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The Structure Of Primycin

Rup Chand Jain

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THE STRUCTURE OF PRIMYCIN

by

Rup Chand Jain

Department of Chemistry

Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Canada
July, 1970
ABSTRACT

Primycin, a metabolite of Streptomyces primyi, has been found to be a non-polyene macrolide, consisting of a mixture of closely related but so far inseparable constituents. It is a monoalkyl-substituted guanidine sulphate and these two functions are responsible for all the sulphur and nitrogen contained in the molecule.

Alkaline hydrolysis of primycin gives as major product an amino acid called Compound F, also found to be a mixture of closely related compounds. The acetylation and ozonolysis studies of Compound F are described in the first chapter, and a partial structure has been proposed for the major constituent of this substance (47b).

Methylation of primycin with methyl iodide and silver oxide gives a neutral compound called $M_1$ (46a) and two basic compounds called $M_2$ (46b) and $M_3$. The ozonolysis studies of Compounds $M_1$ and $M_2$ are described in the second chapter and structural proposals have been made for the major constituent of each of these compounds. Compound $M_1$ has been shown to differ from Compound $M_2$ in having a trimethylated urea moiety in the place of the trimethylated guanidine grouping. Compound $M_3$ has not been extensively investigated.

Finally, based on these studies, a structural proposal has been made for the major constituent of primycin (46c). It has a molecular formula of $(C_{55}H_{103}N_{17}O_{17})_2H_2SO_4$ and consists of a 35 membered
carbocyclic lactone ring having 13 hydroxyl groups, 6 C-methyls, 2 carbon-carbon double bonds, and a mono alkyl substituted guanidine sulphate moiety. It also possesses a D(-)-furanosyl arabinose unit attached to it through a glycoside linkage having the α-anomeric configuration.
ACKNOWLEDGMENTS

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I feel greatly indebted to my wife Kamlesh and my daughter Kapila whose encouragement and patience allowed me to carry out this work.
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Macrolide Antibiotics

General Introduction:

One of the most fascinating classes of antibiotics is the "macrolides", so named by Woodward (1). Primycin, to be described in this thesis, can now be classified in this category of antibiotics. These substances are characterised by a many-membered, highly-substituted lactone ring, which contains a number of double bonds, often conjugated. The macrocyclic ring does not contain nitrogen atoms, but often carries one or more sugar residues which can be amino sugars, ordinary sugars or both, connected to the lactone ring through a glycoside linkage.

Origin, Biological Activity and Classification

The macrolide antibiotics represent members of a group of structurally related compounds, produced by the actinomycetes (streptomyces) organisms which have yielded nearly one half of the antibiotic principles discovered to date. Besides the macrolides, the other antibiotics produced by these organisms include among many others, streptomycin (2), the tetracyclines (3), chloroamphenicol (4), and the actinomycins (5). As a rule, the macrolide antibiotics are active against gram-positive micro-organisms, although a few also possess antiprotozoal properties (6). However, due to toxicity only about 5 per cent of the antibiotics discovered so far can be used clinically. Among the other macrolide antibiotics, erythromycin (7), spiramycin (8) and oleandomycin (9) have been used clinically as well as supplementarily in animal feeding.

Since the discovery of the first macrolide antibiotic, pikromycin in 1950, a large number of macrolide antibiotics or suspected macrolides
have been discovered (10). In fact, the literature has grown so enormous that a sub-classification of these compounds has been warranted (10, 11) which divides them into polyene and non-polyene macrolides. As a rule the non-polyene macrolides are antibacterial and the polyenes are antifungal agents. Many of the Streptomyces species which produce macrolide antibiotics, produce two or more of these compounds which are usually distinguished for purposes of nomenclature by the addition of letters or figures to the name of the original complex antibiotics, e.g. erythromycin A, B and C.

Structure Determination

Because of the diversity of functionalities and molecular complexity these antibiotics offer a challenge to the structural organic chemist. Among the non-polyene macrolides, methymycin \( \sim \) was the first whose structure was determined (12). Since then numerous members of this series have had their structures elucidated, characteristic examples being magnamycin \( \sim \) (13), oleandomycin (14), and pikromycin \( \sim \) (15).

In the past ten years the polyene macrolides have been among the most studied antibiotics. The following general methods in conjunction with mass spectrometry have been used to determine their carbon skeletons. The first approach involves conversion of the aglycone moiety into the corresponding saturated hydrocarbon by successive treatment with lithium aluminum hydride, hydroiodic acid-red phosphorous and lithium aluminum hydride reduction of the perhydro aglycone. This method was initially developed by Cope and has now been successfully
applied to determine the carbon skeleton of a number of macrolides, including fungichromin (16), pimaricin (17), (the structure of which has been recently revised [18]), filipin (19), lucensomycin (20), rimocidin (21, 22), and flavofungin (23). The second approach which involves high pressure hydrogenation of the aglycone, was used initially by Ceder for pimaricin (17), then by Cope (22, 24), and more recently by Djerassi (25) for the structure determination of nystatin. The structure of this latter compound, which represents the largest ring size polyene macrolide, has recently been revised (26) to structure 4.

The determination of the exact molecular composition of such large molecules is a problem in itself. The elemental analysis and conventional methods for determination of molecular wt., e.g., titration equivalents are not sufficiently accurate. This is partly because these compounds are usually highly solvated and occlude impurities. In fact, a large number of macrolide antibiotics have had their molecular formula assigned only after their complete structural determination by the methods of chemical degradation. Magnamycin (13) is one such example and primycin has now been found to belong to this class. However, with the advent of high resolution mass spectrometry, determination of the molecular wts. and molecular formulas of relatively small macrolide antibiotics has become possible. More often, the compounds used for such purposes are the peracetyl or pertrimethylsilyl ether derivatives. The pertrimethylsilyl ether derivatives of pimaricin (18), flavofungin (23), nystatin (26), lagosin, filipin and lucensomycin (27, 20) have
been successfully used to determine their molecular wts. and in some
cases the correct molecular compositions. Mass spectrometry has also
been invaluable in the structure determination of such complex classes
of compounds. In contrast to comparable fields in recent years, x-ray
crystallography has not been used for the structure determination of
such compounds. This is primarily because it is very hard to obtain
the macrolides as pure crystals.

Stereocchemistry

The stereocchemistry of the macrolide aglycones is a point of
current interest. In general, each of the known macrolide structures
contains several asymmetric centres within the aglycone. As a rule all
of these compounds possess an asymmetric centre $\alpha$ to the ring oxygen
atom and with some exceptions, e.g. magnamycin and pimaricin, another
in the $\beta$-position. Although the absolute configuration has been
established at a few centres of a large number of macrolides including
magnamycin (13), neomethymycin (28), erythromycin (28) and fungichromin
(16), the complete stereocchemistry has been established for only
oleandomycin (29) and methymycin $\downarrow$ (30). However, the stereocchemistry
of the sugar components of the macrolides has now been completely
established by the usual processes of chemical degradation to compounds
of known stereocchemistry and synthesis and through the physical methods
of n.m.r. and x-ray crystallography (10). In general the sugar com-
ponents are in the pyranose form and all are 6-deoxy sugars.
Biogenesis

The study of biogenesis of the macrolides (31, 32, 10) has not only been of interest from a mechanistic point of view of the buildup of the rings, but also has illustrated the relation these molecules bear with other natural products, such as the tetracyclines. Most of the known macrolide structures resemble one another as polysubstituted linear acids closed into large ring lactones. An examination of their oxygenation pattern and their co-occurrence in nature with other secondary metabolites such as phenolics, tetracyclines and numerous anthraquinone derivatives (33) has led to two postulates, primarily due to Birch (34, 35). These are respectively, the build-up of the macrolide ring by the linear condensation of acetic acid units and methylation of the poly-β-ketomethylene skeleton via methionine, choline or an equivalent one-carbon atom donor system and secondly, the incorporation of propionic acid units during the formation of the carbon skeleton. A consideration of the biogenesis of macrolides thus involves the study of the acetogenins, especially the fatty acids.

The primary building block of acetogenins is a hypothetical linear poly-β-ketomethylene chain. The first step in the construction of such a chain is the formation, from acetic acid, of acetyl co-enzyme A. The latter is then carboxylated with carbon dioxide through the agencies of biotin, adenosine triphosphate (ATP) and magnesium ion (36) giving the malonyl co-enzyme A. The malonyl residue is then transferred to a new enzyme system which after Claisen condensation with an acetyl co-enzyme A unit and subsequent decarboxylation leads to acetoacetyl co-enzyme A.
This compound may then react with another malonyl unit in the same way to provide further linear extension of the chain to give finally a poly-\(\beta\)-ketomethylene chain. The reactions involved are shown in fig. 1.

This process has been studied in detail only for the biosynthesis of saturated fatty acids, in which case reduction of the \(\beta\)-ketone precedes further chain extension with malonyl units, so that the actual existence, in nature, of a poly-\(\beta\)-ketomethylene chain remains an open question. This chain may undergo C or O-substitution by methyl, methylene, isopentenyl, hydroxyl, or sugars. Besides this, it may cyclize in aldol-type condensation by virtue of its enolic reactivity to give a variety of products whose details can be obtained elsewhere (33).

Although the enzyme system requires acetyl co-enzyme A to make the malonic acid unit, other small saturated fatty acids can also serve as chain initiators, being extended by malonyl units. Thus propionyl, butyryl, hexanoyl, isobutyryl and isovaleryl co-enzyme A initiators are also known to be incorporated in the polyketomethylene chain (37), though the latter four with more difficulty.

The working hypotheses for the biosynthesis of macrolides as for other acetogenins have been tested by tracer studies. In the case of methymycin, the carbon skeleton has been demonstrated (38) to be built-up of five propionic acid units and one acetic acid unit. Incorporation of sodium (carboxy-C\(^{14}\)) propionate in the fermentation culture led to the labelling of the carbon atoms shown in structure 1. Similarly, erythronolide (the aglycone of erythromycin) has been shown to be derived entirely from seven propionate units (39).
Although a sizeable amount of work has been done to unravel the mechanism of the build-up of the complete carbon skeletons of macrolide antibiotics, the problem is still unsolved for many cases. For example, in magnamycin the mode of formation of the aldehyde function, is still open to speculation.

Though the primycin antibiotic is a macrolide, it is significantly different from the known antibiotics which have had their structures established so far. This will become overwhelmingly obvious when the complete structure of primycin is written.
Primycin

Introduction

Primycin, the antibiotic agent of an actinomycetes strain known as Streptomyces primycinii originating from Galeria melonela was first isolated in 1954 by Szilagyi et al. (40). It was obtained as a white microcrystalline powder m.p. 192-195° (dec.) and appeared homogeneous by paper chromatography, partition column chromatography, counter current (Craig) distribution and fractional crystallization (41). In vitro, primycin was found to exhibit a bacteriostatic effect on Gram-positive organisms, both pathogenic and non-pathogenic, as well as on mycobacteria (42, 43). However, since in animal experiments it appeared to be toxic, only superficial infections of man have been treated with it, but with good results.

The antibiotic forms complexes with poly-deoxyribonucleotides and polyribonucleotides (44), a property also exhibited by streptomycin. It did not reduce Fehling's solution or react with aldehyde reagents, but exhibited a positive Sakaguchi* reaction (45, 46, 47). Based on elemental analysis, Szilagyi proposed the empirical formula C₁₈H₁₇N₇O₇ for the antibiotic, later revised to C₁₈H₁₇N₈ (48). The infrared spectrum of primycin (49, 50, 51) was not very informative except that it showed an intense absorption at ca 3300 cm⁻¹ due to strongly hydrogen bonded hydroxyls. A strong band at 1675 cm⁻¹ was attributed in part to the guanidino function (52), although the presence of a carbonyl function

* A specific test for monosubstituted guanidines.
could not be ruled out from the spectrum.

In the ultraviolet spectrum, primycin exhibited only end absorption \( \varepsilon_{205} = 14,000, \ \varepsilon_{200} = 22,000 \), for \( M = 1500 \), which was initially attributed entirely to the guanidino function (53). However, recently by comparing the extinction coefficient at 200 m\( \mu \) of primycin with that of cyclohexyl guanidine \( \varepsilon_{200} = 5,000 \). Aberhart (49) clearly demonstrated the presence of carbon-carbon double bonds in primycin. He also found that primycin readily consumed two moles of bromine for an equivalent wt. of about 750 for the antibiotic. Catalytic hydrogenation of primycin, however, could not be attempted because of its lack of solubility in a suitable hydrogenation solvent (49).

The work described in the following introduction is that reported by Aberhart (49) unless otherwise indicated.

The n.m.r. spectrum of primycin in dimethylsulfoxide (insoluble in most other solvents) was found to be devoid of structural features except that it showed the presence of a number of C-methyl \( \delta : 1.22 \) ppm protons, a number of methylene and methine protons \( \delta : 1.63, 1.92 \) ppm and the protons due to the guanidino function \( \delta : 7.95, 8.62 \) ppm.

Zeisel determination on primycin showed the complete absence of methoxyl groups. Sodium fusion of primycin showed the presence of nitrogen and sulphur and the absence of halogens. Primycin formed a precipitate with barium chloride giving barium sulfate, characterised by its infrared spectrum. This showed that the sulfur was present as a sulfate anion. Quantitative barium perchlorate titration of the sulfate, assuming one sulfate anion per mole of primycin, gave a molecular
wt. of 2285 for the antibiotic.

Potentiometric titration of primycin as a free base, obtained by percolating primycin in a methanol solution through a column of Dowex 1 - X 2, 50-100 mesh, OH form; gave an equivalent wt. of 1320 (average of two determinations).

Vigorous permanganate oxidation of primycin gave guanidine. This evidence in conjunction with the Sakaguchi reaction and the basicity of primycin (pKa, 11.2) was taken to indicate the presence of a mono-substituted guanidine in the antibiotic. Alkaline fusion of primycin gave n-caproic acid as the only major volatile acid.

Primycin formed a microcrystalline picrate (m.p. 166-168°C), a non-crystalline hydrobromide and a microcrystalline perchlorate (no definite m.p. because of decomposition). However, a definite empirical formula of primycin could not be derived from the elemental analysis of these compounds.

Szilagyi in 1963 (48) found that under acidic conditions, primycin gave a biologically inactive, but Sakaguchi positive aglycone and a sugar considered to be arabinose, isolated only as the p-nitrophenyl-hydrazone. More recently Aberhart (49) repeated these studies and identified the sugar as D(-)-arabinose. The aglycone part called nor-primycin was isolated as its amorphous hydrochloride which had molecular formula C_{55}H_{103}N_3O_{14}Cl from its analysis. This molecular composition of nor-primycin hydrochloride must be considered inexact since from its alkaline hydrolysis Aberhart isolated methyl β-D(-)-arabinopyranoside which must have been present as an admixture in the aglycone.
From the alkaline hydrolysis, the aglycone also gave n-caproic acid as obtained from primycin. The aglycone had 5.8% C-methyls (mol. wt. 1000) from Kuhn-Roth determination. It formed a microcrystalline perchlorate (m.p. 154-155°). However, it did not give a crystalline picrate.

The infrared and the n.m.r. spectra of norprimycin salts were practically identical with those of the primycin salts, giving no new information. However, in the ultraviolet spectrum the norprimycin hydrochloride gave besides strong end absorption as found in primycin, weaker absorptions at longer wavelengths which have not been explained. Thus it was concluded that more than simple hydrolysis of a glycoside linkage had taken place during the acid hydrolysis of primycin.

Norprimycin hydrochloride readily consumed two moles (for M = 1000) of hydrogen over platinum catalyst, and the tetrahydro derivative gave a u.v. spectrum typical of a mono alkyl guanidine hydrochloride. Thus, norprimycin was shown to contain two carbon-carbon double bonds.

In contrast to primycin, norprimycin hydrochloride reacted with periodate reagent and had an uptake of ~ 1.4 moles of the periodate after 48 hours and ~ 1.9 moles (for M = 1000) after 120 hours. The uptake was found to be strongly dependent on the pH of the medium. Using norprimycin hydroxide the uptake was ~ 2 moles in 24 hours and ~ 4 moles in a week. Two tentative conclusions were drawn from these results. Firstly, acid hydrolysis of primycin liberated a hydroxyl function adjacent to at least one other hydroxyl group or alternatively primycin possessed an epoxide linkage which produced a vicinal glycol unit under acidic conditions. Secondly, the dependence on pH indicated that a
second site of attack of periodate was being liberated in a base cata-
lysed reaction, e.g. lactone ring opening in 5 to 6. Such behaviour
had been observed for fungichromin 7 (16). However, the quantita-
tive periodate results should only be considered tentative because of
the contamination of the glycone with methyl β-D(-)-arabinopyranoside.

Acetylation of norprimycin hydrochloride with acetic anhydride and
pyridine at room temperature gave two products called acetate 1 and
acetate 2. By u.v. spectra these were shown to differ in having a
diacetyl and monoacetyl guanidine function respectively. This was also
shown by converting acetate 2 into acetate 1 by merely refluxing
acetate 2 in a mixture of acetic anhydride and acetic acid. In the
n.m.r. spectra these acetates did not give new structural information
except to show the presence of vinylic protons (δ: 5.3 ppm) and 7-9
acetyl groups (δ: 2.02 ppm) assuming 6 C-methyls.

An attempt to strip norprimycin of all the functional groups and
obtain a saturated hydrocarbon containing all the carbon atoms of the
main skeleton by an approach used to determine the carbon skeleton of
the polyene macrolides (16), gave instead of mainly one saturated hydro-
carbon, a spectrum of products without any major component. Tetra-
hydonorprimycin gave approximately the same results.

To summarise the work of Aberhart, the following functional groups
(fig. 2) in primycin were proposed by him. This study of the primycin
functional groups was invaluable as a guide to further work to be
described in this thesis.
as SO₄

salt

— OH ~ 5–8, not

including arabinose

— D(−) arabinose as glycoside

— two C=C

— ca. 6

in norprimycin

possible in norprimycin

Fig 2 Primycin: Functional Groups
Structure of Primycin

Results and Discussion

The foregoing discussion represents the complete knowledge of the chemistry of primycin when the work described here was begun. Though the nature of some functional groups in the molecule had been established by Aberhart, not enough definitive results had been obtained to allow a logical proposal for the arrangement of functional groups in this molecule.

Some of the results described below were obtained originally by Dr. T. Fehr, who was working on the primycin problem concurrently with myself, and are included so that the solution of the problem could be presented in a logical fashion. Dr. Fehr's work is identified as such where appropriate.

In the work described in this thesis, primycin has been shown to be a mixture of a number of compounds. Two approaches, namely, the alkaline hydrolysis studies of primycin described in the first chapter and the methylation studies of primycin described in the second chapter have been taken to propose a structure for the major component of primycin.
Chapter I

Alkaline Hydrolysis Studies of Primycin

Dr. Fehr found that hydrolysis of primycin with 5N potassium hydroxide under reflux for 4 hours afforded besides a large number of other products, a major product now called Compound F isolated in 42% yield by silica gel chromatography. It was an amorphous glass soluble in most of the common hydroxylic solvents such as water and alcohols and insoluble in non-hydroxylic solvents such as benzene, ether, chloroform and dioxane. It gave a negative Sakaguchi reaction, showing that a guanidine function was not present in the molecule. This was further substantiated by its infrared spectrum in which the strong band at 1675cm\(^{-1}\) due to the guanidine moiety present in that of primycin was completely absent. A strong band at 3300cm\(^{-1}\) was attributed to strongly hydrogen bonded hydroxyls. There was also a strong absorption band at 1560cm\(^{-1}\) and a weak band at 1650cm\(^{-1}\). The latter was assigned to carbon-carbon double bonds. Compound F had a molecular weight of 1100 by osmometry (methanol solvent).

The n.m.r. (A-60) spectrum of Compound F like those of primycin and norprimycin was not very informative. F consumed two moles of hydrogen over a platinum catalyst in 26 hours for a molecular weight of 1010. However, the hydrogen uptake continued after 26 hours, though at
a very slow rate, and a clear cut end-point could not be achieved.

Acetylation of Compound F with acetic anhydride and pyridine at room temperature gave a colorless sticky glass now called F-polyacetate. It analysed correctly for C\textsubscript{84}H\textsubscript{133}NO\textsubscript{33} (Mol. wt. 1683) and gave a molecular weight of 1700 by osmometry.

The infrared spectrum showed a weak absorption band at 3400cm\textsuperscript{-1} due to an amine or hydroxyl function. A strong band at 1730cm\textsuperscript{-1} and a broad and strong band centered at 1225cm\textsuperscript{-1} were attributed to acetoxyl groups (54). A strong band at 1660cm\textsuperscript{-1} was assigned to an amide carbonyl group.

These results of Dr. Fehr could now be extended. The n.m.r. (A-60) chemical shifts and possible assignments of the various resonance signals of F-polyacetate are given in Table I. The integration was based on the assumption that there were 15 acetyl methyls in the molecule, since a quantitative estimation of the number of acetyl groups in F-polyacetate gave a value of ca. 15 (for M = 1700) in which one might be an N-acetyl.

F-polyacetate could be directly titrated with alkali (0.4N) giving an equivalent wt. of 1605, showing that it contained a free carboxylic group. This observation was supported by the fact that it reacted with diazomethane to give a less polar product, a colorless syrup now called F-polyacetyl methyl ester which analysed correctly for C\textsubscript{85}H\textsubscript{135}NO\textsubscript{33} (Mol. wt. 1697).

In the infrared spectrum it showed the presence of a secondary amide (strong band at 1665cm\textsuperscript{-1} and weak bands at 3450, 1520cm\textsuperscript{-1}) and
### Table 1

**N.m.r. Chemical Shifts of F-Polyacetate**

<table>
<thead>
<tr>
<th>$\delta$ (ppm from TMS)</th>
<th>No # protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.88</td>
<td>ca. 12</td>
<td>(An apparent broad doublet $J \sim 6.5$ cps) 4, C-methyls</td>
</tr>
<tr>
<td>1.15 - 1.85</td>
<td>-</td>
<td>(multiplet) methylene and methine protons.</td>
</tr>
<tr>
<td>1.98, 2.0, 2.07</td>
<td>ca. 45</td>
<td>(three singlets) 15, O-acetyl methyls including the N-acetyl methyl.</td>
</tr>
<tr>
<td>4.30</td>
<td>2-3</td>
<td>(multiplet) -</td>
</tr>
<tr>
<td>4.55 - 5.2</td>
<td>ca. 16</td>
<td>(multiplet) partly due to $\gamma$CH-OAc and vinylic protons.</td>
</tr>
</tbody>
</table>
acetoxyl functions (broad and strong bands at 1730, 1250 cm\(^{-1}\)) in the molecule. However, the presence of a free hydroxyl group could not be ruled out from the spectrum. The n.m.r. (A-60) spectrum was quite similar to that of F-polyacetate except that a sharp three proton singlet at 3.70 ppm and an apparent one proton triplet at 6.30 ppm were present in the spectrum of the methyl ester. These were assigned respectively to methyl of the carbomethoxy group and to the N-H proton of the secondary amide probably having a methylene group adjacent to it. It may be noted that a triplet at 6.3 ppm in the n.m.r. spectrum of F-polyacetate was not observed. This might be due to a fast exchange between the N-H and the carboxylic acid proton which caused broadening of the bands and thus the signal could not be seen. In the ultraviolet spectrum the F-polyacetyl methyl ester showed only a strong end absorption (\(\varepsilon_{199} = 16,960\) for \(M = 1700\)).

