ANALOGS AND HOMOLOGS OF PROLINE AND HYDROXYPROLINE

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CONTENTS

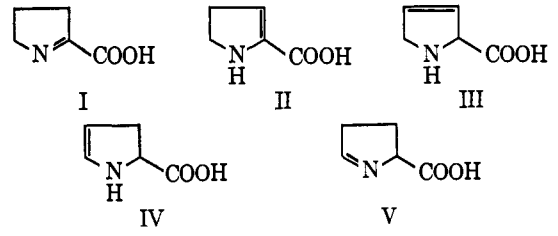
I. INTRODUCTION

L-Proline and 4-hydroxy-L-proline are major building stones of collagen, the protein of connective tissue which constitutes one-third of the total body protein. While proline occurs in all proteins, *trans*-4-hydroxy-L-proline, together with a small amount of *trans*-3-hydroxy-L-proline, is generally found only in collagen. Because of the accurate and facile analytical methods for detection, hydroxyproline offers a convenient handle for following biosynthesis and degradation of collagen. In recent years biochemical, metabolic, and clinical studies have focused attention on metabolites, analogs, and homologs of proline. In addition novel proline derivatives have been isolated from plants and bacterial antibiotics. This review summarizes some of the recent advances in this field.

II. PROLINE ANALOGS HAVING A DOUBLE BOND IN THE RING

A. INTRODUCTION: TYPES OF OLEFINIC PROLINES (I–V)

Unsaturation in pyrrolidine-2-carboxylic acid can theoretically occur in five distinct locations, as in structures I–V. However, the enamines II and IV are stable



only if there is a substituent on the nitrogen atom to prevent isomerization to the imines I or V, respectively. In solution, I and V exist in equilibrium with δ-amino-α-ketovaleric acid and glutamic γ-semialdehyde, respectively. By contrast 3,4-dehydroproline is stable.

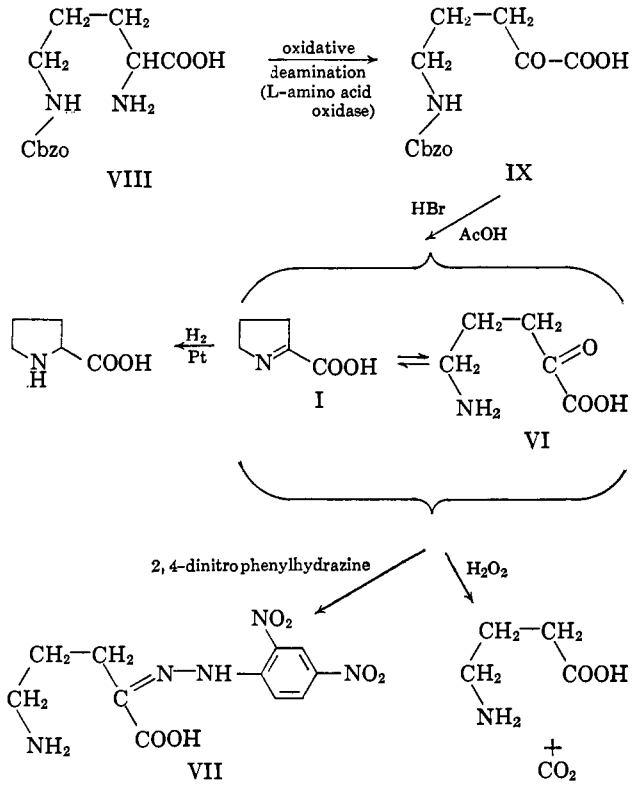
B. COMPOUNDS OF STRUCTURAL TYPE I

1. Δ¹-Pyrroline-2-carboxylic Acid

Oxidation of D-proline by sheep kidney D-amino acid oxidase (138) and of L-proline by rat kidney L-amino acid oxidase (33) gives an equilibrium mixture of Δ¹-pyrroline-2-carboxylic acid (I) and δ-amino-α-ketovaleric acid (VI); the latter was characterized as its 2,4-dinitrophenylhydrazone (VII).

For the isolation of Δ¹-pyrroline-2-carboxylic acid (160), δ-N-carbobenzyloxy-L-ornithine (VIII) was oxidatively deaminated using the venom of *Crotalus adamanteus* (159), giving δ-N-carbobenzyloxyamino-α-ketovaleric acid (IX) (m.p. 121°; 2,4-dinitrophenylhydrazone, m.p. 194°). This product on decarbobenzoxylation with hydrogen bromide in acetic acid furnished Δ¹-pyrroline-2-carboxylic acid hydrobromide, m.p. 211–212° (dec.).

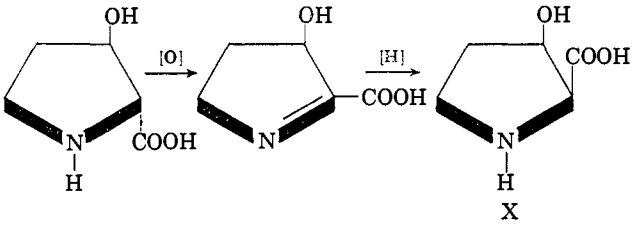
Δ¹-Pyrroline-2-carboxylic acid gave a yellow color with *o*-aminobenzaldehyde; this reaction made it visible on paper chromatograms and distinguished it from the isomeric Δ¹-pyrroline-5-carboxylic acid (V). The synthesis and some typical reactions of I are summarized below (160).



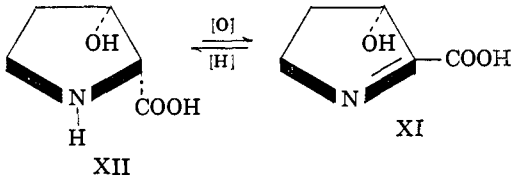
Δ¹-Pyrroline-2-carboxylic acid failed to support the growth of *E. coli*, mutant 55-1 (which responds to L-proline), 55-25 (which responds to L-proline or to Δ¹-pyrroline-5-carboxylic acid), or 160-37 (which responds to L-ornithine, L-arginine, or L-citrulline) (160).

2. 3-Hydroxy-Δ¹-pyrroline-2-carboxylic Acid

Dehydrogenation of *trans*-3-hydroxy-DL-proline by D-amino acid oxidase produces a dehydro compound which may be reduced to *cis*-3-hydroxy-L-proline (X).



The analogous oxidation of *cis*-3-hydroxy-DL-proline gave (*S*)-3-hydroxy-Δ¹-pyrroline-2-carboxylic acid (XI) which was separated from unchanged *cis*-3-hydroxy-L-proline by ion-exchange techniques and stereoselectively reduced by sodium borohydride to *cis*-3-hydroxy-D-proline (XII) (113).



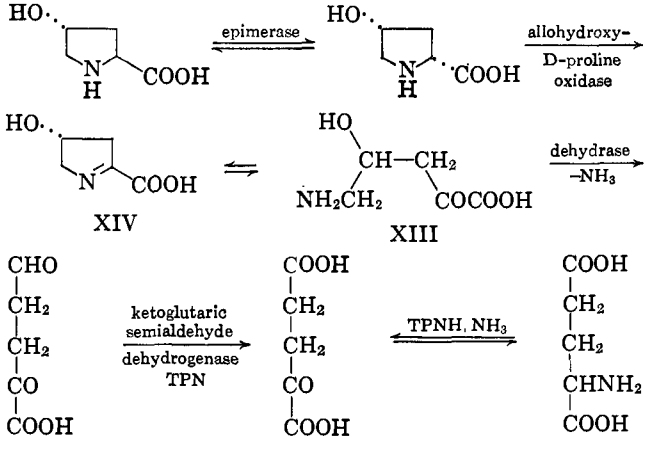
XII XI

Both L- and D-*trans*-3-hydroxyproline are nonenzymatically dehydrogenated by hydrogen peroxide and cupric ions. Thus *trans*-3-hydroxy-DL-proline, upon oxidation and subsequent reduction, gave *cis*-3-hydroxy-DL-proline (113).

3. *4-Hydroxy-Δ¹-pyrroline-2-carboxylic Acid*

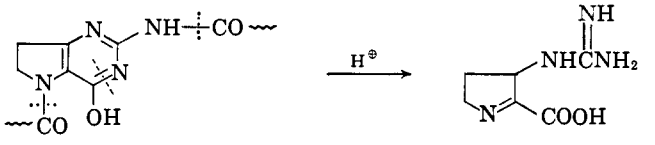
This substance was postulated as an intermediate in the metabolic breakdown of hydroxyproline to aspartic and glutamic acids (84, 249) which was known to proceed *via* δ-amino-γ-hydroxy-α-ketovaleric acid (XIII) (33, 133, 136–138, 244). It is formed when kidney D-amino acid oxidase acts upon hydroxy-D-proline and allohydroxy-D-proline (206, 207) and when allohydroxy-D-proline oxidase from *Pseudomonas striata* acts upon allohydroxy-D-proline (7). 4-Hydroxy-Δ¹-pyrroline-2-carboxylic acid is rapidly converted to pyrrole-2-carboxylic acid, which had been encountered previously in studies of the metabolism of hydroxyproline (206, 207).

Conversion of hydroxy-L-proline to α-ketoglutarate and L-glutamate *via* 4-hydroxy-Δ¹-pyrroline-2-carboxylic acid (XIV) is outlined below (7).

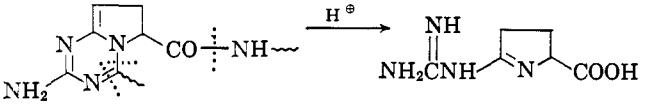


4. *Viomycidine*

Viomycidine, a product of acid hydrolysis of the antibiotic viomycin (21, 71, 96), was first assigned the structure 3-guanido-Δ¹-pyrroline-2-carboxylic acid (35, 36, 66, 199), believed to arise from a dihydropyrrolopyrimidine unit in the intact antibiotic (35, 36).



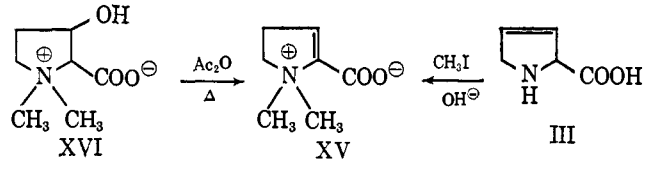
Recently alternative structures for viomycidine (67) and viomycin (68) have been proposed; viomycidine (2-guanido-Δ¹-pyrroline-5-carboxylic acid) is interpreted as arising from a dihydropyrrolotriazine unit in viomycin (68).



C. COMPOUNDS OF STRUCTURAL TYPE II

Δ²-Pyrroline-2-carboxylic acid is known only as N-substituted derivatives such as the betaine and N-acetyl derivative.

The betaine XV (prisms, m.p. 235°) was obtained by dehydration of *cis*- or *trans*-3-hydroxystachydrine (XVI) using hot acetic anhydride (54) and by methylation of 3,4-dehydroproline (III) using methyl iodide and alkali (213). Catalytic hydrogenation of XV gave DL-stachydrine and some δ-dimethylaminovaleric acid (54). The 2,3-position of the double bond was confirmed by the n.m.r. spectrum (213) in which one olefinic proton and two equivalent methyl groups were observed.



XVI XV III

N-Acetyl-Δ²-pyrroline-2-carboxylic acid (m.p. 135°, $\lambda_{max}^{CH_3OH}$ 256 mμ (ε 2900)) was prepared (98) by acetylation of δ-amino-α-ketovaleric acid and obtained in 21% yield.

D. COMPOUNDS OF STRUCTURAL TYPE III

1. *3,4-Dehydroproline*

a. Synthesis

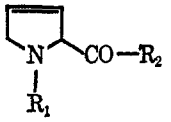
3,4-Dehydroproline so far has not been found in nature. It was first synthesized by reduction of pyrrole-2-carboxamide with phosphonium iodide in hydriodic acid (sp. gr. 1.96) and isolated as the copper salt in 25% yield (73).

Recently this procedure was improved (210, 212); reduction of pyrrole-2-carboxamide at room temperature with phosphonium iodide in aqueous hydriodic acid (saturated at −20° with hydrogen iodide) and separation of the products by ion-exchange techniques afforded 3,4-dehydroprolinamide and 3,4-dehydroproline in yields of 70 and 10%, respectively.

b. Properties (212)

3,4-Dehydro-DL-proline formed prisms, m.p. 236–237°. The 60-Mc./sec. n.m.r. spectrum (solvent, TFA–TMS) showed peaks for protons on carbon at τ 3.88 (2 protons), 4.66 (1 proton), and 5.49 (2 protons).

TABLE I



DERIVATIVES OF 3,4-DEHYDRO-DL-PROLINE

R_1	R_2	M.p., °C.	Crystal form	Ref.
H	OH	236–237	Prisms	212
H	NH_2	95–96	Needles	212
H·HCl	OMe	134–136	...	212
H·HCl	NH_2	190–192	Needles	213
Ac	OH	128–130	Needles	155
Ac	NH_2	192–193	Needles	155
Cbzo	OH	...	Oil	117
Cbzo	NH_2	132–133	...	117
Cbzo	gly-OH	168.5–169.5	...	117
C_6H_5CO	OH	137–141	Prisms	117
C_6H_5CO	NH_2	189–191	Plates	117
C_6H_5CO	gly-OH	160–161	...	117
Tos	OH	141–144	Prisms	117
Tos	OMe	97.5–98.5	...	117
Tos	NH_2	202–204	Prisms	117
Tos	gly-OH	236–237	...	117
Tos	gly-OEt	84–85	...	117
PAB[a]	OMe	112–114	...	213
PAB[a]	NH_2	204.5	Plates	213
CO—NH		153–155	...	213

[a] PAB = *p*-phenylazobenzoyl.

The amide had peaks of similar relative area at τ 3.87, 4.66, and 5.50. The amino acid could be detected on paper chromatograms by the yellow-orange color formed upon treatment with ninhydrin. For the derivatives, see Table I.

c. Stereochemistry (212)

3,4-Dehydro-DL-proline was resolved either enzymatically, by the action of hog kidney amidase (method, 31) on 3,4-dehydro-DL-prolinamide or, alternatively, by fractional crystallization of the (+)-α-bromocamphor-π-sulfonate of 3,4-dehydro-DL-prolinamide. The less-soluble salt afforded (+)-3,4-dehydroprolinamide having $[\alpha]^{20}D$ +300° (*c* 2.0, H_2O), which upon catalytic hydrogenation gave prolinamide hydrochloride, $[\alpha]^{20}D$ +59° (*c* 2.0, EtOH).

Production of 3,4-dehydro-L-proline by the action of hog kidney amidase on 3,4-dehydro-DL-prolinamide exceeded 50% (approximately 75%), the first occasion on which this theoretical possibility had been experimentally realized. This phenomenon results from the greater optical lability of the amide compared with the amino acid. Spontaneous racemization of the D isomer proceeds simultaneously with enzymic cleavage of 3,4-dehydro-L-prolinamide.

3,4-Dehydroproline, $[\alpha]^{20}D$ −385° (*c* 1.0, H_2O) and −257° (*c* 1.0, 5 *N* HCl) (Lutz–Jirgensons rule (148, 149) obeyed), contained only 3% of 3,4-dehydro-D-proline (manometric assay with D-amino acid oxidase) and on catalytic hydrogenation gave L-proline, $[\alpha]^{20}D$ −82° (*c* 2.0, H_2O).

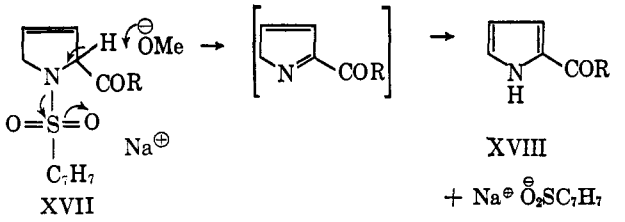
d. Optical Stability (212)

3,4-Dehydro-L-proline (2%) in water (pH 6) was unchanged in rotation after 1 week at 20° but at pH 11.5 (NH_4OH) and pH 14 (NaOH) racemized with half-lifetimes of 17 and 9.5 days, respectively. On the steam bath in 1.0 *N* sodium hydroxide, racemization was complete within 30 min.

3,4-Dehydro-D-prolinamide in water (pH 9.5) racemized at 20° with a half-lifetime of 48 hr.; on the steam bath racemization was complete after 30 min.

e. Chemical Reactions of 3,4-Dehydroproline

N-*p*-Toluenesulfonyl-3,4-dehydroproline methyl ester, amide, or glycine peptide (but not the free acid) undergoes elimination of *p*-toluenesulfinic acid in alkali or methoxide, as demonstrated spectroscopically by the appearance of a new maximum at 265 mμ due to formation of the corresponding pyrrole (213). The methyl ester XVII gave methyl pyrrole-2-carboxylate (XVIII, R = OCH_3) in high yield.



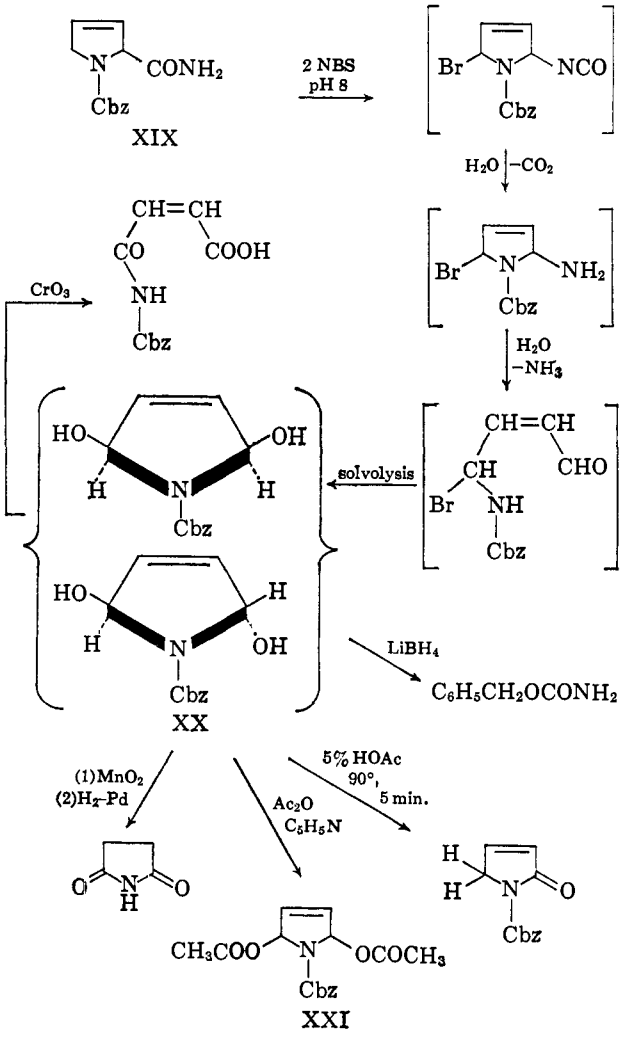
XVII (R = OCH_3, NH_2, or $NHCH_2COOH$)

XVIII + $Na^{\oplus}\,\overset{\ominus}{O}_2SC_7H_7$

The hydantoin of 3,4-dehydroproline was prepared in the usual way with cyanate, and also by the action of hot aqueous alkali upon N-carbobenzyloxy-3,4-dehydro-DL-prolinamide (213); in both cases n.m.r. evidence showed that the double bond remained in the 3,4-position. The racemic hydantoin had a melting point of 153–155°.

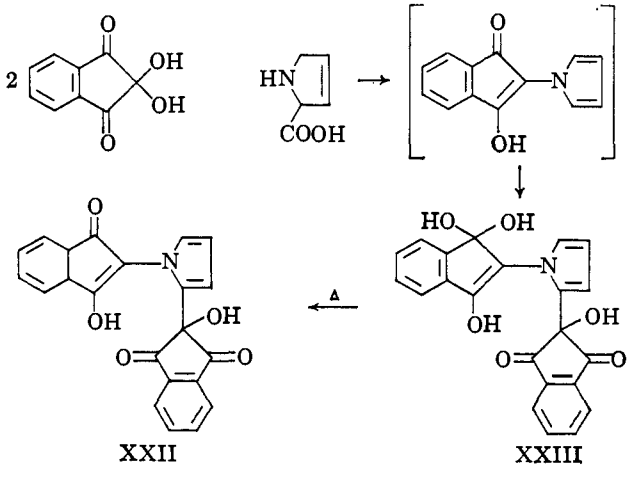
The most remarkable feature of the chemistry of 3,4-dehydroproline emerged from a study of the action of N-bromosuccinimide upon N-carbobenzyloxy-3,4-dehydro-DL-prolinamide (XIX) (117, 213). Consumption of 2 moles of reagent was accompanied by evolution of 1 mole of ammonia and the formation, in 60% yield, of the unusual dicarbinolamide, N-carbobenzyloxy-2,5-dihydroxy-Δ^3-pyrroline (XX). The analogous N-*p*-toluenesulfonyl- and N-benzoyl-3,4-dehydroprolinamides did not give corresponding products.

The formation and reactions of the dicarbinolamide XX, which formed a di-O-acetyl derivative (XXI), are summarized below. XX was obtained in two crystalline forms, m.p. 81–82 and 122–124°.

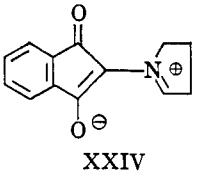


The hydroboration of N-carbobenzyloxy-3,4-dehydro-DL-proline methyl ester and the synthesis of *trans*-3-hydroxy- and -4-hydroxyprolines thereby (113) will be described in the section on 3-hydroxyprolines (III, B1).

3,4-Dehydroproline gives a yellow color with ninhydrin; the product (XXII) and a colorless form (XXIII) have been isolated (214).



The initial reaction is probably similar to that of proline with ninhydrin (120), but the intermediate is a pyrrole which combines with a second ninhydrin molecule. The enol betaine structure XXIV proposed for ninhydrin–proline yellow (120) is supported by the n.m.r. spectrum (155).



f. Biological Studies

3,4-Dehydroproline is a potent inhibitor of protein synthesis in growing carrot phloem explant tissue cultures (241, 242), being ten times as potent an inhibitor as hydroxy-L-proline in this system. The inhibition is reversed by L-proline.

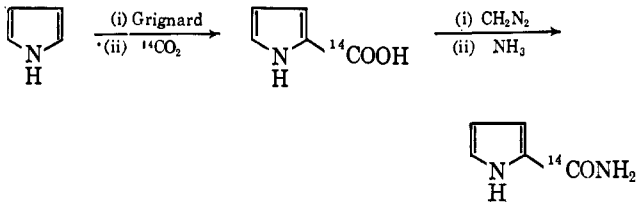
3,4-Dehydro-DL-proline was shown to be a powerful inhibitor of the growth of *L. arabinosus*, *S. lactis*, *P. cerevisiae*, *L. dextranicum*, and *E. coli*. The inhibition was competitively reversed by proline and by various proline peptides (234, 236).

The effect of 3,4-dehydroproline upon the growth of mung bean seedlings and of *E. coli* cultures was studied (74); it proved to be a growth inhibitor, severely so in the case of the bacterium. In seedlings up to 15% of proline residues were replaced by dehydroproline, while in *E. coli* replacement of 60% of proline residues was observed, amounting to almost complete replacement in those protein molecules synthesized after addition of dehydroproline.

