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STRUCTURAL STUDIES ON PELIOMYCIN

by

© Willem Enterman

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled

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submitted by Willem Enterman in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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This dissertation is dedicated to
my wife Martha for her encouragement
and understanding throughout the past
decade, and to Catharina and John.

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ABSTRACT

The suspect macrolide peliomycin was subjected to investigation by physical methods and fragmentation analysis. The molecular formula of $C_{52}H_{80}O_{14}$ was arrived at from a consideration of the carbon to oxygen ratio and percentage hydrogen obtained from duplicate elemental analyses (C,H and O). The molecular weight was limited by integration and methyl group count of the 220 MHz p.m.r. spectra of peliomycin and mass spectral analyses of peliomycin and hexatrimethylsilylpeliomycin.

Quantitative oxidation of peliomycin with the periodate-permanganate reagent and chromium trioxide-acetic acid reagent showed the uptake of 16 oxygen atoms per mole of peliomycin. The periodate-permanganate oxidation did not lead to isolable products other than acetic and formic acid.

Oxidative degradation (Jones, then periodate-permanganate) of peliomycin provided dimethyl 2-methylsuccinate and dimethyl 2*R*,3*s*,4*S*-2,4-dimethyl-3-acetoxyglutarate. The identity of both compounds was proven by synthesis. The assignment of the asymmetric centers of this latter compound is based on conformational analyses (p.m.r.) of the diastereoisomers of the corresponding 5-*O*-acetyl-2,4-dimethyl-1,3-*O*-isopropylidene-pentane-1,3,5-triols. When peliomycin was oxidized with the periodate-osmium tetroxide reagent, the 2,4-dinitrophenyl-hydrazone of glycolaldehyde was apparently isolated.

Oxidative degradation (Jones, then Baeyer-Villiger) of perhydropeliomycin led to the isolation of dimethyl 2-methylglutarate, methyl 4-methyl-5-oxoheptanoate, methyl 2-methyldodecanoate, the methyl ester of the mono carboxylic acid $C_{16}H_{30}O_3$ and dimethyl *R*-2,4-dimethyl-pent-2-en-1,5-dioate. All these compounds, except the above C_{16} acid, were characterized by comparison with an authentic synthetic sample.

Peliomycin appears to contain six hydroxyl groups indicated by the crystalline pentaacetate derivative and mass spectral analysis of the fully trimethylsilylated derivative. It was established that peliomycin contains an α,β-unsaturated ester function and two kinds of ketone groups in saturated environments. The molecule is very sensitive to base which caused extensive conjugation as observed in the ultraviolet spectrum (λ_{max} 353 mμ). No evidence for a glycosidic structure was obtained on acid hydrolysis of peliomycin. Extensive spin decoupling studies (p.m.r.) of peliomycin and penta-*O*-acetylpeliomycin established the environment of many protons, which fact was helpful in constructing an approximate formula for peliomycin.

Spectral evidence suggest that the antibiotic oligomycin B (64,65), if not the same, is structurally closely related to peliomycin. The available evidence suggests that these antibiotics may differ in their antimicrobial activity.

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I INTRODUCTION

A. Historical Notes

The antimicrobial properties displayed by certain microorganisms were used to advantage more than 2500 years ago. The Chinese (1) were aware of the therapeutic value of moldy curd of soybeans, and they applied this material as standard treatment of carbuncles, boils and similar diseases. There are many recordings (2) in medical history of infections cured with soil or plants, which were probably sources of antibiotic-forming molds and bacteria.

Pasteur and Joubert (3) were the first to recognize the clinical uses of antibiotics when they noted that experimental anthrax in susceptible animals could be repressed by inoculations with non-pathogenic bacteria. These investigators speculated on the therapeutic potentialities of natural microbial forces to conquer microbial diseases.

The discovery of the chemotherapeutic effects on bacterial infections displayed by penicillin (4,5) and streptomycin (6) announced an era of active search for microbial metabolites showing antimicrobial properties. These chemical substances were called antibiotics (7), a name derived from antibiosis, first used by Vuillemin in 1889 (8). The investigations led to the isolation of a large number of antibiotics which showed activity against a variety of organisms. In particular,

numerous metabolites produced by actinomycetes of the genus *Streptomyces* have been found to possess this activity; indeed, a recent publication (9) lists more than 725 such antibiotics. Many antibiotics have certain structural features in common and are classified accordingly, *viz.*: tetracycline, polypeptide, polyene, aminoglycoside, macrolide, etc., antibiotics (9).

This dissertation is concerned with the structure of peliomycin, a metabolite of *Streptomyces luteogriseus*, which probably belongs to the macrolide antibiotics.

B. Macrolide Structural Features

The macrocyclic lactones are members of a structurally related group of antibiotics produced by *Streptomyces* strains. The peptide macrocyclic lactones (10) belonging to the Streptogramin family will not be discussed here. The former antibiotics feature medium or large-sized lactone rings containing from 11 to 37 carbon atoms in the macrocyclic nucleus. Methyl groups are the usual substituents, and the distribution of oxygen often follows a complicated pattern (13). With few exceptions (11,12) the lactone ring is closed between the carboxyl group at one end and the hydroxyl group farthest toward the opposite end of the chain.

The antibiotics which possess a lactone ring with more than ten carbon atoms have been divided into two distinct groups (13), on the basis of their microbial behavior and

certain structural features of their lactone rings. The members of one group are classified as polyene antibiotics and are active against fungi and yeasts. They are further characterized by a chromophore of conjugated olefinic bonds which ranges from tetraene to heptaene. Otherwise, the polyenes resemble structurally the members of the second group, designated as macrolide antibiotics by Woodward (14).

The macrolides, are generally effective against Gram-positive bacteria, and cross resistance for different macrolides has been reported (15). In contrast to the polyene antibiotics, these macrolides have a maximum of two conjugated olefinic bonds. To date, all macrolides investigated have been found to possess a keto group, a feature which is often absent in the polyene antibiotics (13). Most macrolide antibiotics (and polyenes) contain mono- or disaccharides joined via an *O*-glycoside bond to a carbon in the lactone ring. In the case of chalcomycin (16), one of the sugars (mycinose) is attached to a side chain. In some macrolides, the sugars are esterified by an acyl function such as an acetyl (11) or an isovaleryl group (17). The sugars can be amino sugars, non-nitrogenous sugars, or both, and as a rule they are of the 6-deoxypyranose type (18). Forosamine (19) is the rare tetradeoxy sugar: 4-dimethylamino-2,3,4,6-tetradeoxypyranose, isolated from spiramycins. Borrelidin (20) is a notable exception among the macrolides, not only in that it lacks

sugars, but also because it carries a cyano group in the macrolide nucleus. Pentaenes of the polyene antibiotics do not contain sugars except pentafungin (21) and tetrin A and B. The latter on hydrolysis liberated 3-amino-3,6-dideoxy-L-mannofuranose.

C. Macrolide Structure Determination

In 1956, the first complete structure of a macrolide antibiotic, methymycin, was published (23). This occurred six years after the discovery of this group of antibiotics with the isolation of picromycin (24). Since that time more of such structures have been elucidated (25). These early structure investigations were quite laborious, and hence, because of time expenditure, were made only on antibiotics of great medical interest (9).

With the increased availability of proton magnetic resonance (p.m.r.) spectroscopy during the last decade, structure determinations have been greatly facilitated. Its usefulness became very apparent in the studies on spiramycins (26) which caused a reconsideration (17) of the "unambiguous" (25) structure of magnamycin as proposed by Woodward (14). P.m.r. spectroscopy also became of great importance (18), together with optical rotatory dispersion (o.r.d.) spectroscopy (27) and acidity constants (28) in stereochemical problems associated with macrolide antibiotics. The refinement in

separation techniques with the introduction of thin-layer and gas chromatography, and the application of mass spectrometry (29) accelerated the progress in structural chemistry of macrolides. High resolution mass spectrometry was found to be of great utility in establishing correct molecular formulas (30), as the classical methods of elemental analysis and molecular weight determination were not sufficiently precise for compounds of high molecular weight. For this purpose, trimethylsilyl ethers(31) have often been used to protect hydroxyl groups. The unprotected alcohols tend to be dehydrated at the temperatures required to obtain an adequate vapor pressure.

X-ray crystal structure analysis, potentially a most powerful and direct method for establishing molecular structure, has so far found only limited application in the macrolide field (32,33). The difficulties encountered in this technique are mainly associated with the preparation of crystalline heavy atom derivatives (34), and the tendency to occlude impurities such as solvent in the crystal lattice (35). When the preparation of an appropriate crystal containing a heavy atom was successful, the utility of X-ray analysis was clearly established. Such was the case with erythromycin A (33), where it was unequivocally shown that the glycosidic linkage of cladinose was of the α configuration, instead of β as previously reported (36).

D. Biogenesis of Macrolides

Since the first macrolide structures were reported, their biogenesis has received considerable attention (13, 37-40), and many radioactive tracer experiments have been performed (13). These studies led to a division of the macrolide antibiotics into three classes according to the biogenesis of their lactone rings (13):

a. lactones arising only from propionate (methylmalonate) precursors;
b. lactones derived from propionate and acetate precursors;
c. lactones arising from propionate, acetate and other precursors, also called compound macrolides (41).

The majority of macrolides are formed from acetate and propionate units (41), whereas compound macrolides are few in number, e.g. magnamycin (17) and the spiramycins (26).

As a result of these biogenetic studies, two major hypotheses have evolved which demonstrates common patterns of carbon skeletons among the macrolides despite individual structural differences.

Vaněk and co-workers (40,41) proposed a biogenetic interrelationship based on a comparison of a dozen macrolides with known structures. They arranged the lactone backbones

side by side and found eight analogous building units. As required, some of these units were omitted in order to comply with the size of the lactone ring. Upon examination of this scheme, a relationship emerged which showed, for instance, that most macrolides carry sugar residues at carbons 3 and 4 of the lactone ring (40). The basis for this proposal was associated with the "specificity" of the pertinent active center of the matrix surface of the enzyme (41), such that formation of the macrolide rings may take place. Such a relationship between structure and enzyme would necessitate configurational agreement between the building units. The lack of this agreement (40) constitutes a serious shortcoming of the Vaněk hypothesis.

A configurational model for both macrolide and polyene antibiotics based on the absolute configuration of oleandomycin was proposed by Celmer (18, 42). This model (Figure I) is portrayed in the Fischer convention, and it is in accord with Gerzon's propionate rule (38) and Klyne's glycoside rule (43).

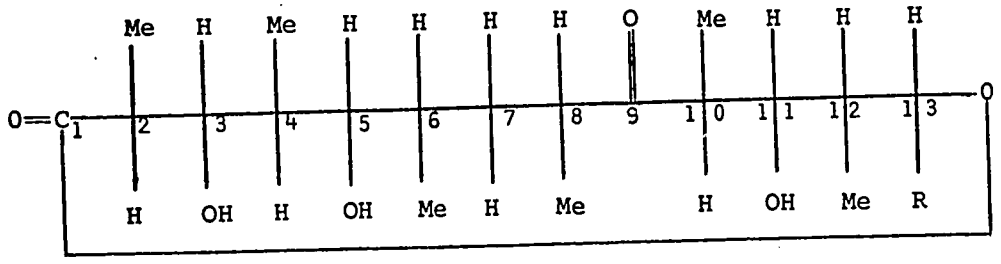


Figure I Configurational Model for Macrolide and Polyene Antibiotics according to Celmer (18,42)

The absolute configurations are based on *R*-glyceraldehyde (44) and were established by chemical degradations to be 2*R*, 3*S*, 4*S*, 5*S*, 6*S*, 8*R*, 10*R*, 11*S*, 12*R*, 13*R*. Both lankamycin (11) and the erythromycins (18) afford lactone rings which are configurationally in complete agreement with Celmer's model. This has been further substantiated in the case of erythromycin A by X-ray analysis (33). The configuration of the sugar substituents of leucomycin, as derived by Celmer (47), has recently been confirmed (28). Even the compound macrolide magnamycin (47) could be related to this proposed model. The relationship between methymycin and narbomycin (46) was confirmed, when it was shown that they possessed identical absolute configurations on corresponding asymmetric centers (40).

This configurational model suffered a weakness in that the assigned structure of picromycin (24) differed from methymycin in the stereochemistry involving C_2 to C_6 (40), although both macrolides are elaborated by the methymycin producers (45). Their structures were found to agree with the biogenetic model proposed by Vaněk (40). However, the structure of picromycin was recently revised simultaneously by Rickard *et al.* (48) and Muxfeldt *et al.* (49), who showed that the antibiotic required one more propionate unit as C_3 and C_4. The absolute configuration of the new asymmetric center (C_4) was not determined, but it appears that the previously (27)

assigned centers of 4*S*,6*R* are in fact 6*S*,8*R*, in full agreement with Celmer's model. Initial studies indicated that the configurational model is also applicable to polyene antibiotics. For instance, Ceder and Hansson (50) found the absolute configuration of C_{25} of pimaricin to be of the *R*-configuration, in agreement with corresponding centers of four other polyenes investigated. So far, all macrolide antibiotics examined possess the *R*-configuration of the carbon atom closing the lactone ring (42,50).

Grisebach (13) demonstrated that methylmalonyl-CoA is used in the biosynthesis of macrocyclic lactones. Lynen (51) showed that fatty-acid synthesase of yeast cannot utilize methylmalonyl-CoA for chain elongation; instead it acts as an inhibitor of fatty acid synthesis by the multiple enzyme-complex. The findings of Grisebach and Lynen appear to suggest that the biogenesis of fatty acids and macrolides are not related in spite of their structural similarities. The same conclusion was reached in biosynthetic studies of erythromycin and magnamycin (52). Corcoran (53) postulated a mutation in the enzyme structure of the actinomycetes such that methylmalonyl-CoA rather than malonyl-CoA is the preferred, or only, substance to produce macrolides.

It has been shown (13), that the formation of many macrolides is initiated by propionyl-CoA instead of acetyl-CoA.

Tracer experiments (13) with propionic acid -[1-^{14}C-3-T] as a precursor proved unequivocally that erythromycin is derived from seven propionate units, and that the methyl groups are not due to methionine. It was further demonstrated (13) with labeled precursors that the biosynthesis of erythronolide (erythromycin aglycone) involves one molecule of propionyl-CoA and six molecules of methylmalonyl-CoA. A scheme for the biosynthesis of erythronolide according to Grisebach (13) is presented in Figure II.

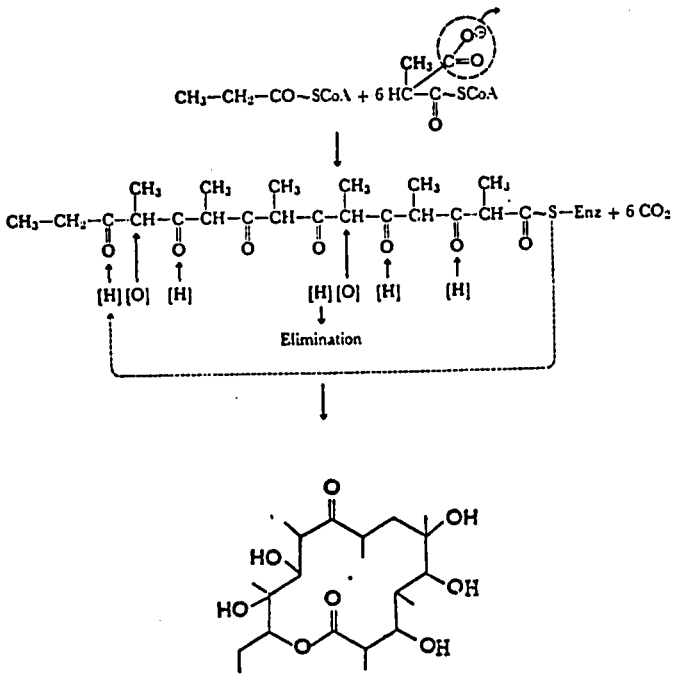


Figure II The Biosynthetic Pathway for Erythronolide

The biosynthetic investigations of methymycin (54) suggested the incorporation of one acetate and five propionate units into the lactone ring. The biosynthesis of the compound macrolide magnamycin was thought to involve a succinate unit in addition to propionate and acetate precursors (13,17). Recent investigations by Grisebach and Weber-Schilling (55), however, have shown that sodium succinate -[1,4-^{14}C] is incorporated to the extent of only 3%. Hence, succinate—CoA is an unlikely precursor of the C_5 to C_8 atoms of magnamycin.

The proposition (40) that the glycosidation reaction in the macrolide biosynthesis is one of the last steps, is supported by the isolation of the sugarfree macrolide, borrelidin (20), and of a sugarless compound (56) closely related to the erythromycin aglycone as a metabolite of *Streptomyces erythreus*. The biogenesis of macrolide sugars has not yet been established, but it appears (42) that D-glucose is the ultimate precursor. Vaněk (40) has presented a tentative pathway for the enzymic reactions employing D-glucose as the precursor. Celmer (42) noted a stereochemical pattern wherein all D-sugars are β-glycosides and all L-sugars are α-glycosides. A scheme was presented to account for the observed stereochemical regularity according to Klyne's rule (43).

Keller-Schierlein and co-workers (57) have studied the relationship between *Streptomyces* species and their products. They found that the microbial products are not species specific;

that is, a particular macrolide may be produced by several species, or a single species or even a strain of *Streptomyces* may elaborate several antibiotics.

E. Mode of Action of Macrolides

Macrolide antibiotics are of clinical importance; they are used in the chemotherapy of malignant tumors and microbial diseases (15). Furthermore they are used for the preservation of organic materials including foods and for the promotion of animal growth (9).

The microbiological activity shown by the macrolides is preferentially directed against Gram-positive bacteria, and these bacteria usually exhibit cross resistance to the macrolides (15). The antibiotics display markedly different inhibitory concentrations *in vitro*, and these differences may vary *in vivo* (15).

It has been shown (58) that the mechanism of action of the macrolides such as erythromycin and oleandomycin is related to the inhibition of protein synthesis by binding to the ribosomes. Vazquez (15) demonstrated that this inhibition may be due to either interference of the macrolide with the formation of the m-RNA-ribosomes-aminoacyl-t-RNA-complex, or with the growth of the peptide on the ribosomes.

Before the neutral macrolides were found, the activity was ascribed to the basicity of the antibiotics (15), i.e.

to the amino sugars. Since neutral macrolides were also shown to possess antibacterial activity (11,16), this hypothesis was abandoned. That sugars do play a rôle was demonstrated by a reduction in the activity upon chemical removal of the sugar moieties (15). Recently, Poralla and Zähner (59) claimed that the sugarless macrolide borrelidin blocks protein synthesis via inhibition of the incorporation of amino acids with t-RNA. Other macrolides so far investigated show inhibition after formation of aminoacyl-t-RNA (15).

Celmer (42) related the alleged common mode of action of the macrolides, despite their structural differences, to their configurational and chirality properties by suggesting a "template or close-fit hypothesis." Thus, differences in inhibitory concentrations between different macrolides (15) were thought to be due to variations in "fit" at some active site (60).

F. Production and Isolation of Peliomycin

Peliomycin (NCS 76455D) is produced by a streptomycete culture *Streptomyces luteogriseus* Sp.n., which was isolated from an alfalfa field in France (61). The organism is on deposit as ATCC No. 15072 at the American Type Culture Collection, Washington, D.C., U.S.A.

This actinomycete produced yellowish-gray aerial mycelium and beige-colored diffusible pigments in

proteinaceous media (61). In addition, the culture was characterized by the formation of catenulate, smooth-walled, ovoid spores. These spores were arranged in compact to extended spirals on short side branches located along axial hyphae (62).

Peliomycin may be produced in shake-flask cultures as described by Schmitz *et al.* (62). The flask fermentation was used to seed production media in fermentators. The toxicity of peliomycin for HeLa cell cultures was used as an assay in its isolation, and showed that most of the activity was present in the mycelium (61). The antibiotic was extracted into methyl alcohol, transferred into ethyl acetate and purified by counter-current distribution in a Craig machine. Peliomycin thus purified could be crystallized from ether-ligroin and from 50% ethyl alcohol-water. The latter system gave colorless hexagonal plates, m.p. 160-164°C, $[\alpha]_D^{25}$ -74.2° ($c = 1$, chloroform) (62).

G. Biological Properties of Peliomycin

Peliomycin is a cytotoxic antibiotic with limited antimicrobial properties (61). *In vitro* experiments demonstrated the agent to be significantly toxic for all six neoplastic tissue lines studied (61). However, peliomycin did not show marked antineoplastic effects in rodents, although highly purified peliomycin may cause

slight inhibition of Sarcoma 180 in Swiss mice (62). The responses tended to be somewhat inconsistent, a fact attributed to the insolubility of the antibiotic in aqueous media.

Weak antibacterial action against microorganisms was displayed by peliomycin. The activity shown is essentially limited to Gram-positive organisms such as *Micrococcus lysodeikticus* (61), whose growth was inhibited by a peliomycin concentration of 1.6 µg/ml; this was the most sensitive bacterial strain tested.

H. Characterization of Peliomycin

The purified peliomycin appeared to be homogeneous as only one zone of inhibition was observed when paper-strip chromatograms in fifteen solvent systems were placed on agar seeded with either *Micrococcus lysodeikticus* or *Kloeckera brevis* (62). Thin-layer chromatograms on silica gel showed a single spot when developed with ligroin: ethyl acetate (1:1) and sprayed with aqueous permanganate or upon exposure to iodine (62). However, the sample of peliomycin which was provided for this investigation in a similar chromatogram developed with chloroform: acetone (9:1) showed a shadow of somewhat lower R_f value than the main component, when charred with aqueous sulfuric acid.

The antibiotic was reported (62) to be very soluble in most organic solvents, slightly soluble in hydrocarbon solvents, but insoluble in water, nitromethane, aqueous sodium hydroxide and aqueous mineral acid. The compound was found (62) to be stable in aqueous alcohol for two hours over a pH range of 1.3 to 9.2; at pH 10.2 the biological activity was considerably reduced.

A solution of peliomycin in chloroform exhibited a shoulder at 295 mμ, and a concentrated sulfuric acid solution produced a maximum at 280 mμ (62). The infrared spectrum showed absorption peaks indicative of hydroxyl and carbonyl groups (62). Solutions of the antibiotic in carbon tetrachloride were found (62) to decolorize bromine, and solutions in acetone decolorized potassium permanganate. The ferric hydroxamate test for esters proved positive (62).

The reported (62) elemental analysis and molecular weight determination of peliomycin gave results corresponding to $C_{46}H_{76}O_{14}$ with a molecular weight of 853.1. Kuhn-Roth oxidations indicated eight C-methyl groups. Similar analysis of the acetate derivative of peliomycin gave results in very good agreement with the above values (62). Evidence from acetyl determination supported a pentaacetate derivative.

Table I shows a comparison of some of the physical constants and the biological activity of the neutral macrolides thus far reported. The structures of chalcomycin (16) and lankamycin (11) have been established.

TABLE I

Comparison of Properties of Neutral Macrolides [d]

	$[\alpha]_D$ in° CH_3OH	m.p. °C	U.V. (mμ, CH_3OH) λ Max.	λ Sh.	Formula or M.W.	Active against	Ref.
Aldgamycin E	-56, 1 [a]	158-161, 173-178	216		769-813	G+	63
Azalomycin B	-48, 1	210-212 dec.	252		$C_{14}H_{24}O_5$	G+	34
Bandamycin A	-59, 2	142-144 dec.	c		∿ 500	G+	9
Bandamycin B	-49, 2	107-109 dec.	218	240,250	∿ 500	G+	9
Cineromycin B	-110, 1	149-150	c		$C_{17}H_{26}O_4$	G+	9
Chalcomycin	-44[b], 1	121-123	218[b]	240[b]	$C_{35}H_{56}O_{14}$	G+ & G-	16
Lankamycin	-94 , 1	147-150, 181-182	289		$C_{42}H_{72}O_{16}$	G+ & G-	11
Megacidin	-51[b], 1	162-164	217		$C_{24}H_{38}O_{10}$	B.mega-therium	9
Mikinomycin	-40[b], -	111-116	219	240,290	-	G+	9
Neutramycin	-35[b], 1	222-223	216	240	$C_{34}H_{56}O_{14}$	G+	9
Oligomycin	-49.5, 1	169-170	220,225,232[b]	240,295[b]	-	Fungi	64
Peliomycin	-54 , 1	160-164	218,228,232	242,282	$C_{52}H_{80}O_{14}$	G+	e

(a) concentration in g/100ml (b) ethyl alcohol (c) end absorption only

(d) includes suspect-macrolides (e) present work

I. Purpose of Present Research

A knowledge of the structures and the configurations of macrolide antibiotics is of interest to the eventual understanding of both their modes of action and their biogenesis. Such detailed comprehension may lead in turn to the development of semi-synthetic antibiotics, or to the modification of existing ones, such that all the advantages, but none of the deleterious effects are retained.

Due to the size and the complexity of the macrolide molecules, and their tendency to give intractable mixtures of products (14,34,65) structure elucidation should be initiated with a thorough investigation of the structural features by all physical methods available prior to controlled degradations. Since X-ray analysis is often not feasible (34,35,65), the physical examination should be followed by a "fragmentation analysis." This procedure consists of degrading the antibiotic to small compounds by chemical reactions, separation of the fragments and the characterization of each by physical means, including high-resolution mass spectrometry and high-resolution nuclear magnetic resonance (n.m.r.) spectroscopy. The identity of each compound should be verified by synthesis. The physical data may prove useful in similar studies with related antibiotics, for instance oligomycin B (64,65). The methods established in this research can be expected to be of importance in this rapidly expanding area of natural products. They should prove useful not only as a prelude to a detailed structural investigation, but also in biogenetic studies employing labeled precursors.

II DISCUSSION OF RESULTS

A. Molecular Formula of Peliomycin

Peliomycin is a crystalline compound which can be recrystallized from aqueous ethyl alcohol to give colorless hexagonal plates which melt at 160-164°C (62). This range can be explained by the strong tendency of the crystal lattice to retain solvent of crystallization. A quartet at τ6.32 in the p.m.r. spectrum run in *deuterio*-chloroform indicated the presence of ethyl alcohol (Figure III). Indeed, after the antibiotic had been dissolved in absolute chloroform and the solvent evaporated, this quartet had disappeared (Figure IV). However, traces of chloroform were also tightly held and were still discernible (p.m.r.) after drying the amorphous compound at 1 mm Hg and 40°C for five days. This observation was verified by a positive chlorine analysis of the amorphous compound, an analysis which proved negative for the original sample of peliomycin prior to the chloroform treatment.

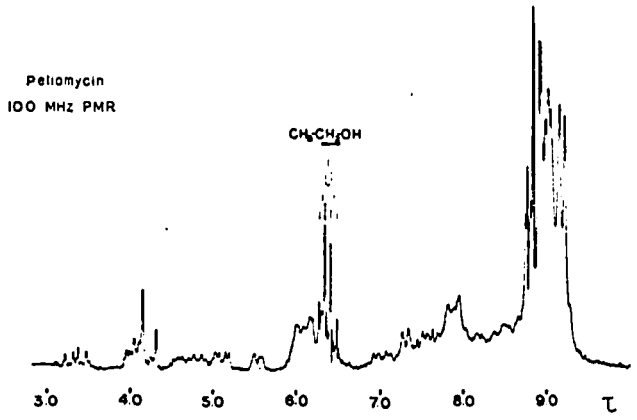


Figure III 100 MHz P.M.R. Spectrum of Peliomycin containing Ethyl Alcohol ($CDCl_3$)

The elemental analyses (C, H, O and Cl) were performed in duplicate on two samples of peliomycin, each treated with chloroform to remove solvent of crystallization. These results are presented in Table II.

TABLE II

Elemental Analyses of the Peliomycin - Chloroform Complex

	% C	% H	% O	% Cl	Total
Sample 1 [a]	63.26	8.73	22.79	3.43	
	63.12	8.63	22.86	-	
Average	63.19	8.68	22.83	3.43	98.13
Sample 2 [b]	64.90	8.28	23.08	1.79	
	64.99	8.42	23.30	1.77	
Average	64.94	8.35	23.19	1.78	98.26

(a) Mr. A.B. Gygli, Microanalyses Laboratory, Toronto, Ontario.

(b) Dr. F. Pascher, Mikroanalytisches Laboratorium, Bonn, Germany.

As seen in Table II, the analyses in both cases account for 98.2% of the sample. It was shown (62) that peliomycin contains only the elements carbon, hydrogen and oxygen. A possible explanation for this discrepancy was the presence of 1.8% of non-analyzable material such as an inorganic salt, traces of which may have chelated with the polyhydroxy compound.

The calculations concerning the elemental composition of peliomycin, discussed below, were based on a sample weight of 100 g. Since the elemental analyses are only used to furnish a carbon to oxygen ratio and a hydrogen content, the former is not affected, while the hydrogen content remains within the limits set below (see Table III).

The presence of chloroform in the analytical samples offered advantage over ethyl alcohol in that it allowed the determination of the percentage solvent from chlorine analysis. These analyses showed that samples 1 and 2 contain 3.85% and 2.00% chloroform, respectively. Calculations based on these values indicate that sample 1 contained 0.39% carbon and 0.32% hydrogen, and sample 2 contained 0.20% carbon and 0.02% hydrogen due to the chloroform. The elemental composition of peliomycin was then computed by adjusting the weight to solvent-free samples. These results, together with the carbon to oxygen ratios are presented in Table III.

TABLE III

Elemental Composition of Peliomycin

	% C	% H	% O	C/O
Sample 1 (100% sample weight)	65.31	8.82	23.74	3.66
" (98.13% sample weight)	66.60	9.16	24.24	3.66
Sample 2 (100% sample weight)	66.06	8.50	23.66	3.72
" (98.26% sample weight)	67.26	8.65	24.09	3.72

Of these samples, the percentages of carbon and oxygen are the most significant. Assuming an error of 1 part in 300 for these determinations, the true carbon to oxygen ratio for peliomycin should be within the range 3.62 to 3.76. A limitation on the molecular formula is furnished by the integration of the 220 MHz p.m.r. spectrum of peliomycin (Figure IV). This spectrum reveals a number of signals with structures and intensities that can only be interpreted as arising from single hydrogens. Integration over the region τ3.0 to τ5.8 showed unequivocally the presence of eight hydrogens. By extending the integration from τ5.8 to τ8.5, where the signal tracing reaches the baseline, at least 35 more hydrogens are present. The methyl region of the 220 MHz spectrum (sweep width of 500 Hz; Figure IV), exhibited signals which represent eleven methyl groups. Therefore, peliomycin contains at least 76 and probably not more than 82 hydrogens, as only some weak signals not attributable to methyl groups are observed above τ8.5. The hydrogen content as determined by elemental analysis (Table III) is of limited value in calculating the molecular formula. However, it may be expected from these determinations that the hydrogen content of peliomycin is in the range of 8.4 to 9.4%.

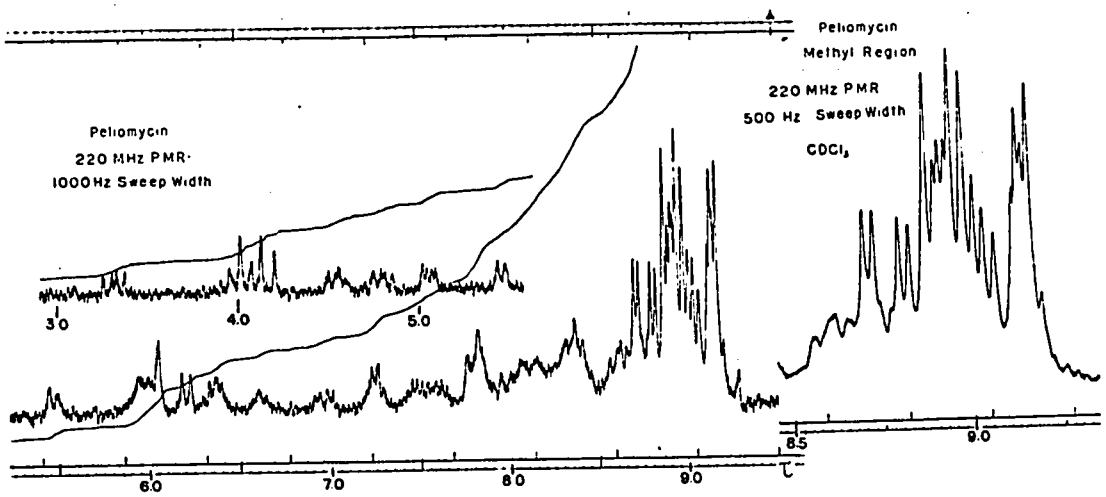


Figure IV 220 MHz P.M.R. Spectrum of Peliomycin ($CDCl_3$, 1000 Hz Sweep Width) and its Methyl Region ($CDCl_3$, 500 Hz Sweep Width)

Based on the above considerations, possible molecular formulas were calculated by solving for X and Y of the following simultaneous equations using the limits set by the carbon to oxygen ratio and the hydrogen content.

$$X/Y = 3.62 \text{ to } 3.76$$

$$\frac{76 \text{ to } 82}{12X + (76 \text{ to } 82) + 16Y} = 8.4 \text{ to } 9.4$$

X = number of carbons and

Y = number of oxygens in the formula for peliomycin.

These calculations require peliomycin to have a molecular formula in the range $C_{46.4}H_{76}O_{12.3}$ to $C_{54.5}H_{82}O_{15.5}$. However, not every possible combination in this range remains within the limitations set above, and only the three molecular formulas which comply with all the restrictions are included in Table IV.

TABLE IV

Possible Molecular Formulas of Peliomycin

No.	C	O	H	Mol. Wt.
1	48	13	76-80	860-864
2	51	14	78-82	914-918
3	52	14	78-82	926-930

It is seen that the molecular weight for peliomycin determined in this way is in good agreement with the reported (62) value of 856, determined by the thermoelectric method. This is especially significant, since it is now known that the original peliomycin contained ethyl alcohol which would result in an apparent smaller molecular weight.

In an attempt to obtain an unequivocal molecular formula, peliomycin was subjected to mass spectral analysis. However, it failed to give a molecular ion, and the fragmentation

pattern was not informative. Nevertheless, the data proved useful in limiting the molecular weight of peliomycin to a value of not less than 786 (Figure V). The reported (65) mass spectrum of oligomycin B, in which the highest peak also appeared at *m/e* 786, showed a close resemblance to that of peliomycin.

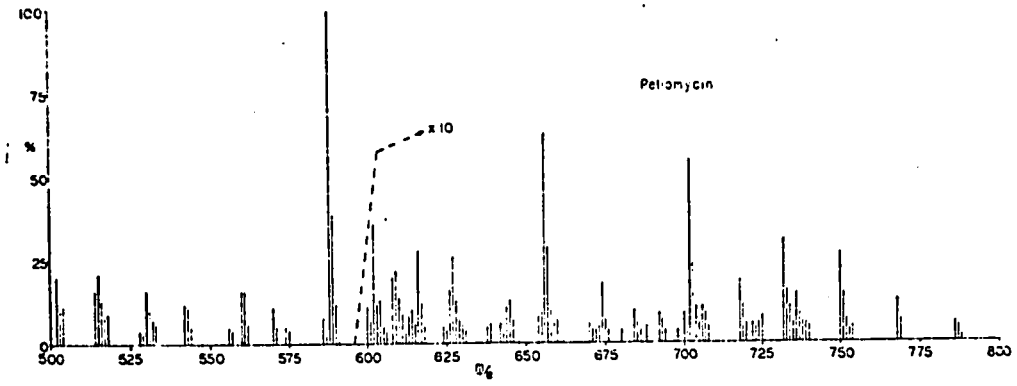
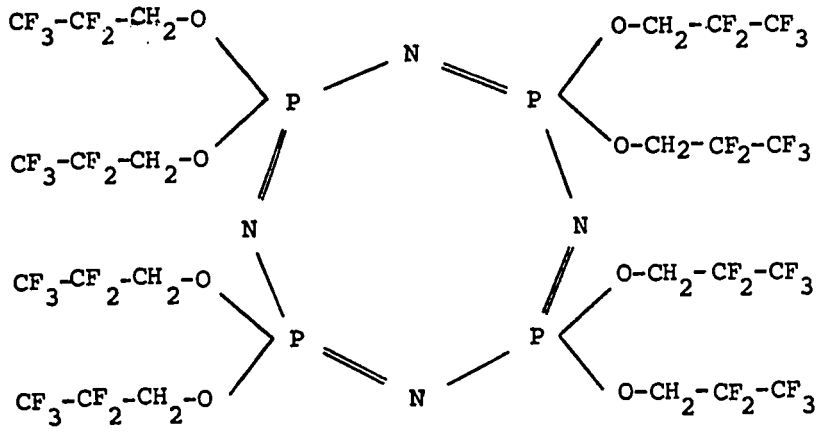


Figure V Mass Spectrum of Peliomycin (Source Temp. 200°C)

A major problem encountered in direct mass spectrometry of many polyhydroxy compounds is the prevention of dehydration and decomposition at temperatures required to obtain an adequate vapor pressure (30). In recent years, the molecular formulas of some polyene antibiotics have been successfully determined via their pertrimethylsilyl ethers (31), and the fragmentation pattern of these derivatives have been well investigated (66,67).

A difficulty which arises in high resolution mass measurements of compounds with high molecular weights is the availability of mass markers. Rickards (30,31) in his studies on polyene antibiotics

used "Fluorolube" (68), but this fluorinated hydrocarbon is no longer available. It was suggested (69) that the undecatrimethylsilyl derivative of raffinose (mol. wt. = 1296) could be used as a convenient mass marker. However, when synthesized, this derivative gave a mass spectrum which proved useless in our hands, as it failed to give a molecular ion or easily identifiable fragments. This problem was solved by using 2,2,4,4,6,6,8,8-octa-(2,2,3,3,3-pentafluoropropyloxy)-cyclotetraphosphazatetraene* (I) as mass marker (mol. wt. = 1372).



I

At first, the pertrimethylsilyl derivative of peliomycin was prepared according to Sweeley (70) and Rickards (30), but infrared analysis of the product showed that conversion was incomplete (hydroxyl band at 3400 cm^{-1}). Schmitz and co-workers (62) have reported hydroxyl absorption by their crystalline penta-*O*-acetyl derivative of peliomycin. Since an acetyl determination (62)

*We are grateful to Dr. G.W.A. Milne, National Heart Institute, N.I.H., Bethesda, Md., for the gift, and information on its fragmentation pattern.

indicated five acetate groups, peliomycin must contain more than five alcohol functions.

Further information on the number of hydroxyl groups was sought from p.m.r. spectroscopy. When peliomycin was thoroughly exchanged by shaking a solution in *deuterio*-chloroform with deuterium oxide, the intensity of the signals in the range τ6.8 to τ8.0 decreased by at least five protons. No further decrease was observed in a second exchange. When the exchanged sample in the *deuterio*-chloroform was repeatedly protonated with water, the peliomycin isolated, dried and the p.m.r. spectrum redetermined in *deuterio*-chloroform, the spectrum of the original sample was reproduced. Therefore, it is concluded that peliomycin in all probability contains not more than six hydroxyl groups. However, the possibility that peliomycin contains more than six hydroxyl groups will be considered subsequently.

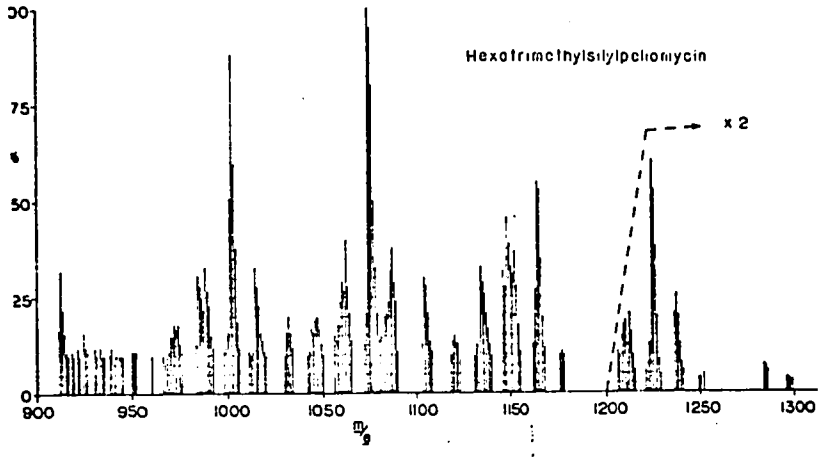


Figure VI Mass Spectrum of Hexatrimethylsilylpeliomycin (Source Temp. 210°C)

When peliomycin was heated under reflux with hexadimethylsilazane in dry dimethylsulfoxide (71), a product was isolated which was free from hydroxyl absorption bands (infrared). The highest peaks observed in the mass spectrum of this compound were at *m/e* 1296 and 1284 (Figure VI). The highest peak most suitable for high resolution mass measurement was at *m/e* 1224 and measured 1224.7046. The possible formulas which fit this measured mass were determined by an IBM 360\67 computer and are presented in Table V. The data with impossible combinations of the elements were discarded. The limit of error permitted was 5 p.p.m., and only the elements C,H,O and Si were considered. The search was limited to a maximum of sixteen oxygen atoms, so as to comply with the carbon to oxygen ratio and the approximate molecular weight range, and seven silicon atoms, for the reasons discussed below.

TABLE V

Mass Spectral Results of Hexatrimethylsilylpeliomycin

Possible Formulas which fit *m/e* 1224.7046

No.	C	H	O	Si	Error-p.p.m.
1	66	108	15	3	4
2	62	112	16	4	1
3	65	112	12	5	4
4	61	116	13	6	1
5	64	116	9	7	3
6	57	120	14	7	0

As seen earlier, peliomycin may contain more than five hydroxyl groups, and the highest peak in the mass spectrum of the trimethylsilyl derivative was found at *m/e* 1296. If seven or more trimethylsilyl groups are present, then peliomycin cannot have a molecular weight greater than 1296-7x72 = 792. The highest peak in the mass spectrum of peliomycin appears at *m/e* 786 which is only six mass units less. This would imply, that with seven or more hydroxyl groups, peliomycin has a similar or greater stability in the mass spectrometer than its trimethylsilyl derivative; such a possibility is very unlikely for polyhydroxy compounds. The anomaly would be even further accentuated if *m/e* 1296 is not the molecular ion, but a breakdown fragment with seven or more trimethylsilyl units. Hence, formulas number 5 and 6 of Table V are discarded, and it appears that peliomycin contains six hydroxyl groups.

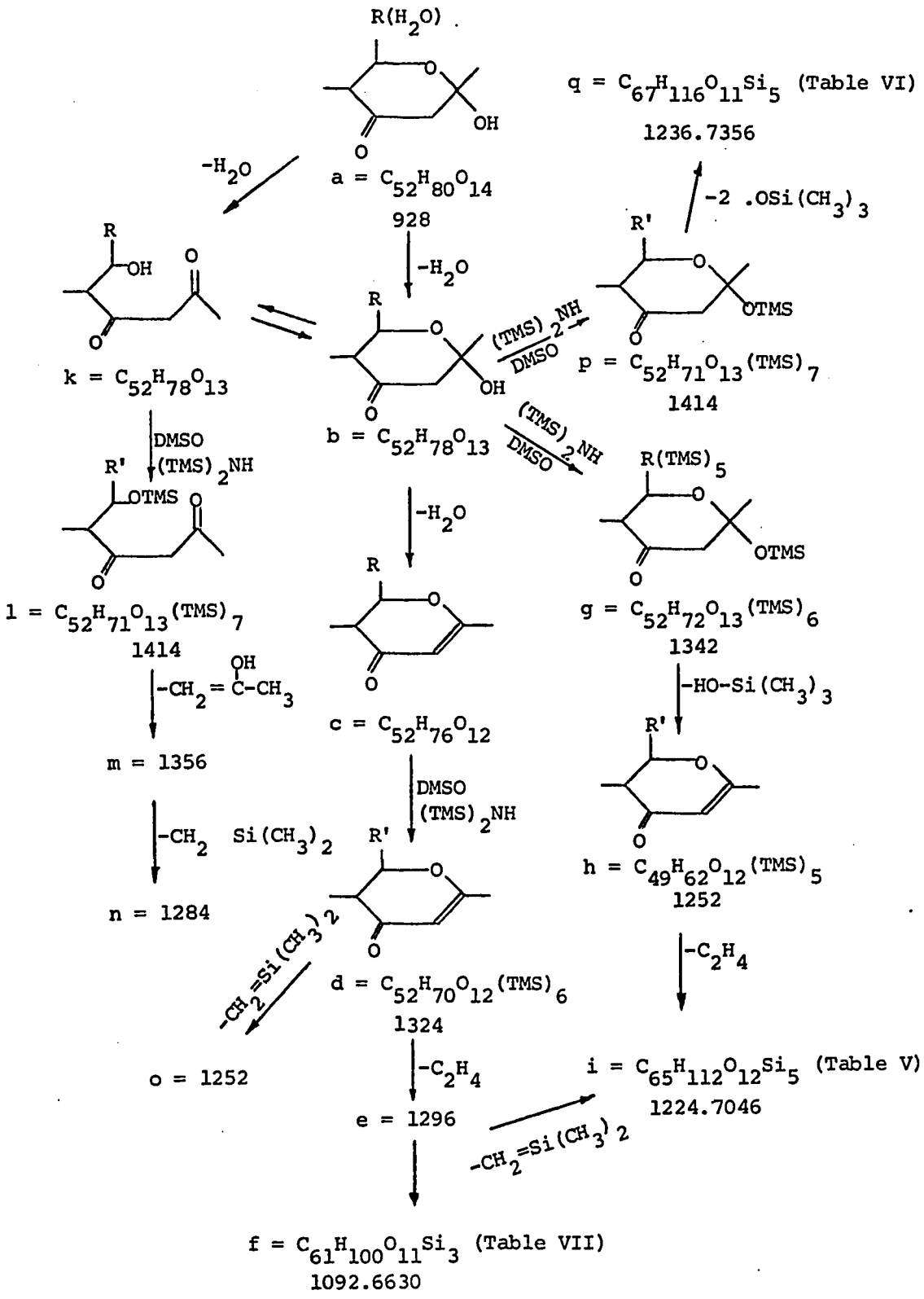
In order to arrive at a possible molecular formula for peliomycin, a correlation must hold such that a trimethylsilyl derivative of a formula listed in Table IV will give a formula in Table V as a reasonable breakdown product. In view of the conditions which led to the formulas in Table IV, formulas 1 and 2 of Table V can be discarded as they contain too many oxygen atoms. Hence, only formulas 3 and 4 of Table V will be considered henceforth. Formulas 1 of Table IV allow the molecular formula for peliomycin

to be $C_{48}H_{80}O_{13}$, in which case the molecular ion of the hexatrimethylsilyl derivative will coincide with the highest peak in the mass spectrum, *viz.*: *m/e* 1296. Only formula 4 of Table V can reasonably be correlated with the above derivative, but this would necessitate the unlikely elimination of the saturated fragment C_5H_{12} to give the fragment ion at *m/e* 1224. This elimination is even more unlikely in the light of p.m.r. observations to be presented later, which, together with degradative evidence, led to the "Heuristic Formula of Peliomycin" (Figure XXXII).

