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STEVENs, Sandra Ruth Abel, 1947-
I. AN APPROACH TO THE SYNTHESIS OF THE
PYRROLO[1,4][2,1c]BENZODIAZEPINE SKELETON
OF ANTHAMYCIN AND RELATED NATURAL PRODUCTS.
II. A PARTIAL SYNTHESIS OF d-BIOTIN.

Rice University, Ph.D., 1977
Chemistry, organic

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RICE UNIVERSITY

I. AN APPROACH TO THE SYNTHESIS OF THE PYRROLO[1,4][2,1c]BENZODIAZEPINE SKELETON OF ANTHRAMYCIN AND RELATED NATURAL PRODUCTS

II. A PARTIAL SYNTHESIS OF d-BIOTIN

by

Sandra Abel Stevens

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

Thesis Director's Signature:

Robert V. Snider

Houston, Texas

May, 1977
DEDICATION

There were times when I imagined (or hoped) I would be a graduate student forever, but now I have finished this phase of my life. I dedicate this work to the future and to that person who will share it with me - my husband, Jim.
ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Robert V. Stevens for his guidance and support, both deeply appreciated. To both Rice University and The Robert A. Welch Foundation I am gratefully indebted for financial assistance.

Hoffmann-La Roche, and specifically Dr. Pat Confalone, of the Nutley, N.J., Laboratories, deserve much thanks for sharing their results with us. Their cooperation was invaluable.

We would also like to thank the Maumee Chemical Company, a subsidiary of the Sherwin-Williams Company for so generously providing us with samples of isatoic anhydride and 5-chloroisatoic anhydride. We are thankful, too, to the Upjohn Corporation for their partial financial support of the anthramycin work and for the biological activity testing.

I am indebted to Dr. Paul Engel for many helpful discussions regarding spectral interpretation, and to Drs. G. Schroepfer and W. E. Billups for reading my thesis and offering suggestions for its improvement.

My largest debt of gratitude is to my friends and co-workers through the years whose experience and advice were of inestimable value. Of these I would especially like to thank Claire Lampard and Jim Hudson - Claire, not only for the painstaking services of typing and proofreading, but also for her warmth and friendship - and Jim, for the many hours of consultation and consolation he shared with me as mass spectral analyst, friend, and confidante. Lastly, I would like to thank Bob Rossen for his love and encouragement.
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Part I. An Approach to the Synthesis of the Pyrrolo[1,4][2,1c]benzodiazepine Skeleton of Anthramycin and Related Natural Products
INTRODUCTION

In recent years extracts from certain strains of the class of micro-organisms known as *Actinomycetes* have yielded compounds with the relatively rare pyrrolo[1,4]benzodiazepine skeleton (1) that possess strong antitumor properties. In most cases antileukemia and antibacterial activity are present also.

![Chemical structure of compound 1](image)

Working on the premise that highly aerobic organisms, such as the *Actinomycetes*, might produce substances that would inhibit the anaerobic energy mechanisms of cancer cells, Dr. Moses Tendler of Yeshiva University, New York City, began to investigate the fermentation broths of thermophilic *Actinomycetes*. When the broth extracts indeed showed antitumor activity, Tendler turned the work of isolation and characterization of the active principles over to researchers at Hoffmann-La Roche, Nutley, New Jersey.

The first of the new antibiotics to be discovered was anthramycin (2), isolated from *Streptomyces refluineus* in 1965. Since

![Chemical structure of anthramycin](image)
that time four additional Actinomycete antitumor agents have been discovered: three by Japanese workers, dextrochrysin (3),\textsuperscript{3} tomaymycin (4),\textsuperscript{4} and neothramycin (5);\textsuperscript{5} and one by Russian workers, sibiromycin (6).\textsuperscript{6} Sibiromycin has the additional unusual feature of an aminoglycoside group. In addition to the structural similarities, all the antibiotics above possess large positive optical rotations.

\begin{align*}
\text{dextrochrysin} & \quad \text{tomaymycin} \\
\text{Streptomyces calvus} & \quad \text{Streptomyces achromogenes} \\
3 & \quad 4 \\
[\alpha]_D^{24^*} +837.9^\circ & \quad [\alpha]_D^{20^*} +423^\circ
\end{align*}

\begin{align*}
\text{neothramycin} & \quad \text{neothramycin} \\
\text{Streptomyces No. MC916-C4} & \quad \text{Streptomyces No. MC916-C4} \\
\text{Streptomycin} & \quad \text{Streptomycin} \\
5 & \quad 5 \\
[\alpha]_D^{26^*} +272^\circ & \quad [\alpha]_D^{26^*} +314^\circ
\end{align*}

\begin{align*}
\text{sibiromycin} & \quad \text{sibiromycin} \\
\text{Streptosporangium sibiricum} & \quad \text{Streptosporangium sibiricum} \\
6 & \quad 6 \\
[\alpha]_D +525^\circ & \quad \text{Further details...}
\end{align*}

\* The natural product is actually "des-methoxy"tomaymycin. A molecule of methanol seems to be incorporated during work-up.

\** These structures are proposed by S. Stevens on the basis of partial structures and further evidence provided in references 3 and 5.
Three other related products have been isolated from molds: cyclopenin (7), cyclopenol (8), and LL-S409β (9). It is not known what antibiotic properties these latter three might possess. The biological properties of the compounds mentioned above are summarized in Table 1.
### Table 1

<table>
<thead>
<tr>
<th></th>
<th>$^{2a,9}$</th>
<th>$^{3}$</th>
<th>$^{4a,10}$</th>
<th>$^{5, A&amp;B}$</th>
<th>$^{6}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>antiphage</td>
<td>+</td>
<td></td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>antiviral</td>
<td></td>
<td>++</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>antileukemia</td>
<td>+</td>
<td></td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>antitumor</td>
<td>++</td>
<td></td>
<td>vitro +</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>vivo -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>antibacterial: gram +</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
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<tr>
<td></td>
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<td>antibacterial: gram -</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>antifungal</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>chemisterilant</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD$_{50}$ intravenous, mg/kg body wt.</td>
<td>$&gt;700$ (crude extr.)</td>
<td>0.75</td>
<td>3</td>
<td>20-30</td>
<td>.058</td>
</tr>
</tbody>
</table>

Both anthramycin$^9$ and tomaymycin$^{10}$ have been found to inhibit nucleic acid synthesis (by polymerase inhibition) by forming complexes with double stranded DNA. The benzodiazepines may either intercalate or form weak covalent bonds to the DNA, but they do not appear to bind along the groove. There is some speculation that the rearrangement$^{11}$ in vivo of anthramycin to actinomycin-type compounds accounts for its antitumor activity.$^2$ Actinomycin (10) is known to intercalate into DNA.

![Chemical Structure](attachment:image.png)
In the case of anthramycin, the same degree of RNA polymerase inhibition is observed with either C-11 epimer, the C-11 O-methyl ether, or the anhydro-compound. Oxidation at C-11 or substitution at C-9 or N-10 decreases inhibition markedly. Compounds in which the side chain at C-2 has been replaced by -OH or O-acetyl also show a loss of activity. Of the five active benzodiazepines mentioned above, it should be noted that, although substitution patterns on the benzene ring and the character of the C-2 side chain differ, they all possess the same carbinolamine moiety at C-10 and N-11.

![Chemical structure of anthramycin]

Probably because of the relative youth of this new class of antibiotics, few attempts at synthesis have been made. One stereo-specific total synthesis of anthramycin has been carried out by Leimgruber's group at Hoffmann-La Roche, beginning with the Schotten-Bauman condensation of 1-hydroxyproline methyl ester and the anthranilic acid derivative, 3-benzylxyloxy-4-methyl-2-nitrobenzoyl chloride. The product was identical in every way with the natural product, including biological activity, and established the stereochemistry beyond doubt. The synthesis is outlined in Scheme 1.

The only snag in the synthesis occurred when compound 11, Leimgruber's first synthetic objective, resisted reduction to the carbinolamine. The lactam remained unchanged when treated with an excess of lithium aluminum hydride in refluxing tetrahydrofuran. It
thus became necessary to obtain the desired end by a more roundabout method.

Scheme 1

\[
\begin{align*}
\text{CH}_3\text{O} & \quad \text{COCl} \\
\text{R} = \text{CH}_2\text{C}_6\text{H}_5 \\
\text{OH} & \quad \text{NH} \\
\text{COCl} & \quad \text{OH} \\
\text{CH}_2\text{Cl}_2 & \quad \text{Et}_3\text{N} \\
\text{r.t.} & \quad \text{Et}_3\text{N} \\
\text{Na}_2\text{S}_2\text{O}_4 & \quad \text{THF/H}_2\text{O} \\
\text{aq. HCl} & \quad 1 \leftarrow 2
\end{align*}
\]

\[
\begin{align*}
\text{THF} & \quad 0^\circ \text{C} \\
\left(\text{C}_2\text{H}_5\text{O}\right)_2\text{PCHCOOC}_2\text{H}_5 & \quad \text{CrO}_3
\end{align*}
\]

1) DIBAL/PhCH\text{3} \\
2) NaHSO\text{3}/KCN

\[
\begin{align*}
\text{CF}_3\text{COOH} & \quad \text{BF}_3\cdot\text{Et}_2\text{O}
\end{align*}
\]

12

\[
\begin{align*}
\text{CF}_3\text{COOH/H}_2\text{O}
\end{align*}
\]

11

\[
\begin{align*}
\text{all trans} & \quad \text{LiAlH}_4 & \quad \text{N.R.}
\end{align*}
\]

4:1 trans:cis
Protection of the phenol group and conversion of the secondary lactam to a more easily reduced tertiary lactam was accomplished in one step by condensation of 12 with benzaldehyde dimethyl acetal at 200°C under nitrogen. The nitrile 13 was hydrolyzed with hot polyphosphoric acid. Subsequent reduction with lithium aluminum hydride in tetrahydrofuran at -60°C or sodium borohydride in methanol at 5°C, followed by hydrolysis of the benzal protecting group with 0.01 N aqueous hydrochloric acid in methanol afforded anthramycin methyl ether (14) in good yield.

The final step in this synthesis involved conversion to the O-methyl ether of anthramycin, which retains all the biological activity of the natural product, and is much more stable. Anthramycin itself is extremely sensitive to heat, acid, and base. Interconversion of both epimers of anthramycin, anhydroanthramycin, and anthramycin methyl ether is readily accomplished by refluxing in different solvents. This same ease of interconversion has also been observed
with tomatmycin\textsuperscript{4a} and neothramycin.\textsuperscript{5}

Synthesis of a degradation product of tomatmycin to establish stereochemistry and substitution pattern was carried out by Kariyone\textsuperscript{4b} in an approach analogous to Leimgruber's. The target, benzodiazepine \textsuperscript{(15)}, was obtained by the route outlined in Scheme 2, again beginning with 1-hydroxyproline methyl ester.

Scheme 2

\[
\begin{align*}
\text{C}_2\text{H}_5\text{O} & \quad \text{COCl} \\
\text{CH}_3 & \quad \text{NO}_2 \\
\text{COOCH}_3 & \quad \text{HCl} \\
\text{CH}_2\text{C}_2\text{O} & \quad \text{NH} \\
\text{CH}_2\text{C}_2\text{O} & \quad \text{O} \\
\text{C}_2\text{H}_5\text{O} & \quad \text{N} \\
\text{CH}_3 & \quad \text{OH} \\
\text{DMF} & \quad \text{CrO}_3 \\
\end{align*}
\]

The only other approach to the pyrrolo[1,4]benzodiazepine system in the literature is that of an Italian group headed by G. De Martino and M. Artico at the University of Rome. In a series of papers beginning in 1969,\textsuperscript{14} the synthesis of a number of compounds containing the benzodiazepine structure joined to a pyrrole ring is described. The Italian approach differs from Leimgruber's in that proline derivatives are avoided, and the condensation step involves o-nitrobenzyl bromide instead of a benzoyl chloride. As a result the C-5 position in the benzodiazepine ring is a saturated carbon atom. The
general method is outlined in Scheme 3.

Scheme 3

No attempt was made to reduce the pyrrole ring. Derivatives were made by acetylation of N-10 before and after ring closure, leading to C-11 substitution (methyl) and N-10 substitution, respectively:
Substitution on the pyrrole ring was achieved by modification of the cyano group in compounds 16 and 17, or by condensations of the product of Vilsmeier-Haack formylation of compound 18 with malonic acid derivatives.

Although only C-2 substituents on the pyrrole ring have been found in the natural series, all of De Martino's work concentrated on the C-3 position. Of the twenty or more compounds synthesized, only 19 showed appreciable antitumor activity. 14d

Our own approach to the synthesis of this intriguing series of antibiotics is centered around anthramycin. 15 Using an activated
anthranilic acid derivative and a suitably substituted $\Delta^2$-pyrroline derivative, we thought it possible to form the pyrrolo[1,4]benzodiazepine system in one step. Our pyrroline would be substituted in such a manner that conversion to the acrylamide side chain of anthramycin itself would require far fewer steps than the number employed by Leimgruber's group. With $-Z$ equal to $-\text{CHCHCONH}_2$, no further modification of the side chain would be necessary.

The condensation of $\alpha$-metallated isonitriles$^{16}$ with activated olefins to produce mixtures of $\Delta^1$- and $\Delta^2$-pyrrolines reported by Saegusa$^{17}$ and Schöllkopf$^{18}$ seemed ideal for the preparation of the
needed pyrrolines:

\[
\text{\begin{align*}
\text{COOC_2H_5} & \quad \text{Cu}_2\text{O or NaOEt}^- \\
\text{N=C} & \quad \text{HN} \\
\text{Z} & \quad \text{Z}
\end{align*}}
\]

Although Saegusa's studies involved CuI and CuII catalysis, and Schöllkopf formed the isonitrile anion directly with sodium ethoxide and later with lithium diisopropylamide, both processes led only to the 2,4-disubstituted pyrrolines. No 2,3-isomers were ever observed. Clearly, the \( \Delta^2 \)-pyrrolines prepared by this method are racemic, while the \( \Delta^1 \)-pyrrolines are diastereomeric. In principle a stereospecific synthesis of anthramycin could be accomplished by resolution of the pyrrolines before condensation with the anthranilic acid analog.

A report\(^{19} \) that the condensation of isotoic anhydride (21) and proline gave a 55% yield of the benzodiazepine assured us that isotoic anhydride would be a good choice for the activated anthranilic acid derivative. Isotoic anhydride itself is easily prepared by

\[
\text{\begin{align*}
\text{\text{NH}_2\cdot\text{HCl}} & \quad \text{COCl}_2 \\
\text{\text{COOH}} & \quad \text{H}_2\text{O}
\end{align*}}
\]

treatment of anthranilic acid hydrochloride in aqueous solution with gaseous phosgene.\(^{20} \) Once our proposed procedure was perfected on the unsubstituted anhydride, it would be fairly easy to obtain the isotoic anhydride analog necessary for the preparation of anthramycin itself,
using the same method.

Admittedly, the condensation of isatoic anhydride and a pyrroline ester would lead to the formation of the dilactam system that was Leimgruber's stumbling block. However, reduction of the secondary lactam had been found to occur with lithium aluminum hydride on pyrrolo[1,4]benzodiazepinone systems in which the benzene ring was unsubstituted, albeit to the amine.\textsuperscript{19} We felt that even on the anthramycin system itself reduction to the carbinolamine would be possible with the right reducing agent. If necessary, as a last resort, we would employ the Leimgruber expedient in order to complete the synthesis.

