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The Total and Partial General Syntheses of the Penicillins

BY JOHN C. SHEEHAN AND KENNETH R. HENERY-LOGAN

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The first total general synthesis of the penicillins and a (chemical) partial synthesis from penicillin G are described. The key synthetic intermediate in both routes is 6-aminopenicillanic acid (6-APA), which was acylated to form both "natural" penicillins and "new" penicillins not obtainable directly by fermentation.

The announcement[1] in March, 1958, that ". . .we have prepared this compound [6-aminopenicillanic acid (6-APA, XI)] *via* a totally synthetic route. . . . We have shown that one can acylate with various acid chlorides and obtain the corresponding penicillins," together with the subsequent development of commercially attractive biochemical routes to 6-aminopenicillanic acid, has been followed by the explosive growth of the "new penicillins." Two are in current medical use[2] and several others are reported to be under extensive clinical trial. At the recent "First Interscience Conference on Antimicrobial Agents and Chemotherapy"[3] thirty-nine papers dealt with the new derivatives of 6-aminopenicillanic acid (6-APA). The medical advantages reported for the "new penicillins" include effectiveness against "penicillin-resistant" staphylococci, a broadened microbiological spectrum (including certain Gram-negative organisms) and increased oral absorption.

In addition to our original totally synthetic route and partial synthesis (by chemical means) from penicillin G[4] the "key intermediate (6-APA)" has been prepared more recently by direct fermentation and by enzymatic splitting of "natural" penicillins (V and G) in four independent laboratories.[5] The commercially available "semi-synthetic" penicillins are made by one of these biochemical preparations of 6-aminopenicillanic acid, followed by chemical acylation of this key intermediate as discovered in this Laboratory. By independent totally synthetic routes, not involving 6-aminopenicillanic acid, this Laboratory has previously prepared both a "natural" penicillin (penicillin V)[6,7] and "new" penicillins[8,9] not obtainable directly by fermentation.

The reaction sequences employed in the syntheses of 6-aminopenicillanic acid (XI) *via* the methyl and benzyl esters of 6-tritylaminopenicillanic acid (VIII and IX) furnish an unambiguous chemical proof for the fused β-lactam-thiazolidine structure of 6-APA. The acylation of 6-aminopenicillanic acid to penicillins G and V and the formation of 6-phthalimidopenicillanic acid under mild conditions unequivocally establishes the structures of both "natural" and "unnatural" penicillins. It is noteworthy that the last eight steps (starting with penicillamine and *t*-butyl phthalimidomalonaldehydate) of the 6-aminopenicillanic acid synthesis (and the additional step to the penicillins) were carried out at, or below, room temperature.

The Total and Partial Syntheses of the Methyl and Benzyl "Desacylpenicilloates" (VI and VII).—In the total synthesis series, the phthaloyl group was removed from *t*-butyl D-α-4-carbomethoxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetate[7] by the action of hydrazine at room temperature, as described for the corresponding DL-isomer,[10] to yield the phthalhydrazide complex isolated by lyophilization. Treatment of the complex with dilute hydrochloric acid afforded D-α-I in high yield. Anhydrous hydrogen chloride at ice-bath temperature rapidly cleaved the *t*-butyl ester to produce the crystalline dihydrochloride of D-α-IV in high yield. Trityl chloride and diethylamine[11] converted the dihydrochloride into D-α-VI.

Similarly, dry hydrogen chloride converted DL-α-I[10] into DL-α-IV, which was tritylated[11] to afford crystalline DL-α-VI in 26% yield. In spite of the known sensitivity of trityl chloride toward hydrolyzing media and despite a controversial statement in the literature,[12] Zervas and Theodoropoulos[11] were able to tritylate amino acids directly in aqueous solution; employing diethylamine they obtained 35–47% tritylamino acid, although with a tertiary base (pyridine or triethylamine) the yield was minute.

(1) J. C. Sheehan in "Amino Acids and Peptides with Antimetabolic Activity," G. E. W. Wolstenholme and C. M. O'Connor, Editors, J. A. Churchill Ltd., London, England, 1958, p. 258. See also J. C. Sheehan, Canadian Patent 610,096 (Dec. 6, 1960) and Canadian Patent 619,205 (April 25, 1961); patent application in the United States March 1, 1957.

(2) These are 6-(α-phenoxypropionamido)-penicillanic acid (phenethicillin) and 6-(2,6-dimethoxybenzamido)-penicillanic acid (methicillin). The penicillanic acid nomenclature, which is now standard in the field, is described in J. C. Sheehan, K. R. Henery-Logan and D. A. Johnson, *J. Am. Chem. Soc.*, **75**, 3292 (1953).

(3) Sponsored by the American Society for Microbiology, held in New York City, Oct. 31–Nov. 2, 1961.

(4) Reported in communication form, J. C. Sheehan and K. R. Henery-Logan, *J. Am. Chem. Soc.*, **81**, 5838 (1959).

(5) F. R. Batchelor, *et al.*, *Nature*, **183**, 257 (1959); C. A. Claridge, A. Gourevitch and J. Lein, *ibid.*, **187**, 237 (1960); H. T. Huang, *et al.*, *J. Am. Chem. Soc.*, **82**, 3790 (1960); W. Kaufman and K. Bauer, *Naturwiss.*, **47**, 474 (1960). In 1950, K. Sakaguchi and S. Murao [*J. Agr. Chem. Soc. Japan*, **23**, 411 (1950)] claimed the isolation of a "penicillin nucleus" after enzymatic removal of phenylacetic acid from penicillin G; however, the constants reported differ markedly from those subsequently determined. K. Kato [*J. Antibiotics (Japan), Ser. A*, **6**, 130, 184 (1953)] suggested that a "penicillin nucleus" was present in fermentation broths grown in the absence of added side chain precursors. However, there is no published indication that any laboratory other than our own has made a serious effort toward the chemical synthesis of 6-aminopenicillanic acid or the penicillins, since the cessation of the very extensive and intensive attempts during World War II (for this latter program see "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson and R. Robinson, editors, Princeton University Press, Princeton, N. J., 1949).

(6) J. C. Sheehan and K. R. Henery-Logan, *J. Am. Chem. Soc.*, **79**, 1262 (1957).

(7) J. C. Sheehan and K. R. Henery-Logan, *ibid.*, **81**, 3089 (1959).

