1 ml. per minute while the stirrer was rotated from 5000–7000 r.p.m. The agitation was continued for 45 minutes longer, after which the contents were forced, *via* a delivery tube under pressure of nitrogen, into a 4-l. erlenmeyer flask one-third full of solid carbon dioxide.

Subsequently the excess sodium was eliminated from this mass by addition of 75 ml. of 95% ethanol. Water (500 ml.) was then added. Care was taken during these operations lest tiny bits of metallic sodium were yet present. An atmosphere of carbon dioxide was maintained by addition of pieces of solid carbon dioxide. After all traces of metal was gone, the mixture was warmed on a steam-bath to break up solid particles. The two layers were separated in a funnel. The organic layer was dried over calcium sulfate and the hydrocarbons analyzed by fractionation and measurement of refractive index. On the small scale used and with the low yields obtained the joint use of the two methods is desirable. With large yields separations are easily made with a good column. No amyl chloride was ever present and the complete removal of the halogen was shown by analyses for sodium chloride as silver chloride in representative experiments.

The aqueous layer was acidified and the acids removed by ether extraction. The ether extract was shaken with ammonium hydroxide and the aqueous layer heated on a steam-bath to remove excess ammonia. An aliquot portion was titrated. Another portion was acidified with sulfuric acid and steam distilled. The two acids came over at different rates and could be determined by the semidistillation values which are 0.946 and 0.048, respectively, for caproic and phenylacetic.

The water layer left from the original acidification was found to contain no butylmalonic acid upon extraction with ethyl acetate.

Examination of the combined residues left after the fractionation of the hydrocarbon fractions from forty experiments, representing the reaction of 690 g. of sodium metal with 2130 g. of amyl chloride in 20 l. of toluene, showed very little material other than *n*-hexylbenzene. Chromatographic adsorption over alumina gave only slight amounts of tar. Fractional distillation yielded 28.6 g. of the hexylbenzene, 5.1 g. of two much smaller fractions and 9.4 g. of residue. The last yielded no easily isolable acid after alkaline permanganate oxidation. If any dimetalation had resulted during the process or any other effect had occurred which could lead to a high-boiling compound, the quantity was less than 1%.

Reaction in Pentane and Cumene–Toluene.—For the pentane experiments, the sodium sand was made in decane. When cooled, the decane was removed in the usual way under nitrogen pressure, and the sand was washed three times each with 150 ml. of pentane. The decane that should be left would be negligible, and any error made would be on the side of a higher yield of decane. All operations otherwise were the same.

For the experiments in the 50–50 mole mixture of cumene-toluene, the sand was made in the mixture. The cumene used in this work was dried over sodium and then fractionated. All other operations were the same as before.

Reactions at Higher Temperature.—The process was in general the same except for the higher temperature and, in one case, different amounts of reagents. At 65° with one-quarter mole each of sodium isopropoxide and sodium, the yield of *n*-hexylbenzene was 73.6%. A trace of decane was found. The phenylacetic acid was 0.9%; caproic acid was 0.1%.

At 75° with sodium 2-pentoxide in the quantities regularly used at 20°, the yield of *n*-hexylbenzene was 49.2%. Those of decane, caproic acid and phenylacetic acid were 3.1, 0.03 and 0.5%, respectively. The residue was 1.4 g. At 60° the respective quantities were 48.4, 3.4, 0.1 and 0.9% and 1.3 g.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

The Synthesis of Substituted Penicillins and Simpler Structural Analogs. II. α-Acylamino β-Lactam-thiazolidines[1]

BY JOHN C. SHEEHAN AND JAMES J. RYAN[2]

The synthesis of several compounds containing the penicillin ring system, including the fused β-lactam-thiazolidine structure with an α-amino function, has been achieved by a rational method. In the key step, phthaloylglycyl chloride and 2-phenyl-2-thiazoline interact in the presence of triethylamine under high dilution conditions to yield 2-phenyl-α-phthalimido-2-thiazolidineacetic acid β-lactam. The chemical and physical properties of these synthetic β-lactam-thiazolidines correspond closely to the behavior of the penicillins. Oxidation with permanganate produced a sulfone and hydrolysis followed by mercuric chloride led to a carbonyl fragment, carbon dioxide and presumably thioethanolamine. Selective alkaline hydrolysis attacked the phthalimide group in preference to the β-lactam ring, and esterification with diazomethane of the acid thus produced led to α-(*o*-carbomethoxybenzamido)-2-phenyl-2-thiazolidineacetic acid β-lactam, a prototype of the penicillins. The infrared spectra of these analogs are similar to that of benzylpenicillin. The corresponding β-lactam-thiazolidine bearing a 3-nitrophthalimide substituent was also prepared and characterized.

The preceding communication[3] of this series described the synthesis of a group of α-acylamino monocyclic β-lactams. We are now reporting the preparation and reactions of several compounds containing the fused β-lactam-thiazolidine rings present in the β-lactam formula for the penicillins. In these compounds a rational synthesis of the combination of this ring system with an acylamino function alpha to the lactam carbonyl has been achieved for the first time.[4]

An important feature of the present work is the formation of a compound, by a synthesis specifically designed to yield a β-lactam, which undergoes two reactions (one of derivatization and one of degradation) exactly analogous to reactions of decisive significance in the chemistry of penicillin itself. Furthermore, the compound possesses an infrared spectrum in the critical range similar to that of benzylpenicillin.