Preliminary investigations of our proposed procedure were begun by Dr. Robert M. Cory. Following Saegusa's procedure, using Cu\textsubscript{2}O as catalyst, Dr. Cory reacted methyl isocyanoacetate\textsuperscript{21} with methyl acrylate and obtained a mixture of the \(\Delta^1\) - and \(\Delta^2\) - isomers (22) as expected.\textsuperscript{17} The product partially decomposed upon distillation. Reaction of the crude pyrroline mixture with one mole of isatoic anhydride in refluxing pyridine overnight gave a 38\% yield of crude benzodiazepine (23).

\[
\begin{align*}
\text{H} & \quad \text{COOCCH}_3 \\
\text{21} & \quad \text{22} \\
\text{O} & \quad \text{COOCCH}_3 \\
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{COOCCH}_3 \\
\text{23} & \quad \text{N} \\
\text{O} & \quad \text{COOCCH}_3 \\
\end{align*}
\]

Encouraged by this result, Dr. Cory repeated the methyl isocyanoacetate condensation with trans-methyl vinylacrylate (24).\textsuperscript{22} The crude pyrroline 25, probably a mixture of \(\Delta^1\) - and \(\Delta^2\) - isomers, was
reacted with isatoic anhydride for two hours in refluxing pyridine. Extensive chromatography resulted in the isolation of a few milligrams of crystalline product whose physical characteristics fit those of the desired compound 26.

Although the yield of 26 was small (ca. 1%), it was clear that the procedure itself held promise, and that further investigation was definitely warranted.
RESULTS AND DISCUSSION

We began the in-depth investigation with a closer look at pyrroline formation. Methyl isocyanatoacetate was reacted with methyl acrylate, acrylonitrile, acrolein, acrolein dimethylacetal, and trans-methyl vinylacrylate. All reactions could be monitored easily by watching the disappearance of the intense isonitrile band at 2160 cm⁻¹ in the ir. The absence of the characteristic foul pungent odor of the isonitrile moiety provided an additional indication of when reaction was complete.

Reaction with methyl acrylate in the presence of a catalytic amount of copper(I) oxide to give 22 was mildly exothermic, and proceeded smoothly at room temperature by shaking or stirring the reactants in dry benzene in a nitrogen atmosphere. Frequently the catalyst would become coated with a reddish oil and reaction would cease until more catalyst was added. This insoluble oil formation was observed with all subsequent substrates to a greater or lesser extent, but the oil was not identified. Shaking seemed to be more effective at keeping the catalyst in suspension. Complete reaction took from three to six hours. The benzene solution was then decanted from the oily film and filtered through Celite filter aid. Evaporation of the solvent gave a nearly quantitative yield of product. The ¹H nmr showed only faint traces of impurities and indicated the product ratio of Δ²- to Δ¹-isomer to be roughly 2.5 to 1. Because the product decomposed appreciably upon distillation, and the ¹H nmr of the distilled product was identical to that of the crude material, the crude product was used in all subsequent reactions. The crude pyrroline mixture could be stored without decomposition under nitrogen
in the freezer, but was usually prepared immediately before use.

The pyrrole \( \text{25} \) from methyl vinylacrylate was prepared in a similar manner (Cu\(_2\)O, benzene, 25°C, \( \text{N}_2 \), 3 hrs.), and no attempt at distillation was made. 92% of an orange oil was the maximum yield obtained.

Condensation of methyl isocyanooacetate with acrylonitrile stabilized with a little diisopropylamine, proved to be more exothermic, and best results were obtained by running the entire reaction at 0°–5°C. The acrylonitrile was added dropwise to a solution of the isonitrile in dry benzene containing the copper(I) oxide catalyst, then stirred four hours under nitrogen. Again, distillation led primarily to decomposition. The \(^1\text{H} \) nmr spectrum of the crude pyrrole \( \text{27} \) showed three methyl peaks in the ratio of 3 to 1 to 1. These are probably the \( \Delta^2 \)-isomer, the \( \Delta^1 \)-isomer, and unreacted isonitrile, respectively.

Attempts to condense methyl isocyanooacetate with acrolein (25°C; simultaneous addition, 5°C) gave complex mixtures of products, probably containing \( \text{28, 29, and 30} \). Both Saegusa\(^{17,23} \) and Schöllkopf\(^{24} \) report that \( \alpha \)-metallated isonitriles condense with aldehydes and ketones to give oxazolines.

Acetal formation inhibited olefin reaction completely, but led to the discovery of an interesting, but useless (for our purposes)
side-reaction. Methyl isocyanoacetate in the presence of a catalytic amount of copper(I) oxide in refluxing benzene will condense with itself to give crystalline dimethyl 4-carboxyl-1-imidazoleacetate (31) in 51\% yield, m.p. 143.5°-145°C (needles, CHCl₃). The structure was verified by ¹H nmr, ir and mass spectral analysis [ir (CHCl₃) 3020, 3000, 1760, 1720, 1550 cm⁻¹; ¹H nmr (CDCl₃) δ 7.49 (broad s, 1, CH), 7.36 (broad s, 1, CH), 4.68 (s, 2, CH₂), 3.75 (s, 3, OCH₃), 3.68 (s, 3, OCH₃); m/e M⁺ 198].

From the preceding results the dicarbomethoxytryproline 22 proved to be the most convenient pyrrole to prepare, and so it was used most often in subsequent studies. Although we could not purify any of the pyrrolines, the crude purity was high in most cases and did not seem to present a serious obstacle to the success of the total scheme.

We next turned our attention to the step involving pyrrole condensation with isatoic anhydride (IA). Cory's reactions with IA had all employed an equimolar ratio of reactants. Formation of methyl anthranilate (32) was found to occur as a side reaction. A molecule of methanol is released in the condensation step and this can
react with IA to give $3$. It was thus necessary to use a twofold excess of IA to replace the material consumed by the side reaction.

\[
\begin{align*}
\text{H} & \quad \text{COOCH}_3 \\
\text{O} & + \quad \text{HN} & + \quad \text{CH}_3\text{OH} \\
\text{O} & \quad \text{N} & \quad \text{X} \\
\text{O} & \quad \text{N} & \quad \text{X} \\
\end{align*}
\]

Cory's work-up consisted of pouring the reaction mixture into sulfuric acid and ice. Filtration of the brown precipitate gave a 33-38% yield in the case of product $3$. Recrystallization from dimethyl sulfoxide/methanol, acetonitrile, or ethyl acetate never gave greater than a 50% recovery. It was thought that a modification of the work-up procedure could lead to isolation of an increased amount of purer product.

The product $3$ is insoluble in most common solvents, except those mentioned above. It is sparingly soluble in chloroform. We found that the crude reaction mixture could be chromatographed on silica gel, after pyridine removal, by dissolving it in a large volume of chloroform, and mixing it with a small amount of silica gel followed by solvent removal on a rotary evaporator. The silica gel impregnated with benzodiazepine was then added to a prepared silica gel column, covered with a short layer of sand, and then eluted with chloroform.
By treating pyrroline 22 with a twofold excess of IA (pyridine), reflux, 20 hrs.), followed by the above described chromatography, it was possible to obtain a 34% yield of a yellow granular solid, analytically pure after one recrystallization from acetonitrile, m.p. 257°-258°C. Two other products were isolated by chromatography: methyl anthranilate (32), ca. 10-15% yield,* and anthraniloylanthranilic acid (33), ca. 7% yield.* The latter product is simply the product of reaction of IA with itself.

When the above procedure was used with pyrroline 25, no benzodiazepine 26 could be isolated.

Repeating the reaction of pyrroline 22 at 60°C resulted in a prolonged reaction time, but no increase in yield. Refluxing in quinoline (b.p. 120°C) instead of pyridine gave a 10% yield. When dimethylformamide was added to the reaction mixture containing pyridine, heating at 130°C for twenty-four hours resulted in an approximate yield of 25%.

Since we were not able to increase the yield of 23 significantly, we began to look at the pyrroline starting material more closely.

* Yield of 32 is based on moles of pyrroline consumed. Yield of 33 is based on moles of IA.
It was thought that the low yields might be due to decomposition of the pyrrolines under the reaction conditions. To test this hypothesis a sample of pyrrole 22 was refluxed in pyridine under nitrogen for twenty-four hours. The oil remaining after solvent evaporation was much darker in color than the starting material, but the $^1$H nmr showed little change.

We had assumed that the presence of $\Delta^1$-pyrrolines in our reaction mixtures would have no effect on the condensation reaction. We supposed that the $\Delta^1$- and $\Delta^2$-isomers existed in equilibrium and that as the $\Delta^2$-isomer reacted with the anhydride, more would be produced from the $\Delta^1$-compound, until all the material had reacted. If this assumption were false, that might account for the modest results. More information was needed.

Pyrrole 25 was reacted with benzoyl chloride in benzene/pyridine. The reaction immediately began to darken at room temperature. The mixture was black after heating for two hours at 70°C. About fifteen products were present by TLC (1:1 ethyl acetate/cyclohexane; silica gel), but none could be identified. Repeating the reaction at 0°-5°C, and gradually allowing the reaction mixture to warm to room temperature, gave slightly better results. Again, the product after work-up was a black-red oil, but TLC showed four major spots - none of which could be identified.

After treatment of pyrrole 22 with o-nitrobenzoyl chloride, with cooling, the mixture was allowed to warm gradually to room temperature. A 73% yield of brown-green oil was obtained which appeared to be mainly one compound. The product was very sensitive to air and decomposed when it was eluted on an alumina column with benzene.
Repeating the reaction in 5% aqueous sodium hydroxide and chloroform (20 min. shaking at r.t.) gave a small yield of yellow green oil composed of several unidentifiable compounds.

Attempts were next made to condense pyrrolone \textsuperscript{22} with o-nitrobenzoic acid in the presence of dicyclohexylcarbodiimide (DCC).\textsuperscript{26} It was hoped that the milder reagents would lead to fewer side reactions. Amide formation in the presence of disubstituted carbodiimides (\textsuperscript{34}) had been found to proceed by initial condensation between the carboxylic acid and the carbodiimide to form the activated intermediate \textsuperscript{35}, which then reacts with the amine to produce the amide and a substituted urea:

\[
R'\text{COOH} + R^2\text{N}=\text{C}NR^2 \rightarrow R^2\text{NHCOCR'}^\text{35}
\]

In our hands the reaction effectively stopped at the initial adduct stage.

o-Nitrobenzoic acid and pyrrolone \textsuperscript{22} were stirred in a 1:1 mixture of anhydrous ether and methylene chloride at room temperature. To this was added dropwise an excess of DCC in the same solvent. After forty-five minutes of stirring at approximately 35°C, water was added to the already cloudy solution. Following an additional ten
minutes of stirring, the mixture was refrigerated overnight. Then the solid urea was filtered off and the phases were separated. The organic phase was washed with 5% aqueous sodium carbonate, 1N hydrochloric acid, and water. Additional urea was removed by filtration, and the solvent was evaporated off to give a yellow semicrystalline solid. Trituration with ethyl acetate followed by filtration yielded a white solid, m.p. 126°-149°C, which TLC (8:2 benzene/methanol; silica gel) indicated to be mostly α-nitrobenzoic acid (m.p.147°-149°C).

Preparative layer chromatography (PLC) of the ethyl acetate solution (8:2 benzene/methanol; silica gel) showed two major product bands. \(^1\text{H}\) nmr of the band at R\(_f\) 0.377 indicated two methyl esters and what appeared to be an ortho-substituted benzene ring. It was somewhat contaminated with dicyclohexylurea. In a later reaction this material was obtained pure and identified as the pyrrole \(^{36}\), formed by oxidation of the pyrrole \(^{22}\). \([\text{ir (CHCl}_3] 3460 (\text{NH}), 2960, 2940, 2860 (\text{CH}), 1550 (\text{C=C}), 1700 (\text{broad C=O}), 1260 \text{cm}^{-1} (\text{C-O}); \]
\(^1\text{H}\) nmr (CDCl\(_3\)) \(\delta 7.55 (\text{m, } 1, \text{ aromatic H}), 7.30 (\text{m, } 1, \text{ aromatic H}), 3.89 (\text{s, } 3, \text{ OCH}_3), 3.88 (\text{s, } 3, \text{ OCH}_3), 1.30 (\text{broad s, } 1, \text{ NH?})\]

\[
\begin{array}{c}
\text{COOCH}_3 \\
\text{CH}_3\text{OOC} \\
\text{N}
\end{array}
\]

\(^{36}\)

The band at R\(_f\) 0.517 slowly crystallized after removal from the plate with ethyl acetate, m.p. 142°-147°C. It was not α-nitrobenzoic acid, although the \(^1\text{H}\) nmr appeared to be a composite of acid and urea. The product was found to be the adduct \(^{37}\). \([\text{ir (CHCl}_3] 3390, 3300 (\text{NH}), 2920, 2860 (\text{CH}), 1695 (\text{s, C=O}), 1650 (\text{s, N=O}), 1510 (\text{broad s, C=N}), \]

\[
\begin{array}{c}
\text{COOCH}_3 \\
\text{CH}_3\text{OOC} \\
\text{N}
\end{array}
\]
1340 cm$^{-1}$ (s, N=O); $^1$H nmr (CDCl$_3$) $\delta$ 8.05 (m, 1, aromatic H), 7.3-7.7 (m, 3, aromatic H), 6.65 (broad, 1, NH?), 0.8-2.2 (broad m, 20, CH$_2$), CH peak too weak to record accurately.]

\[
\begin{align*}
\text{NO}_2 & \\
\text{O} & \\
\text{N} & \\
\text{H} & \\
\end{align*}
\]

The reaction was repeated with a four-day reaction time, using an identical work-up. The residual yellow oil was taken up in hot ethanol and it deposited white needles, m.p. 149°-152°C, upon cooling. These were identical to product 37. The filtrate showed some adduct 37, some of the pyrrole 36, some o-nitrobenzoic acid, and some unreacted pyrroline 22. $^1$H nmr spectroscopy of this residue strongly resembled that of the product of reaction of pyrroline 22 with o-nitrobenzoyl chloride.

The DCC reaction was repeated once more using dry refluxing tetrahydrofuran as solvent (5 hrs.). When again the adduct 37 was the major product, it became very clear that a new tactic was required.

2-Trifluoromethyl-4H-3,1,4-benzoazinone 38* was a known compound. We hoped that it would react in a manner analogous to that of isatoic anhydride, but better, because of its increased reactivity. Unfortunately, this reactivity made the benzoazinone very sensitive to water,

* The trifluoroacetamide group present after reaction can be easily hydrolyzed with aqueous methanolic potassium carbonate.
and it was difficult to obtain free of the acid* 39, because it hydrolyzed so readily.

A series of reactions were run as follows in the presence of non-nucleophilic bases. Under anhydrous conditions, pyrroline 22 and the trifluoromethylbenzoxazinone 38 were refluxed in benzene. No reaction was noted after 18 hours when tetramethylquainidine (40) was

* The best method of purification is vacuum sublimation.
used as the base. In the presence of potassium tert-amyloxide, after 7 hours reflux, extensive decomposition of 38 had occurred, but 22 was still present (by TLC). Stirring in dimethylsulfoxide overnight with potassium tert-amyloxide produced the same result.

Four hours of refluxing in pyridine provided more interesting results. When the reaction mixture was poured into 1N aqueous hydrochloric acid, a gummy tan precipitate resulted. One crystallization from chloroform gave yellow granules, m.p. 167-177°C. Mass spectral analysis showed a molecular ion at 400, the correct weight for the uncyclized product 41. The base peak, however, was 233, and indicated

![Chemical Structure](image)

the presence of a great deal of hydrolysis product 39. Indeed, an additional recrystallization from chloroform produced white needles, m.p. 185°-185.5°C (sealed capillary) confirming that the product did contain α-trifluoroacetamidobenzoic acid. Preparative layer chromatography of the chloroform extract of the acid solution above (9:1 chloroform/methanol, silica gel) led to the isolation of a yellow solid (R_f 0.432) whose spectra strongly resembled that of one of the compounds isolated in the DCC reactions described earlier. By ir on a KBr disc, we were able to identify the compound as a pyrrole, specifically pyrrole 36. Other compounds separated by preparative layer chromatography were more of product 41 (R_f .371), unreacted
benzoxazinone (Rf .589), and a green fluorescing product (GFP) isolated earlier during an attempt at preparation of \( \Delta^2 \) (also in pyridine). Although well characterized, the GFP could not be identified. [m.p. 172.5°C, needles, EtOH, \( \text{ir (CHCl}_3 \) 1770 (s), 1737 (broad s), 1610 (s), 1160 (s), 1000 cm\(^{-1}\) (m); \( \text{^1H nmr (CDCl}_3, \text{TMS ext.} \) \( \delta 13.06 \) (broad, 1), 8.36 (d, aromatic H, \( \text{J}=3\text{Hz} \)), 8.20 (d, aromatic H, \( \text{J}=3\text{Hz} \)), 7.82 (broad), 6.50-6.75 (m, aromatic H), soluble undiluted base, insoluble acid, \( \text{m/e M}^+ 334 \), obs: C 56.09, H 2.74, N 8.09, F 17.16 calc. for \( \text{C}_{16}\text{H}_9\text{N}_2\text{F}_3\text{O}_3 \), C 57.53, H 2.70, N 8.39, F 17.06] When pyrroline \( \Delta^2 \) and benzoxazinone \( \Delta^2 \) were refluxed in a mixture of benzene and pyridine, formation of the GFP was suppressed, but pyrrole formation seemed to be the major reaction, even though the reaction was run under nitrogen.