3,4-Dehydro-L-proline was a substrate for snake venom L-amino acid oxidase and was converted to pyrrole-2-carboxylic acid (280). D-Amino acid oxidase oxidized both DL and L forms of 3,4-dehydroproline completely to pyrrole-2-carboxylic acid (281); the possibility of racemization during the reaction was excluded.

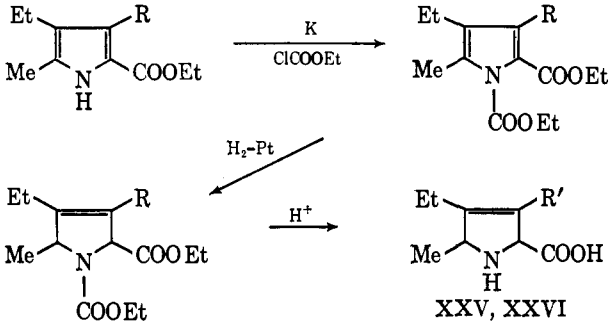
The normal composition (128) of the actinomycin complex produced by *Streptomyces antibioticus* was altered in favor of actinomycin II (containing, *inter alia*, four sarcosines and no proline (121)) and actinomycin III (containing, *inter alia*, three sarcosines and one proline (121)) at the expense of actinomycin IV (two sarcosines and two prolines (44)) in the presence of 3,4-dehydroproline (131). The over-all inhibition of actinomycin production was small.

3,4-Dehydro-DL-proline carboxyl-^{14}C has been synthesized from pyrrole-2-carboxamide labeled in the carboxamide group as follows (215).



2. *Alkyl-Substituted 3,4-Dehydroprolines*

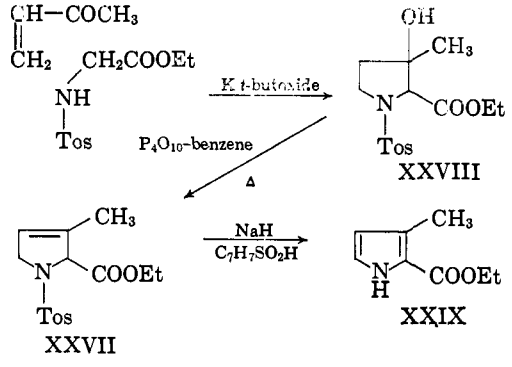
The synthesis of 4-ethyl-3,5-dimethyl-3,4-dehydroproline (XXV) and 3-carboxymethyl-4-ethyl-5-methyl-3,4-dehydroproline (XXVI) (295) is summarized as



XXV: R′ = Me; needles, m.p. 230° (dec.); N-acetyl derivative, needles, m.p. 154° (*via* R = Me)

XXVI: R′ = CH_2COOH; needles, m.p. 249° (dec.) (*via* R = CH_2COOEt)

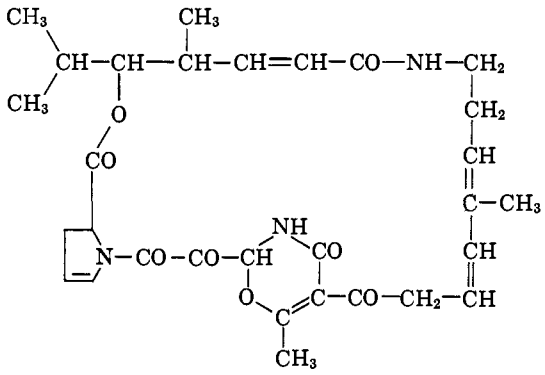
3-Methyl-N-*p*-toluenesulfonyl-3,4-dehydroproline ethyl ester (XXVII) was prepared by dehydration of the alcohol XXVIII obtained by base-catalyzed condensation of N-*p*-toluenesulfonylglycine ethyl ester with methyl vinyl ketone (119). It eliminated *p*-toluenesulfinic acid in the presence of strong base giving ethyl 3-methylpyrrole-2-carboxylate (XXIX).



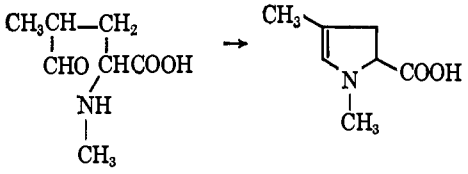
E. COMPOUNDS OF STRUCTURAL TYPE IV

(i) 4,5-Dehydroproline is not known, but it occurs bound in the antibiotic ostreogrycin A, for which the structure shown below has been proposed (257). Hydrolysis of perhydroostreogrycin A gave D-proline, while ozonolysis followed by oxidation and hydrolysis furnished aspartic acid, which indicated the 4,5-position for the double bond.

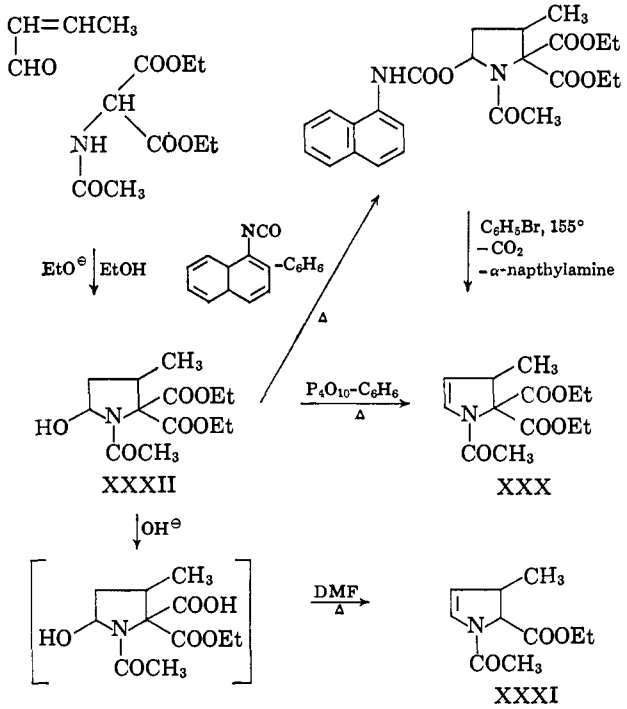
(ii) N,4-Dimethyl-4,5-dehydroproline was found among the hydrolysis products of the antibiotic ilamycin (252, 253); it arises by cyclization from the N,4-dimethylglutamic 4-semialdehyde residue in the cyclopeptide. Specific cleavage of the peptide was achieved through solvolysis of the O-mesyldihydro derivative (115) of rufomycin A (76), which is identical with ilamycin (252, 253).



ostreogrycin A



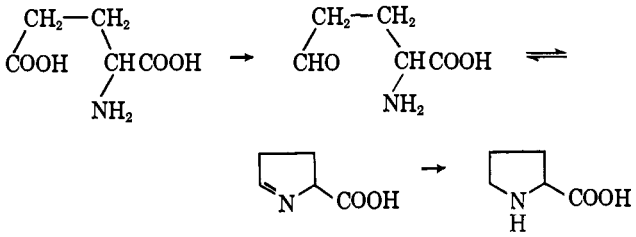
(iii) N-Acetyl-5,5-diethoxycarbonyl-4-methyl-Δ^2-pyrroline (XXX) and N-acetyl-4,5-dehydro-3-methylproline ethyl ester (XXXI) have been synthesized from N-acetyl-2,2-diethoxycarbonyl-5-hydroxy-3-methylpyrrolidine (XXXII) as outlined below; XXXI was separated into *cis* and *trans* racemates by silica gel chromatography (see part IV, C1b) (155).



F. COMPOUNDS OF STRUCTURAL TYPE V

1. Δ^1-*Pyrroline-5-carboxylic Acid*

Δ^1-Pyrroline-5-carboxylic acid is related to glutamic γ-semialdehyde in the same way that Δ^1-pyrroline-2-carboxylic acid is related to δ-amino-α-ketovaleric acid; it has long been postulated as an intermediate in the biosynthesis of proline from glutamic acid (84, 143, 229, 238, 249, 276).



L-Δ^1-Pyrroline-5-carboxylic acid was identified with a *substance A* which accumulated in the culture filtrate of a proline-requiring mutant of *Escherichia coli* (55-1) and which was believed to be a proline precursor (276). *Substance A* supported the growth of another proline auxotroph (55–25). Mutant 22-64 responded to proline, *substance A*, or glutamic acid. Although *substance A* could not be isolated pure from mutant 55-1, its identity with L-Δ^1-pyrroline-5-carboxylic acid was demonstrated by paper chromatography (yellow color with *o*-aminobenzaldehyde) and by bioassay with mutant 55-25. DL-Δ^1-Pyrroline-5-carboxylic acid was prepared (276), but not characterized, by acid hydrolysis of γ,γ-dicarbethoxy-γ-acetamidobutyraldehyde (173); its catalytic hydrogenation gave DL-proline.

A purer preparation of DL-Δ^1-pyrroline-5-carboxylic acid was obtained by the same route and the use of ion-exchange chromatography (243). Its hydrochloride, a granular solid which darkened at 150°, was stable over a desiccant at 5°. On paper electrophoresis it migrated as a single spot (pink color with ninhydrin) distinguishable from Δ^1-pyrroline-2-carboxylic acid. Reduction with sodium borohydride gave DL-proline. Unusual instability in solution indicates both equilibrium with glutamic γ-semialdehyde and polymerization. By reaction with 2,4-dinitrophenylhydrazine, DL-glutamic γ-semialdehyde 2,4-dinitrophenylhydrazone (hydrochloride m.p. 170–171°) was obtained.

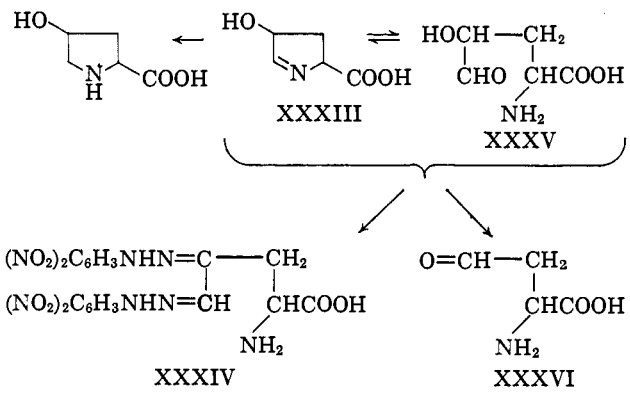
DL-Δ^1-Pyrroline-5-carboxylic acid, m.p. 140–142° (dec.), has also been synthesized by the action of hot aqueous baryta upon 3,3-dichloro-2-pyridone (194, 195).

Enzymes catalyzing reduction of Δ^1-pyrroline-5-carboxylic acid to proline have been described in microorganisms (161, 297) and in rat tissues (161, 235). An enzyme from calf liver which catalyzes the reduction of V to proline by di- or triphosphopyridine nucleotide has been purified (198); it is specific for L-Δ^1-pyrroline-5-carboxylic acid. The reduction is irreversible, indicating that the oxidation and reduction pathways are different.

2. *3-Hydroxy-Δ^1-pyrroline-5-carboxylic Acid*

L-3-Hydroxy-Δ^1-pyrroline-5-carboxylic acid was prepared by enzymic oxidation of hydroxy-L-proline with a beef kidney homogenate (6, 8); it was separated from unchanged hydroxy-L-proline by ion-exchange chromatography, purified further on columns of ion-exchange resin and Norite, and obtained as an unstable, pale-yellow glass. On paper chromatograms it appeared as a single spot on treatment with ninhydrin (purple or brown), alloxan (orange or red), isatin (pale blue), or *o*-aminobenzaldehyde (deep yellow). In dilute solution at −10° it was stable for months. In the infrared spectrum a peak at 6 μ was ascribed to C=N stretching by analogy with 2,5-dimethyl-Δ^1-pyrroline (70) and myosmine (290).

L-3-Hydroxy-Δ^1-pyrroline-5-carboxylic acid (XXXIII) formed a crystalline reineckate, m.p. 226–232° (dec.), while reaction with 2,4-dinitrophenylhydrazine afforded a 2,4-dinitrophenylosazone (XXXIV) of L-γ-hydroxyglutamic γ-semialdehyde (XXXV). Reduction of XXXIII with sodium borohydride or hydrogen over platinum gave hydroxy-L-proline; other isomers were not detected. Oxidation of XXXIII with sodium periodate gave L-aspartic semialdehyde (XXXVI).



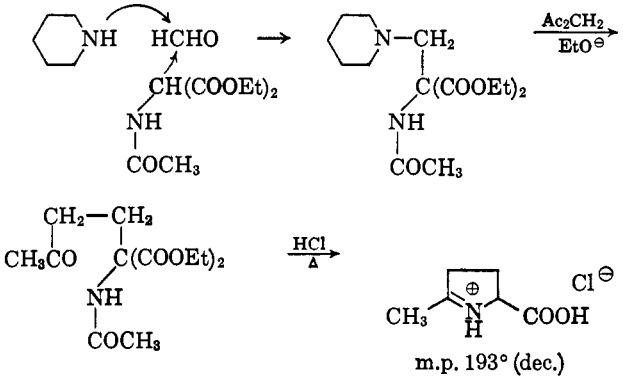
In the equilibrium between XXXIII and XXXV the cyclic form is favored; the borohydride reduction gives hydroxy-L-proline and no γ,δ-dihydroxy-L-norvaline. Moreover, L-3-hydroxy-Δ^1-pyrroline-5-carboxylic acid behaves on Dowex-1 acetate like an acidic, rather than neutral, amino acid; clearly the nitrogen function is only weakly basic (6, 8).

3. *Alkyl-Substituted Δ^1-Pyrroline-5-carboxylic Acids*

2-Methyl-Δ^1-pyrroline-5-carboxylic acid hydrochloride has been synthesized by routes A, B, and C below.

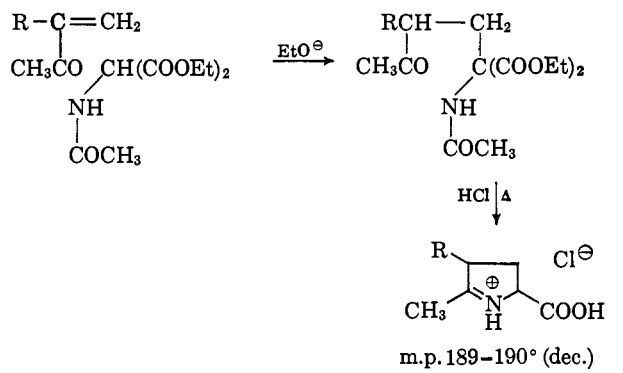
2,3-Dimethyl-Δ^1-pyrroline-5-carboxylic acid hydrochloride, m.p. 153–154°, was also prepared by route B (R = CH_3) (81).

Route A (217)



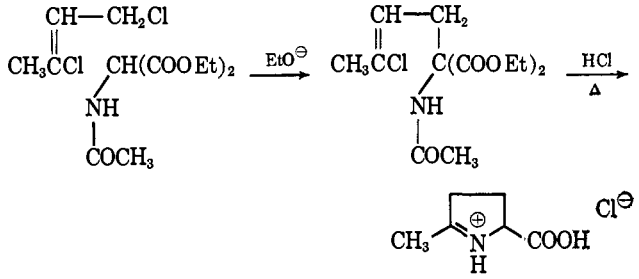
m.p. 193° (dec.)

Route B (81) (R = H)



m.p. 189–190° (dec.)

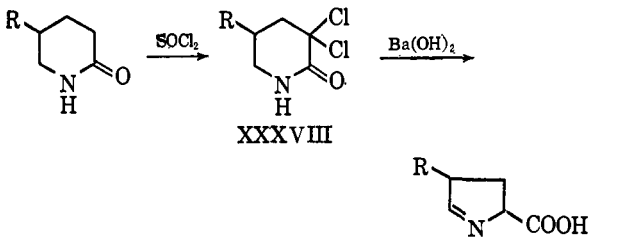
Route C (82)



m.p. 193.5–195° (dec.)

3-Alkyl-Δ^1-pyrroline-5-carboxylic acids (XXXVII) have been synthesized from 5-alkyl-3,3-dichloro-2-piperidones (XXXVIII) (194, 195).

Route D

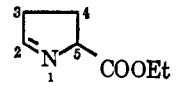


XXXVIII

XXXVII, R = isopropyl, m.p. 130° (dec.)

XXXVII, R = *n*-propyl and R = 1-methyl-2-ethoxyethyl, were also prepared and hydrogenated directly to the corresponding proline analogs (part IV, C2).

TABLE II

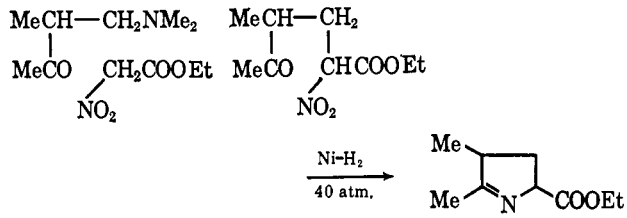


SUBSITUTED ETHYL Δ^1-PYRROLINE-5-CARBOXYLATES

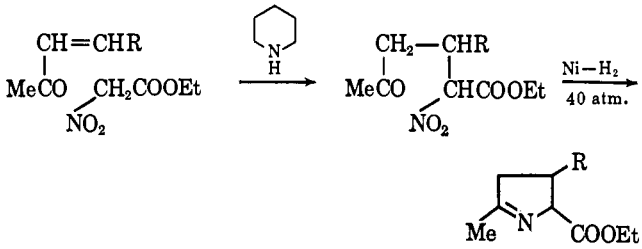
2-	3-	4-	Characterization	Ref.
Methyl	Methyl	H	Picrolonate, m.p. 140°	193
Methyl	H	Methyl	Picrolonate, m.p. 135°	193
Methyl	H	Isopropyl	Picrolonate, m.p. 198°	193
Phenyl	H	Phenyl	Picrate, m.p. 155–156°	61

A series of substituted ethyl Δ^1-pyrroline-5-carboxylates (see Table II) were synthesized as follows (82).

Route E



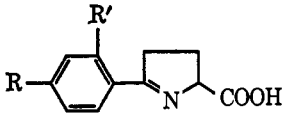
Route F (R = methyl or isopropyl)



4. *Aryl-Substituted Δ^1-Pyrroline-5-carboxylic Acids*

2-Phenyl-Δ^1-pyrroline-5-carboxylic acid (see Table III) was first synthesized (81) by route B above (R = H, phenyl in place of methyl).

TABLE III



SUBSTITUTED 2-PHENYL-Δ^1-PYRROLINE-5-CARBOXYLIC ACIDS (83)

R	R'	Hydrochlorides, m.p., °C.	Dihydro derivatives,[a] m.p., °C.
H	H	169–173	115–117
F	H	105–110	140
Cl	H	104–109	148–153
Br	H	192–194	93
CH_3	H	70	108–111
C_6H_5	H	90	190
CH_3O	H	105–108	175–179
C_6H_5O	H	123	182–190
$NH_2 \cdot HCl$	H	252	137
F	F	111–114	174–177
Cl	Cl	171–174	203–205

[a] 5-Arylproline analogs (see part IV, D2).

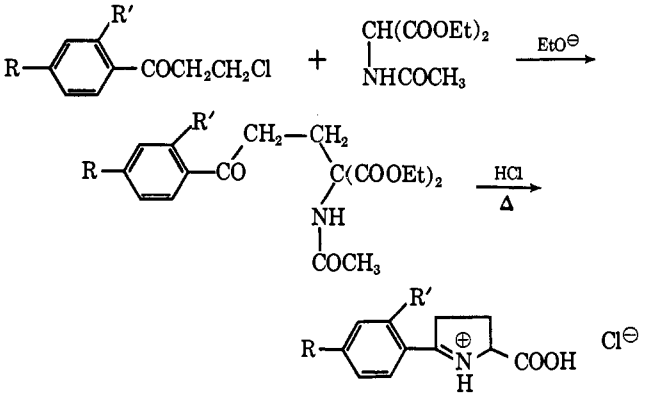
TABLE IV

ISOMERS OF 3-HYDROXYPROLINE

Source	Isomer	M.p., °C.	Rotation	Solvent	Rotation	Solvent	Ref.
Sponge	L-*trans*	>200	$[\alpha]^{20}D$ −17.4°	*c* 1.0, H_2O	$[\alpha]^{20}D$ +13.3°	*c* 0.5, 1 *N* HCl	113
Telomycin	L-*trans*	...	$[\alpha]^{20}D$ −15.3°	*c* 1.0, H_2O	$[\alpha]^{20}D$ +17.4°	*c* 0.5, 1 *N* HCl	113
Synthetic	L-*trans*	...	$[\alpha]^{20}D$ −17.9°	*c* 0.34, H_2O	...	...	177
Telomycin	L-*cis*	...	$[\alpha]^{20}D$ −91.5°	*c* 0.61, H_2O	$[\alpha]^{20}D$ −54.3°	*c* 0.5, 1 *N* HCl	113
Synthetic	L-*cis*	...	$[\alpha]^{20}D$ −99.0°	*c* 0.20, H_2O	...	...	177
Synthetic	L-*cis*	245–255 (dec.)	$[\alpha]^{25}D$ −90.3°	H_2O	$[\alpha]^{25}D$ −47.9°	5 *N* HCl	227
Synthetic	DL-*trans*	224–230 (dec.)	...	...	...	...	227
Synthetic	DL-*cis*	225–235 (dec.)	...	...	...	...	227
Synthetic	DL-*trans*	222–224 (dec.)	...	...	...	...	32
Synthetic	DL-*cis*	223–225 (dec.)	...	...	...	...	32

Ethyl 2,4-diphenyl-Δ^1-pyrroline-5-carboxylate (see Table II) was prepared (61) *via* a variant of route F above.

2-Substitution in Δ^1-pyrroline-5-carboxylic acids enhances stability; a series of 2-aryl-Δ^1-pyrroline-5-carboxylic acids (see Table III) has been synthesized (83) by the following general route.



None of the compounds in the series possessed antitumor activity (S-180, L-1210, and C-755 or Ehrlich ascites).

III. PROLINE ANALOGS HAVING OXYGEN-, HALOGEN-, NITROGEN-, OR SULFUR-CONTAINING SUBSTITUENTS

A. INTRODUCTION

In addition to the well-known occurrence of hydroxy-L-proline in nature, allohydroxy-L-proline has been found both in the free state and in peptides, and allohydroxy-D-proline and 4-oxo-L-proline occur in peptide antibiotics. Recently *trans*-3-hydroxy-L-proline has been isolated from collagens, and both *cis*- and *trans*-3-hydroxy-L-prolines have been identified in a peptide antibiotic. Various betaines of 3- and 4-hydroxyproline also occur naturally. Synthetic studies have kept pace with these developments.

The analogs having halogen-, nitrogen-, and sulfur-containing substituents are synthetic.

B. OXYGENATED PROLINES

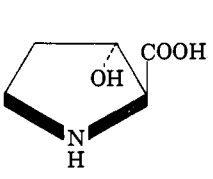
1. *3-Hydroxyproline*

a. Occurrence and Isolation

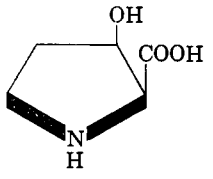
A new amino acid which had been observed (202, 203) in hydrolysates of collagen from spongins A and B was identified as *trans*-3-hydroxyproline by comparison with a synthetic sample (113) in the automatic amino acid analyzer (237).