The two remaining formulas 2 and 3 of Table IV remain to be considered. The hexatrimethylsilyl derivative of formulas 2 cannot reasonably be correlated with formula 3 of Table V, since it requires the loss of a trimethylsilanol unit and the elements $CH_{4-8}O$. The latter could be attributed to loss of methyl alcohol, but most examples known involve methyl esters (72) and methyl ethers (78). The p.m.r. spectrum of peliomycin does not support these structures (no singlets at τ6.3 and τ6.6), therefore a breakdown of this type is unlikely for peliomycin. Formula 4 of Table V can accomodate the hexatrimethylsilyl ethers of formulas 2 of Table IV via the loss of $C_8H_{10-14}O$. This fragmentation seems less probable on the basis of p.m.r. spectroscopic studies to be discussed later and the extensive oxygenation of peliomycin. For these reasons, formulas 3 of Table IV

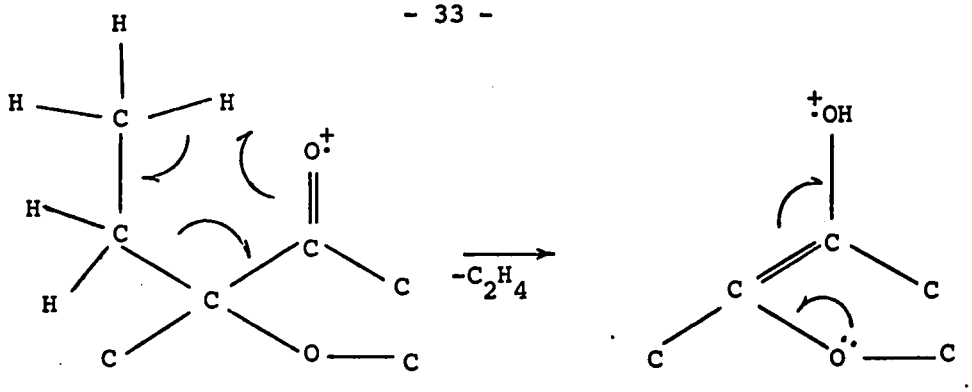
are the remaining alternatives.

The pertrimethylsilyl derivatives of formulas 3 of Table IV can be correlated with formula 3 of Table V by the loss of $C_9H_{10-14}O$. This elimination from the parent ion does not seem probable on the basis of p.m.r. evidence and the distribution of oxygen in the molecule, as mentioned before. The hexatrimethylsilyl ether ($C_{70}H_{128}O_{14}Si_6$, mol. wt. 1360) of $C_{52}H_{80}O_{14}$ can give rise to formula 3 of Table V for *m/e* 1224 by the elimination of a trimethylsilanol unit and the elements C_2H_6O, consisting of an ethylene group and a water molecule. As shown in Scheme I, this breakdown may follow at least two routes compatible with the formation of *m/e* 1224 (route a-e, i and route a,b,g-i), one of which (route a-e, i) leads also to the highest observed peak at *m/e* 1296. The latter peak may have arisen from the molecular ion by the loss of two water molecules and an ethylene group. The dehydration may have occurred during the preparation of the fully trimethylsilylated peliomycin by heating under reflux in dimethylsulfoxide. The ejection of ethylene may have occurred in the mass spectrometer via a McLafferty rearrangement (73). This is quite possible in partial structure II, where the ethereal oxygen can stabilize the resulting structure III.



Scheme I Proposed Fragmentation Scheme for Hexatrimethylsilylpeliomycin

R' = Hexatrimethylsilyl residue
TMS = Trimethylsilyl group



II III

Evidence for the ethyl group is supplied by the 220 MHz p.m.r. spectrum (Figure VII) which exhibits a well resolved triplet at τ9.17 (pyridine-d_5) and a quartet at τ6.35 ($CDCl_3$).

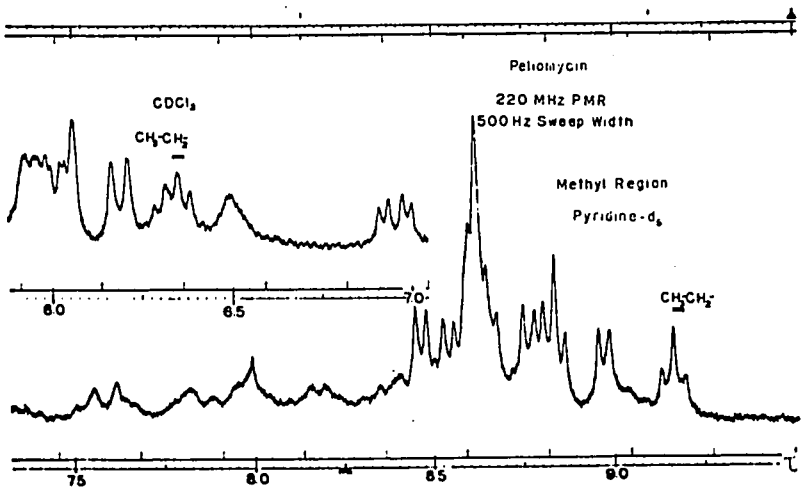
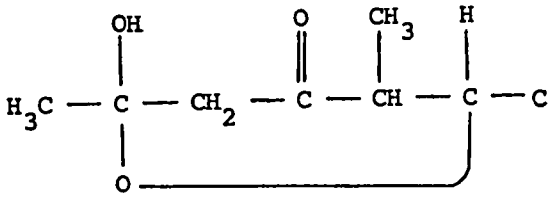


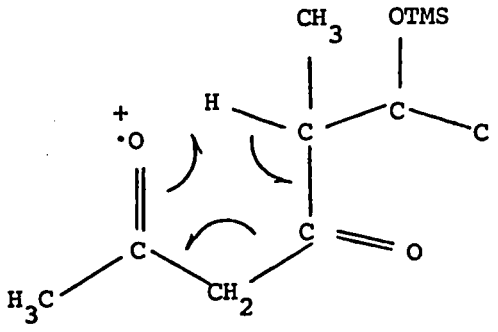
Figure VII 220 MHz P.M.R. Spectra of Methyl Region (Pyridine-d_5) and τ6 - τ7 Region (Insert, $CDCl_3$) of Peliomycin

The peak at *m/e* 1284 may have arisen via the loss of an acetone function in addition to an ethylene and a water molecule (Scheme I, route a,k-n). The presence of an acetyl group is indicated by the liberation of acetic acid when peliomycin is treated with the periodate-permanganate oxidation couple (74, 75). This reagent readily cleaves olefinic bonds; however, no olefinic methyl group could be observed in the p.m.r. spectrum. Peliomycin gave a negative iodoform-test, which may be due to an adjacent, highly activated methylene group (76). The acetyl group is further suggested by the intense peak at *m/e* 43 in the mass spectrum of free peliomycin (73). It is possible that the acetyl function is part of a hemi-ketal ring structure, as this would account for the absence of the acetyl methyl signal at τ8 in the p.m.r. spectrum. Rather, the singlet of relative intensity 3 at τ8.8 could arise from the methyl group in such a structural unit IV (77).



IV

This structural group should readily eliminate water, especially under the strong dehydrating conditions of the trimethylsilylation reaction. When present in the open chain form, the resulting trimethylsilylated methyl ketone can easily eject acetone (73) as shown in V.



V

Although less intense than *m/e* 1224, the peak at *m/e* 1236 was also measured and analyzed by computer under the same conditions as for *m/e* 1224 (Table V). The results are presented in Table VI.

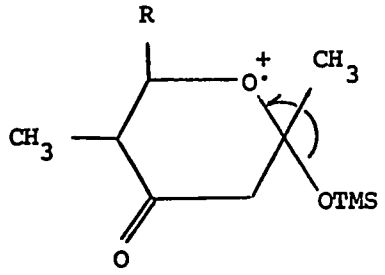
TABLE VI

Mass Spectral Results of Hexatrimethylsilylpeliomycin

Possible Formulas which fit *m/e* 1236.7310

No.	C	H	O	Si	Error-p.p.m.
1	75	108	9	3	0
2	68	112	14	3	3
3	71	112	10	4	1
4	67	116	11	5	3
5	66	120	8	7	4

Computations showed that formulas 1,2,3 and 5 of this table cannot be associated with any of the molecular formulas of Table IV, because they contain either too many carbon, oxygen or silicon atoms. Only formula 4 of Table VI can be correlated with the molecular ion postulated ($C_{52}H_{80}O_{14}$). The possible heptatrimethylsilyl ether of this molecular ion could lose trimethylsilyloxy fragments from two such structural units as shown in partial structure VI and in Scheme I, route a,b,p,q.



VI

Although no studies appear to have been made on this type of compound, the driving force for this reaction could be due to the stability of the resulting ring structure.

The mass of *m/e* 1092 was determined, because it was found as the highest intense peak in the mass spectrum of hexatrimethylsilylpeliomycin (Figure VI) prepared according to Sweeley (70) and Rickards (30). The measured mass was again analyzed using the limitations set for *m/e* 1224 of Table V. The results of the analysis are recorded in Table VII.

TABLE VII

Mass Spectral Results of Hexatrimethylsilylpeliomycin

Possible Formulas which fit *m/e* 1092.6601

No.	C	H	O	Si	Error-p.p.m.
1	61	100	11	3	2
2	54	104	16	3	2
3	57	104	12	4	0
4	60	104	8	5	2
5	53	108	13	6	2
6	56	108	9	6	2
7	52	112	10	7	3

The only reasonable correlation possible between a formula of Table VII and one of those in Tables V or VI is the elimination of two $CH_2{=}Si(CH_3)_2$ units from formula 4 of Table VI to give formula 1 of Table VII (Scheme I, route a-f). As seen before, formula 4 of Table VI was also the only formula that could be associated with the hexatrimethylsilyl derivative of the molecular ion $C_{70}H_{128}O_{14}Si_6$. The elimination of $CH_2{=}Si(CH_3)_2$, which involves a Si-O bond, rather than a C-O bond cleavage, is often observed for trimethylsilyl ethers (66).

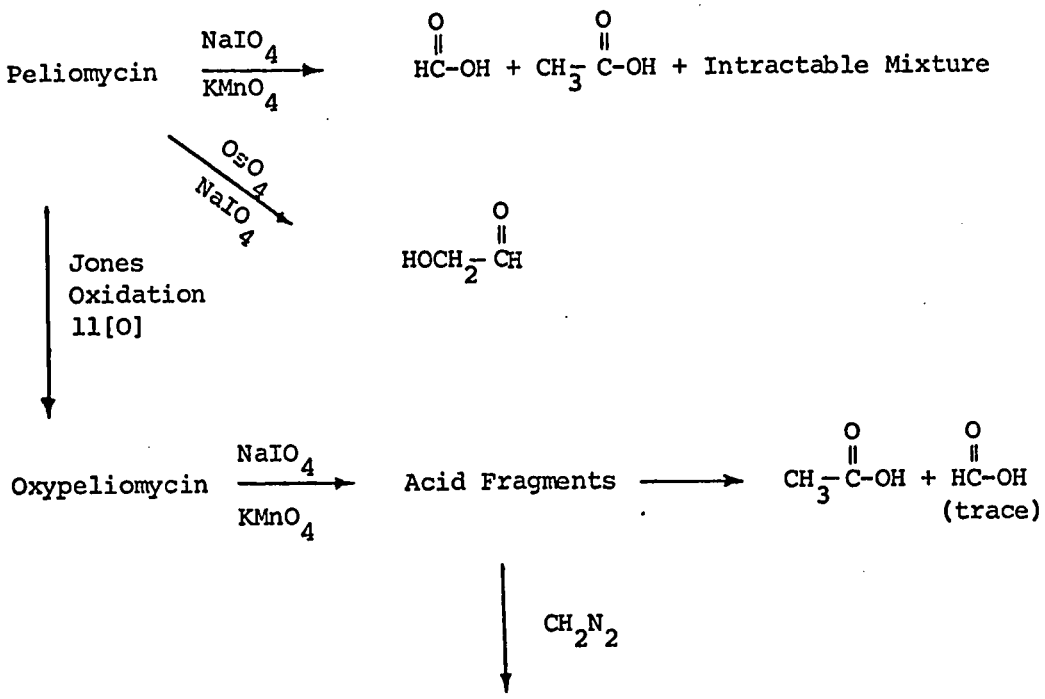
The remaining peaks in the mass spectrum of hexatrimethylsilylpeliomycin (Figure VI) can be explained (66, 67) in terms of successive losses of 90 [HO-Si-$(CH_3)_3$], 72 [$CH_2{=}Si(CH_3)_2$] or 15 (CH_3) mass units from the peaks

established in Scheme I.

In view of the discussions above, it is *tentatively* proposed that peliomycin has the molecular formula $C_{52}H_{80}O_{14}$ with a molecular weight of 928.

B. Degradative Studies of Peliomycin

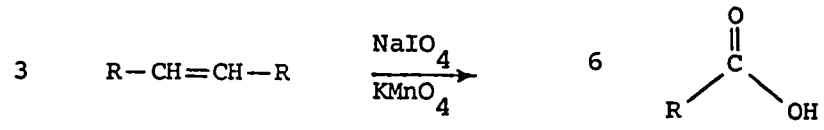
The foregoing evidence suggests that peliomycin, with a molecular formula of perhaps $C_{52}H_{80}O_{14}$, has a structure of very considerable complexity. Therefore, it seemed desirable to subject the molecule to deep-seated fragmentation, and obtain, through preparative gas chromatography, compounds whose structures could be characterized by modern physical methods. Since only 3.5 grams of peliomycin were available for these studies, the degradations were necessarily conducted on a semi-micro scale. The following scheme was employed for these fragmentation analyses of peliomycin.



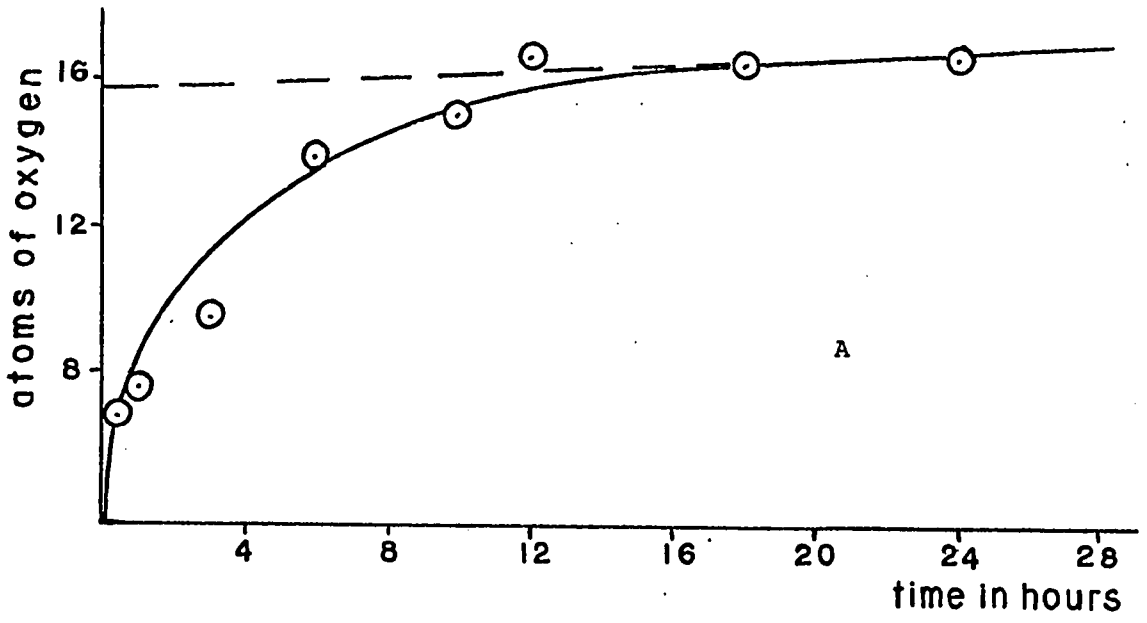
Scheme II Oxidative Degradation of Peliomycin

Peliomycin was shown to contain olefinic bonds (62). This observation was substantiated by catalytic hydrogenation, which caused the disappearance of 6 protons below τ4.9 in the p.m.r. spectrum, as will be seen later. For this reason, the degradative attack was directed towards the olefinic bonds which are readily cleaved by sodium *meta*-periodate containing a catalytic amount of potassium permanganate and buffered at pH 7.7 (74). In the absence of other oxidizable functions (74, 79), the amount of oxygen consumed in this reaction should be indicative of the extent of cleavage.

When peliomycin was subjected to the above oxidation couple, the uptake of oxygen was found to level off at 15 atoms of oxygen per mole of peliomycin (Figure VIII) with consistent results. Since the oxidation proceeds to the carboxylic acid stage (74), twelve oxygen atoms are required for the six olefinic protons shown to be present.



The remaining three oxygens may be ascribed to other oxidizable groups such as β-diketones, acyloins and others (79).



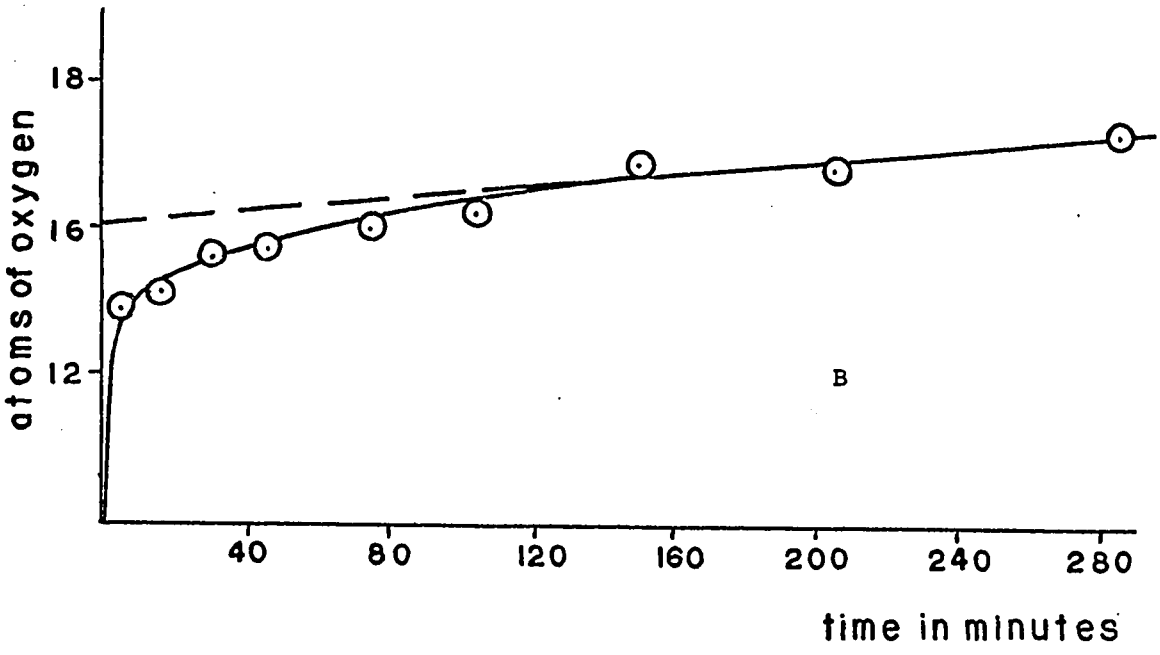


Figure VIII Quantitative Oxidation of Peliomycin.

A. Periodate-Permanganate Oxidation

B. Chromium Trioxide-Acetic Acid Oxidation

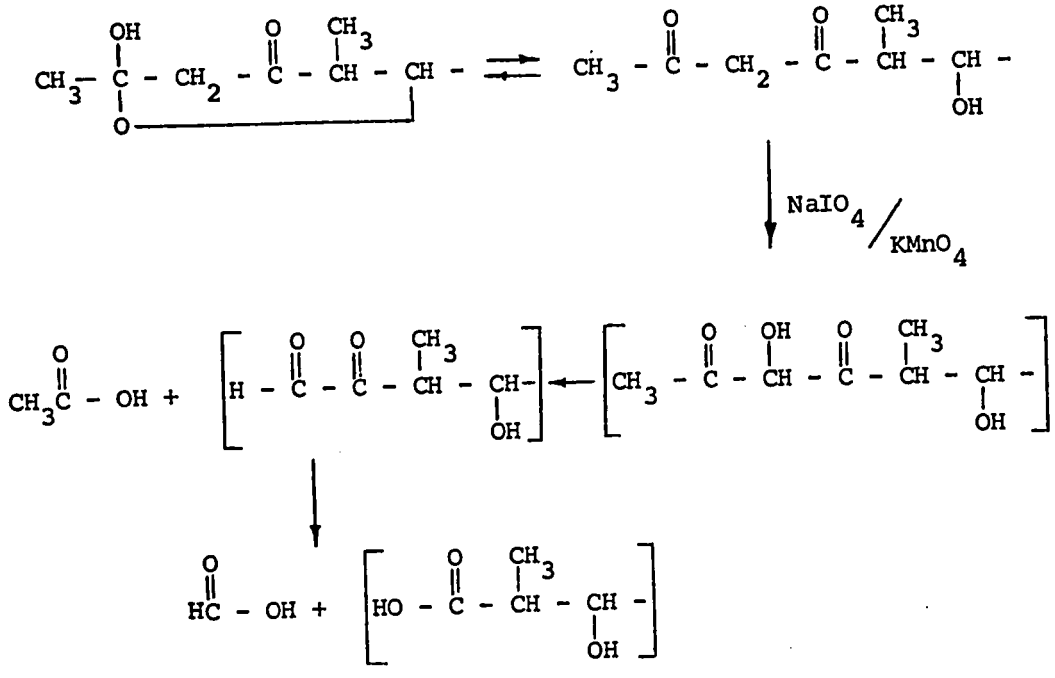
Further support for the reliability of the oxygen uptake was furnished when peliomycin was oxidized with chromium trioxide in acetic acid (80). In this reaction, one mole of peliomycin consumed sixteen atoms of oxygen (Figure VIII), which is in good agreement with the value obtained with the periodate-permanganate oxidation couple.

On the basis of these preliminary studies, peliomycin was oxidized with the periodate-permanganate reagent in aqueous *tert*-butyl alcohol (75), but initial experiments indicated extensive degradation.

In an attempt to prevent over-oxidation, the amount of periodate necessary was based on the uptake of sixteen oxygen atoms, as shown in Figure VIII. The oxidation mixture was allowed to stand at room temperature for fifteen hours, whereupon the permanganate color was wholly discharged. When additional permanganate did not reduce within thirty minutes, the oxidation was considered complete. After removal of solvent, which was shown not to contain any volatile fragments, the residue was acidified with sulfuric acid. The distillate obtained by freeze-drying this mixture was titrated with sodium hydroxide and found to consume 0.4 moles of base per mole of peliomycin. The resulting salt solution was concentrated to dryness, exchanged with deuterium oxide and subjected to p.m.r. and infrared analyses. When compared with an

authentic mixture, it was shown that the product obtained was an equimolar mixture of formic and acetic acids. The base consumption established that not more than one mole of each of formic and acetic acid was liberated in the oxidation reaction.

The formation of acetic acid may have been caused by a masked methyl ketone suitably situated, since p.m.r. studies showed that no olefinic methyl groups are present in peliomycin. As was postulated in the discussion on mass spectral analysis, the methyl ketone may be present in the hemi-ketal structure given, which may give rise to acetic acid and formic acid as shown.



From the residual fraction, after separation of the formic and acetic acids, no identifiable fragments could be obtained. This fraction was subjected to partition chromatography (81) followed by further separation employing thin layer chromatography. In the latter technique, buffered plates were used for the free acids of the oxidation mixture, but extensive streaking was still observed. Attempts to separate the fragments as acetylated or trimethylsilylated methyl esters failed. Extensive thin layer chromatography of the methyl esters using both Silica Gel and microcrystalline cellulose as supports allowed the separation of twenty six fractions. These were further purified by sublimation and then submitted for mass spectral analysis. In no case could a molecular ion be detected. Each fraction gave a different fragmentation pattern of which the intensity of the peaks gradually decreased giving ions of at least *m/e* 300. This result seems best explained by extensive condensation of reaction intermediates.

Although the periodate-permanganate reagent has been successfully applied in the macrolide field (16), other labile functions such as acyloins, reactive methylenes, etc. (79), besides olefinic bonds, may also be attacked. Other anomalies have also been observed. von Rudloff (82) noted oxide formation when he subjected occidentalol, which contains a 1,3-diene system, to this oxidation

technique. This fact had previously been encountered in the oxidation with permanganate of geranyl acetate (83), an acyclic 1,5-diene.

Another mild oxidizing agent for attacking double bonds is sodium *meta*-periodate containing catalytic amounts of osmium tetroxide (84). It differs from the permanganate-periodate reagent in that the oxidation proceeds to the aldehyde stage only. When peliomycin was subjected to the osmium tetroxide oxidation couple, the crude product obtained was treated directly with 2,4-dinitrophenylhydrazine in aqueous hydrochloric acid. The resulting crystalline solid was chromatographed on a silicic acid column; of the five bands separated, only one of these compounds could be characterized. High resolution mass measurement of the intense peak at *m/e* 240, the highest one observed in the mass spectrum of the isolated product (Figure IX), gave an elemental composition required for the 2,4-dinitrophenylhydrazone of glycolaldehyde. Although several attempts failed to provide an authentic sample of the 2,4-dinitrophenylhydrazone of glycolaldehyde, the compound isolated must have the assigned structure. It is not possible to conceive of any other structure which can yield *m/e* 240 of the composition measured.

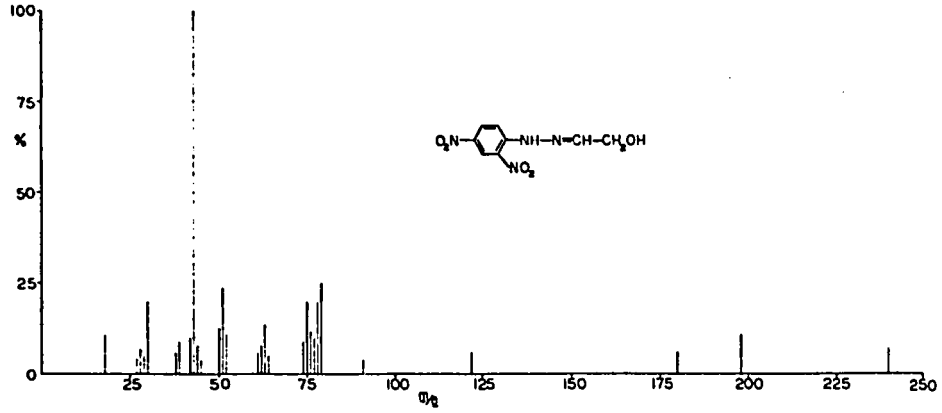
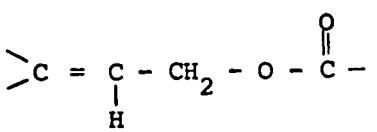


Figure IX Mass Spectrum of the 2,4-Dinitrophenylhydrazone of Glycolaldehyde (Source Temp. 200°C)

For glycolaldehyde to have survived the osmium tetroxide-periodate oxidation, the hydroxyl group must have been substituted in the course of this reaction. The ethereal oxygen of an ester function may likely have been involved in preventing the cleavage of the glycolaldehyde residue by the periodate. Such an ester may be that of the lactone grouping of the suspect-macrolide, or it may have been formed in the course of a periodate oxidation of an α-hydroxyketone in the hemi-ketal form. Although the latter possibility could not be eliminated, the resistance of peliomycin to periodate appears to exclude the presence of an α-hydroxyketone in peliomycin. Since no evidence was obtained for an enolic ether, which could have been oxidized to an ester function by the osmium tetroxide reagent, it is surmised that peliomycin contains the grouping XII.

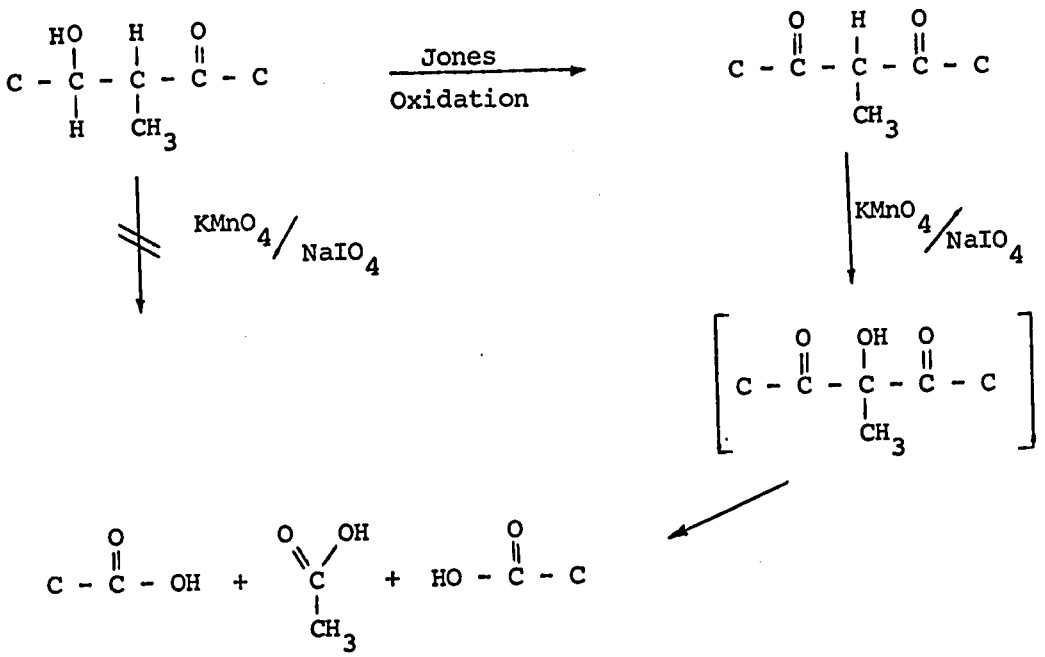


XII

As will be seen later, hydrogenation of peliomycin must have involved hydrogenolysis leading eventually to the formation of an alkyl chain. The ready hydrogenolysis of allylic esters (85) would help to account for this observation.

In view of the intractable mixture of products obtained on periodate-permanganate oxidation of peliomycin, it was decided to subject the antibiotic to a Jones oxidation (86), in the hope of preventing many of the side reactions observed above in the subsequent oxidation with the periodate-permanganate reagent.

When peliomycin was oxidized with the Jones reagent, ten oxygen atoms were consumed. The product, termed oxypeliomycin, was subjected to the periodate-permanganate oxidation couple for 14 hours, whereupon the solvent was removed. Acidification of the residue yielded a distillate on freeze-drying which consumed 2.2 moles of base per mole of peliomycin. P.m.r. analysis of the salts in deuterium oxide indicated acetic acid (singlet at τ8.15) and a trace ($\sim$5%) of formic acid (singlet at τ1.67). Apparently, the oxidation of the alcohol groups decreased the formation of formic acid, but increased the production of acetic acid by a factor of about 10 as compared to the amount formed on oxidation of peliomycin. Possible reasons for these observations are outlined below.



After separation of the volatile acids, the residue from the periodate-permanganate oxidation was esterified with diazomethane and subjected to gas chromatography. This technique allowed the separation of two components which could be characterized and synthesized as described below.

The simplicity of the p.m.r. spectrum of fragment VII, dimethyl 2-methylsuccinate, greatly facilitated its characterization. The 100 MHz p.m.r. spectrum of this compound (Figure X) showed: τ6.32 (d, 6H), τ7-7.7 (m, 3H) and τ8.8 (d, 3H). From this was inferred that the compound was dimethyl 2-methylsuccinate or a possible higher homologue. The infrared was in agreement with this assignment: no hydroxyl bands, but an intense carbonyl stretching band absorped at 1732 cm^{-1}.

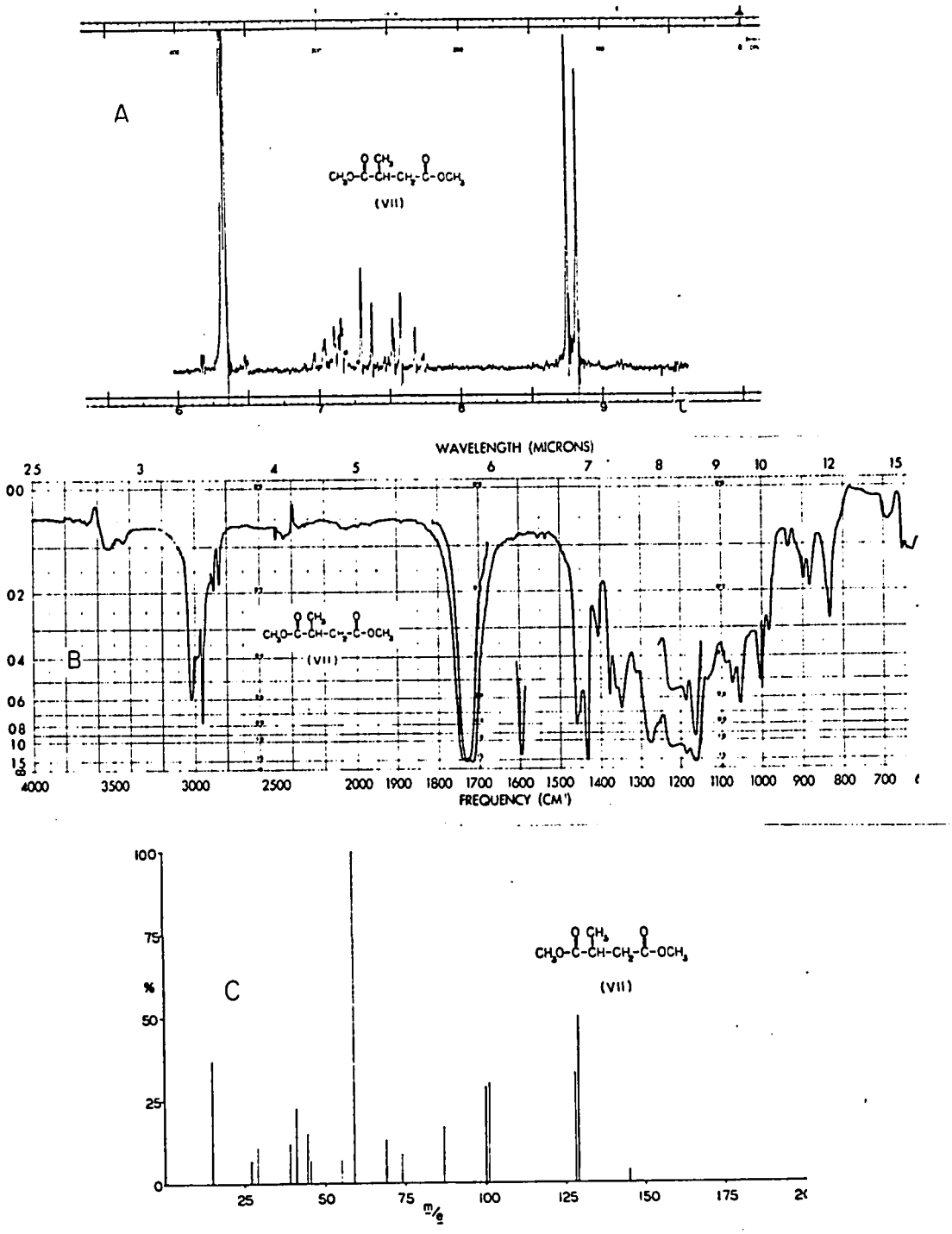
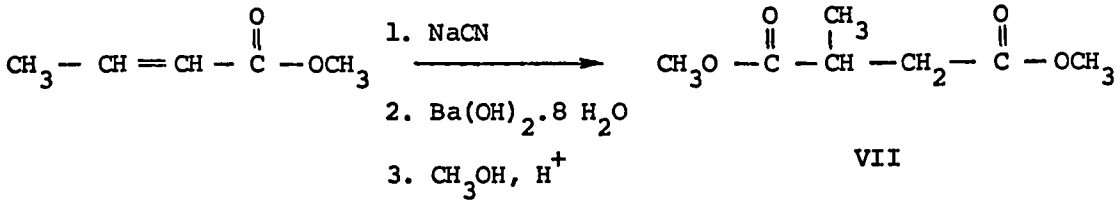


Figure X Spectra of Dimethyl 2-Methylsuccinate (VII). A. 100 MHz P.M.R. Spectrum of VII ($CDCl_3$). B. Infrared Spectrum of VII ($CDCl_3$). C. Mass Spectrum of VII (Source Temp. 150°C).

The possibility of a higher homologue was discounted by the mass spectrum of VII. Since low molecular weight dimethyl esters do not give molecular ions (87), none was expected. The spectrum did show a meta stable peak at *m/e* 102.4 (160-32 → 128) which confirmed a molecular weight of 160 for dimethyl 2-methylsuccinate. Other significant peaks were displayed at *m/e* 129 (M-31), 128 (M-32), 101 (M-59) and 100 (M-60), which were all in agreement with the proposed structure. This assignment was substantiated by direct comparison of the infrared, p.m.r. and mass spectra of fragment VII with those of an authentic sample (Figure X) synthesized as follows.



VII

The 100 MHz p.m.r. spectrum of dimethyl 3-acetoxy-2,4-dimethylglutarate (Fragment VIII) was relatively simple (Figure XI, Table VIII). Initially, however, there was some difficulty in explaining the triplet at τ4.51.

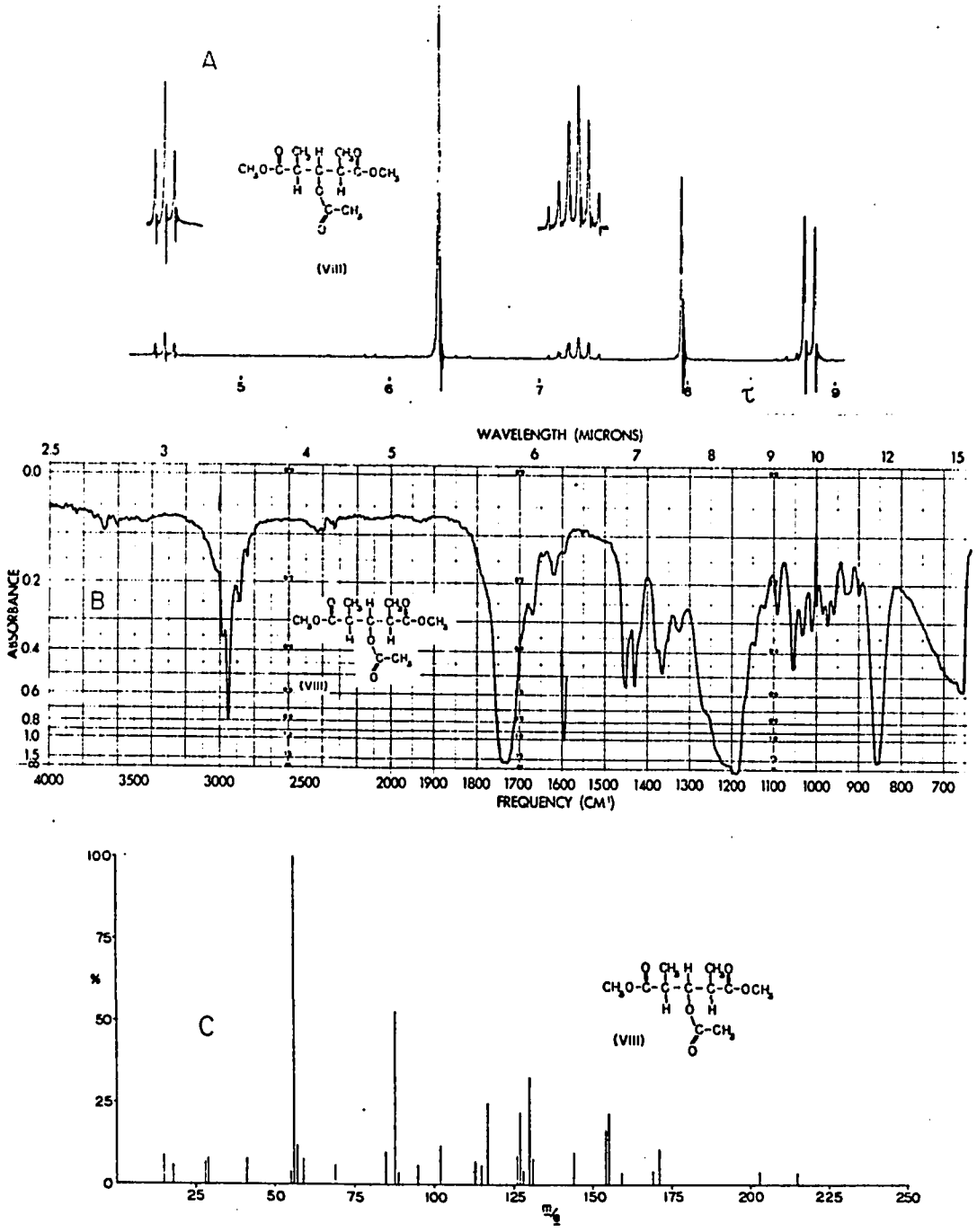


Figure XI Spectra of Dimethyl 3-Acetoxy-2,4-dimethylglutarate (VIII). A. 100 MHz P.M.R. Spectrum of VIII ($CDCl_3$). B. Infrared Spectrum of VIII ($CDCl_3$). C. Mass Spectrum of VIII (Source Temp. 160°C).

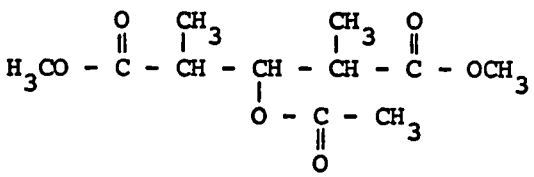
TABLE VIII

100 MHz P.M.R. Data of Dimethyl 3-Acetoxy-2,4-dimethylglutarate

Chemical Shift, τ	Signal	Integral - H
4.51	triplet	1
6.32	singlet	6
7.25	multiplet	2-3
7.88	singlet	3
8.83	doublet	3

At first, the low field signal was assigned to an olefinic proton and the signal at τ7.88 to an olefinic methyl group. Although the infrared displayed weak bands at 1675, 1625 and 860 cm^{-1} in accordance with an olefinic moiety, the signal at τ7.88 exhibited no coupling. Upon irradiating the multiplet, the signal at τ4.51 and the methyl doublet at τ8.83 each collapsed to a singlet. Irradiation of the latter doublet caused the multiplet to collapse to a doublet (J = 6.5 Hz). Hence, both the triplet at τ4.51 and the doublet at τ8.83 are coupled to the multiplet, but not to each other. The intense carbonyl absorption band at 1735 cm^{-1} in the infrared spectrum (Figure XI) was assigned to an ester function in agreement with the methyl ester absorption peak at τ6.32 in the p.m.r. spectrum.

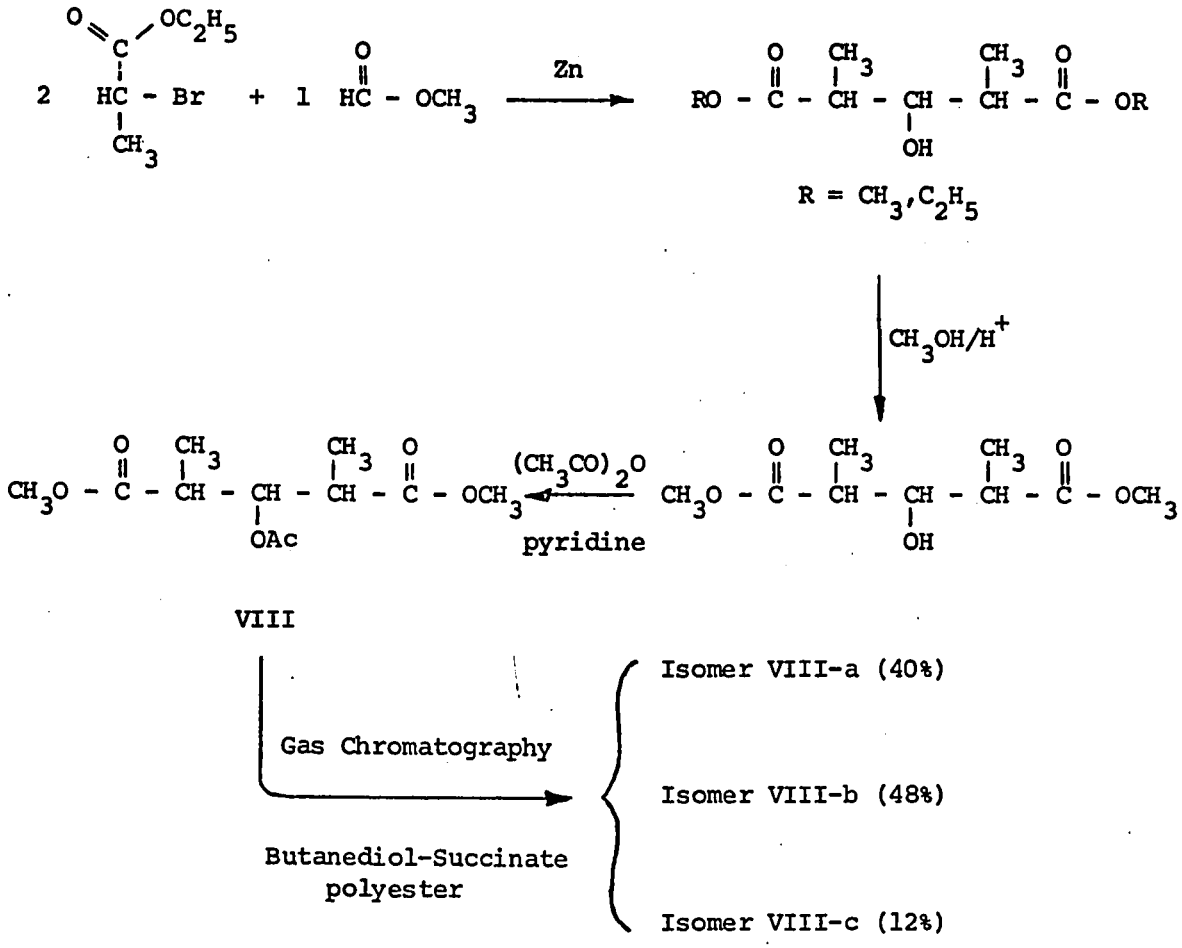
The mass spectrum (Figure XI) did not display a molecular ion, but the first discernible peak was found at *m/e* 171. A weak metastable peak at *m/e* 127.5(186 → 154) suggested a molecular ion at *m/e* 186, because of the expulsion of 32 mass units (methyl alcohol). In the p.m.r. spectrum, fragment VIII displayed a three proton singlet at τ7.88 in the region typical of methyl carbonyl groups. Although a methyl ketone could not be excluded *a priori*, an *O*-acetyl function was more in line with mass spectral observations. In this spectrum, a weak metastable ion at *m/e* 111.7 was found corresponding to a loss of 60 mass units (215 → 155) which was attributed to the expulsion of the elements of acetic acid. The metastable peaks corresponding to 186-32 = 154 and 215-60 = 155 suggest a molecular weight of 186 + 60 = 246. From symmetry considerations, and the presence of the acetate function in the p.m.r. spectrum, the following structure (VIII) seemed probable.