Mild alkaline hydrolysis of F-polyacetyl methyl ester gave essentially one product now called F-N-acetyl methyl ester obtained as a colorless sticky froth. The n.m.r. (HA-100) spectrum (in pyridine-d\(_5\) solvent) was not very informative. However, it did show the presence of a three proton singlet at 2.03 ppm assigned to an N-acetyl methyl and a three proton singlet at 3.69 ppm assigned to a carbomethoxy methyl, besides a multiplet at 4.0-6.0 ppm partly due to a number of hydroxyl and vinylic protons and a broad one proton triplet at 8.34 ppm (disappeared on adding D\(_2\)O) assigned to the secondary amide N-H proton. The strong absorption bands at 0.83, 1.0 ppm and 1.38-2.0 ppm were assigned respectively to a number of C-methyls and methylene, methine protons.
F-N-acetyl methyl ester being a sticky froth and insoluble in a suitable solvent, its infrared spectrum could not be obtained.

From the n.m.r. spectrum of F-N-acetyl ester, it was concluded that it differed from the F-polyacetyl methyl ester in having free hydroxyl groups in place of the O-acetyl groups.

Acid hydrolysis of F-N-acetyl methyl ester gave D(-)-arabinose, isolated as its p-nitrophenylhydrazone. It may be noted that a physical contamination of the F-N-acetyl methyl ester with D(-)-arabinose was very unlikely because the F-N-acetyl methyl ester showed no trace of arabinose by t.l.c., although they had different R_f values. Thus it was reasonable to conclude that the F-N-acetyl methyl ester contained D(-)-arabinose attached to it through a glycoside linkage.

Periodate titration of F-N-acetyl methyl ester in aqueous methanol consumed 0.45 moles (for M = 1100) of periodate after 24 hours and appeared to stop at this point (0.45 moles after 70.5 hours). However, the lead tetraacetate titration in acetic acid consumed 0.45 moles in 10 hours; 0.75 moles in 48.5 hours and 0.88 moles in 97 hours. One tentative conclusion was drawn from these results, i.e. the F-N-acetyl methyl ester contained at the most one vicinal glycol unit.

Since F-N-acetyl methyl ester possessed arabinose attached to it through a glycoside linkage, the uptake of one mole of lead tetraacetate suggested that the arabinose was in the furanose form.

The next objective was to determine the correct molecular wt. of Compound F or a suitable derivative by mass spectrometry. However, since the F-polyacetyl methyl ester could not be volatilised in the mass
spectrometer, methylation of the F-N-acetyl methyl ester was taken as the second alternative.

Methylation of F-N-acetyl methyl ester with methyl iodide and silver oxide in dimethylformamide (55) gave F-N-acetyl methyl ester polymethyl ether in low yield. This was obtained as a colorless syrup which analysed correctly for C\textsubscript{72}H\textsubscript{137}N\textsubscript{19}O\textsubscript{9} (Mol. wt. 1319).

The mass spectrum* in the high mass region showed the presence of the molecular ion at m/e 1319 in low intensity and a quite intense peak at m/e 1287 possibly due to the loss of a methanol molecule from the molecular ion 1319. However, there were also relatively weak peaks at 1333, ~ 1345 and as high as m/e 1364-1366. The spectrum being correctly countable only up to 1320 mu, the exact mass of the latter peaks could not be determined. These ions might possibly be due to the higher carbon homologues of the molecular ion 1319.

In the infrared spectrum (neat film) F-N-acetyl methyl ester polymethyl ether showed the presence of an ester (strong band at 1740cm\textsuperscript{-1} and a weak band at 1190cm\textsuperscript{-1}), a tertiary amide (strong band at 1650cm\textsuperscript{-1}, no absorption in the amino or hydroxyl region) and ether functions (broad and strong band centered at 1090cm\textsuperscript{-1}).

The n.m.r. (A-60) spectrum showed complex multiplets between 0.80-1.10 ppm corresponding to about 12 protons due to ca. four C-methyls; 1.1-1.7 ppm due to a number of methylene and methine protons. A broad singlet at 1.61 ppm might be due to C-methyls on carbons bearing a

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* We are indebted to Dr. B.C. Das (Gif-Sur-Yvette) for this valuable determination.
double bond (56). A three proton broad singlet at 2.07 ppm was assigned to an N-acetyl methyl and a three proton broad singlet at 3.0 to an N-methyl. There were also three apparent singlets at 3.35, 3.39 and 3.41 ppm respectively, integrated for ca. 39-42 protons assigned to 13-14 O-methyls. A three proton singlet at 3.70 ppm was assigned to a carbomethoxy methyl and a 4.8-5.18 ppm multiplet was partly due to a number of methine protons on carbons bearing a methoxyl group. There was also a broad apparent doublet centered at 5.35 ppm (J~10 cps) corresponding to two protons. This was assigned to vinylic protons.

A flow sheet showing the derivation of F and the related compounds is given in fig. 3.

Although the foregoing discussion clearly demonstrated the probable nature of the functional groups present in the F-polyacetyl methyl ester and the related compounds, their exact positions in the molecules could not be established. To achieve this end, ozonolysis of the F-polyacetyl methyl ester into simpler fragments was taken as the next objective since Compound F (and hence F-polyacetyl methyl ester) contained two double bonds.

Dr. Fehr found that ozonolysis of the F-polyacetyl methyl ester at -78°C in methanol solution followed by reduction of the ozonide with sodium borohydride (57, 58) in situ and then reacetylation of the products mixture gave a mixture of four major products now called Compounds A, "B", C and D. These were efficiently separated by silica gel chromatography, purified and characterized by the author.
Fig. 3

Derivation of F and the Related Compounds
Structure of Compound A

A flow sheet showing the derivation of A and related compounds to be discussed in this section is given in fig. 4.

Compound A was obtained as a colorless viscous oil which analysed correctly for $C_{33}H_{56}O_{12}$ (Mol. wt. 644), confirmed by mass spectrometry. The mass spectrum is shown in fig. 5. The infrared spectrum showed the presence of acetoxy functions (broad and strong bands at 1725, 1230 cm$^{-1}$) in Compound A. However, the presence of an ester function besides the acetoxyds could not be ruled out from the spectrum. There was no absorption in the hydroxyl region. The n.m.r. spectrum (fig. 6) is discussed at the end of this section.

Quantitative estimation of the number of O-acetyl groups in Compound A gave a value of ca. 5.

Mild alkaline hydrolysis of Compound A followed by methylation with diazomethane gave a microcrystalline compound m.p. 101-103° now called polyl A-1, which analysed correctly for $C_{23}H_{40}O_{7}$. The infrared spectrum of polyl A-1 showed the presence of strongly hydrogen bonded hydroxyls (band at 3380 cm$^{-1}$) and an ester function (a strong band at 1725 cm$^{-1}$ and a weak band at 1170 cm$^{-1}$).

The chemical shifts of the various resonance signals in the n.m.r. (HA-100) spectrum in pyridine-$d_5$ solvent of polyl A-1 and their possible assignments are given in Table 2. The one proton multiplet at 2.7-3.1 ppm might be due to a methine proton on a carbon α to the carboxymethoxy group.
Fig. 4

Derivation of A and the Related Compounds
On periodate titration, Polyol A-1 readily consumed about one mole of periodate. In a qualitative periodate reaction, it liberated acetaldehyde isolated as its dimedone derivative.

These results showed the presence of a part structure 8 (p. 39) in that of polyol A-1. The other periodate oxidation product now called seco A-1 is described later.

In an attempt to find out if a hydroxyl group \( \xi \) to the carbomethoxy group was present in polyol A-1, Compound A was hydrolysed with mild alkali to give the corresponding polyol carboxylic acid called Compound A-2 (treatment of Compound A-2 with diazomethane gave polyol A-1). Treatment of Compound A-2 with lead tetra-acetate in acetic acid gave one compound which reacted with diazomethane to give a less polar product identical in behaviour by t.l.c. with seco A-1. Thus, an hydroxyl group at a position \( \zeta \) to the carbomethoxy group in polyol A-1 was not present*.

Compound A-2 on heating under nitrogen from 30-200° for 45 minutes failed to lactonise. This showed that a hydroxyl group at a position \( \gamma \) to the carboxylic group in Compound A-2 was not present*.

Polyol A-1 on treatment with acetic anhydride and fused sodium acetate under reflux for 24 hours, followed by mild alkaline hydrolysis of the reaction products and then diazomethane treatment gave three products. These were separated by t.l.c. and accounted for the total weight of the starting material. The major product (ca. 75-80%)

* I should like to thank Dr. O. Motl for these observations.
### Table 2

**N.m.r. Chemical Shifts of Polyol A-1**

<table>
<thead>
<tr>
<th>$\delta$ (ppm from T.M.S.)</th>
<th>No. # Protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.82</td>
<td>3</td>
<td>(Apparent triplet, $J \sim 6$ cps) $-\text{CH}_2-\text{CH}_3$</td>
</tr>
<tr>
<td>1.12</td>
<td>3</td>
<td>(doublet, $J = 6$ cps) $&gt;\text{CH-CH}_3$</td>
</tr>
<tr>
<td>1.35</td>
<td>3</td>
<td>(doublet, $J = 6$ cps) $&gt;\text{CH-CH}_3$</td>
</tr>
<tr>
<td>1.44 - 2.42</td>
<td>-</td>
<td>(multiplet) methylene and methine protons</td>
</tr>
<tr>
<td>2.7 - 3.1</td>
<td>1</td>
<td>(multiplet) $-$</td>
</tr>
<tr>
<td>3.69</td>
<td>3</td>
<td>(sharp singlet) $-\text{COOCH}_3$</td>
</tr>
<tr>
<td>2.73 - 3.03</td>
<td>5</td>
<td>(multiplet) $5 &gt;\text{CH-OH}$</td>
</tr>
<tr>
<td>5.48</td>
<td>5</td>
<td>(broad singlet, disappeared on adding $\text{D}_2\text{O}$) $5 \text{ O-H}$</td>
</tr>
</tbody>
</table>
exhibited the same t.l.c. and u.v. behaviour as polyol A-1. The second major product (ca. 15-20%), less polar than polyol A-1 exhibited in the u.v. spectrum a clear maximum at 217 m\(\mu\) \((\epsilon \approx 9000)\) due to an \(\alpha,\beta\) unsaturated ester chromophore. The third minor product was not investigated.

When the first major product was resubjected to the above conditions, similar results were obtained. These results suggested that a hydroxyl group \(\beta\) to the carbomethoxy group was present in the polyol.

After fixing the position of three of the five hydroxyl groups of polyol A-1, attention was directed towards the periodate oxidation product.

Compound seco A-1 prepared from the periodate oxidation of polyol A-1 was obtained as a colorless viscous oil and appeared homogeneous by t.l.c. The infrared spectrum of seco A-1 was quite similar to that of polyol A-1 except that a strong band at 1715 cm\(^{-1}\) due to the carbonyl group generated by the periodate reaction was present in the spectrum of seco A-1. It had a molecular weight of 388 and molecular formula \(C_{21}H_{40}O_6\) by high resolution mass spectrometry.

The n.m.r. (HA-100) spectrum in pyridine-d\(_5\) solvent showed the presence of a primary C-methyl at 0.85 ppm (3H, an apparent triplet, J \(\approx 6\) cps) and a sec. C-methyl at 1.15 ppm (3H, doublet, J 6 cps) as found in the spectrum of polyol A-1. However, the secondary C-methyl at 1.35 ppm present in that of polyol A-1 was completely absent in the spectrum of seco A-1. This observation was in agreement with the loss of a methyl group (as acetaldehyde) from polyol A-1 during the
periodate treatment. There were also strong absorption signals at 1.2 - 2.5 ppm (multiplet) assigned to a number of methylene and methine protons; a 2.7 - 3.1 ppm (1H) multiplet possibly due to the methine proton on a carbon α to the carbomethoxy group; a complex 3.3 - 3.6 ppm ( ~ 1H) multiplet; a 3.74 - 4.10 ppm (2H) multiplet due to the methine protons on carbons bearing a hydroxyl group; a three proton singlet at 3.69 ppm assigned to the carbomethoxy methyl; a 4.1 - 4.45 ppm (1H) multiplet; a 4.85 - 5.05 ppm (1H) multiplet; a broad singlet at 5.58 ppm corresponding to about 1H and a hump at 5.7 - 6.1 ppm due to a number of hydroxyl groups. There was no absorption in the aldehyde proton region (9 - 10 ppm). A spectrum in CDCl₃ solvent is discussed in detail at the end of this section.

The presence of half proton signals in various regions of the n.m.r. spectrum suggested that seco A-1 was a mixture. It is possible that an aldehyde function was generated by the periodate oxidation of polyol A-1, but that it cyclised with an hydroxyl group within the molecule to form either a five or a six membered ring hemiacetal, giving two possible anomers in each case. Such a behaviour is very common in sugars.

The modified Huang-Minlon reduction (59) of seco A-1 followed by diazomethane treatment of the reaction products gave besides methyl n-caproate (identical v.p.c. behaviour with authentic methyl n-caproate) a mixture of two products now called Compounds A-3 and A-4. These two were separated by silica gel chromatography and their structures were determined as follows:
Structure of Compound A-3

Compound A-3 was obtained as colorless needles, m.p. 106-107°. The infrared spectrum (KBr) showed a strong band at 3340 cm⁻¹ due to strongly hydrogen bonded hydroxyls. The strong bands at 1385, 1360 cm⁻¹ were possibly due to a gem-dimethyl group (54). There was no absorption in the carbonyl region.

The n.m.r. (HA-100) spectrum in pyridine-d₅ solvent showed the presence of a six proton doublet at 0.88 ppm (J = 6 cps) assigned to two sec. C-methyls; an apparent three proton triplet at 0.88 ppm (J ~ 6 cps) assigned to a C-methyl attached to a saturated alkyl chain; a 1.07 - 2.07 ppm (17H) multiplet due to methylene and methine protons; a 3.59 - 3.92 ppm (2H) multiplet possibly due to the hydrogens attached to carbons bearing a hydroxyl group (60) and a broad hump at 4.74 - 5.07 ppm due to a number of hydroxyl protons including water (pyridine-d₅ not being dry).

The mass spectrum (fig. 7) of Compound A-3 gave a weak molecular ion peak at m/e 230 and a relatively strong peak at m/e 229 (M⁺-1) corresponding to a composition of C₁₄H₂₉O₂. Thus a molecular formula of C₁₄H₃₀O₂ was derived for Compound A-3.

Acetylation of Compound A-3 with acetic anhydride and pyridine at room temperature gave a diacetate obtained as a colorless liquid. In the infrared spectrum it had strong absorptions at 1740, 1250 (broad) cm⁻¹ assigned to acetoxy groups. There was no absorption in the hydroxyl region showing that there was no tertiary hydroxyl group in Compound A-3.
Fig. 7. Mass Spectrum of Compound A-3
The n.m.r. (HA-100) spectrum (fig. 8) showed the presence of a doublet at 0.88 ppm (J = 6 cps) assigned to two sec. C-methyls; an apparent triplet at 0.86 due to a C-methyl attached to the end of a saturated alkyl chain; a 1.06 – 1.72 ppm multiplet (17H) due to methylene and methine protons; a six proton singlet at 2.02 ppm assigned to two O-acetyl methyls and a 4.68 – 4.98 ppm (2H) multiplet assigned to the hydrogens attached to the carbons bearing an acetoxy group. Since there are only two such protons, both of the acetates must be secondary.

The mass spectrum gave a molecular weight of 314. However, a peak of relatively weak intensity at m/e 342 was also present in the mass spectrum. This was probably due to a two carbon homologue of Compound A-3 diacetate.

Structure 2 proposed for Compound A-3 was derived from its fragmentation pattern (primary cleavages from either end of the molecule shown) and the foregoing n.m.r. information. The fragmentation ions marked with an asterisk have had their compositions established by high resolution measurements.

Structure of Seco A-1

The origin of methyl caproate and that of Compound A-3 was found when it was discovered that these two were produced in roughly equi-molar proportion and the number of carbon atoms of these two compounds added together accounted for the total number of carbon atoms in seco A-1. It thus became apparent that these simply represented two frag-
ments of the seco A-1. Furthermore, the yield of these two fragments depended strongly on the severity of the reaction conditions in that, the stronger the reflux and the longer the reaction period, the greater the amount of these compounds and the less the amount of Compound A-4 was produced. This, therefore, suggested that the caproic acid and Compound A-3 were formed at the expense of Compound A-4. Also, since seco A-1 contained only two C-methyls, one being primary and the other being secondary, whereas the caproic acid and Compound A-3 contained a total of four C-methyls, two of these being primary and the other two being secondary, a possible mechanism must account for the formation of one primary and one secondary C-methyl. Furthermore, it should not involve oxygen at any stage, because when the reaction was carried out in the presence of a nitrogen atmosphere, the caproic acid and Compound A-3 were still formed in the usual amounts.

This information suggested structure 10 for seco A-1. A mechanism which explains the formation of n-caproic acid and Compound A-3 from the proposed structure 10 of seco A-1 is shown in fig. 9. The first step involves the formation of a hydrazone of the aldehyde function (in equilibrium with the hemiacetal) of seco A-1 followed by reduction of the hydrazone to the methyl group prior to the retroaldolization step, because, as pointed out before, the Compound A-3 and the n-caproic acid appeared to be formed at the expense of Compound A-4 which is produced from seco A-1 by reduction of the aldehyde group to a methyl (see later discussion). The retroaldolization might then proceed by a cyclic path as shown or by an intermolecular path involving the stronger base.
Mechanism of Formation of n-caproic Acid and Compound A-3 from Compound Seco A-1.
11 \sim a, R=H \\
11 \sim b, R=Ac

12 \sim
hydroxide giving an aldehyde group which then is reduced to give Compound A-3.

The part structure \( \text{IIa} \) present in the proposed structure 10 of seco A-1 is in agreement with the n.m.r. spectrum of seco A-1 shown in fig. 10. The one proton multiplet at 2.4 - 2.7 ppm is assigned to the C-2 methine proton and the apparent triplet at 3.55 ppm (\( J \sim 5.5 \text{ cps} \)) is assigned to the C-3 methine proton and these are shown to be coupled with each other, since irradiation at 2.54 ppm collapsed the C-3 methine triplet to a broad doublet (\( J \sim 5.5 \text{ cps} \)), this also required a methine proton on the C-4 carbon atom. Irradiation in turn at 3.51 ppm collapsed the C-2 methine multiplet to an apparent broad triplet. This therefore showed that the C-2 methine proton was not only coupled to the C-3 methine proton, but also with methylene protons. Irradiation at 1.45 ppm, i.e., the methylene and C-4 methine proton region, partially decoupled the C-4 methyl doublet, also collapsed the C-3 methine triplet, and also simplified the C-2 methine multiplet. These results, therefore, required the partial structure \( \text{IIa} \) in that of seco A-1.

The 2.85 - 3.28 ppm multiplet (disappeared on adding \( \text{D}_2\text{O} \)) is assigned to hydroxyl protons. The C-7 and C-11 methine protons are present in the 3.3 - 3.6 ppm and 3.8 - 4.1 ppm regions, since irradiation at 1.45 ppm, i.e., the methylene region very much simplified absorptions in these regions to give broad singlets at 3.44 and 3.99 ppm. A broad doublet centered at 4.68 ppm and a broad singlet at 5.28 ppm are assigned to the anomeric methine proton (a mixture of \( \alpha \) and \( \beta \) anomers) at C-15. The anomeric hydroxyl proton may be present
in the 4.15 - 4.45 ppm hump (disappeared on adding D₂O). The sec.
C-methyl is present at .88 ppm (d, J, 6 cps) and the primary C-methyl
at .87 ppm (apparent triplet J ~ 6 cps).

**Structure of Compound A-4**

Compound A-4 was isolated in poor yields. Since the caproic acid
and Compound A-3 were simply the two fragments of seco A-1, and these
appeared to be formed at the cost of Compound A-4, it was thought that
Compound A-4 might be the normal reduction product of seco A-1. In
view of this hypothesis Compound A-4 should have the structure 12.
This was supported by its n.m.r. (HA-100) spectrum and that of its tri-
acetate (fig. 11) summarised in Table 3.

The mass spectrum of Compound A-4 gave a molecular weight of 374.
However, a peak at m/e 382 of relatively weak intensity was also present
in the mass spectrum, which has not been explained. The structure 12
proposed for Compound A-4 was further confirmed by its fragmentation
pattern (primary cleavages from either end of the molecule shown).

Since seco A-1 was obtained by the loss of an acetaldehyde mole-
cule from the periodate reaction of polyol A-1, it became possible to
propose structure 13a for polyol A-1 and hence structure 13b for
Compound A. The proposed structure 13b for Compound A is consistent
with its n.m.r. (HA-100) spectrum taken in different solvents. The
chemical shifts are given in Table 4.

The part structures 11b and 14 in that of Compound A could also be
obtained from double irradiation experiments on Compound A as described
Table 3

N.m.r. Chemical Shifts of Compound A-4 and its Triacetate

\[
\begin{align*}
\text{OR} & \quad \text{OR} & \quad \text{OR} \\
\text{CH}_3 & \quad \text{CH}_3 & \quad \text{CH}_3 \\
\text{(CH}_2\text{)}_2 & \quad \text{(CH}_2\text{)}_2 & \quad \text{(CH}_2\text{)}_2 \\
\text{CH}_3 & \quad \text{CH}_3 & \quad \text{CH}_3
\end{align*}
\]

\(12\)

a, Compound A-4, \(R = \text{H}\)

b, Compound A-4 triacetate, \(R = \text{Ac}\)

<table>
<thead>
<tr>
<th>Proton</th>
<th>Compound A-4 (Pyridine-(d_5) solvent)</th>
<th>Compound A-4 triacetate (CDCl(_3) solvent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>2.59-2.87 (multiplet)</td>
<td>2.5-2.75 (multiplet)</td>
</tr>
<tr>
<td>c and methylenes</td>
<td>1.25-2.07 (multiplet)</td>
<td>1.05-1.75 (multiplet)</td>
</tr>
<tr>
<td>f or g</td>
<td>0.82 (triplet, (J \sim 6) cps), 0.87 (triplet, (J \sim 6) cps)</td>
<td>0.88 (apparent triplet (J \sim 6) cps)</td>
</tr>
<tr>
<td>h</td>
<td>1.16 (doublet, (J, 6.5) cps)</td>
<td>0.88 (apparent doublet (J \sim 6.5) cps)</td>
</tr>
<tr>
<td>i</td>
<td>3.71 (singlet)</td>
<td>3.66 (singlet)</td>
</tr>
<tr>
<td>d, e</td>
<td>3.55-3.95 (multiplet)</td>
<td>4.7-4.94 (multiplet)</td>
</tr>
<tr>
<td>b</td>
<td>3.55-3.95 (multiplet)</td>
<td>5.03 (triplet, (J \sim 6.5)cps)</td>
</tr>
<tr>
<td>OH</td>
<td>5.09 (broad singlet)</td>
<td>---</td>
</tr>
<tr>
<td>3,0-acetyl methyl</td>
<td>---</td>
<td>2.03, 2.04 (two singlets)</td>
</tr>
</tbody>
</table>
13

a, \( R = R' = H \)

b, \( R = \text{Ac} , \ R' = H \)

c, \( R = \text{Ac} , \ R' = D \)
Table 4

N.m.r. Chemical Shifts of Compound A

\[(\text{CH}_3)_2\text{C-CH-CH-CH-(CH}_2\text{)}_2\text{-CH-CH-(CH}_2\text{)}_3\text{-CH-CH-CH}_3\text{(h)}\]
\[(\text{CH}_2\text{)}_3\text{CH}\text{(i)}\]

<table>
<thead>
<tr>
<th>Proton</th>
<th>(\delta) (ppm from T.M.S.)</th>
<th>Solvent ((\text{CDCl}_3))</th>
<th>Solvent ((\text{C}_6\text{D}_6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>2.5-2.8 (multiplet)</td>
<td>2.6-2.85 (multiplet)</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>4.66-5.15 (multiplet)</td>
<td>5.33 (triplet, J, 6.5 cps)</td>
<td></td>
</tr>
<tr>
<td>d, e, f, g</td>
<td>4.66-5.15 (multiplet)</td>
<td>4.8-5.2 (multiplet)</td>
<td></td>
</tr>
<tr>
<td>c and methylenes</td>
<td>1.1-1.8 (multiplet)</td>
<td>1.15-1.7 (multiplet)</td>
<td></td>
</tr>
<tr>
<td>h</td>
<td>1.15 (doublet, J, 6.5 cps)</td>
<td>1.07 (doublet, J, 6.5 cps)</td>
<td></td>
</tr>
<tr>
<td>i</td>
<td>0.88 (doublet, J, 6.5 cps)</td>
<td>0.83 (doublet, J, 6.5 cps)</td>
<td></td>
</tr>
<tr>
<td>j</td>
<td>0.86 (triplet, J, 6.5 cps)</td>
<td>0.82 (triplet, J, 6.5 cps)</td>
<td></td>
</tr>
<tr>
<td>k</td>
<td>3.63 (singlet)</td>
<td>3.46 (singlet)</td>
<td></td>
</tr>
<tr>
<td>5, O-acetyl methyls</td>
<td>2.01, 2.02, 2.03, 2.04</td>
<td>1.74, 1.76, 1.78, 1.81</td>
<td>(all singlets)</td>
</tr>
</tbody>
</table>
for seco A-1.