During a study of the peptides formed by partial enzymic hydrolysis of collagen, a tripeptide gly-X-hypro was found, X being a hitherto unknown amino acid (190, 191); X was later isolated from hydrolysates of cattle achilles tendon collagen, in which it occurs to the extent of 0.26%, by ion-exchange chromatography (192). X gave a yellow ninhydrin color, could be reduced to proline by phosphorus and hydriodic acid, and gave β-alanine on permanganate oxidation. The amino acid was identical with synthetic 3-hydroxyproline (192).

trans-3-Hydroxy-L-proline (XXXIX) was isolated from hydrolysates of Mediterranean sponge (111, 113) and both *trans*- and *cis*-3-hydroxy-L-proline (XL) were



XXXIX

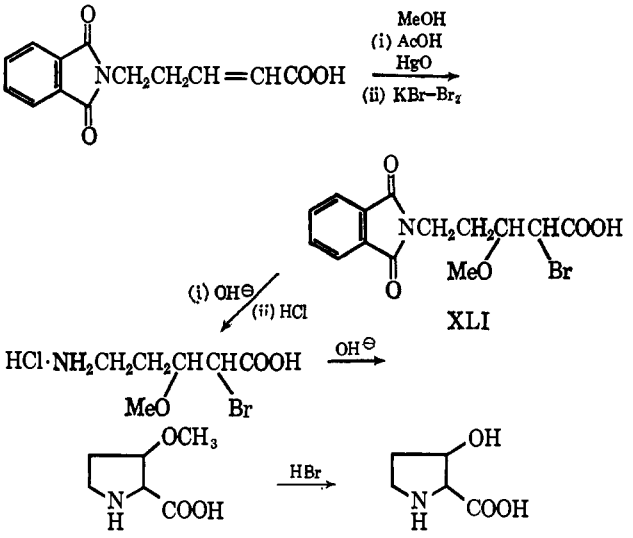


XL

isolated (112, 113) from the peptide antibiotic telomycin (164), separated by ion-exchange chromatography, and identified by synthesis (111, 112, 177). Their properties are summarized in Table IV. In an independent study of telomycin, for which a structure has been proposed (226), the two cyclic imino acids were identified (225, 227) by comparison with synthetic *cis*- and *trans*-3-hydroxy-DL-prolines.

b. Synthesis and Chemistry of 3-Hydroxyprolines

(i) *cis*- and *trans*-3-hydroxy-DL-prolines were formed *via cis*- and *trans*-3-methoxy-DL-prolines (225, 227).



The separation of 2-bromo-3-methoxy-5-phthalimidopentanoic acid (XLI) into its diastereoisomers A and B was achieved by fractional recrystallization. Isomer A gave rise to *cis*-3-methoxy-DL-proline, while isomer B gave a mixture of this and *trans*-3-methoxy-DL-proline which were separated through the copper salts. The respective L isomers were obtained through the action of D-amino acid oxidase on the two racemates. The properties of the various 3-methoxyprolines are recorded in Table V.

trans-3-Methoxy-L-proline was oxidized by permanganate to a methylsuccinic acid which was converted to L-methoxysuccinamide, providing proof of stereochemistry. Thus the stereochemistry of the 3-hydroxyprolines obtained by demethylation was established, and it was shown that the "slow-moving" and "fast-moving" 3-hydroxyprolines in telomycin were *trans* and *cis*, respectively.

(ii) A synthesis of racemic 3-hydroxyproline by a route similar to the foregoing scheme was reported (192).

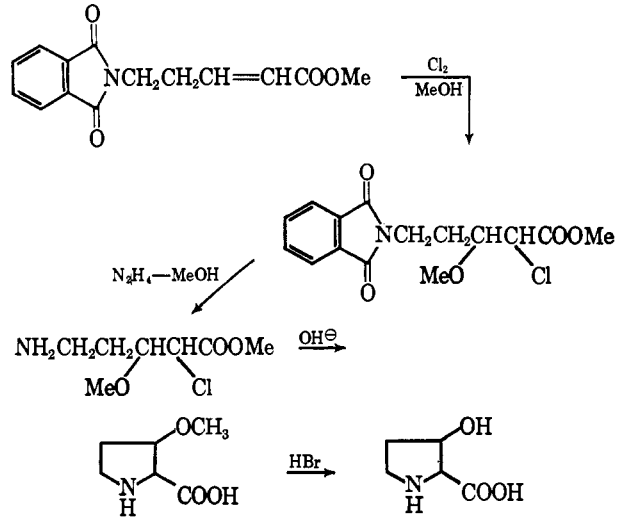
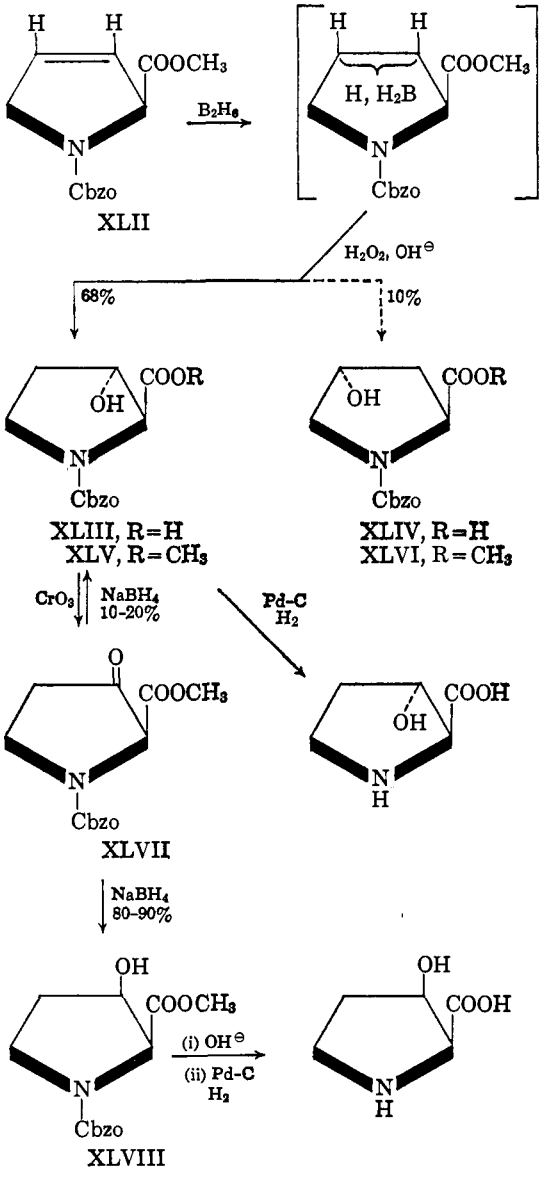


TABLE V

PROPERTIES OF 3-METHOXYPROLINES (227)

Isomer	M.p., °C.	$[\alpha]^{25}D$, c 1, H_2O	$[\alpha]^{25}D$, c 1, 5 N HCl
DL-*cis*	205.5–206.5	...	...
DL-*trans*	184–185	...	...
L-*cis*	212–214	− 110.8°	− 65.7°
L-*trans*	216.5–219	− 25.3°	+7.8°

(iii) Hydroboration of N-carbobenzyloxy-3,4-dehydro-DL-proline methyl ester (XLII) gave a mixture of hydroxyproline derivatives XLIII and XLIV. After hydrogenolytic removal of the carbobenzyloxy groups, the products were *trans*-3-hydroxy-DL-proline (68%), 4-hydroxy-DL-proline (10%), and a trace of 4-allohydroxy-DL-proline (113). In an alternate procedure, by the use of potassium carbonate in place of sodium



(L forms only are shown)

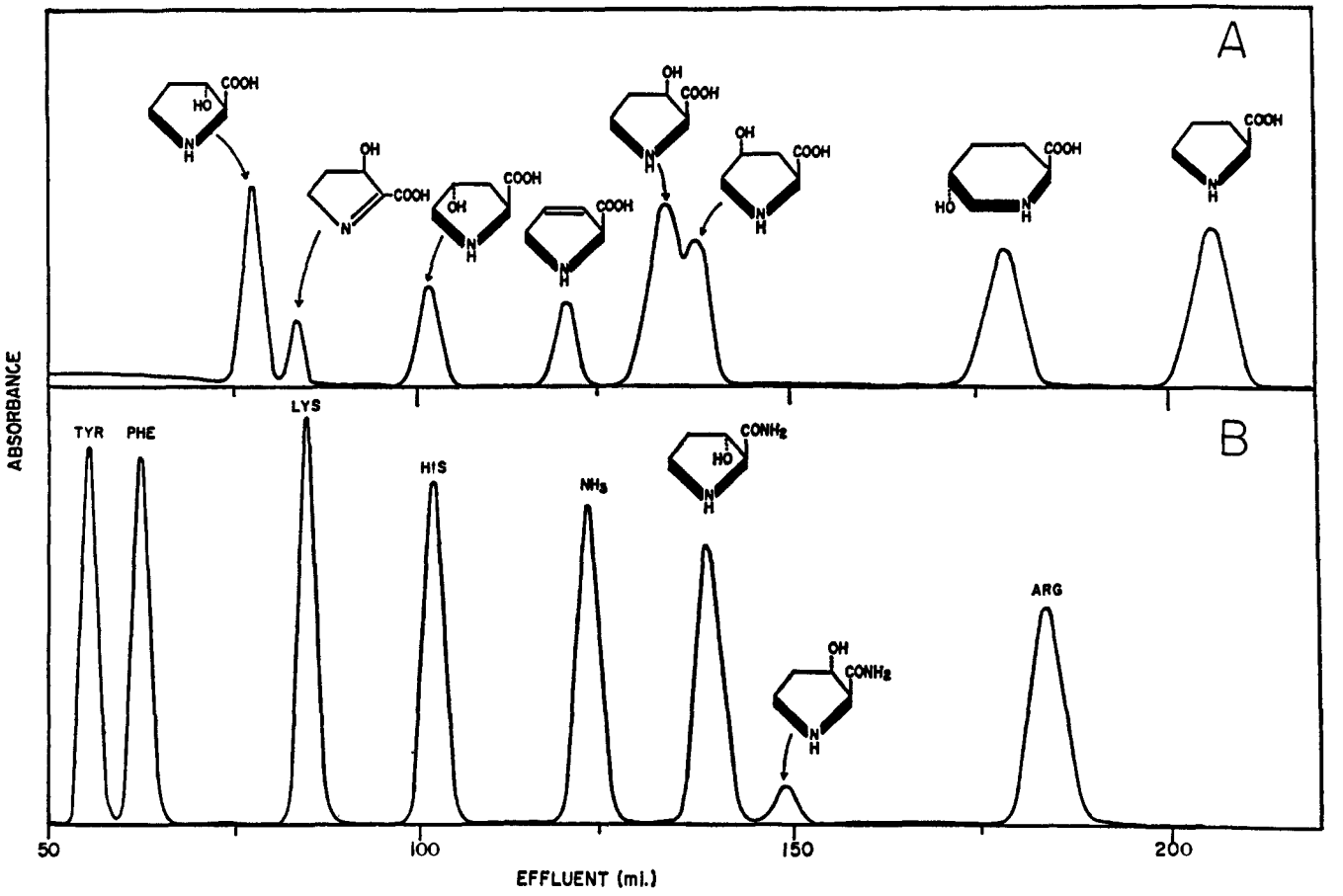


Figure 1.—Position of the cyclic secondary amino acids on the automatic amino acid analyzer: A, the pyrrolidine- and piperidinecarboxylic acid derivatives were eluted from a 150-cm. column of Amberlite IR-120 at 30 and 50°, with 0.2 *N* sodium citrate, pH 3.25 and 4.25. The ninhydrin values of the following amino acids were determined at λ 440 mμ: *trans*-3-hydroxy-L-proline (1 mμ), 3-hydroxy-Δ^1-pyrroline-2-carboxylic acid (0.77 mμ), *trans*-4-hydroxy-L-proline (1 mμ), 3,4-dehydro-DL-proline (4 mμ), *cis*-3-hydroxy-L-proline (1 mμ), *cis*-4-hydroxy-D-proline (1 mμ), 5-hydroxy-L-pipecolic acid (2 mμ). DL-Pipecolic acid (4 mμ) was read at 570 mμ. B, the basic amino acids (0.25 μmole of the markers, ~4 μmoles of the *trans*- and *cis*-3-hydroxyproline amide) were eluted from a 50-cm. column of Amberlite IR-120 at 50°, with 0.7 *N* sodium citrate (pH 5.28). Ninhydrin colors were read at λ 570 mμ.

hydroxide, saponification did not occur and the esters XLV and XLVI were obtained and separated by silicic acid chromatography. Thus Brown's observations (43) on the stereospecificity of the hydroboration reaction were extended to the heterocyclic series. These results parallel the hydroboration of N-carbobenzyloxybaikiain methyl ester, which gave rise to *trans*-5-hydroxy-DL-pipecolic acid (72%) and *trans*-4-hydroxy-DL-pipecolic acid (28%) (77).

cis-3-Hydroxy-DL-proline was synthesized *via* sodium borohydride reduction of N-carbobenzyloxy-3-ketoproline methyl ester (XLVII), obtained by chromic acid oxidation of XLV. The reduction products XLV and XLVIII were separated by chromatography on silicic acid and the protecting groups removed, giving 80–90% *cis*- and 10–20% *trans*-3-hydroxy-DL-prolines. The formation of predominantly *cis*-3-hydroxyproline is analogous to the reduction of carbobenzyloxy-4-ketoproline to carbobenzyloxy-4-allohydroxyproline by sodium borohydride (196). Attack by borohydride ions from the less hindered side of the molecule is only one of the several aspects to be considered.

During oxidation of carbobenzyloxy-3-hydroxyproline methyl ester (mixture of *cis* and *trans* racemates), it was shown by vapor phase chromatography that the *cis* form was oxidized more rapidly than the *trans* owing to release of Pitzer strain. On the other hand, saponification of carbobenzyloxy-3-hydroxyproline methyl ester was expectedly more rapid for the *trans* isomer than for the *cis*.

In the n.m.r. spectra coupling between the C-2 and C-3 protons was greater in the *cis* case than the *trans;* the C-2 proton signals appeared as doublets, $J = 4$ and 1 c.p.s. in the *cis* and *trans* isomers, respectively.

The enzymatic and chemical oxidation of *cis*- and *trans*-3-hydroxyprolines to 3-hydroxy-Δ^1-pyrroline-2-carboxylic acid has been described in part II.

The natural *cis*- and *trans*-3-hydroxyprolines were compared with the synthetic racemates with regard to paper chromatography, infrared spectra of the N-*p*-toluenesulfonyl methyl esters, and by the use of the automatic amino acid analyzer (237) (see Figure 1).

Racemic *cis*- and *trans*-3-hydroxyprolines were resolved into their optical antipodes by the action of

TABLE VI

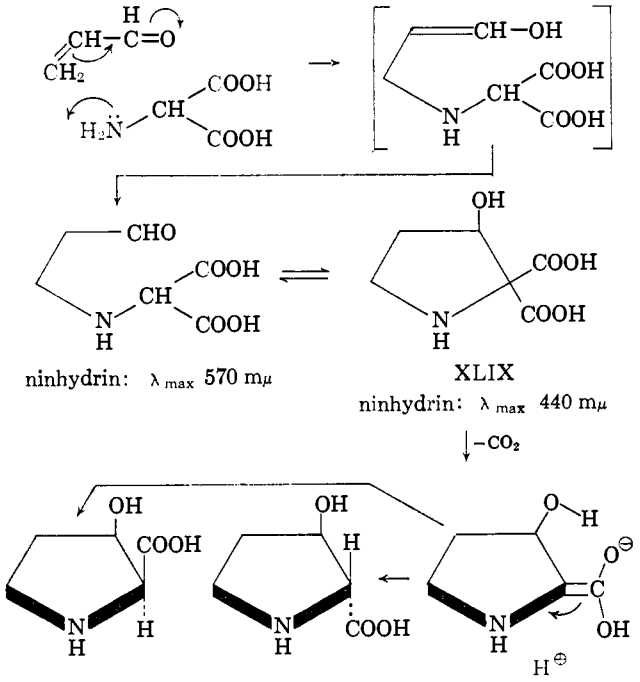
ANALYSIS OF *cis*- AND *trans*-3- AND -4-HYDROXYPROLINE DERIVATIVES BY VAPOR PHASE CHROMATOGRAPHY

Conditions	Compound	Retention time, min.
2% QF-1, gaschrom A, 210°, 170 × 0.4 cm., argon, 10 p.s.i.	N-Cbz-$\Delta^{3,4}$-dehydroproline methyl ester	1.5
	N-Cbz-*trans*-3-hydroxyproline methyl ester	3.3
	N-Cbz-*cis*-3-hydroxyproline methyl ester	3.8
1% SE-30, gaschrom P, 187°, 170 × 0.4 cm., N_2, 24 ml./min.	N-Cbz-O-acetyl derivatives of	
	trans-3-Hydroxyproline methyl ester	9.0
	cis-3-Hydroxyproline methyl ester	10.1
	4-Hydroxyproline methyl ester	10.3
	Allo-4-hydroxyproline methyl ester	11.2
1% SE-30, gaschrom P, 187°, 170 × 0.4 cm., N_2, 24 ml./min.	Carbobenzyloxy-O-trifluoroacetyl derivatives of	
	trans-3-Hydroxyproline methyl ester	3.8
	cis-3-Hydroxyproline methyl ester	4.8
	4-Hydroxyproline methyl ester	4.4
	Allo-4-hydroxyproline methyl ester	5.2
2% NGS[a] gaschrom P, 192°, 170 × 0.4 cm., argon, 10 p.s.i	N-Acetylproline methyl ester	1.2
	N,O-Diacetyl-*trans*-3-hydroxyproline methyl ester	5.0
	N,O-Diacetyl-*cis*-3-hydroxyproline methyl ester	6.3
	N,O-Diacetyl-allo-4-hydroxyproline methyl ester	7.5
2% NGS[a] gaschrom P, 170 × 0.4 cm., N_2, 33 ml./min.	N,O-Bistrifluoroacetyl derivatives of	
	trans-3-Hydroxyproline methyl ester	5.7 (138°)
		4.0 (150°)
	cis-3-Hydroxyproline methyl ester	12.4 (139°)
		6.4 (150°)
	4-Hydroxyproline methyl ester	7.6 (143°)
	Allo-4-hydroxyproline methyl ester[b]	13.5 (143°)

[a] NGS: neopentylglycol succinate. [b] The treatment of allo-4-hydroxyproline with trifluoroacetic anhydride probably does not cause lactonization judging from the comparable retention times of the two 3-hydroxyprolines.

leucine aminopeptidase on the respective amides (177). The optical rotations of the synthetic and natural products are comparable (Table IV).

Stereospecifically tritium-labeled *cis*- and *trans*-3-hydroxy-DL-prolines-3-H^3 were synthesized by reduction of carbobenzyloxy-3-keto-DL-proline methyl ester with sodium borotritide followed by removal of the protecting groups and chromatographic separation (113).



(iv) A one-step synthesis of 3-hydroxyproline was developed from the reaction of acrolein, as its sodium metabisulfite adduct, with aminomalonic acid (177).

The yield of 3-hydroxyprolines was 42% under optimum conditions, in pyridine buffer at 60°, when the *cis–trans* ratio was approximately 1:1. In potassium acetate buffer (pH 4.5) at 60° the yield was 18% and the *cis–trans* ratio 2:1. On the other hand, when the purified intermediate XLIX was kept in acetate buffer (pH 3.5) at room temperature spontaneous decarboxylation gave a *cis–trans* ratio of 1:2 (see above).

Vapor phase chromatographic analysis of *cis*- and *trans*-3- and -4-hydroxyproline derivatives is summarized in Table VI.

(v) 3-Hydroxyprolines (*cis–trans* ratio 1:2) were synthesized *via* sodium borohydride reductions of N-ethoxycarbonyl-3-ketoproline ethyl ester (L) or the corresponding nitrile (LI) which were obtained by Dieckmann condensations as shown below (32).

The mixture of *cis* and *trans* racemates was separated by ion-exchange chromatography. Either diastereoisomer on heating under reflux in aqueous barium hydroxide reverted to the original isomeric mixture, *cis–trans* ratio 1:2.

In the infrared spectra the O–H stretching absorption occurred at the same wave number (3590 $cm.^{-1}$) in both isomers, indicating little or no intramolecular hydrogen bonding in the *cis* isomer.

In the n.m.r. spectra $J_{2,3}$ values were 4 and <1 c.p.s. for the *cis* and *trans* isomers, respectively (in D_2O);

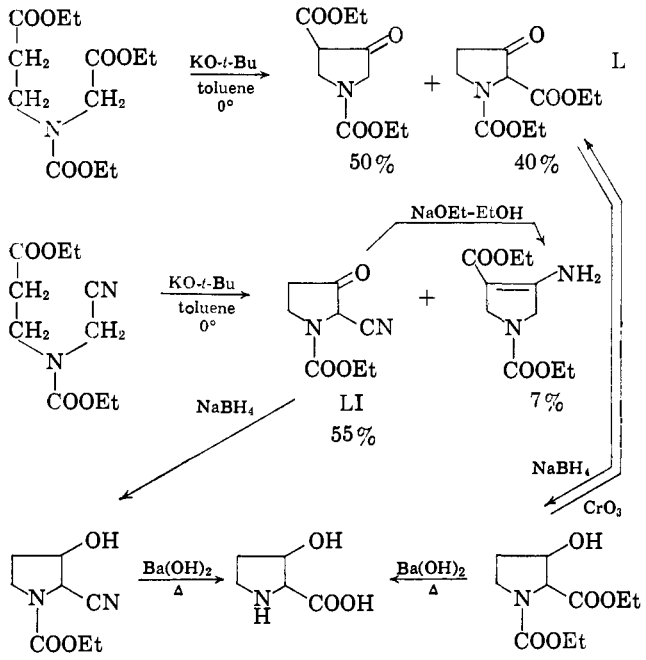
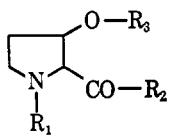


TABLE VII



DERIVATIVES OF 3-HYDROXYPROLINE

R_1	R_2	R_3	Isomer	M.p., °C.	Ref.
H·HCl	OEt	H	L-*trans*[a]	125–130	192
H	OH	Ac	L-*trans*[a]	205 (dec.)	192
H	OH	Me	L-*trans*	216.5–219	227
H	OH	Me	L-*cis*	212–214	227
H	OH	Me	DL-*trans*	184–185	227
H	OH	Me	DL-*cis*	205.5–206.5	227
Cbzo	OMe	H	DL-*trans*	81–82	113
Cbzo	NH_2	H	DL-*cis*	166–167	177
Tos	OH	H	DL-*trans*	164–168	32
Tos	OH	H	DL-*cis*	160–164	32
Tos	OMe	H	DL-*trans*	99–100	113
				101–102	32
Tos	OMe	H	L-*trans*	101–102	113
Tos	OMe	H	DL-*cis*	127–129	32
2,4-DNP	OH	H	DL-*trans*	89–91	32
2,4-DNP	OH	H	DL-*cis*	173–176	32

[a] From collagen. Other derivatives in this table are synthetic.

in D_2O–CF_3COOH the corresponding values were 3.7 and 2.3 c.p.s. (see Table VII for derivatives).

c. Biochemical Studies

Proline-C^{14} was converted into protein-bound *trans*-3-hydroxy-L-proline-C^{14} in the intact chick embryo (124). In chick embryo collagen one residue of *trans*-3-hydroxy-L-proline was found for every 70 residues of hydroxy-L-proline.

