SYNTHETIC STUDIES RELATED TO PENICILLIN

by

David A. Johnson

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July, 1952

Signature of Author .....

Signature of Research Supervisor .....

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

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David A. Johnson

Submitted for the degree of Doctor of Philosophy
in the Department of Chemistry on July 18, 1952.

Abstract

By a cyclization procedure, a thiazolidine-β-lactam has been synthesized, which has the complete structure of the natural penicillins, except for the substitution of a phthalimido group for the phenylacetylamino side chain. The cyclization was possible because azlactonization was precluded by the presence of the phthaloyl blocking function on the amino group of a -methyl penicilloate. The required free acid was prepared from penicillamine and t-butyl phththalimidoacetate by a four-step synthesis.

The sodium salt of α-benzyl-α-thiol-DL-benzylpenicilloate was prepared and subjected to thermal treatment in an effort to obtain the sodium salt of benzylpenicillin by elimination of benzyl mercaptan. No pure products were identified from this reaction. Preliminary measurements for biological activity indicated slight activity or no activity.

A new thiazolidine-β-lactam derived from cysteine has been prepared in 34% yield by an extension of the reaction of a diacylamino acid chloride with a thiazoline.

This doctoral thesis
has been examined by a Committee
of the Department of Chemistry
as follows:

Professor George H. Buchi, Chairman.....

Professor John C. Sheehan, Research Supervisor.....

Professor C. Gardner Swain.....

Professor David N. Hume.....

Professor Clark C. Stephenson.....

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Financial assistance is gratefully acknowledged from the Department of Chemistry of The Massachusetts Institute of Technology, from Bristol Laboratories Inc., Syracuse, New York and from The Du Pont Company, Wilmington, Delaware.

Bristol Laboratories is also to be credited for the numerous bioassays.

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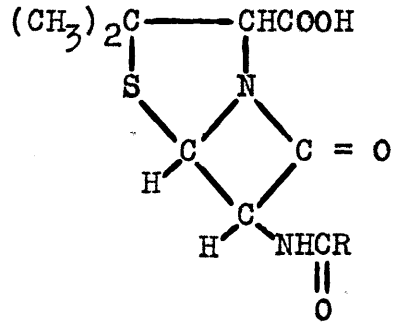
Part I

ATTEMPTED SYNTHESES OF THIAZOLIDINE-β-LACTAMS BY RING CLOSURE OF THE β-LACTAM

INTRODUCTION

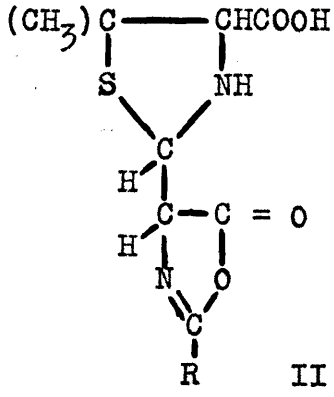
This portion of the thesis deals with two new attempts to prepare the fused thiazolidine-β-lactam nucleus of penicillin by ring closure formation of the β-lactam in the last step.

Penicillin was first discovered by Fleming in 1929,[1] but it was not until early in the 1940's that significant advances were made toward elucidation of its structure. Recognition of the possible military importance of penicillin in 1943 initiated a cooperative effort by British and American academic and industrial laboratories, unparallelled in chemical investigation. During the course of this work, many formulas were suggested, but the possibilities were soon reduced to the following three (I,II,III).



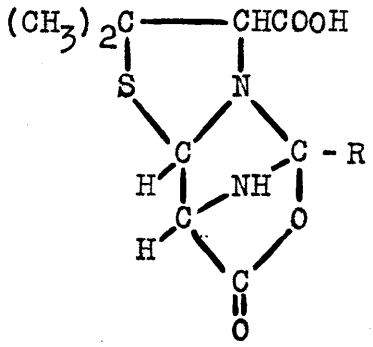
I

β-Lactam Formula



II

Azlactone Formula



III

Tricyclic Formula

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1. A. Fleming, Brit. J. Exp. Path., 10, 226 (1929).

Evidence for their support and reports of the subsequent physical and chemical findings which ultimately led to the general acceptance of the β-lactam formula for penicillin can be found in two comprehensive reviews.[2,3]

Having determined the structure with as much certainty as attends degradative methods, the next logical step was a rational synthesis. The synthesis of penicillin had become of great importance since the sudden demand created by the widespread application of the antibiotic in medicine could not be satisfied immediately by the fermentation sources. The importance of a synthesis of penicillin is further evidenced by the great number of attempts to prepare a compound having structure II before later investigations indicated structure I as the preferred possibility.[4]

The first isolation of a pure synthetic penicillin is credited to Du Vigneaud and his collaborators.[5] They were able to isolate the crystalline triethylammonium salt of benzylpenicillin in minute yield (0.02%) from the reaction

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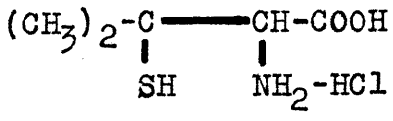
2. H. T. Clarke, J. R. Johnson and R. Robinson, editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N.J.(1949).

3. H. W. Florey, E. Chain, N. C. Heatley, M. A. Jennings, A. G. Saunders, E. P. Abraham and M. E. Florey, editors, "Antibiotics," Oxford University Press, London (1949), E. Chain, Volume II, Chapter 28, p. 957.

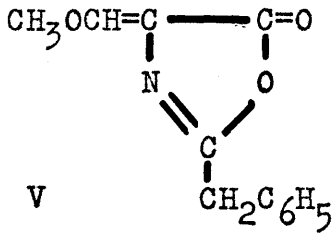
4. W. E. Bachmann and M. W. Cronyn, Reference 2, Chapter XXII, p. 867.

5. V. Du Vigneaud, F. H. Carpenter, R. W. Holley, A. H. Livermore and J. R. Rachele, Reference 2, Chapter XXVIII, p.1018.

products of penicillamine hydrochloride (IV) and 2-benzyl-4-methoxymethylene-5-oxazolone (V). Intensive efforts to improve the reaction failed to increase the yield significantly.

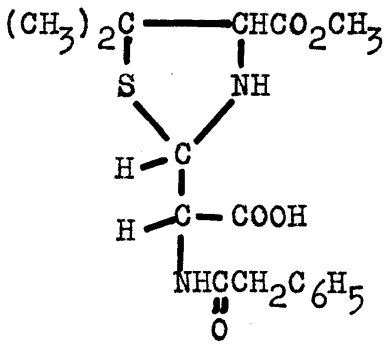


IV

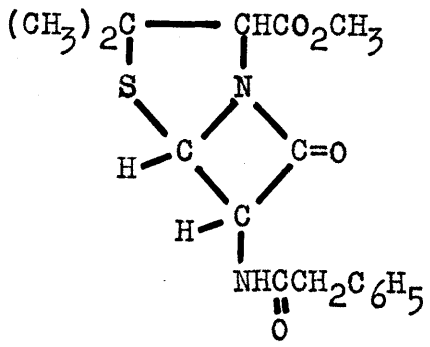


V

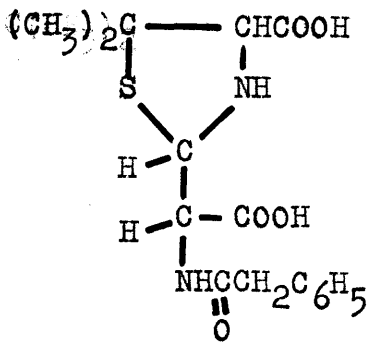
The most frequently used intermediate for the numerous attempted syntheses of either the β-lactam (I) or the azlactone (II) formula has been β-methyl-α-benzylpenicilloate (VI). The methyl ester of VI had been prepared synthetically and shown to be identical with the methanol inactivation product of methyl benzylpenicillinate (VII). Numerous dehydrating or acid chloride forming agents were applied to VI in attempts to eliminate the elements of water. The crude products were then tested for penicillin by biological assay.[6]



VI



VII



VIII

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6. Reference 4, p. 861.

In several instances, traces of activity were reported. Recently, a second isolation of a synthetic penicillin was reportedly obtained from the products of the reaction of phosphorous trichloride on the diacid VIII.[7] The yield in this case was also extremely small.

While the synthetic approaches to penicillin were being exhausted, great advances were being made in the fermentation process, so that it would now be difficult for a synthetic method to offer serious competition. However, in view of the small yields from the two reported syntheses, a rational synthesis in fair yield remains of interest as a final proof of structure and also as a possible opening to structural analogs possessing biological activity.

There are two serious handicaps in attempting to prepare I from VI. The first is that the acid decarboxylates readily even under mild conditions.[8] Secondly, formation of the azlactone structure (II) with immediate isomerization to the penicillenate (IX) may account for the failure to obtain the β-lactam (I). In a few cases where products of the reaction were characterized, they were found to be penicillenates (IX).[9] The oxazolidonethiazolidine structure (II) is believed to be only a transitory intermediate.[10,11]

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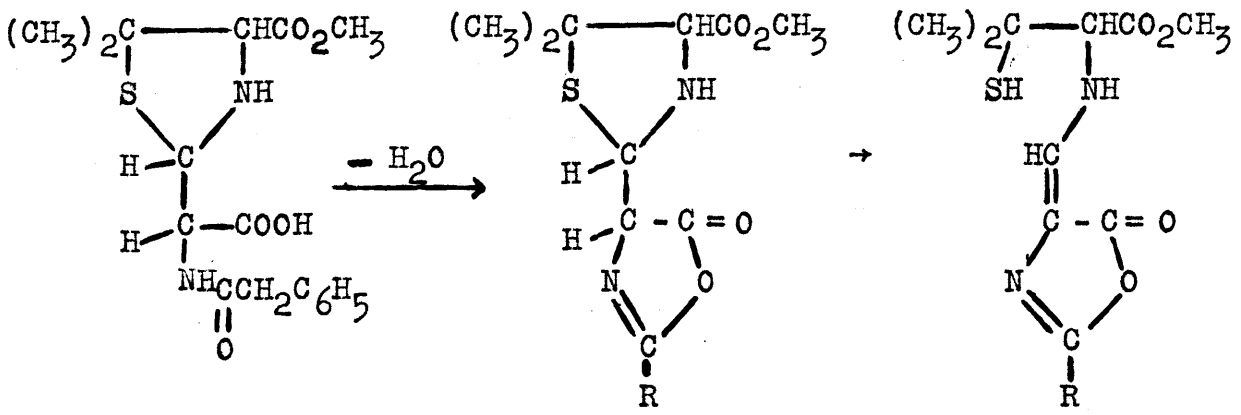
7. O. Sus. Ann., 571, 201 (1951).
8. R. Mozingo and K. Folkers, Reference 2, Chapter XVIII, p.605.
9. Reference 4, p. 851.
10. J. H. Hunter, J. W. Hinman and H. E. Carter, Reference 2, Chapter XXIV, p. 909.
11. J. R. Johnson, R. B. Woodward, and R. Robinson, Reference 2, Chapter XV, p. 440.



VI II IX

These results are not surprising, since the energy of the transition state in forming a fused thiazolidine-β-lactam would probably be considerably greater than that in forming an unfused five-membered ring.

It therefore appears necessary to prepare an intermediate which is unable to azlactonize, but which still contains a carboxyl function sufficiently reactive to acylate the secondary amino group of the thiazolidine. Compounds X and XI were prepared and subjected to the ring closure experiments on the assumption that these amides would be incapable of azlactonization.[12] No biologically active products were obtained and from recent reports, it appears likely that these N-alkylated penicilloates may have formed azlactones (XII) also.

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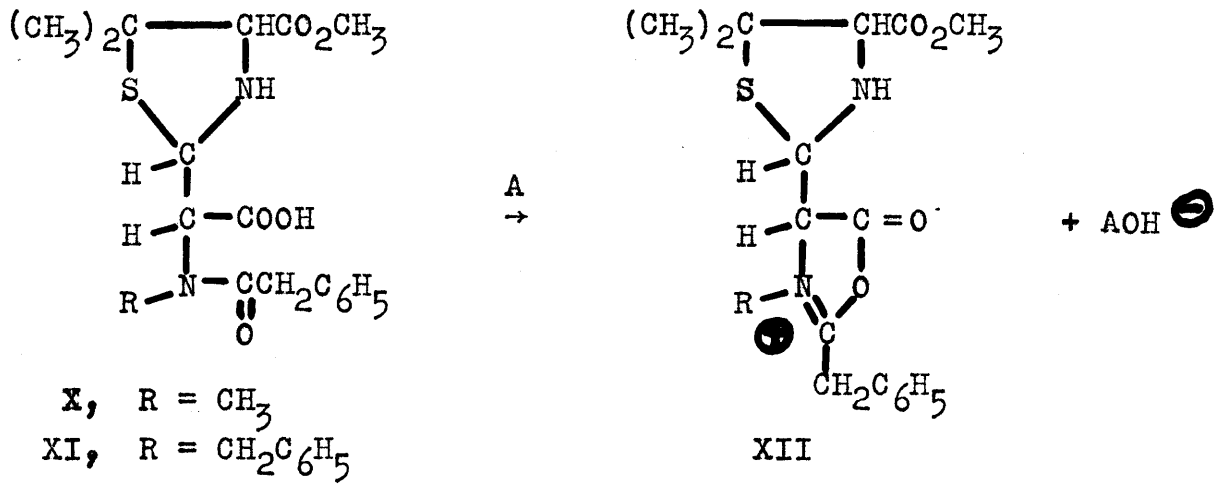
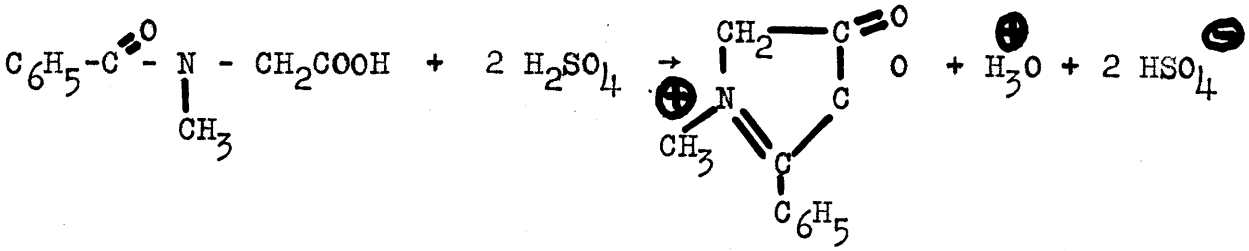
12. Reference 4, p. 852.



X, R = CH_3
XI, R = $CH_2C_6H_5$

XII

It has been reported that benzoylsarcosine (XIII) gives a van't Hoff "i" factor of 3.8 in concentrated sulfuric acid, thus indicating it is nearly completely cyclized to XIV.[13] This would tend to indicate that simple alkylation of the amide nitrogen is insufficient to prevent azlactonization.



XIII XIV

Section A of this part of the thesis describes the preparation of compound XV, in which azlactonization during attempted ring closure reactions is precluded. This fact is borne out by the successful conversion of XV to the bicylic β-lactam, which was characterized as its sulfone (XVI). Compound XVI is the first thiazolidine-β-lactam prepared in

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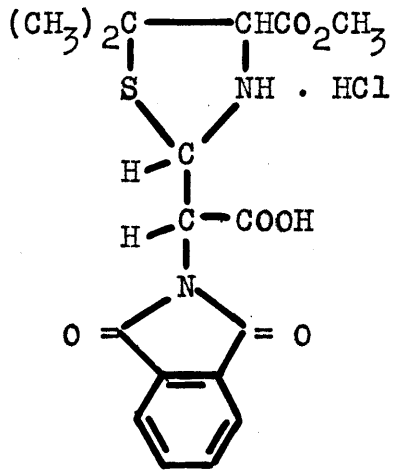
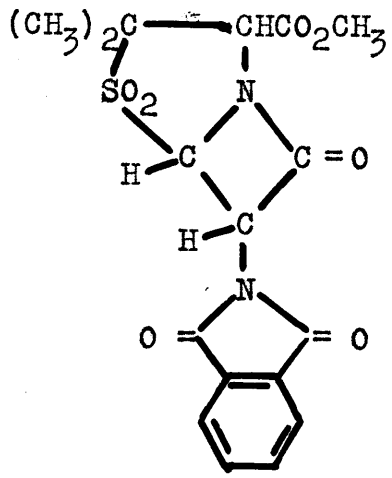
13. J. L. O'Brien and C. Niemann, J. Am. Chem. Soc., 72, 5348 (1950).

fair yield by closure of the β-lactam ring in the last step and it is the only thiazolidine-β-lactam known, exclusive of penicillin, which has a hydrogen atom attached to the carbon atom common to both rings.



XV



XVI

The other alternative principle which may be applied to overcome the problem of azlactonization, is to leave the acylamino side chain of the penicilloate intact while using a carboxyl function which is active as an acylating agent, but which does not azlactonize. The azide group would appear to fulfill these requirements, since azides of α-acylamino acids can frequently be prepared. However, it is reported that when the azide XVII, prepared from the corresponding hydrazide and nitrous acid, was stored in chloroform overnight, a low yield of the crystalline penicillenate (XVIII) was the only product isolated.[14] This is readily understood

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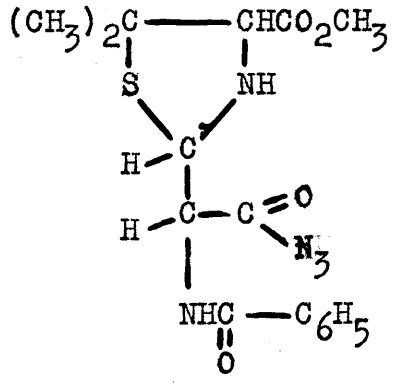
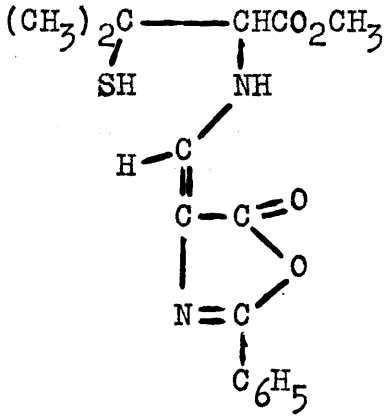
14. J. W. Cornforth, Reference 2, Chapter XXI, p. 767.

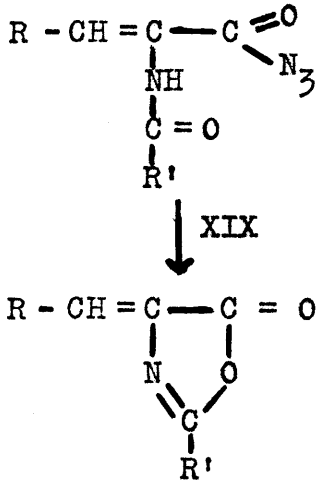
from reports that azides of type XIX are readily converted to oxazolones (XX) but that the reaction could not be extended to those of type XXI.[15] The unsaturated oxazolones are more easily prepared and are more stable than their saturated counterparts.



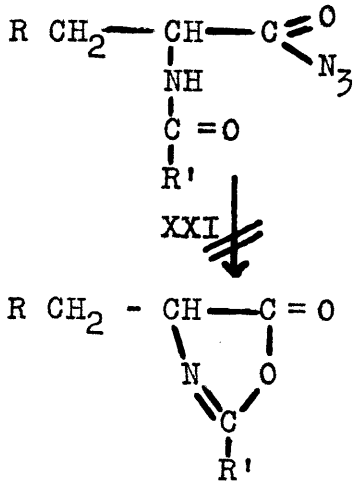
XVII



XVIII



XX



XXII

A study of the reaction of benzylpenaldic acid azide (XXIII) with penicillamine (XXIV) has been reported recently.[16]

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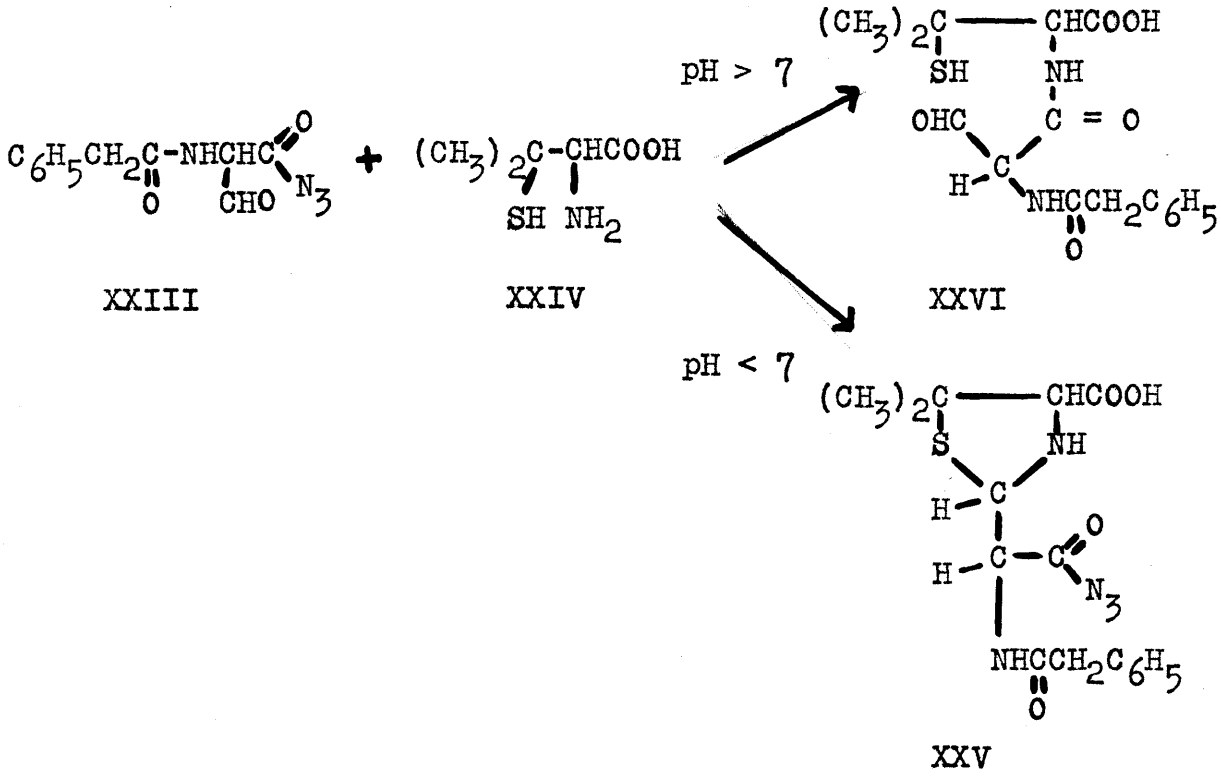
15. Reference 14, p. 731.

16. A Butenandt, H. Jatzkewitz and U. Schiedt, Z. Physiol. Chem., 288, 63 (1951).

If the pH is maintained below 7, compound XXV is the predominant product, while XXVI is obtained when the pH is greater than 7.



