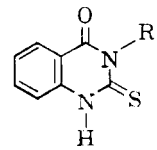
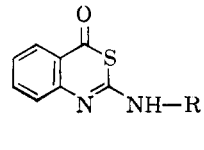
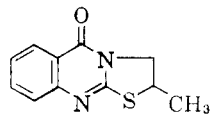


I. R = C_6H_5
III. R = $CH_2-CH=CH_2$



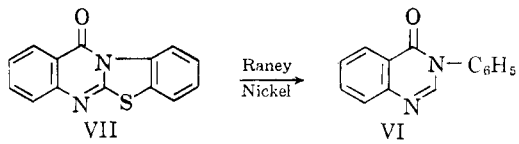
II. R = C_6H_5
IV. R = $CH_2-CH=CH_2$



V

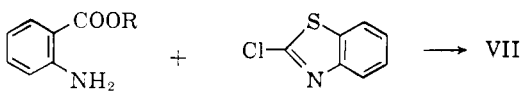
that reported for II by Ghosh, but its properties were not in accord with the assigned structure. The infrared spectrum showed no absorption in the N—H stretching region (3500–3300 cm.$^{-1}$)[4] as would be predicted for II,[4] and desulfurization with Raney nickel[5] afforded 3-phenyl-3*H*-quinazoline-4-one in good yield.

We were led to the tentative conclusion that the product of this reaction has the benzo[*d*]thiazolo-[2,3-*b*]quinazoline-11-one structure (VII). This



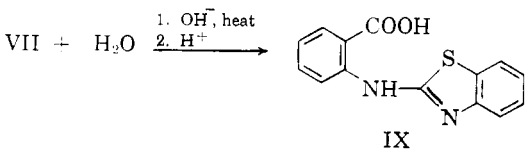
VII VI

compound has previously been prepared by Bose and Pathak[6a] (from anthranilic acid (VIIIa) and 2-chlorobenzothiazole) and by Katz[6b] (from ethyl anthranilate (VIIIb) and 2-chlorobenzothiazole).



VIII a. R = H
b. R = C_2H_5

Katz[6b] has also described the basic hydrolysis of the amide linkage present in VII to form *N*-(2-benzothiazolyl)anthranilic acid (IX).



IX

The Katz preparation[6a] of VII was repeated in a slightly modified form and the product was indistinguishable from the compound prepared by the method of Ghosh.[1] The melting points and mixed melting points were identical, and the infrared absorption spectra were the same. The hydrolysis described by Katz[6b] was performed on the two samples of VII and the melting points and mixed melting points were the same.

(4) F. A. Miller in H. Gilman, "Organic Chemistry, An Advanced Treatise," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 122.

(5)(a) R. Mozingo, D. E. Wolf, W. A. Harris, and K. Folkers, *J. Am. Chem. Soc.*, **65**, 1013 (1943); (b) R. O. Robbins, Jr., J. O. Lampen, J. P. English, Q. P. Cole, and J. R. Vaughn, Jr., *ibid.*, **67**, 290 (1945); (c) J. A. Zderic, W. A. Bonner, and T. W. Greenlee, *ibid.*, **79**, 1696 (1957).

(6)(a) P. K. Bose and K. B. Pathak, *J. Indian Chem. Soc.*, **11**, 463 (1934); (b) L. Katz, *J. Am. Chem. Soc.*, **75**, 712 (1953).

Experimental[7]

Benzo[*d*]thiazolo[2,3-*b*]quinazoline-11-one (VII).—Method 1. This compound was prepared by the method of Ghosh[1] in a 46% yield, m.p. 190–191°, reported[1] 184–185°. 3-Phenyl-1*H*,3*H*-quinazoline-2,4-dione, m.p. 276–277° (reported,[8] 275–277°), was also isolated in a 41% yield.