VIII

The lack of optical rotation (o.r.d.) indicated that this structure had either the 2*R*,3*r*,4*S* or the 2*R*,3*s*,4*S*-configuration. The syntheses and configurational analyses described in the following pages established the structure of VIII as dimethyl 2*R*,3*s*,4*S*-3-acetoxy-2,4-dimethylglutarate.

The synthesis of fragment VIII and the separation of its isomers (VIII-a, VIII-b, and VIII-c) was achieved as outlined in Scheme III, which is essentially that reported by Favorsky (88) in 1895.



By comparing the gas chromatographic retention time and the 100 MHz p.m.r. spectrum of each isomer (Figure XII) with the data of fragment VIII isolated from peliomycin, it was found that isomer VIII-a is identical to fragment VIII from peliomycin in both respects. However, its absolute configuration remained to be assigned.

Gerzon and co-workers (38) in their study on erythromycin isolated 2,4-dimethyl-3-hydroxyglutaric acid, which was confirmed by a synthesis also employing the Favorsky method (88). Although this synthesis should yield all three isomers, the above workers claim the isolation of a *meso* product. In fact, on reduction of the *meso* compound to a triol, a compound was isolated which had a melting point in accordance with the 2*R*,3*s*,4*S* (*xylo*)-configuration, *vide infra*. The isolated hydroxy-diacid from erythromycin was assigned the *xylo* (2*R*,3*s*,4*S*)-configuration based on its stability to dehydration in the course of acid and base treatment and not on direct observation (38).

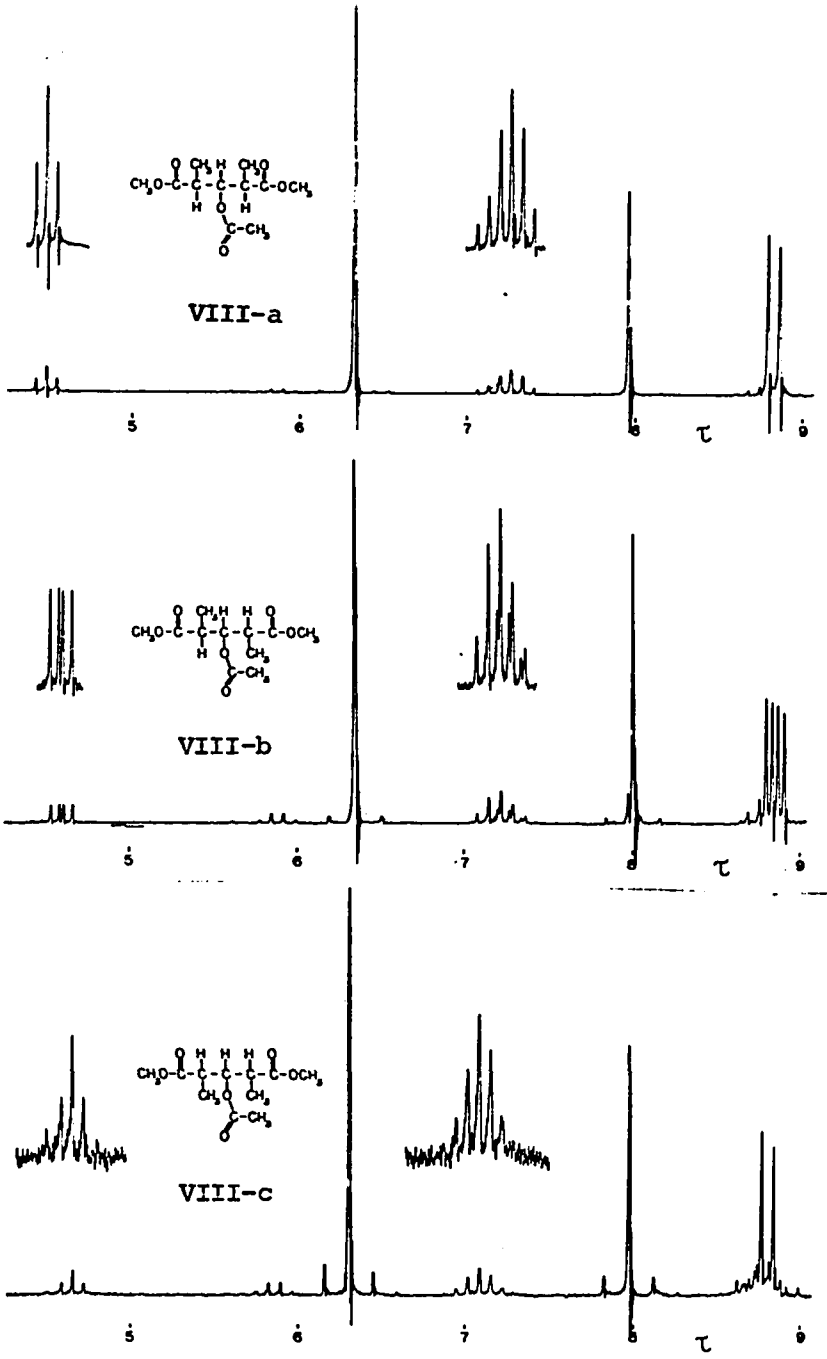


Figure XII 100 MHz P.M.R. Spectra of Diastereoisomers VIII-a, VIII-b and VIII-c of Dimethyl 3-Acetoxy-2,4-dimethylglutarate ($CDCl_3$).

The configurational assignment was achieved in the following way. Isomers VIII-a, VIII-b and VIII-c were reduced with lithium aluminum hydride in ether to give hygroscopic crystalline products IX-a, IX-b and IX-c, respectively (Table IX).

TABLE IX

Melting Points of Diastereoisomers IX-a, IX-b and IX-c of 2,4-Dimethylpentane-1,3,5-triol

Isomer	Trihydroxy Cpd. m.p., °C	Isopropylidene Der. m.p., °C
IX-a	74.5 - 76	oil
IX-b	89 - 89.5	oil
IX-c	86 - 86.5	36 - 38

In order to fix three carbons of each isomer as part of six-membered rings, these compounds were converted to 1,3-*O*-isopropylidene derivatives and characterized as their mono-5-*O*-acetates by p.m.r. analysis (Figure XIII and Tables X and XI).

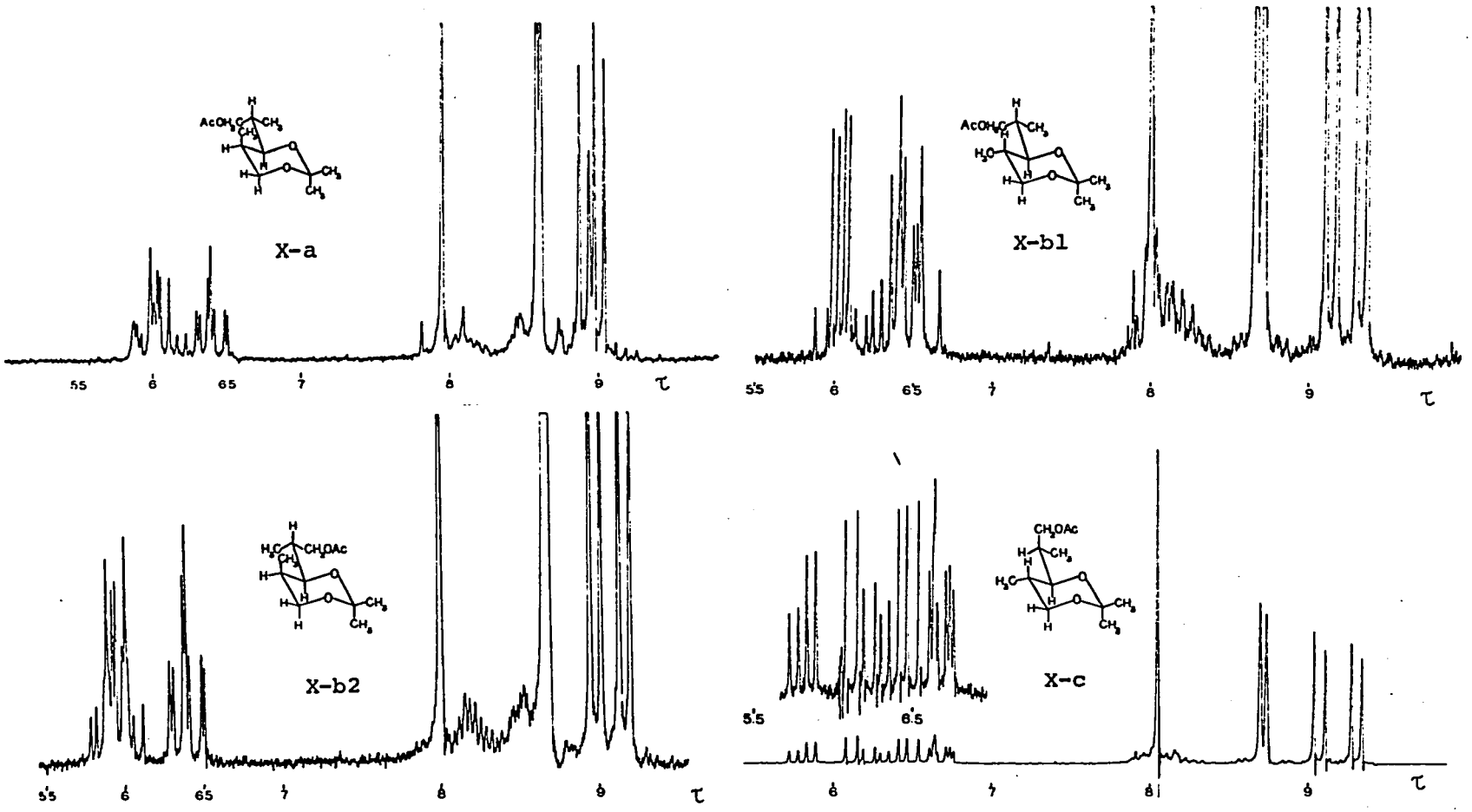


Figure XIII 100 MHz P.M.R. Spectra of the Diastereoisomers X-a, X-b1, X-b2 and X-c of 5-*O*-Acetyl-2,4-dimethyl-1,3-*O*-isopropylidenepentane-1,3,5-triol ($CDCl_3$).

TABLE X

100 MHz P.M.R. Data of Isomers of

5-*O*-Acetyl-2,4-dimethyl-1,3-*O*-isopropylidenepentane-1,3,5-triol

Chemical Shift, τ						
X-a	X-b1	X-b2	X-c	Signal[a]	Integral	Assignment
6.14[b]	6.42	6.14[c]	6.31	q	1	H_1 equatorial
6.23[b]	6.47	6.23[c]	6.44	q	1	H_1 axial
6.35	6.46	6.34	6.61	q	1	H_3
6.51	6.03	5.94	5.84	q	1	H_5' or H_5''
6.57	6.05	5.97	5.97	q	1	H_5'' or H_5'
7.94	7.94	7.96	7.96	s	3	$CH_3 - \overset{O}{\overset{\|}{C}} - O$
8.1	8.0	8.2	8.1	m	1	H_4
8.5	8.2	8.5	7.9	m	1	H_2
8.59	8.63	8.64	8.63	d	6	isopropylidene gr.
8.90	9.28	8.95	8.98	d	3	$C_2 - CH_3$
9.00	9.19	9.15	9.22	d	3	$C_4 - CH_3$

(a) s, d, q and m denotes singlet, doublet, quartet and multiplet, respectively.

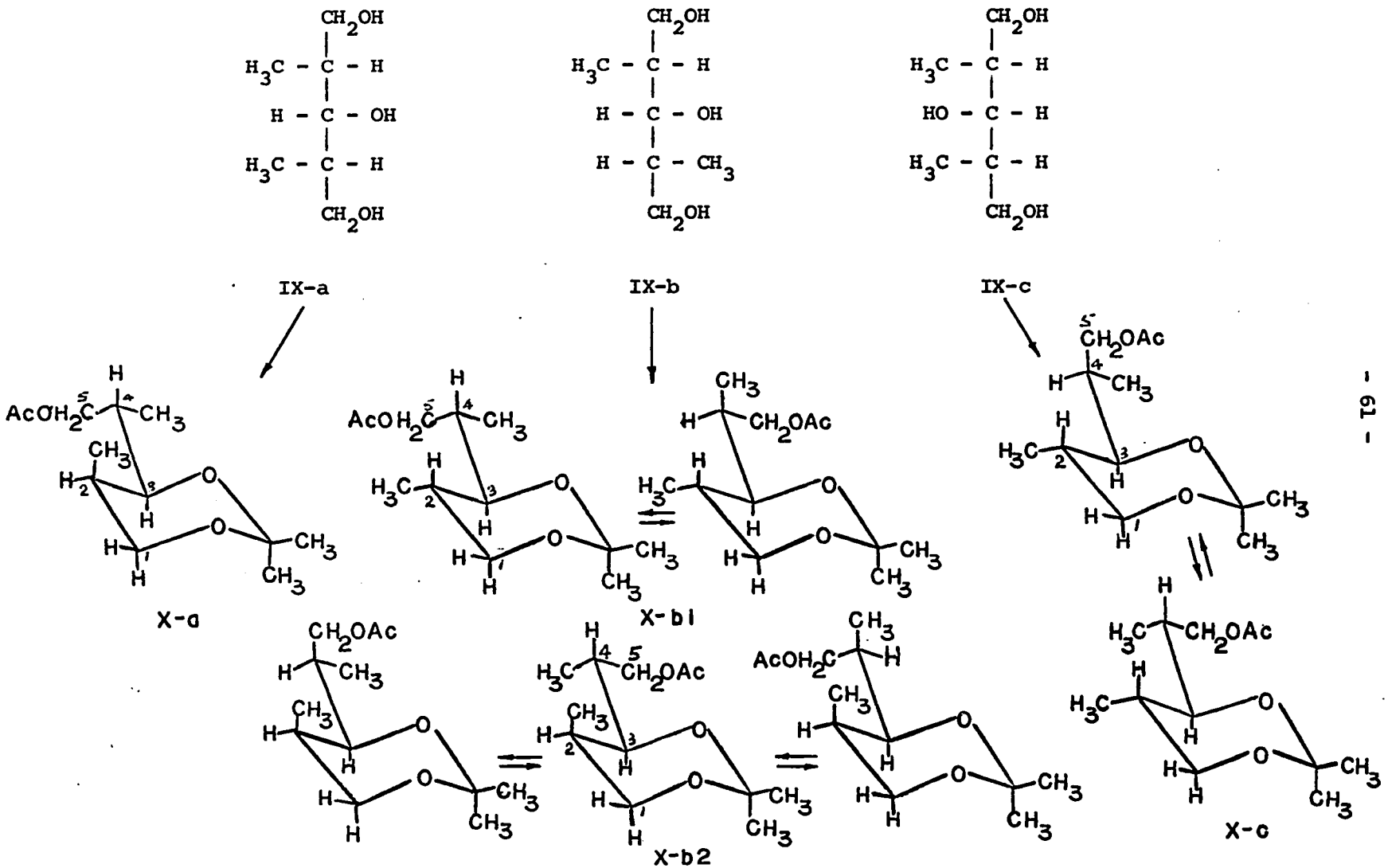
(b) and (c) Assignments of H_1 axial and H_1 equatorial may be reversed due to deshielding effects of axial methyl groups on axial protons (H_1 axial) by approximately 0.2 τ values. [H. Booth, Tetrahedron, 22, 615 (1966)]

TABLE XI

Coupling Constants observed in the 100 MHz P.M.R. Spectra of the Isomers

5-*O*-Acetyl-2,4-dimethyl-1,3-*O*-isopropylidenepentane-1,3,5-triol

Isomer	Hydrogens	Coupling Constants, Hz
X-a	H_1 axial - H_2 equat.	1.5
	H_1 equat. - H_2 equat.	2
	H_3 axial - H_2 equat.	2.3
	H_3 axial - H_4	10
X-b1	H_1 axial - H_2 axial	11.5
	H_1 equat. - H_2 axial	5.4
	H_3 axial - H_2 axial	10.5
	H_3 axial - H_4	2.3
X-b2	H_1 axial - H_2 equat.	1.5
	H_1 equat. - H_2 equat.	2.5
	H_3 axial - H_2 equat.	2.5
	H_3 axial - H_4	7
X-c	H_1 axial - H_2 axial	7
	H_1 equat. - H_2 axial	5.3
	H_3 axial - H_2 axial	10.5
	H_3 axial - H_4	2.5



Scheme III Formation of the 5-*O*-Acetyl-1,3-*O*-isopropylidene Derivatives of the Diastereoisomers of 2,4-Dimethylpentane-1,3,5-triol

The configuration of the derivatives shown in Scheme IV are those determined by p.m.r. analysis of which the pertinent data have been recorded in Tables X and XI. The configurations assigned to each isomer are presented at this time to facilitate the discussion.

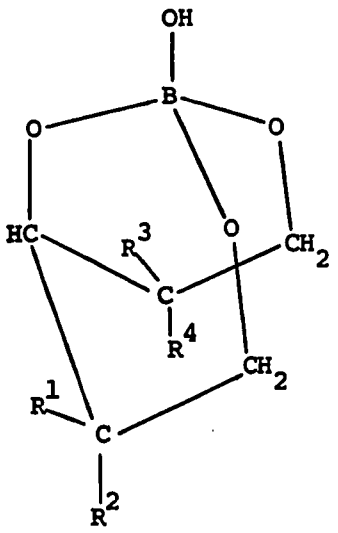
It may be expected that the D, L - triol, namely that isomer with the *arabino* or 2*R*,4*R*-configuration, will provide two isomeric isopropylidene-acetoxy derivatives. In agreement, only triol IX-b gave the stereoisomeric compounds X-b1 and X-b2 (ratio 4:1, respectively), which were separated by gas chromatography. For this reason, compounds IX-a and IX-c must be the *meso*-triols. The structure assigned to X-b1 is based on the large coupling constant of 12 Hz observed for H_1- axial and H_2. As expected, the isomer of common origin, X-b2, shows relatively small coupling constants (<2.5 Hz) appropriate to *gauche* relationship (H_1- axial - H_2 and H_1- equatorial - H_2). For compound X-b1, that conformation which has the $-CH_2OAc$ opposing the C_2-CH_3 group should be much less stable than that staggered orientation where the C_2-CH_3 group is opposed by a hydrogen. Therefore, the coupling constant of 2.5 Hz for H_3-H_4 is in agreement for that observed for hydrogens in *gauche* relationship (89). The coupling for these hydrogens of 7 Hz in isomer X-b2 requires a contribution of about 40% by that conformer

which has the C_2-CH_3 group opposed by the methyl group of the side chain. The third possible orientation for C_4-substituents can be assumed to make negligible contribution. Thus, the analyses of the p.m.r. spectra are compatible with the assignment of the *arabino* (2*R*,4*R*)-configuration to IX-b.

As a result of this assignment, either isomer X-a or X-c should have a large coupling constant for the axial H_1 and H_2. Indeed, isomer X-c exhibited a coupling of 7 Hz for these two protons, whereas the corresponding coupling constant of isomer X-a was found to be 1.5 Hz. Therefore, the *xylo* and *ribo*-configurations are attributed to compounds X-a and X-c, respectively. These assignments received confirmation from the relative magnitudes of the coupling constants for H_3 and H_4. The side chain must be held rigidly in the conformation shown for X-a with a coupling constant in the range expected for *trans* hydrogens (89). The coupling of 10 Hz observed for this compound was in agreement with the assigned conformation. On the other hand, the coupling for the H_3 and H_4 protons of X-c was only 2.5 Hz, a value which appears to reflect a greater repulsive interaction between the $-CH_2OAc$ group and the ring oxygen than between a methyl group and the ring oxygen. This situation may arise in view of the evidence (90) that the carbonyl oxygen of an acetoxy

group in the preferred conformation for the acetoxymethylene group is in close proximity to the methylene hydrogens, and therefore also in a position to be repelled electrostatically by the ring oxygen. Furthermore, the freedom of rotation of the acetoxymethyl group must be more restricted in that conformation wherein H_3 and H_4 are in a *trans* relationship than in the other two staggered orientations. Since fragment VIII isolated from peliomycin was identical to compound VIII-a from which X-a was derived, the configuration of this structural unit in peliomycin must be *xylo* or 2*R*,3*s*,4*S*. Therefore, because of the agreement in melting points between the triol VIII-a and the fragment isolated from erythromycin by Gerzon and co-workers (38) the ascribed configuration (38, 60) of 2*R*,4*S* has been verified.

It is well established that tridentate borate complexes, for instance those of cis-inositols (91), are relatively highly stable. Consequently, polyols capable of forming such complexes display much greater electrophoretic mobilities than those compounds which have only two hydroxyl groups available for complex formation (91). Therefore, it can be expected that the triols IX-a, IX-b and IX-c may tend to form tridentate complexes of structure XI to different extents.



XI

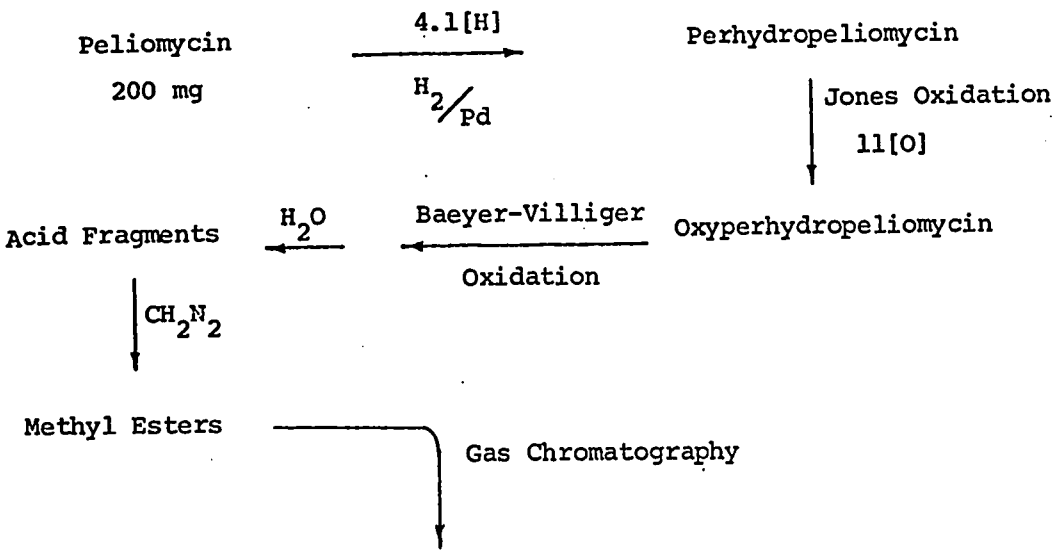
$R^1 = R^3 = CH_3$ and $R^2 = R^4 = H$; *ribo*-configuration

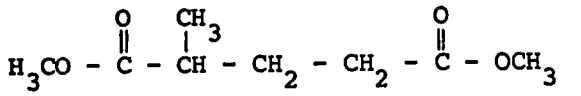
$R^2 = R^3 = CH_3$ and $R^1 = R^4 = H$; *arabino*-configuration

$R^2 = R^4 = CH_3$ and $R^1 = R^3 = H$; *xylo*-configuration

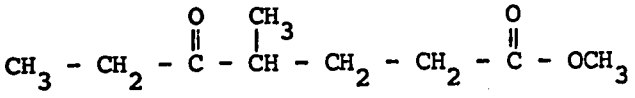
On this basis, only the *xylo* (2*R*,3*s*,4*S*)-configuration is obviously unfavorable for tridentate complexing, due to 1,3-diaxial interaction of the methyl substituents. Hence, when the triols IX-a, IX-b and IX-c were subjected to paper electrophoresis using borax as the complex-forming electrolyte (92), they showed mobilities (with respect to glucose) of 0.10, 0.46 and 0.47, respectively. These results substantiate the *xylo* (2*R*,3*s*,4*S*)-configuration assigned to triol IX-a on the basis of p.m.r. analysis of its *O*-isopropylidene-*O*-acetyl derivative (X-a).

The foregoing degradative experiments resulted in the isolation of fragments VII and VIII, which represent fourteen carbon and at least five oxygen atoms of the peliomycin molecule. In the following degradation reactions outlined in Scheme IV, the olefinic bonds were reduced to hydrocarbon prior to oxidation. After the alcohol groups were oxidized with the Jones reagent (86), the carbon skeleton was cleaved at the ketonic positions by means of the Baeyer-Villiger reaction (93). The acidic fragments obtained were converted to methyl esters for gas-chromatographic separation. As seen in Scheme IV, this approach led to the isolation of five additional fragmentation products. None of these fragments carried hydroxyl functions, which may be due to the low vapor pressure of alcohols and the fact that only a non-polar column packing (silicone grease) was capable of giving an adequate separation.

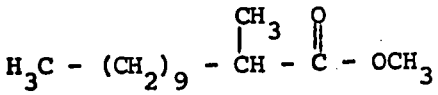




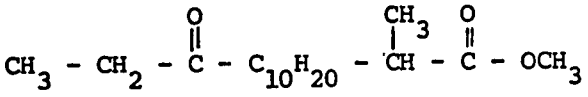
XIII



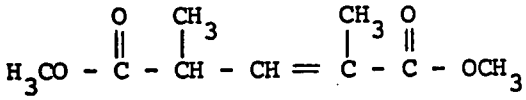
XIV



XV



XVI



XVII

Scheme IV Oxidative Degradation of Perhydropeliomcyin

The characterization of dimethyl 2-methylglutarate (fragment XIII) was achieved as follows. The 100 MHz p.m.r. spectrum of fragment XIII (Figure XIV) showed a strong methoxy-carbonyl peak at τ6.32, multiple peaks from τ7.4 to τ8.5, and a well defined doublet at τ8.83. The integration clearly established that τ6.32: τ8.83 = 2:1, while the multiple peak region did not contain more than six protons. The position of the doublet at τ8.83 appeared at lower field than a secondary methyl group in a saturated environment, such as an isobutyl group (94), by about 30 Hz. By irradiating the doublet at τ8.83 it was found that the methine proton coupled to the doublet was situated at τ7.5. From these considerations a tentative structure was assigned as the dimethyl ester of a dibasic acid having one methyl group next to the methoxy-carbonyl group, or a higher homologue.

The mass spectrum was void of a molecular ion (Figure XIV), as no peak of even mass number could be discerned. The highest peak in the spectrum was at *m/e* 143. By comparing the fragmentation pattern with that of dimethyl glutarate (86) the resemblance was very good, except that the peaks in the unknown were greater by fourteen mass units. The base peak at *m/e* 88 is typical of a McLafferty rearrangement of a 2-methyl substituted methyl ester (95).

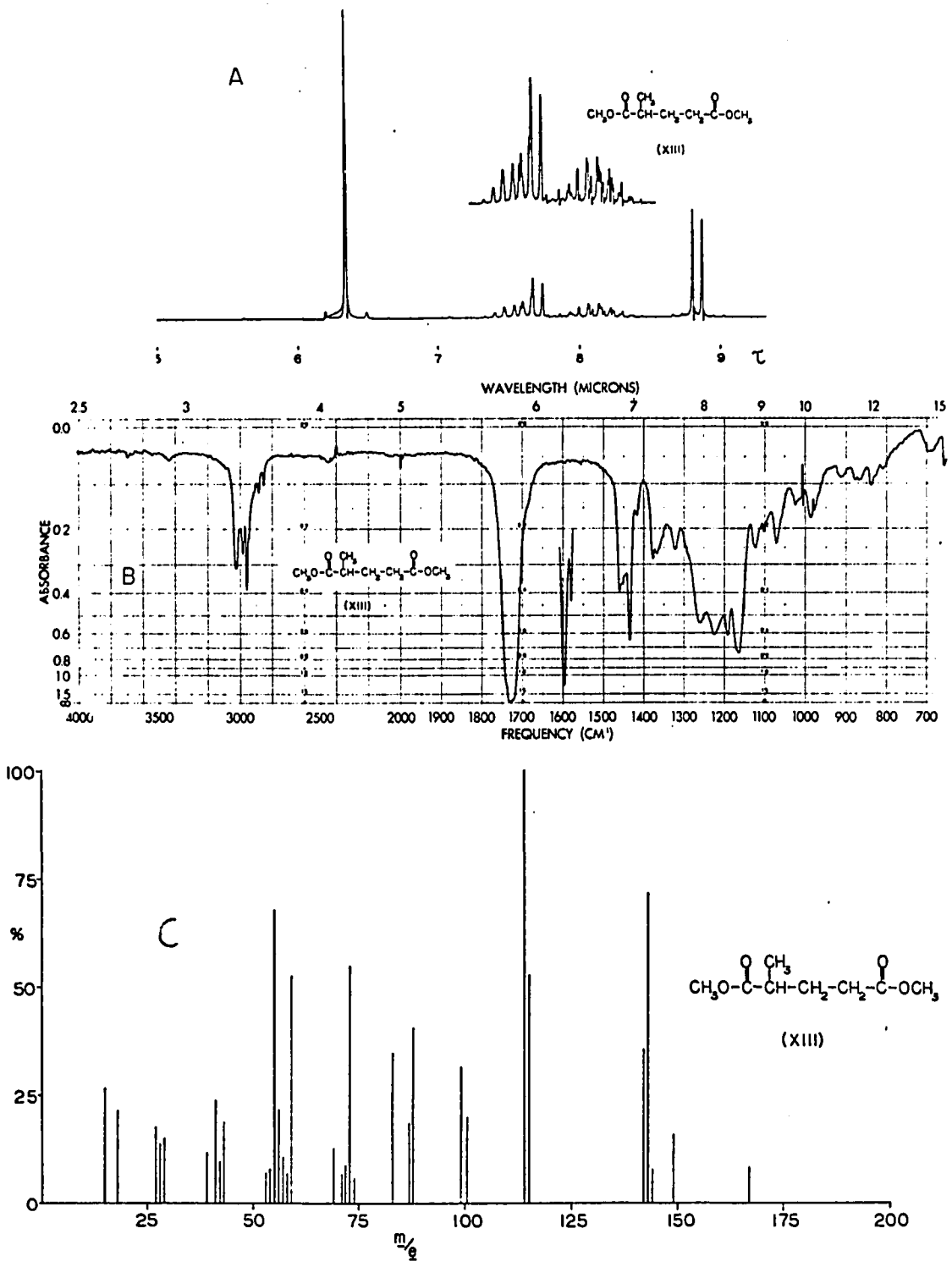
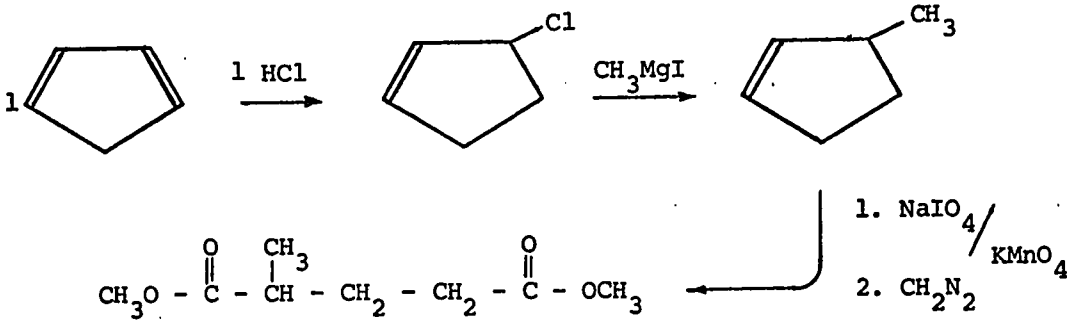


Figure XIV Spectra of Dimethyl 2-Methylglutarate (XIII). A. 100 MHz P.M.R. Spectrum of XIII ($CDCl_3$). B. Infrared Spectrum of XIII ($CDCl_3$). C. Mass Spectrum of XIII (Source Temp. 195°C).

The absorption bands in the infrared spectrum supported the observations made above. Besides the methyl and methylene stretching vibrations at 2960 and 2900 cm^{-1}, respectively, a strong carbonyl ester band was seen at 1730 cm^{-1}.

From the physical data collected, the structure that suggested itself was that of dimethyl 2-methylglutarate. The identity of this fraction was established by direct comparison of its spectral properties (Figure XIV) to those of an authentic sample synthesized as follows.



XIII

As no optical rotation was observed for fragment XIII, it is possible that two enantiomers were present in near equimolar quantities. The presence of a racemate may have arisen during catalytic hydrogenation of peliomycin prior to degradation. This seems unlikely in this case, however, since no olefinic methyl protons were observed in the p.m.r. spectrum of peliomycin. A more plausible explanation is the epimerization of the asymmetric center in the course of the degradative reactions.

The identity of methyl 4-methyl-5-oxoheptanoate (Fragment XIV) was deduced from its spectral properties (Figure XV) and confirmed by synthesis. Inspection of the 100 MHz p.m.r. spectrum of XIV (Figure XV) indicated a methoxy-carbonyl peak at τ6.3, multiple peaks at τ7.5 to τ8.8 and an overlapping doublet centered at τ8.94 and triplet at τ8.99. The integration of this spectrum established that the ratio of τ6.3: τ8.8-9.1 = 1:2. On the assumption that one methoxy-carbonyl was present, then six methyl protons were in the region of τ8.8-9.1. Due to background noise in the spectrum, the integration of the multiple peaks in the region τ7.5-8.8 could not be established clearly, but most probably did not contain more than eight protons. The infrared spectrum was most revealing in the carbonyl region. A very strong band was present at 1730 cm^{-1} and a lesser band at 1712 cm^{-1} (Figure XV), which was attributed to a ketone functional group.

A molecular ion could be observed in the mass spectrum (Figure XV) of this compound at *m/e* 172. By measuring this peak at high resolution a mass of 172.1103 was found which agrees with 172.1100 for the formula $C_9H_{16}O_3$. As the infrared spectrum indicated a ketone function (1712 cm^{-1}), the peaks in the mass spectrum at *m/e* 143 (M-29) could be assigned to the presence of an ethyl ketone group because

of the fragment $C_2H_5^+$. On the same basis, the loss of 57 mass units [peaks at *m/e* 57 and 115 (M-57)] could be due to $C_2H_5C \equiv O^+$. The peaks at *m/e* 88 and *m/e* 74 were of about similar intensities, and no decision could be made from this information whether the branched methyl group was in the α-position to the methoxy-carbonyl or to the ketone carbonyl. The elemental composition of the molecular ion showed two degrees of unsaturation, one of which must be assigned to the methoxy-carbonyl group. If the remaining unsaturation was due to an olefinic bond, then the third oxygen had to be present as an alcohol or as an ether functional group. The p.m.r. spectrum lacked olefinic protons and the infrared spectrum was void of an olefinic stretching band in the 1600-1700 cm^{-1} and alcoholic bands in the 3200-3600 cm^{-1} region. On this basis, it was very tempting to assign the unsaturation to a carbonyl function. An aldehyde group seemed unlikely, because of the position of the extra band in the carbonyl region (1712 cm^{-1}) of the infrared spectrum, and no aldehyde proton could be found at low field in the p.m.r. spectrum.

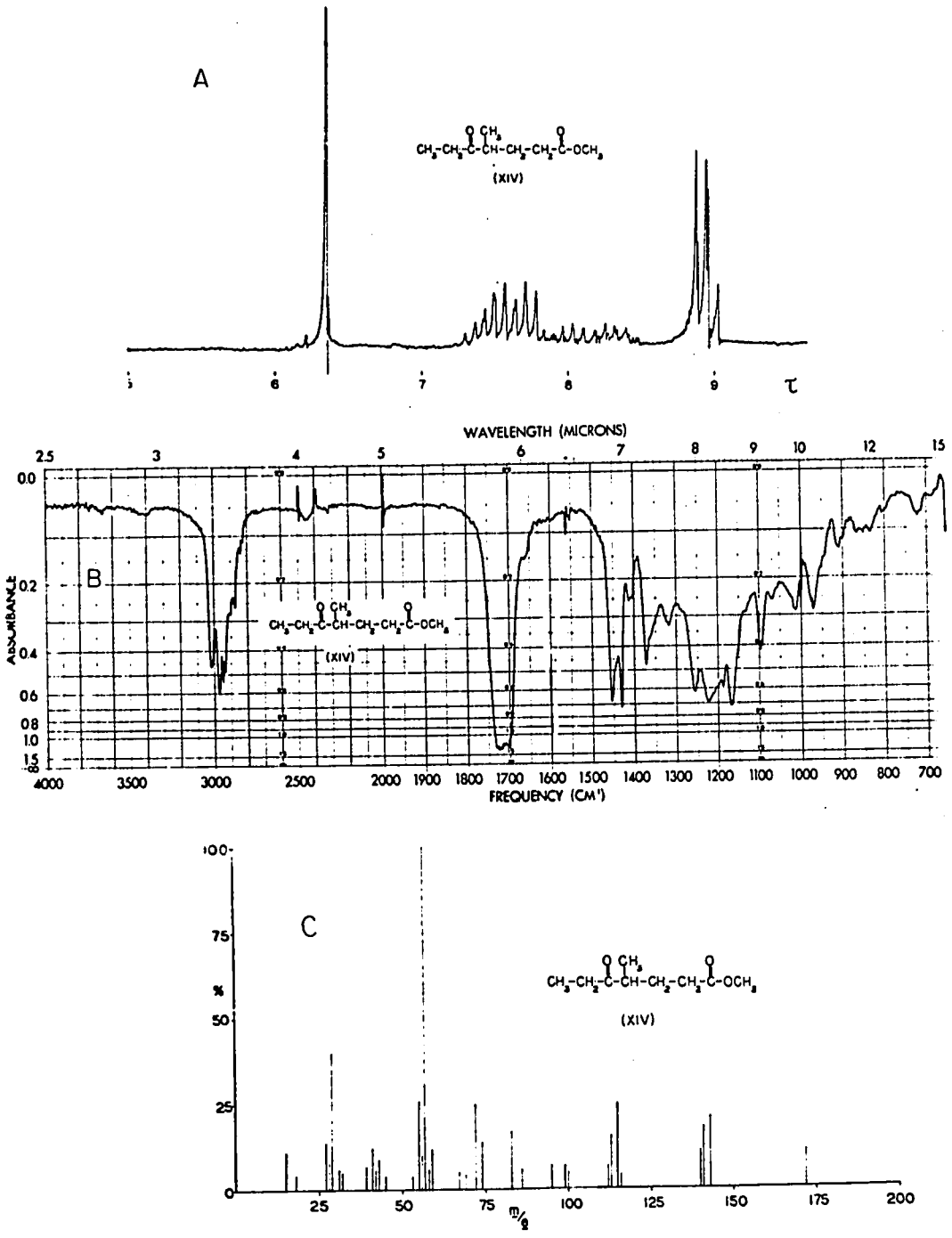
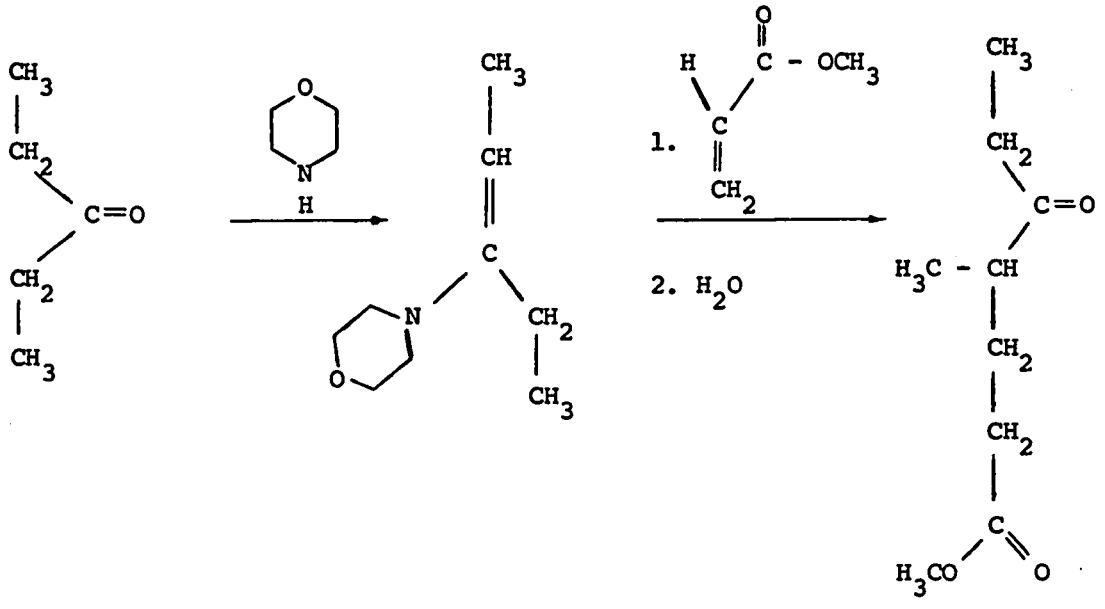


Figure XV Spectra of Methyl 4-Methyl-5-oxoheptanoate (XIV). A. 100 MHz P.M.R. Spectrum of XIV ($CDCl_3$). B. Infrared Spectrum of XIV ($CDCl_3$). C. Mass Spectrum of XIV (Source Temp. 185°C).

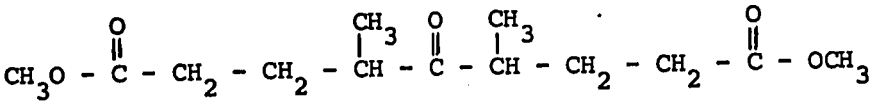
The combined physical data indicated that the compound in question was either methyl 2-methyl-5-oxoheptanoate or methyl 4-methyl-5-oxoheptanoate. These two compounds differ in the position of the branched methyl group only, otherwise both fit the spectra considered. Syntheses of the above compounds showed conclusively that methyl 4-methyl-5-oxoheptanoate is in complete agreement with the spectral data of fragment XIV. The lack of optical rotation of fragment XIV may likely be due to epimerization of the asymmetric center during the degradative reactions, as mentioned in the discussion on fragment XIII.

The synthesis of methyl 4-methyl-5-oxoheptanoate was achieved by reacting the morpholine enamine of 3-pentanone with methyl acrylate, followed by hydrolysis.



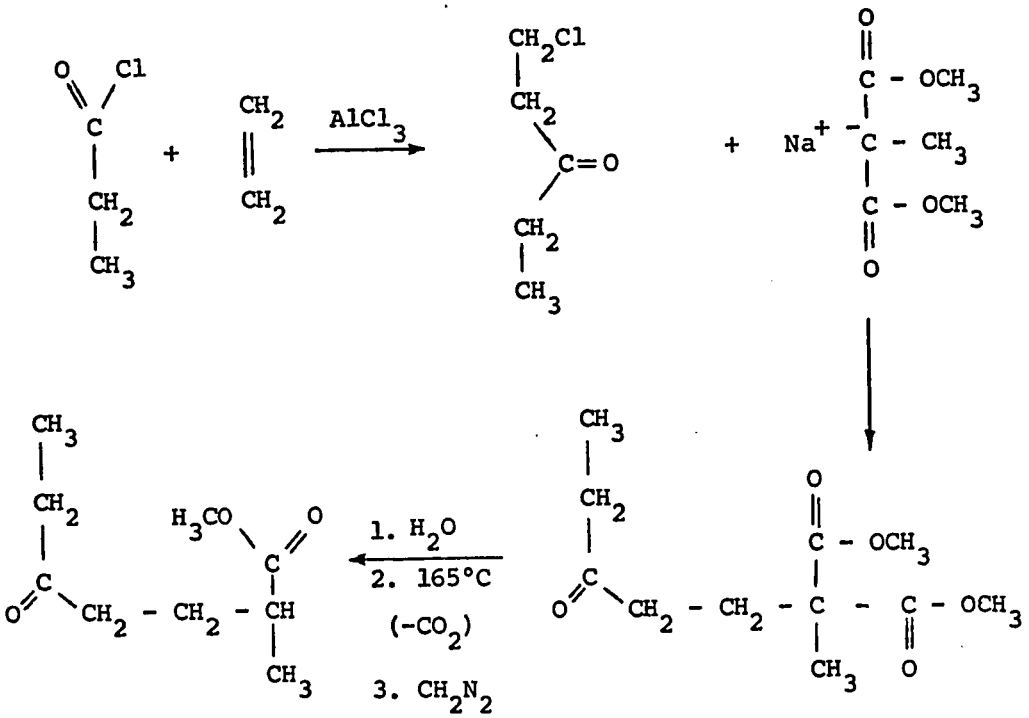
XIV

When pyrrolidine was used in the enamine synthesis instead of morpholine, the hitherto unreported dimethyl 4,6-dimethyl-5-oxoazelate (XVIII) was isolated. Gas chromatography (20% silicone grease) of the pure reaction product indicated two stereoisomers in a 1:1 ratio, 4*R*,6*S* and 4*R*,6*R*.



