acid was added until the mixture was just barely alkaline. The resulting solid was filtered, washed well with water, and was then refluxed in 2.5 l. of acetone, and filtered. The insoluble residue so collected consisted of silicates as shown by infrared analysis. The filtrate was evaporated to a solid which was slurried with acetone to give 3.68 g. of solid. This material was dissolved in 300 ml. of tetrahydrofuran, 3.5 g. of lithium aluminum hydride was added, and the mixture was refluxed for 2 hr. Ethyl acetate was added cautiously and when the lithium aluminum hydride had decomposed, the mixture was filtered and the filter cake was washed thoroughly with boiling acetone and ethyl acetate. The combined filtrates and washings were evaporated to give a white solid which was refluxed in 1 l. of methylene chloride and filtered. The insoluble residue was discarded, and the filtrate was evaporated to a solid which was slurried with petroleum ether to afford 2.6 g. of solid. Several crystallizations from acetone–petroleum ether gave material melting over a broad and inconsistent range (m.p. 176–194°).

A portion (200 mg.) of this material was dissolved in 30 ml. of methanol to which was added 3 ml. of 8% (v./v.) sulfuric acid. The solution was refluxed for 0.5 hr., water was added and the turbid mixture was evaporated until solid formed. This was filtered off and washed well with water to yield 100 mg. of Xa. Four crystallizations from acetone–petroleum ether gave 40 mg. of Xa, m.p. 163–164°; λ_{max} 241 mμ (ε 16,800); ν_{max} 3510, 1717, 1667, and 1613 cm.$^{-1}$; $[\alpha]^{25}D$ +160° (chloroform).

Anal. Calcd. for $C_{22}H_{32}O_3$ (344.48): C, 76.70; H, 9.36. Found: C, 76.32; H, 9.41.

16α-*p*-Toluenesulfonyloxypregn-4-ene-3,20-dione (Xb).—To a solution of 250 mg. of Xa in 1.5 ml. of pyridine was added 250 mg. of *p*-toluenesulfonyl chloride at −5°, and the mixture was allowed to stand at −5° for 24 hr. Water was added, and the crude solid obtained was collected and recrystallized from acetone–petroleum ether to give 240 mg. of Xb, m.p. 203–205°; λ_{max} 228 mμ (ε 21,600), 240 mμ (ε 16,400); ν_{max} 1708, 1670, 1625, 1603, 1178, and 945 cm.$^{-1}$; $[\alpha]^{25}D$ +98° (chloroform).

Anal. Calcd. for $C_{29}H_{38}O_5S$ (498.65): C, 69.85; H, 7.68; S, 6.41. Found: C, 69.39; H, 7.80; S, 6.71.

16α-Iodomethylpregn-4-ene-3,20-dione (Xc).—To a solution of 210 mg. of the tosyl compound Xb in 25 ml. of acetone was added 400 mg. of sodium iodide. The mixture was refluxed for 9 hr., concentrated, and water was added. The resulting solid was collected and washed with water to give 130 mg. of Xc; m.p. 118–120°. Recrystallization from acetone–petroleum ether afforded 50 mg., m.p. 120–125°; ν_{max} 1700, 1670, and 1615 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{31}O_2I$ (454.37): C, 58.15; H, 6.88; I, 27.93. Found: C, 57.62; H, 7.05; I, 27.55.

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The Preparation of 4-*H*^{3}-Hydroxy- and 4-*H*^{3}-Allohydroxy-L-proline

A. V. ROBERTSON,[1] E. KATZ,[2] AND B. WITKOP

National Institute of Arthritis and Metabolic Diseases, and the National Heart Institute, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Md.

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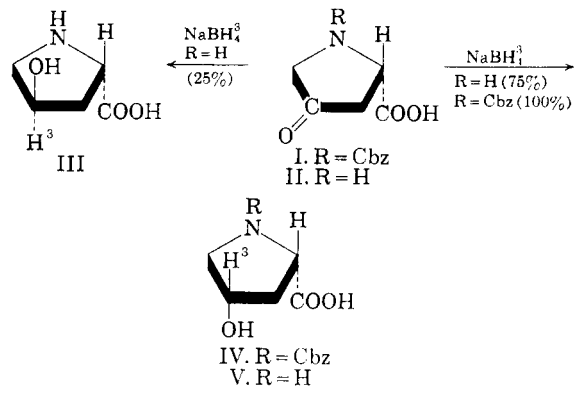
For the study of the incorporation of amino acids into the peptide part of the actinomycins[3] elaborated by *Streptomyces antibioticus*[4,5] a selectively labeled hydroxyproline was needed.