Several reviews are available[5] which summarize the extensive efforts made during the war and early post-war periods toward the elucidation of the structure and the synthesis of the penicillins.

(1) This communication is from part of a thesis submitted by one of us (J. J. R.) to the Graduate School of the Massachusetts Institute of Technology in partial fulfillment of requirements for the Ph.D. degree, April, 1949.

(2) Bristol Laboratories Fellow, 1948–1949.

(3) J. C. Sheehan and J. J. Ryan, THIS JOURNAL, **73**, 1204 (1951).

(4) A preliminary communication reported the extension of this synthesis to the preparation of a 5-phenyl pencillin: methyl 5-phenyl-(2-carbomethoxyethyl)-penicillinate, J. C. Sheehan, E. L. Buhle, E. J. Corey, G. D. Laubach and J. J. Ryan, *ibid.*, **72**, 3828 (1950).

(5) (a) "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson and R. Robinson, editors, Princeton University Press, Princeton, N. J., 1949; (b) E. Chain, *Ann. Rev. Biochem.*, **17**, 657 (1948); (c) "Antibiotics," Florey and Others, Oxford University Press, London, 1949; (d) E. Chain, *Endeavour*, **7**, 83, 152 (1948); (e) N. R. Trenner, *Anal. Chem.*, **22**, 405 (1950).

The vast amount of chemical and physico-chemical evidence uncovered has led to general acceptance of the β-lactam formula in preference to the azlactone-thiazolidine structure which had been favored earlier. However, interpretation of the chemical and physical properties of the penicillins in terms of the β-lactam formula has been handicapped by the absence of molecules of known structure which embody the key features to be compared.

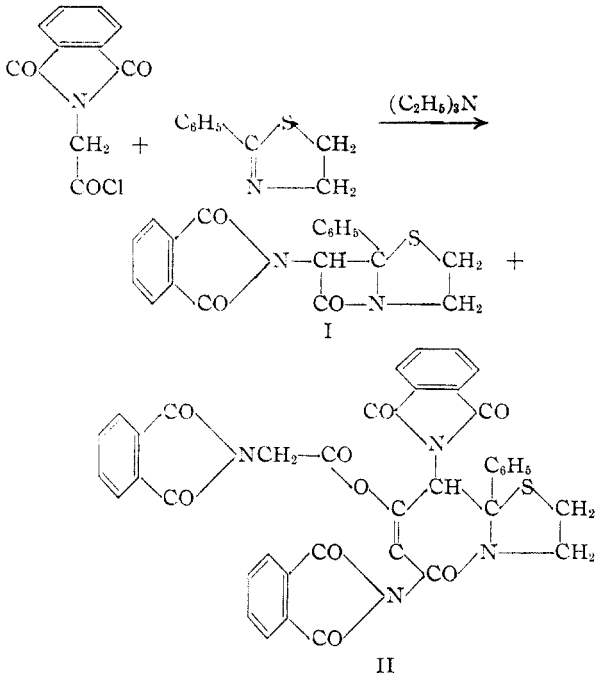
The Merck[6a] and Oxford[6b] groups independently detected benzylpenicillin as a trace constituent of the reaction product formed from penicillamine and oxazolones.[7] This microformation of benzylpenicillin cannot be interpreted as evidence for any particular formula and it is obviously of very little value as a preparative method.

By an extension of the methods pioneered by Staudinger[8a] three fused thiazoline-β-lactams[8b] have been prepared. Diphenyl ketene and 2-phenyl-2-thiazoline interact to form the β-lactam of thiazolidinediphenylacetic acid. Dimethyl ketene reacts with 2-methyl-2-thiazoline and 2-phenyl-2-thiazoline to give the corresponding piperidinediones, which are convertible to the β-lactams by partial hydrolysis followed by pyrolysis.

Although these highly substituted β-lactams failed to show the facile chemical reactions characteristic of the penicillins, they nevertheless provided valuable experimental confirmation for the assignment of the 5.62 μ infrared absorption band of the penicillins to a fused β-lactam carbonyl group. All other recorded attempts to synthesize benzylpenicillin or penicillin-like structures have failed.[9] Fourteen reported attempts by five different laboratories to add ketene itself to thiazolines led to no identifiable crystalline product and no evidence of β-lactam formation.[10] Many other widely different approaches, notably the recylization of β-penicilloates, were all unsuccessful.[11]

We have prepared acylamino thiazoline-β-lactams by a technique similar to that reported in the first communication in this series.[3] By the interaction of phthaloylglycyl chloride, 2-phenyl-2-thiazoline and triethylamine, in ether or benzene, was formed 2-phenyl-α-phthalimido-2-thiazolidineacetic acid β-lactam (I), plus a high yield of triethylammonium chloride and a quantity of a crystalline compound with properties best interpreted in terms of structure II.