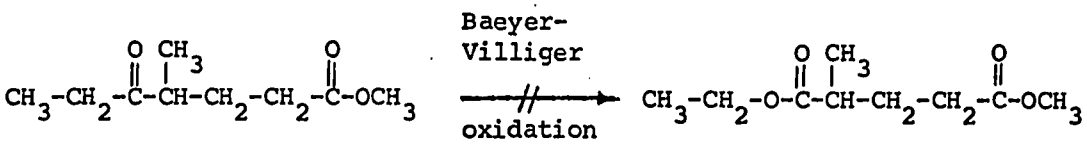
XVIII

The synthesis of methyl 2-methyl-5-oxoheptanoate was achieved using the following Friedel-Crafts reaction.



XIX

Since synthesis showed unequivocally that methyl 4-methyl-5-oxoheptanoate is identical to fragment XIV isolated from peliomycin, it was tempting to infer that the Baeyer-Villiger oxidation employed in the degradation sequence caused XIII to arise from XIV.



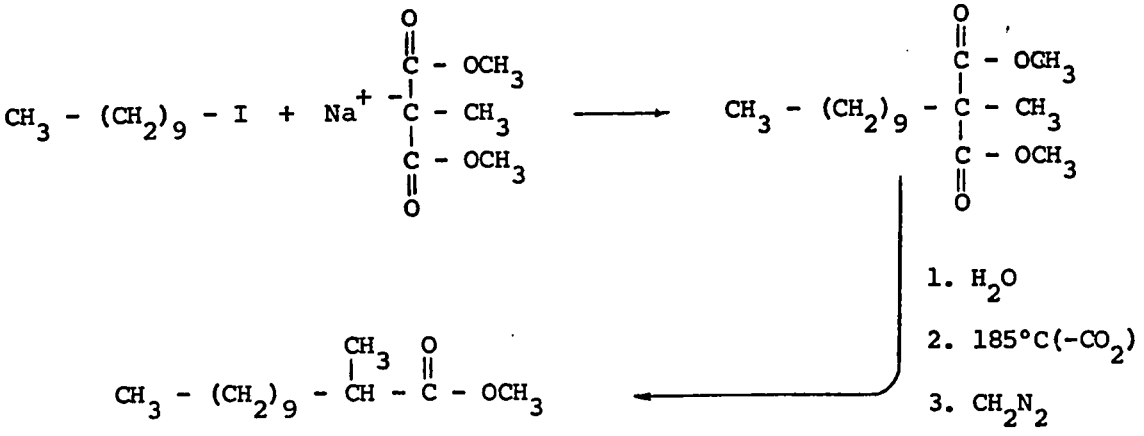
XIV

The mechanism postulated for this reaction involves an electron-deficient intermediate; thus, the migratory aptitude in the alkyl series is tertiary>secondary>primary (96). Indeed, when 4-methyl-5-oxoheptanoate (XIV) was subjected to the Baeyer-Villiger reaction, only methyl propionate could be isolated by transesterification of the oxidation product. Hence, XIII could not have arisen from XIV in the course of the Baeyer-Villiger oxidation reaction.

The yield of methyl 2-methyldodecanoate (XV, Scheme IV) was very small and a 100 MHz p.m.r. spectrum exhibited a poor signal to noise ratio. A better spectrum was obtained with the aid of a time-average computer (C.A.T.). The signals appeared at about τ9.15 (t), τ8.9 (d), τ8.8 (s), τ7.7 (m) and at τ6.4 (s) which integrated approximately as 3:3:16:1:3. The large singlet at τ8.8 together with the

methoxy-carbonyl signal at τ6.4 suggested a fatty ester with a long alkyl chain terminating in a methyl group as indicated by the poorly resolved triplet at τ9.15 (97). Based on observations of compounds VII and XIII, the doublet at τ8.9 was assigned to a methyl group α to the carboxy function.

The latter assignment found support in the strong peak at *m/e* 88 in the mass spectrum. This peak is typical of a McLafferty rearrangement of 2-methyl substituted long chain esters (98). Further analysis of the mass spectrum yielded a molecular ion at *m/e* 228 which measured 228.2090 and calculated 228.2089 for $C_{14}H_{28}O_2$. The peak at *m/e* 101 is due $CH_3O\text{-}\overset{O}{\overset{\|}{C}}\text{-}\overset{CH_3}{\overset{|}{CH}}\text{-}CH_2$ (98). These observations from mass spectral analysis indicate that the p.m.r. integration is in error by two methylene protons. Otherwise all physical data (Figure XVI) pointed towards methyl 2-methyldodecanoate which was substantiated by synthesis:



XV

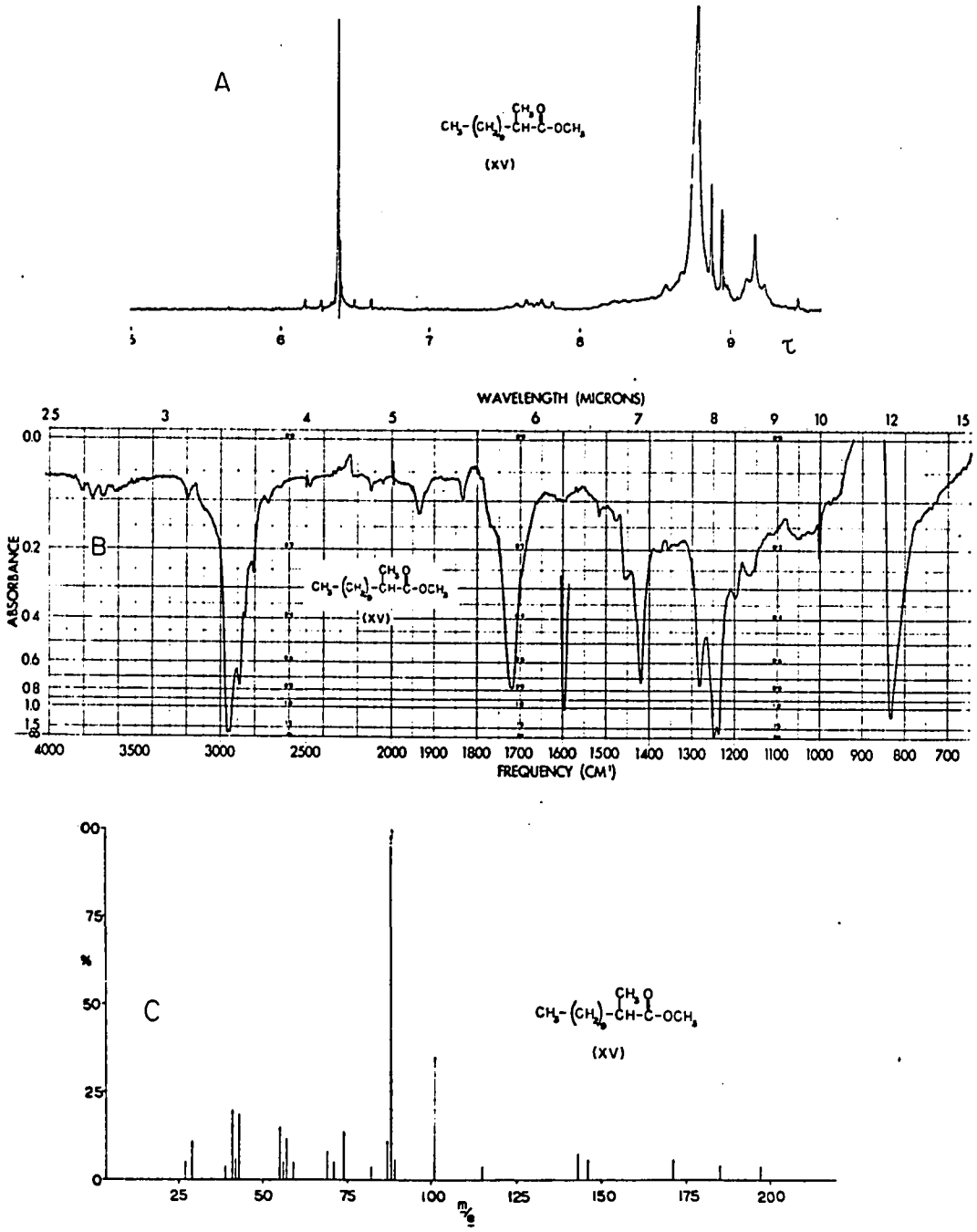


Figure XVI Spectra of Methyl 2-Methyldodecanoate (XV). A. 100 MHz P.M.R. Spectrum of XV ($CDCl_3$). B. Infrared Spectrum of XV ($CDCl_3$). C. Mass Spectrum of XV (Source Temp. 160°C).

Fragment XVI was not completely characterized (Figure XVII). Like fragment XV, the signal to noise ratio in the p.m.r. spectrum was poor, but could be improved by the C.A.T. technique. The results are tabulated in Table XII, but are only approximations; the integral values are based on the presence of one methyl ester = 3H.

TABLE XII

100 MHz P.M.R. Data of Fragment XVI isolated from Perhydropeliomycin

Chemical Shift τ	Signal	Integral-H
9.15	triplet	3
8.95	triplet	3
8.85	doublet	3
8.75	singlet (broad)	10
7.5-7.8	multiplet	5
6.35	doublet	3

The signal at τ9.15 displays a poorly resolved triplet and is assigned to a long alkyl chain (97). The methyl triplet and methyl doublet at τ8.95 and τ8.85 must be deshielded by a carbonyl or oxygen function due to their chemical shifts. The infrared spectrum of this fraction lacks hydroxyl absorption, but displays carbonyl stretching absorption at 1730 cm^{-1} with a shoulder at about 1715 cm^{-1}. From their frequencies and intensities, these bands may be attributed to an ester and ketone function, respectively (99).

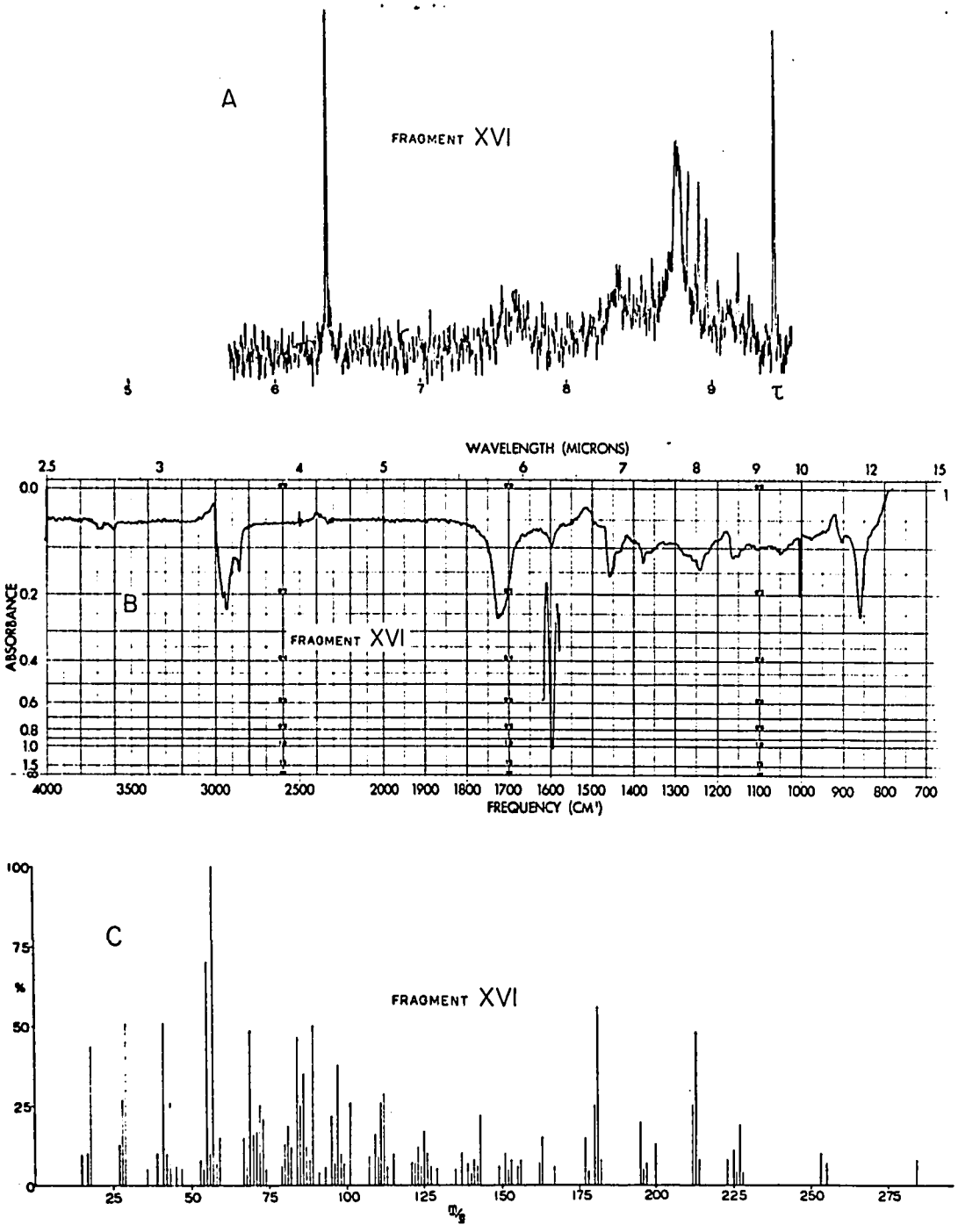


Figure XVII Spectra of Fragment XVI isolated from Perhydropeliomycin. A. 100 MHz P.M.R. Spectrum of XVI ($CDCl_3$). B. Infrared Spectrum of XVI ($CDCl_3$). C. Mass Spectrum of XVI (Source Temp. 150°C).

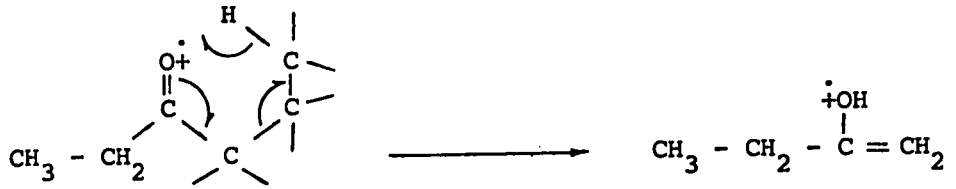
Mass spectral analysis of this compound (Figure XVII) yields a molecular ion at *m/e* 284; this peak and four other peaks at high mass were measured and the values recorded in Table XIII.

TABLE XIII

Mass Spectral Measurements of Fragment XVI isolated from Perhydropeliomycin

Formulas	Calculated	Measured
$C_{17}H_{32}O_3(M^+)$	284.2352	284.2353
$C_{13}H_{25}O_2$	213.1855	213.1855
$C_{13}H_{24}O_2$	212.1776	212.1776
$C_{12}H_{21}O$	181.1593	181.1595
C_9H_{17}	125.1327	125.1330
$C_8H_{13}O$	125.0967	125.0966

There were no significant peaks at *m/e* 74 or 88 attributable to a McLafferty rearrangement of a 2-unsubstituted or 2-methyl substituted methyl ester, respectively (95). However, a moderate peak (25% of base peak) was noticed at *m/e* 72, which may have arisen from a McLafferty rearrangement of an ethyl ketone function as in structure XX.



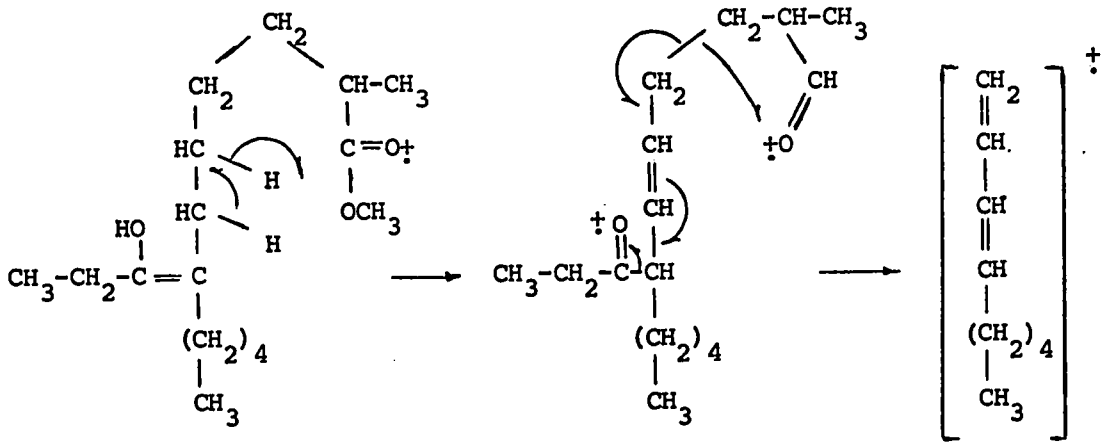
XX

m/e 72

Supporting evidence for this assignment comes from the p.m.r. analysis: triplet at τ8.95; and mass spectral analysis: M-29 and M-57. Furthermore, mass measurement of *m/e* 212 (M-72) is in agreement with a loss of C_4H_8O from the molecular ion. Hence, it is reasonable to assume that *m/e* 213 arises from a cleavage of the C_4-C_5 bond without hydrogen migration. The metastable peaks at *m/e* 152.8 and 153.9 indicate that both *m/e* 212 and 213 fragments have the methoxy carbonyl intact [loss of CH_3OH (72)]. The doublet at τ8.85 is again assigned to a 2-methyl substituted ester as in the structure of fragment VIII. The absence of a McLafferty rearrangement peak (no *m/e* 88) of the methyl 2-methylester may be due to other fragmentations which became energetically more favorable (100).

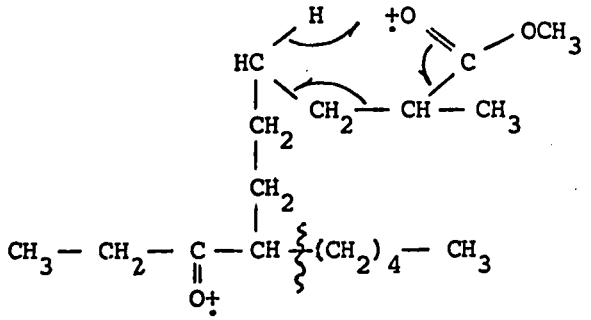
Since there appear to be only three olefinic bonds in peliomycin (see discussion on Structural Features), and the fact that the p.m.r. spectrum of the antibiotic does not support many methylene hydrogens, it becomes necessary to incorporate methyl 2-methyldodecanoate (XV) in the

structure to be written for fragment XVI. The multiplet at *m/e* 125 (Table XIII) yielded two identifiable ions C_9H_{17} and $C_8H_{13}O$ which may have been generated by the following mechanism, if fragment XVI possesses the structure XXI.

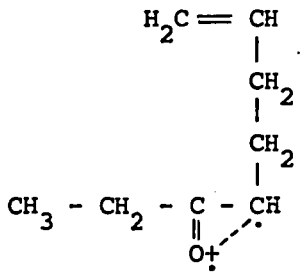


XXI (enol form) XXI-a C_9H_{17}

The loss of methoxide ion may be aided by the formation of a six-membered transition state with hydrogen radical transfer from C-5. The resulting radical should readily eliminate the ethyl acylium ion with olefinic bond formation. In intermediate XXI-a the cleavage of the allylic bond between C_3 and C_4 of the alkyl chain is aided by the formation of a cyclic oxonium ion (101) to give C_9H_{17}. The assigned structure of fragment XVI (XXI) can give $C_8H_{13}O$ by the fragmentation indicated below.



XXI



$C_8H_{13}O$

The genesis of $C_8H_{13}O$ may be visualized by a McLafferty rearrangement of the ester moiety with the elimination of $C_4H_8O_2$. The driving force for the ejection of the side chain C_5H_{11} may be oxirane formation, rather than a McLafferty rearrangement. Structure XXI cannot account for the occurrence of *m/e* 72 and *m/e* 212 (M-72) which was attributed to a McLafferty rearrangement, as discussed above (XX). Furthermore, the optical rotatory dispersion curve of this compound is void of a Cotton effect expected for the ketone group (102). For these reasons, no synthesis was attempted.

Characterization of dimethyl 4*R*-2,4-dimethyl-pent-2-en-1,5-dioate (fragment XVII) was also achieved by a consideration of its spectral properties (Figure XVIII). The 100 MHz p.m.r. spectrum of this fragment showed two singlets at τ6.25 and τ6.30. The chemical shift is typical of methyl esters, but in different chemical environments. Unsaturation in the molecule was suggested by a doublet at τ8.11 (J = 1.5 Hz) which is indicative of an olefinic methyl group (103). However, during the initial investigations no olefinic proton(s)

could be distinguished, but by searching a proton was found buried in the noise at τ3.23. The signal of this proton showed a quartet with approximate coupling constants of 9.5 and 1.5 Hz. Irradiation of this signal caused the collapse of the methyl doublet at τ8.11. The spectrum displayed one more doublet at τ8.70 (J = 7 Hz), which was attributed to a deshielded methyl group. Integration of the major signals indicated that τ6.28: τ8.11: τ8.70 = 6:3:3. The infrared spectrum exhibited carbonyl absorption at 1717 and 1737 cm^{-1} in almost equal intensities. A weak olefinic stretching band absorbed at 1650 cm^{-1}, and a strong olefinic C-H bending band at 840 cm^{-1}, representative of a trisubstituted olefin (99).

No readily identifiable molecular ion could be discerned in the mass spectrum, although it showed rather intense peaks at *m/e* 154 and 155. High resolution measurement of the latter peak measured 155.0707 which calculated 155.0708 for $C_8H_{11}O_3$. A metastable peak at *m/e* 127.4 was due to the loss of 32 mass units [methyl alcohol (72)] from *m/e* 186 giving *m/e* 154. This indicated a possible molecular ion at *m/e* 186 which could have given rise to *m/e* 155 by losing $^{+}OCH_3$. If so, then the molecular ion should be $C_8H_{11}O_3 + CH_3O = C_9H_{14}O_4$ with three degrees of unsaturation. Other significant peaks at *m/e* 126 and 127 corresponded to a loss of 28 mass units from *m/e* 154 and 155, respectively. A metastable peak at *m/e* 103.0 confirmed this explanation at least for *m/e* 154 and 126.

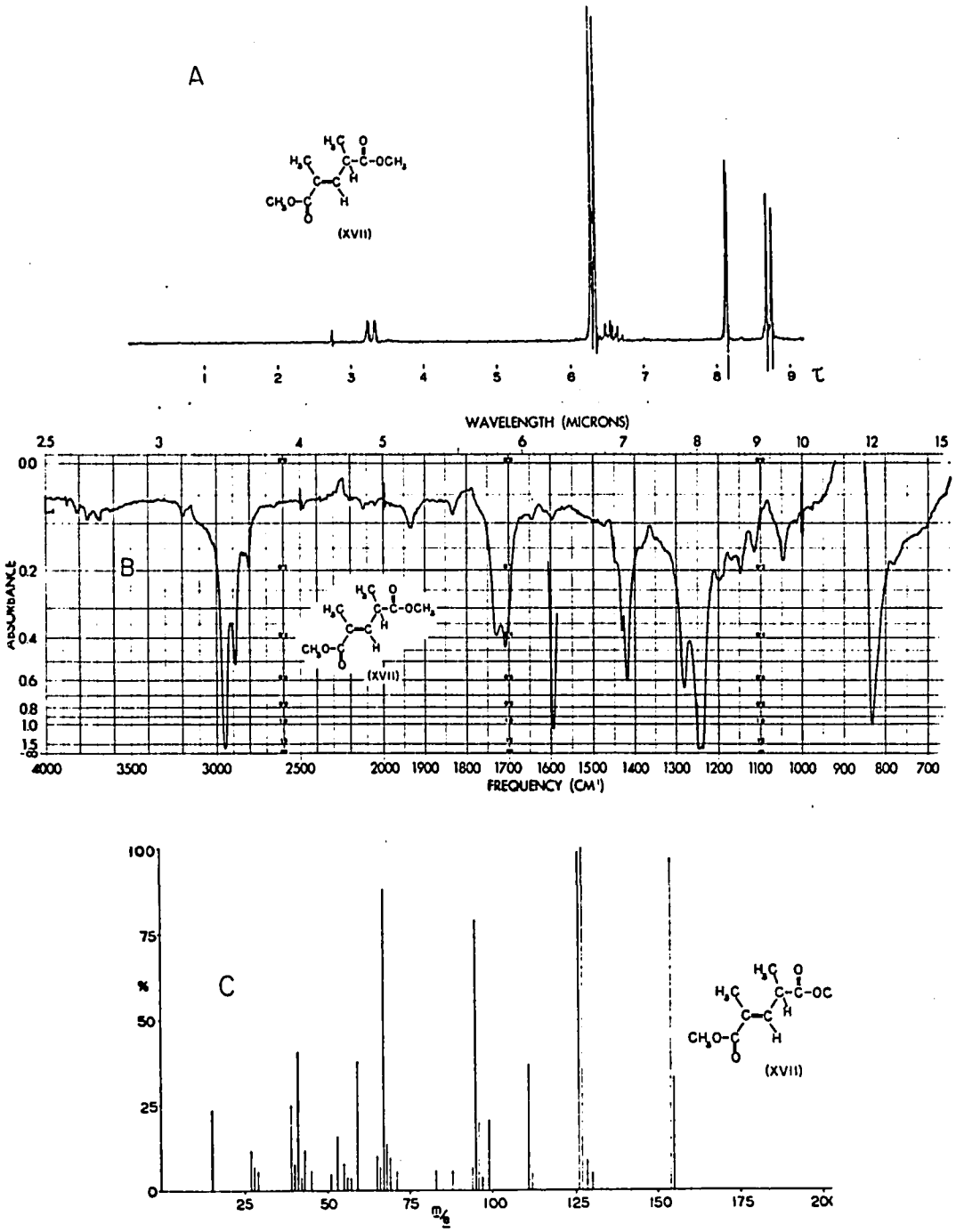
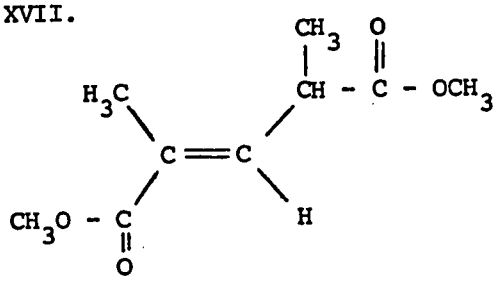


Figure XVIII Spectra of Dimethyl 2,4-Dimethyl-pent-2-en-1,5-dioate (XVII). A. 100 MHz P.M.R. Spectrum of XVII ($CDCl_3$). B. Infrared Spectrum of XVII ($CDCl_3$). C. Mass Spectrum of XVII (Source Temp. 150°C).

The combined physical data point towards an unsaturated dicarboxylic acid with an olefinic and a saturated methyl group having a molecular weight of 186, for XVII.

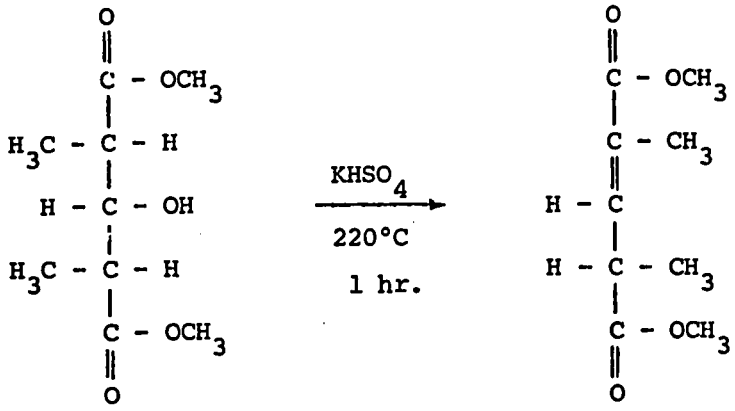


XVII

The reason for placing the methyl groups β to each other rests on biogenetic observations (13), and one of the coupling constants of the olefinic proton is 9.5 Hz which cannot be explained otherwise (103). The *cis* relationship of the olefinic proton and the carboxymethyl group is suggested by the low field position of the olefinic proton caused by the deshielding effect of the carbonyl group (104).

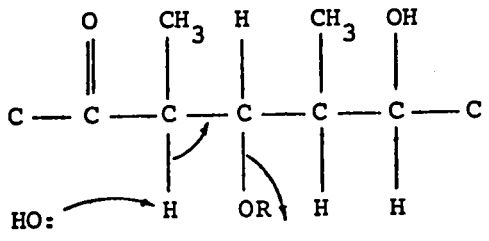
The optical rotatory dispersion curve shows a plain positive curve over the whole spectral region (200-400 mμ). Therefore, the asymmetric center has the *R*-configuration, and fragment XVII is identified as dimethyl 4*R*-2,4-dimethyl-pent-2-en-1,5-dioate. The synthesis of this compound was achieved by dehydrating dimethyl 2,4-dimethyl-3-hydroxy-glutarate, an intermediate in the synthesis of structure VIII. The physical data of this product, except for the optical

rotation, were in complete agreement with fragment IX.



IX

In view of the isolation of fragment VIII, it seems more than likely that XVII may have been produced by the former via a simple dehydration. However, such a dehydration must have occurred mainly in one direction, since XVII shows a positive optical rotation. It could be argued, that, in peliomycin, VIII or XVII was present as shown in partial structure XXII, projected in the Fischer convention.



XXII

In XXII, the dehydration takes place more readily via a β-hydroxy elimination than from the other end of the chain which does not have such a labile hydrogen.

The degradation of perhydropeliomycin resulted in the isolation of five fragments (XIII - XVII). Four of these structures could be characterized and proven by synthesis. By assuming that fragments XIII and XV have arisen from fragments XIV and XVI, respectively, the combined fragmentation products obtained from this degradation sequence represents at least 31 of the 52 carbon atoms and at least 6 of the 14 oxygen atoms postulated for the molecular formula of peliomycin ($C_{52}H_{80}O_{14}$). If indeed, fragment XVII was formed by dehydration of VIII, as discussed above, then two more carbon atoms and one more oxygen atom can be added to the total. By including the dimethyl 2-methylsuccinate and the two moles of acetic acid obtained as degradation products from peliomycin, then at least 42 carbon atoms and 11 oxygen atoms of the molecular formula are accounted for. An attempt to incorporate these fragments, obtained in the degradation experiments, in one structure, will be made in the presentation of the heuristic formula of peliomycin (Figure XXXII).

C. Structural Features of Peliomycin

In the discussion of structural features, extensive reference will be made to p.m.r. spectral analyses of peliomycin and penta-*O*-acetylpeliomycin at 100 MHz, the spectra of which are presented in Figure XIX and Figure XX, respectively. The spectra were determined in *deuterio*-chloroform, unless otherwise stated. The data obtained from these spectra by first-order analyses and spin decoupling studies are tabulated in Tables XIV and XV. In the interpretation of the spectral data, much reference will be made to the 220 MHz spectrum of peliomycin (Figure XXI) which displays a far superior resolution over the whole spectrum, thus demonstrating the great value of this recent development in p.m.r. spectroscopy, especially in the case of complicated molecules.

Further support for the structural features will be sought in infrared and ultraviolet spectroscopy, together with optical rotatory dispersion and circular dichroism measurements. Where pertinent, results of chemical reactions will be introduced to expand on a particular structure.

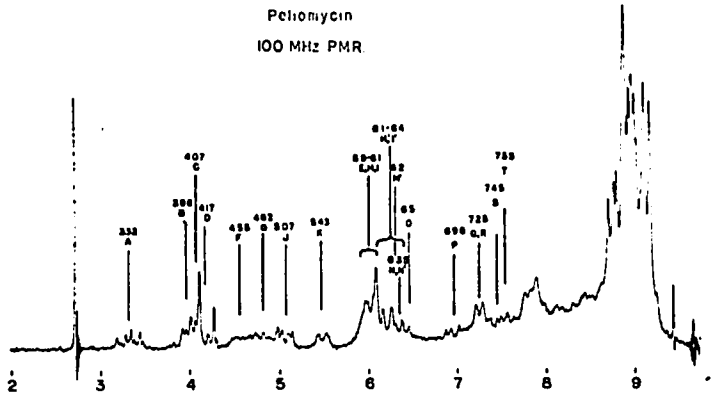


Figure XIX 100 MHz P.M.R. Spectrum of Peliomycin ($CDCl_3$)

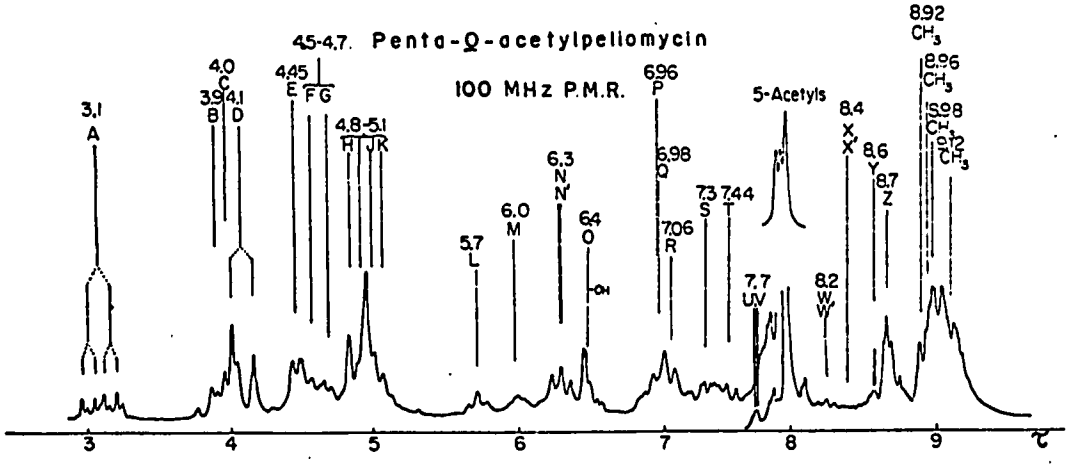


Figure XX 100 MHz P.M.R. Spectrum of Penta-*O*-acetylpeliomycin ($CDCl_3$)

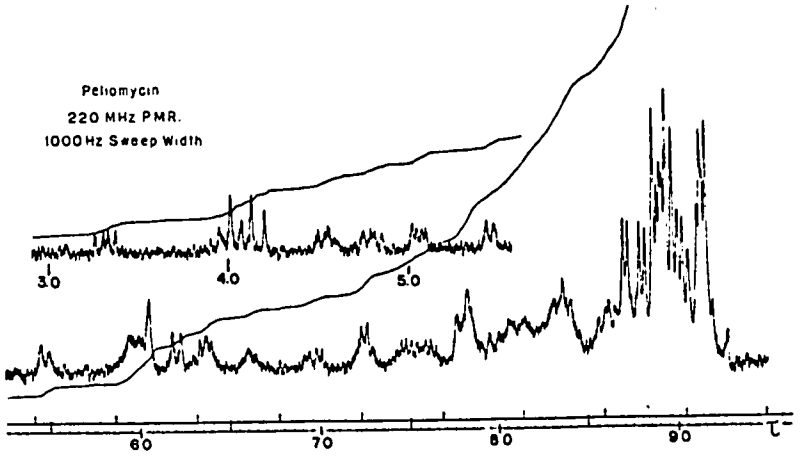


Figure XXI 220 MHz P.M.R. Spectrum of Peliomycin ($CDCl_3$)

TABLE XIV

The P.M.R. Spectra of Peliomycin and Penta-*O*-acetylpeliomycin in *deuterio*-Chloroform in the Range τ3 - τ7.5

Hydrogen	Chemical Shift, τ Peliomycin	Pentaacetate	Signal	Spacings, Hz
A	3.32	3.15	quartet	10, 15.5
B	3.96	3.92	quartet	11, 14
C	4.07	4.00	quartet	11, 14
D	4.17	4.09	doublet	15.5
E	5.9-6.1	4.45	quartet	<1, 5.7
F	4.55	4.5	quartet	10, 14
G	4.82	4.7	quartet	10, 14
H, I	5.9-6.1	4.8-4.9	multiplet	-
H',I'	6.1-6.4	6.1-6.4	multiplet	-
J	5.07	5.0-5.1	quartet	4.5, 11
K	5.43	5.0.5.1	doublet	10
L	-	5.72	triplet	6
M	-	6.0	triplet	6
M'	6.2	-	doublet	10
N, N'	6.35	6.3	multiplet	5.2, 7.2, -
O	6.5	6.4	singlet	
P	6.96	6.96	quartet	6, 14
Q	7.25	7.01	multiplet	7, -
R	7.25	7.10	multiplet	7, <1, -
S	7.45	7.30	multiplet	10, -
T	7.55	7.44	multiplet	4.5, -

TABLE XV

Spin-Spin Couplings Observed in the P.M.R. Spectrum of Penta-*O*-acetylpeliomycin in *deuterio*-Chloroform

		Spin-coupled to		
Hydrogen	Chem. Shift, τ	Hydrogen	Chem. Shift, τ	J, Hz
A	3.15	D	4.09	15.5
		S	7.30	10
B	3.92	C*	4.00	11
		F	4.5	14†
C	4.00	B	3.92	11
		G	4.7	14
D	4.09	A	3.15	15
E	4.45	N	6.31	5.7
		R	7.10	< 1
F	4.5	B	3.92	14†
		U,V	7.9-8.15	
G	4.7	C	4.00	
		U,V	7.9-8.15	
J	5.07*	T*	7.41	11
		V'*	7.82	4.5
K	5.43*	W"*	8.27	10

* observed in peliomycin ($CDCl_3$)

† observed in peliomycin ($DMSO\text{-}d_6$)

TABLE XV (Cont'd)

Spin-Spin Couplings Observed in the P.M.R. Spectrum of Penta-*O*-acetylpeliomycin in *deuterio*-Chloroform

		Spin-coupled to		
Hydrogen	Chem. Shift, τ	Hydrogen	Chem. Shift, τ	J, Hz
L	5.72	W,W'	8.23	6, 6
M	5.99	X,X'	8.4	6, 6
N	6.31	E	4.45	5.7
		X	8.4	7.2
P	6.96*	U,V*	7.8	5.5, 14
Q	7.01	U,V	7.8	
		CH_3	8.87	7
R	7.10	U,V	7.8	
		CH_3	8.96	7
S	7.30	A	3.15	10
		CH_3	8.98	
T	7.44	J*	5.04	11
		CH_3	9.12	6.5
W,W'	8.23	L	5.72	6
X	8.4	M	5.99	6
		N	6.31	7.2
Z	8.7	CH_3	8.8-9.2	

* observed in peliomycin ($CDCl_3$)

† observed in peliomycin (DMSO-d_6)

It was established in the derivation of a molecular formula from pertrimethylsilylpeliomycin via mass spectral analysis, that the antibiotic probably contains six hydroxyl groups. Acetyl determination of the *O*-acetyl derivative of peliomycin showed five acetate groups (62). This observation was confirmed by integration of the p.m.r. spectrum of this derivative (Figure XX), which displayed three singlets at τ8. The sixth hydroxyl function was indicated by the hydroxyl band in the infrared (3400 cm^{-1}) of penta-*O*-acetylpeliomycin, and the disappearance of a singlet of intensity one at τ6.3 when the latter compound in *deuterio*-chloroform was exchanged with deuterium oxide. Masamune (64) had observed a free hydroxyl group in the infrared spectrum of acetylated oligomycin B, which he attributed to a tertiary alcohol function.

It is well established (103), that acetylation of a secondary alcohol group causes strong deshielding of the methine proton by about 1.5 τ-values from τ6. On the other hand (103), the deshielding of the methylene protons of a primary alcohol function upon acetylation amounts only to about 0.5 τ-values. The 220 MHz spectrum of peliomycin showed five protons by integration in the region τ5.9 - τ6.1. Three of these protons moved to lower field (τ4.3 - τ4.9) after acetylation, and are therefore assigned to secondary alcohol groups. These three protons are designated as

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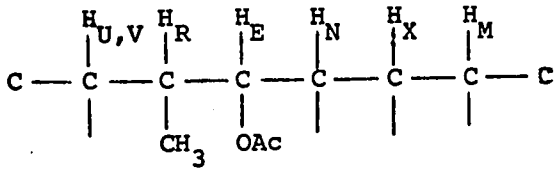
protons E, H and I in Table XIV and XV and Figures XIX and XX.

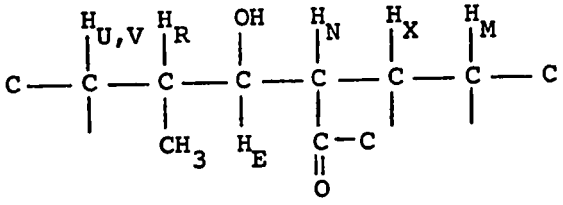
Spin decoupling experiments provided some more information on the environment of these protons. Proton E of the penta-*O*-acetyl derivative absorbed at τ4.45 as a quartet. It was found to be coupled to proton N at τ6.31 (J_{EN} = 5.7 Hz) and to proton R at τ7.10 (J_{ER} < 1 Hz). Further searching revealed that proton N was coupled to a proton (X) at τ8.4, which, upon irradiation, caused the signal of N to collapse to a doublet (J_{NX} = 7 Hz). Triple irradiation at protons E and X gave a singlet for N. When the quartet of proton R was similarly investigated, it collapsed to a singlet upon triple irradiation in the U,V-region (τ7.8) and in the methyl region (τ8.96). The coupling constant $J_{R-U,V}$ could not be determined, but J_{R-CH_3} is approximately 7 Hz. The foregoing information suggests partial structure XXIII.



XXIII

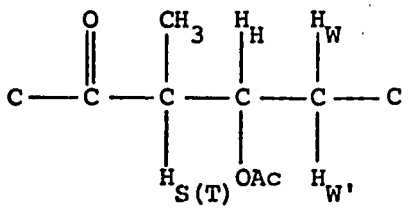
The chemical shift of proton X is in accordance with that of a hydrogen of a methylene group. Although other protons such as proton M at τ6.0 in the pentaacetate spectrum are coupled to X, no further information could be obtained either about proton M or proton X. The chemical shift of proton N (τ6.31)

necessitates the incorporation of a vicinal electron-withdrawing group other than hydroxyl, since peliomycin was resistant to periodate oxidation. On the basis of these results, proton N seems best rationalized as arising from a β-hydroxy carbonyl structure as indicated in partial structure XXIV.

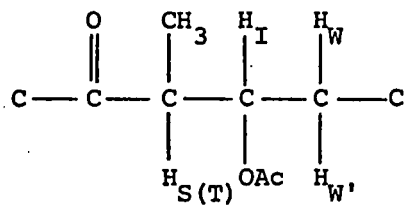


XXIV

Double resonance studies indicated that protons H and I (Table XV) are in very similar environments. Their chemical shifts could not individually be assigned, but they absorbed in the τ4.8 to τ4.9 region of the spectrum for the pentaacetate. The signals were disturbed when the protons in the S,T-region (τ7.3-7.4) and at τ8.2 (W,W' protons) were irradiated. The S,T protons appear to be coupled to protons in the methyl region. Since proton S was affected by reducing peliomycin with sodium borohydride, and considering also the chemical shifts of protons S and T, it seems likely that partial structures XXV and XXVa are present.

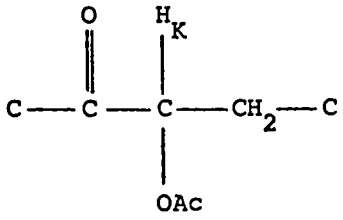


XXV



XXVa

The deshielding of proton K from τ5.43 in peliomycin to τ5.0-5.1 in the penta-*O*-acetyl derivative (see Table XIV) is not as big as seen above for protons E,H and I. Its chemical shift in the pentaacetate spectrum could not be exactly established as it was part of a large multiplet (protons E-J). In peliomycin proton K exhibits a doublet with a spacing of 10 Hz due to coupling with proton W" which absorbs at τ8.27. This doublet of proton K is not sharp, and most probably K is coupled to some other protons as well. The signal of proton K disappears when peliomycin is reduced with sodium borohydride, therefore, the chemical environment of K may be as in partial structure XXVI.



XXVI

Further information on the types of hydroxyl group (104) present in peliomycin was sought from the p.m.r. spectrum in which *hexadeuterio*-dimethylsulfoxide was used as the solvent. This approach failed, however, as only a broad absorption band was obtained in the region τ5.5 to τ6.8. Exchange with deuterium oxide caused this band to decrease in intensity and a broad singlet to appear at τ6.4, which is due to DOH in *hexadeuterio*-dimethylsulfoxide (Figure XXII); the singlet shifted to lower τ-values upon addition of some more deuterium oxide. Integration of the spectrum in the region τ5.5 to τ6.8 showed the presence of only four protons immediately after exchange. After 24 hours, the integration over the same region accounted for 5 protons; and 5.6 protons and 7.8 protons after 48 and 144 hours, respectively. It appears that peliomycin contains four readily accessible hydroxyl groups for proton exchange. The resistance to exchange exhibited by two of the hydroxyl groups cannot be explained. However, for a molecule with the apparent complexity of peliomycin which is highly hydrophobic, it is conceivable that strong intramolecular hydrogen-bonded hydroxyl groups are present, both in dimethylsulfoxide and in chloroform solutions. As a consequence, the slow exchange could arise as well from an inaccessibility of the hydroxyl groups to the deuterium oxide as a resistance to cleavage of the hydrogen bond.

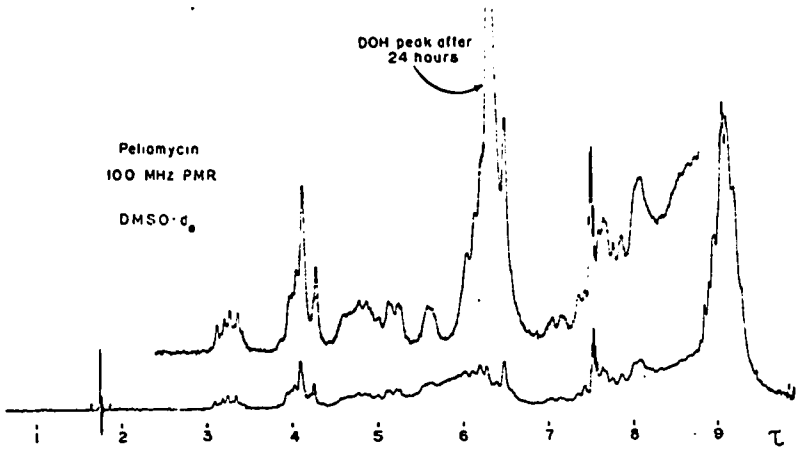


Figure XXII 100 MHz P.M.R. Spectrum of Peliomycin (DMSO-d_6)

When peliomycin was treated with sodium hydroxide in aqueous methyl alcohol at room temperature for twenty-four hours, one equivalent of base was consumed per 616 g of peliomycin. Since the molecular weight of peliomycin appears to be 928, the antibiotic must contain at most one ester grouping and liberated about 0.3 moles of additional acid during the alkaline treatment. The product of this reaction yielded two fractions, a neutral and an acidic one. Examination of the methyl esters of the latter fraction by gas chromatography revealed two major components whose structures could not be established. As will be seen later, these two structures are chemically closely related and both are monocarboxylic acid derivatives.

