PART I

FORMATION OF β-LACTAMS AND

RELATED COMPOUNDS

PART II

ALKYL PHOSPHATES AND THIOPHOSPHATES

by

Roy William Roth
B.S., Massachusetts Institute of Technology (1950)
M.S., University of Michigan (1951)

Submitted in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

February, 1955

Signature of Author..... Signature redacted

Signature of Research Supervisor..... Signature redacted

Signature of Chairman of
Departmental Committee on
Graduate Students..... Signature redacted

# FORMATION OF β-LACTAMS AND RELATED COMPOUNDS

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This Doctoral Thesis Has Been Examined by a
Committee of the Department of Chemistry
as Follows:

Prof. George H. Buchi, Chairman. Signature redacted

Prof. John C. Sheehan, Supervisor Signature redacted

Prof. Arthur C. Cope... Signature redacted

Prof. Walter C. Schumb..... Signature redacted

Prof. Charles D. Coryell..... Signature redacted

Prof. Richard C. Lord.....

Houghton, Nov 22, 1955

Part I

# THE FORMATION OF β-LACTAMS AND RELATED COMPOUNDS

Part II

# ALKYL PHOSPHATES AND THIOPHOSPHATES

Roy William Roth

Submitted to the Department of Chemistry

in partial fulfillment of the requirements for the

degree of Doctor of Philosophy

## Abstract

The reaction between phthaloyl-L-phenylalanyl-chloride and benzalaniline in the presence of triethylamine is described. The products have been shown to be critically dependent upon the mode of addition of the reactants and upon reaction conditions. By varying conditions, three types of products have been formed. The course of the reaction has been shown to be dependent not only upon the use of anhydrous conditions but also upon the use of high-dilution techniques.

By using an optically active acyl chloride, the possibility of the existence of a ketene intermediate in the reaction of an acyl chloride with an imine in the presence of a tertiary base to form a β-lactam has been strengthened. The existence of a ketene "molecular complex" which had been earlier postulated as an intermediate has been disproved.

By using an alpha-disubstituted acyl chloride, two isomeric β-lactams have been obtained. Also, two isomeric piperidiones have been isolated by varying reaction conditions.

The action of hydrazoic acid on 2-cyclohexene-1-one, under normal Schmidt reaction conditions, has been shown to yield cyclohexadione-1,2. A suggestion as to the possible mechanism of the reaction is made. The synthesis of 3-azidocyclohexanone and its conversion to cyclohexadione-1,2 is described, An improved synthesis of 2-cyclohexene-1-one is also outlined.

Pyrrolidine-2-acetic acid was synthesized, but the attempted closure to a bicyclic β-lactam was unsuccessful. A direct attempt at closure of ethyl pyrrolidine-2-acetate to a bicyclic β-lactam was unsuccessful.

In Part II of this Thesis is described the preparation of several alkyl phosphates, but no alkyl thiophosphate similar to that postulated as an intermediate in coenzyme A activity was obtained.

Thesis Supervisor: John C. Sheehan
Title: Professor of Organic Chemistry

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Professor John C. Sheehan for his assistance and advice in the preparation of this work.

He is also indebted to Dr. S. N. Nagy and associates for the microanalytical determinations and infrared measurements.

Financial assistance is gratefully acknowledged from the Department of Chemistry of the Massachusetts Institute of Technology, from Swift and Co., and from the National Institute of Health.

TO BARBARA

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C. Synthesis and Attempted Cyclization of Ethyl 2-Pyrrolidine Acetate



**PART** II: Alkyl Phosphates and Thiophosphates

# PART I

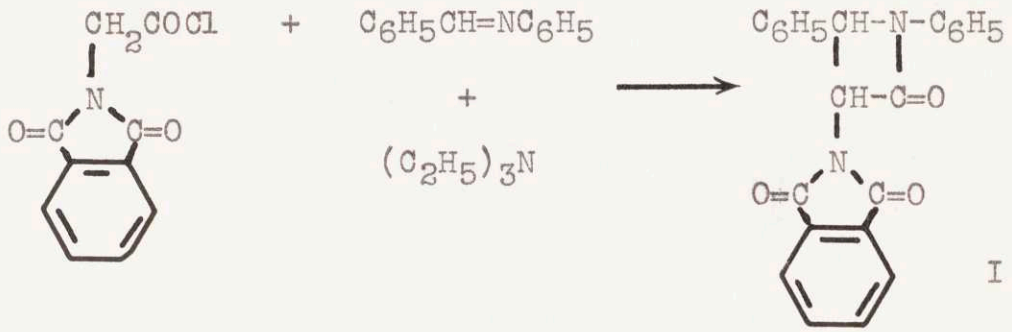
## FORMATION OF β-LACTAMS AND RELATED COMPOUNDS

## SECTION A

## REACTIONS OF OPTICALLY ACTIVE ACYL CHLORIDES WITH BENZALANILINE AND ANILINE

## INTRODUCTION

The mechanism of the reaction of acyl chlorides with amines and imines in the presence of a tertiary amine to give amides and β-lactams is not known. The fundamental reaction in the latter case is illustrated by the preparation of 1,4-diphenyl-3-phthalimido-2-azetidinone (I).[1]




I

The work described in this section involves a study of the reaction of phthaloyl-L-phenylalanyl chloride with aniline and with benzalaniline in the presence of triethylamine with the view of gaining information about the scope of the reaction.

Although no aldoketenes have been made by the dehydrohalogenation of an acid chloride with tertiary amines, it is possible to visualize the reaction of an acyl chloride with an imine in the presence of triethylamine as proceeding by way of a ketene intermediate.

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1. J. C. Sheehan and J. J. Ryan, J. Am. Chem. Soc., 73, 1204 (1951).

Because ketenes are known to react with imines to give β-lactams,[2] the idea of a ketene intermediate is helpful in explaining the products. Since ketenes may be made by the dehydrohalogenation of acid chlorides and since ketenes may be condensed with amines to form amides and with imines to form β-lactams, the mechanism involving a ketene intermediate is a natural conclusion. Opposing this mechanism are the facts that tertiary amine salts are reported[3] to be catalysts for the dimerization of ketenes, and that only one aldoketene (ketene itself) has been condensed with an imine to give a β-lactam.

Another possible mechanism involves the acylation of the tertiary amine to give a quaternary salt, followed by the reaction of this salt with the amine or imine. Quaternary addition compounds of this type are known.[4] It was reported that relatively strong bases

$$R'CH_2COCl + R_3N \longrightarrow R'CH_2\overset{O}{\overset{\|}{C}}-\overset{+}{N}R_3 \quad Cl^-$$

such as triethylamine favored dehydrohalogenation rather than complex formation.[4] Addition compounds with triethylamine could be isolated at low temperatures, however.

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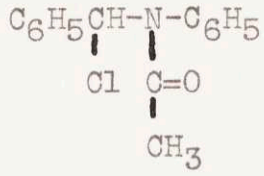
2. H. Staudinger, Ber., 40, 1145 (1907).

3. H. Staudinger and H. W. Klever, Ber., 41, 594 (1908).

4. H. Adkins and Q. E. Thompson, J. Am. Chem. Soc., 71, 2242 (1949).

Garzarolli-Thurnlackh[5] observed that acetyl chloride also formed an addition compound with benzalaniline. The structure for this addition compound was given as II.



II

The compound was precipitated from ether solution and was readily hydrolyzed to give benzaldehyde, acetanilide and hydrogen chloride.

If the model acyl chloride contains an optically active center at the alpha carbon atom, it would be expected that a ketene intermediate would lead to a racemized product. Such need not be the case if the intermediate is an addition complex. Retention of optical activity would therefore exclude a ketene intermediate. It was also of interest to extend the imine-acyl chloride reaction to include a disubstituted acyl chloride. This should make possible the formation of a β-lactam which would be disubstituted on the alpha carbon atom. Phthaloyl-L-phenylalanyl chloride is a disubstituted acyl chloride, and its reaction with an imine might show whether a ketene intermediate is involved in the reaction, since an optically active product would exclude a ketene intermediate.

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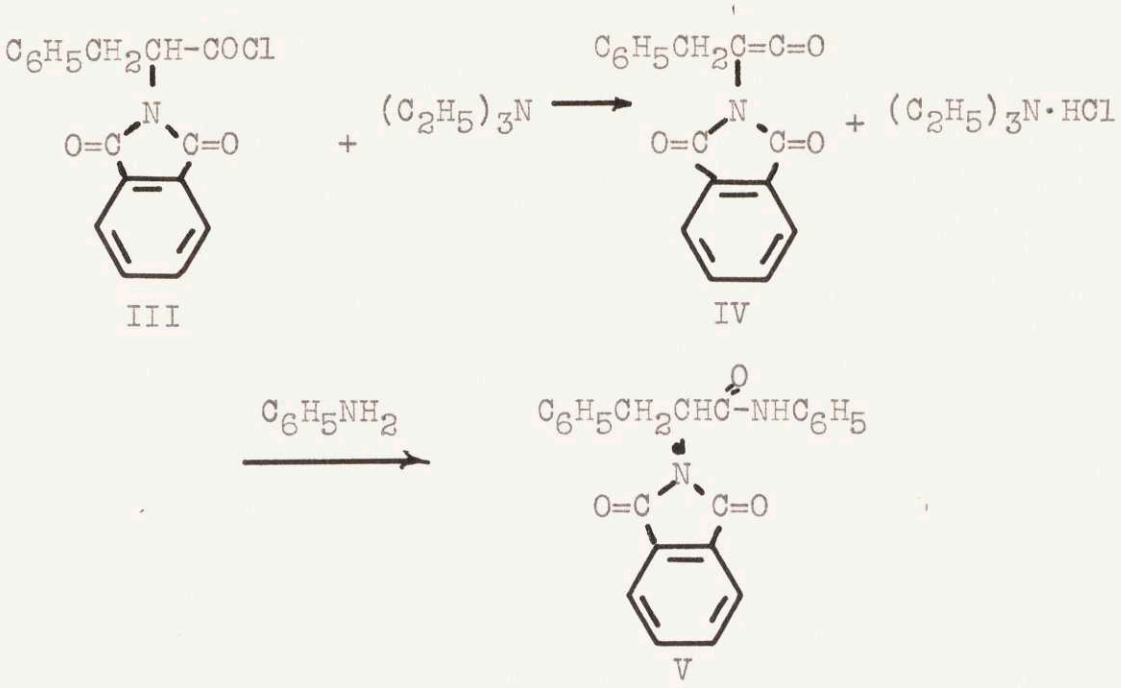
5. K. Garzarolli-Thurnlackh, Ber., 32, 2277 (1899).

## DISCUSSION

In order to study further the reaction between an acyl chloride and an imine or an amine, phthaloyl-L-phenylalanyl chloride was prepared and treated with benzalaniline and with aniline in the presence of triethylamine. It was found that the optical activity of the product critically depended on the mode of addition, and that varying conditions led to different products. The order of addition of the three reactants was varied to determine its effect upon the product. Also, various solvents were used -- benzene or ether, in which triethylamine hydrochloride is insoluble, and methylene chloride, in which triethylamine hydrochloride is soluble.

On treating phthaloyl-L-phenylalanyl chloride (III) with triethylamine in benzene solution, triethylamine hydrochloride was immediately precipitated, and the solution became yellow. Addition of aniline then produced phthaloyl phenylalanylanilide (V). Whereas the reported[12] specific rotation of phthaloyl-L-phenylalanylanilide is -104°, the product in the above reaction had a specific rotation of only -6°, indicating that almost complete racemization had taken place. This result tends to support the existence of a ketene intermediate (IV).




If the order of addition was changed, different results were observed. If a solution of phthaloyl-L-phenylalanyl chloride in methylene chloride was added to a solution of triethylamine and aniline in methylene chloride, there was no yellow coloration, and the isolated phthaloyl-L-phenylalanylanilide had a specific rotation of -101°, showing that practically no racemization had taken place. There must be a direct reaction between the acyl chloride and aniline, as is the case if a base is not present. A ketene intermediate is therefore excluded if the acyl chloride is treated directly with aniline or with a mixture of aniline and triethylamine. There is no dehydrohalogenation of the acyl chloride prior to reaction with aniline.

To determine whether this situation exists in forming a β-lactam by treating an acyl chloride with benzalaniline, phthaloyl-L-phenylalanyl chloride with triethylamine in methylene chloride solution was treated with benzalaniline. The isolated products were phthaloyl phenylalanylchloride (specific rotation -6°) and benzaldehyde (identified as its 2,4-dinitrophenylhydrazone). Again, racemization had taken place when the acyl chloride was treated with the tertiary amine before adding, in this case, the imine. When the acyl chloride was mixed with benzalaniline prior to adding triethylamine, the isolated phthaloyl-L-phenylalanylanilide showed a specific rotation of -102°. Racemization had not taken place, and therefore a ketene is not the intermediate.

In either case, however, no β-lactam was isolated, either because of hydrolysis of the β-lactam during its isolation or because of hydrolysis before cyclization to the β-lactam could take place. It was concluded that the latter was the case, because a solution of phthaloyl-L-phenylalanyl chloride and benzalaniline in benzene exposed to the atmosphere readily formed phthaloyl-L-phenylalanylanilide.

Smith[6] has shown that acyl chlorides form an addition complex (VI) with benzalaniline. It is this complex which is then readily hydrolyzed under non-anhydrous

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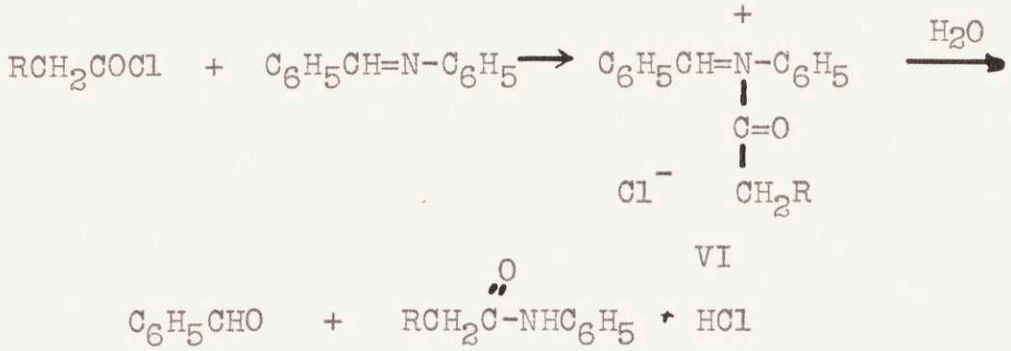
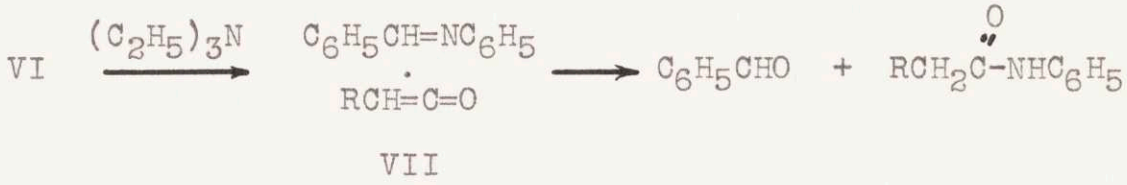
6. Howard L. Smith, Ph.D. Thesis, M.I.T., September, 1951.

conditions.

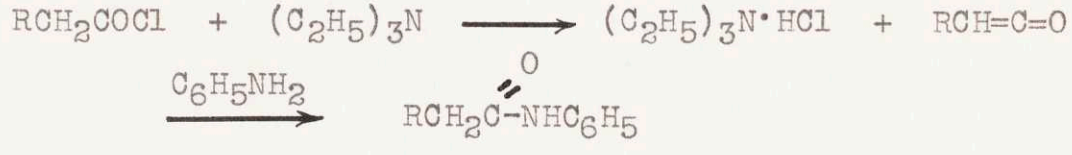


Since the anilide is optically active, there is no racemization in this sequence of reactions.

