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The Ohio State University, Ph.D., 1974
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II. ANTIMICROBIAL AGENTS FROM HIGHER PLANTS. ISOLATION OF CANTHIN-6-ONE FROM ZANTHOXYLUM ELEPHANTIASIS AND APPROACHES TO ITS SYNTHESIS AND ANALOGS.

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By
Howard Daniel Hollis Showalter, B.A.

* * * * *

The Ohio State University
1974

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To my mother and father
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PART I

CHEMICAL IONIZATION MASS SPECTROMETRY OF MACROLIDE ANTIBIOTICS, β-LACTAM ANTIBIOTICS, AND MODEL COMPOUNDS.
INTRODUCTION

Chemical ionization mass spectrometry (CIMS) is a recent addition to available means of ion production such as electron impact and field ionization mass spectrometry. Ion production by chemical ionization consists of reaction of the substance under investigation with a known and pre-selected set of ionizing reactant ions. Thus the reactant ions are to chemical ionization what the electrons are to electron impact ionization (EI) (1).

Chemical ionization mass spectrometry had its beginnings in 1966 when Munson and Field (2) published their introductory paper on this subject. All work published in chemical ionization up to 1969 was performed on the Esso chemical physics mass spectrometer described by Field and Munson (2,3,4). Soon after, several mass spectrometers designed for electron impact ionization were modified to permit the use of instruments alternatively in the chemical and electron impact modes of ionization (5,6,7,8). Also chemical ionization mass spectra of biomolecules obtained by vaporizing the samples directly into the ion source rather than into a heated inlet system were reported (9,10). Furthermore, accurate mass measurements leading to the
assignment of elemental compositions to ions produced by chemical ionization were reported at that time (9). In 1970, yet another instrument was modified and used to introduce a different approach to gas chromatography-mass spectrometry, that of gas chromatography-chemical ionization mass spectrometry in which the carrier gas for gas chromatography is used as the reactant gas for chemical ionization mass spectrometry (11). Various modifications and applications of this technique are now commonplace (12,13,14).

Chemical ionization was discovered (2) as a result of Field and Munson's (3,4) long-standing interest and active studies in ion-molecule reactions in the gas phase. The chemical reactions of ions in binary collisions with neutral molecules are defined as ion-molecule reactions (15). These reactions are extremely rapid compared with ordinary chemical reactions. The most common ion-molecule reaction taking place under the low pressure conditions that usually prevail in a mass spectrometer ion source while the mass spectrum of an organic compound is being measured is the abstraction of a hydrogen radical by the molecule ion. This chemical reaction gives rise to a peak in the mass spectrum which occurs 1 atomic mass unit* higher than the molecular ion peak (1).

*Henceforth a.m.u.
The collision of ions with neutral molecules is relatively rare under the low pressure conditions (less than $10^{-5}$ Torr) which usually prevail in routine mass spectrometry. In contrast, gas phase ion-molecule reaction studies are carried out at relatively high ion source pressures in the range of $10^{-4}$ Torr to several Torr. The low pressure mass spectrum of a compound is usually considerably altered when the pressure is increased.

Figure I gives a schematic diagram of the source of a standard MS-9 mass spectrometer modified for CI conditions (7). In order to operate a chemical ionization source at 1 Torr, an ionization chamber was constructed with only three apertures. A large aperture in the back of the chamber admits the reagent gas and the sample. The ion exit slit in the front of the box is 0.05 X 3.2 mm and the electron entrance is a circular hole 0.35 mm in diameter. A pressure differential of about $10^{-4}$ Torr can be maintained across each of the two exits and the housing if a pumping speed of about 500 liters/sec is used on the source housing. The above arrangement ensures that the filament and the ion accelerating plates are in the low pressure region.

In general, the combined EI-CI source must be gas-tight (1). Gas tightness is necessary to allow the pressure within the ion source to be on the order of 1 Torr while the reactant gas flow rate is 2-15 atm-ml/min and the pressure in the ion source region is less than $10^{-3}$ Torr. The ion
FIG. 1.— Schematic diagram of the source of a standard MS-9 mass spectrometer modified for CI conditions (7).
source and analyzer regions should have their own diffusion pumps and the connection between the two regions should be gas-tight except for the slit necessary to allow the fully accelerated ions to enter the analyzer region. The pressure requirements in the analyzer region are highly dependent on the instrument.

The potential between the filament and the shell of the combined ion source must be made variable to 500 V. This increase in electron energy is necessary to enable electrons to penetrate sufficiently in the source held at 1 Torr pressure.

The differences between CI mass spectra, with the exception of charge transfer CI mass spectra, and other mass spectra are due to three factors (16):

(a) Unlike photoionization and EI ionization, chemical ionization is not governed by Frank-Condon principles, but involves slow equilibrium adjustments of electronic states and atom positions.

(b) The initial product ions in chemical ionization have an even number of electrons, whereas electron impact, field and photoionization give rise initially to odd-electron ions.

(c) The amount of energy transferred to the initial product ions by chemical ionization
is low by mass spectrometry standards, being highly dependent on the reactant gas used.

In conventional electron impact mass spectrometry, the energy of the electrons (70eV) produces extensive fragmentation of the molecules. The resultant positive ions are separated in the mass spectrometer to give a characteristic spectrum for a given molecule. The extensive fragmentation of the molecule produces complex mass spectra. This complexity can be an important asset, but it can also be a severe handicap, particularly when analyzing mixtures or impure samples. Compared to EI mass spectrometry, CIMS offers the advantages of simpler fragmentation patterns, intense quasi-molecular ions, and relatively easy to interpret spectra. It also provides the same high sensitivities as EI (17,18,19). CIMS is not a replacement for EIMS, but is a highly efficient and useful complement which provides a convenient means to identify molecular weights and observe fragment ions generated by processes considerably different from those of EIMS. Table I illustrates some of the similarities and differences of the two methods as observed in molecules with highly electronegative functions (e.g., ketone or amine) and a proton-source ionizing gas such as methane.

Methane has been the reagent gas most commonly used in CIMS, partly because it was the first one tried, but
also because it generally gives information about molecular weight and structure from quasi-molecular and fragment ions.

At an ion source pressure of 1 Torr of methane (4), the reactant ions formed are as follows:

(a) Primary electron impact reaction

\[
\text{CH}_4^+ + e^- \rightarrow \text{CH}_4^+ , \text{CH}_3^+ , \text{CH}_2^+ , \text{CH}^+ , \text{C}^+ , \text{H}_2^+ , \text{H}^+ + 2e^- \tag{1}
\]

(b) Secondary ion-molecule reactions

\[
\begin{align*}
\text{CH}_4^+ &+ \text{CH}_4* \rightarrow \text{m:H}_5^+ + \text{CH}_3 \\
\text{C}_2\text{H}_4^+ &+ \text{H}_2 \rightarrow \text{C}_2\text{H}_3^+ + \text{H}_2 \\
\text{C}_2\text{H}_2^+ &+ \text{CH}_4 \rightarrow \text{polymer}
\end{align*}
\tag{2}
\]

(c) Tertiary ion-molecule reactions

\[
\begin{align*}
\text{C}_2\text{H}_5^+ &+ \text{CH}_4 \rightarrow \text{C}_3\text{H}_7^+ + \text{H}_2 \\
\text{C}_2\text{H}_3^+ &+ \text{CH}_4 \rightarrow \text{C}_3\text{H}_5^+ + \text{H}_2 \\
\text{C}_2\text{H}_2^+ &+ \text{CH}_4 \rightarrow \text{polymer}
\end{align*}
\tag{7-9}
\]

Of the above processes, 95% of the total ionization is due to \(\text{CH}_5^+\) (48%), \(\text{C}_2\text{H}_5^+\) (41%) \(\tag{3}\), and \(\text{C}_3\text{H}_5^+\) (6%), and the mass spectrum mainly shows three peaks at m/e 17, 29, and 41. All three may react as either Bronsted or Lewis acids \(\tag{2,20-23}\). Reactions between hypothetical sample molecules, BH and methane reactant ions, can be limited to the reactant
<table>
<thead>
<tr>
<th>Category</th>
<th>EIMS</th>
<th>CIMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conditions</td>
<td>High vacuum; heated inlet</td>
<td>Positive pressure of a reagent gas; heated inlet</td>
</tr>
<tr>
<td>Sample size required</td>
<td>Micrograms</td>
<td>c.a. 10 micrograms</td>
</tr>
<tr>
<td>Process for generation of &quot;parent&quot; ion</td>
<td>Electron capture</td>
<td>Proton or carbonium ion addition; hydride abstraction</td>
</tr>
<tr>
<td>Intensity of &quot;parent&quot; ion</td>
<td>Usually weak</td>
<td>Usually strong</td>
</tr>
<tr>
<td>Main fragmentation mode</td>
<td>Radical ion</td>
<td>Protonated or addition ion (both positive species)</td>
</tr>
<tr>
<td>Mechanistic parallel</td>
<td>Free radical 3°&gt;2°&gt;1°</td>
<td>Gas phase and acid solution chemistry (retro-aldol, C-X cleavage, X=hetero atom).</td>
</tr>
<tr>
<td>Location of primary event</td>
<td>Non-bonded electrons, e.g. R-NH₂ → R-NH₂</td>
<td>Non-bonded electrons, e.g.</td>
</tr>
<tr>
<td></td>
<td>+2e</td>
<td>[ R-\ddot{\text{N}}_2 \xrightarrow{\text{H}^+} R-\text{NH}_2 ]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[ \text{or H} ]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[ R-\ddot{\text{N}}_2 \xrightarrow{R^{+}} R-\text{NH}_2 ]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[ \text{or } R' ]</td>
</tr>
<tr>
<td>Fragmentation</td>
<td>Extensive</td>
<td>Relatively slight</td>
</tr>
<tr>
<td>C-C cleavage</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Molecular weight determination</td>
<td>Usually difficult</td>
<td>Usually facile</td>
</tr>
</tbody>
</table>
ions \( \text{CH}_5^+ \) and \( \text{C}_2\text{H}_5^+ \) since these species account for 89% of the total ionization at 1 Torr, and since the minor methane reactant ions react much in the same manner as the major ones do. Reactions of these species may be written as follows:

\[
\text{CH}_5^+ + \text{BH}+\text{BH}_2^+ + \text{CH}_4
\]

(10)

\[
\text{C}_2\text{H}_5^+ + \text{BH}+\text{BH}_2^+ + \text{C}_2\text{H}_4
\]

(11)

\[
\text{C}_2\text{H}_5^+ + \text{BH}+\text{B}^+ + \text{C}_2\text{H}_6
\]

(12)

The ionic species \( \text{BH}_2^+ \) (10 and 11) and \( \text{B}^+ \) (12) formed initially may be sufficiently energetic to decompose as follows:

\[
\text{BH}_2^+ \rightarrow \text{B}^+ + \text{H}_2
\]

(13)

\[
\text{BH}_2^+ \rightarrow \text{A}_i^+ + \text{C}_i
\]

(14)

\[
\text{B}^+ \rightarrow \text{A}_i^+ + \text{C}_i
\]

(15)

\( \text{A}_i^+ \) (14) and \( \text{A}_i^+ \) (15) each represent one of several possible fragment ions. \( \text{C}_i \) and \( \text{C}_i^- \) are the corresponding neutral fragments (1). It is possible to use many different gases as reagents and thus produce spectra from reactant ions of different energies (23). Some of the reagent gases used and the major ions formed under CI conditions are shown in Table II (24).

Methane CI spectra show considerably more fragmentation than is observed when isobutane is the reagent gas. In general, there are fewer ions in the isobutane CI mass
Table II. Reagent gases and ions generated in CIMS

<table>
<thead>
<tr>
<th>Reagent gas</th>
<th>Intense ions at about 1 Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td>( \text{CH}_5^+, \text{C}_2\text{H}_5^+, \text{C}_3\text{H}_5^+ )</td>
</tr>
<tr>
<td>Propane</td>
<td>( \text{C}_3\text{H}_7^+, \text{C}_3\text{H}_8^+ )</td>
</tr>
<tr>
<td>Isobutane</td>
<td>( \text{C}_4\text{H}_9^+ )</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>( \text{H}_3^+ )</td>
</tr>
<tr>
<td>Ammonia</td>
<td>( \text{NH}_4^+, (\text{NH}_3)_2\text{H}^+, (\text{NH}_3)_3\text{H}^+ )</td>
</tr>
<tr>
<td>Water</td>
<td>( \text{H}_3\text{O}^+ )</td>
</tr>
<tr>
<td>Tetramethyilsilane</td>
<td>( \text{Me}_3\text{Si}^+ )</td>
</tr>
<tr>
<td>Dimethylamine</td>
<td>( \text{Me}_2\text{NH}_2^+, (\text{Me}_2\text{NH})_2\text{H}^+, \text{C}_3\text{N}_8\text{N}^+ )</td>
</tr>
<tr>
<td>Helium</td>
<td>( \text{He}^+ )</td>
</tr>
</tbody>
</table>

spectrogram than in its methane counterpart (25-28). Fig. II shows the principle reactions occurring under isobutane CI conditions, with subsequent protonation of alcohol ROH and elimination of water (24).

\[
\text{ROH} + \text{CH}_3^+ \rightarrow \text{ROH}_2^+ + \text{CH}_3\text{C} = \text{CH}_2
\]  

(17)

\[
\text{ROH}_2^+ \rightarrow \text{R}^+ + \text{H}_2\text{O}
\]  

(18)

Fig. II.--Principal reagent gas ions generated in isobutane CIMS.
The t-C₄H₉⁺ ion accounts for over 90% of the total ionization (25,29), and reacts mainly as a weak Bronsted acid (25), but may also function as a hydride abstractor (16).

Propane is intermediate between methane and isobutane in observed fragmentation. As protonation by H₃⁺ is generally highly exothermic (23), hydrogen CIMS shows fragmentation leading to numbers of ions approaching that observed in EIMS. Ammonia and water are interesting reagent gases just beginning to be applied (19,30). Since water is a major component of biological samples, it may be possible to inject body fluid samples directly into a CI mass spectrometer and use the endogenous water as the reactant gas (24).

With tetramethylsilane as the reagent gas trimethylsilylation occurs instead of protonation. Dimethylamine appears to cause selective ionization of carbonyl compounds. For example, when equal amounts of cholesterol and androstenedione were introduced into the mass spectrometer with dimethylamine as the reagent gas, the mass spectrum showed a very intense ion resulting from attachment of protonated dimethylamine to the androstenedione, but no ions resulting from ionization of the cholesterol molecules (24). When helium (31), argon (31,32) neon (31,32), xenon (31,32), nitrogen (31,33), carbon monoxide (31), carbon dioxide (31), or nitrous oxide (31,34) is the reagent gas, the sample
molecules are ionized by charge exchange rather than proton transfer. For xenon one can illustrate this process with hypothetical molecule :BH.

\[
\text{Xe}^+ + :\text{BH} \rightarrow \text{Xe}: + \text{BH}^+ \quad (19)
\]

Rare gas CI mass spectra of substances tend to be somewhat similar to their EI mass spectra, although the degree of fragmentation varies considerably from one gas to another, being the least for xenon and the most for helium (16). Studies of ion-molecule reactions with mixtures of argon-water (30), methane-water (35), and propane-water (36), have also been made. Such mixtures provide a convenient method for generating CI mass spectra which exhibit features characteristic of both CI and EI modes of operation.

The effect of temperature on CI mass spectra has been examined in some detail (25-27,37,38,39-42). In general, the intensity of the abundant high-mass ions present in CI mass spectra decreases with increasing source temperature. The extent of the temperature effect is dependent on the reactant gas.

Systematic studies have been made of several classes of compounds: alkanes (38,43), aromatic hydrocarbons (39,44,45), alkenes and alkynes (46), dienes (45), amines (33,47-49), esters (25-27, 37,40,42,50,51), alcohols (28, 52), alkaloids (10,53), dimeric cyclic ketones (54,55), amino acids and their derivatives (56,57), drugs (58-66), drug
metabolites (24,66,67), monosaccharides and their simple glycosides (8,68,69), macrolide antibiotics (70,71), aminocyclitol antibiotics (72-75), tetracycline antibiotics (76), and β-lactam antibiotics (77). Work has also been carried out for the sequencing of simple peptides (17) and phenylthiohydantoin derivatives of simple peptides formed by Edman degradation (18).

Less extensive studies have been made on tricyclic flavanoids (78), conjugated ketones (24,79), substituted benzophenones (5,80), aryl ketones (81), 17-hydroxysteroids (82), borazine (83), phospholipids (24), nucleosides (19), and peptide antibiotics (84).

Extensive mechanistic studies have been carried out with labelled compounds (17,43,45,56,85-87). It has also been possible to determine the number of active hydrogens present in organic compounds with CIMS by use of D₂O or CH₃OD as the reagent gas (88).

Objectives of research.--Interest in the therapeutic properties of various classes of antibiotics remains high and new chemical entities are being constantly described. Recent discoveries frequently have been mixtures of closely related antibiotics differing from one another in some cases by one functional group in the molecule. Differentiation of these products from each other and from previously known substances, especially when only very limited quantities of some of the minor components are available, presents a
substantial analytical challenge. Electron impact mass spectrometry has proven extremely useful in this context, but suffers from complex fragmentation patterns and often the absence of a discernible molecular ion.

Chemical ionization mass spectrometry was applied to representative examples of 14- and 16-membered ring macrolide antibiotics (70,71), aminocyclitol antibiotics (72), tetracycline antibiotics (76), and β-lactam antibiotics (77) in order that basic fragmentation patterns could be deciphered. In cases where the mode of fragmentation was not apparent, mechanistic studies were carried out, either with high resolution mass measurements (77) or on isotopically labelled or unlabelled model compounds approximating some functional groups within a class of antibiotics (86).

Special emphasis was placed on obtaining CI spectra for the above classes of antibiotics, not only because of their therapeutic value, but also because some of these molecules represent the highest molecular weight compounds yet to be examined by CI mass spectrometry.
EXPERIMENTAL

Samples of macrolide antibiotics erythromycin A, erythromycin B, dihydroerythromycin B, 5-O-desosaminylerthronolide B, lankamycin and niddamycin, were obtained from Mr. L. Theriault, Drs. P. H. Jones, J. R. Martin and T. J. Perun, Abbott Laboratories, Chicago, Illinois. Laidlomycin was received from Mr. Fumio Kitame of the School of Medicine, Tohoku University, Sendai, Japan. Spiramycin I, spiramycin III, antibiotic XK-41-A, and antibiotic XK-41-B were obtained from Dr. T. Nara of the Kyowa Hakko Kogyo Company (Japan). Oleandomycin was received from Dr. W. Celmer, Pfizer Laboratories, Inc.

Samples of β-lactam antibiotics were obtained from several suppliers: sodium cloxacillin, sodium methicillin, and hetacillin from Bristol Laboratories, Syracuse, New York; ampicillin, potassium penicillin V, and potassium penicillin G from Wyeth Laboratories, Inc., Philadelphia, Pennsylvania; 6-aminopenicillanic acid from Aldrich Chemical Co., Inc.

Instrumentation.—Infrared spectra were obtained with a Perkin-Elmer model-257 grating spectrophotometer. NMR spectra were recorded with a Varian A-60A nmr spectrometer.
at 60 MHz using tetramethylsilane as an internal standard. The circular dichroism spectra were run at ambient temperature on a Durrum-Jasco model ORD/UV-5 instrument equipped with a circular dichroism attachment modified by Sproul Instruments according to model number SS-20. The chemical ionization mass spectra were recorded by Dr. Roger L. Foltz on one of two spectrometers at Battelle-Columbus Laboratories, Columbus, Ohio. (a) An AEI MS-9 mass spectrometer equipped with a SRIC CIS-2 chemical ionization source. The isobutane pressure within the ion block was maintained at approximately 1 Torr. The mass spectrometer was operated at 450 eV ionization voltage, 200 mA filament emission, 0 V ion repeller, and 8 KV accelerating voltage. The samples were introduced into the ion source by means of a heated probe and volatilized at probe temperatures of approximately 250°. The ion source was maintained at 200°. The mass spectra were recorded both on magnetic tape with subsequent computer processing, and on an oscillographic recorder. (b) A Finnigan 1015 quadrupole mass spectrometer equipped with a chemical ionization source. The isobutane pressure within the ion block was maintained at 0.5 Torr. The mass spectrometer was operated at 100 eV ionization voltage, 200 μA filament emission, 2-3V ion repeller, 40V lens voltage, and 3V ion energy. The samples were introduced into the ion source by means of a heated probe. The probe temperature was increased until maximum volatilization occurred. The ion
source temperature was maintained at 180°. Spectra were recorded on a Systems Industries 250 data system coupled to the spectrometer. This system utilizes a PDP8e computer.

Coupled to the Finnigan 1015 spectrometer was a Varian model 1740 VPC unit. Methane was used as the carrier gas. Mixtures were chromatographed on a 6 ft x 2 mm glass column packed with 3% OV-17 on Gas Chrom Q, 100/120 mesh. The column was programmed from 80° to 250° at 10°/min. Sample size was variable.

Synthesis of N,N-dimethylamino compounds.—

General procedure: To the primary aminoalcohol at ice bath temperature were added dropwise and with stirring approximately 5.0 equivalents of formic acid (from 90% formic acid solution, Baker Chemical Co.) and then about 2.2 equivalents of formaldehyde (from 37% formaldehyde solution, Baker Chemical Co.). The mixture was refluxed over a steam bath for 3.5-4.0 hr. After cooling in ice, concentrated aqueous HCl was added dropwise (1 ml/g aminoalcohol) and extraction was carried out thrice with CHCl₃.