The relative proportions of the lactam I and the product II formed seemed to be critically dependent on the manner in which the reaction was run. The highest yield of I was obtained when an equimolar solution of the acyl chloride and thiazoline in ether was heated to rapid reflux in a dilution



cycle[12] and a solution of an equivalent amount of triethylamine in ether then slowly added so that each drop of amine solution was mixed with a large volume of reflux, and thus entered the reaction flask in a highly diluted state. In this way a 40% yield of the lactam I was isolated while the amount of II formed was negligible. In an entirely analogous manner α-(3-nitrophthalimido)-2-phenyl-2-thiazolidineacetic acid β-lactam was prepared.

By contrast, when a benzene solution of phthaloylglycyl chloride was added directly to a solution of triethylamine and 2-phenyl-2-thiazoline in benzene, the yield of compound II was 36% while only 5% of the β-lactam I was obtained.

The analysis of compound II corresponds to a combination of three "phthaloylglycyl residues" with one thiazoline molecule and could arise from addition of two acyl residues to the thiazoline to form a six-membered ring, a piperidinedione. This piperidinedione contains an enolic hydrogen which could presumably react with a third acyl moiety under the conditions of the experiment. Successful application of the reaction therefore depended upon selection of the correct conditions to favor formation of the four-membered ring by one-to-one addition, rather than the competing tendency toward two-to-one addition to give the six-membered ring. Piperidinedione formation has been noted previously in the reaction of ketoketenes with thiazolines[8b] and with Schiff bases.[8a]

Oxidation of the crystalline β-lactam I with potassium permanganate in dioxane–water–acetic acid led to the sulfone III in 59% yield. The conversion of benzylpenicillin methyl ester to a sulfone (X) was one of the early arguments in support of the β-lactam expression and against the azlactone-thiazolidine formula. A study of many model compounds showed that permanganate converted N-acylated thiazolidines to sulfones, but caused deep-seated oxidation when the nitrogen was un-

(6) (a) Reference 5a, pp. 8, 893; *Merck Report*, **M 10**, 12, January, 1944; (b) Reference 5a, pp. 8, 893; *Oxford Report*, CPS 35, March, 1944.

(7) A few milligrams of crystalline triethylammonium benzylpenicillinate was later isolated from essentially this same reaction mixture by counter-current extraction methods: V. du Vigneaud, F. H. Carpenter, R. W. Holley, A. H. Livermore and J. R. Rachele, *Science*, **104**, 431 (1946).

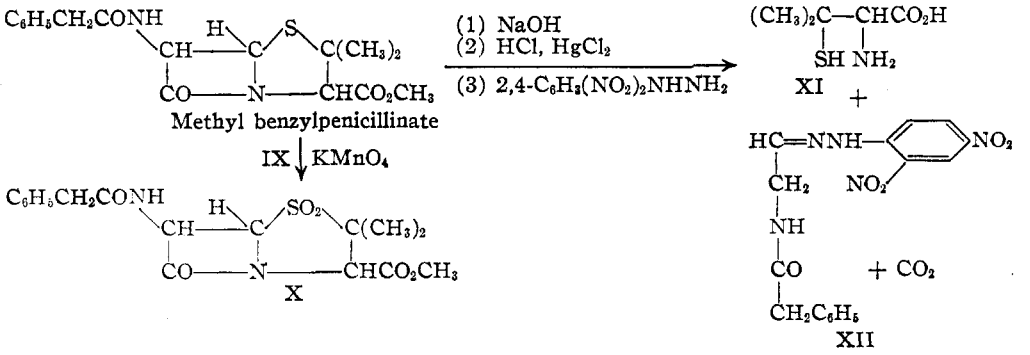
(8) (a) H. Staudinger, "Die Ketene," Enke, Stuttgart, 1912; (b) Reference 5a, pp. 996, 997 and 1000. Shell reports, Sh. 7, p. 81; Sh. 8, 106; Sh. 14, p. 211.

(9) Reference 5a, Chapter XXII.

(10) Reference 5a, p. 988.

(11) Reference 5a, p. 851.

(12) A. C. Cope and E. C. Herrick, THIS JOURNAL, **72**, 983 (1950)



protected.[13] The inherent instability of α-amino sulfones had been suspected earlier.[14]

Mild hydrolysis of the lactam I with an equivalent amount of dilute sodium hydroxide gave 2-phenyl - α - (*o* - carboxybenzamido) - 2 - thiazolidineacetic acid β-lactam (IV) in 75% yield. Evidence that the phthalimido group was the point of attack and not the β-lactam ring was provided by successful reclosure of the acid IV to the original lactam (I) by use of acetic anhydride. The easy elimination of water by such *o*-carboxybenzamides, even by simple heating above the melting point, is well known. However, many attempts[11] to cyclize β-penicilloates using a wide variety of dehydrating agents, including acetic anhydride, all failed. The phthaloyl group is known to be very susceptible to alkaline hydrolysis.

An additional indication that the phthalimide ring was opened preferentially was provided by a determination of the *p*H titration curve, together with an approximate *pK* of the acid IV. The titration was made in acetone–water solution, using a glass electrode. A smooth curve with one sharp break was obtained and no indication was observed of the buffering action which might be expected of the amino acid product from cleavage of the β-lactam ring. After application of a correction factor for use of the aqueous acetone medium (determined by a titration of benzoic acid under exactly the same conditions) the *pK* was determined from the titration mid-point as 3.8. The recorded *pK* of benzoic acid (25°) is 4.2; some increased acidity is reasonable for the acid IV as a result of the negative ortho substituent.