Structure \( 13b \) of Compound A was also confirmed by its fragmentation pattern (primary cleavages from either end of the molecule given) as shown. The ions marked with an asterisk have had their compositions established. The m/e 273 ion peak being too weak in intensity (\(< 0.5\%\)) has not been shown in the plot in fig. 5 (p.29).

It is to be noted that Compound A is possibly a mixture of three carbon homologues as has been noted in the mass spectrum of some of the derivatives of Compound A. Structure \( 13a \) represents the predominant homologue. This evidence is also presented in Chapter 2. At present the exact site of homologation is not known. The possibility of homologation in the \( n-C_4H_9 \) chain to \( n-C_5H_{11} \) or \( n-C_6H_{13} \) chains is at least ruled out because, in the Huang-Minlon reduction of seco A-1, only methyl \( n \)-caproate was detected. There was no peak corresponding to authentic methyl \( n \)-caprylate or a peak between methyl \( n \)-caprylate and methyl \( n \)-caproate in the v.p.c. of the ester mixture.

"Compound B"

"Compound B" appeared homogeneous by t.l.c., but on mild alkaline hydrolysis gave two polyols (see fig. 17, p. 71). These were separated by silica gel chromatography and are called Compound B-1 and Compound C-1. That the two products were not due to a chemical transformation was demonstrated when the mixture on reacetylation gave on t.l.c. one spot identical in \( R_f \) value with that of "Compound B".
Basic Functionalities of Compound C-1

Compound C-1 was obtained as colorless microplates m.p. 161-163°. The infrared spectrum (KBr) showed the presence of strongly hydrogen bonded hydroxyls (3300 cm⁻¹), but no absorption in the carbonyl region.

The mass spectrum gave a peak of highest mass at m/e 343 which has not been explained. There were also peaks of relatively high intensity at m/e 332 (M⁺-2H₂O); 314 (M⁺-3H₂O); 301 (M⁺-CH₂OH); 296 (M⁺-4H₂O); 278 (M⁺-5H₂O) and 260 (M⁺-6H₂O). The molecular weight 368 assumed here was calculated from the molecular formula of Compound C-1 polyacetate to be described later.

On periodate titration, Compound C-1 consumed 1.98 moles of periodate in 1.5 hours for a mol. wt. of 368. This showed the presence of two vicinal glycol units in Compound C-1.

Acetylation of Compound C-1 with acetic anhydride and pyridine at room temperature gave a polyacetate as a colorless syrup called Compound C-1 polyacetate. The infrared spectrum showed the presence of-acetoxyl groups (strong bands at 1730, 1230 cm⁻¹). There was no absorption in the hydroxyl region, showing that there was no tertiary hydroxyl group in the molecule. It analysed satisfactorily for C_{33}H_{52}O_{16} (Mol. wt. 704), confirmed by mass spectrometry.

The n.m.r. (HA-100) spectrum (fig. 12) showed the presence of a three proton doublet at 1.18 ppm (J, 6.5 cps) assigned to a sec. C-methyl; a 1.26 - 1.89 ppm (ca. 16H) multiplet assigned to 8 methylene protons; four singlets at 2.0, 2.02, 2.05 and 2.09 ppm corresponding to 24 protons, assigned to eight O-acetyl methyls; a 3.86 - 4.39 ppm (2H)
multiplet which appeared like an AB part of an ABX system, assigned to the methylene protons on a carbon bearing an acetoxy group; a 4.7 - 5.05 ppm (5H) multiplet due to the methine protons on carbons bearing an acetoxy group and a 5.05 - 5.25 ppm (2H) multiplet also assigned to the methine protons on carbons bearing an acetoxy group.

Since from the n.m.r. spectrum there were eight O-acetates (none of them could be tertiary), but only nine hydrogens at the carbons bearing an acetoxy group, seven of the acetates must be secondary, and one must be primary. The eight O-acetates in Compound C-1 polyacetate accounted for all the oxygen functions in the molecule, showing that the Compound C-1 polyacetate was a saturated compound.

The presence of a part structure 14 in that of Compound C-1 polyacetate was shown by decouping experiments. Thus, irradiation at 4.91 ppm collapsed the methyl doublet to a singlet. Irradiation in turn at 1.18 ppm simplified the 4.7 - 5.05 ppm region.

The foregoing results thus showed that the Compound C-1 was a saturated octahydroxy alcohol having seven secondary hydroxyls and one primary hydroxyl group. It also contained two vicinal glycol units, eight methylenes and one C-methyl in a part structure 14 (corresponding alcohol).

The structure of Compound C-1 is proposed later in the section describing the structure of Compound C.

Basic Functionalities of Compound B-1

Compound B-1 was obtained as white microcrystals m.p. 159-161°.
The infrared spectrum (KBr) showed the presence of strongly hydrogen bonded hydroxyls (3300 cm\(^{-1}\)). There was no absorption in the carbonyl region.

The mass spectrum gave a molecular weight of 352 and molecular formula \(C_{17}H_{36}O_7\). There were also very intense peaks at m/e 319 (\(M^+ - \text{CH}_3\_\text{H}_2\text{O}\)) and 298 (\(M^+ - 3\text{H}_2\text{O}\)).

Compound B-1 did not consume any periodate under exactly the same conditions as described for Compound C-1. Thus, it did not contain a vicinal glycol unit.

Acetylation with acetic anhydride and pyridine at room temperature gave a polyacetate called Compound B-1 polyacetate obtained as a colorless syrup. The infrared spectrum did not show absorption in the hydroxyl region, showing that there was no tertiary hydroxyl group in the molecule. The strong absorption bands at 1725 cm\(^{-1}\) (broad) and 1230 cm\(^{-1}\) (broad) were assigned to acetoxy groups. It analysed satisfactorily for \(C_{31}H_{50}O_{14}\) (Mol. wt. 646). This result was confirmed by mass spectrometry.

The n.m.r. (HA-100) spectrum (fig. 13) showed the presence of a three proton doublet at 1.18 ppm (\(J = 6.5\) cps) assigned to a secondary C-methyl; a 1.25 - 1.94 ppm (ca. 18H) multiplet due to 9 methylene protons; three singlets at 2.0, 2.01 and 2.03 corresponding to 21 protons, assigned to seven O-acetyl methyls; a two proton triplet at 4.07 ppm (\(J = 6.5\) cps) assigned to protons on a methylene bearing an acetoxy group and having a methylene group adjacent to it (part structure 15) a 4.72 - 5.12 ppm (6H) multiplet assigned to the methine
Fig. 13. 100 MHz NMR spectrum of Compound B-1 polyacrylate in CDCl3.
protons on carbons bearing an acetoxy group.

The presence of part structure \( \text{\textit{14}} \) in that of Compound B-1 polyacetate was found by double irradiation experiments as described for Compound C-1 polyacetate.

Compound B-1 polyacetate thus contained seven acetoxy groups, six being secondary and one being primary. The seven acetates accounted for all the oxygen functions in the molecule showing that it was a saturated compound. Hence, the Compound B-1 was a hepta hydroxy alcohol having six secondary and one primary alcoholic groups. Further, it contained 9 methylenes and the part structures \( \text{\textit{14}} \) and \( \text{\textit{15}} \) for the corresponding alcohols. It had no vicinal glycol units.

A possible structure for Compound B-1 polyacetate will be proposed following the section on the structure of Compound C-1.

**Partial Structure of Compound C**

Compound C was obtained as a colorless syrup which analysed satisfactorily for \( \text{C}_{42}\text{H}_{64}\text{O}_{22} \) (Mol. wt. 920), confirmed by mass spectrometry. The infrared spectrum showed the presence of acetoxy groups (strong bands at 1735, 1235 cm\(^{-1}\)). There was no absorption in the hydroxyl region.

The n.m.r. (HA-100) spectrum (fig. 14) showed the presence of a three proton doublet at 1.18 ppm (J, 6.5 cps) assigned to a secondary C-methyl; a 1.26 - 1.89 ppm (16H) multiplet due to 8 methylene protons; four singlets at 2.00, 2.05, 2.09 and 2.10 ppm corresponding to 30 protons assigned to ten O-acetyl methyls; a 3.84 - 4.54 ppm (6H)
Fig. 14. 100 MHz NMR Spectrum of Compound C in CDCl$_3$
multiplet partly due to methylene protons on carbons bearing an acetoxy group; a 4.74 - 5.07 ppm (6H) multiplet and a doublet at 5.10 ppm (2H, J, 1.5 cps) assigned to methine protons on carbons bearing an acetoxy group; a one proton singlet at 5.22 ppm.

A quantitative estimation of the number of O-acetates by determination of the saponification equivalent of Compound C gave a value of ca. 10, in good agreement with the n.m.r. results. However, the ten O-acetates accounted for only 20 oxygen atoms out of the total 22 found in Compound C.

The nature of the 2 remaining oxygen atoms could be found when it was discovered that Compound C contained D(-)-arabinose attached to it through a glycoside linkage. This is described in the later part of this section.

Compound C was hydrolysed with alkali and the reaction mixture neutralised by passing through a cation exchanger column. The products were then chromatographed on Kieselgel to give two polyols. One of these polyols had m.p. 161-163° and did not depress the m.p. of Compound C-1 on mixed melting point. It showed identical t.l.c., i.r. and periodate titration behaviour with that of Compound C-1 and also gave a polyacetate that was identical (t.l.c., i.r., n.m.r., analysis and mass spec.) with Compound C-1 polyacetate. The other polyol is henceforth called "Compound C-2".

**Structure of Compound C-1**

The reaction of Compound C-1 with periodate gave about one mole of
formaldehyde (obtained as its dimedone derivative). This evidence in conjunction with the presence of two vicinal glycol units (described before) suggested the presence of a partial structure 16 in Compound C-1. The other periodate oxidation product of Compound C-1 being unstable was reduced with sodium borohydride in situ to give a white microcrystalline polyol, m.p. 128-130°, designated as seco C-1.

Structure of Seco C-1

The infrared spectrum (KBr) of Compound Seco C-1 showed the presence of strongly hydrogen bonded hydroxyls (3300 cm⁻¹). There was no absorption in the carbonyl region.

The mass spectrum gave a molecular weight of 308.

Acetylation of Compound Seco C-1 with acetic anhydride and pyridine at room temperature gave a polyacetate called seco C-1 polyacetate obtained as a colorless syrup. The infrared spectrum showed the presence of acetoxy groups (strong and broad bands at 1725 cm⁻¹ and 1240 cm⁻¹). There was no absorption in the hydroxyl region, showing that there was no tertiary hydroxyl group in the molecule.

The mass spectrum (fig. 15) gave a molecular weight of 560* and molecular formula C₂₇H₄₄O₁₂.

The n.m.r. (HA-100) spectrum (fig. 16) showed the presence of a three proton doublet at 1.18 ppm (J, 6.5 cps) assigned to a sec.

* The molecular ion peak at m/e 560 being too weak (<0.5% of the base peak) in intensity has not been shown in the plot in fig. 15.
FIG. 16. 100 MHz NMR Spectrum of Compound 1 Seco C-1 polyacetalate in CDCl₃.
C-methyl; a 1.25 - 1.93 ppm (16H) multiplet due to methylene protons; three singlets at 1.99, 2.0 and 2.02 ppm corresponding to 18 protons, assigned to six O-acetates; a two proton triplet at 4.07 ppm (J, 6.5 cps) assigned to protons on a methylene, bearing an acetoxy group and coupled to a methylene protons (part structure 15); a 4.72 - 5.11 ppm (5H) multiplet assigned to the methine protons on carbons bearing an acetoxy group.

The presence of part structure 14 in that of seco C-1 polyacetate was found by n.m.r. decoupling experiments as described for Compound B-1 polyacetate. Thus, the positions of two of the six acetoxy, two of the eight methylene and the secondary methyl groups could be fixed.

The structure 17 was proposed for seco C-1 polyacetate from its fragmentation pattern (primary cleavages from either end of the molecule given) as shown. The ions marked with an asterisk have had their compositions established by high resolution measurements. Thus, seco C-1 would have the corresponding alcohol structure.

From the structure of Compound Seco C-1, and the presence of a part structure 16 in that of Compound C-1, it became possible to propose structure 18 for Compound C-1 and the corresponding acetate for Compound C-1 polyacetate.

Structure of Compound B-1 polyacetate

A comparison of the \( \text{C}_{31}\text{H}_{50}\text{O}_{14} \) molecular formula of Compound B-1 polyacetate with that \( \text{C}_{27}\text{H}_{44}\text{O}_{12} \) of seco C-1 polyacetate revealed that these two differed by a composition of \( \text{C}_{4}\text{H}_{6}\text{O}_{2} \). The n.m.r. spectroscopic
data of these two compounds showed that Compound B-1 polyacetate contained both a -CH₂ and a -CHOAc unit more than the seco C-1 polyacetate. Since these two units accounted for the difference of C₄H₆O₂ between the molecular compositions of these two molecules and both of these compounds had the same part structures 14 and 15 and also approximately the same pattern in the methylene region in their n.m.r. spectra (see fig. 13, 16) it became possible to propose a tentative structure 19 for Compound B-1 polyacetate and hence, the corresponding alcohol for Compound B-1.

This structure of Compound B-1 polyacetate was confirmed by its fragmentation pattern (primary cleavages from either end of the molecule given) as shown. The ions marked with an asterisk have had their compositions established by high resolution measurements.

"Compound C-2"

It was obtained as white microcrystals m.p. 151-153° and appeared homogeneous by t.l.c. The amount of "Compound C-2" obtained was approximately 33% (w/w) of that of Compound C-1.

On periodate titration, "Compound C-2" readily consumed two moles of periodate for a molecular weight of about 343. For an uptake of three moles of periodate a molecular weight of about 515 was calculated.

The infrared spectrum (KBr) showed the presence of strongly hydrogen bonded hydroxyls (3300cm⁻¹). There was no absorption in the carbonyl region.

Acetylation of Compound C-2 with acetic anhydride and pyridine at
room temperature or with heating gave a colorless syrup which exhibited two almost overlapping spots on a t.l.c. plate. One of them was identical in $R_f$ value with that of Compound C and the other was from a more polar product.

The structure and origin of "Compound C-2" is not understood at present.

**Partial Structure of Compound C**

The next objective was to get the polyol corresponding to Compound C. Compound C-1 obviously was formed as a result of a chemical reaction of this polyol during its contact with the cation exchanger, since, Compound C-1 polyacetate had a molecular composition different from that of Compound C. A different work-up procedure therefore should give the desired product. Accordingly, hydrolysis of Compound C was followed by careful neutralization with acetic acid and evaporation to dryness. Extraction of the product with dry pyridine gave the desired compound, now called Compound C polyol. It was further purified by silica gel chromatography to give a slightly yellow sticky solid which failed to crystallise from the common solvents. However, on t.l.c., it gave essentially one spot having an $R_f$ value different from that of Compounds C-1 and C-2. Also on acetylation, it gave one compound identical in $R_f$ value with that of Compound C, showing that it was most probably the corresponding alcohol of Compound C.

Hydrolysis of Compound C polyol with hydrochloric acid gave two compounds. One of them was identical with arabinose, identified as its
p-nitrophenylhydrazone and its acetate by t.l.c. However, the amount being small these derivatives could not be isolated. The other compound had an $R_f$ value identical with that of Compound C-1.

It was thus reasonable to conclude that the Compound C polyol had arabinose attached to it through a glycoside linkage. Since the precursor F-N-acetyl methyl ester (hence also F-polyacetyl methyl ester) of Compound C had D(-)-arabinose attached to it, it was assumed that the arabinose in Compound C polyol had the same configuration.

From the acid hydrolysis of Compound C polyol it also became evident that the Compound C-1 was derived from Compound C polyol by a loss of D(-)-arabinose unit during its contact with the cation exchanger. However, since the arabinose was not obtained as one of the products, it must have been destroyed by the cation exchanger. That this was possible was shown by percolating an authentic sample of D(-)-arabinose through the cation exchanger, when the arabinose was completely destroyed.

Although the quantitative periodate titration of Compound C polyol was not done (it not being possible to remove inorganic impurities from Compound C polyol either by extraction into dry pyridine or by chromatography on silica gel or ion exchange resins), a qualitative t.l.c. correlation of the periodate reaction products of Compounds C-1, "C-2" and C polyol was worth noting.

Compounds C-1, "C-2" and C polyol were separately treated with the periodate reagent under exactly the same conditions and the products
analysed by t.l.c.; after 1.5 hours each of the Compounds C-1 and "C-2" disappeared completely and gave the same product as judged by the \( R_f \) value. Compound C polyol appeared to have reacted up to the extent of only ca. 20% and gave a different (more polar) oxidation product from that obtained in the other two cases. After 24 hours, it appeared to have reacted completely giving the same oxidation product as obtained after 1.5 hours.

Two tentative conclusions were drawn from these qualitative results. Firstly, (in contrast to the attractive possibility of the identity of Compounds "C-2" and C polyol based on their genesis) the above results indicated that the Compounds "C-2" and C polyol were different. Secondly, the arabinose in Compound C polyol was not attached to the terminal methylene carbon of Compound C-1.

At this point it is important to mention that the Compound C used for the derivation of the corresponding polyol (i.e. Compound C polyol) was a different column chromatographic fraction than that used to give Compounds C-1 and "C-2". Thus, the possibility of Compound C being a mixture could not be ruled out.

For a complete structure of Compound C, it then remained to establish the site of attachment of the arabinose moiety and the configuration at the anomeric center. The possibility of the arabinose unit being in the furanose form as indicated by the periodate or lead tetraacetate titration of the F-N-acetyl methyl ester is in good agreement with the slow reaction of the Compound C polyol with the
periodate reagent, because the hydroxyl groups in the arabinofuranose form at carbon atoms C₂ and C₃ are trans to each other. Furthermore, the uptake of about one mole of lead tetraacetate by Compound F-N-acetyl methyl ester required the arabinose at either the second or the third carbon atom of the vicinal triol unit in Compound C-I.

The configuration at the anomeric center of Compound C could be derived from its n.m.r spectrum (fig. 14, p. 57). The n.m.r. spectrum of Compound C as described before showed the presence of a one proton singlet at 5.22 ppm which is absent in that of Compound C-I polyacetate (the aglycone of Compound C). This, therefore, was assigned to the anomeric proton of the arabinose moiety. The presence of a singlet for the anomeric proton of arabinose suggested the arabinose in the furanose form with the glycoside linkage in the α configuration. These assignments are in agreement with the J₁₂ coupling constants of <0.5 cps for the methyl α-D-arabinofuranoside derivatives reported by Stevens (61).

These results thus allowed a partial structure 20a for Compound C. The complete structure of Compound C was derived from the methylation studies of primycin described in the second chapter.

An interpretation of "Compound B" as a mixture of the polyacetates of Compounds B-I and C-I was now readily acceptable. Compound C-I polyacetate must have been formed by the loss of the arabinose moiety from Compound C because the Compound C had undergone hydrochloric acid treatment during its derivation from F-polyacetyl methyl ester.
20, $R = \text{Ac}$

a, $R' = \text{H}$

b, $R = \text{D}$

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Derivation of the Compounds from Compounds "B" and C
A flow sheet showing the derivation of the compounds from Compounds "B" and C is given in fig. 17.

**Structure of Compound D**

Compound D was obtained as a colorless syrup, $[\kappa]_D^{28} + 11.2^\circ \pm 0.5^\circ$ (c, 3.72, MeOH). The infrared spectrum showed the presence of a secondary amide (bands at 3440, 1665, 1515 cm$^{-1}$), and acetoxy groups (broad and strong bands at 1730, 1235 cm$^{-1}$).

Compound D analysed correctly for $C_{18}H_{31}NO_7$ (Mol. wt. 373), confirmed by mass spectrometry.

The structure $\tilde{21}$ (R=R'=H) proposed for Compound D was derived from its n.m.r. spectrum (fig. 18) and the double irradiation experiments. The chemical shifts of the various protons are given in Table 5.

(i) Irradiation at 6.89 ppm (proton i) collapsed the apparent quartet at 3.21 ppm (proton h) to a triplet ($J$, 6 cps). Irradiation in turn at 3.23 ppm collapsed the broad triplet at 6.85 ppm to a singlet and also simplified the 1.35 - 1.77 ppm (protons g, f) multiplet, but there was no effect on the signals due to the protons c, e and a.

These results showed that the methylene proton h was coupled to proton i and the methylene protons g or f and thus required the partial structure $\tilde{22}$.