Catalytic hydrogenation of peliomycin over palladium on charcoal caused the rapid uptake of 4.1 moles of hydrogen per mole of peliomycin (mol. wt. 928). The p.m.r. spectrum of this product lacked absorption peaks below τ4.9. Hence, the region τ3.1 to τ4.9 represents six olefinic protons (A-D, F and G of Table XIV), as shown by integration. Two of these protons (A and D) are part of an α,β-unsaturated lactone for which evidence is presented in Table XVI. The p.m.r. spectrum of perhydropeliomycin is reproduced in Figure XXIII.

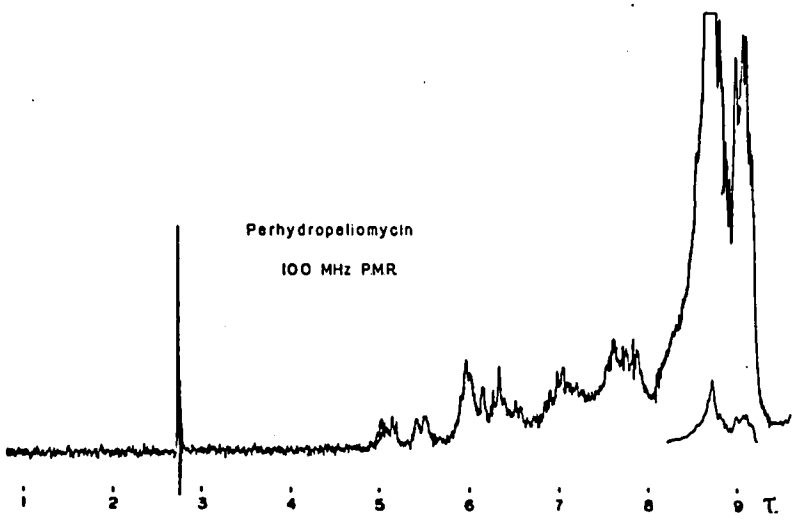


Figure XXIII 100 MHz P.M.R. Spectrum of Perhydropeliomycin ($CDCl_3$)

TABLE XVI

Evidence for the α,β-Unsaturated Lactone Group in Peliomycin

	Peliomycin	Perhydropeliomycin
p.m.r. ($CDCl_3$)	τ3.32* (quartet; 10,15.5 Hz)	none
	τ4.17** (doublet; 15.5 Hz)	none
infrared ($CHCl_3$)	1707 cm^{-1}	1727 cm^{-1}
ultraviolet (CH_3OH)	218 mμ	none

*proton A of Table XIV

**proton D of Table XIV

The infrared spectrum of peliomycin (Figure XXIV), determined in chloroform, shows a strong absorption band at 1707 cm^{-1} and a definite shoulder at 1732 cm^{-1}. The former band moved to 1727 cm^{-1} upon catalytic hydrogenation. These frequencies correspond to those reported (1712 and 1724 cm^{-1}) for the α,β-unsaturated lactone of the macrolide chalcomycin (16) and the polyene antibiotic pimaricin (105). Although sodium borohydride (Figure XXV) reduced the whole carbonyl region in the infrared by 66%, this reaction caused the shoulder at 218 mμ in the ultraviolet spectrum to disappear. This absorption band suffered the same fate when peliomycin was treated with dilute alkali [0.05 *M* NaOH/CH_3OH (99%)]. The absorption position at 218 mμ is in agreement with α,β-unsaturated esters (106).

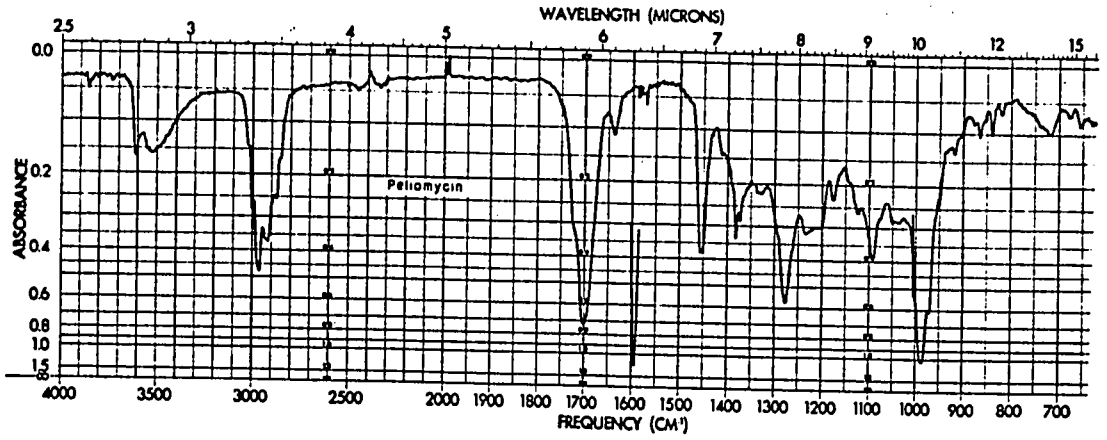


Figure XXIV The Infrared Spectrum of Peliomycin ($CHCl_3$)

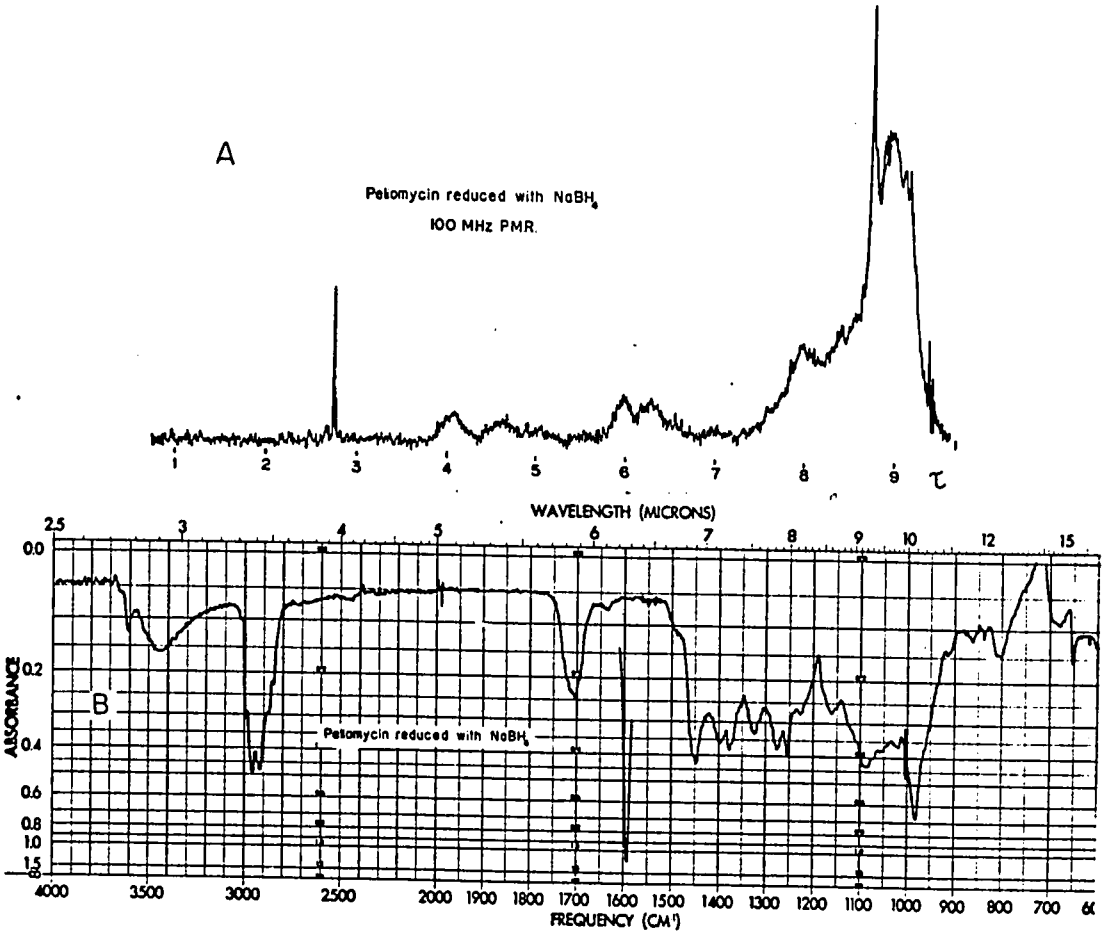
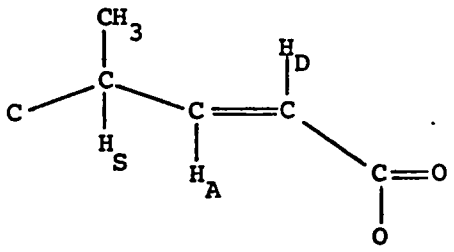


Figure XXV Spectra of Peliomycin reduced with Sodium Borohydride. A. 100 MHz P.M.R. Spectrum ($CDCl_3$). B. Infrared Spectrum ($CHCl_3$).

The chemical shift and the coupling constants of protons A and D are typical of α,β-unsaturated esters of which the β-hydrogen is *cis* to the carbonyl group (107). These values are in agreement with those reported for methyl *trans*-2-pentenoate (108) and methyl *trans*-crotonate (109). Furthermore, the p.m.r. values reported for the α,β-unsaturated lactone function of chalcomycin (16) agree closely with those of peliomycin. Therefore, the following partial structure (XXVII) may be written:

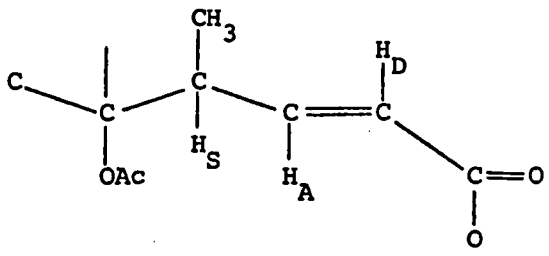


XXVII

Spin decoupling studies (Table XV) showed that irradiating proton S at τ7.42 caused the signal of proton A to collapse to a doublet (J_{AS} = 10 Hz). Proton S was also found coupled to protons in the methyl region (τ8.98); triple irradiation at proton A and at τ8.98 did not collapse the signal of S to a singlet and must therefore be coupled to other protons as well.

Examination of the spectrum of penta-*O*-acetylpeliomycin showed that acetylation had caused protons A,D and S to move

to lower field, *viz.*: τ3.15, 4.07 and 7.30, respectively. This shift may be explained by the deshielding effect of an acetoxy group (103) as in partial structure XXVIII:



XXVIII

Besides the lactone function, peliomycin contains two kinds of ketone group(s) as indicated by infrared spectral analysis. In chloroform, the spectrum displayed a shoulder at 1732 cm^{-1} (Figure XXIV), in addition to the intense lactone band at 1707 cm^{-1}. Upon changing the solvent to carbon tetrachloride, the lactone band moved to 1727 cm^{-1} and thereby revealed a shoulder at 1700 cm^{-1}. No aldehyde functions are present in peliomycin, as all the p.m.r. spectra lacked absorption peaks below τ3.

The presence of ketone functions was confirmed by optical rotatory dispersion (o.r.d.) and circular dichroism (c.d.) measurements. The o.r.d. curve of peliomycin determined in methyl alcohol (Figure XXVI) displays a prominent negative Cotton effect with inversion point at 292 mμ. Furthermore, a shoulder is observed at 265 mμ, and a weakly positive Cotton effect in the 345 mμ region, which is partly masked by the background

curve. The latter Cotton effect was better revealed in the c.d. curve (Figure XXVI) at 330 mμ, which curve also exhibited a negative minimum at 292 mμ. Upon changing the solvent to dioxane, only a slight bathochromic shift (1-5 mμ) was observed due to change in polarity of the solvent (110). These shifts and some variations in the molecular ellipticity [θ], the molecular rotation [φ], and the molecular amplitude (a) due to solvent effects are presented in Table XVII.

TABLE XVII

Optical Rotatory Dispersion and Circular Dichroism Data of Peliomycin

	CH_3OH	*p*-Dioxane
$[\theta]_{292}$	- 10,000	- 13,500
$[\theta]_{330}$	+ 630	+ 462
$[\phi]_{270}$	+ 11,500	+ 15,500
$[\phi]_{310}$	- 7,500	- 9,000
a	- 190†	- 240†
a^*_{292}	- 122†	- 165†
a^*_{330}	- 7.7	+ 5.6

$a^* = 0.0122\ [\theta]$ (111)

† = extremum was not reached (111)

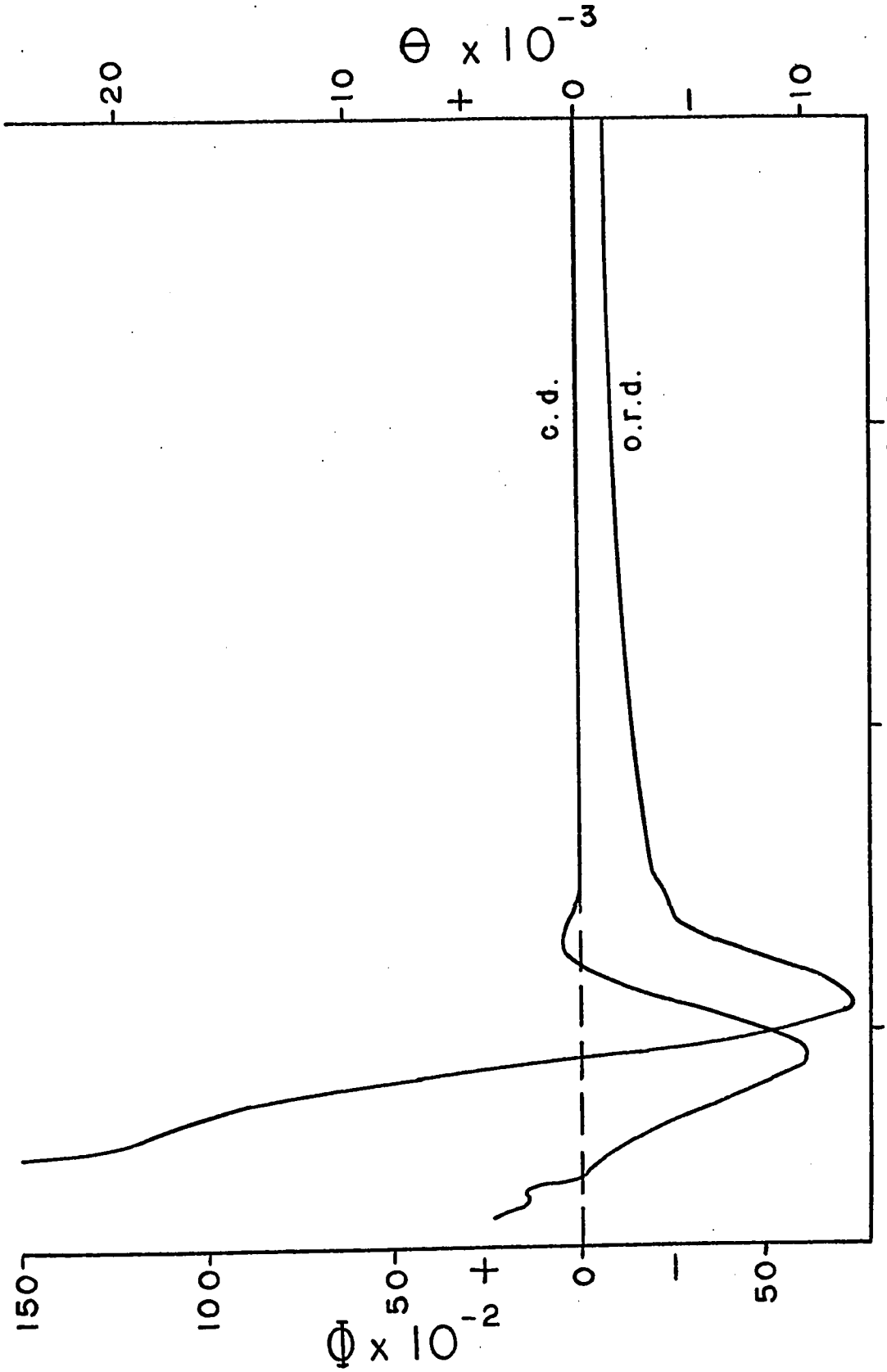
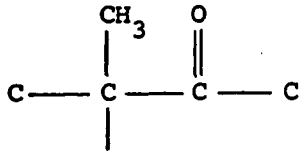


Figure XXVI The Optical Rotatory Dispersion (o.r.d.) and Circular Dichroism (c.d.) Curves of Peliomycin (CH_3OH)

The starred values may be somewhat more accurate than the corresponding unstarred values. The latter are only approximate as they were based on the shoulder at 270 mμ (Figure XXVI), since the second extremum was not reached.

The observed Cotton effect with inversion point at 292 mμ is consistent with saturated ketones perturbed by an asymmetric center within two carbon atoms of this chromophore (112). The spectra were not affected by solvent polarity changes (113), nor were there any changes observed when the methanolic solution was acidified with a drop of concentrated hydrochloric acid. Hence, hemi-ketal formation (114) did not take place, which suggests (114) that the ketone groups in peliomycin have a structural environment as in XXIX.



XXIX

This partial structure is common in macrolide antibiotics (40) and can be explained by the propionate rule (38) in the biosynthesis of these compounds.

The absence of α,β-unsaturated ketones was indicated by the following observations. The o.r.d. and c.d. data were not affected by changes in the polarity of the

solvent (113). The ultraviolet spectrum did not display absorption peaks attributable to this grouping, nor were any of them altered when peliomycin was reduced with sodium borohydride, except at 218 mμ. Furthermore, the infrared spectrum lacked carbonyl absorption bands below 1700 cm^{-1} (99) associated with α,β-unsaturated ketones.

Although the extremum at low wave length of the rotatory dispersion curve was not reached, the c.d. curve indicated further absorption below 250 mμ. The shoulder at 250 mμ is in accordance with an α,β-unsaturated ester (115); this band could be observed just before the instrument fails to register, and is part of a Cotton effect which extends to lower wavelength. An explanation is not apparent for the small Cotton effect in the c.d. curve at 330 mμ. No corresponding absorption peaks were observed in the ultraviolet spectrum, not even at high concentration of the antibiotic. Perhaps, the recent development of magnetic circular dichroism and optical rotatory dispersion measurements (116) can give more conclusive evidence. This technique appears to be promising in resolving overlapping Cotton effects.

Although catalytic reduction indicated the presence of four double bonds, an epoxide may be present, as suggested by the disappearance of absorption bands at

1260, 960 and 820 cm^{-1} (99). When peliomycin was subjected to the Bodforss reaction (117), no extension of conjugation could be observed in the ultraviolet spectrum. Thus, if an epoxide is present, it is not γ,δ to an α,β-unsaturated carbonyl function (105). The p.m.r. spectrum is not conclusive with respect to an epoxide function. Although signals are observed around $\tau 7$, where epoxides absorb (118), no definite conclusions can be given.

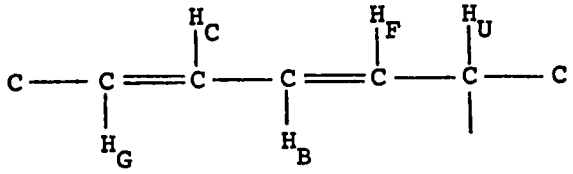
When peliomycin was treated with sodium borohydride in methyl alcohol, a product was obtained which showed p.m.r. and infrared spectra which are reproduced in Figure XXV. There is no evidence of protons A and D in the p.m.r. spectrum and the carbonyl region in the infrared spectrum (1700-1735 cm^{-1}) of this product has been reduced by 66% as compared with peliomycin. Therefore the presumed α,β-unsaturated lactone group of the peliomycin molecule was altered. Since the solution was buffered with sodium bicarbonate, it seems unlikely that hydrolysis had affected protons A and D in the p.m.r. spectrum, but that reduction of the lactone group had taken place. Esters are not normally reduced with sodium borohydride (119), although the reduction of the ester function of methyl 3α-hydroxy-12-keto-9(11)-cholenate has been reported (120). On the other hand, sugar uronates are

readily reduced to the corresponding hydroxy methyl groups (121). This easy reduction may be attributed to the electron-withdrawing ability of the ring oxygen. Recent work of Ishizumi and co-workers (122) led them to conclude that an increase in the electrophilicity of the carbonyl carbon atom facilitates sodium borohydride reduction of esters.

The spectroscopic investigations confirmed the presence of unsaturation in peliomycin as indicated by chemical tests (62) during its characterization. The weak band at 1645 cm^{-1} in the infrared spectrum, as well as the absorption bands in the ultraviolet spectrum, disappeared on catalytic hydrogenation. As seen earlier, the olefinic protons in the p.m.r. spectrum (< τ4.9) were similarly affected. Two of these protons have been accounted for (A and D).

The remaining four olefinic protons appear to be present in one structural unit and were clearly displayed in the 220 MHz spectrum. These protons are designated as protons B,C,F and G (Table XIV). The signal for proton B at τ3.96 is a quartet which is overlapped by another quartet of proton C at τ4.17. In *deuterio*-chloroform, no coupling could be discerned between protons B,C and F,G. In *hexadeuterio*-dimethylsulfoxide, it was established that

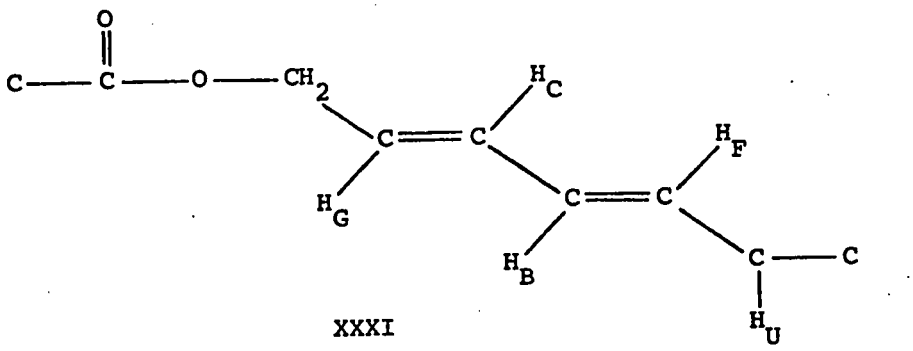
protons B and C are coupled to protons F (τ4.55) and G (τ4.82), respectively. In addition, it was found that proton F is coupled to proton U at τ7.75, which, when irradiated, caused the signal of proton F to collapse to a rough doublet such that J_{BF} = 14 Hz. The chemical shift is typical of an allylic proton (123), which was not found in perhydropeliomycin. Triple irradiation of protons A and (F+G) produced three singlets for protons B,C and D, proving that the splittings are not due to any other protons. On the basis of the above data, partial structure XXX is presented.



XXX

The assigned *trans*-olefinic configuration is based on the relatively large coupling constants observed in the signals of protons B,C,F and G (J_{BF} = J_{CG} = 14 Hz).

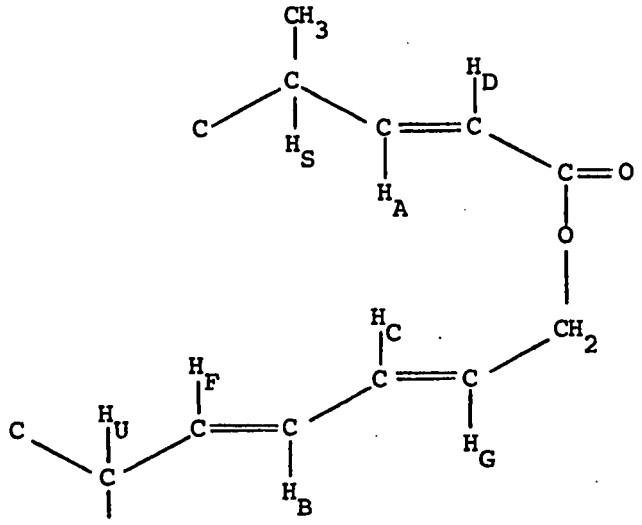
The proton(s) responsible for the remaining splitting of proton G could not be found. In view of the detection of glycolaldehyde (Scheme II) as a product of the oxidation of peliomycin with the osmium tetroxide-periodate reagent (84), it can be surmised that G is coupled to methylene protons as in partial structure XXXI.



XXXI

If so, proton G would be very weakly coupled to one of the protons of the vicinal methylene group, since the signal of G was a rough quartet. As will be seen later, catalytic hydrogenation of peliomycin gives rise to an alkyl (probably *n*-hexyl) group. It is therefore conceivable that this group was generated by hydrogenolysis of the allylic ester grouping (85) of partial structure XXXI, prior to saturation of the olefinic linkage. Furthermore, the p.m.r. spectrum has provided evidence for only three olefinic bonds in peliomycin, but hydrogenation consumed 4.1 moles of hydrogen per mole of peliomycin. For these reasons, the presence of an epoxide ring in the antibiotic, which also undergoes hydrogenolysis, seems to be precluded. As seen before, no p.m.r. evidence for an epoxide function could be supplied.

The saponification equivalent determination of peliomycin showed only one ester grouping present; thus structural unit XXXI can be expanded to partial structure XXXII:



XXXII

In the discussion of the carbonyl environment, the suspected Cotton effect observed just below 250 mμ in the c.d. curve (Figure XXVI) was attributed to the α,β-unsaturated ester. However, optically active dienes are also expected to display a Cotton effect in this region (124). For this to occur, a skew pattern for the diene would be required (124).

As seen before, the absorption band at 218 mμ in the ultraviolet spectrum disappeared upon base treatment. The ultraviolet spectrum of peliomycin (Figure XXVII) showed extended conjugation when determined in 0.05*M* sodium hydroxide in methyl alcohol 99% (Figure XXVII). The pertinent data are presented in Table XVIII.

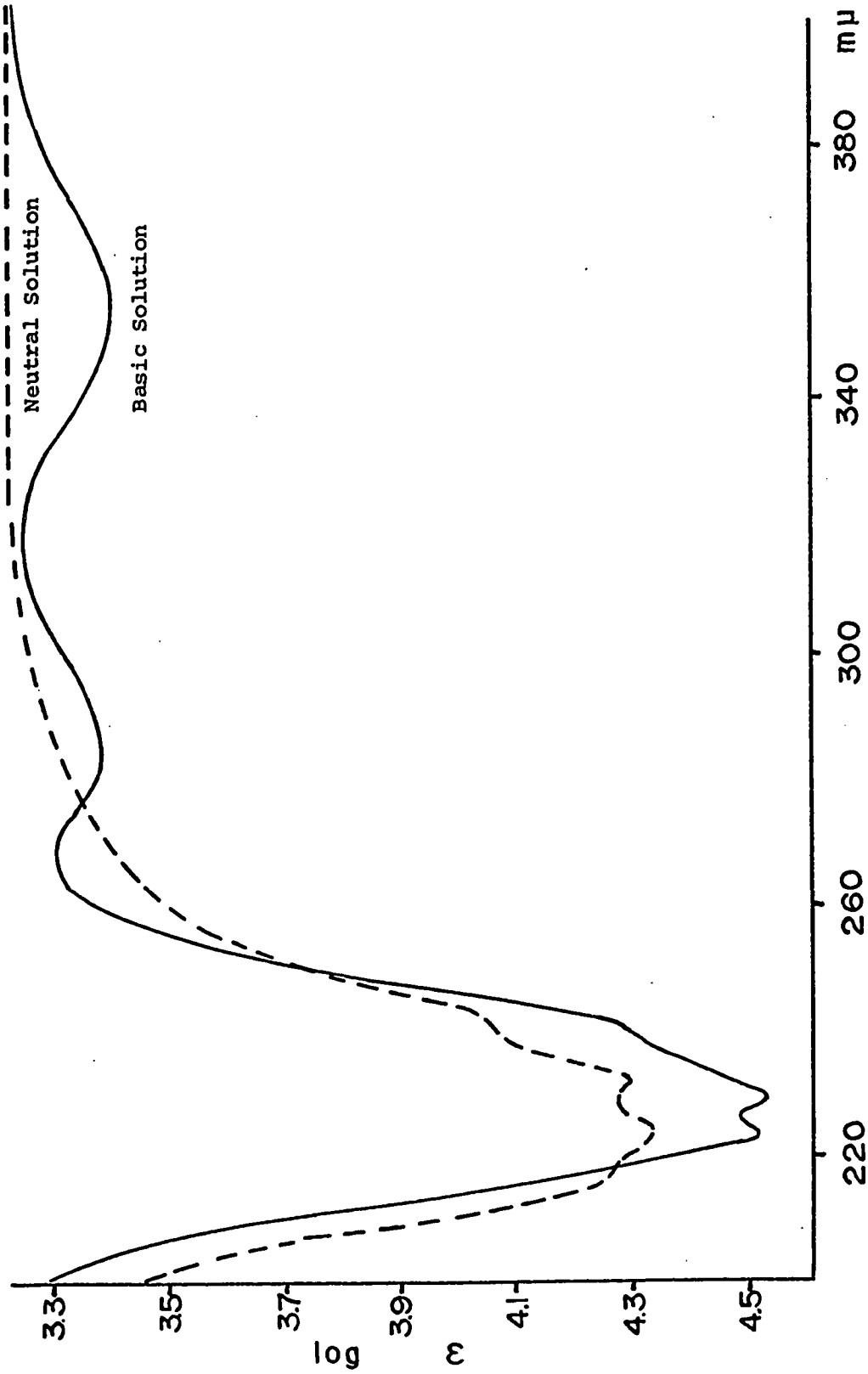


Figure XXVII The Ultraviolet Spectrum of Peliomycin in Neutral (CH_3OH) and Basic [0.05*M* NaOH/CH_3OH (99%)] Solution

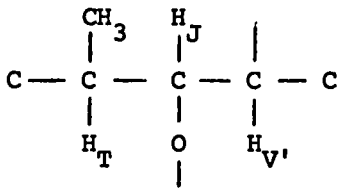
TABLE XVIII

Ultraviolet Spectral Data of Peliomycin in Neutral and Bacic Media

CH_3OH (99%)		0.05*M* NaOH/CH_3OH (99%)		
λ max, mμ	log ε	λ max, mμ	log ε (0.5 hr)	log ε (18 hrs.)
218 (sh)	4.29	223	4.50	4.51
228	4.35	229	4.51	4.50
232	4.30	238 (sh)	4.37	4.34
240 (sh)	4.07	282	3.40	-
		353	3.40	3.74

The extension of conjugation changed with time. At first, an absorption peak appeared at 282 mμ which vanished when a peak at 353 mμ presented itself. Upon neutralization with methanolic hydrochloric acid, the latter band disappeared, but no peak at 218 mμ was observed, while the remainder of the spectrum had returned to its original state. Rebasification, however, failed to extend the conjugation again. The reason for this extended conjugation is not understood, and even the Woodward rules (125) are not helpful. It appears that a chemical reaction had taken place in the alkaline solution which caused an irreversible transformation which was finalized by the addition of acid. An extensive literature search (126, 127) failed to reveal any precedent for this observation.

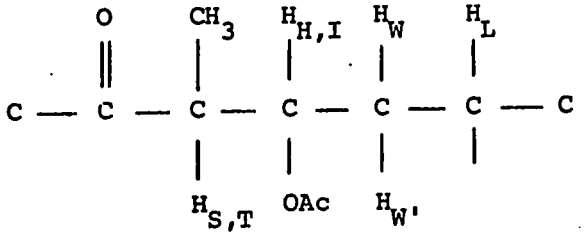
The remainder of this discussion on the structural features of peliomycin pertains mainly to the p.m.r. analysis. The most deshielded proton not affected by catalytic hydrogenation is proton J which absorbs at τ5.07 as a quartet. Spin decoupling studies showed that irradiation of proton T at τ7.41 collapsed the signal of J to a doublet with a spacing of 4.5 Hz. A larger doublet of 11 Hz was obtained when proton V' at τ7.82 was irradiated, hence J_{JT} = 11 Hz and $J_{JV'}$ = 4.5 Hz. Upon investigating proton T, this quartet was found coupled to a methyl group at τ9.12. Irradiation of the latter position collapsed T to a doublet of 11 Hz, which is in fact J_{JT}. Like protons A and D, the signal of proton J was not found in the product from the sodium borohydride reduction of peliomycin. It seems therefore, that proton J is a methine proton as shown in partial structure XXXIII.



XXXIII

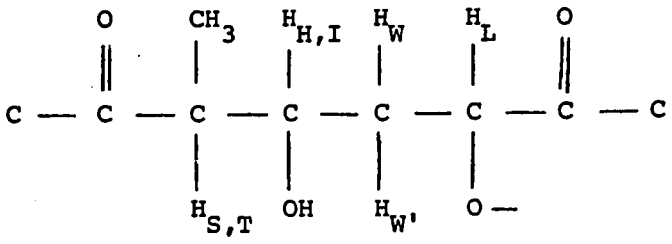
The oxygen atom could be a carbonyl group or part of a hemiketal function, as both would provide a basis for the low field absorption as well as for the change in chemical shift caused by the borohydride reduction.

The absorption of proton L at τ5.72 was detected only in the pentaacetate spectrum, where it appeared as a triplet. Irradiation at τ8.23 collapsed the signal to a singlet, hence there are two protons W and W' with $J_{LW} \simeq J_{LW'} \simeq 6$ Hz. Since the methine protons H and I of the secondary alcohol groups as represented in structural unit XXV are also coupled to protons in the τ8.2 region, it appears reasonable to extend this structure to partial structure XXXIV.



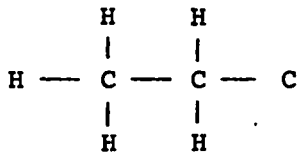
XXXIV

Since L represents only one proton, either H or I and S or T may be related to proton L. The chemical shift of proton L and its triplet character are in agreement with partial structure XXXV, especially if the neighboring carbonyl group is masked in a hemi-ketal ring.



XXXV

The doublet at τ6.2 in the 220 MHz spectrum was not observed before as it was most probably buried in the large multiplet at τ6 in the 100 MHz spectrum. It showed a spacing of 10 Hz and integrated for one proton. In the same spectrum (220 MHz), a two proton quartet was displayed at τ6.35 with spacings of 7 Hz. This signal is attributed to the methylene protons of an ethyl group (XXXVI).

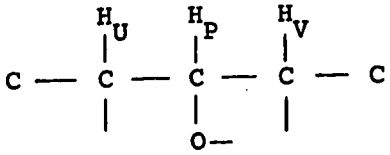


XXXVI

Finally, this spectrum shows a broad singlet at τ6.5, which is most probably due to a hydroxyl proton.

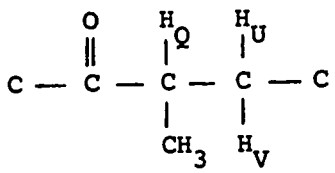
The quartet at τ6.96 was denoted as proton P, and displayed spacings of 5.5 and 14 Hz. The signal collapsed to a singlet upon irradiation at τ7.84. Hence, there are two protons, *viz.*: U and V, and which have a chemical shift smaller than the band width of the signal used for the double irradiation experiment. Proton P was affected by sodium borohydride reductions and appears to be situated near a carbonyl or a hemi-ketal function. Its chemical shift is not unlike epoxide protons (118), but perhydropeliomycin still displays this signal. In the spectrum of the penta-*O*-acetyl derivative, proton P is

not shifted, but is badly obscured by signals of protons Q and R. Proton P may be present as in partial structure XXXVII.

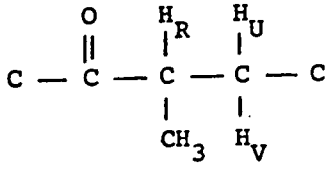


XXXVII

The rough doublet at τ7.24 displays a spacing of 7 Hz (Figure XXI) and integrates for three protons: Q, R and a wide multiplet. The signal is affected by reductions with sodium borohydride, but not by hydrogenation; therefore, the signals are not epoxide protons. In the pentaacetate spectrum, proton Q absorbs at τ7.01 and R at τ7.10. Irradiations showed coupling of Q and R with protons in the U,V-region (τ7.8) and with protons at τ8.87 and τ8.96, respectively. No information could be obtained regarding $J_{Q-U,V}$, but $J_{Q-CH_3} = J_{R-CH_3} = 7$ Hz. Triple irradiation of the U,V region and at τ8.9 reduced the signals of protons P,Q and R to three singlets. These data imply that the environment of Q and R are similar, and structure XXXVIIIa and XXXVIIIb should be representative:

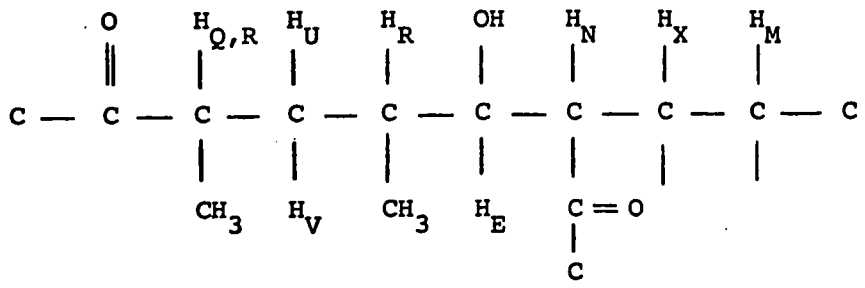


XXXVIII-a



XXXVIII-b

In view of the fact that both partial structures XXIV and XXXVIII have protons U and/or V in common, an expanded structure XXXIX could be written:



XXXIX

The 220 MHz spectrum of peliomycin was very informative concerning the methyl region. By employing a 500 Hz sweep width and the solvents indicated (Figure XXVIII), the methyl region lends itself to the following assignment: 1, triplet, 9 doublets and 1 singlet for a total of eleven C-methyl groups. The results obtained via the Kuhn-Roth oxidations (62) analyzed for eight C-methyl groups.

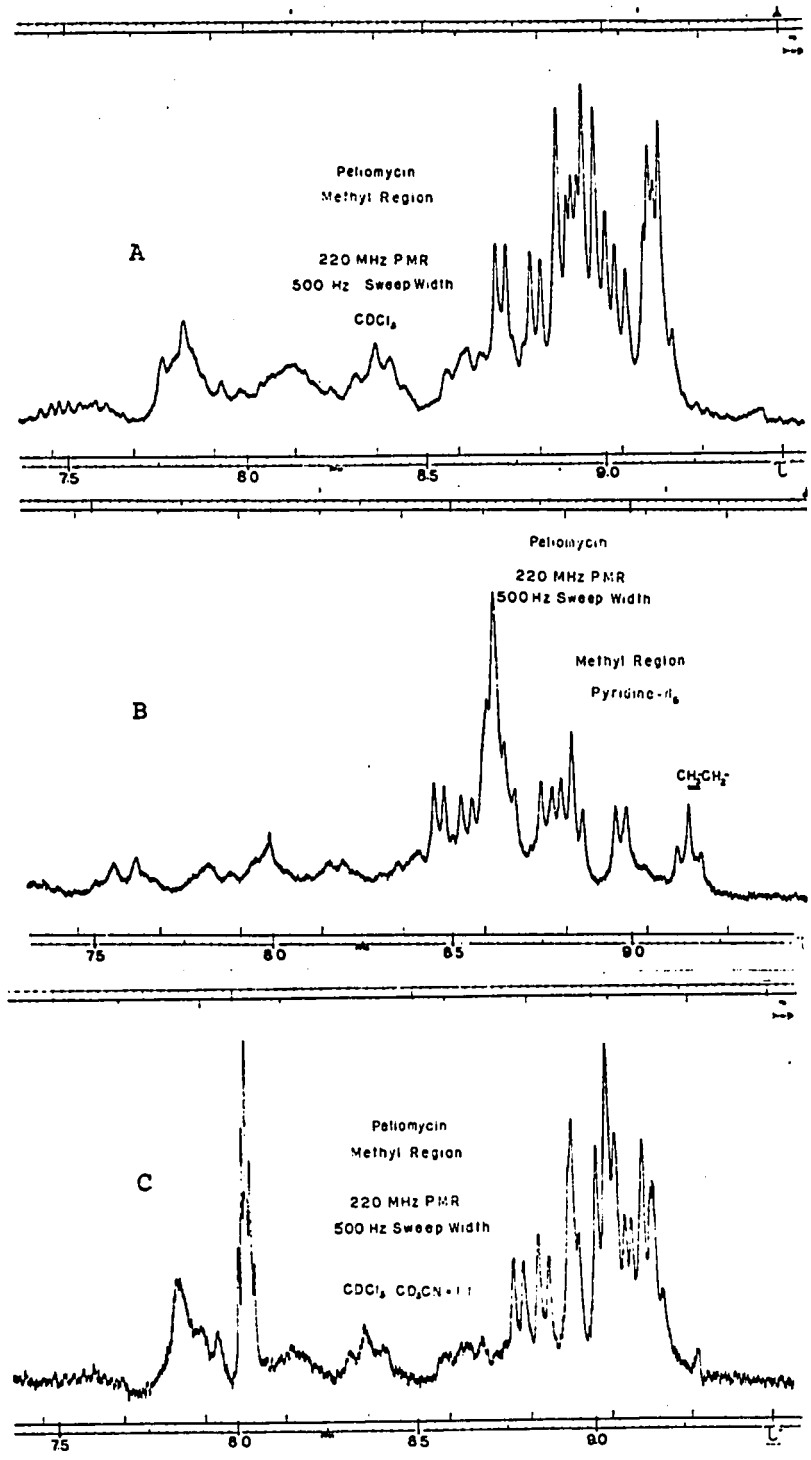


Figure XXVIII 220 MHz P.M.R. Spectra of the Methyl Region of Peliomycin in $CDCl_3$ (A), Pyridine-d_5 (B) and CD_3CN:$CDCl_3$ = 1:1 (C).

All the macrolide antibiotics, which have been structurally investigated, contain sugar residues with the exception of borilidin (20). Since sugars yield readily to acid hydrolysis by cleavage of their glycosidic bonds, this reaction facilitates structure investigation by liberating a definite fragment of the molecule which can be isolated and characterized. However, when peliomycin was subjected to acid hydrolysis under various conditions of acid strength (from 50% acetic acid to 3*N* hydrochloric acid in acetic acid) for periods from 3 to 14.5 hours up to 100°C, no sugars could be detected. Under mild acid conditions the antibiotic is stable (t.l.c.), but stronger acid concentrations causes complete breakdown of the molecule without liberating sugars as shown by paper chromatography of the products and employing the alcoholic silver nitrate method, as a means of detection (128). These results prove that peliomycin lacks sugar substituents, a fact which it has in common with oligomycin B (65).

As the hydroxamic acid test for esters proved positive (62), it was of great interest to determine how many ester functions are present in peliomycin. Furthermore, this approach, barring the presence of other base sensitive centers in the molecule, should give information whether peliomycin in fact belongs to the macrolide family of antibiotics (14). Initial saponification experiments were carried out in 0.006*N* and 0.025*N* sodium hydroxide

up to 100°C and periods of twelve hours. Acidification of the alkaline solution and extraction yielded a compound on solvent removal which lacked the carbonyl stretching band at 1707 cm^{-1}. Instead of this band, which is assigned to the α,β-unsaturated ester in the discussion on structural features, a weak band had appeared at 1760 cm^{-1} which suggested lactone formation (99) to some extent. Thin layer chromatograms of the products showed only one spot when developed in four different solvent systems. In each case the R_f value was somewhat different than that for peliomycin. Mass spectral analysis of the trimethylsilyl derivative prepared according to Sweeley (70), showed the highest peak at about *m/e* 1274 and a completely different fragmentation pattern from that observed for peliomycin, trimethylsilylated in similar manner. No accurate mass measurements were made, but the t.l.c. display of one component together with the mass spectrum result point strongly towards a macrolide structure for peliomycin with one ester function present.

In order to get support for the contention of one ester group in the peliomycin molecule, both peliomycin and the perhydro derivative were subjected to saponification-equivalent determination. The solutions were kept at room temperature in *M* methanolic sodium hydroxide until the change in rotation became negligible (24 hours). Peliomycin

immediately turned yellow when dissolved in the base and changed to deep-red at the end of the reaction. Titration with dilute hydrochloric acid to pH 6 changed the color back to yellow, and the difference in titer of the sample and a reagent blank indicated a consumption of 1.3 moles of base per mole of peliomycin (mol. wt. 928). Perhydropeliomycin did not change color so drastically, but the neutralized solution was still yellow after consuming 1.2 moles of base per mole of perhydropeliomycin.

Each solution was extracted with chloroform to give a neutral fraction and an aqueous fraction containing the salts. The p.m.r. spectra of the salt solutions, after exchanging with deuterium oxide, indicated methyl ketones (τ8.0) for both peliomycin and perhydropeliomycin, and an aldehyde proton (τ0.35) for the latter only. That the signal at τ8.0 was due to a methyl ketone rather than an acetoxy group was shown by reduction of these products with sodium borohydride and the subsequent disappearance of this signal. The neutral fractions of both hydrolysates lacked methyl ketones, but exhibited aldehyde protons at τ0.3. These fractions were mainly one component (t.l.c.), but no useful products could be obtained from them. The degradation attempts consisted of oxidizing the compound with the Jones reagent (86), followed by treating the

product with the periodate-permanganate reagent (74, 75). Thin layer and gas chromatography of the methyl esters yielded negative results.

The salt solution of perhydropeliomycin after acidification, extraction and esterification similarly resisted separation of the multiple components indicated by the t.l.c. However, these attempts with the corresponding fraction of peliomycin were more successful. Probably the absence of an aldehyde function only in this fraction prevented extensive rearrangements. Gas chromatography of the product after acidifaction, extraction and esterification yielded two components in a 1:1 ratio with a small difference in retention times. The infrared and mass spectra (Figure XXIX) of these components were nearly identical and only small differences were observed in the p.m.r. spectra. In the interpretation, the components are considered isomeric and dealt with as one species.