The acid IV was converted to the corresponding methyl ester V in 54% yield using diazomethane. This ester (V) and the parent acid (IV) are the prototypes of a group of compounds which includes the closest synthetic analogs of the penicillins thus far reported.[3]

A key reaction of the penicillin molecule, and one discovered very early in the investigation of penicillin, is a degradation[15] by hydrolysis and treatment with mercuric chloride into three components, penicillamine (XI), penilloaldehyde (XII) and carbon dioxide.

We have subjected the lactam-acid (IV) to an exactly parallel degradation. Hydrolysis of IV (or the lactam I) with 2 *N* sodium hydroxide, followed by treatment with mercuric chloride, gave immediately a heavy, white precipitate which contained mercury. Filtration followed by addition of 2,4-dinitrophenylhydrazine to the filtrate precipitated orange crystals of the 2,4-dinitrophenylhydrazone (VI) of N-phenacylphthalamic acid, which was identical with an authentic synthetic sample.

The independent synthesis of the dinitrophenylhydrazone VI was by known reactions starting with phenacyl bromide and potassium phthalimide to give ω-phthalimidoacetophenone (VII). Alkaline hydrolysis of VII and acidification yielded N-phenacylphthalamic acid, which was readily converted to the phenylhydrazone VI.

No effort was made to identify the mercury-containing precipitate but it is presumably a mercaptide of thioethanolamine. Nor was any attempt made to isolate the compound corresponding to penaldic acid, from which the aldehyde moiety of XII is derived by loss of carbon dioxide, although some evidence of its formation was noted. Alkaline hydrolysis of I or IV and acidification produced a precipitate of spongy character which tended to float because of inclusion and adhesion of small gas bubbles—presumably carbon dioxide.

The infrared study of penicillin, its derivatives, and possible models of penicillin has been extensive[16] and has provided a firm physical basis for the β-lactam formulation.[17] Evidence that the β-lactam IV is cogeneric with the penicillins is furnished by a comparison of infrared spectra. Four spectra are shown in Fig. 1, those of the phthalimido-β-lactam I, the *o*-carboxybenzamido-β-lactam IV, methyl benzylpenicillinate and N,N-diethylphthaloylglycyl amide.

In connection with present work absorption peaks of methyl benzylpenicillinate (in tetrachloroethane) and their assignments which are significant are: 5.62 μ, the carbonyl group of the β-lactam ring; 5.7 μ, the carbomethoxyl carbonyl; 5.94 μ and 6.64 μ, bands characteristic of the open-chain monosubstituted amide group; 2.9–3.0 μ, the N–H stretching frequency; and 7.6 μ, a band associated with reported fused β-lactam-thiazolidine structures. Similar bands of comparable relative intensities are found in the spectrum of the *o*-carboxybenzamido-β-lactam IV (in dioxane): 5.61 μ, the β-lactam carbonyl; 2.9 μ, 5.93 μ and 6.6 μ, due to the acylic monosubstituted amide; and the 7.6 μ band attributed to the fused ring system.

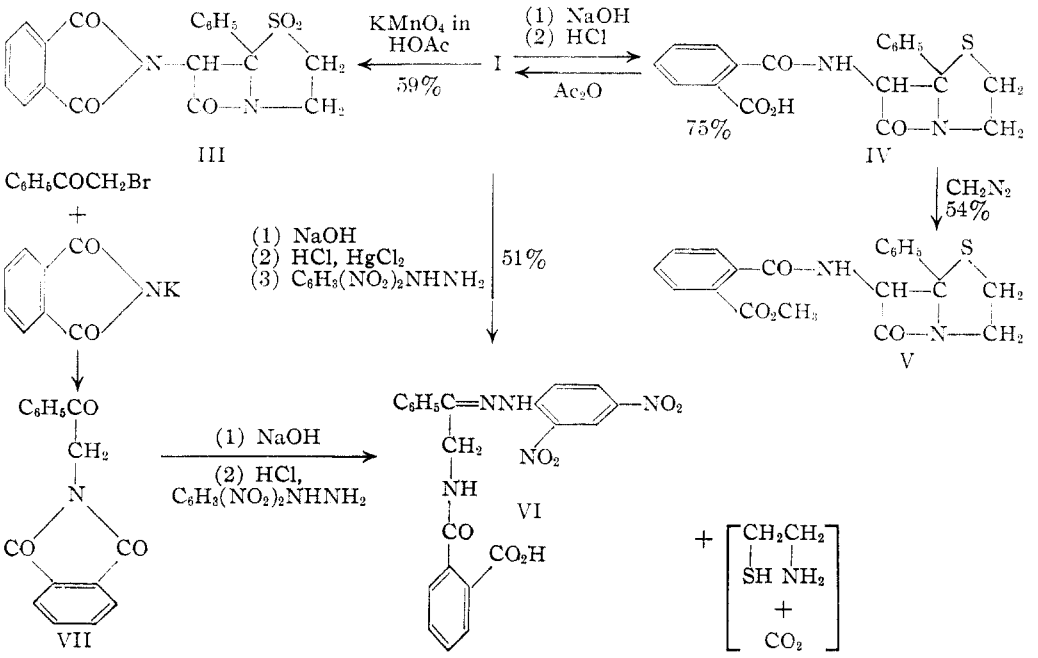
(13) Ref. 5a, p. 153.

