The Synthesis and Stereochemistry of Substituted 1,4-Thiazepines Related to the Penicillins[1]

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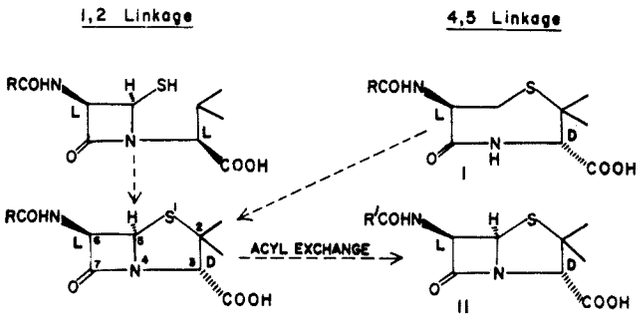
The monocyclic analog of benzylpenicillin, 3D-carboxy-2,2-dimethyl-5-oxo-6L-phenylacetamidoperhydro-1,4-thiazepine (V), and its 3D,6D diastereomer VI, and the monocyclic analog of 6-aminopenicillanic acid, 6L-amino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (VII), and its 6D,3D diastereomer VIII, have been synthesized by separate routes. Condensations between D-penicillamine and appropriate methyl α-amidoacrylates gave the seven-membered ring compounds in a single step. Pairs of diastereomers were separated by simple fractional crystallizations. The stereochemistry of the 6-phenylacetamido compounds was established by desulfurizations to straight-chain products, which were identified with synthetic materials of known configurations. The 6L-amino VII was related to the 6L-phenylacetamido V by N-acylation with phenylacetyl chloride; in like manner the 6D-amino was related to the 6D-phenylacetamido compound. Configurational assignments made for both pairs of diastereomers were corroborated by hydrolysis to the skeletal S-(L-2-amino-2-carboxyethyl)-D-penicillamine (XVI) and S-(D-2-amino-2-carboxyethyl)-D-penicillamine (XVII), which were easily distinguishable from each other through changes in specific rotations, upon acidification, in a positive and a negative direction, respectively.

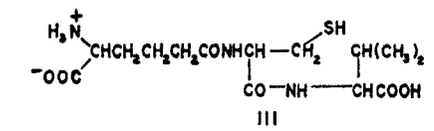
Elucidation of the biosynthetic route to the penicillins II has commanded active and continuing interest.[2-5]

Through a series of experiments using isotopically labeled compounds, Arnstein and his co-workers[6] have shown that the fused bicyclic nucleus of penicillin is probably constructed from L-cysteine and L-valine (in preference to D-valine). However, the question remains[7-9] as to whether the 1,2[10] or the 4,5 linkage (Scheme I),[8,11-15] or indeed the 4,7 linkage,[16,17] is the last formed to create the bicyclic system.

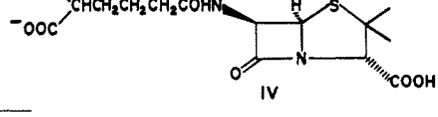
The point at which the acyl group becomes attached to the two amino acid moieties which appear in the final molecule has not been established, although many different penicillins have been produced biosynthetically in cultures enriched with various acid derivatives.[18] It is not known, for example, whether a particular acyl group is attached to the cyst(e)inyl nitrogen throughout the biosynthetic sequence and then remains or is finally exchanged[3,9] with the aid of an acylase,[19,20] or whether 6-aminopenicillanic acid (6-APA) is formed[21,22] and is acylated in a final step. The isolation of a minute quantity of the tripeptide δ-(α-aminoadipoyl)cyst(e)inylvaline (III) from the mycelium of *Penicillium chrysogenum*[23] and the discovery of a small amount of isopenicillin N (IV) in the

SCHEME I





III



IV

(1) This work was supported in part by a research grant (U. S. PHS-GM-05829) from the National Institutes of Health, U. S. Public Health Service, to whom we are pleased to acknowledge our thanks.

(2) A. L. Demain, *Advan. Appl. Microbiol.*, **1**, 23 (1959).

(3) (a) E. P. Abraham, *Pharm. Rev.*, **14**, 473 (1962); (b) E. P. Abraham, G. G. F. Newton, and S. C. Warren, "Chemistry of Microbial Products," Institute of Applied Microbiology, University of Tokyo, Japan, 1963, pp 79–96.

(4) D. J. D. Hockenhull, in "Biochemistry of Industrial Micro-organisms," C. Rainbow and A. H. Rose, Ed., Academic Press Inc., New York, N. Y., 1963, pp 228–246.

(5) H. Umezawa, "Recent Advances in Chemistry and Biochemistry of Antibiotics," Microbial Chemistry Research Foundation, Tokyo, Japan, 1964, Chapter VI.

(6) See H. R. V. Arnstein and H. Margreiter, *Biochem. J.*, **68**, 339 (1958), and earlier papers.

(7) E. P. Abraham and G. G. F. Newton, "Biochemistry of Antibiotics," Vol. V, Proceedings of the 4th International Congress of Biochemistry, Vienna, Pergamon Press, Inc., New York, N. Y., 1958, pp 54–55 and 62–63.

(8) H. R. V. Arnstein and M. E. Clubb, *Biochem. J.*, **68**, 528 (1958).

(9) H. R. V. Arnstein and D. Morris, *ibid.*, **76**, 323, 357 (1960).

(10) The recent celebrated synthesis of cephalosporin C is based upon an analogous closure of the six-membered ring in that molecule; see R. B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan, and H. Vorbrüggen, *J. Am. Chem. Soc.*, **88**, 852 (1966).

(11) D. J. D. Hockenhull, K. Ramachandran, and T. K. Walker, *Arch. Biochem.*, **23**, 160 (1949).

(12) J. C. Sheehan and A. J. Birch, in discussion reported in Ciba Foundation Symposium on Amino Acids and Peptides with Antimetabolic Activity, G. E. W. Wolstenholme and C. M. O'Connor, Ed., Little, Brown and Co., Boston, Mass., 1958, p 259.

(13) I. L. Knunyants, O. V. Kil'disheva, M. P. Krasuskaya, M. G. Lin'kova, V. V. Shokina, Z. V. Benevolenskaya, and L. P. Rasteikene, *Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 1702 (1959); *Chem. Abstr.*, **54**, 8843 (1960).

(14) N. J. Leonard and G. E. Wilson, Jr., *J. Am. Chem. Soc.*, **86**, 5307 (1964).

(15) B. Sjöberg, H. Thelin, L. Nathorst-Westfelt, E. E. van Tamelen, and E. R. Wagner, *Tetrahedron Letters*, 281 (1965).

(16) The premier synthesis of the penicillins followed this route; see J. C. Sheehan and K. R. Henery-Logan, *J. Am. Chem. Soc.*, **79**, 1262 (1957); **81**, 3089, 5838 (1959).

(17) 5,6 linkage, employed in an intriguing photochemical synthesis of the ring system of the penicillins, is not a candidate for consideration in the biosynthetic route; see E. J. Corey and A. M. Felix, *ibid.*, **87**, 2518 (1965).

(18) O. K. Behrens, in "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson, and Sir Robert Robinson, Ed., Princeton University Press, Princeton, N. J., 1949, Chapter XIX.

(19) (a) K. Sakaguchi and S. Murao, *J. Agr. Chem. Soc. Japan*, **23**, 411 (1950); (b) S. Murao, *ibid.*, **29**, 400, 404 (1955).

(20) G. N. Rolinson, F. R. Batchelor, D. Butterworth, J. Cameron-Wood, M. Cole, G. C. Eustace, M. V. Hart, M. Richards, and E. B. Chain, *Nature*, **187**, 236 (1960).

(21) K. Kato, *J. Antibiotics* (Tokyo), *Sect. A*, **6**, 130 (1953).

(22) F. R. Batchelor, F. P. Doyle, J. H. C. Nayler, and G. N. Rolinson, *Nature*, **183**, 257 (1959).