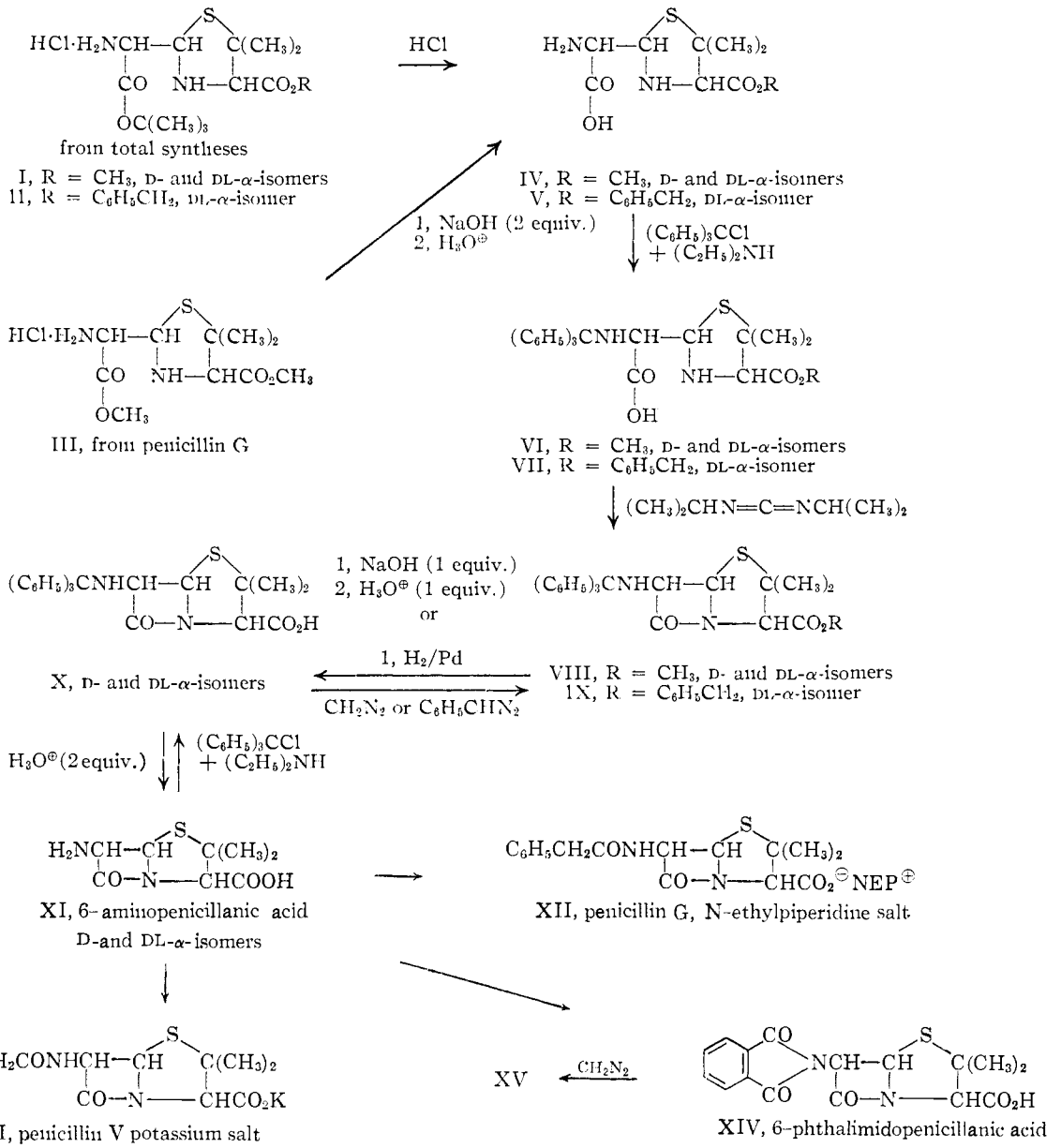
(8) J. C. Sheehan and D. R. Hoff, *ibid.*, **79**, 237 (1957).

(9) W. A. Bolhofer, J. C. Sheehan and E. L. A. Abrams, *ibid.*, **82**, 3437 (1960).

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

(11) L. Zervas and D. M. Theodoropoulos, *ibid.*, **78**, 1359 (1956).

(12) A. Hillmann-Elies, G. Hillmann and H. Jatzkewitz, *Z. Naturforsch.*, **8b**, 445 (1953).



For the partial synthesis route to VI, the starting material was methyl D-α-4-carbomethoxy-5,5-dimethyl-α-amino-2-thiazolidineacetate hydrochloride (III), which had been obtained previously by Sheehan and Ferris[13] from penicillin G following chemical removal of the phenylacetic acid side chain. The free base from III was preferentially saponified[14] with one equivalent of sodium hydroxide to give D-α-IV, which was tritylated[11] immediately to afford D-α-VI in 21% over-all yield.

The benzyl ester series was initiated with *t*-butyl DL - α - 4 - carbobenzyloxy - 5,5 - dimethyl-α-amino-2-thiazolidineacetate hydrochloride (II)[8]; this *t*-butyl ester was cleaved in nitromethane at ice-bath temperature with dry hydrogen chloride to give the crystalline hydrochloride of V in 88% yield. The hydrochloride was treated with trityl chloride and diethylamine in water and tetrahydrofuran and, after countercurrent distribution between sodium carbonate solution and ether, VII was isolated as an analytically pure white solid in 25% yield.

Several explanations can be put forward to account for monotritylation on the primary nitrogen rather than the thiazolidine nitrogen. Most important, the nitrogen in thiazolidine esters is very feebly basic (pK 4.0)[16] compared with the primary amine in α-amino acids (pK_2 9.6). Steric factors would also favor the primary amine in tritylation. Thus in the reaction of DL-4-carboxy-5,5-dimethyl-α-amino-2-thiazolidineacetic acid with

(13) J. C. Sheehan and J. P. Ferris, *J. Am. Chem. Soc.*, **81**, 2912 (1959).

(14) The β-esters of the penicilloic acids are obtained generally by the selective partial saponification of the corresponding diesters.[15]

(15) H. T. Clarke, J. R. Johnson and R. Robinson, editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 548.

(16) S. Ratner and H. T. Clarke, *J. Am. Chem. Soc.*, **59**, 200 (1937)

methyl phenylthioacetate, acylation was ascribed to the primary nitrogen.[17]

β-Lactam Cyclizations Using Carbodiimides.—The desacylpenicilloates VI and VII were rapidly cyclized at ice-bath temperatures in dioxane–water solution to the β-lactams VIII and IX, respectively. The β-lactam closures were effected readily in both the optically active and racemic series using one and a quarter equivalents of N,N′-diisopropyl carbodiimide; crystalline products were obtained after chromatography over alumina. This same carbodiimide was utilized previously in the cyclization of penicillin V.[7]

Many efforts directed toward the synthesis of penicillins have failed in the last step, the attempted cyclization of the corresponding penicilloates.[18] Utilizing the trityl protective group, the competing azlactonization reaction[19] is structurally precluded. The formation of the β-lactam proceeded particularly well in the benzyl ester series where the yield of crystalline IX was 67%. It is well known that the presence of bulky groups facilitates the formation of small rings, and it may be that this unusually high cyclization yield to form a β-lactam is due to the steric effect of the tritylamino and carbobenzyloxy groups. The totally synthetic benzyl ester DL-α-IX and the optically active D-α-IX (obtained from 6-APA) both had infrared absorption maxima at 5.68 and 5.73 μ attributable to the β-lactam and ester carbonyls, respectively, and the two infrared spectra (potassium bromide) were in fact identical. The replacement of the acylamino side chain of the penicillins by the tritylamino group has displaced the β-lactam carbonyl band from 5.62[20] to 5.68 μ.

In the optically active methyl ester series, crystalline methyl 6-tritylaminopenicillanate (D-α-VIII), m.p. 166–167°, was prepared *via* three sequences. Cyclization of totally synthetic D-α-VI with N,N′-diisopropylcarbodiimide gave the β-lactam D-α-VIII in 27% yield. A sample of D-α-VI, obtained by the partial synthetic route from penicillin G, was similarly cyclized. In addition, natural 6-APA was tritylated[11] and the product was esterified with diazomethane to give D-α-VIII in 25% over-all yield. The triple mixture melting point of these three β-lactams was undepressed, the optical rotations were the same, and the infrared spectra (potassium bromide) were identical in the position and intensity of all peaks and shoulders. Preparation of the same compound by these three routes provides convincing evidence that VIII is in the natural D-α-series. In the unlikely event that the tritylation of D-α-IV had occurred on the thiazolidine nitrogen (*vide ante*), intermolecular cyclization with carbodiimide would have produced a dimer of VIII (a substituted diketopiperazine); the molecular weight (Rast), however, is consistent with formula VIII only.

Via the totally synthetic DL-series, the β-amino acid VI was cyclized in similar yield (28%) to afford the crystalline β-lactam DL-α-VIII. The infrared spectrum (potassium bromide) of the racemic β-lactam VIII was identical with the spectra of the corresponding optically active compounds prepared by three routes. A by-product, isolated in low yield from the reaction of DL-α-VI with N,N′-diisopropylcarbodiimide, had an elementary analysis consistent with the N-acylurea derivative of VI.

Two other carbodiimides were investigated as cyclizing agents in the methyl ester series. Parallel β-lactam closures were run under the standard conditions using N-*n*-propyl-N′-*t*-butylcarbodiimide[21] and N,N′-diisopropylcarbodiimide; comparison of intensities of the β-lactam carbonyl bands in the crude lyophilized products indicated that the former had effected cyclization in about 5% yield. In a similar experiment, it was estimated that N,N′-di-*t*-butylcarbodiimide[21] cyclized D-α-VI in approximately 1% yield.