The aqueous phase was basified to pH 10 with 50% aqueous NaOH solution, saturated with NaCl, and extracted five times with CHCl₃. The organic phase was dried (anhydrous Na₂SO₄), filtered, and evaporated in vacuo to dryness to afford an oil which was distilled through a short-path column.

N,N-dimethyl-2-amino-1-ethanol.—2-Amino-1-ethanol (15.0 g,
0.246 mole, Eastman Organics, Inc.) when allowed to react with formic acid and formaldehyde gave upon distillation (35°/20 mm) 4.757 g (22%) of a clear oil; ir cm⁻¹ (neat): 3400 (broad), 2950, 2870, 2830, 2790, 1465, 1270, 1160, 1088, 1042, 945; nmr (CDCl₃): δ 2.26 (s, 6H, -N(CH₃)₂), δ 2.44 (t, 2H, J=6.0Hz, -CH₂-N=C), δ 3.62 (t, 2H, J=6.0Hz, -O-CH₂-), δ 4.91 (broad s, 1H, -OH)*.

N,N-dimethyl-3-amino-1-propanol.—3-Amino-1-propanol (15.0g, 0.2 mole, Aldrich Chemicals, Inc.) when allowed to react with formic acid and formaldehyde gave upon distillation (68°/20 mm) 12.571 g (61%) of a clear oil; ir cm⁻¹ (neat): 3400 (broad), 2950, 2865, 2825, 2785, 1465, 1385, 1262, 1160, 1045, 832; nmr (CDCl₃): δ 1.68 (distorted q, 2H, J=6.0Hz, -CH₂-C-), δ 2.25 (s, 6H, -N(CH₃)₂), δ 2.48 (distorted t, 2H, J=6.0Hz, -CH₂-N <), δ 3.69 (distorted t, 2H, J=6.0Hz, -O-CH₂-), δ 5.4 (broad s, 1H, -OH)*.

N,N-dimethyl-5-amino-1-pentanol.—5-Amino-1-pentanol (5.0g, 0.049 mole, Aldrich Chemicals, Inc.) when allowed to react with formic acid and formaldehyde gave upon distillation (110°/20 mm) 3.817g (60%) of a clear oil; ir cm⁻¹ (neat): 3400 (broad), 2940, 2870, 2825, 2785, 1475, 1380, 1265, 1175, 1065, 1040, 835; nmr (CDCl₃): δ 1.45 (m, 6H, -O-CH₂-(CH₂)₃-CH₂-N <), δ 8.22 (s overlapping m, 8H, -CH₂-N(CH₃)₂),

*With addition of D₂O signal sharpens and moves upfield.
δ3.57 (distorted t, 2H, -O-CH$_2$-), δ4.9 (broad s, 1H, -OH)*.

**Synthesis of THP-ethers of tertiary aminoalcohols.**

**General procedure:** To a stirred suspension of anhydrous p-toluenesulfonic acid (K and K Laboratories, Inc., 1.1 equivalents) and dry CHCl$_3$ (40 ml/g aminoalcohol) at ice bath temperature was added dropwise the tertiary aminoalcohol (1 equivalent). After the resulting solution had warmed to 25°, dihydropyran (1.1 equivalent, Aldrich Chemicals, Inc.) was added dropwise and stirring was carried out for 30 min.

The solution was cooled in ice and washed with 10% aqueous NaOH until the aqueous layer remained at pH 10. The organic phase was dried (anhydrous Na$_2$SO$_4$), filtered, and evaporated in vacuo to dryness to afford an oil which was chromatographed over alumina (Woelm, activity III) and distilled through a short-path column.

**THP-ether of N,N-dimethyl-2-amino-1-ethanol.**—N,N-dimethyl-2-amino-1-ethanol (2.0g, 0.022 mole) when allowed to react with anhydrous p-toluenesulfonic acid and dihydropyran gave upon chromatography and distillation (90°/20 mm) 1.031g (27%) of a clear oil; ir cm$^{-1}$ (neat): 2950, 2885, 2830, 2780, 1457, 1445, 1390, 1355, 1270, 1210, 1135, 1040; nmr (CDCl$_3$): δ1.6 (m, 6H, CH$_2$), δ2.28 (s,6H,-N(CH$_3$)$_2$),

---

*Addition of D$_2$O diminishes peak intensity.*
62.5 (t, 2H, J=6.0 Hz, -CH<sub>2</sub>-N<sup>-</sup>), 63.6 (m, 4H,

δ4.58 (broad s, 1H, anomeric H).

**THP-ether of N,N-dimethyl-3-amino-1-propanol.**—N,N-dimethyl-
3-amino-1-propanol (2.22 g, 0.022 mole) when allowed to react
with anhydrous p-toluenesulfonic acid and dihydropyran gave
upon chromatography and distillation (25°/0.15 mm) 2.221 g
(55%) of a clear oil; ir cm<sup>-1</sup> (neat): 2940, 2870, 2810,
2770, 1460, 1440, 1355, 1325, 1265, 1145, 1070, 1030; nmr
(CDCl<sub>3</sub>): 61.65 (m, 8H, and -CH<sub>2</sub>-N(CH<sub>3</sub>)<sub>2</sub>), 62.35
(s overlapping distorted t, 8H, -CH<sub>2</sub>-N(CH<sub>3</sub>)<sub>2</sub>), 63.6 (m,
4H, ), δ4.58 (broad s, 1H, anomeric H).

**THP-ether of N,N-dimethyl-5-amino-1-pentanol.**—N,N-dimethyl-
5-amino-1-pentanol (1.62 g, 0.022 mole) when allowed to react
with anhydrous p-toluenesulfonic acid and dihydropyran gave
upon chromatography and distillation (84°/1.7 mm) 1.711 g
(50%) of a clear oil; ir cm<sup>-1</sup> (neat): 2940, 2860, 2820,
2765, 1470, 1463, 1385, 1355, 1275, 1205, 1140, 1125, 1080,
1040, 905, 870, 815; nmr (CDCl<sub>3</sub>): 61.55 (m, 12H,

and -(CH<sub>3</sub>)<sub>3</sub>CH<sub>2</sub>N<sup>-</sup>), 62.25 (s overlapping
distorted t, 8H, -CH<sub>2</sub>-N(CH<sub>3</sub>)<sub>2</sub>), 63.3 (m, 4H, )

, δ4.58 (broad s, 1H, anomeric H).
21

δ4.58 (broad s, 1H, anomeric H).

**THP-ether of 4-hydroxy-N-methylpiperidine.** (prepared by Allison F. Fentiman, Jr., Battelle-Columbus Laboratories, Columbus, Ohio). 4-Hydroxy-N-methylpiperidine (4.0g, 0.035 mole, Aldrich Chemicals, Inc.) when allowed to react with anhydrous p-toluenesulfonic acid and dihydropyran gave upon distillation (86°/1.4 mm) 3.6g(51%) of a clear oil; ir cm⁻¹ (neat): 2940, 2850, 2790, 1450, 1350, 1275, 1200, 1140, 1120, 1075, 1065, 1040, 1030, 1000, 970, 915, 875, 815, 780.

**Deuterated THP-ether of 4-hydroxy-N-methylpiperidine.** To a stirred solution of trifluoroacetic anhydride (4.043g, 0.019 mole, Aldrich Chemicals, Inc.) in 5 ml dry CHCl₃ was added dropwise 0.385g (0.019 mole, Diaprep, Inc.) of 99.8% deuterium oxide in 20 ml dry CHCl₃ and 5 ml dry THF. After stirring for 1.0 hr at 25°, the solution was cooled in ice and 4-hydroxy-N-methylpiperidine (4.0g, 0.035 mole, Aldrich Chemicals, Inc.) in 20 ml dry CHCl₃ was added dropwise. After warming to 25°, dihydropyran (4.0g, 0.047 mole) in 10 ml dry CHCl₃ was added dropwise and the resulting mixture was stirred for 3 hr. Workup as described before afforded upon distillation (68°/1.25 mm) 1.311g (19%) of a clear oil; ir (neat) was the same as the undeuterated analog with additional peaks at 2740 cm⁻¹ and 2680 cm⁻¹.

**Preparation of free acids of various β-lactam antibiotics.** To 50 mg of the salt of the β-lactam antibiotic were added
25 ml each of distilled H\textsubscript{2}O and EtOAc. A Sargent glass electrode was immersed into the stirred mixture at 0\degree and dilute aqueous HCl was added until the desired pH was achieved (penicillin G and methicillin, pH 3.5; penicillin V and cloxacillin, pH 3; cephalothin, pH 2.5). After draining off the H\textsubscript{2}O layer, the organic layer was washed with cold H\textsubscript{2}O, dried (anhydrous Na\textsubscript{2}SO\textsubscript{4}) and evaporated in vacuo at 25\degree to afford an oily residue which was dried at 1.0 mm/25\degree for 10 min.

Preparation of methyl esters of various penicillins.—General procedure: (89) A weighed quantity of the antibiotic salt was suspended in 2 ml dry CH\textsubscript{2}Cl\textsubscript{2} and pyridine hydrochloride (1 equivalent) was added. After cooling the cleared suspension in an ice bath, absolute CH\textsubscript{3}OH (4 equivalents) was added and then N,N'-dicyclohexylcarbodiimide (1 equivalent) in 0.5 ml dry CH\textsubscript{2}Cl\textsubscript{2} was added dropwise. Stirring was carried out for 18 hr at 25\degree during which dry solvent was added to maintain level.

The mixture was filtered, washed (5% aqueous NaHCO\textsubscript{3} and H\textsubscript{2}O), dried (anhydrous MgSO\textsubscript{4}), and concentrated to dryness in vacuo. The residue was purified by preparative thick-layer chromatography (silica gel G) to afford an oily product containing a small amount of crystalline residue (probably an adduct of DCC) which could be removed by successive crystallizations from 50% benzene:petroleum ether
Penicillin G methyl ester.—Penicillin G potassium salt (100 mg, 0.268 mmole) when reacted with pyridine hydrochloride (31.0 mg), absolute CH₃OH (34.4 mg), and DCC (55.3 mg) gave upon workup 69.6 mg (75%) of an oil; ir cm⁻¹ (CHCl₃): 3410 (broad), 3038, 2960, 2915, 2860, 1780, 1750, 1650, 1495, 1455, 1438, 1295 (broad); nmr (CDCl₃): δ 1.43 (s, 3H, 2α-CH₃), δ 1.45 (s, 3H, 2β-CH₃), δ 3.6 (s, 2H, CH₂-), δ 3.72 (s, 3H, -OCH₃), δ 4.38 (s, 1H, H-3), δ 5.4-5.8 (overlapping m, 2H, H-5 and H-6), δ 6.4 (d, 1H, NH), δ 7.29 (s, 5H, aromatic H's).

Penicillin V methyl ester.—Penicillin V potassium salt (100 mg, 0.258 mmole) when reacted with pyridine hydrochloride (29.8 mg), absolute CH₃OH (32.8 mg), and DCC (53.2 mg) gave upon workup 68.2 mg (73%) of an oil; ir cm⁻¹ (CHCl₃): 3405, 3180, 2960, 2930, 1785, 1748, 1680, 1600, 1515, 1495, 1440, 1300, 1240; nmr (CDCl₃): δ 1.49 (s, 3H, 2α-CH₃), δ 1.58 (s, 3H, 2β-CH₃), 3.76 (s, 3H, -OCH₃), δ 4.48 (s, 1H, H-3), δ 4.54 (s, 2H, -OCH₂-), δ 5.5-5.8 (overlapping m, 2H, H-5 and H-6), δ 6.8-7.5 (complex m, 5H, aromatic H's).

Cloxacillin methyl ester.—Cloxacillin sodium salt (100 mg, 0.219 mmole) when reacted with pyridine hydrochloride (25.3 mg), absolute CH₃OH (28.0 mg), and DCC (45.1 mg) gave upon workup 85.0 mg (87%) of a viscous oil; ir cm⁻¹ (CHCl₃): 3400 (broad), 3018, 3005, 2960, 2923, 2860, 1785,
1745, 1670, 1600, 1510, 1475, 1295; nmr (CDCl₃): δ 1.38 (s, 3H, 2α-CH₃), δ 1.40 (s, 3H, 2β-CH₃), δ 2.78 (s, 3H, isoxazole CH₃), δ 3.73 (s, 3H, -OCH₃), δ 4.30 (s, 1H, H-3), δ 5.4–5.9 (overlapping m, 2H, H-5 and H-6), δ 6.0 (d, 1H, NH) δ 7.49 (s, 4H, aromatic H's).

Preparation of penicilloic acid methyl esters.—
General procedure: To 100 mg of the free acid in CH₃OH was added excess ethereal CH₂N₂. The solution stood overnight at 25°. Excess solvent was evaporated and the residual oil was purified by silica gel G thick-layer chromatography.

Penicillin G penicilloic acid methyl ester.—Penicillin G (100 mg, 0.299 mmole) when reacted with ethereal CH₂N₂ upon workup gave 83.6 mg (74%) of an oil; ir cm⁻¹ (CHCl₃): 3410, 3350, 3035, 2960, 2925, 2850, 1735, 1665, 1510, 1495, 1435, 1370, 1330, 1240; nmr (CDCl₃): δ 1.12 (s, 3H, 2-CH₃), δ 1.45 (s, 3H, 2-CH₃), δ 3.38 (m, 2H, thiazolidine NH and H-3), δ 3.62 (s, 2H, β-CH₂-), δ 3.68 (s, 3H, C₇-CO₂CH₃), δ 3.70 (s, 3H, C₃-CO₂CH₃), δ 4.62 (q, J=8.3Hz, 4Hz, 1H, H-6), δ 5.08 (distorted d, J=4Hz, H-5) δ 6.55 (broad d, J=8.3Hz, 1H, amide NH), δ 7.28 (s, 5H, aromatic H's).

Penicillin V penicilloic acid methyl ester.—Penicillin V (100 mg, 0.286 mmole) when reacted with ethereal CH₂N₂ upon workup gave 96.1 mg (85%) of an oil; ir cm⁻¹ (CHCl₃): 3410, 3350, 3030, 3000, 2960, 2930, 1738, 1660, 1600, 1512, 1493, 1438, 1370, 1330, 1240; nmr (CDCl₃): δ 1.19 (s, 3H,
2-CH₃), δ1.50 (s,3H,2-CH₃), δ3.73 (s,6H,C₃-CO₂CH₃ and
C₇-CO₂CH₃), δ4.7 (q overlapping s, 3H,H-6 and φ-O-CH₂⁻),
δ5.18(d,J=4.5,1H,H-5), δ7.1 (complex m, 5H, aromatic H's).

**Attempted preparation of O¹⁸-carboxy penicillin V.**—To
penicillin V (5.0 mg, 0.014 mmole) was added 0.33 ml of 40% solution of O¹⁸-H₂O (Koch Isotopes, Inc., Lincoln Park, N.J.) and a trace of concentrated aqueous HCl. The mixture was stirred for 3 hr at 4°. Excess H₂O was recovered by vacuum transfer at 10⁻³ mm/⁻196°. After twice repeating this sequence, the product was examined by silica gel G tlc (developed in 50% CH₃OH:acetone (90)), and displayed an Rf identical with starting material; [φ]₂₃₂(CH₃OH) +47,250 (91).
RESULTS AND DISCUSSION

Chemical ionization mass spectrometry is now recognized as a useful adjunct to electron impact mass spectrometry primarily because of its ability to give prominent peaks in the molecular ion region. This characteristic is indeed a useful feature, especially when working with biomedical compounds which frequently give complex electron impact mass spectra in which the molecule ion peaks are frequently weak or absent altogether.

More than 2000 distinct naturally occurring antibiotics have been described and about 100 new ones are being recorded each year. This number includes at least 40 macrolides and these frequently occur in mixtures of complex and closely related substances with minor components often as interesting as the major ones, especially in the 16-membered ring sub-group. Rapid identification and differentiation presents a formidable analytical challenge, especially when only small amounts of material are available.

CIMS of 14- and 16-membered macrolide antibiotics and model compounds.—The electron impact mass spectra of macrolide antibiotics are quite complex with nearly all of the abundant ions in the low mass region. Consequently the
spectra are difficult to interpret. However, this study shows that the chemical ionization mass spectra of macrolide antibiotics normally show prominent protonated molecular ion peaks when isobutane is used as the reactant gas. The fragmentation patterns are relatively simple, but provide important structural information. The glycosidic bonds are particularly susceptible to cleavage under chemical ionization conditions, giving abundant ions for sugar fragments as well as for the aglyconic moiety. Most of the remaining prominent ions are attributed to loss of oxygenated substituents as neutral molecules, such as water, methanol, and acetic acid. Most processes involve cleavage of carbon-hetero atom bonds. However, a few important fragmentations involving carbon-carbon bonds have been observed in this work for the first time and have well established solution chemistry counterparts.

Fourteen-membered ring macrolide antibiotics have been thoroughly investigated (70). Fig. III compares the CI mass spectrum of erythromycin B with its EI mass spectrum (92). The base peak at m/e 718 is the protonated molecule ion, while the peaks at m/e 756 and 774 are due to adduct ions typically observed in isobutane CI mass spectra ($\text{M}^+ + \text{C}_3\text{H}_4$ and $\text{M}^+ + \text{C}_4\text{H}_9$ respectively). Consequently, assignment of the molecular weight is no problem. The direct loss of neutral cladinose from the protonated molecule ion, either with or without its glycosidic oxygen gives peaks at
Fig. III.— Comparison of EI mass spectrum and isobutane CI mass spectrum of erythromycin B.
m/e 542 and 560 respectively. These fragmentations are substantiated by metastable ion peaks at the appropriate mass values (409.1 and 436.8). The peak at m/e 524 results from loss of H₂O from the m/e 542 fragment. Loss of a neutral desosamine fragment from the protonated molecule ion is not observed. However, the presence of desosamine is indicated by the peak at m/e 158 corresponding to fragment 20.

Ions due to cladinose (21-23) are also observed in the low mass region of the spectrum and provide evidence for the sugar's OH and OMe substituents.

The abundant MH⁺-H₂O ion (m/e 700) appears to be primarily due to a reaction sequence (Fig. IV) which is analogous to a known acid-catalyzed reaction which occurs rapidly in solution in those macrolides having a C(6)-OH conformationally suited for attack of the C(9)-carbonyl (93).

Fig. IV.— Acid-catalyzed attack of C(9)-carbonyl by C(6)-OH in 14-membered macrolides.
The intense peak at m/e 99 can be rationalized as resulting from protonation of the C(9)-ketone, McLafferty ring-opening at the lactone linkage to give 28, and cleavage of the C(10)-C(11) bond via a retro-aldol type fragmentation to give ion 30 shown in Fig. V.

Fig. V.— Fragments resulting from McLafferty rearrangement of protonated molecule ion followed by retro-aldol reaction.
The peak at m/e 602 could arise from ion 25 via the double McLafferty rearrangement shown in Fig. VI.

\[ \text{\textbf{OH OH}} \]
\[ \text{\textbf{McLafferty Rearrangement}} \]
\[ \text{\textbf{OR}} \]
\[ \text{\textbf{OR}} \]
\[ \text{\textbf{25, m/e 718}} \]
\[ \text{\textbf{R}}_1 = \text{desosamine} \]
\[ \text{\textbf{R}}_2 = \text{cladinose} \]

\[ \text{\textbf{HO}} \]
\[ \text{\textbf{Rearrangement (-H2O)}} \]
\[ \text{\textbf{33, m/e 602}} \]

\[ \text{\textbf{HO}} \]
\[ \text{\textbf{32}} \]

\[ \text{\textbf{Fig. VI.-- Fragments resulting from double McLafferty rearrangement of the protonated molecule ion.}} \]

In the isobutane CI mass spectrum of 5-O-desosaminyl-erythronolide B (Fig. VII), the protonated molecule ion m/e 560) is again the base peak. The MH\textsuperscript{+}-H\textsubscript{2}O peak (m/e 542)
Fig. VII.— Isobutane CI mass spectrum of 5-0-desosaminylerythronolide B.
is more prominent than that observed with erythromycin B. This might be expected since the process shown in Fig. IV would be more favorable with greater conformational flexibility due to the absence of cladinose. An ion attributed to desosamine is observed at m/e 158 and the retro-aldol process outlined in Fig. V results in ion m/e 99. The peak at m/e

![Diagram of molecular structure and rearrangement]

Fig. VIII.— Alternate scheme for double McLafferty rearrangement of the protonated molecule ion. 444 represents the process shown in Fig. VI, and the one at m/e 462 could arise from a similar process in which protonation has occurred at a remote site (e.g., the amino sugar). Double McLafferty rearrangement could then be shown to occur by the scheme outlined in Fig. VIII above.
The schemes shown in Figs. VI and VIII represent fundamentally the same process. The one that predominates will be dictated by the site of protonation. The ion at m/e 116 is not readily interpretable, but possibly arises via an EI process.

The isobutane CI mass spectrum of erythronolide B (Fig. IX) is especially noteworthy because of the absence of a protonated molecule ion. This lends support to the notion that initial protonation occurs predominantly on an amino function when it is present. Protonation of the C(9)-carbonyl concomitant with loss of H_2O gives ion m/e 385. This process should now be extremely favorable because of even greater conformational flexibility due to the absence of both sugars. Prominent peaks (m/e 367, 349) result from successive losses of H_2O from the base peak fragment. The retro-aldol process gives ion m/e 99 and double McLafferty rearrangement, concerted with loss of H_2O, gives ion m/e 287. Prominent peaks (m/e 269, 251) represent successive losses of H_2O from this ion. Formation of fragment ion 38 could be shown to occur by the scheme given in Fig. X, as shown on page 36.