(iii) Irradiation at 1.57 ppm (protons g, f) collapsed the apparent quartet at 3.21 ppm (proton h) to a doublet ($J$, 6 cps). This showed that the proton h was coupled to the methylene protons g or f. The irradiation also simplified the multiplet at 4.87 ppm (proton e) which
Compound D, \( R = R' = H \)

**Table 5**

**N.m.r. Chemical Shifts of Compound D**

<table>
<thead>
<tr>
<th>Proton</th>
<th>( \text{CDCl}_3 ) (( \delta ): ppm from TMS)</th>
<th>( \text{C}_6\text{D}_6 ) (( \delta ): ppm from TMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>3.86 (doublet, ( J ), 6 cps)</td>
<td>AB part of an ABX system centered at 3.90, JAX, 7 cps, JBX, 6 cps, JAB, 11 cps.</td>
</tr>
<tr>
<td>b</td>
<td>1.82-2.38 (multiplet)</td>
<td>1.7-2.35 (multiplet)</td>
</tr>
<tr>
<td>c</td>
<td>4.96 (doublet of a doublet ( J ), 4.5 and 7 cps)</td>
<td>5.15 (doublet of a doublet, ( J=4.5 ) and 7 cps)</td>
</tr>
<tr>
<td>d</td>
<td>1.82-2.38 (multiplet)</td>
<td>1.7-2.35 (multiplet)</td>
</tr>
<tr>
<td>e</td>
<td>4.87 (multiplet)</td>
<td>5.02 (multiplet)</td>
</tr>
<tr>
<td>f, g</td>
<td>1.35-1.77 (multiplet)</td>
<td>1.3-1.7 (multiplet)</td>
</tr>
<tr>
<td>h</td>
<td>3.21 (apparent quartet ( J ), 6 cps)</td>
<td>3.21 (apparent quartet ( J ), 6 cps)</td>
</tr>
<tr>
<td>i</td>
<td>6.85 (broad triplet ( J ), 6 cps)</td>
<td>6.5 (multiplet)</td>
</tr>
<tr>
<td>j</td>
<td>1.95 (singlet)</td>
<td>1.77 (singlet)</td>
</tr>
<tr>
<td>k, l</td>
<td>.93 (doublet, ( J ), 7 cps)</td>
<td>.85 (doublet, ( J ), 7 cps)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.92 (doublet, ( J ), 7 cps)</td>
</tr>
<tr>
<td>3, O-acetyl methyls</td>
<td>2.03, 2.06 (two singlets)</td>
<td>1.83 (broad singlet)</td>
</tr>
</tbody>
</table>
required the protons of one methylene to be coupled to proton e. These results required the presence of a partial structure 23. However, since there was no effect on the absorption signals due to the protons a and c, the presence of partial structures 24 and 25 could be excluded.

(iii) Irradiation at 2.30 ppm or 2.07 ppm (proton b or d) collapsed the C-methyls doublet at 0.93 ppm; the multiplet at 4.87 (proton e) became an apparent triplet; the doublet of a doublet at 4.96 ppm (proton c) became a singlet and also, the doublet at 3.86 ppm (protons a) became a rough singlet. These results showed that the proton b or d was coupled to the C-methyl protons, proton e, protons c and the protons a and, thus, required the presence of partial structures 26 and 27.

(iv) Irradiation at 4.93 ppm (proton c or e) simplified the 1.82 - 2.38 ppm multiplet due to the protons b and d. This also simplified the 1.35 - 1.77 ppm signals due to the methylene protons g and f. These results thus required the presence of a partial structure 28.

(v) Irradiation at 3.91 ppm (protons a) simplified the 1.28 - 2.38 ppm multiplet due to the methine protons b and d, but there was no effect on the absorption signals due to the methylene protons g and f, and thus supported the presence of the partial structure 27.

These irradiation experiments are summarised in fig. 19. From these partial structures, the complete structure of Compound D was derived as follows:

The partial structures 26 and 27 could now be joined to give the extended partial structure 29. Since the proton e was coupled to the
Double Irradiation Experiments on Compound D
methylenic protons g or f (presence of partial structure 23) and the proton c was not coupled to these methylenic protons (absence of structure 25), the partial structure 29 could be extended to give the partial structure 30. This argument also excluded the possibility of joining the partial structure 26 to 27 by the reverse end from that used to give the structure 29.

The partial structure 22 required it to be joined to the partial structure 30 to give the extended partial structure 31. For a complete structure of Compound D, a methylene group (g or f) remained to be fixed in the structure 31. Since the methylenic protons g or f were not coupled to either proton a (absence of the partial structure 24) or proton c (absence of the partial structure 25), the only place in which the methylene group could be placed was, therefore, as shown in the complete structure 21 of Compound D.

Alkaline hydrolysis of Compound D gave a triol called Compound D polyol obtained as a colorless syrup, [α]$_D^{28}$ + 8.05° ± 0.10° (c, 4.00, MeOH). The infrared spectrum showed the presence of a secondary amide (bands at 3300, 1635, 1550 cm$^{-1}$) and strongly hydrogen bonded hydroxyls (3300 cm$^{-1}$).

The mass spectrum of Compound D polyol gave a molecular weight of 247 and molecular formula C$_{12}$H$_{25}$NO$_4$.

The n.m.r. (HA-100) spectrum shown in fig. 20 (Table 6) is consistent with the corresponding alcohol structure (21, R$^1$ = H) of Compound D for Compound D polyol. Thus, it supported the proposed structure 21 of Compound D.
Table 6

N.m.r. Chemical Shifts of Compound D-polyol

<table>
<thead>
<tr>
<th>Proton</th>
<th>( \delta (ppm \text{ from TMS}) ) in Pyridine-( d_5 )</th>
</tr>
</thead>
</table>
| a      | An apparent AB part of an ABX pattern  
\( \delta_A = 3.94 \),  \( \delta_B = 3.85 \),  \( J_{AX} = 5 \text{ cps} \);  \( J_{BX} = 5.5 \text{ cps} \)  
and  \( J_{AB} = 10.5 \text{ cps} \) |
| b      | 1.86-2.4 (multiplet) |
| c      | 4.18 (doublet of a doublet; \( J = 4.5 \text{ and } 7 \text{ cps} \)) |
| d      | 1.86-2.4 (multiplet) |
| e      | 4.06 (multiplet) |
| f/g    | 1.45-1.86 (multiplet) |
| h      | 3.47 (an apparent broad quartet; \( J \sim 7 \text{ cps} \)) |
| i      | 8.30 (broad triplet) disappeared on adding \( D_2O \)  
(not shown on the chart) |
| j      | 2.01 (singlet) |
| k or l | 1.29 (doublet, \( J = 7 \text{ cps} \)) |
| k or l | 1.36 (doublet, \( J = 7 \text{ cps} \)) |
| 3 x OH | 5.14 (broad singlet) disappeared on adding \( D_2O \) |
The structure 21 (R = R' = H) of Compound D was also derived from its fragmentation pattern (primary cleavages from either end of the molecule given) as shown (p. 70). The ions marked with an asterisk have had their compositions established by high resolution measurements. The mass spectrum is shown in fig. 21 (p. 81). The fragmentation ions at m/e 200, 201 and 273 being too weak in intensity (1.2, 0.5 and 0.6% respectively of the base peak) have not been shown in the plot in fig. 21.

Partial Structure of F-polyacetyl methyl ester

Since the F-polyacetyl methyl ester contained only two double bonds, only three products were expected from its ozonolysis reaction. However, the formation of four products suggested that it was possibly a mixture. The possibility of Compound B-1 polyacetate being an integral part of the F-polyacetyl methyl ester could be excluded because it was not obtained in a mole proportion of any of the Compounds A, C and D.

After establishing the structures of the ozonolysis products of F-polyacetyl methyl ester, the next objective was to determine the sites of attachments of these products in the F-polyacetyl methyl ester. This objective was achieved as follows:

The ozonolysis of F-polyacetyl methyl ester (n.m.r. fig. 22) (prepared by reacetylation of F-N-acetyl methyl ester) as described before except now reduction of the ozonide with sodium borodeuteride and careful neutralisation of the reaction mixture with hydrochloric
Fig. 22. 100MHz NMR Spectrum of Compound F-Polyacetylmethyl ester in CDCl₃
acid at 0° followed by immediate work-up and reacetylation of the mixture gave a mixture of three products. These were separated by silica gel chromatography and are designated as Compounds A-d₁, C-d₂ and D-d₁.

Structure of Compound A-d₁

It was identical with Compound A by t.l.c. The n.m.r. (A-60) spectrum of Compound A-d₁ differed from that of Compound A in having a C-methyl singlet at 1.18 ppm instead of a doublet in that of Compound A. Also the number of protons in the 4.6 - 5.18 ppm region of the spectrum were four as compared to five in that of Compound A.

Thus, it became evident that the Compound A-d₁ differed from Compound A in having a deuterium atom in the place of a hydrogen atom at the carbon bearing a methyl and an acetoxyl group. This allowed us to propose the structure 13c (p. 48) for Compound A-d₁. Thus, the site of attachment in Compound A with one of the other two compounds could be established.

Partial Structure of Compound C-d₂

It was identical with Compound C by t.l.c. The n.m.r. (A-60) spectrum of Compound C-d₂ apparently differed from that of Compound C in having a C-methyl singlet at 1.18 ppm instead of a doublet in that of Compound C. Also, the number of protons in the 3.9 - 4.5 ppm and 4.7 - 5.3 ppm region of the spectrum were five and eight respectively as compared to six and nine respectively in that of Compound C.

The Compound C-d₂ thus differed from Compound C in having two deuterium atoms in the place of two hydrogen atoms. One of the
deuterium atoms certainly was on a carbon atom bearing a methyl and an acetoxy group. The other was most probably on the terminal methylene because the proton replaced by this deuterium atom lay in the 3.9 - 4.5 ppm region of the spectrum of Compound C. This region most probably contained the methylene protons attached to carbons bearing an acetoxy group and the methine protons on carbons bearing an ether linkage (60).

Thus, a tentative structure $20 (R^1 = D, \ p. \ 70)$ could be proposed for Compound C-$d_2$. This, therefore, established the sites of attachment in Compound C with Compounds A and D.

Structure of Compound D-$d_1$

Compound D-$d_1$ was identical with Compound D by t.l.c. The n.m.r. (HA-100) spectrum of Compound D-$d_1$ differed from that of Compound D only in having a pair of doublets centered at 3.85 and 3.89 ppm respectively, corresponding to one proton in place of a two proton doublet at 3.86 ppm in that of Compound D. These results thus required Compound D-$d_1$ to differ from Compound D in having a deuterium atom in place of a hydrogen atom at the terminal methylene carbon.

The presence of a pair of doublets at 3.85 and 3.89 ppm respectively in the spectrum of Compound D-$d_1$ suggested that it was a mixture of two diaastereoisomers, differing in the configuration of the terminal carbon atom. These diaastereoisomers thus would have the structures $21 (p. \ 70)$ with $R = H$, $R^1 = D$ and $R = D$, $R^1 = H$.

This, therefore, allowed us to fix the site of attachment in Compound D with Compound C.
The sodium borodeuteride experiment not only fixed the sites of attachment in Compounds A, C and D (except the relative orientation of Compound C with respect to the other two) in F-polyacetyl methyl ester but also showed that these compounds were the only integral parts of F-polyacetyl methyl ester because a compound similar to "Compound B" obtained from the ozonolysis of the F-polyacetyl methyl ester described before was not detected at all. This suggested that the F-polyacetyl methyl ester used before was a mixture.

At this point it became possible to propose a partial structure for the major constituent of Compound F-polyacetyl methyl ester. A complete structure of Compound F-polyacetyl methyl ester is proposed in the methylation studies of primycin described in the second chapter.

In concluding the study of the alkaline hydrolysis of primycin, it is important to mention that the ozonolysis of Compound F-polyacetyl methyl ester, prepared from crude Compound F gave besides the four compounds A, "B", C and D another compound now called Compound iso-D. A comparison of the n.m.r. (HA-100) spectra of Compound D and that of iso-D (Table 7) in CDCl₃ solvent showed that these compounds had the same gross structure. However, a downfield shift by 1.67 ppm of the N-H absorption band and a slight shift in that of N-acetyl methyl and various other absorption bands in the spectrum of Compound iso-D relative to that of Compound D suggested that these two compounds possibly differed in the configuration at one or the other asymmetric center.
$32, \quad R = Ac$
### Table 7

N.m.r. Chemical Shifts of Compound iso-D in CDCl₃

<table>
<thead>
<tr>
<th>δ (ppm)</th>
<th>No # protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>.91</td>
<td>6</td>
<td>(doublet, J = 7 cps) two sec. O-methyls</td>
</tr>
<tr>
<td>1.34-1.74</td>
<td>4H</td>
<td>(multiplet) methylene protons</td>
</tr>
<tr>
<td>2.0, 2.03, 2.10</td>
<td>12</td>
<td>(three singlets) three 0-acetyl methyls; one N-acetyl methyl</td>
</tr>
<tr>
<td>1.82-2.4</td>
<td>2 (excluding the 0-acetyl and N-acetyl methyls)</td>
<td>(multiplet) methine protons</td>
</tr>
<tr>
<td>3.28</td>
<td>2</td>
<td>(an apparent quartet, J, 6 cps) Ac-NH-CH₂⁻</td>
</tr>
<tr>
<td>3.86</td>
<td>2</td>
<td>(doublet, J = 6 cps) CH-CH₂-OAc</td>
</tr>
<tr>
<td>4.92</td>
<td>1</td>
<td>(multiplet) CH-OAc</td>
</tr>
<tr>
<td>4.98</td>
<td>1</td>
<td>(doublet of a doublet J, 4.5 and 7 cps) OAc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH-CH-CH&lt;</td>
</tr>
<tr>
<td>8.56</td>
<td>1</td>
<td>(apparent triplet, J, 6 cps) Ac-NH-CH₂⁻</td>
</tr>
</tbody>
</table>
Chapter 2

Methylation Studies of Primycin

In the foregoing discussion it was possible to establish the complete structures of Compound D, that of one of the homologues of Compound A, and the partial structure of Compound C. However, it was not possible to establish the relative orientation of Compound C with respect to Compounds A and D in the F-polyacetyl methyl ester.

Besides this, since Compound F was derived from the strong alkaline treatment of primycin, the other problem was to find whether, besides the conversion of the guanidine moiety of primycin to the free amine of Compound F, an integral part of primycin had been lost during this transformation. Moreover, the mode of derivation from primycin of the free carboxylic group in Compound F remained an integral part of the above problem. These problems were successfully investigated in the methylation studies of primycin to be described in this chapter.

Although the methylation of norprimycin with methyl iodide and silver oxide in dimethylformamide solvent did not meet with success as found by Aberhart (49), Dr. Fehr found that the methylation of primycin under these conditions gave a mixture of three main products now called $M_1$, $M_2$ and $M_3$. These were separated by silica gel chromatography and were obtained as almost colorless oils.
Partial Structure of Compound M₁

Compound M₁ was neutral and appeared homogeneous by t.l.c. The infrared spectrum showed strong absorption bands at 1720 and 1645 cm⁻¹. These could be due respectively to an ester carbonyl (Primycin does not contain an aldehyde or a ketone function) and an amide carbonyl. However, since there was no absorption in the N-H region, the amide was possibly tertiary. There was also a very strong band at 1090 cm⁻¹ (broad) due to the ether functions in the molecule.

Compound M₁ analysed satisfactorily for C₇₁H₁₃₄N₂O₁₈. This was based on the assumption that there were two nitrogen atoms in the molecule. However, since Compound M₁ could not be volatalized in the mass spectrometer a confirmation of this molecular formula was not possible.

The n.m.r (HA-100) spectrum (fig. 23) of Compound M₁ was not structurally revealing. It showed a 0.78 - 1.03 ppm multiplet assigned to ca. four (assumed) C-methyls; a 1.13 - 1.83 ppm multiplet due to methylene and methine protons; a broad singlet at 1.62 ppm possibly due to C-methyls on carbons bearing a double bond; a broad singlet at 2.79 ppm corresponding to 9 protons relative to the C-methyls, assigned to three N-methyls; singlets at 3.24, 3.30, 3.33, 3.34, 3.38, 3.39, 3.42 and 3.49 ppm overlapping with a multiplet were assigned to a number of methyls of methyl ethers; a broad singlet at 5.00 on a multiplet, and the weak absorption bands at 5.13 - 5.35 which were nicely separated in the 220 MHz spectrum*(5.0 - 5.31 ppm region shown) might be due respectively to the anomeric proton of arabinose and vinylic protons.

* Spectrum ran by Dr. M.C. Woods at the Ontario Research Foundation, Toronto.
Fig. 23. 100 MHz NMR spectrum of Compound M in CDCl₃
A flow sheet showing the derivation of $M_1$ and the related compounds is shown in fig. 24.

Partial ozonolysis of Compound $M_1$ followed by reduction of the ozonide with sodium borohydride and then work-up as described in the case of F-polyacetyl methyl ester, but without reacetylation of the product mixture, gave a mixture of two main products now called "Compound $M_1-a$" and Compound $M_1-b$. These were separated by silica gel chromatography.

"Compound $M_1-a$"

It was a colorless viscous liquid and appeared homogeneous by t.l.c., but on acetylation with acetic anhydride and pyridine at room temperature gave a mixture of two products now called Compound $M_1-a_1$ and Compound $M_1-a_2$. These were separated on silica gel and were obtained as colorless liquids.

Structure of Compound $M_1-a_1$

The infrared spectrum of Compound $M_1-a_1$ showed strong absorption bands at 1735 and 1240 cm$^{-1}$ assigned to acetoxy functions. However, the presence of any other ester carbonyl group could not be ruled out from this spectrum. A strong band at 1645 cm$^{-1}$ was assigned to an amide carbonyl group and was possibly due to a tertiary amide because there was no absorption in the N-H region. The presence of ether functions in the molecule was shown by a strong absorption band at 1090 cm$^{-1}$.

The chemical shifts of the various resonance signals in the n.m.r.
Fig. 24
Derivation of M₁ and the related compounds
(HA-100) spectrum (fig. 25) of Compound M₁-a₁ and their possible assignments are given in Table 8. From Table 8 it is clear that Compound M₁-a₁ contained five C-methyls, two O-acetates, three N-methyls and five O-methyls. The presence of two broad doublets at 3.91 and 4.05 ppm due to two protons showed that the methylene carbon bearing an acetoxy group had an asymmetric center adjacent to it so that the two methylene protons were magnetically non-equivalent. The presence of a two proton multiplet centered at 5 ppm showed that besides a methine proton on a carbon bearing an acetoxy group, there was a methine proton on a carbon bearing an ester function other than the acetoxy group.

The presence of part structures -CH₂OAc and >CH-OAc in Compound M₁-a₁ suggested that these were the sites of attachment of Compound M₁-a₁ with the other fragments of Compound M₁. Furthermore, the presence of a sec. C-methyl at 1.20 ppm in the spectrum of Compound M₁-a₁ which was absent in that of Compound M₁ showed that this methyl group in Compound M₁ was on a carbon bearing a double bond. This is in agreement with the results obtained from the ozonolysis and sodium borodeuteride reduction experiment of F-polyacetyl methyl ester.

These results suggested that Compound M₁-a₁ contained the methyl ether derivative corresponding to Compounds A and D of F-polyacetyl methyl ester joined through an ester linkage.

Since the Compound A carried the carboxylic function of Compound F, it was reasonable to assume that the same fragment in the ether series of compounds carried the carboxylic function and was involved in ester formation with one of the secondary hydroxyl groups of the fragment
Table 8

N.m.r. Chemical Shifts of Compound M<sub>1</sub>-a<sub>1</sub>

<table>
<thead>
<tr>
<th>δ (ppm from TMS)</th>
<th>No # protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>.85-1.09</td>
<td>12</td>
<td>(multiplet), 4, C-CH&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>1.20</td>
<td>3</td>
<td>(doublet, J = 6.5 cps) sec. C-CH&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>1.25-2.0</td>
<td>ca. 29</td>
<td>(multiplet) methylene and methine protons</td>
</tr>
<tr>
<td>2.04, 2.05</td>
<td>6</td>
<td>(two singlets) 2, 0-acetyl methyls</td>
</tr>
<tr>
<td>2.78, 2.80, 2.81</td>
<td>9</td>
<td>(three singlets) 3, N-methyls</td>
</tr>
</tbody>
</table>
| 2.90-3.28        | 7            | (multiplet)  \[
\text{OCH}_3 \quad 0 \\
5, \quad \text{CH}_2 \quad 1, \quad \text{C-N-CH}_2 \\
\]
| 3.31, 3.33, 3.42, 3.43 | ca. 15 | (four singlets) 5, 0-methyls |
| 3.91, 4.05       | 2            | (two broad doublets centered at) -CH<sub>2</sub>-OAc |
| 5.0              | 2            | (broad multiplet centered at) 1, -CH-OAc; 1, -CH-O-C=O |
analogous to Compound D. But which one of the two possible hydroxyl
groups was involved in the ester formation could not be answered from
the n.m.r. data.

Compound M₁-a₁ analysed satisfactorily for C₄₅H₈₆O₁₂N₂ (Mol. wt.
846), confirmed by mass spectrometry. However, besides the molecular
ion at 846 m/e, the mass spectrum (fig. 26) showed the presence of two
relatively weak ions at m/e 860 and 874. These were obviously the one
and two carbon homologues of the molecular ion. There were also quite
intense peaks at m/e 831 (M⁺-CH₃) composition C₄₄H₈₆N₂O₂; 774 (M⁺-C₃H₆NO
i.e. -C=N(CH₃) composition C₄₂H₈₀NO₁₁.

The complete structure of Compound M₁-a₁ could be derived from
its fragmentation pattern. The primary cleavages from either end of
the molecule are shown. The ions marked with an asterisk have had their
compositions established by high resolution measurements.

Compound M₁-a₂

The infrared spectrum of Compound M₁-a₂ was very similar to that of
Compound M₁-a₁, except that the intensities of the absorption bands at
2930 and 1090 cm⁻¹ were relatively higher than in those of Compound
M₁-a₁. These results suggested that Compound M₁-a₂ had a larger number
of methylenes and methoxyl groups than Compound M₁-a₁.

The chemical shifts in the n.m.r. (A-60) spectrum of Compound M₁-a₂
and their possible assignments are given in Table 9. From Table 9 it
became apparent that Compound M₁-a₂ represented the Compound M₁ in which
<table>
<thead>
<tr>
<th>δ (ppm)</th>
<th>No # protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.85-1.10</td>
<td>12</td>
<td>(multiplet) four C-CH$_3$</td>
</tr>
<tr>
<td>1.20</td>
<td>3</td>
<td>(doublet, J = 6.5 cps) sec. C-CH$_3$</td>
</tr>
<tr>
<td>1.25-1.92</td>
<td>45-46</td>
<td>(multiplet, with a broad singlet at 1.63 ppm lower in height as compared to a similar singlet in Compound M$_1$ itself) partly due to methylene and methine protons.</td>
</tr>
<tr>
<td>2.01, 2.03</td>
<td>6H</td>
<td>(two singlets) 2, O-acetyl-methyls</td>
</tr>
<tr>
<td>2.09-2.75</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.81</td>
<td>9</td>
<td>(singlet) 3, N-methyls</td>
</tr>
<tr>
<td>3.10-3.55</td>
<td>ca. 39</td>
<td>(multiplet with singlets at 3.20, 3.35, 3.41 ppm and 3.5) ca. 13, O-methyls</td>
</tr>
<tr>
<td>3.55-4.75</td>
<td>-</td>
<td>(multiplet) partly due to a number of -CH-OCH$_3$</td>
</tr>
<tr>
<td>4.75-5.55</td>
<td>4</td>
<td>(multiplet) partly due to -CH-OAc. A broad singlet at $\sim$ 5 ppm and a broad doublet at 5.36 ppm (J $\sim$ 10 cps)</td>
</tr>
</tbody>
</table>
33  \( R = \text{CH}_3 \)
only one double bond was cleaved during the ozonolysis experiment and
the diol produced acetylated. The presence of a double bond in Compound
$M_1-a_2$ was indicated by a broad doublet ($J \approx 10$ cps) at 5.36 ppm assigned
to vinylic protons. The presence of a sec. methyl doublet at 1.20 ppm
as in the case of Compound $M_1-a_1$ substantiated the hypothesis that
Compound $M_1-a_2$ was formed by the cleavage of at least one double bond
in Compound $M_1$. The presence of a broad singlet at 1.63 ppm also
present in the spectrum of Compound $M_1$ suggested that there was at
least one methyl group on a carbon bearing a double bond in Compound
$M_1-a_2$.

