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

The Total Synthesis of Penicillin V

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Potassium phenoxymethylpenicillinate (VIII), synthesized totally in a series of reactions from D-penicillamine (D-II) and *t*-butyl phthalimidomalonaldehydate (I), has been shown to be identical to natural penicillin V (potassum salt) in physical and biological properties. In the key step, the monopotassium salt of the penicilloic acid (D-α-VII) was cyclized by means of N,N′-dicyclohexylcarbodiimide. A similar series of reactions starting with DL-penicillamine (DL-II) produced crystalline DL-penicillin V, which was resolved to give the hitherto unknown L-penicillin V having less than 1% of the bioactivity of the natural enantiomer. The natural and synthetic series were related stereochemically at three points, *viz.*, IV, VII and VIII. A promising intermediate for the synthesis of penicillins, penicillin analogs and penicillanic acids, *viz.*, *t*-butyl D-α-4-carboxy-5,5-dimethyl-α-amino-2-thiazolidineacetate hydrochloride (V), was prepared. Acylation of D- and DL-α-V with phenoxyacetyl chloride, followed by cleavage of the *t*-butyl ester, afforded the penultimate penicilloic acids D- AND DL-α-VII.

Penicillin was discovered by Fleming in 1929.[1] The remarkable *in vivo* activity of the antibiotic against a variety of pathogenic organisms was first demonstrated by Chain, Florey and co-workers.[2] Extensive degradative and physical studies during the wartime coöperative program between American and British scientists culminated in the proposal of the fused β-lactamthiazolidine structure for the penicillins (VIII).[3] This same intensive collaborative effort directed toward the synthesis of penicillin resulted, however, in the formation of penicillin only in minute yield.[4]

In 1948, this laboratory embarked on a substantial program, the objective of which was to devise synthetic methods powerful and selective enough to overcome the "diabolic concatenations of reactive groupings"[5] and thereby to make possible a total synthesis of the penicillins and simpler structural analogs. Within a few years three new β-lactam syntheses[6-8] were developed including one which led to the formation of a 5-phenylpenicillin[8] having many of the chemical and physical properties of the natural penicillins. Recently, the synthesis of a biologically active "sulfonyl analog" of benzylpenicillin (penicillin G) has been reported.[9] We now wish to record the first rational synthesis of a natural penicillin.[10]

Many attempts directed toward the cyclization of penicilloates of type VII with acid halide- and acid anhydride-forming reagents (*e.g.*, thionyl chloride, phosphorus trichloride, acetyl chloride and acetic anhydride) have failed,[11] which is not surprising in view of the known instability of the desired product (penicillin) in the presence of acidic reagents and byproducts of the reaction. The discovery that aliphatic carbodiimides are capable of forming amide bonds in aqueous solution directly from the amine and carboxyl components under very mild conditions[12] suggested the use of these reagents for the cyclization of a penicilloic acid (VII) to a penicillin (VIII). By the use of N,N′-dicyclohexylcarbodiimide cyclization was effected readily at room temperature to give totally synthetic penicillin V in both the natural and racemic series.

This communication also describes the preparation of important intermediates for a penicillin synthesis carried through without a blocking group on the β-carboxyl of the penicilloic acid VII. This feature obviates the necessity of removing a protective group (*e.g.*, a benzyl ester by catalytic hydrogenolysis) in a last step.[9] Thus the key intermediate V presents attractive possibilities for synthesis of a variety of natural and unnatural penicillins differing in the acyl substituent on the side-chain amino group.

Stereoisomerism of Penicilloic Acids.—In the condensation of I with D-II two new asymmetric centers are formed and it is necessary to determine which, if either, of the two thiazolidines formed (of the four theoretically possible) corresponds in configuration to the natural D-α-penicilloates. Comparisons were made at three points in the synthetic sequence, *viz.*, at D-α-IV, −VII and −VIII. The DL-α-IV isomer had been shown previously by Sheehan and Cruickshank[13] to correspond in configuration to the natural D-α-penicilloates; this assignment was confirmed in the present work by the conversion of DL-α-III into DL-α-VIII, the natural diastereomer.

Assignments of configuration to D-α-IV and D-γ-IV were made on the basis of detailed comparison of infrared spectra with those of DL-α-IV and DL-γ-IV. The crystalline α-isomers also melted 50° higher than the corresponding γ-isomers. The diacid hydrate D-α-VII was shown to be identical with the penicilloate obtained from the alkaline hydrolysis of penicillin V in physical properties, including optical rotation.

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

(2) E. Chain, H. W. Florey, A. D. Gardner, N. G. Heatley, M. A. Jennings, J. Orr-Ewing and A. G. Sanders, *Lancet*, **239**, 226 (1940).

(3) H. T. Clarke, J. R. Johnson and R. Robinson, editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 454.

(4) Penicillamine and 2-benzyl-4-methoxymethylene-5(4)-oxazolone condense to form trace amounts (0.03–0.08% by bioassay, 0.008% isolated) of penicillin G (benzylpenicillin). For a recent review of this reaction see Karl Folkers in "Perspectives in Organic Chemistry," Sir Alexander Todd, Editor, Interscience Publishers, Inc., New York, N. Y., 1956, p. 409.

(5) R. B. Woodward in "Perspectives in Organic Chemistry," Sir Alexander Todd, Editor, Interscience Publishers, Inc., New York, N. Y., 1956, p. 160.

(6) J. C. Sheehan and P. T. Izzo, THIS JOURNAL, **70**, 1985 (1948); **71**, 4059 (1949).

(7) J. C. Sheehan and A. K. Bose, *ibid.*, **72**, 5158 (1950).

(8) J. C. Sheehan, E. L. Buhle, E. J. Corey, G. D. Laubach and J. J. Ryan, *ibid.*, **72**, 3828 (1950); J. C. Sheehan and G. D. Laubach, *ibid.*, **73**, 4376 (1951).

