methylammonium, was converted into the trans product IV, m.p. 202-204°, C, 78.9; H, 8.33, in 69% yield. Alkaline peroxide oxidation transformed IV into V (R = HI) which was converted with diazomethane into the ester V (R = CH3), and cyclized with potassium t-butoxide in benzene. The resulting keto ester was decarboxylmethylated with hydrochloric and acetic acid to give the dl-ketone VI, m.p. 158.5-161.5°. The infrared spectrum of this material was indistinguishable from that of authentic 3-hydroxy-9,11-dehydroandrostane-17-one.

(5) At this stage the 3-hydroxyl group was protected as the tetrahydropropyranol ether (cf. ref. 3).


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THE TOTAL SYNTHESIS OF PENICILLIN V

Sir:
The ability of aliphatic carbodiimides to form amide bonds in aqueous solution directly from the amine and carboxylic components under very mild conditions suggested the use of these reagents for the cyclization of a penicillic acid to a penicillin. We have prepared by total synthesis in good over-all yield the penicilloic acid corresponding to penicillin V (phenoxymethylpenicillin). By use of N,N'-dicyclohexylcarbodiimide, one of the more soluble (ethanol-water) forms of the natural 3,S-hydroxyl-9,11-dehydroandrostane-17-one (potassium phenoxymethylpenicillinate). The loss of the 3-hydroxyl group was isomerized in high yield in the d-a-form as in the d-l-ester series, thus providing a stereomerically efficient synthesis. Hydroxylaminoysis of I, followed by acidification with hydrochloric acid, produced the t-butyld-a-4-carboxy-5,5-dimethyl-a-phthalimido-2-thiazolidineacetate (I), C6H3N2O4S, m.p. 161° dec. [Found: C, 57.45; H, 6.96; N, 5.76; a25° + 54° (c, 1 in acetic acid)] as described for the corresponding dl-a-acid. The a, or natural, configuration of the more soluble (ethanol–water) form of I was established chemically by relationship to the a-natural dimethyl d-α-benzylpenicilloate. The less soluble d-γ-isomer may be isomerized in high yield to the a-form as in the dl-ester series, thus providing a stereomerically efficient synthesis. Hydroxylaminoysis of I, followed by acidification with hydrochloric acid, produced the t-butyld-a-4-carboxy-5,5-dimethyl-a-amino-2-thiazolidineacetate hydrochloride (II), C6H3N2O4SCl, in 87% yield; m.p. 172° dec. [Found: C, 46.88; H, 6.19; Cl, 10.87; a25° + 111° (c, 1 in methanol)].

Phenoxyacetyl chloride and triethylamine converted II to α-t-butyld-a-phenoxyphospholycopine (III), C6H2N2O4S, in 75% yield; m.p. 120-122° dec. [Found: C, 56.88; H, 6.86; N, 6.59; a25° + 67° (c, 1 in methanol)]. Cleavage of the t-butyld ester with dry hydrogen chloride, followed by crystallization from acetone-water containing an equivalent of pyridine, led to 75% of d-a-phenoxymethylpenicilloic acid hydrate (IV), C6H3N2O4SH2O; m.p. 129° dec. [Found: C, 49.61; H, 5.77; N, 6.94; a25° + 94° (c, 1 in methanol)]. Identity with a sample prepared by saponification of natural penicillin V was established by comparison of m.p., infrared spectra (KBr), optical rotation and mixed m.p.

Treatment with N,N'-dicyclohexylcarbodiimide in dioxane-water (20 min. at 25°) cyclized IV as the monopotassium salt in 10-12% yield. By partition between methyl isobutyl ketone and pH 5.5 phosphate buffer (two funnels) the totally synthetic crystalline potassium salt of penicillin V was isolated. The natural and synthetic potassium salts were shown to be identical by microbiological assay, optical rotation (synthetic, a25° + 223° (c, 0.2 in water); natural, a25° + 223° (c, 0.2 in water); reported, a25° + 223° (c, 1 in water)), infrared spectra (KBr), m.p. 263° dec. (reported, 256-260° uncorr., undepressed upon admixture.