(23) H. R. V. Arnstein, M. Artman, D. Morris, and E. J. Toms, *Biochem. J.*, **76**, 353 (1960).

broth of *P. chrysogenum* grown on a simple medium deprived of side-chain precursors[24] have contributed to the belief that the RCO group in the structures in Scheme I is that of α-aminoadipic acid.[3,4,23]

Since intact cell suspensions have to be used in experiments involving suspected precursors in the biosynthesis of penicillins,[2] the permeability of such substrates through the cell walls becomes a severe limiting factor. In the absence of permeability data, no meaningful conclusion can be drawn from negative incorporation results.[8,15] In any continuing examination of the possible transannular synthesis (4,5 linkage) of the bicyclic system present in penicillin it would be desirable to have available several isomeric pairs of appropriately substituted 1,4-thiazepine derivatives (3D,6L of type I and the related 3D,6D),[25] with stereochemistry at the asymmetric centers clearly established, and obtainable by a practical synthetic sequence. The synthesis of the first such pair (I, R = CH_3, and its 3D,6D diastereomer), fully characterized as to stereochemistry, has been described by Sjöberg and van Tamelen and their co-workers,[15] who improved a route followed earlier by Arnstein and Clubb[8] for the preparation of 6-acetamido-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine of unspecified stereochemistry. Since the acetyl side chain is foreign to the natural penicillins, the synthesis of the monocyclic compound related to penicillin G, 3D-carboxy-2,2-dimethyl-5-oxo-6L-phenylacetamidoperhydro-1,4-thiazepine (V) continued to be a desirable goal, especially since we had made derivatives and established the stereochemistry of its diastereomer, 3D-carboxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (VI).[14,26] We are now able to report the synthesis of the 6L- and 6D-phenylacetamido compounds and, of possibly greater importance, the synthesis and stereochemical characterization of the monocyclic compound related to 6-APA (I, H in place of RCO), 6L-amino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (VII), and its 6D,3D diastereomer VIII.

The approach[8,15] involving condensation of D-penicillamine with appropriate α-aminoacrylate (dehydroalanine) derivatives was employed in the synthesis of the two pairs of diastereomers. Experimental conditions were sought which would provide the optimum in simplicity and yield. Methyl α-phenylacetamidoacrylate (IX) was prepared by condensation between phenylacetamide and pyruvic acid under anhydrous conditions,[27] followed by controlled esterification of α-phenylacetamidoacrylic acid with diazomethane.[28] N-Carbobenzyloxydehydroalanine methyl ester (X) was prepared by a base-catalyzed β-elimination reaction of N-carbobenzyloxy-O-tosyl-DL-serine methyl ester[29,30] in 68% over-all yield starting with DL-serine methyl ester hydrochloride. This synthesis (Scheme II) represents an improvement over the route (17% over-all yield) previously reported,[8] employing condensation between benzyl carbamate and pyruvic acid. Seven-membered ring formation was accomplished in one laboratory operation by the reaction of methyl α-phenylacetamidoacrylate with D-penicillamine in methanolic solution at room temperature, in the presence of triethylamine.[15,31,32] A mixture of 3D-carboxy-2,2-dimethyl-5-oxo-6L-phenylacetamidoperhydro-1,4-thiazepine (V) and the 3D,6D isomer VI was obtained. In a similar manner, condensation of N-carbobenzyloxydehydroalanine methyl ester (X) with D-penicillamine gave a mixture of 6L-carbobenzyloxyamino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (XI) and the 6D,3D isomer XII. Both the 6L-phenylacetamido V and the 6L-carbobenzyloxyamino XI isomers have higher melting points, 212–214 and 222–222.5°, respectively, and are more readily crystallizable than the corresponding 6D isomers VI and XII, which melt indefinitely in the ranges 100–120 and 125–150°, respectively. This difference in properties permitted separation of the two pairs of diastereomers by selective crystallizations. Configurational assignments in the 6-phenylacetamido pair V and VI were made through desulfurization experiments. Raney nickel desulfurization of V gave phenylacetyl-L-alanyl-D-valine (XIII), mp 205–206°, $[\alpha]^{25}D$ −41.5° (lit.[33] mp 206–207°, $[\alpha]^{25}D$ −42°), and a decarboxylated product, the N-isobutylamide of phenylacetyl-L-alanine (XIV), mp 167–168°, $[\alpha]^{25}D$ −55° (lit.[33] mp 165–166°, $[\alpha]^{25}D$ −59°). These are the same two of the three major products obtained from the desulfurization of benzylpenicillin itself under identical conditions.[33] In further confirmation of structure XIII, this product gave no depression in melting point when mixed with a synthetic sample.[34] Esterification of VI with diazomethane gave the crystalline known[14,26] methyl ester, 3D-carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (XV). The configurations of the asymmetric centers in this ester have been established by Leonard and Wilson through desulfurization to methyl phenylacetyl-D-alanyl-D-valinate. The carbobenzyloxy protective groups of 6L- and 6D-carbobenzyloxyamino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (XI and XII) were readily removed by mild treatment with hydrogen bromide in glacial acetic acid to give the versatile free amino VII and VIII, respectively. This acid treatment did not affect the lactam linkage to any appreciable extent. The configurations in the 6L-carbobenzyloxyamino XI and 6L-amino VII were related to the 6L-phenylacetamido analog V by N-acylation of VII with phenylacetyl chloride to give V. Likewise the 6D isomers were related through the phenylacetylation of the 6D-amino compound.

An interesting corroboration of these configurational assignments is provided by the hydrolysis of the 1,4-thiazepines to the skeletal S-(2-amino-2-carboxyethyl)-D-penicillamines (β,β-dimethyllanthionines). S-(L-2-Amino-2-carboxyethyl)-D-penicillamine (XVI) is known[35] but the corresponding D,D isomer XVII is not.

(24) E. H. Flynn, M. H. McCormick, M. S. Stamper, H. DeValeria, and C. W. Godzeski, *J. Am. Chem. Soc.*, **84**, 4594 (1962).

(25) Positions 5, 6, and 7 in the thiazepine ring correspond to positions 7, 6, and 5, respectively, in the penicillin nucleus.

(26) N. J. Leonard and G. E. Wilson, Jr., *Tetrahedron Letters*, 1465 (1964).

(27) T. Wieland, G. Ohnacker, and W. Ziegler, *Ber.*, **90**, 194 (1957).

(28) M. Brenner and K. Rüfenacht, *Helv. Chim. Acta*, **36**, 1832 (1953).

(29) I. Photaki, *J. Am. Chem. Soc.*, **85**, 1123 (1963).

(30) S. Ginsburg and I. B. Wilson, *ibid.*, **86**, 4716 (1964).

(31) B. C. Barrass and D. T. Elmore, *J. Chem. Soc.*, 4830 (1957).

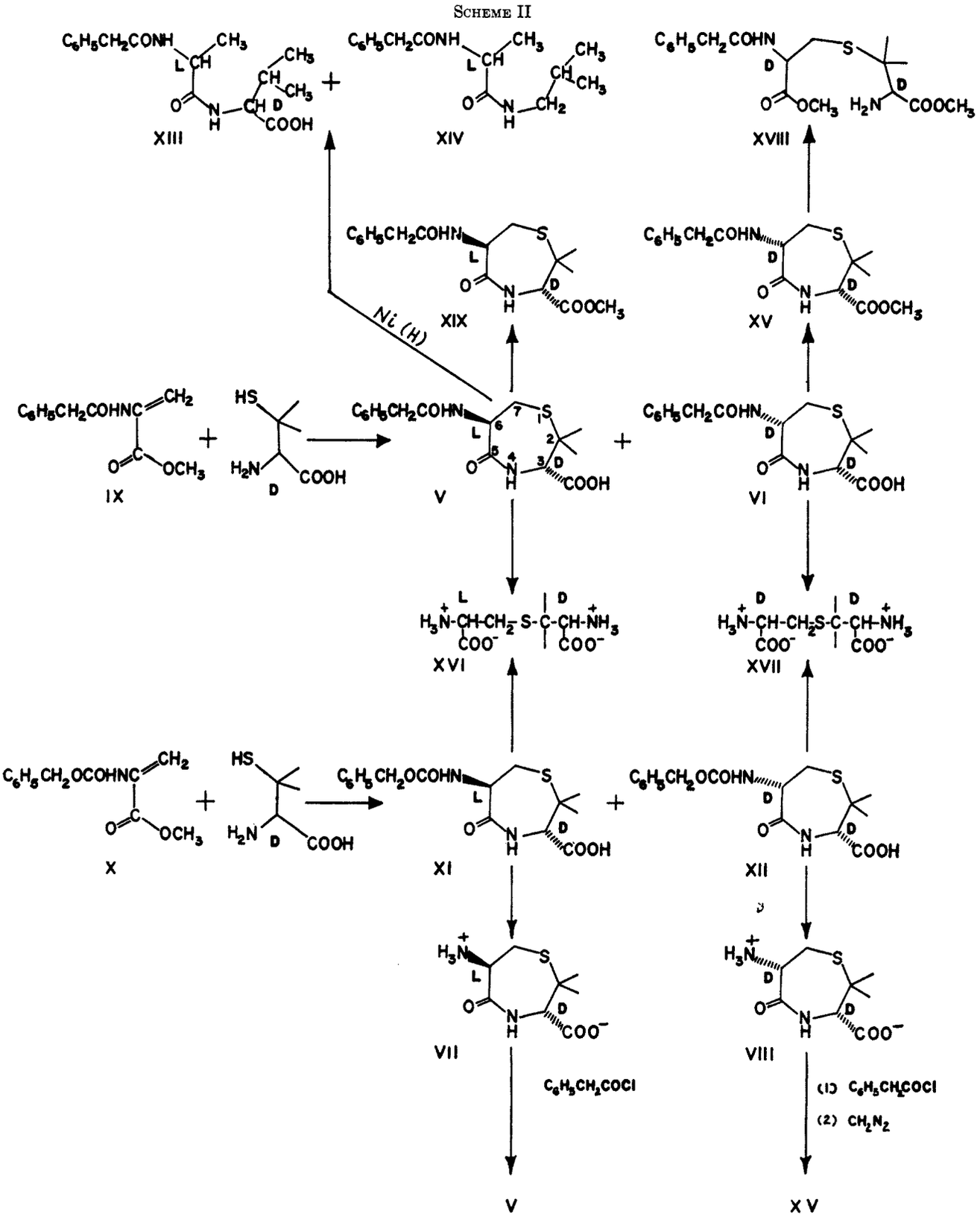
(32) See the Experimental Section for modifications in procedure.