XXIII XXIV XXVI XXV

When the same reaction was carried out in formamide solution, a low order of antibiotic activity was observed. However, this activity was not diminished by penicillinase, the enzyme which possesses a highly selective destructive action against penicillin.[16]

Although it is a well known fact that thiolesters react with amines to give amides, it is only recently that this reaction has been suggested as a general method of peptide syntheses.[17] The method involves the reaction of a

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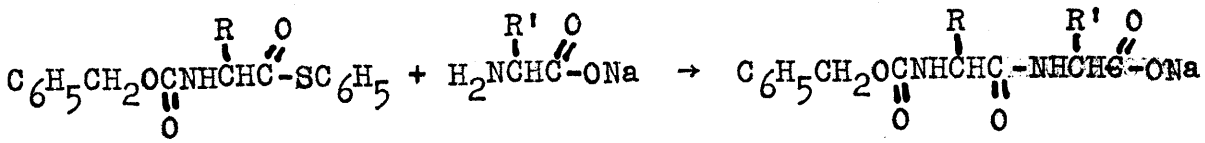
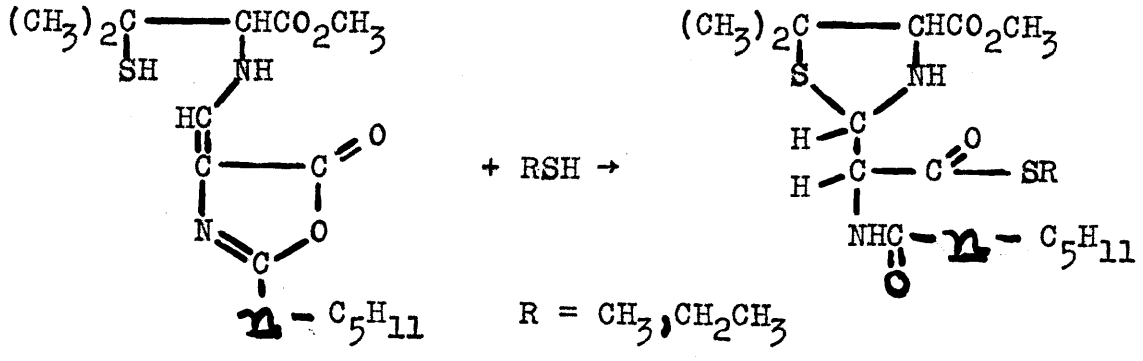
17. T. Wieland, W. Schafer and E. Bokelmann, Ann., 573, 99 (1951).

N-carbobenzoxyamino acid thiophenyl ester (XXVII) with the sodium salt of an amino acid (XXVIII). Heating for several hours gives good yields of the peptide (XXIX).



XXVII XXVIII XXIX

This type of reaction would appear to be well adapted to the synthesis of penicillin, since the product would be the sodium salt, one of the most stable derivatives of penicillin known. It reportedly can be heated at 100° for several hours without loss of activity.[18] Despite the exhaustive attempts to close the β-lactam ring of penicillin, apparently this reaction has not been tried. Several thiol-penicilloates (XXXI) are reported to have been obtained by the action of mercaptans on a penicillenate (XXX),[19] but there are no reports of attempts to cause them to cyclize.

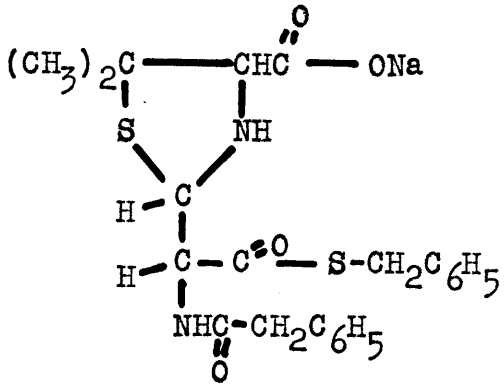


XXX R = CH_3, CH_2CH_3 XXXI

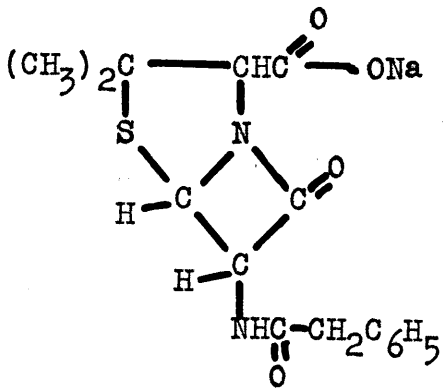
18. O. Wintersteiner and co-authors, Reference 2, Chapter V, p. 87.

19. Reference 8, p. 552.

We have prepared the sodium salt of α-benzyl-α-thiol-DL-benzylpenicilloate (XXXII). The preparation of XXXII and attempts to convert it to the sodium salt of benzylpenicillin (XXXIII) by thermal elimination of benzyl mercaptan are described in Section B of this part of the thesis.



XXXII



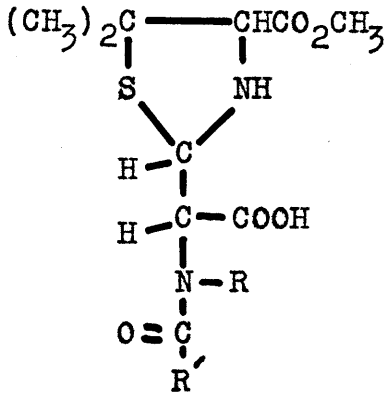
XXXIII

SECTION A

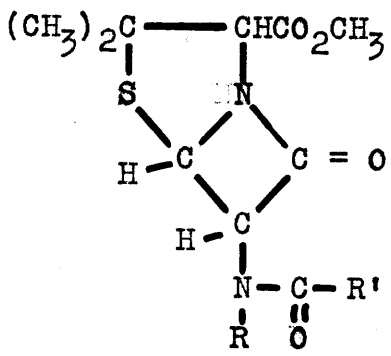
SYNTHESIS OF A THIAZOLIDINE-β-LACTAM BY RING CLOSURE OF THE β-LACTAM

DISCUSSION

The immediate objective of this work was to prepare a compound having the structural features of XXXIV in which R is any group capable of precluding azlactonization from occurring under dehydrating reaction conditions. Attempts could then be made toward cyclization of the acid to the thiazolidine-β-lactam (XXXV).



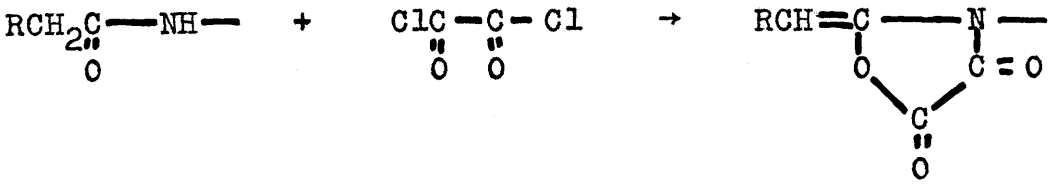
XXXIV



XXXV

In the most favorable circumstances, R should be an effective blocking group, which can be readily replaced by hydrogen after ring closure to form the penicillin (R = H). These are especially stringent conditions in view of the reactivity of the β-lactam in penicillin. This section is devoted to our application of two blocking groups to the solution of this problem.

Oxalyl chloride is known to react with monosubstituted amides to form oxazolidinediones (XXXVI)[20] which can be converted back to the amides under comparatively mild conditions.[21]



XXXVI

Evidence that this heterocyclic system is an effective blocking group is provided by the reported preparation of XXXVII from the corresponding carboxylic acid with phosphorous pentachloride.[22] The reaction of XXXVII with 2-phenyl-2-thiazoline (XXXVIII) is reported to give the β-lactam XXXIX.[23] Treatment of XXXIX with benzylamine, affords the α-acylamino-β-lactams XL and XLI. The success of the cleavage in this case may be due to the 2-phenyl substituent, since lactams of this type appear to be more resistant to ring cleavage than does penicillin.

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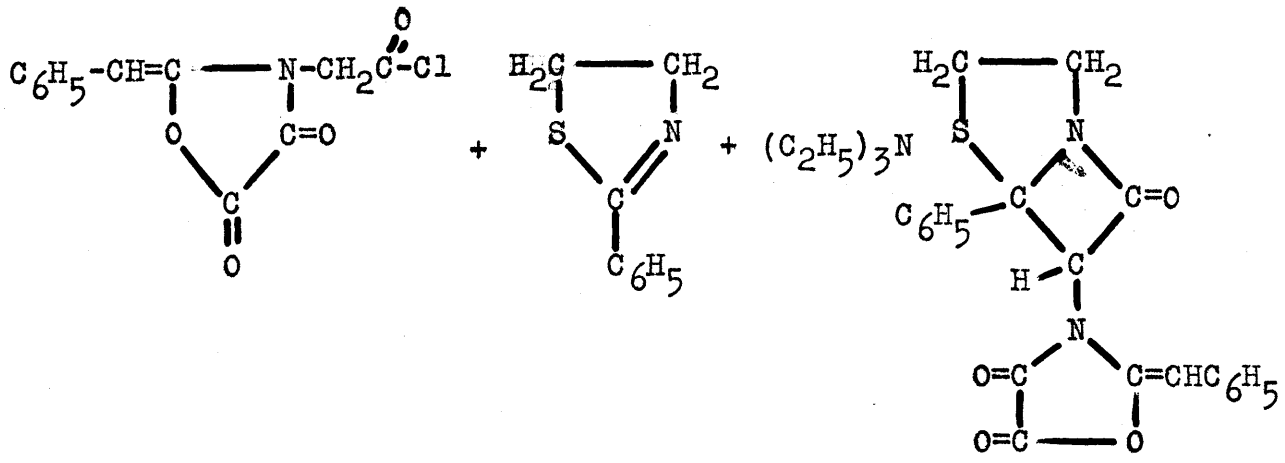
20. R. Stolle and M. Luther, Ber., 53, 314 (1920).

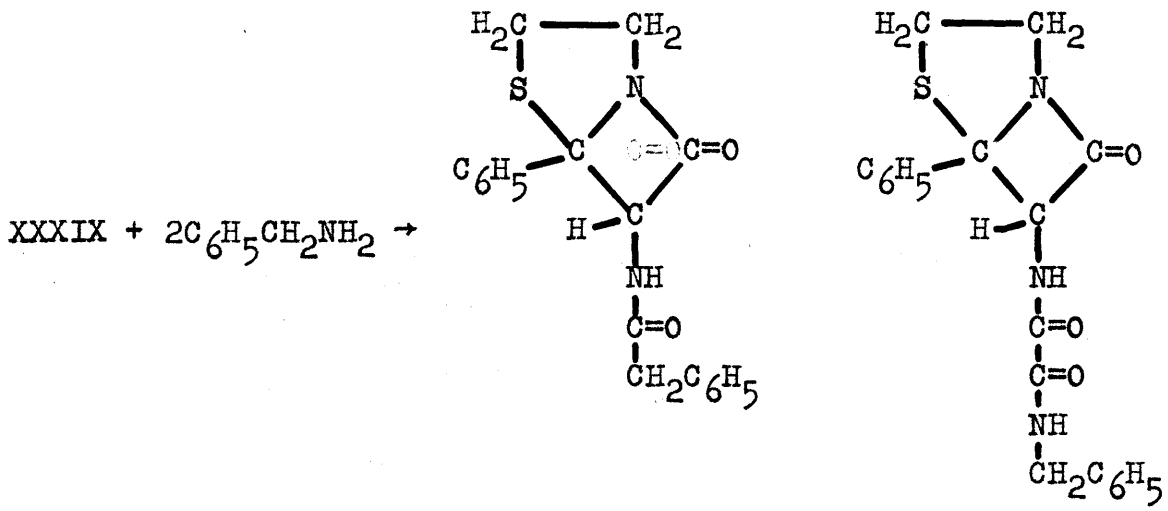
21. J. C. Sheehan and E. J. Corey, J. Am. Chem. Soc., 74, 360 (1952).

22. G. B. Brown, Arch. Biochem., 24, 429 (1949).

23. J. C. Sheehan and E. J. Corey, J. Am. Chem. Soc., 73, 4756 (1951).



XXXVII XXXVIII XXXIX



XL 31.7% XLI 21%

Figure I outlines the attempted preparation of the oxazolidinedione XLIV. Methyl benzylpenicillinate (VII) was obtained[24] by acidification in the cold of potassium benzylpenicillin (XLII), followed by treatment with ethereal diazomethane. It has been reported that an equivalent of sodium benzoate is a suitable catalyst for the methanolysis of VII

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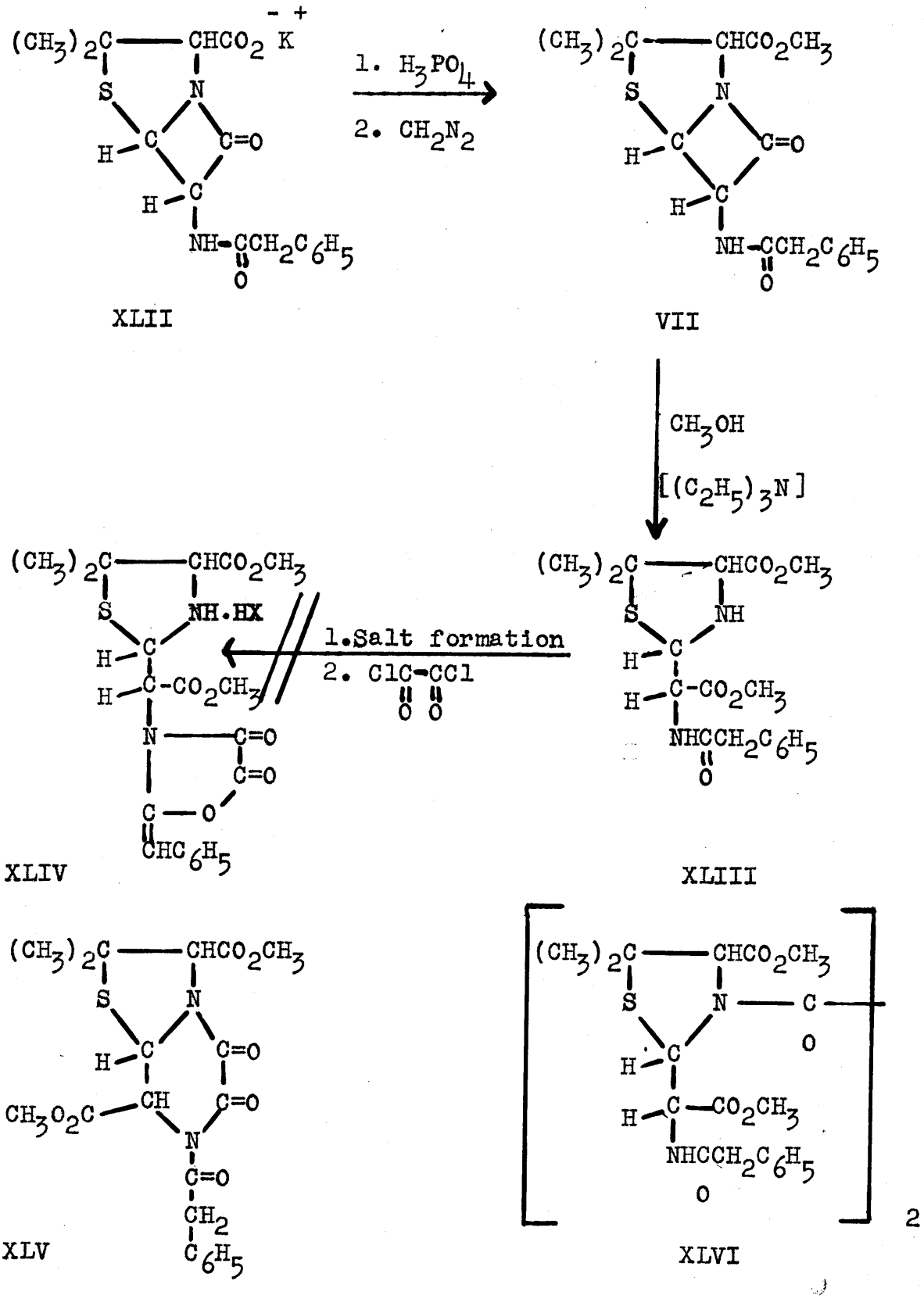
24. Reference 18, p. 93.

Figure I


1. H_3PO_4
2. CH_2N_2
CH_3OH
$[(C_2H_5)_3N]$
1.Salt formation
2. ClC-CCl
XLII
VII
XLIV
XLIII
XLV
XLVI
2

to XLIII.[25] However, a small amount of triethylamine was found to be just as effective and more convenient. In this way, a 61% overall yield of dimethyl D-α-benzylpenicilloate (XLIII) was obtained from XLII.

In the contemplated reaction of XLIII with oxalyl chloride, the danger of acylation on the amino group of the penicilloate was recognized. For this reason, the known hydrochloride, p-toluenesulfonate and perchlorate salts of XLIII were prepared.[25] It seemed reasonable that the ammonium salts would have sufficient positive character to resist acylation under acidic conditions. Amino alcohols have been esterified in this manner in good yields.[26]

When these salts were treated with oxalyl chloride in dioxane or methylene chloride, the characteristic yellow colors of oxazolidinediones were produced, but only non-crystallizing oils were obtained when the solvents were removed. Essentially the same results were obtained if a dioxane or methylene chloride solution of the salt was first saturated with hydrogen chloride and then treated with oxalyl chloride.

The only crystalline products obtained from these reactions were compounds XLV and XLVI. When a dioxane solution of the hydrochloride of XLIII was treated with a

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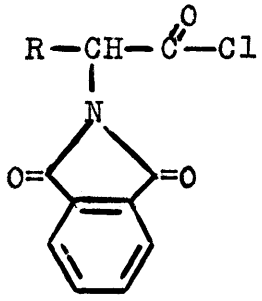
25. Reference 8, p. 612.

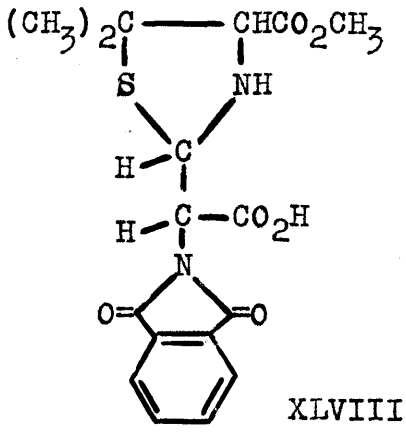
26. A. C. Cope and E. M. Hancock, J. Am. Chem. Soc., 66, 1738 (1944).

large excess of oxalyl chloride, a 50% yield of XLV was obtained. Lack of acidic or basic properties coupled with the absence of any bands in the region 2.6μ to 3.2μ of the infrared spectrum (Plate I) suggested the assigned structure. When the p-toluenesulfonate of XLIII in methylene chloride was treated with two equivalents of oxalyl chloride, a 65% yield of crude XLVI was isolated. This compound also showed no acidic or basic properties. However, the infrared spectrum exhibited bands at 3.02μ, 5.92μ and 6.46μ, all indicative of a monosubstituted amide grouping. The elemental analysis is also in agreement with the formula XLVI. Neither of these compounds responded to tests for antibiotic activity.

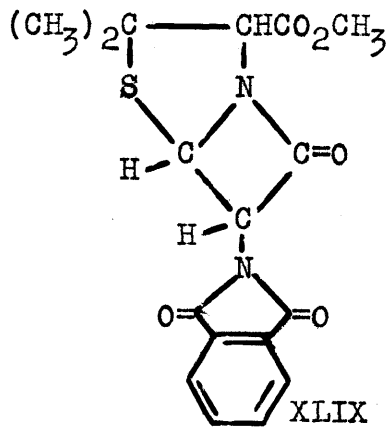
Concurrently with the work just described, a second blocking group was investigated. The success of the phthaloyl blocking group in peptide syntheses is well established. Not only does it permit preparation of acid chlorides of type XLVII, but it also generally confers favorable crystallizing properties to the molecule. These attributes led us to consider the synthesis of XLVIII, a promising intermediate for the formation of XLIX by ring closure reactions.



XLVII



XLVIII



XLIX

While it is questionable whether the phthaloyl group of XLIX could be removed or opened without destruction of the β-lactam, its synthesis alone would be significant. With the exception of penicillin, all the fused thiazolidine-β-lactams known have an alkyl or aryl substituent on the bridge position between the rings. This may be an important reason for the greater stability of the ring systems as compared to penicillin. This greater stability in turn may account for the failure of any synthetic lactams to exhibit significant antibiotic activity as measured by routine penicillin assay methods.

The sequence of reactions to be discussed in this section is presented in Figure II. The crucial step of this synthesis is preparation of the aldehydes (LIII-LV). The Penicillin Monograph states that attempts to prepare α-phthalimidomalonaldehydic esters either by reactions of potassium phthalimide with bromomalonaldehydic esters or by formylation of phthalimidoacetic esters were unsuccessful.[27] In contrast, we have been markedly successful using the latter method.

Two methods are recorded in the literature for the preparation of methyl phthalimidoacetate (L). One recommends heating for 23 hours at reflux a solution of phthaloylglycine in methanol and sulfuric acid.[28] The second proceeds via the

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27. E. V. Brown, Reference 2, Chapter XVII, p. 493.

28. G. B. Crippa and P. Galimberti, Gazz. chim. ital., 63, 81 (1933).

acid chloride.[29] We have found it expedient to use methanolic hydrogen chloride which resulted in a 98.5% yield of the ester. The condensation of methyl phthalimidoacetate (L) and methyl formate in the presence of sodium methoxide afforded the aldehyde LIII in 46.5% yield. The yellow, crystalline solid showed reducing properties and gave a positive ferric chloride test. The infrared spectrum exhibits a weak, broad band in the region 3.0 μ - 3.3 μ, attributed to the enolic form of the aldehyde; two bands at 5.62 μ and 5.80 μ, which are characteristic of the phthalimide system, a shoulder at 5.70 μ, assigned to the ester function, and a sharp band at 5.96 μ due to the formyl group.

The reaction to produce the thiazolidines (LVI-LVIII) was conducted in 50% aqueous ethanol from which the products generally crystallized directly. A stereoisomeric mixture of 4-carboxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetic acid methyl ester (LVI) was obtained in 81.3% yield by this method, when DL-penicillamine hydrochloride was used. No attempt was made to separate the mixture. When D-penicillamine hydrochloride was employed, a 75.0% yield of D-LVI was isolated. In this case, fractional crystallization was used to isolate two isomeric products, which were purified to constant optical rotation. Theoretically, four stereoisomers can be obtained in this reaction, but the general indications

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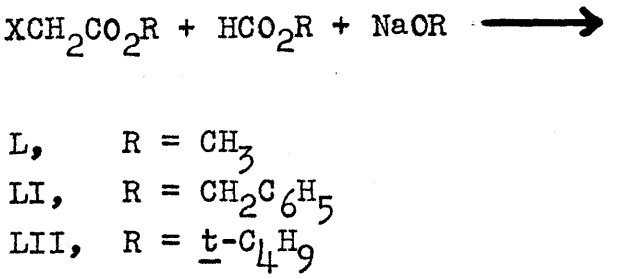
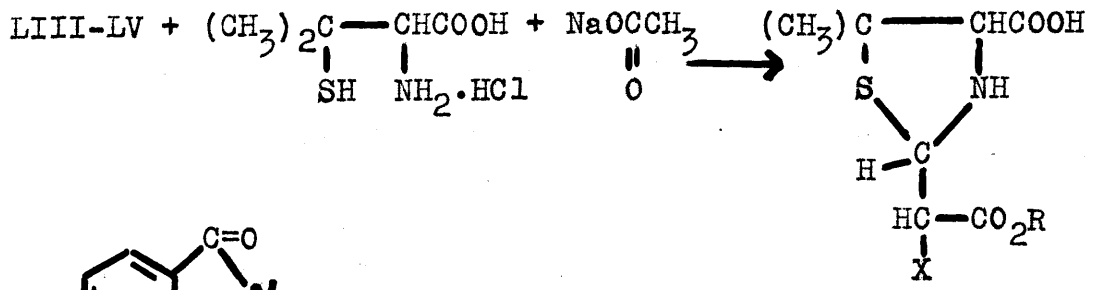
29. J. Pascual and R. R. Rebollo, Anales soc. espan. fis. quim., 32 374 (1934).