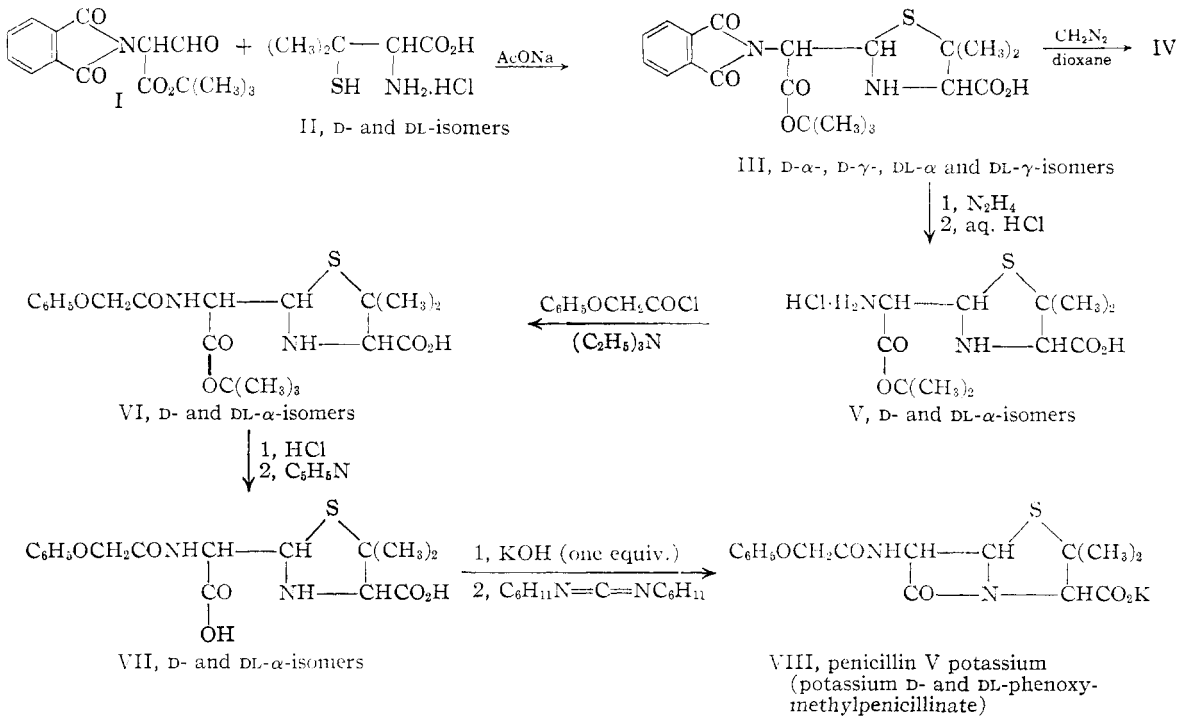
(9) J. C. Sheehan and D. R. Hoff, *ibid.*, **79**, 237 (1957).

(10) A preliminary communication has been published, J. C. Sheehan and K. R. Henery-Logan, *ibid.*, **79**, 1262 (1957).

(11) Reference 3, p. 861.

(12) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955).

(13) J. C. Sheehan and P. A. Cruickshank, *ibid.*, **78**, 3677 (1956).



The third comparison was made by showing the identity of totally synthetic D-VIII with natural penicillin V potassium salt (*vide infra*). Incidentally, the conversion of natural D-α-VII to VIII established for the first time that the reverse reaction, the alkaline hydrolysis of VIII, occurred without epimerization. Consequently the many wartime attempts at penicilloic acid cyclization did not fail because of operating in the wrong stereochemical series.

Synthesis of D- and DL-α-Penicilloic Acids.—The interaction of D-penicillamine hydrochloride (D-II) and *t*-butyl phthalimidomalonaldehydate (I) in sodium acetate buffered aqueous ethanol afforded directly the crystalline thiazolidine D-γ-III (30%). The α-isomer, which separated only on addition of water, appeared to be uncontaminated with the γ-isomer and was isolated in slightly larger amount (24%) than in the DL-series.[13]

Additional quantities of the desired D-α-isomer of III were prepared by heating a pyridine solution of the γ-isomer. This procedure established an equilibrium consisting of about 25% of D-α-III, which crystallized directly on cooling the solution. Additional quantities of the α-isomer could be obtained by recycling the filtrate. The DL-γ-isomer was also isomerized in pyridine to DL-α-III.

Removal of the phthaloyl group from D-α-III was accomplished by the action of hydrazine below room temperature to yield the phthalhydrazide complex isolated by lyophilization. A suspension of the complex in acetic acid was treated with hydrochloric acid to afford D-α-V in 82% yield. Similar treatment of the DL-isomer with hydrazine gave DL-α-V in 85% yield.

The D-isomer of V, upon treatment with one equivalent of phenoxyacetyl chloride and two equivalents of triethylamine at 0°, afforded in 70% yield the phenoxymethylpenicilloate D-α-VI. Similarly, the DL-isomer of V gave the racemic thiazolidine DL-α-VI in 79% yield.

The marked lability of *t*-butyl esters toward anhydrous acids[14] permits the facile cleavage of the carbo-*t*-butoxy group in compounds VI to give the β-amino acid hydrochlorides. Treatment of methylene chloride solutions of D- and DL-α-VI with anhydrous hydrogen chloride at 0° liberated the α-carboxyl function in almost quantitative yield. Recrystallization of these hydrochlorides from acetone-water containing an equivalent of pyridine afforded the penicilloic acids D- and DL-α-VII. Identity of the D-α-phenoxymethylpenicilloic acid hydrate (D-α-VII) with a sample prepared by saponification of natural penicillin V was established by comparison of m.p., mixed m.p., infrared spectra (potassium bromide) and optical rotation.

Cyclization of Penicilloic Acids to Penicillin V and DL-Penicillin V.—Cyclization of the penicilloates VII as the monopotassium (or monosodium) salts was found to take place readily at room temperature in dioxane-water solution. The β-lactam ring closure could be effected rapidly (20 min.) with one equivalent of N,N′-dicyclohexylcarbodiimide. Higher yields were achieved with longer reaction times in very dilute solutions (0.3%), using four equivalents of carbodiimide. In an experiment run for 33 hours using natural phenoxymethylpenicilloic acid hydrate (D-α-VII)[15] the yield of penicil-

(14) J. C. Sheehan and G. D. Laubach, THIS JOURNAL, **73**, 4752 (1951).