It has been shown previously that sodium borohydride reduction of N-carbobenzyloxy-4-keto-L-proline (I) yields exclusively N-carbobenzyloxy-4-allohydroxy-L-proline (IV).[6] However, in marked



contrast to results from another laboratory,[6a] we have now found that a mixture of V and 4-hydroxy-L-proline (III) is obtained by the action of sodium borohydride (or in this study by tritium-labeled sodium borohydride) on 4-keto-L-proline (II). This effect of the ring nitrogen on the stereochemistry of reduction by complex metal hydrides is also operative in the reductions of N-carbobenzyloxy-5-keto-L-pipecolic, 5-keto-DL-pipecolic acid and 4-keto-L-pipecolic acid (Table I), and may be rationalized in terms of participation of the nitrogen[6] in an intermediate complex of the metal hydride with the carbonyl group.[7]

The quantitative assay of this mixture of 4-tritiated diastereoisomeric hydroxyprolines (III and V) on a column of ion exchange resin[8,9] showed the presence of three parts of allohydroxy- and one part of hydroxy-L-proline. On a preparative scale the diastereoisomers were separated by elution with ethanolic–aqueous ammonium acetate buffer from a column of Amberlite CG-120.[10]

Beyond its use in actinomycin studies 4-H^3-hydroxy-L-proline will permit the study of the reversibility of the reduction of 4-keto-L-proline by a special hydrogenase present in rat-kidney homo-

(1) Associate in the Visiting Program of the USPHS, present address: Dept. of Chemistry, University of Sydney, Australia.

(2) Special Fellow, USPHS.

(3) E. Katz, D. Prockop, and S. Udenfriend, Precursors of the Hydroxyproline and Ketoproline in the Actinomycins, *J. Biol. Chem.*, in press.

(4) *Cf.* E. Katz and L. H. Pugh, *Applied Microbiology*, **9**, 263 (1961).

(5) H. Brockmann and J. H. Manegold, *Chem. Ber.*, **93**, 2971 (1960).

(6) A. A. Patchett and B. Witkop, *J. Am. Chem. Soc.*, **79**, 185 (1957).

(6a) H. C. Beyerman, *Rec. trav. chim.* **80**, 556 (1961).

(7) *Cf.* D. M. S. Wheeler and J. W. Huffman, *Experientia*, **16**, 516 (1960).

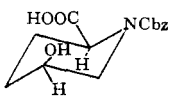
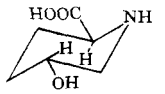
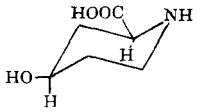
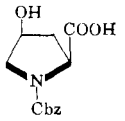
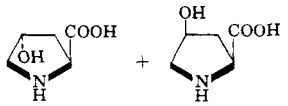
(8) K. A. Piez, *J. Biol. Chem.*, **207**, 77 (1954).

(9) *Cf.* B. Witkop and T. Beiler, *J. Am. Chem. Soc.*, **78**, 2882 (1956).

(10) We are greatly obliged to Dr. S. M. Birnbaum, NCI, for directions on this extremely useful unpublished procedure.

