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Prepared by

Robert E. Lyle

Assisted by, Dr. P. S. Halwe, Walter Gay, and John Thomas

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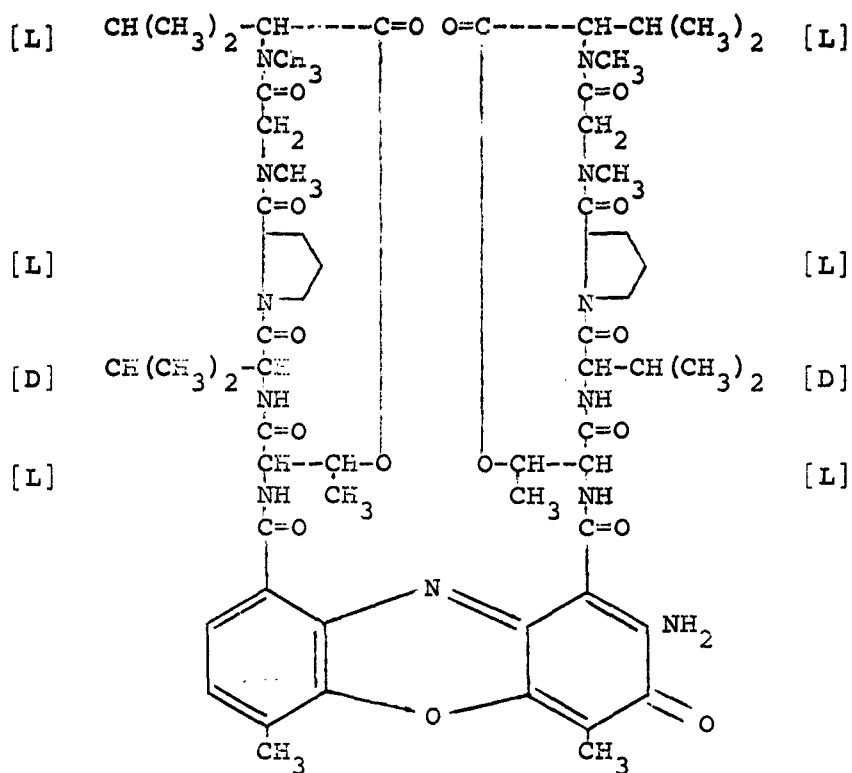
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INTRODUCTION

The report in 1940 by Waksman of the isolation of an antibiotic from the culture broth of Actinomyces (now Streptomyces) antibioticus gave the pharmacologist a very interesting compound. Actinomycin, as this antibiotic was called, was extremely toxic; however, it showed very selective effects on certain organs of experimental animals. Thus, although the high toxicity prevented the use of this antibiotic for antibacterial purposes, the selective nature of its action suggested potential anticancer therapy. During the twenty years since its discovery actinomycin has been investigated extensively as a potential chemotherapeutic agent in cancer research.¹

From an organic chemical point of view, the actinomycin molecule proved to be a very interesting one in terms of structure. It combines the two most common organic systems found in antibiotics, that is, a heterocyclic nucleus and a polypeptide system. This very interesting structure was deciphered by the excellent work of Brockmann who recently has been able to report a total synthesis of the compound.²



ACTINOMYCIN IV

The chemical and pharmacological interest in actinomycin suggested the importance of a project of synthesis of compounds containing these two systems analogous to those found in actinomycin. It would be the obvious desire that in certain of these analogous structures the antibiotic and toxic natures of the natural material could be separated. The importance of this approach has been evidenced by recent publications of the groups of Baker³, Takahayashi⁴, Predvoditeleva⁵ and Okumura⁶.