Smith had postulated that triethylamine removes the elements of hydrogen chloride from the salt (VI) to form a molecular complex (VII), which is then hydrolyzed during product isolation. Such an intermediate must be

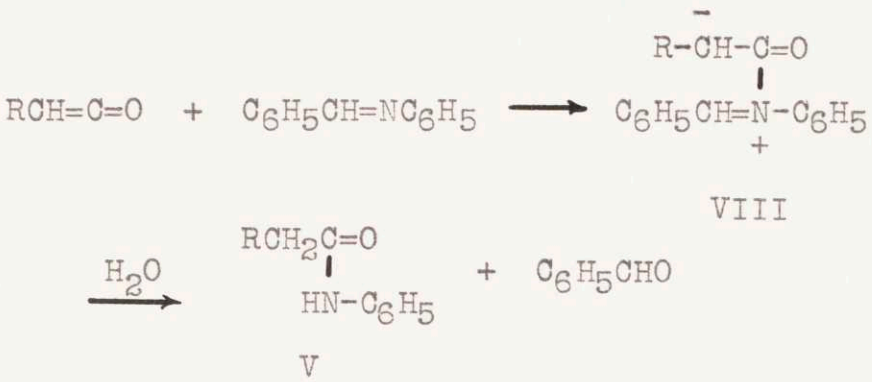


excluded since it would lead to a racemized product. Triethylamine hydrochloride must be formed after the addition salt (VI) has been hydrolyzed to the anilide. The formation of a ketene is possible if the acyl chloride is treated with a base prior to treatment with aniline.



Isolation of the racemized anilide substantiates this suggestion.

It is harder to explain how an inactive anilide also arises when benzalaniline is treated with a solution of the acid chloride and triethylamine. Assuming initial formation of a ketene, an intermediate (VIII) might be formed which is easily hydrolyzed.

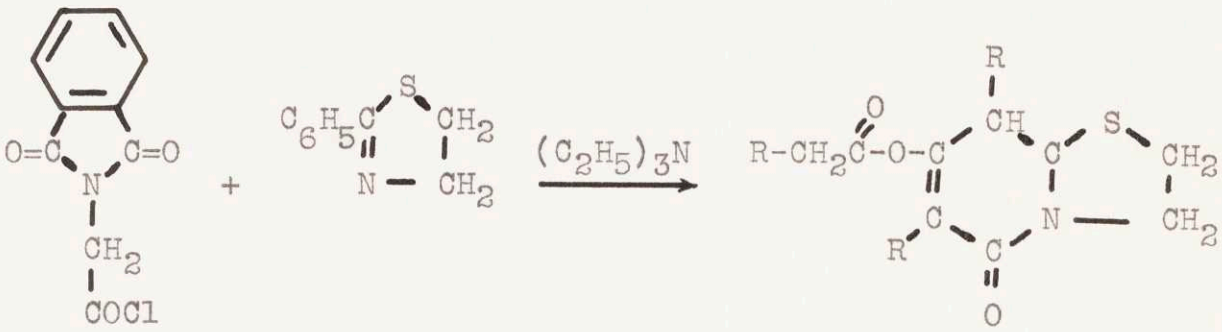


Before it can cyclize to a β-lactam, the intermediate VIII must be hydrolyzed to the anilide and benzaldehyde. Since strictly anhydrous conditions were not adhered to in the above experiments, the conditions were repeated with the exclusion of moisture.

It was found that the anilide (V) was not formed under anhydrous conditions. Instead, at ice-bath temperature, two isomeric compounds of empirical formula $C_{47}H_{33}N_3O_6$ were formed. The infrared absorption curves of these two compounds indicate the absence of a β-lactam (only the two phthaloyl peaks at 5.65 mu and 5.86 mu below 6 mu) but suggest the presence of an amide linkage (6.02 mu).

Several cases have been noted where piperidiones have resulted in the reaction of ketoketenes with thiazolines[7] and with Schiff bases.[8]

Sheehan and Ryan[9] have also shown a similar type compound to result from phthaloylglycyl chloride, 2-phenyl-2-thiazoline and triethylamine.



R = phthalimide

Analogously, the structure best fitting the analysis and infrared absorption spectrum of the isolated isomers is IX, resulting from the addition of two molecules of acyl chloride to one molecule of benzalaniline.

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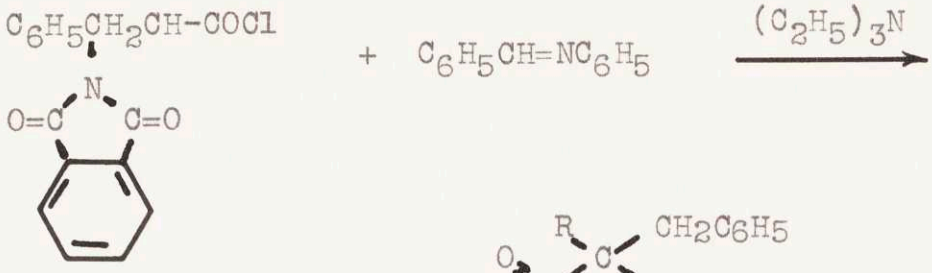
7. "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson and R. Robinson, editors, Princeton University Press, Princeton, N. J., 1949, pp. 996, 997, 1000.

8. H. Staudinger, "Die Ketene," Enke, Stuttgart, 1912.

9. J. C. Sheehan and J. J. Ryan, J. Am. Chem. Soc., 73, 4367 (1951).

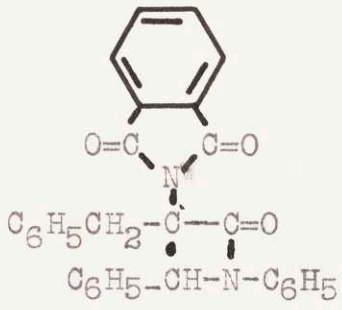


R = phthalimido

IX

Except for different melting points, and slight differences in the infrared absorption above 8 mu, the two compounds appear similar and are probably geometric isomers.

The product IX could almost be excluded from the reaction products if the reaction was run under high-dilution conditions. The relative proportions of the desired lactam X and the product IX formed seemed to be critically dependent on the manner in which the reaction was run. The highest yield of X was obtained when an



X

equimolar solution of the acyl chloride and benzalaniline in benzene was heated to rapid reflux in a dilution cycle[10] and a solution of an equivalent amount of triethylamine in benzene then slowly added so that each drop of amine was mixed with a large volume of reflux, thus entering the reaction flask in a highly diluted state.

The β-lactam X was isolated in two isomeric forms. Since they are both racemic, they must represent the two geometric isomers possible, depending on whether the phenyl and benzyl groups on carbon atoms 3 and 4 are cis or trans.

On treating the β-lactam X under conditions known to open the phthaloyl group,[11] i.e., in contact with hydrazine at room temperature for 20 hours, the product no longer has the intact phthaloyl group, as shown by the loss of the 5.65 mu and 5.84 mu peaks in the infrared absorption spectrum, but a new compound is formed whose analysis most closely corresponds to the addition of one equivalent of hydrazine. The infrared absorption spectrum of this new product shows strong absorption at 6.05 mu and weaker absorption at 3.05 mu and 3.15 mu, besides retaining the β-lactam peak at 5.75 mu. Refluxing this

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10. A. C. Cope and E. C. Herrick, J. Am. Chem. Soc., 72 983 (1950).

11. P. A. Cruikshank, Ph.D. Thesis, M.I.T., December, 1954.

material in 5% sodium carbonate regenerates the original phthalimido β-lactam (X). Clearly, hydrazinolysis of X is abnormal under the usual conditions, so no further attempts were made to remove the phthaloyl group.

## EXPERIMENTAL

Phthaloyl-L-phenylalanyl Chloride (III). - A mixture of phthaloyl-L-phenylalanine[12] (3.14 g., 0.011 mole, $\alpha]_D^{25}$ = -208°) and phosphorus pentachloride (2.71 g., 0.013 mole) in 60 ml. of dry benzene was heated to 50-55° for one hour. The resulting solution was concentrated to dryness at reduced pressure, flushed twice with dry toluene, and dissolved in 25 ml. of benzene. Adding 100 ml. of petroleum ether (b.p. 30-60°) afforded 3.51 g. (100%) of crystalline material, m.p. 83-84° (reported m.p. 82-83°)[12], $\alpha]_D^{25}$ = -222°.

Phthaloyl-L-phenylalanyl Anilide (V). (a) From aniline. - To a cooled solution (0-5°) of aniline (0.16 g., 1.70 millimoles) and triethylamine (0.17 g., 1.70 millimoles) in 25 ml. of methylene chloride was added with stirring during forty-five minutes, a solution of phthaloyl-L-phenylalanyl chloride (0.50 g., 1.59 millimoles) in 25 ml. of methylene chloride. After one hour the clear solution was evaporated at reduced pressure to a syrupy liquid which was crystallized from methanol. The yield of fine, colorless needles was 0.41 g. (70%), m.p. 205.2-206.4° (reported m.p. 203-205°)[12], $\alpha]_D^{25}$ = -101.1°.

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12. Douglas W. Chapman, Ph.D. Thesis, M.I.T., September, 1951.

(b) From benzalaniline. - To a solution of phthaloyl-L-phenylalanyl chloride (0.3 g., 0.95 millimole) and benzalaniline (0.17 g., 0.95 millimole) in 50 ml. of dry benzene cooled to 5° was added over a period of forty-five minutes a solution of triethylamine (0.10 g., 1.0 millimole) in 25 ml. of benzene. After two hours the triethylamine hydrochloride was removed by filtration and the filtrate evaporated to dryness at reduced pressure. The residue was recrystallized from methanol, yield 0.23 g. (65%), m.p. 204.8-205.6°, $\alpha]_D^{25}$ = -102.6°. Adding 2,4-dinitrohydrazine reagent to the filtrate produced the 2,4-dinitrophenylhydrazone of benzaldehyde, m.p. 236.5-237.5° (reported m.p. 237°)

Racemized Phthaloyl Phenylalanyl Anilide. (a) From aniline. - To a solution of phthaloyl-L-phenylalanyl chloride (0.5 g., 1.59 millimoles) and triethylamine (0.30 ml., 2.2 millimoles) in 50 ml. of methylene chloride cooled to -40° by a dry ice-acetone bath was added with stirring a solution of aniline (0.15 g., 1.6 millimoles) in 30 ml. of methylene chloride over a period of thirty minutes. After a total of forty-five minutes the solvent was evaporated at reduced pressure and the residue crystallized from methanol, affording 0.45 g. (76%), m.p. 206-207°, $\alpha]_D^{25}$ = -6°. A m.p. of 194-198° was observed on admixture of a sample of phthaloyl-L-phenylalanyl anilide;

and a m.p. of 204.4-207.8 was observed on admixture of a sample of phthaloyl-D,L-phenylalanyl anilide.

(b) From benzalaniline. - To a solution of phthaloyl-L-phenylalanyl chloride (0.3 g., 0.95 millimole) and triethylamine (0.2 ml., 1.4 millimoles) in 30 ml. of methylene chloride cooled to -40° by a dry ice-acetone bath was added, with stirring, a solution of benzalaniline (0.17 g., 0.95 millimole) in 20 ml. of methylene chloride. After a total of thirty minutes, the clear solution was allowed to come to room temperature and the solvent removed at reduced pressure. The solid residue was taken up in a small amount of methylene chloride, washed with dilute sodium bicarbonate, water and dried over anhydrous sodium sulfate. The filtered solution was evaporated at reduced pressure and the residue crystallized from methanol, affording 0.2 g. (57%), m.p. 206-207°, $[\alpha]_D^{25} = -6°$.

1,2-Diphenyl-3,5-dibenzyl-3,5-diphthalimido Piperidione-4,6 (IX). - To a solution of phthaloyl-D,L-phenylalanyl chloride (3.13 g., 0.01 mole) and benzalaniline (1.81 g., 0.01 mole) in 75 ml. of dry benzene cooled in an ice-bath was added a solution of triethylamine (1.57 g., 0.015 mole) in 25 ml. of dry benzene. After stirring under an atmosphere of nitrogen for thirty minutes, triethylamine hydrochloride was removed by filtration (yield, 1.34 g., 98%). The yellow filtrate was evaporated under reduced pressure and the taffy

residue triturated with warm methanol. Fractional crystallization from ethyl acetate-ethanol afforded two isomeric forms of the piperidione, m.p. 224.7-224.8°, 0.43 g.; m.p. 259°, 0.83 g.; and a small amount of phthaloyl-D,L-phenylalanylaniline, m.p. 212-213°, 0.27 g.

Infrared absorption of the piperidiones showed absorption peaks at 5.65 mu and 5.85 mu due to the phthaloyl group, and at 6.02 mu due to the amide group.

Anal. Calcd. for $C_{47}H_{33}N_3O_6$: C, 76.72; H, 4.52; N, 5.71. Found: C, 76.47; H, 4.70; N, 5.51.

1,4-Diphenyl-3-benzyl-3-phthalimido-2-azetidinone (X). - A solution of phthaloyl-L-phenylalanyl chloride (3.13 g., 0.01 mole) and benzalaniline (1.81 g., 0.01 mole) in 100 ml. of dry benzene was prepared in a 200 ml. three-necked flask equipped with a nitrogen-inlet tube, magnetic stirrer and a small high-dilution cycle tube which carried an efficient reflux condenser and a dropping funnel. A solution of triethylamine (1.01 g., 0.01 mole) in 50 ml. of dry benzene was added dropwise over a period of six hours through the cycle tube while a rapid reflux was maintained. After cooling to room temperature, triethylamine hydrochloride was removed by filtration, yield 1.27 g. (93%). The filtrate was evaporated at reduced pressure and fractionally crystallized from methanol to yield two isomeric solids, m.p. 182.4-182.9°, 0.82 g., $\alpha]_D^{25} = -1.08°$, and m.p. 212.0-213.3°, 1.38 g., $\alpha]_D^{25} = -2.09°$. A second crop of material

was obtained on concentration of the mother liquors, weight 0.38 g. The total weight represents a yield of 82% of β-lactam.

Anal. Calcd. for $C_{30}H_{22}N_2O_3$: C, 78.58; H, 4.83; N, 6.11. Found: (lower melting isomer) C, 78.79; H, 4.93; N, 6.30. (higher melting isomer) C, 78.41; H, 5.14; N, 5.85.

Treatment of 1,4-Diphenyl-3-benzyl-3-phthalimido-2-azetidinone with Hydrazine. - To a solution of the β-lactam (0.31 g., 0.67 millimole) in 15 ml. of purified dioxane was added 0.70 ml. (1.40 millimoles) of a 2.0 M solution of hydrazine hydrate in ethanol, and the clear solution was stored at room temperature for 19 hours. The solution was lyophilized and the remaining white solid shaken with 6 ml. of 0.13 N hydrochloric acid. The insoluble material was removed by filtration, weight 0.36 g., and the filtrate lyophilized. No material was recovered from the lyophilized filtrate.

The material insoluble in hydrochloric acid was crystallized from methanol to a constant melting point, 174.3-175.0°. Infrared absorption spectrum showed a disappearance of the two phthaloyl peaks at 5.65 mu and 5.87 mu with the appearance of a strong absorption at 6.05 mu and weaker absorption at 3.05 mu and 3.15 mu.

Anal. Found: C, 73.82: H, 6.20; N, 11.08.

On refluxing this material with 5% aqueous sodium carbonate, the original phthalimido-β-lactam was regenerated as shown by mixed melting point with the starting lactam, and by infrared absorption.