(15) All samples of natural phenoxymethylpenicilloic acid hydrate made by the alkaline hydrolysis (pH 11.5) of penicillin V by the procedure described in the Experimental section contained no penicillin V as determined by bioassay[16] and by chemical assay.[17]

(16) The synthetic samples were compared to standard natural penicillin V in a plate diffusion assay carried out under the supervision of Dr. J. Lein, Bristol Laboratories, Syracuse, N. Y. Bioassays of crude samples of synthetic penicillin V tend to decrease on storage, even at 5°, which may account for the discrepancy between chemical and microbiological assay.

lin V in the partially purified product was 11% by chemical assay[17] and 9% by bioassay.[16] In a larger scale cyclization (4.6 g.) carried out for 22 hours the yield by chemical assay was 9% and by bioassay 6% and, after purification by partition between methyl isobutyl ketone and two phosphate buffers, pure crystalline potassium phenoxymethylpenicillinate was isolated in 5.4% yield. The natural and synthetic potassium salts of penicillin V were shown to be identical by microbiological assay (99.7% of the activity of natural penicillin V) and by physical properties. These yields are considered representative of the developed cyclization and isolation procedure, since they have been obtained consistently on a macro scale. A somewhat less efficient process (first chronologically) was employed in the totally synthetic and DL-series.

In a similar manner, cyclization of D-α-VII, obtained from D-penicillamine, proceeded rapidly (25 min.) at room temperature with one equivalent of N,N′-dicyclohexylcarbodiimide to give a 4[16]–5%[17] yield of D-VIII. This sample of totally synthetic potassium salt of penicillin V was shown to be identical with the natural potassium salt by microbiological assay[16] (108 ± 10% of the bioactivity of penicillin V potassium), optical rotation [synthetic $\alpha^{25}D$ + 223° (*c* 0.2 in water); natural, $\alpha^{20}D$ + 223° (*C* 1 in water)], infrared spectra (40 peaks and shoulders in potassium bromide), m.p. 263° dec. (reported[18] 256–260° uncor.), undepressed upon admixture.

Formation of the β-lactam ring was also carried out in the racemic series. Thus DL-α-VII cyclized to form an optically inactive penicillin V potassium salt, m.p. 244° dec. This salt exhibited 51% of the bioactivity[16] of natural penicillin V, strongly suggesting that the unnatural L-penicillin is devoid of antibiotic activity. The infrared spectrum (potassium bromide) had a strong band at 5.64 μ, characteristic of the fused β-lactam-thiazolidine carbonyl function, and was identical with the spectrum of the natural potassium salt of penicillin V.

In parallel experiments N,N′-diisopropylcarbodiimide promoted cyclization of the D-α-penicilloate in essentially the same yield as with N,N′-dicyclohexylcarbodiimide. It was also found possible to effect β-lactam closure with other amide-bond forming reagents, which, like carbodiimides, have the property of being neutral themselves and giving rise to neutral by-products. Ethoxyacetylene has been used by Arens[19] for the formation of peptides in anhydrous solvents. Sheehan and Hlavka,[20] attempting to form a peptide in aqueous solution at room temperature, found that phthaloylglycine and ethoxyacetylene formed a reactive isolable peptide intermediate, which could interact with ethyl glycinate to form phthaloylglycylglycine ethyl ester. Ethoxyacetylene cyclized the monosodium salt of the D-α-penicilloate in 0.29% yield. Pentamethyleneketene cyclohexylamine[21] also effected cyclization in 0.19% yield. N,N′-Carbonyldiimidazole, a new peptide-forming reagent,[22] gave a product with no biological activity.[16] For cyclizations with reagents other than carbodiimides, however, no attempt was made to develop optimum conditions for the reactions. The monosodium salt of the penicilloic acid corresponding to penicillin G was also cyclized with N,N′-dicyclohexylcarbodiimide to penicillin G but in much lower yield.[23]

Resolution of DL-Penicillin V.—The racemic amine *erythro*-1,2-diphenyl-2-methylaminoethanol is readily resolved since the *levo*-isomer forms a sparingly soluble salt with penicillin G[24]; hence it follows that the optically active amine would resolve penicillin G. Natural penicillin V formed a salt with the *levo*-amine[25] which immediately crystallized from water or *n*-butyl acetate. Reaction of DL-penicillin V with the *levo*-amine in water solution gave a salt of the D-acid but only after standing many days at 5° (even with initial seeding with traces of the natural salt). This salt was, however, readily crystallizable from *n*-butyl acetate. The filtrate, containing the L-penicillin, was treated with the *dextro*-amine[25] in *n*-butyl acetate solution to give rapid crystallization of the L-penicillin salt. The D-penicillin *levo*-amine and L-penicillin *dextro*-amine salts had opposite rotations but were identical in other physical properties.

The salt of D-penicillin from the resolution gave the same bioassay value as the salt of natural penicillin V. The salt of L-penicillin, however, showed only 0.7% of the bioactivity expected for the salt of natural penicillin. It is entirely possible that a trace of D-isomer, present as a contaminant, is sufficient to account for the very low bioactivity observed, and that pure L-penicillin V might show no biological activity.

We are indebted to Bristol Laboratories of Syracuse, N. Y., for financial support, to Merck and Co., Inc., of Rahway, N. J., for the preparation of substantial quantities of certain key intermediates and to Mr. Sergey V. Chodsky for technical assistance.

Experimental[26]

***t*-Butyl D- and DL-4-Carboxy-5,5-dimethyl-α-phthalimido-2-Thiazolidineacetate (III).**—To an ethanol solution (300 ml.) of 42 g. (0.146 mole) of *t*-butyl α-phthalimidomalonaldehydate[27] (I) was added a solution of 27.2 g. (0.146 mole) of D-penicillamine hydrochloride[28] (D-II) and sodium acetate trihydrate (29.9 g., 0.22 mole) in 300 ml. of water. After

(17) J. H. Ford, *Anal. Chem.*, **19**, 1004 (1947). The procedure was modified only by replacing the one volume of phosphate buffer and one volume of acetate buffer with two volumes of phosphate buffer; this change did not affect the color yield from the sodium salt of penicillin V.

(18) E. Brandl and H. Margreiter, *Österr. Chem. Ztg.*, **55**, 11 (1954).

(19) J. F. Arens, *Rec. trav. chim.*, **74**, 769 (1955).

