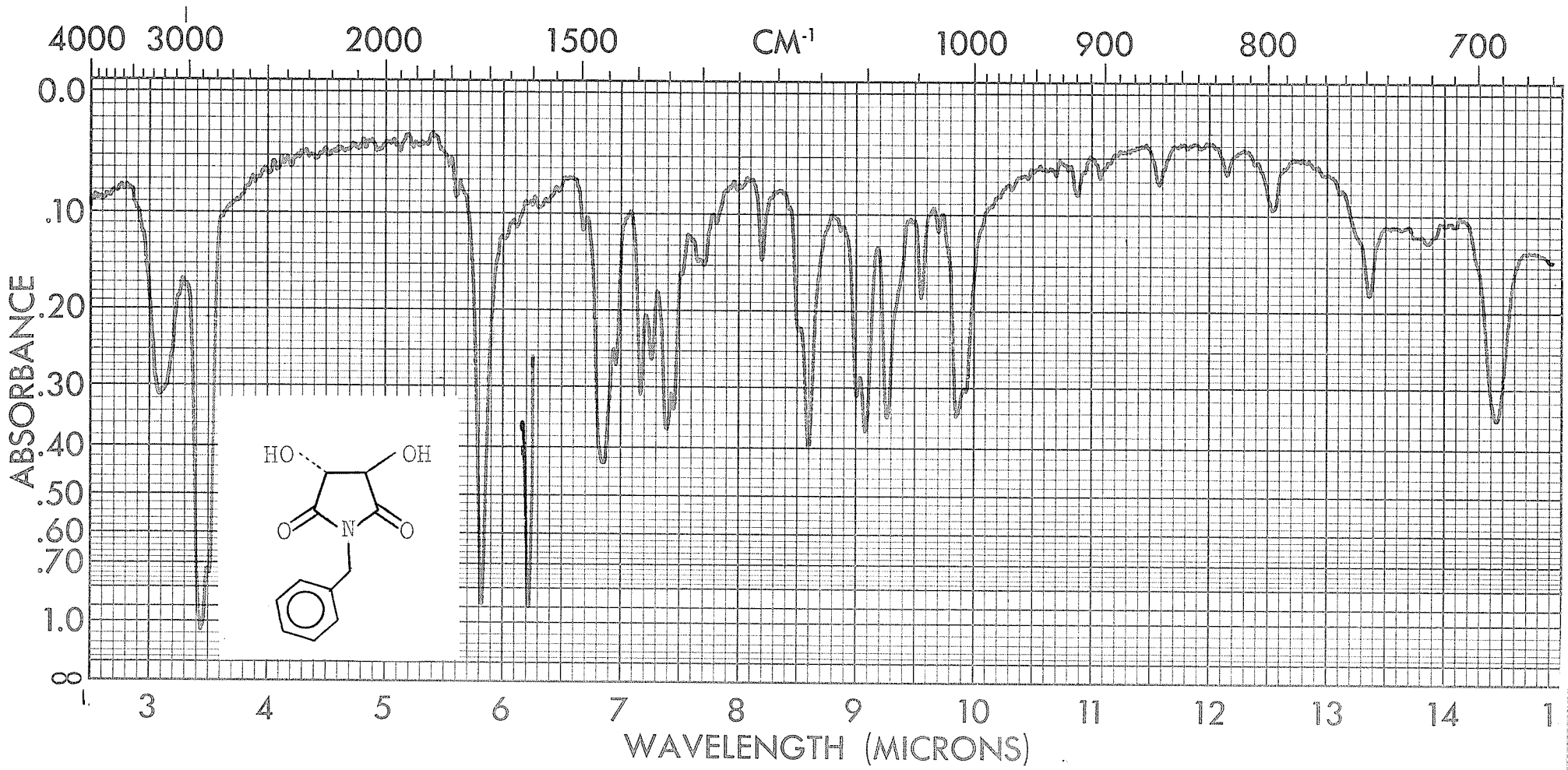
Infrared spectrum figure 2. Compound 21 (CH_2Cl_2)



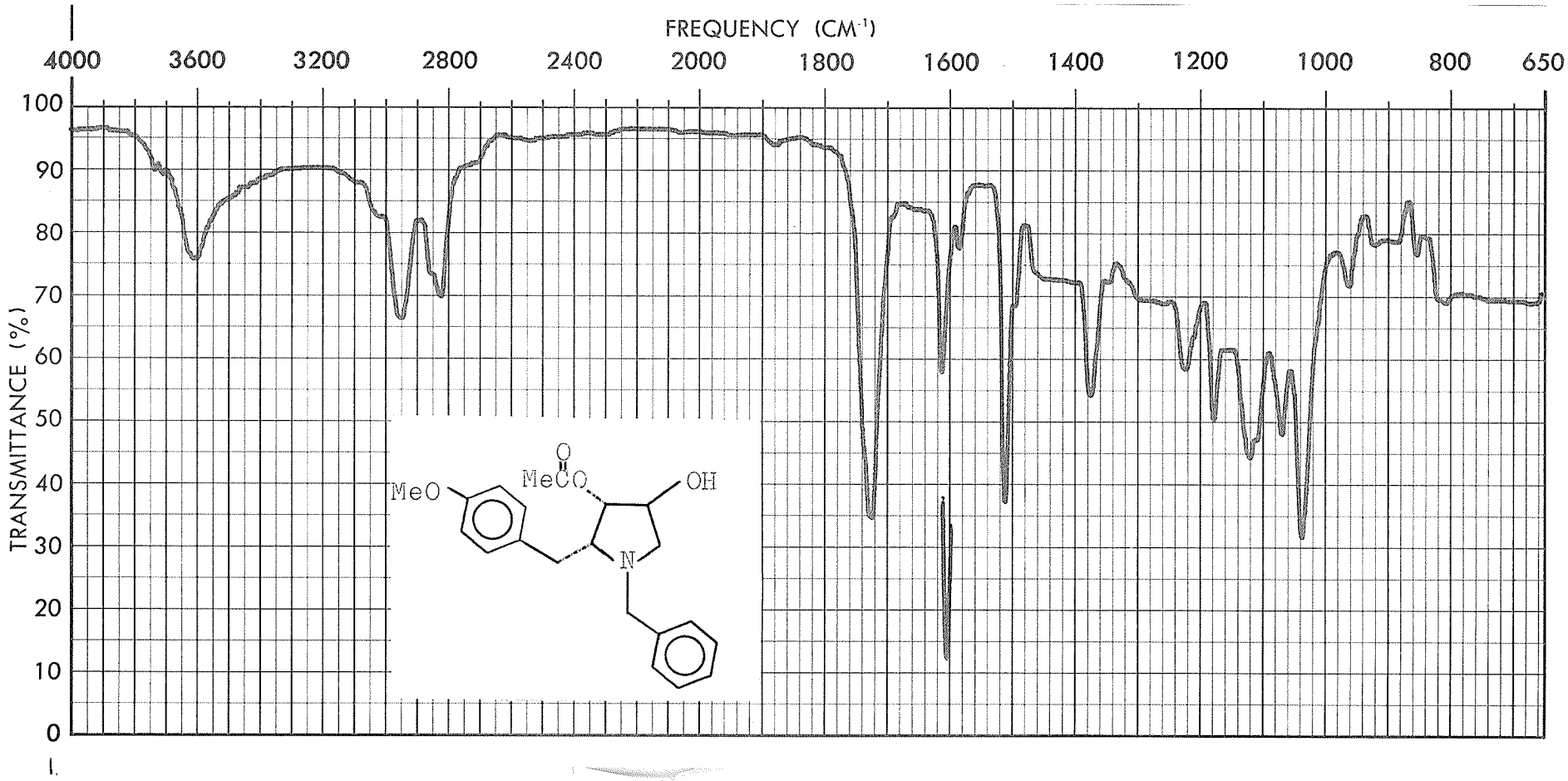
Infrared spectrum figure 3. Compound 20 (CH_2Cl_2)



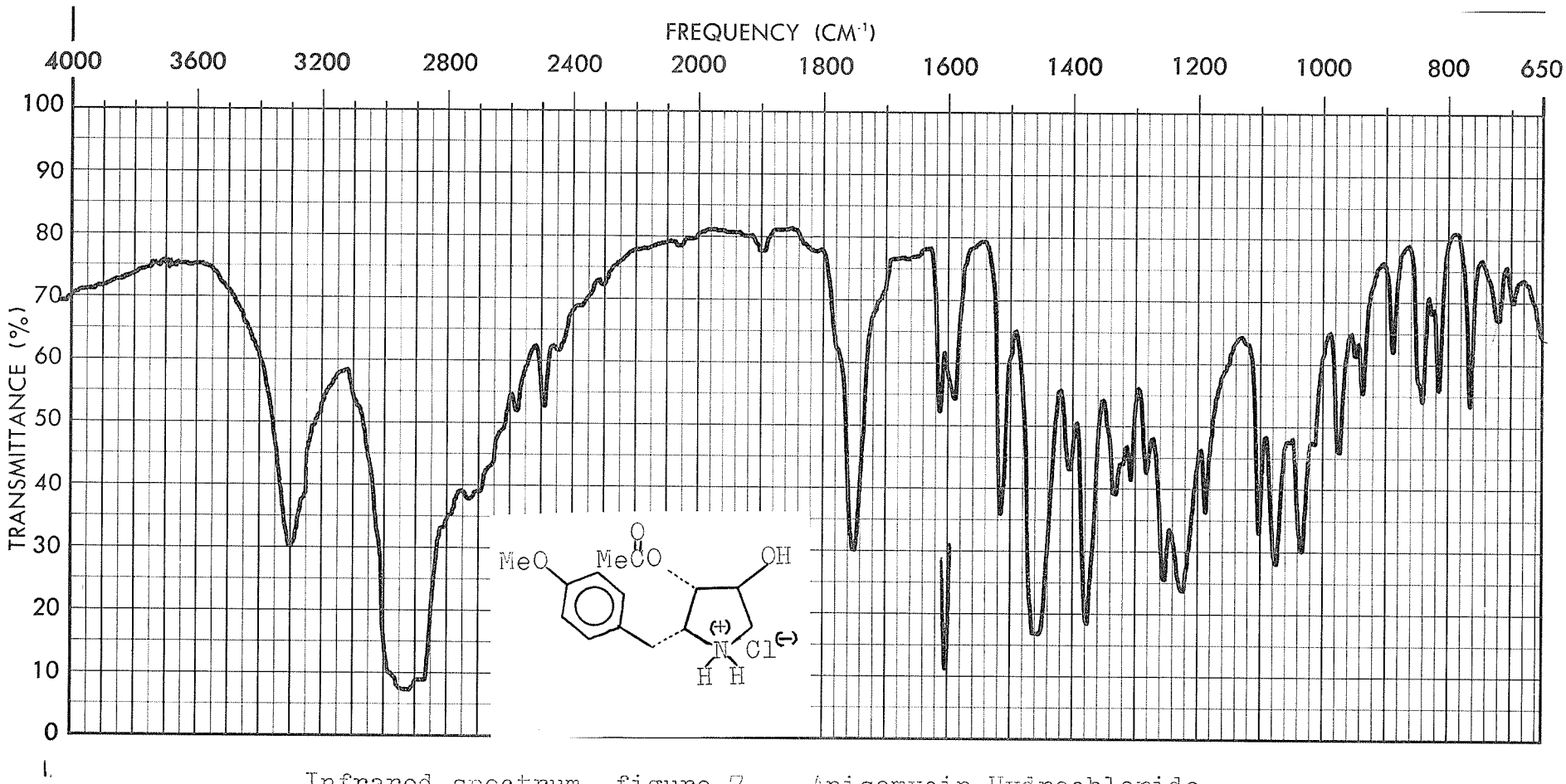
Infrared spectrum figure 4. Compound 22 (CH_2Cl_2)



Infrared spectrum figure 5. Compound 16 (Nujol mull)

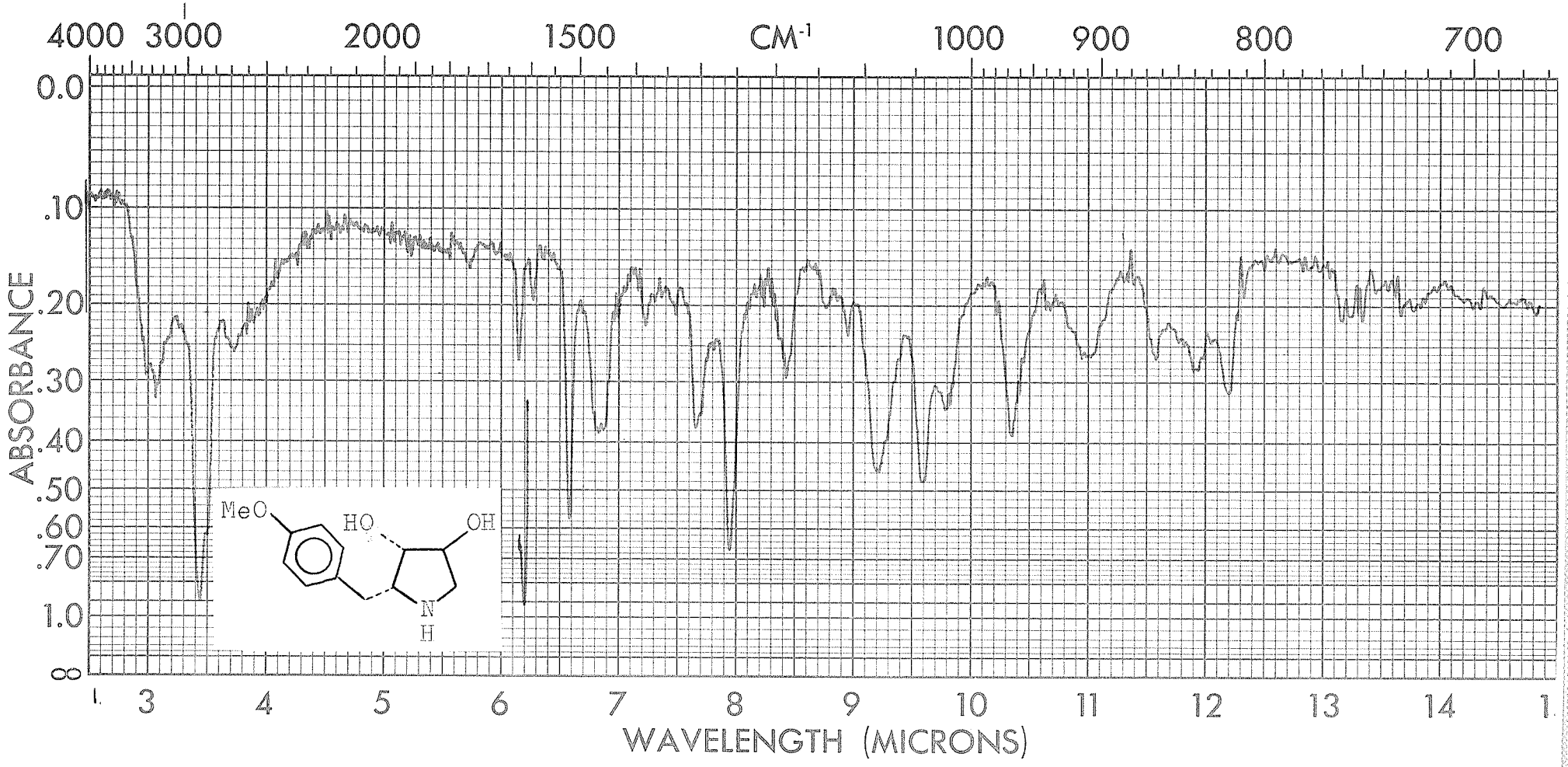


Infrared spectrum figure 6. Compound 23 (CH_2Cl_2)

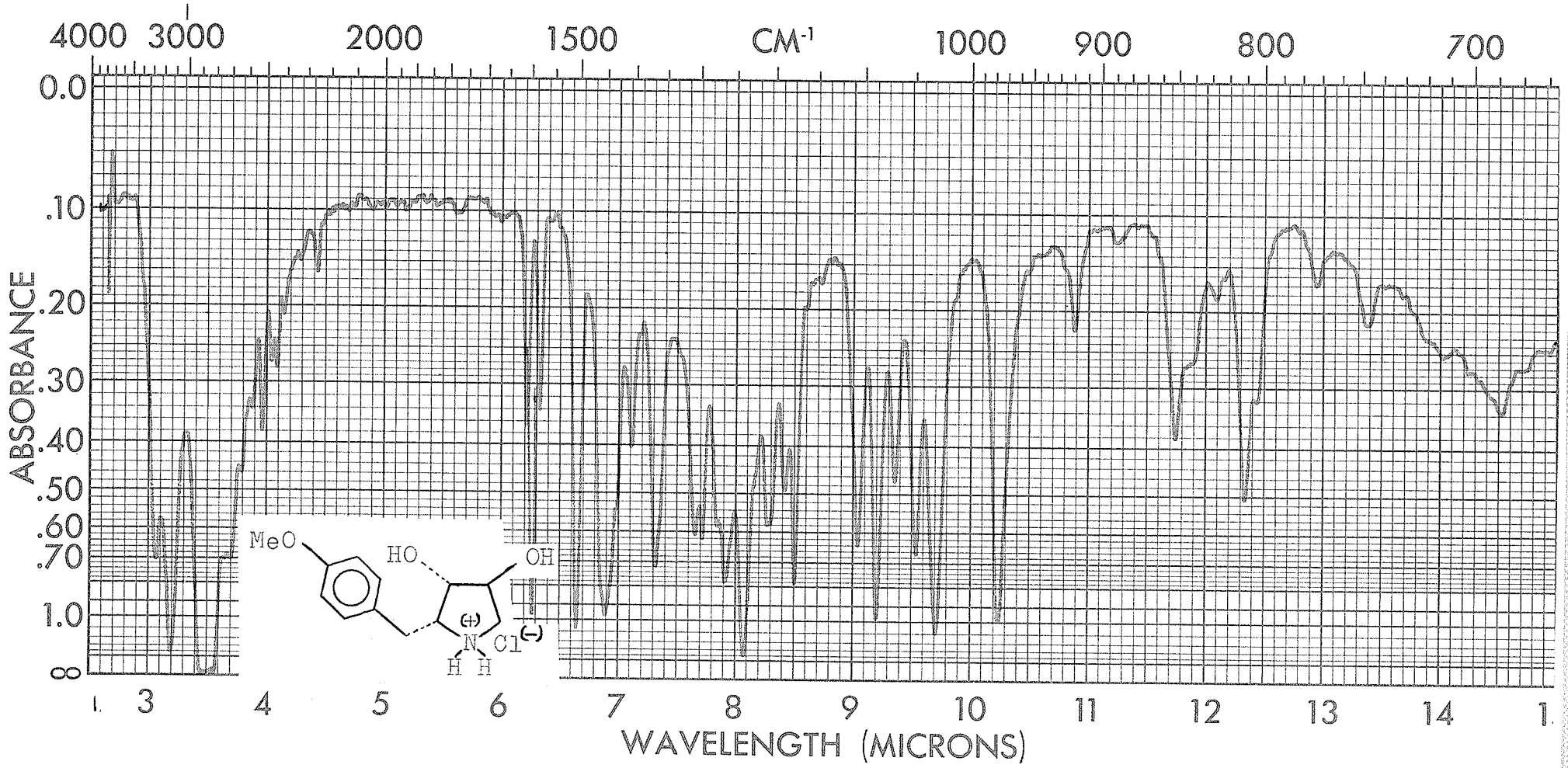


Infrared spectrum figure 7. Anisomycin Hydrochloride

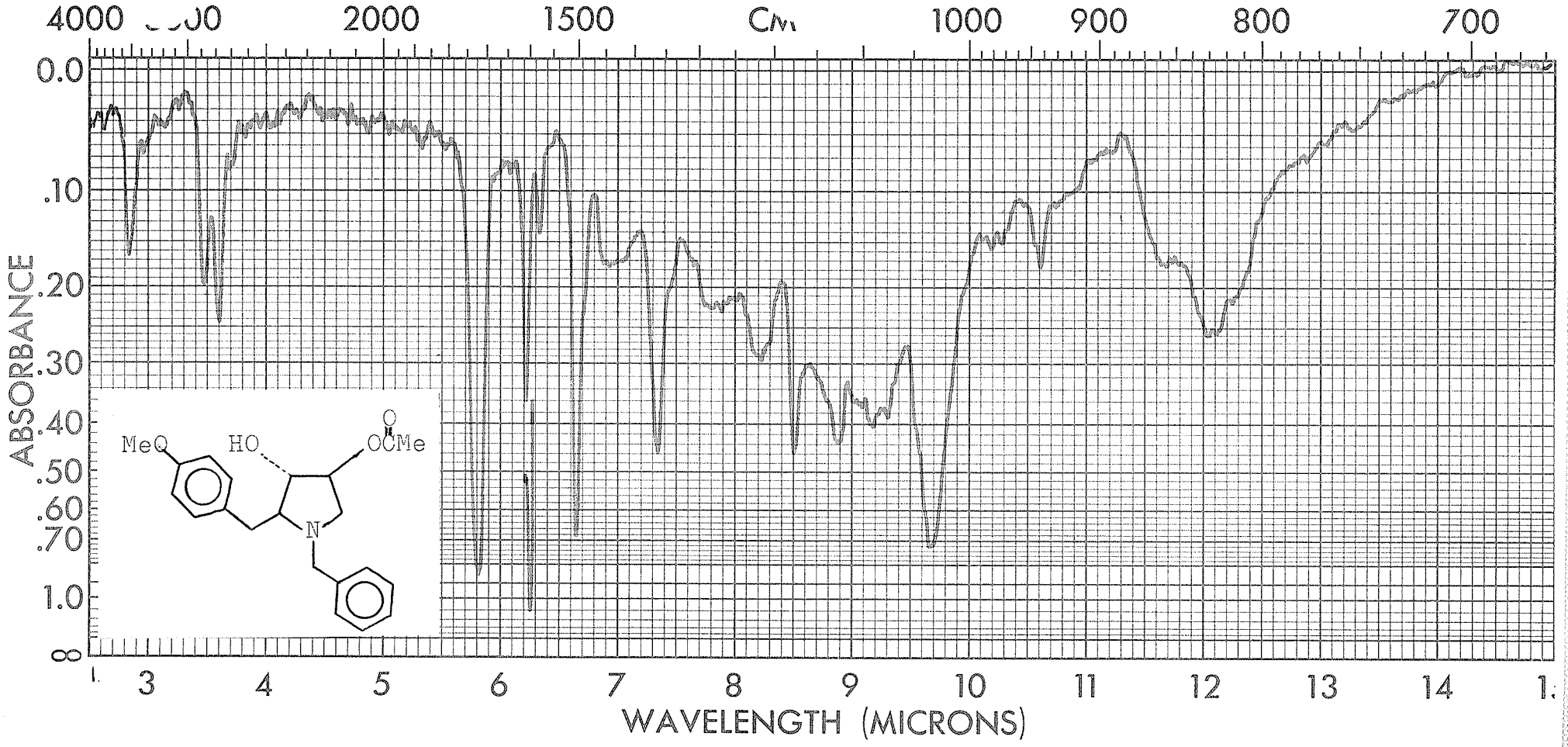
(Nujol mull)