Plate I

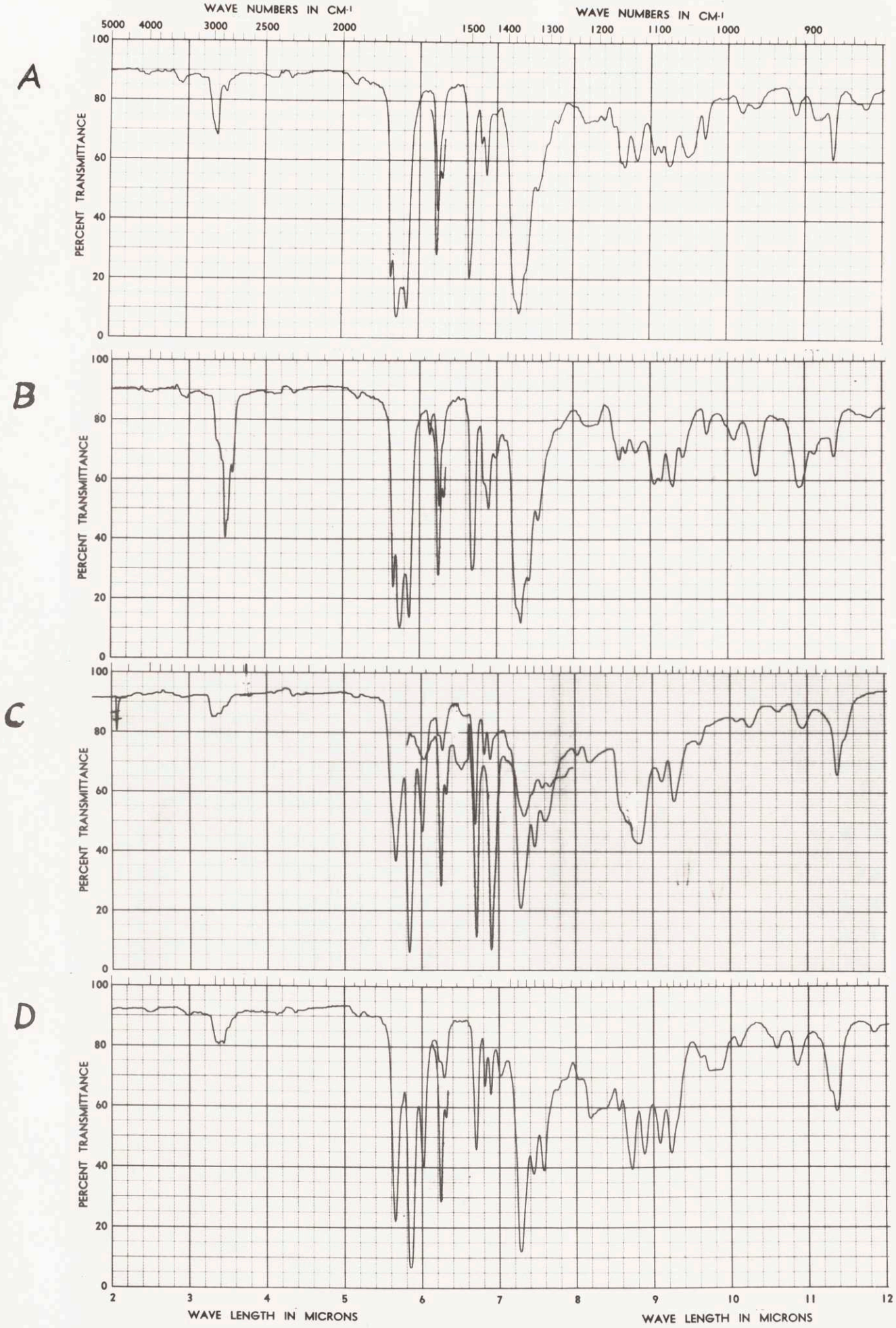
Curve A: 1,2-Diphenyl-3,5-dibenzyl-3,5-diphthalimido Piperidione-4,6 (IX), m.p. 224.7-224.8°, 10% in tetrachloroethane.

Curve B: 1,2-Diphenyl-3,5-dibenzyl-3,5-diphthalimido Piperidione-4,6 (IX), m.p. 259°, 10% in tetrachloroethane.

Curve C: 1,4-Diphenyl-3-benzyl-3-phthalimido-2-azetidinone (X), m.p. 182.4-182.9°, 10% in tetrachloroethane.

Curve D: 1,4-Diphenyl-3-benzyl-3-phthalimido-2-azetidinone (X), m.p. 212.0-213.3°, 10% in tetrachloroethane.

The infrared absorption spectra were determined with a Baird Infrared Recording Spectrophotometer, Model B. Solution spectra were determined in a cell of thickness 0.10 mm.


A
B
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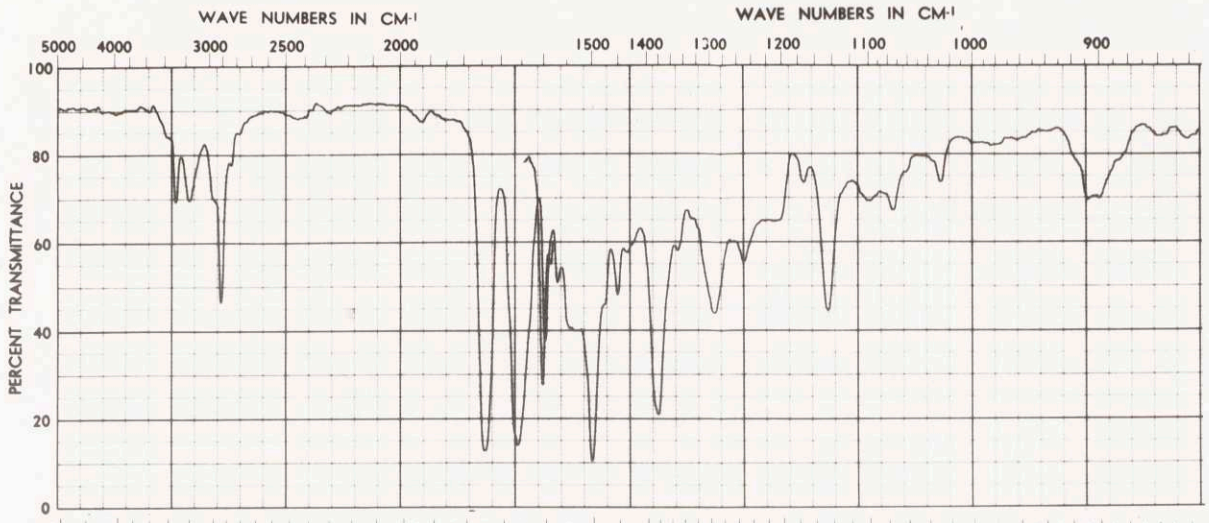
D
WAVE NUMBERS IN CM-1
5000 4000 3000 2500 2000
1500 1400 1300 1200 1100 1000 900
PERCENT TRANSMITTANCE
100
80
60
40
20
0
2 3 4 5 6 7 8 9 10 11 12
WAVE LENGTH IN MICRONS

Plate II

Curve E: 1,4-Diphenyl-3-benzyl-3-phthalimido-2-azetidinone after treatment with hydrazine at room temperature for 19 hours.

Curve F: After refluxing the above material with 5% aqueous sodium carbonate.

E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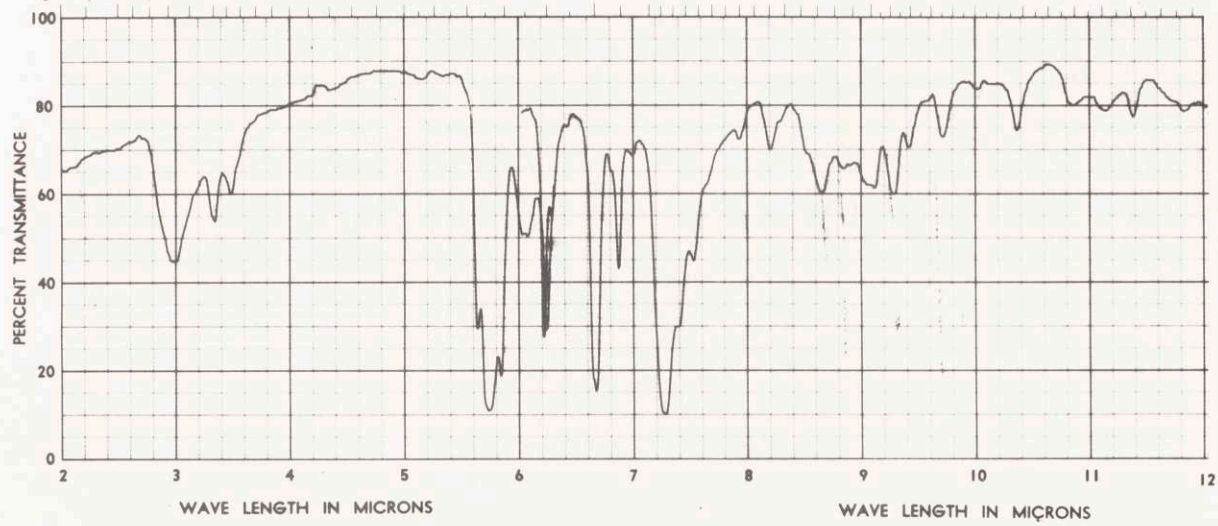
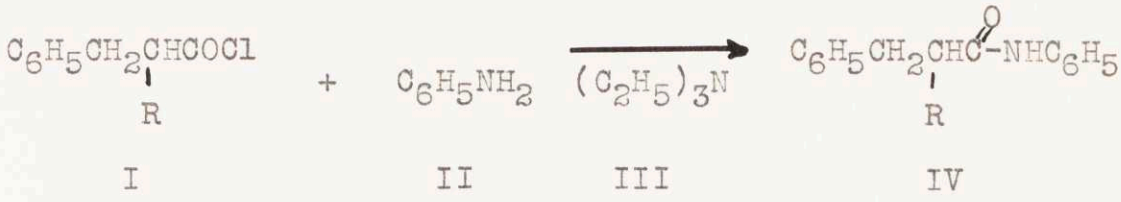
100 80 60 40 20 0

F


PERCENT TRANSMITTANCE
100 80 60 40 20 0
2 3 4 5 6 7 8 9 10 11 12
WAVE LENGTH IN MICRONS
WAVE LENGTH IN MICRONS

## SUMMARY OF EXPERIMENTAL RESULTS

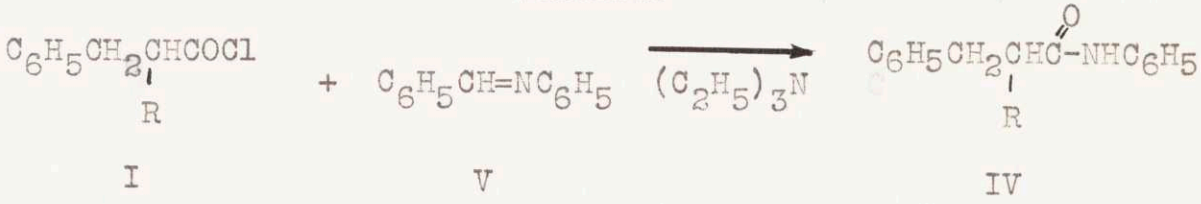
A. Non-anhydrous Conditions. -



I II III IV

R = phthalimido

| Reactants | Additive | Solvent | Temp. | $\alpha_D^{25}$ |
|---|---|---|---|---|
| I(D,L) & III | II | Ether | -20° | |
| I(D,L) & II | None | Benzene | 25° | -- |
| II & III | I(L) | Methylene Chloride | 5° | -101° |
| I(L) & III | II | Methylene Chloride | -40° | -6° |

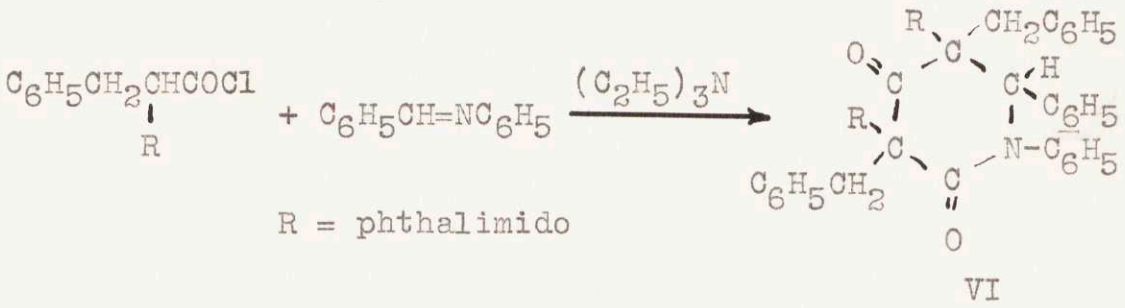


I V IV

R = phthalimido

| Reactants | Additive | Solvent | Temp. | $\alpha_D^{25}$ |
|---|---|---|---|---|
| I(D,L) & III | V | Ether | -20° | -- |
| I(L) & V | III | Benzene | 5-10° | -102° |
| I(L) & III | V | Methylene Chloride | -40° | -6° |

B. Anhydrous Conditions. -

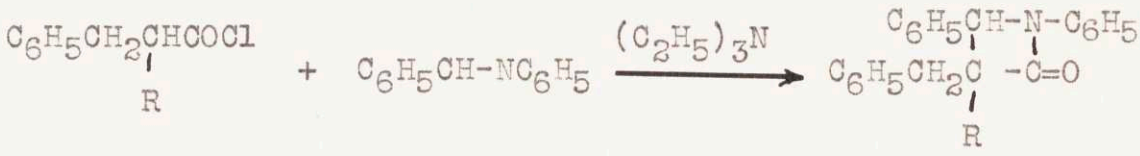


R = phthalimido

VI

The piperidione (VI) was racemized regardless of the mode of addition. Two isomers of the piperidione (VI) were isolated.

C. Anhydrous, High-dilution Conditions



VII

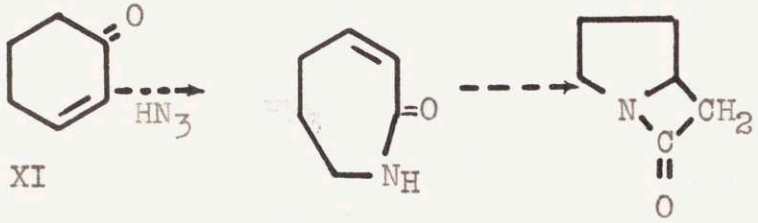
The β-lactam (VII) was racemized regardless of the mode of addition. Two isomers of the β-lactam were isolated.

## SECTION B

# SYNTHESIS OF 2-CYCLOHEXENE-1-ONE AND ITS REACTION WITH HYDRAZOIC ACID

## INTRODUCTION

One possible route to the synthesis of a β-lactam fused to a five-membered ring is by an internal Michael-type condensation of an α,β-unsaturated caprolactam (XII). Since the synthesis of this compound has not been reported, a Schmidt reaction on 2-cyclohexene-1-one (XI) was investigated.




XI XII

Benzalacetone (XIII) undergoes the Schmidt reaction, i.e., the addition of hydrazoic acid, to form N-methyl-cinnamate (XIV).[13] This reaction appears to follow the normal mechanism described[14] for the Schmidt reaction with the hydrazoic acid adding to the carbonyl group, with the subsequent loss of nitrogen and rearrangement.

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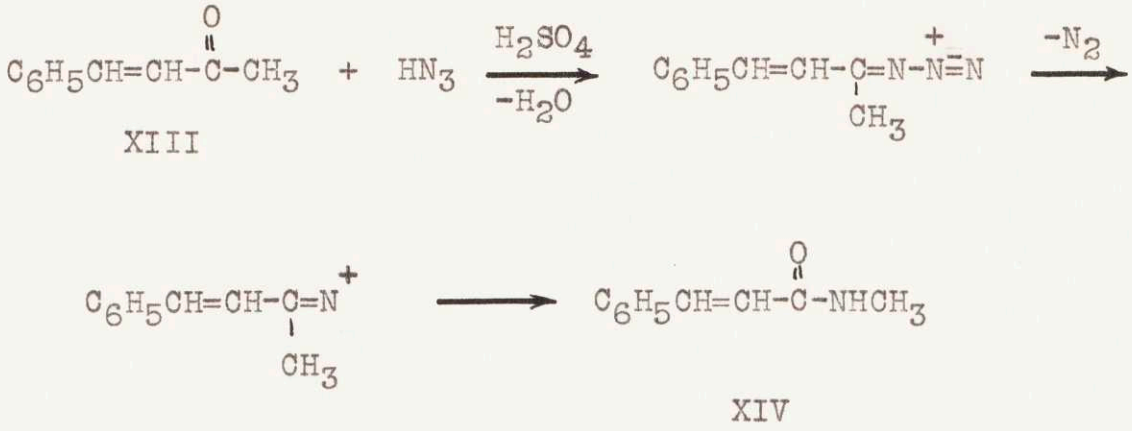
13. P. A. S. Smith and J. P. Horwitz, J. Am. Chem. Soc., 72, 3718 (1950).