This process appears to be general and requires the presence of a free OH at the C(3) and C(5) positions. Other fragment ions (m/e 185, 167) can be shown to occur through combinations of fragmentations given in Figs. V, VI, VIII, and X.
Fig. IX.--- Isobutane CI mass spectrum of erythronolide B.
The isobutane CI mass spectrum of 9-dihydroerythronolide B (Fig. XI) shows a moderate protonated molecule ion at m/e 405. Whereas no protonated molecule ion was observed in erythronolide B, due to the protonated C(9)-carbonyl function directing formation of a protonated enol ether moiety, dihydroerythronolide B shows a protonated molecule ion resulting from protonation of either the lactone or a hydroxyl function. However, the intensity of this

![Double McLafferty Rearrangement](image)

Fig. X.— Fragments formed from double McLafferty rearrangement of erythronolide B.

ion is considerably less than that for erythromycin B or 5-O-desosaminylerthyronolide B due to the absence of an amino sugar moiety. Because enol ether formation is now forbidden, the MH⁺-H₂O peak intensity is likewise diminished.

Successive losses of H₂O from the protonated molecule ion give fragment ions at m/e 387, 369, 351, and 333.
Fig. XI.— Isobutane CI mass spectrum of 9-dihydroerythronolide B.
Formation of the base peak ion at m/e 267 results from a double McLafferty rearrangement analogous to that shown in Fig. X.

Ions 42 (m/e 157) and 43 (m/e 139) could be shown to arise through the process above.

The isobutane CI mass spectrum of oleandomycin (94,95) (Fig. XII) shows an intense protonated molecule ion at m/e 688. Especially noteworthy is the virtual absence of a MH⁺-H₂O ion. This is not surprising since there is no C(6)-OH suitable for attack of the C(9)-carbonyl as is the case for the erythromycins. The ion at m/e 604 arises from a double McLafferty rearrangement of the protonated molecule ion (c.f., Fig. VIII). The base peak at m/e 85 represents
Fig. XII. -- Isobutane CI mass spectrum of oleandomycin.
a McLafferty rearrangement followed by retro-aldolization. This decrease in m/e value (14 a.m.u.) over erythromycin B and its derivatives shows the utility of this general process in serving as an effective mass marker for this section of the aglyconic moiety.

Successive losses of the neutral sugars oleandrose and desosamine leads to fragments of m/e 544 and 387 respectively with further loss of H₂O from the aglyconic moiety giving ion m/e 369. Loss of neutral sugar moieties can be hypothesized to occur by one or both of the four-centered mechanisms depicted in Fig. XIII for the sugar oleandrose.

In general, event (b) is observed to occur and may represent a more favorable process from thermodynamic and steric considerations (shown by subsequent mechanistic studies).

Protonation at the glycosidic oxygen followed by detachment of charged sugar moieties leads to ions of m/e 158 and 145 for desosamine and oleandrose respectively, with loss of MeOH from the latter ion giving the conjugated ion m/e 113. The weak peak at m/e 460 represents initial loss of neutral oleandrose followed by double McLafferty rearrangement (c.f., Fig. VIII).

The isobutane spectrum of lankamycin (96,97) (Fig. XIV) shows the presence of a very weak MH⁺ peak (m/e 833) and MH⁺-H₂O peak (m/e 815). One undoubtedly can in part attribute these diminutions of peak intensity to the
(a) Abstraction of proton from aglyconic moiety

(b) Abstraction of proton from sugar moiety

Fig. XIII.—Hypothesized mechanisms of loss of neutral sugars from the aglyconic moiety.
Fig. XIV.-- Isobutane CI mass spectrum of lankamycin.
absence of an amino sugar and a C(6)-OH group. Loss of sugar fragments along with MeOH, H$_2$O, and AcOH by standard processes as outlined for previous examples gives rise to the remaining fragments shown. It is especially noteworthy that

\[
\text{HQ} \quad \text{HO} \quad \text{OR}
\]

\[
\text{desosamine} \quad \text{cladinose}
\]

no ion corresponds to the retro-aldol process outlined in Fig. V. This is totally within expectations since a C(11)-acetate function would vitiate this process.

Erythromycin A (98,99) represents yet another type of 14-membered ring macrolide. Its structure is the same as
erythromycin B except for a C(12)-OH group. This additional feature manifests its presence in two ways in the CI mass spectrum of erythromycin A (Fig. XV). (1) In addition to an intense protonated molecule ion (m/e 734) and MH$^+\text{-H}_2\text{O}$ ion (m/e 716), one also notices prominent ions at m/e 676 and 658, the latter being the base peak. This ion could be hypothesized to arise via a mechanism observed in EIMS for those 14-membered ring macrolides possessing a C(12)-OH group (100). Cleavage between the OH's of glycols is quite common in mass spectrometry. (See page 43.) The former ion (m/e 676) could be shown to arise in like manner from the protonated molecule ion. (2) The retro-aldol process found to be prevalent in erythromycin B derivatives and oleandomycin now displays itself at ion m/e 115 (16 additional a.m.u. than in Erythromycin B) and in high mass ions (m/e 602, 620) deficient 114 a.m.u. The former ion is easily explained on the basis of the mechanism shown in Fig. V and the latter ions reflect the processes shown in Figs. VI and VIII respectively. Various peaks in the spectrum (m/e 558, 518, 500, 444) reflect loss of cladinose from higher mass fragments. Ions for cladinose (m/e 159) and desosamine (m/e 158) along with loss of small molecules confirm the nature of the sugars. Significantly intense ions at m/e 169 and 555 cannot be rationalized as yet.

The megalomicins (101, 102) are a new family of macrolide antibiotics elaborated by *Micromonospora Megalomicea*
Fig. XV.— Isobutane CI mass spectrum of erythromycin A.
sp.n. and have been shown to possess the erythronolide A aglyconic moiety with glycosidic linkages to sugars at C(3), C(5), and C(11).

Antibiotics XK-41-A and XK-41-B have recently been isolated by the Kyowa Hakko Company and have been characterized as 3',4"-diacetylmeagalomacin A and 4"-acetylmeagalomicin A respectively (103). The isobutane CI mass spectrum of XK-41-A (Fig. XVI) shows a base peak protonated molecule ion at m/e 961. A relatively intense peak at m/e 973 represents an apparent homolog impurity (presumably XK-41-A1, 3"-acetyl-4"-propionylmeagalomicin A). This peak was observed in a preliminary scan and is not recorded in Fig. XVI. Loss of H₂O gives a small peak at m/e 943. Further elimination of neutral diacetylcladinose and desosamine (or rhodosamine) from this fragment gives ion m/e 558, with subsequent loss of H₂O giving ion m/e 540. Loss of neutral diacetylcladinose from the protonated molecule ion through a 4-centered process as outlined in Fig. XIII(b) would give rise to ion m/e 733. Loss of a neutral desosamine or rhodosamine unit would give fragment m/e 804 with further loss of H₂O resulting in ion m/e 786. Concomitant loss of both desosamine and rhodosamine by this mechanism does not occur as evidenced by lack of a fragment ion at m/e 647. This seems to indicate that the site of protonation is predominately on one amino sugar followed by facile elimination of the other from the aglyconic moiety. Also double protonation does not appear to occur as evidenced by lack of a M+2H⁺
Fig. XVI.—Isobutane CI mass spectrum of antibiotic XK-41-A.
Peaks corresponding to each sugar moiety are seen for diacetylcladinose (m/e 229) and desosamine or rhodosamine (m/e 158). Loss of small molecule fragments gives rise to the other assigned peaks (m/e 187, 169, 140, 109). Especially noteworthy are major peaks at m/e 227, 197, and 182. These defy explanation at present.

The CI mass spectrum of antibiotic XK-41-B (Fig. XVII) shows a prominent protonated molecule ion (m/e 919) and a weak ion corresponding to loss of H²O (m/e 901). Prevalent in this spectrum, as was seen for erythromycin A, are peaks corresponding to loss of 58 a.m.u. (m/e 861, 675, 657) either from the protonated molecule ion or high mass fragment ions. Peaks at m/e 762 and 733 represent loss of neutral desosamine (or rhodosamine) and 4”-acetylcladinose respectively. Other peaks in the spectrum can be assigned to sugar moieties (m/e 187, 158) and to the loss of small molecules from these units (m/e 169, 127, 109) or from larger fragments (m/e 744, 715). Intense peaks at m/e 383, 381, 273 and 137 are presently unexplicable and could represent contaminant ions.

Because of the higher molecular weights and greater complexity of many of the 16-membered ring macrolide antibiotics, it was of interest to see if CIMS would be useful in characterizing selected members of this group of antibiotics (71).
Fig. XVII. -- Isobutane CI mass spectrum of antibiotic XK-41-B.
The CI mass spectrum of spiramycin I (104) (Fig. XVIII) shows a relatively intense protonated molecule ion at m/e 843. The small peak at m/e 899 results from attachment of a t-butyl carbonium ion to the spiramycin molecule. Adduct ions of this type are normal to CIMS as was seen for 14-membered ring macrolides. The most abundant fragment ions result from cleavage at glycosidic bonds, thereby facilitating identification of the sugar residues. The peak at m/e 336 indicates that cladinose and mycaminose exist as a dissacharide unit, thus conveniently providing sugar sequencing information. Peaks at m/e 699 and 558 indicate that forosamine and cladinose are lost sequentially via a four-centered mechanism (*vide infra*). Peaks at m/e 142 and 127 are corresponding charged fragments. Loss of all sugars as neutral fragments does not occur. These observations implicate mycaminose as the primary site of protonation for those molecules undergoing fragmentation.

Since the spiramycins have two basic centers relatively remote from each other, one might expect to see evidence for double protonation. This apparently does not occur since at high amplification no peak was detected at m/e 422.5 corresponding to the $^{13}$C-isotope peak of MH$_2$$^{++}$.

Spiramycin III (104) differs from spiramycin I in having a propionyl ester $\beta$ to the lactone carbonyl rather than a hydroxyl group. This kind of substituent difference is common among the 16-membered ring macrolides; for example
Fig. XVIII.—Isobutane CI mass spectrum of spiramycin I.
the leucomycins and maridomycins (105,106). Under isobutane CIMS conditions, fragmentation involving loss of propionic acid competes effectively with loss of the terminal sugars. Consequently the CI mass spectrum (Fig. IXX) contains a series of prominent peaks (m/e 825, 758, 755, 684, 681, 614 and 540) corresponding to all possible combinations of losses of the propionic acid and the two terminal sugars from the protonated molecule ion (m/e 899). Therefore, the fragment ions not only identify the acyl group, but also clearly indicate that the acyl group is not attached to one of the sugars. This latter observation is further substantiated in that ions m/e 336, 142, and 127 are identical to those seen in spiramycin I. Attachment of various acyl functions to the terminal sugar of a variety of macrolide antibiotics in this class is quite common and the ease with which this distinction can be made is quite apparent.

The isobutane (CI) mass spectrum of niddamycin (107) (Fig. XX) is comparatively simple, partly because only two sugars are present. Loss of the terminal sugar from the abundant protonated molecule ion (m/e 784) gives the intense m/e 556 ion. Additional structural information regarding the terminal sugar is provided by the prominent series of peaks consisting of m/e 229, 211, and 109, which arise through mechanisms previously discussed. These peaks indicate that the 2-methylbutarate ester function must be located on the sugar cladinose and not on mycaminose or the
Fig. IXX.— Isobutane CI mass spectrum of spiramycin III.
Fig. XX. -- Isobutane Cl mass spectrum of mtdamycin.
aglyconic moiety. The prominent peak at m/e 420 establishes that the sugar units are linked as a disaccharide. The intensity of the M+2 ion (m/e 786) indicates that there is some dihydroniddamycin present in this sample.

Laidlomycin is an antibiotic recently isolated by F. Kitame of the Tohoku University School of Medicine, Sendai, Japan. It is a narrow spectrum antibiotic and currently is being subject to chemical and spectroscopic analyses in order to determine its structure. Initial nmr data (100 MHz) along with hydrolysis data seem to indicate this antibiotic to be erythromycin A with two new neutral sugars replacing desosamine and cladinose. However, its isobutane CI mass spectrum (Fig. XXI) suggests this notion as dubious, and leads one to suspect that it probably is not a macrolide. This basis of this assertion rests primarily in the absence of a retro-aldol fragment at m/e 115, commonly seen in macrolides containing the erythronolide A aglyconic moiety.

The intense ion at m/e 721 could be the protonated molecule ion or could represent a fragment resulting from elimination of a small molecule(s). If the sugars are nonamino in nature and are attached to the aglyconic unit as monosaccharides, one would expect to see an intense ion for each fragment at odd m/e values as a result of cleavage following protonation on the glycosidic oxygen. Intense ions are seen at m/e 273, 271, 257, and 255, but defy
Fig. XXI. -- Isobutane CI mass spectrum of laidlomycin.
assignment since they do not correspond to any neutral sugars presently found in macrolide antibiotics. Furthermore, prominent \( \text{MH}^+ \)-sugar ions should be seen; none of the above values when subtracted from m/e 721 gives an observed ion. Hence it must be concluded that either a protonated molecule ion is absent or else the sugars are not simply mono- or disaccharide in nature, or both. The base peak at m/e 399 is likewise unexplicable.

For the CI mass spectra of those macrolides containing a sugar moiety(s), it has been observed that prominent fragment ions corresponding to loss of one or more neutral sugars from the aglyconic moiety are usually present (70, 71). This process assumes protonation at a site remote from the glycosidic linkage, followed by a 4-centered fragmentation as depicted in Fig. XIII. This avoids the necessity of involving a double protonation for which there is no evidence. To lend support to this hypothesized mechanism, a series of tetrahydropyranyl ethers of some amino alcohols were synthesized (86). These compounds were chosen because they contain a basic amino function to serve as a strong proton acceptor and a ketal function comparable to the glycosidic linkage in the macrolide antibiotics. The length of the amino side chain was varied to see if there would be selective protonation on the amino function as the length was increased, and hence result in more selective fragmentation events.
Fig. XXII. -- Isobutane CI mass spectrum of THP-ether of 4-hydroxy-N-methylpiperidine.
The isobutane CI mass spectrum of 4-hydroxy-N-methylpiperidine-THP-ether is shown in Fig. XXII. It should be noted that protonation can occur on each heteroatom in the molecule. Fragmentation leading to smaller charged units would result from protonation on either the nitrogen or on the glycosidic oxygen. Fig. XXIII gives the hypothesized fragmentation scheme accounting for each observed ion.

Loss of dihydropyran via a 4-centered process from ion 54 (m/e 200) would result in ion 55 (m/e 116). Subsequent loss of H₂O would lead to ion 58 (m/e 98). On the other hand a 4-centered mechanism involving hydrogen transfer from the N-methylpiperidinol moiety could likewise give ion 58. Initial protonation on the glycosidic oxygen followed by extrusion of 4-hydroxy-N-methylpiperidine would give oxonium ion of m/e 85. Ion 62 (m/e 57) formed by retro Diels-Alder cleavage of 61 is isobaric with the tert-butyl carbonium ion, so it is not detected in isobutane CI mass spectra. However, it is prominent in methane CI mass spectra of other THP-ethers examined.

Metastable ion peaks were observed at the calculated masses for fragmentation (y) in Fig. XXIII(a) (m/e 200 [67.3] 116 [82.8] 98), thereby indicating that fragment ion 55 is formed directly from the protonated molecule ion and not by protonation of the neutral amino alcohol 60. Consequently, one of the hydrogen atoms in 54 must have migrated from the tetrahydropyranyl nucleus. In order to
(a) Protonation on nitrogen

\[
\text{Me-N}^+ \xrightarrow{(x)} \text{Me-N}^+ \xrightarrow{(y)} \text{Me-N}^+ \]

\[
\begin{align*}
\text{m/e 200 (R=H)} \\
\text{m/e 201 (R=D)} \\
\text{m/e 116 (R=H)} \\
\text{m/e 117 (R=D)} \\
\text{m/e 98}
\end{align*}
\]

(b) Protonation on glycosidic oxygen

\[
\text{Me-N}^+ \xrightarrow{(x)} \text{Me-N}^+ \]

\[
\begin{align*}
\text{m/e 200} \\
\text{m/e 57}
\end{align*}
\]

Fig. XXIII.— Fragments resulting from protonation on nitrogen or glycosidic oxygen of THP-ether of 4-hydroxy-N-methylpiperidine.
verify the origin of the migrating hydrogen, compound 54 (R=D) was prepared containing deuterium in the 3-position of the tetrahydropyranyl ring, and its methane CI mass spectrum was examined. The results are given in Table III.

Table III. Comparison of ion-abundance ratios for deuterated and undeuterated 54.

<table>
<thead>
<tr>
<th>Ion-abundance ratios</th>
<th>Measured ratios on natural abundance isotope</th>
<th>Measured ratios containing deuterium</th>
<th>% of ions containing one deuterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>201/200</td>
<td>0.114±0.006</td>
<td>0.340±0.012</td>
<td>22.6</td>
</tr>
<tr>
<td>117/116</td>
<td>0.073±0.002</td>
<td>0.178±0.005</td>
<td>10.5</td>
</tr>
</tbody>
</table>

If pathway (y) is the dominant mechanism for the formation of fragment ion 55, the abundance of the deuterium-containing ion 55 (R=D, m/e 117) relative to the undeuterated ion 55 (R=H, m/e 116) should be one-half the abundance of the deuterium-containing protonated molecule ion (m/e 201) relative to the undeuterated protonated molecule ion (m/e 200), since either of the two hydrogens at the 3-position could migrate. Within the accuracy of the experiment, this is exactly what is observed, thus indicating that not only does protonation occur predominately on

*The ± values represent the standard deviation for three consecutively recorded mass spectra.
nitrogen, but also that the source of the migrating hydrogen is predominately the tetrahydropyranyl nucleus. Furthermore, 58 formed from 55 is substantially deuterium-free.

Figs. XXIV-XXVI give the methane CI mass spectra of the THP-ethers of N,N-dimethyl-2-amino-1-ethanol, N,N-dimethyl-3-amino-1-propanol, and N,N-dimethyl-5-amino-1-pentanol, respectively. Methane as the reagent gas gives greater fragmentation and correspondingly smaller MH⁺ peaks. In each case the oxonium ion at m/e 85 forms the base peak. The fragmentation pattern is essentially the same as for the isobutane spectrum of the cyclic analog with the exception of intense M-1⁺ peaks (and their corresponding fragment, CH₂=NMε₂⁺), both of which are documented processes for methane CIMS (38,53). The retro Diels-Alder fragment at m/e 57 is now quite visible. Increasing the length of the side chain seems to increase the intensity of the MH⁺ and M-1⁺ ions relative to the other ions, and can be attributed to the increasing hydrocarbon character of the side chain (38).

The effect of the distance between the primary protonation center and the fragmentation processes discussed previously is negligible and appears to support the independent existence of two major fragmentation pathways and the lack of double protonation events.
Fig. XXIV. — Methane CI mass spectrum of THP-ether of N,N-dimethyl-2-amino-1-ethanol.
Fig. XXV.— Methane Cl mass spectrum of THP-ether of N,N-dimethyl-3-amino-1-propanol.
Fig. XXVI.— Methane CI mass spectrum of THP-ether of N,N-dimethyl-5-amino-1-pentanol.
CIMS of β-lactam antibiotics.—Relatively few accounts of the mechanisms involved in the EI mass spectra of β-lactam antibiotics have appeared in the literature (108,109). In order for sample volatilization to occur, it was necessary to carry out studies on methyl ester derivatives. Molecular ions are reasonably intense, but interpretation of the origin of fragment ions is difficult due to the abundance of ions in the low mass region. This study (77), however, shows that CIMS can be carried out on both the free acids and ester derivatives of β-lactam antibiotics. The isobutane CI mass spectra of the acids give poor to good protonated molecule ion peaks and fragmentation is moderately extensive. However, most of the fragment ions can be shown to arise through loss of simple molecules such as H₂O, HCN, CO₂, H₂S, and CO along with well-established molecular rearrangement processes and some reactions with solution counterparts. The isobutane and ammonia CI mass spectra of ester derivatives are even less complex giving moderate to very intense protonated molecular ions.