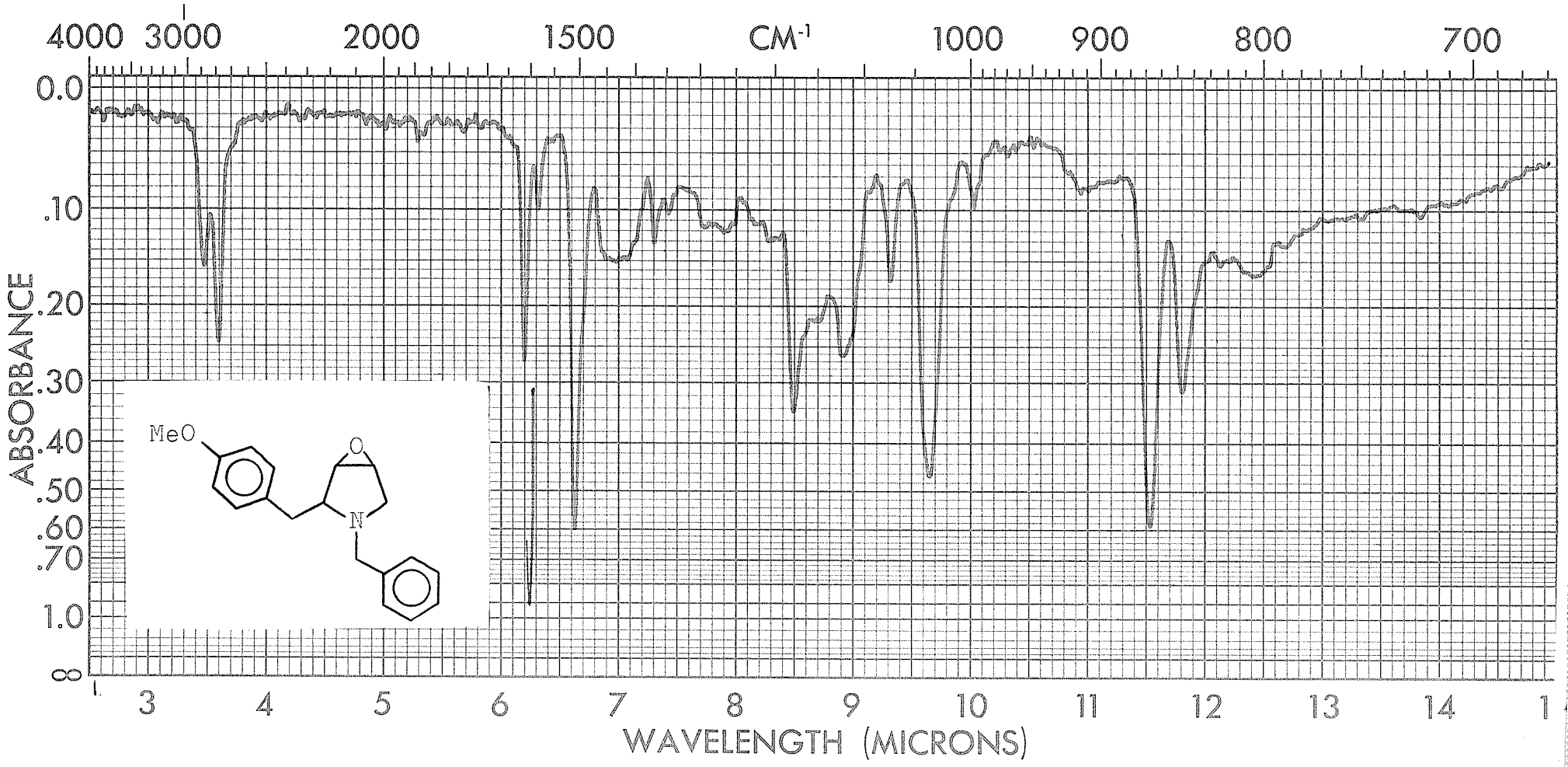
Infrared spectrum figure 8. Compound 4. (Nujol mull)



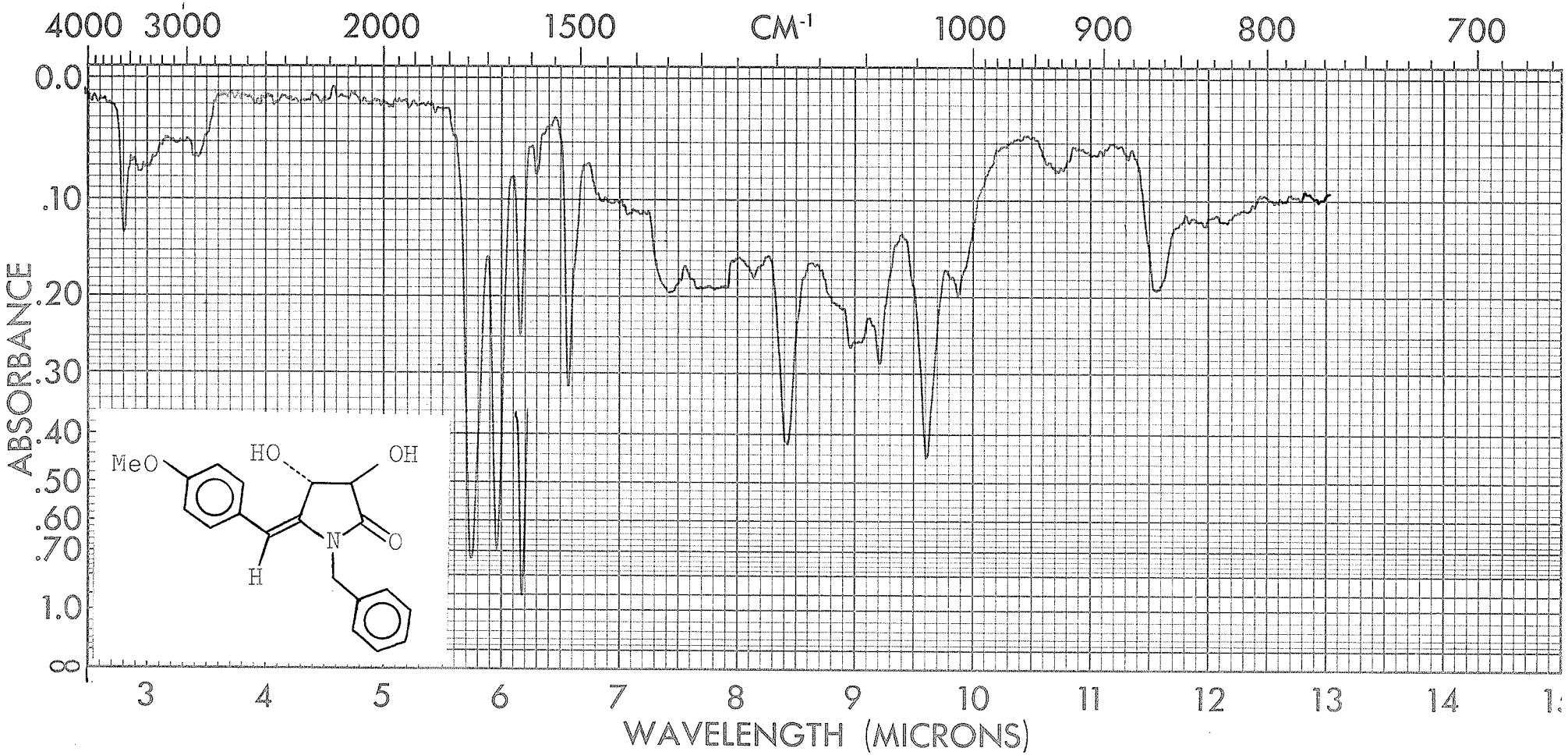
Infrared spectrum figure 9. Compound 4.(Hydrochloride Salt) (Nujol mull)



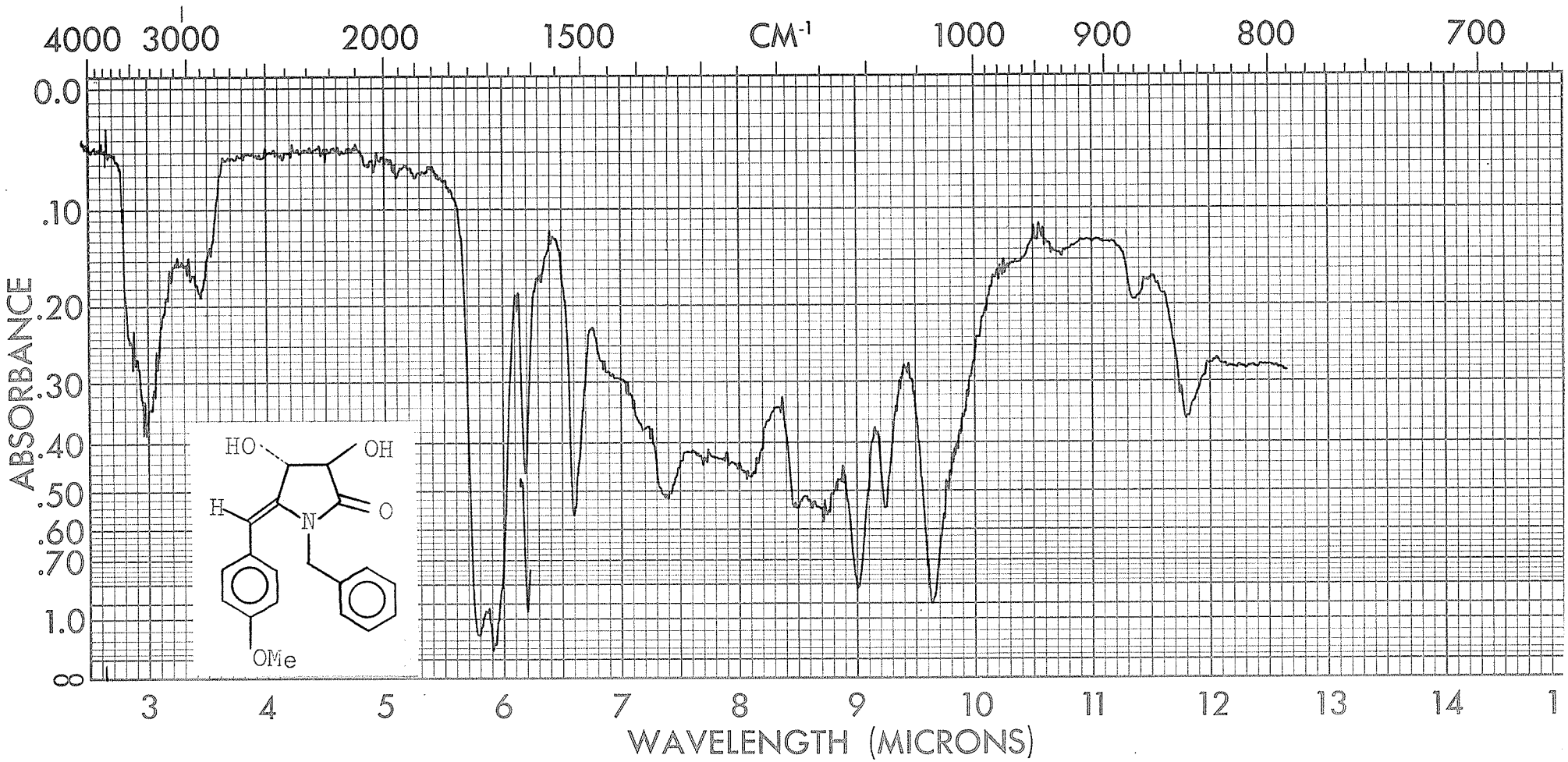
Infrared spectrum figure 10. Compound 24 (CH_2Cl_2)



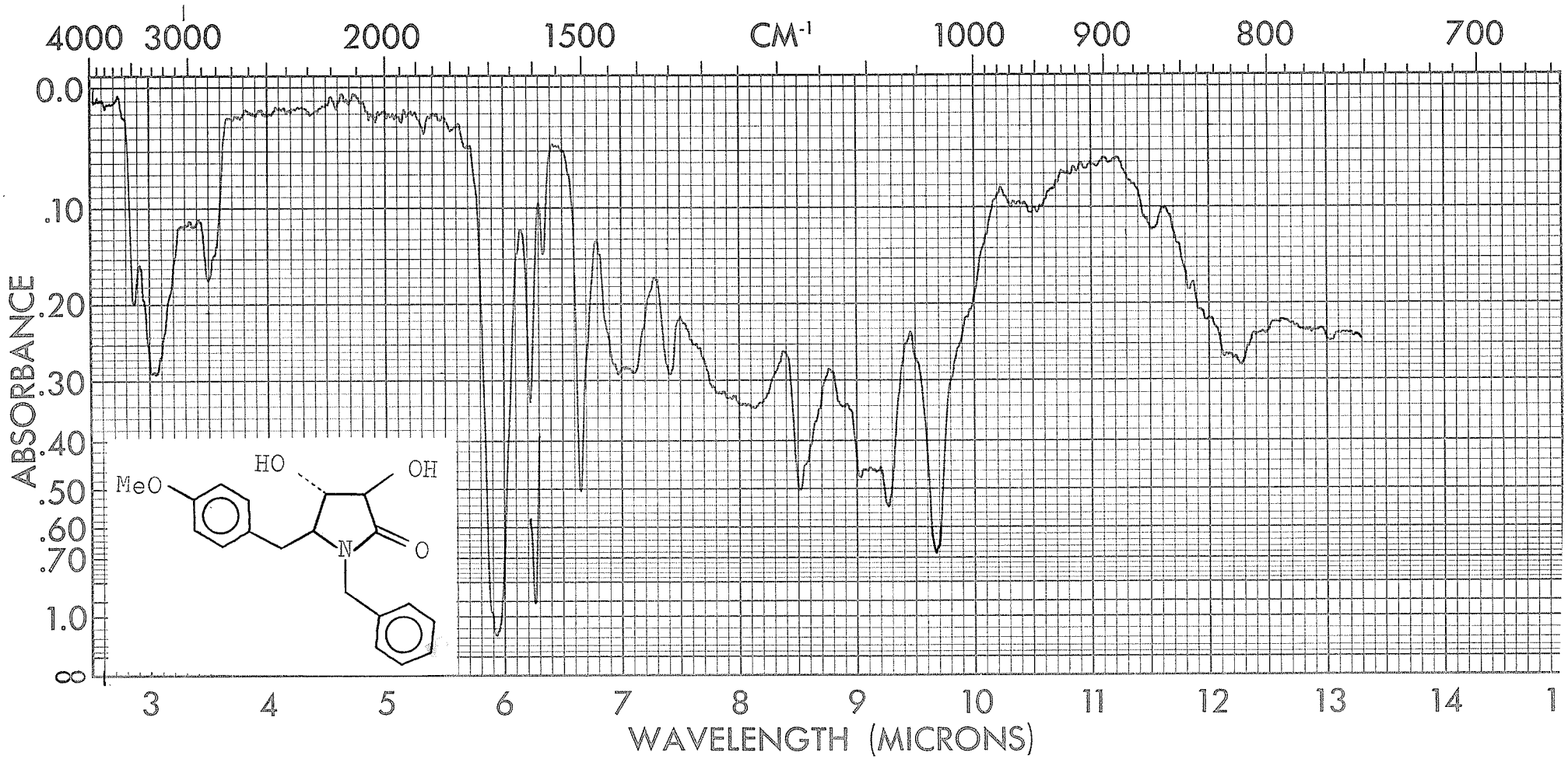
Infrared spectrum figure 11. Compound 25 (CH_2Cl_2)



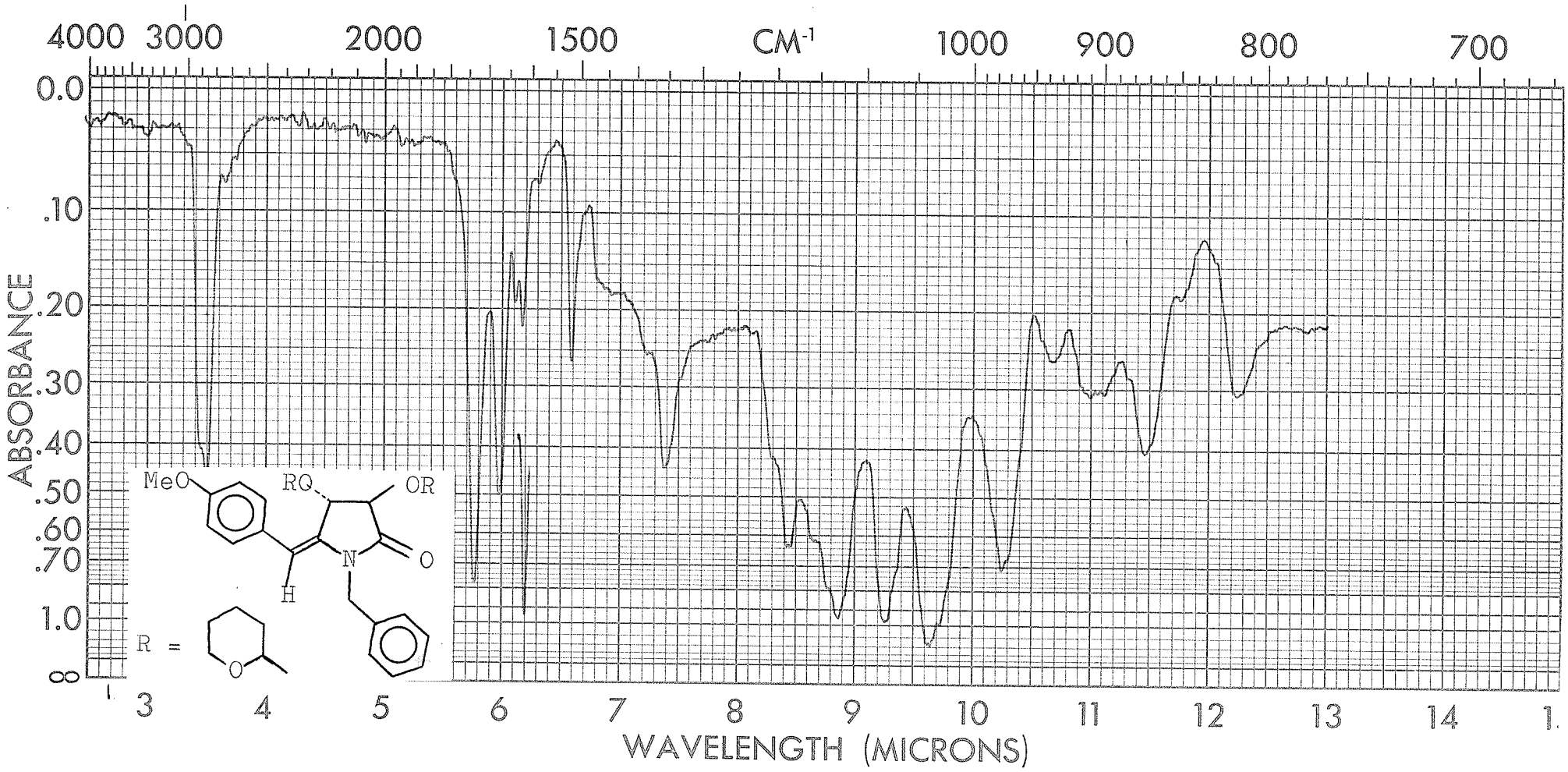
Infrared spectrum figure 12. Compound 29 (CH_2Cl_2)



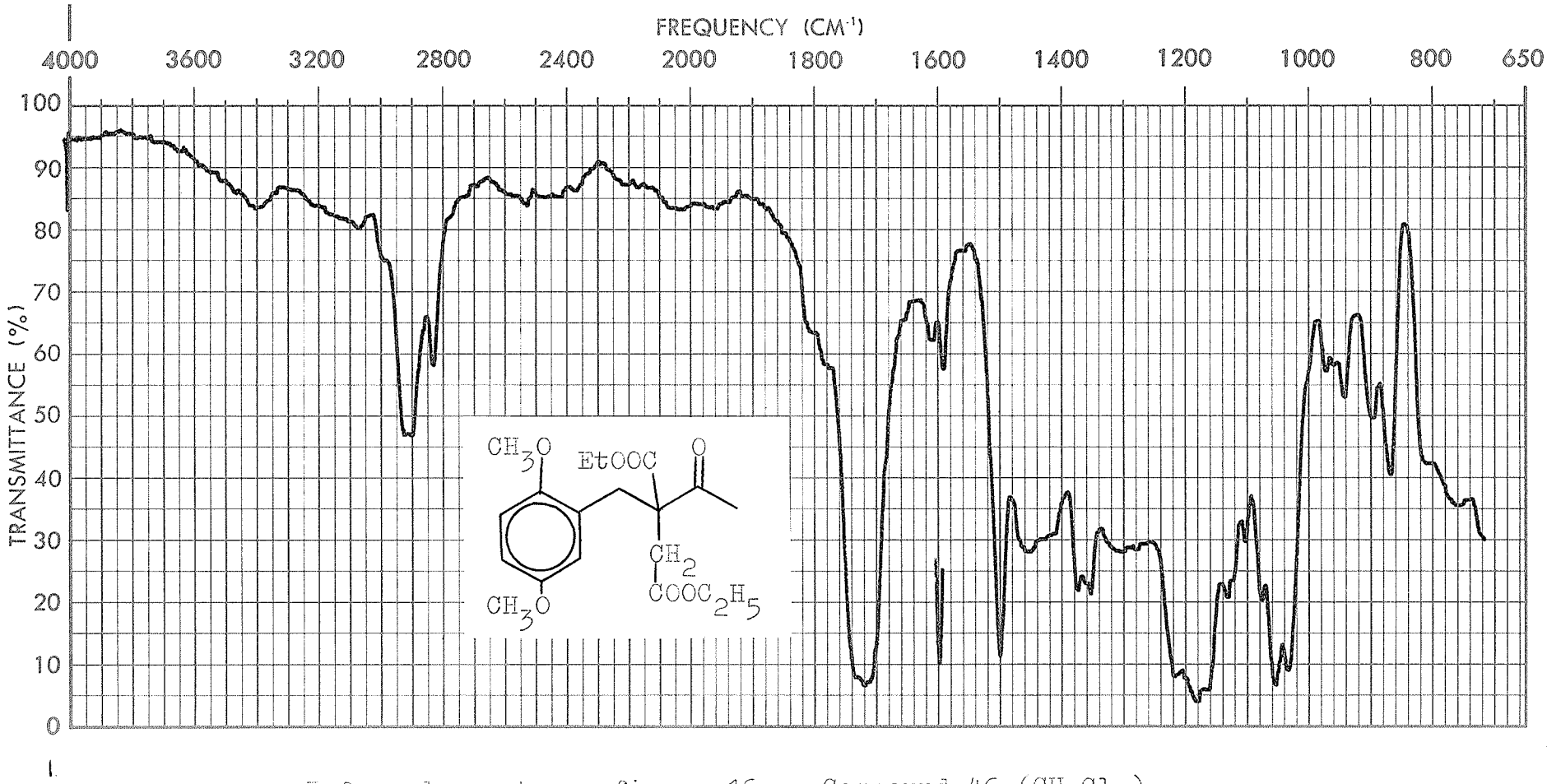
Infrared spectrum figure 13. Compound 30 (CH_2Cl_2)