De-esterifications of the Methyl and Benzyl 6-Tritylaminopenicillanates VIII and IX.—The methyl esters of VIII were preferentially saponified to 6-tritylaminopenicillanic acid (X), which was also obtained by catalytic hydrogenolysis of IX. Compound X was also prepared by direct tritylation of 6-aminopenicillanic acid.

Selective saponification of the methyl ester in VIII in the presence of the alkali-sensitive β-lactam may appear to be a formidable task. However, it is known that esters of N-tritylamino acids are resistant to saponification,[11] presumably due to the shielding effect of the bulky trityl group. It would likewise be expected that the trityl group would deactivate the β-lactam. In any event, the methyl ester in totally synthetic DL-α-VIII was saponified and, after countercurrent distribution, X was isolated as the crystalline diethylamine salt in 17% yield. The identical diethylamine salt of DL-α-X was independently obtained by treatment of IX with hydrogen and palladium catalyst. These experiments furnish evidence that epimerization has not occurred during the basic hydrolysis of the methyl ester.

The optically active ester VIII was similarly monosaponified in the same yield to afford the crystalline diethylamine salt of D-α-X. Tritylation of 6-aminopenicillanic acid proceeded in 27% yield to afford the identical compound. The infrared spectra (potassium bromide) of racemic and optically active 6-tritylaminopenicillanic acid (X) were identical. The identity of the infrared spectra of X, produced by four reaction sequences, provides compelling evidence that all have the configuration of the natural or α-series.

Detritylation of Tritylaminopenicillanic Acid to 6-APA.—Attempted application of literature methods for removing the trityl protective group to DL-α-6-tritylaminopenicillanic acid (X) resulted in extensive destruction of the β-lactam. Following treatment of a solution of D-α-X in isopropyl alcohol with two equivalents of *N* hydrochloric acid for 22 hours at room temperature, however, there was isolated crystalline 6-aminopenicillanic acid in a conversion of 31%. Taking into account the recovered starting material, the isolated yield of D-α-6-APA was 44%. This sample of synthetic XI was shown to be identical with natural 6-amino-

(17) Reference 15, pp. 543, 578.
(18) Reference 15, p. 861.
(19) Reference 15, p. 851.
(20) Reference 15, Chapter XIII.
(21) John W. Frankenfeld, Ph.D. Thesis, M.I.T., 1961.

penicillanic acid[22] by melting point, 207–208° dec. (reported[5] 208–209° dec.), undepressed mixture melting point, optical rotation and infrared spectrum (potassium bromide). Synthetic XI was compared to natural XI, in parallel determinations involving phenylacetylation followed by microbiological assay of the penicillin G formed, and shown to contain 99 ± 10% of 6-aminopenicillanic acid. In the totally synthetic DL-series, X was also converted to DL-α-6-aminopenicillanic acid.

Synthesis of "Natural" and "New" Penicillins from 6-Aminopenicillanic Acid.—Treatment of 6-aminopenicillanic acid with phenylacetyl chloride in aqueous acetone containing sodium bicarbonate at ice-bath temperature readily gave penicillin G, isolated in 77% yield as the crystalline N-ethylpiperidine salt (XII), m.p. 153–155° dec. Identity of this sample with the corresponding salt[23] obtained directly from fermentation was established rigorously by melting point, mixture melting point, optical rotation, infrared spectrum (potassium bromide) and microbiological assay.

Penicillin V, prepared in 80% yield by phenoxyacetylation of 6-aminopenicillanic acid, was isolated as the potassium salt XIII, which showed 99% of the microbiological activity of fermentation-produced penicillin V. Comparison of physical properties and infrared spectra demonstrated that XIII obtained from 6-aminopenicillanic acid was identical with "natural" XIII and with the potassium salt of penicillin V made previously by total synthesis.[6,7] Similarly, 6-APA was converted to the "new penicillin" 6-phenylsulfonamidopenicillanic acid (XVI),[9] isolated as the N-ethylpiperidine salt.

Employing N-carboethoxyphthalimide, which has been used recently for the preparation of phthaloylamino acids,[24] XI was converted into D-α-6-phthalimidopenicillanic acid (XIV), isolated in 66% yield as the crystalline free acid. The infrared spectrum (dioxane) of XIV shows broad bands with strong maxima at 5.59, 5.65 and 5.74 μ. The 5.74 μ band is assigned to a combination of the intense phthalimido and carboxyl bands, while the other two bands are attributed to the combination of the medium intensity phthalimido and the strong β-lactam bands. Compound XIV was inactive when tested by routine microbiological assay.

Diazomethane converted XIV into methyl D-α-6-phthalimidopenicillanate (XV). It is interesting to note that synthesis of XV, by cyclization of 4 - carbomethoxy - 5,5 - dimethyl - α-phthalimido-2-thiazolidineacetic acid, failed in the DL-α-series but was accomplished in the diastereomeric β-series to give a 36% yield of DL-β-XV.[25] The infrared spectra (chloroform) of D-α- and DL-β-XV were identical in the carbonyl region but were distinctly different in the region between 7 and 15 μ.

(22) A sample of natural 6-aminopenicillanic acid was kindly furnished by Bristol Laboratories, Syracuse, N. Y.

(23) J. C. Sheehan, W. J. Mader and D. J. Cram, *J. Am. Chem. Soc.*, **68**, 2407 (1946).

(24) G. H. L. Nefkens, *Nature*, **185**, 309 (1960).

(25) J. C. Sheehan and P. A. Cruickshank, *J. Am. Chem. Soc.*, **78**, 3680 (1956).

It is obvious that similar acylations of DL-α-6-aminopenicillanic acid would produce the corresponding DL-penicillins, and that resolution of these racemic penicillins would give the L-enantiomers; the one presently reported L-penicillin, however, has little or no biological activity.[7]

We are indebted to Bristol Laboratories of Syracuse, N. Y., for financial support and for bioassays, and to Mrs. Aviva R. Tepley and Mr. Edward J. Hessler for excellent technical assistance.

Experimental[26]

***t*-Butyl D-α-4-Carbomethoxy-5,5-dimethyl-α-amino-2-thiazolidineacetate Hydrochloride** (I).—A solution of 12.51 g. (0.0288 mole) of *t*-butyl D-α-4-carbomethoxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetate[7] and 1.80 g. (0.036 mole) of hydrazine hydrate in 190 ml. of pure dioxane was stored at room temperature for 20 hours, after which solvent and excess hydrazine were removed by lyophilization. The phthalhydrazide complex was decomposed by shaking with 180 ml. of 0.2 *N* hydrochloric acid at room temperature for 2 hours. After cooling in an ice-bath for an additional hour, 5.3 g. (114%) of crude phthalhydrazide was removed by filtration, and the filtrate was lyophilized. Crystallization from methanol–ether afforded 3.79 g. of needles, m.p. 178.5–180° dec.; further addition of ether gave a second crop of 4.19 g., m.p. 172–174° dec. The total yield of I was 7.98 g. (81%). Two recrystallizations gave an analytical sample, m.p. 183–184° dec., $[\alpha]^{31}D$ +91° (*c* 1.2 in methanol).

Anal. Calcd. for $C_{13}H_{25}N_2O_4SCl$: C, 45.79; H, 7.39; N, 8.22. Found: C, 45.65; H, 7.61; N, 8.31.

D- and DL-α-4-Carbomethoxy-5,5-dimethyl-α-amino-2-thiazolidineacetic Acid Dihydrochloride (IV).—Anhydrous hydrogen chloride was passed through a suspension of 0.50 g. of D-α-I in 20 ml. of dry nitromethane at 0°; most of the solid went very rapidly into solution. A small amount of insoluble solid was removed by centrifugation and discarded. After a further 10 minutes at 0°, 0.364 g. (78%) of crystalline D-α-IV had separated, m.p. 90–93° dec. (in bath 75°), $[\alpha]^{29}D$ +82° (*c* 0.5 in 6 *N* hydrochloric acid).