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 synthetic potassium penicillin V and 2-potassium phenoxymethylpenicillinate.

The same results were obtained using IV derived from natural penicillin V. The entire series also has been carried through starting with 3-penicillamine. The crystalline d-penicillamine V potassium salt showed 51.4% (514u/mg.) of the bioactivity of natural penicillin V, indicating that L-penicillin V has little, if any, antibiotic activity. Cyclization of the penicilloate also was effected, but in lower yield, by ethoxyacetylene and a ketenimine (pentamethyleneketene cyclohexylimines). It is interesting to note that the entire reaction sequence starting with penicillamine was conducted at or below room temperature.

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 synthetic potassium penicillin V and 2-potassium phenoxymethylpenicillinate.

(2) Penicillinammonium and 2-benzyl-4-methoxybenzylmethylamine 3-(4) esterone condense to form trace amounts (0.03 to 0.08%) of 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., Vol. V, p. 408.
(5) Kindly furnished by Eli Lilly & Company, Indianapolis, Ind.
(6) Synthetic potassium penicillin V had a potency of 1078 u/mg. ± 10% (107.8% ± 10%) compared to standard natural penicillin V in a plate diffusion assay carried out under the supervision of Dr. J. Lein, Burroughs Wellcome Laboratories, Syracuse, N.Y.
(8) Directions for the preparation of this ketenimine were furnished by Dr. C. L. Stevens, Wayne University, private communication.
Sir:

The effect of added neutral salts upon the velocity of the second order of the ion-dipole aromatic nucleophilic substitution reactions of lithium, sodium and potassium methoxides with 2,4-dinitrochlorobenzene has been investigated at 25°. The rates were studied in absolute methanol solvent as a function of reactant (LiOCH₃, NaOCH₃, and KOCH₃) in the presence of added cations (Li⁺, Na⁺, and K⁺) and added anions (Cl⁻, Br⁻, ClO₄⁻, I⁻, and NO₃⁻). The reaction of NaOCH₃ in the presence of added LiClO₄·3H₂O also was studied in a 50 volume % methanol-benzene solvent.

For reactions without added salts, the rate constants (1 mole⁻¹ sec⁻¹) were: LiOCH₃, 0.0242; NaOCH₃, 0.0202; KOCH₃, 0.0278. A consistent pattern of salt effects is typified by the data for the LiOCH₃ reaction shown in Fig. 1. At low concentrations of added salt, each cation exhibits an individual effect, added to that of the cation introduced along with the reactant methoxide. The anions cause an additional secondary effect. The reaction rate increases for acetate > Cl⁻, Br⁻ > I⁻, NO₃⁻ > ClO₄⁻. Salt effects are more pronounced in solvents of lower dielectric constant. The observed effects cannot be correlated with changes in ionic strength of the reaction medium as found by Bolto and Miller.

A qualitative explanation of the effect of lithium salts assumes the equilibrium

\[ \text{LiOCH}_3 + \text{Li}^+ \rightarrow \text{Li}^+ + \text{OCH}_3^- \]

The addition of a salt providing Li⁺ as a common ion should shift this equilibrium to decrease the concentration of the reactant, OCH₃⁻. Since the effective concentration of added Li⁺ will depend on the degree to which it remains associated with the added anion, the rate will differ with different added salts. This assumes that the ion pair reacts at a negligible rate compared to that for the ion. A similar interpretation has been used to account for the variation in rate of decarboxylation of trichloroacetic acid. The observed effect of anions on reaction rate thus can be interpreted to suggest that the order of attraction for lithium ions in methanol is Ac⁻ > Cl⁻, Br⁻ > NO₃⁻, I⁻ > ClO₄⁻.