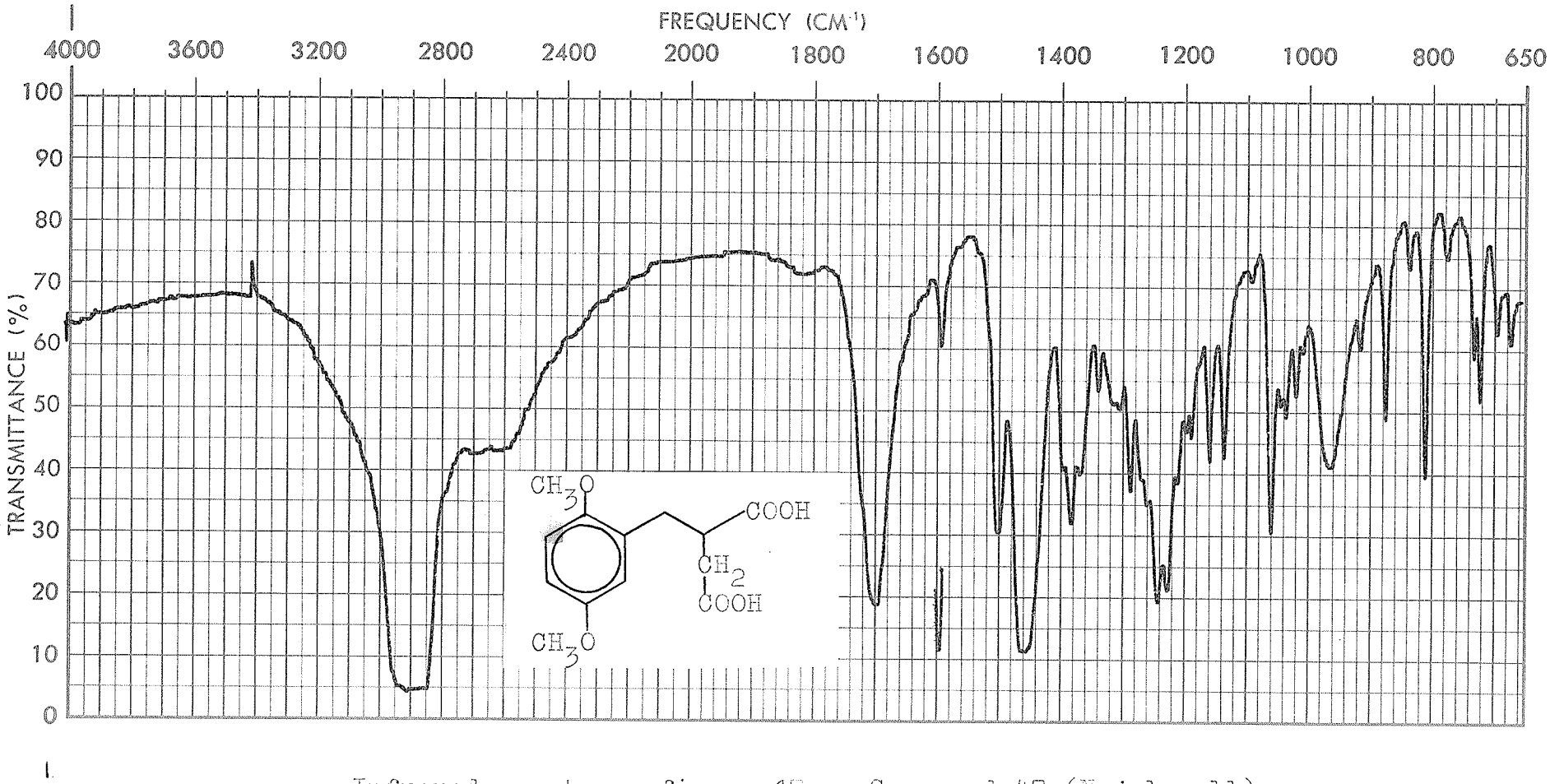
Infrared spectrum figure 14. Compound 31 (CH_2Cl_2)



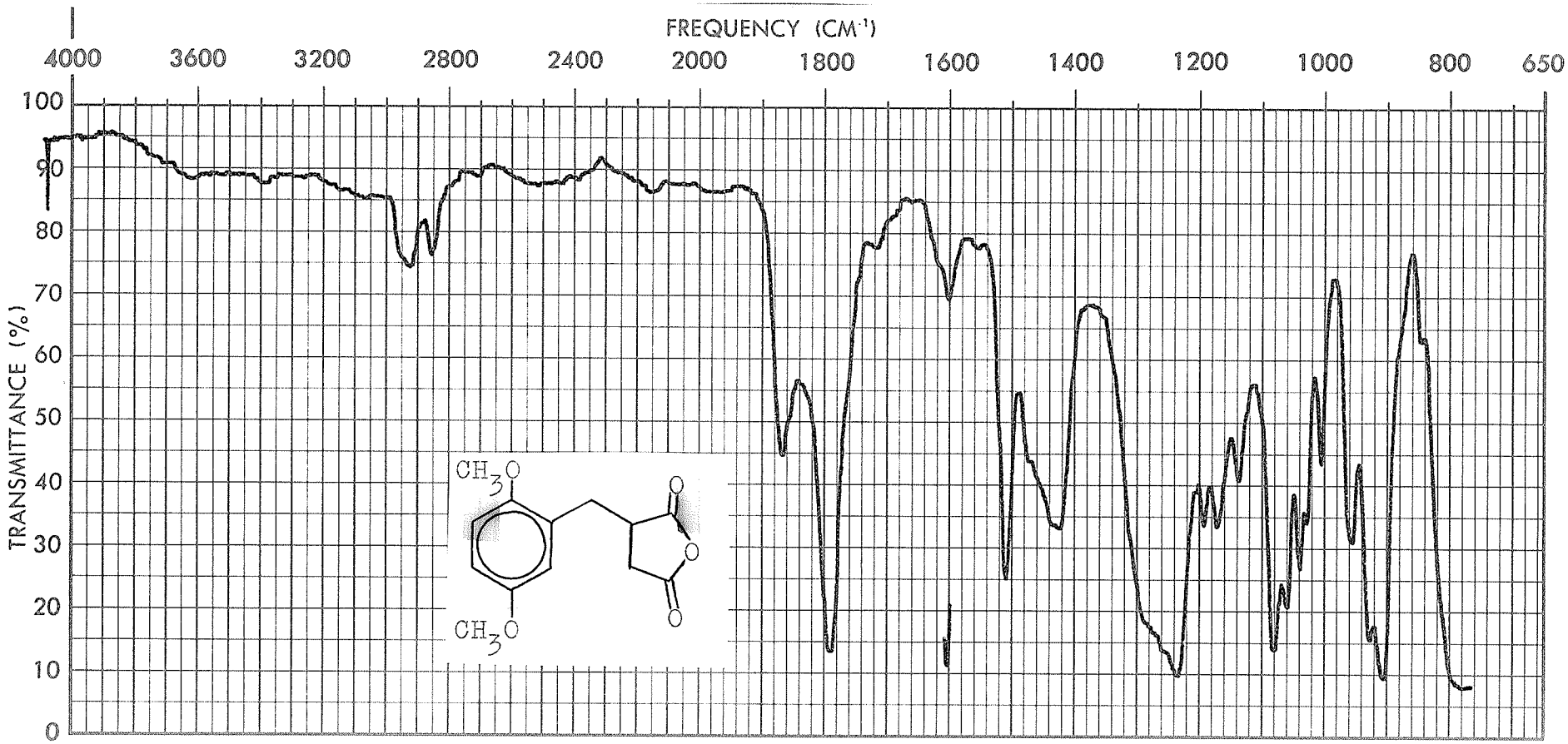
Infrared spectrum figure 15. Pyranyl ether of (29).(CH_2Cl_2)



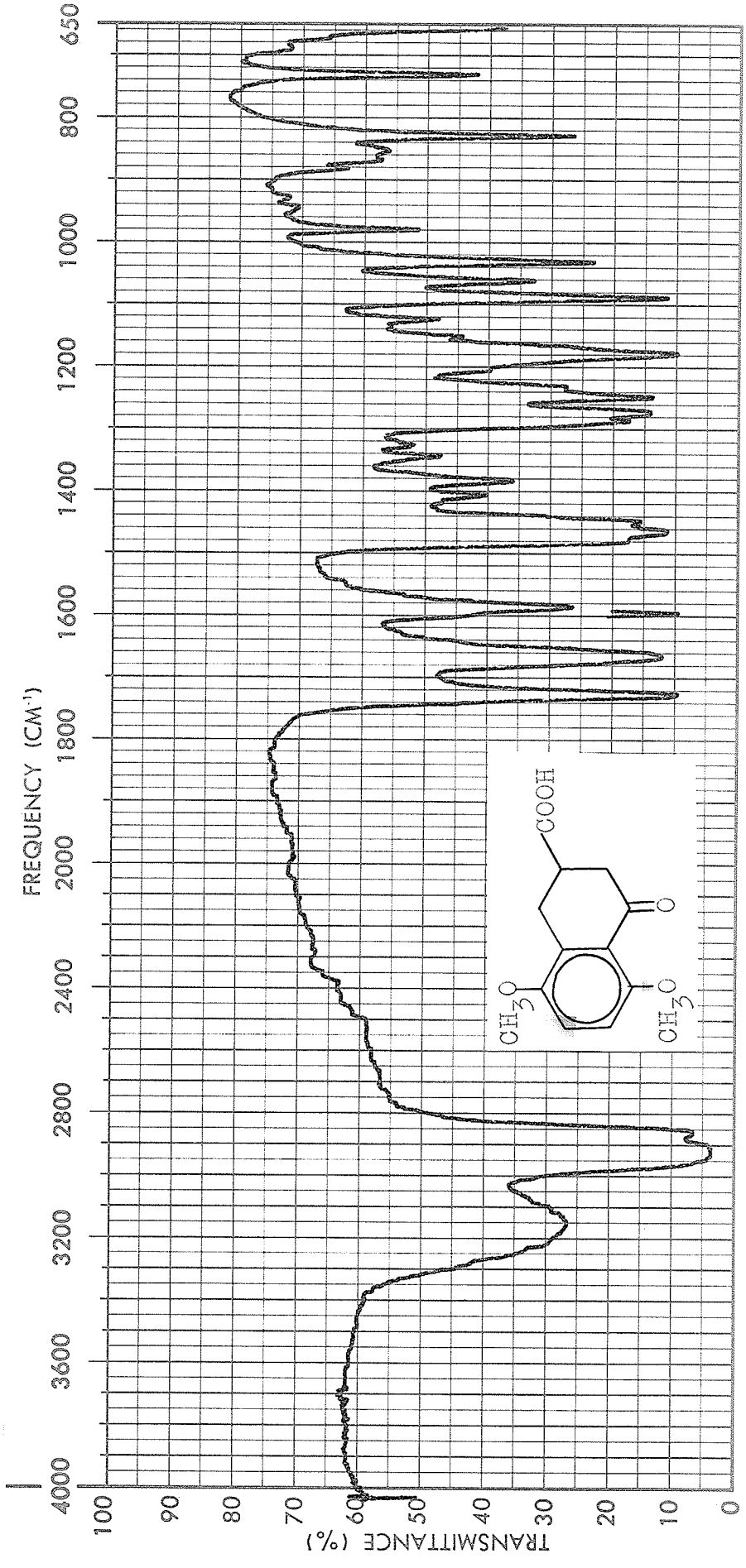
Infrared spectrum figure 16. Compound 46 (CH_2Cl_2)



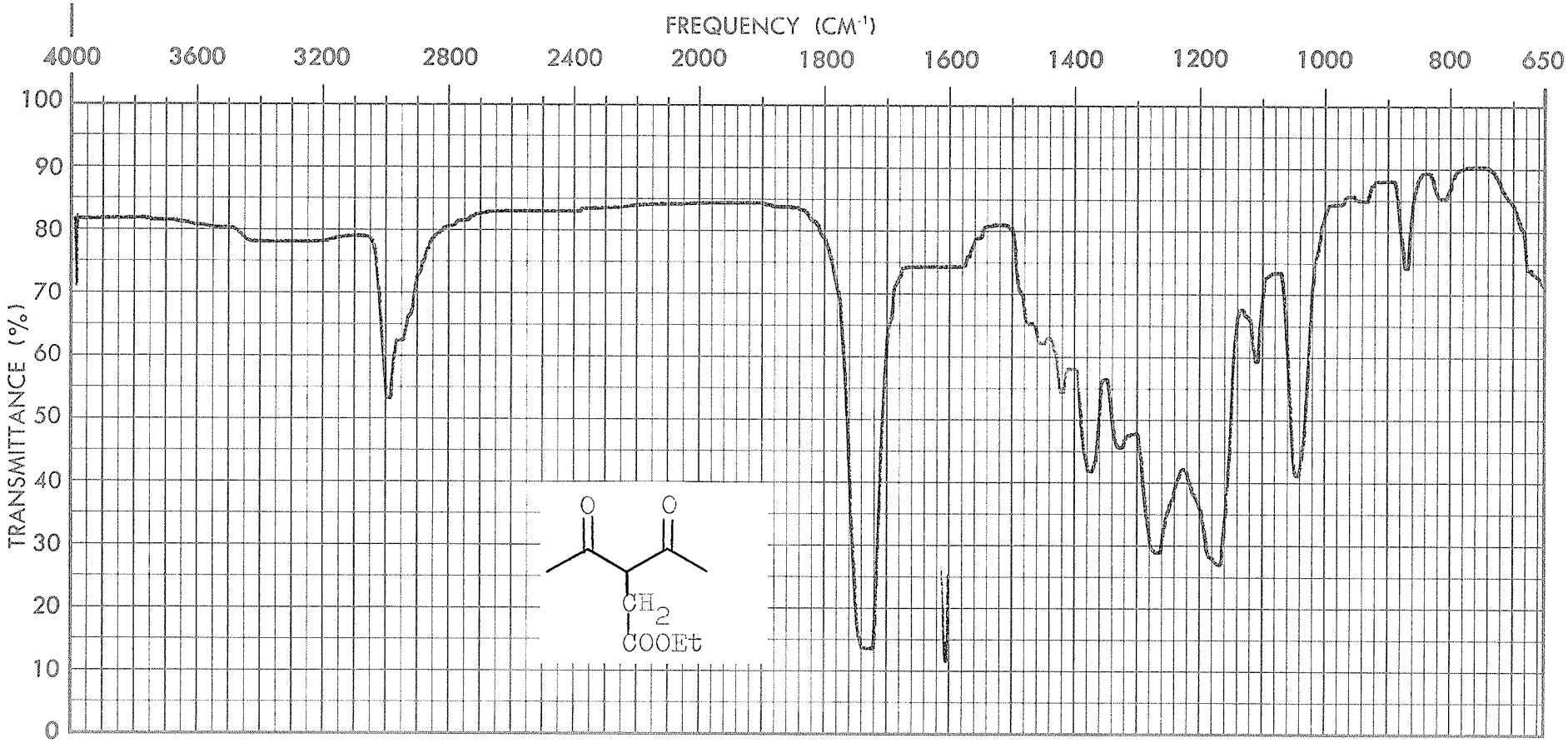
Infrared spectrum figure 17. Compound 47 (Nujol mull)



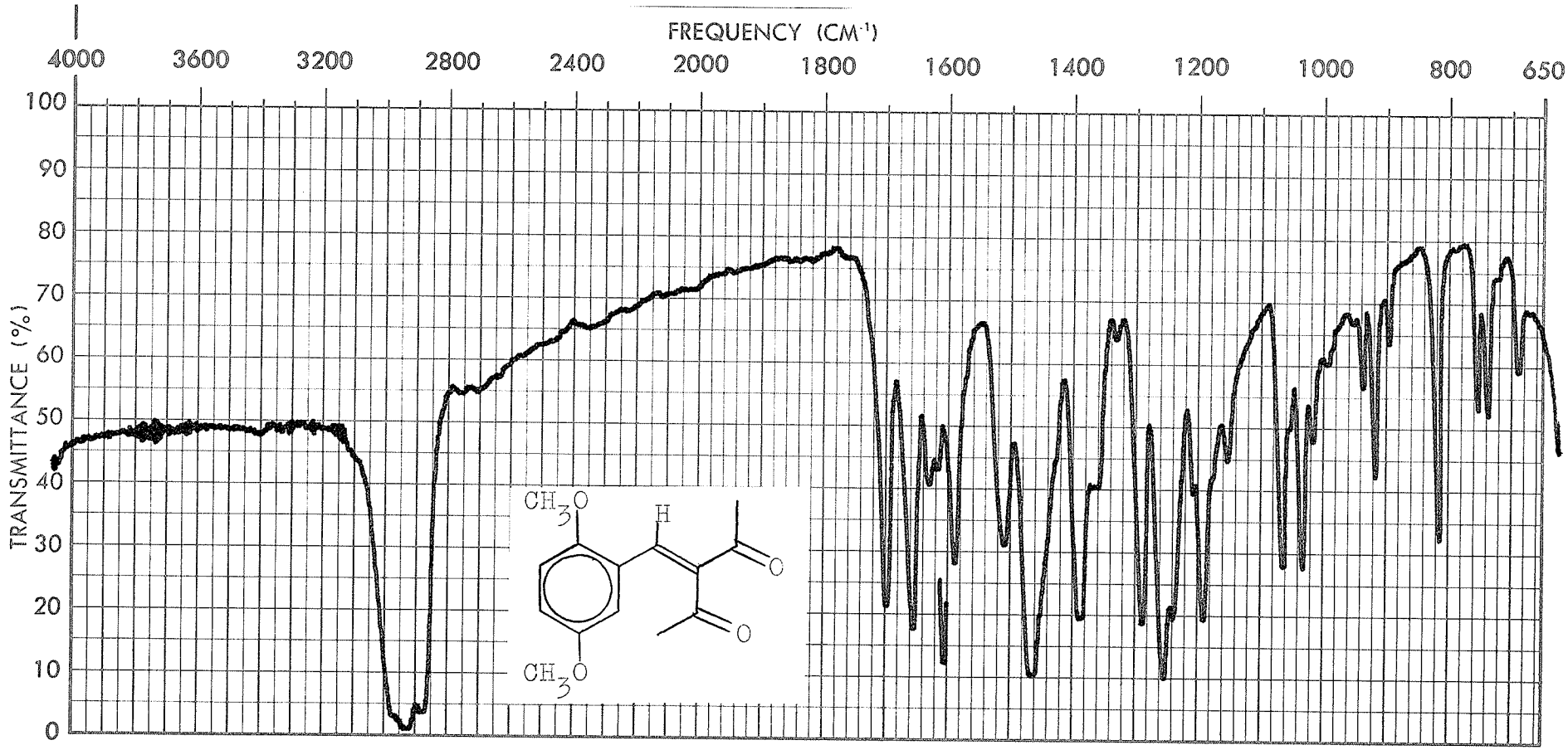
Infrared spectrum figure 18. Compound 48 (CH_2Cl_2)



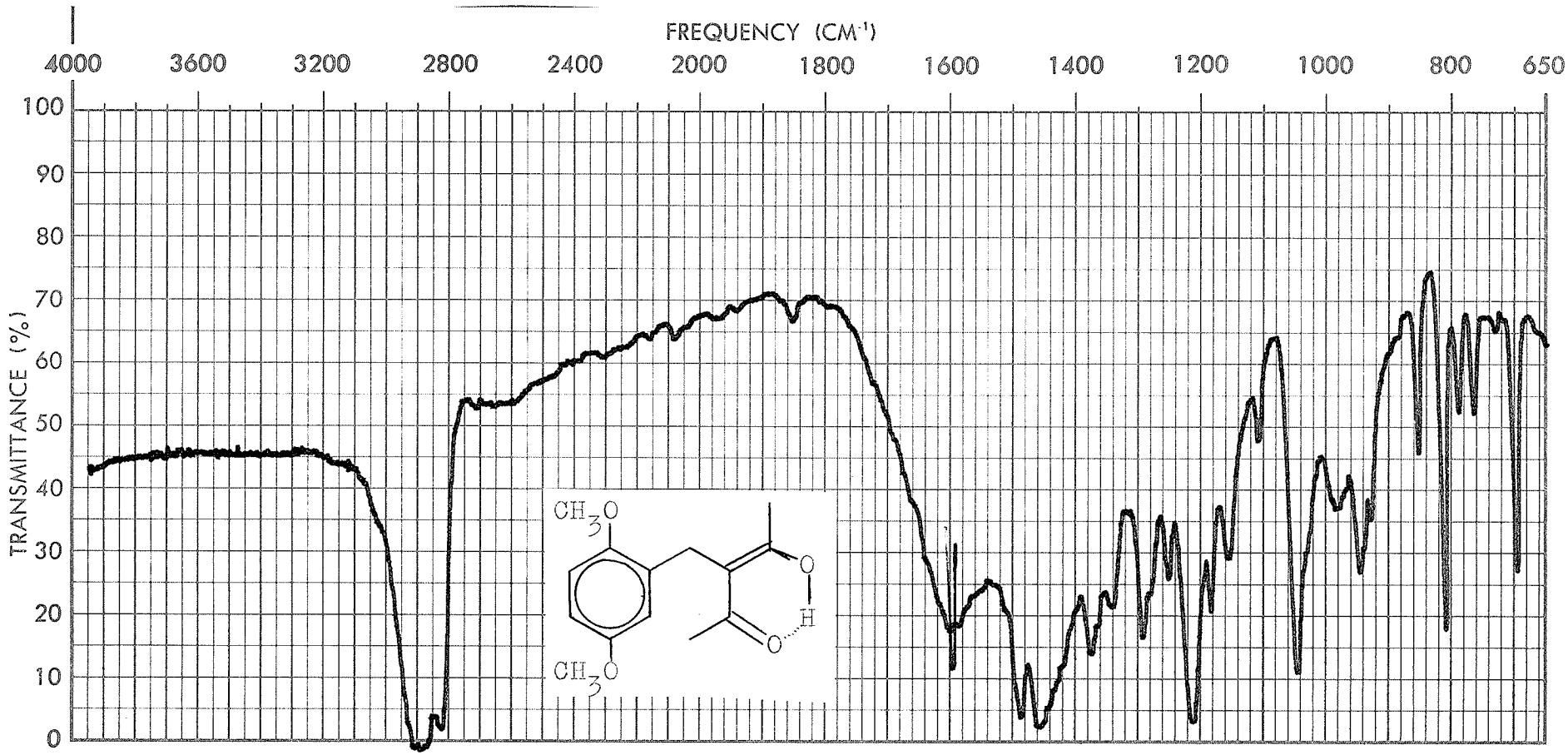
Infrared spectrum figure 19. Compound 49 (Nujol mull)



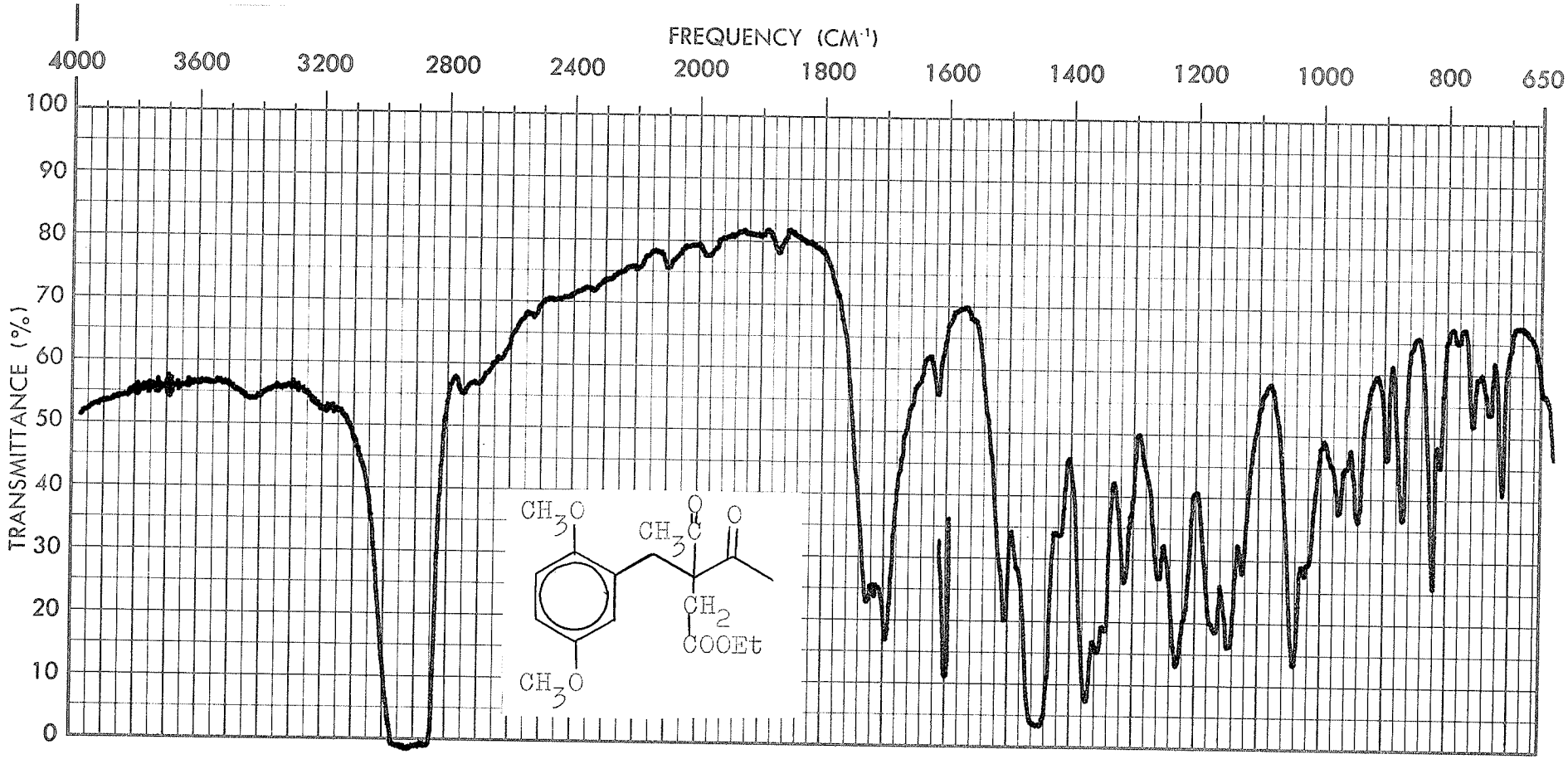
Infrared spectrum figure 20. Compound 50 (liquid film)