The 100 MHz p.m.r. spectrum of fragment XL (Figure XXIX) displays a simple spectrum. Although the signal to noise ratio is poor, integration shows one olefinic proton at τ3.62, a methoxy carbonyl group at τ6.32 and two 3-proton singlets at τ7.65 and τ8.24. The multiplet centered at τ8.9 appears as two doublets with spacings of 7.0 Hz each. The doublet at τ8.92 displays a signal which is twice as intense as the doublet at τ8.81. Integration of the multiplet indicates a maximum of seven protons; hence, because of the difference in intensities, two methyl groups are not involved.

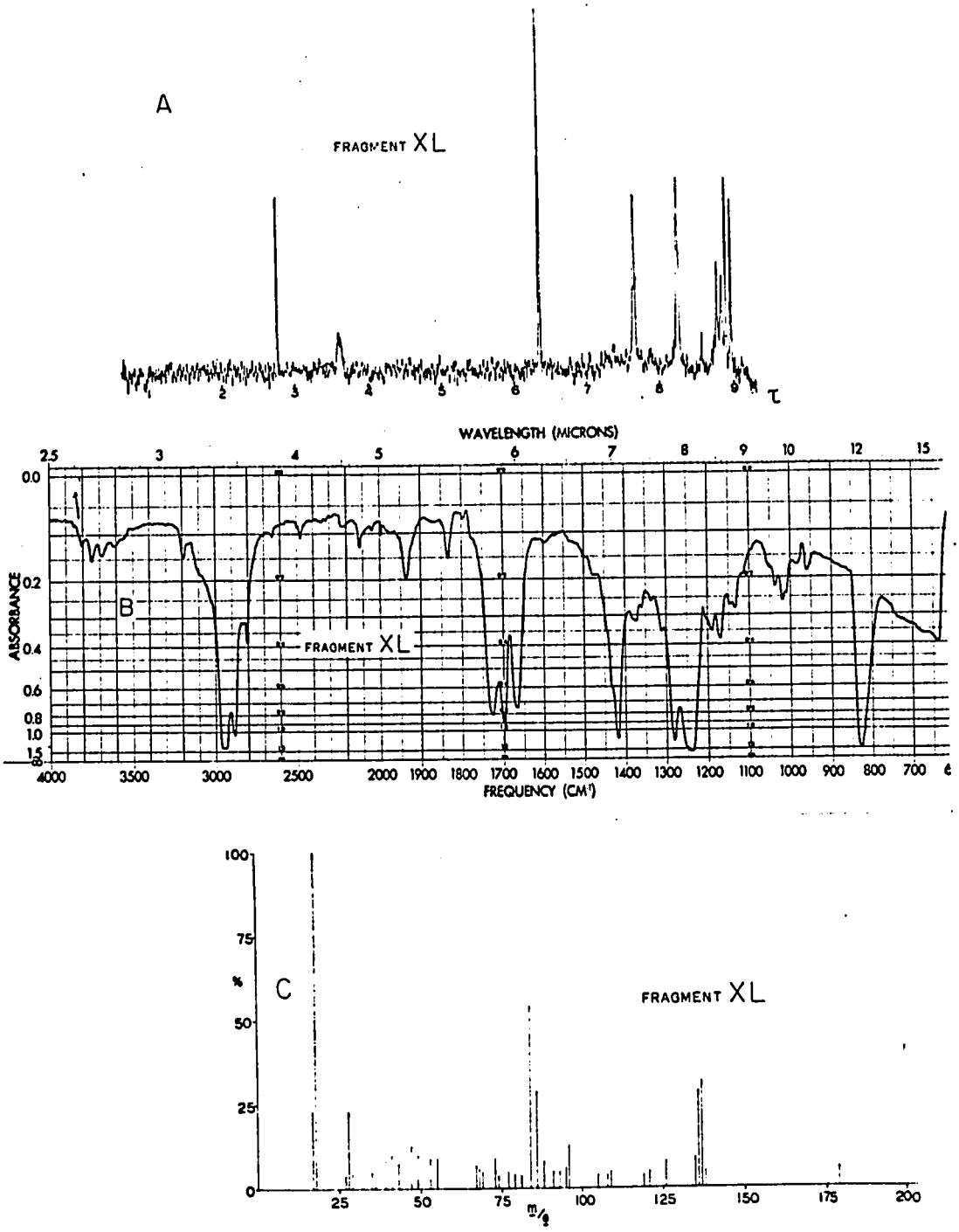


Figure XXIX Spectra of Fragment XL isolated from Peliomycin.

A. 100 MHz P.M.R. Spectrum of XL ($CDCl_3$). B. Infrared Spectrum of XL ($CDCl_3$). C. Mass Spectrum of XL (Source Temp. 180°C).

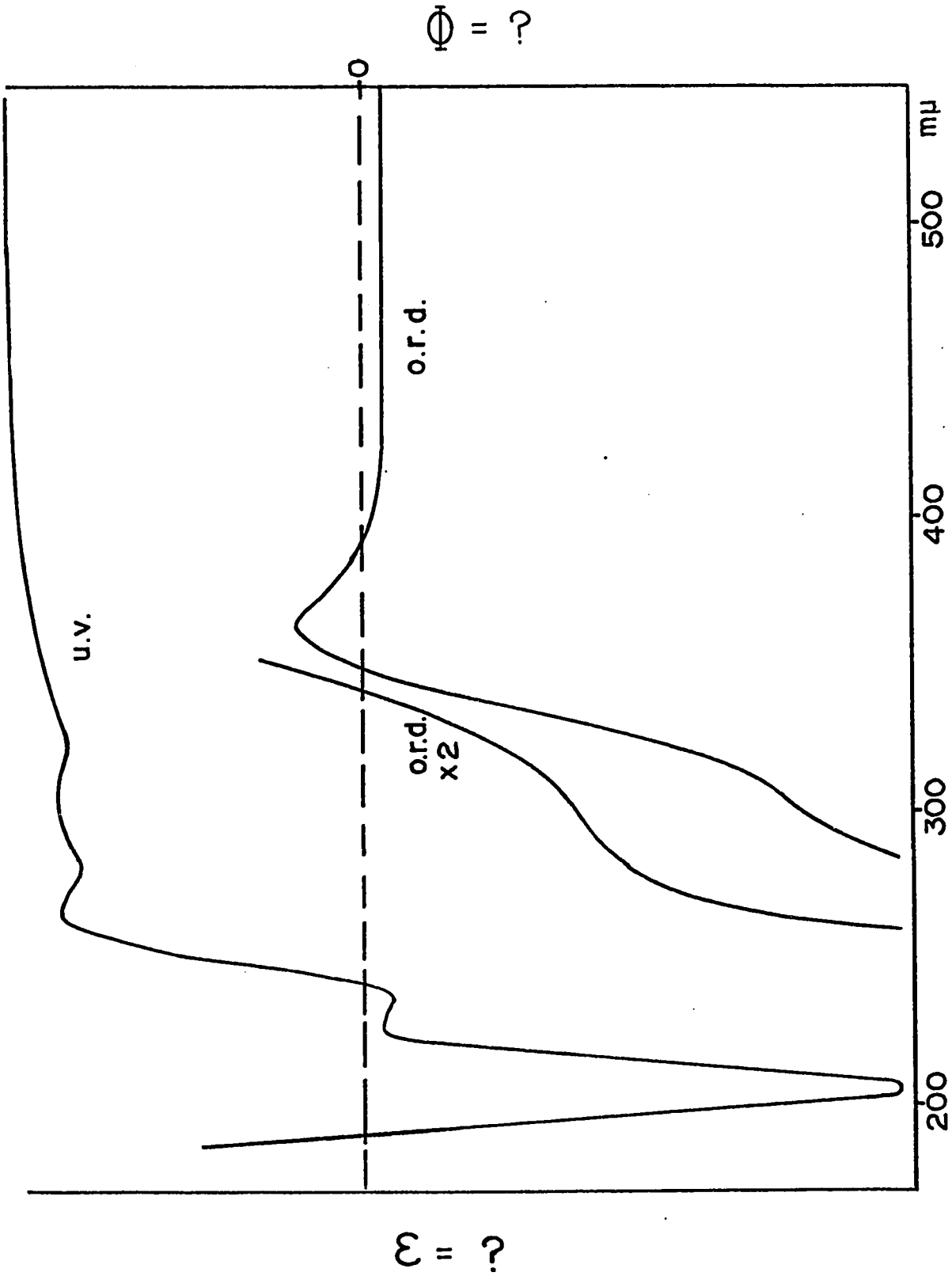


Figure XXX The Ultraviolet and Optical Rotatory Dispersion Curves of Fragment XL isolated from Peliomycin ($CDCl_3$)

A molecular ion was observed at *m/e* 210 in the mass spectrum, in addition to strong peaks at *m/e* 137 and 136 and a moderate peak at *m/e* 96. The results obtained from high resolution mass measurements are presented in Table XIX.

TABLE XIX

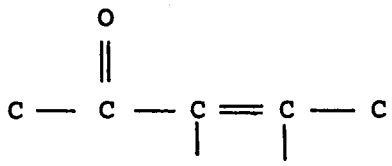
Mass Spectral Data of Fragment XL isolated from Peliomycin

Formula	Measured	Found
$C_{12}H_{18}O_3$	210.1255	210.1256
$C_9H_{13}O$	137.0961	137.0966
C_6H_8O	96.0575	96.0575

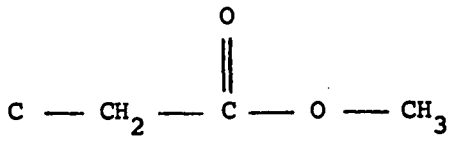
The loss of 73 mass units from the molecular ion corresponds to $C_3H_5O_2$ and is typical (129) for a structure of the kind $RCH_2-\overset{O}{\overset{\|}{C}}-OCH_3$. The carbon to hydrogen ratio in the molecular ion indicates that XL has four degrees of unsaturation, two of which are accounted for by the ester group and the olefinic proton observed in the p.m.r. spectrum.

The infrared spectrum of XL gave some hints. It was void of hydroxyl bands, but it showed strong absorptions at 1731, 1702 and 1669 cm^{-1}. The band at 1731 cm^{-1} is assigned to the methyl ester function and the band at 1669 cm^{-1} to an α,β-unsaturated ketone (99). Furthermore, the ultraviolet spectrum (Figure XXX) shows absorption maxima at 205,

236 and 321 mμ of which the latter two maxima together with their relative extinction coefficients are typical of the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively, of an α,β-unsaturated ketone (106). Finally, the presence of this structural moiety is substantiated by the positive Cotton effect (Figure XXX) with an inversion point at 348 mμ in the optical rotatory dispersion curve (110). Hence, three of the four degrees of unsaturation are accounted for, and the following structural units have been indicated by the spectral data.

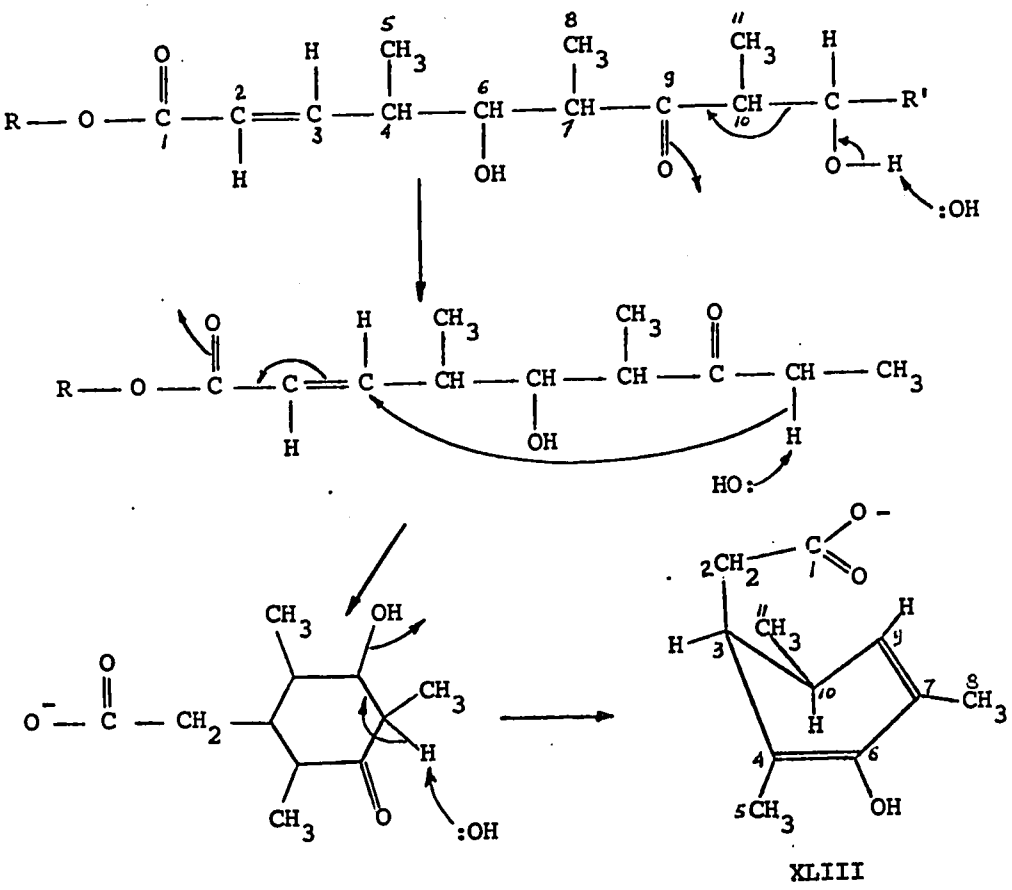


XLI



XLII

Since the isolation of the 11C-acid was probably caused by a retroaldol reaction during the alkali treatment, it appears plausible that the isolated fraction cyclized, as shown below, to account for the fragmentation product $C_{12}H_{18}O_3$.



XLIII

The p.m.r. data noted before is in good agreement with structure XLIII. The 3-proton singlets at τ7.65 and τ8.24 are reasonably assigned to the methyl groups C_5 and C_8, respectively. The signal at τ3.62 is due to the olefinic proton at C_9 and the 3-proton doublet at τ8.92 may arise from the methyl group of C_{11}. The singlet at τ6.32 is, of course, from the methyl ester which was introduced prior to the isolation of the fragment. The methine protons of C_3 and C_{10} are expected to be effectively lost in the noise, in view of their extensive coupling. The doublet at τ8.81 has an intensity much closer to a 2-proton signal than to a methyl group. It is conceivable, in fact, that this

signal (τ8.81) could arise from a product with the configuration and conformation shown, since this would bring the methylene group of C_2 over the shielding region of the diene structure. If the large grouping was situated in the equatorial position at C_3, it would strongly interact with the methyl group of C_5 for reasons of the $A^{(1,2)}$ effect (130, 131).

In spite of the fact that most of the spectral data agrees with the structure proposed, no synthesis was attempted for the following reasons. The two carbonyl stretching frequencies at 1731 and 1669 cm^{-1} have been correlated with structural features of XL in the keto-form. However, no such assignment is possible for the intense band at 1702 cm^{-1}, other than that it may be due to a Fermi resonance (132), a nonfundamental absorption band. Structure XL seems to suggest, however, a diene structure from its p.m.r. data which is not in line with the observed infrared, ultraviolet and o.r.d. spectra.

In the discussion on Molecular Formula, the analysis of the data on hand suggests a possible molecular formula of $C_{52}H_{80}O_{14}$ for peliomycin. For further support for the number of carbon atoms present, and for the contention that peliomycin indeed has a macrocyclic lactone group, a total reduction of the antibiotic to the hydrocarbon backbone was attempted. This reduction sequence, as developed by Ceder (133), consists of catalytic hydrogenation

of olefinic bonds and reduction of all carbonyl functions with lithium aluminum hydride to the polyol. The polyols are in turn reduced by refluxing in hydriodic acid and red phosphorus followed by purification with lithium aluminum hydride and catalytic hydrogenation to reduce any iodides formed. The final hydrocarbon should be representative of the carbon skeleton of the antibiotic. When peliomycin was reduced in this manner no pure product was obtained, as the final reduction product gave no response on gas chromatography. Mass spectral analysis of the oil did not indicate a molecular ion, but the peaks decreased slowly in intensity and reached values of *m/e* 636. The reason for this poor result is not clear. It may be assumed that any ether group would have been cleaved by the hydriodic acid-red phosphorus reaction under the conditions employed. Furthermore, it was shown that hydroxyl and carbonyl groups (infrared) and iodides (ultraviolet) were absent in the final product. However, the mass spectral observation does not disprove the contention that the lactone function in peliomycin is of a macrocyclic nature, although small molecular weight fragments could have escaped.

In the derivation of a possible molecular formula for peliomycin, it was mentioned that mass spectroscopy of the antibiotic failed to produce a molecular ion. Furthermore, the fragmentation pattern (Figure XXIX) was

of no assistance in arriving at a molecular formula, but it did limit the molecular weight of peliomycin to at least 786. Some of the more important ions, many of which were also present in most of the degradation products obtained by oxidizing peliomycin with the periodate-permanganate reagent, are presented in Table XX.

TABLE XX

Mass Spectral Data of Peliomycin (Figure XXXI)

Formula	Measured	Calculated
C_7H_{11}	95.0857	95.0860
C_6H_9O (65%)	97.0651*	97.0653
C_7H_{13} (35%)	97.1014*	97.1017
$C_5H_7O_2$ (10%)	99.0444*	99.0446
$C_6H_{11}O$ (90%)	99.0808*	99.0809
$C_{10}H_{13}O$ (55%)	149.0967*	149.0966
$C_{11}H_{17}$ (45%)	149.1327*	149.1330
$C_{34}H_{52}O_8$	588.3645	588.3662

*Composite peaks

The highest peak in the mass spectrum of peliomycin suitable for high resolution mass measurement was at *m/e* 588. In the discussion of the heuristic formula (Figure XXXII), an attempt will be made to assign the peaks recorded in Table XXII.

The mass spectrum of oligomycin B (65) together with that of peliomycin are reproduced in Figure XXXI. Only the high mass end of these spectra are displayed. Many similarities are evident, especially the intense peak at *m/e* 588.

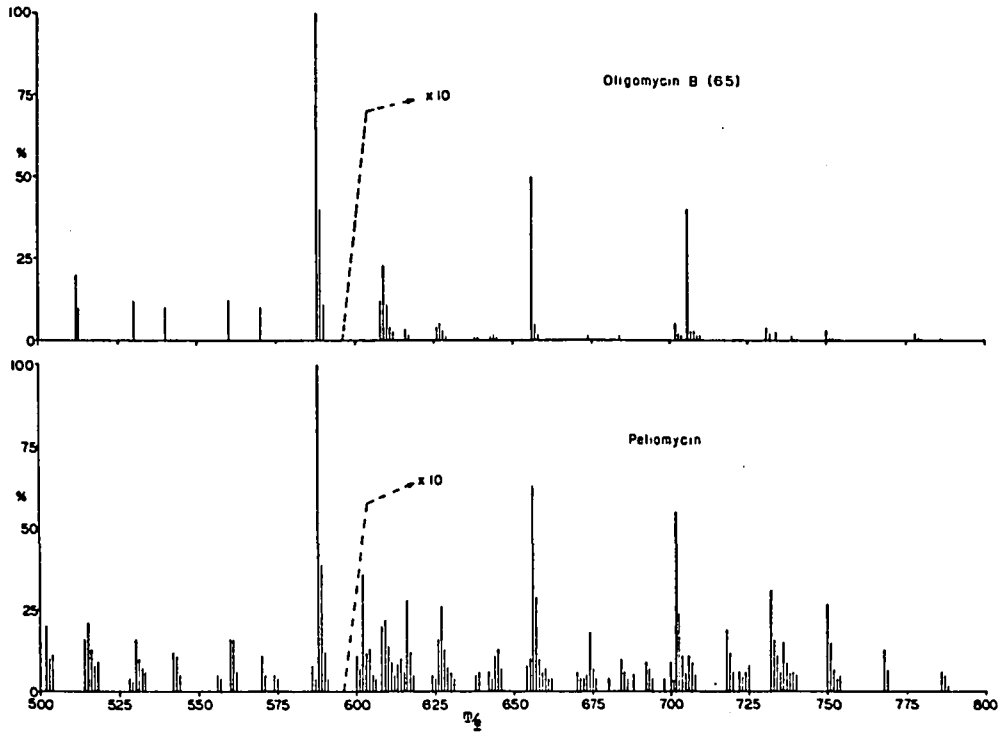


Figure XXXI A Comparison of the Mass Spectra of Peliomycin (Source Temp. 200°C) and Oligomycin B (65)

The close, if not identical, relationship is further demonstrated by the infrared spectrum (Figure XXXIII) and ultraviolet spectrum (Figure XXXIV) of oligomycin B when compared with those of peliomycin (Figure XXV and XXVII, respectively).

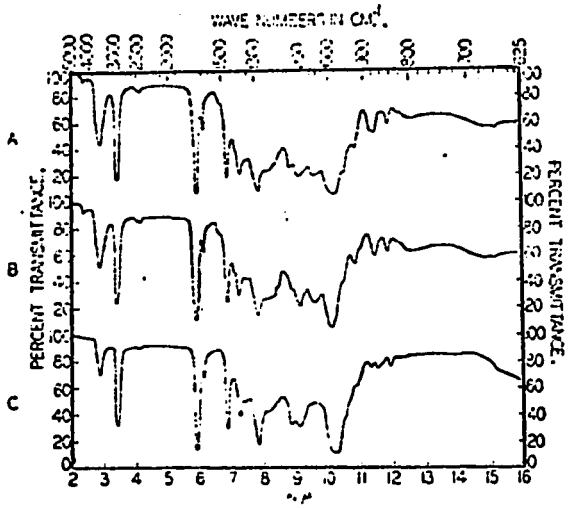


Figure XXXIII The Infrared Spectra of Oligomycin A, B and C (64)

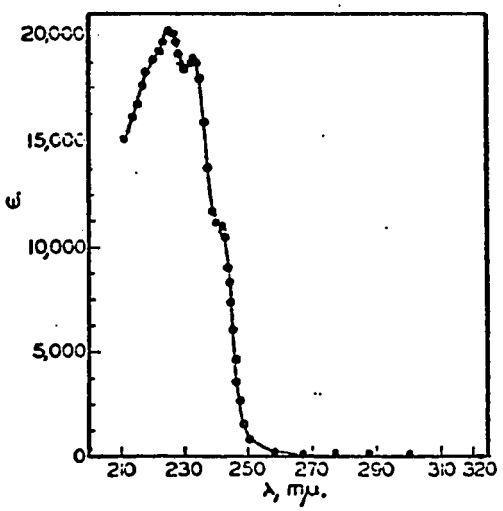


Figure XXXIV The Ultraviolet Spectrum of Oligomycin B (64)

The structural similarities are especially evident from a comparison of the 100 MHz p.m.r. spectrum of oligomycin B (Figure XXXV) with that of peliomycin (Figure XXXVI) both recorded using *hexadeuterio*-acetone as the solvent.

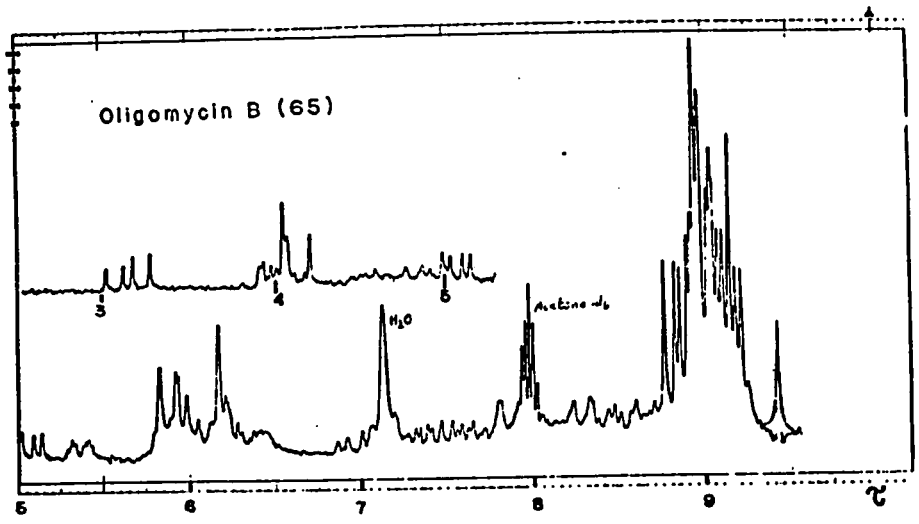


Figure XXV The P.M.R. Spectrum of Oligomycin B (Acetone-d_6) (65)

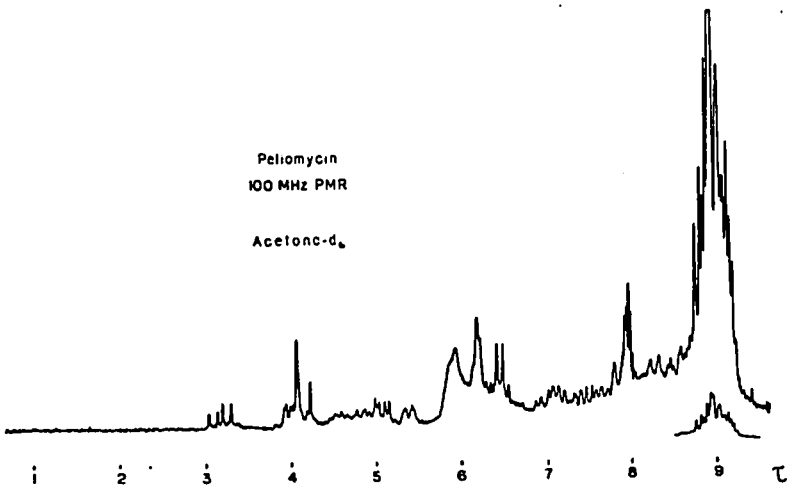


Figure XXXVI The P.M.R. Spectrum of Peliomycin (Acetone-d_6)

As was stated in the Introduction, the aim of this research was to develop a technique for structure elucidation of complex molecules, such as peliomycin, encompassing "fragmentation analysis" and a thorough investigation by modern physical methods. The immediate goal was to acquire, as a first stage in the structural elucidation of peliomycin, sufficient insight into the molecular structure to permit efficient planning of future experiments. To be useful, this information should allow the writing of what has been termed a heuristic formula of peliomycin as depicted in Figure XXXII. This highly tentative structure is then a basis for the selection of experiments which in turn will allow the alteration of the formula towards the correct structure. Indeed, many macrolide antibiotics have been subjected to structural investigation (9), and only a limited number of structures have been elucidated. Those structures that have been established are not always unequivocal, but undergo revision as a consequence of further work (17, 48, 49). Thus, the establishment of these complex structures by chemical means often comprises a series of approximations.

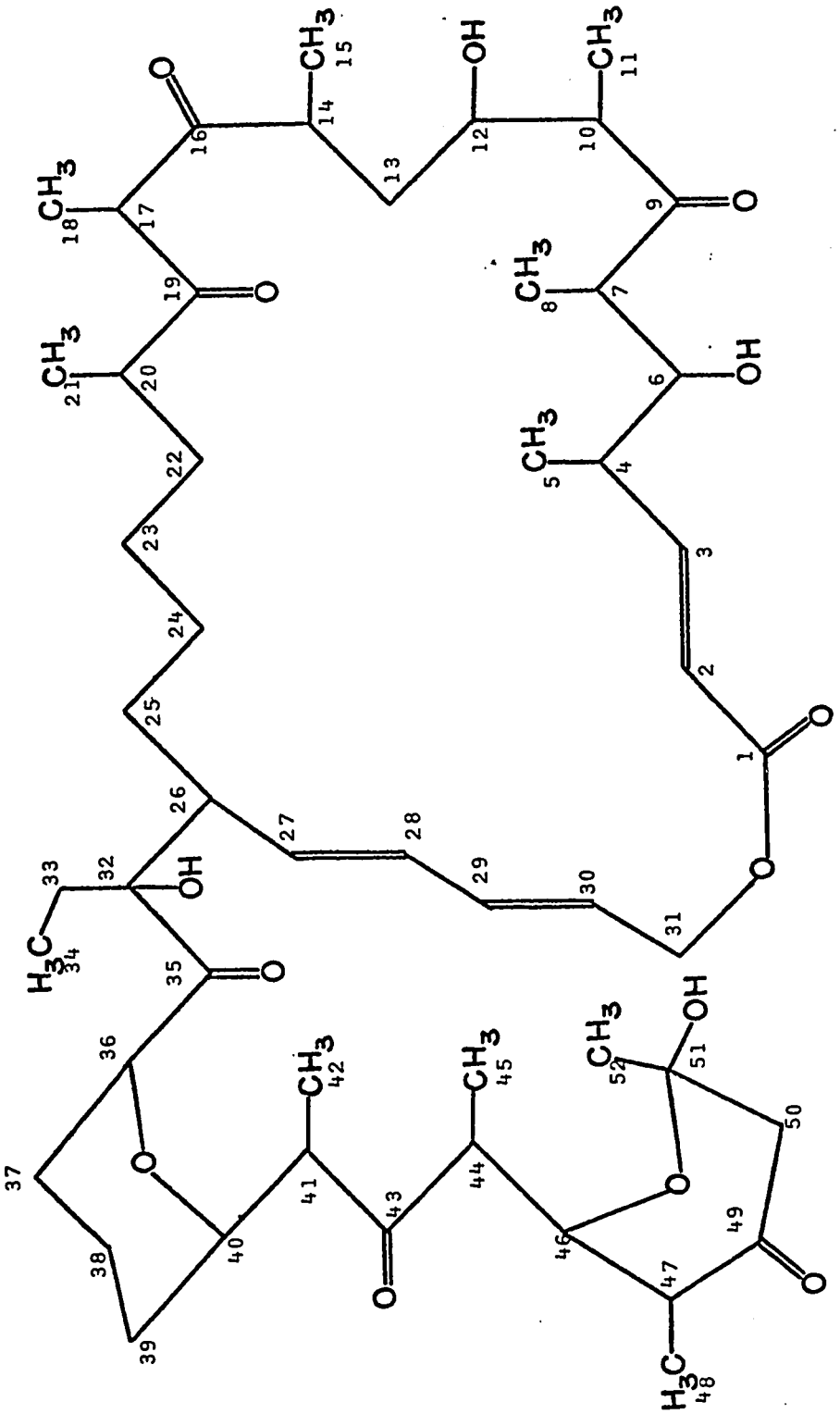


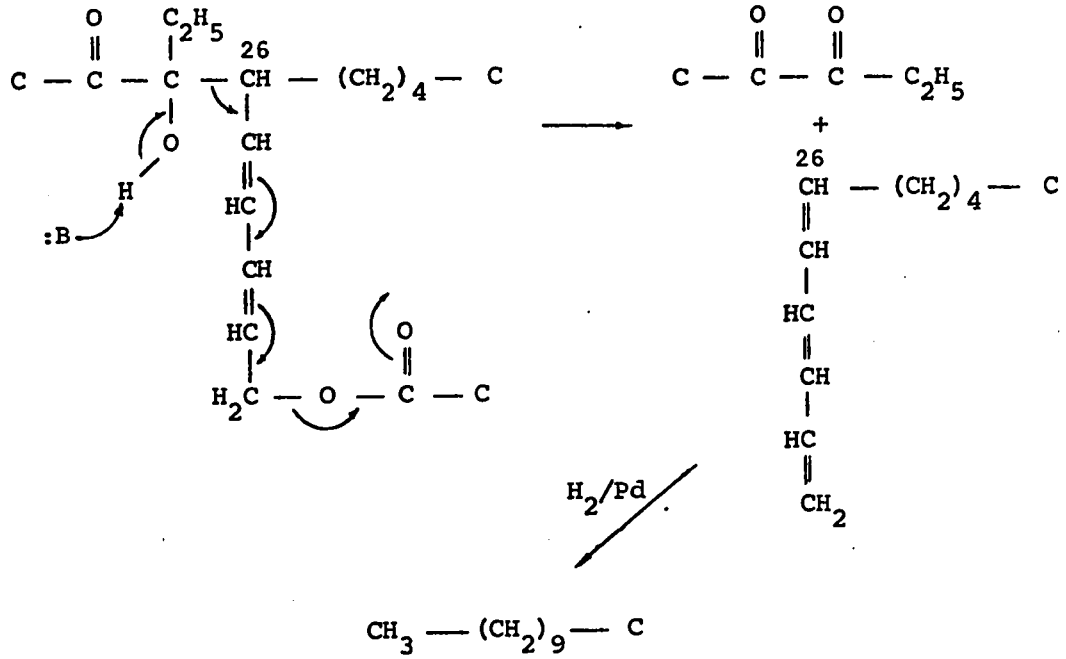
Figure XXXII The Heuristic Formula of Peliomycin

The heuristic formula (Figure XXXII) arrived at as a result of this research must be considered a first approximation. A substantial effort has been made to incorporate in this formula all the information on hand. As was seen in the study of the structural features of peliomycin, the carbons 1 to 5 are assigned to the α,β-unsaturated lactone (XXVII) for which spectral data (Table XVI) is offered. The structural unit containing carbons 4 to 6 is based only on p.m.r. observations (XXVIII). The carbon 7 to 12 were arranged to allow a retroaldol condensation to yield the fragment XL. This fragment can cyclisize effectively to help shift the equilibrium for this proposed retroaldol reaction. The proposed partial structure involving carbons 12 to 19 affords a structural unit that can be expected to provide readily the 2-methylsuccinic acid (VII) formed on oxidation of peliomycin (Scheme II). Since carbon 16 and 17 are strongly activated by the neighboring carbonyl groups, they are, therefore, readily oxidizable. The oxidation of carbon 10 and 17 may now lead to a facile cleavage of the C_{10}-C_{12} and C_{16}-C_{17} bonds by the oxidant. These oxidations would lead to two moles of acetic acid, in line with observations (experiment E-1-a).

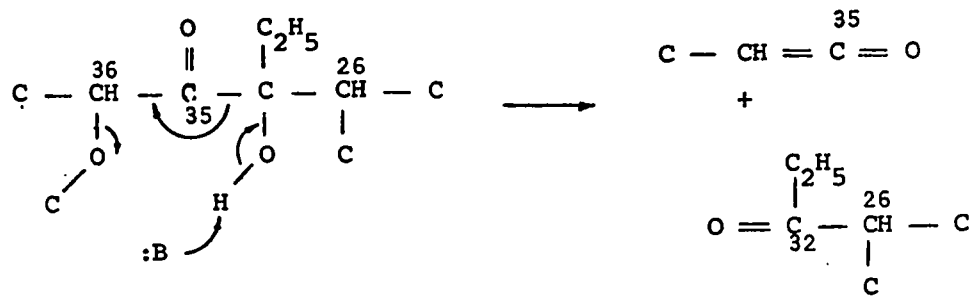
The isolation of 2-methyldodecanoic acid (XV) on oxidation of perhydropeliomycin requires the presence of a chain of ten consecutive methylene groups in perhydropeliomycin.

However, the p.m.r. spectrum of peliomycin (Figure IV) is not compatible with as many as 20 methylene protons. Therefore, a part of this long chain hydrocarbon must have been formed in the hydrogenation of peliomycin. For this reason the diene grouping (XXX) is connected to carbon 26. Hydrogenation of peliomycin will then result in the long chain hydrocarbon C_{20} to C_{31}. The terminal methyl group of the 2-methyldodecanoic acid (XV) would have been generated by hydrogenolysis of the C_{31} allylic ester bond (85). Indeed, as was shown, oxidation of peliomycin with the osmium tetroxide-periodate reagent appeared to yield glycolaldehyde (experiment D-2-a) which would have arisen from carbons 30 and 31 of the heuristic formula.

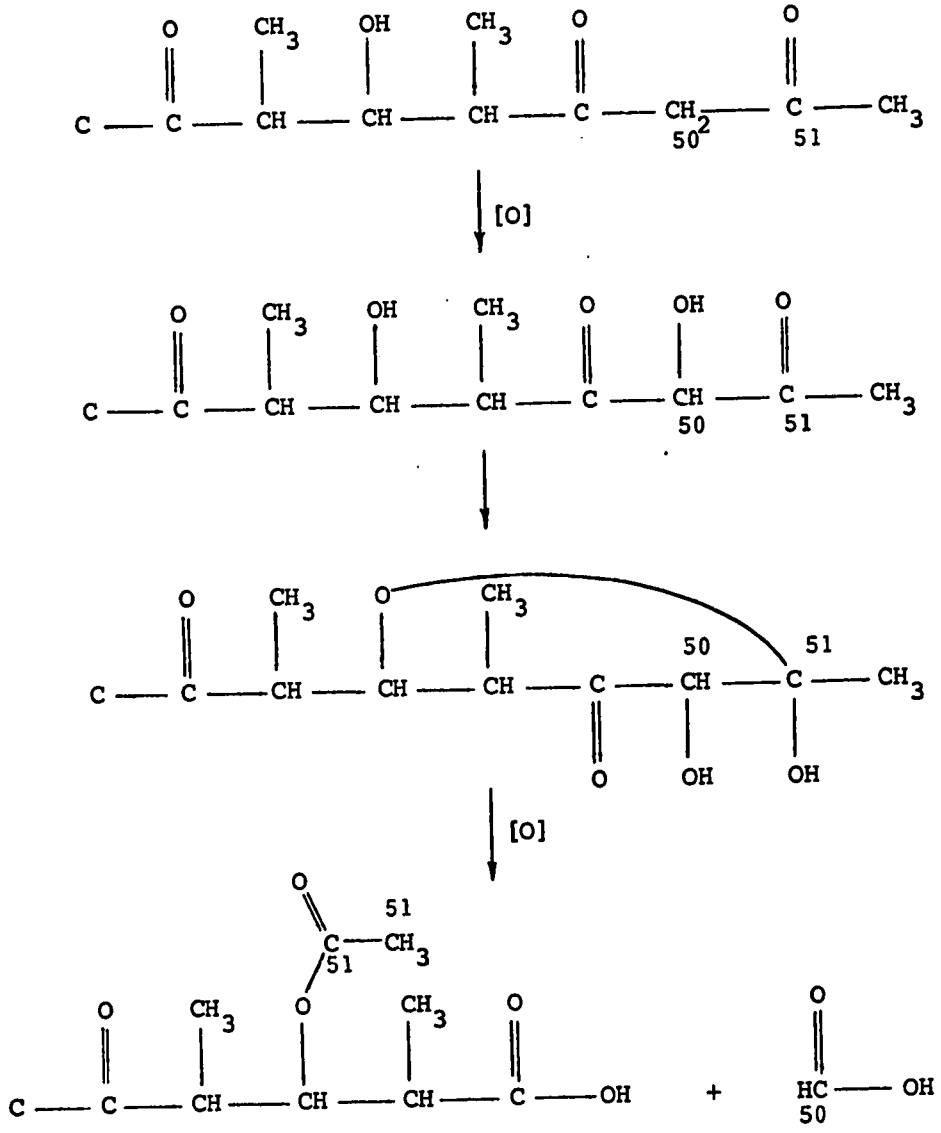
A main problem in accounting for the presence of 2-methyldodecanoic acid (XV) in the product of oxidation was the isolation of fragment XVI from the same degradation sequence (Scheme IV). Since the latter fragment also contained a large hydrocarbon moiety, in addition to a propionyl side chain, the compound must necessarily be closely related to fragment XV. In order to rationalize this hypothesis, the tertiary alcohol structure involving C_{32} is proposed. Thus, C_{26} could conceivably be converted to a methylene group by way of a reaction sequence similar to that of a tertiary ketol reaction, as shown.



Since the structural unit involving C_{32} must also be compatible with the formation of fragment XVI as well as fragment XV, the heuristic formula must account for both possibilities. The rationale that both reactions may take place is based on a competition between the C_{26}-C_{32} and the C_{32}-C_{35} bonds. Should hydrogenolysis of the lactone ring and saturation of carbons 27 to 30 occur prior to the cleavage of the C_{26}-C_{32} bond, then it is plausible that the alkaline treatment will give rise to the formation of the propionyl group of fragment XVI.



A main consideration in the construction of the heuristic formula was the observation in the mass spectrum of the intense peak at *m/e* 588, which analyzed for $C_{34}H_{52}O_8$ (Table XX). Furthermore, the mass spectrum of hexatrimethylsilylpeliomycin was best interpreted by a fragmentation involving the loss of ethylene (II→III). The structural features about C_{32} may give rise to both of these observations. The partial structure C_{36} to C_{41} is extremely speculative; indeed, it is mainly formulated to provide an oxygen atom at both C_{36} and C_{40}. The three methylene groups at C_{37}-C_{39} are written to comply with the molecular formula $C_{52}H_{80}O_{14}$ without involving more C-methyl groups. The heuristic formula allows the fragment XVII to be generated either from carbons 3 to 9 or from carbons 43 to 49. The formation of the acetate ester grouping of fragment IX from peliomycin via the Jones oxidation, followed by the periodate-permanganate oxidation, seems best rationalized via the hemi-ketal structure involving carbons 43 to 52, as shown. Thus, the formation of formic acid is also accounted for in the periodate-permanganate oxidation of peliomycin, since activated methylene groups are known to be attacked by this reagent (79). The oxidative cleavage of the C_{41}-C_{43} bond would perhaps take place by enolization of the C_{41}-C_{43} grouping. Another possibility, that fragment XVII may have arisen from carbons 3 to 9, requires a macrocyclic hemi-ketal type structure involving the C_6-hydroxyl group.



The heuristic formula displays four hydroxyl groups which may be expected to exchange readily with deuterium oxide, as was observed. The formation of a penta-*O*-acetyl derivative of peliomycin may be accounted for by enolization of the β-diketone at C_{16}-C_{19}. The enolization followed by acetylation will not give rise to olefinic protons in the

p.m.r. spectrum. The hexatrimethylsilyl derivative of peliomycin may be the result of enolization of other β-diketones such as C_{49}-C_{51} and/or involving the carbonyl groups at C_9 and C_{43}. The arrangement of β-diketones is further justified by the failure to isolate hydroxy acids from the oxidation of perhydropeliomycin (Scheme IV). It may be surmised that the Baeyer-Villiger reaction was redundant, the Jones oxidation proving sufficient to cause the cleavage reactions.

The mass spectral results recorded in Table XX are intelligible on the basis of the heuristic formula. The C_7H_{11} and C_7H_{13} ions may be generated by cleavage of the branch points at C_{20} and C_{26} to yield carbons 20 to 26. The structural unit involving carbons 26 to 31 together with the ethereal oxygen of the lactone group, may have generated the C_6H_9O and the $C_6H_{11}O$ ions. In all these reactions, hydrogen migration is assumed (151). Similarly, the $C_{10}H_{13}O$ fragment may involve carbons 22 to 31 and the C_{31}-oxygen atom. Loss of the methyl group of C_{21} may yield the $C_{11}H_{17}$ fragment which consists of C_{20} and C_{22}-C_{31}.

The extent of conjugation, observed in the ultraviolet spectrum of peliomycin in basic solution (Table XVIII, Figure XXVII), may be expected from a single dehydration of the C_{32}-alcohol function to give a chromophore C_{31}-C_{35}.

The development of the heuristic formula should be useful in contemplating further controlled degradations. For instance, to remove the sensitivity of the molecule to basic media, peliomycin should be subjected to catalytic hydrogenation, followed by a sodium borohydride reduction. Such a reaction sequence deactivates acidic methylenes and destroys hemi-ketal ring structures. Both these reactions were shown to leave the molecule intact, but should make the C_{32}-C_{35} bond much more susceptible to cleavage by sodium *meta*-periodate or lead tetraacetate. On the basis of these results, the heuristic formula will then have to be altered to explain the new evidence. It may be expected that the approach to structure elucidation as presented in this dissertation should also be useful for related antibiotics, such as the suspect-macrolide oligomycin B, which closely resembles peliomycin in its spectral properties, but possesses different antimicrobial activity (64). It is noteworthy that, in spite of all efforts (65, 152), the complete structure of oligomycin B remains unsolved.

III EXPERIMENTAL

A. Physical Methods of Analysis and Techniques of Separation

Except where stated otherwise, all elementary analyses were performed and spectra recorded by the departmental service laboratories. The refractive indices were measured with a Bausch and Lomb Optical Co. refractometer (Model 33-45-58).

Optical rotations were determined with a Perkin-Elmer (Model 141) polarimeter. The optical rotatory dispersion (o.r.d.) and circular dichroism (c.d.) spectra were recorded with a Jasco Model ORD/UV-5 optical rotatory dispersion recorder with circular dichroism attachment, and adjusted to zero by means of solvent or air.

The ultraviolet spectra were recorded with a Cary recording photospectrometer, Model 14M, using methyl alcohol as solvent.

The infrared spectra were obtained with a Perkin-Elmer 421 grating spectrometer using chloroform as solvent, unless mentioned otherwise. The 1601 cm^{-1} band of polystyrene sample was recorded on the spectra as a point of reference.

The proton magnetic resonance (p.m.r.) spectra at 60 MHz and 100 MHz were recorded with a Varian A-60 and Varian HA-100 spectrometer, respectively. The 220 MHz spectra were determined at Varian Associates, Palo Alto, California, through the courtesy of Dr. LeRoy F. Johnson.

Chemical shifts are reported as τ (tau) values with tetramethylsilane as internal standard. Double and triple-resonance experiments were performed using the frequency sweep technique (134). On occasion, due to low concentration of sample, the time-average computer (C.A.T.) was employed.

The electrophoresis of borate complexes was performed with an apparatus made by Savant Instruments Inc., Hucksville, N.Y., which is capable of delivering a potential difference of 1500 volts. The melting points were taken on a Leitz micro heating stage Model 350 and are uncorrected.

Preparative and analytical gas chromatography (g.c.) were performed on a F. & M. Model 500, Programmed Temperature Gas Chromatograph, with a detector bridge current of 150 mA and helium as the carrier gas. The column packings were prepared in the laboratory by dissolving the liquid phase in chloroform. The required amount of solid support (Chromosorb W, 60-80 mesh) was added to the solution, and the solvent removed on the rotary evaporator. After thorough drying in high vacuum, the dry column packing was introduced into the column made of copper tubing (6' x 0.25" o.d., unless otherwise stated), packed by means of a vibrator and coiled.