The fact that NaOCH₃ and KOCH₃ react faster suggests that the corresponding equilibria involving these methoxides is shifted more to the right, providing a greater effective concentration of OCH₃⁻. Conductivity data suggest that more ion association occurs for LiOCH₃ than for KOCH₃ or NaOCH₃ in methanol. Potassium salts are strong electrolytes in methanol with dissociation constants of about 0.1 to 0.02. It is known that potassium salts are stronger electrolytes than are lithium salts in acetone. If a similar order of electrolyte strength holds for methanol solutions, then the effect of added potassium salts on the LiOCH₃ ⇌ Li⁺ + OCH₃⁻ equilibrium would be to supply anions which would tend to associate more readily with Li⁺ so that the equilibrium would be shifted to provide a greater concentration of OCH₃⁻. This accounts for the increase in rate of the reaction. Sodium salts are not as effective as potassium salts, and the anion effects are consistent with those observed in the presence of Li⁺ alone.

(1) E. C. Evers and A. G. Knox, THIS JOURNAL, 73, 1739 (1951).
(3) E. C. Evers and A. G. Knox, THIS JOURNAL, 73, 1739 (1951).
(8) J. Wright, to be published.
Potassium phenoxymethylpenicillinate (VIII), synthesized totally in a series of reactions from D-penicillamine (D-II) and t-buty1 phthalimidomalonaldehydate (I), has been shown to be identical to natural penicillin V (potassium salt) in physical and biological properties. In the key step, the monopotassium salt of the penicilloic acid (D-CY-VII) was isolated, which led to the formation of a 5-phenylpenicillin (penicillin G) 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. We now wish to record the first rational synthesis of a natural penicillin.

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, 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 suggested the use of these reagents for the cyclization of a penicillic 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 "y-carboxyl of the penicillic acid VII. This feature obviates the necessity of removing a protective group (e.g., a benzyl ester by catalytic hydrogenolysis) in a last step. 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-a-penicilloates. Comparisons were made at three points in the synthetic sequence, viz., at D-a-IV, -VII and -VIII. The D-l-a-IV isomer had been shown previously by Sheehan and Cruickshank to correspond in configuration to the natural D-a-penicilloates; this assignment was confirmed in the present work by the conversion of D-l-a-III into D-l-a-VIII, the natural diastereomer.

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

**Synthesis of d- and DL-α-Penicilloic Acids.**—The interaction of d-penicillamine hydrochloride (d-11) and t-butyl phthalimidomalonaldehyde (I) in sodium acetate buffered aqueous ethanol afforded directly the crystalline thiazolidine d-7-11 (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 (23%) than in the DL-series. Additional quantities of the desired d-α-isomer of VIII 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 80% yield. Higher yields were achieved with longer reaction times in very dilute solutions (0.37G) using four equivalents of carbodiimide. In an experiment run for 33 hours using natural phenoxymethylpenicilloic acid hydrate (d-α-VII), the yield of penicillin V was 79% and DL-penicillin V in 76% yield.

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 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°C 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-phenoxyethylpenicilloic 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), the yield of penicillin 

(14) J. C. Sheehan and G. D. Laubach, This Journal, 73, 4792 (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 and by chemical assay.

(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°C, which may account for the discrepancy between chemical and microbiological assay.
lin V in the partially purified product was 11% by chemical assay and 9% by bioassay. 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 natu-
ral 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 con-
sidered 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 em-
ployed in the totally synthetic and dl-series.

In a similar manner, cyclization of d-α-VII, ob-
tained from n-penicillamine, proceeded rapidly (25
min.) at room temperature with one equivalent of
N,N'-dicyclohexylcarbodiimide to give a 48–
5% 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 (108 ± 10% of the bioac-
tivity of penicillin V potassium), optical rotation [synthetic αD + 228° (C 0.2 in water): natural,
αD + 229° (C 1 in water)], infrared spectra (40
peaks and shoulders in potassium bromide), m.p.
293° dec. (reported 250-260° uncorr.), underpres-
ad mixture.

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. 294° dec. This salt exhibited 31% of the bioactivity 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, char-
acteristic of the fused β-lactam-thiazolidine
carbonyl function, and was identical with the spec-
trum of the natural potassium salt of penicillin V.