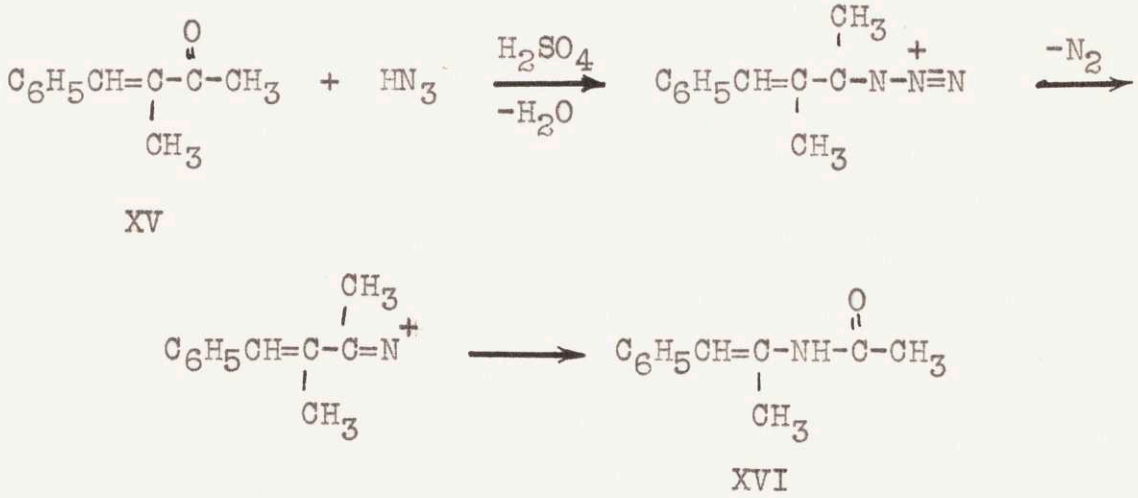
14. R. Adams, W. E. Bachmann, L. F. Fieser, J. R. Johnson, and H. R. Snyder, editors, "Organic Reactions," Vol. III, John Wiley & Sons, Inc. (1946).



XIII

XIV

α-Methyl-β-phenylvinyl methyl ketone (XV), however, produces the acetamide derivative XVI.[13] In this case the unsaturated portion of the molecule migrates.



XV

XVI

As is explained by Alexander,[15] the product appears to depend on the bulkiness of the migrating groups and not on their migrational aptitude.

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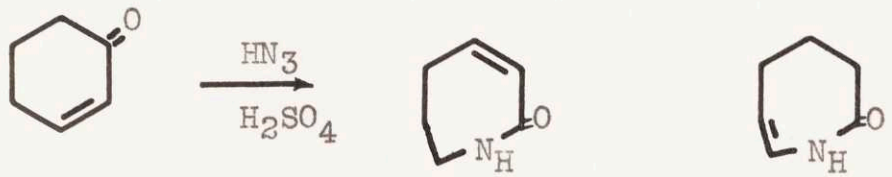
15. E. R. Alexander, "Ionic Organic Reactions," John Wiley & Sons, Inc., N.Y. (1950).

2-Cyclohexene-1-one (XI) is a cyclic, α,β-unsaturated carbonyl compound which, depending on the course of the reaction could conceivably yield either of the unsaturated caprolactams XII or XVII, according to which group migrates in the above mechanism.



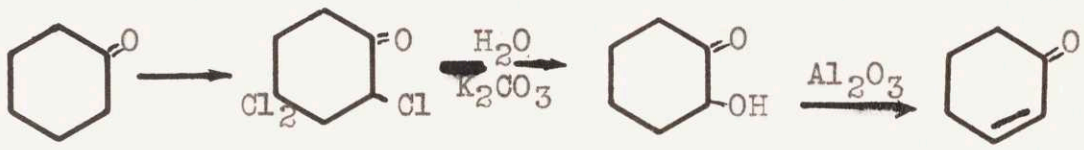

XI XII XVII

2-Cyclohexene-1-one was synthesized and subjected to the conditions of the Schmidt reaction.

## DISCUSSION

There is no report that a purely aliphatic α,β-unsaturated carbonyl compound has been subjected to the conditions of the Schmidt reaction. We have synthesized 2-cyclohexene-1-one in order to study its reaction with hydrazoic acid. An unsaturated caprolactam might result or perhaps other products would be obtained.

Previous methods for the preparation of 2-cyclohexene-1-one have given poor yields.[16-24] Perhaps the best preparation previously reported[16] involves the dehydration of 2-hydroxycyclohexanone, made via 2-chlorocyclohexanone.

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16. A. Kotz and C. Gotz, Ann., 358, 183 (1908).

17. A. Kotz and T. Grethe, J. prakt. Chem., 2 , 80, 473 (1909).

18. A. Kotz, K. Blendermann, F. Mahnert and R. Rosenbusch, Ann., 400, 72 (1913).

19. A. Kotz and K. Richter, J. prakt. Chem., 111, 373 (1925).

20. A. Giullemontat, Ann. Chim., 11, 143 (1939).

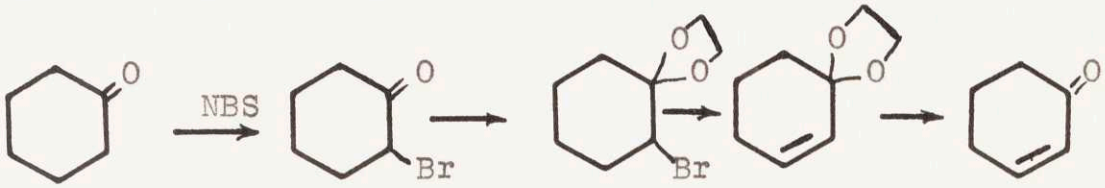
21. C. Courtot and J. Pierron, Bull. soc. chim., 45, 286 (1925).

22. C. S. Marvel and W. L. Walton, J. Org. Chem., 7, 88 (1942).

23. H. Schmid and P. Karrer, Helv. Chim. Acta, 28, 573 (1940).

24. P. D. Bartlett and G. Woods, J. Am. Chem. Soc., 62, 2933 (1940).

2-Cyclohexene-1-one has been synthesized in the present work in considerably better yields by two different methods. The first involves dehydrobromination of 2-bromocyclohexanone with alcoholic potassium hydroxide after rendering the carbonyl group inert by protecting it as an ethylene ketal.[25]




XVIII XIX XX XXI

Cyclohexanone has been brominated with N-bromosuccinimide (NBS) but no yields were mentioned.[23] Following the normal Wolff-Ziegler conditions, 2-bromocyclohexanone was prepared in good yield. In order to dehydrobrominate this bromoketone (XVIII) with potassium hydroxide, the carbonyl group must be masked so that the alpha methylene group will not enter into self-condensation reactions. To do this, the ethylene ketal was formed. The bromoketal (XIX) was then dehydrobrominated with alcoholic potassium hydroxide, and the masking ketal group removed with acid. By this route, the overall yield of 2-cyclohexene-1-one was 32%. It was found that a better yield could be obtained simply

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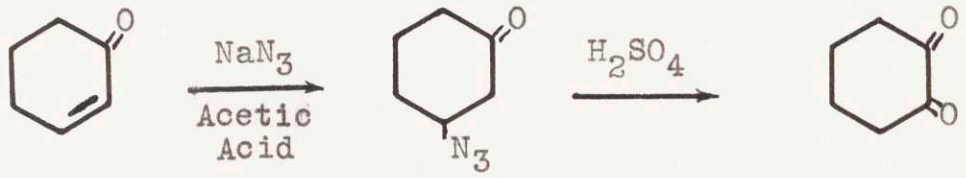
25. E. A. Youngman, Ph.D. Thesis, University of Washington, 1952.

by dehydrobrominating 2-bromocyclohexanone in refluxing triethylamine. In this manner an overall yield of 65% was obtained.

2-Cyclohexene-1-one was treated with hydrazoic acid and concentrated sulfuric acid under conditions of the Schmidt reaction. The product unexpectedly contained no nitrogen and analyzed for the empirical formula $C_6H_8O_2$. The infrared absorption spectrum of this material showed two absorption peaks in the carbonyl region (5.85 mu and 6.05 mu) and strong absorption at 3.00 mu, suggesting the presence of a hydroxyl group.

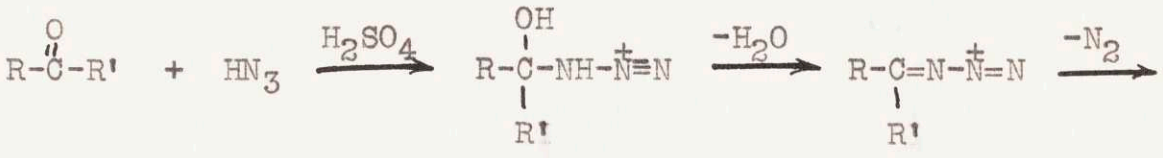
The material reduced Fehling's solution and Tollen's reagent, and gave a violet color with ferric chloride solution. The compound formed dicarbonyl derivatives, and the dioxime gave a bright red precipitate with nickel salts. All the chemical and physical evidence fits the compound cyclohexadione-1,2, and an authentic sample of the dioxime of cyclohexadione-1,2 (nioxime) showed no depression in melting point when mixed with the dioxime of the product obtained from the Schmidt reaction on 2-cyclohexene-1-one. This is the first recorded case in which an α,β-unsaturated carbonyl compound yields an α-diketone on treatment with hydrazoic acid. That the hydrazoic acid is necessary in the reaction was shown by repeating the experiment with no hydrazoic acid. In this case, only starting material was obtained.

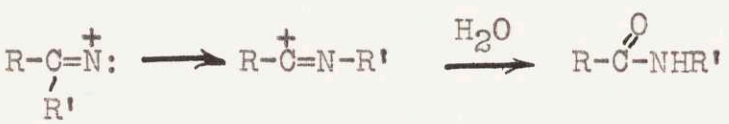
With the establishment of the product as the α-diketone, it is of interest to postulate a reasonable mechanism. Since α,β-unsaturated carbonyl compounds are known to add hydrazoic acid under mildly acidic conditions to yield the β-azido carbonyl derivative,[26] it seemed reasonable to assume that β-azidocyclohexanone (XXII) is the first product formed in the reaction. This compound was synthesized and, indeed, it was shown to form cyclohexadione-1,2 (XXIII) upon treatment with sulfuric acid.




XI XXII XXIII

In the normal Schmidt reaction, after addition of hydrazoic acid to a carbonyl compound, the next step is the loss of water, followed by the loss of nitrogen, to give the unstable intermediate XXIV which has only six outer electrons.[15]



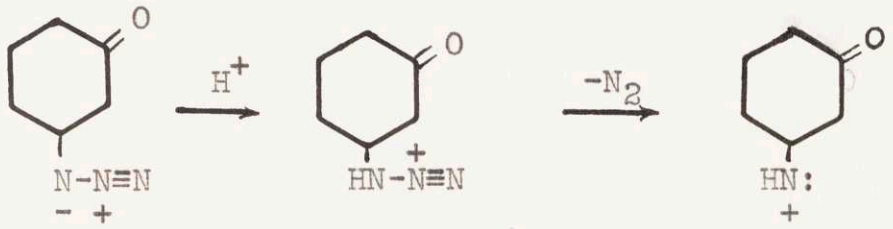




XXIV XXV

26. J. H. Boyer and F. C. Canter, Chem. Rev., 54, 1 (1954).

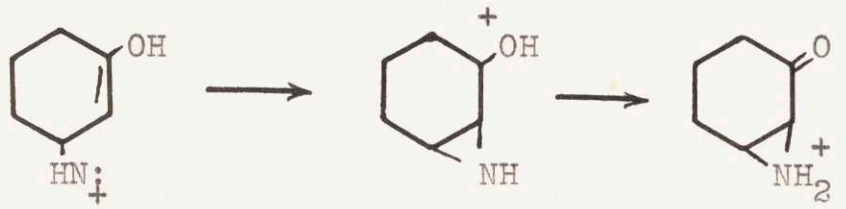
Migration of an alkyl group gives XXV, which is then hydrolyzed to the amide.

3-Azidocyclohexanone (XXII) cannot lose a molecule of water in this manner, but it can lose nitrogen to form an intermediate XXVII which is similar to XXIV in that it has a nitrogen atom with only six outer electrons. The nitrogen can fill its outer shell




XXII XXVI XXVII

with eight electrons by taking the pair of electrons available in the enolized form (XXVIII) of XXVII to form an ethylenimine type intermediate XXIX. This system would not be expected to be stable in acid media




XXVIII XXIX

and the ethylenimine ring might open to form either XXX or XXXI. Cromwell[27] has shown that in an ionizing medium

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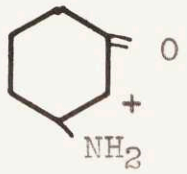
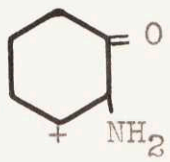
27. N. H. Cromwell, G. V. Hudson, R. A. Wankel and P. J. Van der Horst, J. Am. Chem. Soc., 75, 5384 (1953).



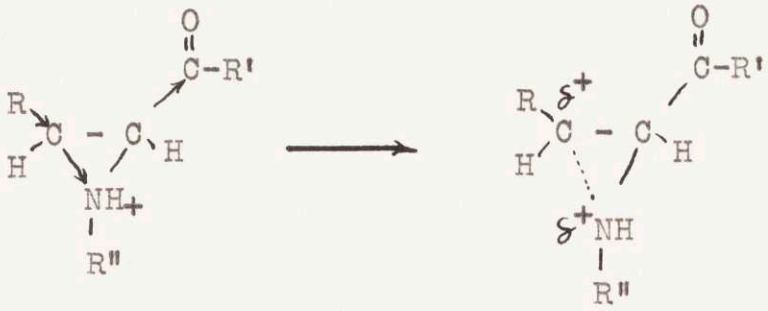
XXX

or



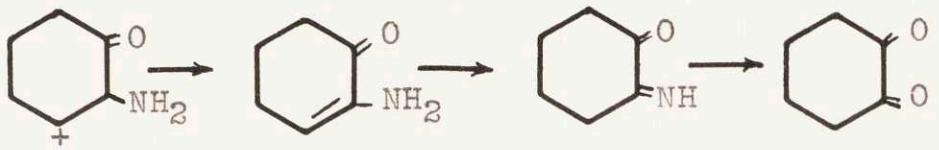
XXXI

an $S_N1$ reaction is favored in the acid cleavage of ethylenimine ketones. The following sequence is postulated.[27]



The carbon assumes part of the positive charge initially held by the nitrogen. Nucleophilic attack would then take place on this partially charged carbon atom, or conversely, it would be the bond between the nitrogen and the β-carbon which would be expected to cleave. Indeed, in polar media, ethylenimine ketones are cleaved by hydrogen chloride to yield the β-chloro-α-amino ketone.[27] Similar reasoning would favor cleavage of the ethylenimine ring of XXIX to form XXXI.

Loss of a proton from XXXI would yield the enolized form of the imine XXXII, which would be readily hydrolyzed to the α-diketone XXIII.