(20) J. C. Sheehan and J. J. Hlavka, *J. Org. Chem.*, **23**, 635 (1958).

(21) Directions for the preparation of this ketenimine were furnished by Dr. C. L. Stevens, Wayne University, private communication.

(22) G. W. Anderson and R. Paul, THIS JOURNAL, **80**, 4423 (1958).

(23) Preliminary experiments in this Laboratory by Dr. P. A. Cruickshank indicated that sodium D-α-benzylpenicilloate was cyclized to sodium benzylpenicillinate (penicillin G) in small amounts (0.3% by bioassay).

(24) V. V. Young, *J. Am. Pharm. Assoc., Sci. Ed.*, **40**, 261 (1951); W. B. Wheatley, W. E. Fitzgibbon and L. C. Cheney, *J. Org. Chem.*, **18**, 1564 (1953).

(25) Samples of *levo*- and *dextro-erythro*-1,2-diphenyl-2-methylaminoethanol hydrochlorides were kindly supplied by Dr. L. C. Cheney, Bristol Laboratories, Syracuse, N. Y.

(26) All melting points are corrected. We are indebted to Dr. S. M. Nagy and his associates for the microanalyses, and to Dr. N. A. Nelson and his associates for the infrared and ultraviolet spectra.

(27) J. C. Sheehan and D. A. Johnson, THIS JOURNAL, **76**, 158 (1954).

(28) J. C. Sheehan, R. Mozingo, K. Folkers and M. Tishler, U. S. Patents 2,496,416 and 2,496,417; B. E. Leach and J. H. Hunter, *Biochem. Preparations*, **3**, 111 (1953).

storage for 10 hours, 18.2 g. (30%) of crystals was collected by filtration, m.p. 145° (dec., in bath at 135°). This crop was essentially pure γ-isomer. Two recrystallizations from methanol-water raised the m.p. to 145–146° dec., $\alpha^{23}D$ +22° (*c* 1 in acetic acid).

Anal. Calcd. for $C_{20}H_{24}N_2O_6S$: C, 57.13; H, 5.75; N, 6.66. Found: C, 57.25; H, 5.79; N, 6.62.

Addition of 75 ml. of water to the aforementioned filtrate caused the slow crystallization of 9.03 g. of α-isomer as colorless needles, m.p. 152–153° (dec., in bath 135°). Addition of a further 60 ml. of water gave 5.63 g., m.p. 148–151° dec. The total yield of α-isomer was 14.66 g. (24%). Three recrystallizations from methanol–water afforded a product with a constant melting point, 159–160° dec., $\alpha^{24}D$ +62° (*c* 1 in acetic acid).

Anal. Calcd. for $C_{20}H_{24}N_2O_6S$: C, 57.13; H, 5.75; N, 6.66. Found: C, 57.45; H, 6.06; N, 6.83.

The condensation under similar conditions of DL-penicillamine hydrochloride[28] and I and the isolation of the less-soluble γ-isomer has been described previously.[13] From the last crop the more soluble (in acetone–water) α-isomer has also now been obtained, m.p. 184–185° dec., mixed m.p. with the γ-isomer was 180–181° dec. (both in bath at 160° with pure γ-isomer, which melted at 183–184° dec.). The two diastereoisomers had infrared spectra (potassium bromide) which were distinctly different in the region between 10 and 13 μ.

Anal. Calcd. for $C_{20}H_{24}N_2O_6S$: C, 57.13; H, 5.75; N, 6.66. Found: C, 57.21; H, 6.03; N, 6.55.

Isomerization of the DL-γ-Isomer to the DL-α-Isomer.[29]—A solution of 200 g. of DL-γ-III in 350 ml. of reagent-grade pyridine was heated on a steam-bath under an atmosphere of prepurified nitrogen for 22 hours. After cooling in a refrigerator overnight, the crystalline DL-α-isomer (80 g.) was collected and washed with two 25-ml. portions of cold pyridine and two 75-ml. portions of cold ethyl ether. Additional DL-γ-isomer (80 g.) was added to the pyridine filtrate which was heated on a steam-bath for 24 hours and cooled as before to give an additional 86 g. Another 80 g. of DL-γ-isomer was added to the filtrate to give after isomerization a further 81 g. The combined product (247 g.) was recrystallized from acetone–water to yield 186 g. (52%), m.p. 186–187° dec.; comparison of infrared spectra (potassium bromide) indicated that this sample was substantially pure DL-α-isomer.

Anal. Calcd. for $C_{20}H_{24}N_2O_6S$: C, 57.13; H, 5.75; N, 6.66. Found: C, 57.21; H, 6.03; N, 6.55.

Isomerization of the D-γ-Isomer to the D-α-Isomer.—In a similar manner, heating a solution of 10 g. of DL-γ-III in 18 ml. of reagent pyridine for 21 hours on a steam-bath yielded 2.76 g. of colorless needles. Recrystallization from 40 ml. of methanol–water (1:1) afforded 1.69 g. of pure D-α-III, m.p. 161° (dec., in bath 135°), mixed m.p. with authentic D-α-III was 160.5° dec., $\alpha^{24}D$ +62° (*c* 1 in acetic acid).

Anal. Calcd. for $C_{20}H_{24}N_2O_6S$: C, 57.13; H, 5.75; N, 6.66. Found: C, 56.87; H, 5.87; N, 6.70.

Concentration of the pyridine mother liquors and recycling three times gave, after recrystallization, 2.10 g., m.p. 159–160° dec. The total yield of D-α-III was 3.79 g. (38%).

***t*-Butyl D- and DL-4-Carbomethoxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetate (IV).**—The preparations of DL-α- and DL-γ-IV have been described previously by Sheehan and Cruickshank.[13]

The first crop of D-III (3.48 g., 8.26 mmoles) was dissolved in 40 ml. of dioxane (heating required) and the solution treated with excess diazomethane. The ester was crystallized from ethanol–water yielding 1.54 g., m.p. 124–125°. Two recrystallizations gave an analytical sample, m.p. 126–127°, $\alpha^{24}D$ −19° (*c* 2 in dioxane). The infrared spectrum (1,1,2,2-tetrachloroethane) was identical with that of the DL-γ-isomer[13]; therefore this ester has been designated as the D-γ-isomer.