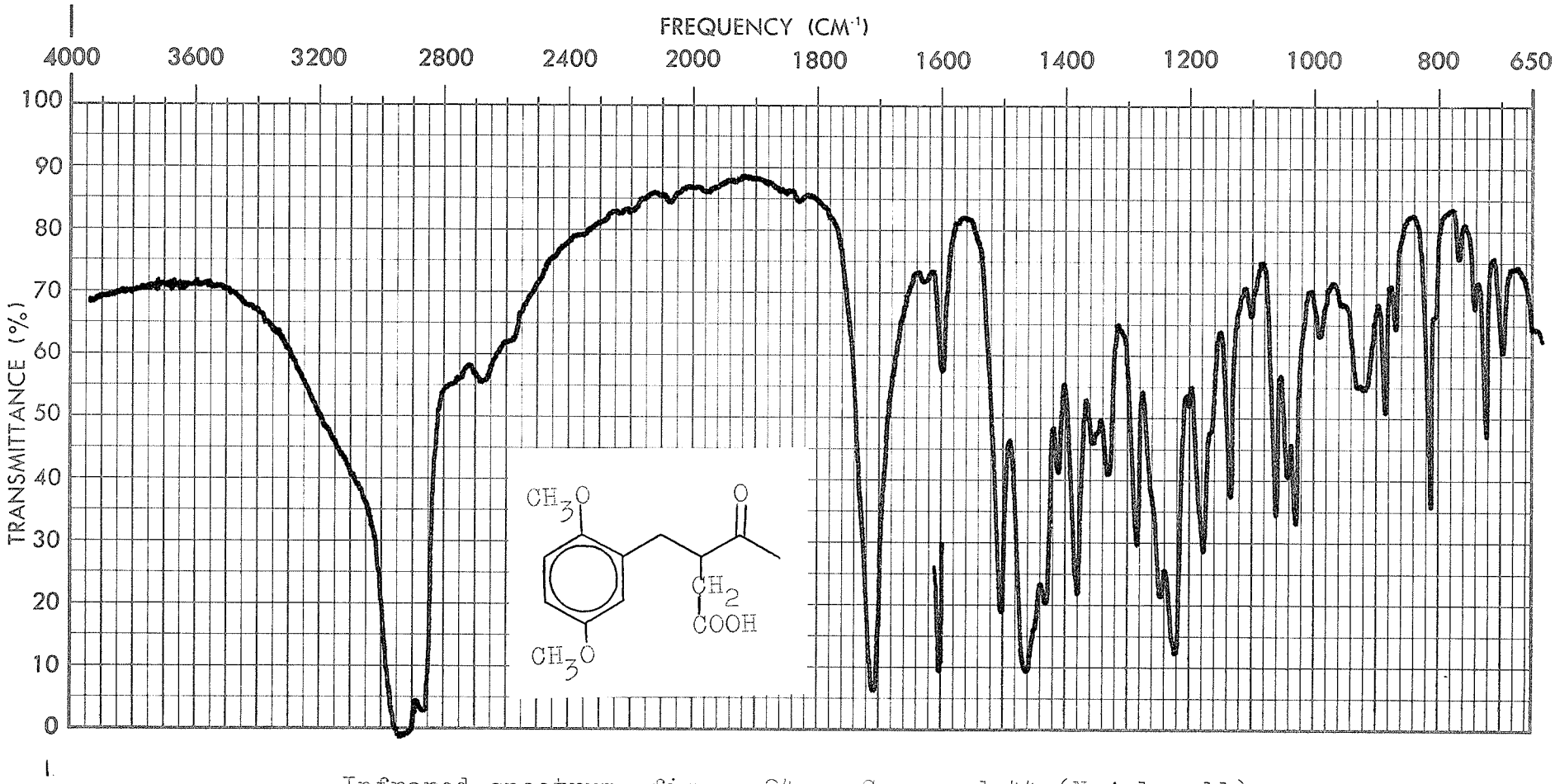
Infrared spectrum figure 21. Compound 52 (Nujol mull)



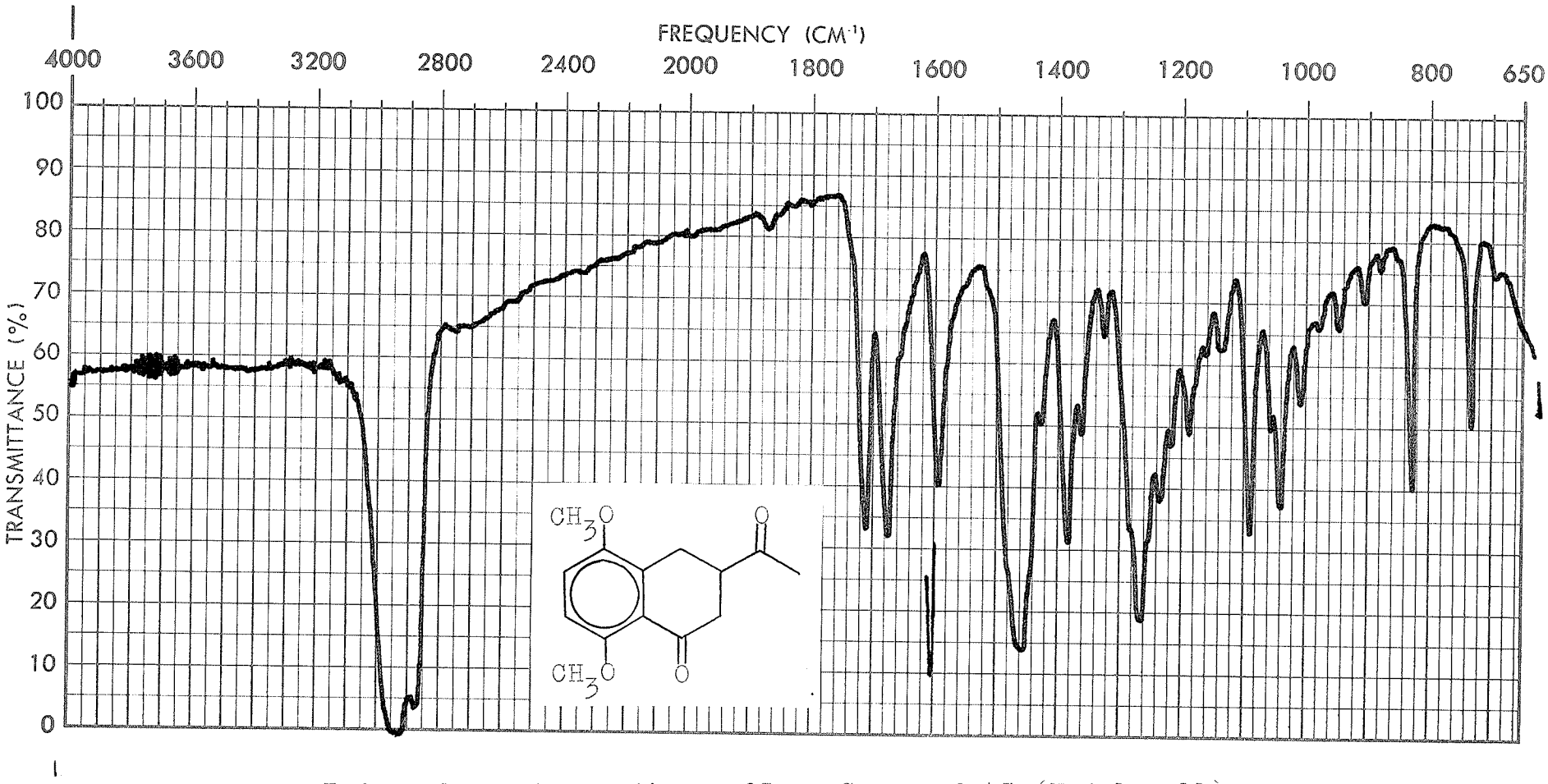
Infrared spectrum figure 22. Compound 53 (Nujol mull)



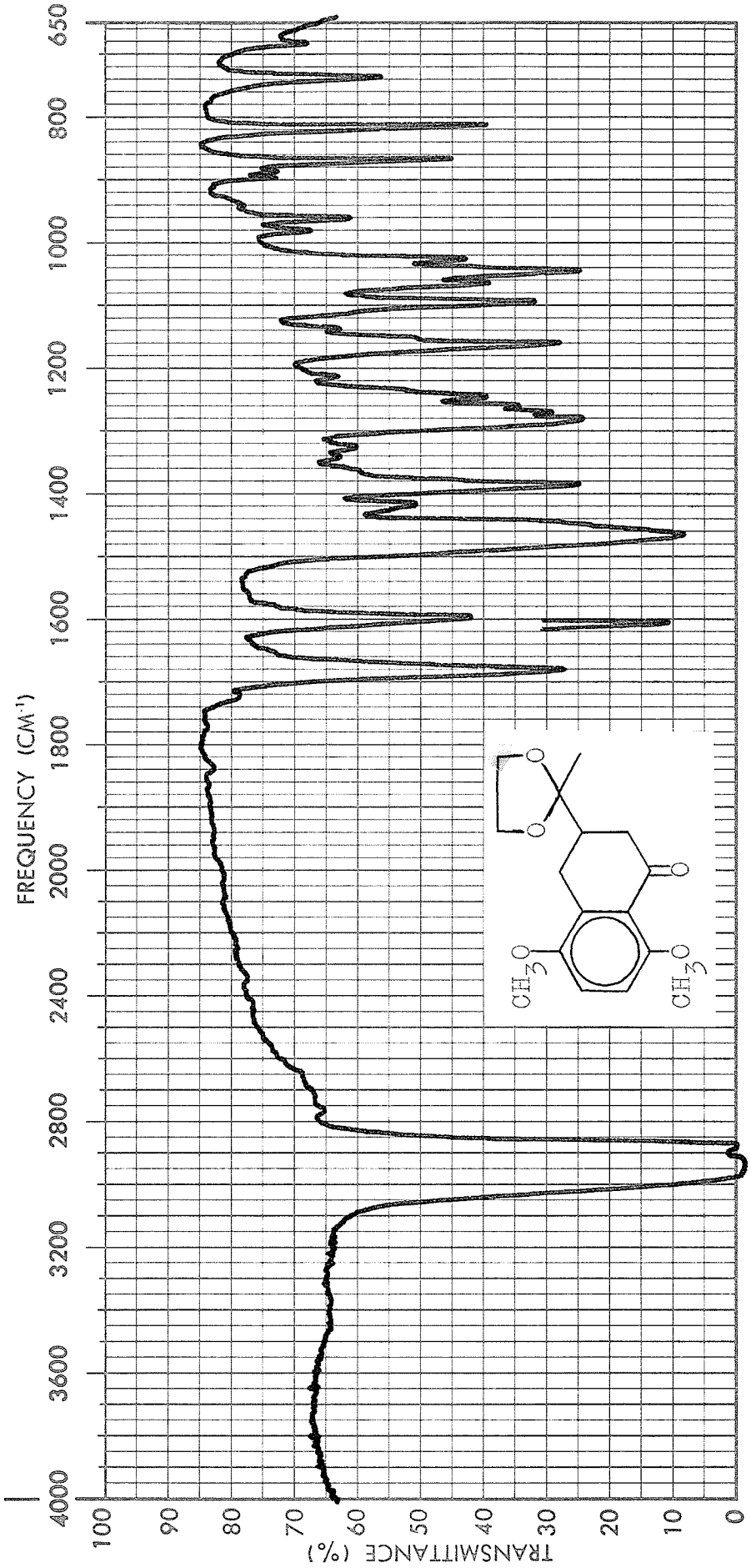
Infrared spectrum figure 23. Compound 51 (Nujol mull)



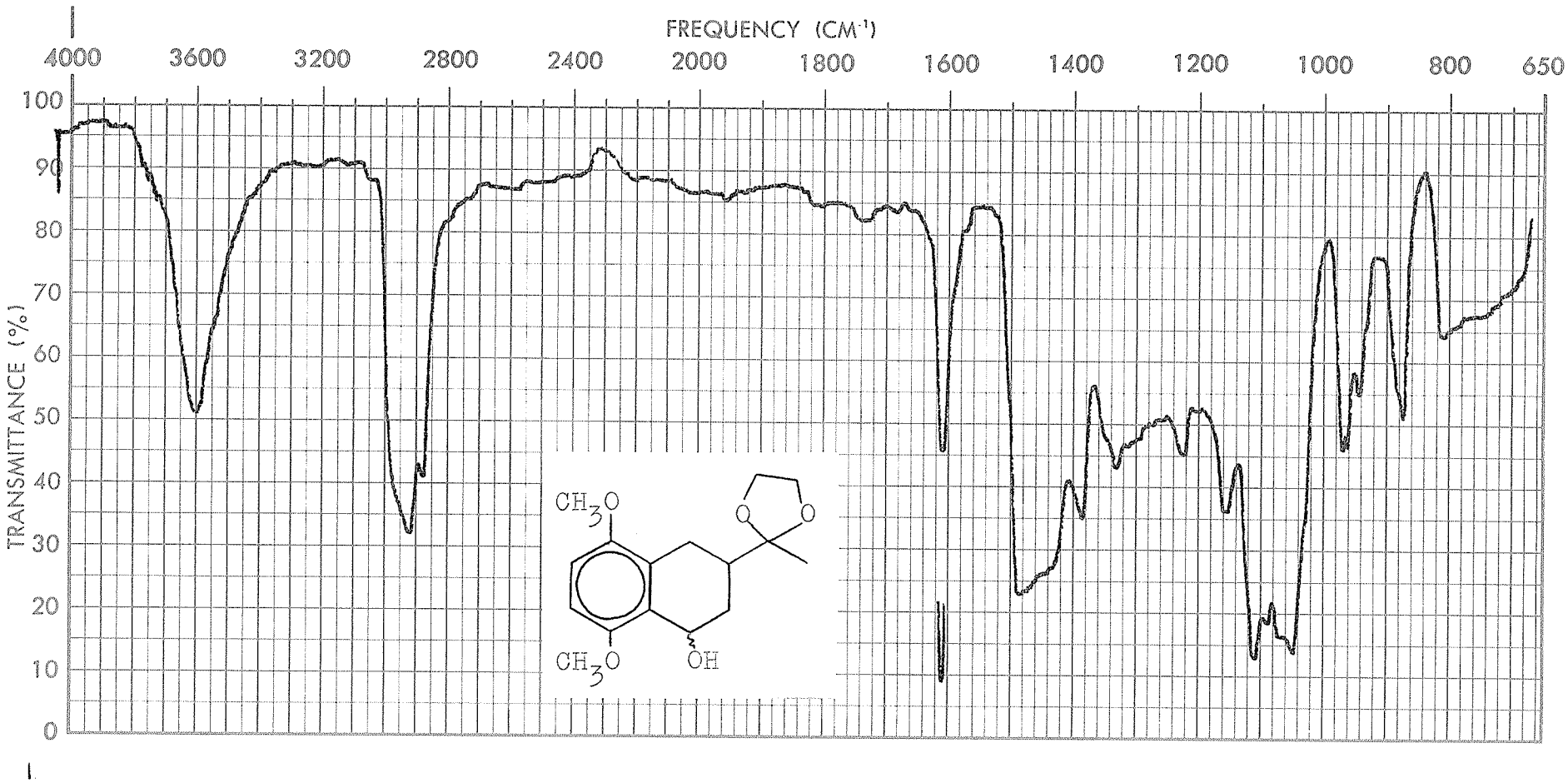
Infrared spectrum figure 24. Compound 44 (Nujol mull)



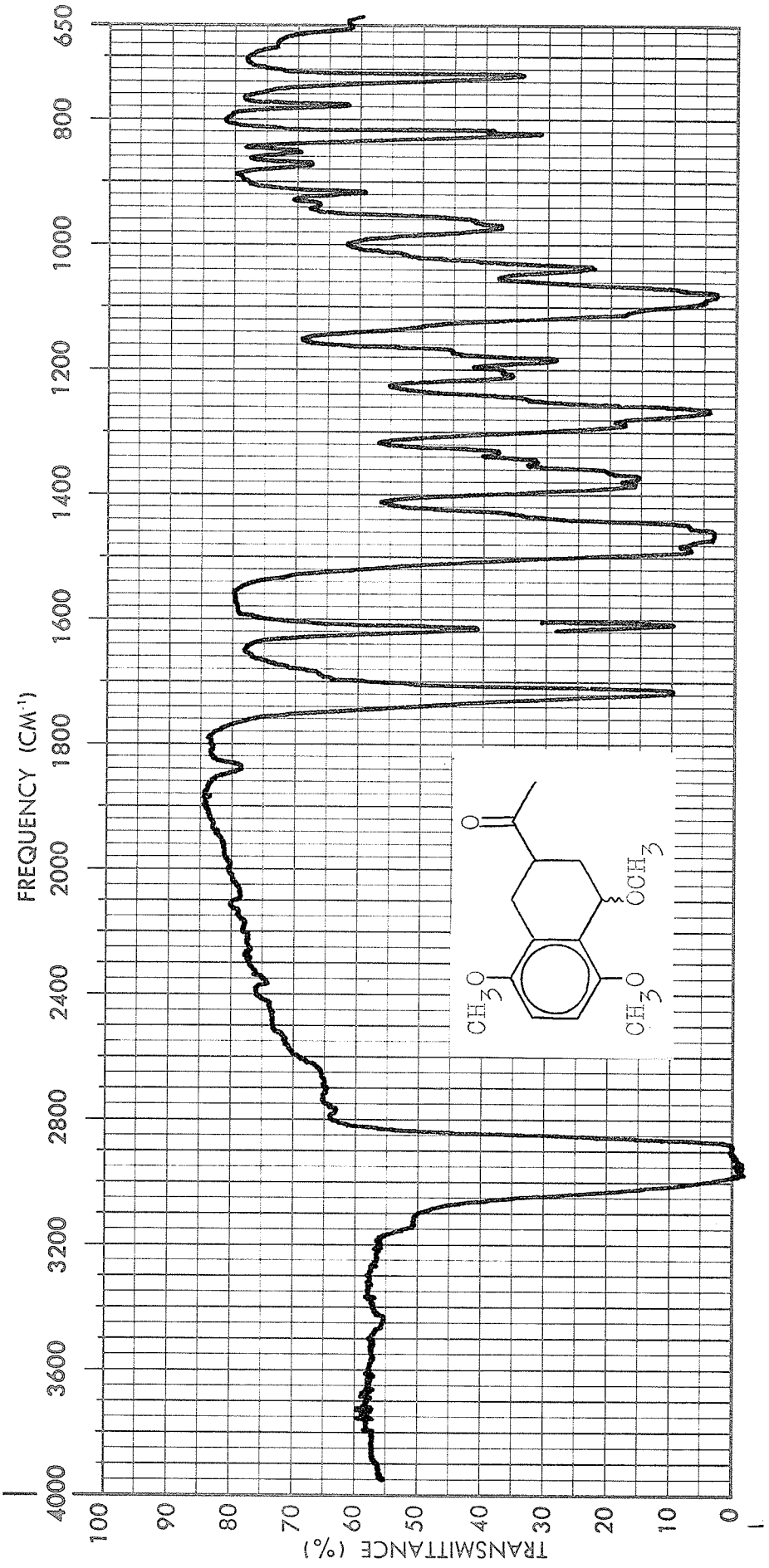
Infrared spectrum figure 25. Compound 45 (Nujol mull)



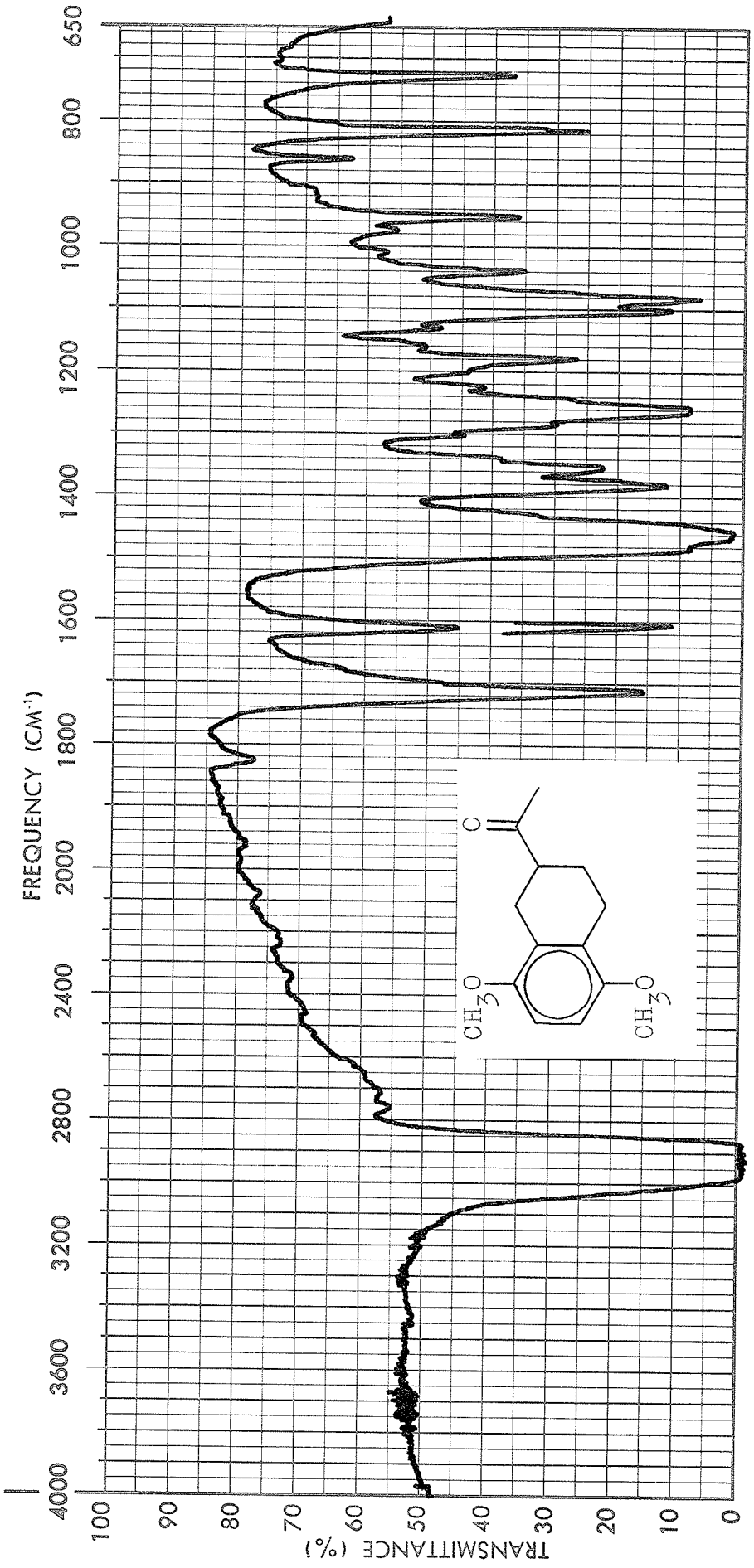
Infrared spectrum figure 26. Compound 55 (Nujol mull)