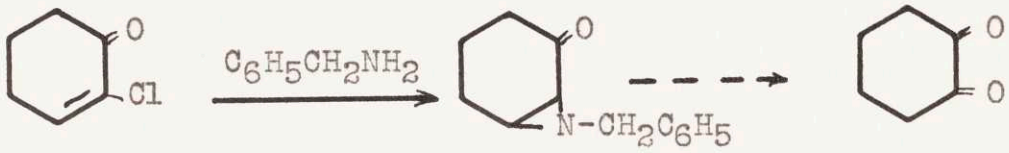
XXXI XXXII XXIII

In 30% sulfuric acid, 2-benzoyl-2-phenyl-1-benzyl ethylenimine (XXXIII) gives, besides what is described as an inner salt of the amine-sulfuric acid, a low yield of benzyl phenyl diketone (XXXIV).[28]

XXXIII XXXIV

This is in agreement with the mechanism described above, and helps to support the mechanism described for the conversion of 2-cyclohexene-1-one to cyclohexadione-1,2.

An attempt was made to prepare an intermediate of the type XXXVI by treating 2-chlorocyclohexenone (XXXV) with benzylamine.



XXXV XXXVI

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28. N. H. Cromwell, R. D. Babson and C. E. Harris, J. Am. Chem. Soc., 65, 312 (1943).

A stable compound was not isolated. On treating the crude reaction mixture with sulfuric acid, only 2-chlorocyclohexenone could be recovered.

## EXPERIMENTAL

2-Bromocyclohexanone (XVIII). - Cyclohexanone (98 g., 1.0 mole), N-bromosuccinimide (180.8 g., 1.0 mole) and carbon tetrachloride (1000 ml.) were placed in a 3-liter flask fitted with an efficient condenser to which was attached a drying tube. After adding 10 drops of piperidine, the reaction was alternately brought to reflux and cooled slightly until bromination began (45 minutes), as evidenced by vigorous boiling, disappearance of the bromine color and appearance of succinimide. Cooling was needed to moderate the initial violent reaction. The flask was then heated on a steam cone until all the brominating agent had disappeared from the bottom of the flask. The reaction was then cooled and magnesium oxide (2 g.) was added to neutralize any hydrogen bromide. Succinimide and magnesium oxide were removed by filtration and washed with carbon tetrachloride. After adding more magnesium oxide (2 g.) the combined filtrate and washings were concentrated at about 50 mm. on a water-bath (30-40°). Fractional distillation of the residue from magnesium oxide through a Vigreux column gave the following fractions:

a. 9.9 g. (10.1%) largely unreacted cyclohexanone, b.p. up to 57° at 0.7 mm.

b. 135 g. (76%) 2-bromocyclohexanone, b.p. 44° at 0.4 mm.

c. 9.7 g. (4%) impure dibromocyclohexanone, b.p. 60-95° at 0.5 mm.

2-Bromocyclohexanone Ethylene Ketal (XIX). - Ethylene glycol (180 g., 2.9 moles), 2-bromocyclohexanone (131 g., 0.74 mole), p-toluene-sulfonic acid monohydrate (0.8 g.) and benzene (600 ml.) were placed in a 1000 ml. round bottomed flask to which was attached a water separator and condenser. The contents of the flask were refluxed in an oil-bath at about 115° for 72 hours. A total of 26 ml. of water was collected. After cooling, the solution was washed with aqueous sodium bicarbonate. The bicarbonate washings were extracted with ether and the combined ethereal extracts and benzene solution was dried over anhydrous magnesium sulfate. After removal of the solvent at reduced pressure, the product was distilled through a Vigreux column to give a small forerun, and 147 g. (90%) of 2-bromocyclohexanone ethylene ketal, b.p. 67° at 0.8 mm. (reported 65° at 0.2 mm.).[25]

2-Cyclohexenone Ethylene Ketal (XX). - Potassium hydroxide (133 g., 2.38 moles) and absolute ethanol (550 ml.) were placed in a 1-liter flask fitted with a stirrer, dropping funnel and reflux condenser to which was attached a calcium chloride drying tube. The mixture was stirred and heated to reflux in a nitrogen atmosphere until almost all the potassium hydroxide was dissolved,

then 2-bromocyclohexanone ethylene ketal (147 g., 0.66 mole) was added dropwise over a period of one hour. The mixture was then stirred and refluxed under nitrogen for a total of 20 hours. The solid was removed by filtration, washed well with ethanol and the filtrate concentrated at reduced pressure to remove the ethanol. Water (500 ml.) was then added and the product extracted with three 100 ml. portions of ether. After drying over anhydrous magnesium sulfate, the ether was evaporated at reduced pressure and the residue fractionated through a Vigreux column. After a small forerun, the product was collected, b.p. 82-86° at 20 mm. (reported 83-84° at 18 mm.),[25] yield 64 g. (69%).

2-Cyclohexene-1-one (XXI). (a) From 2-cyclohexenone-ethylene ketal. - A mixture of 2-cyclohexenone ethylene ketal (64 g., 0.45 mole) and water (340 ml.) containing 9 drops of concentrated hydrochloric acid in a 500 ml. flask was swirled occasionally during a period of two and one-half hours. The solution was then continuously extracted with ether for 18 hours. After drying over anhydrous magnesium sulfate the ether was evaporated at reduced pressure and the residue fractionated. After a small forerun, the product was collected, b.p. 65-68° at 20 mm. (reported 66-68° at 22 mm.); 28.6 g. (66%); $n_D^{25}$ = 1.4847 (reported $n_D^{25}$ = 1.4845)[25]; $\lambda_{max}$ = 226 mu; 2,4-dinitrophenylhydrazone, m.p. 165.5-167.2° (reported

m.p. 163°).[24] The overall yield from cyclohexanone was 31%.

(b) From 2-bromocyclohexanone. - 2-Bromocyclohexanone (130 g., 0.73 mole) was refluxed with 300 ml. of triethylamine for 12 hours. After cooling, triethylamine hydrochloride was removed by filtration (115.5 g., 87%) and excess triethylamine evaporated at reduced pressure. The residue was distilled, collecting the fraction, b.p. 57-60° at 15 mm., yield 62.5 g. (88%); $n_D^{25}$ = 1.4840; $\lambda_{max}$ = 226 mu; 2,4-dinitrophenylhydrazone, m.p. 165.9-167.2° (on admixture of the 2,4-dinitrophenylhydrazone of 2-cyclohexene-1-one made via the ethylene ketal, m.p. 165.2-166.0°). The overall yield from cyclohexanone was 67%.

Treatment of 2-Cyclohexene-1-one with Hydrazoic Acid. - A solution of hydrazoic acid (8.19 g., 0.19 mole) in 106 ml. of chloroform[29] was added dropwise over a period of one and three-quarter hours to a stirred mixture of 2-cyclohexene-1-one (19.2 g., 0.20 mole), concentrated sulfuric acid (70 ml.) and chloroform (200 ml.) in a 500 ml. 3-necked flask cooled in an ice-bath. After addition was complete, stirring was continued for an additional three hours, allowing the reaction to come to room temperature. The reaction mixture was poured onto ice, diluted with water and continuously extracted with chloroform for

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29. Reference 14, p. 327.

36 hours. After drying the chloroform layer over anhydrous magnesium sulfate, the chloroform was evaporated at reduced pressure and the product distilled, collecting the fraction, b.p. 75-78° at 20 mm., yield 12 g. (56%), $n_D^{24} = 1.5122$. The material solidified if cooled in a refrigerator.

Anal. Calcd. for $C_6H_8O_2$: C, 64.27; H, 7.19. Found: C, 64.37; H, 7.15.

The product reduced Fehling's solution and Tollen's reagent, gave a violet color with aqueous ferric chloride solution, and formed a diphenylhydrazone, m.p. 153.0-154.2° (reported 152-153°).[30]

Anal. Calcd. for $C_{18}H_{20}N_4$: C, 74.16; H, 6.91; N, 19.23. Found: C, 73.99; H, 6.92; N, 19.30.

The product formed a dioxime, m.p. 188.5-189.7° (reported 189-190°)[30] which formed a red precipitate with nickel salts. On admixture of an authentic sample of the dioxime of cyclohexadione-1,2[31] (m.p. 186-190°), the m.p. was 186.5-189.5°.

Anal. Calcd. for $C_6H_{10}O_2N_2$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.35; H, 7.07; N, 19.53.

The 2,4-dinitrophenylhydrazone has m.p. 227.0-227.5° (reported m.p. 227-228°).[32]

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30. O. Wallach, Ann., 437, 173 (1924).

31. The author wishes to express his gratitude to Prof. Harvey Diehl (Iowa State College) for supplying a sample of the dioxime of cyclohexadione-1,2 (nioxime).

32. F. Ramirez and A. F. Kirby, J. Am. Chem. Soc., 74, 4331 (1952).

When the conditions of this experiment were repeated without the use of hydrazoic acid, only starting material was recovered.

3-Azidocyclohexanone (XXII). - To a solution of 2-cyclohexene-1-one (11 g., 0.11 mole) in 17 ml. of glacial acetic acid was added a solution of sodium azide (11.2 g., 0.17 mole) in 45 ml. of water. The mixture was stored at room temperature for one and one-half hours with occasional shaking and then extracted with ether. The ethereal extracts were washed with 5% sodium carbonate solution and dried over anhydrous magnesium sulfate. After evaporation of the ether at reduced pressure, the residue was distilled, collecting the following fractions:

a. 2.24 g., b.p. up to 70° at 1.5 mm., mostly unreacted 2-cyclohexene-1-one.

b. 11.15 g. (88%), b.p. 71-73° at 1.1 mm., $n_D^{25} = 1.4923$. Infrared absorption spectrum showed strong absorption at 4.75 mu and 5.87 mu.

Anal. Calcd. for $C_6H_9ON_3$: C, 51.79; H, 6.52; N, 30.20. Found: C, 51.73; H, 6.61; N, 30.47.

Treatment of 3-Azidocyclohexanone under Conditions of the Schmidt Reaction. - A solution of 3-azidocyclohexanone (11.1 g., 0.08 mole) in 45 ml. of chloroform was added dropwise over a period of 75 minutes to a stirred mixture of 30 ml. of concentrated sulfuric acid and 100 ml.

of chloroform cooled in an ice-bath. After stirring for an additional three hours, allowing the reaction to come to room temperature, the reaction mixture was poured onto ice, diluted with water and continuously extracted with chloroform for 60 hours. After drying over anhydrous magnesium sulfate, the solvent was evaporated at reduced pressure and the product distilled, collecting the fraction, b.p. 60-62° at 20 mm., yield 3.9 g. (44%), $n_D^{25}$ = 1.5126. The infrared absorption spectrum showed the same absorption maxima shown by cyclohexadione-1,2. The material reduced Fehling's solution and gave a diphenylhydrazone, m.p. 152.0-152.9° (on admixture of the diphenylhydrazone of cyclohexadione-1,2, m.p. 151.5-153°), and 2,4-dinitrophenylhydrazone, m.p. 224-225°. The product was in all ways identical with cyclohexadione-1,2.

2-Chlorocyclohexenone (XXXV). - To 285 ml. of a solution of 0.35 M hypochlorous acid[33] was added 9.6 g. (0.1 mole) of cyclohexene-1-one. After storage at room temperature for two hours, the solution was saturated with sodium chloride and extracted with ether. After drying over anhydrous magnesium sulfate, the ether was evaporated at reduced pressure, leaving 13.4 g. of a yellow oil. This

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33. H. Gilman et al., "Organic Synthesis," Coll. Vol. I, John Wiley & Sons, Inc., N.Y., 1941, p. 158.

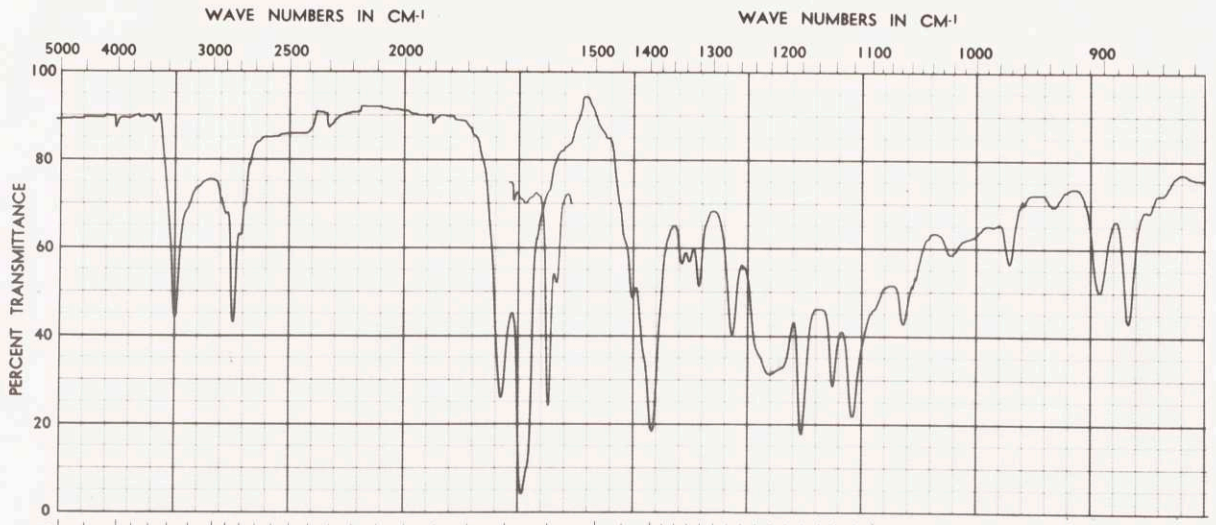
oil was warmed at 70° for 30 minutes with 30 ml. of 30% sulfuric acid. After cooling in a refrigerator overnight, the product was collected by filtration, washed with water and dried. The yield of tan platelets was 6.7 g. (51%), m.p. 62-70°, recrystallized from petroleum ether (30-60°), m.p. 72.2-73.5° (reported m.p. 70°).[19] Care must be used in handling this material because it causes severe burns if brought in contact with the skin.

Plate III

Curve G: Cyclohexadione-1,2 (XXIII), from the treatment of 2-cyclohexene-1-one with hydrazoic acid.

Curve H: 3-Azidocyclohexanone.

G


WAVE NUMBERS IN CM-1
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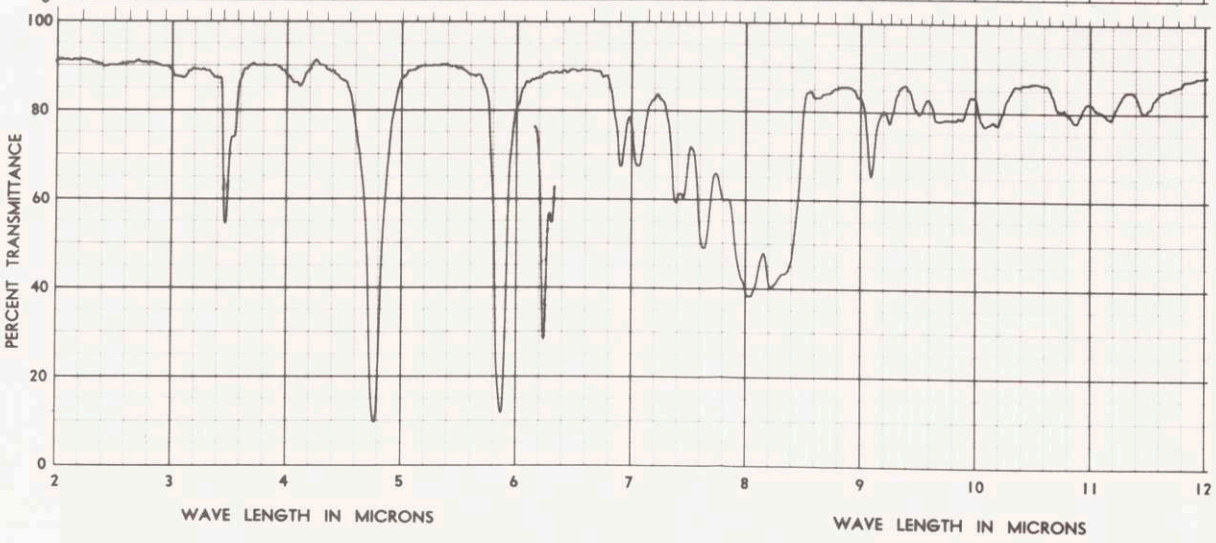
1500 1400 1300 1200 1100 1000 900
PERCENT TRANSMITTANCE
100
80
60
40
20
0

H


PERCENT TRANSMITTANCE
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WAVE LENGTH IN MICRONS

# SECTION C

# SYNTHESIS AND ATTEMPTED CYCLIZATION OF ETHYL 2-PYRROLIDINE ACETATE

# INTRODUCTION

In the reaction with alkali, the β-lactam portion of benzyl penicillin is hydrolyzed much more rapidly than model β-lactams that have been synthesized and subjected to hydrolysis rate studies.[34,35,36] Table I lists a few of the substituted β-lactams that have been synthesized, together with their relative rate constants in reactivity toward base. From this table it can be seen that none of the synthesized β-lactams approach benzyl penicillin's rapid reactivity toward base.