The approach of these authors has largely been concerned with the preparation of polypeptides having a terminal unit containing hydroxyanthranilic acids. It is evident that these compounds could on mild oxidation be converted to the phenoxazine system found in natural actinomycin. The approach

to this problem in this laboratory has been more general in nature being concerned with the preparation of polypeptide derivatives of a variety of heterocyclic acids. This approach has been based on the reports of a variety of pharmacodynamic effects of derivatives of a variety of heterocyclic nuclei. Thus, phenoxathiin derivatives have been shown to be bacteriostatic toward streptococci and also have insecticidal nature⁷. Derivatives of phenothiazine have shown dramatic effects on the central nervous system.³ Phenazine derivatives have been isolated from bacterial cultures, and these derivatives have been shown to be powerful bacteriostats attacking tuberculosis and leprosy among others, but again the materials are quite toxic.⁹ Because of the extremely important role that pyridine derivatives play in vitamin chemistry and as other medicinal agents, it seemed of importance to include this heterocyclic nucleus for investigation. As a result of the high incidence of activity in these heterocyclic derivatives, it was deemed important to prepare the polypeptide derivatives of a number of heterocyclic carboxylic acids.

DISCUSSION

The nature of the products of this research required a division of the effort into two sections. One is concerned with the synthesis of heterocyclic carboxylic acids and the second the preparation of polypeptide units. With the exception of the pyridine carboxylic acids the acid derivatives of the other heterocycles were not commercially available. The synthesis of these acids then required either substitution of the carboxyl group on the preformed heterocyclic nucleus or cyclization of a fragment containing the carboxyl group in the starting material. Because of the commercial availability of phenoxathiin and phenothiazine, the possibility of introducing the carboxylic group via the metalation reaction was attempted with these two heterocyclic nuclei. Since phenazine cannot be obtained commercially in the quantities desired, cyclization procedures may be used to produce the parent heterocycle or the desired carboxylic acids. Both synthetic procedures are under investigation. The facile cyclization of ortho-aminophenols to produce phenoxazines has led to the preparation of phenoxazine carboxylic acids via cyclization of hydroxyanthranilic acids. The major drawback to this approach of synthesis has been the unavailability of the hydroxyanthranilic acids or related starting materials for the preparation of these compounds. Thus one aspect of this project has been concerned with the preparation of 3-hydroxyanthranilic acids.

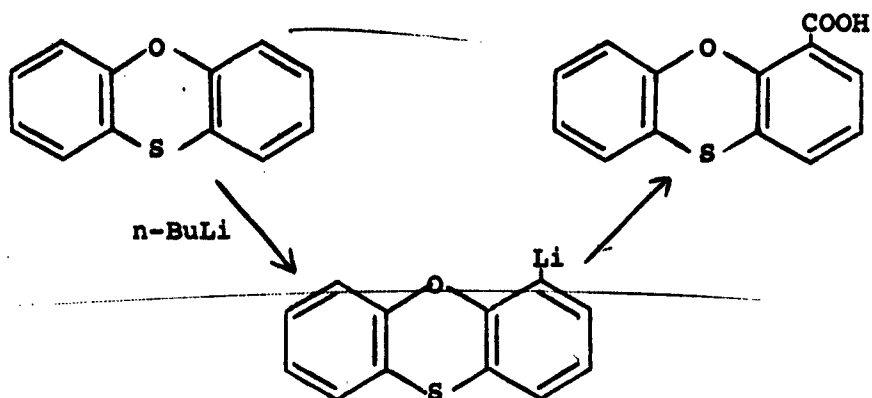
Metalation of Phenoxathiin

The metalation of phenoxathiin with n-butyllithium

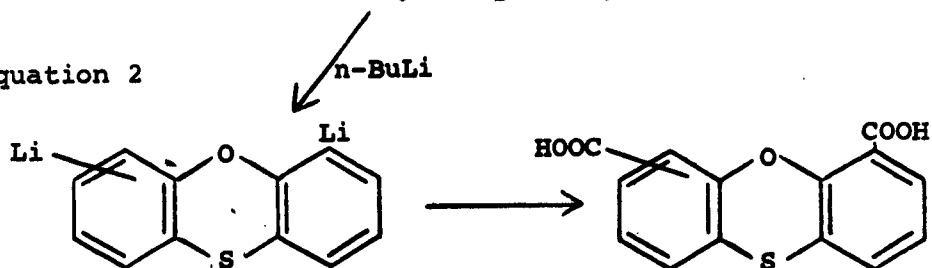
has been reported by Gilman¹⁰ to yield the 1-lithio derivative which on carbonation yields the 1-carboxylic acid. If an excess of n-butyllithium were used, polycarboxylic acids were isolated. Since we will be interested in obtaining polycarboxylic acids as well as the monocarboxylic acids, we have repeated the work of Gilman varying not only the relative molar ratios of reactants but also the temperature of the reaction. It has been found that the monocarboxylic acid can be obtained in purified yields of better than 50% by the reaction of one mole of butyllithium with one mole of phenoxathiin at room temperature or below in diethylether (Equation 1). At higher temperatures and higher molar quantities a mixture of polycarboxylic acids is obtained. An investigation of the structures of these polycarboxylic acid compounds has not yet been made due to the questionable reproducibility of their formation.