Two facts concerning our pyrrolines had by now become very obvious. (1) The dihydropyrroles had a strong propensity toward aromatization. (2) The nitrogen atom was not very nucleophilic.

The oxidation of the pyrroline occurred most readily in the presence of a nitro group, which can act as an oxidizing agent. It was also more evident after prolonged reaction times at high temperature. More rigorous exclusion of air should combat the problem in the second case.

The \( \Delta^2 \)-pyrrolines are also vinlylogous carbamates, and as such would be expected to be less reactive at nitrogen than amines, due to the electron withdrawing effect of the ester through the double bond. By the same effect, the hydrogen attached to the nitrogen should be fairly acidic. Using a strong, non-nucleophilic base, it should be possible to remove this hydrogen, and thus increase the nucleophilicity
at nitrogen. We thought it possible to remove the hydrogen at C-3 in the Δ¹-pyrroline (A), also, and assumed that the majority of negative charge would then rest on the nitrogen (B).

\[
\begin{align*}
\text{COOCH}_3 & \quad \leftrightarrow \quad \text{COOCH}_3 \\
\text{HN} & \quad \text{N} \\
\text{A} & \quad \text{B}
\end{align*}
\]

Lithium diisopropylamide (LDA) was chosen as base. Addition of pyrroline 22 to LDA (in THF) at -78°C resulted in an immediate color change and formation of a slight precipitate. After stirring three hours at -78°C, the solution was red-brown. When the reaction was quenched with glacial acetic acid, the color immediately returned to a pale yellow. The pyrroline 22 was recovered as a yellow-orange oil, unchanged by TLC and ¹H nmr. It was not possible to tell whether isomerization of Δ¹ to Δ² had taken place.

In a perhaps overly optimistic vein, we decided to attempt pyrrolobenzodiazepine formation in a one-pot synthesis, starting with methyl isocyanatoacetate and using LDA to catalyze pyrroline formation with acrylonitrile. This is essentially the approach used by Schöllkopf.¹⁹ The mechanism outlined has been proposed by both Schöllkopf¹⁹a,b and Saegusa.¹⁸a Hydrogen abstraction by 27a is assumed to be intermolecular, either from solvent, unreacted isonitrile, or the conjugate acid of the base used to generate the isonitrile.
anion. In our case the proton would be provided by diisopropylamine or possibly a molecule of \(27\) already protonated.

Subsequent addition of \(38\) to the pyrroline anion (\(27c\)) should result in formation of product \(42\). As in the case with isatoic anhydride, \(38\) should be used in twofold excess to react with the methoxide liberated in the reaction.

The reaction was actually run in dry tetrahydrofuran at \(-60^\circ\text{C}\). A precipitate formed when methyl isocyanooacetate was added to the solution of LDA. The color deepened as acrylonitrile was added. After two hours of stirring at \(-60^\circ\text{C}\), the benzoazinone was added and the temperature was allowed to rise slowly to \(0^\circ\text{C}-5^\circ\text{C}\). A precipitate was still present which appeared to be polyacrylonitrile.
After stirring overnight at room temperature the reaction was worked up by extraction and PLC (1:1 ethyl acetate/cyclohexane, silica gel). 17% of a sweet smelling pale yellow oil that gradually crystallized was isolated. Mass spectral analysis gave a molecular weight of 315. The product appears to result from the reaction of \( \text{38} \) with methyl isocyanatoacetate anion directly. It is possibly compound \( \text{43} \). [\( \text{ir} (\text{CHCl}_3) 3240 \) (broad w), 1730 (s), shoulder at 1700 (s), 1200 (s), 1585 (m), 1525 (m), 1365 (m), 1285 cm\(^{-1}\) (m); \( m/e \) \( \text{M}^+ 315; \) \( ^1\text{H nmr} \) (CDCl\(_3\)) \( \delta \) 7.91-7.4 (m, 5, aromatic H), 4.0 (s, 3, OCH\(_3\)), no CH\(_2\) or CH protons.]

![Chemical structure](image)

Trifluoroacetimidobenzoic acid was recovered also. No pyrroline could be detected. It appears that under the reaction conditions the initial desired condensation did not take place, or, if it did, it was reversible.

The reaction was repeated with preformed pyrroline \( \text{22} \), in tetrahydrofuran (THF) at -70°C with hexamethyl phosphoramidate (HMPA) added
to keep the pyrroline anion in solution. TLC at the end of the reaction indicated ten to fifteen products. None were identified.

We were heartened by the apparent ease with which the pyrroline anion formed and decided to try reaction with another substrate, namely, o-nitrobenezoyl chloride. Addition of the acid chloride to a solution of the anion in THF at -60°C triggered a series of dramatic color changes. After one hour at -60°C, the reaction was worked up to give a 94% yield of a red oil. A distinct doublet of doublets in the $^1$H nmr at 5.14 $\delta^*$ together with the other spectral data strongly implied the presence of a substituted pyrroline. Chromatography on silica gel (9:1 benzene/ethyl acetate) gave 47% of a fairly pure material that was confirmed by mass spectral analysis (m/e M$^+$334) as the desired adduct 44. The ir of the purified material was essentially the same as that of the crude. About ten mole percent of the product oil was pyrrole 36. Catalytic hydrogenation of 44 over 10% Pd/C in ethyl acetate gave a yellow oil which was definitely an amine (45),
ir (film) 3500 (m, NH), 3400 cm\(^{-1}\) (m, NH). TLC showed one major spot and three minor spots. Treatment of the crude amine with 1N aqueous hydrochloric acid gave a 48% overall yield of pearly white crystals, identical in every respect with the benzodiazepine \(\text{23}\) prepared from isatoic anhydride. Repetition of the latter two reactions on a larger scale (i.e., > 0.2 mmol), should increase the yield of \(\text{23}\) considerably.

The LDA reaction was next tried with pyrrole \(\text{25}\) under the same reaction conditions. The product obtained was immediately subjected to catalytic hydrogenation over 10% Pd/C in ethyl acetate. Although a 50% hydrogen uptake occurred, a graph of hydrogen uptake versus time indicated some over-reduction may have taken place. The product was extracted with 1N aqueous hydrochloric acid and chromatographed on silica gel prep plates (1:1 ethyl acetate/cyclohexane). Tan needles were obtained from the band at \(R_f\) 0.44. These were identified by spectral data and mass spectral analysis as pyrrole \(\text{46}\). [\(\text{ir (CHCl}_3\) 3430 (s, NH), 3000 (m, =C-H), 2920 (m), 1700 (broad s, C=O), 1635 (s, =C-H) 1465 (m), 1300 (broad m, C-O), 970 cm\(^{-1}\) (w); m/e M\(^+\) 209; \(^1\text{H nmr (CDCl}_3\), 7.48 \(\delta\) (d, 1, vinyl H, \(\text{J}=15\))}, 7.10-6.91 (m, 2, aromatic H), 6.08 (d, 1, vinyl H, \(\text{J}=15\)), 3.82 (s, 3, OCH\(_3\)), 3.70 (s, 3, OCH\(_3\)), 1.68 (broad s, 1, NH)]

The residue in ethyl acetate solution was chromatographed on a silica gel column and eluted with chloroform, then ether. Mass spectral analysis of a semicrystalline sample obtained from the column indicated
the presence of a small amount of the desired product \(47\) (\(m/e\) \(M^+\) \(330\)), but the major component of the sample was the pyrrole \(46\).

The LDA reaction was repeated and a portion of the nitro-product was purified by PLC as above. Four major bands were present. None had the appearance of product \(48\). However, more pyrrole was isolated.

The remaining nitro-product was reduced with sodium hydrosulfite (\(Na_2S_2O_4 \cdot 2H_2O\)) and ammonium hydroxide. Mass spectral analysis of the crude product gave peaks at \(m/e\) 330 and 220. Although it is possible for \(47\) to fragment to a product of molecular weight 220 (loss of \(HC=C-CH=CH-COOC_3\)), most of the peak at 220 is probably due to the presence of \(49\), the reduced product of the condensation between LDA and \(\alpha\)-nitrobenzoyl chloride. Its presence is supported by other spectral data, namely, the presence of an intense methyl doublet (\(J=6\) Hz) at 1.48 \(\delta\) in the \(^1H\) nmr of the sample in which \(46\) was first detected. Diisopropylamine absorbed at 1.15 \(\delta\) (\(J=6\) Hz). Anomalous methyl peaks (1.60 \(\delta\), \(J=6\) Hz) were also present in the \(^1H\) nmr of the
crude nitro compound before reduction. [Diisopropylamine was at 1.18 \( \delta \) \((\ddot{J}=6\text{Hz})\).]

Comparative TLC of the pyrroline starting material with pyrrole \( \approx \) showed a substantial amount of the pyrrole already present. LDA, then, had probably been added in excess. Decreasing the amount of LDA in the reaction mixture should increase the proportion of \( \approx \) formed. This reaction should be investigated further, but was not, due to lack of time. The possibility of reaction between the pyrroline anions and isatoic anhydride should also be explored.

One last study was taken on in this project. Two other biologically active compounds existed which possessed the benzodiazepine nucleus without the pyrrolo ring, namely, \( \approx \) and \( \approx \). They are best known by their trade names of Valium\(^{29}\) and Librium\(^{30}\) respectively.

\[
\begin{align*}
\text{50} & \quad \text{51} \\
\begin{array}{c}
\text{Cl} \\
\text{N} \\
\text{O} \\
\end{array} & \begin{array}{c}
\text{Cl} \\
\text{N} \\
\text{O} \\
\end{array}
\end{align*}
\]

Widely used as tranquilizers, they have no antibiotic activity. Since most of our products were to be tested for biological activity, we were very curious as to what effect the introduction of a chloro-group on the benzene ring would have on their activity.

The condensation of pyrroline \( \approx \) with 5-chloroisatoic anhydride proceeded smoothly to give a 43\% yield of \( \approx \), purified by chroma-
tography. Unfortunately, none of the compounds submitted* for testing possessed any biological activity whatsoever, neither as antibiotics nor as tranquilizers.

While our total scheme suffers slightly from our inability to turn the isatoic anhydride condensation into a high yield step, it still shows considerable merit as a fast, easy approach to the pyrrolobenzodiazepine nucleus. It is possible, too, that substitution on the benzene ring could improve the condensation step. Introduction of a chloro-substituent was seen to increase the yield of product approximately 10%.

A possible reason for the low yields, not previously mentioned, might be failure of the intermediate 53 to cyclize further. Although

* Biological tests were run by Upjohn Pharmaceutical Co., Kalamazoo, Michigan.
we identified none of these intermediates in the reaction with isatoic anhydride, Ermili and Filacchioni\textsuperscript{20} found aminobenzoylphenylalanine(54) to be the major product when they reacted phenylalanine with isatoic anhydride in the presence of sodium hydroxide. Cyclization was effected by fusion of the crystalline amino acid at 100°C.

\[
\begin{array}{c}
\text{N} \\
\text{H} \\
\text{NH} \\
\text{C} \\
\text{O} \\
\text{O} \\
\text{COOH} \\
\text{N} \\
\text{H} \\
\text{CH}_2 \phi \\
\end{array}
\quad
\begin{array}{c}
\text{N} \\
\text{H} \\
\text{NH} \\
\text{C} \\
\text{O} \\
\text{O} \\
\text{COOH} \\
\text{N} \\
\text{H} \\
\text{CH}_2 \phi \\
\end{array}
\quad
\begin{array}{c}
\text{NaOH} \\
\text{N} \\
\text{H} \\
\text{NH} \\
\text{C} \\
\text{O} \\
\text{O} \\
\text{COOH} \\
\text{N} \\
\text{H} \\
\text{CH}_2 \phi \\
\end{array}
\quad
\begin{array}{c}
\text{N} \\
\text{H} \\
\text{NH} \\
\text{C} \\
\text{O} \\
\text{O} \\
\text{COOH} \\
\text{N} \\
\text{H} \\
\text{CH}_2 \phi \\
\end{array}
\Rightarrow
\]

In a model study reaction between the benzoxazinone\textsuperscript{38} and glycine methyl ester hydrochloride, 55 was produced in approximately 90% yield (pyridine, 50°C, 24 hrs.). Treatment of 55 with p-toluene-sulfonic acid in toluene (111°C, 7 d.) produced a 40% yield of a product tentatively identified as 56. [\text{ir (CHCl\textsubscript{3}) 1750 (m), 1690 (s), 1600 (m), 1130 cm}^{-1} (s); m/e M^+ 286; \text{\textsuperscript{1}H nmr (CDCl\textsubscript{3}) 8.40 \delta (d, 1, aromatic H, J=10Hz), 8.10-7.38 (m, 3, aromatic H), 4.99 (s, 2, CH}_2, 3.81 (s, 3, CH}_3)] No benzodiazepine could be isolated.

\[
\begin{array}{c}
\text{N} \\
\text{H} \\
\text{NHCOCF}_3 \\
\text{CONHCH}_2\text{COOCH}_3 \\
\text{PhCH}_3 \\
\text{pTsOH} \\
\end{array}
\quad
\Rightarrow
\begin{array}{c}
\text{N} \\
\text{H} \\
\text{CF}_3 \\
\text{N} \\
\text{CH}_2\text{COOCH}_3 \\
\end{array}
\Rightarrow
\]
Due to lack of time it was not possible to go back and search specifically for the uncyclized adducts in the isatoic anhydride reaction. If they are present, it should be possible to find conditions that would effect ring closure.

Another useful result of our research, although not appreciated at the time, is the discovery of the relatively easy oxidation of pyrrolines to pyrroles. Pyrrolines made by the methods of Saegusa or Schöllkopf could provide a useful route to substituted pyrroles difficult to obtain by other means. Van Leusen et al.\textsuperscript{31} has reported pyrrole synthesis via elimination of tosylate from pyrrolines made for p-toluene sulfonylmethyl isocyanide (57) and activated olefins with sodium hydride as base. One of Schöllkopf's students observed

\[
\begin{align*}
\text{NC} & \quad \text{X} \quad \text{NaH} & \quad \text{BH} & \quad \text{B}^- \quad \text{R} \quad \text{Tos}^- \quad \text{H} \quad \text{HN} \quad \text{X} \\
\begin{aligned}
\text{Tos} & \quad \text{X} \quad \text{R} \\
57
\end{aligned}
\end{align*}
\]

pyrrole formation with elimination of nitrite from the adducts formed from nitroolefins and isocyanooacetic esters.\textsuperscript{32} No one apparently has treated the pyrrolines with simple oxidizing agents. Bubbling oxygen should be sufficient to effect aromatization and would inhibit radical initiated polymerization.