It is not known definitely what structural features of penicillin lead to this lability toward base. Woodward[34] mentions that the resonance form XXXVIII accounts for the stability of a normal amide bond.

XXXVII XXXVIII

In a β-lactam, such resonance is excluded because of the

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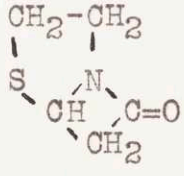
34. "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson and R. Robinson, editors, Princeton University Press, Princeton, N. J., 1949, pp. 443, 996.

35. A. D. Holley and R. W. Holley, J. Am. Chem. Soc., 73, 3172 (1951).

36. A. D. Holley and R. W. Holley, J. Am. Chem. Soc., 72, 2771 (1950).

necessity of the double bond's being in a four-membered ring. However, the β-lactam ring alone is not responsible for the labile character of penicillin, because desthiopenicillin is relatively resistant to cleavage. The increased reactivity of penicillin appears to be due either to the sulfur substituent or to fusion of the β-lactam with a five-membered ring, or to both. The synthesis and rate study of compound XLIII (Table I) shows it to be less reactive toward base than the corresponding desthio compound XLII. Therefore, the methylmercapto substituent present in XLIII does not facilitate the hydrolysis of the β-lactam.

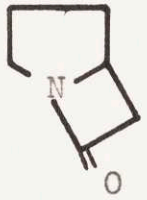
The fusion of the thiazolidine and β-lactam rings seems to be responsible for the reactivity of penicillin. Hydrolysis of compound XLI was shown to be relatively slow in comparison to penicillin,[36] but the methyl groups, not present in penicillin, were shown to decrease the rate of hydrolysis, i.e., XXXIX was much more reactive toward base than XL. Therefore, it may be inferred that the unknown β-lactam XLIV would be more reactive than 2-azetidinone



XLIV

toward alkali and that its reactivity would approach that of benzyl penicillin. This is consistant with the view that alkaline hydrolysis of benzyl penicillin is greatly facilitated by the sulfur bonded to the β-lactam and fusion of the β-lactam ring with a five-membered ring.

No reported compounds have been studied which incorporate the fusion of the β-lactam ring with a five-membered ring which does not contain sulfur. Such a compound should be studied to determine whether the lability of penicillin is due to the fused ring system in conjunction with the sulfur atom or whether it is due to the fused system alone. To learn which of these structural features is the more important, an investigation was undertaken to prepare the pyrrolidine-β-lactam XLV.




XLV

Table I

HYDROLYSIS RATE STUDIES OF VARIOUS β-LACTAM SYSTEMS

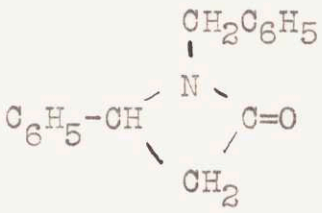
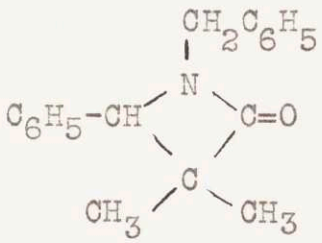
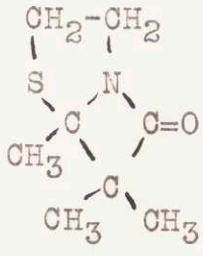
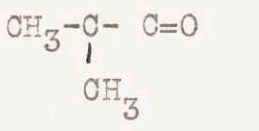
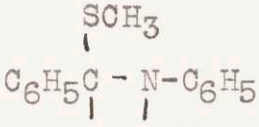
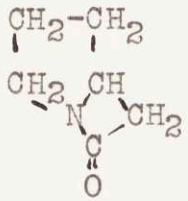
| Compound | Time | Temp. | Alkali % reacted | k x $10^2$ |
|---|---|---|---|---|
| Benzyl penicillin | 7 min. | 0° | 20 | 36.0 |
| | 25 " | 0° | 48 | 37.0 |
| 2-Azetidinone | 15 " | 50° | 48 | 13.0 |
| | 20 " | 50° | 56 | |
| | 1050 " | 0° | 38 | 0.12 |
| Desthiopenicillin | 30 " | 50° | 35 | 4.0 |
| | 60 " | 50° | 52 | 4.0 |
|  XXXIX | 180 " | 50° | 43 | 1.0 |
| | 300 " | 50° | 55 | |
|  XL | 2340 " | 50° | 24 | 0.04 |
| | 3900 " | 50° | 40 | |
|  XLI | 3300 " | 0° | 20 | 0.016 |
| | 10,000 " | 0° | 43 | 0.015 |

Table (Cont'd)

| Compound | Time | Temp. | Alkali % reacted | k x $10^2$ |
|---|---|---|---|---|
|  | 4 hrs. | | 13 | |
|  | 10.5 " | | 22.5 | |
| XLII | | | | |
|  | 21 hrs. | | 14 | |
| | 48 " | | 38 | |
| XLIII | | | | |

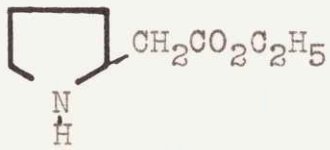
## DISCUSSION

In order to learn whether the alkaline lability of penicillin is due to a combination of the sulfur bonded to the β-lactam ring and the fusion of the β-lactam ring with a five-membered ring, or only to the fusion of the β-lactam ring with a five-membered ring, an investigation was begun to synthesize the pyrrolidine-β-lactam XLV.

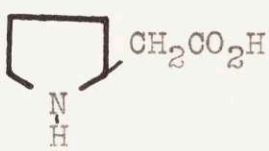


XLV

Of the known methods to prepare β-lactams, the ones which appeared most attractive to synthesize this pyrrolidine-β-lactam system involved ethyl 2-pyrrolidine acetate (XLVI) or pyrrolidine acetic acid (XLVII) as an intermediate.



XLVI



XLVII

The latter, XLVII, had been prepared[37] by a lengthy

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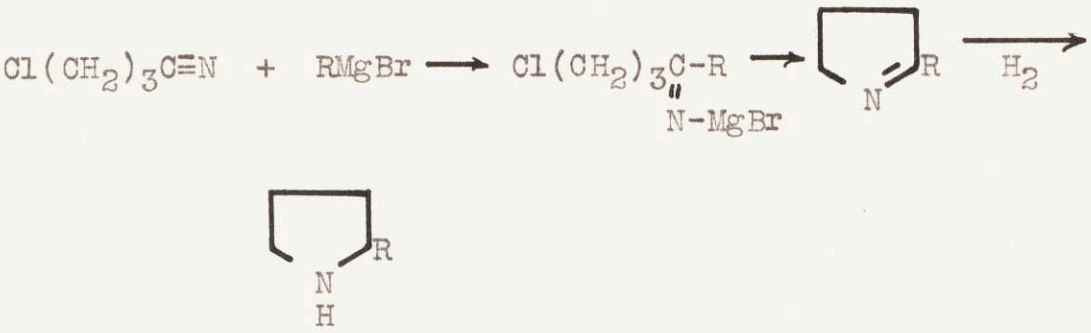
37. B. R. Baker, R. E. Schaub and J. H. Williams, J. Org. Chem., 17, 123 (1952).

nine-step synthesis involving a malonic ester intermediate. Rather than repeat this procedure, a new procedure was investigated.

Alkylated pyrrolidines have been prepared[38] by the action of a Grignard reagent on a γ-chloro nitrile.



Certain α-bromoesters have been shown to condense with nitriles under conditions of a Reformatsky reaction to give the corresponding β-keto ester.[39]

Several attempts were made to condense γ-chloro butyronitrile with ethyl bromoacetate under conditions of the Reformatsky reaction. Only a very poor yield of a β-keto ester was obtained. This route was therefore abandoned.

Several pyrrole derivatives have been treated with ethyl diazoacetate to yield the corresponding ethyl

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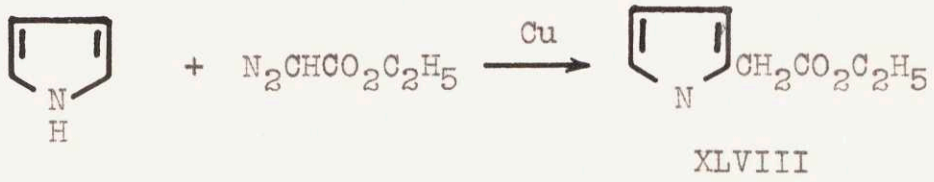
38. L. C. Craig, H. Bulbrock and R. M. Hixon, J. Am. Chem. Soc., 53, 1831 (1931).

39. A. Horeau and J. Jacques, Bull. soc. chim., 57, 58 (1947).

pyrrole acetate.[40,41,42] Ethyl pyrrole-2-acetate (XLVIII) was prepared by treating pyrrole with ethyl diazoacetate in the presence of copper powder.



XLVIII

That the addition takes place at the alpha position has been shown by hydrolysis and decarboxylation to a known methyl pyrrole derivative.[43]

The hydrogenation of the pyrrole nucleus is reported to be very difficult when the nitrogen atom is not substituted.[44,45] 1-Phenyl pyrrole and 1-carbethoxy pyrrole are reduced to the corresponding pyrrolidine much more easily than is pyrrole itself.[46] To facilitate hydrogenation, an attempt was made to prepare the carbethoxy

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40. G. R. Clemo and T. P. Metcalfe, J. Chem. Soc., 606 (1936).

41. W. E. Sohl and R. L. Shriner, J. Am. Chem. Soc., 55, 3823 (1933).

42. F. Blicke, R. J. Warzynski, J. A. Faust and J. E. Gearing, J. Am. Chem. Soc., 66, 1675 (1944).

43. C. D. Nenitzescu and E. Solomonica, Ber., 64B, 1924 (1931).

44. E. Ochiai, J. Pharm. Soc. Japan, 61, 298 (1941).

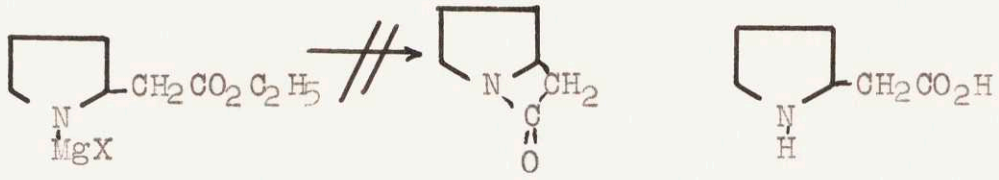
45. J. L. Rainey and H. Adkins, J. Am. Chem. Soc., 61, 1104 (1939).

46. H. Adkins and H. L. Coonradt, J. Am. Chem. Soc., 63, 1563 (1941).

derivative of ethyl 2-pyrrole acetate by condensing the sodium salt with ethyl chloro carbonate. The expected N-substituted product could not be isolated, probably owing to the benzenoid character of the pyrrole nucleus, thus making the α-hydrogen of the acetate portion of the molecule similar to the α-hydrogen of ethyl phenyl acetate. It is probably this hydrogen and/or rather than the amine hydrogen which reacts with sodium.

Ethyl pyrrole-2-acetate (XLVIII) was hydrogenated over platinum oxide in acetic acid to ethyl pyrrolidine-2-acetate, although in poor yield (30%). The observation was made that the acetate salt of the reduced pyrrolidine could be isolated in a pure state in satisfactory yield (70%) from the hydrogenation mixture, but it was the conversion of this to the free base which accounted for the poor overall yield.

Three attempts were made to cyclize the β-amino ester by methods known to produce monocyclic β-lactams. Ethyl pyrrolidine acetate was treated with an equivalent amount of ethyl magnesium bromide and also with methyl magnesium iodide. An addition complex was obtained, presumably XLIX, but this could not be made to react intramolecularly with the ester group.



XLIX XLX

A possible side reaction in the synthesis of β-lactams by this method is the enolization of the α-hydrogen of the ester followed by self-condensation. It has been reported[47] that diethylamino magnesium bromide causes self-condensation of ethyl propionate, with the formation of only a little amide.

The ester group was next hydrolyzed to the acid XLX and cyclizations to the β-lactam attempted with thionyl chloride and phosphorus oxychloride, reagents known to form monocyclic β-lactams and bicyclic β-lactams containing a thiazolidine-β-lactam ring system.[11,48,49] In each case only the amino acid hydrochloride could be isolated.

From these results, it is concluded that the fused pyrrolidine-β-lactam system would be highly strained, or at least, that the carboxyl and amine functions are not

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47. F. C. Frostick and C. R. Hauserm, J. Am. Chem. Soc., 71, 1350 (1949).

48. J. R. Courtright, S. M. Thesis, M.I.T., September, 1954.

49. R. J. Gaul, Ph.D. Thesis, M.I.T., June, 1954.

close enough in the molecule to allow an intramolecular reaction to take place readily. Since such a reaction does proceed if the pyrrolidine ring is replaced by a substituted thiazolidine ring,[49] the larger sulfur atom must bring the reaction centers closer together. Also, since in the bicyclic β-lactams formed, there have been alkyl groups in either or both of the rings, it may be necessary to have some crowding around the amine ester to bring the reacting groups closer.

## EXPERIMENTAL

Ethyl Glycinate Hydrochloride. - Anhydrous hydrogen chloride was passed through a stirred suspension of glycine (500 g., 6.66 moles) in 3000 ml. of absolute ethanol. After solution was complete (one to two hours) the alcohol was refluxed for three hours. After cooling in a refrigerator overnight, the product which crystallized was collected by filtration. The yield, collected in two crops, was 885 g. (95%), m.p. 140-144° (reported m.p. 142-143°).[50] This material was sufficiently pure for the next step.

Ethyl Diazoacetate. - The following procedure was found to be superior to that reported in "Organic Synthesis."[51] A solution of 140 g. (1 mole) of ethyl glycinate hydrochloride in 250 ml. of water was mixed with 600 ml. of methylene chloride in a 2-liter, 3-necked round bottomed flask, fitted with a stirrer, dropping funnel, thermometer and nitrogen inlet tube, and cooled to -5°. After flushing with nitrogen, an ice-cold solution of 83 g. (1.2 moles) of sodium nitrite in 250 ml. of water was added with stirring. The temperature was lowered to -9° and 95 g. of 5% by weight of

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50. R. Adams et al., "Organic Synthesis," Coll. Vol. II, John Wiley & Sons, Inc., N.Y., 1943, p. 311.

51. N. L. Drake et al., "Organic Synthesis," Vol. 24, John Wiley & Sons, Inc., N. Y., 1944, p. 56.

sulfuric acid added from a dropping funnel during a period of 3 minutes. The methylene chloride layer was separated and shaken with 1000 ml. of saturated bicarbonate solution until no acid remained. After drying over anhydrous magnesium sulfate, the methylene chloride was evaporated at reduced pressure and the remaining oil used for the next step; yield 107 g. (93%).