Method 2. The procedure followed was essentially that of Katz.[6b] To 5.0 g. of methyl anthranilate, 5.0 g. of 2-chlorobenzothiazole was added, and the mixture was heated cautiously until a vigorous exothermic reaction was initiated. Thereafter the reaction vessel was cooled with a jet of air as necessary to keep the reaction under control. When the reaction had moderated, the crude product was powdered and washed with dilute sodium bicarbonate solution. To ensure complete reaction, the air-dried powder was heated above 200° for 10 min. Two crystallizations from isopropyl alcohol gave 6.5 g. of white needles, m.p. 190–191°, reported 189°[6a] and 193°.[6b] A mixed melting point of this compound prepared by method 1 with that prepared by method 2 was 189.5–191°. The infrared spectra of these two were identical, each showing maxima at 1695, 1590, 767, and 750 cm.$^{-1}$.[9]

***N*-(2-Benzothiazolyl)anthranilic Acid (IX).**—This compound was prepared by the method of Katz[6b] in a 72% yield, m.p. 194–195° (reported, 187°[6a] and 195–196°[6b]).

Raney Nickel Desulfurization of Benzo[*d*]thiazolo[2,3-*b*]-quinazoline-11-one (VII).—To a solution of 2.5 g. of VII in 200 ml. of absolute ethanol, 10 g. of W-6 Raney nickel[10] was added. The resulting mixture was heated under reflux for 2 hr. The nickel was removed by filtration, and the ethanol by evaporation, to give 1.6 g. of white needles. Two crystallizations (aqueous ethanol) gave 1.1 g. of 3-phenyl-3*H*-quinazoline-4-one (VI), m.p. 138–139°, reported 139°[11] and 142–142.5°.[3b]

Acknowledgment.—The author is greatly indebted to Dr. J. Allinger for determining the infrared spectra, and to Drs. J. and N. L. Allinger for helpful discussions. The author also wishes to thank the National Science Foundation for financial support of part of this work.

(7) All melting points were determined in capillary melting point tubes and are uncorrected.

(8) B. Pawleski, *Chem. Ber.*, **38**, 130 (1905).

(9) These spectra were determined in carbon disulfide solution and recorded on a Beckman Model IR4 spectrophotometer.

(10) H. R. Billica and H. Adkins, *Org. Syn.*, Coll. Vol. III, 176 (1955).

(11) C. Paul and M. Busch, *Chem. Ber.*, **22**, 2863 (1899).

The Preparation of 16α-Hydroxymethylprogesterone

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Received January 24, 1962

In view of the recent disclosure[1] of the preparation of 16α-substituted methyl corticoids we wish

(1) P. F. Beal and J. E. Pike, *J. Org. Chem.*, **26**, 3887 (1961).

to record here the use of a similar set of reactions to prepare *authentic* 16α-hydroxymethylprogesterone (Xa).

For preparing pregnanes with the desired 17β-acetyl-16α-substituent it was decided to proceed from 16α-cyano-3β-hydroxypregn-5-en-20-one (I)[2] protected from the possibilities of inversion[3] by an ethylene ketal grouping at C-20. Accordingly, reaction of I with ethylene glycol and *p*-toluenesulfonic acid afforded 16α-cyano-20-ethylenedioxypregn-5-en-3β-ol (II). Strong alkaline hydrolysis of the latter gave 16α-carboxy-20-ethylenedioxypregn-5-en-3β-ol (III), which upon hydrolysis with dilute sulfuric acid was converted into 16α-carboxy-3β-hydroxypregn-5-en-20-one (IV), markedly different in properties from the compound synthesized by Romo[2] with this assigned structure. This latter compound must, presumably, be the 16β-carboxy-17α-acetyl isomer. The correctness of the stereochemistry of the 16α-carboxylic acid IV was further confirmed by treatment of IV with sodium borohydride followed by acetylation to give 3β,20β-diacetoxypregn-5-ene-16α-carboxylic acid (Vb), identical to an authentic sample.[4]

Lithium aluminum hydride treatment of 16α-carboxy-20-ethylenedioxypregn-5-en-3β-ol (III) gave the 16α-hydroxymethyl compound VI as a gelatinous solid. Acid hydrolysis of the latter afforded 16α-hydroxymethyl-3β-hydroxypregn-5-en-20-one (VII). Oppenauer oxidation of either the 20-ketal VI or the 20-one VII was not fruitful, and, consequently, an alternate pathway was investigated.