Table IV compares the peak intensity for ions common to most of the penicillin free acids examined by isobutane CIMS. There is considerable variation in peak intensity for analogous ions and this can usually be rationalized with the relative ease one presumes that the molecule can follow a given pathway. More than one mechanism can
<table>
<thead>
<tr>
<th>Compound</th>
<th>MH⁺</th>
<th>MH⁺</th>
<th>MH⁺</th>
<th>MH⁺</th>
<th>MH⁺</th>
<th>MH⁺</th>
<th>m/e</th>
<th>m/e</th>
<th>m/e</th>
<th>m/e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>335</td>
<td>317</td>
<td>291</td>
<td>273</td>
<td>220</td>
<td>194</td>
<td>176</td>
<td>100</td>
<td>15.6%</td>
<td>10.8%</td>
</tr>
<tr>
<td>Penicillin V</td>
<td>351</td>
<td>333</td>
<td>307</td>
<td>289</td>
<td>236</td>
<td>210</td>
<td>192</td>
<td>100</td>
<td>16.4%</td>
<td>39.2%</td>
</tr>
<tr>
<td>Methicillin</td>
<td>381</td>
<td>363</td>
<td>337</td>
<td>319</td>
<td>266</td>
<td>240</td>
<td>222</td>
<td>6.8%</td>
<td>16.4%</td>
<td>98.4%</td>
</tr>
<tr>
<td>6-APA</td>
<td>217</td>
<td>217</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>436</td>
<td>418</td>
<td>392</td>
<td>374</td>
<td>321</td>
<td>295</td>
<td>277</td>
<td>66.4%</td>
<td>22.8%</td>
<td>17.6%</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>350</td>
<td>332</td>
<td>306</td>
<td>235</td>
<td>191</td>
<td></td>
<td>5%</td>
<td>2%</td>
<td>2.4%</td>
<td>5.2%</td>
</tr>
<tr>
<td>Metacillin</td>
<td>390</td>
<td>249</td>
<td>233</td>
<td>210</td>
<td>191</td>
<td>171</td>
<td>5.2%</td>
<td>2%</td>
<td>8%</td>
<td>28.8%</td>
</tr>
</tbody>
</table>

* Acquired on Finnigan 1015 quadrupole mass spectrometer with isobutane as the reagent gas.
Fig. XXVII.— Isobutane CI mass spectrum of penicillin G.
usually be invoked for any single event in mass spectrometry unless considerable study is undertaken. The "simplest" or most likely mechanisms will be advanced in this discussion. Verification of any proposed route will be offered in future studies with high resolution mass measurements and/or labelled antibiotics (77).

The isobutane CI mass spectrum of penicillin G (Fig. XXVII) displays fragmentation patterns that typify most of those observed for the other penicillins listed in Table IV. Hence an extensive discussion will be advanced for this spectrum to delineate common mechanisms. A relatively weak protonated molecule ion is seen at m/e 335. Ions due to loss of simple neutral molecules H$_2$O (m/e 317), H$_2$S (m/e 301), CO$_2$ (m/e 291), or combinations thereof (m/e 283, 273, 257), are present as weak to very weak peaks. Loss of H$_2$O is a frequent event for compounds in this study giving a weak to moderate ion at MH$^+$-18. The

![Proposed mechanism for loss of H$_2$O from the protonated molecule ion of penicillin G in isobutane CIMS.](image)
mechanism advanced for loss of H₂O in penicillin G is shown in Fig. XXVIII. This mechanism requires that loss of H₂O via initial protonation on the carboxy function is not a common process, but rather initial protonation occurs elsewhere, formulated here as occurring on the basic lactam nitrogen, followed by formation of stable ketene 64. Furthermore, if initial protonation did occur on the carboxyl function, followed by loss of H₂O and formation of an acylium ion, one would also expect to see a prominent ion attributed to subsequent loss of CO. This generally is not observed. Loss of CO₂ from the protonated molecule ion is usually not a prominent event as evidenced by extremely small MH⁺-44 ions for

Fig. XXIX.--- Possible mechanism for concerted loss of H₂O and CO₂ from the protonated molecule ion of penicillin G in isobutane CIMS.
most of the compounds listed in Table IV. However, loss of CO₂ is quite common when in concert with loss of H₂O as evidenced by moderately intense MH⁺-62 ions. Fig. XXIX shows a possible mechanism for this process for penicillin G. It should be noted in this regard that one could not show loss of CO₂ from ion 64 and hence separate mechanisms are invoked. The ion at MH⁺-115 is a prominent feature for those compounds shown in Table IV and could represent initial protonation on the β-lactam nitrogen, followed by eventual extrusion of an aziridine moiety as shown in Fig. XXX for penicillin G.

The fragment at MH⁺-141 is quite intense and represents
Fig. XXXI.-- Possible mechanism for formation of $\text{MH}^+ - 141$ moiety from the protonated molecule ion of penicillin G in isobutane CIMS. A process quite favorable to most compounds listed in Table IV. Its proposed mechanism of formation invokes protonation on the side chain amide carboxyl followed by successive rearrangements to a thiazolidinium moiety as depicted in Fig. XXXI for penicillin G.

The $\beta$-lactam ring in both the penicillins and
cephalosporins is quite strained and thus subject to facile molecular rearrangement. Accordingly, the ion seen at m/e 160 for all but one compound listed in Table IV usually represents the base peak. Its formation can be rationalized by invoking the rearrangement processes given in Fig. XXXII for penicillin G. Initial protonation can be invoked either on the β-lactam nitrogen or the sulfur atom of the thiazolidine ring. Note that this four-centered fragmentation is quite analogous to that invoked for fragmentation of 70 to 71.

Process (b) implies that the facility of formation of 75' is highly dependent on the nature of the amide side chain moiety. This requirement would be analogous to that used to explain the acid stability of various penicillin derivatives (110). Alternatively, the same rearrangement process shown in Fig. XXXII can occur after initial protonation on the side chain amide moiety to give an $\text{MH}^+ - 159$ ion. This ion can be either weak or quite intense depending on the nature of the functionality in the side chain (e.g., amino functions enhance peak intensity) and serves as a useful diagnostic tool for this region of the molecule. Figure XXXIII demonstrates this process for penicillin G. It should be pointed out that the facile 4-centered rearrangement processes outlined in Figs. XXXII and XXXIII could also occur by an alternate "horizontal" mechanism,
(a) 2+2 Retro-Diels-Alder rearrangement following protonation on sulfur.

(b) Rearrangement with anchimeric assistance from the side chain following protonation on β-lactam nitrogen.

Fig. XXXII.—Proposed mechanisms for formation of ion m/e 160 in isobutane CI MS of penicillins.
leading not to fragmentation, but contributing to protonated molecule ion intensity only (c.f., Fig. XXXI).

Ions at m/e 142, 133, 126, and 116 can form directly from ion 75 by respective extrusion of H$_2$O, HCN, H$_2$S, and CO$_2$. Figure XXXIV outlines these pathways.

A moderately intense ion found in most of the spectra of the penicillins shown in Table IV is that at m/e 101. Its mechanism of formation is not immediately obvious. One can postulate rearrangement of the protonated molecule ion 82 to a neutral nitrene species and charged fragment as shown in Fig. XXXV for penicillin G.

The isobutane CI mass spectrum of penicillin V (Fig. XXXVI) features fragmentation ions closely analogous to those seen with penicillin G. One again sees a relatively weak protonated molecule ion at m/e 351 and weak ions at m/e 333, 317, 307 corresponding to loss of H$_2$O,
Fig. XXXIV.---Proposed mechanism of formation of ions of m/e 142, 133, 126, and 116 in isobutane CIMS of penicillins.
Fig. XXXV.—Proposed mechanism of formation of ion m/e 101 in isobutane CIMS of penicillins.

H₂S, and CO₂, respectively, along with combination ions at m/e 299, 289, and 273. The peaks at m/e 236 (MH⁺-115), 210 (MH⁺-141), 192 (MH⁺-159), m/e 160, m/e 142, m/e 133, m/e 126, m/e 116, and m/e 101 probably arise via the mechanisms outlined in Figs. XXVIII-XXXV and do not require further comment. A moderately intense ion at m/e 219 (MH⁺-132) is analogous to a weak ion seen at m/e 203 for penicillin G. Its formation can be rationalized as follows:
Fig. XXXVI. — Isobutane CI mass spectrum of penicillin V.
In an attempt to corroborate or destroy the mechanisms proposed in Figs. XXVIII-XXXV with labelling studies, an attempt was made to prepare $^{18}$O-carboxypenicillin V by repeated lyophilization of 40% $^{18}$O$_2$ solutions containing penicillin V. However, its isobutane CI mass spectrum did not show the anticipated mass shifts for any of the prominent ions. Presumably exchange with $^{18}$O$_2$ occurred to some extent. If so, it was so low that it escaped detection by this technique.

In the isobutane CI mass spectrum of methicillin (Fig. XXXVII), a considerably more intense protonated molecule ion than observed for previous penicillins is seen at m/e 381. The standard mechanistic processes previously outlined rationalize the occurrence of most prominent ions in the high mass range. Conspicuously absent is ion $^{~75}$ (m/e 160) which quite frequently forms the base peak ion. However, a base peak ion is now found at m/e 165 and probably this arises via cleavage of the side chain amide bond to give acylium ion $^{~88}$ and 6-APA as shown on page 81. Methicillin is the only molecule in this study to show this cleavage. The formation of $^{~88}$ is analogous to the very facile hydrolysis of certain aromatic esters in which acyloxygen fission occurs to form a stable acylium ion (111). Both steric and electronic factors appear to be
Fig. XXXVII.-- Isobutane CI mass spectrum of methicillin.
operative (112). The unusual absence of ion 75 (m/e 160) suggests that pathway (b) given in Fig. XXXII might be predominately operative in the formation of this ion, or else the spectrum of this penicillin is idiosyncratic with respect to the unusual observation. The presence of ions at m/e 133, 126, and 116 does not vitiate the mechanisms hypothesized for their formation in Fig. XXXIV. This simply suggests that their pathways of formation involve different sequences of events. Initial protonation of the thiazolidine sulfur followed by ring opening to an imminium fragment would give a species isoatomic with the protonated molecule ion. Bond cleavage with extrusion of the neutral
\[ \beta\text{-lactam ring fragment would give ion } 79 \text{ (m/e 133).} \]
Alternatively, prior loss of H\textsubscript{2}O or CO\textsubscript{2} followed by a 4-centered retro-Diels-Alder rearrangement outlined in Fig. XXXII would result in fragment 80 (m/e 126) and another fragment isoatomic with 81 (m/e 116). The enhanced intensity of the latter ion over the previous penicillins discussed is somewhat surprising and not easily rationalized. One should note the absence of a peak at m/e 142. This suggests that ion 75 (m/e 160) is an obligatory intermediate in its formation (vide infra, Fig. XXXIV). Further evidence that formation of ion 75 might be highly dependent on the nature of the side chain is implicated in the isobutane CI mass spectrum of 6-aminopenicillanic acid (6-APA) (Fig. XXXVIII). The m/e 160 ion is present but of considerably lower intensity, presumably due in part to the lack of an amide side chain and to the presence of a primary amino function which can control protonation events. The resulting base peak is the protonated molecule ion at m/e 217. An intense ion at m/e 189 represents loss of CO, a process not seen in other free acid derivatives. Its formation can be proposed as shown in Fig. XXXIX. This makes this spectrum idiosyncratic among the penicillin free acids in this study.

The isobutane CI mass spectrum of cloxacillin (Fig. XL) shows a very weak protonated molecule ion at m/e 436. However, prominent ions corresponding to those seen for penicillins G and V as outlined in Figs. XXVIII-XXXV are
Fig. XXXVIII.---Isobutane CI mass spectrum of 6-aminopenicillanic acid (6-APA).
A primary reason for the search for new semi-synthetic penicillins has been the need for antibiotics effective against a wider range of pathogens than before. Older penicillins show little activity against Gram-negative organisms. It has been found that an amino group in the side chain contributes to effectiveness against some Gram-negative pathogens (113). The most widely used semi-
Fig. XL.-- Isobutane CI mass spectrum of cloxacillin.
synthetic penicillin, ampicillin, is an example. Hetacillin, a prodrug of ampicillin, has been reported to give a more prolonged action (114). The isobutane CI mass spectra of ampicillin and hetacillin are shown in Figs. XLI and XLII, respectively. Both spectra show relatively intense protonated molecule ions at m/e 350 and 390, respectively. Both show few or no high mass fragments corresponding to loss of $\text{H}_2\text{O}, \text{H}_2\text{S}$, or $\text{CO}_2$. This is within expectation since the side chain amino function now predominately controls initial protonation events. Accordingly, both spectra also show prominent $\text{MH}^+-159$ ions at m/e 191 for ampicillin and at m/e 231 for hetacillin (see Fig. XXXIII). In each spectrum are lower mass fragment ions whose formation can be rationalized on the basis of the mechanisms outlined in Figs. XXX-XXXV. One exception in this regard is the absence of a $\text{MH}^+-115$ ion for hetacillin and a $\text{MH}^+-141$ ion for ampicillin. Moderately prominent fragments at m/e 204 and 150 in ampicillin are without precedent and are presently unexplicable.

Since the isobutane CI mass spectra of penicillins G and V (Figs. XXVII and XXXVI) and cloxacillin (Fig. XXXIX) show quite weak protonated molecule ions, it was decided to prepare their methyl esters by conventional means (89) in order to increase volatilization in the sample probe. Also the ester derivatives were examined to see if mass shifts could be observed for various hypothetical fragments. The
Fig. XLI.— Isobutane CI mass spectrum of ampicillin.
Fig. XLII.—Isobutane CI mass spectrum of hetacillin.
spectra of the resulting esters are considerably less complex and result from lower energy processes. Weak to moderately intense ions seen at m/e 89 are artifactual and arise from EtOAc which was used as an extracting solvent in the synthesis of the ester derivatives.

The isobutane CI mass spectrum of penicillin G methyl ester is shown in Fig. XLIII. The protonated molecule ion (m/e 349) is of moderate intensity and allows for ready determination of molecular weight. A very weak ion at m/e 321 corresponds to loss of CO and could arise via the mechanism shown in Fig. XXXIX. Moderately weak fragments at m/e 225 and 212 are presently unexplicable. Determination of their origin awaits high-resolution mass measurements (77). The base peak ion at m/e 174 is 14 a.m.u. greater than fragment 75 and hence lends credence to the mechanism outlined in Fig. XXXII.

The isobutane CI mass spectrum of penicillin V methyl ester (Fig. XLIV) is analogous to that of penicillin G methyl ester despite a diminution in relative intensity of the protonated molecule ion at m/e 365. However, its ammonia CI mass spectrum (Fig. XLV) shows an increase in intensity of the protonated molecule ion along with an enhancement of the m/e 225 peak. The m/e 212 ion is now not seen. In the isobutane CI mass spectrum of cloxacillin methyl ester (Fig. XLVI), one sees essentially the same processes occurring with the exception of a moderately
Fig. XLIII.-- Isobutane CI mass spectrum of penicillin G methyl ester.
Fig. XLIV. — Isobutane CI mass spectrum of penicillin V methyl ester.
Fig. XLV.-- Ammonia CI mass spectrum of penicillin V methyl ester.
Fig. XLVI.— Isobutane CI mass spectrum of cloxacillin methyl ester.
intense peak at m/e 277 which could arise from initial protonation on the isoxazole ring nitrogen followed by a rearrangement process analogous to that outlined in Fig. XXXII.

The instability of penicillins to extremes of both acidic and basic conditions is well documented (110). Acid treatment results in rapid rearrangement to penillic acids (pH 2) and penicillenic acids (pH 4) with anchimeric assistance from the side chain amide function. Consequently, penicillin G undergoes extensive degradation when it comes in contact with the acidic milieu of the stomach. This requires oral doses at least 5 times the recommended parenteral dose for therapeutic effectiveness (115). In the presence of alkali, penicillins convert to penicilloic acids. The isobutane CI mass spectrum of penicilloic acid G dimethyl ester is given in Fig. XLVII. The spectrum is extremely simple with a base peak protonated molecule ion at m/e 381. One can attribute this enhancement of protonated molecule ion intensity over that observed for the penicillin free acids and methyl esters to both an increase in sample volatility and the presence in the thiazolidine moiety of a free secondary amino function which would control the site of protonation. Less strain should also be present in the molecule due to absence of the β-lactam ring. A moderately intense fragment at m/e 174 could be shown to form as depicted in Fig. XLVIII following
Fig. XLVII. — Isobutane CI mass spectrum of penicilloic acid G dimethyl ester.
protonation on the side chain ester carbonyl.

Fig. XLVIII.—Proposed formation of ion m/e 174 in isobutane CI MS of penicilloic acid dimethyl esters.

The isobutane CI mass spectrum of penicilloic acid V dimethyl ester (Fig. XLIX) likewise shows a base peak protonated molecule ion at m/e 397. The peak at m/e 174 can be explained by the mechanism given in Fig. XLVIII and the less intense idiosyncratic peak at m/e 152 can be explained by initial protonation on the amide carbonyl followed by a McLafferty rearrangement shown on page 98.

The cephalosporin antibiotics are of interest because of their marked bactericidal activity and excellent stability to the action of acid and penicillinase (116). A synthetic
Fig. XLIX. -- Isobutane CI mass spectrum of penicilloic acid V dimethyl ester.
chemical program based on modification of the cephalosporin nucleus, 7-aminocephalosporanic acid (7-ACA), has yielded many new antibiotics including cephalothin (117, 118). Cephalothin exhibits in vitro activity against both Gram-positive and Gram-negative bacteria. Cephalothin is not absorbed in humans after oral administration and must be given parenterally. Its isobutane CI mass spectrum is shown in Fig. 1. A preliminary scan indicated that no protonated molecule ion was observed at m/e 397 and hence this portion of the mass scale was not recorded. The ion of m/e 337 is of moderate intensity and represents loss of AcOH following protonation on the acetate moiety. Further extrusion of CO₂ from fragment 99 leads to ion of m/e 293.
Fig. L. -- Isobutane CI mass spectrum of cephalothin.
These events are outlined in Fig. LI. Protonation on the lactam nitrogen followed by rearrangement processes and extrusion of $C_2H_2$ as shown in Fig. LII account for ions of m/e 226 and 200, the former being the base peak.

Another site of protonation is on the side chain thiophene ring. Protonation here followed by a 4-centered rearrangement as outlined in Fig. XXXIII for the penicillins would lead to the observed ion at m/e 182. It is interesting to note in this context that a companion ion

Fig. LI. — Proposed fragmentation events following presumed protonation on acetate moiety in isobutane CIMS of cephalothin.
Fig. LII.-- Proposed fragmentation events following presumed protonation on lactam nitrogen in isobutane CIMS of cephalothin.
corresponding to the same process as delineated in Fig. XXXII for the penicillins is not seen for cephalothin. It remains to be seen if this phenomenon is a general characteristic of the cephalosporins. The origin of weak to moderate ions at m/e 226 and 142 is not presently understood.

From initial data it appears that the same basic fragmentation processes are operative for both the penicillins and cephalosporins. The intensity of the protonated molecule ion makes molecular weight determination easy only in those cases in which free amino groups are present or when acid moieties are esterified. The modes of fragmentation are usually fairly decipherable and in many cases have solution chemistry counterparts.

The EI (108) and CI mass spectra of penicillin G and penicillin V methyl esters both show base peak ions at m/e 174 presumed to arise via mechanisms proposed in Fig. XXXII. Both types of spectra show moderately intense molecule or quasi-molecule ions although the peak intensity is slightly greater in the former case. This is somewhat surprising. The CI mass spectra, however, are considerably less complex, making spectral interpretation easier. In general, from the isobutane CI mass spectra of both the penicillin acids and ester derivatives examined, it appears that peaks in the mass region above m/e 160 (174 for esters) are diagnostic of the amide side chain substituent. This serves as an effective "fingerprinting" tool. The
region below m/e 160 (174) is diagnostic of the thiazolidine moiety. With respect to these general characteristics, CIMS of the β-lactam antibiotics is quite similar to that for the macrolides, making this tool a useful adjunct to the more classical techniques of IR, NMR, EIMS, and UV in structural determination.
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PART II

ANTIMICROBIAL AGENTS FROM HIGHER PLANTS. ISOLATION
OF CANTHIN-6-ONE FROM ZANTHOXYLUM ELEPHANTIASIS
AND APPROACHES TO ITS SYNTHESIS AND ANALOGS.
INTRODUCTION

Over the last twenty-five years since the introduction of penicillin G into clinical use, man has been making intensive efforts at uncovering new, clinically useful antibiotics. Heretofore, most of his efforts have centered around lower plant forms and bacteria, with special emphasis on various Streptomyces strains and a few fungi. Despite the significant chemotherapeutic impact of drugs from these sources, certain disease entities remain ubiquitous and not satisfactorily treated by major antibiotics at hand.

Of the approximately two dozen clinically utilized antibiotics (1), many have considerable drawbacks in terms of limited antimicrobial spectrum or side effects. Especially needed are safe and efficacious antibiotics for clinical infections caused by Gram-negative organisms, fungi, viruses, and mycobacteria.

Recently a few potentially clinically useful antibiotics have been reported from other microbial sources such as Micromonspora, Nocardia, Microspora, and certain fungi. The structures of these agents are frequently analogous to those of the Streptomyces-derived antibiotics, but there are some notable variations (e.g., chelocardin from
Nocardia sulphurea (2), kanamycin from Streptomyces kanamyceticus, and gentamicin from Micromonospora purpuracens). In addition to these sources, new antibacterial agents from higher plant sources have been described (3-7) and are now being isolated at an increasing rate in an attempt to provide clinically and commercially significant new antibiotics with activities supplemental to and structures widely different from traditional agents.

Systematic screening studies for antimicrobial activity in higher plants have been numerous. Osborn (1943) surveyed more than 2300 different species of plants collected in England and tested them against S. aureus and E. coli (8). Several other workers (9-18) have reported antibacterial activity for plant extracts from a wide variety of terrestrial sources. Burkholder et al. (19) and later Su and co-workers (20) have examined antimicrobials from aquatic sources including some higher plants. Special studies (21-24) have been directed toward finding plant sources of antibacterial activity against Mycobacterium tuberculosis.