Anal. Calcd. for $C_9H_{18}N_2O_4SCl_2$: C, 33.66; H, 5.65; N, 8.73. Found: C, 33.72; H, 5.88; N, 8.93.

A rapid stream of anhydrous hydrogen chloride was passed through a suspension of 10 g. of DL-α-I[10] in 100 ml. of dry nitromethane at 0° over a period of 25 minutes. Some insoluble solid (0.543 g.) was removed by filtration and discarded. The filtrate was resaturated with hydrogen chloride and stored at 0° for 75 minutes. The excess hydrogen chloride was removed from the cloudy solution with a rotating concentrator (15 minutes) and the precipitated solid was collected by filtration, washed twice with ether and vacuum dried; yield 8.253 g., m.p. 110–114° dec. Analyses for carbon, hydrogen, nitrogen and chlorine indicated that the product was a mixture of the mono- and dihydrochlorides of DL-α-IV.

DL-α-4-Carbobenzyloxy-5,5-dimethyl-α-amino-2-thiazolidineacetic Acid Monohydrochloride (V).—Anhydrous hydrogen chloride was passed through a suspension of 0.209 g. (0.0005 mole) of DL-α-II[8] in 7 ml. of dry nitromethane at 0°; most of the solid went into solution within 2 minutes. The insoluble portion was removed by centrifugation. After storage for 27 hours at 0–5°, the crystalline product was collected by filtration, washed twice with nitromethane and three times with ether. This product, which amounted to 0.159 g. (88%), m.p. 109–112° dec., was analytically pure.

Anal. Calcd. for $C_{15}H_{21}N_2O_4SCl$: C, 49.92; H, 5.86; N, 7.76; Cl, 9.83. Found: C, 49.45; H, 6.07; N, 7.87; Cl, 9.40.

D- and DL-α-4-Carbomethoxy-5,5-dimethyl-α-tritylamino-2-thiazolidineacetic Acid (VI). A. Total Syntheses.—To a cold solution of 2.56 g. (7.98 mmoles) of DL-α-IV in 22.5 ml. of water and 45 ml. of reagent isopropyl alcohol was added 4.12 ml. (2.92 g., 40 mmoles) of diethylamine. This solution was stirred vigorously, at room temperature, with a

(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 spectra.

magnetic stirrer during the addition of 2.9 g. (10.4 mmoles) of trityl chloride in fifteen portions over 30 minutes. After a further 15 minutes stirring, 187 ml. of water was added and the precipitate was removed by filtration (Celite) and discarded. To the cooled (0–5°) filtrate was added, gradually with swirling, 12 ml. of 2 *N* acetic acid; the resulting colorless solid was collected by filtration, washed with water and dried. The yield of DL-α-VI of sufficient purity for conversion to the lactam (*viz.*, DL-α-VIII), was 2.276 g. (58%).

A portion of this product (0.984 g., 2 mmoles) was purified further by dissolving in a 100-ml. portion of cold ether and washing with 200 ml. of cold 0.15 *M* phosphate buffer (*p*H 5.5) followed by 200 ml. of cold water. The product was then extracted into three 400-ml. portions of cold 1% potassium bicarbonate solution. The combined aqueous layer was washed with 50 ml. of ether (which was discarded), covered with 500 ml. of cold ether, and acidified to *p*H 6 with 10% phosphoric acid (50 ml.). After the aqueous layer was extracted with a further 250-ml. portion of cold ether, the combined ethereal layers were washed with 50 ml. of water, dried over magnesium sulfate and concentrated to dryness. The colorless foam was taken up in 5 ml. of benzene whereupon it crystallized spontaneously yielding 0.432 g. (26%), m.p. 103–110°. Two recrystallizations from ether–petroleum ether and another recrystallization from ether afforded an analytical sample of DL-α-VI, m.p. 150–154° dec.

Anal. Calcd. for $C_{28}H_{30}N_2O_4S$: C, 68.55; H, 6.16; N, 5.71. Found: C, 68.45; H, 6.17; N, 5.63.

Similar tritylation of 1.233 g. (3.84 mmoles) of D-α-IV (from D-α-I) gave 0.79 g. (42%) of solid D-α-VI, which was used without further purification in the next synthetic step (*viz.*, conversion to D-α-VIII).

B. Partial Synthesis of D-α-VI (from Penicillin G).—The starting material for this partial synthesis was methyl D-α-4-carbomethoxy-5,5-dimethyl-α-amino-2-thiazolidine hydrochloride (III) prepared from penicillin G by the method of Sheehan and Ferris.[13,27] To a solution of 6 g. (20 mmoles) of III in 10 ml. of water was added with stirring 10 ml. of 2 *N* (20 mmoles) sodium hydroxide in one portion followed by the dropwise addition of an additional 10 ml. of 2 *N* sodium hydroxide over 10 minutes; this solution was left at room temperature for a further 20 minutes (*p*H about 9) and 20 ml. of *N* hydrochloric acid (20 mmoles) was then added (*p*H about 4). The monocarboxylic acid, D-α-IV, was immediately tritylated without prior isolation.

To the above solution was added 100 ml. of reagent isopropyl alcohol and then 6.2 ml. (4.38 g., 60 mmoles) of diethylamine. Trityl chloride (5.58 g., 20 mmoles) was added in about 15 portions over a 30-minute period with vigorous stirring (magnetic stirrer). After an additional 15 minutes of stirring at room temperature, 360 ml. of water was added. The precipitate was discarded. The filtrate was cooled to 2° and 2 *N* acetic acid was added until the *p*H was 6.0 (22 ml. was required). The resulting colorless solid weighed 2.02 g. (21%).

Most (1.92 g.) of this product was purified by distribution between ethereal and aqueous phases as described in the total synthesis of DL-α-VI. The colorless lyophilized solid (1.28 g.) was analytically pure, $[\alpha]^{31}D$ +45° (*c* 1.3 in *n*-butyl acetate).

Anal. Calcd. for $C_{28}H_{30}N_2O_4S$: C, 68.55; H, 6.16; N, 5.71. Found: C, 68.74; H, 6.28; N, 5.55.

DL-α-4-Carbobenzyloxy-5,5-dimethyl-α-tritylamino-2-thiazolidineacetic Acid (VII).—To a cold well-stirred solution of 3.91 g. (9.85 mmoles) of DL-α-V in 24 ml. of water and 52 ml. of reagent tetrahydrofuran was added 3.6 ml. (2.55 g., 35 mmoles) of diethylamine. Trityl chloride (3.85 g., 13.8 mmoles) was added portionwise to this vigorously-stirred solution at room temperature over a 35-minute period; the reaction mixture was stirred for an additional 1-hour period then placed at 5° for 12 hours. The tetrahydrofuran was removed *in vacuo* at room temperature and the suspension shaken with 300 ml. of ether and 200 ml. of 2% potassium bicarbonate; the aqueous layer was discarded and the crystalline portion was collected (257 mg., m.p. 164–166° dec.; analyses of the recrystallized material indicated that it was the hydrate of the free base of DL-α-V. *Anal.* Calcd. for $C_{15}H_{22}N_2SO_5$: C, 52.62; H, 6.48; N, 8.18. Found: C, 53.47; H, 6.54; N, 8.11). The organic layer was concentrated to dryness at room temperature and the residue (5.44 g.) taken up in 200 ml. of ether and purified by a two-funnel distribution between ether and 2% sodium carbonate. The second funnel contained 100 ml. of ether and the two funnels were extracted successively with three 800-ml. portions of sodium carbonate. The combined aqueous layers were cooled in an ice-bath, covered with 600 ml. of cold ether and acidified with 400 ml. of 10% phosphoric acid (*p*H about 6). The aqueous layer was extracted with an additional 400 ml. of ether and the combined ether layers were dried over magnesium sulfate and concentrated to dryness at room temperature. The residue was lyophilized from 50 ml. of benzene to yield 1.37 g. (25%) of analytically pure DL-α-VII as a white solid.

Anal. Calcd. for $C_{34}H_{34}N_2O_4S$: C, 72.06; H, 6.05; N, 4.94. Found: C, 71.99; H, 6.19; N, 4.78.