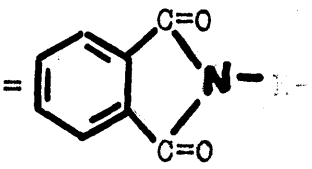


L, R = CH_3
LI, R = $CH_2C_6H_5$
LII, R = t-C_4H_9

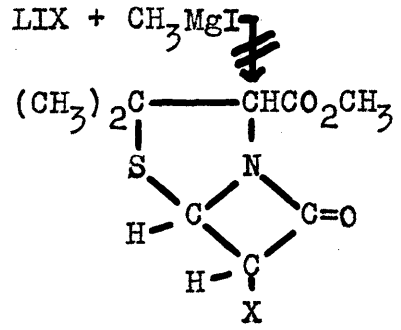
LIII, R = CH_3
LIV, R = $CH_2C_6H_5$
LV, R = t-C_4H_9



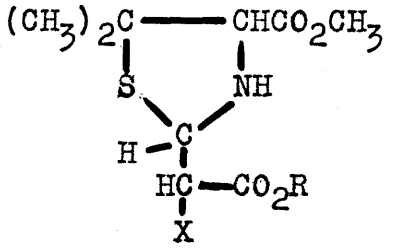
X =



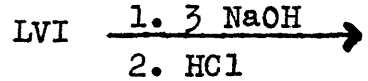
LVI, R = CH_3
LVII, R = $CH_2C_6H_5$
LVIII, R = t-C_4H_9

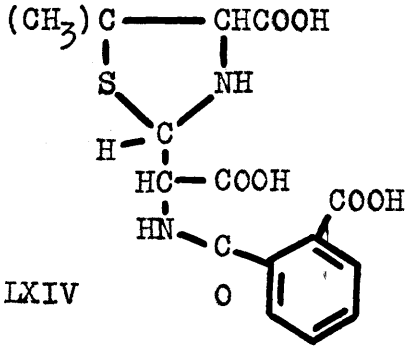


XLIX



LIX, R = CH_3
LX, R = $CH_2C_6H_5$
LXI, R = t-C_4H_9

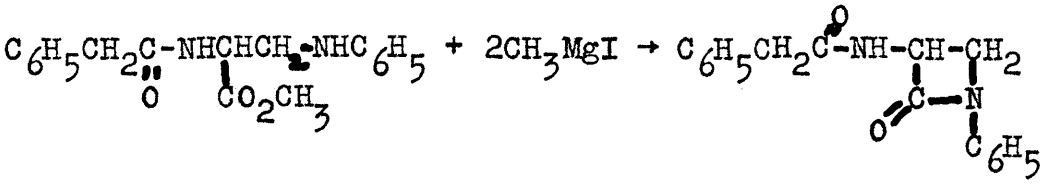




LXIV

are that only two are formed in significant amounts. Esterification with diazomethane generated the corresponding methyl esters (LIX).

The successful synthesis of LIX offered an opportunity to attempt a ring closure to XLIX by use of a Grignard Reagent.[30] The cyclization of several β-amino acid esters to β-lactams was accomplished in this way during the penicillin synthesis program.[31] Methyl β-anilino-α-phenylacetamidopropionate (LXII) gave the lactam LXIII in 35% yield when treated with two equivalents of methylmagnesium iodide.[32]



LXII LXIII

A stereoisomeric mixture of DL-LIX has been treated with methylmagnesium iodide under several sets of conditions. Starting material was the only crystalline product recovered from the reaction mixtures. Infrared analysis of the crude products showed no detectable quantity of β-lactam present.

Treatment of LVI with 3 equivalents of sodium hydroxide and subsequent acidification gave a small amount of crystalline material, the analysis of which corresponds to the formula

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30. Breckpot, Bull. Soc. Chim. Belgique, 32, 412 (1923).

31. S. A. Ballard, D. S. Melstrom and C. W. Smith, Reference 2, Chapter XXVI, p. 976.

32. Reference 28, p. 993.

for the triacid LXIV. Because of the sensitivity of the phthaloyl group towards aqueous alkalies, it seemed improbable that the acid XLVIII could be obtained by saponification of LIX. We therefore turned our attention to esters which are readily cleaved under mild (preferably anhydrous) conditions. Two such esters have been considered.

Since the reduction of benzyl phthalimidoacetate (LI) to phthaloylglycine takes place very readily under mild conditions,[33] the benzyl ester series was prepared (Figure II, R = benzyl). Benzylphthalimidoacetate has been prepared recently from the acid chloride in 88% yield.[33] We have obtained a 96% yield directly from the acid and benzyl alcohol in refluxing toluene using 1% p-toluenesulfonic acid as catalyst and removing the water formed azeotropically. The formylation, using benzyl formate and sodium benzyloxide, has been accomplished in 44.5% yield. This aldehyde (LIV), obtained as a colorless, crystalline solid, exhibited chemical properties similar to the corresponding methyl ester. The infrared spectra of the two aldehydes in the carbonyl region were nearly identical. Condensation with penicillamine hydrochloride gave an oil plus crystalline LVII. Attempted reduction of LVII in dioxane using 100% Pd-C (30%) catalyst was unsuccessful. When glacial acetic acid was used as the solvent and several drops of perchloric acid added,[34]

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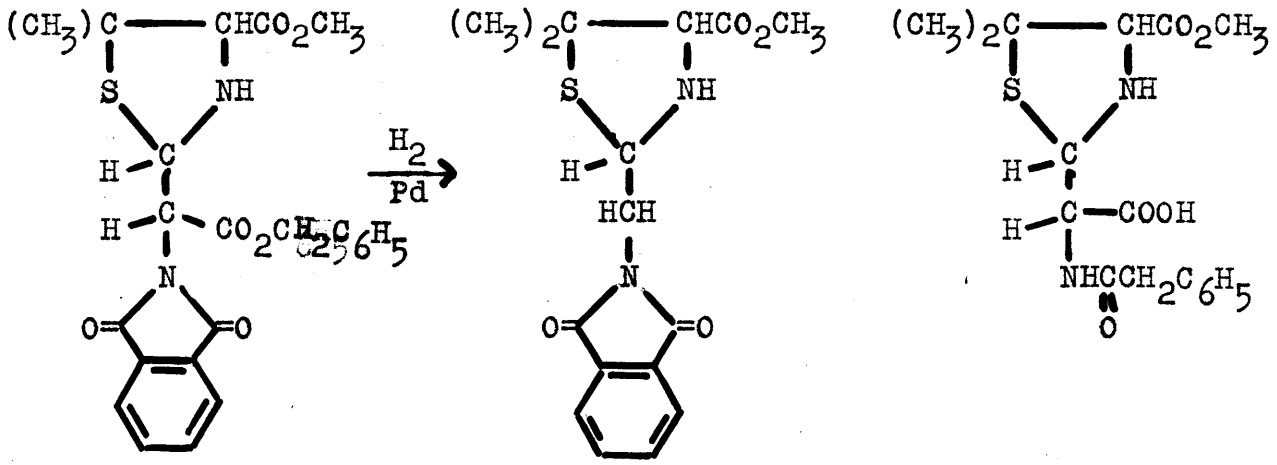
33. E. J. Corey, Ph.D. Thesis, M.I.T., January, 1951.
34. K. W. Rosenmund and E. Kury, Ber., 75, 1850 (1942).

hydrogen was taken up slowly. The only crystalline material isolated, however, was a small quantity of starting acid. A similar reduction carried out on the benzylmethyl diester (LX) yielded a small amount of a non-acidic crystalline solid, the analysis of which corresponds to the formula for the decarboxylated product LXV. Only a small portion of the total crude product was extractable by aqueous becarbonate solution, thus indicating extensive decarboxylation. This is not entirely unexpected, since β-methyl-α-benzylpenicilloate (VI) decarboxylates under mild conditions.[8]



LX LXV VI

Simultaneously, the use of a t-butyl ester as a temporary mask for the carboxyl group was being investigated. The cleavage of a t-butyl ester to the acid under anhydrous conditions has been reported.[35] Treatment of LXVI with dry hydrogen chloride in dioxane is reported to give a 70% yield

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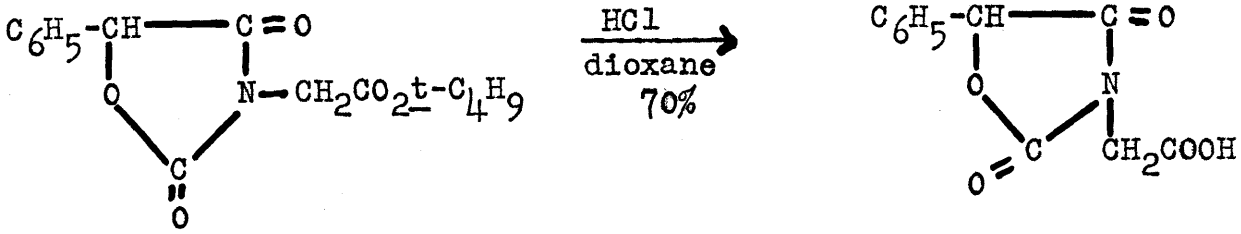
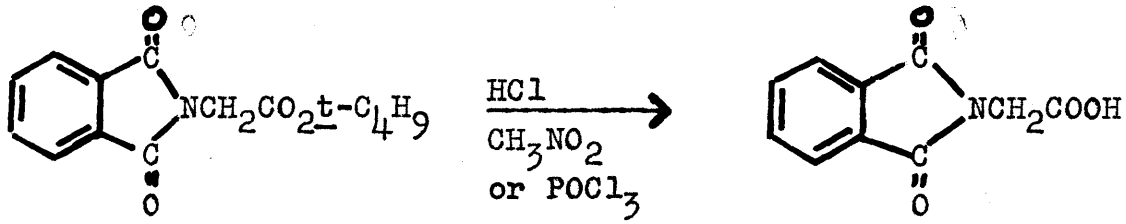
35. J. C. Sheehan and G. D. Laubach, J. Am. Chem. Soc., 73, 4752 (1951).

of the acid LXVII.



LXVI LXVII

t-Butyl phthalimidoacetate (LII) was first prepared as a model with which the cleavage conditions could be studied. It was prepared in 94.5% yield from phthaloylglycyl chloride[36] and t-butanol in the presence of triethylamine. Dry hydrogen chloride in dioxane or diethyl ether had no apparent affect on t-butyl phthalimidoacetate after 48 hours at room temperature. When benzene or chloroform was used as the solvent, cleavage to phthaloylglycine (LXVIII) occurred slowly. In nitromethane or phosphorous oxychloride, the reaction appears to be nearly instantaneous.



LII LXVIII

The in situ preparation of the acid chloride was also studied. When a solution of t-butyl phthalimidoacetate in phosphorous oxychloride was treated with an equivalent of

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36. J. C. Sheehan and V. S. Franck, J. Am. Chem. Soc., 71, 1856 (1949).

phosphorous pentachloride either before or after saturation with hydrogen chloride gas, the acid chloride was formed readily. When no hydrogen chloride was added, the reaction proceeded at a diminished rate.

The successful cleavage studies of the model compound prompted the preparation of the t-butyl ester series (Figure II, R = t-butyl). The preparation of the t-butyl formate, which is necessary for the introduction of the formyl group, was carried out as described in the Penicillin Monograph.[37] In our hands, the product obtained appeared to be a mixture of t-butyl formate and t-butanol. However, this crude t-butyl formate has been used to obtain a 13% yield of the aldehyde LV. An 83.5% yield of the thiazolidine LVIII was produced by interaction with penicillamine hydrochloride. The t-butylmethyl diester LXI was obtained by esterification with diazomethane.

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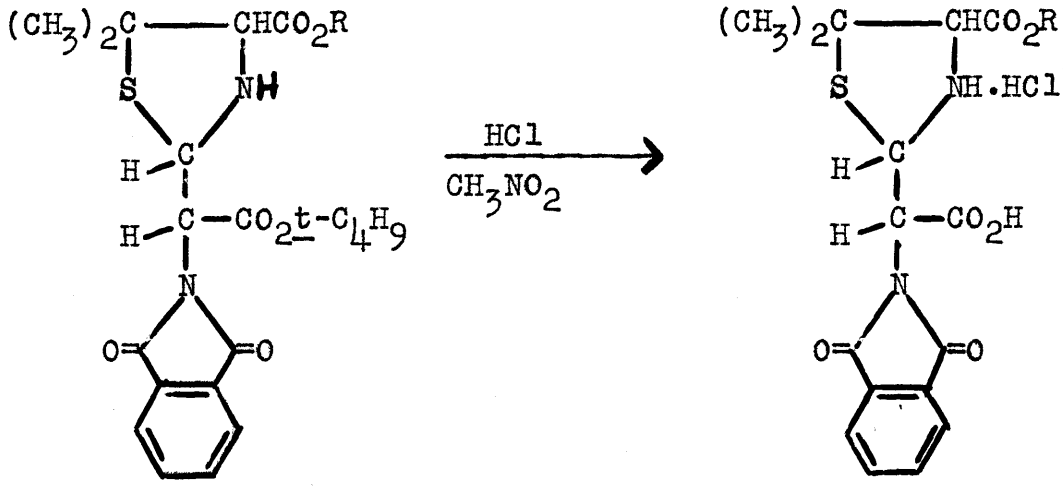
37. Reference 25, p. 533. According to this method, a mixture of formic acid and t-butanol are distilled through a good fractionating column, giving product-water azeotrope which is dried over magnesium sulfate and redistilled yielding about 60% of a liquid collected at 75-76°. No analysis or refractive index are given. These workers were apparently unaware that the preparation of t-butyl formate in low yield from t-butyl chloride and calcium formate had been reported previously (W. Taylor, J. Chem. Soc., 1852 (1937)). In this communication, the boiling point of an analytical sample was reported as 82.5-83.5/757 mm. No refractive index was given. We have repeated the more recent report and have obtained a 47% yield of liquid, b.p. 76-79°. On redistillation, a middle fraction was submitted for elemental analysis and infrared spectrum determination. There was an intense band in the hydroxyl region of the infrared spectrum and the elemental analysis also indicated the presence of an appreciable quantity of t-butanol.

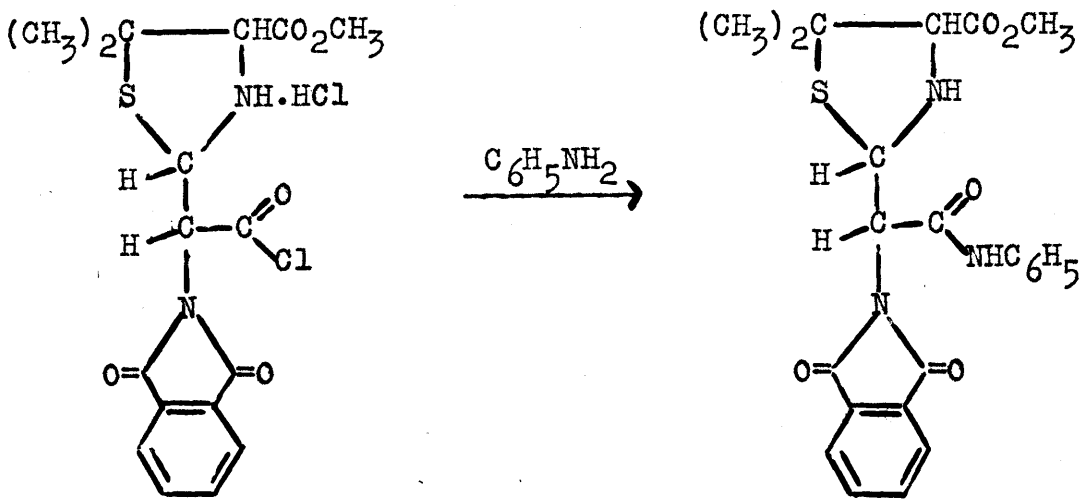
The cleavage of the t-butyl ester LXI to yield XV was accomplished by treatment with hydrogen chloride in nitromethane. The diacid LXIX was obtained by similar treatment of LVIII.



LXI, R = CH_3 XV, R = CH_3

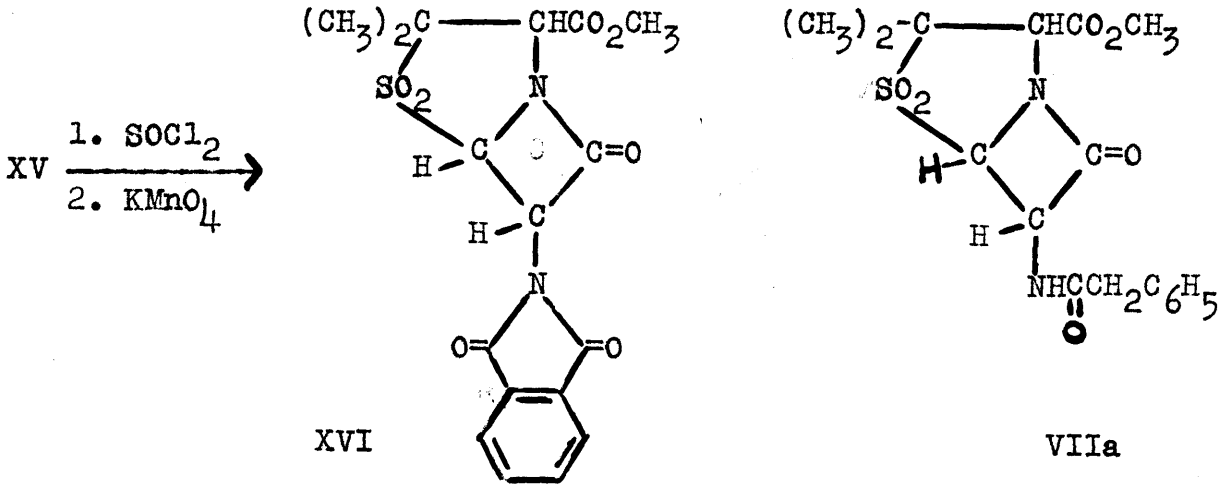
LVIII, R = H LXIX, R = H

When a solution of LXI in phosphorous oxychloride was saturated with hydrogen chloride and phosphorous pentachloride added, a yellow glass was obtained, which appeared to be the acid chloride hydrochloride LXX since on treatment with aniline, the anilide (LXXI) was obtained. Several unsuccessful attempts have been made to obtain the lactam XLIX from LXX.



LXX LXXI

The successful closure of the β-lactam ring was accomplished, however, by the action of thionyl chloride in refluxing benzene. The β-lactam was isolated by oxidation to the corresponding sulfone (XVI), which was characterized by infrared determination and elemental analysis. The overall yield of XVI from XV was 13%. The infrared spectra (Plate II) of XVI and of methyl benzylpenicillinate sulfone (VIIa) both exhibit a strong band at 5.54 μ for the carbonyl group of the β-lactam.



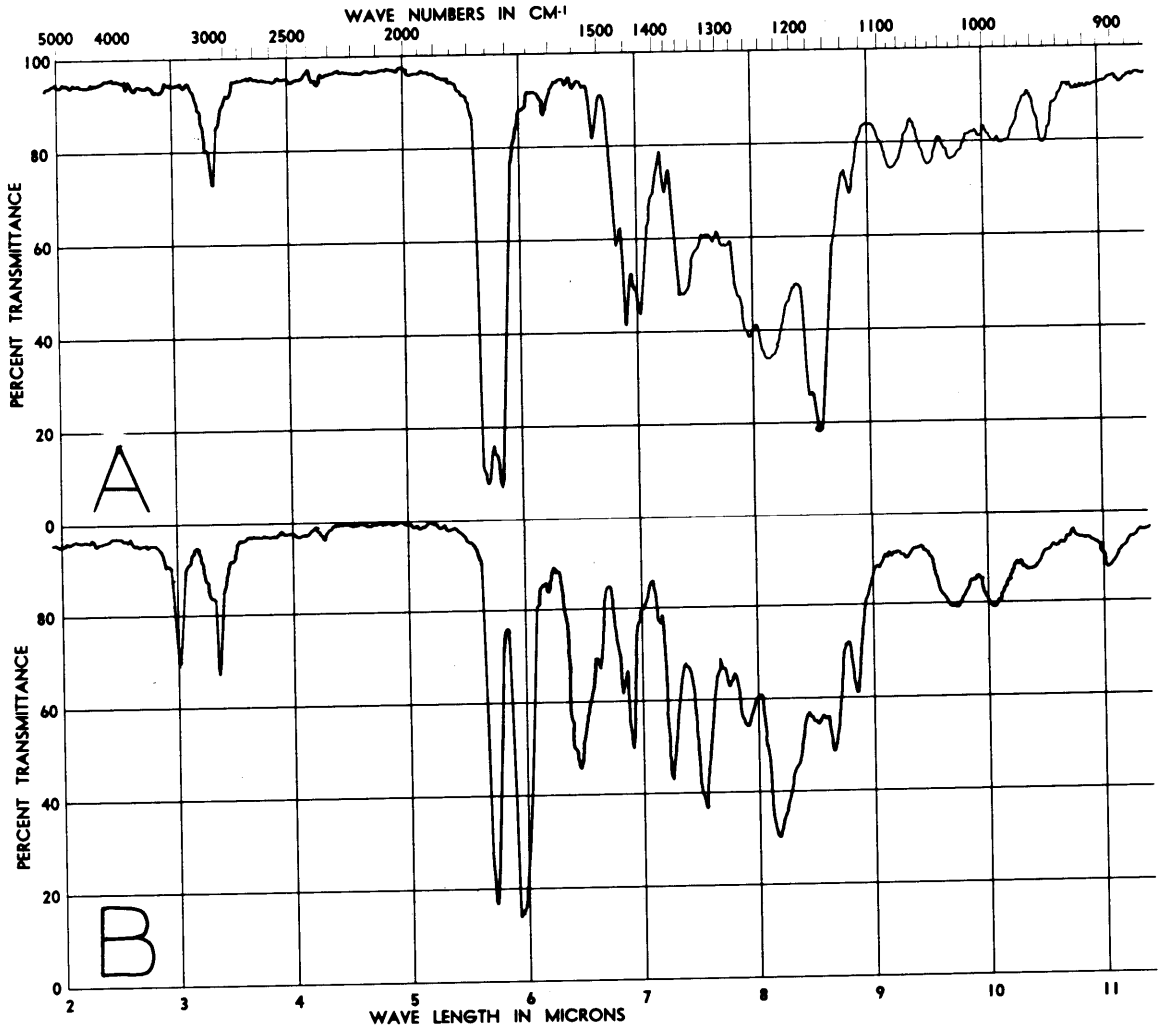
XVI VIIa

Preparation of XVI provides stimulus to attempts to remove or modify the phthaloyl group of XVI so as to cause it to more closely resemble the penicillins. Also, this series of reactions may be applied where some other effective blocking group is employed. Time did not permit this work to be undertaken.

Plate I

Curve A: Compound XLV, 5% solution in tetrachloroethane.