2. 3-Hydroxyproline Betaines

a. Occurrence and Isolation

Two isomeric 3-hydroxystachydrines were isolated from the husks of the mature fruit of *Courbonia virgata* (54) (their properties are shown in Table VIII): 3-hydroxystachydrine-"a" hydrochloride, needles, m.p. 196–197° (dec.); picrate, m.p. 160°; 3-hydroxystachydrine-"b" hydrochloride, prisms, m.p. 210–202° (dec.).

b. Structure Determination

Both isomers on treatment with hot acetic anhydride gave the same anhydro compound, 2,3-dehydroproline betaine (see part II, section C), which had no optical rotation and which gave DL-stachydrine upon catalytic hydrogenation. This evidence sufficed to establish the structure, but it was not known whether isomers "a" and "b" were epimeric at C-2 or C-3.

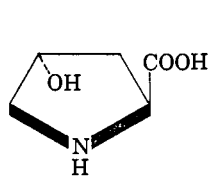
c. Synthesis

cis- and *trans*-3-hydroxy-L-prolines were each converted to the silver salts and treated with methyl iodide, a method which avoids epimerization (216). The *cis*- and *trans*-3-hydroxystachydrines so obtained were identical with the natural products (Table VIII). Both isomers showed weak inhibitory activity in the system acetylcholinesterase–acetylcholine.

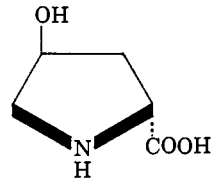
In an independent investigation (228) *cis*- and *trans*-3-hydroxy-DL-stachydrines were synthesized by the same procedure from the two racemic diastereoisomers of 3-hydroxyproline: *trans*-3-hydroxy-DL-stachydrine, prisms, m.p. 232.5–223°; *cis*-3-hydroxy-DL-stachydrine, prisms, m.p. 222–222.5°.

These synthetic studies established that the naturally occurring isomers "a" and "b" were *trans*- and *cis*-3-hydroxy-L-stachydrines, respectively.

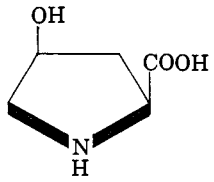
3. 4-Hydroxyproline



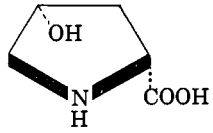
Hydroxy-L-proline



Hydroxy-D-proline



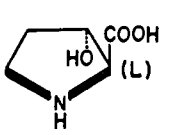
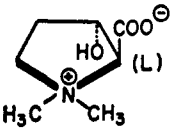
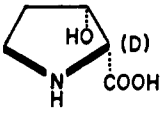
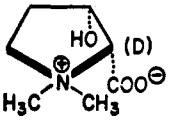
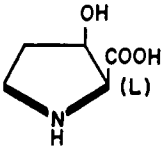
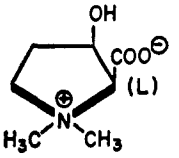
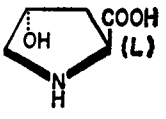
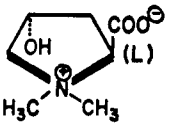
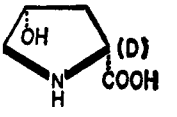
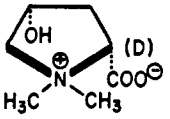
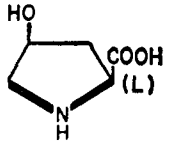
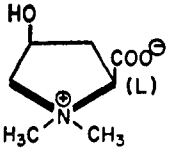
Allohydroxy-L-proline



Allohydroxy-D-proline

TABLE VIII

BETAINES OF 3- AND 4-HYDROXYPROLINES

Parent amino acid	Betaine	M.p., °C.	$[\alpha]^{20}D$ in H_2O	$[\alpha]^{20}D$ in NaOH	Effect on cholinesterase[a]	Ref.
		250 (natural)	+10.0° (30°)		$1.07 \pm 0.01 \times 10^{-4}$	54
trans-3-Hydroxy-L-proline	3-Hydroxystachydrine a	−245 (synthetic)	+9.6°	+33.9– +6.9° (24 hr.)		
		209–210 (natural)	+53.0° (22°)	...	$8.84 \pm 0.57 \times 10^{-4}$	54
cis-3-Hydroxy-D-proline	3-Hydroxystachydrine b					
		210–211 (synthetic)	−46.0°	−32.9– −8.7° (24 hr.)	16×10^{-4}	216
cis-3-Hydroxy-L-proline	*cis*-3-Hydroxy-L-stachydrine (synthetic)					
		252–253	−34.2°	−36.0– 0° (24 hr.)	$8.5 \pm 0.4 \times 10^{-4}$	113
4-Hydroxy-L-proline	L-Betonicine (natural)					
		259–260	+37.8°	+51.1– 0° (24 hr.)	...	196
4-Allohydroxy-D-proline	D-Turicine (natural)					
		252–254	−39.0°	...	No inhibitor	*Cf.* choline: 4.5×10^{-4}; stachydrine: 5.4×10^{-4}
4-Allohydroxy-L-proline	L-Turicine (synthetic)					

[a] K_I (competitive).

a. Occurrence

Hydroxy-L-proline was discovered in gelatin hydrolysate in 1902 (72). Apart from its well-known presence in gelatins and collagens (90, 91), it occurs bound in alfalfa protein (240), sugar-beet protein (231), *Sarcina lutea* (23), dentine protein (147), horseradish peroxidase (152), proteins of insect cuticle (92), and in the antibiotic actinomycin Xoβ (37, 40).

Hydroxy-L-proline occurs in the free state in pollen (19), prunes (122), the haemolymph of *Drososophila melanogaster* (20), the sporulation medium of *Bacillus globigii* (62), and the blood and Malpighian tubes of the larvae of *Bombyx mori* infected with polyhedral disease (65).

Allohydroxy-L-proline occurs in the free state in the flowers, fruit, and leaves of sandal (*Santalum album* L.)

(205, 208), and bound in the toxic cyclopeptides from *Amanita phalloides*, such as phalloidin (150) from which it was first isolated (283), phallacidin, and amanitin (284–287).

Allohydroxy-D-proline was isolated (97) from the hydrolysate of the peptide antibiotic viridogrisein (22), subsequently found to be identical with etamycin (99), a novel peptide lactone (223, 224).

b. Properties

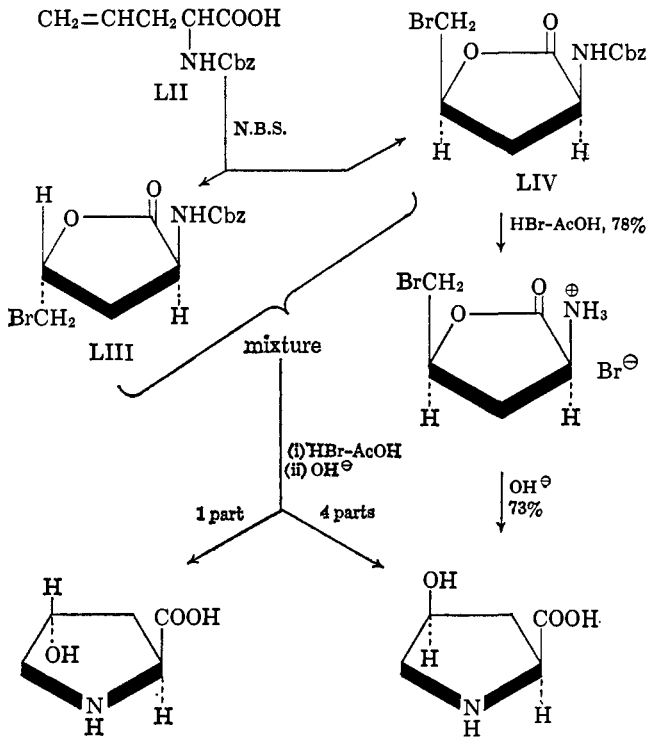
Properties of the 4-hydroxyprolines and their derivatives are summarized in Table IX. The optical rotatory dispersion of the cyclohexylamine salt of N-phenylthioacetyl-hydroxy-L-proline has been described (232). Mass spectra of hydroxy-L-proline and its N-acetyl derivative have been recorded and interpreted (101).

The proton resonance spectra of hydroxyproline and allohydroxyproline have been studied (2–4). Coupling constants of adjacent protons attached to carbon were determined, and, using the Karplus equation (126) dihedral angles were calculated and hence conformations for the molecules proposed. However, doubts have been cast on the validity of the Karplus equation in complex systems (93, 127); caution should be exercised in its application to conformational problems.

c. Synthesis

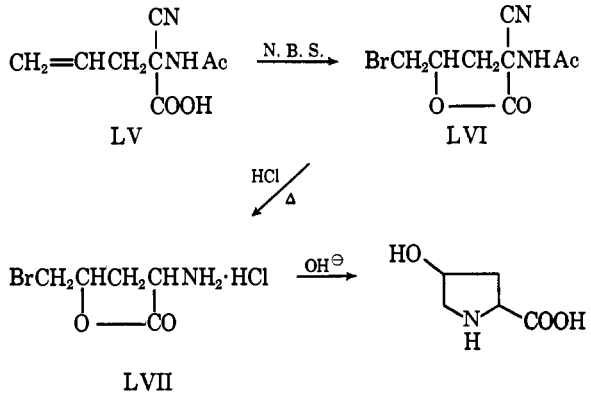
Classical methods for the synthesis and stereochemical interconversion of the various 4-hydroxyprolines have been reviewed elsewhere (88).

Reaction of carbobenzyloxyallylglycine (LII) with N-bromosuccinimide gave two isomeric bromolactones

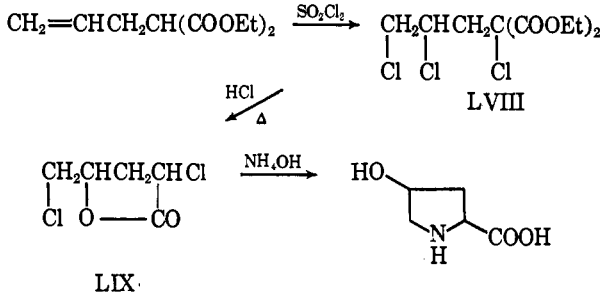


LIII and LIV, which after decarbobenzyloxylation and treatment with alkali afforded hydroxy-DL-proline and allohydroxy-DL-proline, respectively (118).

In a related route acetamidoallylcyanoacetic acid LV was converted to the bromolactone LVI with N-bromosuccinimide; the derived aminobromolactone LVII, upon treatment with alkali, gave hydroxy-DL-proline and allohydroxy-DL-proline in a ratio of 1:2 (13).



Treatment of diethyl allylmalonate with sulfuryl chloride gave a trichloro ester LVIII which on acid hydrolysis afforded the dichlorolactone LIX. This lactone in aqueous ammonia gave hydroxy-DL-proline and allohydroxy-DL-proline in a ratio of 7:5 (13). An improved procedure for preparative separation of hydroxylproline from allohydroxyproline was described (13).

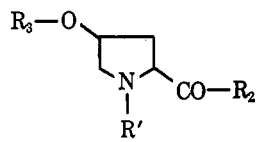


Allohydroxy-DL-proline was synthesized by stereoselective reduction of 4-ketoproline hydrobromide by sodium borohydride (28).

4-H³-Hydroxy- and 4-H³-allohydroxy-L-prolines were prepared in a 1:3 ratio by reduction of 4-keto-L-proline hydrobromide with sodium borohydride-H³ (211). The stereospecificity of the reduction of cyclic ketoimino acids and their carbobenzyloxy derivatives is summarized in Table X.

Hydroxyprolines have been employed in the synthesis of stereospecifically tritium-labeled *cis*- and *trans*-L-prolines-4-H³ (78). O-*p*-Toluenesulfonyl groups were displaced by tritide ion ($LiAlT_4$), with complete inversion of configuration, according to Figure 2.

TABLE IX[a]



DERIVATIVES OF 4-HYDROXYPROLINE

R_1	R_2	R_3	Isomer	M.p., °C.	Rotation	Concentration and solvent	Ref.
H	OH	H	L	274	$[\alpha]^{26}D$ −74.6°	H_2O	145
H	OH	H	D	274	$[\alpha]^{21}D$ +75.2°	H_2O	145
H	OH	H	L-Allo	238–241	$[\alpha]^{18}D$ −58.1°	H_2O	145
H	OH	H	D-Allo	237–241	$[\alpha]^{18}D$ +58.6°	H_2O	145
H	OH	Ac	L	179–181 (dec.)	...	...	133
H	OH	COC_6H_5	L	...	$[\alpha]^{22}D$ −5.4°	H_2O	247
H	OH	Tos	L	163–165	...	...	142
H·HCl	OMe	H	L	162–164 (dec.)	...	...	233
H·HCl	OEt	H	L	147–148	...	...	123
H·HCl	OEt	H	L-Allo	148–151	...	...	5
H·HCl	OBz	H	L	147–150	...	...	233
H·TosOH	OBz	H	L	107–109	$[\alpha]^{13}D$ −21.8°	*c* 2.0; H_2O	116
H·HBr	Lactone		L-Allo	193–194	...	...	196
H·HBr	Lactone		D-Allo	188 (dec.)	...	...	196
H	OH	Me	L	202	$[\alpha]D$ +56.0°	*c* 1.0; H_2O	187
H·HCl	OEt	Me	L	150–152	...	...	5
Ac	OH	H	L	133–134	$[\alpha]^{20}D$ −116.5°	H_2O	247
Ac	OH	H	L-Allo	144–145	$[\alpha]D$ −91.5°	*c* 2.0; H_2O	187
Ac	OH	H	D-Allo	145.5	$[\alpha]D$ +91.0°	*c* 2.0; H_2O	87
Ac	OH	H	DL-Allo	143–144	...	...	80
Ac	OMe	H	L	78	...	...	187
Ac	OMe	H	DL-Allo	79–80	...	...	80
Ac	OH	COC_6H_5	L	185–186	$[\alpha]^{20}D$ −42.9°	EtOH	247
Ac	OH	Tos	L	181–182	...	...	187
				168.5–170	...	...	196
Ac	OH	Tos	D-Allo	143.5	$[\alpha]D$ +30.5°	*c* 1; EtOH	87
Ac	OH	Tos	DL-Allo	85–86	...	...	80
Ac	OMe	Tos	L	60	...	...	187
				71–73	...	...	196
Ac	OMe	Tos	D-Allo	143.5	$[\alpha]^{25}D$ +32.0°	*c* 1; EtOH	87
Ac	OMe	Tos	DL-Allo	119–120	...	...	80
Ac	Lactone		L-Allo	99–101	$[\alpha]D$ +61.1°	*c* 1.0; $CHCl_3$	196
Ac	OH	Me	L	152–153	$[\alpha]D^{20}$ −104.3°	*c* 3; H_2O	248
Ac	OMe	Me	L	76–77	$[\alpha]^{18}D$ −81°	*c* 4.5; EtOH	248
Cbzo	OH	H	L	106–107	$[\alpha]^{20}D$ −72.0°	*c* 1.0; $CHCl_3$	196
Cbzo	OH	H	L-Allo	110–111	$[\alpha]^{20}D$ −23.7°	*c* 1.0; $CHCl_3$	196
Cbzo	OH	H	D-Allo	110.5–111.5	$[\alpha]^{20}D$ +26.3°	*c* 1.0; $CHCl_3$	196
Cbzo	$NHNH_2$	H	L	149–149.5	...	...	233
Cbzo	OH	Tos	L-Allo	100–101.5	$[\alpha]D$ −20.0°	*c* 1.0; MeOH	196
Cbzo	OMe	Tos	L	76–78	$[\alpha]^{20}D$ −32.4°	*c* 1.0; MeOH	196
Cbzo	OMe	Tos	L-Allo	138–139	$[\alpha]^{20}D$ −25.4°	*c* 1.0; $CHCl_3$	196
Cbzo	OMe	Nis	L	97–98	...	...	78
Cbzo	Lactone		L-Allo	102–103	...	...	196
Cbzo	Lactone		D-Allo	102–103	$[\alpha]D$ −30.9°	*c* 1.0; $CHCl_3$	196
p-NO_2·Cbzo	OH	H	L	136.5–139	$[\alpha]^{26}D$ −41.6°	*c* 1.0; NaOH	48
$C_6H_5SO_2$	OH	H	L	143–144	$[\alpha]^{24}D$ −40.6°	*c* 1.0; NaOH	163
Tos	OH	H	L	153	...	...	157
Tos	OH	H	L-Allo	146–147	$[\alpha]^{20}D$ −43.9°	*c* 1.0; EtOH	78
Tos	OMe	H	L	104	...	...	12
Tos	OMe	H	L-Allo	103–104	$[\alpha]^{20}D$ −66.5°	*c* 1.0; $CHCl_3$	12
Tos	NH_2	H	L	204	...	...	12
Tos	OH	Tos	L	95	...	...	12
Tos	OMe	Tos	L	94–95.5	$[\alpha]^{20}D$ −54.1°	*c* 1.0; $CHCl_3$	78
Tos	OMe	Tos	L-Allo	123–124	$[\alpha]^{20}D$ −25.0°	*c* 1.0; $CHCl_3$	78
Tos	OMe	Nis	L	141–142	$[\alpha]^{20}D$ −53.8°	*c* 1.0; $CHCl_3$	78
Tos	Lactone		L-Allo	107	...	...	12
Nis	OH	H	L	179–181	...	...	78

Table IX (*Continued*)

R_1	R_2	R_3	Isomer	M.p., °C.	Rotation	Concentration and solvent	Ref.
2,4-DNP	OH	H	L	174–175	...	...	209
2,4-DNP	OH	H	L-Allo	Hygroscopic	...	...	209
3,5-DNB	OH	H	DL	203–206	...	...	135
				205–207			274
3,5-DNB	OH	H	L	95–108	$[\alpha]_D$ −147°	*c* 1; 50% EtOH	274
							135
TFA	OMe	H	L	B.p. 114 (0.2 mm.)	...	...	153
TFA	OMe	TFA	L	B.p. 99 (1.2 mm.)	...	...	153
$ClCH_2CO$	OH	H	L	160	...	...	1
4C2NP	OH	H	L	171–174	$[\alpha]^{23}_D$ −114.0°	*c* 0.5; MeOH	104
PAPC	OH	H	L	201	...	...	299
BTC	OEt	H	L	Monohydrate, 83–84	...	...	69

[a] Ac = acetyl; Tos = *p*-toluenesulfonyl; Cbzo = carbobenzyloxy; Bz = benzyl; Nis = *p*-nitrobenzenesulfonyl; DNP = dinitrophenyl; DNB = dinitrobenzoyl; TFA = trifluoroacetyl; 4C2NP = 4-carbomethoxy-2-nitrophenyl; PAPC = *p*-phenylazophenylcarbamoyl; BTC = benzoylthiocarbamoyl.

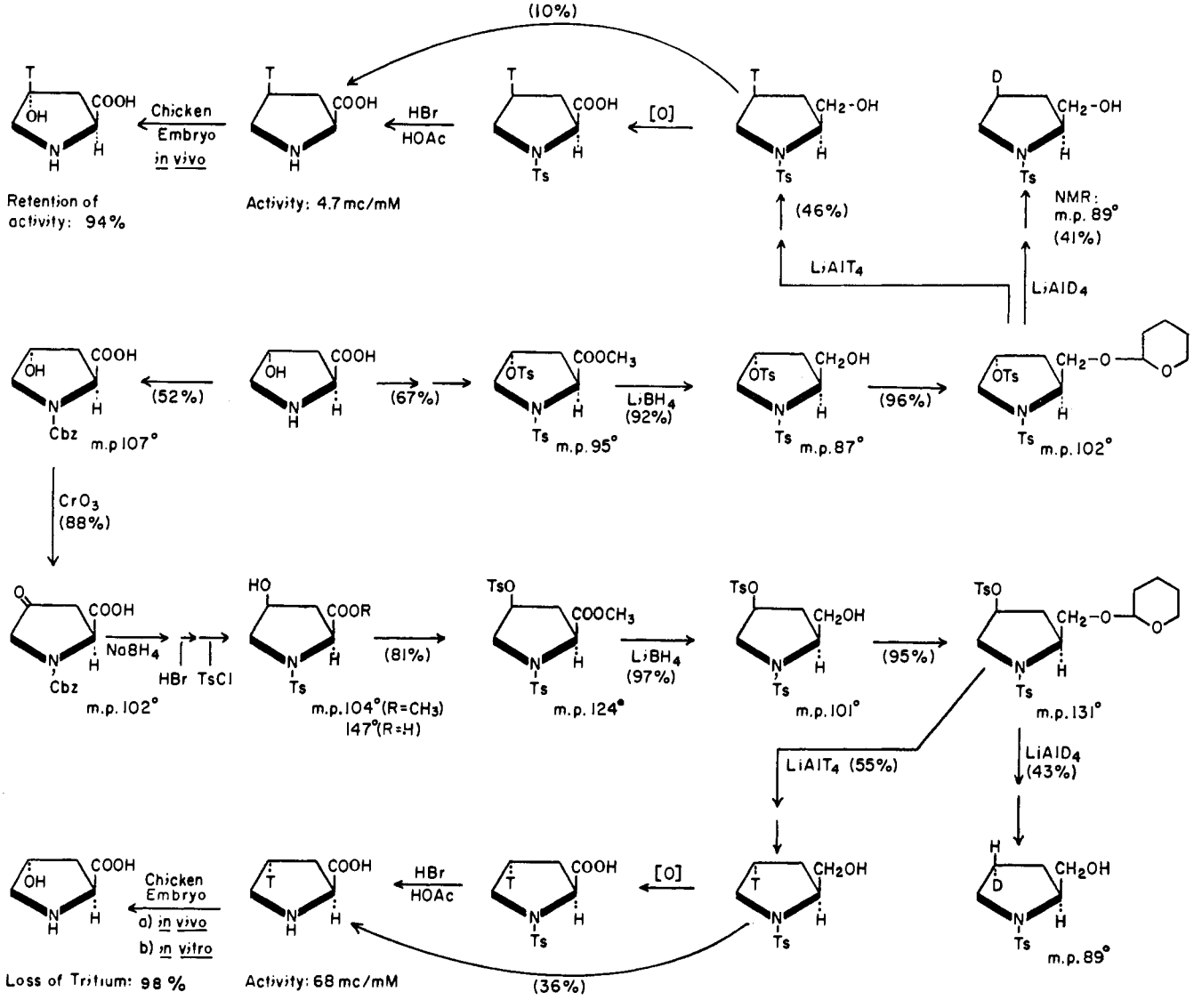
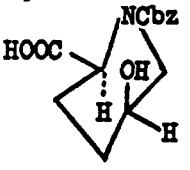
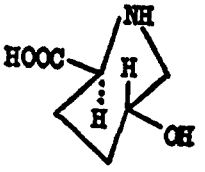
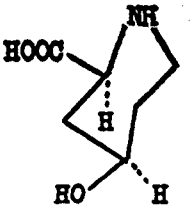
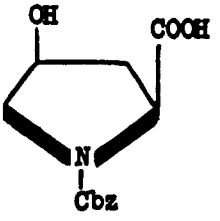
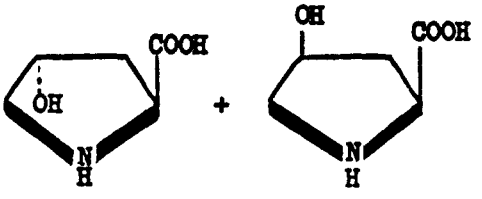


Figure 2.