Methyl D- and DL-α-6-Tritylaminopenicillanate (VIII), A. Total Synthesis of DL-α-VIII.—To a solution of 2.778 g. (5.67 mmoles) of DL-α-VI in 78 ml. of reagent dioxane was added 49 ml. of water. To this solution, which was stirred in an ice-bath, was added a solution of 1.15 ml. (0.91 g., 7.2 mmoles) of N,N′-diisopropylcarbodiimide in 49 ml. of dioxane in one portion. After an additional 35 minutes in an ice-bath and 2 hours at room temperature the pale yellow solution was lyophilized. The residue was taken up in 30 ml. of benzene (0.298 g. of an insoluble gummy solid was discarded) and chromatographed over 84 g. of Brockman activity III ethyl acetate-neutralized alumina, with benzene as the eluent. Residues from the first five 30-ml. fractions of eluate were crystallized from ether–petroleum ether to afford 0.745 g. (28%) of colorless prisms, m.p. 135–138°. A sample of this DL-α-VIII recrystallized twice from ethyl acetate–petroleum ether had m.p. 152–153°. The infrared spectrum (potassium bromide) of DL-α-VIII was identical with the spectra of D-α-VIII prepared *via* three sequences as described below.

Anal. Calcd. for $C_{28}H_{28}N_2O_3S$: C, 71.17; H, 5.97; N, 5.93. Found: C, 71.28; H, 6.06; N, 5.89.

The residue from the sixth 30-ml. chromatography fraction was an oil (0.12 g.) which crystallized slowly from ether–petroleum ether to give 0.018 g. of colorless prisms, DL-α-XVII, m.p. 136–138°; the mixed m.p. with authentic DL-α-VIII was 117–125°, and the infrared spectrum showed no absorption at 5.6 μ. Elution with an additional 150 ml. of benzene afforded, in a similar manner, an additional 0.162 g. of XVII. A sample recrystallized three times from ether–petroleum ether had m.p. 135–136.3° and an analysis consistent with an N-acylurea derivative of VI.

Anal. Calcd. for $C_{35}H_{44}N_4O_4S$: C, 68.16; H, 7.19; N, 9.09. Found: C, 68.47; H, 7.12; N, 9.47.

B. Total Synthesis of D-α-VIII.—Water (14 ml.) was added to a solution of 790 mg. (1.6 mmoles) of crude D-α-VI (from D-α-I *via* D-α-IV) in 22 ml. of reagent dioxane and, after cooling in an ice-bath, a solution of 0.32 ml. (253 mg., 2 mmoles) of N,N′-diisopropylcarbodiimide in 14 ml. of dioxane was added in one portion with stirring continued in an ice-bath for 35 minutes, and then for an additional 2 hours at room temperature. The pale yellow solid from the lyophilization was dissolved in 10 ml. of benzene and chromatographed over 24 g. of Brockman activity III ethyl acetate-neutralized alumina, with benzene as the eluent. Residue from the first 45 ml. of eluate was crystallized from ether to yield 219 mg. (27%) of crystals, m.p. 163–165°. Recrystallizations from ethyl acetate–petroleum ether followed by acetone–water gave an analytical sample of D-α-VIII, m.p. 166–167°, $[\alpha]^{26}D$ + 101° (*c* 1 in *n*-butyl acetate). A triple mixture melting point of this sample of totally synthetic D-α-VIII with the compounds (*vide infra*) prepared from penicillin G and from 6-aminopenicillanic acid was undepressed and the infrared spectra of all three samples of D-α-VIII were identical in the positions and intensity of all peaks and shoulders.

Anal. Calcd. for $C_{28}H_{28}N_2O_3S$: C, 71.17; H, 5.97; N, 5.93. Found: C, 71.01; H, 5.89; N, 5.91.

C. Partial Synthesis of D-α-VIII (from Penicillin G).—In a similar manner 1.47 g. (3 mmoles) of D-α-VI (obtained from penicillin G) was cyclized to yield 0.297 g. (22%) of D-α-VIII, m.p. 163.3–164.8°. One recrystallization afforded an analytical sample, m.p. 166–167°, $[\alpha]^{31}D$ +96° (*c* 1 in *n*-butyl acetate).

(27) We are indebted to Donald N. McGregor for the preparation of a substantial quantity of this intermediate.

Anal. Calcd. for $C_{28}H_{28}N_2O_3S$: C, 71.17; H, 5.97; N, 5.93; mol. wt., 472. Found: C, 70.96; H, 5.84; N, 6.11; mol. wt., 440 (Rast).

D. Partial Synthesis of D-α-VIII from 6-Aminopenicillanic Acid.—To a suspension of 3.24 g. (15 mmoles) of 6-aminopenicillanic acid[22] in 6 ml. of water and 12 ml. of reagent isopropyl alcohol in an ice-bath was added 4.63 ml. (3.29 g., 45 mmoles) of diethylamine over a period of 2 minutes and the solution was stirred for an additional 5 minutes. The ice-bath was removed and 5.44 g. (19.5 mmoles) of trityl chloride was added in 12 portions over a period of 35 minutes with vigorous stirring. Stirring was continued an additional hour at room temperature. After dilution with 37 ml. of water and cooling in an ice-bath the precipitate was discarded. The clear solution was cooled to 2° and 12 ml. of *N* acetic acid was added (*p*H 6). The supernatant was decanted, and the taffy-like semi-solid was triturated four times with water and dried to give 3.42 g. of crude D-α-X.

A portion (2.87 g.) of this crude acid was dissolved in 42 ml. of methylene chloride and the solution was treated with excess diazomethane. The crude ester was dissolved in 20 ml. of benzene and chromatographed over 80 g. of Brockman activity VI ethyl acetate-neutralized alumina, with benzene as the eluent. The residue from the second 50 ml. of eluate, crystallized from ether–petroleum ether and recrystallized from ethyl acetate–petroleum ether, afforded 1.45 g. (25%) of colorless needles, m.p. 165–166.5°. An analytical sample recrystallized from acetone–water melted at 166–167°, $[\alpha]^{28}D$ +100° (*c* 1 in *n*-butyl acetate).

Anal. Calcd. for $C_{28}H_{28}N_2O_3S$: C, 71.17; H, 5.97; N, 5.93. Found: C, 71.29; H, 6.07; N, 5.73.

Benzyl D- and DL-α-6-Tritylaminopenicillanate (IX). A. Total Synthesis.—To a cold solution of 1.303 g. (2.3 mmoles) of DL-α-VII in 32 ml. of dioxane and 20 ml. of water was added a solution of 0.585 ml. (0.462 g., 3.66 mmoles) of N,N′-diisopropylcarbodiimide in 20 ml. of dioxane in one portion. The solution was stirred an additional 40 minutes in an ice-bath then for 2 hours at room temperature. The lyophilized solid was dissolved in 25 ml. of benzene and chromatographed over 39 g. of Brockman activity III ethyl acetate-neutralized alumina, with benzene as the eluent. Residue from the first 100 ml. of eluate was crystallized from benzene–ether to afford 0.849 g. (67%) of colorless crystals, m.p. 196–197° dec. Three recrystallizations from benzene–ether gave an analytical sample, m.p. 200–201° dec. The infrared spectrum (potassium bromide) was identical with the spectrum of D-α-IX; λ_{max}^{KBr} 5.68 μ was ascribed to the β-lactam group, and λ_{max}^{KBr} 5.73 μ to the ester carbonyl.

Anal. Calcd. for $C_{34}H_{32}N_2O_3S$: C, 74.51; H, 5.88; N, 5.10. Found: C, 74.40; H, 5.85; N, 5.14.

B. Partial Synthesis (from 6-Aminopenicillanic Acid).—The crystalline diethylamine salt of D-α-X, prepared (*vide infra*) from 6-aminopenicillanic acid, was employed in this synthesis; 3.004 g. (5.65 mmoles) was dissolved in 180 ml. of reagent benzene and shaken well with two 120-ml. portions of 1% phosphoric acid followed by three 60-ml. portions of water. The benzene layer was dried by filtration through paper and then lyophilized. Relyophilization from 12 ml. of benzene afforded 2.619 g. (101%) of the free acid D-α-X.