Ethyl Pyrrole-2-acetate (XLVIII). - To a stirred mixture of pyrrole (173 g., 2.58 moles) and finely divided copper powder (8 g.) heated to 100° was added dropwise ethyl diazoacetate (147 g., 1.29 moles) over a period of two hours. The temperature of the reaction mixture was kept between 100° and 110° by controlling the rate of addition. After stirring for an additional thirty minutes at 105°, the copper was removed by filtration and the dark filtrate distilled to give the following fractions:

a. 123 g. (71%) pyrrole, b.p. 64° at 20 mm.

b. 80.4 g. (70% based on reacted pyrrole) ethyl pyrrole-2-acetate, b.p. 61° at 0.25 mm. $n_D^{28}$ = 1.4919 (reported $n_D^{19}$ = 1.4962, b.p. 129° at 15 mm.);[43] saponification number 157 (calculated 153).

c. 10.3 g. (5.8%), assumed to be a disubstituted product, b.p. 130-136° at 0.6 mm., $n_D^{28}$ = 1.4890; saponification number 244 (calculated 239).

Anal. Calcd. for $C_8H_{11}O_2N$: C, 62.71; H, 7.23; N, 9.15. Found: C, 62.70; H, 7.23; N, 8.96.

Ethyl Pyrrolidine-2-acetate Acetate. - A solution of ethyl pyrrole-2-acetate (26.9 g., 0.17 mole) in 35 ml. of absolute ethanol and 35 ml. of glacial acetic acid was shaken for 24 hours with 0.4 g. of platinum oxide and 0.4 g. of charcoal in the presence of hydrogen initially at 350 lbs. per sq. in. Another 0.4 g. of platinum oxide was added and shaking continued for 60 hours. The liquid was filtered and the solvents removed at reduced pressure. The residue was distilled, collecting the fraction, b.p. 62-65° at 0.1 mm., yield 26.2 g. (71%), $n_D^{27} = 1.4700$.

Anal. Calcd. for $C_{10}H_{19}O_4N$: C, 55.28; H, 8.81; N, 6.49. Found: C, 54.91; H, 8.83; N, 6.14.

Ethyl Pyrrolidine-2-acetate. (a) Hydrogenation in ethanolic hydrogen chloride. - A solution of ethyl pyrrole-2-acetate (10 g., 0.065 mole) in 100 ml. of absolute ethanol and 6 ml. of concentrated hydrochloric acid was shaken for 23 hours with 0.2 g. of platinum oxide in the presence of hydrogen initially at 56 lbs. per sq. in. The filtered solution was evaporated at reduced pressure and the residue extracted with ether after adding a little dilute hydrochloric acid. Evaporation of the ethereal extracts gave no unchanged pyrrole ester. The aqueous solution was concentrated and the residue basified with

saturated potassium carbonate solution to pH 8 and extracted with ether. Evaporation of the ether and distillation of the residue afforded 4.28 g. (42%) of ethyl pyrrolidine-2-acetate, b.p. 103-104° at 20 mm., $n_D^{25} = 1.4477$.

(b) Hydrogenation in ethanolic glacial acetic acid. - A solution of ethyl pyrrole-2-acetate (10.6 g., 0.069 mole) in 10 ml. of absolute ethanol and 20 ml. of glacial acetic acid was shaken for 24 hours with 0.2 g. of platinum oxide and 0.1 g. of charcoal in the presence of hydrogen initially at 100 lbs. per sq. in. Another 0.2 g. of platinum oxide was then added and shaking continued for 24 hours. After filtration and evaporation of the solvents at reduced pressure, the residue was acidified with dilute hydrochloric acid and extracted with ether to remove any unchanged pyrrole ester. The aqueous solution was evaporated and basified to pH 8 with saturated potassium carbonate solution and extracted with ether; then basified to pH 9.5 with potassium hydroxide and again extracted with ether. After drying the combined ethereal extracts over anhydrous magnesium sulfate, the ether was evaporated and the residue distilled, collecting the fraction, b.p. 50-53° at 0.4 mm., yield 4.2 g. (39%)., $n_D^{22} = 1.4520$.

Anal. Calcd. for $C_8H_{15}O_2N$: C, 61.19; H, 9.62; N, 8.92. Found: C, 60.79; H, 9.68; N, 8.81.

Picronolate had m.p. 153.6-154.8° (reported m.p. 146°).[52]

Anal. Calcd. for $C_{18}H_{23}O_7N_5$: C, 51.30; H, 5.50; N, 16.62. Found: C, 51.50; H, 5.72; N, 16.65.

Pyrrolidine-2-acetic Acid (XLX). - A mixture of ethyl pyrrolidine-2-acetate (2.1 g., 0.0134 mole) and barium hydroxide octahydrate (4.42 g., 0.013 mole) in 80 ml. of water was refluxed for five hours. Dry ice was then added to the solution and after standing for several minutes the precipitated barium carbonate was removed by filtration and the filtrate evaporated to dryness at reduced pressure. The residue was treated with 50 ml. of hot, absolute ethanol and filtered. After concentration of the filtrate to about 10 ml., 30 ml. of acetone and 30 ml. of ether were added and the solution stored in a refrigerator. The yield of colorless crystals was 1.73 g. (100%), m.p. 164.5-166°, benzoyl derivative had m.p. 136-137° (reported m.p. 133-134°).[37]

Attempted Cyclization of Pyrrolidine-2-acetic Acid. (a) Treatment with thionyl chloride. - To a suspension of pyrrolidine-2-acetic acid (1.0 g., 7.75 millimoles) in 50 ml. of methylene chloride were added 10 ml. of purified thionyl chloride and the clear solution refluxed for one and one-half hours. After concentration at reduced pressure, the residue was flushed with benzene, and then dissolved in methylene chloride, washed with 5% sodium bicarbonate and dried over anhydrous magnesium sulfate. Evaporation of the methylene chloride left only

a small residue weighing about 20 mg., which was not purified further. The bicarbonate washings were evaporated at reduced pressure and the solid residue treated with 30 ml. of boiling ethanol and filtered. After evaporation of the ethanol at reduced pressure, the residue was dissolved in dilute hydrochloric acid and evaporated. The solid residue was crystallized from a mixture of ethanol, acetone and ether. The yield of colorless crystals was 1.06 g. (84%), m.p. 175.5-176.8°.

Anal. Calcd. for $C_6H_{12}O_2NCl$: C, 43.51, H, 7.31; N, 8.46. Found: C, 43.87; H, 7.45; N, 8.82.

(b) Treatment with phosphorus oxychloride. - Phosphorus oxychloride (1.1 ml.) was added to a suspension of pyrrolidine-2-acetic acid (0.30 g., 2.3 millimoles) in 10 ml. of dry methylene chloride. After refluxing on a steam cone for thirty minutes, the solvent and excess phosphorus oxychloride were removed at reduced pressure. The residue was almost totally dissolved in 10 ml. of water. The water was evaporated at reduced pressure and the residue crystallized from ethanol-ether. The yield of colorless crystals was 0.25 g., m.p. 172-175°. The material was soluble in water, giving a precipitate with aqueous silver nitrate.

Attempted Cyclization of Ethyl Pyrrolidine-2-acetate. - To a solution of ethyl pyrrolidine-2-acetate (8.10 g., 0.051 mole) in a mixture of 50 ml. of benzene

and 50 ml. of ether were added dropwise 25.5 ml. of a 2.0 N solution of methyl magnesium iodide. After stirring for an additional hour the magnesium compounds were decomposed by the addition of 10% ammonium chloride solution. The aqueous solution was separated and extracted with methylene chloride. The combined organic layers were washed with 2 N hydrochloric acid and 5% sodium bicarbonate and dried over anhydrous magnesium sulfate. Evaporation of the solvent at reduced pressure left only a small residue which was not further purified. The acid washings were made basic with saturated potassium carbonate solution and extracted with methylene chloride. Evaporation of the methylene chloride at reduced pressure left 1.0 g. of a dark oil which was not purified further. Infrared absorption spectrum of this crude oil showed no absorption between 5.00 mu and 5.90 mu, indicating the absence of a β-lactam group.

# PART II

## ALKYL PHOSPHATES AND THIOPHOSPHATES

# INTRODUCTION

In 1946, a new cofactor necessary for the acetylation of sulfanilamide and choline in biological systems was reported.[1,2] Purification and structural studies[3,4,5] on the coenzyme indicated that it contained pantothenic acid, adenine and thioethanolamine in the molecule. Further study[6] showed that one phosphorus was present in the molecule as a monoester, while two others probably formed a pyrophosphate bridge. It was also found[7,8,9] that enzymatic cleavage of the coenzyme yielded a phosphorus-free fraction which was identified as the Lactobacillus bulgaricus factor found in biological systems, and a phosphorus-containing fraction which was similar in biological activity but was also a growth factor for Acetobacter suboxydans. This general

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1. F. Lipmann, J. Biol. Chem., 160, 173 (1945).
2. F. Lipmann and N. O. Kaplan, J. Biol. Chem., 162,743 (1946).
3. F. Lipmann, N. O. Kaplan, G. Novelli, N. Tuttle and B. Guirard, J. Biol. Chem., 167, 869 (1947).
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6. G. Novelli, J. Gregory, R. Flynn and F. Schmetz, Fed. Proc., 10, 229 (1951).
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8. J. Baddiley and E. Thain, J. Chem. Soc., 3783 (1952).
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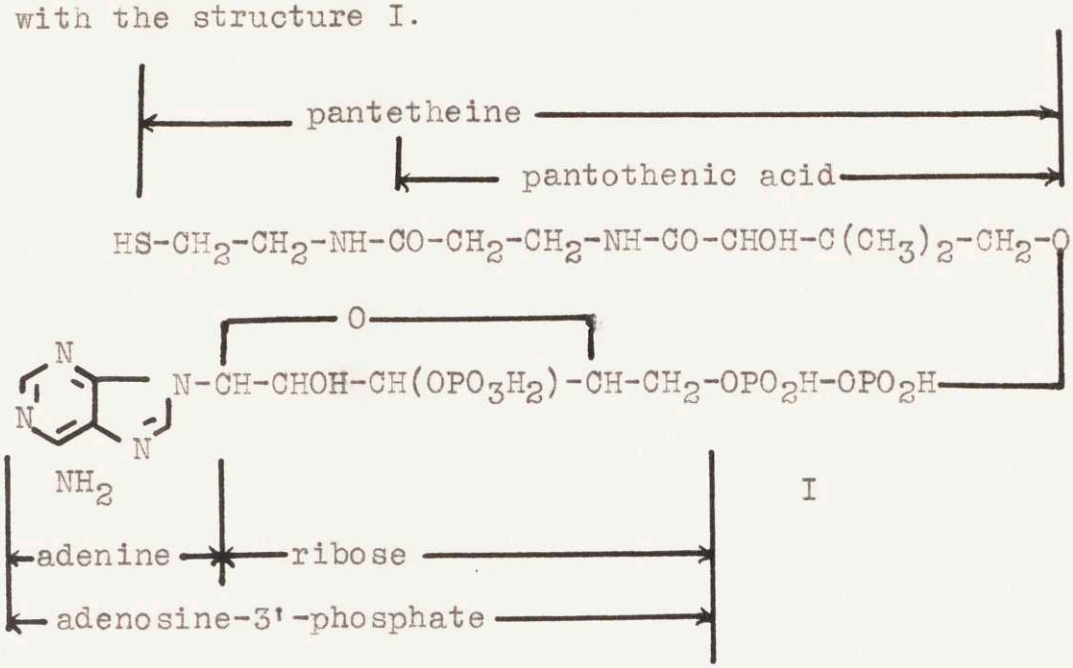
chemical and biological evidence is in agreement[9,10] with the structure I.




I

An independent synthesis[11] proved the pantetheine portion of coenzyme A.

Coenzyme A is known to act as an "active acetate" source in biological systems. An abundance of indirect evidence has shown that acetyl-coenzyme A serves as the intermediate acetyl group carrier between various acetyl acceptor enzyme systems. Direct proof for the existence of the hypothetical acetyl-coenzyme was obtained when Lynen[12] succeeded is isolating this substance from yeast

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10. G. Novelli, F. Schmetz and N. Kaplan, J. Biol. Chem., 206, 533 (1954).

11. E. Snell et al., J. Am. Chem. Soc., 72, 5349 (1950).

12. F. Lynen and E. Reichert, Angew. Chem., 63, 47 (1951).

juice. Lynen further showed that the acylating activity of the coenzyme involves the terminal thiol group. He suggests that the coenzyme accepts an acetyl group at this thiol group and transfers it to the substrate. In its S-acetyl form, coenzyme A presumably contains the acetyl in an "energy rich" state. This discovery has led to the preparation and study of model S-acetyl compounds including derivatives of S-acetylthioethylamines,[13] S-acetylpanthetheine,[14-17] and acetyl-coenzyme itself.[18] S-Acetylthioethanolamine was shown[13] to be an active acetylating agent, e.g., the acetyl group reacted with hydroxylamine to give acethydroxamic acid, thus supporting the suggestion by Lynen that coenzyme A brings about acetylation by acetyl transfer through its thiol group.

Equilibrium data[19] have shown that the energy of the acetyl-S bond in acetyl-coenzyme A is about 10,000-

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13. J. Baddiley and E. Thain, J. Chem. Soc., 3425 (1951).
14. E. Walton, A. Wilson, F. Holly and K. Folkers, J. Am. Chem. Soc., 76, 1146 (1954).
15. T. King, C. Stewart and V. Cheldelin, Science, 117, 439 (1953).
16. J. Baddiley and E. Thain, Science, 117, 439 (1953).
17. R. Schwyzer, Helv. Chim. Acta, 35, 1903 (1953).
18. I. Wilson, J. Am. Chem. Soc., 74, 3205 (1952).
19. J. Stern, S. Ochoa and F. Lynen, J. Biol. Chem., 198, 313 (1952).

12,000 calories. It has not been ascertained how this "high energy" bond is formed in biological systems. Its formation must be in combination with other high-energy-yielding reactions. The driving force for the formation of acetyl-coenzyme A is attributed to the free energy supplied by intermediate phosphorylated compounds. The postulated mechanism involving adenosine triphosphate (ATP) is[20]:

1. ATP + Co A $\longrightarrow$ ADP + Co A-phosphate
2. Co A-phosphate + acetate $\longrightarrow$ acetyl-Co A phosphate

Acetyl-coenzyme A then plays an important part in numerous biological reactions.[21] To support the above mechanism postulated by Lynen, it would be desirable to isolate the intermediate phosphorylated coenzyme A. Alternatively, if a phosphorylated sulfhydryl compound could be prepared, reaction 2 could then be tested by studying the conversion to the corresponding thioacetyl compound.

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20. F. Lynen, E. Reichert and L. Rueff, Ann., 574, 1 (1951).

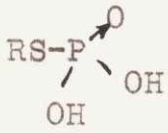
21. E. Stadtman, J. Cellular Comp. Physiol., 41, suppl. 1, 89 (1953).

## DISCUSSION

The isolation of thiolacetyl-coenzyme A[12] has shown that the thiol group of coenzyme A is involved in transferring the acetyl function required in various biological reactions. The mode of formation of this thioester is unknown, but intermediate thiophosphorylated compounds have been proposed.[20]

$$\text{Co A-SH} + HPO_4^{=} \longrightarrow \text{Co A-S}\sim PO_3^{=} + H_2O$$

These thiophosphates have not been isolated or synthetically prepared in pure form. A program was initiated to prepare a compound of structure II and to study its stability and reactivity. Alternatively, the di- and tri-alkyl thiophosphates would be of some interest.