In parallel experiments N,N'-dissopropilcarbo-
diimide promoted cyclization of the d-α-penicilloate
in essentially the same yield as with N,N'-dicyclo-
hexylcarbodiimide. 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. Ethoxycetacene has
been used by Arens for the formation of peptides
in anhydrous solvents. Sheehan and Hlavka, attempt-
ing to form a peptide in aqueous solution at
room temperature, found that phthaloylglycine and
ethoxyacetene formed a reactive isolable peptide
intermediate, which could interact with ethyl gly-
cinate to form phthaloylalginic ethyl ester. Ethoxyacetylene cyclized the monocarboxyl salt of
the d-α-penicilloate in 0.29% yield. Pentamethyl-
ekenetene cyclohexylamine also effected cycliza-
tion in 0.19% yield. N,N'-Carboxyldimidazole,
a new peptide-forming reagent, gave a product with
no biological activity.

For cyclizations with re-
agents other than carbodiimides, however, no at-
tempt was made to develop optimum conditions
for the reactions. The monosodium salt of the penicillolic acid corresponding to penicillin G was
also cyclized with N,N'-dicyclohexylcarbodiimide
to penicillin G but in much lower yield.

Resolution of dl-Penicillin V.—The racemic
amine erythro-1,2-diphenyl-2-methylaminoethanol
is readily resolved since the lev-o-isomer forms a
sparingly soluble salt with penicillin G; hence it
follows that the optically active amine would re-
solve penicillin G. Natural penicillin V formed a
salt with the levo-amine, which immediately crys-
tallized from water or n-butyl acetate. Reaction of
dl-penicillin V with the levo-amine in water solu-
tion 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 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 iden-
tical in other physical properties.

The salt of d-penicillin from the resolution gave
the same bioassay value as the salt of natural peni-
cillin 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 suf-
cient to account for the very low bioactivity ob-
served, and that pure L-penicillin V might show no
biological activity.

We are indebted to Bristol Laboratories of Syra-
cuse, 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. Chodsdy for technical assis-
tance.

Experimental

β-Butyl p- and dl-4-Carboxy-5,5-dimethyl-o-phenilal-
2-Thiazolidinediacetate (III).—To an ethanol solution (300
mL) of 42 g. (0.146 mole) of β-butyl o-phenilalmaid-
aldehyde (I) was added a solution of 27.2 g. (0.146 mole)
of phenilalmandiacetoxychloride (II) and sodium acetate
trihydrate (29.9 g., 0.22 mole) in 300 mL of water. After
nished by Dr. C. L. Stevens, Wayne University, private communica-
tion.

(22) G. W. Anderson and R. Paul, THIS JOURNAL, 60, 4420 (1938).

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

1564 (1953).

(25) Samples of levo- and dextro-erythro-1,2-diphenyl-2-methylamino-
ethanol hydrochlorides were kindly supplied by Dr. L. C.
Young, J. Am. Pharm. Assoc., Sci. Ed., 40, 261 (1951); W.
1564 (1953).

(26) All melting points are corrected. We are indebted to Dr. S. M.
Nagly and his associates for the microanalyses, and to
Dr. A. Johnson, THIS JOURNAL, 76, 158 (1954).

Patents 2,496,410 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 \( \gamma \)-isomer. Two recrystallizations from methanol-water raised the m.p. to 145-146° dec., \( \alpha \)-isomer (98%) was essentially pure \( \gamma \)-isomer.

The condensation under similar conditions of \( \alpha \)-penicillinamide hydrochloride and I and the isolation of the less-soluble \( \gamma \)-isomer has been described previously. From the cillamine hydrochloride and I and the isolation of the less-soluble \( \gamma \)-isomer, caused the slow crystallization of 9.03 g. of \( \alpha \)-isomer as 6.66. Found: C, 57.45; H, 6.06; N, 6.83.