Relatively recent screening reports describe the study of individual plants for antimicrobial activity. Notable amongst these are the discovery and characterization of the antimicrobials uliginosin A and B from Hypericum uliginosum HBK (25-27) and isohumolone from Humulus lupulis (28). Tschesche's work on extracts from Hedera sp., Avena sp., Convallaria sp., Solanum sp., Sarsaparilla sp., Cyclamen
sp., and Spinacea sp. have shown that the isolated saponins possess marked antimicrobial activity (29). Another quite different group of antimicrobials are the glycosides with a lactone-forming aglycone, which are isolated from or found to be present in a large number of plants such as Tulipa sp., Pyrus sp., and various species of the Ranunculaceae (29). The study of the antimicrobial activity of essential oils also has been of interest (30-35). Similarly, fungistatic action from diseased safflower (36), broad bean (37), Dalbergia sp. (38), and Juglans sp. (39) has been reported.

Mitscher et al. have carried out extensive investigations into the isolation of antimicrobials from Thalictrum rugosum (40-42) and Thalictrum polygamum (43). Fractionation of various extracts revealed the presence of the active compounds berberine, obamegine, thalidasine, thalrugosine, thalrugosidine, and thalrugosamine. The first three were previously known structurally although their potential antitubercular activity had not been reported. The latter three are weakly active and represent new bis-benzylisoquinoline alkaloids. Mitscher also has examined the alkaloidal extracts of Ptelea trifoliata L. and has found the presence of new quaternary quinoline alkaloids, one of which shows activity against Mycobacterium smegmatis and Candida albicans (44).

Vichkanova et al. (45) have recently screened 33 coumarins from higher plants and have found 4-methyl-7-
hydroxycoumarin, pimpinellin, isopimpinellin, and prangolarin to be the most active against a series of organisms. In an earlier screening procedure (46), a series of fatty acid esters extracted from *Tropaeolum major*, *Phytolacca americana*, and *Ochillea salicifolia* likewise displayed antimicrobial activity.

Schemes designed for the total synthesis of antibiotics have been applied primarily to new, exotic structures elaborated by various fungi and bacteria. However, efforts directed toward the synthesis of antimicrobials from higher plants and structurally related analogs are becoming more commonplace.

Barnes et al. (47) synthesized 2,3',4,5'-tetrahydroxystilbene to confirm the structure of a compound that was isolated from *Taxylon pomiferum* and found to have significant antifungal activity.

Szczepanski et al. (48) have recently synthesized an unusual antimicrobial sulfur compound isolated from *Petiveria alliacea* L.

Mitscher et al. have given special attention to efficient syntheses of various compounds containing the canthin-6-one ring system (49-51) and the quinoline and furoquinoline nucleus (52).

The purpose of the research.—The objective of this research was to isolate and characterize the antimicrobial agent(s)
from *Zanthoxylum elephantiasis* Macf. This work was subsequently published (53). Through a large-scale screening program for antimicrobial agents (16), it was found that the neutral and alkaloidal extracts of the bark of *Zanthoxylum elephantiasis* had reproducible activity against *Staphylococcus aureus* (ATCC 13708), *Klebsiella pneumoniae* AD (ATCC 10031), *Mycobacterium smegmatis* (ATCC 607) and *Candida albicans* (ATCC 10231), all at 100 μg/ml. The neutral fraction was further purified as described in this dissertation to give the active component canthin-6-one (1).

New synthetic routes to canthin-6-one, starting with readily available compounds, were carried out. Additional efforts were directed toward the generation of analogs in an attempt to better understand the minimum structural requirements conferring bioactivity to this molecule.

Previous studies on *Zanthoxylum elephantiasis* and of synthetic approaches to canthin-6-one and its analogs.—The genus *Zanthoxylum* represents one of the 140 genera constituting the fairly large Rutaceae family and contains more than 200 species dispersed primarily over tropical and subtropical regions of the world. From a purely chemical viewpoint, *Zanthoxylum* is a fascinating genus because, with respect to its constituents, it is probably one of the most versatile and heterogenous genera known.

Price (54) in a review of the distribution of alkaloids in the Rutaceae alludes to the nomenclature ambiguity
between the genera *Zanthoxylum* and *Fagara*. Indeed *Z. elephantiasis* was at one time placed in the genus *Fagara* (55), but this dichotomy has been reasonably well resolved so that less confusion now exists (56).

*Zanthoxylum elephantiasis* Macf. (*Z. aromaticum* DC., *Fagara elephantiasis* Kr. and Urb.) is indigenous to the Caribbean region predominating in Costa Rica, Mexico, Cuba, Hispaniola, and in Jamaica where it is known popularly as "yellow sanders." It is a tree of moderate size, 15-40 ft in height, which is conspicuous in having corky conical spiny knobs on the bark around the base of the trunk (55).

Special attention has been directed toward the isolation of the alkaloidal components of the bark of *Z. elephantiasis*. Awad et al. (57-59) described the isolation of canthin-6-one (1), 5-methoxycanthin-6-one (2), laurifoline chloride (3), candidine chloride (4), all from the alkaloidal fraction, and xanthoxyletin (5) from the petroleum ether extract (Fig. I).

The occurrence of canthin-6-one in plants was first reported by Haynes, Nelson, and Price (60) in their report on the alkaloids of *Pentaceras australis* Hook (Rutaceae). In subsequent studies there was reported the isolation of canthin-6-one from *Z. suberosum* C.T. White (61), *Z. dominicanum* Merr. and Perry (62), *Fagara viridis* A. Cheval (Rutaceae) (63), and *Z. ololifolium* (64).
Fig. I.— Canthine ring system and compounds isolated from Zanthoxylum elephantiasis Macf.
Other naturally-occurring canthin-6-one analogs have been isolated from various plant sources and their formulae are given in Fig. II along with the bibliographic citations. The synthesis of various canthin-6-one analogs has been carried out both by total and by semi-synthetic means. Fig. III lists almost all analogs made to date, along with their corresponding bibliographic citations.

Fig. II.— Canthin-6-one analogs isolated from various plant sources.
Fig. III  Some canthin-6-one compounds derived from synthesis.
A formal synthesis of canthin-6-one was first reported by Bartlett and Taylor (71) and is outlined in the following scheme:

\[
\text{harmane} \xrightarrow{(1) \text{n-butyllithium}} \xrightarrow{(2) (\text{CO}_2\text{Et})_2} \text{5-hydroxy-canthin-6-one (18)}
\]

\[
\text{canthin-6-one (1)} \xrightleftharpoons{\text{AcOH, Zn}} \xrightarrow{(\text{Ref. 65})} \text{4,5-dihydrocanthin-6-one (34)}
\]

Rosenkranz et al. (72) carried out the first total synthesis of canthin-6-one from $\beta-N$-succinoyl tryptophan, but the yield was poor. This synthesis is delineated in the following scheme:

\[
\text{tryptophan} \xrightarrow{\text{pyridine}} \beta-N\text{-succinoyl tryptophan}
\]

\[
\text{canthin-6-one (4 parts)} + \text{4,5-dihydrocanthin-6-one (1 part)} \xrightarrow{\text{PPA, V}_2\text{O}_5, \text{POCl}_3} \]
EXPERIMENTAL

Methodology.—

1. The infrared spectra were taken on a Perkin-Elmer Model 257 infrared spectrophotometer.
2. Ultraviolet spectra were determined on a Cary Model 15 recording spectrophotometer.
3. Proton magnetic resonance spectra were determined in CDCl₃, trifluoroacetic acid, or d₆-DMSO solutions with tetramethyilsilane as an internal standard on a Varian Model A-60A instrument and are reported in δ(ppm) units.
4. Mass spectra were measured with an MS-9 or DuPont Model 21-491 mass spectrometer and were kindly provided by Mr. C. R. Weisenberger and Mr. Edward Fairchild of The Ohio State University.
5. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected.
6. All reported temperatures are in centigrade degrees.
7. Microanalyses were determined by Midwest Micro-laboratories, Indianapolis, Indiana.
8. Thin-layer chromatography was performed either on silica gel G (2.5 X 7.5 cm Eastman Strips, No. 6060, with
fluorescent indicator, or 5 X 20 cm glass plates spread with EM Reagents silica gel G, Cat. 7731), or alumina (2.5 X 7.5 cm Eastman Strips, No. 6063, with fluorescent indicator).

9. Thick-layer chromatography was performed on silica gel G, EM Reagents, Cat. 7731, spread on 20 X 20 cm glass plates.

10. Absorption column chromatography was carried out with neutral alumina (Fisher Scientific or Woelm, grade I or grade III), silica gel 60 (EM Reagents, particle size 0.063-0.200, 70-230 mesh ASTM), or 100 mesh silicic acid (Mallinckrodt).

11. Antibacterial testing was carried out by Mr. R. P. Leu of the College of Pharmacy, The Ohio State University, Mr. Roger White and Mr. Maurice Marietti of the Rohm and Haas Company, Warren-Teed Pharmaceuticals Division, or Dr. F. Denison, Jr., of Abbott Laboratories.

Purity of reagents.—All reagents used in this investigation were of analytical purity, unless otherwise specified.

Source of plant material.—The bark used in this investigation was collected during the summer of 1962 at the foot of Long Mountain in Jamaica and the identification of the plant as Zanthoxylum elephantiasis Macf. was verified by Dr. Dennis Adams, Department of Botany, University of the West Indies.
Extraction and initial fractionation procedures (Fig. IV).—Pulverized bark (500 g) of Zanthoxylum elephantiasis was packed into a glass percolator and allowed to macerate in 95% EtOH at 25° for 12 hr. Percolation was then carried out at a flow rate of 2-3 ml/min until the initially dark green extract became almost colorless. A total of 7.5 l of solvent was required. A small amount of crystalline material (2.7 g) separated from the percolate. This was antimicrobially inactive and was subsequently identified as sucrose by tlc comparison and by comparison of the x-ray powder diagrams of the respective octaacetate derivatives*.

The percolate was concentrated in vacuo to a thick syrup (87 g) at 40°. The residue (A) was active against Staphylococcus aureus (ATCC 13708), Klebsiella pneumoniae AD (ATCC 10031), Mycobacterium smegmatis (ATCC 607) and Candida albicans (ATCC 10231) all at 1000 mcg/ml in vitro using the agar dilution-streak method (16). Residue A was partitioned between 900 ml of 2% aqueous citric acid and three 300 ml volumes of CHCl₃. The combined CHCl₃ layers were dried over anhydrous Na₂SO₄, filtered and evaporated to give 33.1 g of a bioactive residue (B). Residue B was active against the four organisms listed above, but at 100 mcg/ml. The citric acid layer was adjusted to pH 9 with aqueous NH₄OH and extracted with CHCl₃. Evaporation of the dried CHCl₃ layer

*Special thanks is given to Prof. D. Horton and D. Baker of the Chemistry Department, The Ohio State University, for this determination.
**Ground Bark (500 g)**
- EtOH percolation
- Evaporation

**Marc**  **Crystals**  **Residue A (87 g)**
- Sucrose (2.7 g)
- 2% citric acid sol'n/chloroform

**Material insoluble in both phases (5.0 g)**  **Chloroform**  **Aqueous acid**
- Residue B (33.1 g)
  - Adjust to pH 9 with ammonia
  - Skelly solve B/90% methanol
  - Extract with chloroform

**Skelly solve B**  **Methanol**  **Chloroform**
- Residue D (5.9 g)
- Residue E (18.4 g)
  - Chromatography
  - Xanthoxyletin (0.482 g)
  - Canthin-6-one (0.740 g)

**Residue C (0.6 g)**

**Zanthoxylum elephantiasis (Rutaceae)**

*Fig. IV. Fractionation scheme for Z. elephantiasis.*
produced 0.6 g of an active residue (C) (100 mcg/ml against the four organisms). Some material (5.0 g) was insoluble in both layers and was found to be bacterially inactive. The aqueous ammonia phase remaining from removal of the alkaloids was inert.

Residue B (33.1 g) was dissolved in 330 ml of 90% CH$_3$OH and extracted with 330 ml portions of petroleum ether until the petroleum ether extracts were colorless. Evaporation of the petroleum ether fraction produced 5.9 g of inert waxes, etc. (residue D). Evaporation of the CH$_3$OH phase produced 18.4 g of a bioactive (100 mcg/ml vs. four organisms) residue E.

Isolation of canthin-6-one (1) and xanthoxyletin (5) from the methanol residue.—Residue E (18.4 g) was chromatographed over silicic acid (400 g) using CHCl$_3$ as the initial solvent and collecting 80 ml fractions. The polarity was gradually increased by addition of increasing amounts of CH$_3$OH (1%, 2%, 4%, 8%, 16% and 50%). Fractions were collected by weight and the analysis is shown in Fig. V. Fraction G (3.39 g) gave 482 mg of yellow crystals of xanthoxyletin (5) on crystallization from CH$_3$OH; mp 131-132° (lit (59) mp 133°). The identity of this product was confirmed by mixture melting point and comparison of ir, nmr, and uv spectra. This fraction was inactive against bacteria. In fact, fraction H was the only bioactive fraction eluted from the column. Evaporation produced 2.97 g of residue
Fig. V.— Elution diagram (wt. in mgs. vs. fraction number) for silicic acid chromatography of residue E.
which crystallized as light yellow needles from CH₃OH; mp 156-157° (lit (58) mp 159-160°). Filtration produced 740 mg of the active constituent which was identical in mixture melting point, tlc, ir, uv, nmr, and mass spectrum with an authentic sample of canthin-6-one (1). Evidently the feeble basicity of the compound and its CHCl₃ solubility led to its distribution into this fraction which would normally contain non-alkaloidal substances.

**Identification of canthin-6-one (1) in the alkaloidal fraction.**—Chromatography of the crude tertiary alkaloid containing fraction (C) was carried out on Woelm neutral alumina (grade III) as previously described (58). Bioactivity was only present in the canthin-6-one containing fractions. The active agent was identified by comparative tlc (silica gel G-EtOAc as developing agent; canthin-6-one has Rf 0.68 in this system) with an authentic sample. The antibacterial properties of canthin-6-one are set forth in Table I in comparison with the data for streptomycin and 5-methoxycanthin-6-one.

**Preparation of 4,5-dihydrocanthin-6-one (34)(60).**—Canthin-6-one (100 mg, 0.45 mmole), granulated zinc (300 mg, 20 mesh, Baker Chemical Co.), and AcOH (5 ml) were heated at reflux with stirring for 10 min. The reaction mixture was diluted with H₂O, filtered, and basified to pH 8 with concentrated aqueous NH₄OH. Extraction was carried out with CHCl₃.
### Table I. Minimum inhibitory concentration (µg/ml) by agar dilution.**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substance</th>
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</thead>
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<tr>
<td></td>
<td>Streptomycin</td>
</tr>
<tr>
<td>Staphylococcus aureus 209P</td>
<td>3.1</td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em> 10541</td>
<td>&gt;100</td>
</tr>
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</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> 8045</td>
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</tr>
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<tr>
<td><em>Pasteurella multicida</em> 10544</td>
<td>6.2</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em> ED #9</td>
<td>12.5</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>12.5</td>
</tr>
<tr>
<td><em>Mycobacterium smegmatis</em> 101434</td>
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</tr>
<tr>
<td><em>Mycobacterium smegmatis</em> 599</td>
<td>1.56</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> Abbott JJ</td>
<td>6.2</td>
</tr>
</tbody>
</table>

*Testing was carried out by Mr. Roger White and Mr. Maurice Marietti of Warren-Teed Pharmaceuticals Division, and Dr. F. Denison, Jr., of Abbott Laboratories.*

**Two-fold serial dilutions were made in brain heart infusion agar.*

***Broth tube dilution in this case.*
(2 X 20 ml), and the combined extracts were dried (anhydrous Na$_2$SO$_4$). Evaporation of solvent in vacuo afforded 119 mg of crude residue which was purified by silica gel G column chromatography (CHCl$_3$ as eluting solvent). Crystallization from CH$_3$OH of the column residue gave 62 mg (62%) of light yellow needles; mp 72-73° (lit (60) mp 74-75°); ir cm$^{-1}$ (CHCl$_3$): 3030, 2958, 1705, 1630, 1430, 1360, 1330, 1165; nmr (CDCl$_3$): δ2.9-3.6 (m, 4H, H-4 and H-5 protons), δ7.2-8.8 (m, 6H, aromatic H's). The uv was the same as previously reported (60).

1-Methoxymethyl-9H-pyrido[3,4-b]indole (39).--(±)-Tryptophan (8.0 g, 0.0392 mole, Eastman Organics, Inc.) and 20 ml of methoxyacetaldehyde diethylacetal (K and K Laboratories) were heated together for 2 hr in 80 ml 80% AcOH on a steam bath in a loosely stoppered flask. The dark brown solution was diluted to 2 l with H$_2$O and boiled for 5 min. Solid K$_2$Cr$_2$O$_7$ (40 g, 0.196 mole, Matheson, Coleman and Bell) was cautiously added and then excess dichromate was destroyed by addition of Na$_2$SO$_3$. After basifying the aqueous layer to pH 9 with 25% aqueous NH$_4$OH, continuous extraction was carried out with Et$_2$O for 24 hr. The Et$_2$O layer was washed, dried (anhydrous Na$_2$SO$_4$), and evaporated to dryness in vacuo to afford 8.5 g of residual material. Crystallization from benzene:petroleum ether (65-110°) gave 7.25 g (87%) of 39; mp 120-122°; further crystallization from EtOH: hexanes gave light yellow prisms; mp 130-132° (lit (74) mp 129-130°
from EtOH-H$_2$O; ir cm$^{-1}$ (KBr): 3300-2500 (broad), 1610, 1560, 1420, 1230; nmr (CDCl$_3$): $\delta$3.35 (s, 3H, -OCH$_3$), $\delta$4.88 (s, 2H, -OCH$_2$O-), $\delta$6.8-8.1 (m, 6H, aromatic H's).

9H-Pyrido[3,4-b]indole-1-carboxaldehyde (40).--Compound 39 (8.0 g, 0.038 mole) was refluxed 1.5 hr with 320 ml 47% HBr (Baker Chemical Co.). The solution was cooled, the volume was reduced to 100 ml, and 200 ml additional H$_2$O was added. After refluxing for an additional 2 hr, the warm aqueous solution was filtered and basified with Na$_2$CO$_3$. The precipitate that settled out was filtered, washed with H$_2$O, and dried in vacuo.

The crude hydroxymethyl compound was suspended in 250 ml CH$_2$Cl$_2$ and active MnO$_2$ (15.0 g, 0.273 mole, (75)) was added. After stirring for 48 hr at 25°, residual MnO$_2$ was filtered through diatomaceous earth and then washed well with a large volume of hot and cold CHCl$_3$. The combined filtrate and washings were evaporated to dryness in vacuo to give a light reddish residue. Chromatography on alumina (elution with CHCl$_3$) afforded 4.2 g (57% from 39) of compound 40 crystallized from EtOH; mp 200-201° (lit (74) mp 198-200°); ir cm$^{-1}$ (KBr): 3380, 3050, 2825, 1680, 1628, 1595, 1495, 1452, 1435, 1360, 1325, 1275, 1200, 1120, 1058, 938; nmr (CDCl$_3$): $\delta$7.2-8.7 (m, 6H, aromatic H's), $\delta$10.30 (s, 1H, aldehyde H); mass spectrum: 197 (14.3%), 196 (M$^+$, 92.1%), 169 (15.0%), 168 (100%), 167 (20.8%), 166 (8.2%), 141 (18.5%), 140 (14.0%), 139 (7.8%), 115 (5.9%), 114 (16.6%), 113 (12.7%).
Conversion of aldehyde 40 to canthin-6-one (1).—Compound 40 (0.49 g, 2.5 mmoles) and malonic acid (0.78 g, 7.5 mmoles, Aldrich Chemicals, Inc.) were dissolved in 10 ml of hot anhydrous pyridine. Piperidine (1 ml) was added and the mixture was heated on a steam bath for 1 hr followed by refluxing for 7 hr (76). The solvent was removed in vacuo and toluene (3 X 10 ml) was added and co-distilled to remove final traces of solvent. The brown residue was chromatographed on alumina (eluted with benzene:CHCl₃, 3:1) to afford 0.17 g (31%) of material crystallized from CH₃OH; mp 150-153° (lit (58) mp 159-160°). This material was identical with an authentic sample of canthin-6-one (mixed mp, comparative ir, nmr, uv and tlc). Further crystallization from CHCl₃:hexanes gave yellow needles; mp 154-156°; ir cm⁻¹ (KBr): 3050, 1672, 1635, 1605, 1465, 1437, 1393, 1333, 1308, 1142, 1060, 845, 800; nmr (CDCl₃): δ6.82 (d,J=9.5 Hz, 1H, H-4), δ7.1-8.8 (m, 7H, H-5 and aromatic H's); mass spectrum: 221 (17%), 220 (M⁺, 100%), 193 (13.5%), 192 (87%), 165 (12%), 164 (17%), 139 (15%), 114 (12%).