A portion (0.999 g., 2.16 mmoles) of this acid was dissolved in 14 ml. of methylene chloride, cooled in an ice-bath and 7 ml. of an ether solution, containing 0.315 g. (2.67 mmoles) of phenyldiazomethane, was added in portions over a period of 3 minutes. The solution was left at 0° for 35 minutes and at room temperature for 30 minutes and the excess phenyldiazomethane was decomposed with glacial acetic acid. The solution was diluted to 40 ml. with methylene chloride, extracted twice with 5% potassium bicarbonate and twice with water, dried over magnesium sulfate and concentrated to 10 ml., then diluted with ether. After storage at 5°, 0.921 g. (78%) of crystals was collected by filtration, m.p. 206–209° dec. Two recrystallizations from methylene chloride–ether gave an analytical sample (dried 65 hours at 60° at 0.1 mm.), m.p. 210–211.5° dec.

Anal. Calcd. for $C_{34}H_{32}N_2O_3S$: C, 74.51; H, 5.88; N, 5.10. Found: C, 74.40; H, 6.03; N, 5.08.

D- and DL-α-6-Tritylaminopenicillanic Acid (X). A. Total Synthesis of DL-α-X *via* Benzyl Ester.—A suspension of 2 g. of palladous oxide[28] in a solution of 1.25 ml. of acetic acid and 3 ml. of ethylene glycol in 24 ml. of purified dioxane was prereduced at room temperature and atmospheric pressure. Benzyl ester (DL-α-IX, 0.501 g.) was added and hydrogenation was continued. The theoretical quantity of hydrogen had been adsorbed at the end of 55 minutes and no further reduction took place after an additional 10 minutes. The filtered solution was concentrated under reduced pressure to a small volume and distributed between 25 ml. of benzene and 25 ml. of water. The aqueous layer was extracted with an additional 25 ml. of benzene, and the combined benzene layers were extracted with another 50 ml. of water. The benzene solution was concentrated to dryness (reduced pressure) and lyophilized from 4 ml. of fresh benzene. The lyophilized solid was soluble in 25 ml. of ether except for a small amount of solid which was discarded. Addition of 0.16 ml. (0.114 mg., 1.56 mmoles) of diethylamine and storage at 5° for 16 hours afforded 0.241 g. (58%) of an analytical sample of the diethylamine salt of DL-α-X, m.p. 166.5–167.5° dec., λ_{max}^{KBr} 5.66 μ attributable to the β-lactam carbonyl group.

Anal. Calcd. for $C_{31}H_{37}N_3O_3S$: C, 70.03; H, 7.02; N, 7.90. Found: C, 69.85; H, 7.05; N, 7.91.

B. Total Synthesis of DL-α-X *via* Methyl Ester.—To a solution of 1.122 g. (2.38 mmoles) of DL-α-VIII in 48 ml. of reagent pyridine was added in one portion with stirring in an ice-bath, a cold solution of 4.77 ml. of 0.5 *N* (2.38 mmoles) sodium hydroxide in 43 ml. of water. After stirring, with ice-bath cooling, for an additional 90 minutes, 250 ml. of cold ether was added and the stirred mixture acidified (*p*H 5.5) with cold 10% phosphoric acid (29 ml.). The aqueous layer was extracted with an additional 250 ml. of cold ether and the combined ether solutions were washed with 250 ml. of cold 0.15 *M* phosphate buffer (*p*H 5.5) followed by 250 ml. of cold water. The product was extracted from the ethereal layer with three cold 250-ml. portions of 1% potassium bicarbonate and each portion of potassium bicarbonate was back extracted in turn with a second 500-ml. portion of cold ether. The combined bicarbonate layers were cooled in an ice-bath, covered with a 500-ml. portion of ether and acidified to about *p*H 5.5 with 10% phosphoric acid (55 ml.). After the addition of 95 ml. of 0.15 *M* phosphate buffer (*p*H 5.5), the layers were separated and the aqueous layer was extracted with an additional 155-ml. portion of ether. The combined organic phase was washed with a 140-ml. portion of cold water, dried over magnesium sulfate, and concentrated by aid of a water aspirator to an oil which was lyophilized from 10 ml. of benzene. To the light yellow residue (0.261 g., 24% crude yield) from the lyophilization, dissolved in 4 ml. of ether, was added 0.12 ml. (0.085 g., 1.16 mmoles) of diethylamine. Storage for 22 hours at 5° afforded 0.215 g. (17%) of the crystalline diethylamine salt of DL-α-X, m.p. 165–166° dec. This salt was further purified by conversion to the free acid (same procedure as used for the formation of D-α-X from the diethylamine salt) and reformation of the crystalline diethylamine salt which melted at 166–167° dec., λ_{max}^{KBr} 5.66 μ. The melting point on admixture with a sample of DL-α-X, prepared by hydrogenolysis of the benzyl ester DL-α-IX, was undepressed; the infrared spectra (potassium bromide) of these compounds were identical with the spectrum of the corresponding optically active compound D-α-X.

C. Conversion of D-α-VIII into D-α-X.—A sample of 1.172 g. (2.48 mmoles) of D-α-VIII was saponified selectively with one equivalent of sodium hydroxide, as described previously for the DL-series, to yield 0.216 g. (16.4%) of the crystalline diethylamine salt of D-α-X, m.p. 162–166° dec. Two recrystallizations from dioxane–ether afforded an analytical sample, m.p. 166–168° dec., $[\alpha]^{29}D$ +89° (*c* 1 in dioxane), λ_{max}^{KBr} 5.66(vs) μ.

Anal. Calcd. for $C_{31}H_{37}N_3O_3S$: C, 70.03; H, 7.02; N, 7.90. Found: C, 69.71; H, 7.00; N, 7.90.

This salt (on an equimolar basis) gave 58% of the color yield obtained from the sodium salt of penicillin V in the quantitative hydroxylamine assay for penicillins[29]; the low value may be ascribed to the incomplete solubility of the diethylamine salt in the aqueous medium, or to lowered β-lactam reactivity due to the bulky trityl group.

The melting point on admixture with a sample of D-α-X, prepared from 6-APA as described below, was not depressed; the infrared spectra (potassium bromide) were identical.

(28) D. Starr and R. M. Hixon, "Organic Syntheses," Coll. Vol. II John Wiley and Sons, Inc., New York, N. Y., 1943, p. 566.

(29) J. H. Ford, *Anal. Chem.*, **19**, 1004 (1947).

D. **Synthesis of D-α-X From 6-Aminopenicillanic Acid.**—The procedure for the preparation of crude D-α-X (described previously in the section on the partial synthesis of D-α-VIII from 6-aminopenicillanic acid) was improved so that the crude product was a solid rather than a semi-solid; in the tritylation of 3.24 g. of 6-APA, the mixture obtained after the addition of the trityl chloride was stirred an additional 30 minutes (instead of an hour) to yield 3.033 g. of solid D-α-X. Part of this crude product (2.49 g.) was distributed between 400 ml. of ether and 400 ml. of 0.15 *M* phosphate buffer (*p*H 5.5), and the organic layer extracted with a further 400 ml. of phosphate buffer followed by 50 ml. of water; the aqueous phases were discarded. A second funnel containing 400 ml. of fresh ether was added and the two funnels extracted successively in countercurrent fashion with three 400-ml. portions of 1% potassium bicarbonate. The combined bicarbonate layers were covered with 300 ml. of ether, cooled in an ice-bath, and acidified with 10% phosphoric acid to *p*H 6.0–6.2; the aqueous layer was extracted with an additional 50-ml. portion of ether. The combined ethereal layers were washed with a 50-ml. portion of water, dried over magnesium sulfate, concentrated under reduced pressure to a foam and lyophilized from 45 ml. of benzene to yield 1.62 g. of residue; the quantitative hydroxylamine test[29] indicated this solid was 94 ± 5% pure. A portion (0.89 g.) of this product was dissolved in 20 ml. of ether and 0.145 g. (2 mmoles) of diethylamine added; spontaneous crystallization afforded 0.955 g. (27%) of the diethylamine salt of D-α-X, m.p. 164–166° dec. Two recrystallizations from dioxane–ether afforded an analytical sample, m.p. 165–167° dec., $[\alpha]^{29}D$ +90° (*c* 1 in dioxane), λ_{max}^{KBr} 5.66(vs) μ. This compound was essentially inactive when tested by routine microbiological assay.