6.66. Found: C, 57.25; H, 5.79; N, 6.62.

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6.66. Found: C, 57.25; H, 5.79; N, 6.62.

from methanol-water afforded a product with a constant melting point, 159-160° dec., \( \alpha \)-isomer (98%) was essentially pure \( \gamma \)-isomer. Two recrystallizations from methanol-water raised the m.p. to 145-146° dec., \( \alpha \)-isomer (98%) was essentially pure \( \gamma \)-isomer.

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6.66. Found: C, 57.25; H, 5.79; N, 6.62.
additional recrystallization from the same solvent combination gave analytically pure d-α-phenoxymethylpenicillanic acid m.p. 129.5° dec., optical rotation and infrared spectrum (pontassium bromide).

Atal. Calcd. for C_{13}H_{17}O_{4}N_{3}S: C, 51.43; H, 5.24; N, 7.18.

In a similar manner 75 g. of DL-α-VII-HCl gave, after one recrystallization from acetone-water, 23 g. of dl-α-phenoxymethylpenicillanic acid, m.p. 133° dec. Recrystallization gave an analytical sample, m.p. 139° dec.

Alkaline Hydrolysis of Natural Penicillin V—To a stirred suspension of 75 g. of DL-α-VII-HCl, m.p. 129° dec., in 750 ml. of water there was added 300 ml. of 20% phosphoric acid and 300 ml. of water. This suspension was brought to pH 11-11.5, the solution was maintained at this pH by the addition of 20% phosphoric acid. After chemical analysis, the solution was brought to pH 7.0 (pH meter). The aqueous phase was lyophilized to yield 327 g. of a white solid. Most of this solid (323 g.), which contained 242 mg. of potassium penicillin V, was removed from the organic layer by successive extractions with seven 400-ml. portions of butyl alcohol and water. The aqueous layer (pH 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 solution was titrated with 0.05 N potassium hydroxide (17.8 ml.) to pH 7.0. The aqueous phase was then extracted with 300 ml. of dichloromethane, and the combined organic layers were separated and a trace of insoluble solid removed by filtration. The final aqueous layer was neutralized to pH 7.0 (pH meter). The aqueous phase was lyophilized to yield 393 mg. of a solid containing 0.22% sodium penicillin V potassium had 268 mp (c 1 in water); ultraviolet spectrum (λ max 268 (e 1250) and 274.5 (e 1600) natural penicillin V potassium had λ max 268 (e 1270) and 274.5 (e 1400).

Analysis. Calcd. for C_{13}H_{17}O_{4}N_{3}S: 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., 20 mg., 0.6 mmole, of DL-Ⅱ-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 solution of 221 mg. 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 mg. (4.40 mmole) of ethoxycetylethane in 8 ml of dioxane. After storage at room temperature for 10 hours, the solution was lyophilized to yield 250 mg. of a solid containing 0.22% (0.22% yield) of penicillin V by bioassay.

To a solution of 212 mg. of dioxane was added 5 ml. of 0.12 N sodium hydroxide. To this solution was added a solution of 0.5 mg. (5.7 mmole) of d-α-VII in 8 ml of dioxane. After storage at room temperature for 10 hours, the solution was lyophilized to yield 256 mg. of a solid containing 0.22% (0.22% yield) of penicillin V by bioassay.

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

(31) A very similar solvent system was used for the separation of penicillin G from its penicillic acid by S. C. Pan, Atal. Chem., 26, 1408 (1954).
Total Synthesis (First Procedure).—To a solution of 2.64 g. (0.09 mole) of DL-α-VII-HCl in 80 ml. of dioxane at 0°C was added 17 ml. of 0.5 N (12.6 mmoles) sodium hydroxide. To this solution of the mono-