Anal. Calcd. for $C_{21}H_{26}N_2O_6S$: C, 58.05; H, 6.03; N, 6.45. Found: C, 57.79; H, 6.02; N, 6.66.

Similar treatment of the second crop of D-III (2.72 g., 6.46 mmoles) from the condensation afforded, after crystallization from 35 ml. of 95% ethanol, 2.22 g. (79%) of an ester, m.p. 176–177.5°. Recrystallization gave an analytical sample, m.p. 177.5–178.5°, $\alpha^{24}D$ −1° (*c* 2 in dioxane). The infrared spectrum (1,1,2,2-tetrachloroethane) was identical with that of the DL-α-isomer, which had been shown previously[13] to correspond to configuration to the natural dimethyl D-α-benzylpenicilloate; therefore this isomer has been designated as the D-α-isomer.

Anal. Calcd. for $C_{21}H_{26}N_2O_6S$: C, 58.05; H, 6.03; N, 6.45. Found: C, 57.96; H, 6.09; N, 6.28.

***t*-Butyl D- and DL-α-4-Carboxy-5,5-dimethyl-α-amino-2-thiazolidineacetate Hydrochloride (V).**—A solution of 14.22 g. (0.0338 mole) of D-α-III in 425 ml. of purified dioxane and 4 ml. of water was cooled to 13° and 3.80 ml. (3.84 g., 0.0768 mole) of hydrazine hydrate added over a 1-minute period with stirring. The solid which precipitated was redissolved by warming to 18°, and the solution was maintained at 13–15° for 3 hours, then at room temperature for 21 hours, after which solvent and excess hydrazine were removed by lyophilization. The phthalhydrazide complex was decomposed by treatment of a suspension in 310 ml. of acetic acid at 13° with 8.15 ml. of concentrated hydrochloric acid, followed by agitation at room temperature for 30 minutes. The lyophilized suspension was digested with 175 ml. of cold methanol; 5.2 g. (95%) of phthalhydrazide was removed by filtration. Concentration of the filtrate to 80 ml. yielded 1.63 g. of hydrazine dihydrochloride, m.p. 199° dec. Addition of 275 ml. of ether yielded 0.93 g. of impure solid of m.p. 90–125° dec. The further gradual addition of 1650 ml. of ether gave 9.07 g. (82%) of analytically pure D-α-V, m.p. 172° dec., $\alpha^{25}D$ +111° (*c* 1 in methanol).

Anal. Calcd. for $C_{12}H_{23}N_2O_4SCl$: C, 44.10; H, 7.09; Cl, 10.85. Found: C, 43.83; H, 7.13; Cl, 10.87.

Similar treatment of 28.44 g. of DL-α-III in a solution of 1360 ml. of dioxane and 10 ml. of water with 7.60 ml. of hydrazine hydrate yielded 18.8 g. (85%) of analytically pure DL-α-V, m.p. 170° dec.

Anal. Calcd. for $C_{12}H_{23}N_2O_4SCl$: C, 44.10; H, 7.09; N, 8.57. Found: C, 43.80; H, 7.33; N, 8.72.

***α-t*-Butyl D- and DL-α-Phenoxymethylpenicilloate (VI).**—To a solution of 20 g. (0.0612 mole) of DL-α-V and 8.6 ml. (6.19 g., 0.0612 mole) of triethylamine in 800 ml. of methylene chloride at 0°, there was added, simultaneously, a solution of 8.6 ml. (6.19 g., 0.0612 mole) of triethylamine in 600 ml. of methylene chloride and a solution of 8.65 ml. (10.47 g., 0.0612 mole) of phenoxyacetyl chloride in 600 ml. of methylene chloride over a period of 1.5 hours in a system protected from moisture. After 20 hours at room temperature, a small amount of solid was removed by filtration, and the filtrate washed with two 1600-ml. portions of a solution containing equal volumes of 0.1 *N* hydrochloric acid and saturated sodium chloride, then with 800 ml. of saturated sodium chloride, dried over magnesium sulfate and concentrated under reduced pressure to a foam. From an ethereal solution there was obtained 20.54 g. (79%) of crystalline product, m.p. 142° dec. Recrystallization from ether–petroleum ether gave an analytical sample, m.p. 143° dec.

Anal. Calcd. for $C_{20}H_{28}N_2O_6S$: C, 56.59; H, 6.65; N, 6.60. Found: C, 56.48; H, 6.86; N, 6.53.

In a similar manner, phenoxyacetyl chloride and triethylamine converted D-α-V into D-α-VI in 70% yield, m.p. 120–122° dec., $\alpha^{25}D$ +67° (*c* 1 in methanol).

Anal. Calcd. for $C_{20}H_{28}N_2O_6S$: C, 56.59; H, 6.65; N, 6.60. Found: C, 56.88; H, 6.86; N, 6.59.

D- and DL-α-Phenoxymethylpenicilloic Acid Hydrochloride (VII·HCl).—A solution of 19.8 g. (0.0466 mole) of DL-α-VI in 800 ml. of methylene chloride, cooled to 0°, was saturated with hydrogen chloride by passing the anhydrous gas through the solution for 20 minutes. Storage at 5° for 29 hours gave 18.1 g. (96%) of colorless crystals, m.p. 204–205° dec.

Anal. Calcd. for $C_{16}H_{21}N_2O_6SCl$: C, 47.46; H, 5.23; N, 6.92. Found: C, 47.37; H, 5.21; N, 7.05.

Analogous treatment of 3.21 g. (7.56 mmoles) of D-α-VI in 52 ml. of methylene chloride with hydrogen chloride afforded 2.87 g. (94%) of D-α-VII·HCl, m.p. 113–116° dec.

D- and DL-α-Phenoxymethylpenicilloic Acid (VII).—To a solution of 147 mg. of D-α-VII·HCl (0.36 mmole) in 0.6 ml. of acetone–water (1:2) was added 35 mg. (0.44 mmole) of pyridine. The crystalline product was recrystallized from acetone–water to yield 113 mg. (81%), m.p. 125° dec. An

(29) This method of isomerization was developed in this Laboratory by V. J. Grenda.

additional recrystallization from the same solvent combination gave analytically pure D-α-phenoxymethylpenicilloic acid hydrate, m.p. 129.5° dec., $\alpha^{25}D$ +94° (*c* 1 in methanol). This penicilloic acid hydrate was shown to be identical with the compound (*vide infra*) obtained from the alkaline hydrolysis of natural penicillin V by comparison of m.p., mixed m.p. (129° dec.), optical rotation and infrared spectrum (potassium bromide).