Since the dicarboxylic acids of phenoxathiin will be desired, a study of the second metalation step has been initiated (Equation 2). Since metalation reactions are electrophilic substitutions, it seemed reasonable to assume that the second metalation could best be accomplished if the carboxyl group were converted to an electron releasing substituent. The most obvious manner of accomplishing this is the reduction to the methylol derivative. Lithium aluminum hydride reduction of phenoxathiin carboxylic acid was difficult at best; however, the reduction of the corresponding ester proceeded without complications to produce 1-hydroxymethyl-phenoxathiin (Equation 3). An investigation of metalation of this compound is in progress.

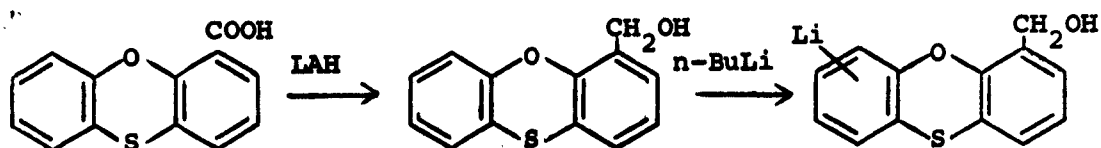
Equation 1



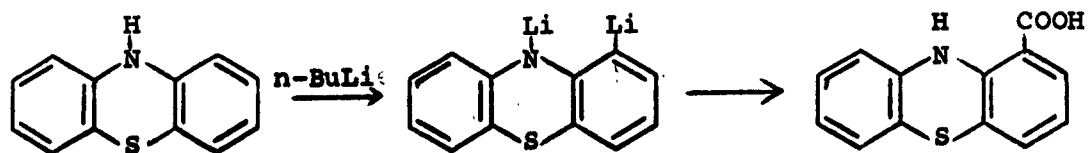
Equation 2



Equation 3



Equation 4

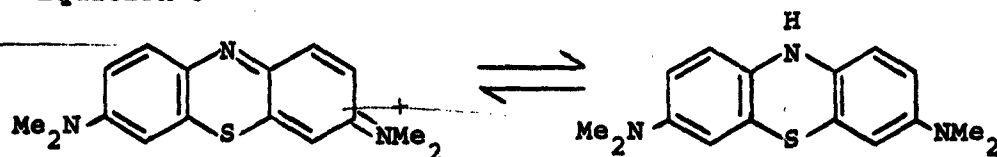


Metalation Investigation of Phenothiazine

The phenothiazine commercially available is extremely impure and initial metalation reactions of this material gave an acidic product with a wide melting range. Attempted purifications were unsuccessful. Thus purification of the phenothiazine was investigated. The most successful procedure was a distillation of the solid and recrystallization of the distillate from a 5:1 mixture of hexane and acetone. This process gave a purified product, m.p. 187.5-189°. The metalation of this material in ether using butyllithium gave a high yield of a carboxylic acid whose melting point corresponded to the previously reported phenothiazine-4-carboxylic acid¹¹. Attempts to reduce the phenothiazine and the corresponding ester with lithium aluminium hydride have not yet been made. (Equation 4)