Anal. Calcd. for $C_{31}H_{37}N_3O_3S$: C, 70.03; H, 7.02; N, 7.90. Found: C, 69.93; H, 6.96; N, 7.90.

D- and DL-α-6-Aminopenicillanic Acid (XI). A. D-Series.—The crystalline diethylamine salt of D-α-X (1.062 g., 2.00 mmoles) was converted into the free acid (using the method described previously in the section on the partial synthesis of D-α-IX from 6-aminopenicillanic acid). All of this acid (0.974 g., 100% yield) was dissolved in 16 ml. of reagent isopropyl alcohol, 4.00 ml. (4.00 mmoles) of *N* hydrochloric acid was added and the solution was stored at room temperature. After 4 minutes a 0.25-ml. aliquot (1.25% of total weight) was removed and distributed between 2 ml. of water and 8 ml. of benzene; the quantitative hydroxylamine assay[29] of the aqueous layer (containing all the 6-APA) indicated 0% lactam, and similar assay of the residue from the benzene layer (containing all the trityl-6-APA) showed 103% lactam. Another aliquot removed after 22.5 hours analyzed for 51% 6-APA and 27% trityl-6-APA; the yield (not isolated) of 6-APA, based on the trityl-6-APA consumed, was therefore 70%. The remaining 19.5 ml. of solution was immediately partitioned between 400 ml. of benzene and 100 ml. of water and the aqueous phase extracted with a 20-ml. portion of *n*-butyl alcohol, which removed most of the color. The filtered aqueous layer was titrated to *p*H 3.65 with 20 ml. (4 mmoles) of 0.2 *N* lithium hydroxide. The aqueous layer, after extraction with a further 20-ml. portion of *n*-butyl alcohol, was concentrated at 0.2 mm. pressure and room temperature to 12 ml. whereupon crystals began to come out of solution. Storage at 5° overnight afforded 0.068 g. of crystalline product, m.p. 200–203° dec. Concentration to a smaller volume and storage at 5° gave additional crystals melting at 197–200° dec. The total yield of D-α-XI was 0.122 g. (29%). Recrystallization was effected by dissolving in an excess of *N* hydrochloric acid, diluting with water, treating with carbon and Celite and adding an equivalent of *N* lithium hydroxide; the analytical sample was dried 5 days at 0.01 mm., m.p. 207–208° dec. (natural,[5] 208–209° dec.), $[\alpha]^{31}D$ +273° (*c* 1.2 in 0.1 *N* hydrochloric acid).

Anal. Calcd. for $C_8H_{12}N_2O_3S$: C, 44.44; H, 5.60; N, 12.96. Found: C, 44.28; H, 5.63; N, 12.80.

The mixture melting point with natural 6-APA[22] was undepressed and the infrared spectra (potassium bromide) were identical. Synthetic XI was compared to natural XI, in parallel determinations involving phenylacetylation followed by microbiological assay of the penicillin G; the samples assayed for 920 μg./mg. and 925 μg./mg., respectively, indicating that the synthetic 6-APA was 99 ± 10% pure.

In a similar detritylation carried out on 2.087 g. (4.56 mmoles) of D-α-X for 22 hours at room temperature there was isolated 0.308 g. (31.3% conversion) of crystalline 6-APA as well as 0.714 g. (1.34 mmoles) of starting material as the crystalline diethylamine salt. The yield isolated of 6-APA, based on unrecovered D-α-X, was therefore 44%.

B. **DL-Series.**—Detritylation of DL-α-X (0.458 g., 0.1 mmole) was carried out for 20.5 hours as described for the D-series. After titration with lithium hydroxide and washing with *n*-butyl alcohol, the aqueous solution was concentrated to dryness to yield 218 mg. of pale yellow crystals. The hydroxylamine test[29] indicated these crystals contained 24% of 6-APA and hence DL-6-APA had been formed in 24% yield. Descending paper chromatography of this product using the *n*-butyl alcohol–acetic acid–water (4:1:5) system, spraying with ninhydrin solution and allowing the color to develop at room temperature gave one spot with a characteristic brown color at R_f 0.46; natural 6-APA, run simultaneously, gave a brown spot at R_f 0.46.

In a similar detritylation of 70 mg. (0.15 mmole) of DL-α-X, the reaction mixture was treated as described above but the neutralized solution was concentrated to 2 ml. and lyophilized to afford 26 mg. of a heterogeneous pale yellow solid. Paper chromatography (descending) of this product in *n*-butyl alcohol–ethanol–water (4:5:1) with a ninhydrin spray produced a brown spot at R_f 0.20; natural 6-APA run on the same paper gave a brown spot at R_f 0.20. Paper chromatography (descending) in phenol–water (4:1), in the presence of hydrogen cyanide vapor, and a ninhydrin spray resulted in three spots (decomposition products): brown at R_f 0.26, gray at R_f 0.9, and brown at R_f 0.96; natural 6-APA, chromatographed on the same sheet, also gave three spots: brown at R_f 0.26, gray at R_f 0.9 and brown at R_f 0.96. A sample of the crude lyophilized product was submitted to activation as penicillin G for bioassay, which indicated a content of 11% of D-6-APA, corresponding to 22% of DL-6-APA.

N-Ethylpiperidine Salt of Penicillin G (XII).—To a solution of 216 mg. (1 mmole) of D-α-XI in 7 ml. (2.8 mmoles) of 4% potassium bicarbonate and 6 ml. of acetone was added dropwise, with stirring and ice-bath cooling, a solution of 0.175 ml. (204 mg., 1.32 mmoles) of phenylacetyl chloride in 3 ml. of acetone over a period of 10 minutes. After an additional 30 minutes stirring the acetone was removed under reduced pressure and the aqueous phase extracted with 15 ml. of ether. The aqueous phase was layered with 10 ml. of fresh ether, cooled in an ice-bath and acidified with 10% phosphoric acid to *p*H 2.5; the aqueous phase was extracted with an additional 10-ml. portion of cold ether. The combined ethereal layers were washed with a 5-ml. portion of cold water and dried rapidly with magnesium sulfate. To the filtered solution was added a solution of 0.137 ml. (113 mg., 1 mmole) of N-ethylpiperidine in 5 ml. of ether. A colorless solid precipitated immediately and, after the addition of a 12-ml. portion of acetone, the mixture was stored overnight at 5°. The crystalline product was analytically pure, 345 mg. (77%), m.p. 152.5–154.5° dec. (in bath 140° and heated 3° per minute; reported[23] 152–154°), $[\alpha]^{28}D$ + 244° (*c* 1 in water) [reported[23] +240° (*c* 1 in water)]. The mixture melting point with an authentic sample of XII[23] was undepressed and the infrared spectra (potassium bromide) were identical. This salt showed 98 ± 10% of the theoretical bioactivity expected for the salt of natural penicillin G.

Anal. Calcd. for $C_{23}H_{33}N_3O_4S$: C, 61.71; H, 7.43; N, 9.39. Found: C, 61.44; H, 7.36; N, 9.46.