5-Carbomethoxycanthin-6-one (41).—Aldehyde 40 (1.0 g, 5.1 mmoles) was dissolved in boiling anhydrous CH₃OH (70 ml). Dimethyl malonate (1.5 g, 11.4 mmoles, Aldrich Chemicals, Inc.) and triethylamine (4 ml) in 30 ml CH₃OH were added to the cooled solution. The reaction mixture on standing overnight at 25°, with occasional shaking, deposited yellowish green
crystals (0.958 g; mp 180-183°) which were removed by filtration. The filtrate was concentrated on a steam bath and on cooling gave an additional 0.266 g of crystals (total yield 83%). Recrystallization from benzene gave crystals of elevated mp 185-187°; ir cm\(^{-1}\) (KBr): 3040, 1745, 1680, 1638, 1560, 1490, 1440, 1345, 1305, 1280, 1215, 1108, 1045; nmr (CDCl\(_3\)): \(\delta\) 4.02 (s, 3H, -CO\(_2\)CH\(_3\)), \(\delta\) 7.3-8.8 (m, 7H, H-4 and aromatic H's); mass spectrum: 279 (21.6%), 278 (M\(^+\), 100%), 248 (16.3%), 247 (97.5%), 221 (16.3%), 220 (92.0%), 192 (18.9%), 191 (27.2%), 164 (27.0%); UV \(\lambda_{\text{max}}^{\text{MeOH}}\): 283 nm (log \(\varepsilon\) 4.10), 308 nm (log \(\varepsilon\) 3.91), 272 nm (log \(\varepsilon\) 4.12), 263 nm (log \(\varepsilon\) 4.08), 234 nm (log \(\varepsilon\) 4.25), 208 nm (log \(\varepsilon\) 4.60);

__Anal:__ Calcd for C\(_{16}\)H\(_{10}\)N\(_2\)O\(_3\): C, 69.06; H, 3.62; N, 10.07; Found: C, 69.26; H, 3.75; N, 9.91.

5-Carboxycanthin-6-one (42).—Ester 41 (500 mg, 1.8 mmoles) in 100 ml 2N aqueous HCl was heated at reflux. Complete solubilization of 41 occurred after 5 min. After 2 hr reaction time the yellow precipitate that had formed was filtered and subsequently dried in vacuo to afford 450 mg (95%) of light green needles; mp 271-273° (decomposition); ir cm\(^{-1}\) (KBr): 3100-2400 (broad), 1730, 1718 (sh), 1618, 1595, 1460, 1430, 1373, 1345, 1215; nmr (TFA): \(\delta\) 7.2-9.0 (m, aromatic H's); mass spectrum: 264 (M\(^+\), 19.0%), 221 (5.2%), 220 (100%), 192 (17.4%);

__Anal:__ Calcd for C\(_{15}\)H\(_8\)N\(_2\)O\(_3\): C, 68.18; H, 3.05; N, 10.60; Found: C, 67.04; H, 3.01; N, 10.41.
Conversion of acid 42 to canthin-6-one (1).—Compound 42
(200 mg, 0.758 mmole), powdered copper (50 mg, U.S. Bronze Powders, Inc.), and anhydrous pyridine (6 ml, distilled from KOH pellets) (77) were heated together under a blanket of N\textsubscript{2} for 5 hr at 80° oil bath temperature. After additional stirring overnight at 25°, the mixture was filtered over diatomaceous earth. After thoroughly washing the diatomaceous earth with CH\textsubscript{3}Cl:CH\textsubscript{3}OH (1:1), the combined washings and filtrate were evaporated to dryness at 1.5 mm/40° to afford 176 mg of dark brown residue corresponding predominately to canthin-6-one as determined on silica gel G tlc. Further purification effected with silica gel G column chromatography (CH\textsubscript{3}Cl as eluting solvent) followed by crystallization from MeOH:CHCl\textsubscript{3} yielded 81 mg (49%) of crystalline material identical with authentic canthin-6-one by tlc, uv, and nmr. Further crystallization from CHCl\textsubscript{3}: hexanes gave yellow needles; mp 155-156°.

1,2,3,5,6,11b-Hexahydro-3-oxo-11H-indole-[3,4,-g] pyrrocoline (43).—L-glutamic acid (1.47 g, 10 mmoles, Sigma Chemical Co.) was dissolved in 100 ml of 0.1N aqueous NaOH solution. Solid chloramine T (2.90 g, 10.3 mmoles, Eastman Organics, Inc.) was slowly added and the resulting solution was heated over a steam bath for 50 min (78). The solution was cooled in ice, filtered, and concentrated in vacuo to 20 ml. To this solution was added glacial \textsubscript{H}CO\textsubscript{H} (80 ml) and
tryptamine (1.60 g, 10 mmoles, Aldrich Chemicals, Inc.). After heating over a steam bath for 4 3/4 hr, excess solvent was removed in vacuo to afford a dark green residue which was diluted with 75 ml H₂O. After adjusting the aqueous phase to pH 2 with concentrated aqueous HCl, extraction was carried out with CHCl₃ (4 X 50 ml).

The acidic aqueous layer was basified to pH 9 and extracted with CHCl₃ (3 X 50 ml). The combined organic extracts were dried (anhydrous Na₂SO₄) and concentrated to dryness in vacuo to afford 0.592 g of unreacted tryptamine.

The combined organic extracts from the initial extraction were washed with dilute aqueous NaHCO₃ until the aqueous phase remained basic, dried (anhydrous Na₂SO₄) and concentrated to dryness in vacuo to afford a dark green residue. Crystallization from CH₃OH afforded 439 mg (31% based on unreacted tryptamine) of light pink crystals; mp 250-253° (lit (79) mp 253-255°); ir cm⁻¹ (KBr): 3255 (broad), 2920, 2850, 1650, 1618, 1490, 1445, 1435, 1418, 1380, 1345, 1308, 1262, 1230, 1160, 903, 750; mass spectrum: 227 (12%), 226 (M⁺, 100%), 225 (88%), 198 (6%), 197 (6%), 182 (9%), 171 (23%), 169 (23%), 168 (12%), 155 (19%), 91 (53%), 78 (70%).

Conversion of tetracyclic lactam 43 to canthin-6-one (1).

Compound 43 (140 mg, 0.62 mmole), mercuric acetate (0.40 g, 1.26 mmoles, Baker Chemical Co.) and 80% AcOH (4 ml) were heated on a steam bath for 47.5 hr. Hydrogen sulfide was
then bubbled through the hot solution for 5 min followed by air for 5 min. The hot solution was filtered through diatomaceous earth (diatomaceous earth washed thoroughly with hot 80% AcOH) and then evaporated in vacuo to a residue which was purified by silica gel G thick-layer chromatography (5% CH₃OH:CHCl₃ as eluting solvent) to afford 9.7 mg (7.9%) of product identical to canthin-6-one by ir, uv, and tlc. Crystallization from CHCl₃: hexanes gave yellow needles; mp 154-156°.

Indole-3-acetic acid methyl ester (44)(80).—Indole-3-acetic acid (15.0 g, 85.7 mmoles, Aldrich Chemicals, Inc.), absolute CH₃OH (150 ml), and Dowex ion exchange resin (4.0 g, 50 W X 8, acid washed and dried at 150°/3 hr, Baker Chemical Co.) were refluxed for 6.5 hr. After filtering off the resin, excess solvent was removed in vacuo to afford 15.84 g (98%) of a brown oil; ir cm⁻¹ (neat): 3425, 3065, 2960, 1735, 1623, 1460, 1440, 1340, 1170, 1100, 1015, 750; nmr (CDCl₃): δ 3.60 (s,3H,-OCH₃), δ 3.70 (s,2H,-CH₂-CO₂Me), δ 6.78 (m,1H,H-2), δ 7.0-7.2 (m,3H,H-4,H-6,H-7), δ 7.4-7.65 (m,1H,H-5), δ 8.0 (broad s, 1H,NH).

3-(2-Methoxy-2-oxoethyl)-γ-oxo-lH-indole-1-butanoic acid (45).—To a stirred dry DMF solution (50 ml) of NaH (1.30 g, 54.2 mmoles, Ventron Corporation) at 25° was added dropwise ester 44 (10.0 g, 52.9 mmoles) in 75 ml dry DMF. After stirring for 1 hr at 25°, succinic anhydride (5.83 g,
Table II.* Antimicrobial testing results of canthin-6-one and related analogs.

The values are minimum inhibitory concentrations in mcg/ml (for activity below 100 mcg/ml).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Staphylococcus aureus ATCC 13799 (gram positive)</th>
<th>Escherichia coli 9637 (gram negative)</th>
<th>Salmonella gallinarum 9494 (gram negative)</th>
<th>Klebsiella pneumoniae AD 10031 (gram negative)</th>
<th>Mycobacterium smegmatis 6078 (acid fast)</th>
<th>Candida albicans (yeast)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canthin-6-one (1)</td>
<td></td>
<td>i</td>
<td>i</td>
<td>i</td>
<td>i</td>
<td>i</td>
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<tr>
<td>5,6-Dihydrocanthin-6-one (34)</td>
<td></td>
<td>i</td>
<td>i</td>
<td>&lt;100</td>
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<td>i</td>
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<td>β-Carboline aldehyde (40) ~</td>
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<td>5-Carbomethoxycanthin-6-one (41)</td>
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<td>i</td>
<td>i</td>
<td>12.5</td>
<td>50</td>
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<td>5-Carboxycanthin-6-one (42) ~</td>
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<td>Acid ester (45)</td>
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<td>Thiazino ester (46) ~</td>
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<td>Keto-lactam (52)</td>
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</table>

*The agar dilute-streak assay was used (16) and was carried out by Mr. R. P. Leu of The Ohio State University.

i = inactive
58.3 mmole, Eastman Organics, Inc.) in 75 ml dry DMF was added dropwise and stirring was continued for 24 hr. The mixture was added to 300 ml H₂O and extracted with EtOAc (2 X 150 ml). The combined extracts were washed with H₂O (5 X 75 ml), dried (anhydrous MgSO₄), and concentrated in vacuo to afford 1.06 g of an oil corresponding to starting materials. The original aqueous layer was acidified to pH 2 with concentrated HCl and extracted with EtOAc (4 X 150 ml). The combined extracts were washed with H₂O (4 x 300 ml) and brine (300 ml), dried (anhydrous MgSO₄), and concentrated to dryness in vacuo to afford 9.53 g of a crystalline residue. Crystallization from 95% EtOH afforded 8.86 g (62% based on recovered starting material) of white needles; mp 151-153°; ir cm⁻¹ (KBr) 3300-2500 (broad), 1740, 1708, 1692, 1608, 1440, 1392, 1265, 1243, 1165, 1070, 915, 755; nmr (d₆-DMSO): δ2.70 (m, 2H, -CH₂CO₂H), δ3.20 (m, 2H, >NCCH₂-), δ3.65 (s, 3H, -CO₂CH₃), δ3.80 (s, 2H, -CH₂CO₂CH₃), δ7.2-7.7 (m, 3H, aromatic H-4, H-5, H-6 (indole nomenclature)), δ7.83 (s, 1H, aromatic H-2), δ8.20-8.42 (m, 1H, aromatic H-7); mass spectrum: 290 (2.7%), 289 (M⁺, 14%), 230 (2%), 190 (5.2%), 189 (42%), 131 (10%), 130 (100%), 101 (6%); UV λₘₐₓ(MeOH): 298 nm (log ε 3.85), 291 nm (log ε 3.80), 238 nm (log ε 4.26); Anal: Calcd for C₁₅H₁₅N₅O₅: C, 62.29; H, 5.22; N, 4.88; O, 27.64; Found: C, 61.79; H, 5.16; N, 4.84; O, 27.56.
Attempted cyclization of acid 45 with trifluoroacetic anhydride.--Compound 45 (100 mg, 0.35 mmole) and trifluoroacetic anhydride (15 ml, Eastman Organics, Inc.) were stirred for 23.5 hr at 25°. Excess solvent was removed in vacuo and the residual oil was added to 30 ml H2O. After basification to pH 10 with concentrated aqueous NH4OH, extraction was carried out with CHCl3 (2 X 20 ml). The combined extracts were dried (anhydrous MgSO4) and concentrated to 2 ml. Column chromatography on silica gel G (CHCl3 as eluting solvent) afforded 16.3 mg of a yellow oil giving a negative 2,4 DNP test. IR analysis (CHCl3) showed the presence of a strong band at 1810 cm\(^{-1}\) and the absence of the carboxyl bands in the 3000 cm\(^{-1}\) region and at 1710 cm\(^{-1}\). The amide band at 1690 cm\(^{-1}\) was still present. NMR analysis (CDCl3) showed that the butanoic side chain protons were no longer present in the δ2.7-3.2 region.

The product was dissolved in 2 ml xylene and refluxed for 3 hr. Evaporation of solvent gave a yellow oil whose nmr was unchanged.

Attempted cyclization of acid 45 with POCl3.--To a xylene (5 ml) solution of compound 45 (25 mg, 0.087 mmole) was added 1 ml of POCl3 (Baker Chemical Co.) and the mixture was refluxed for 0.5 hr. Xylene and POCl3 were removed in vacuo and ice (5 g) and concentrated aqueous NH4OH (25 ml) were added to the residue. After standing for 0.5 hr, extraction was carried out with CHCl3 (2 X 25 ml). The
combined extracts were washed with $H_2O$, dried (anhydrous MgSO$_4$), and evaporated to dryness in vacuo to afford a greenish residue showing 4 spots on silica gel G tlc. An ir (KBr) of the product mixture showed that the amide band at 1692 cm$^{-1}$ was no longer present presumably due to cleavage of the butanoic acid side chain.

**Attempted cyclization of acid 45 with P$_2$O$_5$.** Compound 45 (100 mg, 0.35 mmole) in dry toluene (2 ml) and P$_2$O$_5$ (1.5 g, Baker Chemical Co.) were refluxed for 5 hr. The mixture was poured into $H_2O$ (75 ml), basified to pH 9 with concentrated aqueous NH$_4$OH, and extracted with CHCl$_3$ (3 X 50 ml). The combined extracts were washed with $H_2O$, dried (anhydrous MgSO$_4$), and evaporated to dryness in vacuo to afford 1.0 mg of residue which was not further characterized because of the small yield.

**Attempted cyclization of acid 45 with HF.** To compound 45 (100 mg, 0.35 mmole) in a polyethylene bottle was added dropwise about 1 g of liquid HF at -33°. After evaporation of excess HF at 25° (18 hr), the dark brown residue was dissolved in CHCl$_3$. The organic solution was washed twice with $H_2O$, twice with dilute aqueous Na$_2$CO$_3$, dried (anhydrous MgSO$_4$), and evaporated to afford 3.1 mg of residual material which was not further characterized because of the small yield.
Attempted cyclization of acid 45 with AlCl₃.—To compound 45 (100 mg, 0.35 mmole) in dry CH₂Cl₂ (40 ml) was added anhydrous AlCl₃ (420 mg, 3.15 mmole, Matheson, Coleman, and Bell) and the mixture was stirred for 55 hr at 25°. After addition of ice, the mixture was extracted with Et₂O (3 X 40 ml). The combined extracts were washed with H₂O, dried (anhydrous MgSO₄), and concentrated in vacuo to afford a crystalline residue shown to be starting material by tlc, ir, and nmr.

Reaction of acid 45 with SOCl₂ - formation of methyl 2-(methoxycarbonyl)-4-oxo-4H-[1,3]thiazino[3,2-a]indole-10-acetate (46).—Compound 45 (500 mg, 1.73 mmole) and SOCl₂ (10 ml, Matheson, Coleman, and Bell) were refluxed for 5 hr during which time the reaction mixture turned a deep red color. After removal of excess SOCl₂ in vacuo, dry CH₃OH (50 ml) was added to the red residue and the mixture was heated over a steam bath for 10 min. Removal of excess CH₃OH in vacuo followed by silica gel G column chromatography (benzene eluting solvent) afforded 136 mg of the major component. Crystallization in EtOAc afforded 116 mg (20%) of bright orange fibrous needles; mp 164-165°; ir cm⁻¹ (KBr): 3080, 2960, 1740, 1728, 1685, 1608, 1588, 1440, 1383, 1308, 1160, 1085, 750; nmr (CDCl₃): δ3.66 (s,2H,-CH₂CO₂Me), δ3.78 (s,3H,-CH₂CO₂CH₃), δ3.90 (s,3H, vinyl CO₂CH₃), δ7.05 (s,1H, vinyl H), δ7.2-7.5 (m,3H, aromatic H-4,H-5,H-6 (indole nomenclature)), δ8.0-8.2 (m,
1H, aromatic H-7); mass spectrum: 333 (5.5%), 332 (7.8%), 331 (M⁺, 41%), 300 (4%), 274 (9%), 273 (19%), 272 (100%), 244 (27.5%), 212 (10%), 185 (16.5%), 115 (19%), 92 (22%), 78 (23%), 71 (23.5%); UV λmaxCl: 433 nm (log ε 3.72), 284 nm (log ε 4.35), 236 nm (log ε 4.00);

Anal: Calcd for C₁₆H₁₃NO₅S: C, 57.99; H, 3.95; N, 4.22; O, 24.14; Found: C, 57.57; H, 4.24; N, 4.25; O, 24.82.

3-methyl-γ-oxo-1H-indole-1-butanoic acid (47).—A dry DMF solution (40 ml) of skatole (5.0 g, 38.2 mmole, Aldrich Chemicals, Inc.) was added dropwise at 25° to a stirred solution of NaH (1.01 g, 42.1 mmole, Ventrion Corporation) in 25 ml dry DMF. After stirring for 1 hr at 25°, succinic anhydride (4.225 g, 42.3 mmole, Eastman Organics, Inc.) in 50 ml dry DMF was added dropwise, and stirring was continued for 15 hr. The mixture was added to 200 ml H₂O and the aqueous phase was acidified to pH 2 with concentrated aqueous HCl, followed by extraction with EtOAc (4 X 100 ml). The combined extracts were washed with brine (5 X 200 ml), dried (anhydrous MgSO₄), and concentrated in vacuo to afford 5.36 g of a crystalline orange-tinted residue. Crystallization from EtOAc afforded 4.93 g (56%) of white needles; mp 148-149°; ir cm⁻¹ (KBr): 3420-3360 (broad) 3250-2400 (broad envelope), 1710, 1682, 1612, 1448, 1408, 1354, 1328, 1265, 1240, 1215, 1180, 1072, 920, 740; nmr (d₆-DMSO): δ2.25 (s, 3H, Ar-CH₃), δ2.73 (m, 2H, -CH₂CO₂H), δ3.2 (m, 2H, >N C CH₂-),
δ6.9-7.8 (m, 4H, aromatic H-2, H-4, H-5, H-6 (indole nomenclature)), δ8.1-8.4 (m, 1H, aromatic H-7); mass spectrum: 232 (3.6%), 231 (M+, 25.3%), 132 (9.6%), 131 (100%), 130 (60.2%), 77 (6.6%); UV λ<sub>max</sub><sub>MeOH</sub>: 301 nm (log ε 3.91), 292 nm (log ε 3.87), 239 nm (log ε 4.33);

Anal: Calcd for C<sub>13</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>: C, 67.52; H, 5.67; N, 6.06; Found: C, 67.35; H, 5.63; N, 5.90.

Reaction of acid 47 with SOCl<sub>2</sub> - formation of methyl 10-methyl-4-oxo-4H-[1,3]thiazino[3,2-a]indole-2-carboxylate (48). Compound 47 (500 mg, 2.2 mmoles) and SOCl<sub>2</sub> (10 ml, Matheson, Coleman, and Bell) were refluxed for 5 hr during which time the reaction mixture turned a deep red color. After removal of excess SOCl<sub>2</sub> in vacuo, dry CH<sub>3</sub>OH (50 ml) was added to the red residue and the mixture was heated over a steam bath for 10 min. Removal of excess CH<sub>3</sub>OH in vacuo followed by silica gel G column chromatography (benzene as eluting solvent) afforded 424 mg of the major component. Crystallization from EtOAc afforded 368 mg (62%) of bright orange fibrous needles; mp 197-198°; ir cm<sup>-1</sup> (KBr): 3060, 2960, 1723, 1688, 1590, 1440, 1382, 1305, 1178, 1010, 920, 850; nmr (TFA): δ1.60 (s, 3H, Ar-CH<sub>3</sub>), δ3.52 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), δ6.38 (s, 1H, vinyl H), δ6.7-6.9 (m, 3H, aromatic H-4, H-5, H-6 (indole nomenclature)), δ7.2-7.5 (m, 1H, aromatic H-7); mass spectrum: 275 (6%), 274 (14%), 273 (M+, 100%), 272 (7%), 244 (12.5%), 242 (7.5%), 214 (7%), 186 (15.5%); UV λ<sub>max</sub><sub>CHCl<sub>3</sub></sub>: 436 nm (log ε 3.85), 287 nm (log ε 4.41), 237 nm (log ε 4.16);
Anal: Calcd for C$_{14}$H$_{11}$NO$_3$S: C, 61.53; H, 4.06; N, 5.12; S, 11.73; Found: C, 61.40; H, 4.10; N, 5.01; S, 11.57.

A minor component of slightly higher R$_f$ was also isolated from the reaction mixture with crystallization from EtOAc giving dark orange needles; mp 218-221°C. Its ir (KBr) showed prominent bands at 1712 cm$^{-1}$, 1695 cm$^{-1}$, 1450 cm$^{-1}$, 1430 cm$^{-1}$, 1375 cm$^{-1}$, and 1260 cm$^{-1}$. Its mass spectrum showed an intense peak at m/e 307 and a very weak peak at m/e 341.

Reaction of thiazinoindole 48 with CH$_3$I.—Compound 48 (300 mg, 1.1 mmole) and CH$_3$I (0.228 g, 1.6 mmole, Baker Chemical Co.) in 35 ml benzene were heated together for 16 hr in a sealed Pyrex ampoule at 100° oil bath temperature. Silica gel G tlc of the reaction mixture after this time showed the presence of only starting material.