TABLE X

INFLUENCE OF THE BASIC NITROGEN ON THE STEREOCHEMISTRY OF REDUCTION OF KETOIMINO ACIDS

Ketoimino acid	Product of reduction with sodium borohydride	Stereospecificity	Ref.
N-Carbobenzyloxy-5-keto-L-pipecolic acid		100% *cis*-N-carbobenzyloxy-5-hydroxy-L-pipecolic acid	291
5-Keto-DL-pipecolic acid		Almost exclusively *trans*-5-hydroxy-DL-pipecolic acid	27
4-Keto-L-pipecolic acid		80% *cis*-4-hydroxy-L-pipecolic acid	53
N-Carbobenzyloxy-4-keto-L-proline		100% *cis*- (or allo-) 4-hydroxy-L-proline	196
4-Keto-L-proline		25% *trans*- and 75% *cis*-4-hydroxy-L-proline	211

d. Biochemical Studies

Biochemical studies of hydroxyproline are numerous and are reviewed here in a selective manner.

Enzymatic hydroxylation of L-proline in chick embryos was studied using the *cis*- and *trans*-L-prolines-4-H^3 mentioned above. Tritium retention of 94% was observed in *cis*-4-H^3-L-proline and 98% loss of tritium was seen in *trans*-4-H^3-L-proline. These results established that enzymatic hydroxylation in this system proceeds by front-side displacement of hydrogen by hydroxyl with retention of configuration (78).

The enzymatic oxidation of hydroxy-L-proline and its metabolism to aspartate and glutamate are mentioned elsewhere (part I, sections B3 and F2). An enzyme from *Pseudomonas striata* which equilibrates epimers of hydroxyproline by inversion of configuration at C-2 has been described (7), purified (9), and found to be free from pyridoxal phosphate (9).

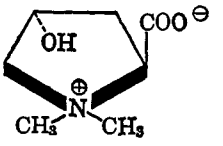
Incorporation of free hydroxy-L-proline into proteins occurs only to a small extent (165, 239); hydroxy-L-proline in body proteins is derived mainly from peptide or protein-bound L-proline (24,165,201,230,238). On the other hand, hydroxy-L-proline-C^{14} was directly incorporated into the peptide of actinomycin I in *Streptomyces antibioticus* (130).

e. N-Methyl-4-hydroxyproline

N-Methyl-4-hydroxyproline was isolated from the bark of *Croton gubouga* as prisms, m.p. 242°, $[\alpha]_D$ −85.4° (*c* 5.23, H_2O); its optical rotation indicates that it is the N-methyl derivative of natural hydroxy-L-proline (85).

cis- and *trans*-N-methyl-4-hydroxy-DL-prolines were synthesized by reaction of the bromochlorolactone LX with methylamine and separated through their copper salts into isomers: (a) m.p. 207–208°, and (b) m.p.226–227° (144).

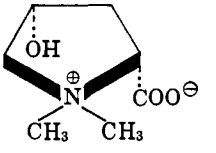
TABLE XI



BETONICINE AND ITS DERIVATIVES

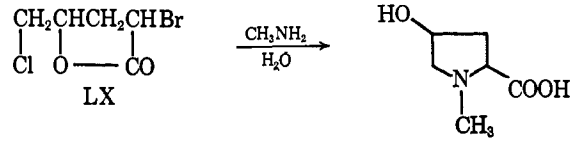
Compound	M.p., °C.	Rotation	Concentration and solvent	Ref.
Natural betonicine	243–244 (dec.)	$[\alpha]^{15}D$ −36.60°	*c* 4.88; H_2O	140
Natural betonicine hydrochloride	222–223	$[\alpha]^{15}D$ −24.97°	*c* 8.58; H_2O	140
Natural betonicine chloroaurate	242	...	...	140
Natural betonicine chloroplatinate	226	...	...	140
Synthetic betonicine	243	...	...	141
Synthetic betonicine hydrochloride	...	$[\alpha]D$ −24.1°	*c* 0.925; H_2O	141
Synthetic betonicine chloroaurate	230–232	...	...	141
Synthetic betonicine chloroplatinate	225	...	...	141
Synthetic betonicine	252 (dec.)	$[\alpha]D$ −35.1°	*c* 3.505; H_2O	85
Synthetic betonicine hydrochloride	227 (dec.)	$[\alpha]D$ −24.8°	*c* 3.59; H_2O	85
Synthetic betonicine chloroaurate	230–232 (dec.)	...	...	85
Synthetic betonicine	252–253	$[\alpha]^{20}D$ −34.2°	*c* 1.0; H_2O	196
Synthetic betonicine O-Ac, HCl	200–201	...	...	196

TABLE XII



TURICINE AND ITS DERIVATIVES

Compound	M.p., °C.	Rotation	Concentration and solvent	Ref.
Natural turicine	249 (dec.)	$[\alpha]D$ +36.26°	H_2O	140
Natural turicine hydrochloride	223	$[\alpha]D$ +24.65°	*c* 7.18; H_2O	140
Natural turicine chloroaurate	232	...	...	140
Natural turicine chloroplatinate	223	...	...	140
Synthetic turicine	249 (dec.)	$[\alpha]D$ +34.97°	*c* 3.46; H_2O	141
Synthetic turicine hydrochloride	222	...	...	141
Synthetic turicine chloroaurate	232	...	...	141
Synthetic turicine	260 (dec.)	$[\alpha]D$ +41°	H_2O	85
Synthetic turicine hydrate	249	...	...	85
Synthetic turicine hydrochloride	224 (dec.)	$[\alpha]D$ +25.7°	*c* 2.8; H_2O	85
Synthetic turicine chloroaurate	230–232 (dec.)	...	...	85
Synthetic turicine	259–260	$[\alpha]^{20}D$ +37.8°	*c* 1.0; H_2O	196



LX

The L form of isomer b was obtainable by methylation of hydroxy-L-proline with methyl iodide and alkali.

4. *4-Hydroxyproline Betaines*

a. Occurrence

Betonicine, the betaine of hydroxy-L-proline, and turicine, the betaine of allohydroxy-D-proline, are found together in *Betonica officinalis* and *Stachys sylvatica L.* (220, 221). Their properties (140) are given in Tables XI and XII. It has been suggested (196) that the isolation of turicine depends on the ready epimerization of betonicine at C-2.

Betonicine has also been found in *Marrubium vulgare* (197); significantly no turicine was found in fresh plant extract.

b. Properties

The properties of betonicine and its derivatives are summarized in Table XI, and of turicine in Table XII.

The n.m.r. spectra of stachydrine, betonicine, and turicine have been reported and discussed (197). In each case the protons of the methyl groups attached to nitrogen possess quite different chemical shifts (betonicine, δ 3.03 and 3.30; turicine, δ 3.18 and 3.24; stachydrine, δ 3.03 and 3.25) due to the effect of the carboxylate group. The 4-hydroxyl group affects only the chemical shift of the N-methyl group to which it bears a *cis* relationship.

c. Synthesis (See Tables XI and XII)

(i) A mixture of betonicine and turicine was synthesized by methylation of hydroxy-L-proline with

methyl iodide and potassium hydroxide in methanol, and separated by fractional crystallization (140).

(ii) Methylation of natural N-methylhydroxy-L-proline, with methyl iodide and potassium hydroxide in methanol, gave a mixture of betonicine and turicine which was separated by fractional crystallization (85).

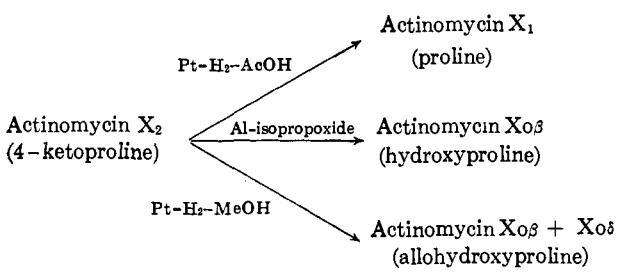
(iii) By reaction of the silver salts of hydroxy-L-proline and allohydroxy-D-proline with methyl iodide, methylation without epimerization was achieved, betonicine and turicine, respectively, being thereby synthesized. O-Acetyl-hydroxy-L-proline by the same procedure gave the labile betaine ester O-acetylbetonicine (196).

5. *4-Ketoproline*

a. Occurrence

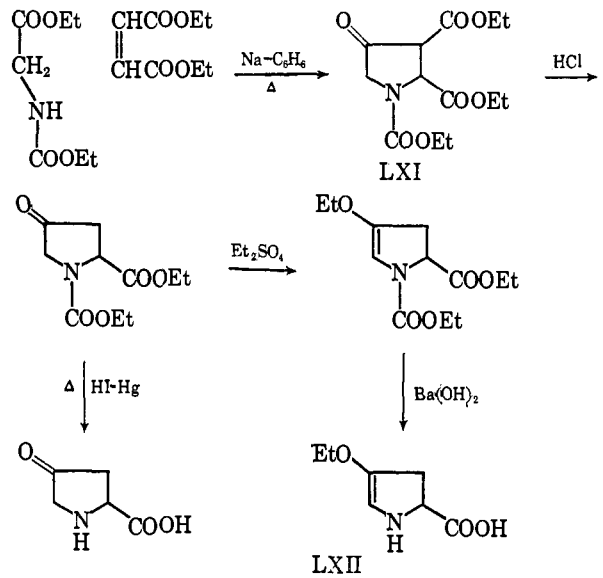
4-Keto-L-proline has been found only in the peptides of actinomycins. Actinomycins, a series of closely related antibiotics from various *Streptomyces* strains, consist of a chromophore, derived from 2-amino-4,6-dimethyl-3-oxophenoxazine-1,9-dicarboxylic acid (actinocin), to which are attached the NH_2 terminals of two pentapeptide lactones (38). In actinomycins A_{IV}, B_{IV}, C_I, D, and X_1, which are identical (89, 275), the amino acid sequence in both peptides is Thr-D-val-Pro-Sar-Meval (44). In actinomycin X_2 one peptide has this sequence, and the other has 4-keto-L-proline in place of L-proline (41). Actinomycins A_V, (129) and B_V are known to be identical with X_2 (275). In actinomycin X_{1a} one peptide has sarcosine in place of L-proline and the other has 4-keto-L-proline in place of L-proline (42).

4-Keto-L-proline was not isolated from actinomycin X_2, but its presence in the hydrolysate was demonstrated by paper chromatography, and it was reduced in the intact antibiotic to L-proline (giving actinomycin X_1) and hydroxy-L-proline (giving actinomycin Xoβ) (41).



b. Synthesis and Chemistry

(i) Condensation of ethyl N-ethoxycarbonylglycinate with diethyl fumarate gave 1,2,3-triethoxycarbonyl-4-pyrrolidone (LXI), from which 4-keto-DL-proline was prepared by acid hydrolysis and isolated as the hydrochloride. An enol ethyl ether LXII, m.p. 199–201°, was also obtained (139).



Catalytic hydrogenation (platinum in methanol) of N-ethoxycarbonyl-4-keto-DL-proline ethyl ester gave N-ethoxycarbonylallohydroxy-DL-proline ethyl ester, while Meerwein–Pondorf reduction (aluminum isopropoxide in isopropyl alcohol) gave allohydroxy-DL-proline (45.5%) and hydroxy-DL-proline (21%) after hydrolysis (139).

(ii) Carbobenzyloxyhydroxy-L-proline was oxidized by chromic acid to carbobenzyloxy-4-keto-L-proline; removal of the carbobenzyloxy group with hydrogen bromide in acetic acid gave 4-keto-L-proline hydrobromide. Likewise carbobenzyloxy-4-keto-D-proline was synthesized starting with allohydroxy-D-proline (196). 4-Ketoprolines were unstable to base owing to intermolecular aldol-type condensations. Sodium borohydride reduction of carbobenzyloxy-4-keto-L-proline and its methyl ester gave the corresponding allohydroxy-L-proline derivatives.

(iii) Chromic acid oxidation of N-*p*-toluenesulfonyl-hydroxy-L-proline and its methyl ester and amide afforded the corresponding derivatives of 4-keto-L-proline (12) (Scheme I).

c. Properties

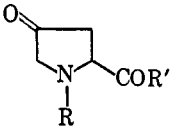
Properties of 4-ketoproline derivatives are given in Table XIII. The ultraviolet spectrum of 4-keto-L-proline hydrobromide had λ_{max} 276 mμ (ϵ 27) in water. The infrared spectrum had λ_{max} 5.65 and 5.81 μ (196).

d. Biochemical Studies

4-Keto-L-proline was inactive in an enzymatic system of a soil bacterium adapted to utilize hydroxy-L-proline as its sole source of nitrogen and carbon (10).

Administration of 4-keto-L-proline to chick embryos resulted in a marked increase in free hydroxyproline without affecting the level of proline. The effect was caused by inhibition of the catabolism of hydroxyproline and by reduction of 4-keto-L-proline to hydroxy-L-proline. A strain of *Achromobacter* grown on hydroxy-

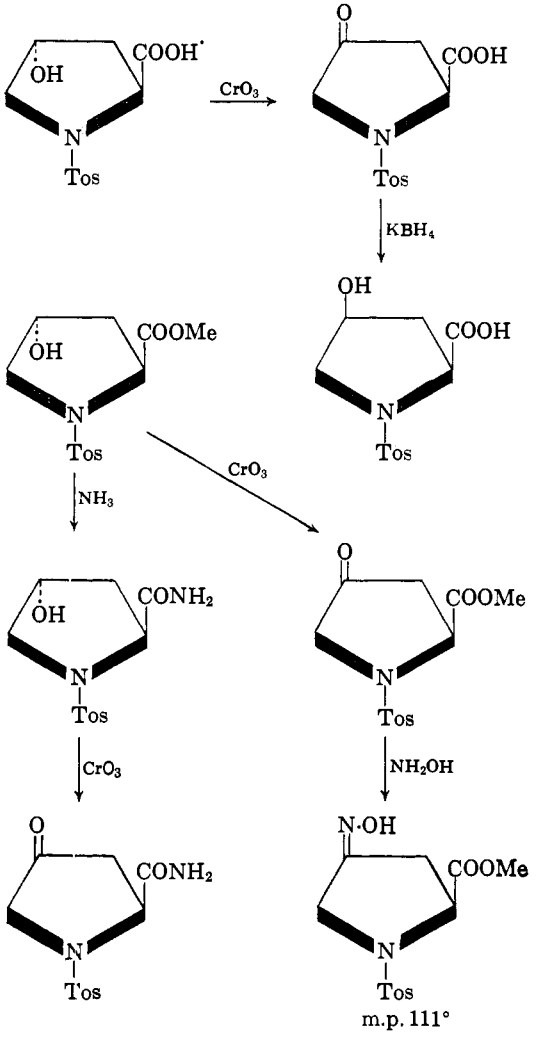
TABLE XIII



4-KETOPROLINE AND ITS DERIVATIVES

R	R′	Isomer	M.p., °C.	Rotation	Concentration and solvent	Ref.
H·HCl	OH	DL	170–172	...	...	139
H·HBr	OH	L	154–156 (dec.)	$[\alpha]^{20}D$ −41°	c 1.0; H_2O	196
Cbzo	OH	L	101–102	$[\alpha]D$ +18.5°	c 1.0; $CHCl_3$	196
Cbzo	OH	D	99–100	$[\alpha]^{20}D$ −19.4°	c 1.0; $CHCl_3$	196
Tos	OH	L	179	...	...	12
Tos	OCH_3	L	104	...	...	12
Tos	NH_2	L	155	...	...	12
EtOCO	OEt	DL	Oil	...	...	139

SCHEME I

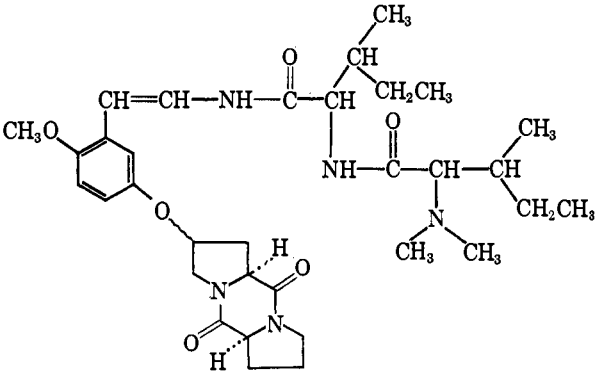


L-proline as sole carbon source metabolized hydroxy-L-proline extensively; addition of 4-keto-L-proline decreased the metabolism of hydroxyproline. However, 4-keto-L-proline was not converted to hydroxy-L-proline in this system. When 4-keto-L-proline was incubated with a dialyzed, soluble fraction of rat kidney homogenate, it was reduced to hydroxy-L-proline, a reaction which required the presence of reduced pyridine nucleotides (166). 4-Ketoproline reductase has been partially purified (236). It had a pH optimum of 6.0–6.5 and a specific requirement for reduced diphosphopyridine nucleotide. Its activity did not appear to be the same as that of other known dehydrogenases. It was suggested that 4-keto-L-proline may be an intermediate in amino acid metabolism.

Uniformly labeled L-proline-C^{14} and tritium-labeled hydroxy-L-proline were used in experiments to test their incorporation into actinomycins A_I, A_{IV}, and A_V in *Streptomyces antibioticus* (130). L-Proline was an excellent precursor for the L-proline, hydroxy-L-proline, and 4-keto-L-proline in these components. Hydroxy-L-proline was a good precursor for the hydroxy-L-proline in A_I but a poor precursor for the 4-keto-L-proline in A_V.

6. *Zizyphin*

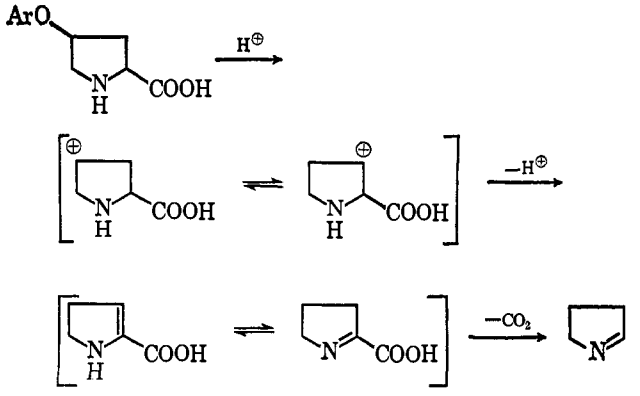
Two basic peptides, zizyphin and zizyphinin, were isolated from the roots of *Zizyphus oenoplia* Mill (162); the following structure has recently been established for zizyphin (298).



M.p. 121° (dec.); $[\alpha]^{24}D$ −465° (c 1, $CHCl_3$); λ_{max} (dioxane) 243 mμ (ε 11,100), 273 mμ (ε 9600), 321 mμ (ε 8950)

Hydroxyproline was obtained after ozonolysis of zizyphin, while pyrolysis gave pyrocoll, the cyclic anhydride of pyrrole-2-carboxylic acid. The formation of

Δ^1-pyrroline upon vigorous acid hydrolysis was explained in the following manner.

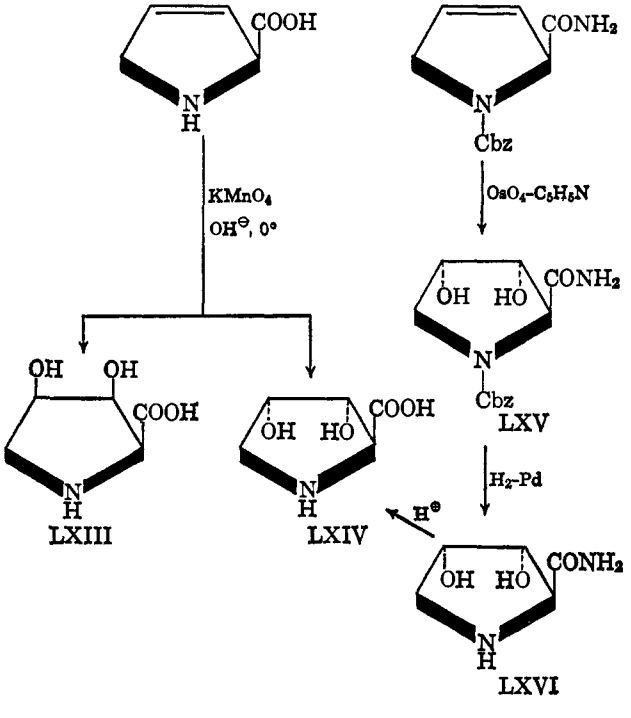


This observation is of interest, since double-bond migrations of this kind were not observed in 3,4-dehydroproline, its N-acyl ester, and hydantoin derivatives (211).

All the amino acid residues in zizyphin, including the 4-aryloxyproline, belong to the L series; the absolute configuration of the alkyloxy substituent at C-4 has not yet been established.

7. *3,4-Dihydroxyproline*

This amino acid has not been found in nature but two of its four possible racemates have recently been synthesized as follows (215). Oxidation of 3,4-dehydro-DL-proline with alkaline potassium permanganate gave a mixture of two diastereoisomeric *cis*-glycols LXIII and LXIV, which were separated through the copper salts. LXIII had m.p. 248–250° (dec.); LXIV, which formed the less soluble copper salt, had m.p. >257° (dec.).



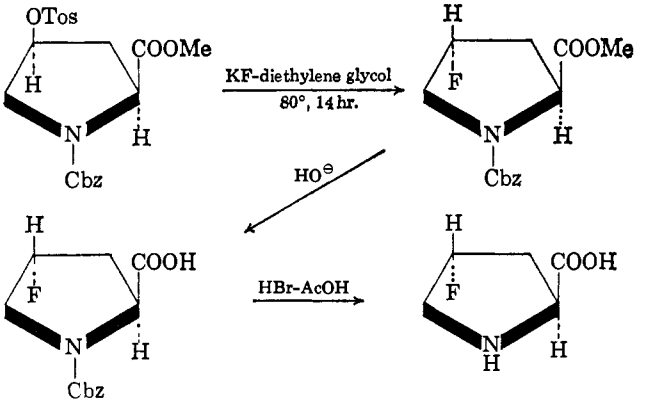
Oxidation of carbobenzyloxy-3,4-dehydro-DL-prolinamide with osmium tetroxide in pyridine gave a *cis* glycol LXV which after hydrogenation over palladium gave a 3,4-dihydroxy-DL-prolinamide LXVI; this amide on hydrolysis afforded a 3,4-dihydroxy-DL-proline identical with LXIV. The latter was presumably the *trans*,[2,3] *cis*[3,4] isomer since the bulky osmium tetroxide would be expected to attack from the less-hindered side of the molecule. It follows that LXIII was the all-*cis* isomer.