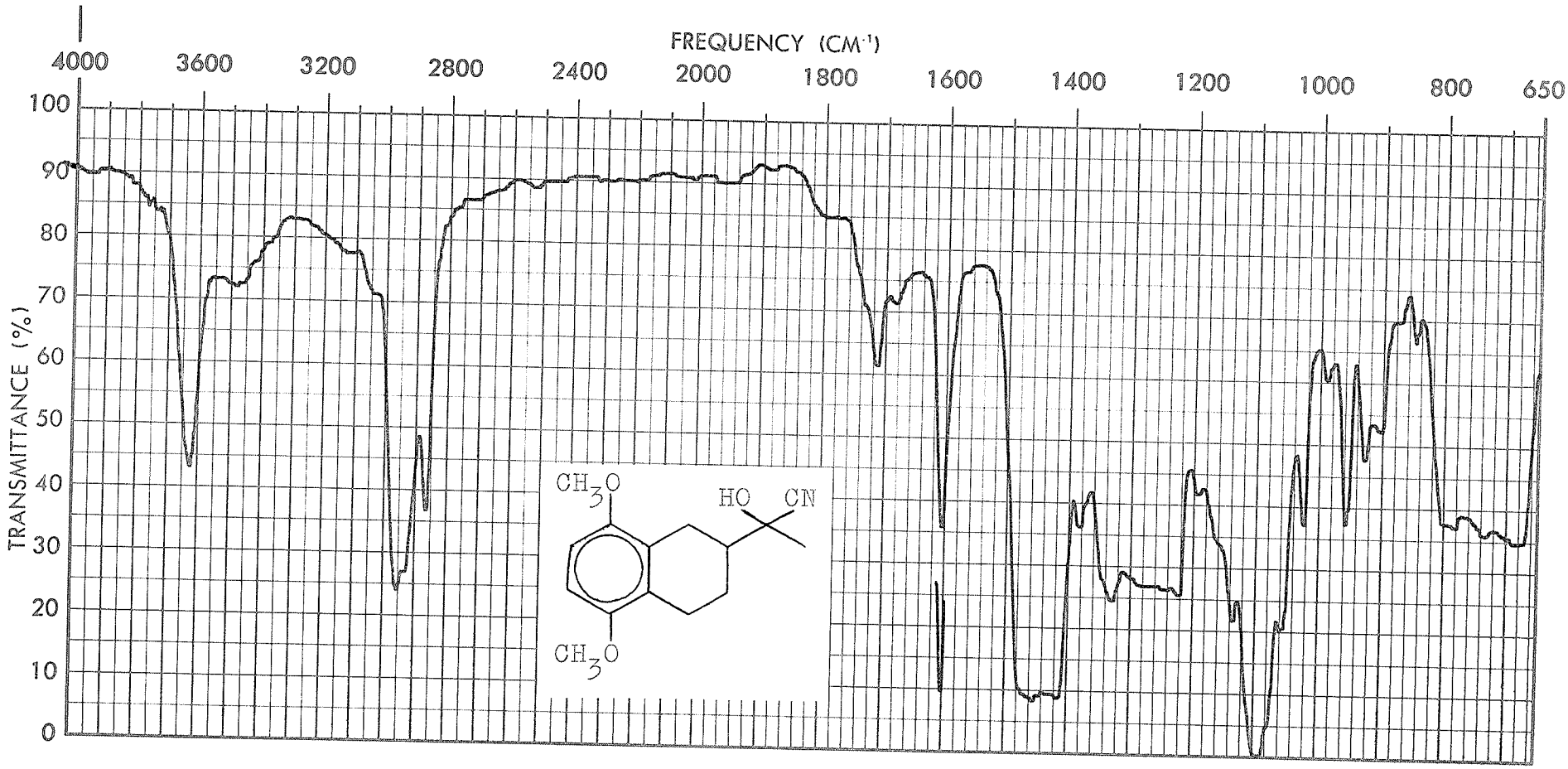
Infrared spectrum figure 27. Compound 56 (CH_2Cl_2)



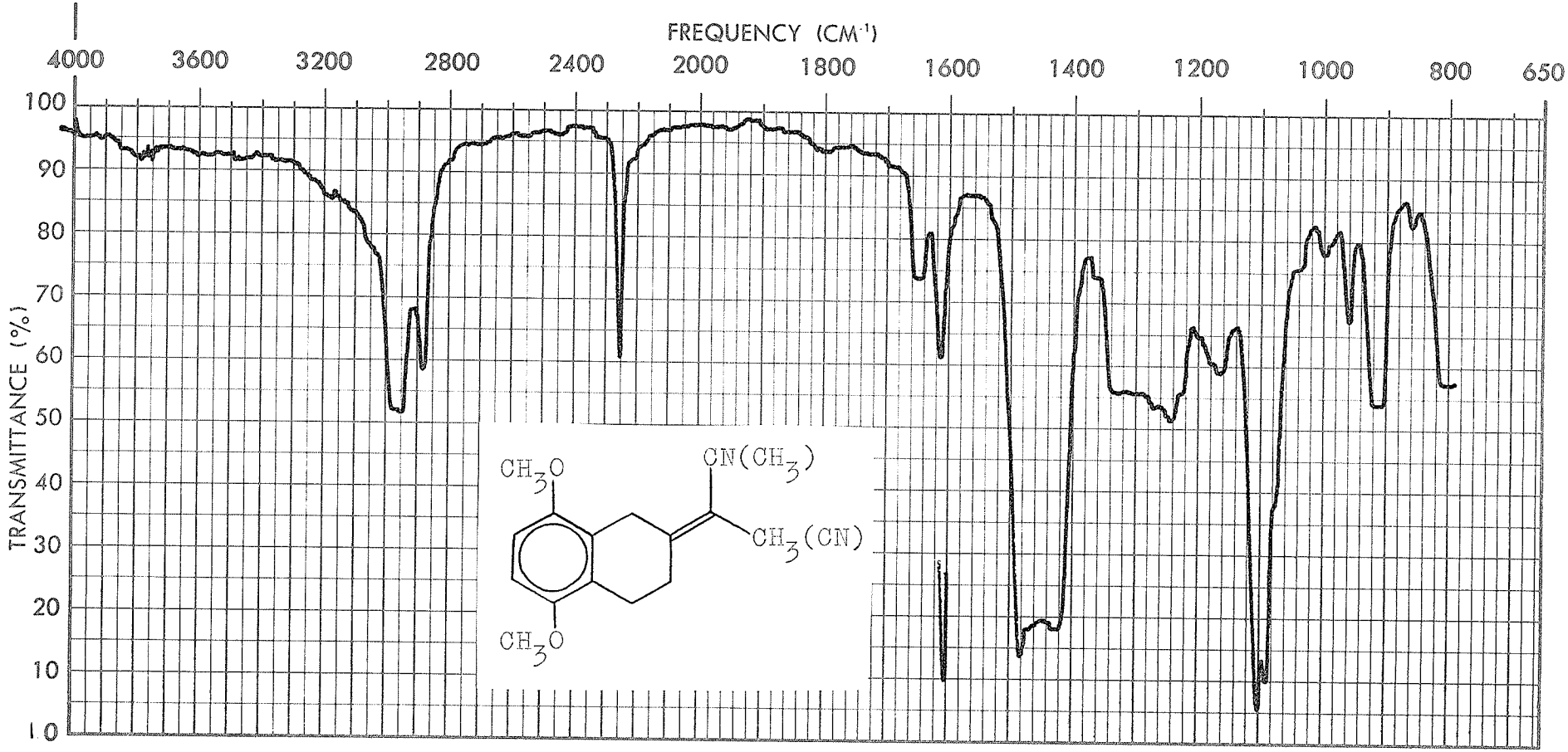
Infrared spectrum figure 28. Compound 57 (Nujol mull)



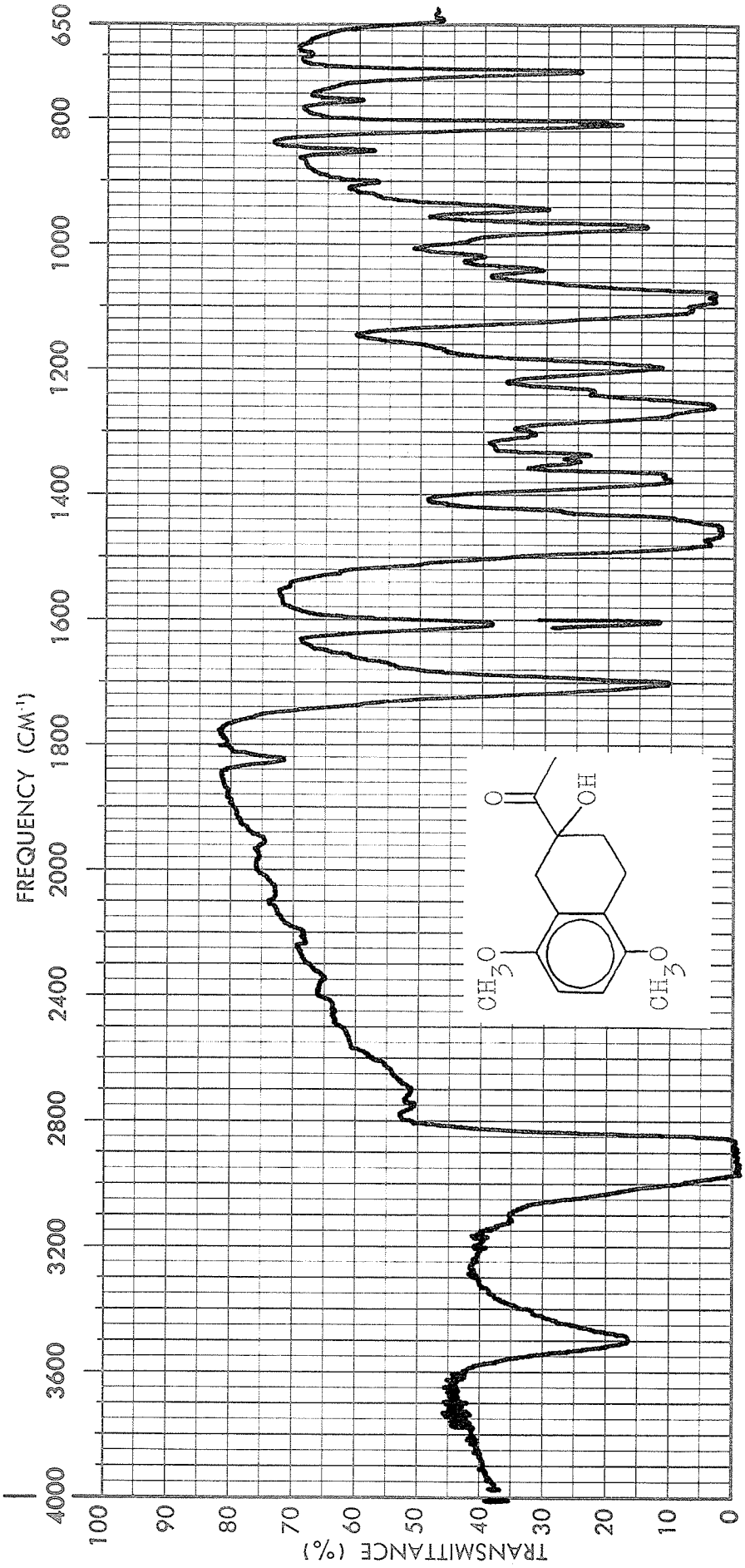
Infrared spectrum figure 29. Compound 58 (Nujol mull)



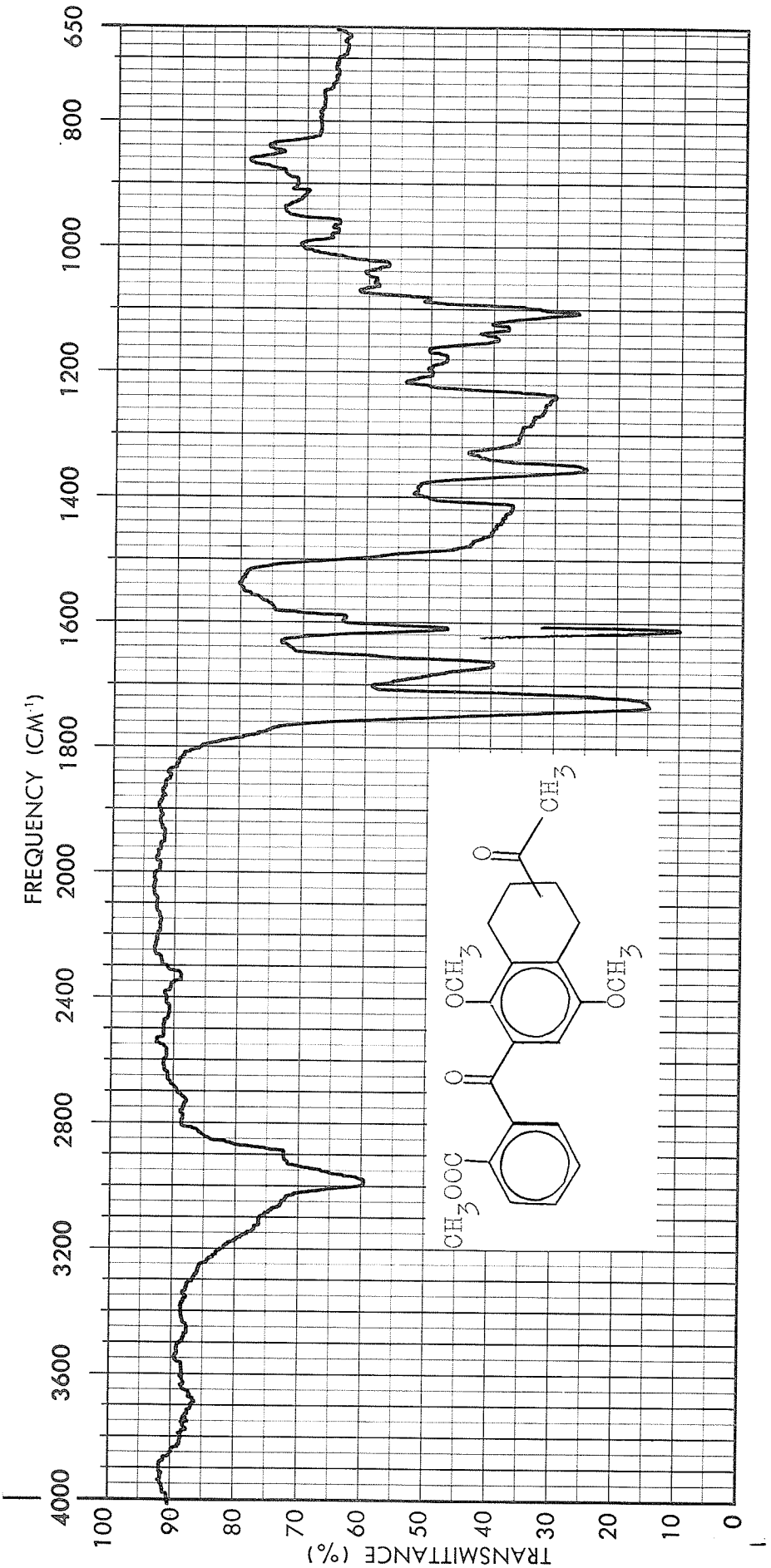
Infrared spectrum figure 30. Compound 59 (CH_2Cl_2)



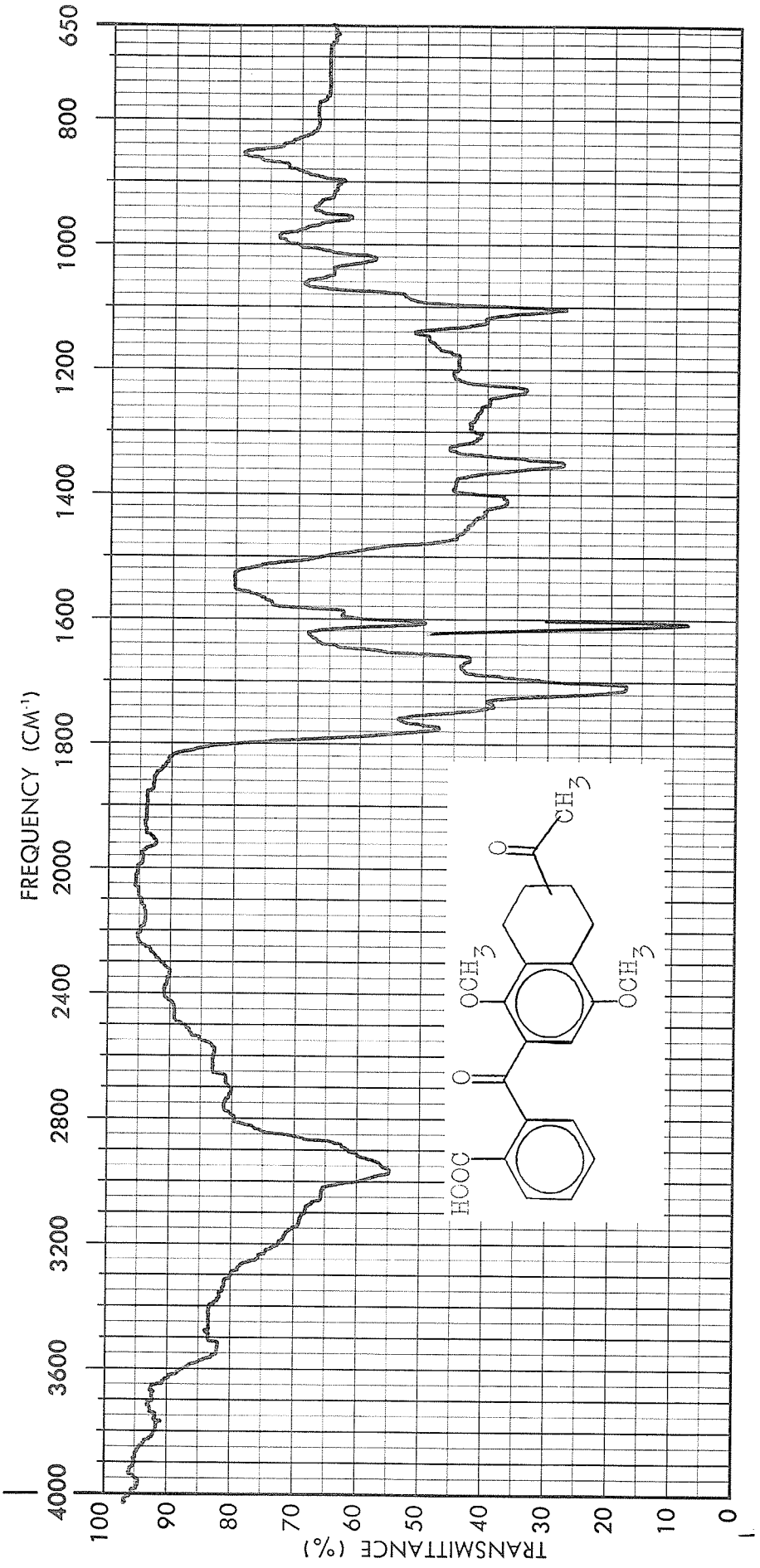
Infrared spectrum figure 31. Compound 60 (CH_2Cl_2)



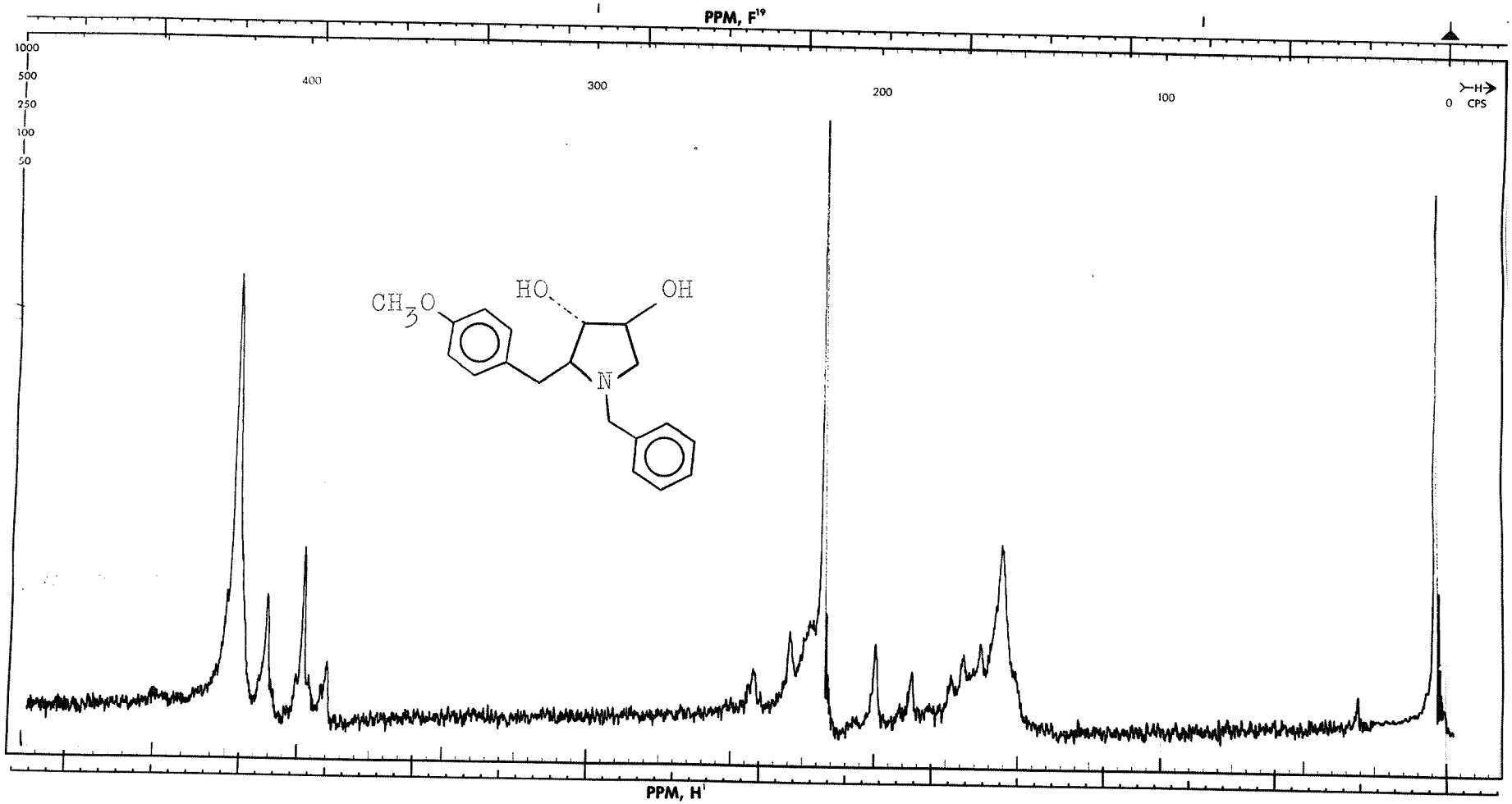
Infrared spectrum figure 32. Compound 61 (Nujol mull)