Anal. Calcd. for $C_{16}H_{20}N_2O_6S \cdot H_2O$: C, 49.74; H, 5.74; N, 7.25. Found: C, 49.61; H, 5.77; N, 6.94.

In a similar manner 73 mg. of DL-α-VII·HCl gave, after one recrystallization from acetone–water, 23 mg. of DL-α-phenoxymethylpenicilloic acid, m.p. 133° dec. Recrystallization gave an analytical sample, m.p. 139° dec.

Anal. Calcd. for $C_{16}H_{20}N_2O_6S$: C, 52.17; H, 5.47; N, 7.61. Found: C, 51.62; H, 5.28; N, 7.50.

Alkaline Hydrolysis of Natural Penicillin V.—To a stirred suspension of 75 g. (0.214 mole) of natural penicillin V[30] in 1000 ml. of water, under an atmosphere of prepurified nitrogen, was added 0.5 *N* sodium hydroxide dropwise until the *p*H was 11–11.5, the solution was maintained at this *p*H by the gradual addition of sodium hydroxide over a period of 2.5 hours (the total amount of sodium hydroxide required was 900 ml., 0.45 mole); the *p*H then remained constant at 11.5 for another hour without the further addition of sodium hydroxide. Hydrochloric acid (450 ml. of *N*, 0.45 mole) was added in portions with stirring over 15 minutes, and the precipitated product was collected on a filter. The wet solid was taken up as quickly as possible in 850 ml. of boiling acetone, concentrated at room temperature to a smaller volume and water added. The resulting solid product was recrystallized by solution in 2800 ml. of boiling acetone, concentration at room temperature to 300 ml. followed by the addition of 300 ml. of water to yield 14.9 g., m.p. 127° dec., $\alpha^{26}D$ +93° (*c* 2 in methanol). Storage of the original aqueous filtrate overnight at 5° yielded a second crop which, after recrystallization, amounted to 13.6 g., m.p. 125°, $\alpha^{26}D$ +94° (*c* 2 in methanol). The total yield was 28.5 g. (35%) of material suitable[15] for cyclization to penicillin V. Recrystallization gave an analytical sample of D-α-phenoxymethylpenicilloic acid hydrate, m.p. 128° dec., $\alpha^{26}D$ +93° (*c* 2 in methanol).

Anal. Calcd. for $C_{16}H_{20}N_2O_6S \cdot H_2O$: C, 49.74; H, 5.74; N, 7.25. Found: C, 49.81; H, 5.98; N, 7.16.

Potassium D-Phenoxymethylpenicillinate (VIII). Relay Syntheses.—To a suspension of 4.64 g. (0.012 mole) of natural D-α-phenoxymethylpenicilloic acid hydrate[15] in 960 ml. of purified dioxane and 600 ml. of water was added with stirring 24 ml. of 0.5 *N* (0.012 mole) sodium hydroxide; after 30 minutes all the solid was in solution. To this stirred solution was added in one portion a solution of 9.31 g. (0.048 mole) of N,N′-dicyclohexylcarbodiimide in 600 ml. of purified dioxane. After 22 hours at room temperature the pale yellow solution was lyophilized. A suspension of the residue in a mixture of 350 ml. of ether and 350 ml. of water was stirred vigorously for 15 minutes. The layers were separated and a trace of insoluble solid removed by filtration. The aqueous layer was extracted with 100 ml. of ether and then lyophilized to yield 3.955 g. of crude water-soluble penicillin V which was 6.8% pure (6.0% yield) by bioassay[16] and 10.5% pure (9.3% yield) by chemical assay.[17]

The crude penicillin V was purified by a two-funnel distribution between methyl isobutyl ketone and two successive phosphate buffers containing different concentrations of ammonium sulfate. A solution of the crude penicillin V in 100 ml. of water and 100 ml. of 1.5 *M* phosphate buffer (*p*H 6.0, dibasic sodium phosphate–monobasic potassium phosphate = 1:2) was covered with 1000 ml. of distilled methyl isobutyl ketone and 800 ml. of saturated ammonium sulfate (70 g./100 ml. water) was added gradually with swirling.[31] The aqueous phase was then transferred to funnel 2, equilibrated with methyl isobutyl ketone and then discarded. The methyl isobutyl ketone layers (each 1000 ml.) in funnels 1 and 2 were then successively extracted with two 1000-ml. portions of buffer "A," which were then discarded as was the small amount of oil which failed to dissolve in either layer. This buffer "A" was prepared from 100 ml. of water, 100 ml. of 1.5 *M* phosphate buffer (*p*H 6.0) and 800 ml. of saturated ammonium sulfate. Funnels 1 and 2 were extracted successively with seven 1000-ml. portions of buffer "B," which was composed of 567 ml. of water, 100 ml. of 1.5 *M* phosphate buffer (*p*H 6.0) and 333 ml. of saturated ammonium sulfate. The 7 l. of buffer "B," containing the penicillin V, was cooled for 1 hour in an ice-bath, covered with 300 ml. of methyl isobutyl ketone, and 240 ml. of 20% phosphoric acid was added gradually with swirling. The aqueous layer was extracted further with two 80-ml. portions of methyl isobutyl ketone. The combined organic layer was immediately shaken with 400 ml. of buffer "A."