sodium salt of DL-α-VII was added with stirring a solution of 34.7 g. (0.18 mole) of N,N'-dicyclohexylcarbodiimide in 1120 ml. of dioxane and 1120 ml. of water was added with stirring 25.1 ml. of 0.5 N (12.6 mmoles) sodium hydroxide. This solution of the mono-sodium salt of DL-α-VII was added with stirring a solution of 0.18 mole of N,N'-dicyclohexylcarbodiimide in 1120 ml. of dioxane. After 25 minutes at room temperature 0.21 g. (16%) of N,N'-dicyclohexylurea was removed by filtration and the filtrate was lyophilized. The residue was taken up in 100 ml. of cold methanol; 100 ml. of water was added followed by 300 ml. of 1.5 M phosphate buffer (pH 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 (pH 2.6) with an additional 150-ml. portion of ether, the combined etheral layers were washed with 125-ml. portion of cold water, which was discarded. Cold water (100 ml.) was added, and the acids were titrated with 0.5 N sodium hydroxide to pH 6.8. The aqueous phase was lyophilized to yield 98 mg. of a colorless solid which contained 65% of potassium phenoxymethylpenicillinate (5.38 δα) and 3.0% yield) and 9.3% pure (3.0% yield) by chemical assay. The aqueous filtrate containing L-penicillin V 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 (pH 2.6) with an additional 150-ml. portion of ether, the combined etheral layers were washed with 125-ml. portion of cold water, which was discarded. Cold water (100 ml.) was added, and the acids were titrated with 0.5 N sodium hydroxide to pH 6.8. The aqueous phase was lyophilized to yield 98 mg. of a colorless solid which contained 65% of potassium phenoxymethylpenicillinate (5.38 δα) and 3.0% yield) and 9.3% pure (3.0% yield) by chemical assay.

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 (5.38 δα) and 3.0% yield) and 9.3% pure (3.0% yield) by chemical assay.

Crystallization from 98% acetone gave a second crop of 165 mg., m.p. 235° dec. The total yield was 41 mg. (96%). Recrystallization from dioxane-ether gave 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 283 mg. (1.4%). 

Potassium DL-Phenoxymethylpenicillinate (VIII).—To a solution of 18.1 g. (0.09 mole) of DL-α-VII-HCl in 1800 ml. of dioxane and 1120 ml. of water was added with stirring 25.1 ml. of 0.5 N (12.6 mmoles) 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. After 25 minutes at room temperature 0.21 g. (16%) of N,N'-dicyclohexylurea was removed by filtration and the filtrate was lyophilized. The residue was taken up in 100 ml. of cold methanol; 100 ml. of water was added followed by 300 ml. of 1.5 M phosphate buffer (pH 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 (pH 2.6) with an additional 150-ml. portion of ether, the combined etheral layers were washed with 125-ml. portion of cold water, which was discarded. Cold water (100 ml.) was added, and the acids were titrated with 0.5 N sodium hydroxide to pH 6.8. The aqueous phase was lyophilized to yield 98 mg. of a colorless solid which contained 65% of potassium phenoxymethylpenicillinate (5.38 δα) and 3.0% yield) and 9.3% pure (3.0% yield) by chemical assay.

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 (5.38 δα) and 3.0% yield) and 9.3% pure (3.0% yield) by chemical assay.

Crystallization from 98% acetone gave a second crop of 165 mg., m.p. 235° dec. The total yield was 41 mg. (96%). Recrystallization from dioxane-ether gave an analytical sample of the dextro-erythro-IX salt of t-VIII, m.p. 175° dec., αC +92° (c 0.2 in N-methyl-2-pyrrolidinone). This compound showed 95 ± 10% (578 μg./mg.) of the theoretical bioactivity (606 μg./mg.) expected for the salt.


**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* (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°. Two recrystallizations from methanol–ether gave an analytical sample, m.p. 181-183° dec., αC +90° (c 0.4 in N-methyl-2-pyrrolidinone). This compound had 95 ± 10% (578 μg./mg.) of the theoretical bioactivity (606 μg./mg.) expected for the salt.


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 44 mg. (0.16 mmole) of the natural penicillin V potassium salt showed 51.4% of the bioactivity (606 μg./mg.) expected for the salt. Comparison of infrared spectra (potassium bromide) showed this compound to be identical with X. This compound was identified by X-ray diffraction, and this crystallography was identical with X. This compound was identical to that of X. This salt of L-penicillin V showed 65% (44%) of the theoretical bioactivity (606 μg./mg.) expected for the salt of natural penicillin V.