C. HALOGEN-, NITROGEN-, AND SULFUR-SUBSTITUTED PROLINES

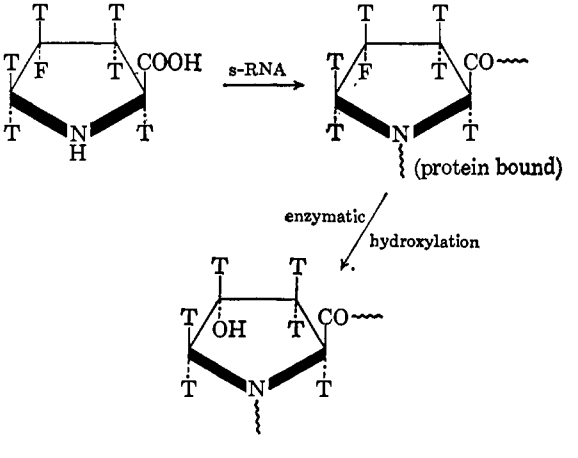
1. *Halogenoprolines*

a. 4-Fluoroprolines

trans-4-Fluoro-L-proline was synthesized from N-carbobenzyloxy-O-*p*-toluenesulfonylallohydroxy-L-proline methyl ester by a displacement reaction with fluoride ion, a reaction which proceeds with complete inversion at C-4. *trans*-4-Fluoro-L-proline had m.p. 243–245°, $[\alpha]^{20}D$ −78.6° (*c* 1.0, H_2O) (79).



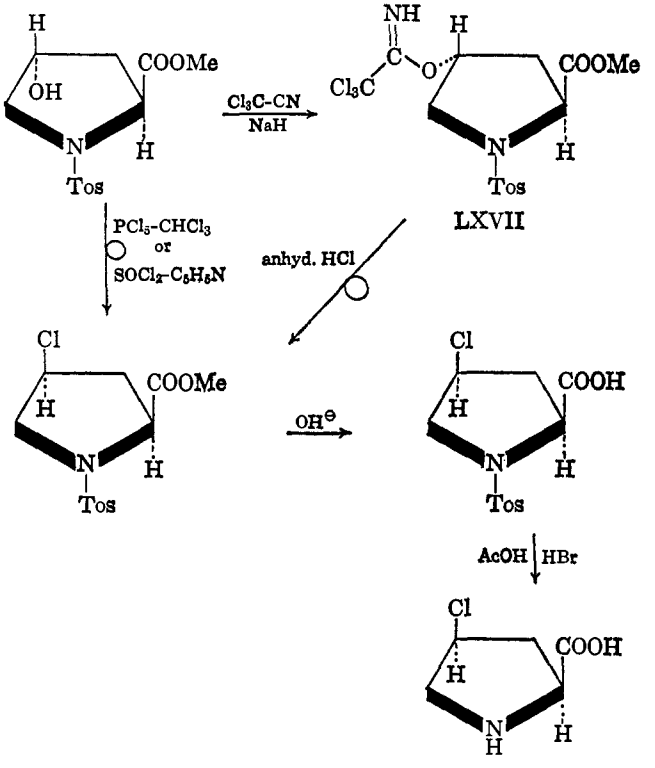
cis-4-Fluoro-L-proline was prepared in the same way from N-carbobenzyloxy-O-*p*-toluenesulfonylhydroxy-L-proline methyl ester. In this case the displacement reaction gave a mixture of *cis* (83%) and *trans* (17%) products. The final product after several recrystallizations was pure *cis*-4-fluoroproline, m.p. 271°, $[\alpha]^{20}D$ −40.2° (*c* 1.0, H_2O).



Both isomers were tritiated by the Wilzbach method. (288). It was shown that *trans*-4-fluoro-L-proline was incorporated into protein as hydroxy-L-proline, by enzymatic displacement of the fluorine by hydroxyl (above). *cis*-4-Fluoro-L-proline was also incorporated into protein, but its enzymatic hydroxylation was not observed.

b. 4-Chloroproline

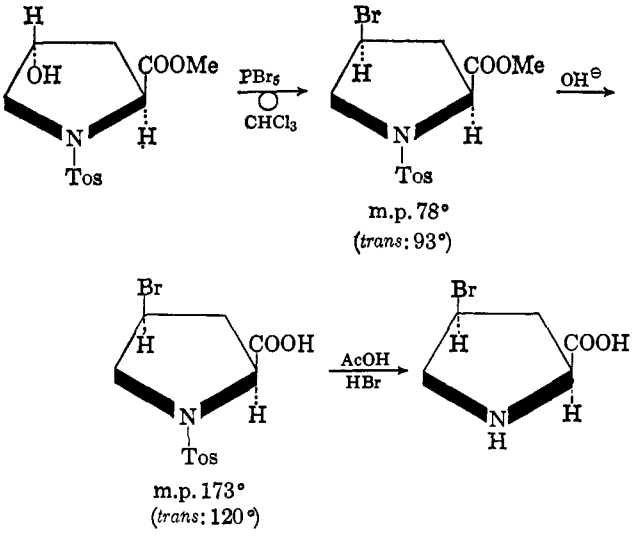
cis-4-Chloro-L-proline has been synthesized *via* displacement of a suitably protected hydroxy-L-proline trichloroacetamidate (56) (LXVII) by chloride ion, according to the following scheme (12).



Recently N-*p*-toluenesulfonyl-*cis*-4-chloro-L-proline methyl ester was prepared as shown above, in one step, from N-*p*-toluenesulfonylhydroxy-L-proline methyl ester. Similarly, N-*p*-toluenesulfonylallohydroxy-L-proline methyl ester was converted to N-*p*-toluenesulfonyl-*trans*-4-chloro-L-proline methyl ester, m.p. 114°, and thence to *trans*-4-chloro-L-proline, m.p. 195° (12).

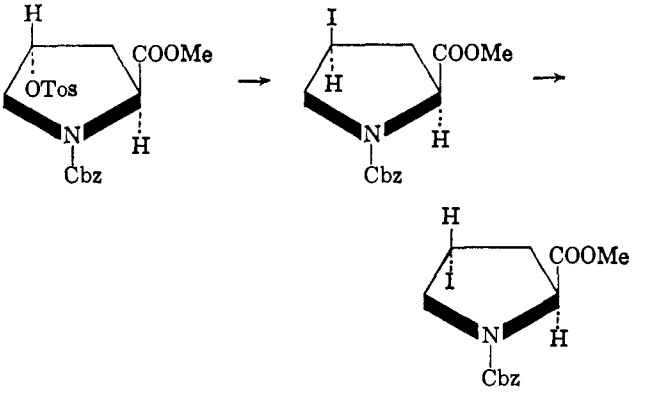
c. 4-Bromoproline

Displacement of *p*-toluenesulfonate groups in N,O-di-*p*-toluenesulfonylhydroxy- and -allohydroxy-L-proline methyl esters by bromide ion (lithium bromide in acetone) gave mixtures of *cis*- and *trans*-4-bromo derivatives. On the other hand, displacement of hydroxyl groups by means of phosphorus pentabromide in chloroform proceeded with complete inversion. *cis*-4-Bromo-L-proline, m.p. 168°, and *trans*-4-bromo-L-proline, m.p. 167° (12), were obtained by analogous routes one of which is shown below.



d. 4-Iodoproline

Displacement of *p*-toluenesulfonyl groups by iodide ion in carbobenzyloxyhydroxy- and carbobenzyloxy-allohydroxy-L-proline methyl esters was studied by vapor phase chromatography. Mixtures of *cis*- and *trans*-4-iodoproline derivatives were usually obtained, the isomeric ratio depending on the reaction conditions (78). The mixtures arise because further displacement of 4-iodo groups by iodide ion occurs with inversion at C-4. In a preparative experiment, crystalline carbobenzyloxy-4-iodo-L-proline *t*-butylammonium salt, m.p. 142–143°, was obtained, having a composition of 90% *cis*, 10% *trans*.



2. Aminoprolines

a. *cis*-3-Aminoproline

This substance was prepared by catalytic hydrogenation of 3-nitropyrrole-2-carboxylic acid, and characterized as a sulfate, m.p. 215–222°, an N,N′-anhydrodibenzoyl derivative, m.p. 193.5–194.5°, and a flavianate salt, m.p. 229–231° (184).

b. *cis*-4-Aminoproline

Two routes for the synthesis of *cis*-4-aminoproline have been used.

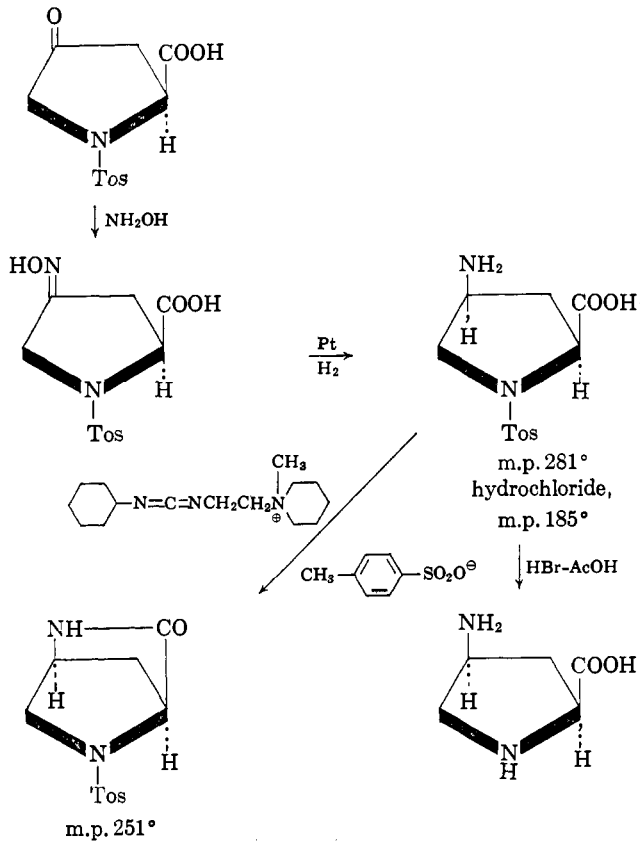
TABLE XIV

4-METHYLPROLINES (60)

Source	Isomer	M.p., °C.	Rotation	Concentration and solvent	Rotation	Concentration and solvent
Apple	L-*trans*	...	$[\alpha]^{16.5}D$ −52°	*c* 0.35; H_2O	$[\alpha]^{19}D$ −21°	*c* 0.19; 3 *N* HCl
I.C.I. 13,959	L-*cis*	...	$[\alpha]^{18}D$ −83°	*c* 0.53; H_2O	$[\alpha]^{18}D$ −43°	*c* 0.51; 3 *N* HCl
Synthetic	L-*trans*	239–240 (dec.)	$[\alpha]^{18}D$ −56.6°	*c* 1.03; H_2O	$[\alpha]^{20}D$ −23.9°	*c* 1.54; 3 *N* HCl
Synthetic	D-*cis*	238–239 (dec.)	$[\alpha]^{20}D$ +85.2°	*c* 1.68; H_2O	$[\alpha]^{20}D$ +47.9°	*c* 1.28; 3 *N* HCl

(i) Catalytic hydrogenation of 4-nitropyrrole-2-carboxylic acid gave *cis*-4-amino-DL-proline, characterized as a sulfate, m.p. 215–222°, an N,N′-anhydrodibenzoyl derivative, m.p. 193.5–194.5°, and a flavianate salt, m.p. 229–231° (184).

(ii) Catalytic hydrogenation of N-*p*-toluenesulfonyl-4-keto-L-proline oxime gave the *cis*-4-amino derivative, as shown by its carbodiimide-induced lactamization (see below); removal of the *p*-toluenesulfonyl group afforded *cis*-4-amino-L-proline, m.p. 193° (12).

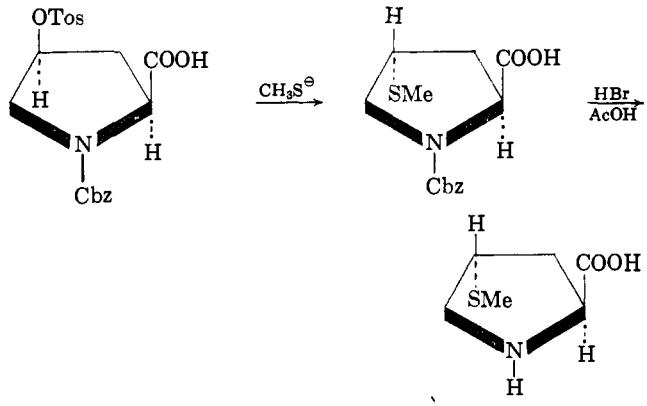


c. 4-Aminopyrrolidine-2,4-dicarboxylic Acid

4-Aminopyrrolidine-2,4-dicarboxylic acid of uncertain configuration was prepared from 1,2-diethoxycarbonyl-4-pyrrolidone. It did not melt but darkened from 300 to 350°, and was characterized as a bis-2,4-dinitrophenyl derivative, m.p. 247–250°, and a flavianate salt, m.p. 250–253° (184). 3-Amino-β-proline was also prepared.

3. Sulfur-Containing Proline Analogs

4-Methylmercapto-L-proline was synthesized by the reaction of N-carbobenzyloxy-O-*p*-toluenesulfonylallohydroxy-L-proline with sodium methylmercaptide (inversion at C-4); its hydrobromide had m.p. 170–172°, $[\alpha]^{20}D$ −24.0° (*c* 1.0, H_2O) (196).



4-Allomethylmercapto-L-proline, m.p. 243–244°, was prepared in the same way from N-carbobenzyloxy-O-*p*-toluenesulfonylhydroxy-L-proline (196).

IV. ALKYL- AND ARYL-SUBSTITUTED PROLINE ANALOGS

A. INTRODUCTION

Proline analogs with 4-alkyl substituents, such as methyl, hydroxymethyl, and methylene, have been isolated from apples, and their stereochemistry has been elucidated by spectrometry and by synthesis. Antibiotics of the lincomycin group contain various N-methyl-4-alkylprolines. Kainic acid, an anthelmintic principle from *Digenea simplex*, is a polysubstituted proline. In addition, the preparation and properties of numerous synthetic alkyl-substituted prolines are reviewed.

B. NATURALLY OCCURRING ALKYLPROLINES

1. 4-Methylproline

a. Occurrence

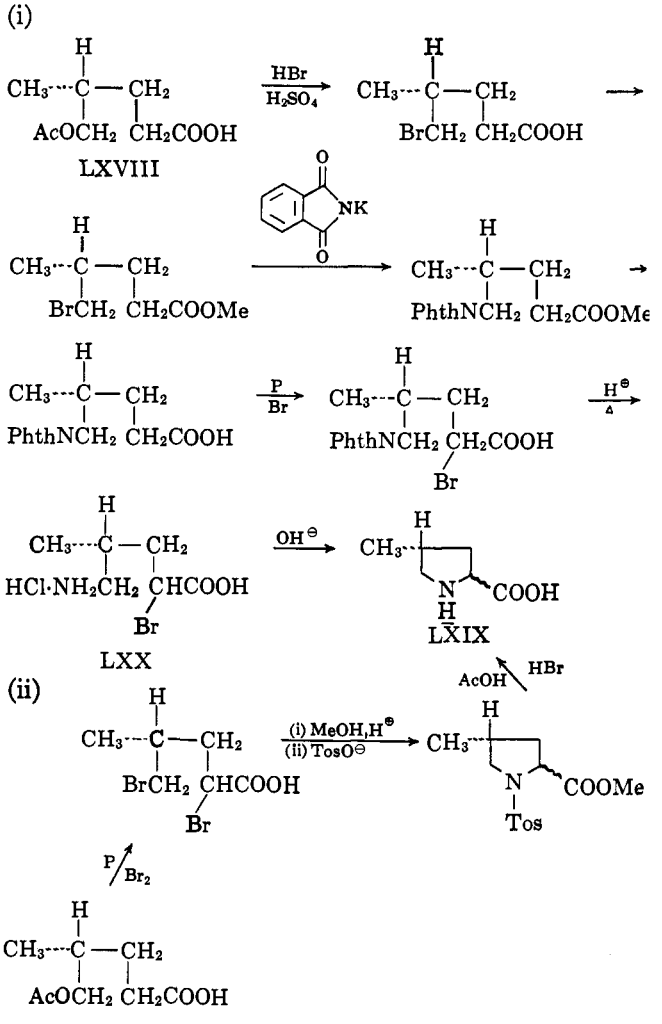
trans-4-Methyl-L-proline was found in young apple fruits (107, 108). *cis*-4-Methyl-L-proline was isolated from the hydrolysate of antibiotic I.C.I. 13,959, which occurs in a strain of *Paecilomyces* (132). 4-Methylproline of unknown configuration was found in the hydrolysate of an actinomycin complex from *Streptomyces antibioticus*, after racemic 4-methylproline had been administered to the culture medium (296).

b. Properties

Properties of natural and synthetic 4-methylprolines (59, 132) are given in Table XIV. The n.m.r. spectra of *cis*- and *trans*-4-methylprolines have been recorded and discussed (2).

c. Synthesis and Stereochemistry

The stereochemistry of the two isomeric natural 4-methylprolines (107, 108, 132) follows from the synthesis of *trans*-4-methyl-L-proline and *cis*-4-methyl-D-proline (60), for which the starting material, (+)-5-acetoxy-4-methylpentanoic acid (LXVIII) of known absolute configuration, was a by-product in the industrial degradation of sapogenins (47). There were two alternate synthetic routes



A partial separation of the final product LXIX into *cis* and *trans* isomers was first achieved through fractionation of the copper salts; pure *trans*-4-methyl-L-proline was thereby obtained. This isomer was also obtainable by oxidative destruction of the D-*cis* isomer in the racemic mixture with D-amino acid oxidase. Repeated recrystallization of 5-amino-2-bromo-4-methylpentanoic acid hydrochloride (LXX) gave a single diastereoisomer which, upon cyclization with base, gave *cis*-4-methyl-D-proline, with inversion at C-2. The properties of these two isomers of 4-methylproline are given in Table XIV.

The infrared spectra of natural *cis*- and *trans*-4-methyl-L-prolines were identical with those of synthetic *cis*-4-methyl-D-proline and *trans*-4-methyl-L-proline, respectively.

Other syntheses of 4-methylproline, in which mixtures of isomers were obtained, are described in section C1.

2. 4-Hydroxymethylproline

a. Occurrence

4-Hydroxymethylproline was found in the twigs of Granny Smith and Delicious apples (158, 273), in the peel of immature Worcester-Pearmain apples (109), and in the spurs of apple trees (29). A study of some 20 species of edible apple (*Malus pumila* and *Pyrus communis*) showed that about half of them contained 4-hydroxymethylproline (46).

b. Structural Investigations

The amino acid $C_6H_{11}NO_3$ from Worcester-Pearmain apples gave a pinkish yellow ninhydrin color and a blue isatin color. The structure of a methylhydroxyproline was suggested (109). The amino acid, m.p. 250°, from twigs of Granny Smith and Delicious apples, was tentatively formulated as hydroxymethylproline (273).

Infrared spectra of these two substances showed that they were identical; however, a decision between the two proposed structures could not be made on the basis of these spectra (110). Mass spectra of the two amino acids confirmed their identity and indicated a 4-hydroxymethylproline structure (30). The ethyl ester gave an intense mass peak at *m/e* 100 (loss of carbethoxy), also one at 31 (CH_2OH) and 142 (M − 31). Since the mass spectrum of the ester was unchanged after the compound was held at 140° for 30 min., conditions considered sufficient to cause lactonization of a *cis*-4-hydroxymethylproline, the *trans* configuration was proposed for the natural product.

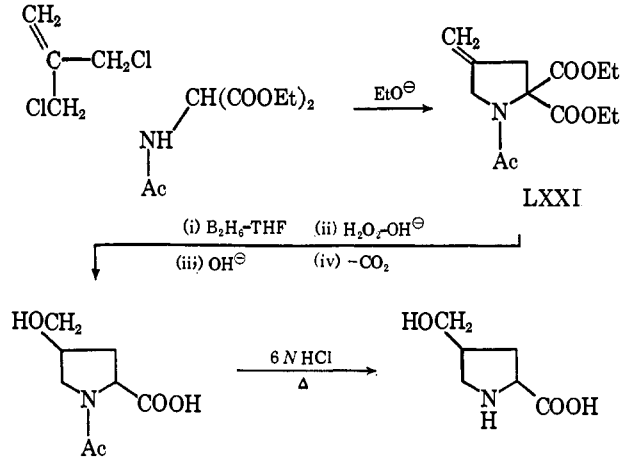
N.m.r. studies (2) confirmed the hydroxymethylproline structure since there were no methyl protons, and a doublet at τ 6.57 was assigned to the $-CH_2O-$ group. However, the *cis* configuration was preferred because the n.m.r. spectrum of the 4-hydroxymethylproline resembled that of *cis*-4-methyl-D-proline and not that of *trans*-4-methyl-L-proline.

The configurational problem was finally settled by synthesis.

c. Synthesis

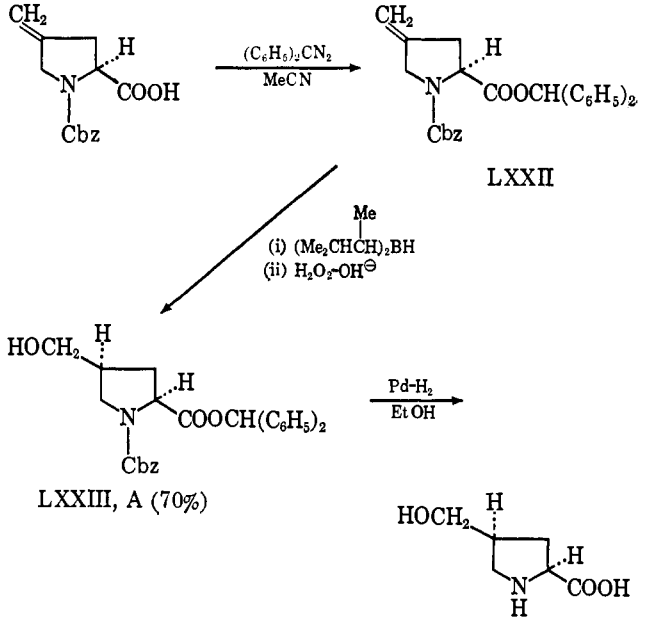
(i) Racemic 4-hydroxymethylproline was synthesized *via* hydroboration of diethyl N-acetyl-4-methyl-

ene pyrrolidine-2,2-dicarboxylate (LXXI), which was prepared by a ring synthetic method as follows (45).

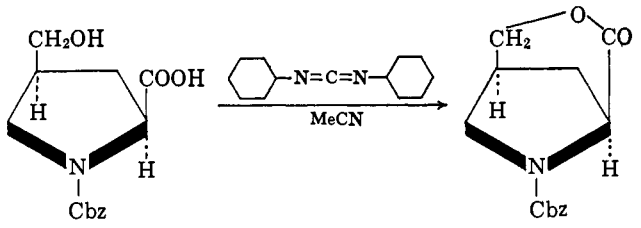


A partial separation into diastereoisomers was achieved through the copper salts; the less soluble copper salt, m.p. 261–264° (dec.), was converted to the free amino acid (microprisms, m.p. 220–221°) which was identical with the natural product in its mass spectrum and paper chromatographic behavior.

(ii) By another hydroboration route carbobenzyloxy-4-methylene-L-proline benzhydryl ester (LXXII) was treated with the bulky diisoamylborane, followed by alkaline hydrogen peroxide, to give what was believed to be a derivative (LXXIII) of *cis*-4-hydroxymethyl-L-proline; after removal of the protecting groups the product (m.p. 257–258° (dec.), $[\alpha]^{19.5}D$ −75.6° (H_2O)) had infrared and n.m.r. spectra identical with those of the natural amino acid (25, 26).