The pyrroles themselves might provide a more active series of anthramycin analogs. Sibiromycin (6), for example, has a pyrrole ring.
EXPERIMENTAL

General

Melting points and boiling points are uncorrected. $^1H$ NMR spectra were recorded as CDCl$_3$ (Merck, Sharp & Dohme, 99.8 atom% D) or DMSO-d$_6$ (Merck, Sharp & Dohme 99.5 atom% D) solutions on a Varian A56/60 or a Perkin-Elmer R-12 instrument. Chemical shifts are reported in parts per million ($\delta$) relative to TMS as an internal standard when CDCl$_3$ was used as solvent. When DMSO-d$_6$ was used as solvent, the DMSO peak at 2.50 $\delta$ was used for standardization.

Infrared spectra were recorded as liquid films, benzene solutions, or KBr pellets on a Beckmann IR-8 instrument. A Consolidated Electrodynamics Corporation 21-1100 high resolution mass spectrometer provided mass spectral data.

Isatoic anhydride and 5-chloroisatoic anhydride were generously provided as gifts from the Maumee Chemical Company, a subsidiary of the Sherwin-Williams Company.
3,5-Dicarbomethoxy-Δ²-pyrroline (22)

1.127 g (0.0114 mol) methyl isocyanacetate, 0.9816 g (0.0114 mol) freshly distilled methyl acrylate, a pinch of Cu₂O, and 25 mls. dry distilled benzene were shaken together in a nitrogen atmosphere for four hours, until the ir isonitrile band at 2160 cm⁻¹ had disappeared. The solution was filtered through Celite filter aid and the solvent removed at reduced pressure. The yellow-orange oily residue (2.032 g, 96%) was used without further purification because the material decomposes appreciably upon distillation. ir (film) 3360 (NH), 1740 (S, C=O), 1670, 1600 cm⁻¹; ¹H nmr (CDCl₃) δ 7.7 (m, N=CH), 7.3 (m, vinyl H), 5.1 (bs, NH), 4.45 (m, NCH), 3.75 (s, OCH₃), 3.65 (s, OCH₃), 2.0-3.2 (m, CH₂, CH). The ratio of Δ² to Δ¹ is roughly 2:5:1 from ¹H nmr.

Methyl 5,10,11,11a-tetrahydro-5,11-dioxo-1H-pyrrolo[2,1-c][1,4]benzdiazepine-2-carboxylate (23) A

1.1620 g (6.29 mmol) 3,5-dicarbomethoxy-Δ²-pyrroline (22) and 3.79 g (19 mmol) isatoic anhydride (21) in 25 mls. dry pyridine were refluxed under nitrogen for 20 hours. The solvent was removed in vacuo. The crude product was dissolved in chloroform and then evaporated onto silica gel. The impregnated silica gel was added to a prepared silica gel column and eluted with chloroform. 0.58 g (34%) of a pale yellow granular solid was obtained. An analytical sample was secured by recrystallization from acetonitrile, m.p. 257°-258°C. Methyl anthranilate (32) and anthraniloylanthranilic acid m.p. 202°-204°C d. (lit²⁵ 203°C d.), picrate m.p. 187°C d. (lit²⁵ 190°C d.) were also recovered.
ir (KBr) 3250 (m, NH), 3110 (=C-H), 1700 (broad s, C=O), 1620 (s),
1265 cm\(^{-1}\) (m). \(^1\)H nmr (DMSO-d\(^6\)) \(\delta\) 8.0-7.1 (m, 5, aromatic + vinyl),
4.9 (dd, 1, CH, \(J_{H_4H_5} = 4\) Hz, \(J_{H_4H_6} = 10.5\) Hz), 3.76 (s, 3, OCH\(_3\)),
3.0-2.7 (broad t, 2, CH\(_2\)); m/e M\(^+\) 272, anal. calc'd for C\(_{14}H_{12}N_2O_4\):
C, 61.76; H, 4.47. Found: C, 60.50; H, 4.47 (probably some DMSO-d\(^6\)
impurity).

2-(2-Vinylcarbomethoxy)-4-carbomethoxy-\(\Delta^2\)-pyrrolino (25)

1.901 g (19.2 mmol) methyl isocyanatoacetate in 30 mls. dry benzene
was stirred vigorously under nitrogen in an ice bath with a spatula
tip of Cu\(_2\)O. 2.18 g (19.4 mmol) of methyl vinylacrylate\(^22\) in 20 mls.
benzene was added over a period of one half hour. The ice bath was
removed and stirring was continued for four hours at room temperature
until the isonitrile band at 2160 cm\(^{-1}\) had almost disappeared from their. More Cu\(_2\)O was added after two hours. The benzene was filtered
through Celite. Solvent evaporation at reduced pressure gave 3.71 g
(92%) of an orange oil. The product is readily air-oxidized and for
this reason is not purified further. ir (film) 3420 (w, NH), 2990,
2950 (s), 1740 (broad s), 1610 (s), 1430 (m), 1200 (s); \(^1\)H nmr (CDCl\(_3\))
\(\delta\) 7.65 (broad s), 6.8 (broad s) 5.85-4.3 (m), 3.75 (s, OCH\(_3\)), 3.68
(s, OCH\(_3\)), 7.25-8.15 (m).

Methyl 5,10,11,11a-tetrahydro-5,11-dioxo-\(\Delta^1\)-
pyrrolo[2,1-c][1,4]benzodiazepine-2-acrylate (26)

To 2.98 g (10.0 mmol) of 2-(2-vinylcarbomethoxy)-4-carbomethoxy-
\(\Delta^2\)-pyrrolo (25) in 30 mls. dry pyridine under nitrogen was added
1.633 g (10.0 mmol) isatoic anhydride (21). The mixture was heated to
reflux with stirring. It was refluxed for two hours, then cooled in an ice bath. Evaporation of the bulk of the solvent and addition of methanol precipitated 0.325 g isatoic anhydride. TLC on silica gel with 1:1 ethyl acetate/cyclohexane indicated the presence of desired product. Extensive column chromatography on alumina using successively methylene chloride, ether, ethyl acetate and 10% methanol in ethyl acetate, led to the isolation of 25 mg. (0.08%) of a white crystalline solid, recrystallized from DMSO/MeOH, fine needles, m.p. 262°-4°C d. After other analyses, not enough material remained for a clear ir spectrum.

^1^H nmr (DMSO-^d^6) δ 7.92-7.05 (m, 5, aromatic and vinyl), 7.58 (d, 1, vinyl; J=7.5H₂), 5.82 (d, 1, vinyl, J=7.5), 4.84 (dd, 1, CH, J_H,CH'=4H₂, J_H,H'=10.5H₂), 3.71 (s, 3, OCH₃), 3.23 (s, H₂O), 3.5-2.7 (m, CH₂), 0.82 (s, impurity); HRMS 298.0950. Calc'd for C₁₆H₁₄N₂O₄: 298.0953.

3-Cyano-5-carbomethoxy-Δ²-pyrroline (27)

3.674 g (37.1 mmol) methyl isocyanoacetate in 10 mls. dry benzene was stirred under nitrogen in an ice-salt bath with a pinch of Cu₂O. To it was added 2.5 mls (1.99 g, 37.6 mmol) of freshly distilled acrylonitrile (stabilized with a little diisopropylamine) in 10 mls. dry benzene dropwise. Addition time lasted approximately forty minutes. The solution was then stirred at 0°-5°C for four hours, until the isonitrile band had almost disappeared from the ir. The cold solution was filtered through Celite and the solvent removed in vacuo. ^1^H nmr showed three methyl peaks in the ratio of 3:1:1. The product was distilled, bp 0.7 mm Hg 136°C. Methyl cyanoacetate
bp_0.4 35°C was also obtained. Some unreacted isonitrile, too, was present. Needles remaining in the distillation flask were tentatively identified as pyrrole. ir (benzene) 3400 (s, NH), 2920, 2180 (s, OCH_3, C=N), 1735 (s, C=O), 1600 (s), 1410 (m), 1200 cm⁻¹ (s); ^1^H nmr (CDCl_3) δ 7.05 (broad s, 1, vinyl), 4.95 (broad s, 1, NH), 4.5 (t, 1, CH, J=9Hz), 3.82 (S, 3, OCH_3), 3.08 (broad d, 2, CH_2, J=9Hz). There does not appear to be any Δ¹ present.

1- α-Nitrobenzoyl-3,5-dicarbomethoxy-Δ²-pyrroline (44)

In a flamed out three-neck flask 0.402 mls. (2.9 mmol) diisopropyl amine (distilled from CaH) in 10 mls. freshly distilled dry THF was cooled to 0°-5°C under nitrogen with vigorous mechanical stirring. 1.34 mls. of 2.2 M butyl lithium in hexane (2.9 mmol) was added very slowly by syringe. After cooling the solution to -60°C with a dry ice-acetone bath, 0.4803 g (2.6 mmol) 3,5-dicarbomethoxy-Δ²-pyrroline (22) in 5 mls. dry THF was added slowly by syringe. Instantly, the solution turned bright red. The solution was stirred at -60°C for one-half hour and then to it was added a solution of 0.545 g (2.9 mmol) α-nitrobenzoyl chloride in 5 mls. dry THF, also by syringe. When two-thirds of the acid chloride had been added, the solution color changed from a deep murky red to a black-green and then gradually cleared and turned orange. The solution was stirred an additional hour at -60°C and then allowed to warm to -10°C. The temperature rose quickly and the color again changed to a dark transparent green.

The cold THF was acidified to pH 5.5 with 5% acetic acid, washed twice with 50 mls. brine each, dried over Na₂SO₄, filtered and
evaporated to give 0.9085 g red oil. The oil was chromatographed on silica gel with benzene, followed by 10% ethyl acetate in benzene. 0.403 g (46.5%) of a yellow oil which darkened rapidly on exposure to air was isolated. TLC indicated a major impurity was 3,5-dicarboxymethoxy-pyrrole (36). By $^1$H nmr spectroscopy the product was about 90 mole percent pure. ir (film) 3100, 3000, 2960, 1740 (s), 1710 (s), 1670 (s), 1630 (s), 1530 (m), 1230 cm$^{-1}$ (s). $^1$H nmr (CDCl$_3$) δ 8.2-7.1 (m, aromatic H), 6.77 (t, 1, viny1 H, $\text{J}=1\text{H}_2$), 5.1 (dd, 1, CH, $\text{J}_{\text{H,H}}=5.5\text{H}_2$, $\text{J}_{\text{H,H}}=12\text{H}_2$), 3.8 (s, 3, OCH$_3$), 5.67 (s, 3, OCH$_3$), 3.3-2.6 (m, 2, CH$_3$).

1-$\alpha$-Aminobenzoyl-3,5-dicarboxylic-D$_2$-pyrrole (45)

0.086 g (0.241 mmol) 1-$\alpha$-nitrobenzoyl-3,5-dicarboxymethoxy-D$_2$-pyrrole was reduced with 10% Pd/C and hydrogen in 9 mls. ethyl acetate at 1 atm. pressure and room temperature. After nine hours 73% of the calculated hydrogen had been taken up. The reaction mixture was filtered through Celite, then the solvent was evaporated to give a yellow oil. ir (film) 3500 (s), 3400 (s), 3140 (w), 2980 (w), 1740 (s), 1710 (s), 1620 (s), 1410 (m), 1230 (s), 760 cm$^{-1}$ (m). TLC on silica gel with 1:1 ethyl acetate/cyclohexane showed one major spot and three minor. 45 was used immediately in the next reaction.

Methyl 5,10,11,11a-tetrahydro-5,11-dioxo-1H-pyrrolo[2,1-c][1,4]benzodiazipine-2-carboxylate (23) B

Cyclization of 1-$\alpha$-aminobenzoyl-3,5-dicarboxylate-D$_2$-pyrrole (45) to methyl 5,10,11,11a-tetrahydro-5,11-dioxo-1H-pyrrolo[2,1-c]-
[1,4]benzodiazepine-2-carboxylate (23) was effected by shaking a chloroform solution of the total amine product above with 5 mls. 1N HCl for about 10 minutes. A small precipitate was filtered off to give 3 mg. pearly white crystals. The chloroform layer was evaporated and the aqueous phase was allowed to stand overnight. A copious crystalline precipitate resulted. This was filtered and washed with methanol. The aqueous solution was evaporated. Methanol was added to the flask, and an additional 4 mg of 23 was recovered. The overall yield was 0.033 g from 44 (48%), m.p. 254°-257°C. A mixed m.p. with the product formed in reaction A was undepressed. 1H nmr and ir spectra were identical to those of 23 prepared from isatoic anhydride (reaction A).

Methyl 5,10,11,11a-tetrahydro-7-chloro-5,11-dioxo-1H-pyrrolo[2,1-c][1,4]benzodiazepine-2-carboxylate (52)

0.663 g (3.58 mmol) 3,5-dicarbomethoxy-Δ2-pyrrole and 2.12 g (10.7 mmol) 5-chloroisatoic anhydride (52) were refluxed with stirring in 25 mls. pyridine under nitrogen for twenty hours and were then stirred at room temperature for two days. Silica gel was added to the solution and pyridine was removed in vacuo at about 50°C. The material adsorbed on the silica gel was added to a prepared silica gel column and eluted with chloroform. 0.473 g (43%) of a pale yellow solid was collected. The solid was triturated with methanol and then recrystallized from chloroform, m.p. 239°-244°C. One impurity was visible by TLC (1:1 EtOAc/cyclohexane, silica gel). ir (KBr) 3200 (=C-H), 3090 (m, =C-H), 2980 (m), 1685 (s), 1655 (broad s), 1475 (m), 1420 (s), 1340 (m), 1290, 1270, 1245 (m), 1080 cm⁻¹ (m); 1H nmr (CDCl₃)
δ 8.8-7.9 (m, aromatic and vinyl), 4.56 (dd, CH, J_H,H′=3.5 Hz, J_H,H″=11 Hz), 3.71 (s, OCH₃), 4.03-3.56 (m, CH₂), 3.25-2.7 (m, CH₂ continued), 1.70 (s, impurity?), 1.28 (s, impurity?), 0.91 (broad singlet, impurity?); HRMS 306.0405. Calc'd for C₁₄H₁₁ClN₂O₄: 306.0407.
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6. (a) M. G. Brazhninova, N. V. Konstantinova and A. S. Mesentsev, ibid., 25, 668 (1972); (b) A. S. Mesentsev, V. V. Kuljaeva and L. M. Rubasheva, ibid., 27, 866 (1974).


Part II. A Partial Synthesis of d-Biotin
INTRODUCTION

Biotin (1), also known as vitamin H, is one of Nature's most essential molecules. Almost every organism, whether plant or animal, requires it for life. As a coenzyme, biotin functions as a CO₂ carrier (2) and is involved in two types of physiological reactions: carboxylations and transcarboxylations.

The vitamin was first isolated as a growth stimulant from yeast and egg yolk, and named by Kögl and Tonnis in 1936. The natural product is optically active \([\alpha]_D^{25} + 91.2^\circ \) (c 1.0, 0.1 N NaOH), and only the d-isomer shown (1) is active biologically. Not until 1942 was the structure of biotin known, when du Vigneaud published his results. The stereochemistry, however, remained a mystery. With
the first total synthesis of biotin in 1943, by Harris, Wolf, and Mozingo, and Folkes, the stereochemistry of the ring junction was established as being cis. One asymmetric center had still to be assigned. Although Harris' work strongly suggested that the hydrogens at C-4, C-5, and C-8 were all cis, this was not proven until 1956, when an X-ray structure determination was done by Traub. The absolute configuration at C-5 was established by Trotter and Hamilton in 1966, again by X-ray, as that shown in 1.