Curve B: Compound XLVI, 5% solution in tetrachloroethane.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PERCENT TRANSMITTANCE
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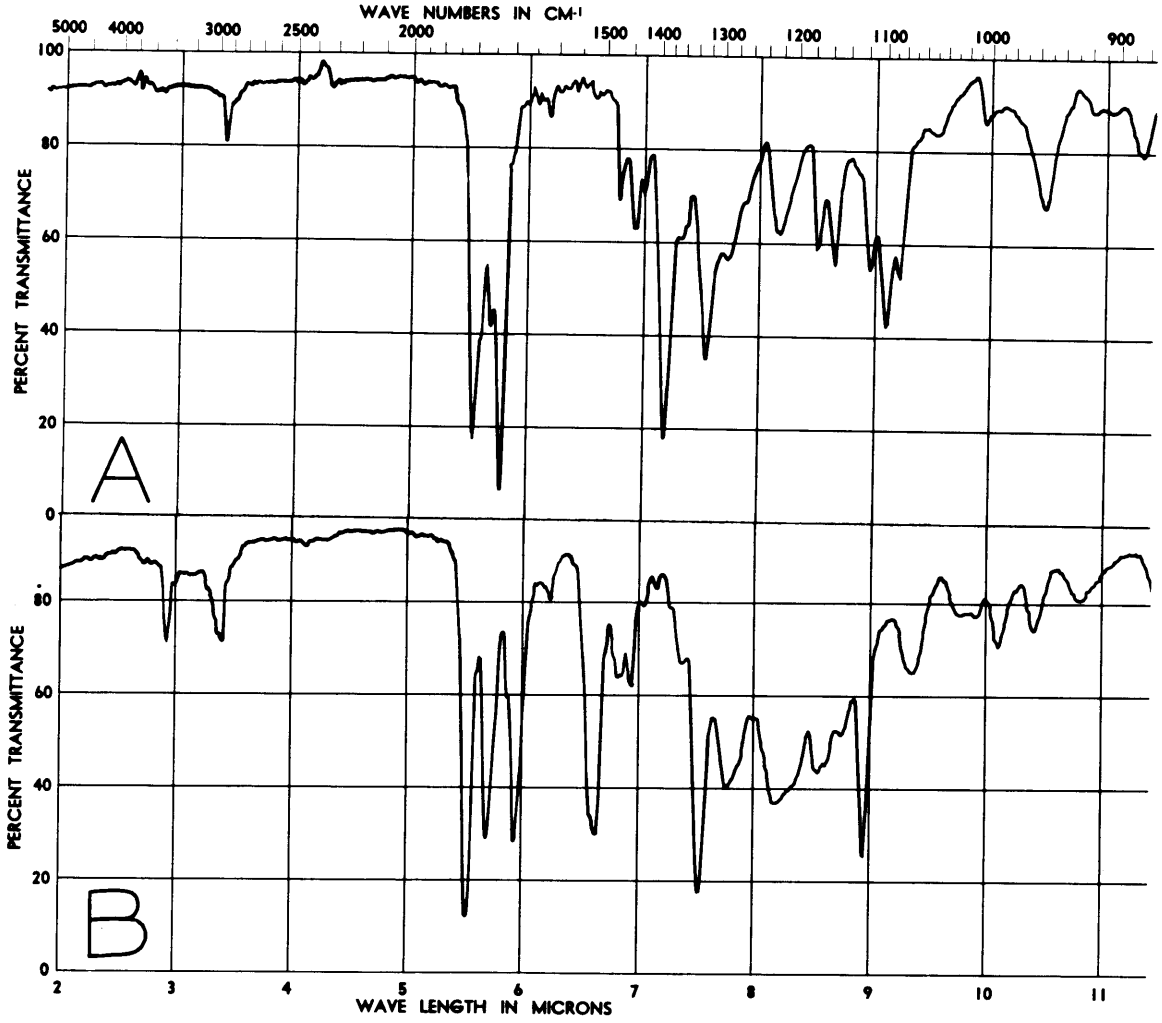
10
11
WAVE LENGTH IN MICRONS

Plate II

Curve A: 4-Carbomethoxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetic Acid β-Lactam Sulfone (XVI), 5% solution in tetrachloroethane.

Curve B: Methyl Benzylpenicillinate Sulfone (VIIa), 5% solution in tetrachloroethane.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WAVE LENGTH IN MICRONS

EXPERIMENTAL[38]

Methyl Benzylpenicillinate (VII).[24] - Potassium benzylpenicillinate (Bristol technical grade, assay 2.92 B.O.U.) (25.0 g., 0.0674 mole) was dissolved in one liter of ice-water and acidified to pH 2 with ice-cold 10% phosphoric acid (100 ml.). The precipitated acid was extracted with 400, 300, and two 200 ml. portions of ether and the combined ethereal extracts were washed with 200 ml. of ice-water and dried over sodium sulfate at 0° for 2 hours. The amber solution was then filtered and treated at 0° with 4 g. of diazomethane in ethereal solution. After storage at 0° for 1½ hours, the solution was allowed to warm to room temperature over a 2 hour period and then extracted with 200 ml. of phosphate buffer of pH 7.1, followed by 200 ml. of water. The amber ethereal solution was dried at 5° over sodium sulfate and concentrated under reduced pressure at room temperature to a tan, crystalline mass. The product was recrystallized by solution in the minimum quantity of ethyl acetate at room temperature followed by dilution with hexane. After crystallization was complete, the product was collected by filtration, washed with hexane and dried to give 17.03 g. (72.5%) of nearly colorless ester,

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38. All melting points are corrected. Infrared spectra were measured with a Baird Infrared Recording Spectrophotometer, Model B. The cell thickness used was 0.10 mm.

m.p. 95.0-96.5° (reported 90-92°, 97-98°),[24] $[\alpha]^{28}_{D}$ in methanol + 297° (c = 2.2).

Dimethyl D-α-benzylpenicilloate (XLIII).[25] - Methyl benzylpenicillinate (15.0 g., 0.043 mole) was dissolved in 500 ml. of commercial absolute methanol and 1 ml. of triethylamine was added. The reaction was followed by observing the drop in optical rotation as follows.

Time	Rotation (c = 3.0)
0	+297°
45 min.	200°
2 hrs.	147°
24 hrs.	87° constant

After the rotation had attained a constant value, the pale amber solution was concentrated under reduced pressure at 30° to a brown oil. The oil was dissolved in 75 ml. of ether and the solution was seeded with a crystal obtained from a previous preparation. After storage at 5° for several hours, the pale tan crystals were collected by filtration, 12.30 g., m.p. 85.5-87.5° (reported 87-89°).[25] By addition of petroleum ether (b.p. 30-60°) to the filtrate, a second crop of 1.46 g., m.p. 83-86° was obtained. The total yield was 84.0%.

Dimethyl D-α-benzylpenicilloate Hydrochloride (XLIIIa).[25] - Dimethyl D-α-benzylpenicilloate (1.00 g.) was dissolved in 50 ml. of absolute ether and dry hydrogen chloride gas was passed into the solution until precipitation was complete. The ether was decanted, the solid was triturated with another

portion of absolute ether, and the product was collected by filtration. The dried, amorphous hydrochloride weighed 1.03 g. (94.5%).

Dimethyl D-α-benzylpenicilloate p-Toluenesulfonate (XLIIIb).[25] - To a solution of crude dimethyl D-α-benzylpenicilloate (from the methanolysis of methyl benzylpenicillinate) in 50 ml. of absolute ether was added an ethereal solution of p-toluenesulfonic acid. The reagent was added until precipitation was complete. The colorless salt was collected by filtration; yield, 4.18 g. (96.0%), m.p. 125-127° (dec., in bath at 124°). Recrystallization from absolute ethanol-ether afforded colorless needles, m.p. 132-135° (dec., in bath at 124°) (reported 134-138°).[25]

Treatment of XLIIIa with Oxalyl Chloride. - Dimethyl D-α-benzylpenicilloate hydrochloride (0.500g.) was dissolved in 25 ml. of dry dioxane and 5 ml. of oxalyl chloride was added. There was an evolution of heat and gas accompanied by the formation of a yellow color. The solution was maintained at 45° for 15 min., concentrated under reduced pressure, and the residue was taken up in chloroform. The yellow solution was washed with water, 10% bicarbonate solution, water and dried over magnesium sulfate. Concentration under reduced pressure yielded an oil, which was crystallized from ether-petroleum ether (b.p. 30-60°) to yield 260 mg. (50%) of pale-yellow crystalline XLV, m.p. 156-158.5°. An analytical sample

crystallized from benzene-petroleum ether (b.p. 30-60°) as rosettes of colorless needles, m.p. 159.4-160.2°, $[\alpha]^{27}$°D in acetone +12.8° (c = 2.11).

Anal. Calcd. for $C_{20}H_{22}N_2O_7S$: C, 55.29; H, 5.10; N, 6.45.
Found: C, 55.03; H, 5.30; N, 6.52.

This compound was insoluble in concentrated hydrochloric acid or 4 N sodium hydroxide. It gave a negative ferric chloride test.

Treatment of XLIIIb with Oxalyl Chloride. - A solution of oxalyl chloride (0.230 g., 1.81 millimoles) in 10 ml. of dry methylene chloride was added in one portion to a solution of dimethyl D-α-benzylpenicilloate p-toluenesulfonate (1.00 g., 1.81 millimoles) in 25 ml. of methylene chloride. After storage at room temperature for 40 hours, the resulting yellow-green solution was concentrated under reduced pressure. Trituration of the residual gum with 30 ml. of absolute ether produced 480 mg. (65%) of crude XLVI. Crystallization from ethyl acetate-petroleum ether (b.p. 30-60°) gave pale-yellow needles, m.p. 214-218.5° (dec.). Additional recrystallization from the same solvent pair afforded colorless platelets, m.p. 227.2-228.0° (dec.), $[\alpha]^{27}$°D in acetone -69.5° (c=2.15).

Anal. Calcd. for $C_{38}H_{46}N_4O_{12}S_2$: C, 56.00; H, 5.69; N, 6.88.
Found: C, 56.12; H, 5.89; N, 6.67.

This compound was insoluble in concentrated hydrochloric acid or 4 N sodium hydroxide. It gave a negative ferric

chloride test.

Methyl Phthalimidoacetate (L). - A suspension of phthaloylglycine (102.6 g., 0.500 mole) in 350 ml. of absolute methanol was treated with a rapid stream of hydrogen chloride gas for 5 min. without cooling, after which time solution was complete. The solution was cooled, and the pure ester which crystallized as colorless needles, was collected by filtration. It amounted to 103.0 g., m.p. 113.5-115.0°. (reported 115-116°).[29] Concentration of the filtrate gave a second crop of 4.1 g., m.p. 113-114°, for a total yield of 98%.

α-Phthalimidomalonaldehydic Acid Methyl Ester (LIII). - Freshly cut sodium (11.50 g., 0.500 mole) and 250 ml. of reagent grade xylene were placed in a 500 ml. three-necked round bottom flask equipped with a dropping funnel, stirrer and reflux condenser with a sidearm takeoff. A stream of dry nitrogen was started over the system and the xylene was heated to reflux. To the stirred suspension was added over a 15 min. period absolute methanol (distilled from magnesium) (22.2 ml., 0.55 mole). Heating was continued for an additional 15 min. and then 50 ml. of distillate was collected from the sidearm takeoff. After cooling to room temperature, the milky suspension was added in small portions over a 30 min. period to a rapidly stirred suspension of methyl phthalimidoacetate (109.5 g., 0.500 mole) in 245 ml. (4.00 moles) of methyl formate (Comm. Solv. redistilled from phosphorous pentoxide) at 0°. Stirring

was continued for another 30 min. and the resulting clear, orange solution was stored at 5° for 16 hours.

The canary yellow mass was treated with 200 ml. of dry benzene containing 30 ml. of glacial acetic acid, stirred rapidly for several minutes and then washed with 250 ml. of N hydrochloric acid. After the layers were separated, the aqueous phase was further acidified to pH 2 with concentrated hydrochloric acid and extracted with two 100 ml. portions of benzene. The combined benzene extracts were washed with two 50 ml. portions of water and concentrated under reduced pressure to a volume of 200 ml. On cooling to 5°, a yellow crystalline solid separated. It was collected by filtration, washed with cold benzene and dried to give 62.30 g. of crude LIII. Concentration of the mother liquors gave an additional 6.20 g. The combined crude product was crystallized from boiling benzene to give 52.00 g. of pale yellow, crystalline powder (green fluorescence), m.p. 140-141°. The second crop amounted to 5.80 g., m.p. 133-138°, bringing the total yield to 46.5%. An analytical sample, recrystallized from benzene, melted at 140.5-142.0°.

Anal. Calcd. for $C_{12}H_9NO_5$: C, 58.30; H, 3.67; N, 5.67.
Found: C, 58.65; H, 3.78; N, 5.61.

The aldehyde gave a wine ferric chloride test and reduced Tollens' Reagent and Fehling's Solution. It dissolved in aqueous sodium bicarbonate solution to give a yellow solution. The 2,4-dinitrophenylhydrazone was formed in the usual manner.

An analytical sample was obtained as yellow prisms from dioxane-water, m.p. 215-216° (dec.).

Anal. Calcd. for $C_{18}H_{13}N_5O_8$: C, 50.59; H, 3.07; N, 16.39.
Found: C, 50.28; H, 3.10; N, 16.55.

4-Carboxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetic Acid Methyl Ester (LVI) - To a hot solution of LIII (24.72 g., 0.100 mole) in 100 ml. of 95% ethanol was added a solution of DL-penicillamine hydrochloride (18.6 g., 0.100 mole) and sodium acetate trihydrate (20.40 g., 0.150 mole) in 100 ml. of water. After 12 hours storage at room temperature, the precipitated product was collected by filtration, washed with 50% aqueous ethanol and dried to give 28.20 g. of LVI as colorless granular crystals, m.p. 183-184° (dec.). After 5 days, the filtrate yielded a second crop of 2.61 g., m.p. 185-186° (dec.); total yield, 81.4%. An analytical sample was obtained as colorless needles by recrystallization from 95% ethanol, m.p. 184.5-185.5° (dec.).

Anal. Calcd. for $C_{17}H_{18}N_2O_6S$: C, 53.96; H, 4.79; N, 7.41.
Found: C, 54.23; H, 4.94; N, 7.29.

When D-penicillamine hydrochloride was used in a similar procedure, a 75% yield of a mixture of the stereoisomeric D-acids was obtained. By collecting the products in small successive fractions as they precipitated, some degree of separation was attained. Since purification of these acids could be followed by changes in optical rotation, attempts were made to separate them by fractional crystallization from

methanol. The least soluble acid was obtained as colorless needles from methanol, m.p. 184.5-185.5° (dec., in bath at 170°), $[\alpha]^{25°}D$ in dioxane -7° (c = 1.3).

Anal. Calcd. for $C_{17}H_{18}N_2O_6S$: C, 53.96; H, 4.79; N, 7.41.
Found: C, 54.23; H, 5.10; N, 7.34.

A more soluble acid was recrystallized to constant optical rotation from methanol-water. An analytical sample of colorless needles melted at 184-185° (dec.), $[\alpha]^{26°}D$ in dioxane +24° (c = 0.8).

Anal. Calcd. for $C_{17}H_{18}N_2O_6S$: C, 53.96; H, 4.79; N, 7.41.
Found: C, 53.94; H, 4.77; N, 7.15.

Esterification of mixtures of the acids with diazomethane led to the isolation of two crystalline esters. One was obtained as fluffy needles from methanol-water, m.p. 146-147°, $[\alpha]^{27°}D$ in methanol -28° (c = 1.7).

Anal. Calcd. for $C_{18}H_{20}N_2O_6S$: C, 55.09; H, 5.14; N. 7.14.
Found: C, 54.90; H, 5.40; N, 7.26.

A second ester, obtained as colorless needles from acetone-water, melted at 138-139°, $[\alpha]^{25°}D$ in methanol -5° (c = 1).

Anal. Calcd. for $C_{18}H_{20}N_2O_6S$: C, 55.09; H, 5.14; N, 7.14.
Found: C, 55.13; H, 5.21; N, 7.27.

The stereochemical relationships of the two acids to the two esters has not been established with certainty.

4-Carbomethoxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetic Acid Methyl Ester (LIX). - A suspension of a stereoisomeric mixture of the DL-acids LVI (3.78 g., 0.0100 mole) in 150 ml. of methylene chloride was cooled in an ice-bath and treated with ethereal diazomethane until the yellow color of the reagent persisted. After 5 min., the solution was decolorized by the dropwise addition of glacial acetic acid, then washed with 50 ml. portions of 5% sodium bicarbonate and water, and dried over magnesium sulfate. Concentration of the solution under reduced pressure left 3.92 g. (100%) of colorless oil. By fractional crystallization of the mixture from methanol-water, a sample of colorless needles was obtained of m.p. 152.5-153.5°. No attempt was made to isolate other isomers.

Anal. Calcd. for $C_{18}H_{20}N_2O_6S$: C, 55.09; H, 5.14; N, 7.14.
Found: C, 55.19; H, 5.36; N, 7.31.

Reaction of DL-LIX with Methylmagnesium Iodide. - A stereoisomeric mixture of DL-LIX (3.92 g., 0.0100 mole) dissolved in 75 ml. of reagent benzene was treated with one equivalent of methylmagnesium iodide in 10 ml. of absolute ether with rapid stirring over a 15 min. period. Evolution of heat and a gas was noted. The yellow-orange suspension was stirred at room temperature for 15 hours. A solution of 4.0 g. of ammonium chloride in 50 ml. of water was then added with rapid stirring. After separation of the layers, the aqueous phase was extracted with 50 ml. of benzene and the combined

organic layers washed with 50 ml. of water. Concentration under reduced pressure led to 2.14 g. of yellow-brown oil. The aqueous layer was acidified to pH 2 with concentrated hydrochloric acid and extracted with two 50 ml. portions of chloroform. The combined extracts were washed with water, dried over magnesium sulfate and concentrated under reduced pressure to a yellow glass; weight, 0.53 g.

In a similar procedure, the solvents were removed from the original Grignard addition product and replaced with toluene. The suspension was then heated at reflux for 15 hours. The products were worked up as in the procedure above to give a neutral fraction of 0.47 g. and an acidic fraction amounting to 1.50 g.

Infrared analysis of the neutral products indicate them to be predominantly starting material.

Alkaline Hydrolysis of LVI. - A stereisomeric mixture of DL-LVI (3.78 g., 0.0100 mole) was added to 65.5 ml. of 0.459 N sodium hydroxide. After storage at room temperature for 4 hours, the solution was neutralized by potentiometric titration with 0.974 N hydrochloric acid. The plotted data showed no sharp breaks in the curve. The resultant clear solution was stored at 5° for 3 weeks, after which time 1.16 g. of crystalline solid had separated, m.p. 127° (dec., in bath 120°). Several recrystallizations from acetone-water gave pure LXIV, m.p. 120-121° (dec.).

Anal. Calcd. for $C_{16}H_{19}N_2O_7S$: C, 50.25; H, 4.74; N, 7.33.
Found: C, 50.38; H, 5.03; N, 7.53.

Benzyl Phthalimidoacetate (LI). - A suspension of phthaloylglycine (102.6 g., 0.500 mole) in benzyl alcohol (56.7 ml., 0.550 mole) and 300 ml. of toluene with 1.0 g. of p-toluenesulfonic acid was heated under a constant water separator. After one hour, solution was complete and after 3 additional hours of heating, the theoretical amount of water had been collected. The solution was allowed to cool to 60° and an equal volume of petroleum ether (b.p. 30-60°) added. Crystallization began immediately, and after storage overnight at 5°, the colorless prisms were collected by filtration, washed with petroleum ether (b.p. 30-60°) and dried to give 141.5 g. (96.0%) of ester melting at 97-101°. Recrystallization from ethanol-water gave colorless platelets (95.5% recovery in one crop), m.p. 102.5-103.5° (reported 102.8-103.1°).[33]

α-Phthalimidomalonaldehydic Acid Benzyl Ester (LIV). - Freshly cut sodium metal (2.30 g., 0.100 mole) was heated in 100 ml. of reagent grade xylene until it became molten. A stream of dry nitrogen was passed through the system while benzyl alcohol (10.3 ml., 0.100 mole) was added with rapid stirring over a 10 min. period. After another 30 min. of heating, the sodium had all reacted. The suspension was cooled to room temperature and added slowly over a 30 min.

period to a mixture of benzyl phthalimidoacetate (29.53 g., 0.100 mole) and 50.4 ml. (0.400 mole) of benzyl formate (Eastman Kodak Pure) with rapid stirring at 5°. The resulting orange solution was stored at room temperature for 18 hours. Ice-water was added (200 ml.) and the mixture shaken vigorously. After separation of the layers, the organic phase was extracted with 50 ml. of water. The combined aqueous extracts were washed with 50 ml. of benzene, cooled in an ice-bath and acidified to pH 2 with concentrated hydrochloric acid while stirring with 100 ml. of methylene chloride. The aqueous layer was extracted further with two 50 ml. portions of methylene chloride. The combined methylene chloride extracts were washed with 50 ml. of water, dried over magnesium sulfate and concentrated to an orange oil. This oil dissolved readily in 50 ml. of ether and on storage overnight at 5°, the product crystallized. It was collected by filtration, washed with ether and dried to give 15.50 g. of colorless LIV, m.p. 129-133°. Recrystallization from ethanol-water gave 14.35 g. (44.5%) of aldehyde, m.p. 137.0-138.0°.

Anal. Calcd. for $C_{18}H_{13}NO_5$: C, 66.87; H, 4.05; N, 4.33.
Found: C, 67.17; H, 4.33; N, 4.58.

This aldehyde reduced Tollens' Reagent and Fehling's Solution and gave a deep wine ferric chloride test. An analytical sample of the 2,4-dinitrophenylhydrazone, obtained as yellow needles from chloroform-cyclohexane, melted at 197.0-198.0°.

Anal. Calcd. for $C_{24}H_{17}N_5O_8$: C, 57.26; H, 3.40; N, 13.91.
Found: C, 56.94; H, 3.56; N, 13.62.

4-Carboxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetic Acid Benzyl Ester (LVII). - A solution of DL-penicillamine hydrochloride (9.30 g., 0.0500 mole) and sodium acetate trihydrate (10.20 g., 0.0750 mole) was added to a warm ethanolic solution (75 ml.) of LIV (16.15 g., 0.0500 mole). After storage for 5 hours at room temperature, a heavy, colorless oil had precipitated. The supernatant solution was decanted and the oil was taken up in ethanol. After 24 hours, 8.00 g. of colorless crystalline material had separated, m.p. 148-152° (dec.). Recrystallization from acetone-water raised the melting point to 158-159° (dec.), 4.87 g. After storage for 8 days, the supernatant provided an additional 3.33 g. of crystalline material, m.p. 153-164° (dec.). The combined filtrates and reaction mixture were extracted with methylene chloride to yield 12.0 g. of amorphous solid which was not purified further. The total yield of the crude products was 89%. Several recrystallizations from acetone-water afforded an analytical sample which melted at 160-161° (dec.).

Anal. Calcd. for $C_{23}H_{22}N_2O_6S$: C, 60.78; H, 4.88; N, 6.17.
Found: C,60.67; H, 5.19; N, 6.05.

4-Carbomethoxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetic Acid Benzyl Ester (LX). - A solution of the acid LVII, as a stereoisomeric mixture, (4.54 g., 0.0100 mole) in methylene

chloride was cooled in an ice-bath and treated with an excess of ethereal diazomethane. After storage for 10 min., the color was discharged with glacial acetic acid, the solution was washed with 50 ml. portions of 5% sodium bicarbonate and water and then dried over magnesium sulfate. Concentration of the solution under reduced pressure provided 4.51 g. (96.5%) of a colorless glass. This product was dissolved readily in 50 ml. of ether, but the crystalline ester began to separate almost immediately. After storage at 5° overnight, the colorless prisms were collected; weight, 2.89 g., m.p. 124-125.5°. An analytical sample recrystallized from acetone-water melted at 125.5-126.0°.