(iii) The above route was repeated in an independent synthesis (272); hydroboration of LXXII, as before, followed by oxidation with alkaline hydrogen peroxide gave two isomeric hydroxymethyl compounds A and B. A (70% of the mixture), m.p. 159.5–160.5, $[\alpha]^{23}D$ −61° ($CHCl_3$) (LXXIII), on catalytic hydrogenation gave *cis*-4-hydroxymethyl-L-proline, m.p. 255–257°, the carbobenzyloxy derivative of which gave a lactone, m.p. 94.5–95.5° (ν_{max} 1705 and 1755 cm.$^{-1}$), as follows.



The lactone formation is proof of *cis* configuration; the corresponding amino acid was identical (infrared and n.m.r. spectra) with the natural product. The *trans* isomer of LXXIII, B (30% of the mixture), m.p. 105–113°, $[\alpha]^{23}D$ −44° ($CHCl_3$), on catalytic hydrogenation gave *trans*-4-hydroxymethyl-L-proline, m.p. 227.5–229°, $[\alpha]_{589.6}$ −48° (H_2O, from O.R.D.), the n.m.r. spectrum of which resembled that of *trans*-4-methyl-L-proline. Carbobenzyloxy-*trans*-4-hydroxymethyl-L-proline could not be lactonized.

It is remarkable that *cis*-4-hydroxymethyl-L-proline and *trans*-4-methyl-L-proline occur together in apples.

3. *4-Methyleneproline*

a. Occurrence

4-Methyleneproline was isolated from the seeds of loquat (*Eriobotyra japonica*) (86).

b. Properties and Structural Investigation

The amino acid $C_6H_9NO_2$ had m.p. 225° (dec.) and no optical rotation; it gave a yellow color with ninhydrin but no color with isatin. It gave aspartic acid and glycine on permanganate oxidation and 4-methylproline on catalytic hydrogenation. The infrared spectrum had λ_{max} 6.1 and 11.2 μ (exocyclic methylene); the n.m.r. spectrum indicated the absence of methyl groups. The 4-methyleneproline structure was proposed (86). It is of interest that the related γ-methyleneglutamic acid has been found in peanut plants (64).

c. Synthesis

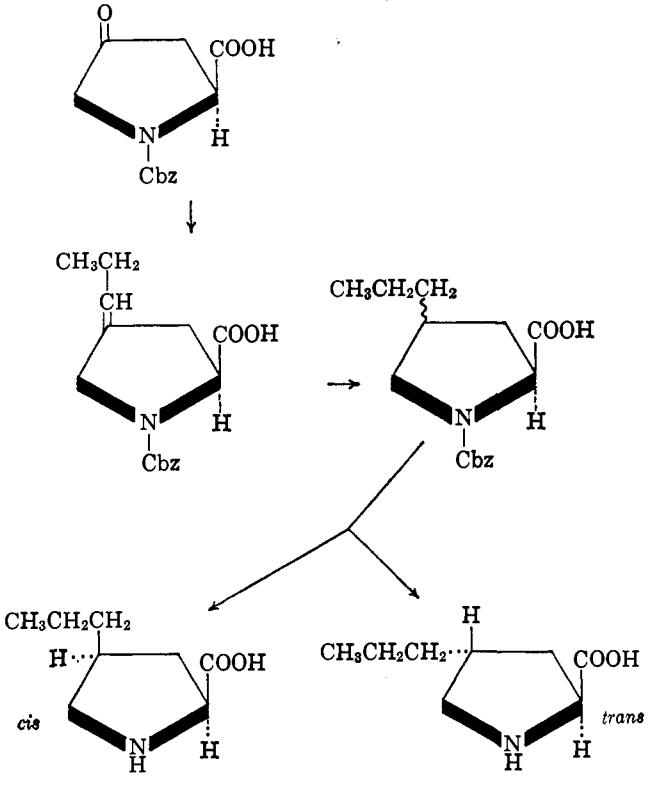
A Wittig reaction between carbobenzyloxy-4-keto-L-proline and methylenetriphenylphosphorane gave carbobenzyloxy-4-methyleneproline as an oil, dicyclohexylammonium salt, m.p. 160–161°, $[\alpha]^{19}D$ −5.5° (*c* 2, $CHCl_3$) (25). The carbobenzyloxydicyclohexylammonium salt of natural 4-methyleneproline had m.p. 139° and no optical rotation. The infrared spectra were different in the solid state but identical in chloroform. The natural amino acid was therefore racemic. This was confirmed by its catalytic hydrogenation to racemic 4-methylproline (mainly *cis*).

4. *N-Methyl-4-alkylprolines of Lincomycins*

The antibiotic lincomycin (57, 94, 95, 100, 146, 154, 258, 277) has structure LXXIV (R = R_2 = CH_3, R_1 = $CH_2CH_2CH_3$ (102)).

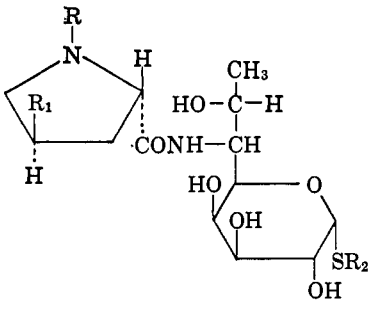
Acid hydrolysis of lincomycin gave *trans*-4-*n*-propyl-L-hygric acid. Hydrazinolysis of lincomycin gave methyl α-thiolincosaminide ($C_9H_{18}NO_5S$) and 4-propylhygric acid hydrazide. Acylation of the former with 4-propylhygric acid *via* a mixed anhydride regenerated lincomycin.

Synthesis of 4-propylhygric acid from carbobenzyloxy-4-keto-L-proline gave two diastereoisomers, one of which, as its amide, was identical with the amide of the lincomycin component. The stereochemistry of the natural amino acid was established by its oxidation to (*R*)-(+)-propylsuccinic acid (102).



Lincomycin is structurally related to celesticetin (103), which has L-hygric acid as the amino acid component.

Four relatives of lincomycin have been isolated; they possess variations of structure as indicated under LXXIV (15–18). U-21,699 occurs in normal lincomycin fermentations, whereas production of U-11,921, U-11,973, and U-20,943 is induced by addition of DL-ethionine, methyl α-thiolincosaminide, and ethyl α-thiolincosaminide, respectively, to fermentation media of *S. lincolnensis*.



LXXIV

U-21,699: R = R_2 = CH_3, R_1 = CH_2CH_3
U-11,921: R = CH_3, R_1 = $CH_2CH_2CH_3$, R_2 = CH_2CH_3
U-11,973: R = H, R_1 = $CH_2CH_2CH_3$, R_2 = CH_3
U-20,943: R = H, R_1 = $CH_2CH_2CH_3$, R_2 = CH_2CH_3

5. *Kainic Acid*

a. Occurrence and Isolation

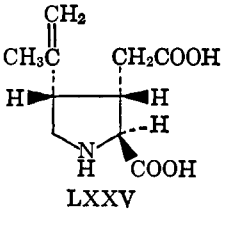
Kainic acid, first named "digenic acid," $C_{10}H_{14}NO_4 \cdot H_2O$, is the anthelmintic principle of *Digenea simplex;* its isolation from an aqueous extract of the dried red algae was achieved by chromatography on alumina. α-Allokainic acid was isolated from the mother liquors of the recrystallization of crude kainic acid (182).

b. Properties

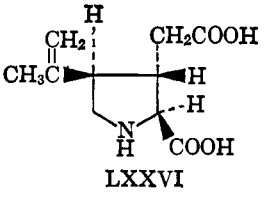
Properties of kainic acid, α-allokainic acid, and their derivatives are given in Table XV.

c. Structure and Stereochemistry

Chemical and degradative studies of kainic acid (106, 169–171, 180, 181, 185, 186, 245, 246, 259–267) are too voluminous to be reviewed here; the structures of dihydrokainic acid (170, 259–267) and hence of kainic acid (171, 180, 181, 259–267) were established and chemical studies indicated 2,3-*trans*-3,4-*cis* stereochemistry for α-kainic acid (LXXV) and 2,3-*trans*-3,4-*trans* stereochemistry for α-allokainic acid (LXXVI) (174–176, 183). These conclusions were supported by X-ray crystallographic studies of zinc kainate dihydrate (188, 278) and α-allokainic acid (279); a review of this subject has appeared (222). The n.m.r. spectra of kainic acid and α-allokainic acid have been recorded and discussed (134).



LXXV



LXXVI

Both α-kainic acid and α-allokainic acid were converted by boiling acetic anhydride to cyclic anhydrides of the corresponding "β" isomers with inversion at C-2.

TABLE XV

KAINIC ACID AND ITS DERIVATIVES

Compound	M.p., °C.	Rotation	Ref.
Kainic acid	251 (dec.)	$[\alpha]^{10}D$ −14.8°	178,179
N-Acetylkainic acid	161–162	$[\alpha]^{21}D$ −53.4°	259–267
β-N-Acetylkainic anhydride	186	$[\alpha]^{16}D$ +38.4°	259–267
N-Acetylkainic diamide	256–258 (dec.)	$[\alpha]^{22}D$ −50.5°	259–267
Kainic acid lactone	276 (dec.)	$[\alpha]^{15}D$ −8.7°	259–267
Monomethyl kainate	232–233 (dec.)	$[\alpha]^{20}D$ −20°	259–267
Kainic acid betaine	205–210 (dec.)	$[\alpha]^{19}D$ +50°	259–267
Dimethyl N-methylkainate methiodide	188–194 (dec.)	$[\alpha]^{17}D$ +3.3°	259–267
α-Allokainic acid	237–238	$[\alpha]^{15}D$ +6.7°	182
N-Acetyl-α-allokainic acid	185	$[\alpha]^{25}D$ −38.1°	182
Dihydro-α-allokainic acid	249–250 (dec.)	$[\alpha]^{25}D$ −19.8°	182
Dihydrokainic acid	272 (dec.)	$[\alpha]^{19}D$ −34°	259–267
N-Acetyldihydrokainic acid	137	...	180, 181
β-N-Acetyldihydrokainic anhydride	182–183	$[\alpha]^{17}D$ +38.7°	259–267
N-Acetyldihydrokainic diamide	252–253 (dec.)	$[\alpha]^{20}D$ −43.5°	259–267
Monomethyl dihydrokainate	250–252 (dec.)	$[\alpha]^{20}D$ −24.9°	259–267
N-Methyldihydrokainic acid	100–110 (dec.)	$[\alpha]^{19}D$ −8°	259–267
Dihydrokainic acid betaine	104	...	259–267
Dimethyl N-methyldihydrokainate methiodide	159–161	$[\alpha]^{15}D$ −16.2°	259–267
Dimethyl dihydrokainate hydrochloride	158 (dec.)	...	259–267
Dimethyl N-benzoyldihydrokainate	94	...	259–267
Dimethyl N-phenylcarbamoyldihydrokainate	154 (dec.)	$[\alpha]^{18}D$ −45.2°	259–267

The structure and synthesis of kainic acid has been comprehensively reviewed elsewhere (255).

d. Synthesis

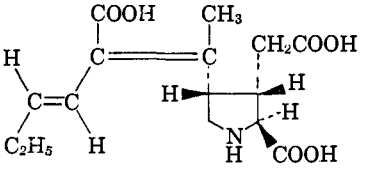
The total synthesis of kainic acid is outlined in Scheme II (268, 270, 271).

Since the introduction of a second asymmetric center gave rise to two diastereoisomers, two forms of LXXVII were obtained, m.p. 219° (dec.) and 235° (dec.). The synthesis was continued with the former, major isomer giving LXXVIII, m.p. 235° (dec.), and finally DL-α-kainic acid which was resolved by means of L-ephedrine; synthetic L-α-kainic acid, m.p. 250° (dec.), $[\alpha]^{17}D$ −15°.

α-Allokainic acid has been synthesized by the route shown in Scheme III (167, 168, 254).

6. *Domoic Acid*

Domoic acid, $C_{15}H_{21}NO_6 \cdot 2H_2O$, the anthelmintic factor of *Chondria armata*, is a close relative of kainic acid. It was isolated from an aqueous extract of the aquatic plant by ion-exchange chromatography and assigned the structure below on the basis of its spectral properties and degradation by ozone to propionaldehyde and L-*arabo*-2-carboxy-3-carboxymethyl-4-acetylpyrrolidine (251).



Domoic acid has m.p. 217° (dec.), $[\alpha]^{12}D$ −109.6° (H_2O), $\lambda_{max}^{H_2O}$ 242 mμ (log ε 4.24); tetrahydrodomoic acid, m.p. 237° (dec.), $[\alpha]^{20}D$ −11.8° (NHCl); N-acetyldomoic acid, m.p. 121–123°, $[\alpha]^{20}D$ −56.0° (H_2O); trimethyldomoate methiodide, m.p. 174°.

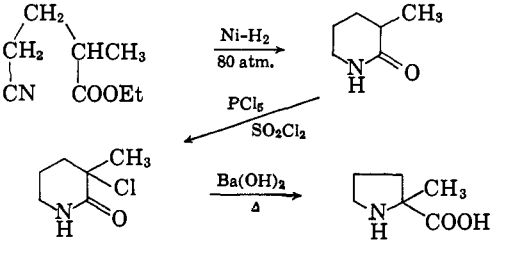
L-*arabo*-2-Carboxy-3-carboxymethyl-4-acetylpyrrolidine, m.p. 197° (dec.), $[\alpha]^{20}D$ +62.6° (H_2O), rearranged on heating in aqueous solution (epimerization at C-4) to L-*xylo*-2-carboxy-3-carboxymethylpyrrolidine, m.p. 211° (dec.), $[\alpha]^{19}D$ −19.8° (H_2O).

C. SYNTHETIC ALKYLPROLINES

1. *2-, 3-, 4-, and 5-Methylprolines*

a. 2-Methyl-DL-proline

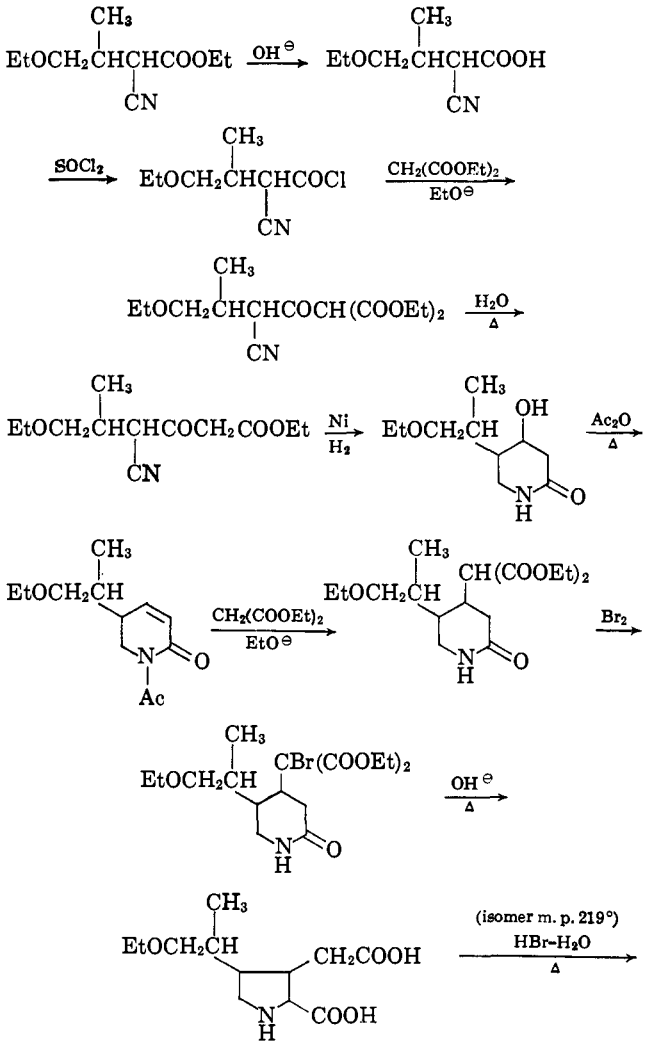
2-Methyl-DL-proline (m.p. 260°) was synthesized *via* 3-methyl-2-pyridone as follows (125).



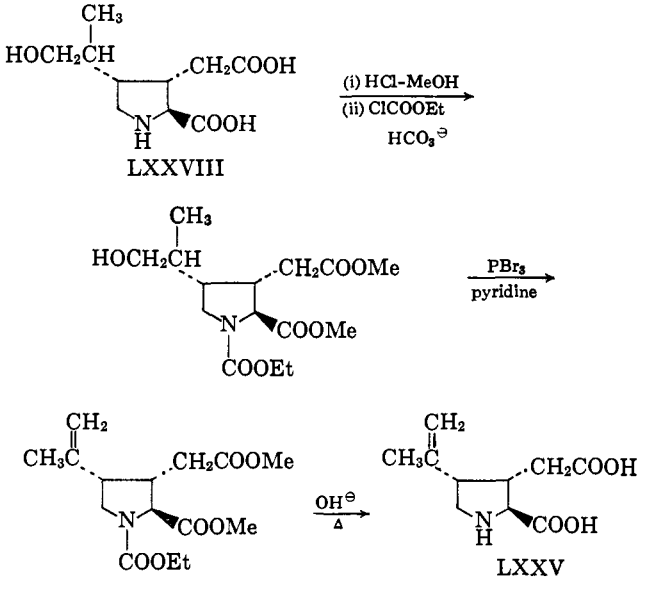
b. 3-Methylproline

(i) Racemic 3-methylproline was first synthesized as its hydrochloride (not crystalline) and ethyl ester (picrate, m.p. 112.5–114°) as outlined below (11).

SCHEME II



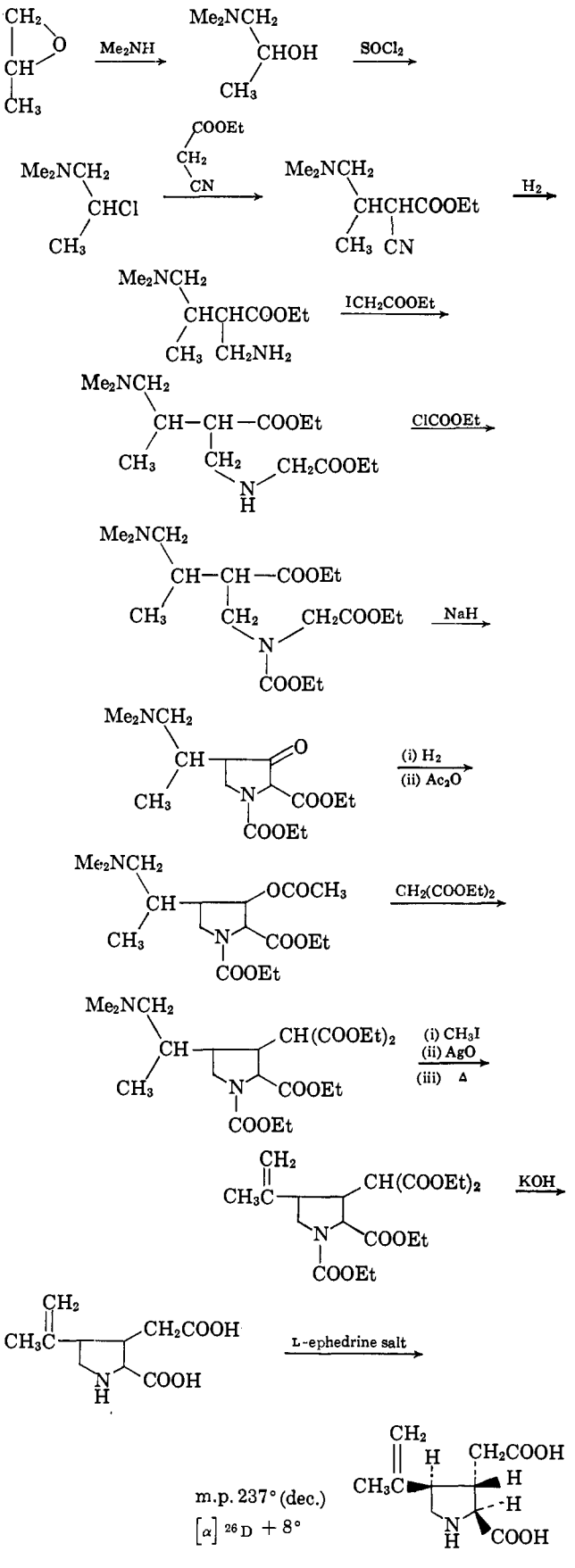
LXXVII (two isomers)



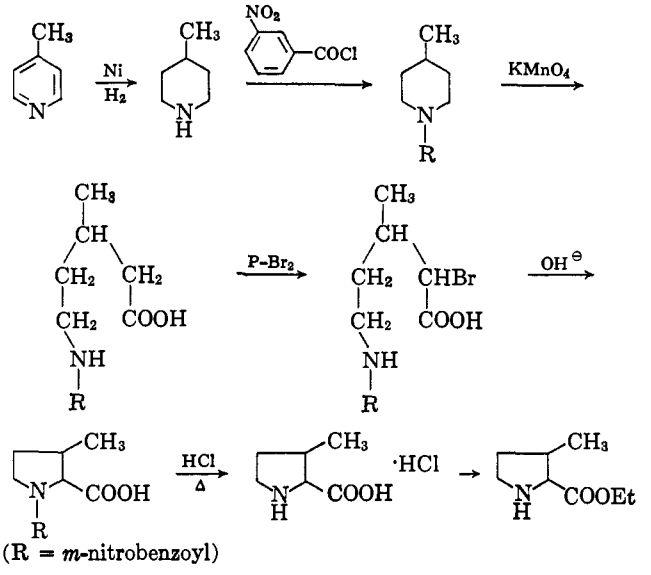
LXXVIII

LXXV

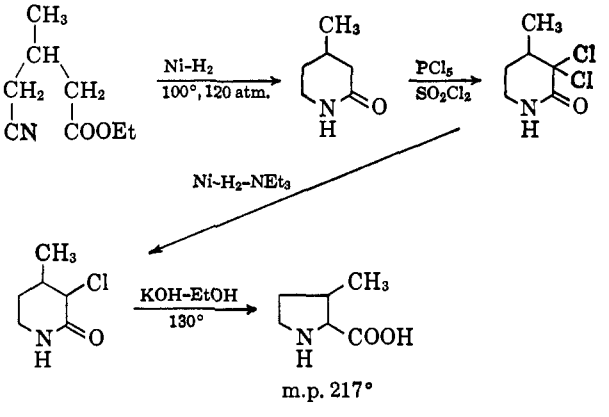
SCHEME III



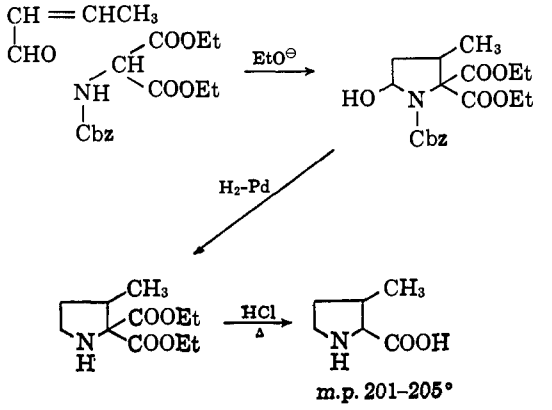
m.p. 237° (dec.)
$[\alpha]^{26}D + 8°$



(ii) Another synthesis of racemic 3-methylproline exemplifies the general route to proline analogs *via* Favorskii rearrangement of substituted 3-chloro-2-piperidones (250).