Du Vigneaud's paper seemed to trigger an explosion of synthetic effort by the major pharmaceutical companies. Four more syntheses of biotin rapidly followed Harris' into print, but only one of these was stereospecific, i.e., it produced d,l-biotin exclusively and not mixtures of the other possible isomers 2, 4, and 5. This synthesis, patented in 1945 by Goldberg and Sternbach at Hoffmann-La Roche, Inc., is still used for industrial production of the vitamin. An outline is presented in Scheme 1. A major drawback to the Goldberg-Sternbach synthesis is that resolution occurs late in
Scheme 1

fumaric acid

\[ \text{1) } \text{Br}_2 \]
\[ \text{2) } \text{RNH}_2 \]
\[ \text{3) } \text{COCl}_2 \]
\[ R = \text{CH}_2 \phi \]

\[ \xrightarrow{\text{Zn}} \]
\[ \xrightarrow{\text{HOAc/AC}_2\text{O}} \]

\[ 6 \]

\[ \text{HOOC} \]
\[ \text{COOH} \]
\[ \text{CH}_3\text{COO} \]
\[ \text{O} \]
\[ \text{O} \]

\[ 7 \]

\[ \text{1) } \text{H}_2\text{S} \]
\[ \xrightarrow{\text{2) } \text{Zn/HCl}} \]

\[ 8 \]

\[ \text{3) } \text{CIMg(CH}_2\text{)}_3\text{OCH}_3\text{RN} \]
\[ \xrightarrow{\text{2) } \text{H}_2\text{O}} \]

\[ \text{CH(CH}_2\text{)}_2\text{OCH}_3 \]

\[ \text{1) } \text{H}_2/\text{PdO} \]
\[ \xrightarrow{\text{2) } \text{HBr}} \]

\[ \text{3) camphor-sulfonic acid} \]

\[ A^- = \text{Br}^-, \text{CH}_2\text{SO}_3^- \]

\[ \text{1) separation} \]
\[ \text{2) } \text{KCN} \]
\[ \text{3) } \text{KOH} \]
\[ \text{4) } \text{Na}/\text{NH}_3 \]

\[ d-\text{biotin} \]
the scheme, decreasing the potential yield of product tremendously. In 1970 von M. Gerecke, J. P. Zimmerman and W. Aschwanden, also of Hoffmann-La Roche (Basel and Paris), published a modification of the original Roche procedure in which 6 is converted first to the symmetrical anhydride and then to the half ester which can be resolved. The unwanted isomer is hydrolyzed and recycled. The desired isomer reenters the scheme as enantiomerically pure 8, and the late resolution step is thus eliminated.

The modified scheme still involves some fourteen steps, and probably for this reason Roche continues active research toward biotin synthesis. Last year they published the first synthesis of d-biotin
which did not require a chemical resolution sequence.

Dr. Pat Confalone's group in Nutley, N.J., chose L (+)-cysteine (9), the biogenetic precursor\(^\text{11}\) of biotin, as their starting material. The synthesis is outlined in Scheme 2.

Scheme 2

\[
\begin{align*}
\text{NH}_2 & \xrightarrow{1) \phi \text{CHO}} \phi & \text{COOH} & \xrightarrow{\text{B}_2\text{H}_6} & \phi & \text{COOCH}_3 \\
\text{H} & \text{COOH} & \xrightarrow{2) \text{ClCOOCH}_3} & \phi & \text{COOH} & \xrightarrow{2) \text{CrO}_3 \cdot \text{Py}} & \phi & \text{CHO} \\
\text{SH} & \text{9} & & & & R = \text{COOCH}_3 & \text{70\% from 9} \\
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2\text{CMgCl} & \quad \phi \quad \text{H} \\
\xrightarrow{\Delta} & \text{Claisen rearr.} & \quad \text{HC(OAc)}_3 & \phi \\
\end{align*}
\]

\[
\begin{align*}
\text{Pyridinium HBr} & \quad \text{Perbromide} \\
\text{11} & \quad \text{60\%} & \quad \text{Br} & \quad i) \text{HBr/HOAc} & \quad \text{Br} & \quad \text{12} & \quad 92\% \\
\quad & \quad \text{COOCH}_3 & \quad & \quad \quad & \quad & \text{12} & \quad \text{92\%} \\
\end{align*}
\]
\[ \text{Br} \quad \text{H} \quad \text{N}_3 \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{Br} \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{Br} \quad \text{H} \quad \text{N} \quad \text{O} \]

\[ 13 \quad \text{H}_2 \quad \text{cat.} \]

\[ \text{NH}_2 \quad \text{H} \quad \text{N} \quad \text{O} \quad \text{N} \quad \text{H} \quad \text{O} \]

\[ 100\% \]

\[ 1) \text{Ba(OH)}_2 \]

\[ 2) \text{COCl}_2 \]

\[ \text{HN} \quad \text{NH} \quad \text{COOH} \]

\[ 1) \text{LiBH} \]

\[ 2) \text{HBr/ HOAc} \]

\[ 80\% \text{ from 13} \]

\[ \text{HN} \quad \text{NH} \quad \text{Br} \quad \text{S} \quad \text{H} \quad \text{N} \quad \text{O} \]

\[ 1) \text{Na}^{+} \]

\[ 2) \text{Ba(OH)}_2 \]

\[ 3) \Delta, \text{H}_2\text{O} \]

\[ \text{d-biotin} \]
The dramatic stereospecific rearrangement of \( \sim 10 \) to \( \sim 11 \) is believed to proceed via anchimeric participation of sulfur to the sulfonium ion \( \sim 14 \), which then expels benzaldehyde to give \( \sim 11 \). The result was correctly predicted by model studies.

Attempts to displace the bromide in \( \sim 11 \) directly with azide resulted in formation of the wrong isomer, compound \( \sim 15 \), presumably arising from an intermediate aziridine \( \sim 16 \). The problem was circumvented by refluxing \( \sim 11 \) in acetic acid until \( \sim 12 \) formed in an analogous reaction. Treatment of \( \sim 12 \) with lithium azide in dimethylformamide gave the desired product \( \sim 13 \), but in low yield. Elimination was the predominant reaction.

Elegant as this synthesis is, it may not be economically feasible as an industrial route due to the relative expense of \( L^{\text{(+)-cysteine}} \).
and the presence of a less than 20% yield step.

Two syntheses of biotin have been published recently which involve the stereospecific reduction of a thiophene as the key step. The first, \(^{12}\) devised by Taguchi's group in Tokyo, begins with the unique starting material 4-methyl-2(1H)-imidazolone (\(^{17}\)). It is summarized in Scheme 3.

Scheme 3

![Scheme 3](image)

The primary importance of this synthesis lies in the generation of thiophene \(^{19}\) from \(^{18}\) in 84% yield by thiol attack on the ketone in the presence of an acid catalyst. Taguchi does not report conditions for the reduction of the thiophene in this paper, although Cheney had reported reduction of \(^{19}\) to \(\delta,\lambda\)-biotin by hydrogenation on \(\text{MoS}_3-\text{Al}_2\text{O}_3\) under high pressure and elevated temperature.\(^{8c}\)
The second synthesis (Scheme 4) is again from Confalone's group. Production of the thiophene 20 was reported earlier by L. C. Cheney and most of the other reagents employed have been used by other groups, but never to such good advantage. Every step, except the catalytic reduction of 21 to the tetrahydrothiouphene 22 proceeded in high yield. The crowning achievement was reduction of the thiophene acid 23 with Pd/C (HOAc, 124 bars, 50°C, 10 hrs.) in greater than 95% yield to give d,l-biotin. It is interesting to note that Cheney rejected palladium catalysis for debenzylation of his thiophene as a possibility out of hand, assuming that sulfur would poison the catalyst totally. Confalone's overall yield was 37%. The only criticism that can be made of this synthesis is that resolution is not possible until the last step.
Scheme 4

\[
\text{CH}_3\text{OOCC}_{\text{NOH}} \xrightarrow{\text{HCl}} \text{CH}_3\text{OOCC}_{\text{NH}_2} \xrightarrow{\text{H}_3\text{O}^+, \Delta} \text{CH}_3\text{OOCC}_{\text{CH}_2\text{CH}_2\text{COOCH}_3} \equiv 20 \quad 97\%
\]

\[
\text{COOH} \xrightarrow{\text{1) } \text{N}_2\text{H}_2} \xrightarrow{\text{2) } \text{HONO}} \xrightarrow{\text{3) } \text{CH}_3\text{OH}} \text{(Curtius rearr.)} \equiv 21 \quad 87\%
\]

\[
2 \quad \text{CICOOC}_2\text{H}_5 \downarrow \equiv 22
\]

\[
\text{OC}_2\text{H}_5 \xrightarrow{\text{1) } \text{N}_3^-} \xrightarrow{\text{2) } \text{CH}_3\text{OH}, \Delta} \xrightarrow{\text{3) } \text{base}} \equiv 23 \quad 80\%
\]

\[
\text{COOC}_2\text{H}_5 \xrightarrow{\text{1)} \text{Pd/C, HOAc}} \xrightarrow{\text{2) } \text{Ba(OH)}_2} \equiv 24
\]

\[
\text{HN} \quad \text{HN} \quad \text{HN} \quad \text{COOH}
\]

\[
\text{HN} \quad \text{HN} \quad \text{HN} \quad \text{COOH}
\]
One last stereospecific synthesis which appeared last year deserves mention. Bory et al. utilized the asymmetry of sulfoxides to introduce the side chain of biotin stereospecifically. A summary is presented in Scheme 5.

**Scheme 5**

\[
\xrightarrow{\text{NaIO}_4} \quad \phi\text{CH}_2\text{N} \quad \text{NCH}_2\phi \quad \phi\text{CH}_2\text{N} \quad \text{NCH}_2\phi \\
\xrightarrow{\text{BuLi/THF}} \quad \phi\text{CH}_2\text{N} \quad \text{NCH}_2\phi \quad \phi\text{CH}_2\text{N} \quad \text{NCH}_2\phi \\
\xrightarrow{\text{t-BuO}_2\text{C(CH}_2)_4\text{I}} \quad 85 : 15 \\
\xrightarrow{\phi_3\text{P/CCl}_4} \quad \phi\text{CH}_2\text{N} \quad \text{NCH}_2\phi \quad \phi\text{CH}_2\text{N} \quad \text{NCH}_2\phi \\
\xrightarrow{(\text{CH}_2)_4\text{COO}^+\text{Bu}^-} \quad \text{d,l-biotin}
\]
Conversion of 24 to 25 had been described by Goldberg and Sternbach. The two sulfoxides (26a and 26b) were produced in nearly quantitative yield from 24 and could be separated by chromatography on silica gel. They were identified by $^1$H nmr coupling constants. Bory and Marquet had shown earlier that alkylation α to a cyclic sulfoxide always occurs trans to the sulfoxide bond. Conversion of 26a to 28 proceeded as predicted, but the yield was only 30%. Reduction of 28 to 29 was quantitative, and 29 was transformed into d,l-biotin by ester hydrolysis followed by debenzylolation. Again, it is unfortunate in this synthesis that resolution cannot be executed until after the low yield step (26a→28). It would seem that the economic utility of this synthesis depends greatly on the availability of the alkylation agent 27.

Most of the existing syntheses of biotin contain flaws which seriously decrease their economic value. Some are not stereospecific. Of those that are, all but one produce d,l-biotin which is resolved late in the synthetic scheme (excepting the von Gerecke modification of the Goldberg-Sternbach method). Most are lengthy (12-14 steps), contain a particularly low yield step, or employ an expensive starting material.

Our goal, then, was to develop a new synthesis of biotin that was high yield, short, and stereospecific. The starting materials and reagents would have to be cheap and readily available, and the resolution step, if necessary, should occur near the beginning of the synthesis. We believe we have found such a synthesis.

The 1,3 dipolar cycloaddition of nitrones to olefins is known to proceed stereoselectively to give syn-addition. A high degree of regioselectivity is also known to occur. By taking advantage of
this selectivity, we felt we could generate all three asymmetric centers with the correct configuration in one dramatic step. Compound 30 should undergo the thermal concerted intramolecular reaction to give racemic tricyclic isoxazolidine 31 possessing the same relative stereochemistry as biotin. (See Figure 1.) The shortness of the side chain precludes attack by the nitrone on the face of the cycloheptene opposite sulfur. Therefore, all three methine protons must be cis-, and cis- to the substituent X.

It is also possible to envision reaction in which the oxygen adds to the olefinic carbon closest to the sulfur atom. (See Figure 2.)
The mode of cycloaddition (regioselectivity) is governed by the kinetics of the reaction. It is sensitive to both steric and electronic effects, but in general steric control predominates, especially in intramolecular reactions, the oxygen atom adding to the more highly substituted carbon atom.

Both electron withdrawing and donating groups activate the double bond. When steric factors are absent, the oxygen adds to the carbon atom bearing a donating group. If a strongly electron withdrawing group is present, the oxygen adds to the opposite carbon atom. Occasionally, but rarely, mixtures are formed.

It can be seen then that the identity of X might be crucial to the direction our reaction takes. If X is a donating group, both steric and electronic factors would favor formation of $\text{3}$. If X is a withdrawing group, these factors would act against each other, but $\text{3}$ would be expected to predominate because steric factors are usually more important. With X equal to H, no prediction could be made based on these criteria alone.

There is, however, an additional consideration - the principle of maximum orbital overlap. Working with molecular models, it can be shown that $\text{30A}$ possesses a high degree of the p-orbital overlap necessary for reaction, while $\text{30B}$ does not. $\text{A}$ is also a lower energy conformation (because sulfur is more nearly equatorial), and thus more likely to occur. In other words, kinetics favors formation of $\text{3}$.

Selective reduction of the nitrogen-oxygen bond would lead to the hydroxy-amino compound $\text{32}$ easily converted to the ketone $\text{33}$. A Beckmann rearrangement on the oxime $\text{34}$ followed by hydrolysis of the
lactam (35) would give 36. Treatment with phosgene and removal of the protective group R would complete the synthesis.

\[ \text{NH}_2\text{OH} \quad \xrightarrow{\text{H}^+} \quad \text{R} \text{NH} \text{NOH} \quad \xrightarrow{\text{H}_3\text{O}^+} \quad \text{R} \text{NH} \text{COOH} \]

It might be possible to resolve the isoxazolidine 31, a mildly basic compound, by salt formation with an optically active acid. If 31 were not basic enough, resolution of 33 should certainly succeed.

There are many ways to make nitrones, but only two of these seemed suitable for generation of the key compound 30:²¹

\[ R' \text{C} = \text{O} + \text{R}^2\text{NH} \text{OH} \xrightarrow{\text{R}^1} R' \text{C} = \text{N}^+ \text{O}^- \]
Both require a carbonyl compound. The second method sometimes leads to the formation of the O-substituted ether, and occasionally it is the only product. The best choice for $R^2$ was benzyl as that could be easily removed in the final step of the synthesis in a known reaction.\textsuperscript{8d}

The aldehyde $\text{37}$ could be made by means of the facile displacement of an allylic halide by sulfide or sulhydryl.\textsuperscript{22} The aldehyde group, however, would have to be protected, because sulfur will also attack carbonyls. Our choice was an acetal.

A literature search revealed that it was not practical to isolate mercaptoacetaldehyde diethyl acetal($\text{38}$), probably due to disulfide formation. But the reaction of acetylmercaptoacetaldehyde diethyl acetal ($\text{39}$) with propyl bromide in the presence of sodium ethoxide was reported to give propylmercaptoacetaldehyde diethyl acetal ($\text{40}$) in 75\% yield.\textsuperscript{23}
The choice of the acetal eliminated the possibility of using $X = \text{alkoxy or trialkylsilyl}$, because these would not be stable under the acid conditions needed for conversion of the acetal to the aldehyde. $X$ was chosen to be cyano.