(33) Reference 18, pp 256–261.

(34) Kindly provided by Dr. Karl Folkers. A generous sample of D-penicillamine was supplied by Dr. Max Tishler of Merck Sharp and Dohme.

(35) C. M. Stevens, P. Vohra, J. E. Moore, and C. W. DeLong, *J. Biol. Chem.*, **210**, 713 (1954).

SCHEME II



With the pair of diastereomeric amino acids in hand, we could compare their properties directly. Commonly L-α-amino acids are identified by positive shifts in rotations upon addition of acid to their aqueous solutions, while D-α-amino acids are identified by shifts in the negative direction.[36] If it be assumed that this rule can be applied just as well to two α-amino acid groups tied to the same short chain, acidification of the aqueous solution of the L,D isomer XVI would be expected to result in a change in specific rotation in the positive or negative direction depending on the relative contributions of the L and the D centers to the over-all rotation, while the acidification of the aqueous solution of the D,D isomer XVII would produce a definite shift

(36) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 1, John Wiley and Sons, Inc., New York, N. Y., 1961, p 85.

in the negative direction. We found that while the specific rotations of the L,D and the D,D isomers are about the same in water, −15 and −14°, respectively, that of the L,D isomer changed to −2° in 1 *N* HCl, a positive shift, and that of the D,D isomer in 1 *N* HCl changed to −29°, a negative shift, clearly in agreement with expectations.

The lability of the lactam linkage in the seven-membered rings in contrast to the side-chain amide is demonstrated by the exclusive ring cleavage (94%) of 3D-carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (XV) to the open-chain diester XVIII when XV was treated with hydrogen chloride saturated methanol at room temperature for 5 hr. We failed to find the conditions of hydrolysis, alcoholysis, or aminolysis, which could convert the 6-phenylacetamido compounds into the corresponding 6-amino compounds without affecting the lactam.

The low melting point, high solubility in chloroform, and poor crystallizing properties of both the 6D-phenylacetamido VI and 6D-carbobenzyloxyamino XII stand in sharp contrast to the properties of the corresponding 6L isomers V and XI. In the free amino series (VII and VIII), the addition of pyridine to an ethanolic solution of the hydrobromide salt of the 6D-amino isomer VIII causes instant crystallization of the free amino carboxylate VIII as grains, while similar treatment of the hydrobromide salt of the 6L-amino isomer VII causes no precipitation. One rationalization for this difference between VII and VIII may be found in the observation that in the 3D,6D isomer VIII the amino and carboxyl groups can come into close proximity to each other, possibly forming an internal zwitterionic pair. The granular VIII can withstand a temperature greater than 320°, whereas VII, which crystallizes in leaves, contains traces of randomly and strongly occluded water and ethanol not removable by prolonged drying and decomposes at 248–249°.

6L-Amino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (VII) may serve as a nucleus to which side chains of interest may be readily attached through the 6-amino group. N-Acylation with phenylacetyl chloride has already been demonstrated. Information derived from the synthesis of δ-(D-α-aminoadipoyl)-glycine[37,38] should be readily adaptable to the synthesis of the monocyclic analog of isopenicillin N (IV) from VII. The nucleus VII itself is a candidate for incorporation experiments in studies on the biosynthesis of penicillins, by virtue of its resemblance to 6-APA. The over-all procedure for the preparation of VII has been made relatively simple and provides reasonable yields, which are important practical considerations for the preparation of labeled materials possessing a high degree of radioactivity.

Experimental Section[39]

α-Phenylacetamidoacrylic Acid.—The procedure of Wieland[27] was followed except that trichloroethylene and an elaborate apparatus for the removal of water were replaced by benzene and a Dean–Stark trap with equal effectiveness, mp 184–186° dec (lit. 184–185°).

Methyl α-Phenylacetamidoacrylate (IX).—Esterification of the acid with diazomethane was carried out by Brenner[28] who also found that diazomethane, if present in excess of 1 equiv, apparently added to the olefinic bond. In our hands, the use of acetonitrile as solvent instead of ether permitted a sharper end point for the addition of diazomethane. The yield was 96%; mp 58–59° (lit. 57–59°); nmr spectrum (10% in $CDCl_3$), τ = 6.42 (2 H, singlet, benzyl CH_2), 6.34 (3 H, singlet, OCH_3), 4.30 and 3.59 (1 H, poorly resolved doublet, and 1 H, singlet, respectively, $=CH_2$), 2.91 (5 H, singlet, phenyl), and 2.38 ppm (1 H, singlet, NH); ν_{max}^{Nujol} 3420 (NH), 1720 ($COOCH_3$), 1680 and 1530 (amide), and 909 cm^{-1} ($=CH_2$).

3D-Carboxy-2,2-dimethyl-5-oxo-6L-phenylacetamidoperhydro-1,4-thiazepine (V) and the D,D Diastereomer VI.—A solution of 5.00 g (22.8 mmoles) of methyl α-phenylacetamidoacrylate (IX), 3.40 g (22.8 mmoles) of D-penicillamine, and 3.75 ml (27 mmoles) of triethylamine in 100 ml of methanol was stirred at room temperature in a stoppered flask under nitrogen for 5 days. The reaction solution was evaporated to a clear, colorless gum. The gum was dissolved in 100 ml of water (pH 8) and extracted with three portions of 50 ml of chloroform, setting aside the chloroform extracts for later treatment. Acidification of the aqueous layer to pH 1 and extractions with chloroform gave a mixture of the two acids. Some L,D isomer sometimes remained undissolved by chloroform but was carried along with it as a suspension. It was found that the triethylamine salt of the D,D isomer was very soluble in chloroform, and a large amount of it was removed from the aqueous layer during the initial chloroform extractions. To recover it, the combined chloroform extracts set aside earlier were evaporated to dryness, and the residue was dissolved in water, acidified to pH 1, and reextracted with chloroform. Evaporation of the chloroform extracts gave a gum, which was dissolved in 40 ml of 5% aqueous sodium bicarbonate. The aqueous bicarbonate solution was washed with chloroform and then acidified to precipitate the D,D acid. The chloroform extracts in all steps were dried over anhydrous sodium sulfate before evaporation to dryness.