The mass spectra were obtained using an A.E.I. Ltd., MS9 double focussing mass spectrometer under the following conditions, ionizing energy: 70 ev; trap current 100 μa;

ion-accelerating voltage: 8 kv below mass 1000, 6 kv above mass 1000. The spectra are plotted in terms of relative abundance with the most intense peak taken as 100%.

Semi-micro preparative gas chromatography was achieved as follows. The exit port of the gas chromatograph (F. & M. Model 500) was fitted with a stainless steel adapter (40 x 7 mm o.d.) machined on the outer end such that it could be fitted with a 12 gauge hypodermic needle which was bent 90°. The adapter was heated with a heating coil to approximately 120°C to minimize condensation of the exit gases. These gases were led into a glass tube (40 cm x 2 mm i.d.) coiled 540° in the middle and cooled in a dry-ice/acetone bath. With repeated injections and subsequent collections enough material could usually be collected for investigations by physical means.

These physical methods started off with a 100 MHz p.m.r. spectrum. To collect the sample, the material was washed out of the coiled-glass collection tube with small amounts of *deuterio*-chloroform, containing 5-10% tetramethylsilane, through a cotton filter into the p.m.r. tube. Subsequent mass spectral analysis was made feasible by repeatedly dropping small quantities of the *deuterio*-chloroform solution into the pin-hole of the probe and blowing of the solvent. Infrared and o.r.d. spectra were run

on the remainder of the solutions in the p.m.r. tube, thereby using *deuterio*-chloroform containing 5-10% tetramethylsilane in the reference cells, where necessary.

Solvents were evaporated on a rotary evaporator under diminished pressure (∿ 15 mm Hg) with a maximum bath temperature of 45°C. In the degradation experiments, where removed solvents had to be investigated for volatile fragments, the technique 'distillation in a closed system' was applied. Use was made of an inverted Y made of glass tubing (20 mm o.d.) with inner joints (℔ 14/20) on each arm and a stopcock at the stem of the Y. The flask containing the mixture was attached to one arm with a receiving flask on the other. After cooling the ether solution in a dry-ice/acetone bath for 30 minutes, the stopcock was opened to the vacuum pump until a pressure of 1 mm of mercury was attained. After closing the stopcock, the semi solidified mixture was removed from the dry-ice/acetone bath, and the receiving flask inserted. By allowing the cooled solution to reach room temperature, solvent was efficiently distilled over.

Thin layer chromatography was performed both on microscope slides and glass plates (20 x 20 cm) with a 0.25 mm thickness of silica Gel G. Unless otherwise mentioned, the thin layer chromatograms were developed with chloroform: methyl alcohol = 95:5. Visualization

was achieved by exposure to iodine and/or spraying with aqueous sulfuric acid (30%) and charring on a hot plate.

B. Reagents and Solvents

Starting materials and solvents were redistilled through a Vigreux column before use. Dry solvents were prepared according to established procedures (135) or by the use of neutral alumina (Woelm, Activity 1). The silica Gel G used in thin layer and preparative chromatography was supplied by E. Merck, Darmstadt, Germany, and the microcrystalline cellulose "Avicel" was obtained from American Viscose Co., Newark, Delaware. Standard solutions were prepared according to Day and Underwood (136).

C. Characterization of Peliomycin

1. Composition and Absorption Spectral Properties of Peliomycin

a. Source and elementary analysis of peliomycin.

The peliomycin (3.5 g) used in this research was kindly provided by Bristol Laboratories, Syracuse, N.Y., U.S.A., as Lot #C4657-W39-G20.

The C,H and O determinations are presented in Table II. As mentioned in the discussion, it was necessary to replace the ethyl alcohol found present in crystalline peliomycin (Figure III) by chloroform prior to the elementary analyses. This was accomplished by dissolving peliomycin (100 mg) in absolute chloroform (5 ml), removing most of the solvent on

the rotary evaporator and drying at 1 mm of Hg and 40°C for 5 days.

b. Physical properties.

The melting point of peliomycin was found to be 158-162°C and $[\alpha]_D^{26}$ - 70.8° (*c* = 1, chloroform); reported (62), m.p. 160-164°C and $[\alpha]_D^{25}$ - 74.2° (*c* = 1, chloroform). Since oligomycin B (64, 65) appears to be a closely related antibiotic with $[\alpha]_D^{23.5}$ - 49.5° (*c* = 1, methyl alcohol), the optical rotation of peliomycin in the same solvent was found to be $[\alpha]_D^{25}$ - 54° (*c* = 1, methyl alcohol).

c. Infrared spectrum.

The infrared spectrum of peliomycin, determined in chloroform, is exhibited in Figure XXIV. The published (64) infrared spectrum of oligomycin B is reproduced in Figure XXXIII.

d. Optical rotatory dispersion, circular dichroism and ultraviolet spectra.

The optical rotatory dispersion and circular dichroism spectra determined in methyl alcohol are recorded in Figure XXVI and the pertinent data are given in Table XVII. The ultraviolet spectra of peliomycin determined in methyl alcohol (99%) and in 0.05*M* sodium hydroxide in methyl alcohol (99%) are reproduced in Figure XXV, and the absorption maxima and extinction coefficients are recorded in Table XVIII. The ultraviolet spectrum of oligomycin B (64) is exhibited in Figure XXXIV.

e. P.m.r. spectra.

The p.m.r. spectra run in *deuterio*-chloroform at 100 MHz and 220 MHz are reproduced in Figures XIX and IV, respectively. The p.m.r. parameters are given in Tables XIV and XV. The p.m.r. spectra of peliomycin run in *hexadeuterio*-dimethylsulfoxide and *hexadeuterio*-acetone are reproduced in Figures XXII and XXXVI, respectively. The latter is included for comparison with the published spectrum of oligomycin B (65) and displayed in Figure XXXV. A sample of crystalline penta-*O*-acetylpeliomycin was also kindly supplied by Bristol Laboratories (64). The p.m.r. spectrum of this compound is shown in Figure XX, and its p.m.r. parameters are recorded in Tables XIV and XV.

2. Mass Spectral Examination of Peliomycin and Hexatrimethylsilylpeliomycin

a. Markers for mass measurement.

Since fluorolube (68) is no longer available, it was suggested (69) to use undecatrimethylsilylraffinose as a mass marker, which was prepared as follows. Anhydrous raffinose (100 mg) was dissolved in dry pyridine (3 ml); and hexamethyldisilazane (1 ml) and chlorotrimethylsilane (0.5 ml) were added (70). The mixture was allowed to stand for 24 hours and then evaporated under reduced pressure. The pyridine was completely removed by co-distillation with carbon tetrachloride (5 x 2 ml). The residue was triturated with hexane, the combined extracts dried over magnesium sulfate and concentrated, whereupon it crystallized. Yield 112 mg, m.p. 115-117°C. For mass spectral purposes, trimethylsilyl derivative of raffinose (50 mg) was sublimed at 150-175°C (10^{-3}-10^{-4} mm Hg).

Since the above compound failed to give a molecular ion or a recognizable fragmentation pattern, 2,2,4,4,6,6,8,8 -octa-(3,3,3,2,2 -pentafluoropropyloxy)-cyclotetraphosphazatetraene (I) was used as a mass marker. This compound gives a strong molecular ion at *m/e* 1372 with intense peaks at *m/e* 1303, 1223 and 1091 which measured 1371.92795, 1302.93274, 1222.92537 and 1090.92553, respectively.

b. Peliomycin.

The mass spectrum of peliomycin is shown in Figure V, and the data from mass measurements of some of the peaks are presented in Table XXII.

c. Hexatrimethylsilylpeliomycin.

The mass spectrum of hexatrimethylsilylpeliomycin is reproduced in Figure VI. The computer results (IBM 360\67) of the mass values *m/e* 1224, 1236 and 1092 are recorded in Tables V, VI and VII, respectively. The conditions set for these determinations are presented in the Discussion (see Table V). The derivative was prepared according to the procedure of Friedman and co-workers (71), as follows.

Peliomycin (25 mg) was dissolved in freshly distilled dimethylsulfoxide (10 ml) which was thoroughly dried over sodium hydride. To this solution was added hexamethyldisilazane (5 ml), and the mixture heated under reflux at 80°C overnight. The solvents were removed on the rotary evaporator, and the residue dried *in vacuo* for five days. The resulting oil (25 mg) was submitted directly for mass spectral analysis. An infrared spectrum lacked all hydroxyl band absorptions, but showed strong $Si-CH_3$ absorption bands at 1250, 840 and 750 cm^{-1}.

When the method of Sweeley (70) and subsequent work-up of Rickard and co-workers (30) was followed, the product thus obtained showed a hydroxyl band in the infrared at 3400 cm^{-1}.

3. Chemical Characterizations of Peliomycin

a. Saponification equivalent.

Peliomycin (201 mg) was dissolved in *N* methanolic sodium hydroxide (90%, 2 ml) with the formation of a yellow color. The solution was kept at room temperature, and the reaction was considered complete when its change in optical rotation became negligible (24 hours). During this period the color had changed to dark red. The solution was titrated with 0.0950*N* aqueous hydrochloric acid to pH 6, while stirring the solution with a stream of nitrogen. The difference in titer with that of a blank determined in the same manner showed in duplicate determination consumptions of 1.3 and 1.35 moles of sodium hydroxide per mole of peliomycin (mol. wt. 928).

b. Catalytic hydrogenation to perhydropeliomycin.

Peliomycin (194.8 mg) was hydrogenated over 5% palladium on charcoal (203 mg) in 95% ethyl alcohol (25 ml) producing a white fluffy material (199.0 mg), which could not be induced to crystallize. In thirty minutes 20.4 ml of hydrogen was taken up, representing 4.1 moles of hydrogen per mole (928) of peliomycin. A 100 MHz p.m.r. spectrum of the product, isolated in the usual manner, indicated the loss of six olefinic protons below τ4.9 (Figure XXIII). The olefinic band at 1650 cm^{-1} in the infrared

spectrum had disappeared, as had the bands perhaps indicative of an epoxide at 1278, 972 and 870 cm^{-1} (99). The α,β-unsaturated carbonyl function at 1708 cm^{-1} had shifted to 1728 cm^{-1}. The ultraviolet spectrum lacked all absorption bands. Mass spectral analysis of the trimethylsilyl derivative of perhydropeliomycin gave a poorly resolved spectrum with the highest peaks at about *m/e* 1300.

c. Sodium borohydride reduction

Peliomycin (21.5 mg) was added to a mixture of sodium borohydride (26.2 mg) and sodium bicarbonate (50.8 mg) in ethyl alcohol (5.0 ml). After stirring at room temperature for twelve hours, the solvent was evaporated and water (5 ml) added. The subsequent chloroform extract (4 x 5 ml) was dried over magnesium sulfate and evaporated to an oil (20.8 mg). A t.l.c. plate, developed in chloroform: acetone = 9:1, showed that R_f-reduced product: R_f-peliomycin = 0.1:0.3. The ultraviolet absorption band at 218 mμ had disappeared. The carbonyl absorption peaks in the infrared spectrum (Figure XXV) had been reduced by approximately 66%, and the 100 MHz p.m.r. spectrum (Figure XXV) showed the loss of many proton signals, e.g. A,D,P and others as mentioned in the discussion. The trimethylsilyl derivative of the reduced product was easily volatilized at 200°C and gave a mass spectrum with a strong peak at *m/e* 1145. No accurate

mass measurements were performed. When the reduced product was again subjected to sodium borohydride reduction, no further change was observed in the infrared spectrum.

d. Qualitative tests for functional groups.

(i) Iodoform reaction.

Peliomycin (42 mg, 0.045*mM*) was dissolved in dry dioxane (2 ml) and 2.5*M* sodium hydroxide (0.4 ml) and *M* iodine in a 20% potassium iodide solution (0.8 ml) were added. This solution was allowed to stand for 36 hours at room temperature. The excess iodine was decolorized with a few drops of a concentrated sodium thiosulfate solution. No iodoform could be detected, although a control of acetone (0.1 ml) in dioxane (2 ml) gave a heavy precipitate under the same reaction conditions. The solution containing peliomycin was acidified with 10% hydrochloric acid whereupon a white precipitate formed. This precipitate was filtered off and thoroughly washed with water. T.l.c. analysis ($CHCl_3$:CH_3OH = 92:8) showed one component (R_f = 0.23). The aqueous fraction was concentrated and showed at least five components in above t.l.c. analysis. A 60 MHz p.m.r. spectrum in *deuterio*-chloroform of the white precipitate showed a low intensity complex band centered at τ4.08. Except for this signal, the spectrum was devoid of absorptions up to τ4.85. One-proton signals appeared at τ5.05 (quartet) and

τ5.48 (multiplet). The remainder of the spectrum was a series of broad bands. No sharp signal appeared in the region τ7.8-8.2 which could be associated with an acetyl group.

(ii) Hydrazine reaction.

Peliomycin (50 mg) was dissolved in warm 95% ethyl alcohol (0.5 ml) to which 85% hydrazine (0.2 ml) was added. The solution was heated under reflux on the steam cone for 3 hours. After cooling, water (5 ml) was added to the mixture which was then extracted with chloroform (5x3 ml). The organic extracts were dried over sodium sulfate, filtered and concentrated *in vacuo*, yielding 27 mg of product. This fraction showed four components on t.l.c. analysis and contained 5.4% nitrogen. The aqueous layer was also concentrated *in vacuo* (2 days), and t.l.c. analysis of the aqueous fraction (25 mg) showed 3 different components. The investigations of both of these fractions were not further pursued.

(iii) Bodforss reaction.

To determine whether peliomycin contains an epoxide function adjacent to a carbonyl or to the α,β-unsaturated lactone group, the Bodforss reaction (117) according to Ceder (130) was followed. To peliomycin (6.9 mg) in glacial acetic acid (2.0 ml) were added a few crystals of

potassium iodide. The mixture was kept at 67°C for 6 minutes, cooled and diluted with ethyl alcohol (7 ml). This solution was filtered through prewashed (water, then ethyl alcohol) Dowex-1 (Cl^-) and its ultraviolet spectrum determined. Except for the absorption band at 242 mμ, a blue shift of 3 mμ was observed for the absorption peaks, and no extension of conjugation was found. The solution was concentrated to dryness under reduced pressure, water (3 ml) added and extracted with ether (4 x 3 ml). Upon concentrating the extract to dryness, the residue was dissolved in methyl alcohol (2 ml) and *N* sodium hydroxide (1 ml) added. The mixture was heated for three minutes at 90°C, cooled and acidified with *N* nitric acid. No precipitate of silver iodide was detected upon addition of 3 drops of *N* silver nitrate.

e. Quantitative oxidations.

(i) Periodate-permanganate oxidation.

At zero time, a known concentration of peliomycin (1.05 mg) in *tert*-butyl alcohol (1 ml) in a 50 ml glass-stoppered Erlenmeyer flask was mixed with sodium *meta*-periodate (197.5*mM*) in water (1 ml), aqueous potassium permanganate (5 mg, 1 ml) and buffered with potassium carbonate (2.5 mg) in water (1 ml). This mixture was made up to 10 ml to give a final solution of 60% *tert*-butyl

alcohol. After known time intervals, the reaction was stopped by the addition of sodium bicarbonate (0.5 g) and 0.1*N* sodium arsenite (5.00 ml). After all the permanganate color was discharged, a few crystals of potassium iodide were added and the solution placed on the steam bath to remove most of the *tert*-butyl alcohol. The excess sodium arsenite was determined by stirring the solution and titrating slowly with iodine solution (0.0161*N*) to a starch endpoint. The same procedure was followed for the reagent blank to determine the amount of oxidant consumed by the peliomycin sample as shown in Table XXI.

TABLE XXI

Periodate-Permanganate Oxidation of Peliomycin at pH 7.7

Time hrs.	0.0161*N* Iodine solution			Atoms of oxygen consumed per mole of peliomycin (mol. wt. 928)
	Sample ml	Blank ml	Difference ml	
0.5	7.62	6.66	0.96	6.84
1	7.97	6.90	1.07	7.62
3	8.30	6.97	1.33	9.45
6	8.90	6.95	1.95	13.9
9	9.10	7.00	2.10	15.0
12	9.65	7.33	2.32	16.5
18	9.40	7.10	2.30	16.4
24	9.42	7.10	2.32	16.5

When the oxygen consumption was plotted vs. time, a curve (Figure VIII) was obtained which on examination suggested that the initial fast uptake of oxidant corresponded to 16 atoms of oxygen per mole of peliomycin. Similar results were obtained when the oxidation was allowed to proceed in a nitrogen atmosphere.

(ii) Chromium trioxide in acetic acid oxidation.

Peliomycin (2.47 mg) was dissolved in acetic acid (5.0 ml), and, at zero time, added to a solution (5.00 ml) of chromium trioxide in acetic acid, prepared by dissolving chromium trioxide (0.1 g) in water (0.2 ml) and diluted to 25 ml with glacial acetic acid . After a given time interval, 0.2*N* sulfuric acid (50 ml) was added followed by potassium iodide (0.5 g). The liberated iodine was determined with 0.0518*N* sodium thiosulfate to the starch endpoint. A blank was determined in a similar way and the difference taken as the amount of oxidant consumed by peliomycin. The results are presented in Table XXII.

TABLE XXII

Chromium Trioxide-Acetic Acid Oxidation of Peliomycin

Time min.	0.0518 N $Na_2S_2O_3$ solution			Atoms of oxygen consumed per mole of peliomycin (mol. wt. 928)
	Blank ml	Sample ml	Difference ml	
5	5.75	4.33	1.42	13.8
15	5.70	4.23	1.47	14.3
30	5.70	4.15	1.55	15.1
45	5.68	4.10	1.58	15.4
75	5.67	4.02	1.65	16.0
105	5.67	3.97	1.70	16.5
150	5.69	3.86	1.83	17.8
210	5.64	3.83	1.81	17.6
285	5.60	3.70	1.90	18.5

Figure VIII, where oxygen consumption was plotted vs. time, indicated that 16 atoms of oxygen are taken up per mole of peliomycin in the initial rapid stages of the reaction.

(iii) Periodate oxidation.

At zero time, aqueous sodium *meta*-periodate (10 ml of 97.5*M*), *tert*-butyl alcohol (25 ml) and water (10 ml) was added to a solution of peliomycin (5.15 mg) dissolved in *tert*-butyl alcohol in a 50 ml volumetric flask to give a solution of 60% *tert*-butyl alcohol. After known time intervals,

10 ml aliquots were withdrawn, and sodium bicarbonate (0.5 g) and 0.1*N* sodium arsenite (5.00 ml) was added. Most of the *tert*-butyl alcohol was removed on the steam cone, and the excess sodium arsenite determined by slow titration with 0.0151*N* iodine to a starch endpoint. After 96 hours a negligible (0.2 atom of oxygen per mole of peliomycin) uptake of oxygen was obtained.

D. Oxidative Degradation of Peliomycin

1. Periodate-Permanganate Oxidation

a. Isolation of acetic acid and formic acid.

Sodium *meta*-periodate (342 mg, 0.16*mM*) was dissolved in water (8 ml) and peliomycin (92 mg $\simeq$ 0.1*mM*) in *tert*-butyl alcohol (15 ml) was added. Potassium carbonate (38.5 mg) in water (1 ml) was added to this mixture together with a catalytic quantity of potassium permanganate (6.2 mg) in water (1 ml). The total solution contained 60% *tert*-butyl alcohol and was allowed to stand at room temperature for 15 hours. After this time the potassium permanganate color was wholly discharged. Additional potassium permanganate was not reduced within thirty minutes, indicating that the oxidation was complete. The solution was concentrated to about 5 ml under freeze-drying conditions. The distillate (i) was investigated for volatile components. No absorption could be detected in the ultraviolet spectrum, nor was a

positive test obtained for acetone or other carbonyl fragments with the 2,4-dinitrophenylhydrazine reagent (137). Negative results were also obtained when nitrogen was bubbled through the aqueous distillate into a solution of 2,4-dinitrophenylhydrazine reagent (138). G.c. analysis did not indicate other components besides the solvents present, using polar as well as non-polar column packings.

The liquid residue from above was acidified with *N* sulfuric acid (1 ml) and concentrated to dryness under freeze-drying conditions yielding distillate (ii) and a solid residue. This distillate (ii) was titrated with 0.010*N* sodium hydroxide (4.30 ml) to the phenolphthalein endpoint. The salt solution was evaporated to dryness and exchanged twice with deuterium oxide (0.5 ml). A 60 MHz p.m.r. spectrum of this salt solution in deuterium oxide showed singlets at τ8.06 and τ1.60 which integrated at 3:1. The infrared spectrum (KBr-disc) showed strong carboxylate anion absorption at 1570 and 1410 cm^{-1}. These data suggest an equimolar mixture of formic and acetic acid. When equimolar quantities of reagent grade formic and acetic acid were mixed, identical p.m.r. and infrared spectra were obtained. Thus 0.1 millimole of peliomycin gave 0.043 millimoles of titrable acids.

b. Other products.

The residue, left after separation of the volatile acids, was separated into a polar and a non-polar fraction by partition chromatography on a celite column, according to Lemieux and Charanduk (81). The non-polar fraction (developed with benzene) appeared to be one homogeneous compound (t.l.c.). The p.m.r. spectrum indicated a long chain hydrocarbon, and the mass spectrum showed peaks gradually decreasing in intensity and reaching values of *m/e* 350, without the formation of a parent peak. The infrared spectrum of this compound lacked hydroxyl absorption, but showed minor carbonyl absorption at 1710 cm^{-1} and strong C-H absorption at 2900 cm^{-1}. The polar fraction, developed with *n*-butyl alcohol, displayed many components by t.l.c., which were acidic (infrared: 2500-2700 cm^{-1}, acidic O-H) and contained hydroxyl and carbonyl groups (infrared: 3200-3600 cm^{-1} and 1700-1750 cm^{-1}, respectively). An attempt to separate the components of this fraction by gas chromatography, employing column packings: 10% Apiezon L, 20% silicone grease and 15% ethyleneglycolsuccinate, met with failure using the following derivatives. a. Esterification of the carboxylic acids with diazomethane (139), and repeated acetylation with acetic anhydride in pyridine (still free -OH at 3400 cm^{-1} in the infrared spectrum). b. Esterification of the carboxylic acids with diazomethane (139), and

trimethylsilylation of the hydroxyl groups (70).

An attempt was made to separate these fractions as the free acids by preparative thin layer chromatography using Silica Gel HF_{254} to permit detection by ultraviolet light. To prevent the heavy trailing observed, the aqueous Silica Gel was buffered at various hydrogen ion concentrations. The buffer solutions were made by adding potassium hydroxide (0.1*M*) to phosphoric acid (0.1*M*) until the desired pH was obtained as indicated by the pH meter. Studies indicated that benzene: *n*-butyl alcohol = 85:15 was the best developing agent on t.l.c. plates buffered at pH 3 giving the least trailing, which was nevertheless still extensive. Initially, eight zones were detected and isolated from the plates (20 x 10 cm, with 1 mm of Silica Gel). After eluting the organic material from the Silica Gel, the purity of each fraction was checked both on Silica Gel G and charred with sulfuric acid and on microcrystalline cellulose (145) using iodine as the developer. Every one of the eight zones were found to be impure and required further chromatography both on Silica Gel and on microcrystalline cellulose. Finally, twenty-six different fractions were isolated which were further purified by sublimation. When submitted to mass spectrometry, no molecular ion could be detected for any of the fractions. The fragmentation of each was different and very complex; the intensity of the lines decreased gradually giving ions

of at least *m/e* 300 without the indication of a parent ion. Further investigations of these fractions were abandoned.

2. Osmium Tetroxide-Periodate Oxidation

a. Isolation of the 2,4-dinitrophenylhydrazone of glycolaldehyde.

The 2,4-dinitrophenylhydrazone of glycolaldehyde was isolated from peliomycin employing the osmium tetroxide-periodate oxidation couple (84). A mixture of peliomycin (11.4 mg) in ether (5 ml), water (5 ml) and osmium tetroxide (16 mg) was stirred while powdered sodium *meta*-periodate (200 mg) was added over a 40 minute period. The temperature was maintained at 24-26°C during the addition and for 80 minutes thereafter to dispel the dark color. The oxidation mixture was extracted with ethyl acetate (6 x 5 ml) and filtered through a little anhydrous sodium sulfate. The solution was treated with 2,4-dinitrophenylhydrazine (600 mg) and two drops of concentrated hydrochloric acid, whereupon it was set aside for twenty hours. The resulting dark crystals were recrystallized by dissolving the crystals on the steam cone in ethyl alcohol (20 ml). After crystallization had gone to completion, the mixture which contained a large amount of unreacted 2,4-dinitrophenylhydrazine, was subjected to chromatography on a silicic acid column in a 25 ml-burette, which was filled to the 10 ml-mark with silicic acid. Definite yellow bands were observed when the mixture

was eluted with chloroform containing 5% ethyl alcohol. Of the five fractions obtained, only the crystalline 2,4-dinitrophenylhydrazone of glycolaldehyde could be characterized, because of the micro amounts, using high resolution mass spectrometry. M.p. 151.5-154.0°C; reported (140)155-156°C. Mass spectrum analysis *m/e* 240, measured 240.0499, calculated for $C_8H_8N_4O_5$, 240.0495.

b. Attempted synthesis of the 2,4-dinitrophenylhydrazone of glycolaldehyde.

Sodium *meta*-periodate (4.3 g, 0.021*M*) in water (30 ml) was added to a cooled, stirred solution of monopropionin (3 g, 0.020*M*) at such a rate (0.5 hr.) that the temperature stayed below 10°C. After standing at 5°C for 3 hours, the solution was allowed to reach room temperature, whereupon barium chloride dihydrate (5.5 g, 0.022*M*) was added. The precipitated salts were filtered off through Celite and the filtrate extracted with chloroform (4 x 25 ml). The combined extract and washings were dried over sodium sulfate and concentrated below 40°C to give 2.1 g of product, which was dried *in vacuo* for two days to remove the formaldehyde produced in the reaction. The p.m.r. spectrum in *deuterio*-chloroform showed the presence of the propionyl group and a multiplet consisting mainly of a sharp peak at its center (τ5.83). No precipitate nor oil was formed when

this product was treated with acidic 2,4-dinitrophenylhydrazine in ethyl alcohol (141) and the solution allowed to stand up to 7 days at room temperature. The mixture was extracted with chloroform (4 x 5 ml), and washed subsequently with water (5 ml), saturated aqueous sodium bicarbonate (5 ml), and water (5 ml). After drying the chloroform extract over sodium sulfate, the solvent was removed, and a syrup obtained which did not crystallize. An attempt to distill the product from the periodate oxidation led to loss of the propionyl group with formation of a high boiling product, b.p. 118-119°C (1 mm Hg). No attempt was made to purify or characterize this compound.

3. Jones Oxidation of Peliomycin (Oxypeliomycin)

The Jones reagent (86) was prepared by dissolving chromium trioxide (26.7 g, 0.267*M*) in concentrated sulfuric acid (23 ml, *d* 1.84) and water (40 ml) while cooling in ice water. This solution was diluted with water to the mark in a 100 ml volumetric flask. Peliomycin (100 mg) was dissolved in acetone (10 ml, freshly distilled from potassium permanganate). The Jones reagent was added dropwise from a microburette while stirring the solution vigorously until a yellow coloration was noticeable (0.30 ml). The reaction mixture was kept in an ice-water bath during the addition of the Jones reagent such that the temperature of

the solution remained below 10°C. It was kept at this temperature for another 20 minutes, whereupon water (10 ml) was added. The solution was extracted with ether (5 x 10 ml) and the combined extract and washings dried over magnesium sulfate. The solvent was removed in a closed system to give a light green syrup, termed oxypeliomycin (101 mg). This distillate was checked by gas chromatography, but only a solvent peak was obtained (20% silicone grease; temperature = 50°C).

E. Oxidative Degradation of Oxypeliomycin (Scheme II)

1. Periodate-Permanganate Oxidation

a. Isolation of acetic acid and formic acid (trace).

Oxypeliomycin (100 mg) was dissolved in freshly distilled *tert*-butyl alcohol (15 ml) to which was added sodium *meta*-periodate (320 mg) in water (8 ml), potassium carbonate (38 mg) in water (1 ml) and potassium permanganate (6.2 mg) in water (1 ml) to give a solution which contained 60% *tert*-butyl alcohol. This mixture was shaken in the dark for 14 hours and distilled under freeze-drying conditions to near dryness. The distillate (i) was examined as described in Experiment E-2-a, but no fragments could be detected. The residual salts were acidified with *N* sulfuric acid (1 ml), then distilled under freeze-drying conditions to complete dryness (overnight) to give a distillate (ii) and residual salts.

This distillate (ii) was titrated with 0.107*N* sodium

hydroxide (2.40 ml) to the phenolphthalein endpoint and consumed 2.2 moles of base per mole of oxypeliomycin. The salt solution was distilled under freeze-drying conditions and exchanged once in similar manner with deuterium oxide (0.3 ml). A 60 MHz p.m.r. spectrum indicated acetic acid at τ8.15 and some formic acid at τ1.67 (∿ 5%) which integrated as 20:1, respectively.

b. Isolation of the methyl esters of fragments VII and VIII.

Water (10 ml) was added to the salt mixture, after separation of the volatile acids, and extracted with ether (8 x 10 ml). The combined extracts were dried over magnesium sulfate and distilled in a closed system to yield a syrup (89 mg). A thin layer chromatogram of the residual fraction indicated two definite components, which were heart-shaped (suggesting ionic character), and some minor impurities. After methylation with diazomethane (139), and solvent removal in a closed system, the residue (76 mg) and distillate were subjected to analysis by gas chromatography using a 20% silicone grease column packing. Only solvent was detected in the distillate, but the residue showed, among some minor impurities, two major peaks with retention times of 8.7 and 17.3 minutes, respectively, while programming the temperature from 80° to 200°C at a rise of 7.9°C/min.

The fragments VII and VIII were isolated by preparative gas chromatography using above column packing and a flow rate of 85 ml/min. of helium. The temperature was programmed from 75° to 200° at a rise of 7.9°C/min. The characterization and spectral data of fragments VII and VIII are presented in the discussion on Degradative Studies and in Figures X and XI, respectively. The syntheses of these compounds are described below.

2. Synthesis and Characterization of Fragments VII and VIII

a. Synthesis and characterization of dimethyl 2-methylsuccinate (VII).

Methyl crotonate was prepared by the Fischer esterification of crotonic acid in absolute methyl alcohol with concentrated sulfuric acid (*d.* 1.84) as the catalyst. The usual work-up yielded a product of b.p. 33-34°C (18 mm Hg), reported (142) 120.2°C (760 mm Hg), which was pure according to a 60 MHz p.m.r. spectrum: τ4.25 (1H, mult.); τ6.32 (3H, s.); τ8.17 (3H, d.).

Dimethyl 2-methylsuccinate was prepared according to Brown (143). A 1-liter round-bottom flask equipped with a condenser was charged with methyl crotonate (29 g, 0.32*M*), methyl alcohol (150 ml) and a solution of 97% sodium cyanide (18 g, 0.33*M*) in water (42 ml). This mixture was heated under reflux on the steam bath for five hours,

whereupon a suspension of barium hydroxide octahydrate (50 g) in hot water (100 ml) was added. The described work-up was followed yielding 23.2 g (56% of theor.) of 2-methylsuccinic acid. M.p. 109-110.5°C; reported (143) 110-111°C.

Methylation of 2-methylsuccinic acid (5 g, 0.034*M*) in absolute methyl alcohol and concentrated sulfuric acid (*d.* 1.84) as catalyst yielded 3.7 g (68% of theor.) of dimethyl 2-methylsuccinate. B.p. 79-80°C (5 mm Hg); n_D^{22} 1.4184. Reported (144) b.p. 196°C (760 mm Hg); n_D^{22} 1.4196. Elemental analysis: calcd.: C, 52.49; H, 7.55; found: C, 52.08; H, 7.55. The 100 MHz p.m.r. spectrum: τ6.31 (6H, d.); τ6.9-τ7.5 (3H, mult.); τ8.75 (3H, d.); and the infrared spectrum: strong C=O absorption at 1735 cm^{-1} (faint doublet) are in agreement with the proposed structure. The mass spectrum showed strong peaks at *m/e* 128 and 129 due to $M^+ - 32$ (CH_3OH) and $M^+ - 31$ (OCH_3^-), respectively.

b. Synthesis and characterization of dimethyl 3-acetoxy-2,4-dimethylglutarate (VIII).

The intermediate dimethyl 2,4-dimethyl-3-hydroxyglutarate was synthesized according to Favorsky (88), a method which had to be slightly modified. A solution of ethyl 2-bromopropionate (50 g, 0.3*M*) and methyl formate (10g, 0.16*M*) was added slowly to zinc (30 mesh, 4 g) in a 3-necked flask (300 ml) equipped with a reflux condenser. The mixture was set aside for three days, as called for, but no hydroxy compound could be

detected upon work-up, by acidifying with 10% sulfuric acid (50 ml), washing with water (50 ml), drying over calcium chloride and distilling in vacuum. However, when the reaction mixture was heated under reflux for seven hours before work-up and by the use of freshly activated zinc, 12.8 g of product was obtained which distilled at 122-133°C (7 mm Hg). A 60 MHz p.m.r. spectrum indicated extensive transesterification and a pronounced hydroxyl peak at τ6.85 which disappeared upon proton exchange with deuterium oxide. The hydroxyl group was also confirmed by the absorption band at 3500 cm^{-1} in the infrared spectrum. The mixed esters were refluxed in absolute methyl alcohol and a catalytic amount of concentrated sulfuric acid (*d.* 1.84). After work-up, dimethyl 2,4-dimethyl-3-hydroxy-glutarate was obtained in 90% (of theor.) yield, b.p. 139-141°C (18 mm Hg). The infrared spectrum showed strong carbonyl absorption at 1730 cm^{-1} and hydroxyl absorption at 3500 cm^{-1}. The 60 MHz p.m.r. spectrum was in agreement with proposed structure: τ5.9 (1H, d.); τ6.3 (6H, s.); τ6.95 (1H, broad s.); τ7.4 (2H, mult.). The signal at τ6.95 disappeared upon shaking the *deuterio*-chloroform solution with deuterium oxide.

The title compound was obtained by acetylation of above hydroxy compound (3.0 g, 0.015*M*) with acetic anhydride (5 ml) and dry pyridine (5 ml) at room temperature for

24 hours. After adding ice/water mixture (25 ml), the solution was extracted with ether (4 x 25 ml). The ether extract was washed subsequently with water (25 ml), *N* sulfuric acid (25 ml), water (25 ml) and dried over magnesium sulfate. After removal of solvent, the residual syrup yielded dimethyl 3-acetoxy-2,4-dimethylglutarate upon distillation. Yield 2.5 g (67% of theor.); b.p. 150-153.5°C (17 mm Hg). Elemental analysis: calcd.: C, 53.65; H, 7.37; found: C, 53.73; H, 7.42.

The infrared spectrum lacked hydroxyl absorption at 3500 cm^{-1}, and the 60 MHz p.m.r. spectrum was in agreement with proposed structure (Table VIII), but indicated a mixture of isomers. These isomers could be separated by gas chromatography on a 20% butanediol-succinate polyester column packing. The temperature was maintained at 150°C and a flow rate of 75 ml/min. yielded fractions VIII-a (40%), VIII-b (48%) and VIII-c (12%), with retention times of 27.2, 31.3 and 34.5 minutes, respectively. Absolute yields were not determined. A comparison of the 100 MHz spectra (Figure XII) of the diastereoisomers VIII-a, VIII-b and VIII-c with that of fragment VIII isolated from peliomycin, showed that isomer VIII-a is identical to fragment VIII from peliomycin (Figure XI).

3. The 2*R*,3*s*,4*S*-Configuration of Dimethyl 3-Acetoxy-2,4-dimethylglutarate (VIII-a) and the Configuration of its Diastereoisomers (VIII-b and VIII-c)

a. Synthesis of dimethyl 2,4-dimethyl-3-trifluoroacetoxyglutarate and separation of its diastereoisomers "VIII-a, VIII-b and VIII-c".

Dimethyl 2,4-dimethyl-3-hydroxyglutarate (3 g, 0.015*M*), as obtained in the synthesis of VIII dissolved in acetonitrile (10 ml), was trifluoroacetylated (146) with trifluoroacetic anhydride (25 ml) and sodium trifluoroacetate (1 g). Considerable heat was evolved during slow addition of trifluoroacetic anhydride. Heating under reflux was continued for 15 minutes after the addition was completed. The solution was cooled, and, after concentrating under reduced pressure, was used as such to separate its isomers as described in the previous experiment. The same experimental conditions were employed except that the temperature was maintained at 135°C and the flow rate increased to 150 ml/min. of helium. To facilitate the isolation of larger fractions, collector flasks belonging to an Aerograph Autoprep Model A700 gas chromatograph were used instead of the coiled glass tubings as mentioned before. Isomers "VIII-a, VIII-b and VIII-c" with retention times of 10, 13.5 and 15 minutes, respectively, were isolated in sufficient amounts to permit further reactions (minimum 200 mg). Isomer "VIII-a" (50 mg) was hydrolyzed in aqueous methyl

alcohol (50%, 10 ml) by heating under reflux on the steam cone for 10 minutes. After cooling the solution to room temperature the solvent was partly (50%) removed and extracted with chloroform (2 x 5 ml). The combined extracts were dried over sodium sulfate, filtered and the solvent removed to yield 30 mg of hydroxy compound. Acetylation of this hydroxy compound with acetic anhydride (3 ml) in pyridine (3 ml) yielded, upon removal of solvents by evaporation and drying *in vacuo* (2 days), a compound with the same retention times as VIII-a and the same 100 MHz p.m.r. spectrum (Figure XII). Each isomer was reacted further as described in experiment E-3-b.

b. Syntheses of the diastereoisomers IX-a, IX-b and IX-c of 2,4-dimethylpentane-1,3,5-triol.

Each isomer obtained in experiment E-3-a was reacted further as described for Isomer "VIII-a" to yield Isomers IX-a, IX-b and IX-c, respectively. Isomer "VIII-a" (400 mg, 1.3*mM*) was dissolved in dry ether (8 ml) and added dropwise to a suspension of lithium aluminum hydride (300 mg) in ether (20 ml) in a 3-necked flask (100 ml), equipped with a condenser capped with a calcium chloride drying tube. The mixture was stirred magnetically and after addition heated under reflux for one hour. Excess lithium aluminum hydride was destroyed with methyl alcohol (2 ml) in ether (3 ml) and acidified with

20% hydrochloric acid (10 ml). This solution was extracted continuously with ethyl acetate for 28 hours. After drying over sodium sulfate, the ethyl acetate was removed under reduced pressure, and the residue treated with Amberlite MB-3. Upon concentrating to dryness, 2*R*,3*s*,4*S*-2,4-dimethylpentane-1,3,5-triol (200 mg, 98%) (IX-a) crystallized after standing at 0°C for one day. M.p. 74.5-76.0 C; reported (38) 75-78°C. Similarly isomers "VIII-b" and "VIII-c" were reduced to triols IX-b and IX-c, which gave crystalline products with melting points of 89-89.5°C and 86-86.5°C, respectively. As was shown in the discussion, Isomer IX-b is the *racemic* mixture with the 2*R*,4*R* *(arabino)*-configuration. Gerzon (38) had isolated the *racemic* isomer (IX-b) from erythromycin with m.p. 86-87°C. The triol IX-c with the 2*R*,3*r*,4*S* *(ribo)*-configuration has not been described before.

c. Syntheses of the diastereoisomers X-a, X-b1, X-b2 and X-c of 5-*O*-acetyl-2,4-dimethyl-1,3-*O*-isopropylidene-pentane-1,3,5-triol.

Each trihydroxy compound (100 mg, 0.67*mM*) obtained in the previous experiment (E-3-b), was heated under reflux in the presence of dry 2,2-dimethoxypropane (90 mg, 0.88*mM*) in dry acetone (3 ml), and a crystal of *p*-toluenesulfonic acid monohydrate. After three hours the reaction had gone to completion (t.l.c.), and a few drops of saturated

sodium bicarbonate solution was added. Evaporation to dryness, extraction with chloroform (3 x 3 ml) from water (1 ml) and drying the combined extracts over sodium sulfate afforded 1,3-*O*-isopropylidene derivative upon removal of solvent under reduced pressure. A 60 MHz p.m.r. spectrum accounted for the six protons of the isopropylidene grouping at τ8.6 (s.). Only the isopropylidene derivative of triol IX-c could be induced to crystallize (36-38°C) after standing in the refrigerator for one week. The isopropylidene derivative of each diastereoisomeric triol was treated as described below and similar yields were obtained.

The isopropylidene derivative (75 mg, 0.38*mM*) of triol IX-a was acetylated with acetic anhydride (0.6 ml) and pyridine (0.7 ml) for 14 hours at room temperature. After extracting the product with chloroform (3 x 3 ml) from ice water (2 ml), the combined extracts were dried over sodium sulfate. The solvent was removed by distillation in a closed system, which procedure was repeated with carbon tetrachloride (4 x 0.5 ml) to remove the pyridine by co-distillation. The 5-*O*-acetyl-1,3-*O*-isopropylidene derivative was obtained in 70% (of theor.) yield (60 mg). Gas chromatographic investigation using a 10% Apiezon L column packing, indicated minor impurities (less than 10%) which were removed by gas chromatography, operated isothermally at 155°C with a flow rate of 150 ml/min. The 5-*O*-acetyl-1,3-*O*-isopropylidene

derivative of triol IX-b gave two products X-b1 and X-b2 as indicated by gas chromatography in a ratio of 4.1:1 with retention times of 6 and 7 minutes, respectively, using the above conditions, except the temperature was maintained at 145°C. Yields were not determined after the final purification step by gas chromatography. The derivatives of the triol, *viz.*: isomers X-a, X-b1, X-b2 and X-c were subjected to configurational analysis by p.m.r. spectroscopy as described in the discussion. The p.m.r. parameters of each diastereoisomer have been presented in Tables VIII, IX, X and XI and their spectra reproduced in Figures XI, XII and XIII.

d. Electrophoresis of the diastereoisomers of IX-a, IX-b and IX-c of 2,4-dimethylpentane-1,3,5-triol.

The experiment was carried out essentially according to the method as described by Frahn and Mills (92), except that the paperstrip was weighted down by means of a glass plate instead of air pressure (92). The apparatus was cooled by tap water and capable of delivering a potential difference of 1500 V. The voltage was kept constant and the current was not allowed to exceed 50 m A (about 2 hrs.). Paper strips of 20 x 62 cm of Whatman No. 1 MM paper were employed to give a potential gradient of approximately 25 V/cm.

After the paper had been dipped in the electrolyte solution (0.05*M* $Na_2B_4O_7 \cdot 10H_2O$, borax) and slightly blotted

to remove the excess, the compounds were applied as an aqueous solution from a fine capillary tube. Upon electrophoresis (2 hours), the paper was dried first with an electric hair dryer, then in an oven maintained at 115°C. The crisp paper was sprayed with a chromium trioxide-permanganate-sulfuric acid reagent (92) to give pale yellow spots on a pink background. The mobilities of the isomers are expressed as R_G values (glucose as reference) and adjusted for electrophoretic mobility using caffeine as a marker (92) for zero migration (Table XXIII)

TABLE XXIII

Electrophoretic Mobilities of Isomers IX-a, IX-b and IX-c of 2,4-Dimethylpentane-1,3,5-triol

Isomer	R_G
IX-a	0.10
IX-b	0.47
IX-c	0.44

F. Oxidative Degradation of Perhydropeliomycin (Scheme IV)

1. Oxidative Procedure

a. Jones oxidation.

The Jones reagent (86) was prepared as described in the preparation of oxypeliomycin (experiment F-2).

Perhydropeliomycin (199 mg) was oxidized under the same conditions as stated in experiment F-2. The compound required 0.70 ml of the Jones reagent and provided 185 mg of oxidized product. Since 13 atoms of oxygen were consumed per mole of perhydropeliomycin, and only six hydroxyl groups are suspected, the remaining oxygen atoms must be responsible for the fragmentation observed by t.l.c. analysis.

b. Baeyer-Villiger Oxidation.

To ensure excess of reactants on the basis of at least six ketone groups, 4.5 millimoles of oxidant was used and the Emmons modified procedure (93) followed. Trifluoroacetic anhydride (1.5 ml) was added dropwise to a stirred suspension of 98% hydrogen peroxide (0.2 ml) in dichloromethane (5 ml) at ice-bath temperature. The resulting solution was added dropwise, over a 20-minute period, to a vigorously stirred suspension of 0.01*M* disodium hydrogen phosphate (1.4 g) in dichloromethane (10 ml) containing the product from the Jones oxidation (185 mg). After addition, the mixture was heated under reflux for 1.5 hours, cooled and poured into water (30 ml). After separation, the aqueous layer was extracted with dichloromethane (8 x 5 ml) and the combined extract and washings dried over sodium sulfate and concentrated below 40°C under reduced pressure, giving

198 mg of product. A t.l.c. plate developed in chloroform containing ethyl alcohol (3%) indicated a similar mixture as the products obtained from the Jones oxidation, except, that a significant spot had appeared at the solvent front.

c. Hydrolysis and esterification of the oxidation products.

The slightly green products from the Baeyer-Villiger oxidation (195 mg) were frozen in water (2 ml) in a dry-ice/acetone bath, and metallic sodium (50 mg) was added. The frozen mixture was allowed to attain room temperature very slowly to give a solution of about *N*. sodium hydroxide. The compound went readily into the alkaline solution, which caused it to turn yellow. After heating under reflux for 1.5 hours at 100°C, the solution had turned dark and was cooled in an ice bath. Upon acidifying with *N* hydrochloric acid, the mixture was concentrated to a syrup in a closed system (24 hours). The distillate appeared to be essentially free from organic materials as shown by g.c. and t.l.c. analyses. The remaining syrup containing the free acids and inorganic salt gave at least eight spots on a t.l.c. plate. The mixture was subjected to esterification with diazomethane according to Schlenk (139). The esterified mixture was filtered through a dropping capillary fitted with a small cotton plug to give the methyl esters in diethyl ether containing 10% methyl alcohol.

d. Isolation of fragments XIII, XIV, XV, XVI and XVII.