Methyl 6,7,8,9-tetrahydro-6,9-dioxopyrido[1,2-a]indole 10-acetate (50).—To acid 45 (7.0 g, 24.2 mmoles) in dry benzene (100 ml) was added oxalyl chloride (10 ml, Aldrich Chemicals, Inc.) and a few drops of triethylamine. After stirring in the dark for 13 hr at 25°C, excess solvent and oxalyl chloride were removed in vacuo to give the orange acid chloride 49. Benzene (20 ml) was added twice to the residue and evaporated to remove traces of HCl. The residue was dissolved in dry CH$_2$Cl$_2$ (130 ml) and added dropwise to a stirred solution of anhydrous AlCl$_3$ (8.12 g, 60.9 mmole,
Matheson, Coleman, and Bell) in 80 ml of dry CH₂Cl₂ at 25°.

Stirring was continued for 60 hr. Excess solvent was evaporated in vacuo and crushed ice was added to the green residue. After addition of concentrated aqueous HCl (20 ml), the aqueous solution was extracted with CHCl₃ (3 X 125 ml). The combined extracts were washed with dilute aqueous Na₂CO₃ (150 ml), dried (anhydrous Na₂SO₄), and concentrated in vacuo to give 5.236 g of a dark green residue showing several spots on silica gel G tlc. The residue was purified by silica gel G chromatography (CHCl₃ as eluting solvent) with fractions giving a positive 2,4-DNP test pooled to afford 3.2 g of brown residue. Two crystallizations from benzene-hexanes (2:1) yielded 1.36 g (21%) of yellow needles; mp 132-133°; ir cm⁻¹ (KBr): 3000, 2950, 1740, 1705, 1670, 1575, 1560, 1435, 1405, 1360, 1328, 1190, 1145, 1012, 987, 760; nmr (CDCl₃): δ3.05 (m, 4H, -N(CH₂)₂-), δ3.72 (s, 3H, -CO₂CH₃), δ4.22 (s, 2H, -CH₂CO₂Me), δ7.2-7.8 (m, 3H, aromatic H-4, H-5, H-6 (indole nomenclature)), δ8.4-8.65 (m, 1H, aromatic H-7); mass spectrum: 272 (6%), 271 (M⁺, 44%), 240 (16%), 239 (68%), 212 (20%), 211 (44%), 188 (14%), 184 (24%); 129 (18%), 128 (26%), 111 (16%), 78 (100%); UV λ_max⁰ MeOH: 308 nm (log ε 4.36), 237 nm (log ε 4.24), 211 nm (log ε 4.30);

Anal: Calcd for C₁₅H₁₃NO₄: C, 66.41; H, 4.83; N, 5.16; O, 23.59; Found: C, 66.61; H, 4.90; N, 5.02; O, 23.50.

The above Friedel-Crafts reaction was repeated with acid chloride 49 (prepared from 45 (200 mg, 0.69 mmole) and
oxalyl chloride) and anhydrous SnCl$_4$ (0.45 g, 1.73 mmole, Baker Chemical Co.). Reaction for 48 hr at 25° followed by workup afforded crystalline material shown to be 45 by nmr, ir, and tlc.

Another Friedel-Crafts reaction at 25° on the same scale as the SnCl$_4$ procedure employing FeCl$_3$ (Allied Chemical Co.) as the Lewis acid afforded upon workup a fluorescent residue that gave a negative 2,4-DNP test and remained near the origin with silica gel G tlc (5% CH$_3$OH:CHCl$_3$ solvent system).

Attempted cyclization of acid 45 with polyphosphoric acid.—Compound 45 (90 mg, 0.31 mmole) and PPA (15 ml, Matheson, Coleman, and Bell) were heated over a steam bath for 1 hr during which the reaction mixture turned to a deep orange color. Crushed ice was added and the aqueous phase was extracted with Et$_2$O (3 X 40 ml). The combined extracts were washed with H$_2$O, dried (anhydrous MgSO$_4$) and evaporated to dryness in vacuo to afford a yellow oil whose silica gel G tlc showed the presence of 4 components with the one of highest R$_f$ giving a positive 2,4-DNP test. Silica gel G column chromatography (CHCl$_3$ as eluting solvent) afforded <1 mg of this product shown to be identical to 50 by ir, uv, and tlc.

Reaction of acid 45 with BF$_3$·Et$_2$O.—To compound 45 (185 mg, 0.64 mmole) in Ac$_2$O (0.2 ml) and AcOH (1.0 ml) was added
0.3 ml BF$_3$·Et$_2$O (Matheson, Coleman, and Bell) at 25°. The solution immediately turned to a light green color. After stirring for 2 hr, excess solvent was removed in vacuo and to the residue was added H$_2$O (20 ml), followed by extraction with CHCl$_3$ (3 x 50 ml). The combined extracts were washed with dilute aqueous NaHCO$_3$, dried (anhydrous Na$_2$SO$_4$) and evaporated to dryness in vacuo to give a light green residue showing a negative 2,4-DNP test and remaining near the origin with silica gel G tlc (5% CH$_3$OH:CHCl$_3$ solvent system).

Reaction of keto-lactam 50 with anhydrous NH$_3$.—Compound 50 (30 mg, 0.11 mmole) in 2 ml of dry THF was added dropwise to 3 ml of dry THF saturated with anhydrous NH$_3$ at 0°. Stirring was carried out for approximately 2 hr at 0° and then excess solvent was evaporated. IR analysis (KBr) of the crystalline residue showed intense bands at 3455 cm$^{-1}$, 3320 cm$^{-1}$, 3200 cm$^{-1}$, and 1650 cm$^{-1}$, all indicating that nucleophilic attack of the lactam band of 50 by NH$_3$ had occurred to give the primary amide.

Reaction of keto-lactam 50 with anhydrous NH$_3$ and TiCl$_4$.—To compound 50 (40 mg, 0.15 mmole) dissolved in 50 ml of dry dioxane was added TiCl$_4$ (15.4 mg, 0.08 mmole, Ventron Corporation). Anhydrous NH$_3$ was bubbled continuously into the solution at reflux. After several hours of heating, silica gel G tlc showed the presence of starting material only.
The oil bath temperature was raised to 150° with addition of dioxane to maintain solvent level. At this temperature the mixture turned green after heating for 6 hr. Excess solvent was evaporated in vacuo and a silica gel TLC showed the presence of starting material and a fluorescent component of higher $R_f$. An IR (CHCl$_3$) of this component indicated that cleavage of the lactam bond had occurred.

Reaction of keto-lactam 50 with benzylamine.— Benzylamine (58.0 mg, 0.54 mmole, Aldrich Chemicals, Inc.) was added dropwise to a CH$_3$OH solution (20 ml) of 50 (62.0 mg, 0.23 mmole) and the mixture was refluxed under a blanket of N$_2$ for 8 hr. Excess solvent was evaporated and the brown residue was dissolved in Et$_2$O. The ethereal solution was washed once each with dilute aqueous HCl, 5% aqueous NaOH, and brine. Drying (anhydrous MgSO$_4$) followed by evaporation to dryness in vacuo afforded 20 mg of a dark green residue crystallized from benzene:hexanes; mp 143-144°; ir cm$^{-1}$ (CHCl$_3$): 3445, 1732 (broad), 1715 (sh); nmr (CDCl$_3$): $\delta$ 2.7 (m, 2H, $\text{C}=$C=C-C=CH$_2$), $\delta$ 3.2 (m, 2H, $\text{-CH}_2\text{CO}_2\text{CH}_3$) $\delta$ 3.69 and $\delta$ 3.71 (2s, 6H, 2-CO$_2$CH$_3$), $\delta$ 4.13 (s, 2H, Ar-CH$_2$), $\delta$ 7.2-7.8 (m, 5H, aromatic H's). The data suggest methanolysis of the lactam bond.

The above procedure was repeated on the same scale but using 10 equivalents of benzylamine and glyme as solvent. After heating at 65° for 8 hr, workup as above afforded
crystalline material shown to be 50 by tlc and nmr. However, when heated at reflux in glyme for 7 hr, cleavage of the lactam bond by benzylamine occurred as evidenced by ir (KBr) bands at 3310 cm\(^{-1}\) and 1640 cm\(^{-1}\), and loss of the lactam band at 1670 cm\(^{-1}\).

Reaction of keto-lactam 50 with benzylamine and catalyst.— Compound 50 (50 mg, 0.18 mmole), benzylamine (197.7 mg, 1.85 mmole), and anhydrous p-toluenesulfonic acid (1.5 mg, 0.05 equivalents, K and K Laboratories) in toluene (10 ml) were refluxed for 6 hr. Workup as described before afforded a residue containing some starting material and 3 products with the nmr of each showing no incorporation of benzylamine.

Repitition of the above procedure using TiCl\(_4\) (17.5 mg, 0.5 equivalents, Ventron Corporation) in benzene for 4.5 hr at 25\(^\circ\) afforded the same results.

Methyl 1-[1,4-dioxo-4-[[(phenylmethyl)amino]butyl]-1H-indole-3-acetate (51).—To acid 45 (1.5 g, 5.19 mmoles) in dry CH\(_2\)Cl\(_2\) (45 ml) at 0\(^\circ\) was added oxalyl chloride (1.5 ml, Aldrich Chemicals, Inc.) and a few drops of triethylamine. After stirring at 0\(^\circ\) for 3 hr, excess solvent and oxalyl chloride were removed in vacuo to afford an orange oil. Dry THF (20 ml) was added twice to the residue and evaporated to remove traces of HCl. The acid chloride 49 was dissolved in 30 ml of dry THF and added dropwise to benzylamine (0.612 g, 5.71 mmoles, Aldrich Chemicals, Inc.)
at -77°. After stirring for 20 min, the mixture was poured into cold 5% aqueous HCl and the aqueous phase was extracted with CHCl₃ (3 X 100 ml). The combined extracts were washed twice with dilute aqueous NaHCO₃, dried (anhydrous Na₂SO₄), and evaporated to dryness in vacuo to afford 1.265 g of an oil crystallized from CH₃OH to afford 861 mg (44%) of light tan crystals; mp 113-114°; ir cm⁻¹ (KBr): 3240 (broad), 3080, 2950, 1728, 1702, 1630, 1570, 1205, 1168, 720 (broad); nmr (CDCl₃): δ2.6 (m,2H, -CH₂N₃CH₂O), δ3.2 (m,2H, -CH₂N₃CH₂), δ3.68 (s overlapping s, 5H, -CH₂CO₂CH₃), δ4.38 (d,J=5.5 Hz,2H, -NHCH₂O), δ6.5 (broad d, 1H, -NH-), δ7.0-7.6 (m,9H, aromatic H's), δ8.2-8.5 (m,1H, aromatic H); mass spectrum: 378.15835290 (M⁺, 1.6%, C₂₂H₂₂N₂O₄ requires 378.15794520), 319 (0.2%), 190 (20.2%), 189 (100%), 131 (7.1%), 130 (70%), 91 (33.3%); UV λₑₒ₉ = 299 nm (log ε 3.90), 282 nm (log ε 3.86), 238 (log ε 4.29).

Reaction of amide 51 with POCl₃.—Compound 51 (80 mg, 0.21 mmole) and POCl₃ (64.9 mg, 0.42 mmole) in 15 ml dry CH₃CN were stirred for 1 hr at 25° and then refluxed for 1 hr. The solvent was removed in vacuo and the residue taken up in CHCl₃. The CHCl₃ solution was washed with two portions of dilute aqueous NaHCO₃, two portions of H₂O, and dried (anhydrous Na₂SO₄). Evaporation of solvent left an oil showing predominately one spot on silica gel G tlc; ir cm⁻¹ (CHCl₃): 3480, 3010, 2960, 2930, 1740 (sh), 1705 (broad), 1490, 1455, 1430, 1400, 1160, 1090, 1015; nmr (CDCl₃):
δ1.25 (s,4), δ2.60 (s,3.6), δ3.7 (m,8), δ4.62 (s,2), δ7.0-
7.7 (m,14), δ8.25 (broad s,1).

Repetition of the above procedure on the same scale with
polyphosphate ester (400 mg (81)) in 15 ml dry CHCl₃
for 3 hr at reflux afforded upon workup (ice addition and
then extraction with Et₂O) an oil giving the same ir and
nmr patterns as previously given.

Methyl 6,9-dihydro-6,9-dioxopyrido[1,2-a]indole 10-acetate

(52).—To keto-lactam 50 (220 mg, 0.81 mmole) in 15 ml
glacial AcOH was added pyridinium hydrobromide perbromide
(248 mg, 0.81 mmole (82)) and the mixture was stirred at
25° for 50 min. Excess solvent was removed in vacuo and
the residue was dissolved in Et₂O. The ethereal solution
was washed with dilute aqueous NaHCO₃, dried (anhydrous
Na₂SO₄), and evaporated to dryness in vacuo to afford a
dark orange residue which was purified by silica gel G
chromatography (CHCl₃ as eluting solvent). The major
component present was collected and crystallized from
CHCl₃:EtOAc to afford 109 mg (50%) of yellow-green needles;
mp 198.5-199.5°; ir cm⁻¹ (KBr): 3060, 2970, 1745, 1695,
1648, 1562, 1440, 1388, 1368, 1348, 1260, 1205, 1160, 750;
nmr (TFA): δ3.45 (s,3H,-CO₂CH₃), δ3.97 (s,2H,-CH₂CO₂Me),
δ6.72 (s,2H, overlapping olefinic H's), δ6.95-7.45 (m,3H,
aromatic H-4,H-5,H-6 (indole nomenclature)), δ7.8-8.1
(m,1H, aromatic H-8); mass spectrum: 270 (9%), 269 (M⁺,
30.7%), 238 (24.5%), 237 (100%), 211 (7.9%), 210 (32.8%),
209 (10.8%), 182 (16.3%), 154 (21.9%), 128 (16.9%), 101
(9.9%), 77 (7.3%); UV λ max
MeOH
: 399 nm (log ε 3.86), 257 nm
(log ε 4.16), 251 nm (log ε 4.15), 217 nm (log ε 4.62).

Methyl 6,7,8,9-tetrahydro-(9-hydroxyimino)-6-oxopyrido
[1,2-a]indole 10-acetate (53).—Keto-lactam 50 (30 mg, 0.11
mmole), pyridine (0.15 ml), hydroxylamine hydrochloride (30 mg,
Baker Chemical Co.), and 1 ml absolute EtOH were refluxed for
2 hr. Excess EtOH and pyridine were removed at 1.0 mm/40°
and the crystalline residue was purified by silica gel G
thick-layer chromatography (two elutions with 5% MeOH:CHCl₃)
to afford 28 mg of product. Crystallization from benzene:
hexanes afforded 25 mg (80%) of light yellow plates; mp 186-
187°; ir cm⁻¹ (KBr): 3460 (broad), 2950, 1726, 1685, 1458,
1432, 1380, 1328, 1265, 1200, 1178, 1025, 920; nmr (TFA):
δ3.4-3.75 (m, 4H, >NC₃H₄CO₂Me), δ4.01 (s, 3H, -CO₂CH₃), δ4.25
(s, 2H, -CH₂CO₂Me), δ7.6-8.1 (m, 3H, aromatic H-4,H-5,H-6
(indole nomenclature)), δ8.5-8.8 (m, 1H, aromatic H-8); mass
spectrum: 287 (17.9%), 286.09575563 (M+, 100%, C₁₅H₁₄N₂O₄
requires 286.09534840), 270 (10.7%), 269 (37.5%), 255 (19.6%),
254 (38.4%), 199 (14.6%), 182 (14.8%), 181 (15.1%), 155
(16.0%), 154 (17.9%), 78 (39.3%), 77 (12.5%), 55 (19.6%); UV
λ max
MeOH
: 303 nm (log ε 4.28), 237 nm (log ε 4.20), 213 nm
(log ε 4.24).
Attempted reduction of oxime 53 with Zn and AcOH.—Compound 53 (5 mg, 0.0174 mmole), glacial AcOH (0.5 ml), and zinc dust (5 mg, Baker Chemical Co.) were heated for 6 hr at 60° oil bath temperature. Unreacted zinc was filtered off and excess solvent was evaporated in vacuo. Silica gel G tlc (5% CH₃OH:CHCl₃ as developing solvent) showed the presence of 4 products; Rf~0.82, Rf~0.29, Rf~0.19 and Rf~0.05. Puri- fication by silica gel G thick-layer chromatography (two elutions with 5% CH₃OH:CHCl₃) afforded ~2 mg of the major product (Rf~0.29). Its ir (CHCl₃) showed the presence of prominent bands at 1715 cm⁻¹ and 1660 cm⁻¹. The lactam band at 1690 cm⁻¹ was no longer present. This product gave a negative test with Dragendorff's spray reagent.

Attempted reduction of oxime 53 with Na(Hg).—To compound 53 (20.0 mg, 0.07 mmole) in 1 ml 95% EtOH at 25° was added 3% sodium amalgam (300 mg, (83)) and glacial AcOH (22 mg) proportionately. The solution immediately turned from a yellow to a green color and metallic Hg separated out. After 1 hr reaction time, the solution was decanted and excess solvent was removed in vacuo. Silica gel G tlc (5% CH₃OH:CHCl₃ as developing solvent) showed the presence of two products, one of Rf > compound 53 and one of Rf < compound 53. The latter product was purified by silica gel G thick-layer chromatography (two elutions with 5% CH₃OH:CHCl₃).
Its ir (CHCl₃) contained major bands at 1720 cm⁻¹ and 1640 cm⁻¹. No lactam band was present at 1690 cm⁻¹.

**Attempted catalytic reduction of oxime 53.**—Oxime 53 (20 mg, 0.07 mmole), 5% Pd/C (15 mg, Engelhard Industries, Inc.), and glacial AcOH (2 ml) were shaken overnight at 25° in a Parr bottle at 40 lbs pressure. Filtration of the catalyst followed by evaporation of solvent in vacuo afforded a residue shown to be 53 by tlc and ir. Like results were obtained when 20 mg of oxime 53, 8 mg Rh/Al₂O₃ (Matheson, Coleman, and Bell) and 2 ml CH₃OH were shaken at 50 lbs pressure for 2 days.

Attempted catalytic reduction of 53 was repeated on the same scale at 25° with glacial AcOH as solvent, once with 15 mg Pt₂O (Engelhard Industries, Inc.) overnight at 40 lbs pressure, and secondly with 10 mg 10% Pd-C (Engelhard Industries, Inc.) at 1 atm pressure. In each case workup afforded a residue whose silica gel G tlc pattern showed the presence of at least 3 products none of which gave a positive test with Dragendorff's spray reagent.

**Methyl 6,7,8,9-tetrahydro-(9-hydroxyiminoacetate-6-oxopyrido [1,2-a]indole 10-acetate (54).**—Oxime 53 (5.0 mg, 0.02 mmole), pyridine (10.6 mg, 0.13 mmole), dry THF (.5 ml) and Ac₂O (35.1 mg, 0.34 mmole) were stirred together for 4.5 hr at 25°. Excess solvent, Ac₂O and pyridine were removed at 1.5 mm/40° to afford 5.7 mg (100%) of an oil showing a single spot on silica
gel G tlc (5% CH₃OH:CHCl₃ as developing solvent); ir cm⁻¹ (CHCl₃): 3150, 3100, 2925, 1765, 1705 (broad envelope), 1565, 1455, 1370, 1345, 1160, 1005, 950; nmr (CDCl₃): δ 2.25 (s, 3H, -OAc), δ 2.8-3.3 (m, 4H, >N=C=CH₂), δ 3.70 (s, 3H, -CO₂CH₃), δ 4.18 (s, 2H, -CH₂CO₂Me), δ 7.2-7.7 (m, 3H, aromatic H-4, H-5, H-6 (indole nomenclature)), δ 8.3-8.5 (m, 1H, aromatic H-7).

Attempted reduction of oximinoacetate 54 with B₂H₆.——Compound 54 (23 mg, 0.07 mmole), B₂H₆ (0.3 ml 1M THF solution, Ventron Corporation) and 1 ml dry THF were refluxed for 24 hr. Excess THF was removed in vacuo and dilute aqueous HCl was added to the residue. After extraction by Et₂O (3 X 30 ml), the combined extracts were washed with H₂O, dried (anhydrous Na₂SO₄) and evaporated to dryness in vacuo to give an oil whose ir (CHCl₃) contained no lactam band. Basification of the aqueous HCl layer with NaHCO₃ followed by extraction with Et₂O (3 X 30 ml), drying of combined organic extracts (anhydrous Na₂SO₄), and evaporation of solvent to dryness in vacuo afforded an oil whose ir (CHCl₃) likewise showed no lactam band.
DISCUSSION

In a screening program (16) designed to uncover new antimicrobials from higher plants, extracts of *Zanthoxylum elephantiasis* Macf. (*Z. aromaticum* DC, *Fagara elephantiasis* Kr. and Urb.) were consistently active against *Staphylococcus aureus*, Smith strain (ATCC 13709), *Klebsiella pneumoniae* AD (ATCC 10031), *Mycobacterium smegmatis* 607B (ATCC 607) and *Candida albicans* (ATCC 10231) when tested *in vitro* using an agar dilution-streak method. Accordingly, fractionation was undertaken to isolate the responsible agent(s) in pure form. Canthin-6-one (1), 6H-indolo[3,2,1-d,e][1,5]naphthyridin-6-one, a well-known alkaloid previously isolated from this species (57,58) but not hitherto known to possess antimicrobial activity, was shown to be the active component in this way.

A supply of the bark of *Z. elephantiasis* Macf. was available as the remnant of a collection made in Jamaica and used in earlier studies (57-59) in which the compounds shown in Fig. I were identified. For this reason extracts of the plant were included in an antimicrobial screening program. Extracts of this plant are characterized by relatively broad spectrum activity. Earlier studies had resulted in the
isolation of both known and new alkaloids from relatively thoroughly studied plants using biological activity as a guide (16). It was hoped, therefore, that study of this plant would lead to the isolation of novel materials. This was not realized for the activity of the plant extract resided entirely in canthin-6-one.