The bulk of the L,D isomer was precipitated from the mixture of the L,D and D,D isomers by dissolving the mixture in a small amount of chloroform and allowing the solution to stand at 5°. Once crystallized, the L,D isomer is fairly insoluble in chloroform. The remaining D,D isomer contaminated with some uncrystallized L,D isomer may be separated by column chromatography on silica gel using ether containing increasing concentration of methanol (up to 5%) as the eluent. Alternatively, the impure D,D isomer may be purified by dissolving in a minimum amount of 5% aqueous $NaHCO_3$; acidification with acetic acid followed by standing in a refrigerator gave crystalline precipitates of L,D isomer and hydrated D,D isomer. Addition of chloroform to this crystalline mixture dissolves only the D,D isomer. The degree of contamination of the D,D isomer by the L,D isomer was readily determinable from the distinctive *gem*-dimethyl signals in the nmr spectra.

The L,D isomer was recrystallized from 1-butanol as colorless needles, mp 212–214°. The total yield was 1.40 g (18.3%); nmr spectrum (DMSO-d_6), τ = 8.59 (6 H, singlet, $2CH_3$), 7.2–7.6 (2 H, multiplet, 7-CH_2), 6.45 (2 H, singlet, benzyl CH_2), 5.98 (1 H, doublet, 3-CH), 5.4–4.9 (1 H, multiplet, 6-CH), 2.68 (5 H, singlet, phenyl), and 2.2 and 1.8 ppm (2 H, doublets, 2NH); ν_{max}^{Nujol} 3310, 3220, 1727, 1643, 1624, 1555, and 1202 cm^{-1} in addition to phenyl absorptions; $[\alpha]^{25}D$ −27° (*c* 1.0, aqueous 5% $NaHCO_3$).

Anal. Calcd for $C_{16}H_{20}N_2O_4S$: C, 57.14; H, 5.99; N, 8.33. Found: C, 56.87, 57.85; H, 6.26, 5.94; N, 8.09, 7.93.

The D,D isomer was precipitated as a colorless, amorphous solid by dissolving in boiling water and cooling, melting point indefinite, softening in the range of 100–130°. Crystallization as

(37) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **58**, 266 (1954).

(38) V. Gut and J. Rudinger, *Collection Czech. Chem. Commun.*, **28**, 2953 (1963).

(39) All melting points are corrected. The nmr spectra were obtained with a Varian Associates Model A-60 spectrometer. Unless otherwise stated, the chemical shift values were measured using tetramethylsilane as an internal standard (τ 10). Whenever practicable, the signals owing to active hydrogens, such as NH and OH, were identified through the loss of these signals after 2 drops of deuterium oxide was added to the nmr sample tube, the mixture was shaken for 5 min or more, and the spectra were remeasured. Infrared spectra were obtained with a Perkin-Elmer automatic recording infrared spectrophotometer Model 521. Ultraviolet spectra were obtained using a Cary Model 15 spectrophotometer. Optical rotations were measured on a Bendix Ericsson ETL-NPL automatic polarimeter, Type 143 A, in a 1-cm cell using a sodium lamp. Unless otherwise noted evaporation of all solvents was conducted under vacuum using a Büchi rotary evaporator at a bath temperature of less than 40°. All solvents used were of reagent grade purity.

microscopic needles may be accomplished by dissolving in a small amount of 5% aqueous $NaHCO_3$, then acidification with acetic acid, followed by standing in the refrigerator: melting point indefinite, softening in the range 100–120°. The total yield was 1.50 g (19.6%); nmr spectrum (DMSO-d_6), τ = 8.81 (3 H, singlet, CH_3), 8.53 (3 H, singlet, CH_3), 7.7–6.9 (2 H, multiplet, 7-CH_2), 6.47 (2 H, singlet, benzyl CH_2), 5.61 (1 H, doublet, 3-CH), 5.3–4.9 (1 H, multiplet, 6-CH), 2.73 (5 H, singlet, phenyl), and 2.8 and 1.8 ppm (2 H, doublets, 2-NH); ν_{max}^{Nujol} 3300 (br), 1730, 1680–1620 (multiple bands), and 1515 and 1240 cm^{-1}; $[\alpha]^{25}D$ −11° (*c* 1.0, aqueous 5% $NaHCO_3$). Analytically pure samples are hard to prepare owing to the tenacity with which hydrogen-bonding solvents such as water, alcohols, and chloroform are held. Residual solvents may be dislodged only after vigorous drying with heat under vacuum.

Anal. Calcd for $C_{16}H_{20}N_2O_4S$: C, 57.14; H, 5.99; N, 8.33. Found: C, 56.95; H, 6.08; N, 8.63.

Desulfurization of 3D-Carboxy-2,2-dimethyl-5-oxo-6L-phenylacetamidoperhydro-1,4-thiazepine (V). Preparation of Phenylacetyl-L-alanyl-D-valine (XIII) and the Isobutylamide of Phenylacetyl-L-alanine (XIV).—A solution of 200 mg of V was prepared by first dissolving the solid in 2 ml of 10% aqueous sodium bicarbonate and then diluting with 25 ml of water. Freshly prepared Raney nickel[40] suspended in water (2 ml, containing about 1.2 g of Ni) was added to the solution, and the mixture was heated rapidly to reflux with a preheated heating mantle. Excessive foaming continued throughout the reaction period, accompanied by the desorption of hydrogen from the nickel. Vigorous stirring was maintained with a vertical stirrer. After 10 min of heating under reflux, the reaction mixture was rapidly cooled with an ice bath. Nickel was collected on a filter and washed six times with 10-ml portions of hot water. The combined filtrates were acidified to pH 1 and lyophilized. The solid residue was extracted thoroughly with chloroform. Evaporation of the chloroform extract gave a film of colorless gum. The gum was dissolved in 20 ml of chloroform and extracted with 20 ml of 10% aqueous sodium bicarbonate. The chloroform layer was washed with water, then dried over anhydrous sodium sulfate, and evaporated to dryness to give 20 mg of XIV. The aqueous bicarbonate layer was acidified and extracted thoroughly with chloroform. The combined chloroform extracts, after drying over sodium sulfate and evaporating, gave 60 mg of XIII.

Characterization of the Isobutylamide of Phenylacetyl-L-alanine (XIV).[41,42]—Recrystallization from hot water gave colorless needles of constant melting point of 167–168° (lit.[33] 165–166°); ν_{max}^{KBr} 3280, 1635 (br), 1540 (br), and 1450 cm^{-1}; $[\alpha]^{25}D$ −55° (*c* 0.3, MeOH) (lit.[33] −59°).

Anal. Calcd for $C_{15}H_{22}N_2O_2$: C, 68.67; H, 8.45; N, 10.68. Found: C, 68.52; H, 8.69; N, 10.40.

Characterization of Phenylacetyl-L-alanyl-D-valine (XIII).—Repeated solution in dilute sodium hydroxide solution and precipitation by acidification gave colorless needles of constant mp 205–206° (lit.[33] 206–207°); $[\alpha]^{25}D$ −41.5 (*c* 1.0, 1 *N* NaOH) (lit.[33] −42 and −37°); ν_{max}^{KBr} 3260 (br), 1730, and 1635 and 1530 (br) cm^{-1}; mixture melting point with an authentic sample of XIII[34] 205–206°.

S-(D-2-Amino-2-carboxyethyl)-D-penicillamine (XVII) from Hydrolysis of 3D-Carboxy-2,2-dimethyl-5-oxo-6D-phenylacetaamidoperhyro-1,4-thiazepine (VI).—A suspension of 336 mg (1.00 mmole) of VI in 6 ml of 20% aqueous hydrochloric acid was heated gently under reflux for 4 hr. A clear solution was formed within the first few minutes of reflux. Upon cooling the clear solution yielded colorless flakes of phenylacetic acid. Dilution of the reaction mixture with water and extractions with chloroform gave 100% yield of almost pure phenylacetic acid, mp 75–77° (lit. 77°); the infrared spectrum was identical with that of an authentic sample. The aqueous layer was filtered and evaporated to dryness, with warming on a steam bath to drive off last traces of moisture. The dry residue was dissolved in 3 ml of absolute ethanol, to which solution pyridine was added dropwise with stirring. A precipitate of XVII formed instantly. Complete precipitation required about 12 drops of pyridine. The precipitate was washed thoroughly with absolute ethanol and was purified by the repeated process of solution in a minimum amount of water and addition of the aqueous solution dropwise to a large excess of absolute ethanol. The yield of colorless amorphous precipitate was 158 mg (67%).