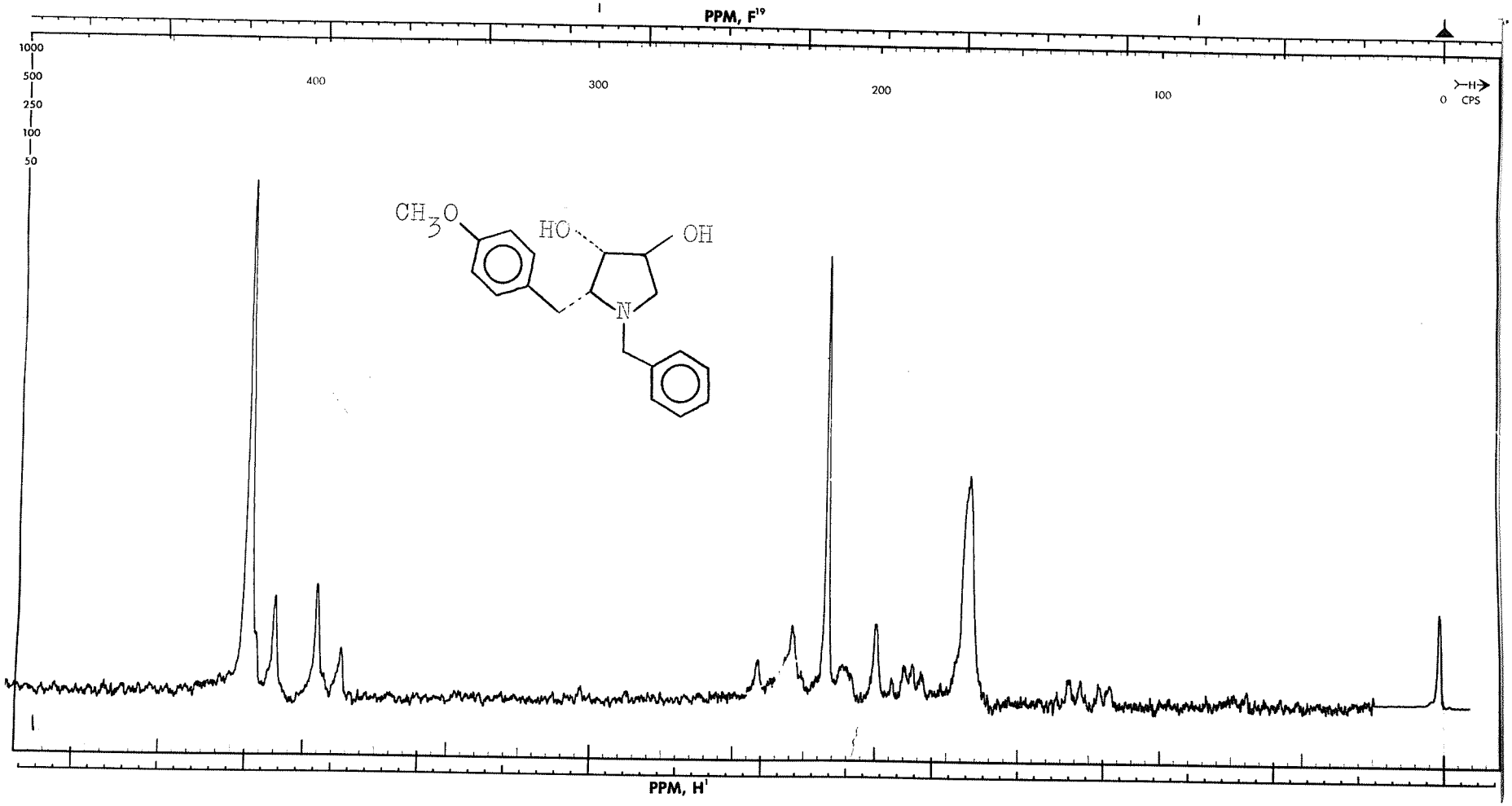
Infrared spectrum figure 33. Compound 62 (CH_2Cl_2)



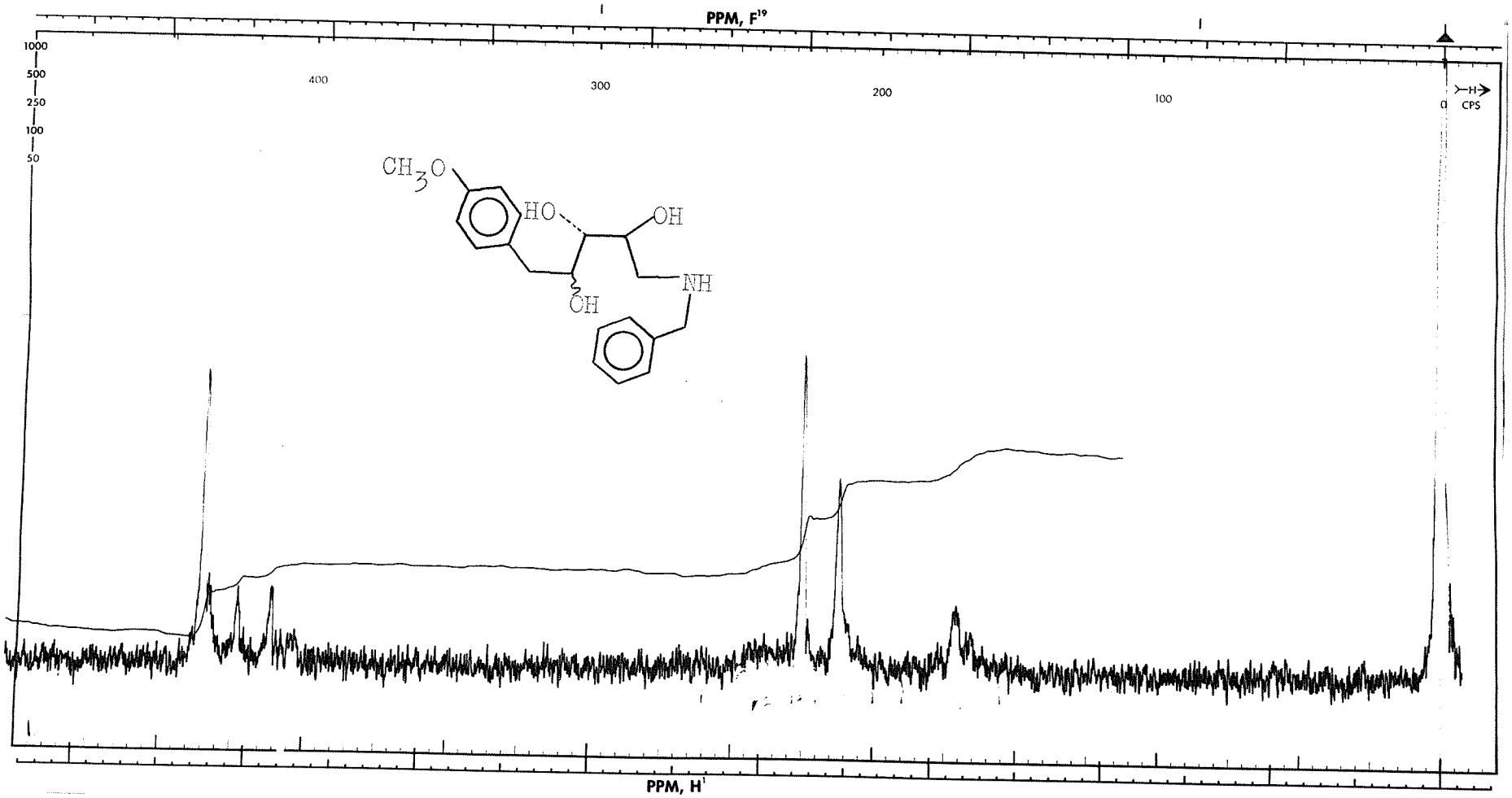
Infrared spectrum figure 34. Compound 63 (CH_2Cl_2)



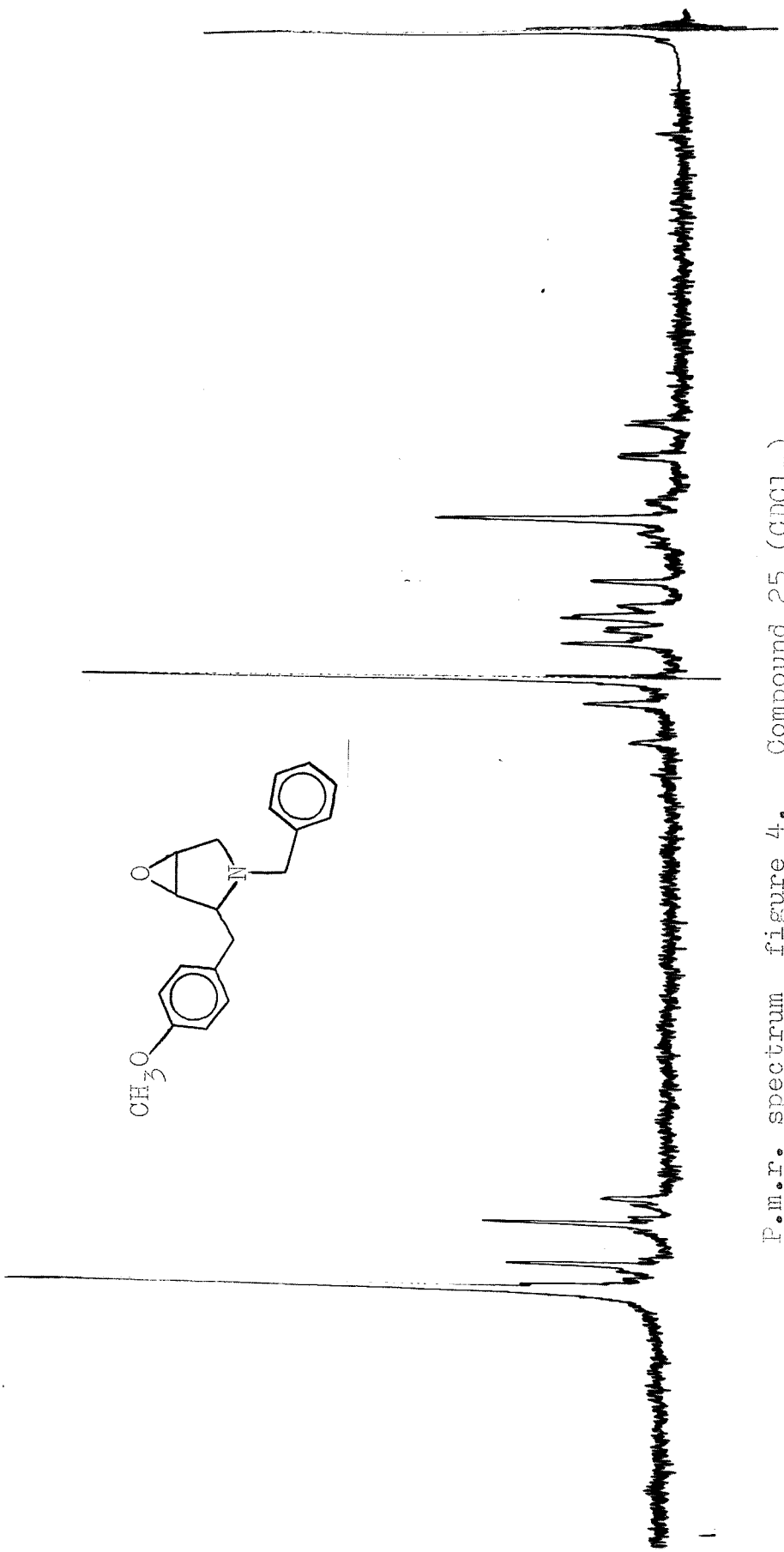
P.m.r. spectrum figure 1. Compound 21 ($CDCl_3$)



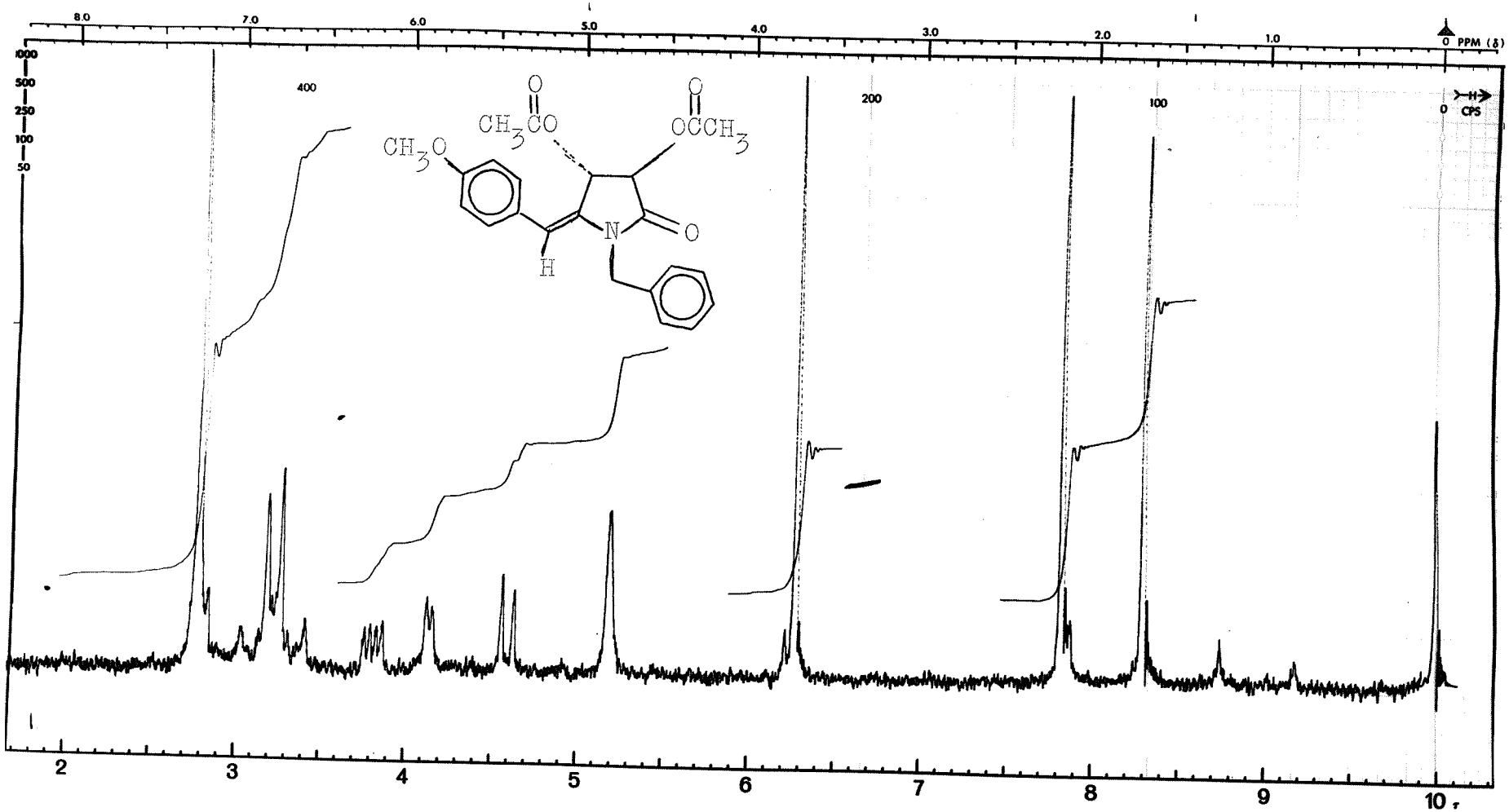
P.m.r. spectrum figure 2. Compound 20 ($CDCl_3$)



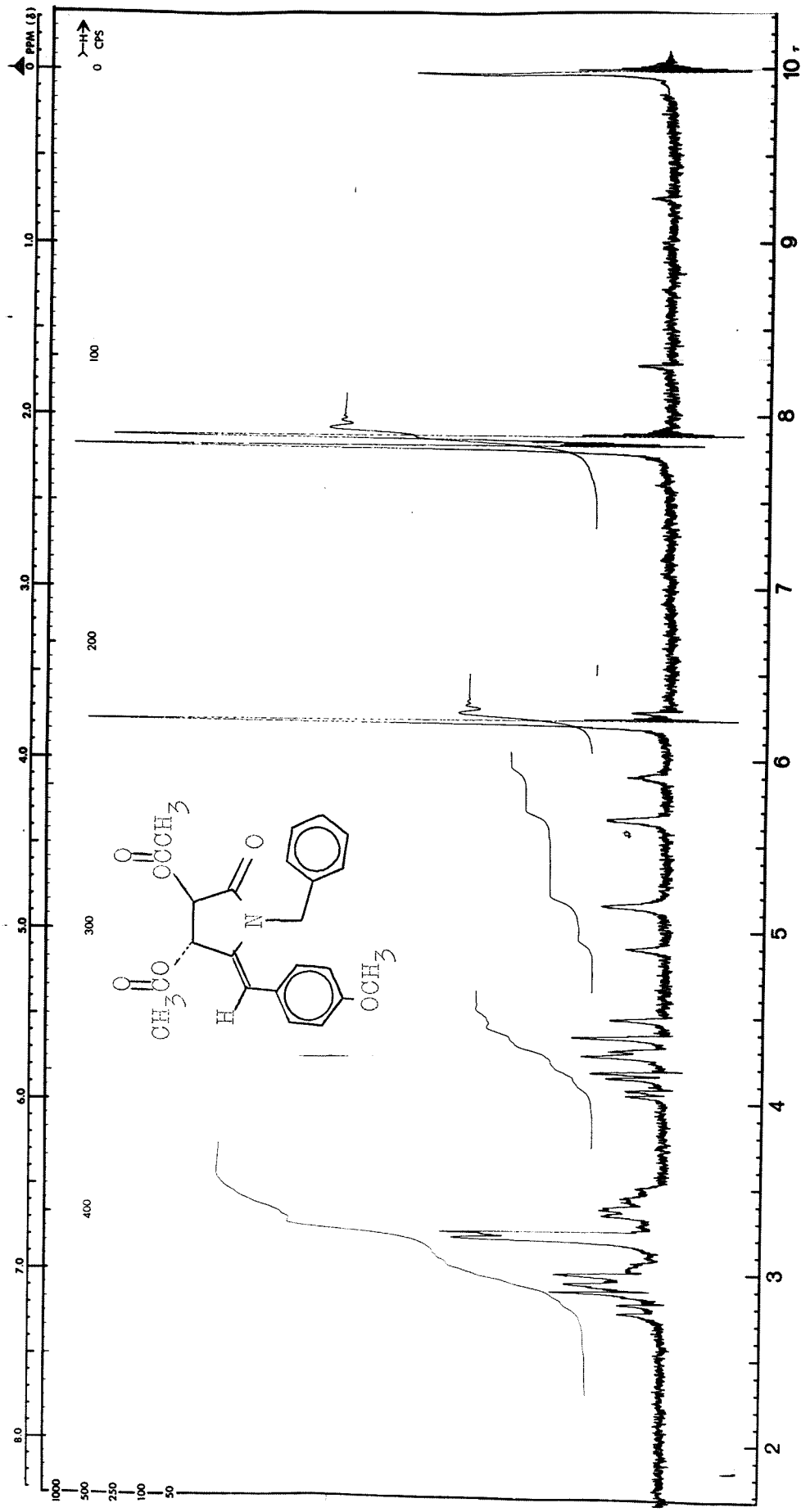
P.m.r. spectrum figure 3. Compound 22 ($CDCl_3$)



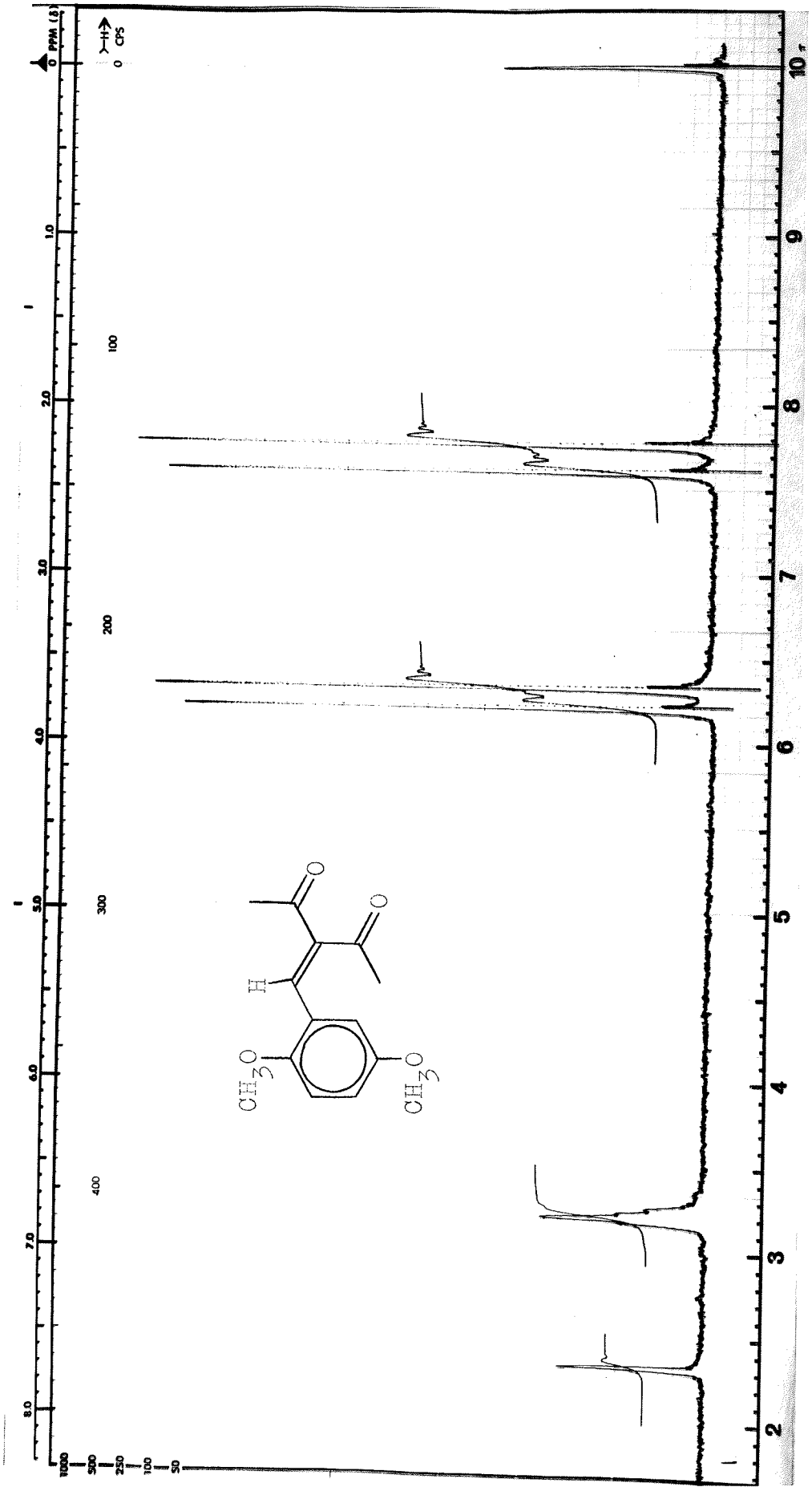
P.m.r. spectrum figure 4. Compound 25 ($CDCl_3$)