Oppenauer oxidation of the ketal nitrile II gave 16α-cyano-20-ethylenedioxypregn-4-en-3-one (VIII) which was converted into the 3,20-bisketal IX. The latter compound was also prepared directly from 16α-cyanoprogesterone.[2] Strong alkaline saponification of IX gave a crude mixture which was treated with lithium aluminum hydride followed by acid to yield 16α-hydroxymethylpregn-4-ene-3,20-dione (Xa). This compound possessed an optical rotation which was considerably more dextrorotatory than that of the compound assigned this structure by Romo.[2] Moreover, it did *not* exhibit hemiketal ring formation in its infrared spectrum as shown by Romo's compound. Indeed, this type of ring formation is most difficult to visualize from molecular models for the assigned structure since the C-20 ketone and the hydroxyl functions are at a considerable distance from each other in the *trans* geometry of a 17β-acetyl-16α-hydroxymethyl molecule. Finally, 16α-hydroxymethylprogesterone (Xa), was converted into the tosylate Xb, which, in turn, was treated with sodium iodide in acetone to afford 16α-iodomethylpregn-4-ene-3,20-dione (Xc).

In the Clauberg progestational assay (subcutaneous route) 16α-hydroxymethylprogesterone (Xa)

(2) J. Romo, *Tetrahedron*, **3**, 37 (1958).

(3) Mazur and Cella,[4] have clearly demonstrated that alkaline hydrolysis of a 16α-cyano-20-ketone system, first prepared and studied by Romo,[2] actually resulted in a double inversion of configuration to afford a 17α-acetyl-16β-carboxylic acid system. This finding was in contrast to the assignment of the 17β-acetyl-16α-carboxylic acid configurations by Romo,[2] and later, by B. Ellis, V. Petrow, and D. Wedlake, *J. Chem. Soc.*, 3748 (1958). This occurrence of a double inversion has been confirmed both chemically[4] and physically (optical rotatory dispersion analysis).[5]

(4) R. H. Mazur and J. A. Cella, *Tetrahedron*, **7**, 130 (1959).

(5) W. A. Struck and R. L. Houtman, *J. Org. Chem.*, **26**, 3883 (1961).

was inactive at a total dose of 1 mg. at which level progesterone exhibits a pronounced response.[6]

Experimental

Melting Points.—All melting points are uncorrected.

Absorption Spectra.—The ultraviolet spectra were determined in methanol; the infrared spectra were determined in a potassium bromide disk.

Petroleum Ether.—The fraction used had a b.p. 60–70°.

16α-Cyano-20-ethylenedioxypregn-5-en-3β-ol (II).—To a solution of 2 g. of 16α-cyano-3β-hydroxypregn-5-en-20-one (I) in 100 ml. of benzene and 3 ml. of ethylene glycol was added 50 mg. of *p*-toluenesulfonic acid monohydrate. The mixture was refluxed for 5–6 hr. with continuous water removal, cooled, and 50 ml. of ethyl acetate were added. The organic layer was separated and washed with aqueous sodium bicarbonate, and then with water until neutral. After being dried, the solution was evaporated to give an oil which crystallized from acetone–petroleum ether to afford 1.6 g. of II, m.p. 184–189°. Several crystallizations from acetone–petroleum ether raised the m.p. to 191–194°; ν_{max} 3560, 2235, and 1050 cm.$^{-1}$; $[\alpha]^{25}D$ −70.5° (chloroform).

Anal. Calcd. for $C_{24}H_{35}O_3N$ (385.53): C, 74.76; H, 9.15; N, 3.63. Found: C, 74.40; H, 8.79; N, 3.67.

16α-Carboxy-20-ethylenedioxypregn-5-en-3β-ol (III).—To a solution of 0.5 g. of the 16α-cyano compound II in 25 ml. of ethylene glycol, was added a solution of 5 g. of potassium hydroxide in 15 ml. of ethylene glycol, and the mixture was refluxed for 24 hr. Addition of water gave a small amount of amorphous solid which was discarded. The filtrate was acidified with dilute hydrochloric acid and extracted with ethyl acetate and methylene chloride. The combined extracts were washed with water until neutral, dried, and evaporated to a semisolid which was crystallized from acetone–petroleum ether to afford 0.24 g. of III, m.p. 239–242°. Two crystallizations from acetone–petroleum ether followed by two crystallizations from acetone gave a m.p. of 245–248°; ν_{max} 3300, 2670, 1710, and 1040 cm.$^{-1}$; $[\alpha]^{25}D$ −36° (chloroform).