Potassium Salt of Penicillin V (XIII).—To a solution of 216 mg. (1 mmole) of D-α-XI in 7 ml. (2.8 mmoles) of 4% potassium bicarbonate and 6 ml. of acetone was added dropwise, with stirring and ice-bath cooling, a solution of 0.2 ml. (250 mg., 1.37 mmoles) of phenoxyacetyl chloride in 3 ml. of acetone over a period of 10 minutes. After stirring at room temperature for an additional 30 minutes, a 35-ml. portion of benzene was added and the organic phase discarded; the aqueous phase was further extracted with a 15-ml. portion of ether which was discarded. The aqueous phase was layered with 15 ml. of ether, cooled in an ice-bath and acidified with 10% phosphoric acid to about *p*H 2.5. The aqueous layer was quickly extracted with two additional cold 5-ml. portions of ether and the combined organic layer washed with a 5-ml. portion of cold water. Cold water (20 ml.) was added and using a *p*H meter the mixture was titrated in an ice-bath with *N* potassium hydroxide (11.7 ml.) to *p*H

7.2. The aqueous phase was concentrated under reduced pressure at room temperature to about 8 ml. and lyophilized. The residue from the lyophilization was taken up in a small volume of water, treated with carbon and Celite, and crystallized by the addition of acetone to yield 308 mg. (80%) of product melting at 252–254° dec. Two recrystallizations from acetone–water afforded pure XIII, m.p. 264–265° dec., not depressed upon admixture with the potassium salt of natural penicillin V, $[\alpha]^{28}D$ +222° (*c* 1 in water). The infrared spectra of natural and synthetic XIII were identical (in 40 peaks and shoulders in potassium bromide) and also identical with the spectrum of totally synthetic XIII made previously by another reaction sequence.[7] In microbiological assay, synthetic XIII showed 99 ± 10% of the bioactivity of natural penicillin V.

D-α-**6-Phthalimidopenicillanic Acid (XIV).**—To a vigorously stirred solution (*p*H 8) of 2.16 g. (10 mmoles) of D-α-XI and 1.06 g. (10 mmoles) of sodium carbonate in 15 ml. of water was added 2.19 g. (10 mmoles) of finely ground N-carboethoxyphthalimide[24] in one portion. At the end of 1.25 hours of vigorous stirring, when essentially all the solid had gone into solution, the mixture was extracted with methylene chloride. A fresh 50-ml. portion of methylene chloride was added to the aqueous phase which was acidified gradually with a total of 20 ml. (20 mmoles) of *N* hydrochloric acid, and the extraction was completed with two additional 25-ml. portions of methylene chloride. The combined organic layers were washed with two 25-ml. portions of water, dried over magnesium sulfate, and decolorized with carbon and Celite. Concentration of the methylene chloride solution with a nitrogen stream afforded 2.29 g. (66%) of colorless crystals, m.p. 145–150° dec. Two recrystallizations from acetone (at or below room temperature) gave an analytical sample of XIV, m.p. 167–170° dec., $[\alpha]^{28}D$ +278° (*c* 1 in *n*-butyl acetate). The infrared spectrum in dioxane solution (5%) had strong carbonyl maxima at 5.59, 5.65 and 5.74 μ. Compound XIV was inactive when tested by routine microbiological assay.

Anal. Calcd. for $C_{16}H_{14}N_2O_5S$: C, 55.49; H, 4.08; N, 8.09. Found: C, 55.41; H, 4.03; N, 8.05.

Methyl D-α-**6-Phthalimidopenicillanate (XV).**—The acid XIV (199 mg., 0.575 mmole) was dissolved in 2.5 ml. of dioxane and the solution treated with an excess of ethereal diazomethane. The ester was crystallized from acetone, giving 121 mg. (59%) of colorless product, m.p. 177–177.5°. An analytical sample recrystallized from the same solvent melted at 177.5–178°, $[\alpha]^{28}D$ +288° (*c* 1.1 in *n*-butyl acetate). The infrared spectrum (chloroform) of XV exhibits a broad intense band at 5.55-5.62 μ due to a combination of β-lactam absorption plus the weak phthalimide band.

Anal. Calcd. for $C_{17}H_{16}N_2O_5S$: C, 56.65; H, 4.48; N, 7.78. Found: C, 56.79; H, 4.73; N, 7.78.

Compound XV was compared with the racemic diastereomer, *viz.*, methyl DL-β-6-phthalimidopenicillanate (reported[25] m.p. 170–172°): the admixture melting point was 145–166°; the infrared spectra (chloroform) of the two compounds were identical in the carbonyl region (5–6 μ), but were distinctly different in the region between 7 and 15 μ.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY, STANFORD, CALIF.]

Alkaloid Studies. XXXVI.[1] The Complete Absolute Configuration of the Diterpene Alkaloids of the *Garrya* and Atisine Groups and their Direct Correlation with the Phyllocladene-type Diterpenes[2]

BY H. VORBRUEGGEN AND CARL DJERASSI

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Conversion of the *Garrya* alkaloids garryfoline (I) and veatchine (II) into the 17-nor-16-ketones VIII and XXII established the absolute configuration at C-8 and C-13, since their rotatory dispersion curves were very similar to that of the 17-nor-16-ketone XI of the phyllocladene (X) series of established absolute configuration. A multistage degradation of garryfoline (I) *via* the azomethine XXVI of cuauchichicine led to the hydrocarbon XXXII, which proved to be identical with a degradation product of steviol (XXXIV) as well as with (−)-"β"-dihydrokaurene obtainable from the diterpene hydrocarbon kaurene (XXXIII). When combined with earlier rotatory dispersion evidence and conformational deductions, these results lead to the complete absolute configurational representations I and II for the principal *Garrya* alkaloids. In view of the chemical interconversion of veatchine (II) with atisine (III), the present absolute configurational assignment also applies to the *Aconitum* alkaloids related to atisine (III). It is noteworthy that these diterpene alkaloids and hence the diterpenes (−)-kaurene (XXXIII) and steviol (XXXIV) represent another group of natural products with the antipodal A/B stereochemistry as compared to the steroids or diterpenoids of the abietic acid class.

The chemical structures of the diterpene alkaloids from *Garrya* (*e.g.*, veatchine and garryfoline) and *Aconitum* (*e.g.*, atisine) species have been settled in recent years.[3] There remained two important outstanding problems—the determination of the absolute configuration of these alkaloids and their experimental interconversion with the diterpene hydrocarbons—and the present paper describes solutions[4] to both of them by a combination of chemical and optical rotatory dispersion[5] approaches.

The starting material for the present investigation was the bark of *Garrya laurifolia* Hartw. from which the alkaloid garryfoline (I) had been isolated earlier.[6] It was now found that varying amounts of veatchine (II)[7] are also present in the plant, but as these two alkaloids are known[6,8] to differ only in the stereochemistry of the C-15 hydroxyl group, they were used interchangeably for the subsequent transformations. It should be

(1) For paper XXXV see A. Sandoval, F. Walls, J. N. Shoolery, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *Tetrahedron Letters*, No. 11 (1962), in press.

(2) Supported by grant No. 2G-682 from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

(3) For reviews see: (a) K. Wiesner and Z. Valenta in L. Zechmeister's "Progress in the Chemistry of Organic Natural Products," Springer, Vienna, 1958, Vol. XVI, pp. 26–89; (b) S. W. Pelletier, *Tetrahedron*, **14**, 76 (1961).

(4) For preliminary communications see: (a) H. Vorbrueggen and C. Djerassi, *Tetrahedron Letters*, 119 (1961); (b) E. Mosettig, P. Quitt, U. Berlinger, J. A. Waters, H. Vorbrueggen and C. Djerassi, *J. Am. Chem. Soc.*, **83**, 3163 (1961); (c) C. Djerassi, P. Quitt, E. Mosettig, R. C. Cambie, P. S. Rutledge and L. H. Briggs, *ibid.*, **83**, 3720 (1961).

(5) See C. Djerassi, "Optical Rotatory Dispersion: Applications to Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1960.

(6) C. Djerassi, C. R. Smith, A. E. Lippman, S. K. Figdor and J. Herran, *J. Am. Chem. Soc.*, **77**, 4801, 6633 (1955).

(7) J. F. Oneto, *J. Am. Pharm. Assoc.*, **35**, 204 (1946).

(8) K. Wiesner and J. A. Edwards, *Experientia*, **11**, 255 (1955); J. A. Edwards, Ph.D Thesis, University of New Brunswick, 1955 (cited on p. 41 of ref. 3a).