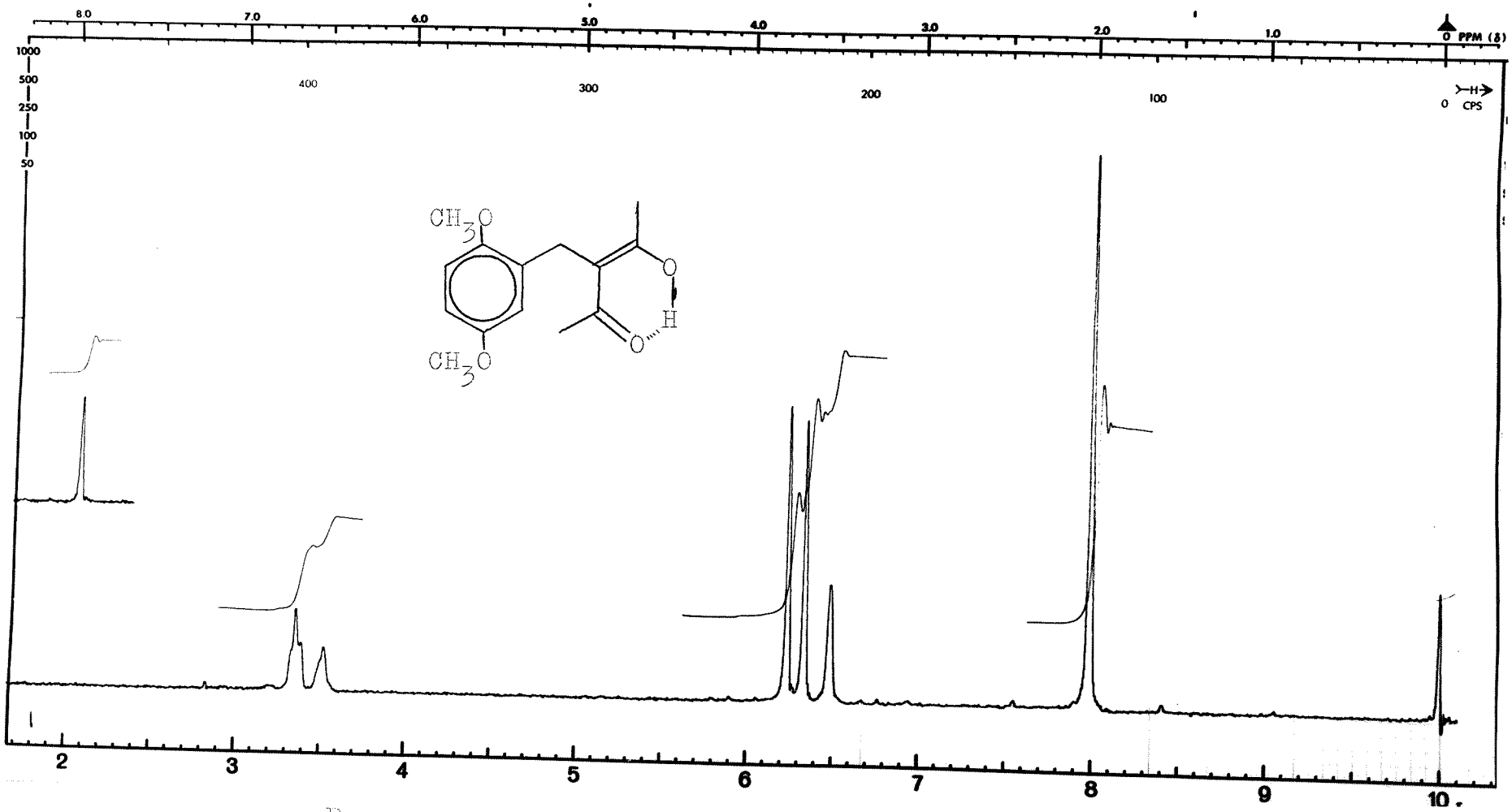
P.m.r. spectrum figure 5. Compound 29 (diacetate) ($CDCl_3$)



P.m.r. spectrum figure 6. Compound 30 (diacetate) ($CDCl_3$)

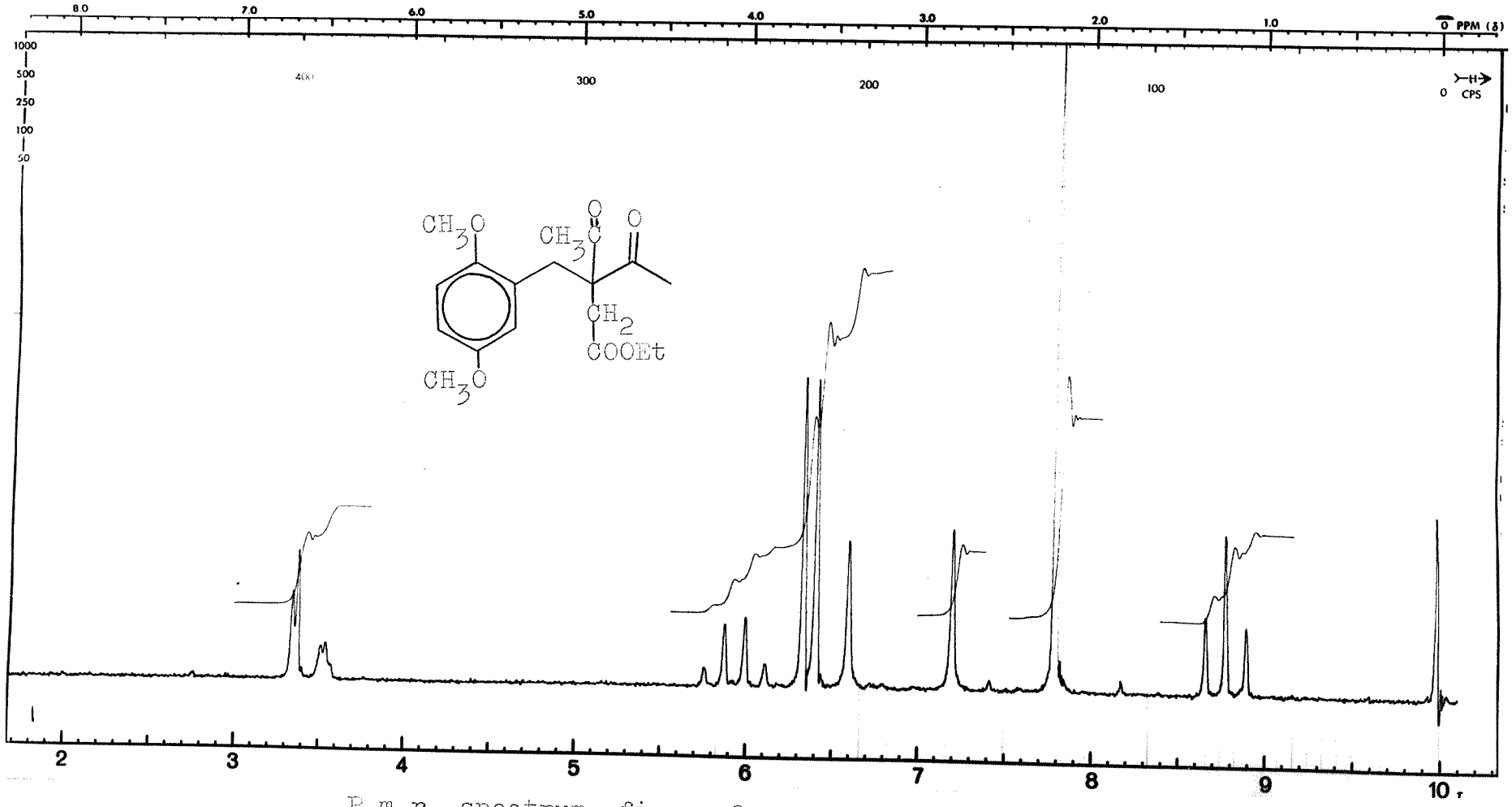


P.m.r. spectrum figure 7. Compound 52 ($CDCl_3$)

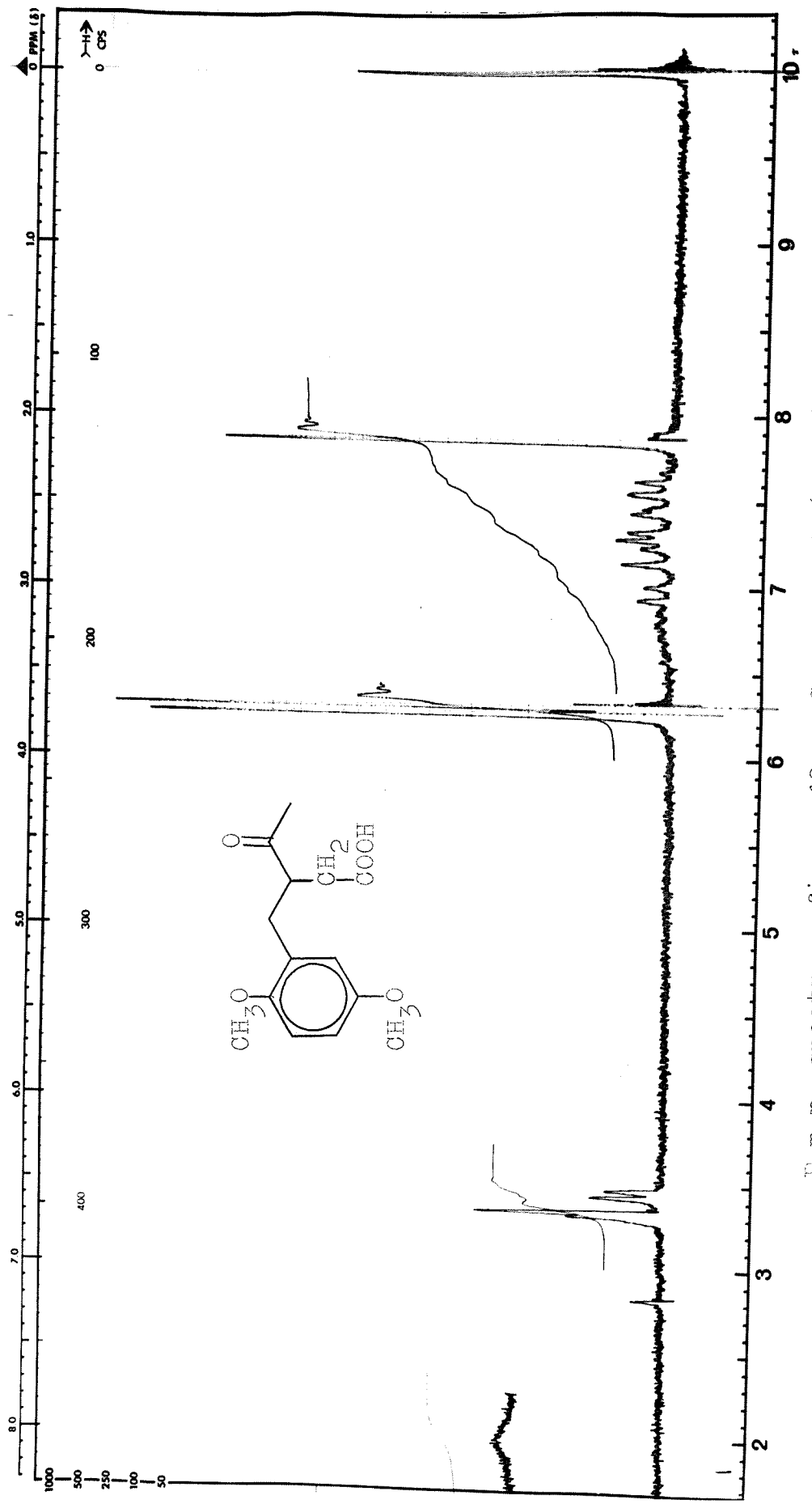


P.m.r. spectrum figure 8. Compound 53 ($CDCl_3$)

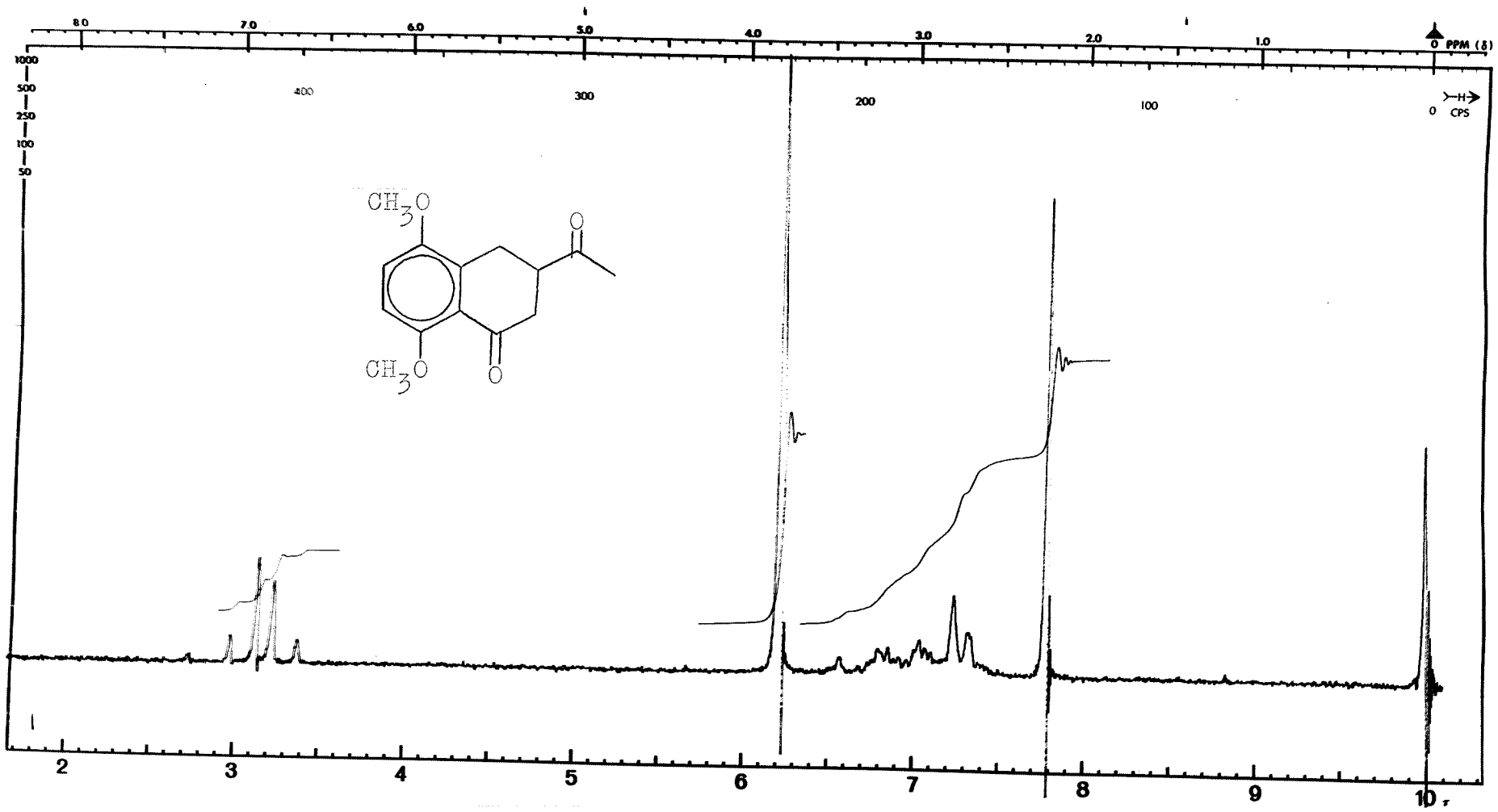
(Sweep Offset 520 cps)



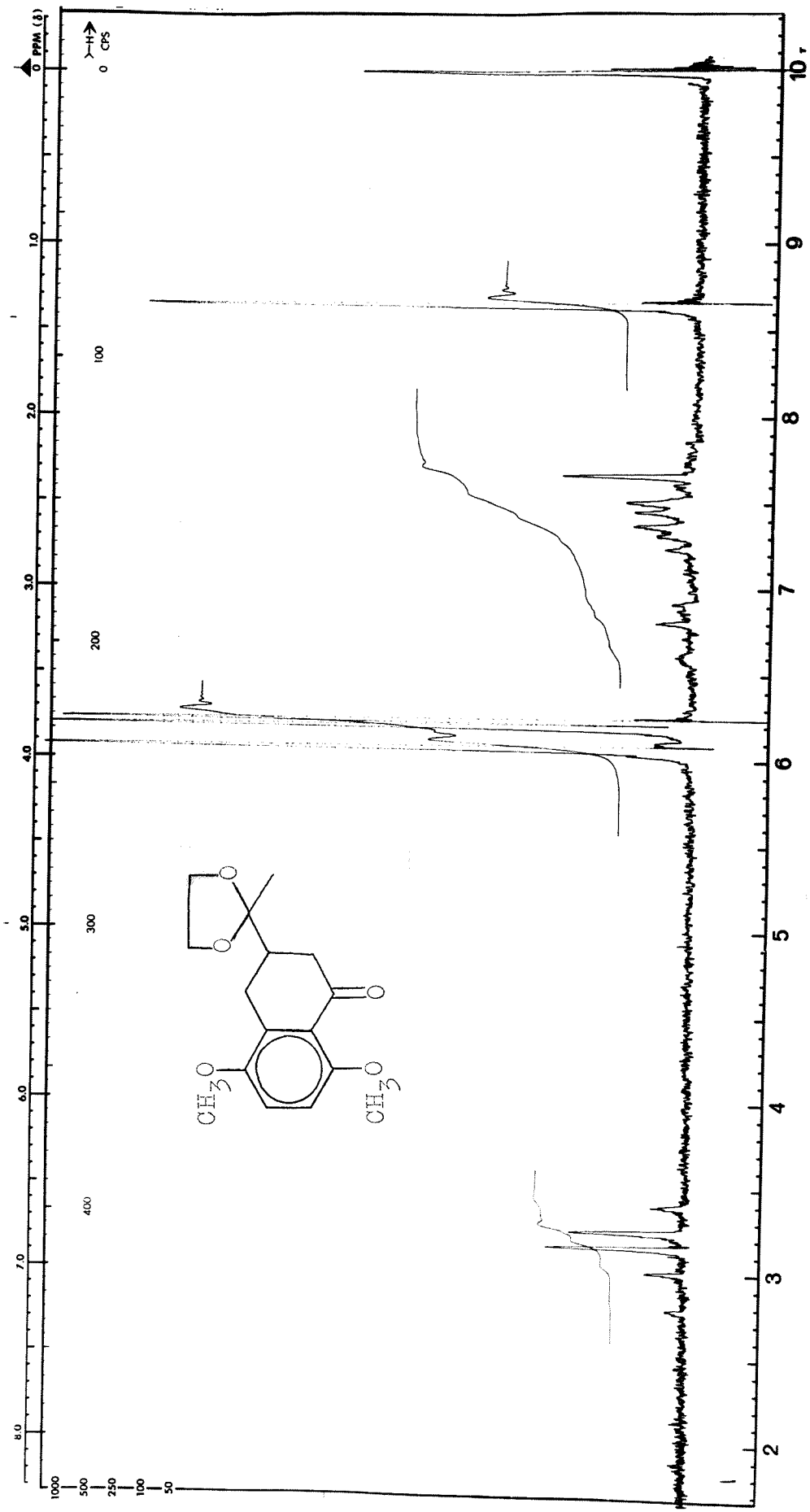
P.m.r. spectrum figure 9. Compound 51 ($CDCl_3$)



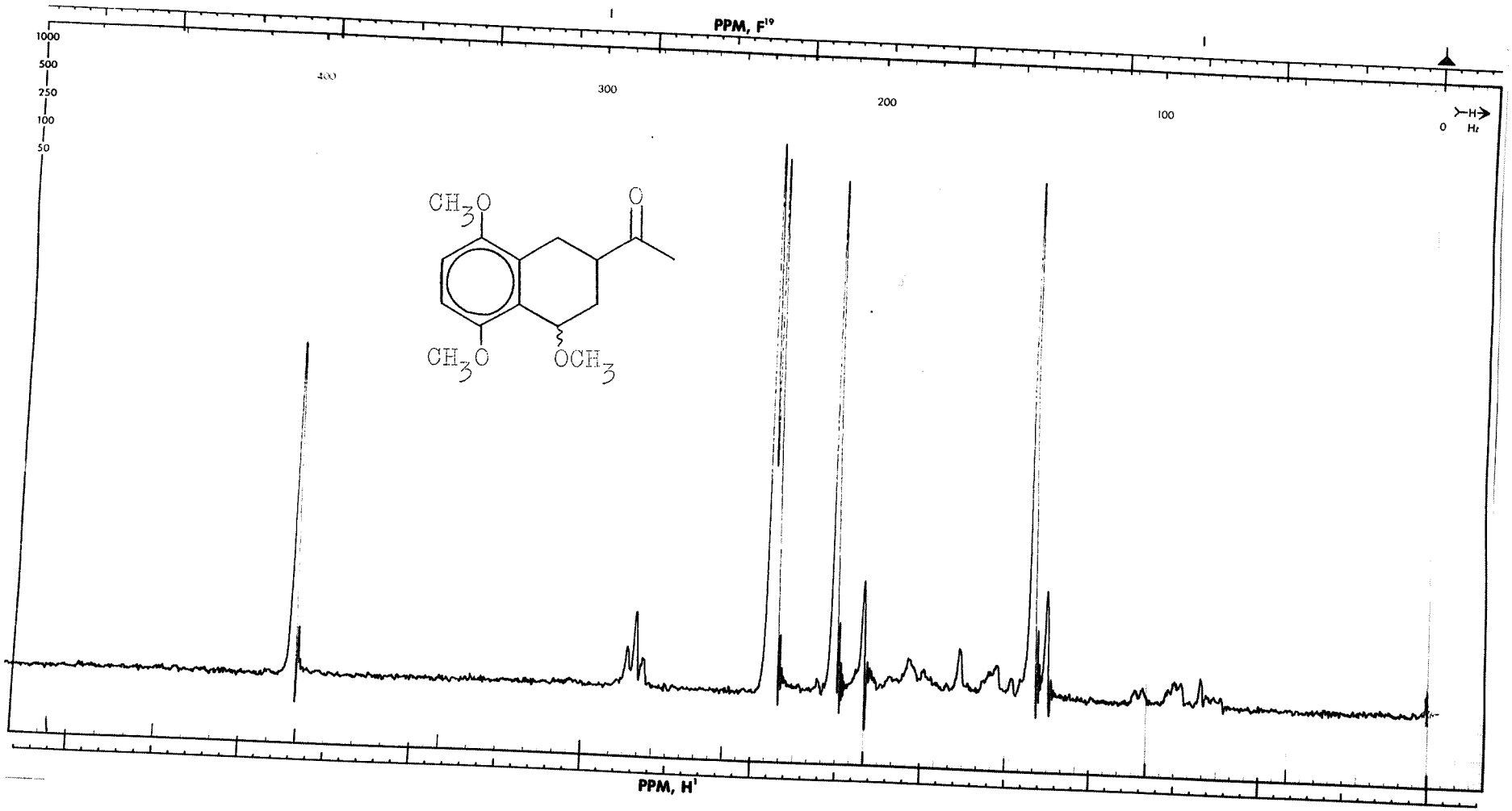
P.m.r. spectrum figure 10. Compound 44 ($CDCl_3$)
(Sweep Offset 170 cps)