Compound $M_1-a_2$ analysed satisfactorily for $C_{75}H_{142}N_{2}O_{22}$. This is
consistent with the proposal that Compound $M_1-a_2$ represented Compound
$M_1$ in which only one double bond was cleaved and the diol acetylated.
It was not investigated any further.

Partial Structure of Compound $M_1-b$

The infrared spectrum of Compound $M_1-b$ showed a broad and strong
absorption band centered at 3440 cm$^{-1}$ due to hydroxyl groups. A very
strong band at 1090 cm$^{-1}$ was assigned to the ether functions.

The chemical shifts of the various resonance signals in the n.m.r.
(A-60) spectrum of Compound $M_1-b$ are given in Table 10. From Table 10
it became evident that the Compound $M_1-b$ was related to Compound C, in
which at least eight O-acetates were replaced by O-methyls and the
others were free hydroxyls.

Acetylation of Compound $M_1-b$ with acetic anhydride and pyridine at
Table 10
N.m.r. Chemical Shifts of Compound M₁-b

<table>
<thead>
<tr>
<th>$\delta$ (ppm from TMS)</th>
<th>No # protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.18</td>
<td>3</td>
<td>(doublet, J = 6.5 cps) sec. C-CH₃</td>
</tr>
<tr>
<td>1.3-1.8</td>
<td>16</td>
<td>(multiplet) methylene protons</td>
</tr>
<tr>
<td>2.4-2.9</td>
<td>ca. 3</td>
<td>(lump, partially disappeared on adding D₂O) partly due to hydroxyl protons</td>
</tr>
<tr>
<td>3.3-3.5</td>
<td>24</td>
<td>(singlets at 3.35, 3.41, 3.45 ppms) 8, O-methyls</td>
</tr>
<tr>
<td>3.5-3.95</td>
<td>9-10</td>
<td>(multiplet) partly due to $\text{CH-OCH}_3$</td>
</tr>
<tr>
<td>4.0-4.3</td>
<td>1</td>
<td>(multiplet) $\text{CH-O-}$</td>
</tr>
<tr>
<td>5.29</td>
<td>1</td>
<td>(broad singlet) $\text{C-O}$</td>
</tr>
</tbody>
</table>


room temperature gave a diacetate called Compound M\textsubscript{1}-b diacetate, obtained as a colorless syrup.

The infrared spectrum showed the presence of acetoxy (strong band at 1735 and 1240 cm\textsuperscript{-1}) and ether functions (strong band at 1090 cm\textsuperscript{-1}). There was no absorption in the hydroxyl region.

The chemical shifts of the various resonance signals in the n.m.r. (HA-100) spectrum of Compound M\textsubscript{1}-b diacetate and their assignments are given in Table II. These n.m.r. data allowed proposal of a partial structure \(\text{34}\) for Compound M\textsubscript{1}-b diacetate and the corresponding diol for Compound M\textsubscript{1}-b. The presence of the arabinose in the furanose form having the \(\alpha\)-glycoside linkage was assumed from the partial structure of Compound C. This was also evident from the presence of a singlet at 5.19 ppm due to the anomeric proton in the spectrum of Compound M\textsubscript{1}-b diacetate.

This partial structure of Compound M\textsubscript{1}-b diacetate confirmed the indication in the first chapter that the terminal carbon atoms bearing the oxygen functions of Compound C were involved in the double bond formation.

In the mass spectrum of Compound M\textsubscript{1}-b diacetate a molecular ion could not be obtained. The peak of highest mass was found at 632 m/e with the composition \(C\text{\textsubscript{32}}H\text{\textsubscript{56}}O\text{\textsubscript{12}}\), which might be formed by the loss of two molecules of methanol from the parent ion expected at 696 m/e. The expected molecular weight 696 was calculated from the molecular formula of Compound C by replacing 8 acetoxy groups by 8 methoxy groups.
<table>
<thead>
<tr>
<th>$\delta$ (ppm from TMS)</th>
<th>No. # protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.19</td>
<td>3</td>
<td>(doublet, J = 6.5 cps) sec. C-CH$_3$</td>
</tr>
<tr>
<td>1.26-1.86</td>
<td>16</td>
<td>(multiplet) methylene protons</td>
</tr>
<tr>
<td>2.0, 2.03</td>
<td>6</td>
<td>(two singlets) 2, 0-acetyl methyls</td>
</tr>
<tr>
<td>2.78</td>
<td>ca. 1</td>
<td>(multiplet)</td>
</tr>
<tr>
<td>3.29-3.46</td>
<td>24</td>
<td>(singlets at 3.30, 3.32, 3.34, 3.38 and 3.42) 8, 0-methyls</td>
</tr>
<tr>
<td>3.46-3.81</td>
<td>ca. 7</td>
<td>(multiplet) -CH-0CH$_3$</td>
</tr>
<tr>
<td>3.93-4.42</td>
<td>4</td>
<td>(multiplet) -CH$_2$-0Ac; 2 -CH-O-</td>
</tr>
<tr>
<td>4.71-5.06</td>
<td>1</td>
<td>-CH-0Ac</td>
</tr>
<tr>
<td>5.19</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

N.m.r. Chemical Shifts of Compound M$_1$-b diacetate
A compound identical with Compound M₁-b was also obtained from the ozonolysis of Compound M₂ to be described later and is studied more thoroughly in that section.

The foregoing discussion showed that the Compound M₁ comprised the compounds M₁-a₁ and M₁-b joined together through two double bonds at the sites indicated in each case. From these results it was thus reasonable to propose the partial structure \( \text{35} \) for Compound M₁.

The urea moiety in Compound M₁ must have been generated by partial hydrolysis of the guanidine moiety of primycin by the weakly basic silver oxide during the methylation reaction.

The partial structure \( \text{35} \) of Compound M₁ gave the first experimental evidence that the primycin was a macrolide. A comparison of the partial structures of Compound M₁ and that of Compound F-polyacetyl methyl ester \( \text{32} \) also suggested that the derivation of Compound F from primycin simply involved opening of the macrolide ring and the complete hydrolysis of the guanidine moiety to the primary amine.

**Structure of Compound M₂**

A flow sheet showing the derivation of the compounds from M₂ hydrochloride to be discussed in this section is shown in fig. 27. These compounds were all obtained as colorless oils.

Compound M₂ was isolated as its hydrochloride. On t.l.c. it gave a long zone rather than a round spot and it was, therefore, not possible to ascertain its purity by this method. The infrared spectrum showed a
Fig. 27

Derivation of Compounds from $M_2$ hydrochloride
broad and strong absorption band centered at 3360 cm\(^{-1}\) due to amino and possibly hydroxyl groups. A strong band at 1720 cm\(^{-1}\) also found in the spectrum of Compound M\(_1\) was assigned to an ester (lactone) carbonyl. The strong bands at 1600 and 1580 cm\(^{-1}\) were assigned to the guanidine moiety. A strong band at 1090 cm\(^{-1}\) was assigned to ether functions in the molecule. A weak band at 1660 cm\(^{-1}\), shoulder of the 1600 cm\(^{-1}\) band might be due to carbon-carbon double bonds.

The n.m.r. (HA-100) spectrum (fig. 28) of Compound M\(_2\)-hydrochloride was quite similar to that of Compound M\(_1\), except that a broad singlet at 2.79 ppm present in that of Compound M\(_1\) was absent in the spectrum of Compound M\(_2\)-hydrochloride and instead a broad singlet at 3.21 ppm appeared, which was assigned to N-methyls. However, no new information could be obtained from this spectrum.

Compound M\(_2\)-hydrochloride analysed correctly for C\(_{71}\)H\(_{135}\)N\(_5\)O\(_8\)·HCl (Mol. wt. 1339). Potentiometric titration of Compound M\(_2\) free-base (methanol solvent) gave an equivalent weight of about 1383 for the hydrochloride. The pH at half the neutralization equivalence point (i.e. \(pK_a\)) was 11.15, in good agreement with that reported by Aberhart (49) for Primycin free base (\(pK_a\) ca. 11.2).

Partial ozonolysis of Compound M\(_2\)-hydrochloride following the conditions for Compound M\(_1\) gave a neutral product, identical by t.l.c., i.r. and n.m.r. spectra with Compound M\(_1\)-b obtained from the ozonolysis of Compound M\(_1\) and two basic products now called Compounds M\(_2\)-a and M\(_2\)-b.
Structure of Compound M₁-b

Compound M₁-b was obtained as a colorless viscous liquid after distillation at 140° under 0.001 mm pressure. It analysed satisfactorily for C₃₀H₆₀O₁₂ and the mass spectrum gave a molecular ion at m/e 612. There were also quite intense peaks at m/e 581 (M⁺-CH₂OH) and 175 (composition C₈H₁₅O₄). The latter fragmentation ion represented the composition of methylated arabinose which had lost the glycosidic oxygen.

Acid hydrolysis of Compound M₁-b afforded a major product now called Nor-M₁-b and two minor products which could not be isolated in the pure state.

Structure of Compound Nor-M₁-b

The infrared spectrum of Compound Nor-M₁-b showed strong bands at 3435 cm⁻¹ (assigned to hydroxyl groups) and 1075 cm⁻¹ (assigned to ether functions). There was no absorption in the carbonyl region.

The chemical shifts of the various resonance signals in the n.m.r. (HA-100) spectrum of Compound Nor-M₁-b and their possible assignments are given in Table 12. The two downfield multiplets containing six and three protons were assigned to the methine protons on carbons bearing a methoxyl and those bearing a hydroxyl group.

The mass spectrum of Compound Nor-M₁-b gave a molecular weight of 438 and molecular formula C₂₂H₄₆O₈. This value is consistent with the loss of a methylated arabinose moiety from Compound M₁-b.

Compound Nor-M₁-b reacted with periodate reagent to give one compound now called Seco Nor-M₁-b. This showed that Compound Nor-M₁-b
Table 12

N.m.r. Chemical Shifts of Compound Nor-M$_1$-b

<table>
<thead>
<tr>
<th>$\delta$ (ppm from TMS)</th>
<th>No # protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.17</td>
<td>3</td>
<td>(d, $J \sim 6.5$ cps) sec. C-methyl</td>
</tr>
<tr>
<td>1.31-1.86</td>
<td>16</td>
<td>(multiplet) methylene protons</td>
</tr>
<tr>
<td>2.99-3.21</td>
<td>3</td>
<td>(lump, disappeared on adding D$_2$O) 3 hydroxyls</td>
</tr>
<tr>
<td>3.32, 3.36</td>
<td>15</td>
<td>(two singlets) 5, 0-methyls</td>
</tr>
<tr>
<td>3.39-3.56</td>
<td>6</td>
<td>(multiplet, a broad singlet at 3.44 ppm)</td>
</tr>
<tr>
<td>3.56-3.83</td>
<td>3</td>
<td>(multiplet with a broad singlet at 3.66 ppm)</td>
</tr>
</tbody>
</table>
contained at least one vicinal glycol unit. Attempts to purify Seco Nor-M1-b on silica gel led to partial decomposition and only a very small amount of the pure product could be isolated. The infrared spectrum showed the presence of an hydroxyl (3440 cm⁻¹), an aldehyde (strong band at 1725 cm⁻¹ and weak bands at 2820 and 2710 cm⁻¹) and ether (strong band at 1090 cm⁻¹) functions.

Since Compound Nor-M1-b contained only three hydroxyl groups (see Table 12) and the periodate oxidation product also contained a hydroxyl group, there could be only one vicinal glycol unit in Compound Nor-M1-b.

The mass spectrum of Seco Nor-M1-b (fig. 29) gave a molecular weight of 406, and molecular formula C₂₁H₄₂O₇. This being 32 mass units lower than that of Nor-M1-b, the presence of a part structure 36 was suggested in that of Compound Nor-M1-b. The structure 37 proposed for Compound Seco Nor-M1-b was deduced from its fragmentation pattern. The primary cleavages from either end of the molecule are shown. The ions marked with an asterisk have had their compositions established by high resolution measurements. The m/e 319 ion peak being too weak in intensity (0.5% of the base peak) has not been shown in the plot in fig. 29.

From the structure of Seco Nor-M1-b, the structures 38 and 39 (R = CH₃, R¹ = H) for Compounds Nor-M1-b and M1-b, respectively, could easily be deduced from the periodate and the acid hydrolysis reactions of Compounds Nor-M1-b and M1-b respectively. The structure of Compound M1-b thus fixed the position of the arabinose unit and also the sites of attachments of Compound M1-b in the parent molecules M₂₂-hydrochloride and M1. This also allowed proposal of the complete structure 39 (R = R¹ = Ac) for Compound C (see Chapter 1).
Fig. 29. Mass Spectrum of Compound Seco Nor-M1-b
$\text{NaIO}_4$ treatment of compound 36 results in the formation of carboxylic acid and formic acid.

$36 \xrightarrow{\text{NaIO}_4} \text{carboxylic acid} + \text{formic acid}$

$\sim 37, R = \text{CH}_3$

$\sim 38, R = \text{CH}_3$
Compound M₂-a

Compound M₂-a was isolated as its hydrochloride. The infrared spectrum showed the presence of an amino and possibly hydroxyl (3300 cm⁻¹) functions; an ester carbonyl (1720 cm⁻¹ as found in Compound M₂ hydrochloride); carbon-carbon double bond(s) (weak band at 1645 cm⁻¹); guanidine (strong bands at 1600 cm⁻¹ and 1575 cm⁻¹), and ether (strong band at 1080 cm⁻¹) functions.

The n.m.r. (A-60) spectrum of Compound M₂-a was very similar to that of Compound M₂ hydrochloride except that it had a three proton doublet at 1.18 ppm (J = 6.5 cps) assigned to a secondary C-methyl. This methyl group was, therefore, on a carbon bearing a double bond in Compound M₂ hydrochloride. This assignment is in agreement with such findings in Compound M₁ and F-polyacetyl methyl ester as described earlier. Besides this a broad hump at 2.5 - 2.8 ppm which disappeared on adding D₂O was present in the spectrum of Compound M₂-a. This was obviously due to hydroxyl protons.

The i.r. and n.m.r. spectra of Compound M₂-a suggested that it was possibly the Compound M₂ hydrochloride with one double bond cleaved.

Alkaline hydrolysis of Compound M₂-a followed by acetylation and then diazomethane treatment afforded a major product now called Compound M₂-a₁ and a minor mixture of two products, which was not examined further.
Structure of Compound $M_2-a_1$

The infrared spectrum of Compound $M_2-a_1$ showed the presence of acetoxyl (strong bands at 1735 and 1245 cm$^{-1}$); an ester (strong band at 1730 cm$^{-1}$ and a weak band at 1185 cm$^{-1}$) other than the acetoxyl; possibly carbon–carbon double bond (weak band at 1645 cm$^{-1}$) and ether (strong band at 1090 cm$^{-1}$) functions. There was no absorption in the hydroxyl region. This i.r. data suggested that the Compound $M_2-a_1$ did not contain the nitrogen component of Compound $M_2-a$.

The chemical shifts of the various resonance signals in the n.m.r. (HA-100) spectrum of Compound $M_2-a_1$ and their possible assignments are given in Table 13. The spectrum being very complex it was not possible to assign all the signals. However, Table 13 shows that Compound $M_2-a_1$ contained the corresponding ethers of Compounds A and C of the F-poly-acetyl methyl ester series joined through a double bond. Besides this it is also clear that Compound $M_2-a_1$ contained only one acetoxyl group.

Mild alkaline hydrolysis of Compound $M_2-a_1$ followed by diazomethane treatment gave the corresponding alcohol which showed the presence of a hydroxyl group in the infrared spectrum. The n.m.r. (HA-100) spectrum of this alcohol was very complex and similar to that of Compound $M_2-a_1$. However, it showed the absence of the acetoxyl methyl singlet present at 2.0 ppm in the spectrum of Compound $M_2-a_1$.

Two phase chromic acid oxidation of Compound $M_2-a_1$ alcohol gave essentially one compound now called Compound $M_2-a_2$. The infrared spectrum showed a strong but unsymmetrical band at 1710 cm$^{-1}$. This was
<table>
<thead>
<tr>
<th>$\delta$ (ppm from TMS)</th>
<th>No # protons</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>.90</td>
<td>6</td>
<td>(an apparent triplet, $J \approx 6.5$ cps) a primary C-CH$_3$ (doublet, $J \approx 6.5$ cps) sec. C-CH$_3$</td>
</tr>
<tr>
<td>1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.18</td>
<td>3</td>
<td>(doublet, $J \approx 6.5$ cps) sec. C-CH$_3$</td>
</tr>
<tr>
<td>1.23-1.82</td>
<td>ca.42</td>
<td>(multiplet with a broad singlet at 1.63 ppm) methylene protons and $\equiv C$-$\overset{{\cdot}}{\text{CH}}_3$</td>
</tr>
<tr>
<td>2.0</td>
<td>3</td>
<td>(singlet) O-acetyl methyl</td>
</tr>
<tr>
<td>2.27</td>
<td>1-2</td>
<td>(an apparent broad triplet, $J \approx 7$ cps)</td>
</tr>
<tr>
<td>2.42-2.68</td>
<td>1</td>
<td>(multiplet)</td>
</tr>
<tr>
<td>3.16</td>
<td>3</td>
<td>(broad singlet) possibly $\overset{\text{O}}{\underset{\text{C-0-CH}}{\text{H}}}_{3}$</td>
</tr>
<tr>
<td>3.24-3.51</td>
<td>ca 36</td>
<td>(multiplet) ca. 12 O-methyls</td>
</tr>
<tr>
<td>3.51-3.77</td>
<td>8-9</td>
<td>(multiplet) $\overset{\text{I}}{\text{CH-OR}}$</td>
</tr>
<tr>
<td>3.99-4.22</td>
<td>1</td>
<td>(multiplet)</td>
</tr>
<tr>
<td>4.50</td>
<td>1</td>
<td>(doublet of a doublet $J$, 5.5 and 9.5 cps)</td>
</tr>
<tr>
<td>4.77-5.02</td>
<td>2</td>
<td>(a singlet at 4.96 ppm and a doublet of a doublet centered at 4.88 ppm $J = 5.5$, 12 cps)</td>
</tr>
<tr>
<td>5.36</td>
<td>1</td>
<td>(broad doublet, $J \approx 10$ cps)</td>
</tr>
</tbody>
</table>
probably due to a ketone carbonyl group. Since excess chromic acid was used, an aldehyde function could be excluded. However, the presence of an ester carbonyl group in addition to the above could not be ruled out.

The n.m.r. (HA-100) spectrum of Compound M₂-a₂ was very complex and quite similar to that of Compound M₂-a₁ alcohol, except that the sec. C-methyl doublet at 1.18 ppm present in the spectrum of the alcohol had disappeared in that of Compound M₂-a₂ and a three proton sharp singlet at 2.10 ppm had appeared. This was obviously due to a methyl of a methyl ketone function. Furthermore, a two proton apparent triplet (J~7 cps) at 2.41 ppm also appeared in the spectrum of Compound M₂-a₂. These results suggested the presence of a part structure CH₃-C-C₆H₄⁻ in that of Compound M₂-a₂ and hence the corresponding alcohol unit in Compound M₂-a₁ alcohol.

Since it was pointed out earlier that Compound M₂-a₁ contained the corresponding ethers of Compounds A and C of the F-polyacetyl methyl ester series, joined together through a double bond, structures 40 and 41 could be proposed for Compound M₂-a₂ and M₂-a₁ respectively. The structure of Compound M₂-a₂ thus fixed the site of attachment of Compound M₁-b with the nitrogen component of the parent molecule M₂ hydrochloride.

Ozonolysis of Compound M₂-a₂ followed by sodium borohydride reduction gave two major products. One of these was identical by t.l.c., i.r. and mass spectrum with Compound M₁-b. The other major product now called "Compound M₂-a₃" showed three almost overlapping spots on t.l.c. An attempt to separate these by chromatography met with no success.
40 \quad R = CH_3, \quad R' = Arabinose

41 \quad R = CH_3, \quad R' = Arabinose
However, the mass spectrum of the mixture showed a quite intense peak at m/e 490 and two relatively weak peaks at m/e 504 and 518. These were obviously the carbon homologues. The peak of mass 490 represented the molecular weight of the polyol A-I (see Chapter 1) in which all the hydroxyl groups except one were methylated. Thus, a possible structure \( \text{\textsuperscript{42}} \) could be proposed for the major constituent of "Compound \( M_2^\text{-a}_3 \)." This was confirmed by mass spectrometry, the primary cleavages on either end of the molecule being shown.

Besides these two products, a third but minor product called Compound \( M_2^\text{-a}_4 \) was also isolated. The amount being small its i.r. and n.m.r. spectra could not be obtained. However, the mass spectrum gave a molecular weight of 348. Compound \( M_2^\text{-a}_4 \) might have been formed from the ozonolysis and sodium borohydride reduction of a compound similar to Compound \( M_2^\text{-a}_2 \), having a double bond in a position \( \alpha, \beta \) to the carboxethoxy group. This compound might have been formed by an elimination of the methoxyl group, \( \beta \) to the carboxethoxy group, from either compound \( M_2^\text{-a} \) or \( M_2^\text{-a}_1 \) during their alkaline treatment and was possibly present in admixture with these compounds. This hypothesis thus required structure \( \text{\textsuperscript{43}} \) for Compound \( M_2^\text{-a}_4 \). This structure for Compound \( M_2^\text{-a}_4 \) was confirmed by mass spectrometry, the primary cleavages from either end of the molecule being shown.

The structures of the ozonolysis products of Compound \( M_2^\text{-a}_2 \) thus confirmed the proposed structures of Compounds \( M_2^\text{-a}_1 \) and \( M_2^\text{-a}_2 \).
$42 \quad R = \text{CH}_3$

$43 \quad R = \text{CH}_3$
Structure of Compound $M_2^-$b

Compound $M_2^-$b was isolated as its hydrochloride. The infrared spectrum showed the presence of an amino and possibly hydroxyl (broad and strong band at 3300 cm$^{-1}$), an ester (strong band at 1725 cm$^{-1}$ and a weak band at $\sim$1160 cm$^{-1}$) and ether (strong band at 1095 cm$^{-1}$) functions.