Anal. Calcd. for $C_{24}H_{24}N_2O_6S$: C, 61.52; H, 5.16; N, 5.98.
Found: C, 61.50; H, 5.20; N, 5.92.

Catalytic Reduction of LX. - The catalyst (1.56 g. of Pd-C (30%)) was pre-reduced in glacial acetic acid (dichromate test). The ester LX (0.468 g., 1.00 millimole) was added, followed by 4 drops of 70% aqueous perchloric acid.[34] The hydrogenation was allowed to proceed for 12 hours, after which time a net uptake of 22.4 ml. of hydrogen was recorded (92%). The catalyst was separated by centrifugation and the colorless solution was lyophylized to give a colorless solid. Crystallization from ethanol-water afforded 50 mg. of material melting at 148-150°. Two more recrystallizations from ethanol-water afforded an analytical sample, m.p. 155.0-156.5°.

Anal. Calcd. for $C_{16}H_{18}N_2O_4S$: C, 57.47; H, 5.42; N, 8.38.
Found: C, 57.55; H, 5.73; N, 8.59.

This analysis corresponds to the formula for the decarboxylated product LXV.

The filtrate from the first crystallization above was extracted with methylene chloride, and the acidic fraction separated by extraction of this solution with aqueous sodium bicarbonate. The methylene chloride extract produced 180 mg. of non-acidic oil, while acidification of the aqueous layer precipitated no solid, even after several weeks storage at 5°.

t-Butyl Phthalimidoacetate (LII). - Phthaloylglycyl chloride[36] (111.8 g., 0.500 mole) and t-butanol (94 ml., 1.0 mole) were dissolved in 300 ml. of dry methylene chloride. The solution was cooled to -10° and triethylamine (70.0 ml., 0.500 mole) added with rapid stirring over a 15 min. period. The ice-bath was removed and stirring continued for an additional hour. The resulting suspension was washed successively with 100 ml. portions of water, 0.1 N hydrochloric acid, 5% sodium bicarbonate and water. The methylene chloride layer was dried over magnesium sulfate and then concentrated under reduced pressure to a light yellow oil which set to a crystalline mass on cooling. It was broken up and dried to yield 123.5 g. (94.5%) of nearly colorless LII, m.p. 94.0-95.5°. Recrystallization from benzene-petroleum ether (b.p. 30-60°) gives colorless needles, m.p. 96.0-97.5°. An analytical

sample was prepared by sublimation at 85°/0.03 mm., m.p. 96.0-97.5°.

Anal. Calcd. for $C_{14}H_{15}NO_4$: C, 64.35; H, 5.79; N, 5.36.
Found: C, 64.17; H, 6.00; N, 5.46.

The Action of Dry Hydrogen Chloride on t-Butyl Phthalimidoacetate. - Hydrogen chloride gas was passed into a solution of t-butyl phthalimidoacetate (2.61 g., 0.0100 mole) in 25 ml. of dry nitromethane until saturation. Crystallization of phthaloyglycine began immediately. After storage for 8 hours at room temperature, the solvent was removed under reduced pressure, and the residue was taken up in 50 ml. of ether and extracted with two 25 ml. portions of 5% sodium bicarbonate. The combined aqueous extracts were acidified with concentrated hydrochloric acid. After chilling thoroughly, the colorless needles were collected by filtration. The yield was 1.88 g. (92%), m.p. 196-197°, undepressed on admixture with authentic phthaloyglycine.

Phthaloyglycyl Chloride from t-Butyl Phthalimidoacetate. - A solution of t-butyl phthalimidoacetate (2.61 g., 0.0100 mole) in 15 ml. of freshly distilled phosphorous oxychloride was saturated with dry hydrogen chloride. Crystallization began after 15 min. Phosphorous pentachloride (2.28 g., 0.0110 mole) was added, causing the solid to dissolve with the evolution of hydrogen chloride. The solution was heated at 60° for 20 min. and then concentrated under reduced pressure at 60° to a

yellow oil. After flushing with 25 ml. of toluene, and cooling, a mass of needles was formed. Absolute methanol (25 ml.) was added and the solid readily dissolved with the evolution of heat. After several min., water was added to the point of turbidity. On storage, 1.82 g. of colorless needles were deposited, m.p. 112-113.5°, undepressed on admixture with authentic methyl phthalimidoacetate. In a similar experiment, the phosphorous pentachloride was added before the hydrogen chloride. The yield in this case was 1.94 g. (88.5%). When no hydrogen chloride was passed in and the reaction conditions above maintained, conversion to the acid chloride appeared to be incomplete.

t-Butyl Formate. - t-Butanol (280 ml., 3.0 moles) and 88% formic acid (130 ml., 3.0 moles) were heated under reflux for 30 min. and then distilled through a 24 inch column of glass helices. Distillate was collected at 68-73° over a 2 hour period. The crude product was dried over two portions of magnesium sulfate and distilled through a 7 inch Vigreaux column. The material which distilled at 76-79° amounted to 145 g. (47%), $n^{27}D$ 1.3786. This product was stored over calcium sulfate and used for the formylation of t-butyl phthalimidoacetate.

α-Phthalimidomalonaldehydic Acid t-Butyl Ester (LV). - Freshly cut sodium (4.60 g., 0.200 mole) in 200 ml. of reagent grade xylene were placed in a 500 ml. three-necked round bottom

flask equipped with a dropping funnel, stirrer and reflux condenser with a sidearm takeoff. A stream of dry nitrogen was started over the system and the xylene was heated gently to reflux. t-Butanol (55 ml., 0.60 mole) was added to the rapidly stirred suspension over a 15 min. period. Heating with stirring was continued for an additional 2 hours, after which time all the sodium had reacted. The sidearm stopcock was opened and 100 ml. of distillate was collected, leaving a nearly clear solution. After cooling to 0-5°, a solution of t-butyl formate (41 g., 0.40 mole) and t-butyl phthalimidoacetate (52.2 g., 0.200 mole) in 100 ml. of benzene was added with rapid stirring over a 30 min. period. The resulting red-brown solution was stored at 5° for 40 hours. Glacial acetic acid (20 ml.) was added and the yellow suspension was washed with 150 ml. of cold N hydrochloric acid. The aqueous layer was extracted with two 100 ml. portions of benzene. The combined organic extracts were washed with 50 ml. of saturated sodium chloride solution and extracted with four 100 ml. portions of 5% sodium bicarbonate solution. The combined bicarbonate extracts were covered with 100 ml. of benzene and acidified to pH 2 with cold 6 N hydrochloric acid. After separation of the layers, the aqueous phase was extracted further with two 50 ml. portions of benzene. The combined benzene extracts were washed with 50 ml. of saturated sodium chloride solution, dried over magnesium sulfate, and concentrated under reduced pressure to a volume of 25 ml. The

crystalline product, which separated on cooling, was collected by filtration, washed with several small portions of cold benzene and dried to yield 3.60 g. of LV, m.p. 146-152° (dec.). After storage for 8 hours, the original organic reaction phase was extracted with sodium bicarbonate solution as before to give 3.14 g. of nearly colorless crystals, m.p. 155-156° (dec.). A third extraction after 24 hours afforded only a trace of crystalline material. The filtrates from the first and second crops were combined and concentrated to yield an additional 0.71 g. of product, m.p. 153-154° (dec.). The total yield was 13%. An analytical sample was obtained as colorless prisms from benzene, m.p. 155.2-156.4° (dec.).

Anal. Calcd. for $C_{15}H_{15}NO_5$: C, 62.27; H, 5.23; N, 4.84.
Found: C, 62.48; H, 5.29; N, 4.70.

This aldehyde also reduced Tollens' Reagent and Fehling's Solution. It dissolved in sodium bicarbonate and gave a deep wine ferric chloride test. The yellow 2,4-dinitrophenylhydrazone was obtained as felted needles from ethanol, m.p. 167-168° (dec., in bath at 150°).

Anal. Calcd. for $C_{21}H_{19}N_5O_8$: C, 53.73; H, 4.08; N, 14.92.
Found: C, 54.00; H, 4.18; N, 15.04.

4-Carboxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetic Acid t-Butyl Ester (LVIII). - To a warm ethanolic solution (40 ml.) of LV (5.78 g., 0.0200 mole) was added a solution of DL-penicillamine hydrochloride (3.72 g., 0.0200 mole) and

sodium acetate trihydrate (4.08 g., 0.0300 mole) in 40 ml. of water. After storage for 4 hours, the mass of colorless needles which had separated was collected by filtration, washed with 50% aqueous ethanol and dried; weight, 4.65 g., m.p. 171-172° (dec.). Three more crops were collected during the following 8 days to give an additional 2.38 g. of product, m.p. 171-174° (dec.). The total yield was 83.5%. Several recrystallizations of the first crop from acetone-water afforded an analytical sample, m.p. 179.5-180.5° (dec.).

Anal. Calcd. for $C_{20}H_{24}N_2O_6S$: C, 57.13; H, 5.75; N, 6.66.
Found: C, 57.20; H, 5.79; N, 6.35.

4-Carbomethoxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetic Acid t-Butyl Ester (LXI). A suspension of the acid LVIII (0.841 g., 2.00 millimoles) in 50 ml. of ether was cooled in an ice-bath and treated with an excess of ethereal diazomethane. After storage for 10 min., the resulting solution was decolorized with several drops of glacial acetic acid, and washed with 25 ml. portions of 5% sodium bicarbonate and water. The dried (sodium sulfate) solution was concentrated under reduced pressure to a colorless oil; weight, 0.840 g. (96.5%). The ester was crystallized from ether-petroleum ether (b.p. 30-60°), to provide 0.650 g. of colorless needles, m.p. 118.5-119.5°. A second crop of 0.130 g., m.p. 119-120° brought the amount of crystalline ester to 90%. An analytical sample was obtained by recrystallization from ethanol-water, m.p. 121.0-122.0°.

Anal. Calcd. for $C_{21}H_{26}N_2O_6S$: C, 58.05; H, 6.03; N, 6.45.
Found: C, 58.02; H, 6.09; N, 6.52.

4-Carbomethoxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetic Acid Hydrochloride (XV). - Hydrogen chloride was passed into a solution of a stereoisomeric mixture of the esters LXI (3.00 g.) in 50 ml. of pure, dry nitromethane.[39] An ice bath was used to maintain the temperature below 30°. After storage for 30 min., colorless needles began to separate from the yellow solution. It was stored at 5° overnight. The mass of colorless needles was collected by filtration, washed with absolute ether and dried in vacuo; weight, 1.90 g., m.p. 157-158° (dec., in bath at 150°).

In a similar experiment, 0.217 g. of analytically pure LXI was treated with hydrogen chloride in 10 ml. of nitromethane. After storage at 5° overnight, the colorless product was collected, washed with nitromethane and dried in vacuo at room temperature to afford 120 mg. of material which melted at 160-161° (dec., in bath at 150°).

Anal. Calcd. for $C_{17}H_{19}N_2O_6SCl$: C,49.21; H,4.62; N,6.75.
Found: C,48.99; H,4.86; N,7.07.

4-Carboxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetic Acid Hydrochloride (LXIX). - A 250 mg. sample of analytically pure LVIII was treated with hydrogen chloride in 10 ml. of nitromethane. After 15 min., crystallization began. The product was collected by filtration after 2 hours, washed

39. H. T. Hays, G. F. Hager, H. M. Engelmann and H. M. Spurlin, J. Am. Chem. Soc., 73, 5369 (1951).

with nitromethane and absolute ether and dried at room temperature *in vacuo*; weight, 0.20 g., m.p. 196.5-197.5° (dec.).

Anal. Calcd. for $C_{16}H_{17}N_2O_6SCl$: C, 47.94; H, 4.28; N, 6.99.
Found: C, 47.45; H, 4.58; N, 7.30.

Reaction of LXI with Hydrogen Chloride and Phosphorous Pentachloride in Phosphorous Oxychloride. - Hydrogen chloride was passed into a solution of LXI (4.34 g., 0.0100 mole) in 25 ml. of freshly distilled phosphorous oxychloride. An ice-bath was used to maintain the temperature below 30°. After one hour, phosphorous pentachloride (2.29 g., 0.0110 mole) was added. Hydrogen chloride was evolved and solution was soon complete. After storage at room temperature for 1 hour, the solution was concentrated under reduced pressure at 55° to an orange oil. The residue was flushed with two 50 ml. portions of toluene and the solvents removed *in vacuo* to leave a residue of 5.77 g. which was taken up in 50 ml. of methylene chloride. A ten ml. portion of this solution was added to a solution of 2 ml. of aniline in 25 ml. of methylene chloride. After storage for 1 hour, the suspension was washed with 25 ml. of water, which caused a solid to separate. The methylene chloride layer yielded an oily solid. The two crude products were combined and crystallized from methanol-water to afford 350 mg. (39%) of tan crystalline powder, m.p. 213-217° (dec., softens at 208°). Recrystallization from ethyl acetate-petroleum ether (b.p. 30-60°) and then from acetone-water provided LXXII as colorless needles, m.p. 222-223° (dec.).

Anal. Calcd. for $C_{23}H_{23}N_3O_5S$: C, 60.90; H, 5.11; N, 9.29.
Found: C, 61.03; H, 5.21; N, 9.03.

This isolation of the anilide in pure form indicated that the acid chloride hydrochloride LXX had been formed. Several preliminary attempts to cause LXX to cyclize have been unsuccessful.

4-Carbomethoxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetic Acid β-Lactam Sulfone (XVI). - A suspension of XV (0.250 g., 0.60 millimole) in 15 ml. of reagent grade benzene and 5 ml. of freshly distilled thionyl chloride was heated under reflux for 15 min. after which time solution was complete. Heating was continued for an additional hour and the yellow solution concentrated under reduced pressure to a yellow glass. Preliminary attempts to obtain a crystalline product were unsuccessful. Therefore, the crude product was dissolved in 10 ml. of glacial acetic acid and treated with a solution of 0.375 g. of potassium permanganate in 3 ml. of water. After storage at room temperature for 30 min., the suspension was decolorized by the dropwise addition of 30% hydrogen peroxide. The clear, colorless solution was diluted to a volume of 75 ml., causing a fine crystalline solid to separate. After storage at 5° for several hours, the product was collected, washed well with water and recrystallized from acetone-water to afford 0.050 g. (13%) of pure XVIa, m.p. 200-201° (dec.). An analytical sample was obtained as colorless

needles from acetone-water, m.p. 200-201° (dec.).

Anal. Calcd. for $C_{17}H_{16}N_2O_7S$: C, 52.03; H, 4.11; N, 7.14.

Found: C, 52.18; H, 4.05; N, 7.27.

SECTION B

APPLICATION OF THE THIOLESTER PEPTIDE SYNTHESIS TO AN ATTEMPTED PREPARATION OF PENICILLIN

DISCUSSION

In this section is discussed the application of the thiolester peptide synthesis[17] to an attempted preparation of penicillin.

The synthetic scheme is outlined on Figure III. Compounds LXXII-LXXVII have been prepared previously. We have selected what appears to be the most convenient route from the numerous syntheses for the intermediate LXXV.

The formylation of glycine ethyl ester hydrochloride was accomplished according to the modified procedure of Jones,[40] which recommends the use of 98% formic acid. The yield of N-formylglycine ethyl ester (LXXII) was 85%. The interaction of LXXII with ethyl formate in the presence of sodium ethoxide afforded the sodium enolate of C-formyl-N-formyl-glycine ethyl ester (LXXIII) in 96% yield. A 37% conversion of the crude enolate to the acetal LXXIV was obtained. Phenylacetylation of LXXIV provided an 88.5% yield of benzylpenaldic acid diethyl acetal (LXXV),[41] which was converted to 2-benzyl-5-methoxy-

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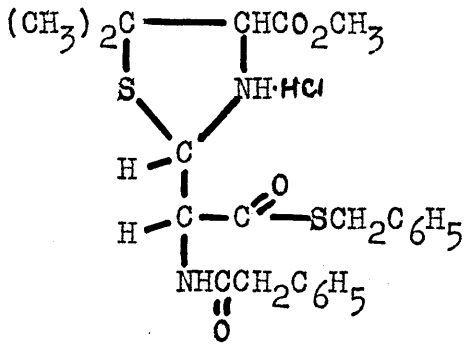
40. R. G. Jones, J. Am. Chem. Soc., 71, 644 (1949).

41. Reference 25, p. 512.

methylene-5-oxazolone by warming with acetic anhydride. This product was hydrolyzed directly with sodium hydroxide to give an 85% yield of 2-benzyl-4-hydroxymethylene-5-oxazolone (LXXVI).[42] Benzyl mercaptan reacted readily with LXXVI to afford a 60% yield of the aldehyde LXXVII.[43] Condensation with DL-penicillamine hydrochloride led to isolation of a 92% yield of amorphous LXXVIII. Esterification with diazomethane gave the methyl ester, which was characterized as its crystalline hydrochloride LXXIX.



LXXIX

Neutralization of the acid LXXVIII with one equivalent of sodium ethoxide in absolute ethanol, followed by the removal of the solvent, afforded the anhydrous, amorphous salt LXXX. This salt was heated under the conditions listed in Figure IV in an effort to eliminate the elements of benzyl mercaptan. After the thermal treatment, the solvents were removed under reduced pressure, the residues taken up in 0.1 M phosphate buffer of pH 7.2 and stored in Dry Ice until they could be

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42. Reference 25, p. 521.

43. Reference 14, p. 825.

assayed for biological activity.

The successful preparation of LXXVIII stimulated interest in possible methods of obtaining the thioacid LXXXI from the thiolester LXXVIII or from an analgous ester obtained from some other mercaptan. Thiol acids are known to be good acylating agents. They generally react with amines at room temperature or with heating to give the amides. In addition, it has been reported[44] recently that amides are obtained when a mixture of the sodium salt of a thioacid and an amine are treated with iodine at 10°. Since the reaction is reported to be very rapid, penicillin would be expected to survive this treatment.

$$RC(=O)SNa + R'NH_2 + I_2 \rightarrow RC(=O)NHR'$$

However, no amino thoacids or their N-acylated derivatives appear to be known. We therefore prepared thiophenaceturic acid (LXXXII) to see whether or not it would azlactonize. The action of dehydrating agents (i.e. attempts to prepare the acid chloride) on phenaceturic acid are reported to produce 2-benzyl-5-oxazolone (LXXXIII) or a salt thereof. The thioacid LXXXII was obtained in 72% yield by extension of the mixed anhydride method for preparation of peptides.[45] Thiolesters have been prepared recently by use of a similar technique.[17]

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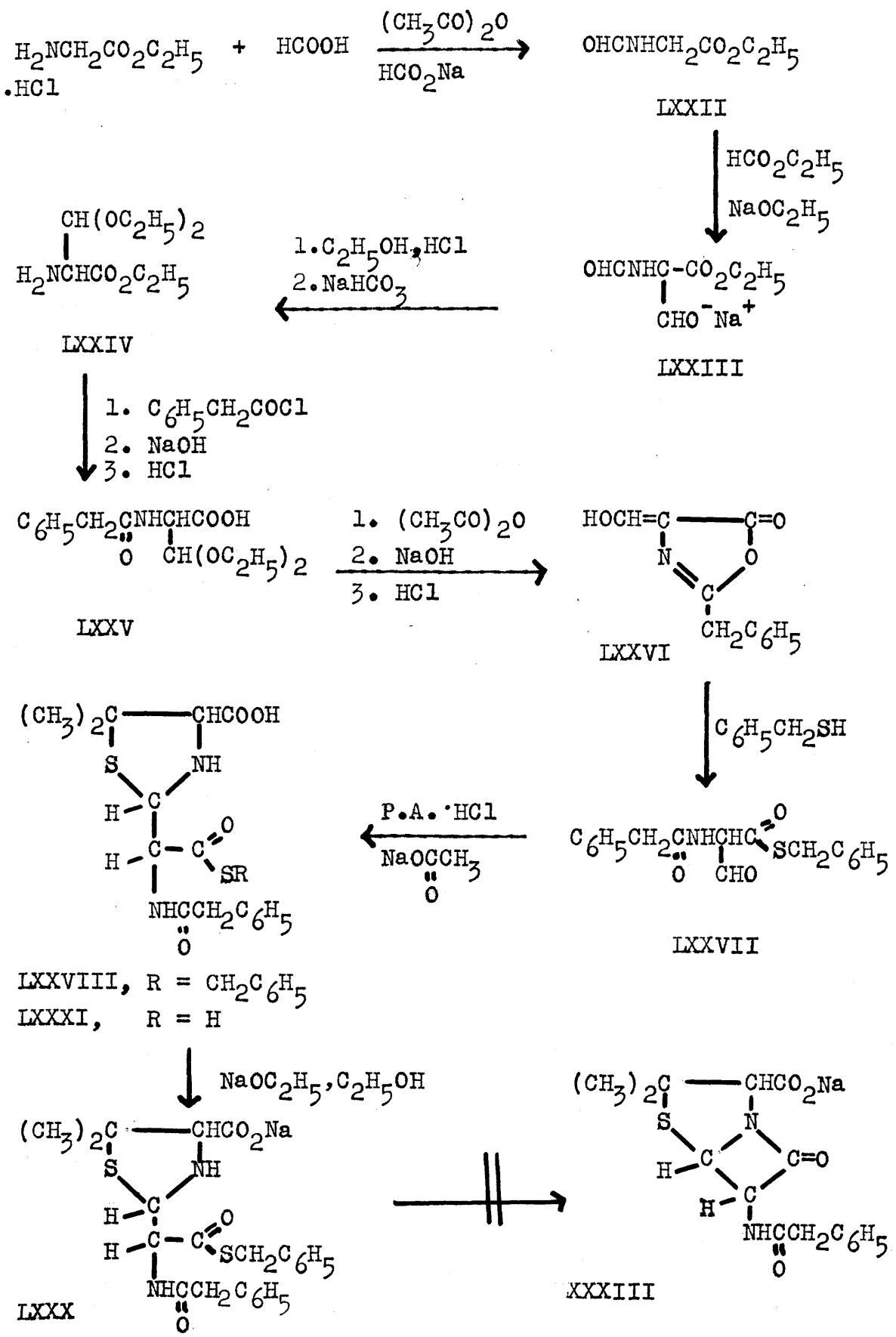
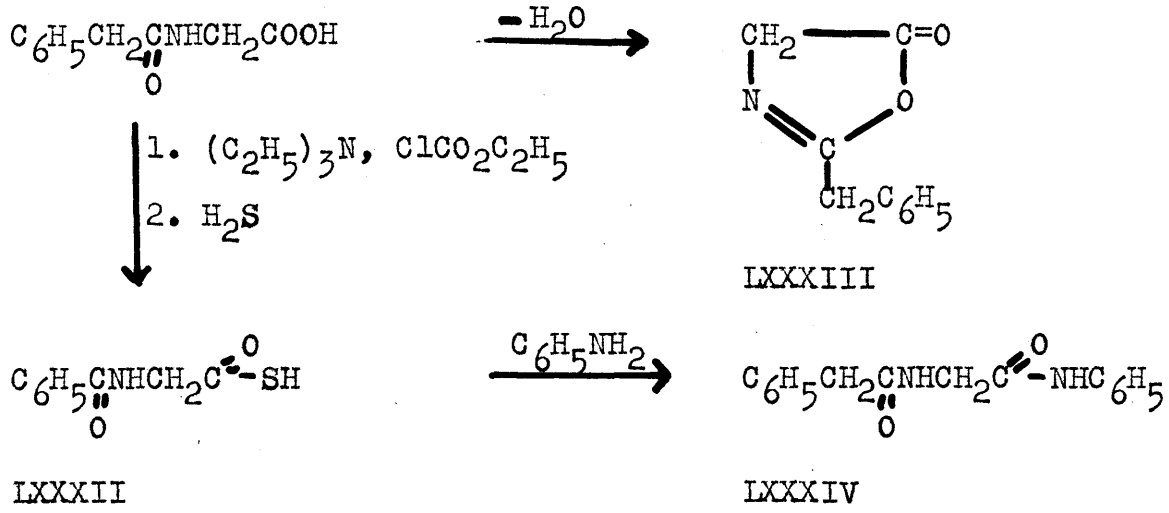
44. G. Alliger, G. E. P. Smith, Jr., E. L. Carr and H. P. Stevens, J. Org. Chem., 14, 962 (1949).