Characterization of S-(D-2-Amino-2-carboxyethyl)-D-penicillamine (XVII).—The colorless, amorphous material is very soluble in water and slightly soluble in ethanol. It retains traces of these solvents even after prolonged drying under high vacuum at 30–50°. Heating at higher temperatures caused decomposition. No acceptable elemental analyses were obtained. The slightly solvated material melted with decomposition and foaming at 186°; nmr chemical shift values (15% solution in 0.08 ml at D_2O and 0.004 ml of CF_3COOH) using the HOD signal as internal standard are τ = +0.54 (1 H, triplet, *J* = 6 cps, CH_2CH), +0.81 [1 H, singlet, $C(CH_3)_2CH$], +1.60 (2 H, br doublet, *J* = 6 cps, CH_2), +3.35 (3 H, singlet, CH_3), and +3.47 ppm (3 H, singlet, CH_3); $[\alpha]^{25}D$ −14° (*c* 1.0, H_2O) and −29° (*c* 1.0, 1 *N* HCl). Ascending chromatogram on Whatman No. 1 paper, using butanol-rich layer of 1-butanol–acetic acid–water (4:1:5) as eluent and ninhydrin spray detection, gave a single purple spot at R_f 0.1. Further characterization was achieved by preparation of the derivative with phenyl isocyanate.

S-(L-2-Amino-2-carboxyethyl)-D-penicillamine (XVI) from Hydrolysis of 3D-Carboxy-2,2-dimethyl-5-oxo-6L-phenylacetamidoperhydro-1,4-thiazepine (V).—Following the same hydrolytic procedure used for the 3D,6D isomer VI, a 100% yield of phenylacetic acid was obtained. Starting with 336 mg (1.0 mole) of V, 175 mg (74%) of XVI was obtained. Compound XVI was purified by solution in hot water (solubility 1–2%) and precipitation by addition of equal volume of ethanol.

Characterization of S-(L-2-Amino-2-carboxyethyl)-D-penicillamine (XVI).—The colorless, amorphous material decomposed at 258–260°, with discoloration occurring above 200°; nmr chemical shift values (15% solution in 0.08 ml of D_2O and 0.004 ml of CF_3COOH) using the HOD signal as internal standard are τ = +0.54 (1 H, triplet, *J* = 6 cps, CH_2CH), +0.78 [1 H, singlet, $C(CH_3)_2CH$], +1.63 (2 H, doublet, *J* = 6 cps, CH_2), +3.30 (3 H, singlet, CH_3), and +3.38 ppm (3 H, singlet, CH_3); $[\alpha]^{25}D$ −15° (*c* 1.0, water) and −2° (*c* 1.0, 1 *N* HCl). Ascending chromatogram on Whatman No. 1 paper, using butanol-rich layer of 1-butanol–acetic acid–water (4:1:5) as eluent and ninhydrin spray detection, gave a single purple spot at R_f 0.1.

Anal. Calcd for $C_8H_{16}N_2O_4S$: C, 40.68; H, 6.83; N, 11.86; S, 13.57. Found: C, 40.82; H, 6.80; N, 12.01; S, 13.40.

The N,N′-Diphenylcarbamoyl Derivative of S-(L-2-Amino-2-carboxyethyl)-D-penicillamine (XVI).—To a suspension of 500 mg (2.12 mmoles) of XVI in 15 ml of water was added 200 mg (5.0 mmoles) of solid sodium hydroxide and 505 mg (4.24 mmoles) of phenyl isocyanate. The mixture was stirred vigorously at room temperature for 40 min. The solution with some suspended solids was filtered, and the filtrate was washed three times with equal volumes of chloroform. Acidification of the aqueous layer with 6 *N* hydrochloric acid precipitated a white solid. The solid was collected on a filter and washed thoroughly with water. After two recrystallizations from ethanol–water, 420 mg (42%) of shiny flakes was obtained: melting point indefinite, shrinking in the range 145–165°; nmr spectrum (15% in CF_3COOH), τ = 8.55 (6 H, singlet, 2-CH_3), 7.0–6.6 (2 H, multiplets, CH_2), 5.31 [1 H, singlet, $C(CH_3)_2CH$], 5.3–4.9 (1 H, multplet, CH_2CH), and 2.59 ppm (10 H, singlet, two phenyls); ν_{max}^{Nujol} 3315, 1720, 1685, 1618, 1583, 1560, 1535, 1490, 1240, 1182, 748, and 680 cm^{-1}.

Anal. Calcd for $C_{22}H_{26}N_4O_6S$: C, 55.68; H, 5.52; N, 11.81; S, 6.74. Found: C, 55.47; H, 5.65; N, 11.73; S, 6.83.

The N,N′-Diphenylcarbamoyl Derivative of S-(D-2-Amino-2-carboxyethyl)-D-penicillamine (XVII).—To a suspension of 500 mg (2.12 mmoles) of XVII in 15 ml of water was added 200 mg (5.0 mmoles) of solid sodium hydroxide and 505 mg (4.24 mmoles) of phenyl isocyanate. The mixture was stirred vigorously at room temperature for 40 min. All traces of oil disappeared, and a colorless solution with some suspended particles was obtained. The solid was removed by fitration and the filtrate was washed three times with equal volumes of chloroform. Acidification of the aqueous layer with 6 *N* hydrochloric acid precipitated a gum. The gum was collected and washed with water by decantation. After draining off the water droplets, the gum was dissolved in 20 ml of benzene and then evaporated to dryness. A residual layer of amorphous solid formed. The solid was purified by dissolving in 100 ml of anhydrous ether, achieved by first dissolving in a minimum amount of acetone and adding the acetone solution to ether, then precipitating it from ether with hexane.

(40) The Raney nickel used was identical with that used for the desulfurization of benzyl penicillin, ref 33.

(41) Yield of the isobutylamide was found to increase greatly if heating with Raney nickel was prolonged.

(42) Sufficient sample was collected from three separate runs.

The colorless, amorphous material, weighing 430 mg (43%), has no sharp melting point, shrinking in the range 130–150°; nmr spectrum (15% in CF_3COOH), τ = 8.56 (3 H, singlet, CH_3), 8.51 (3 H, singlet, CH_3), 7.0–6.4 (2 H, multiplets, CH_2), 5.30 [1 H, singlet, $C(CH_3)_2CH$], 5.2–4.9 (1 H, multiplet, CH_2CH), and 2.56 ppm (10 H, singlet, two phenyls); ν_{max}^{Nujol} 3340, 1720, 1650, 1600, 1553, 1500, 1315, 1230, 750, and 690 cm^{-1}.

Anal. Calcd for $C_{22}H_{26}N_4O_6S$: C, 55.68; H, 5.52; N, 11.81; S, 6.74. Found: C, 55.62; H, 5.70; N, 11.63; S, 6.79.

3D-Carbomethoxy-2,2-dimethyl-5-oxo-6L-phenylacetamidoperhydro-1,4-thiazepine (XIX).—A partial solution of 200 mg (0.60 mmole) of the free acid V was effected in a mixture of methanol and ether. The suspension was treated with ethereal diazomethane solution until the yellow color persisted and the solution turned clear. The solvent was evaporated to give a light yellow oil. Crystallization was induced from a small volume of ethanol at −15° over a period of 2 days. The total yield of the methyl ester was 170 mg (82%); mp 129–130°; $[\alpha]^{25}D$ −57.1° (*c* 0.6, ethanol); ν_{max}^{Nujol} 3400, 1740, 1680, 1660, and 1540 cm^{-1}; nmr spectrum (15% in $CDCl_3$), τ = 8.62 [6 H, singlet, $(CH_3)_2$], 7.19 (2 H, doublet, 7-CH_2), 6.43 (2 H, singlet, benzyl CH_2), 6.23 (3 H, singlet, OCH_3), 5.77 (1 H, doublet, 3-CH), 5.1–5.4 (1 H, multiplet, 6-CH), 3.15 (1 H, doublet, NH), 2.68 (1 H, doublet, NH), and 2.72 ppm (5 H, singlet, phenyl).

Anal. Calcd for $C_{17}H_{22}N_2O_4S$: C, 58.26; H, 6.33; N, 7.99. Found: C, 57.99; H, 6.28; N, 7.83.