The n.m.r. (A-60) spectrum was very complex because of extensive overlapping signals. However, there were absorption signals at 0.82 - 1.05 ppm (12H, multiplet) assigned to four C-methyls; 1.15 ppm (3H, doublet, J = 6.5 cps) assigned to a sec. C-methyl; 1.25 - 2.25 ppm (hump) assigned to methylene and methine protons; 2.4 - 2.70 (hump, partially disappeared on adding D$_2$O) assigned partly to hydroxyl and possibly N-H protons; 3.1 ppm (broad singlet) assigned to N-methyls; 3.25 - 3.55 ppm (multiplet with broad singlets at 3.33 and 3.43 ppm) assigned to a number of O-methyls; 3.55 - 4.2 ppm (multiplet) assigned to the methine protons on carbons bearing an ether function; and 4.85 - 5.3 ppm ($\sim$1H, multiplet) possibly due to a methine proton on a carbon bearing an ester function.

Alkaline hydrolysis of Compound $M_2^-$b followed by diazomethane treatment gave three neutral products. One of these was identical by t.l.c. and mass spectrometry with "Compound $M_2^-$a$_3$" (described earlier). The second compound (obtained in low yield) on acetylation with acetic anhydride and pyridine gave a compound identical by t.l.c. and mass spectrometry with Compound $M_1^-$a$_1$.

The third compound now called Compound $M_2^-$b$_1$ showed in the infrared
spectrum, the presence of an N-H and possibly O-H (strong band at 3420 cm\(^{-1}\)); an amide carbonyl (strong band at 1620 cm\(^{-1}\)) and ether (1080 cm\(^{-1}\)) functions.

Acetylation of Compound \(M_2-b_1\) gave a diacetate. The infrared spectrum showed the presence of acetoxy (strong bands at 1725 cm\(^{-1}\) and 1240 cm\(^{-1}\)), an amide carbonyl (strong band at 1620 cm\(^{-1}\)) and ether (1085 cm\(^{-1}\)) functions.

Structure proposed for Compound \(M_2-b_1\) diacetate could be derived from its n.m.r (HA-100) spec. (fig. 30). The chemical shifts of the various protons are given in Table 14. The presence of a one proton doublet of a doublet at 5.11 ppm required an acetoxy group at the carbon bearing the methine proton c. The presence of a two proton multiplet at 3.81 - 4.01 ppm, which appeared to be the AB part of an ABX pattern, required an acetoxy group at the terminal methylene having an asymmetric carbon with a methine proton adjacent to it. Thus, the position of the methoxyl group could be fixed. These n.m.r. data required structure for Compound \(M_2-b_1\) diacetate.

The mass spectrum gave a molecular weight of 388 and molecular formula \(C_{19}H_{36}N_2O_6\) in agreement with the total proton count from the n.m.r. spectrum. The fragmentation pattern (the primary cleavages from either end of the molecule being shown) confirmed the proposed structure. The ions marked with an asterisk have had their compositions established.

The structures of the alkaline hydrolysis products of Compound \(M_2-b\),
\[
44, \quad R = \text{Ac} \quad R' = \text{CH}_3
\]

Table 14

N.m.r. Chemical Shifts of Compound $M_2-b_1$ diacetate

<table>
<thead>
<tr>
<th>Proton</th>
<th>( \delta ) (ppm from TMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>3.81-4.01 (multiplet, may be an AB part of an ABX system)</td>
</tr>
<tr>
<td>b, d</td>
<td>1.81-2.4 (excluding the singlet at 2.03 ppm)</td>
</tr>
<tr>
<td>c</td>
<td>5.11 (doublet of a doublet ( J = 4, 7 ) cps)</td>
</tr>
<tr>
<td>e, h</td>
<td>2.96-3.27 (multiplet)</td>
</tr>
<tr>
<td>f, g</td>
<td>1.31-1.71 (multiplet)</td>
</tr>
<tr>
<td>i, j, k</td>
<td>2.78 and 2.80 (two singlets)</td>
</tr>
<tr>
<td>l, m</td>
<td>.88, .91 (two doublets, ( J = 7 ) cps)</td>
</tr>
<tr>
<td>n</td>
<td>3.32 (singlet)</td>
</tr>
<tr>
<td>2, O-acetyl methys</td>
<td>2.03 (broad singlet)</td>
</tr>
</tbody>
</table>

---

The table provides the n.m.r. chemical shifts for various protons in the compound $M_2-b_1$ diacetate, with specific ranges and notes on the multiplicity and coupling constants. The shifts are measured in parts per million (ppm) from tetramethylsilane (TMS) as the internal reference.
FIG. 30. 100 MHz NMR Spectrum of Compound $M_2$ by diacete in CDCl$_3$. 

-7.5 ppm (5)
-5.0
-3.0
-1.5
0.0
1.5
2.0
2.5
3.0
3.5
4.0
5.0
5.25
5.5

-2.5

125
thus suggested structure 45 for the major constituent of Compound M₂-b. hydrochloride. The possibility of attachment of the ester linkage with the terminal carbon atom of the nitrogen component has already been excluded from the structure of Compound D-d₁ described in the first chapter and from the structure of Compound M₁-a₁ described in the section of Compound M₁.

The structure of Compound M₂-b. hydrochloride thus fixed the site of the lactone ring in the parent molecule M₂. hydrochloride. Further, the structures of Compounds M₁-a, M₂-a₁ and M₂-b. hydrochloride allowed deduction of complete structures for Compound M₂, hydrochloride and M₁. These are shown by structures 46b and 46a respectively. Compound M₁ thus differed from Compound M₂, hydrochloride in having a trimethylated urea moiety in the place of the trimethylated guanidine hydrochloride grouping. Compound M₂, hydrochloride on treatment with 85% hydrazine hydrate using the procedure of Shemyakin (62) could be converted into Compound M₁ in high yield*. This, therefore, substantiated the correlation between the structures of Compound M₁ and that of Compound M₂, hydrochloride.

From the structure of Compound M₁ or that of M₂, hydrochloride it also became possible to propose a complete structure 47a for Compound F-polyacetyl methyl ester, hence that of Compound F (47b).

At this point it is important to point out that the structures 46a, 46b, 47a, 47b represent the structures of the lowest of the three

* I should like to thank Dr. Motl for this observation
\[ R = \text{CH}_3 \]
\[ \text{a, } R = \text{CH}_3, \quad R = \text{CH}_3 \quad \text{N} \quad \text{H} \quad \text{CH}_3 \]

\[ \text{b, } R = \text{CH}_3, \quad R = \text{CH}_3 \quad \text{N} \quad \text{H} \quad \text{CH}_3 \quad \text{Cl} \quad \text{H}_2 \]

\[ \text{c, } R = \text{CH}_3, \quad R = \text{CH}_3 \quad \text{N} \quad \text{H} \quad \text{CH}_3 \quad \frac{1}{2} \left[ \text{SO}_4 \text{H}^+ \right] \quad \text{H}_2 \]
\[ n-C_4H_9 \]

\[ \text{47} \quad Y = \begin{array}{c} \text{CH}_3 \text{OR} \\ \text{OR} \end{array} \begin{array}{c} \text{OR} \\ \text{CH}_3 \end{array} \]

\[ \begin{array}{c} \text{CH} \text{CH} \begin{array}{c} \text{CH}_2 \end{array} \text{N} \text{R}^\prime \\ \text{R} \end{array} \]

\[ a, \quad R = R'' = \text{AC} \]

\[ R = \text{CH}_3, \quad R = H \]

\[ b, \quad R = R = R = R = H \]
possible carbon homologues of Compound M₁, M₂ hydrochloride, F-polyacetyl methyl ester and Compound F respectively. Similarly, the structures of all other compounds which showed the presence of homologation represent the structure of the lowest carbon homologue.

Structural proposals for the other homologues of these compounds cannot be made at this point. This is because the exact site of homologation is not known at present, although from the foregoing discussion about the structure of Compounds F-polyacetyl methyl ester, M₁ and M₂ hydrochloride, it is clear that the homologation occurs in the component containing the carbonyl carbon of the lactone grouping.

Although the representation of three homologues of a compound by the same name of the compound, e.g. Compound M₁ is not justified, it has been done to avoid the introduction of more complex nomenclature than that already used.

**Compound M₃**

Compound M₃ was isolated as a hydrochloride. On t.l.c. it gave a long zone rather than a round spot and it was, therefore, not possible to ascertain its purity by this method.

The potentiometric titration of Compound M₃ (methanol solvent) using the procedure of Compound M₂ gave an equivalent weight of ca. 1467 and pKₐ ca. 11.35.

Compound M₃ was not further investigated.
Structure of the Major Constituent of Primycin

The largest compound from primycin whose structure has been determined is \( M_2 \)-hydrochloride. If it is assumed that no loss of small fragments has occurred during the methylation then the major constituent of primycin should also have the structure \( \sim 46c \). The equivalent weight of the primycin represented by structure \( \sim 46c \) is 1127. This is in good agreement with the value of 1143 ± 2 reported for primycin by Aberhart (49).

The most likely fragments which could have been lost during the methylation of primycin are carboxylic acids if involved in ester linkages with the hydroxyl groups of primycin. Since, if a guanidine moiety could be hydrolysed to urea during the methylation (formation of Compound \( M_1 \)), then esters could also be hydrolysed to the corresponding acids and alcohols, although the macrolide ring appeared to have resisted the ring opening. Further, since alkaline hydrolysis of primycin has been reported by Aberhart (49) to give \( n \)-caproic acid as the only major volatile acid, the only likely acid fragment lost from the major constituent of primycin would be the \( n \)-caproic acid.

At present it is not known whether the \( n \)-caproic acid isolated from the alkaline hydrolysis of primycin was really involved in an ester linkage or was produced by retro-aldolization of the \( \beta \)-hydroxy lactone moiety of primycin. In view of the fact that the yield of the \( n \)-caproic acid depended on the severity of the alkaline conditions and the maximum yield was ca. 16% under the conditions used for the preparation of Compound F, the caproic acid does not seem to be
involved in ester linkage in the major constituent of primycin.

These results, thus, required 46c to be the most likely structure for the major constituent of primycin. This structure also accommodates almost all the functional groups of primycin reported by Aberhart (49).

Primycin is thus a non-polyene macrolide, having the largest ring size reported so far for such a class of antibiotics. The presence of a guanidine moiety and that of an arabinoose unit also makes it unique amongst the known macrolides whose structures have been established to date.
EXPERIMENTAL

Melting points were obtained on a Reichert Kofler hot stage and are uncorrected. The infrared (i.r.) and ultraviolet (u.v.) spectra were recorded by means of Beckman IR 10 and Cary Model 14M spectrometers respectively. The solvents for i.r. and u.v. spectra are indicated in parenthesis. Nuclear magnetic resonance spectra (n.m.r.) were recorded on Varian HA-100 and Varian A-60 spectrometers in deuterochloroform solvent unless otherwise indicated. The chemical shifts are reported as $\delta$ values in ppm from tetramethylsilane, used as an internal standard. Mass spectra were recorded by Professor D.B. MacLean on a Model CEC 110B Mass spectrometer at McMaster University, Hamilton. Thin layer chromatography (t.l.c.) was carried out on Camag Kieselgel DF-5 and Merck Silica gel GF-254 and the same materials were used for column chromatographic separations. The solvent systems used for thin layer and column chromatography are indicated in brackets. Gas chromatographic (v.p.c.) analyses were made using an F & M Model 700 instrument equipped with 6' x $\frac{1}{8}$" columns packed with 10% FFAP on 60-80 mesh Chromosorb P. Solvents were removed under reduced pressure using a rotary evaporator and sodium sulphate was used as a drying agent. Unless noted otherwise, the samples for analysis were dried at room temperature in vacuo for two to five days and were analysed by A.B. Gygli in Toronto. Molecular weights by osmometer were determined
on a Hewlett-Packard Model 301A vapor pressure osmometer.

The reactions marked with an asterisk were originally worked out by Dr. T. Fehr and his method was used as the basis for the experiments performed by the author.
Chapter 1

Alkaline Hydrolysis Studies of Primycin

*Compound F*

Primycin (5 g), potassium hydroxide (150 ml, 5N aq.) and n-butanol (a few drops, to avoid frothing) were refluxed under nitrogen for 4 hr. On cooling, the solution was made detectably acidic to pH paper with hydrochloric acid (conc.) and evaporated to dryness in vacuo. The residue was extracted with a mixture of CHCl₃:MeOH (4:1) and the extract was evaporated to dryness in vacuo. Column chromatography on silica gel (GF-254, 400 g, eluant: CHCl₃:MeOH : conc. ammonia 12:8:1) gave the major product (2.137 g, 42.7 wt. %) as a colorless froth, now called Compound F. \( \nu_{\text{max}} \) (Nujol mull) 3300 (broad and strong), 1650 (weak), 1560, 1075 and 975 cm\(^{-1}\). Molecular wt. by osmometer (MeOH solvent): 1100.

Anal. Found: C, 57.78; H, 9.77; N, 1.56.

*Catalytic Hydrogenation of Compound F*

Compound F (86 mg) was hydrogenated over pre-reduced platinum oxide (30 mg) in glacial acetic acid (5 ml) at atmospheric pressure. After 26 hr the hydrogen uptake was 4.44 ml at S.T.P. (a molecular wt. of 1010 for a 2 mole uptake requires 4.44 ml at S.T.P.) and still
continued at a very slow rate as the reaction period was extended.

*Acetylation of Compound F*

A mixture of Compound F (600 mg), dry pyridine (5 ml) and acetic anhydride (5 ml) was kept at room temperature for 16 hr and then evaporated to dryness in vacuo. The residue (syrup) was treated with water and the product extracted into chloroform. The chloroform extract was washed successively with hydrochloric acid (1N), aq. sodium bicarbonate (5%), finally water and then dried and evaporated to dryness in vacuo giving a syrup (969 mg). Column chromatography on silica gel (GF-254, 40 g, eluant: CHCl₃:MeOH 9:1) yielded the main product (717 mg) as a nearly colorless sticky glass now called F-Polyacetate. \( \nu_{\text{max}}^{\text{CHCl}_3} \) 3400, 2940, 2850, 1730, 1660, 1440, 1320, 1225 and 1020 cm⁻¹.

Anal. Calcd. for C₈₄H₁₃₃NO₃ (mol. wt. 1683): C, 59.87; H, 7.95; N, 0.83. Found: C, 59.79; H, 8.09; N, 0.89.

Molecular wt. by osmometer (MeOH solvent): 1700.

**Estimation of Number of O-Acetates in F-Polyacetate**

F-Polyacetate (54.8 mg) was treated with sodium hydroxide solution (5 ml, 0.2N) at room temperature for 16 hr. The excess base was back titrated potentiometrically with 0.1N HCl.

Equivalence point = 5.25 ml

Blank titre = 9.85 ml

The difference 4.6 ml was calculated to be equivalent to 14.3 equivalents of alkali per mole of F-Polyacetate
(mol. wt., 1700)

Two other determinations gave 14.4 and 15.97 equivalents respectively.

*Potentiometric Titration of F-Polyacetate

F-Polyacetate (57.9 mg) was dissolved in methanol (a few ml) and percolated through a 10 X 1 cm. column of Dowex 50W-X2, 50-100 mesh, H⁺ form. The column was washed with more methanol (100 ml) and the combined effluent solution was titrated potentiometrically with .1N NaOH.

Titre = 0.90 ml

1 equivalent F-Polyacetate = 1605

Another experiment gave equivalent wt. = 1602

*F-Polyacetate methyl ester

Treatment of F-Polyacetate with diazomethane in ether solution gave a less polar product (colorless syrup) denoted as F-Polyacetate methyl ester. $\nu_{\text{max}}$ (CHCl₃) 3450, 3030, 2950, 2870, 1730, 1665, 1520, 1430, 1375, 1250, 1110, 1020 and 970 cm⁻¹. $\lambda_{\text{max}}$ MeOH 199 mλ (end absorption, $\epsilon = 16,960$ for M = 1700). N.m.r. (A-60) spectrum S:

0.88 (12H, broad doublet, J ~ 6.5 cps); 1.15-1.85 (multiplet); 1.98, 2.00, 2.07 (45H, each singlet); 3.68 (3H, s); 4.30 (2-3H, multiplet); 4.55-5.2 (~16H, multiplet) and 6.30 (1H, broad triplet, J ~ 6 cps).

The number of O-acetates in F-Polyacetate methyl ester determined as for F-Polyacetate were found to be 15.2 and 15.0 respectively in two different estimations, assuming mol. wt. 1700.
F-N-acetyl methyl ester

The solutions (after estimation of number of O-acetates) from the alkaline hydrolysis of F-Polyacetate methyl ester were combined and made detectably acidic with hydrochloric acid (2N) and extracted with n-butanol. The n-butanol extract was washed with water, dried and evaporated in vacuo giving a colorless froth, which exhibited two spots on t.l.c. The froth on diazomethane treatment gave essentially one spot on t.l.c., showing that a partial hydrolysis of the methyl ester had taken place. The diazomethane treated product was purified on silica gel (GF-254, eluant: CHCl₃:MeOH 2:1) giving a colorless sticky froth, homogeneous by t.l.c. This product is called F-N-acetyl methyl ester. N.m.r. (HA-100) spectrum (pyridine-d₅) δ: 0.83 (multiplet); 1.0 (multiplet); 1.38-2.0 (multiplet); 2.03 (3H, singlet); 3.68 (3H, singlet); 4.0-6.0 (multiplet, partially disappeared on adding D₂O), an apparent one proton broad triplet at 8.34 ppm (J~ 6 cps) disappeared on adding D₂O.

Periodate Titrations of F-N-acetyl methyl ester

F-N-acetyl methyl ester (25.95 mg) was dissolved in methanol (15 ml) and an aqueous solution of sodium metaperiodate (5 ml, 0.05M) was added. The volume was made 100 ml by adding water (slightly milky solution). The solution was then allowed to stand at room temperature in the dark. After intervals, 10 ml aliquots were taken, made alkaline with saturated aqueous sodium bicarbonate solution (5 ml) and then a standard sodium arsenite solution (5 ml, 0.007M) was added followed by
aq. potassium iodide solution (1 ml, 20% aq.). The resulting solution was allowed to stand in the dark for 5-10 min, and then titrated with standard 0.01N I₂ (B.D.H.) solution to a slight yellow color end point. The results are summarized in the table below.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Sample (ml)</th>
<th>Blank (ml)</th>
<th>Difference (ml)</th>
<th>Moles (10^{-4}) for (M = 1100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2.22</td>
<td>2.12</td>
<td>0.10</td>
<td>0.20</td>
</tr>
<tr>
<td>10.5</td>
<td>2.35</td>
<td>2.22</td>
<td>0.13</td>
<td>0.27</td>
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<tr>
<td>24</td>
<td>2.60</td>
<td>2.38</td>
<td>0.22</td>
<td>0.45</td>
</tr>
<tr>
<td>48</td>
<td>2.92</td>
<td>2.70</td>
<td>0.22</td>
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<tr>
<td>70.5</td>
<td>3.20</td>
<td>2.98</td>
<td>0.22</td>
<td>0.45</td>
</tr>
</tbody>
</table>

In a second determination similar results were obtained in that in 24 hr 0.40 moles of \(10^{-4}\) were consumed per mole of F-N-acetyl methyl ester taken and remained constant up to 70.5 hr of the reaction period.

The untitrated reaction mixture was extracted with \(n\)-butanol. The \(n\)-butanol extract was washed with water, dried and evaporated to dryness in vacuo to give a sticky solid residue. On t.l.c. this residue showed two spots, one of them (\(~50\)% looked similar to that of the starting material and the other, due to a less polar product ran in a streak. On acetylation, the mixture gave one spot having the same \(R_f\) value as that of F-Polyacetaate methyl ester (obtained by reacetylation of F-N-acetyl methyl ester).

Titrations of F-N-acetyl methyl ester with lead tetraacetate in
acetic acid solvent gave an uptake of .45 moles lead tetraacetate per mole of F-N-acetyl methyl ester in 10 hr, .75 moles in 48.5 hr and 0.88 moles in 97 hr. The products recovered from the untitrated reaction mixture after 97 hr reaction period exhibited the same t.l.c. behaviour as in the periodate case, except that the compound similar to the starting material was now present in smaller amounts. Also the acetylation results were exactly identical with the periodate case.

**Acid Hydrolysis of F-N-acetyl methyl ester**

A mixture of F-N-acetyl methyl ester (19.8 mg), methanol (1 ml) and hydrochloric acid (1 ml, 0.2N) was flushed with nitrogen and then heated in a closed flask on a steam bath for 5 hr. The mixture was carefully neutralised with sodium hydroxide (0.2N) and then evaporated to dryness in vacuo. The residue on cellulose t.l.c., using aniline phthalate spray, showed a red brown spot identical in R_f value with that of an authentic sample of D(-)-arabinose.

To a major portion of the residue in methanol (2 ml), p-nitrophenylhydrazine (5 mg) was added and then heated on a steam bath for 20 min. The mixture on t.l.c. showed the presence of a spot having an R_f value identical with that of an authentic sample of D(-)-arabinose p-nitrophenylhydrazone. This was separated by t.l.c. (eluant: CHCl_3: MeOH 4:1) and crystallized from methanol to give yellow needles (0.1 mg) m.p. 183-185°. The mixed m.p. with an authentic sample of the D(-)-arabinose p-nitrophenylhydrazine derivative was undepressed whereas the mixed m.p. with an authentic sample of the L(+) arabinose
The minor portion of the residue was acetylated with acetic anhydride and pyridine at room temperature. The acetylated mixture on t.l.c. showed a spot (eluant: CHCl₃:MeOH 97:3) having the same R₇ value and charring color (sulfuric acid spray) as that of an authentic sample of D(-)-arabinose tetraacetate.

*Methylation of F-N-acetyl methyl ester

A mixture of F-N-acetyl methyl ester (233 mg), dimethylformamide (10 ml), methyl iodide (2 g) and silver oxide was stirred at room temperature for 4 hr. After adding 2 g more of methyl iodide, the stirring was continued for an additional 16 hr. The precipitate formed was centrifuged and washed repeatedly with dimethylformamide. The centrifugate was combined with the washings and then diluted with water (50 ml). After adding sodium cyanide (1 g), the products were extracted into chloroform. The chloroform extract was washed with water, dried and evaporated in vacuo to give an oily residue (274 mg) which showed strong 0-H band in the i.r. The reaction was repeated first for 1 day, and again for 3 days. The work-up in each case was as above. The oily residue (230 mg) after the third treatment was chromatographed on silica gel (GF-254, 50 g, eluant: CHCl₃:MeOH:Petrol 3:1:6) to give F-N-acetyl methyl ester polymethyl ether (76 mg) as a colorless syrup. \( \nu_{\max} \) (neat film) 2930, 2870, 1740, 1650, 1460, 1375, 1270 (weak), 1235 (weak), 1190 and 1090 cm⁻¹.

Anal. Calcd. for C₇₂H₁₃₇NO₁₉ (mol. wt. 1319): C, 65.5; H, 10.38;
N, 1.06. Found: C, 65.46; H, 10.51; N, 1.20.

*Ozonolysis of F-polyacetyl mether ester

F-polyacetyl methyl ester (813 mg) was dissolved in methanol (20 ml) and cooled in a mixture of dry ice and acetone. A slow stream of ozone was passed through the mixture until a slight blue color persisted in the solution. Sodium borohydride (2.5 g) in methanol (20 ml) was added in portions and the mixture was then kept at room temperature for 20 min. The solution was made acidic with hydrochloric acid (2N) and extracted with chloroform. The chloroform extract was washed with water, dried and evaporated in vacuo to give a viscous oil (776 mg) which was acetylated with acetic anhydride and pyridine. The acetylated products mixture was chromatographed on silica gel (GF-254, 350 g, eluant: CHCl₃:MeOH 9:1) to give four major products A (258 mg), "B" (61 mg), C (218 mg) and D (142 mg), all as colorless oils.