The availability of methylene blue, a phenothiazine derivative, led to a consideration of this compound as a starting material in the preparation of a phenothiazine carboxylic acid. The extremely low electron density of the phenothiazonium salts would suggest that metalation would not occur easily. In an attempt to avoid this difficulty the methylene blue could be reduced to the phenothiazine derivative which on reaction with butyllithium would produce an anion highly susceptible to a metalation reaction. Thus an investigation of the reduction of methylene blue to its leuco form was attempted. The standard reductive procedures on a large scale were highly unsatisfactory. Other reductive procedures are being investigated. (Equation 5)

Equation 5



Investigation of Phenazine Derivatives

The commercial unavailability of phenazine required the investigation of cyclization procedures for the preparation of this kind of compound. The most obvious preparation seemed to be the oxidative coupling of catechol with ortho-phenylene diamine. Various experimental conditions have been employed in an attempt to cause this reaction; however, in no case was a phenazine isolated. The starting materials will be prepared by the condensation of cyclohexane-1,2-diones with ortho-phenylenediamine.

Preparation of Phenoxazine Derivatives

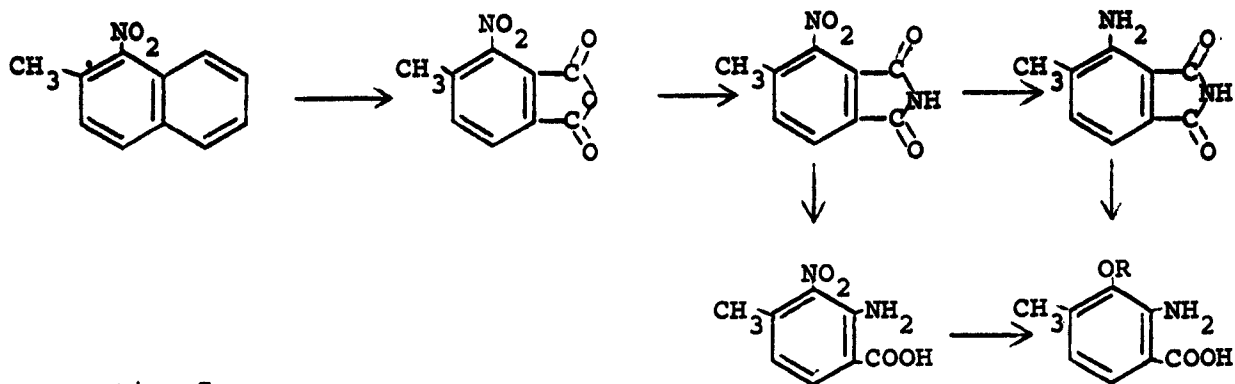
The unavailability of the parent phenoxazine and the facile cyclization of hydroxyanthranilic acids to these heterocycles has led to the investigation of the preparation of phenoxazine carboxylic acids by cyclization procedures. The starting material for the preparation of a phenoxazine identical with the heterocyclic moiety of natural actinomycin would require as starting material 4-methyl-3-hydroxyanthranilic acid. The preparations of this material recorded in the literature^{2,3} require 4-methyl-3-hydroxy (or 3-amino) benzoic acid as a starting material, and this compound is not available commercially. It seemed expedient, therefore, to investigate some novel methods for the preparation of these

anthranilic acids. Two such procedures have received some attention. The oxidation of 2-methyl-1-nitronaphthalene to produce the methylnitrophthalic anhydride which would be converted to an anthranilic acid derivative has been investigated. The conversion of an α -pyrone to 4-methyl-3-hydroxy-2-nitrobenzoic acid has been studied.