3D-Carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (XV).—A solution of 200 mg (0.60 mmole) of the free acid VI in 10 ml of chloroform was treated with an ethereal solution of diazomethane until the yellow color persisted. The solution was evaporated to dryness, and the solid was recrystallized from hot ethanol to give 180 mg (87%) of colorless needles of the methyl ester XV, mp 171–173°. The specific rotation and infrared and nmr spectra were identical with those of the known XV.[14,26]

S-(D-2-Phenylacetamido-2-carbomethoxyethyl)-D-penicillamine Methyl Ester (XVIII) from Controlled Methanolysis of 3D-Carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (XV).—A solution of 350 mg of XV in 10 ml of methanol, which had been saturated with anhydrous hydrogen chloride at room temperature, was allowed to stand at room temperature for 5.5 hr, then evaporated to dryness at 30°. The residual gum was dissolved in 20 ml of cold water and neutralized immediately with aqueous sodium bicarbonate. Acidification of the aqueous solution to pH 1 and extractions with chloroform gave a trace of unchanged starting material. The acid aqueous layer was basified with 6 *N* aqueous ammonia and extracted with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and evaporated at 20°. The amino diester XVIII was obtained as a colorless oil, yield 360 mg (94%). The structure XVIII is assigned on the basis of nmr and infrared spectra, and the reasonable purity of the product was indicated by the lack of extraneous signals in the nmr spectrum ($CDCl_3$), τ = 8.80 (3 H, singlet, CH_3), 8.73 (3 H, singlet, CH_3), 7.82 (2 H, singlet, NH_2), 7.04 (2 H, doublet, SCH_2), 6.63 (1 H, singlet, CH of amino end), 6.40 (2 H, singlet, benzyl CH_2), 6.31 (3 H, singlet, OCH_3), 6.28 (3 H, singlet, OCH_3), 5.5–5.0 (1 H, multiplet, CH of amide end), 3.67–3.25 (1 H, br, NH), and 2.69 ppm (5 H, singlet, phenyl); $\nu_{max}^{CHCl_3}$ 3410 (br, NH_2 overlapping NH), 1745 ($2COOCH_3$, more intense than the amide band), and 1675 cm^{-1} (amide). The amino diester gave positive ninhydrin spot tests. Even under refrigeration, it turned to a solid mass on standing, apparently through self-condensation.

N-Carbobenzyloxy-DL-serine Methyl Ester.—A heterogeneous mixture of 100 g (0.645 mole) of DL-serine methyl ester hydrochloride, 360 g of potassium bicarbonate (3.6 moles), 50 ml of water, and 1.5 l. of ethyl acetate was stirred with an efficient stirrer at room temperature, and 120 g (0.700 mole) of carbobenzyloxy chloride[43] was added slowly over a period of 2 hr. After an additional 1 hr of stirring upon completion of addition, evolution of carbon dioxide slowed to a trickle, 100 ml of water was added, and stirring was continued for an additional 1 hr. Excess carbobenzyloxy chloride was destroyed with 31 ml (0.40 mole) of pyridine, then the ethyl acetate solution was washed successively with water, 1 *N* hydrochloric acid, and water, followed by drying over anhydrous sodium sulfate. Evaporation of the solvent gave a syrup, which, after pumping at room temperature under 0.1 mm, weighed 149 g (92%); nmr spectrum (CCl_4), τ = 6.38 (3 H, singlet, CH_3), 6.23 (2 H, partially obscured AB doublets, CH_2OH), 5.9–5.4 (2 H, multiplets, CH and OH), 4.98 (2 H, singlet, benzyl CH_2), 3.92 (1 H, doublet, J = 8 cps, NH), and 2.76 ppm (5 H, singlet, phenyl); ν_{max}^{film} 3375 (strong, br), 2950, 1720 (strong, br), 1530, 1460, 1445, 1350, 1215, 1060, 776, 755, 740, and 700 cm^{-1}.

N-Carbobenzyloxy-O-tosyl-DL-serine Methyl Ester.—The procedure of Photaki[39] was used. The yields of the tosylate, as colorless grains, varied from 53 to 74%; mp 100–101.5°; nmr spectrum ($CDCl_3$), τ = 7.60 (3 H, singlet, tosyl CH_3), 6.32 (3 H, singlet, carboxyl CH_3), 5.7–5.3 (3 H, multiplet, methylene and methine H of serine), 4.92 (2 H, singlet, benzyl CH_2), 4.5–4.2 (1 H, hump, NH), 2.65 (5 H, singlet, C_6H_5), and 2.8–2.1 ppm (4 H, multiplet, C_6H_4); ν_{max}^{Nujol} 3250, 1750, 1690, 1550, 1360, 1340, 1265, 1175, 1075, 965, 930, 890, and 770 cm^{-1}.

Anal. Calcd for $C_{18}H_{21}NO_7S$: C, 56.00; H, 5.19; N, 3.44. Found: C, 55.83; H, 5.30; N, 3.14.

N-Carbobenzyloxydehydroalanine Methyl Ester (X).—To a solution of 25 g (0.061 mole) of N-carbobenzyloxy-O-tosyl-DL-serine methyl ester in 150 ml of chloroform, 16.8 ml (0.12 mole) of triethylamine[44] was added in one portion. A mildly exothermic reaction took place. After cooling, the clear, colorless solution was allowed to stand at room temperature for 10 hr. Evaporation of chloroform gave an oil, which upon trituration with 150 ml anhydrous ether, gave 17 g (100%) of the crystalline, hydroscopic triethylamine salt of *p*-toluenesulfonic acid. The ethereal filtrate and washings were combined and evaporated to give an oil. A chloroform solution of this oil was washed successively with 1 *N* hydrochloric acid and water, then dried over anhydrous sodium sulfate, and evaporated. The yield of X as a colorless oil after pumping at 0.05 mm at room temperature was 14.8 g (100%); λ_{max} (methanol–water, 4:1) 244 mμ (ϵ 5400); ν_{max}^{film} 3360, 2950, 1715 (strong, br), 1635, 1515, 1440, 1320, 1065, 890, 800, and 690 cm^{-1}; nmr spectrum (CCl_4), τ = 6.21 (3 H, singlet, CH_3), 4.90 (2 H, singlet, benzyl CH_2), 4.30 and 3.72 (1 H, doublet, J = 1.5 cps and 1 H, singlet, respectively, nonequivalent protons of $=CH_2$), 2.8 (1 H, br, NH), and 2.68 ppm (5 H, singlet, phenyl).

Anal. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95. Found: C, 61.32; H, 5.62; N, 6.02.

The oil solidified to flat needles upon refrigeration; the melting point was about 35°.

6L-Carbobenzyloxyamino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (XI) and the D,D Diastereomer XII.—A solution of 100 g (0.425 mole) of carbobenzyloxydehydroalanine methyl ester (X), 63.3 g (0.425 mole) of D-penicillamine, and 72 ml (0.51 mole) of triethylamine in 200 ml of methanol was stirred at room temperature in a stoppered flask under nitrogen until clear and was then allowed to stand for 6 days. The solution was evaporated to dryness, giving a clear, colorless gum. The gum was treated as in the preparation of phenylacetyl analogs. It was found that in addition to the triethylamine salt of the D,D acid, a considerable amount of the L,D acid triethylamine salt was also extracted by chloroform from the aqueous layer containing triethylamine. The combined mixture of the two acids obtained from chloroform extractions of acidified aqueous layers was found to contain a large amount of a ninhydrin-positive material, which apparently was very soluble, in the hydrochloride form, in chloroform. The L,D isomer present in the mixture precipitated in an amorphous form when the mixture was dissolved in a small amount of chloroform, without heating, and the chloroform solution was allowed to stand at 5°. A second crop was obtained by evaporation of the combined filtrate and chloroform washings to dryness, and solution of the gummy residue in a minimum amount of benzene with slight warming, followed by standing at 5°. A total of about 90% of the L,D isomer present in the mixture was isolated. The benzene mother liquor and washings were combined and washed thoroughly with 1 *N* hydrochloric acid, then with water, to remove all of the ninhydrin-positive contaminant. Drying of the benzene layer

(43) Several commercial samples of carbobenzyloxy chloride, even though colorless and well packaged, were found to be only 50% or lower in purity. Nmr spectral determinations [(in CCl_4) τ 4.79 and 2.68 ppm for the methylene and the phenyl protons, respectively] and conversion to benzyl carbamate with aqueous ammonia are convenient methods of assay. The material used here was better than 90% pure.