45. R. A. Boissonnas, Helv. Chim. Acta, 34, 874 (1951).

Figure III


H2NCH2CO2C2H5 .HCl + HCOOH
(CH3CO)2O
HCO2Na
OHCNHCH2CO2C2H5
LXXII
HCO2C2H5
NaOC2H5
OHCNHC-CO2C2H5
CHO⁻Na⁺
LXXIII
1.C2H5OH,HCl
2.NaHCO3
CH(OC2H5)2
H2NCHCO2C2H5
LXXIV
1. C6H5CH2COCl
2. NaOH
3. HCl
C6H5CH2CNHCHCOOH
O CH(OC2H5)2
LXXV
1. (CH3CO)2O
2. NaOH
3. HCl
HOCH=C—C=O
N O
C
CH2C6H5
LXXVI
C6H5CH2SH
C6H5CH2CNHCHC—SCH2C6H5
O CHO O
LXXVII
P.A.·HCl
NaOCCH3
O
(CH3)2C—CHCOOH
S NH
H C
H C—C=O
SR
NHCCH2C6H5
O
LXXVIII, R = CH2C6H5
LXXXI, R = H
NaOC2H5,C2H5OH
(CH3)2C—CHCO2Na
S NH
H C
H C—C=O
SCH2C6H5
NHCCH2C6H5
O
LXXX
(CH3)2C—CHCO2Na
S N
H C C=O
H C
NHCCH2C6H5
O
XXXIII

Thiophenaceturic acid is a stable, crystalline solid. It reacted with aniline in ethanolic solution at room temperature to give the phenaceturanilide (LXXXIV) in good yield.



LXXXIII

LXXXII

LXXXIV

Benzyl thiophenaceturate (LXXXV), also prepared by the mixed anhydride procedure, was used as a model to study the effect of the reagents known to cleave benzyl esters. The Action of sodium in liquid ammonia led to phenaceturamide (LXXXVI) as the sole product isolated. Attempted catalytic reduction of LXXXV using an equal weight of catalyst (Pd-C) was also unsuccessful. Phosphonium iodide[46] in acetic acid similarily was uneffective.

In view of the rapid cleavage of a t-butyl ester to the acid by anhydrous hydrogen chloride, as reported in Part I of this thesis, we have prepared t-butyl phthalimidothiolacetate (LXXXVII) from phthaloylglycyl chloride and t-butyl mercaptan

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46. C. R. Harrington and T. H. Mead, Biochem. J., 29, 1605 (1935).

in the presence of triethylamine and subjected it to similar conditions. Hydrogen chloride in nitromethane had no affect on this compound, whereas the oxygen analog was cleaved immediately under identical conditions. The anticipated thioacid LXXXVIII was obtained *via* the mixed anhydride procedure.

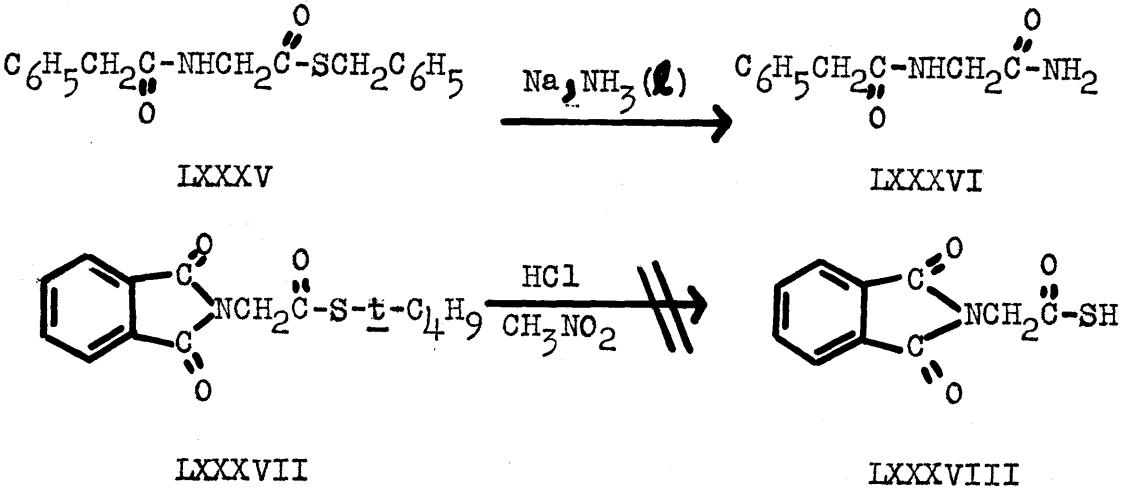

Figure IV

The sodium salt LXXX (0.200 g. portions) was heated under the following conditions. After heating, the solvents were removed under reduced pressure and the residues taken up in 10 ml. of 0.1 M phosphate buffer, pH 7.2. These samples were stored in Dry Ice until they could be bioassayed.

Solvent	Temperature	Time	Appearance After Heating	Bioassay
none	--	--	blank sample	Preliminary reports indicate slight activity or no activity.
xylene	70°	13 hrs.	pale-yellow, clear	
xylene	reflux	1 hr.	yellow, turbid	
nitromethane	70°	13 hrs.	pale-yellow, turbid	
nitromethane	reflux	2 hrs.	yellow, clear	
acetic acid	70°	4 hrs.	yellow, clear	
acetic acid	reflux	2 hrs.	nearly colorless, clear	
pyridine	70°	10 hrs.	light-yellow, turbid	
pyridine	reflux	1 hr.	pale-yellow, clear	
dimethyl formamide	70°	12 hrs.	pale-yellow, clear	
dimethyl formamide	reflux	15 min.	dark-brown	
no solvent	130°	15 min.	brown glass	
dimethyl formamide	65°	16 hrs.	light-yellow, clear	
dimethyl formamide	75°	16 hrs.	light-yellow, clear	
no solvent	110°/0.5mm.	30 min.	yellow glass	
	120°/0.5mm	20 min.	yellow glass	
	135°/0.5mm	20 min.	yellow glass	

EXPERIMENTAL

N-Formylglycine Ethyl Ester (LXXII).[40] - Sodium formate (204 g., 3.00 moles) was dissolved in 300 ml. of 98% formic acid and added to a solution of glycine ethyl ester hydrochloride (349 g., 2.50 moles) in 355 ml. of 98% formic acid. After storage at room temperature for 1 hour, the suspension was filtered through Celite. The filter cake was washed with 700 ml. of acetic anhydride and the washings were then added in portions to the original filtrate. After the vigorous reaction had subsided (1 hour), the solution was heated on a steam cone for 30 min. and concentrated under reduced pressure to an oil. One liter of dry acetone was added and the precipitated salts filtered. The filtrate was concentrated under reduced pressure to a yellow liquid which, when distilled through a 6 inch Vigreaux column, gave 278 g. (85%) of clear, colorless LXXII boiling at 115-117°/2 mm. (reported 110°/1 mm.)[40], $n^{25.4}D$ 1.4510.

Sodium Enolate of C-Formyl-N-formylglycine Ethyl Ester (LXXIII).[41] - Freshly cut sodium (23.0 g., 1.00 mole) and 500 ml. of reagent grade xylene were placed in a 1-l. three-necked round bottom flask equipped with a reflux condenser with a distilling side arm, a stirrer, and a dropping funnel. A constant flow of dry nitrogen was maintained over the system

at all times. The xylene was heated until the sodium became molten, the stirrer started and absolute ethanol (66 ml., 1.1 moles) was added dropwise over a 30 min. period. The suspension was heated under reflux for 10 min. and then 50 ml. of distillate was collected from the side arm. The colorless suspension was cooled to room temperature and added in portions over a 30 min. period to a rapidly stirred solution of N-formylglycine ethyl ester (131 g., 1.00 mole) and 321 ml. (4.00 moles) of ethyl formate (E. K. Pure, dried over calcium sulfate and redistilled) at 0°. The resulting clear, pale-yellow solution was stored at 5° for 17 hours after which time a yellow gum had separated. The supernatant liquid was decanted and the gum triturated with 500 ml. of absolute ether. The mixture was stirred until all the lumps had been broken and the salt was collected by filtration, washed with two 100 ml. portions of absolute ether and dried to constant weight at 0.05 mm. to give 174 g. (96.0%) of LXXIII as a pale-yellow powder.

β,β-Diethoxyalanine Ethyl Ester (LXXIV).[41] - Dry hydrogen chloride gas (200 g.) was passed into 1400 ml. of absolute ethanol contained in a 2-l, three-necked flask equipped with a drying tube and gas inlet. The solution was cooled to 0° and the LXXIII (174 g., 0.960 mole) was added with rapid stirring over a 20 min. period. Stirring was continued for an additional 15 min. and the suspension stored at room temperature overnight. After concentration to a yellow magma under reduced

pressure, the residue was taken up in 1 l. of chloroform and 225 g. of sodium bicarbonate was added with rapid stirring. Stirring was continued for 3 hours after which time the suspension was filtered. The salt cake was washed well with chloroform and the combined filtrate and washings was extracted with 300 ml. of 5% sodium bicarbonate solution, then washed with 300 ml. of saturated sodium chloride solution. Concentration of the chloroform layer under reduced pressure yielded a dark-brown oil. A preliminary distillation at 76-90°/0.08 mm. gave 75.0 g. of colorless liquid which was redistilled through a 6 inch Vigreaux column to yield 72.0 g. (36.9%) of LXXIV as a colorless liquid boiling at 72-73°/0.1 mm. (reported 71°/0.1 mm.),[41] $n^{25.6}D$ 1.4296.

Benzylpenaldic Acid Diethyl Acetal (LXXV).[41] - To a solution of β,β-diethoxyalanine ethyl ester (69.8 g., 0.340 mole) in 800 ml. of chloroform was added to a solution of sodium bicarbonate (67 g., 0.80 mole) in 175 ml. of water. The mixture was cooled to 0-5° and phenylacetyl chloride (45.0 ml., 0.340 mole) was added dropwise with vigorous stirring over a 30 min. period. Stirring was continued for 30 min. at 0° and then for 1 hour after warming to room temperature with a water-bath. After separation of the layers, the chloroform solution was washed with 200 ml. of water and then concentrated under reduced pressure to a light-yellow oil, which was added in one portion to a solution of sodium hydroxide (13.6 g., 0.340 mole) in 750 ml. of 95% ethanol. After storage

at room temperature for 5 hours, the solution was concentrated under reduced pressure to a light yellow oil. The oily salt was dissolved in 400 ml. of water and the solution extracted with 200 ml. of ether. After cooling the aqueous solution in an ice-bath, it was acidified to congo red with 6 N hydrochloric acid. The cream-colored, crystalline precipitate was collected by filtration, washed well with water and dried in vacuo to afford 89.0 g. (88.5%) of LXXV, m.p. 109-110° (dec.). It was recrystallized by solution in 300 ml. of hot benzene and addition of 300 ml. of petroleum ether (b.p. 30-60°). On cooling, 86.8 g. (97.5% recovery) of colorless needles separated, m.p. 111.5-112.0° (dec.) (reported 112-113°).[41]

2-Benzyl-4-hydroxymethylene-5-oxazolone (LXXVI).[42] - Benzylpenaldic acid diethyl acetal (29.53 g., 0.100 mole) was suspended in 100 ml. of acetic anhydride and heated at 70° for 15 min. after solution was complete. The clear, light-yellow solution was concentrated under reduced pressure at 70° (finally at 0.5 mm.) to a cherry-red oil. The oil was shaken for 15 min. with 4.0 g. of sodium hydroxide dissolved in 250 ml. of water. Additional base was added as needed to maintain the solution alkaline to phenolphthalein. The orange solution was extracted with 50 ml. of ether, cooled in an ice-bath and acidified to pH 3 with cold 20% hydrochloric acid. The crystalline product was collected immediately by filtration, washed with four 50 ml. portions of ice-cold water and dried in vacuo to give 17.20 g. (85%) of LXXVI as a pale-yellow powder, m.p. 112.0-112.5° (dec.)

in bath at 100°) (reported 130-132°).[42] Material of this degree of purity is generally used for subsequent reactions, since large losses attend recrystallization.

Benzyl Benzylthiolpenaldate (LXXVII).[43] - 2-Benzyl-4-hydroxymethylene-5-oxazolone (8.12 g., 0.0400 mole) was suspended in 15 ml. of benzyl mercaptan and heated on a steam cone for 5 min. This treatment produced a clear, red solution (accompanied by some evolution of gas) which gave a wine-colored test with ferric chloride rather than the blue color characteristic of the oxazolone. The solution was diluted with 50 ml. of ether and extracted with two 50 ml. portions of 10% sodium carbonate solution. The combined aqueous extracts were washed with 50 ml. of ether, cooled in an ice-bath and acidified to pH 2 with cold concentrated hydrochloric acid, causing the formation of a cream-colored crystalline precipitate, which was collected by filtration. The product was washed well with water and dried in vacuo to give 7.90 g. (60.5%) of crude LXXVII, m.p. 91-100° (dec.). Recrystallization from methylene chloride-ether gave colorless needles, m.p. 112.0-113.5° (dec.). Two more recrystallizations from chloroform-ether provided analytically pure material melting at 115.0-115.8° (reported 113-114°).[43]

Anal. Calcd. for $C_{18}H_{17}NO_3S$: C, 66.03; H, 5.23; S, 9.79.
Found: C, 66.22; H, 5.33; S, 9.65.

This compound gave a wine-colored ferric chloride test. An analytical sample of the yellow-orange 2,4-dinitrophenyl-

hydrazone, crystallized from chloroform-cyclohexane, melted at 171-172.5° (dec.) (reported 158-160°).[43]

Anal. Calcd. for $C_{24}H_{21}N_5O_6S$: C, 56.79; H, 4.17; N, 13.80.
Found: C, 56.70; H, 4.26; N, 13.83.

α-Benzyl α-thiol-DL-benzylpenicilloate (LXXVIII). - To a warm solution of LXXVII (9.15 g., 0.0280 mole) in 100 ml. of 95% ethanol was added a solution of DL-penicillamine hydrochloride (5.21 g., 0.0280 mole) and sodium acetate trihydrate (5.72 g., 0.042 mole) in 100 ml. of water. After storage of the solution at room temperature for 40 hours, a colorless oil had separated. This was extracted into two 50 ml. portions of methylene chloride. The combined methylene chloride extracts were washed with three 50 ml. portions of water, dried over magnesium sulfate and concentrated under reduced pressure to a colorless fluffy glass; 11.8 g. (92%).

Sodium Salt of α-Benzyl α-Thiol-DL-benzylpenicilloate (LXXX). - A neutralization equivalent on the amorphous LXXVIII gave a value of 448 (calcd. 459). Therefore, 4.48 g. of the acid was assumed to be 0.010 mole. This amount of material was dissolved in 50 ml. of absolute ethanol, the solution cooled in an ice-bath and treated with 11.15 ml. of 0.895 N. sodium ethoxide in absolute ethanol. The pale-yellow solution was diluted with an equal volume of benzene and then concentrated under reduced pressure to a pale-yellow, fluffy glass. This was dried further for 20 hours at room temperature at

0.5 mm. The fine, pale-yellow hygroscopic powder weighed 4.66 g. (100%).

α-Benzyl-β-methyl α-Thiol-DL-benzylpenicilloate Hydrochloride (LXXIX). - The amorphous LXXVIII (0.46 g., 1.0 millimole) was dissolved in 50 ml. of methylene chloride, the solution was cooled in an ice-bath and ethereal diazomethane was added until the yellow color of the reagent persisted. After 5 minutes, this color was discharged by the dropwise addition of glacial acetic acid. The colorless solution was washed with 25 ml. portions of 5% sodium bicarbonate and water. After drying over magnesium sulfate, the solution was concentrated under reduced pressure to a pale-yellow oil. The yield was 0.46 g. (97%). Since the crude ester did not crystallize readily, it was dissolved in absolute ether and precipitated as its hydrochloride. After two recrystallizations from absolute ethanol-ether, 0.20 g. of colorless needles were obtained, m.p. 160-162° (dec.). An analytical sample, recrystallized from absolute ethanol-petroleum ether (b.p. 30-60°), melted at 160-161° (dec.).

Anal. Calcd. for $C_{24}H_{29}N_2O_4S_2Cl$: C, 56.62; H, 5.74; N, 5.50.
Found: C, 56.46; H, 5.92; N, 5.27.

Thiophenaceturic Acid (LXXXII). Triethylamine (14.0 ml., 0.100 mole) was added to a suspension of phenaceturic acid (19.32 g., 0.100 mole) in 150 ml. of dry methylene chloride. The resulting solution was cooled to -10° and ethyl

chlorocarbonate (9.55 ml., 0.100 mole)(E. K. Pure) was added with rapid stirring over a 10 min. period. A heavy white precipitate formed in the blue solution. Stirring was continued at -10° for 10 min. and then hydrogen sulfide gas was passed in while allowing the reaction mixture to warm to room temperature. The mixture was stored at room temperature overnight, after which time solution was again complete. When the solution was washed with 100 ml. of water, pale-yellow platelets began to crystallize. After several min., the two phase system was filtered through a suction filter to provide 12.30 g. of LXXXII, m.p. 112-113.5° (dec.). The aqueous layer was extracted with 50 ml. of methylene chloride, and the combined organic extracts washed with 50 ml. of water. After drying over magnesium sulfate, the solution was concentrated under reduced pressure to a volume of 50 ml. Addition of ligroin (b.p. 90-100°) caused the separation of a solid, which after crystallization from chloroform-ligroin gave 2.75 g. of additional acid, m.p. 107-109° (dec.), to bring the total yield to 72%. Recrystallization from acetone-water and then acetone-cyclohexane afforded an analytical sample, m.p. 116.5-118.0° (dec.).

Anal. Calcd. for $C_{10}H_{11}NO_2S$: C, 57.39; H, 5.30; N, 6.69.
Found: C, 57.45; H, 5.33; N, 6.75.

Phenaceturanilide (LXXXIV) from Thiophenaceturic Acid. - The thioacid LXXXII (1.05 g., 5.00 millimoles) and aniline

(0.46 ml., 5.0 millimoles) were dissolved in 10 ml. of 50-50 95% ethanol-0.1M phosphate buffer of pH 7.5, and stored at room temperature. The evolution of hydrogen sulfide was detected at once. After 1 hour, crystals began to separate. The reaction mixture was stored at room temperature for 18 hours after which time the product was collected by filtration; weight, 1.04 g. (77.5%), m.p. 163-164° (reported 162.5-163.5°).[35] No attempt was made to obtain a second crop.

Benzyl Thiophenaceturate (LXXXV). - Triethylamine (14.0 ml., 0.100 mole) was added to a suspension of phenaceturic acid (19.32 g., 0.100 mole) in 150 ml. of dry methylene chloride. The resulting solution was cooled to -25° and ethyl chlorocarbonate (9.55 ml., 0.100 mole) was added with rapid stirring over a 10 min. period. Stirring was continued for 20 min. as the suspension was allowed to warm to -5°. Benzyl mercaptan (11.7 ml., 0.100 moles) was added and the mixture was allowed to come to room temperature with stirring over a 1½ hour period. The solution was washed with 100 ml. of water, 50 ml. of 5% sodium bicarbonate solution, and finally again with 50 ml. of water. After drying over magnesium sulfate, the solution was concentrated under reduced pressure to an amber oil. Absolute ether (50 ml.) was added and the solution was stored at 5° overnight. The product was collected by filtration, washed with ether and dried to give 8.60 g. of LXXXV as colorless needles, m.p. 78-85°. Addition of ligroin

(b.p. 90-100°) to the filtrate afforded a second crop of 3.00 g., m.p. 70-75°. Recrystallization from ethyl acetate-petroleum ether (b.p. 30-60°) provided 9.60 g. of colorless needles, m.p. 84-86°. Two recrystallizations from acetone-water afforded analytically pure ester, m.p. 86.5-87.5°.

Anal. Calcd. for $C_{17}H_{17}NO_2S$: C, 68.20; H, 5.70; S, 10.76.
Found: C, 68.26; H, 5.66; S, 10.97.

The Action of Sodium in Liquid Ammonia on Benzyl Thiolphenaceturate. - The thiolester LXXXV (2.99 g., 0.0100 mole) was added to 40 ml. of liquid ammonia. The solution was stirred rapidly and sodium metal (0.460 g., 0.0200 mole) was added. The initial blue color was discharged almost immediately. An additional 0.33 g. (0.01 mole plus 0.10 g.) portion of sodium was added, which caused the color to persist. After 10 min., 25 mg. of solid ammonium chloride was added and the color soon disappeared. Assuming no moisture was present, this indicated that 3 equivalents of sodium had been consumed by the thiolester. The ammonia was boiled out by warming the flask in a pan of warm water. Methylene chloride (25 ml.) was added and flushed off under reduced pressure. The residue was shaken with 75 ml. of methylene chloride and 75 ml. of water. Some material remained undissolved. This was combined with a trace of material obtained from the methylene chloride extract and crystallized from ethanol to give 0.400 g. of colorless needles, m.p. 177-178°, (reported for phenaceturamide 176-177°).[47] The combined aqueous extracts were

acidified to pH 2 with concentrated hydrochloric acid. There was a strong evolution of hydrogen sulfide. Extraction of the turbid solution with methylene chloride yielded only a trace of material.

When 1.00 g. of the thiolester in 20 ml. of ethanol was treated with anhydrous ammonia overnight, 0.400 g. of colorless plates had separated, m.p. 178-179°.

t-Butyl Phthalimidothiolacetate (LXXXVII). - Triethylamine (14.0 ml., 0.100 mole) was added over a 15 min. period to a rapidly stirred solution of phthaloylglycyl chloride (22.36 g., 0.100 mole) and t-butyl mercaptan (18.0 g., 0.200 mole) in 150 ml. of dry methylene chloride at -10°. The cooling bath was removed and stirring continued for 1 hour. The suspension was washed with successive 50 ml. portions of water, 0.1 N hydrochloric acid, 5% sodium bicarbonate solution and water. After drying over magnesium sulfate, the solution was concentrated under reduced pressure to a clear oil which soon set to a crystalline mass. The product was pulverized and dried further in vacuo to yield 25.38 g. (91.5%) of LXXXVII, m.p. 108.5-110°. Several recrystallizations from acetone-water provided an analytical sample, m.p. 113.5-114.5°.

Anal. Calcd. for $C_{14}H_{15}NO_3S$: C, 60.63; H, 5.45; S, 11.56.
Found: C, 60.75; H, 5.54; S, 11.97.