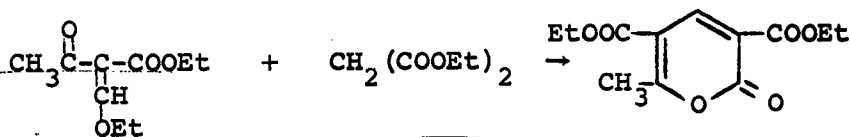
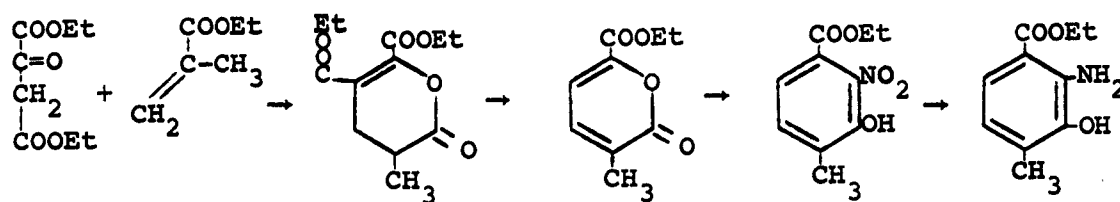
The nitration of 2-methylnaphthalene has been reported to yield 1-nitro-2-methylnaphthalene and this reaction has been duplicated. During our investigation, a source of this methylnaphthalene was found. The oxidation of the aromatic ring of this compound was attempted with sulfuric acid, nitric acid, various peroxides and related chemical oxidizing agents. None of these reactions was successful in causing the rupture of the unsubstituted aromatic ring without producing further reaction of the substituted ring. Thus this synthetic procedure has been temporarily abandoned. (Equation 6)

The reactions of various pyrylium salts with nitromethane to give nitrobenzenes have been reported.¹² Such a reaction with a pyrone to give a nitrophenol, however, has not been described. The α -pyrone desired for this investigation should result from the condensation of methyl α -methyl acrylate and ethyl oxalacetate. This condensation reaction was attempted under various conditions without success in preparing the desired α -pyrone. In order to test the feasibility of this approach the α -pyrone from ethoxymethylene acetoacetic ester and malonic ester was prepared according to the literature¹³ to yield diethyl 6-methyl- α -pyrone-3,5-dicarboxylate. The reaction of this compound with nitromethane under a variety of basic conditions did not yield the desired nitrophenol derivative. Attempts were made to introduce nitromethane in the original condensation reaction mixture; however, this procedure also failed to yield a nitrophenol. (Equation 7)

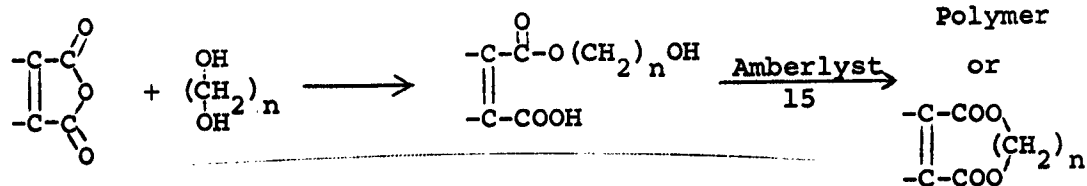
Equation 6



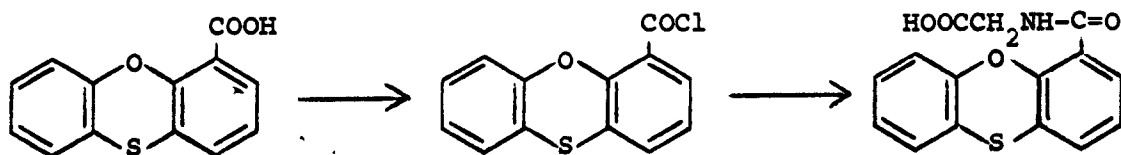
Equation 7



Equation 8



Equation 9



Studies in Polypeptide Synthesis

The synthesis of a nearly identical analog of actinomycin presents two problems: 1) the combination of a series of amino acids to provide correct polypeptide chain and 2) the cyclization of this chain into a lactone system by the condensation of a carboxyl and hydroxyl group. It would be of some interest to prepare analogs of actinomycin in which the polypeptide portion of the molecule was composed of repeating amino acid units. Thus a third phase of this investigation has been concerned with a polymerization of amino-acid derivatives in such a way that a hetero-aroyle function was attached at the terminal position of the polypeptide chain.