**Compound A**

It had $\nu_{\text{max}}$ (CHCl₃) 3000, 2960, 2870, 1725, 1460, 1440, 1375, 1230 (broad and strong) and 1025 cm⁻¹.

Anal. Calcd. for C₃₃H₅₆O₁₂: C, 61.47; H, 8.75. Found: C, 62.02; H, 8.89.

**Hydrolysis of Compound A**

The number of O-acetates in Compound A by a method similar to that described for F-polyacetate methyl ester was found to be 5.15 for a molecular wt. of 644.
The solution (after the estimation of number of O-acetates) from the alkaline hydrolysis of Compound A was made acidic with hydrochloric acid (2N) and then extracted with a mixture of chloroform/n-butanol (9:1). The organic extract was washed with water, dried and evaporated in vacuo to give a solid residue which exhibited two spots on t.l.c. (eluant: CHCl₃:MeOH 4:1). Treatment of the mixture with diazomethane gave only one spot showing that a partial hydrolysis of the methyl ester group had taken place. The diazomethane treated product was crystallized from chloroform/petrol (b.p. 60-80°) to give a white microcrystalline polyol A-1 m.p. 101-103°, $\nu_{\text{max}}$ (CHCl₃) 3380 (broad and strong), 2930, 2860, 1725, 1460, 1440, 1380, 1170 and 900 cm⁻¹.

Anal. Calcd. for $C_{23}H_{46}O_7$: C, 63.56; H, 10.67. Found: C, 63.91; H, 10.52.

**Periodate Titrations of Polyol A-1**

Periodate titrations of polyol A-1 were done exactly as described for F-N-acetyl mether ester. It consumed 0.65 moles of periodate per mole after 1 hr; 0.67 moles after 5 hr; 0.73 moles after 7 hr and again after 15 hr.

**Acetaldehyde from the Periodate Reaction of Polyol A-1**

Polyol A-1 (22.3 mg, $5.15 \times 10^{-5}$ moles) in a 5 ml round bottomed flask equipped with a nitrogen inlet tube was dissolved in pure dioxane (0.5 ml) by warming on a steam bath. After cooling, nitrogen was bubbled very slowly for 20 min and then an aqueous solution of sodium metaperiodate (0.3 ml, .3 M) followed by water (0.5 ml) was introduced
through the nitrogen inlet tube. A slow stream of nitrogen was passed through the mixture and the acetaldehyde formed was trapped in a hot aqueous dioxane solution of dimedone (30.2 mg, 1 ml dioxane, 10 ml water). After 1 hr the reaction mixture showed one spot on t.l.c., different from the starting material. The excess periodate reagent was then destroyed by introducing a solution of 6.5 mg sodium arsenite in 0.5 ml water into the reaction mixture. Nitrogen was passed for an additional 3 hr and then the crystals formed in the dimedone solution were filtered off, washed with water and dried at room temperature in vacuo, yield 4 mg white crystals m.p. 136-138°. Recrystallization from aqueous ethanol gave white microplates m.p. 138-140°, undepressed on admixture with an authentic dimedone derivative of acetaldehyde. The filtrate was concentrated in vacuo to ca. 0.5 ml and then water (0.5 ml) was added, followed by ethanol (0.5 ml) and the mixture was cooled in ice water. The crystals formed were filtered off and then recrystallized in aqueous ethanol to give 2 mg more of the derivative.

Alkaline Hydrolysis of Compound A

A mixture of Compound A (39.6 mg) and potassium hydroxide (5 ml, 0.5N in ethanol:water 1:1) was refluxed under nitrogen for 5 hr. The solution was concentrated in vacuo, diluted with water and then made acidic with hydrochloric acid (dil.). The hydrolysed product was extracted into a mixture of chloroform:n-butanol (4:1) and the extract was washed with water, dried and evaporated in vacuo to give Compound A-2 as a sticky yellow glass (22 mg). T.l.c. analysis of the residue
showed one main product different from polyol A-1. Treatment of a small portion with diazomethane showed on t.l.c., mainly one spot identical in Rf value (eluant: CHCl3:MeOH 4:1) with that of polyol A-1.

Reaction of Compound A-2 with Lead tetraacetate

A mixture of Compound A-2 (6 mg), acetic acid (0.2 ml, glacial) and lead tetraacetate (12 mg in 0.3 ml acetic acid) was kept at room temperature in the dark for 2 hr. The mixture was diluted with water (0.3 ml) and then the excess reagent was destroyed by mannitol (5 mg). The mixture was evaporated to dryness in vacuo and the residue was extracted with chloroform. The chloroform extracte was washed with water, dried and evaporated in vacuo to give a viscous oil, which showed one spot on t.l.c. (eluant: CHCl3:MeOH 9:1) different from that of Compound A-2. The residue reacted with diazomethane to give a less polar product having an Rf value identical with that of Seco A-1 (obtained by the periodate reaction of polyol A-1) showing that the carboxyl group was still present in the viscous oily product.

Attempt to Lactonise Compound A-2

Compound A-2 (2 mg) was heated under nitrogen from 30-200° for 45 min. The residue on t.l.c. looked identical with the starting material and also reacted with diazomethane to give a compound having an Rf value identical with that of polyol A-1.

Reaction of Polyol A-1 with Acetic anhydride and Sodium acetate

A mixture of polyol A-1 (2.3 mg), fused sodium acetate (51.3 mg)
and acetic anhydride (1 ml) was refluxed for 24 hr. The mixture was evaporated in vacuo and the residue was treated with potassium hydroxide (1 ml, 0.5 N in MeOH:water 1:1) at room temperature for 24 hr. The mixture was diluted with water and after making acidic with hydrochloric acid (dil.), extracted with a mixture of chloroform: n-butanol (1:1) to give a solid residue (2.1 mg) which was subsequently treated with diazomethane. T.l.c. analysis (eluant: CHCl₃:MeOH 4:1) of the mixture showed three spots, the major spot (ca. 75-80%) being similar to that of polyol A-1 and the other two (ca. 15-20% and ca. 5%) being due to less polar products. Separation of the mixture by t.l.c. (eluant: CHCl₃:MeOH 4:1) gave 1.5 mg of the major product and 0.3 mg of a compound having $\lambda_{\text{max}}^{\text{MeOH}} = 217 \text{ m}\mu \ (\varepsilon \sim 9000)$. The 1.5 mg major product was treated with sodium acetate and acetic anhydride followed by potassium hydroxide, etc. exactly as described above. T.l.c. analysis of the mixture showed identical results with those obtained above. Repetition of the reaction for 4 days resulted in a complex mixture of products. A control experiment with Compound A and potassium hydroxide gave only ca. 5-10% of the elimination product showing that in the above experiment, part of the elimination was effected with potassium hydroxide.

**Treatment of Polyol A-1 with sodium metaperiodate**

Preparation of seco A-1:

A mixture of polyol A-1 (830 mg), methanol (5 ml) and sodium metaperiodate (450 mg in 5 ml water) was kept at room temperature in the
dark for 30 min. The excess periodate reagent was destroyed by adding 150 mg mannitol. The mixture was concentrated at room temperature in vacuo to remove most of the methanol. The residual solution was diluted with water and then extracted with chloroform to give an oily residue. Chromatography on Kieselgel (DF-5, 75 g, eluant: CHCl₃:MeOH 98:2) yielded Seco A-1 (644 mg) as a colorless viscous oil, homogeneous by t.l.c. \( V_{\text{max}} \) (neat film) 3400 (broad and strong), 2940, 2860, 1735, 1715, 1460, 1360, 1275, 1200, 1110, 1070, 1035, 990 cm⁻¹.

**Huang-Minlon Reduction of Seco A-1**

In a typical experiment, a mixture of Seco A-1 (109.8 mg), ethylene glycol (4 ml, Fisher Scientific), hydrazine hydrate (4 ml, 85%, Fisher Scientific) was heated at 100° for 1 hr. After cooling 660 mg solid potassium hydroxide was added in portions and after removing the condenser, the temperature was slowly raised to ca. 200°. The mixture was then refluxed at this temperature for 2.5 hr. The solution was cooled and then extracted thrice with 20 ml portions of chloroform. The chloroform extract was washed with water, dried and evaporated at room temperature in vacuo to give a solid residue (32.3 mg). The residue was sublimed at 80° under 0.01 mm pressure to give a white sublimate (26 mg) which on t.l.c. (eluant: CHCl₃:MeOH 95:5) showed essentially one spot. Crystallization from chloroform/petrol (b.p. 60-80°) afforded Compound A-3 as colorless needles (20 mg) m.p. 106-107°.

The aqueous solution was made acidic with hydrochloric acid (2N) and then extracted with four 15 ml portions of chloroform. The chloroform
extract was washed with water, dried and distilled on a steam bath using a small fractionating column, until ca. 3 ml solution was left. After treatment with diazomethane in ether, the solution was analysed by v.p.c. Only one major peak identical in retention time (ascertained by mixed injection) with that of an authentic sample of methyl n-caproate was detected. Methyl n-caprylate, n-valerate, propionate and acetate were not detected at all. However, the presence of methyl n-butyrate also could not be excluded, since the chloroform peak came at the same place. Using methyl n-caprylate as the internal standard, 9.72 mg of (75 mole % of Compound A-3) caproic acid was estimated in the mixture.

The solution remaining after the v.p.c. analysis was concentrated on a steam bath and then cooled in ice water. White crystals formed and were filtered off, yield 27.3 mg. Recrystallization from chloroform/petrol (b.p. 60-80°) afforded Compound A-4 as colorless microplates m.p. 95-98°, homogeneous by t.l.c.

**Compound A-3**

It had $\nu_{\text{max}}$ (KBr) 3340, 3230, 2900, 1450, 1385, 1360, 1140, 1110, 1070 and 935 cm$^{-1}$.

**Acetylation of Compound A-3**

Compound A-3 (24 mg) was acetylated as usual to give an oily product which was purified on Kieselgel (eluant: CHCl$_3$:MeOH 98:2) to give Compound A-3 diacetate (22 mg) as a colorless liquid. $\nu_{\text{max}}$ (neat film) 2960, 2880, 1740, 1470, 1375 (doublet), 1250, 1130, 1030 and 950 cm$^{-1}$.
Compound A-4

Acetylation of Compound A-4 as usual gave Compound A-4 triacetate as a colorless viscous liquid.

Huang-Minlon Reduction of Seco A-1 under Nitrogen

The Huang-Minlon reduction of Seco A-1 exactly as described before except using nitrogen throughout the experiment gave the same two major products, i.e. Compound A-3 and Compound A-4, besides the n-caproic acid.

"Compound B"

"Compound B", a colorless syrup, appeared homogeneous by t.l.c., but gave two products on alkaline hydrolysis as described below.

A mixture of "Compound B" (303.6 mg), methanol (10 ml) and aqueous potassium hydroxide (10 ml, 0.5N) was kept at room temperature for 23 hr. The mixture was percolated through a 30 x 1 cm column of Dowex 50W-X2, H⁺ form, 50-100 mesh and the column was washed with 200 ml methanol followed by 200 ml of a mixture of methanol:water (1:1). The effluent combined with the washings was evaporated in vacuo giving a solid residue (166.5 mg). T.l.c. analysis of the residue exhibited two spots (the mixture before passing through the cation exchanger showed the same two spots). When 1 mg of this residue was acetylated only one spot identical in Rf value with that of "Compound B" was obtained. The rest of the residue was chromatographed on Kieselgel (DF-5; eluant: CHCl₃:MeOH:Conc. ammonia 16:4:1) giving 56.4 mg of Compound B-1 and 69.1 mg Compound C-1.
Compound C-1

It was crystallized from methanol/isopropyl ether to give colorless microcrystals m.p. 161-163°. $\nu_{\text{max}}^\text{(KBr)}$ 3300 (broad and strong), 2930, 2860, 1630 (broad and weak), 1465, 1420, 1380, 1345, 1080 and 920 cm$^{-1}$. In the mass spectrum the peak of highest mass was obtained at m/e 343. There were also ions at m/e 332 ($M^+\text{-2H}_2\text{O}$), 314 ($M^+\text{-3H}_2\text{O}$), 301 ($332-\text{CH}_2\text{OH}$), 296 ($M^+\text{-4H}_2\text{O}$), 278 ($M^+\text{-5H}_2\text{O}$) and 260 ($M^+\text{-6H}_2\text{O}$).

Periodate Titrations of Compound C-1

Periodate titrations of Compound C-1 were carried out in aqueous solution using a procedure similar to that described for Compound A-1. In this case the standard reagents used were 0.05M NaIO$_4$; 0.007M NaAsO$_2$; $98.52 \times 10^{-4}$ N I$_2$ (B.D.H.) and starch was used as the indicator. The following results were obtained:

For a molecular wt. of 368 for Compound C-1, after 1.5 hr there was an uptake of 1.98 moles of periodate per mole of Compound C-1; after 10.25 hr, 2.0 moles and the uptake remained constant when the reaction period was extended to 24 hr.

Acetylation of Compound C-1

Compound C-1 was acetylated with acetic anhydride and pyridine as usual to give Compound C-1 polyacetate as a colorless syrup. $\nu_{\text{max}}$ (neat film) 2920, 2850, 1730, 1425, 1365, 1230 (broad and strong), 1120, 1020 and 940 cm$^{-1}$. The mass spectrum gave a molecular wt. of 704 and a molecular formula of C$_{33}$H$_{52}$O$_{16}$.

Anal. Calcd. for C$_{33}$H$_{52}$O$_{16}$ (mol. wt. 704): C, 56.21; H, 7.44.

Found: C, 55.85; H, 7.03.
Compound C-1 was also obtained from Compound C and is described later.

**Compound B-1**

It was crystallized from methanol/isopropyl ether to give white microcrystals m.p. 159-161°. $\nu_{\text{max}}$ (KBr) 3300 (broad and strong), 2930, 1630 (broad and weak), 1465, 1425, 1380, 1345, 1145, 1085, 1055 and 920 cm$^{-1}$. The mass spectrum gave a molecular wt. of 352 and a molecular composition of C$_{17}$H$_{36}$O$_{7}$. There were also very intense peaks at m/e 319 (M$^{+}$-H$_{2}$O-CH$_{3}$), 298 (M$^{+}$-3H$_{2}$O), 280 (M$^{+}$-4H$_{2}$O) and 262 (M$^{+}$-5H$_{2}$O).

Compound B-1 did not consume any periodate under exactly the same conditions as described for Compound C-1.

**Acetylation of Compound B-1**

Compound B-1 was acetylated with acetic anhydride and pyridine as usual to give Compound B-1 polyacetate as a colorless syrup. $\nu_{\text{max}}$ (neat film) 2920, 2850, 1725, 1425, 1360, 1230 (broad and strong) 1120, 1020 and 940 cm$^{-1}$. The mass spectrum gave a molecular wt. of 646 and a molecular formula of C$_{31}$H$_{50}$O$_{14}$.

Anal. Calc. for C$_{31}$H$_{50}$O$_{14}$ (mol. wt. 646): C, 57.57; H, 7.79.

Found: C, 56.97; H, 8.14.

**Compound C**

It was a colorless syrup. $\nu_{\text{max}}$ (CHCl$_{3}$) 3000, 2915, 1735, 1430, 1370, 1235 (broad and strong), 1040, 1025 and 980 cm$^{-1}$.

Anal. Calcd. for C$_{42}$H$_{64}$O$_{22}$ (mol. wt. 920): C, 54.77; H, 7.00.

Found: C, 54.68; H, 7.10.
The number of O-acetates determined as described for Compound A was found to be 9.5.

Alkaline Hydrolysis of Compound C

A mixture of Compound C (1.8180 g) and potassium hydroxide (60 ml, 0.5N) was kept at room temperature under nitrogen for 48 hr. The hydrolysed product not being extractable with n-butanol, the mixture was percolated through a 50 x 2 cm column of Dowex 50W-X2, H⁺ form, 50-100 mesh. The column was washed with 300 ml methanol followed by 200 ml of a mixture of MeOH:H₂O (1:1). The effluent combined with the washings was evaporated in vacuo giving a solid residue (1.0807 g). T.l.c. analysis of the mixture revealed two main products (the mixture before passing through the cation exchanger showed one main product different from these two products, (eluant: CHCl₃:MeOH:conc. ammonia 12:8:1). When 1 mg of this mixture was acetylated three spots were observed on t.l.c., one of them being identical in Rf value with that of the starting Compound C, showing that the hydrolysed Compound C had undergone a chemical transformation during its contact with the cation exchanger. The rest of the residue was chromatographed on Kieselgel (DF-5, 50 g, eluant: CHCl₃:MeOH:conc. ammonia 16:4:1) giving 545.5 mg of Compound C-1 and 187 mg of "Compound C-2".

Compound C-1

It was crystallized from methanol/isopropyl ether to give white microcrystals m.p. 161-163°, mixed m.p. with Compound C-1 obtained from the alkaline hydrolysis of "Compound B" was undepressed. They were
also identical by t.l.c. and infrared spectrum.

Periodate titrations of Compound C-1 obtained from Compound C, carried out exactly as described for Compound C-1 obtained from the "Compound B", showed after 1.5 hr an uptake of 1.95 moles of periodate for a molecular wt. of 368. The uptake remained constant when the reaction period was extended to 24 hr.

**Formaldehyde from the Periodate Reaction of Compound C-1**

The procedure used is a slight modification of that used by R.E. Reeves (63).

A mixture of Compound C-1 (9.2 mg, \(2.5 \times 10^{-5}\) moles for \(M = 368\)), water (2 ml) and aqueous sodium metaperiodate (0.25 ml, 0.3M, i.e. \(7.5 \times 10^{-5}\) moles) in a test tube was kept at room temperature in the dark for 30 min. Hydrochloric acid (0.2 ml, 1N) was then added followed by an aqueous solution of sodium arsenite (0.4 ml, 0.6M containing an equal volume of 0.6M NaOH). The alkaline solution was carefully acidified with hydrochloric acid (1N), when the solution assumed a dark yellow color. More sodium arsenite solution (.6M) was then added dropwise until the yellow color just disappeared (at this point the solution was neutral to pH paper). After this an aqueous solution of sodium acetate (2 ml, 1N) was added followed by 80 mg dimedone dissolved in 1 ml 95% ethanol (distilled from dimedone) and the mixture was heated on a water bath (temp. \(\sim 60^\circ\)) for 1 hr and then kept at room temperature overnight. The white crystals formed were filtered off, washed with water and then dried in hot air, yield 6.7 mg (91.8% of theoretical for 1 mole of
formaldehyde per mole of Compound C-1), m.p. 190–191°, undepressed on admixture with an authentic sample of formaldoxime.

In a blank experiment, no precipitate was obtained.

Acetylation of Compound C-1

It was acetylated with acetic anhydride and pyridine as usual. The product was a colorless syrup having t.l.c. behaviour, i.r. and n.m.r. spectra identical with those of Compound C-1 polyacetate obtained from the alkaline hydrolysis of "Compound B". The mass spectrum gave a molecular wt. of 704. The mass spectrum fragmentation pattern was also identical with that of the Compound obtained earlier.

Anal. Calcd, for C_{33}H_{52}O_{16} (mol. wt. 704): C, 56.21; H, 7.44. Found: C, 56.07, H, 7.76.

Preparation of Seco C-1*

A mixture of Compound C-1 (87.6 mg), water (5 ml) and aqueous sodium metaperiodate (12.5 ml, 0.05M) was kept at room temperature in the dark for 3 hr. Sodium borohydride (600 mg) was then added in portions and the mixture was kept at room temperature for another 2 hr. The mixture was just neutralized with hydrochloric acid (2N) and then extracted four times with 25 ml portions of n-butanol. The n-butanol extract was washed three times with 20 ml portions of water, dried and evaporated at room temperature in vacuo to give a dark brown residue.

* The periodate oxidation product being unstable, it was reduced with sodium borohydride in situ to give Seco C-1.
(80.4 mg). This was dissolved in methanol (few ml) and after adding a few crystals of sodium thiosulfate, the mixture was evaporated at room temperature in vacuo. The solid residue was extracted with a mixture of CHCl₃:MeOH (1:1) to give 79 mg of an almost colorless solid. Purification on Kieselgel (DF-5, 10 g, eluant: CHCl₃:MeOH 4:1) gave a white solid (64.8 mg) called Seco C-1. Crystallization (three times) from methanol/isopropyl ether gave white microcrystals (45 mg) m.p. 128-130°. \( \nu_{\text{max}} \) (KBr) 3300 (broad and strong), 2900, 1620 (very weak), 1450, 1410, 1370, 1330, 1125, 1065, 1050 and 920 cm\(^{-1}\).

**Acetylation of Seco C-1**

It was acetylated with acetic anhydride and pyridine as usual to give Seco C-1 polyacetate as a colorless syrup. \( \nu_{\text{max}} \) (neat film) 2910, 2850, 1725, 1425, 1365, 1240 (broad and strong), 1120, 1020 and 940 cm\(^{-1}\).

"**Compound C-2**"

It was crystallized from methanol/isopropyl ether to give white microcrystals m.p. 151-153°, homogeneous by t.l.c. \( \nu_{\text{max}} \) (KBr) 3300 (broad and strong), 2910, 1620 (broad), 1450, 1400, 1370, 1335, 1140, 1070, 1005 and 910 cm\(^{-1}\).

Periodate titrations of Compound C-2 were carried out exactly as described for Compound C-1. In 1.5 hr for a molecular wt. of 343, two moles of periodate per mole of "Compound C-2" were consumed. The uptake remained constant when the reaction period was extended to 24 hr. For
an uptake of three moles of periodate per mole of "Compound C-2" a molecular wt. of 515 was calculated.

Acetylation of "Compound C-2" with acetic anhydride and pyridine either at room temperature or with heating gave a syrup which exhibited two spots on t.l.c., one of them being identical in R_f value with that of Compound C, the other indicating a more polar product.

Alkaline Hydrolysis of Compound C to Compound C Polyol

A mixture of Compound C (86.9 mg), methanol (5 ml) and potassium hydroxide (5 ml, 0.5N in MeOH:H_2O 1:1) was kept at room temperature under nitrogen for 28 hr. The mixture was diluted with water (20 ml), neutralized carefully with acetic acid and then evaporated at room temperature in vacuo. The solid residue was extracted with dry pyridine to give 55 mg solid (containing some inorganic material). T.l.c. analysis showed one spot different from that of Compounds C-1 and "C-2". Acetylation of 1 mg of the product gave one spot identical in R_f value with that of Compound C. The remainder was chromatographed on Kieselgel (DF-5, 5 g, eluant: CHCl_3:MeOH:conc. ammonia 14:16:1) to give 29.5 mg of Compound C polyol having a slight yellow color. Attempts to crystallize this met with no success.

Acid Hydrolysis of Compound C Polyol

Compound C polyol (6.4 mg) was treated with hydrochloric acid exactly as described for Compound F-N-acetyl methyl ester. T.l.c. analysis of the mixture on cellulose using aniline phthalate spray, showed the presence of arabinose in the mixture. The residue was
divided into two parts as for the F-N-acetyl methyl ester case. The major part was treated with p-nitrophenylhydrazine as described before. The mixture on t.l.c. analysis showed the presence of a spot identical in $R_f$ value with that of an authentic sample of D(-)-arabinose p-nitrophenylhydrazone. However, an attempt to isolate this compound did not give a sufficiently pure sample.