1-Cyanocycloheptene (41) and 39 were both prepared by methods in the literature, 41 from cycloheptanone cyanohydrin via the bisulfite addition product of cycloheptanone, and 39 from potassium thioacetate and bromoacetaldehyde diethyl acetal.

\[ \begin{align*}
\text{CH}_3\text{CS}^-\text{K}^+ & \xrightarrow{\text{BrCH}_2\text{CH(OC}_2\text{H}_5)_2} \text{C}_2\text{H}_5\text{OH} \\
& \xrightarrow{\text{SOCl}_2/\text{py}} \text{CH}_3\text{CSCH}_2\text{CH(OC}_2\text{H}_5)_2
\end{align*} \]

The allylic bromination of 41 with N-bromosuccinimide completes our antithetic analysis of the problem.
RESULTS AND DISCUSSION

Examples of the allylic bromination of α,β-unsaturated nitriles are rare in the literature. Yields with N-bromosuccinimide (NBS) are generally lower than those using other substrates. By following the suggestions of Horner and Winkelman in their review of NBS reactions, it was possible to obtain the bromide \( \text{42} \) in consistent yields of 80%. It proved crucial to the success of the reaction that freshly prepared NBS (by the method of Ziegler) be used. Large amounts (5-10 mole %) of dibenzoyl peroxide were necessary to catalyze the bromination.

\[
\text{CN} \quad \text{Br} \quad \text{CN} \quad \text{Br} \quad \text{CN}
\]

The product obtained was purified by distillation at reduced pressure and it discolored rapidly on exposure to air and heat. The \( ^1\text{H} \) nmr spectroscopy indicated the crude product to be isomer \( \text{42} \), with none of the 7-bromo-compound \( \text{43} \) present. When old NBS was used, yields of \( \text{42} \) were lower (13-25%) and large amounts of a compound suspected to be \( \text{44} \) or a polymer thereof remained in the distillation flask. Impurities present in the NBS (H\(_2\)O\(_2\)) or the distillation apparatus no doubt catalyzed hydrogen bromide evolution.

When treated with two moles of sodium ethoxide, thioacetate reacted rapidly with \( \text{42} \) to give a mixture of the thioethers \( \text{45} \) and \( \text{46} \) in a 46% distilled yield [bp 86°-108°C (0.4 mm Hg)]. It is probable
that 46 is formed from 45 via the anion resulting from the presence of excess ethoxide in the reaction mixture. Curiously, a bright blue-green color was observed during reaction.

Decreasing the amount of base used in the initial reaction to one mole gave a crude yield of 91% that was now a 4:1 mixture of 45 to 46. The blue color did not appear. Pyrolysis occurred upon distillation yielding tar and the enol ether 47. \(^1^H\) nmr (CDCl\textsubscript{3}) \(\delta\)

6.61 (s, HOC\textsubscript{2}H\textsubscript{3}) 6.06 (d, 1, EtOCH=CH, \(J=11\text{H}_2\)), 6.0 (m, imp?), 5.71 (d, 0.5, CHCH=CCN, \(J=5\text{H}_2\)), 4.63 (d, 1, EtOCH=CH, \(J=11\text{H}_2\)), 4.24 (d, 0.5, SC=CHCHCN, \(J=5\text{H}_2\)), 3.32 (q, OCH\textsubscript{2}CH\textsubscript{3}, \(J=6\text{H}_2\)), 3.22 (q, HOCH\textsubscript{2}CH\textsubscript{3}, \(J=6\text{H}_2\)), 3.2 (m, SCH, CHCN), 2.20-1.64 (broad, -CH\textsubscript{2}-), 1.64-.95 (broad, -CH\textsubscript{2}-), 0.78 (t, CH\textsubscript{2}CH\textsubscript{3}, HOCH\textsubscript{2}CH\textsubscript{3}, \(J=6\text{H}_2\)).
When the reaction was repeated using an 8% excess of thioacetate and 6.5% excess of sodium ethoxide, a 97% crude yield of 45 was obtained. No 46 was present. Chromatography on alumina (activity III) with 50% benzene in ligroin gave an 84% yield of 45 as a colorless nonviscous oil. Chromatography on silica gel greatly decreased the yield of product, probably by acid catalyzed elimination of ethanol to give 47 with subsequent polymerization.

Attempts at transformation of acetal 45 into aldehyde 48 produced some strange results. Simple hydrolysis of the 1:1 mixture of 45 and 46 with 1N aqueous hydrochloric acid in p-dioxane at room temperature gave exclusively 1-cyanocycloheptene 41! This amazing product may be the result of a radical cleavage reaction initiated either by peroxides present in the dioxane or a disulfide impurity. However, addition of radical inhibitors did not change the course of reaction. No reaction occurred when 5% aqueous acetic acid was substituted for the hydrochloric acid.

Acetal exchange reactions with acetone and 2-butanone catalyzed by p-toluenesulfonic acid failed to go to completion. Better results were obtained with acetone and oxalic acid, but were not reproducible.

p-Toluene sulfonic acid did have the curious effect of transforming the 1:1 mixture of 45 and 46 into a 4:1 mixture. This result was confirmed by allowing the 1:1 mixture to stand in benzene alone with a catalytic amount of acid overnight. The same reequilibration was observed.

Complete conversion to aldehyde was finally achieved by heating the acetal in 97-100% formic acid on a steam bath for 10 minutes. Although the product was a brown oil, TLC (5% methanol/benzene, silica
gel) and ^1^H nmr spectroscopy showed it to be of high purity. The procedure was improved by the addition of benzene. Stirring the two-phase system overnight under nitrogen gave a 97% yield of pale orange oil, again of high purity by ^1^H nmr and TLC. Further purification was not possible by chromatography or distillation. Both led to complete decomposition. It is possible that the aldehyde could be obtained analytically pure via either its 1,1-dimethylhydrazone derivative or its bisulfite addition product.

A sample of aldehyde contaminated with acetal was treated with hydroxylamine hydrochloride. The mixture of syn- and anti-oximes (49) obtained was then reacted with sodium ethoxide followed by addition of benzyl chloride. Although none of the characteristic nitroene absorptions [1540-1620 (C=N), 1150-1270 cm\(^{-1}\) (N=O)]\(^{27}\) were visible in the ir, some reaction did occur. More than one type of phenyl group was visible by ^1^H nmr spectroscopy.

Refluxing aldehyde 48 with impure benzylhydroxylamine hydrochloride\(^ {27}\) in pyridine resulted in formation of a black oil. A small amount of material with m/e M\(^+\) 300 was isolated by preparative layer chromatography (PLC) (8:2 methylene chloride/ether, silica gel). This might have been the cycloadduct, isoxazolidine 50.

The reaction was repeated in tetrahydrofuran in the presence of anhydrous potassium carbonate. No reaction occurred until the
solution was heated, and then it discolored rapidly. No products were isolated.

The benzylhydroxylamine was released from its salt and recrystallized from cycloheptane. It decomposes rapidly and was kept in the freezer when not in use.

Reaction with the aldehyde was repeated with the free hydroxylamine in aqueous acetic acid. Solid sodium carbonate was added until the solution was faintly basic. No reaction was noted at room temperature and, again, heating caused rapid discoloration. Three major spots were visible by TLC. All three reacted with 2,4-dinitrophenylhydrazine (2,4-DNP) spray reagent, but were not characterized further. Reaction in hot ethanol again gave a dark mixture of products.

At this point the difficulties encountered in the purification of intermediates, namely, 45 and 48, and our failure to detect nitrone formation from the aldehyde led us to suspect the nitrile substituent as the source of our troubles. In an effort to simplify the reaction scheme, the previous series of reactions was repeated starting with cycloheptene.

Allylic bromination with NBS gave a 40-52% yield of 3-bromocycloheptene 51 which was even more sensitive to air and heat than the cyanobromide. Reaction with 39 gave a 98% crude yield of acetal 52 which could be distilled without incident (86%). Acetal hydrolysis in 1N aqueous hydrochloric acid and tetrahydrofuran at room temperature produced aldehyde 53 which distilled smoothly to give product in a yield of 57%.

While the last series of reactions were being run, model studies were undertaken to work out conditions for nitrone formation. Methyl-
mercaptoacetaldehyde diethyl acetal (54) was synthesized from methyl iodide and acetylmercaptoacetaldehyde diethyl acetal (39). The aldehyde could be obtained from the acetal by hydrolysis with 1N aqueous hydrochloric acid.

\[
\begin{align*}
\text{CH}_3\text{CSCH}_2\text{CHR}_2 & \xrightarrow{\text{NaR}} \text{CH}_3\text{SCH}_2\text{CHR}_2 \xrightarrow{2\text{CH}_3\text{I}} \text{CH}_3\text{SCH}_2\text{CHO} \\
R &= \left(\text{OC}_2\text{H}_5\right)
\end{align*}
\]

The literature indicated that reactions between aldehydes and primary alkylhydroxylamines were generally high yield and proceeded quickly under very mild conditions. It was possible that our conditions had been too harsh. We were curious, too, as to whether hydroxylamines could be made to react directly with acetals to give nitrone formation. For this reason acetal 54 was treated with benzylhydroxylamine in aqueous 1N hydrochloric acid. With shaking, the two-phase system quickly became homogeneous, and the pungent smell characteristic of aldehyde was readily noticeable. Within ten minutes a precipitate was present. After five hours the pearly white crystals were filtered off, m.p. 203-206°d. The aldehyde smell was then very faint.

The crystals were insoluble in all the common solvents, including dimethyl sulfoxide. (Nitrones can be recrystallized from ligroin.) An attempt was made to take an \(^1\)H nmr spectrum in trifluoroacetic acid,
but the product appeared to react. The product is probably a nitrone dimer. Dimer formation has been frequently observed.\textsuperscript{21}

The reaction with the acetal was repeated twice more, once in aqueous acetic acid and once in concentrated formic acid. Precipitates similar in appearance to that above were isolated in both cases, although reaction was slower and not as clean.

Treatment of aldehyde 55 with benzylhydroxylamine in absolute ethanol at room temperature caused the solution to become warm and cloudy almost immediately. The reaction was followed by TLC and appeared to stop after 48 hours. The pale yellow crystals were filtered and dried, m.p. 198°C d. Addition of water gave a second crop of white crystals. These crystals are also insoluble in most common solvents and may be the same compound as that isolated from the acetal above, i.e., the nitrone dimer. (m/e \textsuperscript{1}M\textsuperscript{+} 195 is correct for the nitrone, however.)

Reaction of aldehyde 53 with benzylhydroxylamine was first tried in absolute ethanol at room temperature. The solution grew warm immediately and after one hour some precipitate was present. TLC showed most of the aldehyde to be gone. After five hours the bright yellow solution was worked up by filtration of the crystals and evaporation of the filtrate. The residual oil crystallized on standing. The \textsuperscript{1}H nmr and ir spectra were inconclusive as to whether or not a nitrone were present. The vinyl protons, however, were still apparent. Therefore no cyclization had occurred.

In Tufariello's synthesis of pseudotropine,\textsuperscript{29} it was necessary to reflux the nitrone olefin 56 in toluene to effect cycloaddition to the adduct 57. For this reason we repeated the reaction with
aldehyde 53 in toluene at room temperature and then heated it to reflux. The toluene grew warm and cloudy upon mixing. After one hour at reflux, the solvent was removed and the crude red product chromatographed on activity III alumina with benzene. $^1$H nmr spectroscopy of the fraction assumed to be product 58 (28%) looked very promising. Vinyl protons were absent, and the shape of the $-\text{CH}_2-$ envelope had changed. What appeared to be two benzyl peaks at first glance was found to be two doublets (CDCl$_3$, 4.28 δ, dd, J$_{H,H'}$=13.5 Hz, J$_{H,H''}$=20.5 Hz) resulting from hindered rotation around the carbon-nitrogen bond.

In an attempt to decrease tar formation, we next treated 53 with benzylhydroxylamine in dry benzene at room temperature and followed the reaction closely by TLC (5% methanol in benzene, silica gel). Soon after reaction began, a large spot appeared near the origin ($R_f$ 0.2) and another smaller spot was visible at $R_f$ 0.8, the same $R_f$ as the products of the ethanol and toluene reactions.
As reaction proceeded, a precipitate was observed and the spot at 
$R_f$ 0.2 decreased in size as the spot at $R_f$ 0.8 grew. It would appear 
likely that the material of lower $R_f$ is the nitrone $\sim$.

By TLC on silica gel with methylene chloride, it was possible to 
show that the recrystallized product ($E$) from the ethanol reaction 
was different from the chromatographed product ($I$) from the toluene 
reaction, and that the benzene reaction produced some of both products. 
Product $E$ was also produced in the toluene reaction. Mass spectral 
analysis showed $E$ and $I$ to be isomeric, with $m/e \text{M}^+ 275$ the correct 
molecular weight for both the nitrone $\sim$ and cycloadduct $\sim$. On the 
basis of other spectral evidence, it is believed that $E$ is actually 
the nitrone dimer, even though no parent peak was found. The dimer 
could dissociate to the nitrone in the mass spectrometer.

In order to maximize the intramolecular reaction and minimize 
competing intermolecular reactions, a high dilution procedure$^{30}$ sug-
gested itself, and did indeed prove successful.

A dilute solution of aldehyde was added slowly to a refluxing 
solution of benzylhydroxylamine in a large volume of benzene. TLC 
showed only one major product. Rapid chromatography on alumina gave 
a 92% yield of an orange oil, which spectral analysis confirmed as 
the cycloaddition product. However, it was not possible to distinguish 
between the two possible structural isomers $\sim$ and $\sim$. 

\[
\begin{align*}
&\phi\text{CH}_2 \quad 58 \\
\end{align*}
\]

\[
\begin{align*}
&\phi\text{CH}_2 \\
\end{align*}
\]
Inquiries at Hoffmann-La Roche, Nutley, N.J., concerning an X-ray structure determination on a suitable derivative brought a surprising result. They were immediately able to confirm the identity of the cycloadduct as compound 58 because they had already done the X-ray study on their own compound, arrived at through a reaction sequence very similar to our own. In fact, they had already tried the Beckmann rearrangement under many different conditions on oxime 61. Ring cleavage with concomitant aziridine formation was the usual result (62), but a small amount of the desired amide 63 was formed when polyphosphoric acid was used as catalyst.\(^3\)

In spite of these negative results, we thought it possible that the Beckmann would succeed as planned on the secondary amineoxime 64. For cleavage to occur it is necessary for the bridgehead carbon to take on a partial positive charge. Initial protonation of the secondary amine, not possible in the carbamate 61, should electrostatically inhibit this cleavage process. (See Figure 3.)
Out of curiosity, the oxime of aldehyde was treated with sodium ethoxide and benzyl chloride. A large number of products were present by TLC, but no was observed.

The cyanoaldehyde was reacted with benzylhydroxylamine under the conditions of high dilution established above. An 85% yield of orange oil which crystallized on standing was recovered by chromatography on activity III alumina. $^1$H nmr and ir spectra of the product were similar to that of. m/e $M^+$ 300 confirmed the product as the isoxazolidine.
Both cycloadducts 50 and 58 resisted catalytic reduction at one atmosphere with an equal weight of palladium catalyst under both neutral and acidic conditions (5% Pd/C, 10% Pd/C, Pd/CaCO₃, PdO). It is possible reduction would succeed with increased pressure. Treatment of 58 with a variety of bases (sodium isopropoxide, sodium hydride, butyl lithium) also failed to effect ring opening. With this method Bapat et al. had succeeded in opening the isoxazolidine ring of the methiodide salt 66, and the free base 67 could be reduced with sodium isopropoxide. However, it should be mentioned that catalytic hydrogenolysis was also effective with these two compounds. Our compounds are subject to a great deal more steric hindrance than those of Bapat.
Since it was possible to form the hydrochloride salt of \(58\), it may also be possible to make the methiodide or preferably the benzyl-iodide salt. Bapat's method should be effective on the acidic substrate \(68\). However, if \(R=\text{methyl}\), it may be the benzyl proton, and hence the benzyl group that is lost in product formation. An attempt at reaction with methyl chloroformate failed.

Reports\(^{33}\) that titanium trichloride cleaved the nitrogen oxygen bond of oximes cleanly and selectively, even in the face of steric hindrance, led us to try this reagent on \(50\). No reaction occurred.

Reduction of \(58\) to the aminoalcohol \(69\) was finally accomplished in high yield (88%) by zinc and refluxing aqueous acetic acid. The alcohol \(69\) could be purified by means of its hydrochloride salt, but the crude yellow oil was pure enough for most purposes.