The Action of Dry Hydrogen Chloride on LXXXVII. - A solution of the thiolester (2.77 g., 0.0100 mole) in 25 ml. of

nitromethane was saturated with hydrogen chloride gas. After storage at room temperature for 8 hours, the solvent was removed under reduced pressure and the residue taken up in 50 ml. of ether. The ethereal extract was washed with two 25 ml. portions of 5% sodium bicarbonate solution and then with 25 ml. of water. Acidification of the aqueous extracts produced only a faint turbidity. The ethereal phase was concentrated to a crystalline solid under reduced pressure. Recrystallization from acetone-water yielded 2.49 g. (90% recovery) of the starting ester, m.p. 111.5-113°.

Phthaloylthioglycine (LXXXVIII). - Phthaloylglycine (20.52 g., 0.100 mole) and triethylamine (14.0 ml., 0.100 mole) were dissolved in 150 ml. of dry methylene chloride. The solution was cooled to -10° and ethyl chlorocarbonate (9.55 ml., 0.100 mole) was added with rapid stirring over a 10 min. period. The solution was stirred for an additional 10 min. and then saturated with hydrogen sulfide gas as the solution assumed room temperature. The suspension was stirred overnight after which time solution was again complete. The solution was washed with 100 ml. of water and extracted with three 50 ml. portions of saturated sodium bicarbonate solution. The aqueous extracts were acidified with concentrated hydrochloric acid, causing a colorless crystalline precipitate to form. The product was collected by filtration; weight, 13.50 g. (61%), m.p. 109-112°. Recrystallization from

acetone-cyclohexane afforded colorless platelets, m.p. 115-116.5°. A second recrystallization from acetone-cyclohexane provided an analytical sample, m.p. 116.5-118.0°.

Anal. Calcd. for $C_{10}H_7NO_3S$: C, 54.29; H, 3.19; N, 6.33.
Found: C, 54.49; H, 3.33; N, 6.36.

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47. H. Finger and W. Zeh, J. fur prakt. Chem. (2) 82, 50 (1910).

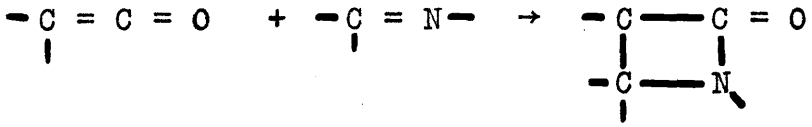
PART II

THE SYNTHESIS OF BICYCLIC β-LACTAMS DERIVED FROM CYSTEINE

INTRODUCTION

This part of the thesis describes the synthesis of a new bicyclic β-lactam.

Monocyclic β-lactams were first prepared by Staudinger as an extension of his investigations of ketenes.[48] They were of little interest, however, until it was indicated that penicillin probably contained a fused thiazolidine-β-lactam nucleus. The chemistry of β-lactams has been treated in a recent review.[49] Staudinger's original synthesis of a β-lactam resulted from the reaction of a ketene with an imine.



The obvious extension to the synthesis of bicyclic β-lactams in general and to penicillin in particular was to use a cyclic imine, for example, a thiazoline. Despite many attempts, only one bicyclic β-lactam has been obtained directly by this method.[49] Diphenylketene (LXXXIX) and 2-phenyl-2-thiazoline (XXXVIII) reacted to give XC.

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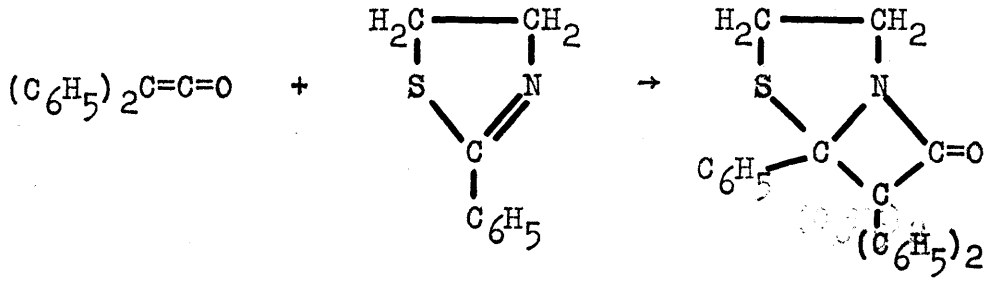
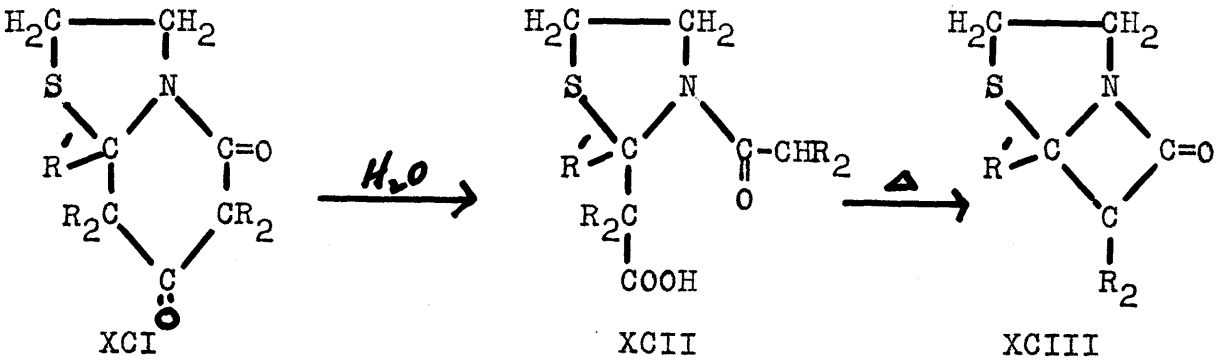
48. H. Staudinger, "Die Ketene," Enke, Stuttgart (1912).

49. Reference 31, p. 973.



LXXXIX XXXVIII XC

In most cases, the products isolated were thiazolidine-2,4-piperidinediones (XCI), formed from 2 molecules of the ketene to one of the thiazoline. In two instances (R = CH_3, R' = CH_3 or C_6H_5), β-lactams were obtained indirectly by hydrolysis of the piperidinediones to the N-isobutyryl-2-thiazolidine-α-butyric acids (XCII) and loss of isobutyric acid accompanied by ring closure to the β-lactam XCIII on heating.



XCI XCII XCIII

Although these three synthetic bicyclic lactams failed to behave like penicillin in many of their chemical reactions, their infrared spectra were highly significant. While the monocyclic β-lactams exhibited a carbonyl band at 5.75 μ, penicillin showed a strong absorption at 5.62 μ.[50] This

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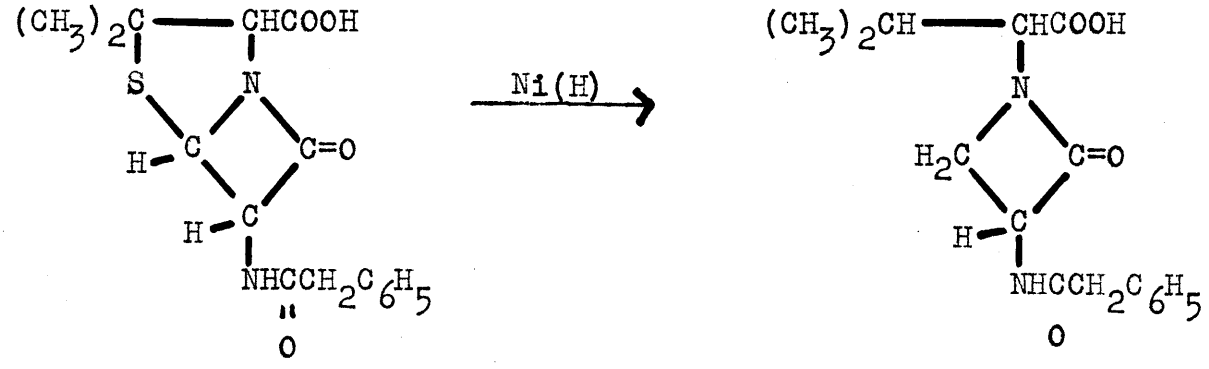
50. H. W. Thompson, R. R. Brattain, H. M. Randall and R. S. Rasmussen, Reference 2, Chapter XIII, p. 382.

apparent discrepancy was eliminated by two developments. Examination of the spectra of the three synthetic bicyclic β-lactams indicated lactam carbonyl absorption at 5.62-5.65 μ, in good agreement with that for penicillin. In addition, the successful desulfurization of benzylpenicillin (XCIV) to desthiobenzylpenicillin (XCV), which contains a monocyclic β-lactam, showed that the carbonyl band had shifted to 5.74 μ.[51]



XCIV XCV

Recently, a new synthesis of bicyclic β-lactams has been reported by Sheehan and Ryan.[52] By allowing 2-phenyl-2-thiazoline (XXXVIII) to react with phthaloylglycyl chloride in the presence of triethylamine under high dilution conditions, they obtained a 32% yield of XCVI.

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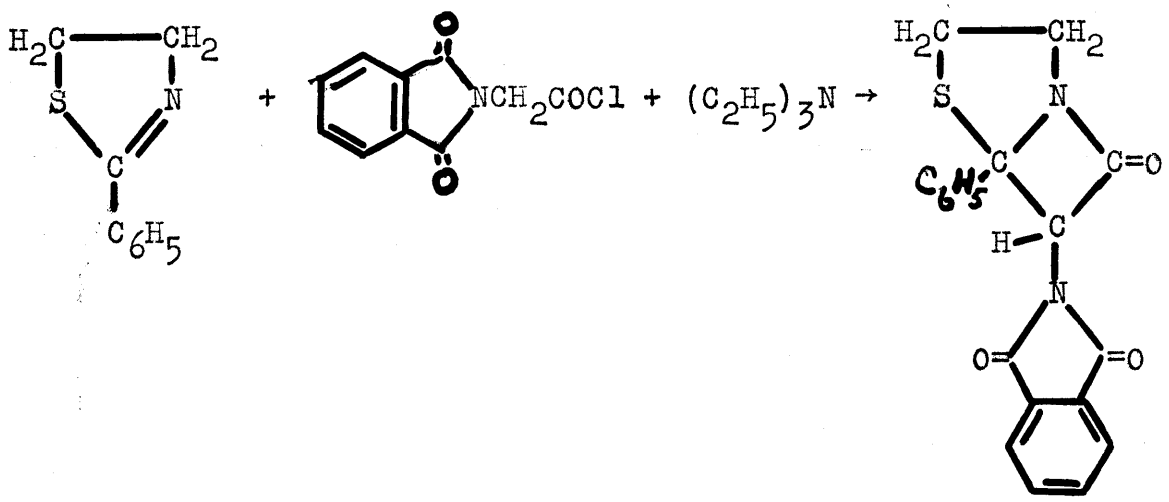
51. E. Kaczka and K. Folkers, Reference 2, Chapter IX, p. 243.

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



XXXVIII XCVI

Concurrent with this work, the reaction has been extended by Sheehan and coworkers[53-57] to the direct synthesis of the bicyclic β-lactams listed in Figure V. In all cases, the β-lactam ring appears to be less susceptible to ring cleavage reactions than penicillin. However, their infrared spectra add further support to the assignment of the 5.62 μ band of penicillin to the carbonyl of the bicyclic β-lactam. None of the compounds prepared were reported to possess antibiotic activity.

We have prepared a new thiazolidine-β-lactam derived from cysteine.

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53. J. C. Sheehan and G. D. Laubach, J. Am. Chem. Soc., 73, 4376 (1951).

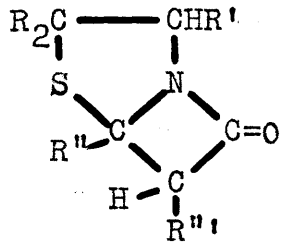
54. J. C. Sheehan and K. L. Henery-Logan, Unpublished results.

55. J. C. Sheehan, H. W. Hill, Jr., and E. L. Buhle, J. Am. Chem. Soc., 73, 4373 (1951).

56. J. C. Sheehan, E. J. Corey, G. D. Laubach and J. J. Ryan, J. Am. Chem. Soc., 72, 3828 (1950).

57. J. C. Sheehan and J. Erikson, Unpublished results.

Figure V



R	R'	R"	R"'	Reference	% Yield
H	H	phenyl	phthalimido	52	31.5
H	H	phenyl	3-nitrophthalimido	52	17
H	H	phenyl	succinimido	53	30
CH_3	H	phenyl	succinimido	53	15.5
CH_3	H	phenyl	phthalimido	53	5
H	H	phenyl	5-phenyloxazolidene-2,4-dione	35	28.4
H	H	p-nitrophenyl	phthalimido	54	65
CH_3	CO_2CH_3	phenyl	phthalimido	55	20.4
CH_3	CO_2CH_3	phenyl	succinimido	56	13
CH_3	CO_2CH_3	m-nitrophenyl	phthalimido	54	25
H	H	phenyl	2-benzylidene-4,5-diketo-3-oxazolidyl	23	44.5
H	H	furyl	phthalimido	57	23
CH_3	CO_2CH_3	furyl	phthalimido	33	17.5

DISCUSSION

This discussion will be centered around the reactions represented in Figure VI. By passing hydrogen chloride gas into a slurry of L(+) cysteine hydrochloride in absolute ethanol, the ethyl ester XCVII) was obtained in 86.5% yield. This ester condensed readily with ethyl benzimidate hydrochloride (XCVIII) in the presence of triethylamine at room temperature to yield 4-carboethoxy-2-phenyl-2-thiazoline (XCIX). This procedure was patterned after one recently reported for the synthesis of 4-carbomethoxy-5,5-dimethyl-2-phenyl-2-thiazoline from penicillamine methyl ester hydrochloride.[55] The thiazoline (XCIX) was obtained as a pale-yellow oil which formed a picrate readily. The optical rotation exhibited by XCIX depends on the treatment during isolation and purification. Racemization occurs slowly at elevated temperatures and is catalyzed by small amounts of triethylamine. The reaction of the DL-thiazoline with phthaloylglycyl chloride in the presence of triethylamine was conducted by means of a high dilution cycle.[58] These conditions afforded a 34% yield of the lactam C. Oxidation with potassium permanganate provided a 75% yield of the sulfone CI. When optically active

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58. A. C. Cope and E. C. Herrick, J. Am. Chem. Soc., 72, 985 (1950).

thiazoline was used, a 10% yield of C was obtained in addition to a non-crystallizing oil which was oxidized to give a 30% yield of crude sulfone (based on thiazoline). Successive recrystallizations raised the melting point to 182.0-183.5° (dec.), $[\alpha]^{26}D$ in chloroform + 11° (c = 1.3). The elemental analysis corresponded to that for the sulfone. A mixed melting point with the pure DL-sulfone (m.p. 184-185° (dec.) was 180.5-181.5° (dec.). The crystallization difficulties are probably due to the presence of both the L and DL forms.

The infrared spectra of C and CI appear on Plate III. The β-lactam carbonyl band at 5.58 μ in C is nearly superimposed on the 5.62 μ band of the phthalimide group. In the sulfone, however, the β-lactam carbonyl band is shifted to 5.52 μ. This shift on oxidation to the sulfone also is observed with methyl benzylpenicillinate and other N-acylated thiazolidines.[59]

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59. Reference 50, p. 409.

Figure VI

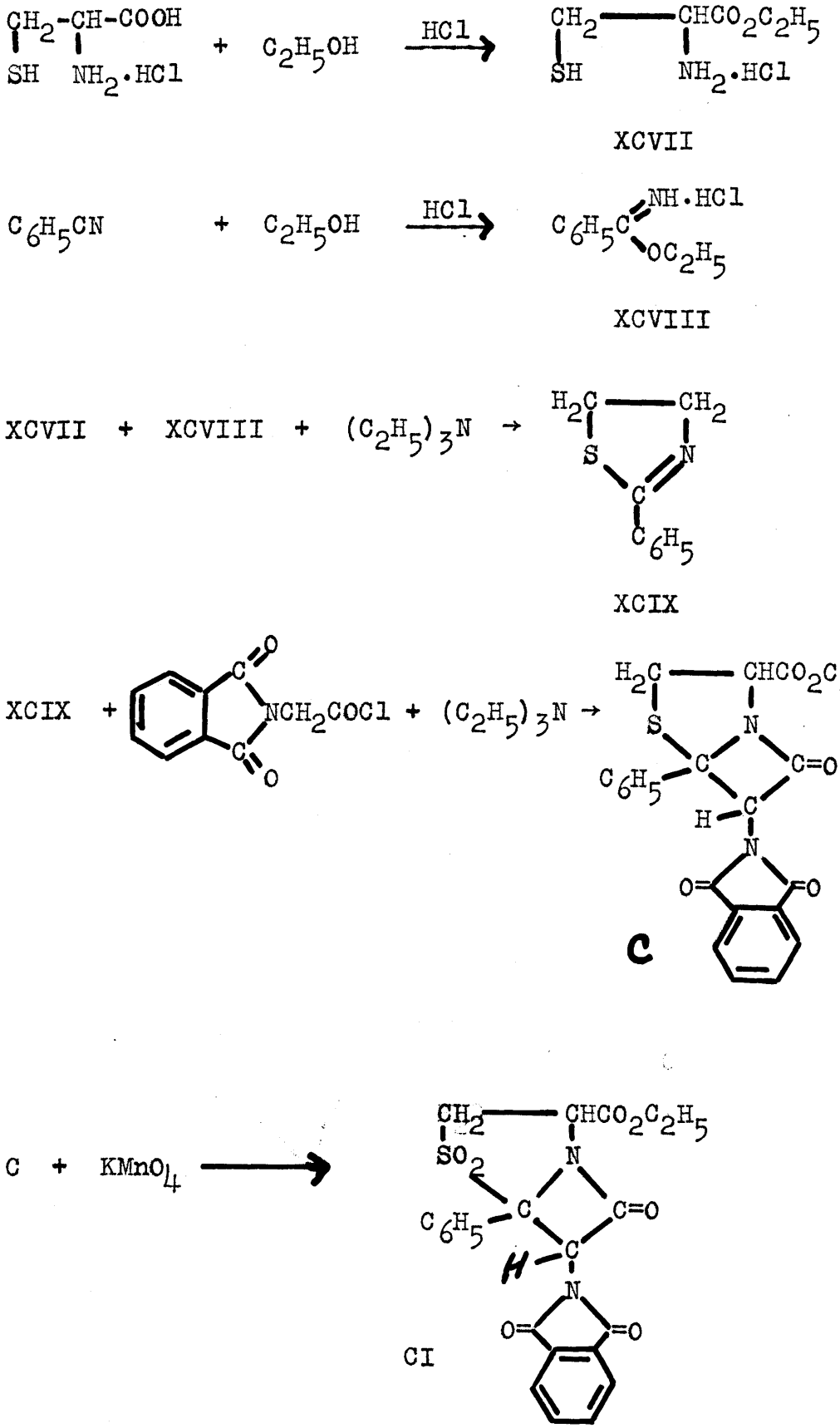
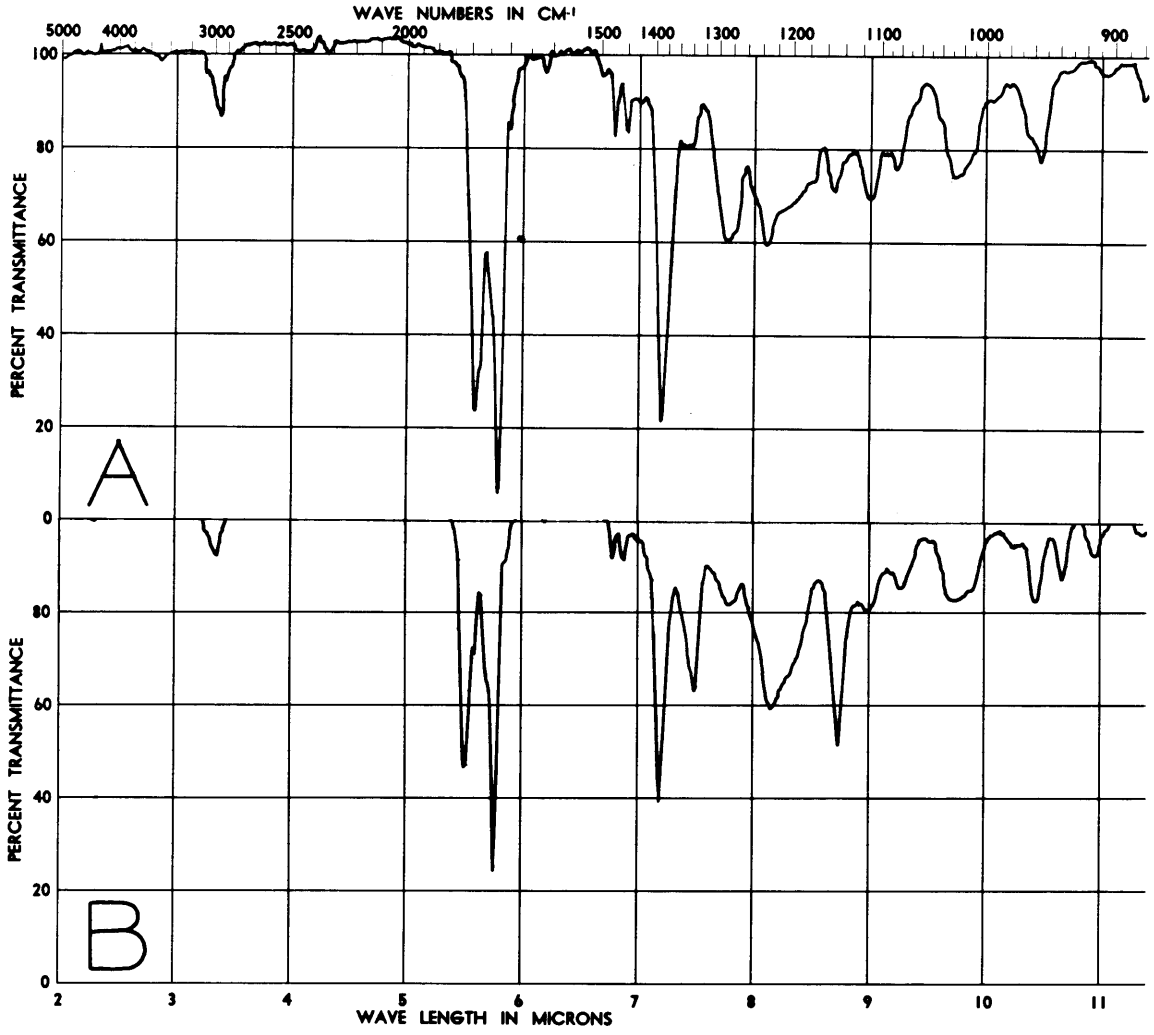

CH2-CH-COOH
SH NH2·HCl
+ C2H5OH
HCl
CH2 CHCO2C2H5
SH NH2·HCl
XCVII
C6H5CN + C2H5OH
HCl
NH·HCl
C6H5C
OC2H5
XCVIII
XCVII + XCVIII + (C2H5)3N →
H2C CH2
S N
C
C6H5
XCIX
XCIX + NCH2COCl + (C2H5)3N →
H2C CHCO2C2H5
S N
C C=O
C6H5
H C
N
O O
C
C + KMnO4 →
CH2 CHCO2C2H5
SO2 N
C C=O
C6H5
H C
N
O O
CI

Plate III

Curve A: 4-Carboethoxy-2-phenyl-α-phthalimido-2-thiazolidineacetic Acid β-Lactam (C), 2% solution in tetrachloroethane.