The minor part was acetylated with acetic anhydride and pyridine. The product on t.l.c. gave two spots, one of these being identical in $R_f$ value with that of an authentic sample of D(-)-arabinose tetraacetate and the other, identical with that of Compound C-1 polyacetate.

**Compound D**

It was a colorless syrup having $\nu_{\text{max}}$ (CHCl$_3$) 3440, 2990 (shoulder at 2880), 1730, 1665, 1515, 1455, 1370, 1200-1250, 1020, 965 and 900 cm$^{-1}$.

Anal. Calcd. for C$_{18}$H$_{31}$NO$_7$: C, 57.89; H, 8.37; N, 3.75. Found: C, 57.49; H, 8.34; N, 3.29.

**Alkaline Hydrolysis of Compound D**

In a typical experiment a mixture of Compound D (88.1 mg), methanol (10 ml) and sodium hydroxide (10 ml, 0.2N) was kept at room temperature overnight. The hydrolysed product could not be extracted either by a mixture of chloroform/t-butanol (4:1) or t-butanol. The solution was made acidic with hydrochloric acid (1N) and then evaporated at room temperature in vacuo. The residue was chromatographed on silica gel (GF-254, 10 g, eluant: CHCl$_3$:MeOH 4:1) to give Compound D polyol (46 mg) as a colorless syrup. $\nu_{\text{max}}$ (neat film) 3300 (broad and strong),
2930 (shoulders at 2970 and 2930); 1635 (broad and strong), 1550, 1440, 1370, 1300, 1190, 1030 and 970 cm⁻¹.

Ozonolysis of F-polyacetyl methyl ester

A mixture of F-polyacetyl methyl ester (128.4 mg, prepared by acetylation of F-N-acetyl methyl ester) and methanol (5 ml) was cooled in a mixture of dry ice and acetone. A slow stream of ozone was passed through the solution until a light blue color persisted, after which 200 mg sodium borodeuteride (Merck Sharp and Dohme of Canada) was added in portions and then allowed to react at room temperature for 30 min. The solution was cooled to 0°, made detectably acidic with hydrochloric acid (2N), and the products were then immediately extracted into chloroform to give after evaporation in vacuo, 120.3 mg of a syrup.

This was acetylated with acetic anhydride and pyridine as usual. T.l.c. analysis showed the presence of three main products which were separated on silica gel (GF-254, 20 g, eluant: CHCl₃:MeOH 98:2) to give Compound A-d₁ (45.6 mg), Compound C-d₂ (66.6 mg) and Compound D-d₃ (24.2 mg) all as colorless oils.

Compound A-d₁

It was identical with Compound A by t.l.c. N.m.r. (A-60) spectrum δ: 0.90 (multiplet) and 1.18 (singlet) integrated for 9 protons; 1.20-1.93 (∼23H, multiplet); 2.05 (15H, broad singlet); 2.4-2.70 (∼1H, multiplet); 3.67 (3H, singlet); 4.60-5.18 (4H, multiplet).
Compound C-d$_2$

It was identical with Compound C by t.l.c. N.m.r. (A-60) spectrum
$\delta$: 1.18 (3H, singlet); 1.25-1.82 (~16H, multiplet); 2.00, 2.04 and
2.08 (30H, three singlets); 3.9-4.5 (5H, multiplet); 4.7-5.30 (8H,
multiplet).

Compound D-d$_1$

It was identical with Compound D by t.l.c. N.m.r. (HA-100) spectrum
$\delta$: 0.93 (6H, doublet, J = 6.5 cps); 1.35-1.78 (4H, multiplet); 1.88-
2.32 (14H, a singlet at 1.97 due to an N-acetyl methyl, two singlets at
2.03, 2.05 due to three O-acetyl methyls); 3.23 (2H, an apparent quartet,
J~6 cps); a pair of broad doublets centered at 3.85 (J~5.5 cps) and
3.89 (J~6.5 cps) integrated for one proton; 4.86 (1H, multiplet);
4.99 (1H, doublet of a doublet J = 5, 6.5 cps); 6.14 (1H, broad lump).
Methylation Studies of Primycin

Methylation of Primycin

A mixture of primycin (3 g), dimethylformamide (100 ml), methyl iodide, and silver oxide (50 g, freshly prepared) was stirred at room temperature for two days. The precipitate formed was centrifuged and washed several times with chloroform. The centrifugate combined with the washings was diluted with water (150 ml) and then shaken with sodium cyanide (10 g). The products were extracted four times with 75 ml portions of chloroform. The chloroform extract was washed with water, dried and then evaporated in vacuo to give an oily residue (4.26 g). The methylation being incomplete (strong O-H band in the i.r.), the reaction was repeated with 100 ml dimethylformamide, 40 ml methyl iodide and 50 g silver oxide for 5 days at room temperature. Working-up as described above gave 4.3 g of an oily residue. The residue in a few ml of methanol was percolated through a 30 x 1 cm column of Dowex 1-X2, OH^- form, 50-100 mesh. The column was washed with 100 ml methanol and the alkaline effluent was neutralised with hydrochloric acid. Evaporation at room temperature in vacuo gave 4.2 g of a viscous oil. T.l.c. analysis (eluant: CHCl_3:MeOH 4:1) showed the presence of three main products now called Compounds M_1, M_2 and M_3, M_1 being the least polar and M_3
being the most polar. Chromatographic separation of the mixture on silica gel (GF-254, 500 g, eluant: CHCl₃:MeOH 95:5 followed by CHCl₃:MeOH 4:1 containing 1% by volume of 2N hydrochloric acid) afforded Compounds M₁ (777 mg); M₂ (2.097 g) and M₃ (5.42 mg) as almost colorless oils.

**Compound M₁**

It was homogeneous by t.l.c. \( \nu_{\text{max}} \) (neat film) 2920, 2810, 1720, 1645, 1490, 1455, 1375, 1090, 1000 and 940 cm\(^{-1}\).

Anal. Calcd. for C₇₁H₁₃₄N₂O₁₈ (mol. wt. 1303.8): C, 65.40; H, 10.35; N, 2.14; Found: C, 65.01; H, 10.29; N, 2.12.

*Ozonolysis of Compound M₁*

Compound M₁ (482 mg) in methanol (30 ml), cooled in a mixture of dry ice and acetone was treated with a slow stream of ozone for 40 min. Sodium borohydride (500 mg) in methanol (10 ml) was then added in portions. After 15 min at room temperature, the mixture was made acidic with hydrochloric acid (2N) and the products were extracted four times with 50 ml portions of chloroform. The chloroform extract after washing with water, drying and then evaporating in vacuo yielded an oily residue (529 mg). T.l.c. analysis showed the presence of two main products now called "Compound M₁-a" and Compound M₁-b. These were separated on silica gel (GF-254, 125 g, eluant: CHCl₃:MeOH 95:5) to give 248 mg of "Compound M₁-a" and 163 mg of Compound M₁-b.
"Compound M\textsubscript{1}-a"

It was a colorless viscous liquid, homogeneous by t.l.c. Acetylation by the usual procedure gave an oil which exhibited two spots on t.l.c. These were separated on silica gel (eluant: CHCl\textsubscript{3}:MeOH 98:2) and are called Compound M\textsubscript{1}-a\textsubscript{1} and Compound M\textsubscript{1}-a\textsubscript{2}, obtained in the ratio of 2:1.

Compound M\textsubscript{1}-a\textsubscript{1}

It was a colorless viscous liquid having $\nu_{\text{max}}$ (neat film) 2930, 2870, 2820, 1735, 1645, 1495, 1460, 1370, 1240, 1160, 1090, 1035 and 920 cm\textsuperscript{-1}.

Anal. Calcd. for C\textsubscript{45}H\textsubscript{86}O\textsubscript{12}N\textsubscript{2} (mol. wt. 846): C, 63.79; H, 10.23; N, 3.31. Found: C, 63.59; H, 10.22; N, 3.21.

Compound M\textsubscript{1}-a\textsubscript{2}

It was a colorless viscous liquid having $\nu_{\text{max}}$ (neat film) 2930, 2820, 1730, 1645, 1490, 1460, 1370, 1240 and 1090 cm\textsuperscript{-1}.

Anal. Calcd. for C\textsubscript{75}H\textsubscript{142}N\textsubscript{2}O\textsubscript{22} (mol. wt. 1423.9): C, 63.26; H, 10.05; N, 1.96. Found: C, 62.86; H, 10.23; N, 2.07.

Compound M\textsubscript{1}-b

It was a colorless viscous liquid having $\nu_{\text{max}}$ (neat film) 3440, 2930, 2820, 1460, 1375, 1190, 1090 and 1000 cm\textsuperscript{-1}.

Acetylation of Compound M\textsubscript{1}-b

Compound M\textsubscript{1}-b was acetylated by the usual method to give a
diacetate as a colorless syrup now called Compound M₁-b diacetate.

\[ \nu_{\text{max}} \text{ (neat film)} \] 2930, 2820, 1735, 1455, 1370, 1240, 1190, 1090 and 1040 cm\(^{-1}\).

Anal. Calcd. for \( C_{34}H_{64}O_{14} \) (mol. wt. 696.8): C, 58.60; H, 9.25. Found: C, 58.82; H, 9.23.

**Compound M₂**

It was a colorless viscous liquid obtained as its hydrochloride. It had \[ \nu_{\text{max}} \text{ (neat film)} \] 3360 (broad), 2920, 2820, 1725, 1660 (weak), 1600, 1580, 1455, 1405, 1370, 1250, 1090, 1000 and 950 cm\(^{-1}\). O.r.d. of Compound M₂-hydrochloride did not show a Cotton effect (260-300 nm region) showing that the carbonyl band at 1725 in the i.r. spectrum was not due to a ketone function in the molecule.

Anal. Calcd. for \( C_{71}H_{135}N_{9}O_{17} \cdot \text{HCl} \) (mol. wt. 1339): C, 63.67; H, 10.23; N, 3.13; Cl, 2.64. Found: C, 63.58; H, 10.57; N, 3.04; Cl, 2.63.

*Potentiometric titration of Compound M₂*

Compound M₂-hydrochloride (231.5 mg) in methanol (a few ml) was percolated through a 20 x 1 cm column of Dowex 1-X2, OH\(^-\) form, 50-100 mesh. The column was washed with 100 ml methanol and the alkaline effluent was directly titrated with standard hydrochloric acid (0.1N) at the potentiometer.

**Titre = 1.67 ml**

This gave an equivalent wt. of 1383 for the hydrochloride.
pH at half the neutralization equivalence point (i.e. pKα) = 11.15.

*Ozonolysis of Compound M₂*

Ozonolysis of Compound M₂ was carried out by the procedure as described for Compound M₁. T.l.c. analysis of the products mixture showed the presence of three main products now called Compounds M₁-b, M₂-a and M₂-b, Compound M₁-b being the least polar and Compound M₂-b being the most polar. The mixture was separated into basic and neutral products by passing its solution in methanol through a column of Dowex 50W-X2. The neutral products which passed through the column were obtained by evaporating the effluent in vacuo. The basic products absorbed in the column were obtained by washing the column with hydrochloric acid (2N) followed by methanol. The acidic effluent was concentrated at room temperature in vacuo and then after diluting with water, made just alkaline with sodium hydroxide (2N) and then extracted with chloroform to give the basic products. The basic as well as the neutral products were further purified by silica gel (GF-254, eluant: CHCl₃: MeOH 4:1 and 9:1 respectively) chromatography. From 944 mg of Compound M₂-hydrochloride, 187 mg Compound M₁-b, 417 mg Compound M₂-a, 170 mg Compound M₂-b and 80 mg of a mixture of the last two compounds were isolated.

Compound M₁-b

It was distilled at 140° under 0.001 mm pressure to give a colorless viscous oil, identical with Compound M₁-b by t.l.c., i.r. and n.m.r. spectra.
Anal. Calcd. for C_{30}H_{60}O_{12} (mol. wt. 612.8): C, 58.80; H, 9.86.
Found: C, 58.22; H, 9.76.

Acid Hydrolysis of Compound M$_1$-b

A mixture of Compound M$_1$-b (75.4 mg), methanol (5 ml) and hydrochloric acid (5 ml, 0.2N) was refluxed on a steam bath for 20 hr. After cooling, the solution was neutralized with sodium hydroxide and then extracted with chloroform. The chloroform extract was washed with water, dried and then evaporated in vacuo to give an oily residue (70.7 mg). Chromatography on Kieselgel (DF-5, 4 g, eluant: CHCl$_3$: MeOH 98:2) gave 49.1 mg of a compound called Nor-M$_1$-b, 7.2 mg starting material and two other products 7.8 mg and 5.6 mg respectively which could not be isolated in the pure state.

Nor-M$_1$-b

It was a colorless viscous liquid, homogeneous by t.l.c. $\nu_{\text{max}}$ (CHCl$_3$) 3435, 2935, 2825, 1455, 1380, 1235, 1180, 1075 cm$^{-1}$.

Seco Nor-M$_1$-b

A mixture of Nor-M$_1$-b (39 mg), dioxane (2 ml), sodium metaperiodate (27.3 mg) and water (0.5 ml) was kept at room temperature in the dark for 1 hr. The products were extracted four times with 10 ml portions of chloroform to give after usual work-up 34.4 mg of an oily residue. On t.l.c. it exhibited essentially one spot. Attempts to purify the product on silica gel resulted in decomposition to give several products and
only 6.5 mg of a pure product called Seco Nor-M$_2$-b could be isolated. $\nu_{\text{max}}$ (CHCl$_3$) 3440, 2990, 2930, 2820, 2710 (weak), 1725, 1460, 1380, 1240, 1185 and 1090 cm$^{-1}$.

**Compound M$_2$-a**

It was a slightly yellow viscous liquid isolated as its hydrochloride. $\nu_{\text{max}}$ (neat film) 3300 (broad), 2930, 2820, 1720, 1645 (very weak), 1600, 1575, 1460, 1405, 1375, 1250, 1080, 950 and 900 cm$^{-1}$.

**Alkaline Hydrolysis of Compound M$_2$-a**

A mixture of Compound M$_2$-a (202 mg), methanol (20 ml) and potassium hydroxide (20 ml, 10N) was refluxed for 4 hr. After cooling the products were extracted into chloroform to give 180 mg of an oily residue after evaporating the chloroform extract in vacuo. The aqueous solution was made acidic with hydrochloric acid (dil.) and then extracted with chloroform to give 14 mg of an oily residue. Both of these extracts exhibited the same t.l.c. behaviour, so were combined and then acetylated with acetic anhydride and pyridine as usual to give 200 mg of a viscous oil. This was treated with diazomethane and then analysed by t.l.c.

Three main products could be seen. An attempt to separate these on silica gel (GF-254, eluant: CHCl$_3$:MeOH 98:2) gave 99 mg of a product now called Compound M$_2$-a$_1$ and 32 mg of a mixture of two products which was not examined further.

**Compound M$_2$-a$_1$**

It was an almost colorless viscous liquid and streaked on t.l.c.
$\nu_{\text{max}}$ (neat film) 2930, 2820, 1735, 1730 (broad), 1645 (very weak), 1455, 1375, 1245, 1185, 1090, 1000 and 950 cm$^{-1}$.

Alkaline Hydrolysis Followed by Chromic Acid Oxidation of Compound M$_2$-a$_1$

A mixture of Compound M$_2$-a$_1$ (72 mg), methanol (5 ml) and potassium hydroxide (5 ml, 0.5N) was kept at room temperature for 12 hr and then worked-up as usual to give an oily residue (69.1 mg) which was then treated with diazomethane. Purification on Kieselgel (DG-5, 20 g, eluant: CHCl$_3$:MeOH 98:2) gave a colorless viscous oil (61.8 mg).

$\nu_{\text{max}}$ (CHCl$_3$) 3440, 3000, 2930, 2820, 1725 (doublet), 1640 (weak), 1450, 1375, 1190, 1090 and 1000 cm$^{-1}$. The n.m.r. (HA-100) spectrum was very complex as that of Compound M$_2$-a$_1$. However, the following obvious changes from the spectrum of Compound M$_2$-a$_1$ were observed: the three proton singlet due to an acetoxy methyl present at 2.00 ppm in that of Compound M$_2$-a$_1$ disappeared and also the two proton doublet of a doublet and a singlet at 4.77-5.02 ppm now became a one proton broad singlet. There were three C-methyls, two of these being at 0.84-.1.01 ppm (multiplet) and the third at 1.18 ppm (doublet, J = 6.5 cps).

The viscous oil (60 mg) obtained above was dissolved in ether (5 ml) and then titrated dropwise with chromic acid [prepared by following the procedure of H.C. Brown (64)] at room temperature until an orange color persisted. After 5 min, the mixture was diluted with water and then extracted with ether. The ether extract was washed with an aqueous solution of sodium bicarbonate (10%), then water, dried and then evaporated in vacuo to give an oily residue (57.4 mg). Purification on
silica gel (eluant: CHCl₃:MeOH 95:5) gave a colorless product (56.3 mg) now called Compound M₂-a₂. \( \nu_{\text{max}} (\text{CHCl}_3) \) 2930, 2830, 1710 (broad), 1455, 1090 and 1010 cm⁻¹.

Ozonolysis of Compound M₂-a₂

Compound M₂-a₂ (35.3 mg) was ozonized and the reaction mixture subjected to borohydride work-up as described earlier. The products (oil, 35.1 mg) on t.l.c. (eluant: CHCl₃:MeOH 95:5) exhibited at least six spots. Chromatography on Kieselgel (DF-5, 6 g, eluant: CHCl₃:MeOH 99:1) gave three products, one of these (17.3 mg) was identical with Compound M₁-b (t.l.c., i.r. and mass spectra). The other two products are called "Compound M₂-a₃" (1.8 mg) and Compound M₂-a₄ (1.3 mg) respectively.

"Compound M₂-a₃" on t.l.c. showed three almost overlapping spots. Compound M₂-a₄, homogeneous by t.l.c., showed the presence of a molecular ion at m/e 348 in the mass spectrum.

Compound M₂-b

Compound M₂-b isolated as its hydrochloride was a viscous liquid having \( \nu_{\text{max}} \) (neat film) 3300 (broad), 2940, 2820, 1725, 1600, 1580, 1460, 1410, 1255, 1220 (weak), 1160 (weak), 1095 (strong), 900 and 755 (very strong) cm⁻¹.

Alkaline Hydrolysis of Compound M₂-b

A mixture of Compound M₂-b (94 mg) and potassium hydroxide (15 ml,
.5N in methanol:water 1:1) was refluxed for 10 hr and the products were isolated by extraction with chloroform as usual. The products were treated with diazomethane and then chromatographed on Kieselgel (DF-5, 20 g, eluant: CHCl₃:MeOH 98:2) to give three main products, called "Compound M₂-a₃" (43 mg), Compound M₁-a₁ diol (5.2 mg) and Compound M₂-b₁ (23.2 mg).

"Compound M₂-a₃" on t.l.c. exhibited three almost overlapping spots, which could not be separated by chromatography. The mixture had \( \nu_{\text{max}} \) (CHCl₃) 3560, 3440, 2990, 2930, 2860, 1720, 1460, 1370, 1225, 1165 and 1085 cm⁻¹. The mass spectrum in the high mass region showed the presence of three molecular ions at 490, 504 and 518 m/e respectively. A similar product was also isolated from the ozonolysis of Compound M₂-a₂ as described earlier.

Compound M₁-a₁ diol had \( \nu_{\text{max}} \) (CHCl₃) 3440, 2990, 2920, 2870, 1720 (shoulder), 1695, 1620, 1500, 1460, 1380, 1205, 1160 and 1090 cm⁻¹. Acetylation with acetic anhydride and pyridine gave a colorless viscous liquid which on t.l.c. had the same \( R_f \) value as that of Compound M₁-a₁ (described earlier). The mass spectrum in the high mass region showed the presence of three molecular ions at 846, 860 and 874 m/e respectively.

\underline{Compound M₂-b₁}

It was a colorless viscous liquid. \( \nu_{\text{max}} \) (CHCl₃) 3420, 2990, 2930, 2870, 1620 (broad and strong), 1495, 1460, 1385, 1225, 1140, 1080, 975 and 920 cm⁻¹.

Acetylation by the usual method gave Compound M₂-b₁ diacetate as a
colorless syrup. \( \nu_{\text{max}} \) 2975, 2925, 1815, 1725, 1620 (broad), 1495, 1455, 1380, 1240, 1145, 1085, 1020 and 960 cm\(^{-1}\).

Reaction of Compound M\(_2\) hydrochloride with Hydrazine Hydrate

A mixture of Compound M\(_2\) hydrochloride (2.60 g), methanol (40 ml), hydrazine hydrate (65 ml, 85%) and water (26 ml) was heated at 95\(^\circ\) under nitrogen for 13 hr. The solvents were removed in vacuo and the residue was extracted with chloroform. The chloroform extract was washed successively with hydrochloric acid (1N), water, sodium bicarbonate (10%) and finally with water, dried and then evaporated in vacuo to give a brown oily residue (2.50 g). Chromatography on silica gel (GF-254, 150 g, eluant: CHCl\(_3\):MeOH 96:4 followed by MeOH containing 1% by volume of 1N HCl) gave an almost colorless oily product (1.076 g) identical with Compound M\(_1\) by t.l.c., i.r. and n.m.r. spectra.


Starting material (1.10 g) was recovered.

Compound M\(_3\)

Potentiometric titration, carried out as described for Compound M\(_2\), gave an equivalent wt. of 1467 and \(pK_a = 11.35\).

Estimation of Caproic Acid from the Alkaline Hydrolysis of Primycin

A mixture of primycin (635.5 mg, 5.7 x 10\(^{-4}\) moles for a mol. wt. of 1100) and potassium hydroxide (20 ml, 5N) was refluxed under a nitrogen
atmosphere for 4 hr. The mixture was allowed to cool to room temperature and then made acidic (pH≈2) with sulfuric acid (2N). The products were steam distilled and about 200 ml of the distillate was collected. The distillate was made alkaline with potassium hydroxide (1N) to phenolphthalein indicator and then evaporated to dryness in vacuo at room temperature. The solid residue was treated with hydrochloric acid (ca. 1N) in absolute methanol to give an acidic solution (pH≈4). This was treated with ethereal diazomethane at room temperature to give a slightly yellow solution (total vol. ca. 2.5 ml). Analysis of the mixture by v.p.c. showed the presence of methyl n-caproate (identical v.p.c. behaviour with authentic methyl n-caproate) as the only volatile ester. Using methyl n-caprylate as the internal standard, 10.82 mg (16.1 mole %) of caproic acid was estimated in the mixture.

In a control steam distillation experiment 14.5 mg (95%) of caproic acid could be accounted for from an initial amount of 15.2 mg.

When primycin was treated with 10% potassium hydroxide in aqueous methanol (50%) and refluxed on a steam bath for 4 hr, about 9.3 mole % of caproic acid was detected. With 1N KOH in aqueous methanol (50%) at 100° for 8 hr about 4.7 mole % of caproic acid was formed. Treatment of primycin with 0.5N KOH in aqueous ethanol (50%) at room temperature for 12 hr gave no caproic acid.
REFERENCES