(14) S. Ratner and H. T. Clarke, THIS JOURNAL, **59**, 200 (1937); Ernst von Meyer, *J. prakt. Chem.*, [2] **63**, 167 (1901); E. P. Kohler and M. Reimer, *Am. Chem. J.*, **31**, 163 (1904).

(15) Ref. 5a, p. 56.

(16) Reference 5a, Chapter XIII.

(17) Reference 5a, Chapter XV.



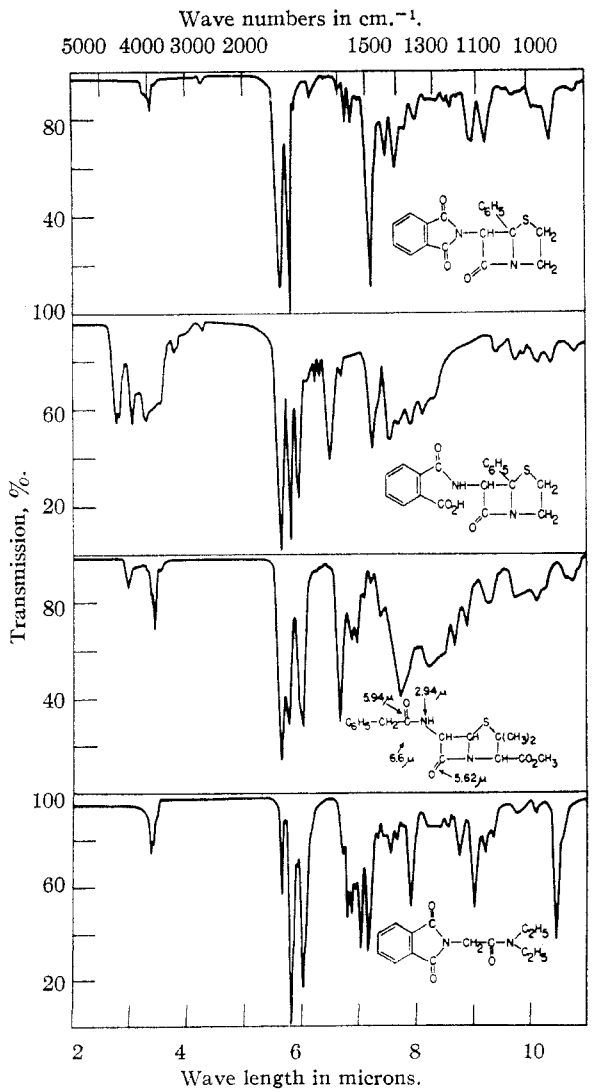


Fig. 1.

The spectrum of the phthalimido-lactam I shows a broad band at 5.60 μ and a narrower band at 5.80 μ. The model compound, N,N-diethylphthaloylglycyl amide, exhibits a band at 5.80 μ, and a sharp band of medium intensity at 5 60 μ, both of which we assign to the phthalimide carbonyl groups. We therefore suggest that the broad, intense band at 5.60 μ of the lactam I is due to a combination of the β-lactam absorption plus the narrow phthalimide band. When the phthalimide group of I was cleaved on transformation to the acid IV, the 5.60 μ band narrowed to the magnitude expected for the lactam carbonyl, the other carbonyl absorptions were shifted to locations characteristic of the open-chain amide and aromatic carbomethoxy groups, and a new band appeared at 6.64 μ due to the N–H bond of the mono-substituted amide. Unfortunately, the difference in band widths at 5.60 μ is not obvious from the reproduced curves, but the effect is unmistakable in the original copy.

We are indebted to Bristol Laboratories of Syracuse, N. Y., for a generous grant for the support of this program.

Experimental[18]

N-(2-Hydroxyethyl)-benzamide.—The method of Wenker[19] was employed, starting with ethanolamine and benzoic acid. The boiling point observed was 135–140° (3.5 mm.); $n^{25}D$ 1.567. The b.p. reported by Wenker, 230–231° (10 mm.), appears to be erroneous.

2-Phenyl-2-thiazoline.—The procedure was a modification of that of Wenker.[19] Cautious heating of a mixture of 77 g. of phosphorus pentasulfide and 142 g. of undistilled N-(2-hydroxyethyl)-benzamide initiated a vigorous reaction which was complete in approximately ten minutes. The mobile, upper layer of crude product was carefully decanted from the partially cooled reaction mixture. The viscous, bottom layer was poured onto a stainless steel sheet (if this material was permitted to cool to room temperature in the reaction vessel a hard cake resulted which was ex-

(18) All melting points are corrected. We are indebted to Mr. S. M. Nagy and his associates for the microanalyses.