II

The methods which appeared most attractive to synthesize a compound having structure II involved treating a mercaptan or its sodium salt with phosphorus oxychloride, and then hydrolyzing the dichlorothiophosphate.

$$RSH + POCl_3 \xrightarrow[2.\ H_2O]{1.\ \text{Base}} (HO)_2P(=O)SR + \text{Base}\cdot HCl$$

$$RSNa + POCl_3 \xrightarrow{H_2O} (HO)_2P(=O)SR + 2\ HCl + NaCl$$

Since mono-, di- and trisubstituted phosphates are possible in these reactions, it was decided to form the trisubstituted thiophosphates first, and then to proceed to prepare the mono- and disubstituted phosphates.

Treatment of phosphorus trichloride with three equivalents of ethyl mercaptan in the presence of dimethylaniline produced trithioethyl phosphite in 92% yield. The reaction proceeded smoothly, and the product was isolated after a reaction time of 5-10 hours. Under similar conditions, treatment of phosphorus oxychloride with three equivalents of ethyl mercaptan in the presence of dimethylaniline produced no pure product. A 40% yield of trithioethyl phosphate was obtained by this reaction after storage at room temperature for three months.

To prepare the latter compound in better yield, it was found necessary to treat phosphorus oxychloride with sodium ethyl mercaptan -- prepared by treating ethyl mercaptan in ethereal solution with metallic sodium. In this manner an 80% yield of trithioethyl phosphate was obtained. Trithioethyl phosphate could also be obtained

by oxidation of the phosphite with a 3% solution of hydrogen peroxide in acetic acid.

Under similar conditions, treatment of phosphorus trichloride with two equivalents of ethyl mercaptan in the presence of dimethylaniline, diethylaniline or triethylamine did not produce any pure disubstituted phosphite. Only the trisubstituted compound was obtained. Also, treatment of phosphorus oxychloride with two equivalents of ethyl mercaptan did not give any isolatable quantity of disubstituted phosphate. The use of one equivalent of the mercaptan, or of one equivalent of the sodium mercaptan similarly offered only the trisubstituted compound. The mono- and disubstituted compounds must be substantially more reactive with the mercaptan than is the unsubstituted chlorophosphate.

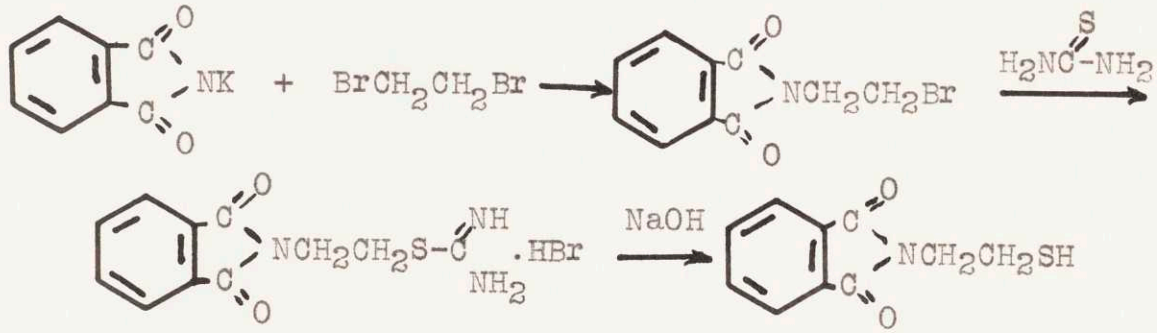
When ethyl alcohol was substituted for ethyl mercaptan, the intermediate mono- and diethyl chlorophosphates could be isolated easily, but when the same conditions were applied to ethyl mercaptan, the only product isolated was a small amount of trithioethyl phosphate. Up to 85% of unreacted ethyl mercaptan could be accounted for by passing the volatile material through a standard iodine solution.

In these experiments using phosphorus oxychloride as an acylating agent, no starting material could be recovered by distillation because of the small amounts used and because of the ready hydrolysis to nondistillable

phosphoric acid. To overcome this difficulty, diethyl chlorophosphate was prepared and used as an acylating agent. In this case after the reaction had taken place, there would be no remaining active chlorine atoms.

On treatment of diethyl chlorophosphate with ethyl mercaptan in the presence of triethylamine, only starting material was recovered. There was no appearance of triethylamine hydrochloride. On treatment of diethyl chlorophosphate with sodium ethyl mercaptan, using ethyl alcohol as a solvent, the only product isolated was triethyl phosphate. The solvent reacted in preference to the mercaptan. Using dimethylformamide as a solvent, no product was isolated.

Since ethyl mercaptan had failed in all attempts to prepare a mono-thiophosphate or dichloro-mono-thiophosphate, another mercaptan was synthesized and subjected to acylating conditions. Phthalimido ethyl mercaptan (III), a solid mercaptan whose formula resembles the thioethylamine portion of coenzyme A, was synthesized according to the following scheme:




III

On treating phthalimido ethyl mercaptan (III) with phosphorus oxychloride in the presence of triethylamine, only starting material was recovered. Similarly, on treating this mercaptan with diethyl chlorophosphate in the presence of triethylamine, there was no formation of triethylamine hydrochloride, and only starting material was recovered. Under similar reaction conditions -- on the treatment of phthalimodo ethyl mercaptan with acetyl chloride or acetic anhydride as the acylating agent, the mercaptan could be acylated easily, producing phthalimido ethyl thioacetic acid. This result shows that the mercaptan is not oxidized to the disulfide, but is still present as the free mercaptan.

In conclusion, the experimental results which have been obtained in this program show that a mono-thiophosphate, either as the free acid or as a substituted phosphate, could not be prepared readily by treating two different mercaptans, one a solid and one a liquid, with phosphorus oxychloride or diethyl chlorophosphate in the presence of a tertiary base as acid acceptor. Likewise, the sodium salt of ethyl mercaptan was not useful in preparing a substituted mono-thiophosphate since a convenient, inert solvent could not be found. However, improved techniques for preparing trialkylthiophosphates and alkylchlorophosphate were developed.

Following the termination of this program, results have been published[22] which repudiate the existence of a phosphorylated coenzyme A as the intermediate possessing the high energy bond which leads to acetyl-coenzyme A. The reaction appears to involve a pyrophosphate split of adenosine triphosphate (ATP), leaving adenosine monophosphate (AMP) and not adenosine diphosphate, as the product of the reaction.

ATP + Co A + acetate $\longrightarrow$ AMP + acetyl-Co A + pyrophosphate

Whereas earlier experiments[23] seemed to support the assumption of coenzyme A phosphate as an intermediate, recent results[22] still leave open the alternative of an enzyme-bound intermediate. Acetyl phosphate was used with transacetylase as acetyl feeder to coenzyme A. The thus-formed acetyl-coenzyme A reacted with AMP and pyrophosphate to form ATP.

1. acetyl-phosphate + Co A $\underset{\text{transacetylase}}{\rightleftharpoons}$ acetyl-Co A

2. acetyl-Co A + pyrophosphate AMP $\longrightarrow$ ATP Co A acetate

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22. F. Lipmann, M. Jones, S. Black and R. Flynn, J. Cellular Comp. Physiol., 41, suppl. 1, 109 (1953).

23. F. Lipmann, M. Jones, S. Black and R. Flynn, J. Am. Chem. Soc., 74, 2384 (1952).

Experiments show that, by way of acetyl-coenzyme A and pyrophosphate, energy-rich acyl bonds may be converted rather easily to energy-rich phosphate bonds of ATP, and not the reverse, as was earlier postulated. The ATP can then be utilized to form acetyl phosphate used in equation 1, thus completing the cycle. The likelihood that acetyl phosphate is the energy-rich intermediate in forming acetyl-coenzyme A has been further advanced by Stadtman.[21]

## EXPERIMENTAL

Trithioethylphosphite. (a) From ethyl mercaptan. - Phosphorus trichloride (3.43 g., 0.025 mole) in 20 ml. of anhydrous ether was added dropwise over a period of 30 minutes to a stirred solution of ethyl mercaptan (4.66 g., 0.075 mole) and dimethylaniline (9.07 g., 0.075 mole) in 50 ml. of anhydrous ether. Stirring was continued for 45 minutes, and the solid dimethylaniline hydrochloride removed by filtration (10.8 g., 91%). The filtrate was washed with dilute hydrochloric acid, water and dried over anhydrous magnesium sulfate. Evaporation of the ether at reduced pressure left 4.8 g. (90%) of colorless liquid. Distillation gave 3.0 g., b.p. 96-102° at 1 mm., $n_D^{24} = 1.5822$ (reported b.p. 140-143 at 18 mm., $n_D^{25} = 1.5689$).[24]

Anal. Calcd. for $C_6H_{15}S_3P$: C, 33.25; H, 7.03. Found: C, 33.62; H, 7.05.

(b) From sodium ethyl mercaptan. - Phosphorus trichloride (10.29 g., 0.075 mole) in 50 ml. of dry ether was added dropwise to a stirred suspension of sodium ethyl mercaptan (18.93 g., 0.225 mole) in 200 ml. of dry ether. After stirring overnight, the solid was removed by filtration, and the filtrate washed with water and dried over anhydrous

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24. A. Lippert and E. Reid, J. Am. Chem. Soc., 60, 2370 (1938).

magnesium sulfate. Evaporation of the ether at reduced pressure gave 14.1 g. (88%) of a yellow liquid, which was distilled, b.p. 82-83° at 0.4 mm., $n_D^{25}$ = 1.5852.

Trithioethylphosphate. - Phosphorus oxychloride (11.49 g., 0.075 mole) in 50 ml. of dry ether was added dropwise to a stirred suspension of sodium ethyl mercaptan (18.93 g., 0.225 mole) in 200 ml. of dry ether. After storage overnight, sodium chloride was removed by filtration, and the filtrate washed with water and dried over anhydrous magnesium sulfate. Evaporation of the ether at reduced pressure left 13.8 g. (80%) of colorless liquid, b.p. 93-102° at 0.25 mm., $n_D^{24}$ = 1.5677 (reported b.p. 165-168° at 15 mm.).[24]

Anal. Calcd. for $C_6H_{15}OPS_3$: C, 31.28; H, 6.56. Found: C, 31.62; H, 6.79.

Oxidation of Trithioethylphosphite to Trithioethylphosphate. - Trithioethylphosphite (5.0 g., 0.023 mole) and 30 ml. of 3% hydrogen peroxide were dissolved in 100 ml. of glacial acetic acid and the resulting solution stirred at room temperature for 14 hours. The solution was neutralized with concentrated ammonium hydroxide and the oil which formed was extracted with ether. After drying over anhydrous magnesium sulfate, the ether was evaporated at reduced pressure. The residue was distilled, yielding a fraction of b.p. 90-93° at 0.24 mm., yield 4.5 g. (84%), $n_D^{24}$ = 1.5677.

Ethyl Dichlorophosphite. - Ethyl alcohol (3.75 g., 0.081 mole) in 50 ml. of dry ether was added dropwise over a period of 30 minutes to a stirred solution of phosphorus trichloride (11.21 g., 0.081 mole) in 50 ml. of dry ether cooled to 0-5°. After stirring for an additional hour, ether was removed at reduced pressure and the product distilled, yielding a fraction of b.p. 38-45° at 47 mm. $n_D^{25}$ = 1.4612 (reported $n_D^{24.5}$ = 1.4610),[25] 6.62 g. (55%).

Diethylchlorophosphate. - A solution of triethylamine (202 g., 2.0 mole) and ethyl alcohol (92 g., 2.0 mole) in 100 ml. of dry ether was added dropwise to a solution of phosphorus oxychloride (153.3 g., 1.0 mole) in 700 ml. of dry ether. After stirring overnight, triethylamine hydrochloride was removed by filtration and the filtrate evaporated at reduced pressure and the residue distilled, yielding a fraction of b.p. 56° at 1.0 mm. (reported 61-63° at 2.5 mm.),[26] $n_D^{24}$ = 1.4162.

Anal. Calcd. for $C_4H_{10}O_3PCl$: C, 27.84; H, 5.85; Cl, 20.55. Found: C, 27.59; H, 5.92; Cl, 20.76.

Ethyldichlorophosphate. - Ethyl alcohol (1.84 g., 0.04 mole) in 20 ml. of dry ether was added dropwise to

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25. F. Zecchini, Gazz. Chim. ital., 24, I, 34 (1894).

26. T. W. Martin, G. R. Norman and E. A. Weilmuenster, J. Am. Chem. Soc., 67, 1662 (1945).

a stirred solution of phosphorus oxychloride (6.14 g., 0.04 mole) in 30 ml. of dry ether at 0-5°. After stirring for two hours, ether was removed at reduced pressure and the product distilled, yielding 5.4 g. (83%) of a colorless liquid, b.p. 41° at 1.3 mm. (reported 64° at 10 mm.),[27] $n_D^{27}$ = 1.4321.

Phthalimido Ethyl Mercaptan. - Sodium hydroxide (0.6 g., 0.151 mole) in 40 ml. of water was added dropwise through a high dilution cycle over a period of four hours to a stirred, refluxing solution of β-isothiourea ethyl phthalimide hydrobromide[28] (5.0 g., 0.151 mole) in 30 ml. of water and 50 ml. of methanol. Methanol was evaporated at reduced pressure, and the residue extracted with benzene. After drying over anhydrous magnesium sulfate the benzene was evaporated at reduced pressure. The oily residue was triturated with petroleum ether to yield 2.4 g. of a colorless solid.

Evaporation of the aqueous solution left an oily residue which, after extraction with benzene and treatment with petroleum ether as before, afforded an additional 0.4 g., making the total yield 2.8 g. (89%).

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27. B. C. Saunders, G. J. Stacey, F. Wild and E. Wilding, J. Chem. Soc., 699 (1948).

28. J. Baddiley and E. M. Thain, J. Chem. Soc., 2258 (1951).

The solid was distilled at 120° and 1 mm. affording a colorless solid, m.p. 61-71°. After one crystallization from petroleum ether (30-60°) the melting point was 78.6-79.5° (reported 78-79°).[28]

Anal. Calcd. for $C_{10}H_9O_2NS$: C, 57.95; H, 4.38; N, 6.76. Found: C, 58.17; H, 4.52; N, 6.56.

β-Thioacetyl Ethyl Phthalimide. - Acetyl chloride (0.85 ml., 11.9 millimoles) was added to a solution of β-phthalimido ethyl mercaptan (1.0 g., 4.82 millimoles) and triethylamine (1.2 g., 11.9 millimoles) in 20 ml. of dry benzene. After removal of the triethylamine hydrochloride, the solvent was evaporated at reduced pressure and the residue crystallized from methanol, affording 0.9 g. (83%), m.p. 113-114°.

Anal. Calcd. for $C_{12}H_{11}O_3NS$: C, 57.81; H, 4.44; N, 5.62. Found: C, 58.00; H, 4.43; N, 5.45.

Treatment of Diethylchlorophosphate with Sodium Ethyl Mercaptan in Ethyl Alcohol. - A solution of sodium ethyl mercaptan (12.6 g., 0.15 mole) in 100 ml. of ethyl alcohol was added to a solution of diethylchlorophosphate (25.9 g., 0.15 mole) in 50 ml. of ethanol alcohol. After stirring overnight, the solid was removed by filtration and the filtrate distilled, collecting the fraction, b.p. 60-75° at 1 mm., yield 20.8 g. (77%), $n_D^{26} = 1.4085$, (reported b.p. 77-79° at 2 mm., $n_D^{20} = 1.4063$ for triethylphosphate).[29] It is concluded that the product is triethylphosphate.

29. A. E. Arbuzov and B. A. Arbuzov, J. prakt. Chem., 130, 110 (1931).

## BIOGRAPHICAL NOTE

The author was born on May 27, 1929, in Collingswood, New Jersey. He attended elementary schools in that city and graduated from Collingswood High School in June, 1946. In September of that year he entered the Massachusetts Institute of Technology, from which he received the Bachelor of Science Degree in June, 1950. After receiving the Master of Science Degree from the University of Michigan in September, 1951, he was admitted to the Graduate School of Chemistry at the Massachusetts Institute of Technology in September, 1951, and completed the requirements for the Ph.D. Degree in February, 1955.