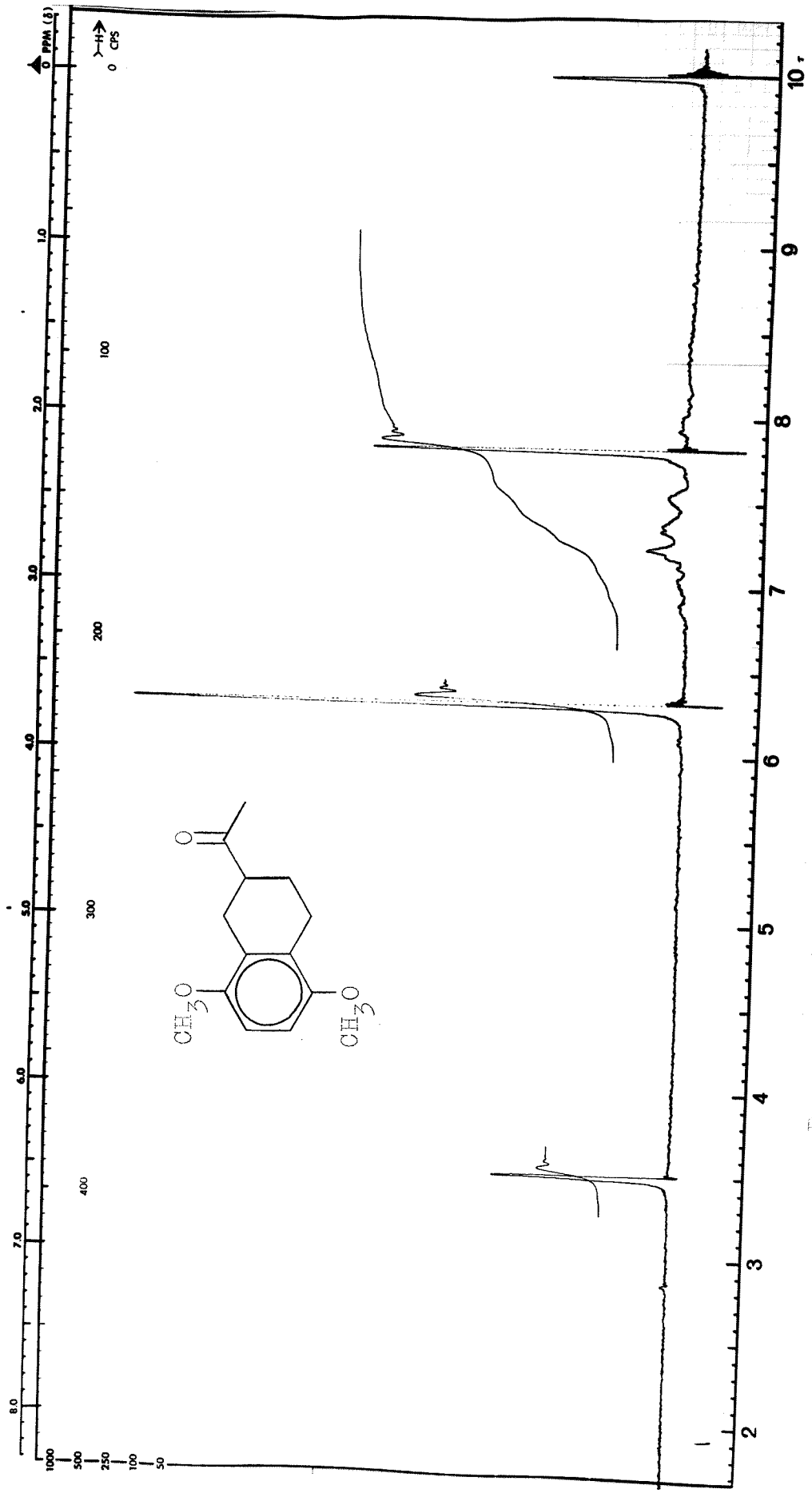
P.m.r. spectrum figure 11. Compound 45 ($CDCl_3$)



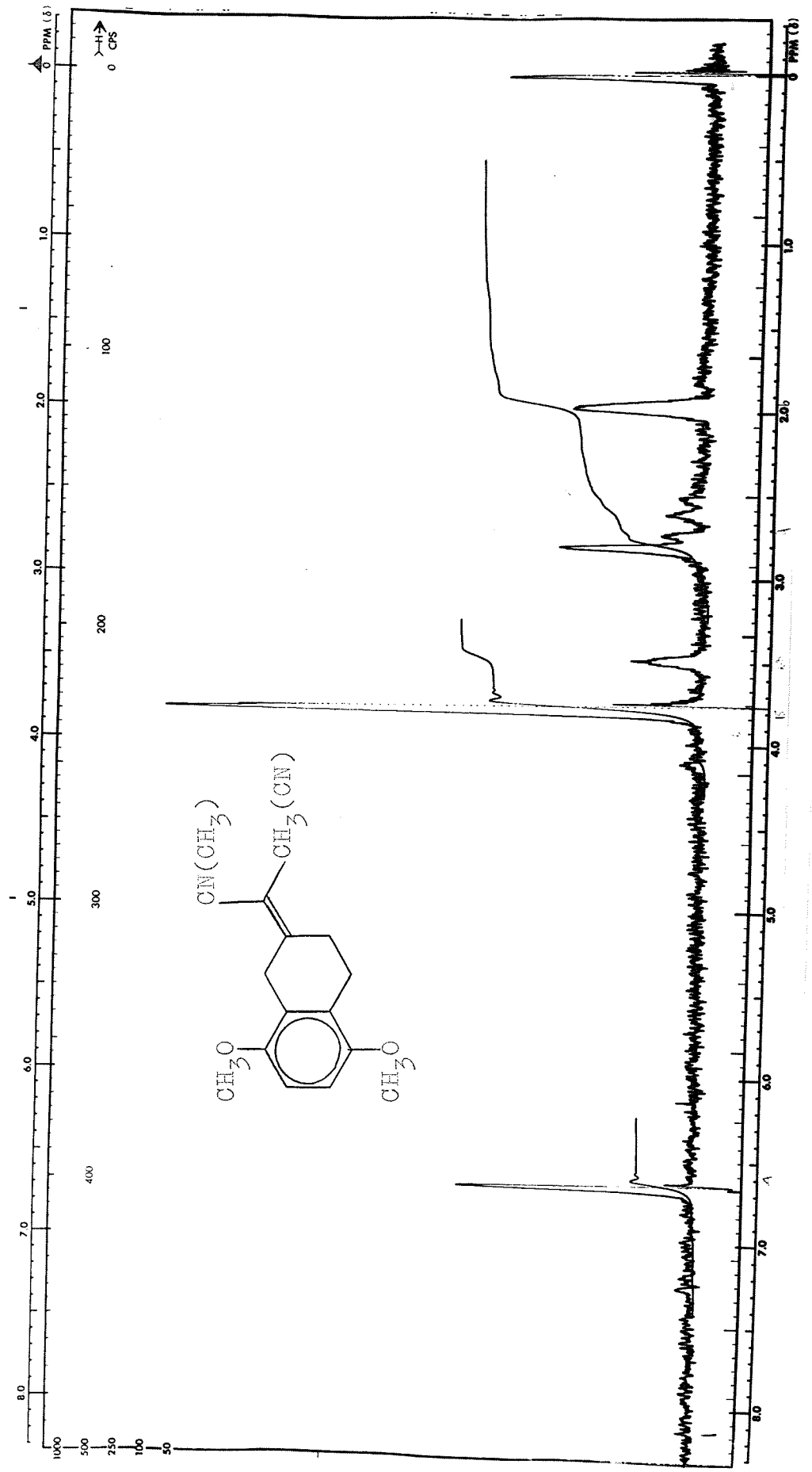
P.m.r. spectrum figure 12. Compound 55 ($CDCl_3$)



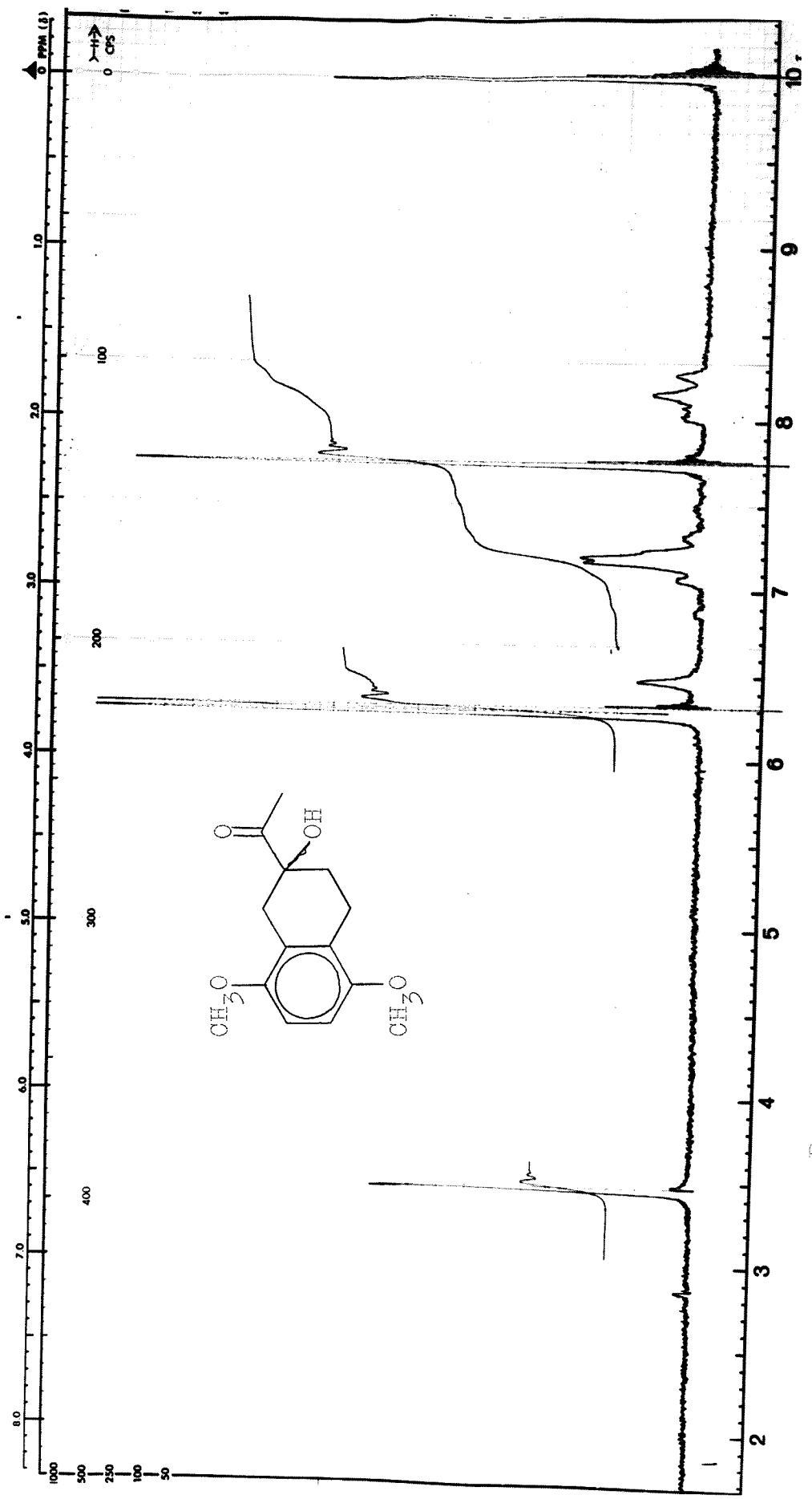
P.m.r. spectrum figure 13. Compound 57 ($CDCl_3$)



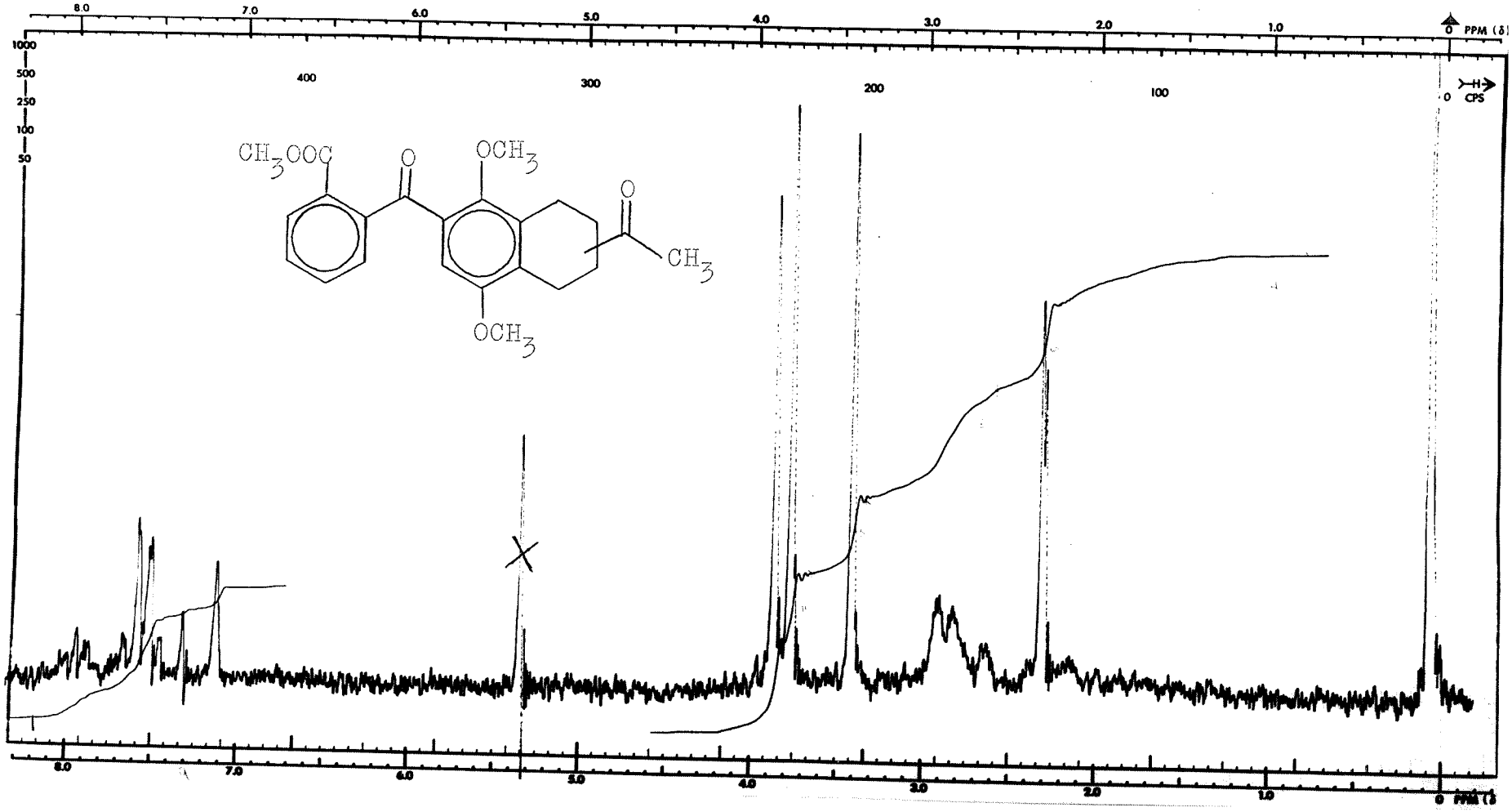
F.m.r. spectrum figure 14. Compound 58 ($CDCl_3$)



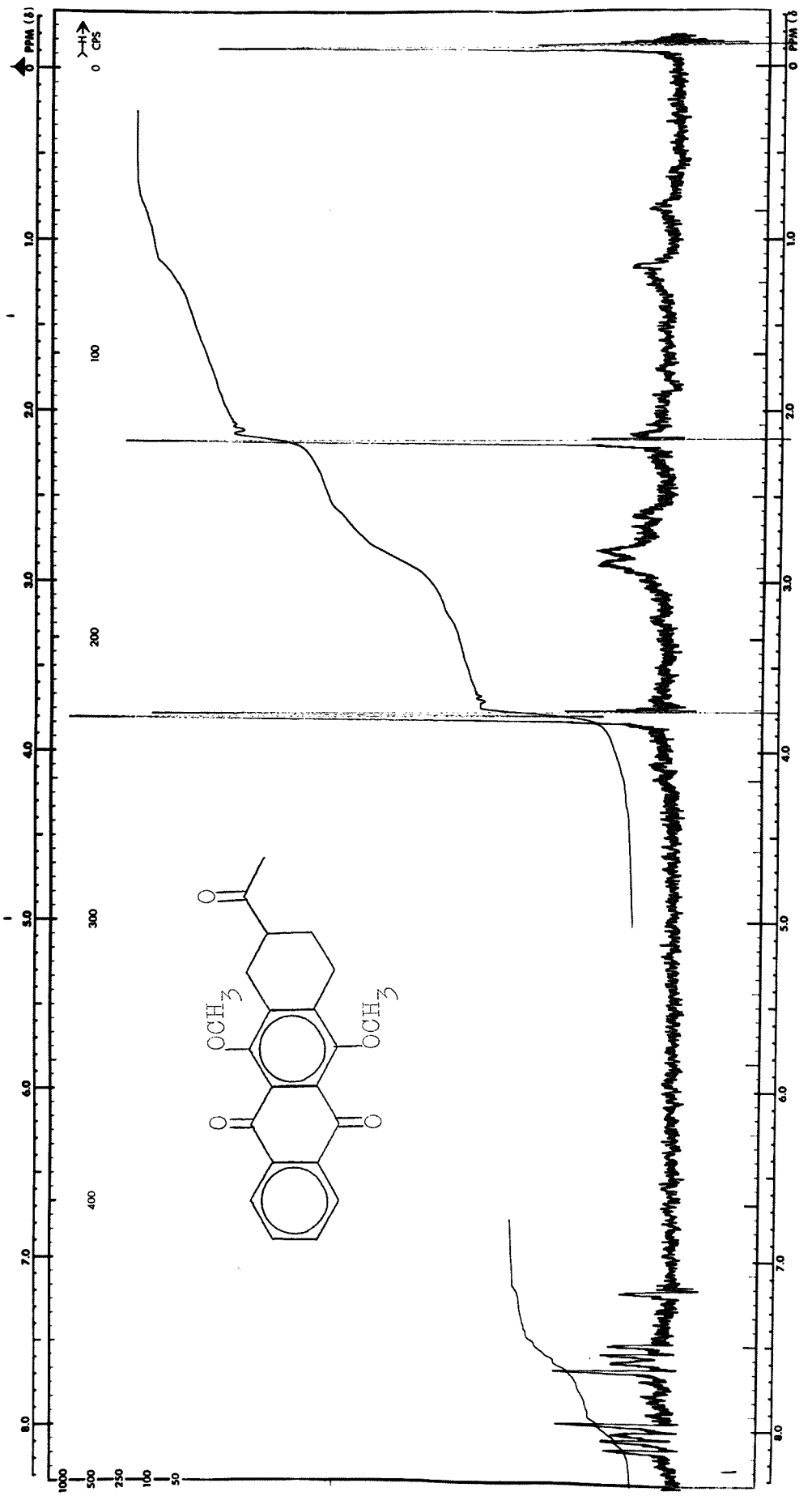
P.m.r. spectrum figure 15. Compound 60 ($CDCl_3$)



P.m.r. spectrum figure 16. Compound 61 ($CDCl_3$)



P.m.r. spectrum figure 17. Compound 62 ($CDCl_3$)



P.m.r. spectrum figure 18. Compound 64 ($CDCl_3$)

REFERENCES

1. B.A. Sobin and F. Tanner, Jr., J. Amer. Chem. Soc. 76, 4053 (1954).
2. A.B. Cue and J.G.H. Diaz, Rev. Invest. biol. Univ. Guadalajara, 1, 94 (1961); G.G. Miranda and e. Urbina, ibid. 1, 95 (1961); J.A. Portilla, ibid. 1, 95 (1961); A.A. Plata, H.B. Zapata and V.A. Munoz, ibid. 1, 96 (1961).
3. J.J. Beerebom, K. Butler, F.C. Pennington, and J.A. Solomons, J. Org. Chem. 30, 2334 (1965).
4. J.P. Schaefer and P.J. Wheatley, Chem. Comm. 1967, 578; J. Org. Chem. 33, 166 (1968).
5. G.H. Alt and D.H.R. Barton, J. Chem. Soc., 4284 (1954).
6. C.M. Wong, Can. J. Chem. 46, 3091 (1968)
7. S. Oida and E. Ohki, Chem. Pharm. Bull. (Tokyo) 16, 2086; 17, 1405 (1969).
8. M. Salmón and F. Walls, Chem. Comm. 1969, 63.
9. J. Buccini, Ph.D. Thesis, University of Manitoba, May, 1970.
10. I. Felner and K. Schenker, Helv. Chim. Acta. 53, 754 (1970).
11. C.M. Wong, J. Buccini, and J. te Raa, Can. J. Chem. 46, 3091 (1968).

12. C.M. Wong, J. Buccini, I. Chang, J. te Raa, and R. Schwenk, Can. J. Chem. 47, 2421 (1969).

13. A. Yokoo and S. Akutagawa, Bull. Chem. Soc. of Japan 35, 644 (1962).

14. Giustiniani, Gazz. Chim. Ital. 24, 224 (1894).

15. A. Queen and A. Reipas, J. Chem. Soc. C. , 245 (1967).

16. W.I. Awad, F.G. Baddar, M.A. Omara, and S.M.A.R. Omran, J. Chem. Soc., 2040 (1965).

17. P.C. Jocelyn and A. Queen, J. Chem. Soc., 4437, (1957).

18. E. Walton, J. Chem. Soc., 438 (1940).

19. R. Lukes and V. Prelog, Coll. Chem. Comm. 1, 119, 282, 334, 461, 617 (1929).

20. M. Sekiya and Y. Terao, Chem. Pharm. Bull. (Tokyo) 19, 391 (1971).

21. A. Di Marco, M. Gaetani, P. Orezzi, B. Scarpinato, R. Silvestrini, M. Soldati, T. Dasdia, and L. Valentini, Nature 201, 706 (1964).

22. Drugs of Choice, 1970-1971, Walter Modell editor, The C.V. Moskey Company, page 561.

23. A. Grein, C. Spalla, A. Di Marco, and G. Canevazzi, Giorn. Microbiol. 11, 109 (1963).

24. F. Arcamone, G. Franceschi, P. Orezzi, G. Cassinelli, W. Barbieri, and R. Mondelli, J. Amer. Chem. Soc. 86, 5334 (1964).

25. F. Arcamone, G. Cassinelli, P. Orezzi, G. Franceschi, and R. Mondelli, J. Amer. Chem. Soc. 86, 5335 (1964).

26. Dubost, Compt. Rend. 257, 1813 (1963).

27. H. Brockmann, Chem. Ber. 96, 1356 (1963).

28. J.P. Marsh, R.H. Iwamoto, and L. Goodman, Chem. Comm. , 1968, 589.

29. F. Arcamone, G. Franceschi, P. Orezzi, S. Penco, and R. Mondelli, Tetrahedron Letters 30, 3349-3352 (1968).

30. R.H. Iwamoto, P. Lim, and N.S. Bhacca, Tetrahedron Letters 36, 3891-3894 (1968).

31. J.P. Marsh, Jr., C.W. Mosher, E.M. Acton, and L. Goodman, Chem. Comm., 973 (1967).

32. A.C. Richardson, Chem. Comm., 627 (1965).

33. F. Arcamone, G. Cassinelli, G. Franceschi, P. Orezzi, and R. Mondelli, Tetrahedron Letters 30, 3353-3356 (1968).

34. C.M. Wong, D. Popien, R. Schwenk, and J. te Raa, Can. J. Chem. 49, 2712 (1971).

35. D. Popien, Master's Thesis, Univ. of Manitoba (1972).

36. B. Schwenk, Ph. D. Thesis, Univ. of Manitoba, (1972).

CURRICULUM VITAE

Candidate's Name:	Arend Jan te Raa
Place and date of birth:	Borculo, The Netherlands April 10, 1940
Permanent address:	Winnipeg, Manitoba
Schools attended:	
Primary education:	Gelselaar, The Netherlands 1946 - 1951
Secondary education:	Zutphen, The Netherlands 1951 - 1956
Universities attended:	University of Manitoba 1962 - 1967 (B.Sc. (Hons.) 1967) University of Manitoba 1967 - 1971