TABLE I

INFLUENCE OF THE BASIC NITROGEN ON THE STEREOCHEMISTRY OF REDUCTION OF KETOAMINO ACIDS TO HYDROXYAMINO ACIDS

Keto-amino acid	Product of reduction with sodium borohydride	Stereospecificity	Reference
N-Carbobenzyloxy-5-keto-L-pipecolic acid		100% *cis*-N-carbobenzyloxy-5-hydroxy-L-pipecolic acid	B. Witkop and C. M. Foltz, *J. Am. Chem. Soc.*, **79**, 192 (1957)
5-Keto-DL-pipecolic acid		Almost exclusively *trans*-5-hydroxy-DL-pipecolic acid	H. C. Beyerman and P. Boekee, *Rec. trav. chim.*, **78**, 648 (1959)
4-Keto-L-pipecolic acid		80% *cis*-4-hydroxy-L-pipecolic acid	J. W. Clark-Lewis and P. L. Mortimer, *J. Chem. Soc.*, 189 (1961)
N-Carbobenzyloxy-4-keto-L-proline		100% *cis* (or allo) 4-hydroxy-L-proline	A. A. Patchett and B. Witkop, *J. Am. Chem. Soc.*, **79**, 185 (1957)
4-Keto-L-proline		25% *trans*- and 75% *cis*-4-hydroxy-L-proline	A. V. Robertson, E. Katz, and B. Witkop, this paper

genate.[11] The loss of tritium in such a case would be an accurate measure of reversion to 4-keto-L-proline.

Experimental

4-Keto-L-proline hydrobromide (35 mg.)[6,12] in 0.5 ml. of water was added drop by drop to a solution of 5 mg. of sodium borohydride-H^3 (100 mC).[13] When the addition was complete the solution was slightly acid, and sufficient cold sodium borohydride (*ca.* 1 mg.) was added to obtain an alkaline solution and hence complete reduction. After 30 min. the reaction mixture was transferred to the top of a column of Amberlite CG-120, type 2 ion exchange resin (70 ml. of 200–400 mesh) prepared in 0.2 *M* ammonium acetate solution in water–ethanol (60:40, v/v). The column was eluted with the same buffer solution and 4-ml. fractions were collected. Aliquots (10 λ) from each tube were spotted on paper and sprayed with isatin reagent. Tubes 13–16 contained 4-hydroxyproline (identified in a cold run by paper electrophoresis). These fractions were combined and the solvent was removed *in vacuo*. The ammonium acetate was then sublimed during 3 hr. at 60°/1 mm. The residue was dissolved in 0.1 ml. of water and 3 ml. of hot ethanol was added. The solution was boiled and acetone was added dropwise until crystallization commenced. After 2 hr. at 0°, 4-H^3-hydroxy-L-proline (5.2 mg.) was collected as colorless needles. Its specific activity was $2.86 \cdot 10^7$ c.p.m./μmole.

Tubes 20–24 contained 4-H^3-4-allohydroxy-L-proline which was worked up in a similar way to give 10.8 mg. of colorless recrystallized product of specific activity $3.24 \cdot 10^7$ c.p.m./μmole. The total recovery of the initial activity present in the sodium borotritide (100 mC) amounts to 1.4%.

(11) C. Mitoma, T. E. Smith, F. M. DaCosta, S. Udenfriend, A. A. Patchett and B. Witkop, *Science*, **129**, 95 (1959).

(12) We are greatly indebted to Dr. Sydney Archer, The Sterling-Winthrop Research Institute, for the preparation of this compound.

(13) Obtained from New England Nuclear Corp., Boston, Mass.

Anomalous Hydrolytic Behavior of Some Basically-Substituted Phthalimides. A Novel Rearrangement of a 4-Aminoquinoline Side Chain[1]

RICHARD M. PECK

Division of Chemotherapy, The Institute for Cancer Research, Philadelphia 11, Pa.

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During the preparation of *N*-(2′-methylaminoethyl)phthalimide (No. 1 in Table I) for use as an intermediate, it was found that its hydrochloride on careful neutralization with alkali in cold water did not yield the free base, but the phthalamic acid; further, the phthalimide obtained by heating the phthalamic acid and distilling gave only a transient alkalinity with water, hydrolyzing to regenerate the phthalamic acid. Investigation showed that this unexpected ease of hydrolysis persisted with homologs containing two-, three-, and four-carbon chains between the two nitrogens, although to a lessening degree. In one other case (No. 4 in Table I) the phthalamic acid also crystallized; three other cases showed a reversal of hydrolysis, in which the base was regenerated on steam cone evaporation of the solution.

All of these phthalimides have been previously reported, either as such or, more generally, as the

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