Canthin-6-one (1) was found to be present in both the alkaloidal fraction and the fraction normally associated with neutral materials (see Figs. IV and V). The latter finding is evidently a consequence of its rather poor basicity and its ready solubility in chloroform. The identification of the alkaloid was straightforward and was based upon a direct comparison of the spectral behavior (mass spectrum, ir, uv, nmr) of the new sample in comparison with an authentic sample. Furthermore, a mixture melting point was undepressed.

The antibacterial properties of canthin-6-one are set forth in Table I in comparison with the data for streptomycin and 5-methoxycanthin-6-one (2), and some additional data are given in Table II. Because of the wide spectrum of antimicrobial activity and the structural simplicity of canthin-6-one, its total synthesis and that of analogs were deemed attractive procedures for assessing structure-activity relationships in this class. It is interesting to note in this regard that meaningful activity
is devoid for 5-methoxycanthin-6-one (2), 4,5-dimethoxy-
canthin-6-one* (9), 4-methylthiocanthin-6-one** (7) and 
benz[4,5]canthin-6-one (6).

Our first two syntheses of canthin-6-one (1) are 
delineated in Fig. VI. The procedure of Bradsher and 
Umans (74) was modified for the synthesis of 9H-pyrido[3,4-b] 
indole-1-carboxaldehyde (40). In the synthesis of 1-methoxy-
methyl-9H-pyrido[3,4-b]indole (39), readily available 
diethylacetal of methoxyacetaldehyde was used instead of 
methoxyacetaldehyde. Hydrolysis of 39 with 47% HBr provided 
the corresponding hydroxymethyl compound which was oxidized 
to compound 40 with active MnO2 (75). The yield of compounds 
39 and 40 were considerably improved over those previously 
reported (74). Aldehyde (40) on condensation (76) with 
malonic acid (Doebner reaction) gave canthin-6-one (1) in 
31% yield identical in all respects with an authentic sample 
derived from natural sources (58).

Treatment of 40 with dimethyl malonate in the presence 
of triethylamine gave 5-carbomethoxycanthin-6-one (41), 
5-carbomethoxy-6H-indolo[3,2,1-de][1,5]naphthyridin-6-one, 
in 83% yield. This product has almost equivalent in vitro 
antimicrobial potency to canthin-6-one itself (see Table II). 
Hydrolysis of compound 41 with 2N aqueous HCl proceeded 
smoothly to afford 5-carboxycanthin-6-one (42) in 95% yield.

*Provided by Dr. G. N. Inamoto and Mr. T. Takahaski.  
**Provided by Dr. J. R. Price.
Fig. VI.-- Synthetic routes to canthin-6-one via 9H-pyrido [3,4-b]indole-1-carboxaldehyde (40).

Decarboxylation of 42 by a modification of the method of Corey and Fraenkel (77), using anhydrous pyridine and copper powder, led to canthin-6-one in moderate yield (49%).
Another route to canthin-6-one is shown in Fig. VII. Reaction of L-glutamic acid with chloramine T, after the method of Dakin (78), resulted in formation of unstable β-aldehydopropionic acid which was then condensed with tryptamine in aqueous AcOH in a Pictet-Spengler manner to give tetracyclic lactam 43 in 31% yield. It should be noted that this route to 43 was tried as an alternative to that of Corsano and Algieri (79) who generated 43 in ~90% yield by the condensation of tryptamine with α-ketoglutaric acid. Rearrangement and aromatization of 43 with 2 equivalents of Hg(OAc)$_2$ (84) after the highly efficient scheme of Shipchandler and Mitscher (49) led directly to canthin-6-one, albeit in poor yield (7.1%), along with many other unidentified products. It should be noted, however, that 43 is at one lower oxidation level than desired (49).
Presumably a precursor molecule with unsaturation in the position α to the lactam carbonyl in 43 would lead to better yields. Fig. VIII gives possible mechanistic schemes for the formation of 1 from lactam 43.

Of the three synthetic schemes to canthin-6-one presented, the most desirable one is that via 5-carbomethoxy-canthin-6-one. The overall yield by this 5-step sequence is 19% from tryptophan and represents a better synthetic route than the other two routes pursued (15% overall in three steps from tryptophan via condensation of 40 with malonic acid, and 6% overall in two steps by Hg(OAc)₂ oxidation of 43 derived by the method of Corsano and Algieri). It also represents a moderate improvement over the yield (12% overall from tryptophan) reported earlier by Rosenkranz et al. (72).

It was observed that 4,5-dihydrocanthin-6-one (34), derived from canthin-6-one by Zn/AcOH reduction (60), was essentially devoid of in vitro antimicrobial activity at 100 μg/ml in agar dilution-streak assay (see Table II). From this and the in vitro test results for some other canthin-6-one analogs (see Tables I and II) it seemed that antimicrobial activity was dependent on the nature of the D ring and its substituents. Hence, it appeared attractive to synthesize simplified analogs incorporating the A, B, and D rings of the canthine moiety to investigate minimum structural requirements for in vitro activity. A secondary goal of this venture was to synthesize the canthin-6-one
Fig. VIII.—Proposed mechanistic schemes for the formation of canthin-6-one by Hg(OAc)$_2$ oxidation of 1,2,3,5,6,11 b-hexahydro-3-oxo-1H-indolo-[3,2-g]pyrrocoline (43).

ring system by a route alternate to classical approaches that employ β-carboline precursors. Fig. IX gives the synthetic scheme used to generate various analogs.

Indole-3-acetic acid was converted to its methyl ester 44 in 98% yield after the method of Stauffer (80). Treatment of the oily product with one equivalent of NaH in DMF followed by the addition of succinic anhydride led to the formation of acid 45, 3-(2-methoxy-2-oxoethyl)-γ-oxo-1H-indole-1-butanoic acid, in 62% yield. The nmr spectrum of this compound was particularly diagnostic of its structure with the protons of the butanoic acid side chain clearly visible as multiplets centered at $\delta$ 2.7 and $\delta$ 3.2. Aromatic H-2 and H-7 protons (indole nomenclature) at $\delta$ 7.83 and $\delta$ 8.20-8.42 respectively are shifted downfield from other aromatic protons largely due to the anisotropic deshielding of the side chain amide carbonyl moiety. Cyclization of compound 45 to keto-lactam 50 occurred only with difficulty, presumably due to the diminution of nucleophilicity at the C-2 position following 1-acylation. Ohki and Nagasaka (85)
indole-3-acetic acid

\[ \text{Dowex resin} \rightarrow \text{CH}_3\text{OH} \rightarrow \text{NaH, DMF, } 25^\circ \rightarrow \text{c}^\phi \text{CH}_3 \]

\[ \text{CH}_2\text{NH}_2, \text{TEA, -77}^\circ \rightarrow \text{AlCl}_3, \text{CH}_2\text{Cl}_2, 25^\circ \]

\[ \text{NH}_2\text{OH-HCl, pyridine, EtOH, } \Delta \rightarrow \text{AcOH, 25}^\circ \]
have observed that 1-acetylation of N-phthaloyltryptophan led to a drastic reduction in reactivity at C-2 and C-3 of the indole moiety. Earlier Szmuszkovicz (86) reported the acylcyclization of the C-4 position of the indole nucleus in 1-acetyl-3-indolesuccinic anhydride by Friedel-Crafts reaction. In view of the latter fact, it would seem that the C-4 position, being very inactive, had become more reactive to some extent as a result of 1-acetylation.

Attempted cyclization of acid 45 with typical cyclodehydration agents such as trifluoroacetic anhydride, POCl₃, P₂O₅, HF, and BF₃·Et₂O led to either unexplicable products or else degradation products in such low yield that characterization was not attempted. Reaction of acid 45 with anhydrous AlCl₃ resulted in the recovery of starting materials. It was then decided to effect ring closure to
compound 50 by formation of the acid chloride 49 followed by Friedel-Crafts reaction with anhydrous AlCl₃. Treatment of 45 with an excess of SOCl₂ at reflux for 5 hr followed by quenching with methanol (to determine if the acid chloride had formed) gave bright orange fibrous needles in 20% yield. Its nmr spectrum showed incorporation of methanol but the substance no longer showed protons in the δ2.7-3.2 region diagnostic of the butanoic acid side chain. However, a sharp singlet at δ7.05, integrating for one proton, suggested that oxidative elimination had occurred. The infrared spectrum indicated the presence of three carbonyl functions, one of which could be assigned to an amide (or lactam) moiety. Mass spectral data along with microanalysis tentatively suggests structure 46, methyl 2-(methoxycarbonyl)-4-oxo-4H-[1,3]thiazino[3,2-a]indole-10-acetate, although structure 66, methyl 2,3-dihydro-2-(2-methoxy-2-oxoethylidene)-3-oxothiazolo[3,2-a]indole-9-acetate, would also be a possibility.

Either one of these compounds represents a novel ring system and hence further chemical investigation was desirable. A similar linear tricyclic ring system has been known since 1929 (87). Formation of compounds 46 and 66 could be hypothesized to occur as shown in Fig. X.
Fig. X.---Hypothesized mechanistic route to formation of compound 46 or 66.
Literature reports of reaction of indoles and 1-acyl-indoles with electrophilic sulfur reagents are quite sparse. In general, reaction occurs at C-2 or C-3. A priori determination for those compounds in which both positions are unsubstituted usually is not possible. Ohki and Nagasaka (85) reported that N-phthaloyl-1-acetyltryptophan reacted with o-nitrophenylsulfenyl chloride to a negligible extent (0.2-0.6%). N-phthaloyltryptophan, on the other hand, reacted to the extent of 81%. Reaction of indoles with SOCl₂ is not an uncommon event. Szmuszkovicz (88) has studied reactions with SOCl₂ and sulfenyl chloride. He found that methyl-1-methylindole-2-carboxylate gave the 3-sulfinyl chloride on reaction with SOCl₂. In light of these prior findings, a satisfactory explanation for the relatively facile formation of 46 is presently lacking. One may speculate that entropy factors would favor formation of intermediate 68 from 67 and thus drive the reaction to completion. This would assume that addition of SOCl₂ to 49 is a reversible process.

To investigate further the generality of this reaction, analog 48 was synthesized through a series of reactions similar to those employed for the synthesis of 46. Acylation of skatole with NaH and succinic anhydride in DMF gave compound 47, 3-methyl-γ-oxo-1H-indole-1-butanoic acid, in 56% yield. The spectral data are similar to that for acid 45 and are fully consistent with the assigned structure.
Reaction of acid 47 with excess SOCl₂ under the same conditions as described before afforded compound 48, methyl 10-methyl-4-oxo-4H-[1,3]thiazino[3,2-a]indole-2-carboxylate, or alternatively the 5-membered analog 71, methyl (9-methyl-3-oxothiazole[3,2-a]indol-2 (3H)-ylidene)acetate, in considerably higher yield (62%) as beautiful orange fibrous needles. The nmr spectrum of this compound shows an almost identical pattern as that observed for compound 46 except for peaks attributable to the substituent in the C-3 position of the indole nucleus. Other spectral data in addition to microanalysis lend support to the structural assignment. Conclusive assignment of the structure as 48 or 71 could presumably be made by ester hydrolysis followed by decarboxylation.
Assignment of the vinyl protons as endocyclic or exocyclic would then be straightforward from infrared and nmr data. Reaction of 48 with CH₃I was also tried in an attempt to form the thiomethyl salt. However, upon heating 48 and CH₃I in a sealed ampoule at 100° for 16 hr, only starting material remained.

In a further attempt to generate acid chloride 49 (see Fig. IX), reaction of acid 45 with excess oxalyl chloride in benzene and a trace of triethylamine was carried out. The oily acid chloride was then cyclicized to the desired tricyclic keto-lactam 50, methyl 6,7,8,9-tetrahydro-6,9-dioxopyrido[1,2-a]indole 10-acetate, under Friedel-Crafts conditions with anhydrous AlCl₃ as the Lewis acid catalyst, albeit in low yield (21%). The reaction mixture showed several spots on silica gel G tlc. The nmr spectrum of the isolated crystalline product 50 displayed an overlapping multiplet centered at δ3.05 diagnostic of the coalescing ring C protons. A sharp multiplet at δ8.4-8.6 is diagnostic of the H-7 proton (indole nomenclature) and is observed downfield from the remaining aromatic protons because of the anisotropic effect of the lactam carbonyl function. The infrared spectrum shows three distinct carbonyl bands. That at 1705 cm⁻¹ is at higher frequency than normally expected for tertiary amides and suggests that this carbonyl is quite ketonic in nature and thus susceptible to facile attack by nucleophiles. The carbonyl band at
1670 cm$^{-1}$ can be assigned to the conjugated ketonic moiety (85,89,90). The lower carbonyl frequency of 2-acylindole derivatives (c.a. 1670-1640 cm$^{-1}$) than that of aryl ketones (c.a. 1690 cm$^{-1}$) strongly suggests that the former are a kind of vinylogous amide similar to 3-acylindoles (91). That cyclization occurred at C-2 instead of C-7 is corroborated by uv data. Intense maxima seen at λ237 nm and λ308 nm are quite characteristic of 2-acylindoles having no substituent on the benzene portion of the indole nucleus (85,89,90,92). Furthermore, cyclization to C-7 would have involved formation of a less favorable 7-membered ring.

The low yield of this reaction is not surprising in light of other documented work (vide infra), and suggests that 1-acylation effectively negated favorable reactivity at the C-2 position of the indole nucleus. In an attempt to enhance yields, reaction of acid chloride 49 under Friedel-Crafts conditions with SnCl$_4$ and FeCl$_3$ as the respective Lewis acid catalysts was carried out. In the former case, only acid 45 was recovered after extensive reaction (48 hr at 25°) and workup. In the latter case, immediate degradation occurred.

Another route to keto-lactam 50 was achieved by cyclo-dehydration of acid 45 by polyphosphoric acid. However, the yield was exceedingly low and thus this approach was abandoned. In general, it appears that formation of compound 50 is best effected through Friedel-Crafts
cyclization of acid chloride 49. Requirements for the Lewis acid catalysis seem somewhat exact from preliminary investigations.

Having synthesized the tricyclic pyridoindole ring system typified by compound 50, it was hoped that facile conversion to the tetracyclic canthine ring system could be effected by formation of an imine (or enamine) followed by nucleophilic attack on the carbomethoxy moiety. However, reaction of 50 in THF with anhydrous NH₃ at 0° after a slightly modified method of Jones (93) gave a crystalline residue whose infrared spectrum clearly indicated that nucleophilic attack of the lactam carbonyl by NH₃ had occurred to give the primary amide. Repetition of the same reaction at dioxane reflux with TiCl₄ as catalyst (94) stabilized the lactam bond to attack by NH₃. However, enamine formation did not occur as evidenced by recovery of starting material upon workup. When the reaction temperature was increased, degradation occurred. Another approach to enamine formation was to employ the method of Kutney et al. (95). Reaction of 50 with benzylamine at methanol reflux for 8 hr afforded upon workup a crystalline product whose nmr spectrum showed the presence of two overlapping methoxyl singlets at δ3.69 and δ3.71, and the absence of the multiplet centered at δ8.5 characteristic of the H-7 proton. The infrared spectrum no longer showed the lactam band at 1705 cm⁻¹, but contained a new band at 1715 cm⁻¹. This data clearly indicated that
methanolation of the lactam band had occurred. Repeated attempts to effect enamine formation with benzylamine and catalyst (p-TsOH or TiCl₄) in nonhydroxylic solvent resulted either in recovery of 50 or products showing no incorporation of benzylamine.

The extreme sensitivity of the lactam bond of 50 to cleavage by nucleophilic reagents is not without precedent. Ohki and Nagasaka (85) noted that 1-acyltryptophan is easily saponified by acid or base. Haynes et al. (60) found upon boiling canthin-6-one with excess Raney nickel in methanol, hydrogenation and methanolation both occurred.

Since it appeared that formation of an imine or enamine from keto-lactam 50 would meet with recalcitrance, it was decided to investigate a possible Bischler-Napieralski reaction for the generation of a canthine ring system. This would obviate Friedel-Crafts cyclization conditions and thus hopefully lead to a better synthesis of the requisite tricyclic system while at the same time introducing the imine moiety. Accordingly, acid chloride 49 was easily converted to diamide 51, methyl 1-[1,4-dioxo-4-[(phenylmethyl)amino]butyl]-1H-indole-3-acetate, under typical Schotten-Bauman conditions, by reaction with benzylamine at -77° (see Fig. IX). The nmr spectrum displayed multiplets centered at δ2.6 and δ3.2 for the protons located between the two amide carboxyls. A doublet at
δ4.38, J=5.5Hz, is assigned to the benzylic protons coupled to the secondary amide proton. The methylene and methoxy protons of the C-3 side chain (indole nomenclature) exist as overlapping singlets at δ3.68. Infrared bands at 3240 cm⁻¹, 1728 cm⁻¹, 1702 cm⁻¹, and 1630 cm⁻¹ further support the assigned structure. Reaction of 51 with either POCI₃ in refluxing CH₃CN (96) or with polyphosphate ester in refluxing chloroform (97) led to the same oily product whose nmr spectrum indicated that the benzylic peak had now collapsed to a singlet at δ4.62. Also the multiplets for the amide side chain protons centered at δ2.6 and δ3.2 in 51 have now disappeared. Instead, sharp singlets are now observed at δ1.25 and δ2.60. Also, a new multiplet is present and centered at δ3.7. The infrared spectrum shows a sharp band at 3480 cm⁻¹ and overlapping carbonyl bands at 1740 cm⁻¹ and 1705 cm⁻¹. From the given data, it is apparent that the expected product did not form. Further attempts at characterization of the oily residue were not pursued.

Having failed to effect formation of the canthine ring system by imine or enamine formation, it was deemed desirable to elevate ring C of keto-lactam 50 to one higher oxidation state. This, in part, would mimic ring D of canthin-6-one and thus would provide an interesting analog for evaluation of antimicrobial activity. Reaction of 50 with pyridinium hydrobromide perbromide in glacial AcOH at 25° gave compound 52, methyl 6,9-dihydro-6,9-dioxopyrido
[1,2-a]indole 10-acetate, directly in 50% yield instead of the anticipated α-bromo product. Extended conjugation is reflected in the infrared spectrum with the lactam and ketonic carbonyl moieties shifted to lower frequency at 1695 cm^{-1} and 1648 cm^{-1}, respectively. The ultraviolet spectrum also shows this phenomenon with a bathochromic shift from λ308 nm to λ399 nm. In vitro testing showed this compound to be devoid of activity (see Table II).

In an attempt to synthesize other analogs, keto-lactam 50 was reacted with hydroxylamine hydrochloride and pyridine in refluxing ethanol which readily gave oxime 53, methyl 6,7,8,9-tetrahydro-(9-hydroxyimino)-6-oxopyrido[1,2-a]indole 10-acetate, as light yellow plates in 80% yield (see Fig. IX). Under these milder conditions of oxime formation the lactam band of 50 remained intact. The infrared spectrum of 53 showed a broad envelope at 3460 cm^{-1} and prominent carbonyl bands at 1726 cm^{-1} (ester) and 1685 cm^{-1} (lactam), both of which exist at considerably lower frequency than observed for 50. The nmr spectrum is quite similar to that for 50 except that the ring C protons are less coalescing. The ultraviolet spectrum shows a hypsochromic shift from λ308 nm in 50 to λ303 nm in 53.

With the generation of oxime 53, an alternate indirect route to the canthine ring system now seemed feasible. Various reductive measures were employed in an attempt to generate analog 65 (see Fig. IX). However, attempted
reduction under a variety of conditions met with failure. Dissolving metal reductions using zinc and glacial AcOH (98) or alternatively 3% amalgamated sodium (99) resulted in multicomponent mixtures. Catalytic hydrogenation with various catalysts including 5% Pd/C in glacial AcOH (100), 10% Pd/C in glacial AcOH, Pt₂O in glacial AcOH (101), and 5% Rh/Al₂O in methanol (102), all at varying hydrogen pressures, resulted in either multicomponent mixtures or recovery of oxime 53. Infrared analysis of isolated products invariably showed no lactam carbonyl band at 1700-1690 cm⁻¹.

Undoubtedly, hydrogenolysis of the lactam bond is a very facile process enabling the indole nucleus to be restored to fuller aromaticity. Conversion of oxime 53 to its corresponding acetate 54, methyl 6,7,8,9-tetrahydro-(9-hydroxyimino-acetate)-6-oxopyrido[1,2-a]indole 10-acetate, by conventional means followed by treatment with diborane at THF reflux (103) likewise proved abortive. Undoubtedly, formation of canthine analog 65 from oxime 53 or oxime acetate 54 will require a reducing reagent quite specific for the oxime function.

Antimicrobial testing results for most of the compounds synthesized in this investigation are shown in Table II. Only canthin-6-one (1) and 5-carbomethoxycanthin-6-one (42) possess meaningful in vitro antimicrobial activity. It is apparent that bioactivity requirements are not a specific function of the canthine ring D structural features alone, but probably represent composite features of the ring system
as a whole. Further structure-activity studies will be required to understand the exact nature of the pharmacophore.

The attempted synthetic route to canthin-6-one analogs, as outlined in Fig. IX, proved to be far inferior to traditional approaches involving elaboration of β-carboline precursors. However, this route is quite useful in enabling one to generate various pyridoindole analogs, all of which appear to be new compounds, and further study of the properties of these compounds would be an intellectually satisfying pursuit.
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