Anal. Calcd. for $C_{24}H_{36}O_5$ (404.53): C, 71.25; H, 8.97. Found: C, 71.36; H, 9.18.

16α-Carboxy-3β-hydroxypregn-5-en-20-one (IV).—To a solution of 300 mg. of 16α-carboxy-20-ethylenedioxypregn-5-en-3β-ol (III) in 25 ml. of methanol was added 3 ml. of 8% (v./v.) sulfuric acid, and the mixture was refluxed for 5 min. Evaporation at 40–45° gave a solid which, after the addition of water, was collected by filtration and was washed well with water to afford 220 mg. of IV. Three crystallizations from acetone–petroleum ether yielded a m.p. 235–238°; ν_{max} 3450 and 1710 (broad) cm.$^{-1}$; $[\alpha]^{25}D$ +14° (chloroform).

Anal. Calcd. for $C_{22}H_{32}O_4$ (360.48): C, 73.30; H, 8.95. Found: C, 73.44; H, 9.16.

Admixture melting point determination with 16β-carboxy-3β-hydroxy-17α-pregn-5-en-20-one[2] (m.p. 233–236°) gave a m.p. of 223–228°.

3β,20β-Diacetoxypregn-5-ene-16α-carboxylic Acid (Vb).—To a solution of 0.41 g. of 16α-carboxy-3β-hydroxypregn-5-en-20-one (IV) in 35 ml. of methanol, was added 1 g. of sodium borohydride and the solution was allowed to stand overnight at room temperature. Water was added and the solution was acidified with hydrochloric acid. The resulting solid was separated by filtration and was washed well with water to give 0.24 g. of Va, m.p. 271–277°. Recrystallization from ethanol afforded 0.10 g., m.p. 283–286°. This material was dissolved in 3 ml. of pyridine to which was added 0.3 ml. of acetic anhydride, and the solution was heated on the steam bath for 1 hr. It was then poured into water and the product was extracted with ethyl acetate. The extract was washed with water, dried, and evaporated to an oil which crystallized from ether–petroleum ether to yield 80 mg. of Vb, m.p. 187–188°. The infrared spectrum was identical to that of an authentic sample.[4,7]

16α-Hydroxymethyl-3β-hydroxypregn-5-en-20-one (VII).—To a solution of 14.1 g. of 16α-carboxy-20-ethylenedioxypregn-5-en-3β-ol (III) in 1.5 l. of tetrahydrofuran was added 14 g. of lithium aluminum hydride, and the mixture was refluxed for 2 hr. Ethyl acetate was added cautiously, the solids were filtered off, and the filter cake was washed thoroughly with hot acetone and ethyl acetate. The combined filtrates and washings were evaporated to give the 16α-hydroxymethyl 20-ketal VI as a gelatinous solid. Approximately 5 g. of the latter was dissolved in 300 ml. of methanol to which was added 30 ml. of 8% (v./v.) sulfuric acid, and the solution was refluxed for 1 hr. Evaporation gave a solid which was separated by filtration, and was washed well with water to yield 2.18 g. of VII, m.p. 225–227°. Two crystallizations from acetone–petroleum ether gave m.p. 226–228°; ν_{max} 3380 and 1703 cm.$^{-1}$; $[\alpha]^{25}D$ +17° (methanol).

Anal. Calcd. for $C_{22}H_{34}O_3$ (346.49): C, 76.26; H, 9.89. Found: C, 76.32; H, 10.02.