Reduction of \(50\) with zinc and aqueous acetic acid led to a mixture of products consisting primarily of \(70, 71, \) and \(72\). Products \(72\) and \(70\) were isolated by PLC (5% methanol/benzene, silica gel). Both gave a positive 2,4-DNP test, and carbonyl bands were present in the ir. There was not enough material for a clear \(^1\)H nmr spectrum of \(72\), but mass spectral analysis was possible, HRMS 170.0762 calculated for \(C_9H_{14}O_5\), 170.0765. Product \(70\) has an \(m/e M^+ 344\). \(^1\)H nmr spectroscopy
shows what appears to be a methyl ester at 4.0 δ (CDCl₃). The structure shown is consistent with the above data.

71 has been identified conclusively from spectra and high resolution mass spectroscopy. [IR (CCl₄) 3460 (w, OH), 3300 (broad m, OH), 3060 (w, C=CH), 3020 (w, C=CH), 2930 (s), 2865 (w), 1675 (broad s, NHCO), 1500 (m), 1435 (m), 1360 (m), 1260 (m), 1020 cm⁻¹ (m); ¹H nmr (dilute, CDCl₃) δ 7.39 (broad s, 5, arom.), 5.38 (s, 2, OCH₂), 3.2-2.6 (broad), 2.3-1.1 (broad); HRMS 303.1289, calc'd for C₁₇H₂₁NO₄S: 303.1293.] It precipitates from the reaction mixture in ~ 25% yield when concentrated ammonium hydroxide is added during workup, m.p. > 235°C d. This unusual product is probably the result of amine attack on the nitrile after the bond cleavage has taken place. Subsequent acid hydrolysis of the imine bond would give 71.
It can be seen by looking at models that when the nitrogen-oxygen bond is cleaved the cycloheptane ring changes conformation in such a way so as to swing the nitrile into almost direct contact with the amine. We hoped that running the zinc reduction in a basic solution would suppress this undesired product.

The reaction proceeded quickly in ethanolic sodium hydroxide to give two compounds, isolated by PLC. Both react with 2,4-DNP spray reagent. $^1$H nmr of one of the products indicates some epimerization may have occurred. A broad carbonyl region is visible in the ir. The reaction should be repeated in pyridine which would be less likely to cause epimerization.

Numerous attempts to oxidize the alcohol 69 to ketone 73 failed. Dimethylsulfoxide (DMSO) and acetic anhydride$^{34}$ appeared to react at sulfur. DMSO and trifluoroacetic anhydride$^{35}$ produced no recognizable products. Treatment with pyridine-sulfur trioxide complex in DMSO$^{36}$ or manganese dioxide in carbon tetrachloride (r.t., 10 d.) led to recovery of the alcohol. The problem with the latter was no doubt steric in nature.

Chromium trioxide-pyridine complex$^{37}$ and pyridinium chlorochromate$^{38}$ both gave a solid compound isomeric with the desired ketone.
(m/e M^+ 275). Vinyl proton resonance visible by ^1H nmr spectroscopy and a red 2,4-DNP derivative indicate a conjugated carbonyl group. 74 is a proposed structure. [ir (film) 3180 (broad w), 3100, 3085, 3040 (w, vinyl or aromatic C-H), 2935 (s), 2860 (m), 1689 (sharp s), 1520 (sharp s), 1450 (s), 1290 (m), 1267 (m), 1205 (m), 1160 (m), 695 cm^{-1} (m); ^1H nmr (CDCl_3) δ 7.32 (d, aromatic H, J=1.5 Hz), 6.84 (m, vinyl H?, J=1 Hz), 4.42 (broad m, NH?), 4.20-3.50 (m), 3.80 (s?), 3.15-2.65 (sharp m), 2.70-1.35 (broad m), 1.30 (s, phthalate imp.)] Oppenhauer oxida-

tion^{39} with aluminum tert-tributoxide also failed--no reaction. Treatment of the aminoalcohol 69 with triisobutylaluminum gave an adduct which, when refluxed with acetone followed by aqueous methanol, decomposed to starting material. We had hoped that reaction would proceed as in Scheme 6, but it is possible that the reaction time was too short (two hours). This reaction should be repeated.

It should be possible to arrive at oxime 64 by a circuitous route. Hoffmann-La Roche tells us that it is possible to oxidize the alcohol 75 to the ketone 76 with DMSO/acetic anhydride.^{31} The protecting group could then be removed from the oxime 61 with barium hydroxide hydrolysis.^{13}
Attempts to synthesize 75 from 69 with methyl chloroformate in pyridine gave inconsistent results. The desired product 75 was observed once, but not isolated.

In refluxing benzene with pyridine added, the disubstituted product 77 was formed. Stirring for a few hours or overnight at room temperature in pyridine resulted in precipitation of crystalline 78 in substantial quantities. The structure of 78 has been confirmed by spectral data and mass spectral analysis. The lactone amide ring of 78 could not be opened by treatment with methoxide at room temperature, but ring-opening to give 75 should be possible at higher temperatures.

Which product is formed probably depends on the temperature of reaction. It was noticed that those reactions which gave 78 in greatest yield were most exothermic upon addition of the methyl chloroformate to
the reaction mixture. Dry, distilled reagents were used in all reactions, but the presence of water may also have some effect on product determination, as may the ratio of chloroformate to alcoholamine. Perseverance cannot fail to give the carbamate alcohol.

It was necessary at this point to discontinue the present research, but the project should by no means be abandoned completely. It should still be possible to find conditions that will reduce the isoxazolidine ring in cleanly to the cyanohydrin, or directly to the ketone. Treatment of 70 with barium hydroxide might produce the ketone without epimerization, or an ester exchange might be possible, to give the cyanohydrin. Compound 71 is not necessarily a dead end either.

The synthetic scheme up to the alcohol 69 and the cyanoisoxazolidine 50 was highly successful. In the nitrile series, the low yield
steps are formation of the starting material 1-cyanocycloheptene (66%), and its bromination (80%), but in both reactions, starting material, i.e., cycloheptanone and 1-cyanocycloheptene, are easily recoverable through distillation. Yields based on percent conversion are actually much higher. Successive steps in the synthesis suffer slightly from purification problems, but we are certain that the crude yields are near quantitative.

The same is true of the des-nitrile series. Cycloheptene can be recovered from the bromination reaction. Production of acetal and aldehyde are assuredly quantitative. It is distillation that lowers the yields.

Hydrochloride salt formation of both the cycloadduct 58 and the derived alcoholamine 69 indicate that resolution via salt formation is feasible at this stage. If the Beckmann rearrangement (or a Schmidt reaction on the ketone 73) can be made to work, we will have an elegant, stereospecific, potentially economically viable synthesis of d-biotin.
EXPERIMENTAL

General

Melting points and boiling points are uncorrected. $^1$H nmr spectra were recorded either as CDCl$_3$ (Merck, Sharpe & Dohme, 99.8 atom% D) or acetone-d$_6$ (Stohler, 99.5 atom% D) solutions using either a Varian A56/60 spectrometer or a Perkin-Elmer R12 instrument. Chemical shifts are reported in parts per million relative to TMS as an internal standard. The $^{13}$C nmr spectrum of 3-N-benzylamino-10-hydroxyoctahydrocyclohepta[b]thiophene (69) was recorded as a CDCl$_3$ solution on a Varian XL-100 spectrometer and is included in the spectral appendix.

Infrared spectra were recorded either as liquid films or KBr pellets on a Beckmann IR-8 instrument. A Finnigan 3300 GC-MS instrument equipped with a 6100 data system provided low resolution mass spectral data, while high resolution data was provided by a Consolidated Electrodyamics Corporation 21-1100 spectrometer.

N-Benzylhydroxylamine was prepared by a modification of Neubauer's$^{27}$ synthesis ("benzaldehyde" should read benzyl chloride) based on Exner's$^{40}$ procedure for N-benzhydrylhydroxylamine: 0.5 mol quantities of acetone oxime, benzyl chloride or benzyl bromide, acetic acid, and water were refluxed until a cooled sample remained homogeneous (ca. 1-1/2 to 2 hrs.). The solution was condensed in vacuo, and the residue was poured into 50 mls. ether and 50 mls. water. The phases were separated. The aqueous phase containing the benzylhydroxylamine salt was washed twice with a little ether and then condensed to half volume. (The salt can be obtained by
evaporating the solvent completely, but it is extremely impure.)
Addition of 10% aq. NaOH (ca. 10-15 mls.) caused the free amine to
to oil out. The oil was extracted immediately into hot cycloheptane.
Repeated extractions were necessary, but a continuous extraction
procedure led to rapid decomposition of the product. The cyclo-
heptane was evaporated off completely, and the residue was re-
crystallized from a minimum of hot cycloheptane. In this manner
pure benzylhydroxylamine could be obtained as feathery white crystals,
m.p. 57-59°C (6-15%). The product must be stored in the freezer.

The low yields are the result of a rapid dismutation reaction
favored by basic catalysis\(^*\) and increased temperature. Benzylamine
and benzaldehyde oxime are the products of dismutation.

Procedures reported to give benzylhydroxylamine in yields of
52% and 70% by reduction of benzaldehyde oxime with diborane\(^*\) and
sodium cyanoborohydride,\(^*\) respectively, could not be reproduced.

1-Cyano-3-bromo-1-cycloheptene (42)

7.01 g (50 mmol) 1-cyanocycloheptene (41) in 70 mls. spectrograde
CCl\(_4\) was refluxed on a steam bath in an apparatus equipped with a
CaCl\(_2\) drying tube with 8.85 g (50 mmol) dry, freshly prepared NBS\(^\text{26}\)
to which 0.6 g (2.5 mmol-5 mole %) dibenzoyl peroxide had been added.
Heating was continued until all the solid present floated (10-30 min.).
If reaction was not proceeding smoothly after 10 minutes, more
peroxide was added.

When reaction was complete, the pale orange solution was cooled
in ice and filtered. Most of the solvent was removed by aspirator,
and the residue was distilled at 0.4 mm Hg, b.p. 80-85°, yield
7.93 g, 80%. The product is a pale yellow liquid at room temperature,
but will crystallize when cooled. It discolors rapidly when exposed
to heat or air. The product is a lacrymator as well as a strong
skin irritant. EXTREME CAUTION SHOULD BE EXERCISED WHEN HANDLING.
WEAR GLOVES AT ALL TIMES. ir (film) 2940 (s), 2865 (m), 2220 (m, CN),
1725 (m, imp.), 1635 (w), 1450 (CH₂, CHBr), 1160 cm⁻¹ (w); ¹H nmr
(CDCl₃) δ 6.73 (d, 1, C=CH, J=6Hz), 5.15-4.56 (broad m, 1, CHBr),
2.94-1.22 (broad m, 8, CH₂).

3-Bromo-1-cycloheptene (51)

See procedure for 42. B.p. 64.5°-65°C (9.0 mm Hg), yield 40-52%.

1-Cyano-3-(mercaptoacetaldehyde diethyl acetal)-1-cycloheptene (45)

1.12 g (49 mmol) sodium was dissolved in 50 mls. anhydrous EtOH
under nitrogen in a flamed-out 200 ml three-neck flask with mechanical
stirrer, reflux condenser, and dropping funnel attached. While the
solution was still warm, 9.64 g (50 mmol) acetylmercaptoacetaldehyde
diethyl acetal (39)²³ was rapidly added to it with stirring. The
mixture was heated briefly on a steam bath, allowed to cool slightly,
and then transferred to the addition funnel.

9.25 g (46 mmol) 1-cyano-3-bromo-1-cycloheptene (42) in 30 mls.
anhydrous EtOH was introduced into the three-neck flask used earlier,
and to it was added quickly with stirring, the sulfide solution con-
tained in the addition funnel. A precipitate (KBr) formed almost
immediately. When addition was complete, the mixture was heated one
hour on a steam bath.
The cooled reaction mixture was poured into 400 ml s. water. After the aqueous solution was saturated with salt, the product was extracted with ether. The dried (MgSO₄) ether solution was evaporated to give 12.0 g (97%) of a pale yellow oil. Of 9.88 g chromatographed on ca. 250 g activity III alumina with 1:1 benzene-ligroin, 8.26 g (84%) were recovered in the first 250 ml s collected, as a colorless light oil. [Attempts at distillation, b.p. 125°-126°C (0.2 mm Hg) usually led to pyrolysis.] ir (film) 2980 (s), 2930 (s), 2880 (shoulder, m), 2220 (m, CN), 1630 (w), 1445 (m), 1370 (m), 1345 (m), 1120 (s), 1050 (s), 1000 cm⁻¹ (m); \(^1\)H nmr (CDCl₃) δ 6.60 (d, 1, C=CH, \(\delta = 6\)Hz), 4.55 (t, 1, CHO₂, \(\delta = 5\)Hz), 4.0-3.25 (m, 5, OCH₂, CHS), 2.67 (d, 2, CH₂S, \(\delta = 5\)Hz), 2.08-2.14 (m, 2, CH₂), 2.14-1.46 (m, 6, CH₂), 1.20 (t, 6, CH₃, \(\delta = 7\)Hz); HRMS 269.1452. Calc'd for C₁₄H₂₃NO₂S 269.1449.

3-(Mercaptoacetaldehyde diethyl acetal)-1-cycloheptene (52)

The procedure was the same as for 45, except that the allylic bromide 51 was added to the hot solution of the mercaptan salt, and stirring was continued at room temperature for two hours. After an identical work-up (crude yield 98%), the product was distilled, b.p. 96°-99°C (0.5 mm Hg) to give an 86% yield of a colorless oil. \(^1\)H nmr (CDCl₃) δ 5.93-5.69 (m, 2, CH=CH), 4.61 (t, 1, CHO₂, \(\delta = 6.0\)Hz), 3.94-3.25 (m, 5, CHS, OCH₂), 2.71 (d, 2, CH₂S, \(\delta = 6.0\)Hz), 2.40-1.46 (m, 8, CH₂), 1.21 (t, 6, CH₃, \(\delta = 7\)Hz).
1-Cyano-3-mercaptoacetaldehyde-1-cycloheptene (48)

1.04 g (3.87 mmol) 1-cyano-3(mercaptoacetaldehyde diethyl acetal)-1-cycloheptene (45) was dissolved in 23 mls. benzene and was treated with 2 mls. 97-100% formic acid. The solution was stirred rapidly overnight, under nitrogen. At first all the acid dissolved, but in the morning a small amount of red liquid had precipitated from the otherwise colorless (or pale yellow) solution. The benzene layer was removed by pipet, and washed twice with 5% aq. Na₂CO₃ (CAUTION!). After drying (MgSO₄), the benzene was removed at reduced pressure, yield 0.733 g (97%) pale orange oil, one spot by TLC (1% methanol/benzene, silica gel). ir (film) 3430 (w, imp.), 3050 (w, C=CH), 2940 (s), 2870 (m), 2840 (shoulder, w, CHO), 2735 (w, CHO), 2220 (m, CN), 1722 (s, CHO), 1635 (w), 1450 (m), 1385 (w), 1160 (w), 1030 cm⁻¹ (w); ¹H nmr (CDCl₃) δ 9.67 (t, 1, CHO, J=3Hz), 6.42 (d, 1, C=CH, J=6Hz), 3.78-3.28 (broad m, 1, CHS), 3.15 (d, 2, CH₂S, J=3Hz), 2.63-2.10 (broad m, 2, CH₂), 2.10-1.46 (broad m, 6, CH₂); HRMS 195.0713. Calc'd for C₁₀H₁₃NOS: 195.0718.

Neutralization of the "red liquid" described above, followed by extraction with ether and TLC of the ether extract showed the red phase to contain some aldehyde 48, and a considerable amount of material with low Rf, that was not identified further.