(19) (a) H. Wenker, THIS JOURNAL, **57**, 1079 (1935); (b) A. P. Phillips and R. Baltzly, *ibid.*, **69**, 200 (1947).

tremely difficult to remove and redissolve), where it solidified into a layer of brittle resin. The pulverized resin was stirred with 500 ml. of 10% sodium hydroxide solution for four hours, and the alkaline liquor was extracted with three 50-ml. portions of ether. The combined ethereal extracts were dried with several successive portions of solid potassium hydroxide. After removal of the solvent, the residue was combined with the product previously obtained by decantation and distilled in a modified Claisen apparatus; yield 58.3 g. (41.6%), b.p. 110° (5 mm.) (reported[19a] 276–277° (760 mm.)) $n^{25}D$ 1.619. After redistillation, the product had the following properties: b.p. 100° (2.0–2.5 mm.); $n^{25}D$ 1.6239; d^{25}_4 1.09.

The picrate was prepared in 94% yield, using stoichiometric quantities in the minimum volume of ethanol; m.p. 174–176° (reported,[19a] 172°).

2-Phenyl-α-phthalimido-2-thiazolidineacetic Acid β-Lactam (I). A. Use of High Dilution.—A solution of phthaloylglycyl chloride, 4.48 g. (0.0200 mole), and 3.36 g. (0.0200 mole) of 2-phenyl-2-thiazoline in 75 ml. of anhydrous ether was prepared in a 200-ml. three-necked flask bearing a mercury-seal stirrer and a small high-dilution cycle tube[12] which carried, in turn, an efficient reflux condenser and a dropping funnel. The cycle tube was filled with ether and the flask was heated so that a very rapid reflux through the cycle tube was maintained. A solution of 2.77 ml. (2.02 g., 0.0200 mole) of triethylamine in 40 ml. of ether was then added over a four-hour period from the dropping funnel. The effect thus achieved was the introduction of the triethylamine into the reaction mixture in a highly diluted state. The solid which formed as the reaction progressed was collected by filtration. The water-soluble portion of this precipitate amounted to 2.54 g., which is equivalent to 93% of the theoretical yield of triethylammonium chloride.

The water-insoluble fraction was a tan solid; m.p. 160–195°, weight 5.64 g. This was digested with two 20-ml. portions of ether (which removed some oily impurity but very little color) and then with 75 ml. of 95% ethanol. Filtration left 2.80 g. (40%) of white, insoluble material which was the semi-pure lactam (I), m.p. 203–215°. This product was sufficiently pure for use in subsequent preparations.

Several recrystallizations from aqueous acetone and one from ethanol gave pure 2-phenyl-α-phthalimido-2-thiazolidineacetic acid β-lactam as colorless prisms, m.p. 215–216.5° (with evolution of gas, to a brown liquid) unchanged on further recrystallization.

Anal. Calcd. for $C_{19}H_{14}N_2O_3S$: C, 65.13; H, 4.03; N, 8.00; S, 9.15. Found: C, 65.07; H, 4.12; N, 7.90; S, 9.14.

The lactam (I) is insoluble in water, moderately soluble in acetone, ethanol and benzene and somewhat more soluble in dioxane. It gives a negative sulfhydryl test and does not react at an appreciable rate with mercuric chloride in ethanol–ether solution.

Purification was complicated by a tendency of the product to retain absorbed, unreacted thiazoline as well as by the presence of a small amount of the more insoluble "thiazolinetriacyl" adduct II.

B. Other Preparative Conditions.—As an illustration of the efficacy of the high-dilution technique our experience with ordinary reaction conditions may be cited.

Dropwise addition over one hour of a solution of 9.4 g. (0.042 mole) of phthaloylglycyl chloride in 65 ml. of benzene to a stirred solution of 7.92 g. (0.049 mole) of 2-phenyl-2-thiazoline and 6.94 ml. (4.91 g., 0.049 mole) of triethylamine in 5 ml. of benzene resulted in a yellow coloration, a temperature rise and formation of a heavy precipitate. After 48 hours the mixture was filtered. The water-soluble portion of this solid was 4.58 g., equivalent to 79% of triethylammonium chloride

Concentration of the benzene filtrates under reduced pressure gave a magma of needle-like crystals and a small amount of red oil. Trituration with an ether–benzene mixture and filtration yielded the crystalline lactam (I), 0.78 g. (5.3%); m.p. 211.5–214.5° (previous sintering at 208°). Reconcentration and evaporative distillation (0.5 mm., 150°) of the filtrate afforded 4.8 g. (61%) of a pale-yellow oil which apeared to be principally the starting 2-phenyl-2-thiazoline.

The portion of the reaction product insoluble in benzene and water proved to be crude triacyl compound (II), weight 4.36 g. (36%). Two recrystallizations from acetone gave colorless needles, m.p. 253–254°.

Anal. Calcd. for $C_{39}H_{24}N_4O_9S$: C, 64.63; H, 3.34; N, 7.73; S, 4.42. Found: C, 64.78; H, 3.35; N, 7.70; S, 4.37.

In an experiment in which the three reactants were mixed directly without use of a solvent, an 11% yield of the lactam (I) was isolated. A double high-dilution run, in which, with ether as the solvent, the acid chloride and amine were added through separate dilution tubes to the thiazoline contained in the flask, gave an approximately 30% yield of the lactam (I).