A second countercurrent distribution was carried out in the same manner with two funnels (400 ml. in each phase). The three 400-ml. portions of buffer "A" were successively equilibrated with funnels 1 and 2 and then were discarded. The penicillin V was then removed from the organic layer by successive extractions with seven 400-ml. portions of buffer "B." The 2800-ml. aqueous layer was cooled in an ice-bath, was covered with 400 ml. of cold ether, and 100 ml. of cold 20% phosphoric acid was added in portions with swirling. The aqueous layer (*p*H 2.5) was extracted with an additional 200-ml. portion of ether. The ether layer was extracted with a 50-ml. portion of cold water, which was discarded. Cold water (150 ml.) was added, and the acids were titrated with 0.05 *N* potassium hydroxide (17.8 ml.) to *p*H 7.0 (*p*H meter). The aqueous phase was lyophilized to yield 327 mg. of a white solid. Most of this solid (323 mg., which contained 242 mg. of potassium penicillin V by chemical assay) was taken up in a solution of 1 ml. of water and 0.7 ml. of acetone. Dilution to 10 ml. with acetone afforded 79 mg. of crystalline product, m.p. 261° dec. Dilution to 40 ml. with acetone gave a second crop of 153 mg., m.p. 257° dec. Work-up of the mother liquors afforded a further 13 mg. The infrared spectra (potassium bromide) of these fractions were identical with the spectrum of natural potassium phenoxymethylpenicillinate. The total isolated yield of crystalline potassium D-phenoxymethylpenicillinate was 245 mg. (5.4%). Recrystallization from water–acetone gave an analytical sample, m.p. 261° dec. (reported[18] 256–260° uncor.), mixed m.p. with natural VIII was 261° dec., $\alpha^{27}D$ +224° (*c* 0.4 in water); reported[18] $\alpha^{20}D$ +223° (*c* 1 in water); ultraviolet spectrum λ_{max}^{water} 268 mμ (ε 1280) and 274.5 mμ (ε 1060) [natural penicillin V potassium had λ_{max}^{water} 268 mμ (ε 1270) and 274.5 mμ (ε 1040)].

Anal. Calcd. for $C_{16}H_{17}N_2O_5SK$: C, 49.46; H, 4.41; N, 7.21; K, 10.06. Found: C, 49.69; H, 4.63; N, 7.52; K, 10.22.

A small scale cyclization (5% of above quantities, *i.e.*, 232 mg., 0.6 mole, of D-α-VII), carried out exactly as above except for a longer period of time (33 hours), afforded 215 mg. of crude water-soluble penicillin V, which was 11% pure (11% yield) by chemical assay and 9% pure (9% yield) by bioassay.

To a suspension of 221 mg. (0.57 mmole) of D-α-VII in 8 ml. of dioxane was added 5 ml. of 0.12 *N* sodium hydroxide. To this solution was added a solution of 0.5 ml. (7 mmoles) of ethoxyacetylene in 5 ml. of dioxane. After storage at room temperature for 19 hours, the solution was lyophilized to yield 259 mg. of a solid containing 0.22% 0.29% yield) of penicillin V by bioassay.

To a solution of 212 mg. (0.6 mmole) of the monolithium salt of D-α-VII in 5 ml. of water and 8 ml. of dioxane was added a solution of 150 mg. (0.78 mmole) of pentamethyleneketene cyclohexylamine[21] in 5 ml. of dioxane. After 18 hours the solution was lyophilized to give 393 mg. of a solid which contained 0.099% (0.19% yield) of penicillin V by bioassay.

To a suspension of 116 mg. (0.3 mmole) of D-α-VII in 4 ml. of dioxane and 1.9 ml. of water was added 0.6 ml. of 0.5 *N* sodium hydroxide. To this solution was added a suspension of 49 mg. (0.3 mmole) of N,N′-carbonyldiimidazole[22] in 2.5 ml. of dioxane. After storage for 1 hour at room temperature, the solution was lyophilized to yield 175 mg. of an oily solid having no biological activity.[16] A second reaction was carried out as above, except that a 195-mg. (1.2 mmoles) portion of N,N′-carbonyldiimidazole suspended in 10 ml. of dioxane was used, and gave a product which also had no biological activity.

(30) Kindly furnished by Eli Lilly and Company, Indianapolis, Indiana.

(31) A very similar solvent system was used for the separation of penicillin G from its penicilloic acid by S. C. Pan, *Anal. Chem.*, **26**, 1438 (1954).

Total Synthesis (First Procedure).—To a solution of 2.54 g. (6.3 mmoles) of D-α-VII·HCl in 86 ml. of dioxane and 28 ml. of water was added with stirring 25.1 ml. of 0.5 *N* (12.6 mmoles) sodium hydroxide. To this solution of the monosodium salt of D-α-VII was added in one portion with stirring a solution of 1.21 g. (6.3 mmoles) of N,N′-dicyclohexylcarbodiimide in 53 ml. of dioxane. After 25 minutes at room temperature 0.21 g. (16%) of N,N′-dicylohexylurea was removed by filtration and the filtrate was lyophilized. The residue was taken up in 100 ml. cold methanol; 100 ml. of water was added followed by 300 ml. of 1.5 *M* phosphate buffer (*p*H 6.4); the water-insoluble material was removed by filtration and discarded. The aqueous layer was covered with 300 ml. of ether and was cooled in an ice-bath; 142 ml. of 20% phosphoric acid was added in portions. After extraction of the aqueous layer (*p*H 2.5) with an additional 150-ml. portion of ether, the combined ethereal layers were washed with a 125-ml. portion of cold water, which was discarded. Cold water (100 ml.) was added, and the acids were titrated with 9.9 ml. of 0.5 *N* sodium hydroxide to *p*H 6.8. The aqueous phase was lyophilized to yield 1.38 g. of crude water-soluble penicillin V which was 6.3% pure (3.8% yield) by bioassay[16] and 9.0% pure (5.3% yield) by chemical assay.[17]

The crude penicillin V (1.34 g.) was purified by two countercurrent distributions between methyl isobutyl ketone and phosphate buffers (as described above for the relay synthesis) to yield 98 mg. of a colorless solid which contained 65% of potassium phenoxymethylpenicillinate (2.8% yield) by chemical assay. Crystallization from 98% acetone gave 30 mg. (1.4% yield), m.p. 263° dec. (unchanged on recrystallization), of the totally synthetic crystalline potassium salt of penicillin V. The natural and synthetic potassium salts were shown to be identical by microbiological assay, optical rotation [synthetic, $\alpha^{25}D$ +223° (*c* 0.2 in water); natural, $\alpha^{25}D$ +223° (*c* 0.2 in water); reported[18] $\alpha^{20}D$ +223° (*c* 1 in water)], infrared spectrum (identical in 40 peaks and shoulders in potassium bromide), ultraviolet spectrum [synthetic, λ_{max}^{water} 268 mμ (ε 1250) and 274.5 mμ (ε 1030); natural, λ_{max}^{water} 268 mμ (ε 1270) and 274.5 mμ (ε 1040)], m.p. 263° dec. (reported[18] 256–260° uncor.) and undepressed mixed m.p.