The oxime 49 was prepared by dissolving the aldehyde 48 in a small amount of ethanol to which a concentrated aqueous solution of NH₂OH·HCl and Na₂CO₃ was added. After standing overnight, the product was extracted with ether. A mixture of syn- and anti-oximes was obtained. ir (film) 3380 (broad s, OH), 3100 (w), 2920 (s), 2880 (m), 2220 (m, CN), 1630 (broad m, C=N), 1445 (s), 1410 (shoulder, m),
950 cm\(^{-1}\) (m); \(^1\)H nmr (acetone-d\(^6\)) \(\delta\) 7.46 (broad t, ~ 0.5, \text{syn CH=N, J=11Hz}), 6.87 (broad t, ~ 1.5, \text{anti CH=N + vinyl H, J=8Hz}), 3.39-3.07 (m), 2.72-2.27 (broad m), 2.27-1.47 (broad m). OH is probably hidden by acetone at 2.07 \(\delta\).

The nitrone "dimer" was prepared by stirring 0.399 g (2.35 mmol) aldehyde 48 and 0.288 g (2.34 mmol) benzylhydroxylamine in 5 mls. anhydrous EtOH under nitrogen for five hours. A precipitate was removed by filtration. The residue after solvent removal formed white needles on standing in the freezer. ir (KBr) 3200 (broad s, OH?), 3060, 3020 (w, =CH), 2910 (s), 2860 (m), 1440 (broad s), 1492, 1364, 1251, 1059 (m), 1022 (s), 718 (m), 685 cm\(^{-1}\) (broad s); \(^1\)H nmr (CDCl\(_3\)) \(\delta\) 7.5-7.17 (m, 5, aromatic + vinyl H?), 5.91-5.42 (m, 2), 5.0-3.41 (m, 4), 3.0-2.8 (m, 1), 2.41-1.36 (m, 8); \(m/e\) M\(^+\) 275.

3-Mercaptoacetaldehyde-1-cycloheptene (53)

3.84 g (15.7 mmol) 3-(mercaptoacetaldehyde diethyl acetal)-1-cycloheptene (52) was dissolved in 40 mls. THF and 5 mls. 1N HCl. The pale yellow solution was allowed to stand 3 d. at room temperature, after which time the yellow color was gone. The reaction can be followed by TLC as above for 48.

The solution was next poured into 25 mls. ether. 6 mls. saturated aq. NaHCO\(_3\) were added, and the ether phase was separated and dried three hours (MgSO\(_4\)). After solvent removal, the residue (2.7 g, 100%) was distilled at 8 mm Hg, but bumped at 94\(^\circ\)C. The distillation was continued at 0.2 mm Hg. 1.53 g (57%) aldehyde 53 were collected (b.p. 58.5\(^\circ\)-62\(^\circ\)C). Much black tar remained in the distillation flask. ir (film) 3420 (w, H\(_2\)O?), 3020 (m, C=CH), 2930
(s), 2860 (m), 2820 (shoulder, CHO), 2730 (w, sharp, CHO), 1720
(s, CHO), 1445 (m), 1390 (m), 1160 (m), 1025 cm⁻¹ (m); ¹H nmr
(CDCl₃) δ 9.53 (t, 1, CHO, J=3Hz), 6.00-5.70 (m, 2, CH=CH), 3.90-3.35
(broad m, 2, CH₅,?), 3.29 (d, 2, CH₂S, J=3Hz), 2.49-1.13 (m, CH₂).

The oxime 65 was prepared from the aldehyde 53 by the same
procedure used for preparation of oxime 49. The solid that pre-
cipitated out was dissolved in ether and washed with 1N HCl. The
product was a mixture of syn- and anti-oximes. ir (film) 3300
(broad s), 3020 (w, =CH), 2920 (s), 2850 (m), 1700 (w, CHO imp.),
1640 (w), 1440 (m), 1405 (m), 1290 (w), 1215 (w), 1040 cm⁻¹ (m);
¹H nmr (CDCl₃) δ 7.42 (t, 0.625, syn-OH=N, J=6Hz), 6.82 (t, 0.375,
anti-OH=N, J=5.5Hz), 6.03-5.66 (m, 2, CH=CH), 4.00-3.10 (m, 2, CH₅,
and EtOH imp.), 3.25 (d, 2, CH₂S, J=6Hz), 2.15 (s, OH), 2.50-1.46
(m, CH₂), 1.22 (t, EtOH imp.).

Methylmercaptoacetaldehyde diethyl acetal (54)

To 2.35 g (10.2 mmol) sodium dissolved in 200 mls. absolute
EtOH in a three-neck flask equipped with nitrogen inlet, dropping
funnel, and reflux condenser was added 20 g (95 mmol) acetylmethylmercap-
toacetaldehyde diethyl acetal. After stirring for 10 minutes 13.48 g
(5.91 mls, 94.9 mmol)methyl iodide was added dropwise. After stir-
ing an additional two hours, the solution was condensed in vacuo
until a sodium iodide precipitate formed. 100 mls.water was added
and the product was extracted with two 100 ml. portions of ether.
The residue after ether removal was distilled at 10 mm Hg, b.p. 70-72°C,
yield 12.85 g (82%), colorless oil. ir (film) 2995 (s), 2940 (s),
2900 (shoulder), 1490 (w), 1450 (m), 1380 (m), 1350 (m), 1215 (m),
1130 (s), 1060 cm\(^{-1}\) (s); \(^1\)H nmr (CDCl\(_3\)) \(\delta 4.52\) (t, 1, CH\(_2\)O, J=5.5 Hz), 3.90-3.22 (m, 4, CH\(_2\)O), 2.59 (d, 2, CH\(_2\)S, J=5.5 Hz), 2.11 (s, 3, CH\(_3\)S), 1.18 (t, 6, CH\(_2\)CH\(_3\), J=7.5 Hz). m/e M\(^+\) 164.

Methylmercaptoacetaldehyde (55)

1.0 g (6.9 mmol) methylmercaptoacetaldehyde diethyl acetal (54) was stirred rapidly in 10 mls. 1N HCl (aq.) for one hour. The aldehyde was extracted with ether. After drying (Na\(_2\)SO\(_4\)) the solvent was removed at reduced pressure to give 0.54 g (87%) of a colorless, strong smelling oil. \(^1\)H nmr (CDCl\(_3\)) \(\delta 9.45\) (t, 1, CHO, J=3 Hz), 3.17 (d, 2, CH\(_2\), J=3 Hz), 2.20 (s, 3, CH\(_3\)).

The nitrone "dimer" was prepared by stirring 0.29 g (3.2 mmol) of aldehyde 55 and 0.39 g (3.2 mmol) benzylhydroxylamine in 10 mls. absolute EtOH under nitrogen for 48 hours, until no further change could be noted by TLC. The precipitate which had formed in the first 10 min. was removed by filtration (29 mg, m.p. 198\(^\circ\)C d). The addition of water and prolonged cooling caused an additional precipitate. The yellow needles were insoluble in most common solvents.

ir (KBr) 3450 (m, OH?), 3070 (w), 3035 (w), 2950 (2), 2920 (w), 2820 (w), 1600 (w), 1500 (sharp m), 1455 (m), 1430 (m), 1357 (m), 1129 (m), 1025 (broad m), 955 (broad m), 822 (m), 720 (m), 690 cm\(^{-1}\) (m); \(^1\)H nmr (CDCl\(_3\)) \(\delta 7.38\) (s, \(\emptyset\)), 7.18-7.5 (m, \(\emptyset\)), 6.75 (t, vinyl H, J=6 Hz), 4.82 (s, \(\emptyset\)CH\(_2\)2-), 4.22-3.32 (m), 2.20 (s), 2.08 (s, CH\(_3\)S) 1.60 (broad s, CH\(_2\)); m/e M\(^+\) 195.
Two liters benzene were placed in a 3 liter, three-neck flask equipped with a mechanical stirrer and a one piece distillation head to which a CaSO₄ drying tube was attached. The flask, insulated with glass wool, was heated slowly in a heating mantle. The first 100 mls. of benzene that distilled over were discarded. 600 mls. "dry" benzene were then collected.

3.1 g (25 mmol) benzylhydroxylamine in 100 mls. of the distilled benzene was added to the benzene remaining in the flask. 4.8 g (25 mmol) l-cyano-3-mercaptoacetaldehyde-l-cycloheptene was dissolved in the remaining dry benzene and one half of this solution was transferred to a 250 ml. Hershberg constant addition funnel. The rest was stored in the refrigerator until needed.

The distillation head was now replaced by a Claisen head which held the constant addition funnel containing the aldehyde solution and a condenser and nitrogen inlet. (See Figure 4.) The bend in the Claisen head serves as a mixing chamber for additional dilution of the aldehyde solution.

The solution in the flask was heated to reflux and the drop rate of the funnel was adjusted to ca. one drop every 9 seconds. Care must be taken not to let solvent vapor reach the funnel inlet, as this will disrupt the addition of aldehyde. When this occurred we found it necessary to remove the insulation from the flask and raise
the funnel by means of a crude extender fashioned from a vacuum adaptor. The addition of aldehyde took four days (lengthened in part by difficulties with the funnel). The solution stored in the refrigerator was added to the funnel when the first portion was exhausted. 

![Diagram of equipment](image)

When addition was complete, the benzene was removed in vacuo. The black-red, semisolid residue (9.5 g, 130%) was chromatographed rapidly on alumina (benzene). 5.5 g (74%) of a dark orange oil was recovered. TLC indicates the product to be a mixture of two compounds. Reaction on a smaller scale (3.76 mmol) proceeded faster and gave a much better product. The crude material showed only one major spot by TLC and was recovered in an 85% yield as a pale orange oil after chromatography on alumina. Both products, however, crystallized on standing in the refrigerator. Two recrystallizations from methanol
gave pale yellow crystals, m.p. 77.5°-79.5°C. ir (film) 3020 (w, C=CH), 2920 (s), 2850 (m), 1500 (w), 1445 (m), 1020 (w), 730 (w), 690 cm⁻¹ (m); ¹H nmr (CDCl₃) δ 7.54 (s, 5, 0), 4.43 (d, 1, OCH₂, J=14H₂), 4.09 (d, 1, OCH₂, J=14H₂), 4.43-3.77 (m, 3, CH), 3.02 (d, 2, CH₂S, J=6.5), 3.1-2.5 (m, 1), 2.50-1.40 (broad d, 7, CH₂, J=12H₂); HRMS 300.1288. Calc'd for C₁₇H₂₀N₂O₅S: 300.1296.

The hydrochloride salt of compound 50 can be formed by bubbling HCl gas through a solution of the cycloadduct 50 in anhydrous ether or chloroform. The salt can be isolated by centrifugation. The solvent is decanted and the solid is dried in a stream of dry nitrogen. Contact with air causes the solid to darken and become gummy. The salt is only sparingly soluble in water, but it is extremely hydroscopic.

58 was prepared by the same procedure as the cyanocycloadduct 50. On a 22 mmol scale, 5.5 g (92%) 58 were obtained as an orange oil after chromatography on alumina (benzene). A few minor impurities were visible by TLC. 58 was never obtained crystalline. ir (film) 3075 (w, C=CH), 3020 (m), 2930 (s), 2860 (s), 1610 (w), 1500 (sharp m), 1455 (s), 1350 (w), 1320 (w), 1230 (w), 1210 (w), 1030 (w), 730 (m), 695 cm⁻¹ (m); ¹H nmr (CDCl₃) δ 7.32 (s, 0), 4.54-3.10 (m, CH), 4.09 (d, 1, OCH₂, J=13H₂), 3.79 (d, 1, OCH₂, J=13H₂), 2.88 (d, 2, CH₂S, J=6H₂), 2.53-1.07 (m, CH₂), HRMS 275.1339. Calc'd for C₁₆H₂₁NOS: 275.1344.
Reduction of cycloadduct 58 (69)

1.7 g (61 mmol) twice chromatographed cycloadduct 58 was reduced in 100 mls. 20% aqueous acetic acid with 0.8 g zinc dust. The suspension was refluxed for 10.5 hours, until TLC of an aliquot basified with conc. \( \text{NH}_4\text{OH} \) and extracted with ether showed reduction to be complete. More zinc was added after 7.5 hrs.

The aqueous solution was saturated with NaCl, cooled in ice and basified (pH 9) with conc. \( \text{NH}_4\text{OH} \). The product was extracted with ether (3x100 mls.). After drying (MgSO\(_4\)) the solvent was removed in vacuo. 1.52 g (89.4%) 69 was obtained as a pale yellow oil. ir (film) 3290 (shoulder), 3150 (broad m), 3070 (w), 3040 (w), 2925 (s), 2860 (s), 2700 (shoulder), 1610 (w), 1500 (m), 1450 (broad m), 1370 (m), 1190 (m), 1050 (m), 728 (m), 695 (m); \( ^1\text{H nmr (CDCl}_3\) \( \delta 7.31 \text{ (s, } \delta) \), 4.50 (dd, 1, \( \text{CHOH}, J_{\text{H},\text{H'}}=4\text{Hz}, J_{\text{H},\text{H''}}=7\text{Hz} \), 3.96 (d, 1, \( \text{ØCH}_2\), \( J=12.5\text{Hz} \), 3.69 (d, 1, \( \text{ØCH}_2\), \( J=12.5\text{Hz} \), 3.71-3.28 (m, CHS), 2.90 (s, 1, OH), 2.89 (d, CH\(_2\)S), 2.48-0.98 (m, CH\(_2\)) \}; HRMS 277.1498. Calc'd for \( \text{C}_{16}\text{H}_{23}\text{NOS} \): 277.1500.

The hydrochloride salt of the alcoholamine 69 can be formed by bubbling HCl through a solution of compound 69 in anhydrous ether, followed by filtration of the resulting needles. The salt can be recrystallized from methanol, but recovery is low and some decomposition appears to occur, m.p. 230-234°C d. Subliming begins ~ 190°C. A better method of purification is to stir the salt with a little chloroform, in which it is only sparingly soluble. The impurities
dissolve.

In this manner it was possible to obtain a 68% overall yield of the hydrochloride of 69 from pure aldehyde 53 with no additional purification of either the cycloadduct 58 or the alcoholamine 69. Trituration with chloroform gave a 79% recovery of white needles, pure by TLC (5% methanol/benzene, silica gel).

The salt is only sparingly soluble in water, but the free alcoholamine can be liberated by rapidly stirring a suspension of the salt in ether with dilute NaOH. Evaporation of the separated (and dried) ether phase gives 69 in high yield.

\[
\phi\text{CH}_2\text{N} = O
\]

To 1.52 g (5.5 mmol) alcoholamine 69 in 10 mls. dry pyridine was added 0.42 mls. (0.52 g, 5.5 mmol) dry, distilled methyl chloroformate dropwise with stirring. A white precipitate formed, but quickly dissolved. The temperature rose to about 40°-50°C, and the color changed to bright yellow, then to orange. When addition was complete, the solution was cooled in ice for about one hour. Stirring was continued overnight.

The pyridine solution was diluted with an equal volume of water, then saturated with salt. The product was extracted with ether, and then the ether was washed three times with 1N HCl. With each successive wash, more precipitate formed on the walls of the separatory funnel. The ether phase with some of the yellow crystals was cooled,
then filtered. The crystals coating the funnel were recrystallized from hot ethyl acetate. 0.47 g (28%) total were collected.  M.p. 156°-156.5°C (methanol).  ir (KBr) 3450 (w, H₂O?), 2930 (m), 2850 (w), 1675 (broad s, NCO₂), 1500 (sharp w), 1445 (s), 1270 (m), 620 cm⁻¹ (m); ¹H nmr (CDCl₃) 7.40 (s, Ø), 5.04 (d, 1, ØCH₂, J=15Hz), 4.52 (dd, 1, CHO, J_H,H'=3.5Hz, J_H,H₉=8Hz), 3.62-3.21 (m, CH₅), 2.83 (d, 2, CH₂S, J=3.5Hz), 2.79-2.15 (m), 2.15-1.29 (m, CH₂); HRMS 303.1292. Calc'd for C₁₇H₂₁NO₂S: 303.1293.
REFERENCES - Biotin


16. D. St. C. Black, R. F. Crozier, and V. C. Davis, *Synthesis*, 1975, 205. This is an excellent review of the 1,3 dipolar cycloaddition reactions of nitrones.


APPENDIX
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