Sulfone of 2-Phenyl-α-phthalimido-2-thiazolidineacetic Acid β-Lactam (III).[20]—Potassium permanganate, 0.34 g. dissolved in a mixture of 10 ml. of glacial acetic acid and 3 ml. of water, was added to a solution of 0.28 g. (0.0080 mole) of the phthalimidolactam (I) in 30 ml. of purified dioxane. The mixture was kept at room temperature for one hour with occasional shaking during which time the permanganate color gradually changed to a brown precipitate. Sulfurous acid (6% solution) was added until the color was discharged (approximately 11 ml. was required), the solution was filtered and 40 ml. of water was then added. After two hours the crop was collected by filtration; yield 0.18 g. (59%), m.p. 215–220° (charring). For purification, the sulfone was recrystallized from dioxane–water. The colorless cubic crystals amounted to 0.16 g., m.p. 225° (some discoloration at 215°).

Anal. Calcd. for $C_{19}H_{14}N_2O_5S$: C, 59.67; H, 3.69; N, 7.34. Found: C, 59.66; H, 3.71; N, 7.35.

α-(*o*-Carboxybenzamido)-2-phenyl-2-thiazolidineacetic Acid β-Lactam (IV).—A solution of 0.35 g. (0.001 mole) of the phthalimidolactam I in 10 ml. of warm dioxane was cooled to room temperature carefully (in order to avoid crystallization) and exactly one milliequivalent of 0.1 *N* sodium hydroxide solution was added. A yellow color developed immediately; the *p*H gradually fell to a constant value of 6 in ten minutes. After 20 minutes a small amount of flocculent precipitate was removed by filtration, and the solution was acidified with 10 ml. of 0.1 *N* hydrochloric acid, which caused disappearance of the yellow color and formation of a white precipitate. Water (20 ml.) was added and after ten minutes the crystalline solid was collected; yield 0.27 g. (74%), m.p. 131.5–133.5°; negative sulfhydryl test. The pure lactam IV was obtained as fine, colorless needles by recrystallization from acetone–dioxane–water; weight 0.075 g., m.p. 141–142° (dec.).

Anal. Calcd. for $C_{19}H_{16}N_2O_4S$: C, 61.98; H, 4.38; N, 7.65; neut. equiv., 368. Found: C, 61.66; H, 4.56; N, 7.37; neut. equiv., 367.

Reclosure of the phthalimide ring by boiling the acid IV with acetic anhydride regenerated the phthalimidolactam I. Twenty-five milligrams of the acid IV was boiled for ten minutes (boiling for only one minute was inadequate) with 0.25 ml. of acetic anhydride. After cooling, the excess anhydride was decomposed by shaking with 1 ml. of water and the colorless, crystalline precipitate was separated by centrifugation. Recrystallization from water–acetone afforded needle-like crystals; m.p. 210–213° (dec.). The melting point on admixture with an authentic sample of the phthalimidolactam (IV), m.p. 213–215°, was undepressed. This experiment is considered evidence that alkaline hydrolysis conducted as described above causes cleavage of the phthalimide and not the β-lactam ring.

α-(*o*-Carbomethoxybenzamido)-2-phenyl-2-thiazolidineacetic Acid β-Lactam (V).—One-tenth gram of the lactam acid IV, suspended in a mixture of 5 ml. of ether and 5 ml. of chloroform, was treated with 2 ml. of a 0.7 *N* ethereal diazomethane solution. Evaporation of the clear solution under reduced pressure gave a white, fluffy powder which was taken up in 15 ml. of anhydrous ether. The ethereal solution was filtered to remove a trace of insoluble material. Concentration and dilution of the solution with pentane afforded the methyl ester V; yield 0.055 g. (53%), m.p. 141–142.5°. Recrystallization from ether–chloroform–pentane gave fine, colorless, rectangular prisms; weight 0.030 g., m.p. 147–148° (depressed on admixture with the acid (IV)).

Anal. Calcd. for $C_{20}H_{18}N_2O_4S$: C, 62.82; H, 4.74; N, 7.33. Found: C, 63.20; H, 4.93; N, 7.26.

(20) For the preparation of the sulfone of methyl benzylpenicillinate (work done by the Merck group) see Reference 5a, p. 177.

Degradation of the β-Lactam (IV) with Formation of the 2,4-Dinitrophenylhydrazone of N-Phenacylphthalamic Acid (VI).—A 0.1-g. portion (0.000272 mole) of the lactam-acid (IV) was heated under reflux for two hours with 6 ml. of *N* sodium hydroxide solution. The resulting yellow solution was acidified with 7 ml. of *N* hydrochloric acid and the precipitate was redissolved by the addition of 7 ml. of ethanol. Treatment with 3 ml. of 0.2 *M* mercuric chloride solution produced an immediate, heavy, colorless precipitate, presumably a mercaptide of thioethanolamine, which was removed by filtration. The solution was treated with 4 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. After five hours, the orange, crystalline 2,4-dinitrophenylhydrazone of VI was collected; 0.064 g. (51%). Recrystallization from aqueous ethanol raised the m.p. to 201–202.5°.

Anal. Calcd. for $C_{22}H_{17}N_5O_7$: C, 57.02; H, 3.70; N, 15.11. Found: C, 57.20; H, 4.03; N, 14.77.