16α-Cyano-20-ethylenedioxypregn-4-en-3-one (VIII). A solution of 4.3 g. of 16α-cyano-20-ethylenedioxypregn-5-en-3β-ol (II) in 500 ml. of toluene and 45 ml. of cyclohexanone was distilled until 25 ml. of distillate was collected. Aluminum isopropoxide solution in toluene (40 ml.; 0.2 g./ml.) was then added, and the reaction mixture was refluxed for 40 min. Rochelle salt (40 g.) in water was added and the mixture was steam distilled until all the cyclohexanone was removed. The cooled aqueous mixture was extracted with ethyl acetate; the combined extracts were washed with water, dried, and evaporated to give 2.85 g. of VIII. Two crystallizations from acetone gave the analytical sample, m.p. 248–250°; λ_{max} 240 mμ (ε 18,700); ν_{max} 2240, 1668, 1620, and 1044 cm.$^{-1}$; $[\alpha]^{25}D$ +44° (chloroform).

Anal. Calcd. for $C_{24}H_{33}O_3N$ (383.51): C, 75.16; 8.67; N, 3.65. Found: C, 74.86; H, 8.70; N, 3.68.

16α-Cyano-3,20-bisethylenedioxypregn-5-ene (IX).—A. To a solution of 18.2 g. of 16α-cyanopregn-4-ene-3,20-dione[2] in 1200 ml. of benzene and 35 ml. of ethylene glycol was added 1 g. of *p*-toluenesulfonic acid monohydrate. The reaction mixture was refluxed for 6 hr. (continuous water removal), cooled, washed with aqueous sodium bicarbonate, and then with water until neutral. Evaporation gave a solid which was recrystallized from acetone to yield 10.3 g. of IX, m.p. 265–270°. Concentration of the mother liquor afforded an additional 2.2 g., m.p. 263–268°. Two crystallizations from acetone gave pure IX, m.p. 267–270°; ν_{max} 2235 and 1100 cm.$^{-1}$; $[\alpha]^{25}D$ −60° (chloroform).

Anal. Calcd. for $C_{26}H_{37}O_4N$ (427.56): C, 73.03; H, 8.72; N, 3.28. Found: C, 73.08; H, 8.76; N, 3.34.

B. To a solution of 2.85 g. of the 20-ketal VIII in 150 ml. of benzene and 4 ml. of ethylene glycol was added 80 mg. of *p*-toluenesulfonic acid monohydrate. The mixture was refluxed for 5–6 hr. (continuous water removal) cooled, washed with aqueous sodium bicarbonate, and then with water until neutral. Evaporation gave 2.7 g. of solid whose infrared spectrum showed an appreciable amount of α,β-unsaturated ketone. Consequently, the reaction in benzene and glycol was repeated, and worked up as described above. This gave 2.4 g. of material which still showed the presence of some α,β-unsaturated ketone. Recrystallization from acetone yielded 1.1 g. of IX; m.p. 260–264°; and whose infrared spectrum was identical to that of IX prepared in A above.

16α-Hydroxymethylpregn-4-ene-3,20-dione (16α-Hydroxymethylprogesterone) (Xa).—A mixture of 12.2 g. of the cyanobisketal IX in 280 ml. of ethylene glycol, was treated with 100 g. of potassium hydroxide in 100 ml. of ethylene glycol. The mixture was refluxed for 24 hr. under nitrogen. It was cooled, diluted with water, and dilute hydrochloric

(6) This assay was carried out by the Endocrine Laboratory, Madison, Wisconsin.

(7) We thank Dr. E. W. Cantrall of these laboratories for this sample.

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.

Acknowledgment.—We wish to thank Louis M. Brancone and associates for the analyses, and William Fulmor and associates for the infrared and ultraviolet absorption spectra and optical rotation data.

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

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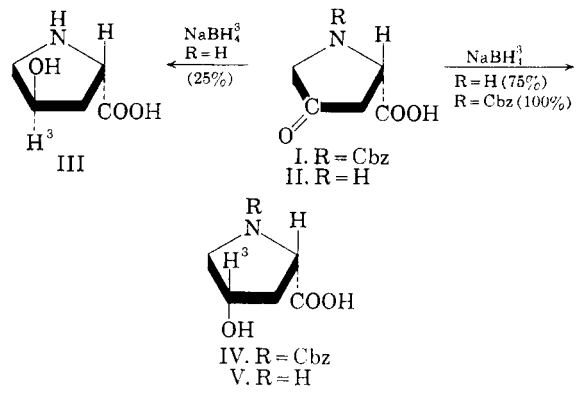
Received January 24, 1962

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.