Curve B: 4-Carboethoxy-2-phenyl-α-phthalimido-2-thiazolidineacetic Acid β-Lactam Sulfone (CI), 2% solution in tetrachloroethane.


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
A
B
2
3
4
5
6
7
8
9
10
11
WAVE LENGTH IN MICRONS

EXPERIMENTAL

L-Cysteine Ethyl Ester Hydrochloride (XCVII). - Dry hydrogen chloride gas was passed into a slurry of L(+) cysteine hydrochloride (47.5 g., 0.300 mole) in 350 ml. of absolute ethanol until a saturated solution was obtained (1½ hours). The clear solution was heated under reflux for 1 hour, cooled to 40°, and again saturated with hydrogen chloride. On cooling at 5°, there was obtained a mass of colorless needles, which was collected by filtration. The dried product weighed 36.68 g., m.p. 124.5-126.5°. The mother liquors were concentrated under reduced pressure until solid began to separate. The residue was flushed with two 50 ml. portions of chloroform and the resulting white solid was crystallized from absolute ethanol-ether to provide 11.85 g. of colorless needles, m.p. 122-125°. The total yield amounted to 86.5%. An analytical sample recrystallized from absolute ethanol-petroleum ether (b.p. 30-60°) melted at 126.8-128.0°, $(\alpha)_D^{25.6°}$ in water -9.9° (c = 2.8) (reported m.p. 115°).[60]

Anal. Calcd. for $C_5H_{12}NO_2SCl$: C, 32.34; H, 6.51; N, 7.54.
Found: C, 32.58; H, 6.68; N, 7.50.

Ethyl Benzimidate Hydrochloride (XCVIII). - This substance

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60. E. Cherbuliez and Pl. Plattner, Helv. Chim. Acta, 12, 328 (1929).

was prepared according to the method of Pinner.[61] A solution of Eastman Pure benzonitrile (51.0 ml., 0.500 mole), absolute ethanol (29.2 ml., 0.500 mole) and 50 ml. of absolute ether was placed in a 250 ml. erlenmeyer flask fitted with a gas inlet tube and an outlet protected by a calcium chloride tube. The solution was maintained at -10° while it was saturated with hydrogen chloride gas over a 15 min. period. After storage at room temperature for 48 hours, the crystalline mass which had separated was collected by filtration, washed with absolute ether and dried in vacuo over potassium hydroxide pellets for 30 hours. The crude product was dissolved in boiling methylene chloride, from which solution there deposited 39.6 g. of colorless prisms on cooling, m.p. 120.5-121.0° (dec.). Addition of absolute ether to the filtrate precipitated a second crop of fine, colorless crystals, 27.6 g., m.p. 120-121° (dec.); total yield 67.2 g. (72.5%).

4-Carboethoxy-2-phenyl-2-thiazoline (XCIX). - A slurry of L-cysteine ethyl ester hydrochloride (9.28 g., 0.050 mole), ethyl benzimidate hydrochloride (9.28 g., 0.0500 mole) and triethylamine (7.00 ml., 0.0500 mole) in 100 ml. of dry methylene chloride was stirred magnetically for 48 hours at room temperature. The white suspension was then concentrated to dryness under reduced pressure, 50 ml. of dry dioxane was added and the suspended salts were removed by filtration;

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61. A. Pinner, Ber., 16, 1643 (1883).

weight, 9.55 g. (100%). The combined dioxane filtrates and washings were concentrated under reduced pressure at 50° to an amber oil. After heating at 50°/0.1 mm. for 2 hours, the residual oil weighed 11.98 g. (102%), $n^{26°}$ D 1.5718, $(\alpha)^{24°}$ D in chloroform +30°. The crude thiazoline (5.00 g.) was evaporatively distilled at 110°/0.005 mm. to give 4.50 g. (corresp. to 92% yield) of pale yellow oil. $n^{26°}$ D 1.5750, $(\alpha)^{24°}$ D in chloroform +40°. Another evaporative distillation afforded pure thiazoline, $n^{25.5°}$ D 1.5758, $(\alpha)^{25°}$ D in chloroform +41° (c = 2.5).

Anal. Calcd. for $C_{12}H_{13}NO_2S$: C, 61.25; H, 5.57; N, 5.95.
Found: C, 61.37; H, 5.63; N, 5.94.

The remainder of the crude product was treated with 0.10 ml. of triethylamine and stored at room temperature overnight. It was then evaporatively distilled at 120°/0.005 mm. to give a yellow oil, $n^{25°}$ D 1.5756, (α) D in chloroform 0.0°, which on redistillation at 110°/0.005 mm. afforded a sample of $n^{25.5°}$ D 1.5758.

The infrared spectra of the L and the DL thiazolines are identical. Treatment of the thiazoline with ethanolic picric acid provided a sample of the yellow picrate, m.p. 101-104°, which was recrystallized from ethanol to give yellow needles, m.p. 103.0-104.4°.

Anal. Calcd. for $C_{18}H_{16}N_4O_9S$: N, 12.07.
Found: N, 12.00

4-Carboethoxy-2-phenyl-α-phthalimido-2-thiazolidineacetic Acid β-Lactam (C). - A solution of DL-XCIX (2.35 g., 0.0100 mole) and phthaloylglycyl chloride (2.24 g., 0.0100 mole) in 100 ml. of dry methylene chloride was placed in a 200 ml. three-necked round bottom flask equipped with a stirrer and a high dilution addition apparatus. The solution was heated to flowing reflux with rapid stirring and triethylamine (1.40 ml., 0.0100 mole) in 50 ml. of methylene chloride was added through the dilution cycle over a 5 hour period. After 15 min. of additional heating, the clear yellow solution was concentrated under reduced pressure to a magma. Dry dioxane (50 ml.) was added and the precipitated solid was collected by filtration and washed well with dioxane. After drying, the colorless solid weighed 1.34 g. (97% theor. triethylammonium chloride). The combined dioxane filtrate and washings were concentrated under reduced pressure to a yellow oil. On storage at 5° overnight, the oil slowly crystallized. It was collected by filtration; weight, 2.92 g. Crystallization from ethyl acetate gave 2.12 g. of colorless needles, m.p. 160-185° (dec.). Recrystallization from chloroform-ethanol provided 1.43 g. (34%) of colorless needles, m.p. 191-194° (dec.). Several more recrystallizations from the same solvent pair gave an analytical sample, m.p. 196.5-197.5° (dec.).

Anal. Calcd. for $C_{22}H_{18}N_2O_5S$: C, 62.55; H, 4.30; N, 6.63.
Found: C, 62.35; H, 4.38; N, 6.48.

4-Carboethoxy-2-phenyl-α-phthalimido-2-thiazolidineacetic Acid β-Lactam Sulfone (CI). - A solution of C (0.200 g., 4.72 millimoles) in 15 ml. of 80% acetic acid was added to a solution of potassium permanganate (0.500 g.) in 6. ml. of water and the mixture was stored at room temperature for 1 hour. The reaction mixture was then decolorized by the dropwise addition of 35% hydrogen peroxide and the clear, colorless solution was diluted with 40 ml. of water, causing a white precipitate to form. This was collected by filtration, washed well with water and dried to give 0.170 g. of white fluffy powder. Recrystallization from acetone-water afforded silvery platelets, 0.160 g. (75%), m.p. 182-186° (dec.). Another recrystallization from the same solvent pair provided an analytical sample, m.p. 184.6-185.8° (dec.).

Anal. Calcd. for $C_{12}H_{18}N_2O_7S$: C, 58.14; H, 3,99; N, 6.17.
Found: C, 58.10; H, 4.13; N, 6.47.

When a sample of L-thiazoline of $(\alpha)D + 41^\circ$ was subjected to the lactam reaction conditions, a 10% yield of optically inactive β-lactam was obtained. The remainder of the material resisted attempts at crystallization, so it was subjected to the conditions for sulfone formation. This yielded 1.67 g. of colorless solid. On crystallization from chloroform-cyclohexane, 1.37 g. (30%) of pale tan crystals were obtained, m.p. 170-174° (dec.). Three more recrystallizations from the same solvent pair raised the melting point to 182.0-183.5° (dec.). $(\alpha)^{26}D$ in chloroform $+11^\circ$ (c = 1.3).

Anal. Calcd. for $C_{12}H_{18}N_2O_7S$: C, 58.14; H, 3.99; N, 6.17.
Found: C, 57.89; H, 4.21; N, 6.29.

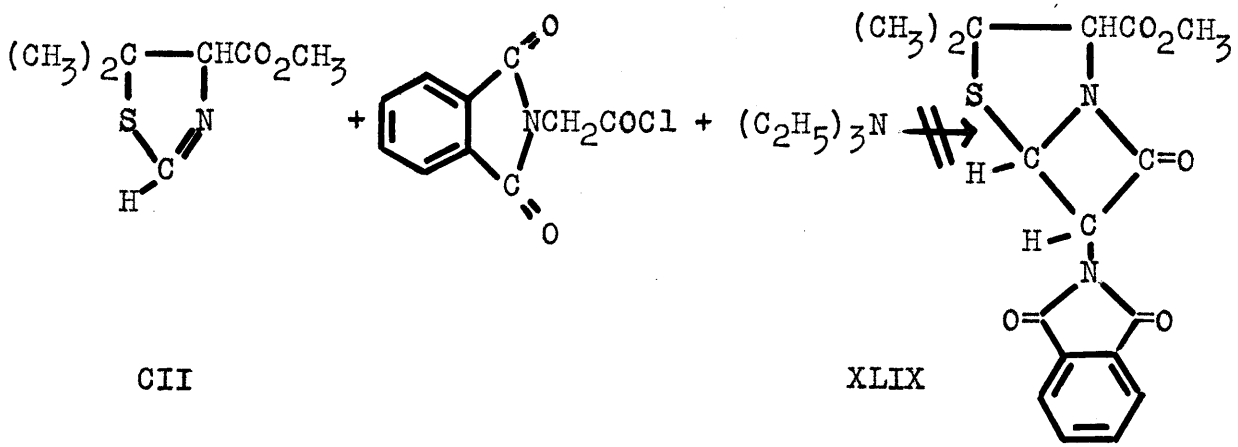
A mixed melting point with pure DL-sulfone (m.p. 184-185° (dec.)) was 180.5-181.5° (dec.).

APPENDIX

THE ATTEMPTED SYNTHESIS OF A 2-CHLOROTHIAZOLIDINE-β-LACTAM

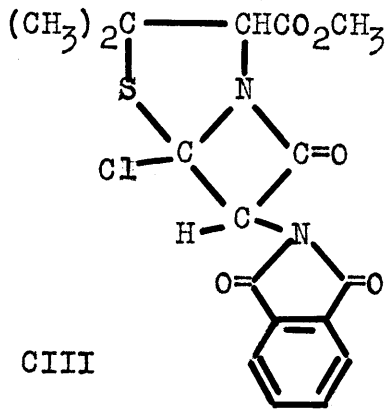
INTRODUCTION

Part II has reviewed development of the synthesis of bicyclic β-lactams recently reported by Sheehan and Ryan.[52] However, as was shown in Figure V, the reaction has yielded the desired product only for 2-aryl substituted thiazolines. The reaction of CII with phthaloylglycyl chloride under the usual conditions for lactam formation has failed to give a significant amount of XLIX. The significance of a synthesis of XLIX was discussed in Part I.



CII XLIX

We have attempted to prepare compound CIII, since it could probably be converted to XLIX by hydrogenolysis.



CIII

DISCUSSION

An outline of the reaction sequence appears on Figure VII. This approach was prompted by the recently reported preparation of the desired thiazoline (CVI) by Cook and coworkers.[62] They prepared the DL-thiazolidone (CV) in 78% yield by addition of a toluene solution of phosgene to a mixture of DL-penicillamine methyl ester hydrochloride (CIV) in chloroform and aqueous sodium bicarbonate solution. By passing phosgene gas into a slurry of CIV in dry methylene chloride, we have increased the yield to 96%.

The reported procedure for preparation of the 2-chloro-2-thiazoline (CVI) does not list a yield or a refractive index. In following the prescribed conditions, we have obtained a 31% yield of a nearly colorless oil, b.p. 95°/0.6 mm. (reported 90°/4 mm.).[62] Although Cook and co-workers reported a satisfactory analysis for CVI, several attempts in this laboratory have indicated values which were high in carbon and nitrogen. This perhaps can be attributed to loss of hydrogen chloride during manipulation, since, as reported, the compound decomposes at room temperature in a sealed tube or, upon exposure to atmospheric moisture, it reverts to the 2-thiazolidone (CV). The oil obtained was insoluble in dilute mineral acids and

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62. A. H. Cook, J. A. Elvidge and G. Shaw, J. Chem. Soc., 2367 (1949).

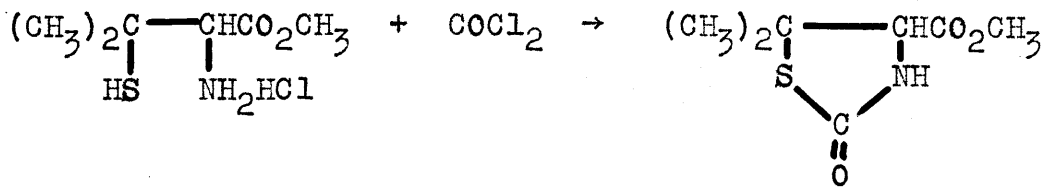
failed to form a picrate, thus indicating the lack of basic properties.

Attempts to obtain the β-lactam CIII with phthaloylglycyl chloride using our preparation of the thiazoline resulted in a 58% recovery of the starting thiazoline.

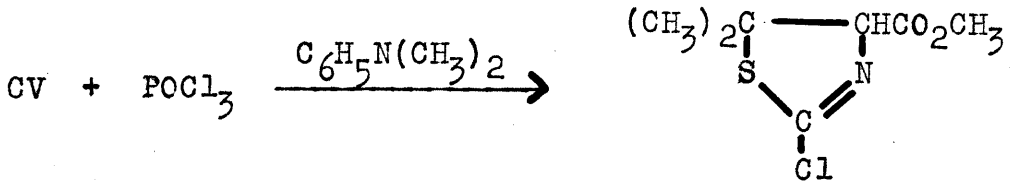
It had been planned to attempt the preparation of CVIII by interaction of CVI with CVII, the acid chloride used successfully by Sheehan and Laubach for the preparation of bicyclic lactam, whose sulfone was hydrogenolyzed to the phenylacetylamino side chain, characteristic of benzylpenicillin.[35] Oxidation of compound CVIII to the sulfone, followed by hydrogenolysis would thus be expected to yield the sulfone of methyl benzylpenicillinate (VIIa).

However, failure of the reaction to proceed with phthaloylglycyl chloride temporarily discouraged further experimental work.

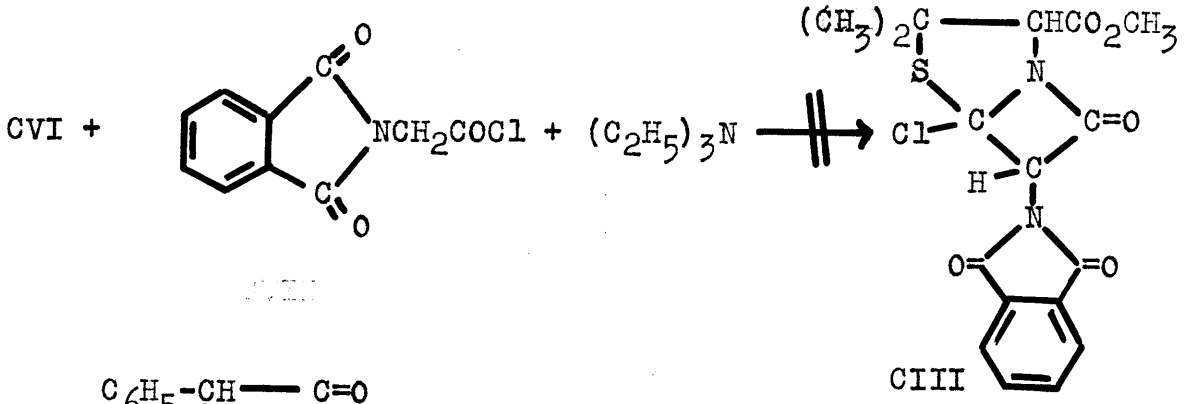
Figure VI



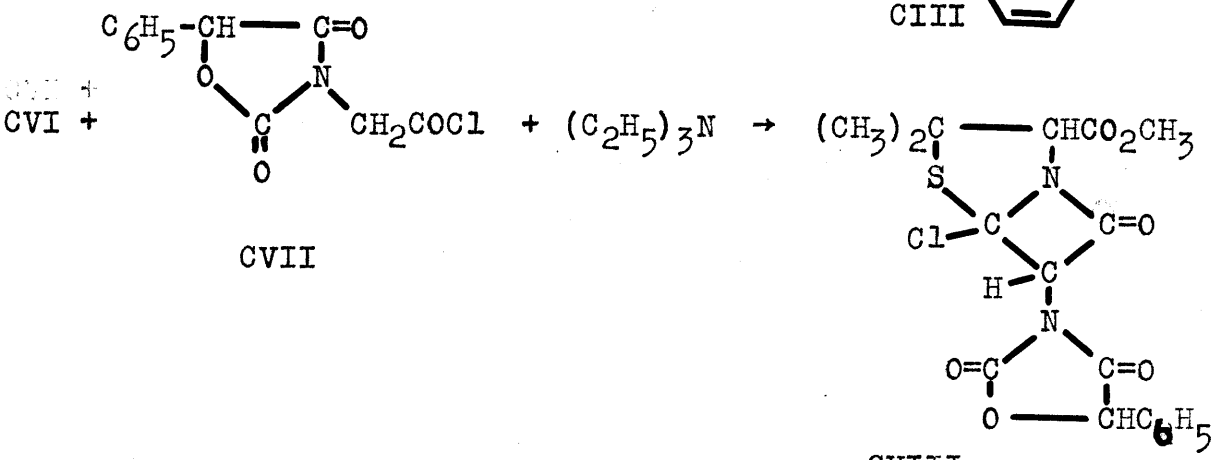
CIV CV



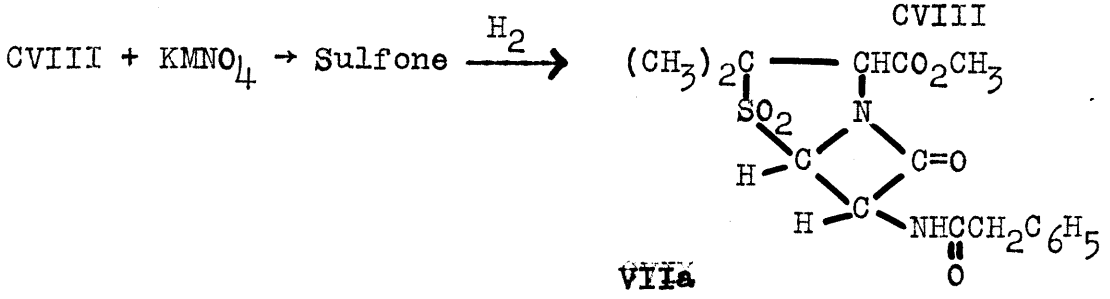
CVI



CIII



CVII CVIII



VIIa

EXPERIMENTAL

D-4-Carbomethoxy-5,5-dimethyl-2-thiazolidone (CV). - This compound was prepared by a modification of the method used by Buhle[63] in this laboratory. Phosgene gas was passed into a magnetically stirred slurry of D-penicillamine methyl ester hydrochloride (15.98 g., 0.0800 mole) in 300 ml. of dry methylene chloride until the solution was saturated. Stirring was continued for 5 hours after which time solution was complete. The solution was again saturated with phosgene and then stored at room temperature overnight. After filtration from a slight turbidity, the solution was concentrated under reduced pressure at 45° to a faintly brown oil which was flushed with three 50 ml. portions of dry benzene. On concentration of the fourth portion of benzene, crystallization began and 100 ml. of ligroin (b.p. 90-100°) was added slowly with swirling. The product was stored at 5° until crystallization was complete, and then it was collected by filtration to afford 14.58 g. (96.3%) of nearly colorless needles, m.p. 92-96°. Recrystallization from benzene-petroleum ether (b.p. 30-60°) provided colorless needles, m.p. 95.5-97.0°, $(\alpha)^{25.8°}_{D}$ in acetone +35.6° (c = 2.5). Buhle reported that an analytical sample melted at 95-97°.

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63. J. C. Sheehan and E. L. Buhle, Unpublished results.

Anal.[63] Calcd. for $C_7H_{11}NO_3S$: C, 44.43; H, 5.86; N, 7.40.
Found: C, 44.71; H, 5.99; N, 7.03.

Using a procedure identical to that above with DL-ester, the DL-thiazolidone was obtained in 96.7% yield, m.p. 78-81° (reported 84-85°).[62]

DL-4-Carbomethoxy-2-chloro-5,5-dimethyl-2-thiazoline (CVI).[62] - A solution of DL-CV (8.00 g., 0.0424 mole) and 16 ml. of dimethylaniline (redistilled from acetic anhydride) in phosphorous oxychloride (32 ml., freshly distilled) was heated under reflux for 3 hours and then concentrated under reduced pressure at 50° to a viscous purple-black oil. After dilution with 5 ml. of chloroform and cooling, it was added slowly to 40 g. of crushed ice with stirring. The suspension was stirred for four minutes and extracted with two 80 ml. portions of ether. The combined ethereal extracts were washed with four 15 ml. portions of ice water, dried over sodium sulfate at 5° for 1 hour and then over calcium sulfate for 30 min. The yellow-orange solution was concentrated under reduced pressure at room temperature and the residual oil was distilled at 95°/0.6 mm. to give 2.72 g. (31%) of nearly colorless oil, $n^{25.0°}$ D 1.5190.

On exposure to atmospheric moisture, this compound reverted to crystalline starting material with the evolution of hydrogen chloride.

Reaction of CVI with Phthaloylglycyl Chloride and Triethylamine. - Freshly distilled CVI (2.70 g., 0.013 mole) and phthaloylglycyl chloride (2.92 g., 0.0130 mole) were dissolved in 100 ml. of dry chloroform (ethanol free) and placed in a 200 ml. three-necked round bottom flask equipped with a stirrer and a high dilution addition apparatus. Triethylamine (2.00 ml., 0.0130 mole +10%) in 50 ml. of chloroform was added to the rapidly refluxing solution through the high dilution cycle over a 6 hour period. Heating was continued for an additional 15 min. and the chloroform was then removed under reduced pressure. The residue was taken up in 50 ml. of dry dioxane and the precipitated salts removed by filtration. The dioxane filtrate and washings were concentrated under reduced pressure to an orange oil. Trituration with two 50 ml. portions of ether caused an oily solid to separate. The ethereal filtrates were concentrated and finally evaporatively distilled to give 1.57 g. (58% recovery) of starting thiazoline. Further trituration of the oily solid with ether afforded 2.95 g. of dry material. The infrared spectrum of this crude material showed no band corresponding to a β-lactam. Bioassay for penicillin-like activity was negative.

BIOGRAPHICAL NOTE

The author was born on June 11, 1926 in Fairfax, Minnesota. He attended the elementary schools in the vicinity of Fairfax and Stewart, Minnesota and was graduated from Stewart Public High School in 1944. After having served for two years in the U. S. Army, he entered the University of Minnesota from which he received the Bachelor of Arts Degree in 1949. The following fall, he was admitted to the Massachusetts Institute of Technology where requirements for the Ph.D. were completed in July of 1952.