The m.p. of a mixture of this 2,4-dinitrophenylhydrazone with an authentic sample of synthetic material (m.p. 202–204°), prepared as described below, was undepressed.

N-Phenacylphthalamic Acid 2,4-Dinitrophenylhydrazone (VI).—N-Phenacylphthalimide (VII) was prepared from potassium phthalimide and phenacyl bromide in 85% yield by an improvement[21] of the method of Gabriel[22] using dimethylformamide as a solvent, m.p. 166–167° (reported,[23] 167° (uncor.)).

A 3-g. portion of N-phenacylphthalimide was refluxed for one hour with 40 ml. of *N* sodium hydroxide. The resulting solution was filtered and acidified to *p*H 2 to yield N-phenacylphthalamic acid; weight 3.0 g., m.p. 160–160.5° (reported[23] 160°). The 2,4-dinitrophenylhydrazone was readily obtained as orange needles, m.p. 206–208°.

3-Nitrophthaloylglycine.—Stephen reported,[24] without giving experimental details, the preparation of 3-nitrophthaloylglycine and 3-nitrophthaloylglycyl chloride by interaction of 3-nitrophthalic anhydride and methyleneaminoacetonitrile, followed by hydrolysis of the resulting 3-nitrophthalimidoacetonitrile and conversion to the acid chloride. The following procedures[25] have proved to be more convenient in our hands.

A pulverized mixture of 1.50 g. (0.02 mole) of glycine and 3.86 g. (0.02 mole) of 3-nitrophthalic anhydride was heated in an oil-bath at 150–160° for 20 minutes. The cooled reaction mixture was recrystallized from water; yield 4.08 g. (81.5%) of pale yellow needles, m.p. 212.3–213.0°. For analysis a sample was recrystallized from water, giving nearly colorless needles, m.p. 213.5–214.2° (reported,[24] 208°).

Anal. Calcd. for $C_{10}H_6O_6N_2$: C, 48.01; H, 2.42; N, 11.20; neut. equiv., 250. Found: C, 47.85; H, 2.57; N, 11.04; neut. equiv., 248.

3-Nitrophthaloylglycyl Chloride.—3-Nitrophthaloylglycine (10 g., 0.04 mole) was dissolved in 200 ml. of dioxane and 8.32 g. (0.04 mole) of phosphorus pentachloride was added. The mixture was warmed on a steam-bath for approximately 30 minutes, the solvent was removed under reduced pressure, and the residue was crystallized from benzene. The crude acid chloride (10.70 g.) was recrystallized from benzene, yielding 7.62 g. (71%) of crystalline acid chloride, m.p. 123–126.5°. A second crop gave 1.18 g. (total yield 82%), m.p. 126–128°. An analytical sample was prepared by recrystallization from toluene; m.p. 127–128° (reported,[24] 119.5°).

Anal. Calcd. for $C_{10}H_5O_5N_2Cl$: Cl, 13.21. Found: Cl, 12.82.

α-(3-Nitrophthalimido)-2-phenyl-2-thiazolidineacetic Acid β-Lactam.—The apparatus used was the same as for the preparation of I. To a refluxing solution of 3-nitrophthaloylglycyl chloride (2.67 g., 0.010 mole) and 1.63 g. (0.010 mole) of 2-phenyl-2-thiazoline in benzene–ether was added through the addition tube 1.40 ml. (1.01 g., 0.010 mole) of triethylamine in 40 ml. of ether over a 45-minute period. After a one hour aging period at room temperature, the solid product was collected by filtration. The water-soluble portion was 1.18 g., equivalent to 86% of the theoretical yield of triethylammonium chloride. The water-insoluble residue amounted to 1.04 g., and was identified as the anhydride of 3-nitrophthaloylglycine. After recrystallization from acetone–pentane the melting point was constant at 237–238° (effervescence).

Anal. Calcd. for $C_{20}H_{10}N_4O_{11}$: C, 49.80; H, 2.09; N, 11.61. Found: C, 49.99; H, 2.06; N, 11.71.

Apparently the anhydride was formed from a trace of moisture accidentally introduced.

The nitrolactam was recovered from the benzene–ether filtrate by removal of the solvent and digestion of the residue with a small portion of acetone to remove colored impurities. Recrystallization from acetone–water and from acetone–pentane gave 0.38 g. (17%) of the nitrolactam pure, m.p. 210–211° (effervescence and charring).

Anal. Calcd. for $C_{19}H_{13}N_3O_5S$: C, 57.72; H, 3.31; N, 10.63. Found: C, 57.75; H, 3.44; N, 10.60.

Infrared Absorption Spectra.—The four infrared spectra shown in Fig. 1 were determined with a Baird Infrared Recording Spectrophotometer, Model B. Tetrachloroethane was the solvent for 2-phenyl-α-phthalimido-2-thiazolidineacetic acid β-lactam (I), methyl benzylpenicillinate and N,N-diethyl phthaloylglycyl amide; the concentrations were 2.5, 5 and 2.5%, respectively. The solvent for α-(*o*-carboxybenzamido)-2-phenyl-2-thiazolidineacetic acid β-lactam was dioxane (concentration, 2%).

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(25) First carried out in this Laboratory by Dr. V. S. Frank.