Studies in the Formation of Macrocyclolactones

In order that the actinomycin analogs produced by this investigation would be available for appropriate screening it would be necessary to have synthetic procedures which could be used to produce macro amounts. Thus, a cyclization of a macrolactone by high dilution techniques would be less appropriate than some other type of procedure. It seemed reasonable that a heterogeneous catalyst for the formation of the lactone function might permit preparation in macro scale. Such a heterogeneous catalyst as an ion exchange resin offered promising possibilities. For this reason the new Amberlyst-15 was investigated as a condensation catalyst for the reaction of maleic and phthalic acids with polymethylene glycols.¹⁴ Initial studies of this reaction seemed promising, for the products of the condensations were indeed esters and showed no hydroxylic absorption in the infrared spectrum nor

were they soluble in sodium bicarbonate. Molecular weight determinations on these compounds, however, showed them to be polymeric. (Equation 8, see page 10)

Attempts to Prepare Repeating Polypeptides with a Heterocyclic Terminal Function

The reported ease with which the anhydrides of N-carboxyamino acids undergo polymerization led to an attempt to produce polymers by catalytic ring opening of the anhydride with an heterocyclic acid. A successful reaction of this type would have led to a repeating polypeptide chain with a hetero-aroyl function at a terminal position. The Leuchs' anhydrides were prepared by the reaction of amino acids with phosgene or the reaction of amino acid esters with ethyl chlorocarbonate. The anhydrides of glycine and L-leucine were prepared. All attempts to cause the polymerization of these anhydrides in the presence of heterocyclic carboxylic acids, such as nicotinic or isonicotinic acids failed to produce polymers containing a heterocyclic residue. A consideration of the probable mechanism of the ring opening of the anhydrides suggested that this was an improbable reaction, and thus attempts to prepare the polymeric materials by this route have been abandoned. Other approaches to the synthesis of this kind of compound are currently under investigation.

The Formation of Amides from Amino Acids and Heterocyclic Carboxylic Acids

The combination of an aromatic acid halide with an amino acid should be a simple reaction between the two substrates in the presence of base. The reaction of benzoyl

chloride with certain amino acids, however, have failed to produce amides under Schotten-Baumann conditions. The notable lack of success with dicarboxylic amino acids and sterically hindered amino acids was noted. With simple amino acids these reaction conditions were found to be successful.

The reaction of (phenoxythiin)-1-carboxylic acid with thionyl chloride produced the corresponding acid chloride which without isolation was found to undergo reaction with glycine under Schotten-Baumann conditions. The corresponding amide gave the correct properties and elemental analyses for the anticipated structure. (Equation 9, see page 10)

The preparation of amino acid amides from nicotinic and isonicotinic acids produced more problems. It was desirable to determine the proper conditions for forming amides of these latter acids since they introduce the additional complexity, anticipated in future work, of having a basic nitrogen in the carboxylic acid residue. Thus, it was found that the isonicotinoyl chloride hydrochloride could be formed readily by the reaction of the amino acid with thionyl chloride; however, this acid chloride hydrochloride was hydrolyzed with base more rapidly than it underwent reaction with an amino acid under Schotten-Baumann conditions and the reactants were not soluble in non-polar solvents. Thus, it seemed necessary to prepare the acid chloride without the formation of the hydrochloride. Reaction conditions which appear promising for this preparation involve the combination of the potassium salt of isonicotinic acid with oxaloyl chloride. This process is presently under investigation.

Summary

This report describes the initial approaches directed at the synthesis of a number of Actinomycin analogs in which the heterocyclic ring system and amino acid units will be varied. Phenoxathiin-1-carboxylic acid and phenothiazine-4-carboxylic acid were prepared by the metalation of the parent heterocycle. The approach to the synthesis of the dicarboxylic acids is described. Two novel and thusfar unsuccessful approaches to the synthesis of 4-methyl-3-hydroxy-anthranilic acid, the intermediate use to form the phenoxazine nucleus of Actinomycin, are reported.

Initial investigations of possible methods of preparing macrocyclic lactones and polypeptides of a single amino acid with a terminal heterocyclic aryl group have proved unsuccessful. Methods for forming amino acid derivatives of phenoxathiin-1-carboxylic, nicotinic, and isonicotinic acids are discussed.

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