Potassium DL-Phenoxymethylpenicillinate (VIII).—To a solution of 18.1 g. (0.045 mole) of DL-α-VII·HCl in 1800 ml. of dioxane and 1120 ml. of water was added with stirring 179 ml. of 0.5 *N* (0.09 mole) sodium hydroxide. To this solution was added with stirring a solution of 34.7 g. (0.18 mole) of N,N′-dicyclohexylcarbodiimide in 1120 ml. of dioxane in one portion. After 26 hours at room temperature the yellow solution was lyophilized. The residue was made completely water-soluble by the procedure described for the total synthesis of D-VIII. The crude water-soluble sodium salt of DL-penicillin V (5.38 g.) was 9.1% pure (bioassay[16] showed the sample contained 4.28% natural penicillin V acid, and assuming the L-form has no biological activity, the sample contains 9.1% DL-penicillin V as the sodium salt) (3.0% yield) and 9.3% pure (3.0% yield) by chemical assay.[17]

The crude DL-penicillin V (5.35 g.) was purified by two countercurrent distributions (as described above for the relay synthesis) to yield 595 mg. of a white solid which contained 53% (1.9% yield) of DL-potassium phenoxymethylpenicillinate by chemical assay. The solid (585 mg.) was taken up in a minimum volume of water and was diluted with acetone to afford 128 mg. of crystalline product, m.p. 239° (dec., in bath 225°); further dilution with acetone gave a second crop of 165 mg., m.p. 235° dec. The total yield of crystalline synthetic potassium DL-phenoxymethylpenicillinate was 293 mg. (1.8%). Two recrystallizations from water–acetone afforded an analytical sample of DL-VIII, m.p. 244° (dec., in bath 225°). The crystalline DL-penicillin V potassium salt showed 51.4% of the bioactivity[16] of natural penicillin V, indicating that L-penicillin V has little, if any, antibiotic activity. The infrared spectrum (potassium bromide) had a strong band at 5.64 μ, characteristic of the fused β-lactam-thiazolidine carbonyl function and was in fact identical (in the position and intensity of 40 peaks and shoulders) with the infrared spectrum of the natural potassium salt of penicillin V.

Anal. Calcd. for $C_{16}H_{17}N_2O_5SK$: C, 49.46; H, 4.41; N, 7.21. Found: C, 49.70; H, 4.61; N, 7.14.

***levo-erythro*-1,2-Diphenyl-2-methylaminoethanol Salt of Penicillin V (X).**—To a stirred solution of 204 mg. (1 mmole) of *levo-erythro*-1,2-diphenyl-2-methylaminoethanol·HCl[24,25] (*levo-erythro*-IX·HCl) in 20 ml. of water was added a solution of 350 mg. (0.9 mmole) of the natural potassium salt of penicillin V in 2 ml. of water. The product crystallized spontaneously and was collected after 20 minutes to afford 464 mg. (89%) of X, m.p. 174–176° dec. Two recrystallizations from methanol–ether gave an analytical sample, m.p. 181–183° dec., $\alpha^{25}D$ +90° (*c* 0.4 in N-methyl-2-pyrrolidone). This compound had 95 ± 10% (573 μ/mg.) of the theoretical bioactivity (606 μ/mg.) expected for the salt.[16]

Anal. Calcd. for $C_{31}H_{35}N_3O_6S$: C, 64.46; H, 6.11; N, 7.28. Found: C, 64.17; H, 6.25; N, 7.25.

The Resolution of Potassium DL-Phenoxymethylpenicillinate (VIII).—To a solution of 78 mg. (0.2 mmole) of DL-VIII in 2.5 ml. of water was added a solution of 43 mg. (0.16 mmole) of *levo-erythro*-IX·HCl[24,25] in 2 ml. of water. Seeding the solution with traces of X failed to induce crystallization, but storage at 5° for 9 days afforded 46 mg. (70%) of crystalline product of m.p. 168° dec. Part of this solid (16 mg., 0.02 mmole) was purified further by shaking with 1.5 ml. of *n*-butyl acetate and 1.5 ml. of 0.7% phosphoric acid until two clear layers resulted. The aqueous layer was discarded, and the organic layer was washed with two 1-ml. portions of water. To this solution was added a solution of 4.6 mg. (0.02 mmole) of *levo-erythro*-IX in 0.4 ml. of *n*-butyl acetate. Storage at 5° overnight afforded 10 mg. (44%) of a crystalline salt, m.p. 175° dec., $\alpha^{25}D$ +92° (*c* 0.2 in N-methyl-2-pyrrolidone). Comparison of infrared spectra (potassium bromide) showed this compound to be identical with X. This compound showed 95 ± 10% (576 μ/mg.) of the theoretical bioactivity expected for the salt.[16]

The aqueous filtrate containing L-penicillin V was covered with 6 ml. of *n*-butyl acetate, and 3.5 ml. of 1% phosphoric acid was added; shaking caused the solid which precipitated to redissolve. The organic layer was washed with two 5-ml. portions of water. To the organic layer was added a solution of 46 mg. (0.2 mmole) of *dextro-erythro*-IX[24,25] in 3 ml. of *n*-butyl acetate. Scratching initiated crystallization and storage for 3 days at 5° afforded 35 mg. of crystals, m.p. 177° dec. A second crop was obtained from the filtrate, m.p. 175° dec. The total yield was 41 mg. (66%). Recrystallization from dioxane–ether gave an analytical sample of the *dextro-erythro*-IX salt of L-VIII, m.p. 175° dec., $\alpha^{25}D$ −86° (*c* 0.4 in N-methyl-2-pyrrolidone). The infrared spectrum (potassium bromide) was identical to that of X. This salt of L-penicillin V showed, 0.7% (4μ/mg.) of the theoretical bioactivity (606 μ/mg.) expected for the salt of natural penicillin V.[16]

Anal. Calcd. for $C_{31}H_{35}N_3O_6S$: C, 64.46; H, 6.11. Found: C, 64.09; H, 6.41.

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