The methylated products obtained from the Baeyer-Villiger oxidation were subjected to distillation in a closed system. After one hour a syrup remained in the reaction flask, which was heated in boiling water for an additional hour to enable less volatile esters to distill over. Both the volatile and residual fractions were subjected to g.c. analysis using a column (7') packed with silicone grease (20%).

The volatile fraction showed two peaks, XIII and XIV, in a ratio of 3:1 and with retention times of 8.5 and 14.7 minutes, respectively, besides minor high boiling components. The flow rate was adjusted to 100 ml/min of helium and the temperature maintained at 120°C. The two components (XIII and XIV were separated by preparative gas chromatography using the semi-micro technique described. The residual fraction, after removal of the volatile esters, showed several peaks by g.c. analysis. Since these peaks overlapped considerably, a twelve foot column, packed with 20% silicone grease, was used. In the semi-micro preparative gas chromatography, the oven temperature was kept isothermally at 150°C for 3 minutes, then allowed to rise to 250°C at a rate of 15°C/min. In this manner, fragments XV, XVI and XVII could be isolated, which had retention times of 16, 18 and 4.5 minutes, respectively, under the conditions stated.

The characterization of the fragments obtained above are

discussed in Degradative Studies, and all structures, except that of fragment XVI were proven by synthesis. The spectral data of fragments XIII, XIV, XV, XVI and XVII have been presented in Figures XIV, XV, XVI, XVII and XVIII, respectively. The syntheses of fragments XIII, XIV, XV and XVII are discussed below.

2. Synthesis and Characterization of Fragments XIII, XIV, XV, and XVII

a. Synthesis and characterization of dimethyl 2-methylglutarate (XIII).

The synthesis required 3-chlorocyclopentene (147) which was prepared by the addition of one mole of hydrochloric acid to one mole of cyclopentadiene and purified by distillation 20-23°C (5 mm Hg). The 3-chlorocyclopentene (39 g, 0.38*M*) was added over a period of one hour to prepared methylmagnesium iodide (0.45*M*) in dry ether (100 ml) in a 3-necked flask (500 ml), which was equipped with a mechanical stirrer and a reflux condenser capped with a calcium chloride drying-tube. The reaction was very vigorous and cooling was necessary. Stirring was continued for one hour after the addition of the chloro-derivative. After pouring the reaction mixture into ice water containing 5% hydrochloric acid, the layers were separated and the aqueous layer extracted with ether (4 x 50 ml); the combined extract and washings were dried over sodium sulfate

and subjected to spinning band distillation yielding 3-methylcyclopentene (20 g, 64% of theor.); b.p. 64.0-64.5°C (690 mm Hg). A 60 MHz p.m.r. spectrum confirmed the assigned structure: τ4.25 (2H, s.); τ7-τ8.8 (5H, mult.); and τ8.96 (3H, d.).

The olefinic bond was cleaved by the periodate-permanganate oxidation couple (74). 3-Methylcyclopentene (2.24 g, 27.3*mM*) was dissolved in dioxane (140 ml) and added to a mixture of potassium carbonate (11.3 g, 82*mM*), potassium permanganate (0.58 g, 3.66*mM*) and sodium *meta*-periodate (46.7 g, 219 *mM*) in water (560 ml). The final solution of 20% dioxane in water was shaken overnight whereupon sodium bisulfite was added to reduce excess oxidant. The solution was concentrated under reduced pressure to approximately 75 ml. Acidification with 20% sulfuric acid to pH 1 was followed by extraction with ether (5 x 25 ml). The combined extract was dried over magnesium sulfate, then concentrated under reduced pressure yielding 2.3 g (58% of theor.) of the 2-methylglutaric acid which crystallized from ethyl acetate. Recrystallized from ethyl acetate and a few drops of Skelly B. M.p. 76-78.5°C. Elemental analysis: calcd.: C, 49.31, H, 6.90; found C, 49.07, H, 7.04.

Methylation of 2-methylglutaric acid (73 mg) was effected with diazomethane according to the procedure of Schlenk (139) yielding 80 mg of the corresponding dimethyl ester (92% of theor.). The mass spectrum did not show a molecular ion and gave a

fragmentation pattern in complete agreement with that of XIII. Both the 100 MHz p.m.r. and the infrared spectrum indicated that the structure of dimethyl 2-methylglutarate postulated for XIII was correct (Figure XIV).

b. Synthesis and characterization of methyl 4-methyl-5-oxoheptanoate (XIV).

The morpholine enamine of 3-pentanone was prepared according to Stork (148) and distilled at 74-75°C (3.5 mm Hg). This enamine (10 g, 0.065*M*) was dissolved in absolute methyl alcohol and added dropwise to freshly distilled methyl acrylate (5.7 g, 0.065*M*) under nitrogen in a 3-necked flask (300 ml), which was equipped with a condenser and stirred magnetically. The solution was heated under reflux for 15 hours. The enamine was then hydrolyzed by adding water (25 ml) and heated under reflux for another hour. The layers were separated and the aqueous layer extracted with benzene (4 x 100 ml) and the combined benzene extracts washed subsequently with 10% hydrochloric acid (2 x 75 ml), water (1 x 50 ml), saturated sodium bicarbonate (1 x 50 ml) and water (1 x 50 ml). After drying over sodium sulfate and solvent removal under reduced pressure, 6.5 g of crude product (57% of theor.) was obtained which distilled at 86-87°C (2.1 mm Hg). n_D^{26} 1.4317. The mass spectrum showed an identical fragmentation pattern as XIV (Figure XV) with a molecular ion at *m/e* 172. The assigned structure was also confirmed by a 100 MHz p.m.r.

spectrum and infrared spectrum (Figure XV). Elemental analysis: calcd.: C, 62.77; H, 9.36; found: C, 62.48; H, 9.64.

c. Baeyer-Villiger oxidation of methyl 4-methyl-5-oxo-heptanoate.

Methyl 4-methyl-5-oxoheptanoate (230 mg, 1.3*mM*) was oxidized with the Emmons modified reagent (93) as described in experiment K-2. Work-up yielded 240 mg (95% of theor.) of methyl 4-propionyloxypentanoate. This compound was pure by g.c. analysis using a column packed with 10% Apiezon L at an isothermal temperature of 125°C (flow 60 ml/min of He). After sublimation, elemental analysis gave: calcd.: C, 57.43; H, 8.57; found: C, 57.80; H, 8.86. A 60 MHz p.m.r. spectrum showed: τ5.08 (1H, mult.); τ6.32 (3H, s.); τ7.4-τ8.3 (6H, mult.); τ8.8 (3H, d.); and τ8.9 (3H, t.). The mass spectrum did not exhibit a molecular ion at *m/e* 188, but its fragmentation pattern was in accordance with proposed structure (M-15, M-31, M-73, M-74), and no M-59 and M-60 could be observed. Methyl 4-propionyloxypentanoate was heated under reflux with 3*N* methanolic hydrochloric acid for two hours. After cooling, the solution was neutralized with carbon dioxide. Distillation in a closed system of this product afforded a distillate which contained only methyl propionate in addition to methyl alcohol upon g.c. analysis, using

the same conditions as described above, but maintaining the temperature at 40°C.

d. Synthesis of dimethyl 4,6-dimethyl-5-oxoazelate (XVIII).

In this synthesis the pyrrolidine enamine of 3-pentanone was synthesized according to Stork (148). It was prepared in dry benzene and distilled at 57-58°C (4.0 mm Hg). The enamine (15.6 g, 0.11*M*) was dissolved in absolute methyl alcohol (100 ml) and added to freshly distilled methyl acrylate (9.7 g, 0.11*M*) in similar fashion as described for the preparation of methyl 4-methyl-5-oxoheptanoate. The same work-up was followed yielding 9.5 g of crude product (68% of theor.). The oil was distilled at 154-170°C (3 mm Hg). G.c. analysis on 20% silicone grease gave two peaks in a 50:50 ratio, indicating two stereoisomers (see elemental analysis). The mass spectrum showed a considerable molecular ion at *m/e* 258 which upon high resolution gave $C_{13}H_{22}O_5$ (calcd. 258.1467, meas. 258.1468). The 60 MHz p.m.r. spectrum conformed to the structure assigned: doublet at τ8.92, methoxycarbonyl at τ6.37 and multiple peaks from τ7 to τ8.7 which integrated as 3:3:5, respectively. The infrared spectrum showed absorption bands at 1738 and 1712 cm^{-1} for the ester and ketone carbonyl, respectively and all other bands were in agreement with the expected structure. Elemental analysis: calcd.: C, 60.45; H, 8.58; found: C, 60.67; H, 8.74.

e. Synthesis of methyl 2-methyl-5-oxoheptanoate (XIX).

1-Chloro-3-pentanone was prepared by a Friedel Crafts reaction of propionyl chloride and ethylene in the presence of aluminum trichloride according to McMahon *et al.* (149). B.p. 43-44°C (2.9 mm Hg). This product was condensed with the sodio derivative of dimethyl methylmalonate according to Blaise and Marie (150).

Dimethyl-methylmalonate (22 g, 0.15*M*) was dissolved in dry ether (120 ml) and finely divided metallic sodium (3.1 g, 1.3*M*) was added. The solution was stirred and slightly warmed until all the sodium had reacted (5 hours). To this mixture was added dropwise 1-chloro-3-pentanone (15.6 g, 0.12*M*) whereupon some heat was evolved. After stirring for two hours at room temperature, the mixture was heated under reflux for another hour. After filtration of the sodium chloride and solvent removal, the oily residue was distilled at 117-125°C (1.3 mm Hg) giving 23 g (83% of theor.) of product. An analytical sample was redistilled at 120.5-121.0°C (1.2 mm Hg). A 60 MHz p.m.r. spectrum of this compound was in accordance with the condensation product [τ6.28 (s.): τ7.7 (mult.): τ8.58 (s.): τ8.94 (s.) = 2:2:1:1]. Elemental analysis: calcd.: C, 57.39; H, 7.83; found: C, 57.61; H,8.16.

Saponification of the dicarboxylic ester was effected by stirring the ester (5.0 g) at room temperature in *N* sodium

hydroxide (50 ml) until the solution was clear (5 hours). Acidification with ice-cold 3*N* hydrochloric acid to pH 2 and extracting with ethyl acetate (4 x 20 ml), drying over magnesium sulfate and solvent removal resulted in 3.35 g of product (77% of theor.). The dicarboxylic acid crystallized very slowly from the syrup after two weeks, m.p. 90-94°C. This compound (1.07 g) was decarboxylated by heating to 165°C until no more carbon dioxide was evolved (barium hydroxide solution). The 2-methyl-4-oxoheptanoic acid (0.80g, 90% of theor.) was pure by p.m.r. analysis.

This monobasic acid (0.71 g) was methylated in dry *N* methanolic hydrochloric acid (18 ml) by heating under reflux for two hours. The solution was poured into water (20 ml) extracted with ether (5 x 10 ml), the combined extracts washed with saturated sodium bicarbonate (10 ml) and water (10 ml). After drying over magnesium sulfate and concentrating under reduced pressure 0.56 g of a slightly yellow oil remained, which was pure for 95% by g.c. analysis on 20% silicone grease. On purification by preparative g.c. using 20% carbowax as the column liquid phase, 0.262 g of product was obtained. B.p. 41-42°C (2.0 mm Hg) n_D^{29} 1.4289.

The 100 MHz p.m.r. spectrum showed a singlet at τ6.35, multiplets centered at τ7.6 and τ8.2, a doublet at τ8.85 and a triplet at τ8.96. The latter two overlapped such that a quartet appeared, but integrated for six protons in

agreement with the overall integration. The infrared spectrum confirmed the structure of the keto ester by an absorption band at 1715 cm^{-1} for the keto carbonyl and one at 1732 cm^{-1} for the ester carbonyl function.

f. Synthesis and characterization of methyl 2-methyl-dodecanoate (XVI).

Dimethyl methylmalonate (5.0 g, 0.035*M*) dissolved in dry ether (50 ml) was treated with finely divided metallic sodium (0.8 g, 0.035*M*) and stirred until all the sodium had reacted (3 hours). *n*-Iododecane (9.4 g, 0.036*M*, b.p. 107°C at 2.0 mm Hg) was added dropwise while stirring the solution. After the addition, the mixture was stirred and heated under reflux overnight. The slightly alkaline solution was acidified with glacial acetic acid and poured into water (25 ml). After separating the ether layer and washing the aqueous layer with ether (2 x 15 ml), the combined extracts were dried over magnesium sulfate. Upon removal of solvent, the residue was distilled yielding 4.2 g of product (43% of theor.); b.p. 136-137°C (2.0 mm Hg); n_D^{28} 1.4386. The 60 MHz p.m.r. spectrum showed a singlet at τ6.3 (6H), doublet at τ8.90 (3H), singlet at τ8.72 (18H) and poorly resolved triplet at τ9.12 (3H). The infrared spectrum showed a strong carbonyl peak at 1720 cm^{-1}. Elemental analysis: calcd.: C, 67.16; H, 10.48; found: C, 67.01; H, 10.71.

Dimethyl *n*-decylmethylmalonate (1.2 g, 42*mM*) was saponified by stirring and heating under reflux in *N* sodium hydroxide (10 ml) overnight. After cooling in ice water and acidification with concentrated hydrochloric acid, the solvent was evaporated. Decarboxylation was effected by heating the residue to 200°C in an oil bath until the evolution of carbon dioxide ceased (185°C). The monobasic acid was separated from the salts by triturating with ethyl acetate (5 x 2 ml), filtering and concentrated under reduced pressure. Yield 780 mg (88% of theor.). The monobasic acid (780 mg, 37*mM*) was heated under reflux overnight in dry *N* methanolic hydrochloric acid. Upon cooling, water (15 ml) was added and extracted with ether (4 x 10 ml). The ether extract was washed with sodium bicarbonate (10 ml), water (10 ml) and dried over magnesium sulfate. Concentrating under reduced pressure yielded 625 mg of a syrup (70% of theor.), b.p. 120-121°C (3.6 mm Hg), n_D^{25} 1.4291. Elemental analysis: calcd.: C, 73.63; H, 12.36; found: C, 73.52; H, 12.05. The spectral data of the final product are in complete agreement with the p.m.r., infrared and mass spectra reproduced in Figure XVI.

g. Synthesis and characterization of dimethyl 2,4-dimethyl-pent-2-en-1,5-dioate (XVII)

The intermediate dimethyl 2,4-dimethyl-3-hydroxyglutarate was prepared from the mixed esters of 2,4-dimethyl-3-hydroxy-

glutaric acid as synthesized in the Favorsky reaction (88) for the synthesis of dimethyl 3-acetoxy-2,4-dimethylglutarate (VIII). Dehydration could be effected by heating the hydroxy compound (5.0 g) with powdered potassium bisulfate (5.0 g) to 220°C for one hour. Distillation in vacuum yielded a fraction of 750 mg (115-120°C, 7 mm Hg). Although the infrared spectrum still showed some hydroxyl absorption at 3500 cm^{-1}, it also indicated an olefinic bond at 1655 cm^{-1}. The olefinic bond was confirmed by a 1-proton doublet at τ3.30 and a 1-proton singlet at τ8.08 in the 60 MHz p.m.r. spectrum.

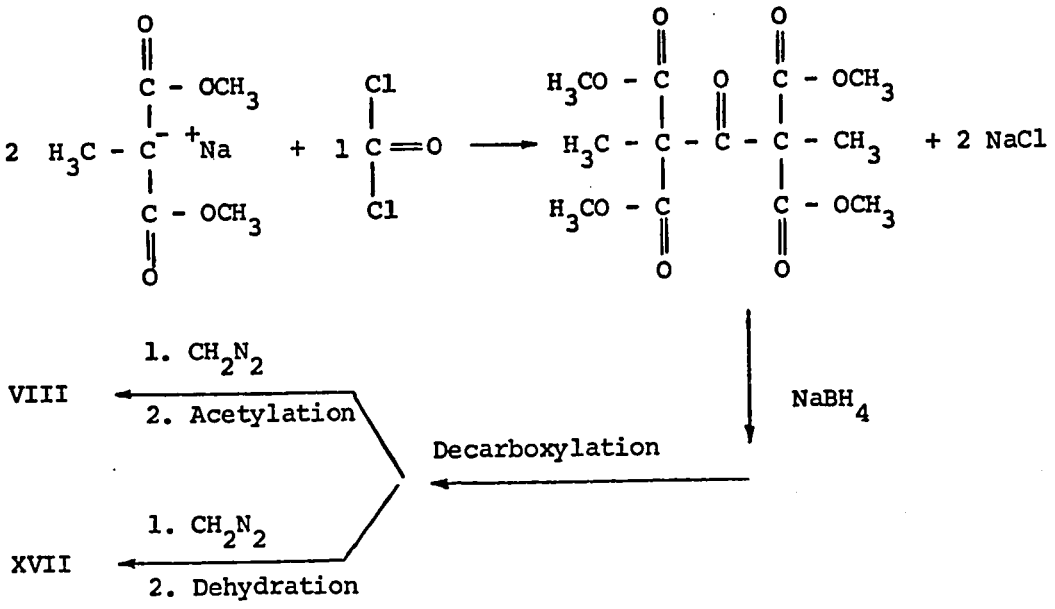
This olefinic compound (750 mg) was saponified in *N* sodium hydroxide (10 ml) by stirring and heating under reflux for two hours. Acidification with concentrated hydrochloric acid to pH 1 was followed by extraction with ethyl acetate (5 x 10 ml). The combined extracts were dried over magnesium sulfate, filtered, and concentrated on the rotary evaporator, whereupon it crystallized to give the olefinic diacid (650 mg). This crude material was triturated with Skelly B to remove any esters and the residue was recrystallized from ethyl acetate-Skelly B mixture. M.p. 144.5-145.2°C. Elemental analysis: calcd.: C, 53.16; H, 6.37; found: C, 53.39; H, 5.94. The 60 MHz p.m.r. spectrum showed olefinic proton absorption at τ3.25 (d),

olefinic methyl at τ8.20 (s) and a saturated methyl group at τ8.76 (d).

Methylation of the dicarboxylic acid (23.3 mg) with diazomethane according to Schlenk (139) furnished the corresponding dimethyl ester (26.3 mg, 96% of theor.). The 100 MHz p.m.r. spectrum (τ3.21, d, 1H; τ6.25, d, 6H; τ8.08, d, 3H; τ8.64, d, 3H) and the infrared spectrum, showing carbonyl absorption at 1737 and 1717 cm^{-1} as well as a weak olefinic bond at 1650 cm^{-1} were in agreement with the structure postulated for XVII. The p.m.r., mass and infrared spectra of XVII are reproduced in Figure XVIII.

h. Synthesis of Dimethyl 2,4-di-(methoxycarbonyl) 2,4-dimethyl-3-oxoglutarate (XVI)

Initially, the syntheses of fragments VIII and XVII were attempted as outlined in the following sequence.



The tetra-ester ketone was readily obtained in 20% yield. However, a clean reduction of the ketone with sodium borohydride could not be achieved, and this approach was abandoned. The use of phosgene for the preparation of symmetrical ketones represents a novel reaction method, which is described below.

Dimethyl methylmalonate (20 g, 0.14*M*), prepared according to standard procedure (150), was dissolved in dry ether (350 ml) in a 3-necked flask (500 ml) which was provided with an efficient stirrer and a condenser capped with a calcium chloride drying-tube. Finely divided metallic sodium (3.0 g, 0.13*M*) was added, and the solution stirred until all the sodium was dissolved (5 hours). Phosgene (6.5 g, 0.065*M*) was condensed in a glass tube (25 cm x 7 mm i.d.) which was cooled in a dry-ice/acetone bath. When the required amount was condensed, the tube was taken out of the dry-ice/acetone bath and put in an ice-water bath and attached with tygon tubing to a gas-inlet tube (4 mm i.d.) which extended to the bottom of the flask containing the sodio-derivative. By allowing the ice-water to warm up very slowly, the phosgene (b.p. 8°C) evaporated into the reaction mixture over a period of 2.5 hours while stirring was maintained. When the addition was completed, the stirring was continued for 1.5 hours at room temperature, then heated under reflux for an additional hour. After cooling to room temperature, water (125 ml) was added and the layers separated. The aqueous phase was

extracted with ether (4 x 50 ml) and the combined ether extracts washed with water. The ether solution dried over magnesium sulfate and removal of the solvent left an oil (24 g). Distillation of this oil gave unreacted dimethyl methylmalonate (15 g) and the expec ted ketone: dimethyl 2,4-di-(methoxycarbonyl)-2,4-dimethyl-3-oxoglutarate (20% of theor.), which was very viscous. B.p. 144-154°C (1.0 mm Hg). An analytical sample was obtained by redistillation, b.p. 143-144°C (0.6 mm Hg), n_D^{23} 1.4556. Elemental analysis: calcd.: C, 49.06; H, 5.66; found: C, 49.15; H, 5.42. The 60 MHz p.m.r. spectrum showed two singlets at τ6.24 and τ8.23 which integrated as 2:1. The infrared spectrum showed strong carbonyl absorption from 1760 cm^{-1} to 1710 cm^{-1} for the ester and ketone functions.

The tetra-ester ketone (3.2 g, 0.02*M*) was added slowly to a solution of sodium borohydride (0.9 g, 0.024*M*), and sodium bicarbonate (500 mg) in 95 methyl alcohol (50 ml). The mixture was stirred at room temperature for 5 hours, then acidified with cold 10% hydrochloric acid to pH 5. After filtering off the salts, the solution was concentrated to about 30 ml, extracted with chloroform (5 x 5 ml) and the combined extracts dried over magnesium sulfate. Removal of the solvent gave a colorless oil, which lacked a hydroxyl band in the infrared spectrum. When the reduction was repeated with a large excess of sodium borohydride and/or

raising the temperature to 50°C for periods up to 20 hours, little improvement in the reduction was noted as indicated by p.m.r. and infrared analyses.

G. Acid Treatment of Peliomycin

In these tests, small quantities of peliomycin were hydrolyzed in sealed tubes under various conditions of acid strength.

Test 1. Peliomycin (1 mg) was heated in 50% acetic acid (2 ml) at 90°C for 3 hours. The solvent was removed under reduced pressure. No by-products could be detected upon t.l.c. analysis. The chromatograms were developed in the following systems: *n*-BuOH:EtOH:H_2O = 5:1:4; Pyridine:*n*-BuOH:AcOH:H_2O = 4:6:1:3; and EtOAc:Pyridine:H_2O = 8:2:1, and charred with aqueous sulfuric acid and heated on a hot plate.

Test 2. Similar negative results were also obtained when two peliomycin samples (2 mg each) were heated with 5% hydrochloric acid in methyl alcohol (2.5 ml) at 100°C for 21 hours and 44 hours, respectively, employing the same developing systems and detection method as described under Test 1.

Test 3. Peliomycin (6 mg) was heated in 3*N* hydrochloric acid in methyl alcohol (4.5 ml) at 100°C for 11 hours, then neutralized with silver carbonate and filtered. Water (3 ml) was added to the filtrate, and the methyl alcohol removed under reduced pressure. The remainder was extracted with

ether (3 x 1 ml) and the water layer concentrated under freeze-drying conditions. T.l.c. analysis ($CHCl_3$: CH_3-CO-CH_3 = 95:5) of the ether extract exhibited a spot with the same R_f value as peliomycin. Paper chromatography of the water extract developed in nBuOH:EtOH:H_2O = 40:11:19 gave no indication of a sugar component upon dipping the chromatogram in alcoholic silver nitrate and spraying with alcoholic sodium hydroxide (128).

Test 4. Peliomycin (6 mg) was dissolved in glacial acetic acid (1.5 ml) and 3*N* hydrochloric acid added until cloudy (6 ml). The solution was heated for 14.5 hours at 100°C. After removal of the solvent under freeze-drying conditions, water (1 ml) was added and the solution again subjected to freeze-drying conditions. This test failed to give any sugar residues employing the paper chromatography technique described under Test 3. T.l.c. analysis (Test 3) indicated extensive decomposition of the peliomycin molecule.

H. Base Treatment of Peliomycin and Perhydropeliomycin

1. Limited Base Hydrolysis of Peliomycin

Peliomycin (2 mg) in 99% methyl alcohol (3 ml) containing sodium hydroxide (7.5 mg, 0.006*N*) was heated in a sealed tube from 85°-100°C for 1.5 hours. The hot mixture was quenched and acidified with concentrated hydrochloric acid (1 drop). After concentrating, the salt mixture was extracted with

carbon tetrachloride (3 x 2 ml) and the combined extracts dried over magnesium sulfate. Thin layer chromatograms developed in the same solvent systems as described in experiment G-test 1, indicated only one spot with somewhat different R_f value than peliomycin.

In another test, peliomycin (2 mg) in 99% methyl alcohol (3 ml) containing sodium hydroxide (30 mg, 0.025*N*) was heated in a sealed tube at 100°C for 12 hours. After quenching and acidifying the solution with concentrated hydrochloric acid (2 drops), the solvent was removed under reduced pressure, and the residue extracted with ether. T.l.c. analyses under the same conditions as described above gave the same results as the first test.

2. Products of Base Degradation of Peliomycin and Perhydropeliomycin

When peliomcyin (201 mg) was subjected to the base treatment as described in the saponification equivalent determination (experiment C-4), the neutralized solution obtained was extracted with chloroform (4 x 5 ml). Due to emulsification, the extraction was repeated (3 x 5 ml). The combined extracts were dried over sodium sulfate and distilled in a closed system to give 164 mg of product.

The aqueous layer was subjected to freeze-drying conditions and exchanged once with deuterium oxide (0.3 ml).

A 60 MHz p.m.r. spectrum of the exchanged material showed olefinic absorption at τ3.2 (s.), singlets at τ8 and τ8.2, and multiplets at τ6.5, τ7.7 and τ9.0. An infrared spectrum showed strong carboxylate absorption at 1560 cm^{-1} and 1415 cm^{-1}. The salt fraction was acidified with *N* sulfuric acid (3 ml), then extracted with chloroform (4 x 1 ml). The combined extracts were dried over sodium sulfate and distilled in a closed system. Yield 45 mg. T.l.c. analysis, with a plate buffered at pH 4 (experiment E-2-b) and developed with EtOAc:$CHCl_3$ = 8:1, indicated one major spot with R_f:0.7 and some minor trailing impurities. The infrared spectrum showed free carboxylic acid absorption at 2500-2700 cm^{-1}, and the solution was methylated with diazomethane (133). After removal of solvent in a closed system, the residual fraction was investigated by gas chromatography. A column packed with 10% Apiezon L indicated two peaks in a 1:1 ratio with retention times of 4 and 5 minutes. These fractions were isolated by semi-micro preparative gas chromatography. The temperature was maintained at 170°C with a flow rate of 150 ml/min of helium. The spectral data (p.m.r., mass and infrared; Figure XXVI) of one component is reproduced since they were nearly identical, and are treated in the discussion as two stereoisomers of one structure. Since the structure could not be determined,

no synthesis was attempted.

When the product obtained from the saponification equivalent determination of perhydropeliomycin was subjected to the same work-up as described for peliomycin, no fragments could be isolated.

I. Attempted Total Reduction of Peliomycin

Perhydropeliomycin (98 mg) was dissolved in dry dioxane (10 ml) and added over a period of 3 hours to a boiling mixture of lithium aluminum hydride (200 mg) in dry dioxane (10 ml). The boiling under reflux was continued for another 4 hours. Upon cooling, a saturated solution of aqueous sodium sulfate (2 ml) was added carefully from a syringe followed by more dry sodium sulfate (1 gm) (11). The resulting salts were filtered under reduced pressure through Celite and the solids washed well with dry dioxane. The filtrate was concentrated under reduced pressure and yielded 82 mg of white fluffy material. An infrared spectrum of this material lacked carbonyl absorption in the 1700-1740 cm^{-1} region. This polyol (82 mg) was dissolved in glacial acetic acid (2 ml) and heated under reflux for twenty hours in the presence of 47% hydriodic acid (10 ml) and red phosphorus (100 mg). Upon adding more red phosphorus (100 mg), the heating under reflux was continued for a total of 45 hours. After cooling the solution, water (10 ml) was added and

extracted with chloroform (8 x 5 ml). This extract was subsequently washed with water (10 ml), 0.1*N* sodium thiosulfate (2 x 10 ml), and water (10 ml). The remaining chloroform extract was dried over sodium sulfate and concentrated under reduced pressure to give a hydroxyl-free (infrared), but iodide (ultraviolet λ_{max} 268 mμ) containing compound (52 mg). This product, dissolved in dry dioxane (5 ml), was added dropwise to a boiling suspension of lithium aluminum hydride in dry dioxane (10 ml) and continued to boil under reflux for a total of 17 hours. This mixture was cooled, and work-up was the same as described above, yielding 35 mg of material. This product was reduced catalytically with 5% palladium on charcoal (100 mg) in hexane (5 ml) for 15 hours. After filtering off and washing the catalyst, the combined filtrate and washings were concentrated to give an oil (25 mg), which lacked hydroxyl and carbonyl absorption in the infrared spectrum, but was contaminated with some silicone grease (1000 - 1100 cm^{-1}). The oil was dissolved in hexane and filtered through Woelm alumina (neutral activity 1). The filtrate was concentrated and the resulting oil (8 mg) subjected to g.c. analysis using a column packed with 3% silicone rubber and temperatures from 125° to 275°C with a flow of 60 to 150 ml/min. but no response could be obtained. An ultraviolet investigation of the product indicated a slight absorption ($\varepsilon \sim 10$) at 228 mμ

and 270 mμ. The latter absorption was ascribed to an alkyl iodide. The alkyl iodide was subjected to hydrogenolysis (85) over a 5% palladium on charcoal (25 mg) and potassium hydroxide (25 mg) in methyl alcohol (12 ml) for 66 hours. The solution was neutralized with concentrated hydrochloric acid and filtered. The filtrate was concentrated, *in vacuo*, giving a colorless oil (4 mg). No absorption bands could be detected in the ultraviolet, but g.c. analysis was unsuccessful. The mass spectrum indicated peaks up to *m/e* 636.

BIBLIOGRAPHY

1. I.M. Rollo in "The Pharmaceutical Basis of Therapeutics," 3rd Ed., Editors L.S. Goodman and A. Gilman, The Macmillan Co., New York, 1965, p. 1171.
2. B. Williams and S. Epstein, "Medicine from Microbes," J. Messner, New York, 1965.
3. H.S. Goldberg and T.D. Luckey in "Antibiotics," Editor H.S. Goldberg, D. van Nostrand Co. Inc., New York, 1959, p. 2.
4. J. Tréfouël, T.J. Tréfouël, F.W. Nitti and D. Bovet, Comp. rend. soc. biol., 120, 756 (1935).
5. A. Fleming, Brit. J. Exptl. Pathol., 10, 226 (1929).
6. E. Chain, H.W. Florey, A.D. Gardner, N.G. Heatley, M.A. Jennings, J. Orr-Ewing and A.G. Saunders, Lancet, II, 226 (1940).
7. A. Schatz, E. Bugie and S.A. Waksman, Proc. Soc. Exp. Biol. Med., 55, 66 (1944).
8. H. Welch in "Antibiotic Therapy," Medical Encyclopedia Inc., Blakiston Co., New York, 1954, p. 1.
9. "Index of Antibiotics from Actinomycetes," Editor H. Umezawa, University of Tokyo Press, Tokyo, 1967.
10. D. Vazquez in "Antibiotics, Mechanism of Action," Vol. I, Editors D. Gottlieb and P.D. Shaw, Springer-Verlag New York Inc., 1967, p. 387.

11. W. Keller-Schierlein and G. Roncari, Helv. Chim. Acta, 45, 138 (1962); 47, 78 (1964).

12. C. Djerassi, O. Halpern, D.I. Williams and E.J. Eisenbraun, Tetrahedron, 4, 369 (1959).

13. H. Grisebach, "Biosynthetic Patterns in Microorganisms and Higher Plants," John Wiley and Sons, Inc., New York, 1967, Ch. 2.

14. R.B. Woodward, Angew. Chem., 69, 50 (1957).

15. Reference 10, p. 366.

16. P.W.K. Woo, H.W. Dion and Q.R. Bartz, J. Am. Chem. Soc., 86, 2724, 2726 (1964).

17. R.B. Woodward, L.S. Weiler and P.C. Dutta, J. Am. Chem. Soc., 87, 4662 (1965).

18. W.D. Celmer, J. Am. Chem. Soc., 87, 1797, 1799, 1801, (1965).

19. R. Paul and S. Tchelitcheff, Bull. soc. chim. France, 1965, 650.

20. W. Keller-Schierlein, Helv. Chim. Acta, 50, 731 (1967).

21. Reference 9, p. 495.

22. K.L. Rinehart, Jr., V.F. German, W.P. Tuchker and D. Gottlieb,Ann. Chem., 668, 77 (1963).

23. C. Djerassi and J.A. Zderic, J. Am. Chem. Soc., 78, 6390 (1956).

24. H. Brockmann and W. Henkel, Naturwissenschaften, 37, 138 (1950).

25. M. Berry, Quart. Rev. (London), 17, 343 (1963).

26. M.E. Kuehne and B. W. Benson, J. Am. Chem. Soc., 87, 4461 (1965).

27. C. Djerassi and O. Halpern, Tetrahedron, 3, 255 (1958).

28. S. Ōmura, H. Ogura and T. Hata, Tetrahedron Letters, 1967, 1267.

29. A.C. Cope, U. Axen, E.P. Burrows and J. Weinlich, J. Am. Chem. Soc., 88, 4228 (1966).

30. B.T. Golding, R.W. Rickards and M. Barber, Tetrahedron Letters, 1964, 2615.

31. G. Gaudiano, P. Bravo, A. Quilico, B.T. Golding and R.W. Rickards, Tetrahedron Letters, 1966, 3567, and references therein.

32. M.L. Dhar, V. Thaller and M.C. Whiting, Proc. Chem. Soc., 1960, 310; J. Chem. Soc., 1964, 842.

33. D.R. Harris, S.G. McGeachin and H.H. Mills, Tetrahedron Letters, 1965, 679.

34. S. Takahashi, M. Arai and E. Ohki, Chem. Pharm. Bull., 15, 1651 (1967).

35. G.A. Simm, Antimicrobial Agents and Chemotherapy, 1965, 958.

36. W. Hofheinz and H. Grisebach, Chem. Ber., 96, 2867 (1963).

37. R.B. Woodward, "Festschrift Arthur Stoll," Vol. II, Birkhauser, Basel, 1957, pp. 524-544.

38. K. Gerzon, E.H. Flynn, M.V. Sigal, Jr., P.F. Wiley, R. Monohan and U.C. Quarck, J. Am. Chem. Soc., 78, 6396 (1956).

39. W.D. Celmer, Antimicrobial Agents and Chemotherapy, 1965, 144.

40. Z. Vaněk and J. Majer in "Antibiotics, Biogenesis," Vol. II, Editors D. Gottlieb and D. Shaw, Springer-Verlag, New York, 1967, p. 154.

41. J. Cudlín, J. Majer, Z. Hošťálek and Z. Vaněk, Folia Microbiol., 11, 399 (1966).

42. W.D. Celmer in "Biogenesis of Antibiotic Substances," Editors Z. Vaněk and Z. Hošťálek, Academic Press, New York, 1965, p. 99.

43. T. Reichstein and E. Weiss in "Advances in Carbohydrate Chemistry," Vol. 17, Editor M.L. Wolfrom, Academic Press, New York, 1962, p. 99.

44. J.N. Bijvoet, A.F. Peerdeman and A.J. van Bommel in E.L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co. Inc., New York, 1962, p. 95.

45. D.E. Devoe, H.B. Renfroe and W.K. Hausmann, Antimicrobial Agents and Chemotherapy, 1963, 125.

46. V. Prelog, A.M. Gold, G. Talbot and A. Zomojski, Helv. Chim. Acta, 45, 5 (1962).

47. C.D. Celmer, J. Am. Chem. Soc., 88, 5028 (1966).

48. R.W. Rickards, R.M. Smith and J. Majer, Chem. Comm., 1968, 1049.

49. H. Muxfeldt, S. Shrader, P. Hansen and H. Brockmann, J. Am. Chem. Soc., 90, 4748 (1968).

50. O. Cedar and B. Hansson, Tetrahedron, 23, 3753 (1967).

51. F. Lynen, as quoted in reference 13.

52. W. Hofheinz and H. Grisebach, Z. Naturforsch., 20B, 43 (1965).

53. J.W. Corcoran in "Biogenesis of Antibiotic Substances," Editors Z. Vaněk and Z. Hošťálek, Academic Press, New York, 1965, p. 131.

54. A.J. Birch, C. Djerassi, J.D. Dutcher, J. Majer, D. Perlman, E. Pride, R.W. Rickards and P.J. Thompson, J. Chem. Soc., 1964, 5274.

55. H. Grisebach and C.A. Weber-Schilling, Z. Naturforsch., 23B, 655 (1968).

56. P.L. Tardrew and M.A. Nyman, U.S. Patent 3. 127, 315 (1964).

57. R. Hütter, W. Keller-Schierlein and H. Zähner, Arch. Mikrobiol., 39, 158 (1961).

58. F.E. Hahn in "Antibiotics, Mechanism of Action," Vol. I, Editors D. Gottlieb and D. Shaw, Springer-Verlag New York Inc., 1967, p. 378.

59. K. Poralla and H. Zähner, Arch. Mikrobiol., 61, 143 (1968).

60. W.D. Celmer, J.Am. Chem. Soc., 87, 1801 (1965).

61. K.E. Price, A. Schlein, W.T. Bradner and J. Lein, Antimicrobial Agents and Chemotherapy, 1963, 95.

62. H. Schmitz, S.B. Deak, K.E. Crook, Jr. and I.R. Hooper, Ibid., 1963, 89.

63. M.P. Kunstmann, L.A. Mitscher and E.L. Patterson, Ibid., 1964, 87.

64. S. Masamune, J.M. Sehgal, E.E. van Tamelen, F.M. Strong and W.H. Peterson, J. Am. Chem. Soc., 80, 6092 (1958).

65. G.A. Foster, Jr. Diss. Abstr. B, 28, 786 (1967).

66. A.G. Sharkey, R.A. Friedel and S.H. Langer, Anal. Chem., 29, 770 (1957).

67. G. Gaudiano, P. Bravo, A. Quilico, B.T. Golding and R.W. Rickards, Gazz. Chim. Ital., 96, 1470 (1966).

68. "Fluorolube" supplied by I.C.I. Ltd., England.

69. Dr. B.T. Golding, E.T.H.S., Zürich, Switzerland, private communication.

70. C.C. Sweeley, R. Bentley, M. Makita and W.W. Wells, J. Am. Chem. Soc., 85, 2497 (1963).

71. S. Friedman, M.L. Kaufman, Anal. Chem., 38, 144 (1966).

72. H. Budzikiewicz, C. Djerassi and D.H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, Inc., San Francisco, 1967, p. 180.

73. Reference 72, Ch. 3-3.

74. R.U. Lemieux and E. von Rudloff, Can. J. Chem., 33, 1701, 1710 (1955)

75. E. von Rudloff, Can. J. Chem., 34, 1413 (1956).

76. R.C. Fuson and C.W. Tullock, J. Am. Chem. Soc., 56, 1638 (1934).

77. Varian NMR Spectra Catalog, Varian Associates, Palo Alto, 1960, Spectrum Number 65.

78. Reference 73, p. 230.

79. E. von Rudloff, Can. J. Chem., 43, 1784 (1965).

80. R.U. Lemieux, Ibid., 31, 396 (1953).

81. R.U. Lemieux and R. Charanduk, Ibid., 29, 759 (1951).

82. E. von Rudloff, Tetrahedron Letters, 1966, 993.

83. E. Klein and W. Rojahn, Tetrahedron, 21, 2353 (1965).

84. R. Pappo, D.S. Allen, Jr., R.U. Lemieux and W.S. Johnson, J. Org. Chem., 21, 478 (1956).

85. R.L. Augustine, "Catalytic Hydrogenation," Marcel Dekker, Inc., New York, 1965 Ch. 6.

86. A. Bowers, T.G. Halsall, E.R.H. Jones and A.J. Lenin, J. Chem. Soc., 1953, 2548.

87. R. Ryhage and E. Stenhagen in "Mass Spectrometry of Organic Ions," Editor F.W. McLafferty, Academic Press, New York, 1963, p. 448.

88. S. Favorsky, Ber., 28, 3262 (1895).

89. R.U. Lemieux, R.K. Kullnig and R.Y. Moir, J. Am. Chem. Soc., 80, 2237 (1958).

90. J.S. Martin, J. - I, Hayami and R.U. Lemieux, Can. J. Chem., *in press*.

91. S.J. Angyal and D.J. McHugh, J. Chem. Soc., 1957, 1423.

92. J.L. Frahn and J.A. Mills, Austr. J. Chem., 12, 65 (1959).

93. M.F. Hawthorne, W.D. Emmons and K.S. McCallum, J. Am. Chem. Soc., 77, 2287 (1955).

94. Reference 77, Spectrum Number 142.

95. Reference 87, p. 403.

96. L.F. Fieser and M. Fieser, "Advanced Organic Chemistry," Reinhold Publishing Corporation, New York, 1961, p. 428.

97. Reference 77, Spectra Numbers 278, 891.

98. R. Ryhage and E. Stenhagen, Arkiv Kemi, 15, 291 (1960).

99. K. Naganishi, "Infrared Absorption Spectroscopy," Holden-Day Inc., San Francisco, 1962, Ch. 2.

100. Reference 73, p.

101. Reference 73, p. 138.

102. C. Djerassi and L.E. Geller, J. Am. Chem. Soc., 81, 2789 (1959).

103. J.R. Dyer, "Applications of Absorption Spectroscopy of Organic Compounds," Prentice-Hall Inc., Englewood Cliffs, N.J., 1965, Ch. 4.

104. A. Segre and J.I. Musher, J. Am. Chem. Soc., 89, 706 (1967).

105. J.B. Patrick, R. Williams, C.F. Wolf and J.S. Webb, J. Am. Chem. Soc., 6688 (1958).

106. Reference 103, Ch. 1.

107. J.W. Emsley, J. Feeney and C.H. Sutcliffe, "High Resolution NMR," Vol. 2, Pergamon Press, New York, 1966, p. 738.

108. R.R. Fraser and D.E. McGreer, Can. J. Chem., 39, 505 (1961).

109. L.M. Jackman and R.H. Wiley, J. Chem. Soc., 1960, 2886.

110. G. Snatzke, Angew. Chemie, Int. Ed., 7, 14 (1968).

111. P. Crabbé, "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," Holden-Day, Inc., San Francisco, 1965, p. 133.

112. C. Djerassi, "Optical Rotatory Dispersion: Applications to Organic Chemistry," McGraw-Hill, New York, 1960, p. 107.

113. Reference 111, Ch. 9.

114. C. Djerassi, Proc. Chem. Soc., 1964, 314.

115. U. Weisz and H. Ziffer, J. Org. Chem., 28, 1248 (1963).

116. B. Briat and C. Djerassi, Nature 217, 918 (1968).

117. S. Bodforss, Ber., 49, 2801 (1916).

118. Reference 77, Spectra Numbers 32, 193.

119. S.W. Chaikin and W.G. Brown, J. Am. Chem. Soc., 71, 122 (1949).

120. H. Heymann and L.F. Fieser, Ibid., 73, 5252 (1951).

121. M.L. Wolfrom and K. Anno, J. Am. Chem. Soc., 74, 5583 (1952).

122. K. Ishizumi, K. Koga, S-I. Yamada, Chem. Pharm. Bull., 16, 492 (1968).

123. Reference 77, Spectra Numbers 210, 337.

124. Reference 111, p. 244.

125. Reference 103, p. 125.

126. A.I. Scott, "Interpretation of Ultraviolet Spectra of Natural Products", The Macmillan Company, New York, 1964.

127. K. Hirayama, "Handbook of Ultraviolet and Visible Absorption Spectra", Plenum Press Data Division, New York, 1967.

128. M.M. Ponpipom, Ph.D. Dissertation, University of Alberta, Edmonton, 1968.

129. Reference 73, p. 183.

130. F. Johnson and S.K. Malhotra, J. Am. Chem. Soc., 87, 5492, 5493 (1965).

131. I.K. O'Neill, Ph.D. Dissertation, University of Alberta, Edmonton, 1966.

132. Reference 99, Ch. 1.

133. O. Ceder, Acta Chem. Scand., 18, 126 (1964).

134. L.F. Johnson, Varian Associates, Technical Bull. Number 87-100-082 (1962).

135. A.I. Vogel, "Practical Organic Chemistry", 3rd Ed., Longmans, Green and Co. Ltd., London, 1965.

136. R.A. Day, Jr., and A.L. Underwood, "Quantitative Analysis", Laboratory Manual, Prentice-Hall Inc., Englewood Cliffs, N.J., 1960.

137. Reference 135, p. 884.

138. W. Keller Schierlein and G. Roncari, Helv. Chim Acta, 47, 78 (1964).

139. H. Schlenk and J.L. Gellerman, Anal. Chem., 32, 1412 (1960).

140. "Dictionary of Organic Compounds", Vol. 3, 4th Ed., Editor G. Harris, Eyre and Spottishwoode Publishers Ltd., London, 1965, p. 1537.

141. R.L. Shriner, R.C. Fuson and D.Y. Curtin, "The Systematic Identification of Organic Compounds", 5th Ed., John Wiley and Sons, Inc., New York, 1965, p. 126.

142. R. Kuhn, C. Grundmann, Ber., 69, 226 (1936).

143. C.B. Brown, Org. Synth., Coll. Vol. III, 615 (1965).

144. G.H. Jeffery and A.I. Vogel, J. Chem. Soc., 1948, 672.

145. M.L. Wolfrom, D.L. Patin and R.M. de Lederkremer, Chem. Ind. (London), 1964, 1065.

146. M. Vilkas, Hui-I-Jan, C. Boussac and M.-C. Bonnard, Tetrahedron Letters, 1966, 1441.

147. R.B. Moffat, Org. Synth., 32, 41 (1952).

148. G. Stork, J. Am. Chem. Soc., 85, 207 (1963).

149. E.M. McMahon, J.N. Roper, Jr., W.P. Utermohlen, Jr., R.H. Hasek, R.C. Harris and J.H. Brant, J. Am. Chem. Soc., 70, 2971 (1948).

150. E.E. Blaise and M. Maire, Bull. soc. chim. France, 3, 413, 421 (1908).

151. Reference 73, p. 55.

152. Dr. S. Masamune, Private Communication.