(44) Diethylamine used by Photaki[39] in the same reaction produced, along with X as major product, a 19% yield of the Michael adduct between diethylamine and X.

over anhydrous sodium sulfate followed by evaporation yielded a gum, which contained primarily the D,D isomer, along with a small amount of the L,D isomer. When a solution of the gum in a minimum amount of ethanol was allowed to stand at room temperature, a crop of 31.2 g of pure D,D isomer separated as short, colorless needles, with no sharp melting point, softening in the range 125–150°. The D,D isomer once crystallized was sparingly soluble in benzene. A second crop of crystals was obtained by concentration of the ethanolic mother liquor and addition of water while hot, to milkiness, then allowing the solution to stand at room temperature accompanied by seeding and scratching. A yield of 13.4 g of a mixture of D,D isomer containing 3.4 g of L,D isomer was obtained. Separation was effected by selective solution of the D,D isomer with chloroform, followed by evaporation of the chloroform solution, and resolution of the residue in benzene to precipitate a trace of the L,D isomer that had dissolved. The D,D isomer was crystallized from aqueous ethanol.

The combined fractions of L,D isomer were recrystallized from 1-butanol as colorless needles: mp 222–222.5°, $[\alpha]^{25}D$ −16° (*c* 1.0, 5% aqueous $NaHCO_3$). The total yield was 32.5 g (22%); nmr spectrum (15% in DMSO-d_6), τ = 8.63 (3 H, singlet, CH_3), 8.57 (3 H, singlet, CH_3), 7.7–7.0 (2 H, multiplets, 7-CH_2), 5.94 (1 H, doublet, J = 8 cps, 3-CH), 5.6–5.2 (1 H, multiplet, 6-CH), 4.96 (2 H, singlet, benzyl CH_2), 2.65 (5 H, singlet, phenyl), and 2.40 and 2.7 ppm (the former a doublet, J = 8 cps, the latter partly obscured by the phenyl peak, probably a doublet, 2-CONH); ν_{max}^{Nujol} 3445, 3310, 1707, 1627, 1424, 1295, 1234, 1214, 1065, 980, 730, and 695 cm^{-1}.

Anal. Calcd for $C_{16}H_{20}N_2O_5S$: C, 54.54; H, 5.72; N, 7.95; S, 9.08. Found: C, 54.33; H, 5.94; N, 7.85; S, 9.13.

Combined fractions of the D,D isomer were recrystallized from ethanol. Total yield was 40.3 g (27%) of colorless, short needles, which gradually softened in the range 125–150°; $[\alpha]^{25}D$ −11° (*c* 1.0, 5% aqueous $NaHCO_3$); nmr spectrum (15% in DMSO-d_6), τ = 8.85 (3 H, singlet, CH_3), 8.50 (3 H, singlet, CH_3), 7.8–6.8 (2 H, multiplets, 7-CH_2), 5.65 (1 H, doublet, J = 9 cps, 3-CH), 5.5–5.2 (1 H, multiplet, 6-CH), 4.96 (2 H, singlet, $C_6H_5CH_2$), 2.87 (1 H, doublet, J = 9 cps, 4-NH), and 2.67 ppm (5H, singlet, phenyl). In addition, two broad signals (τ = 4.7–3.8 and 3.0–2.6 ppm) were observed. These are attributed to the hydrogen-bonded NH and COOH on the side chains. The spectrum taken in $CDCl_3$ showed considerable change in chemical shifts and the structures of some signals: nmr spectrum (15% in $CDCl_3$), τ = 8.72 (3 H, singlet, CH_3), 8.50 (3 H, singlet, CH_3), 7.3–6.8 (2 H, multiplets, 7-CH_2), 5.54 (1 H, doublet, J = 7.5 cps, 3-CH), 5.4–5.1 (1 H, multiplet, 6-CH), 4.89 (2 H, singlet, benzyl CH_2), 3.77 (1 H, br, COOH), 2.69 (5 H, singlet, phenyl), 2.45 (1 H, br, NH), and 1.70 ppm (1 H, doublet, J = 7.5 cps, NH); ν_{max}^{Nujol} 3415, 3340, 1735, 1704, 1620, 1498, 1273, 1236, 1204, 1057, 757, and 701 cm^{-1}.

Anal. Calcd for $C_{16}H_{20}N_2O_5S$: C, 54.54; H, 5.72; N, 7.95; S, 9.08. Found: C, 54.34; H, 5.72; N, 7.65; S, 8.88.

Total Hydrolysis of 6L-Carbobenzyloxyamino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (XI) to S-(L-2-Amino-2-carboxyethyl)-D-penicillamine (XVI) and of the D,D Isomer XII to S-(D-2-Amino-2-carboxyethyl)-D-penicillamine (XVII).—A suspension of 352 mg (1.0 mmole) of 6L-carbobenzyloxyamino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (XI) in 7 ml of aqueous 6 *N* hydrochloric acid in a test tube was heated on a steam bath for 15 hr. The slightly discolored but clear aqueous solution was washed four times with chloroform then evaporated to dryness. The residual hydrochloride salt was dissolved in 6 ml of absolute ethanol and was precipitated by addition of 10 drops of pyridine. Compound XVI obtained was reluctantly soluble only in boiling water. After purification by suspension and washing in ethanol, then precipitation from hot water solution with ethanol, it weighed 220 mg (93%), mp 250–251° dec. Known decomposition points vary between 248 and 258°. The infrared spectrum in Nujol was identical with that of an authentic sample, and so were specific rotations in water and 1 *N* hydrochloric acid.

Following identical procedure, the D,D isomer XII gave XVII in 62% yield, identical with authentic XVII in its melting point, infrared spectrum, and specific rotations in neutral and acid aqueous solutions.

6L-Amino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine Hydrobromide Salt and the Free Amino Acid VII.—To 250 ml of 1 *M* HBr in glacial acetic acid in a flask open to the atmosphere through a drying tube was added 18.4 g (52.2 mmoles) of 6L-carbobenzyloxyamino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (XI). The solid dissolved slowly accompanied by visible evolution of carbon dioxide. A clear solution formed in 30 min at room temperature, followed shortly by crystallization of a copious amount of the hydrobromide salt of VII as thin needles. After a total of 2 hr at room temperature, the needles were filtered and washed thoroughly with ether. This crude salt was precipitated once from ethanolic solution with anhydrous ether, then recrystallized by dissolving in a small amount of ethanol and adding excess chloroform. Total yield of colorless microplates was 15.5 g (99%); mp 241–243° dec; nmr spectrum (15% in DMSO-d_6), τ = 8.58 (3 H, singlet, CH_3), 8.48 (3 H, singlet, CH_3), 7.3–6.7 (2 H, multiplet, 7-CH_2), 5.98 (1 H, doublet, J = 8 cps, 3-CH), 5.63–5.28 (1 H, multiplet, 6-CH), and 1.67 ppm (4 H, doublet, J = 8 cps, superimposed on broad base, NH, N^+H_3); ν_{max}^{Nujol} 3580, 3520, 3100 (br), 1738, 1711, 1662, 1398, 1202, 1162, 1119, 1081, 1037, 818, 802, and 754 cm^{-1}.

Anal. Calcd for $C_8H_{15}BrN_2O_3S$: C, 32.12; H, 5.05; N, 9.36; S, 10.72. Found: C, 32.12; H, 4.93; N, 9.12; S, 10.42.

Addition of pyridine to an ethanolic solution of the hydrobromide salt gave no precipitation of the free amino acid. This was in sharp contrast to the same treatment of the D,D isomer. Addition of chloroform to the ethanolic solution containing an excess of pyridine, however, precipitated a mixture of the free amino acid and hydrobromide salt. Recrystallizations of the mixture from aqueous ethanol gave the free amino acid as shiny leaves, mp 248–249° dec, which gave negative silver nitrate spot test; nmr spectrum (15% in CF_3COOH), τ = 8.30 (6 H, overlapping singlets with less than 2 cps difference in shift, 2-CH_3), 7.1–6.0 (2 H, multiplets, 7-CH_2), 5.49 (1 H, doublet, J = 8 cps, 3-CH), 4.7–4.2 (1 H, broad hump, 6-CH), 2.4–2.0 (3 H, broad singlet, N^+H_3), and 1.80 ppm (1 H, doublet, J = 8 cps, NH); ν_{max}^{KBr} 2970, 2920, 1650 (br), 1610 (br), 1470, 1375, 1280, 1120, 1045, 875, and 765 cm^{-1}; ν_{max}^{Nujol} 3630, 3540, 3230, 3100, 1660, 1640, 1585, 1520, 1290, 1120, 1050, 875, and 760 cm^{-1}. Even after prolonged drying, elemental analysis showed random occlusion of traces of water and ethanol. After correcting for the occluded solvents, $[\alpha]^{25}D$ −121° (*c* 1.0, H_2O) and −101° (*c* 0.5, 1 *N* HCl). Thin layer chromatograms on silica gel, using the butanol-rich layer of 1-butanol–acetic acid–water (4:1:5) as the eluent and ninhydrin spray detection, gave single yellow spots at R_f 0.44, turning brown on standing.

6D-Amino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (VIII).—Starting with 2.1 g (6.0 mmoles) of 6D-carbobenzyloxyamino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (XII), the procedure described for the L,D isomer was followed exactly. The hydrobromide salt of VIII did not precipitate however in this case. After 2 hr at room temperature, the solution containing 1 *N* HBr in acetic acid was lyophilized. Pyridine treatment of the ethanolic solution of the hydrobromide salt in this case gave instant precipitation of VIII as grains, which were washed thoroughly with ethanol followed by ether. The yield was 0.96 g (73%), stable to above 320° with only slight discoloration; $[\alpha]^{25}D$ −42° (*c* 1.0, H_2O) and −84° (*c* 0.5, 1 *N* HCl); thin layer chromatogaphy, as described for the L,D isomer VII, gave single yellow spots, turning brown on standing, at R_f 0.36; ν_{max}^{KBr} 2970, 2930, 1665 (br), 1620 (br), 1515, 1465, 1370, 1290, 1130, 780, and 715 cm^{-1}; ν_{max}^{Nujol} 3360 (sh), 3330, 1685 (br), 1600 (br), 1525, 1290, 1270, 1245, 1130, 960, 785, and 720 cm^{-1}. Nmr spectrum of a 15% solution in trifluoroacetic acid showed signals at τ = 8.61 (3 H, singlet, CH_3), 8.34 (3 H, singlet, CH_3), 6.9–6.5 (2 H, single hump, 7-CH_2), 5.36 (1 H, doublet, J = 8 cps, 3-CH), 5.2–4.7 (1 H, broad hump, 6-CH), 2.6–2.1 (3 H, single hump, N^+H_3), and 1.88 ppm (1 H, doublet, J = 8 cps, NH).

Anal. Calcd for $C_8H_{14}N_2O_3S$: C, 44.03; H, 6.47; N, 12.84; S, 14.66. Found: C, 44.32; H, 6.55; N, 12.52; S, 14.30.

3D-Carboxy-2,2-dimethyl-5-oxo-6L-phenylacetamidoperhydro-1,4-thiazepine (V) from 6L-Amino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (VII).—A solution of 60 mg (0.28 mmole) of VII in 10 ml of water containing 1 mmole of sodium bicarbonate and 1 mmole of sodium carbonate was stirred at room temperature with 155 mg (1.0 mmole) of phenylacetyl chloride for 7 hr. The mixture was washed with chloroform followed by acidification to pH 1 and extractions with chloroform. A mixture of the acylated product and phenylacetic acid was obtained. Ether was added to the gummy acid mixture to give a cloudy solution. Upon standing overnight at room temperature, V crystallized as bundles of needles weighing 70 mg (76%); mp 214–217°;

after recrystallization from 1-butanol, mp 221–222°, mixture melting point with an authentic sample 220–221°. The infrared spectrum was identical with that of the known.

3D-Carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (XV) from 6D-Amino-3D-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (VIII).—With 60 mg (0.28 mmole) of VIII and following the same procedure described for the L,D isomer VII, a mixture of VI and phenylacetic acid was obtained. Solution in ether and standing, however, gave no crystalline material. The ether was then evaporated, and the acid mixture was dissolved in 10 ml of chloroform and treated with a slight excess of ethereal diazomethane. The solution was evaporated to give an oily mixture of esters. Compound XV readily crystallized as needles upon addition of ether: yield 80 mg (83% over-all), mp 170–172°. After one recrystallization from ethanol, it had mp 172–173° and was identical with the known compound; mixture melting point was 172–173°. The infrared spectra of the two were identical.

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Heterocyclic Steroids. VIII. Steroidal Oxazines and 2-Aza-A-nor Steroids[1,2]

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The use of 17β-hydroxy-4-oxo-1,3-seco-2-nor-5α-estran-1-oic acid (**1a**) has been extended for the synthesis of additional heterocyclic steroids having 4-aza-2-oxa moieties. Introduction of a nitrogen replacing C-4 was accomplished *via* Beckmann rearrangement of the acetyl moiety. In addition, 2-benzyl-2-aza-A-nor steroids have been formed from acetyl acid **1a** and diacid **1i**.

Thus far the 17β-hydroxy-4-oxo-1,3-seco-2-nor-5α-estran-1-oic acid[5] (**1a**) (Chart I) has proved to be a versatile intermediate for the syntheses of a variety of heterocyclic steroids. By condensation with hydrazine, 2,3-diaza steroids[5,6] (steroidal pyridazinones) were prepared. Conversion of the acetyl moiety to a C-5 ketone gave a β-keto ester which was utilized for the construction of 2,4-diaza steroids[7] (steroidal pyrimidines). Functionalization[1] of the acetyl methyl by the introduction of a hydroxyl and subsequent ring closure led to a 2-oxa steroid **7**. During these latter studies certain interesting observations on the course of bromination of the acetyl group were made.

In the previous syntheses of steroid analogs having two heteroatoms in ring A, only nitrogens were introduced. It appeared that by appropriate modifications the intermediate **1a** can also be used for the construction of analogs having different heteroatoms in ring A. To create molecules of this type we undertook the syntheses of analogs having an oxygen atom at C-2 and a nitrogen at C-4. The projected route involved using the carboxylic acid group as the source of the oxygen atom which was to replace C-2 and introducing a nitrogen at C-4. Then ring A could be reconstructed. Although various approaches can be employed to introduce a nitrogen at C-4, we chose to explore the Beckmann rearrangement.[8]

The acetyl ester **1b** was therefore condensed with hydroxylamine to yield a mixture of oximes (see below), which was rearranged by phosphorous oxychloride–pyridine. The major product, acetamide **1c**, was identified by its elemental analysis, infrared spectrum, and nmr spectrum. In the nmr spectrum a singlet for the methyl of the acetamide moiety was observed at 115 cps. In addition, the nitrogen proton was coupled to the 5α proton and gave a doublet at 350.5 cps (J = 9.0 cps). Upon thin layer chromatography of the mother liquors a minor amount of the alternate amide **1d** was found. Although this product has an infrared spectrum similar to acetamide **1c**, its structure was deduced from its nmr spectrum. The absence of an acetamide methyl signal and the appearance of a doublet at 164 cps (J = 5.0 cps) for a methyl on a nitrogen clearly established the structure. In addition, a broad multiplet centered at 339 cps for the proton on the nitrogen was found. Upon exchange of the nitrogen proton for deuterium the signal at 339 cps vanished and the doublet collapsed into a singlet, as expected. These results indicate the presence of two oximes, the major being the isomer *syn* to the acetyl methyl. A similar sequence was performed on the corresponding 17-nitrate **1e** to yield **1f**.

Now with the acetamide **1c** prepared we had a potential intermediate for the 4-aza-2-oxa steroids. For example, lithium aluminum hydride reduction of **1c** would lead to the 1-hydroxy-5-N-ethylamine (**2a**). Alternately, hydrolysis of the acetamide followed by lithium aluminum hydride reduction would provide the unsubstituted 1-hydroxy-5-amine (**2c**). The first route to be undertaken was the direct reduction of **1c**, which gave **2a** in good yield. In an nmr spectrum, *inter alia*, a triplet appeared at 65 cps (J = 7.0 cps) for the methyl of the ethylamine portion of the molecule. Acetylation of the ethylamine **2a** gave **2b**.

The synthesis of steroids containing a tetrahydro-1,3-oxazine ring required insertion of a carbon atom between the amine and the hydroxyl groups of **2a**.

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