(iii) A third route of general application to 3- and 4-alkylprolines is illustrated by the following synthesis of racemic 3-methylproline (55).

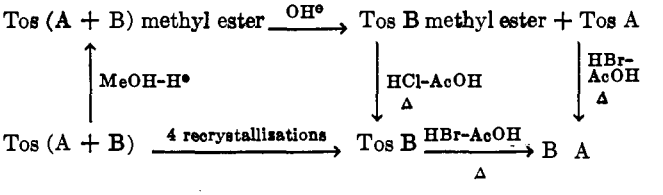


An n.m.r. spectrum of the isomeric mixture of 3-methylprolines in D_2O indicated an approximately equal abundance of the two diastereoisomers. Repeated recrystallization afforded a single diastereoisomer A, while recrystallization of the *p*-toluenesulfonyl derivative gave the derivative of the other diastereoisomer B; A and B were readily distinguished by their n.m.r. spectra, but this evidence was insufficient to decide which isomer was *cis* and which *trans*.

Isomer A (trans): m.p. 218–219°, n.m.r. (D_2O): methyl doublet at τ 8.97 (J = 6.5 c.p.s.); *p*-toluenesulfonate: m.p. 114.5–115.5°, n.m.r. ($CDCl_3$): methyl doublet at τ 9.03 (J = 6.5 c.p.s.), C-2 doublet at τ 6.05 (J = 4.6 c.p.s.).

Isomer B (cis): m.p. 210–211°, n.m.r. (D_2O): methyl doublet at τ 9.04 (J = 6.9 c.p.s.), C-2 doublet at τ 5.91 (J = 7.2 c.p.s.); *p*-toluenesulfonate: m.p. 183–185°, n.m.r. ($CDCl_3$): methyl doublet at τ 8.91 (J = 6.6 c.p.s.), C-2 doublet at τ 5.60 (J = 8.4 c.p.s.).

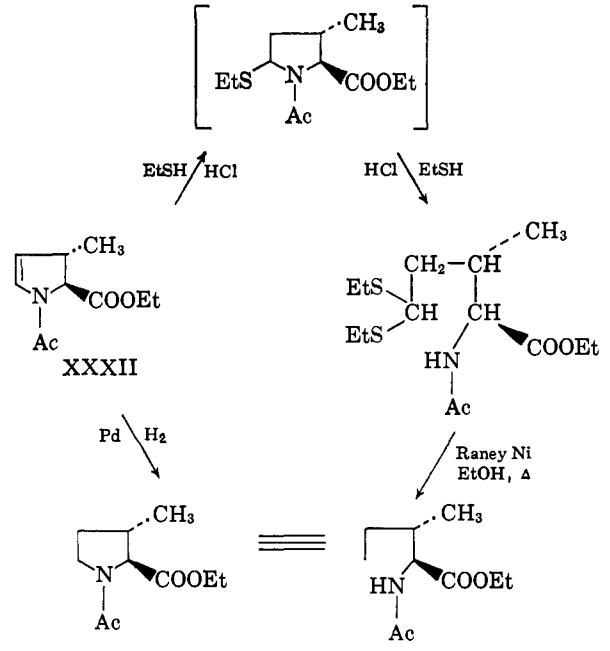
(iv) The separation and identification of the isomers of 3-methylproline have been further studied (114); preparative separation into diastereoisomers A and B was achieved on an ion-exchange column. Saponification of N-*p*-toluenesulfonyl-3-methylproline (A + B) methyl ester was followed by vapor phase chromatography; after the virtual disappearance of peak A the ester and acid fractions were separated. The acid fraction was shown, after re-esterification, to consist almost entirely of the B derivative.



This convenient procedure for the separation of A and B depends on the far slower saponification rate of the sterically hindered *cis* isomer, hence A is *trans* and B is *cis*. An analogous observation was made in the 3-hydroxyproline case (75). Also, the coupling constants $J_{2,3}$ in *cis* are greater than in the *trans* isomers both in the proton magnetic resonance spectra of 3-hydroxy- (75) and 3-methylprolines (55).

To confirm this stereochemical assignment, isomer "A" was related to isoleucine *via* N-acetyl-4,5-dehydro-3-methylproline ethyl ester (XXXII). A single racemic diastereoisomer of the latter substance, the preparation of which is outlined in part II, section E(iii), was assigned the *trans* configuration on the basis of its conversion (see below) to N-acetyl-DL-isoleucine ethyl ester, which was distinguished from the corresponding alloisoleucine derivative by infrared and n.m.r. spectroscopy.

Catalytic hydrogenation of *trans*-XXXII gave N-acetyl-*trans*-3-methyl-DL-proline ethyl ester compa-



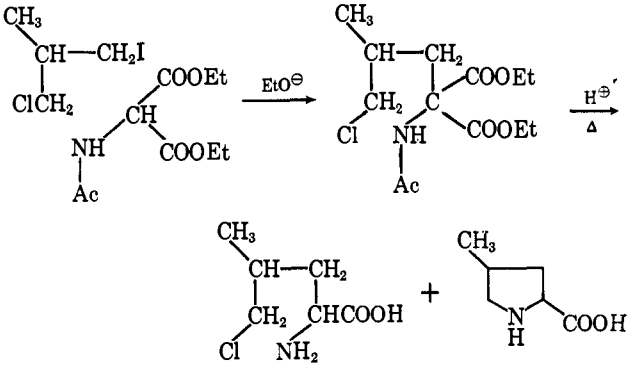
(L forms only shown)

rable with the derivative prepared from *trans*-3-methyl-DL-proline.

(v) 3-Methylproline is a highly potent inhibitor of actinomycin production when added to the culture medium of *Streptomyces antibioticus* (296). Using a mixture of isomers, inhibition was total at 0.5–1.0 μg./ml. and 50% at 0.1 μg./ml. The *cis* isomer was more inhibitory than the *trans;* concentrations required for 50% inhibition were 0.04 and 0.6 μg./ml., respectively. Inhibition of actinomycin synthesis was accompanied by acceleration of protein and cell synthesis.

c. 4-Methylproline

(i) Racemic 4-methylproline was obtained, together with glycine and δ-chloroleucine, by acid hydrolysis of the crude condensate of sodio diethyl acetamidomalonate and 1-chloro-3-iodoisobutane; it was separated *via* the reineckate or by extraction of the mixed amino acids with hot ethanol (58): 4-methyl-

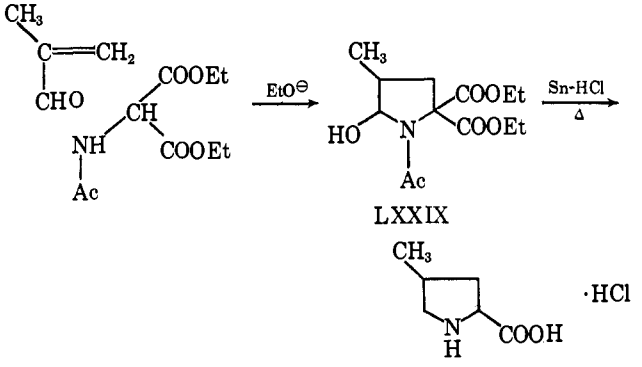


proline, m.p. 230° (dec.); reineckate, m.p. 158–160° (dec.); rhodanilate, m.p. 135–136°; phenylcarbamoyl derivative, m.p. 182–185°; N-phenylhydantoin, m.p. 104–105°.

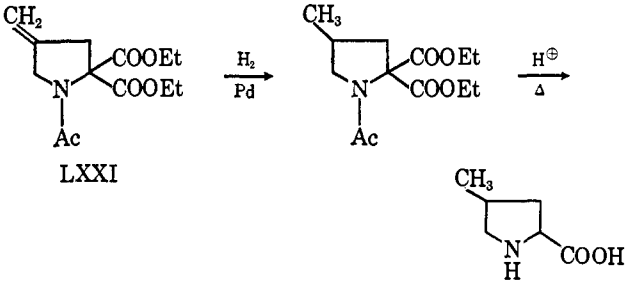
(ii) 4-Methylproline, m.p. 219°, was synthesized (125) from 3-chloro-5-methyl-2-piperidone by the method already described (125, 250) for 2-methyl- and 3-methylprolines.

(iii) The synthesis of *cis*-4-methyl-D-proline and *trans*-4-methyl-L-proline has been discussed in part IV, section B1 (60).

(iv) Racemic 4-methylproline hydrochloride, m.p. 192–193°, was prepared by Michael condensation of diethyl acetamidomalonate with methacrolein to the cyclic product LXXIX, followed by hydrolysis and reduction by tin in boiling hydrochloric acid (60).



(v) 4-Methylproline was prepared by reduction of the intermediate LXXI used (see part IV, section B2) in a synthesis of 4-hydroxymethylproline (45): 4-



methylproline, m.p. 204–206° (dec.); phenylurethane, m.p. 166–167°.

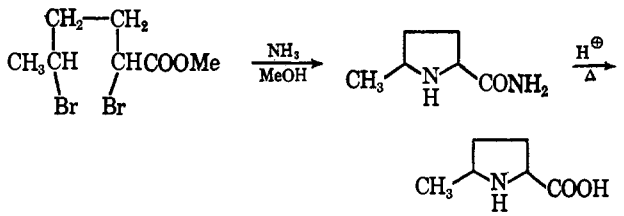
(vi) 4-Methylproline was synthesized by method iii described for 3-methylproline, starting with diethyl benzyloxycarbonylaminomalonate and methacrolein. Acid hydrolysis of diethyl 4-methylpyrrolidine-2,2-dicarboxylate gave racemic 4-methylproline, m.p. 218–225° (dec.); *p*-toluenesulfonyl derivative, m.p. 132–134° (55).

(vii) 4-Methylproline was a somewhat less potent inhibitor of actinomycin production in *Streptomyces antibioticus* than 3-methylproline. Unlike 3-methylproline, it was incorporated into the peptides of the antibiotic in place of proline (296).

d. 5-Methylproline

(i) Racemic 5-methylproline was first synthesized by ammonolysis of methyl α,δ-dibromocaproate; N,5-

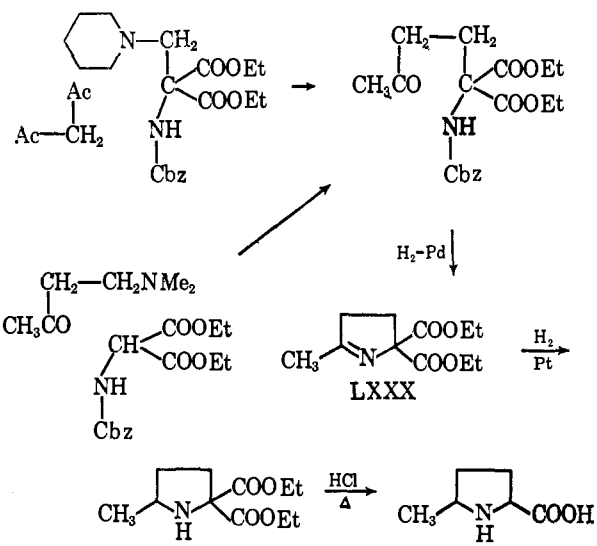
dimethylproline was prepared with methylamine in place of ammonia (289): 5-methylproline, m.p. 207°;



amide, m.p. 193°; copper salt, m.p. 228°; reineckate, m.p. 159°; phenylthiohydantoin, m.p. 130°; ethyl ester chloroplatinate, m.p. 130°; N,5-dimethylproline, m.p. 123–125°; reineckate, m.p. 156° (dec.).

(ii) 5-Methylproline has been synthesized by catalytic hydrogenation of 2-methyl-Δ^1-pyrroline-5-carboxylic acid or its ethyl ester (81, 82, 217), three independent syntheses of which are described in part II, section F3: 5-methylproline, m.p. 188° (217), 188–189° (81); hydrochloride, m.p. 186–187° (217), 191–192° (81).

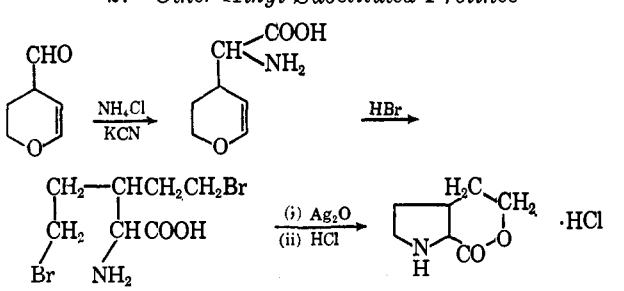
(iii) Racemic 5-methylproline, m.p. 207°, was synthesized *via* diethyl 2-methyl-Δ^1-pyrroline-5,5-dicarboxylate (LXXX) as follows (217).



This synthetic route gave a different diastereoisomer from that obtained by method ii (217) above.

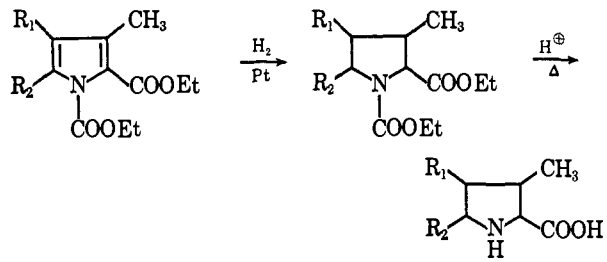
(iv) 5-Methylproline inhibited actinomycin production in *Streptomyces antibioticus* less than either 3- or 4-methylproline (296).

2. *Other Alkyl-Substituted Prolines*



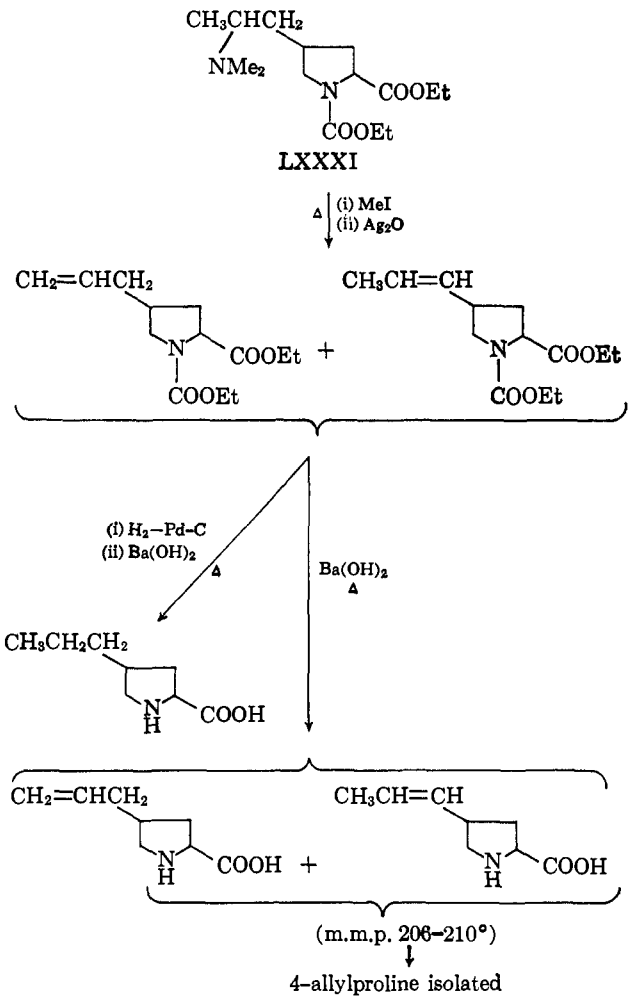
3-β-Hydroxyethylproline lactone hydrochloride, m.p. 260–262° (dec.) was synthesized as shown above (204).

3,4-Dimethylproline was synthesized starting with a substituted pyrrole (295); 3,4-dimethylproline (R_1 = methyl, R_2 = H), m.p. 231°; N-acetyl derivative, m.p. 136°.



3,5-Dimethylproline, m.p. 226°, was prepared as above (in 3,4-dimethylproline), R_1 = H, R_2 = methyl (295). Its ethyl ester, b.p. 105° (23 mm.), was prepared by catalytic hydrogenation of ethyl 2,4-dimethyl-Δ^1-pyrroline-5-carboxylate, the synthesis of which is given in part II, section F3 (193).

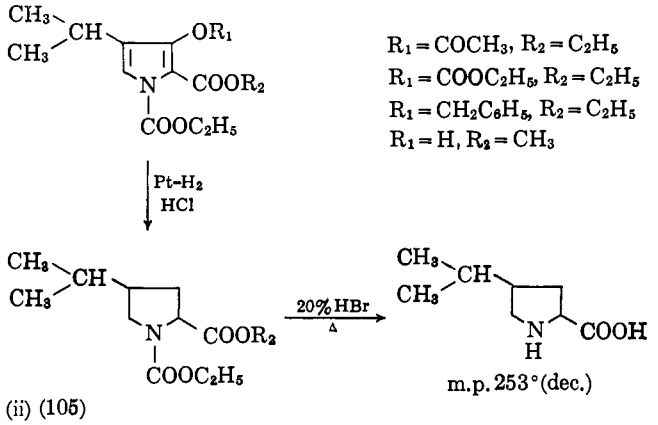
4-n-Propyl-, *4-propenyl-*, and *4-allylproline* were synthesized *via* intermediate LXXXI, which was prepared from the appropriate 3,3-dichloro-5-alkyl-2-piperidone (195): 4-*n*-propylproline, m.p. 216–218°



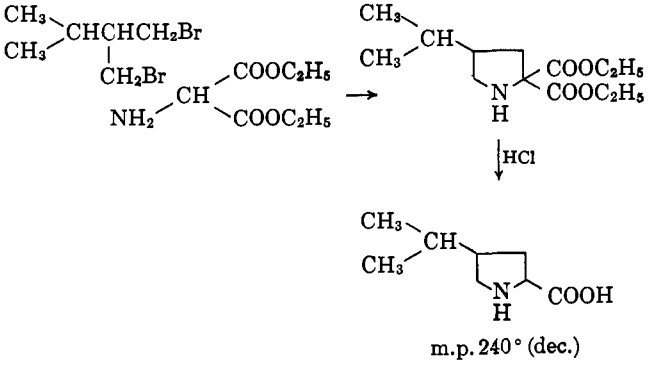
(dec.); picrate, m.p. 142°; 4-allylproline, m.p. 223° (dec.).

4-Isopropylproline has been synthesized by four routes.

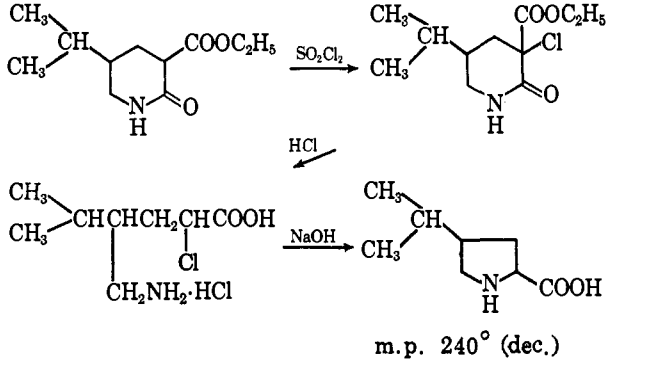
(i) (172)



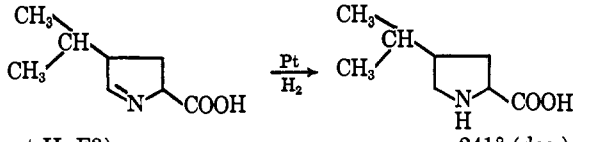
(ii) (105)



(iii) (105)



(iv) (194)



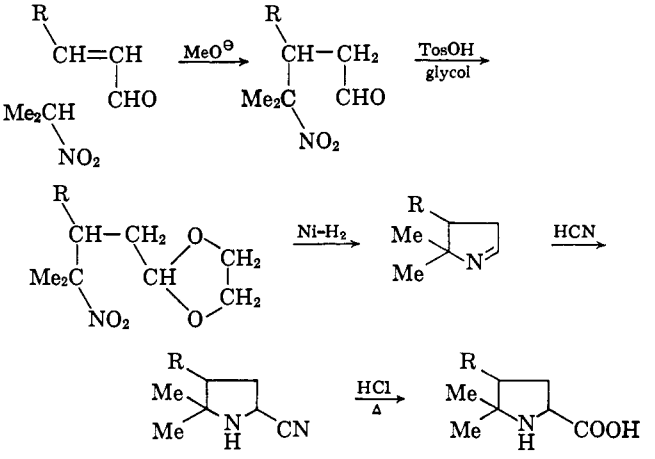
(see part II, F3)

m.p. 241° (dec.)
picrate, m.p. 172°

4-n-Propyl- and *4-(1-methyl-2-ethoxyethyl)proline* were prepared by route iv above from the appropriate Δ^1-pyrrolines (see part II, F3) (194): 4-*n*-propylproline, m.p. 222° (dec.); picrate, m.p. 142°; 4-(1-methyl-2-ethoxyethyl)proline, m.p. 175–177° (dec.).

4-Isopropenylproline, another model for the kainic acid synthesis, was prepared by the action of hydrobromic acid upon N-acetyl-4-(1-methyl-2-ethoxyethyl)-proline methyl ester available by the 3-chloro-2-piperidone route (269).

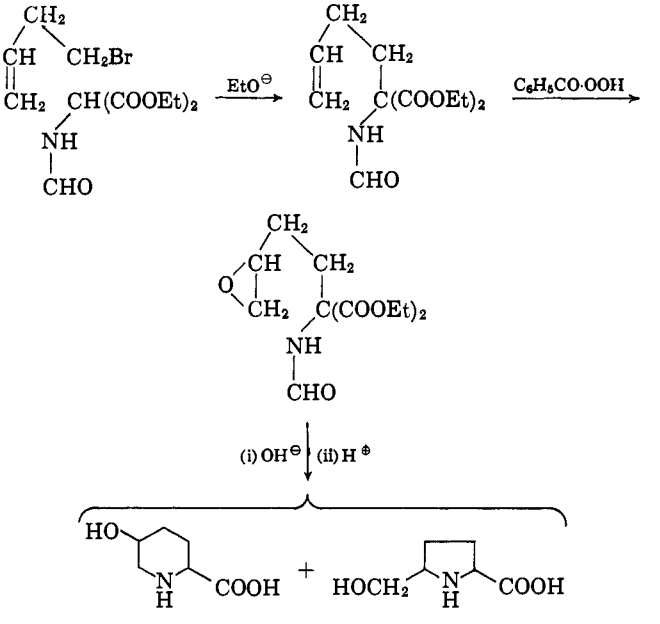
5,5-Dimethylproline and *4,5,5-trimethylproline* were synthesized *via* alkyl-Δ^1-pyrrolines (34): 5,5-dimethyl-



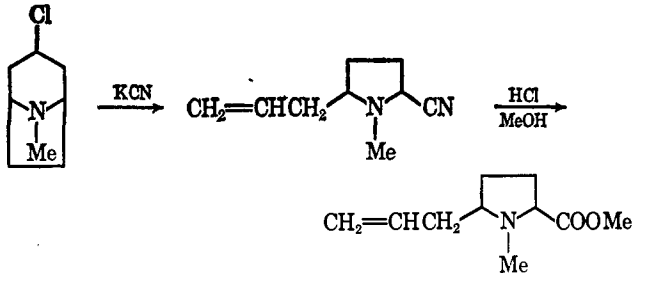
proline (R = H), m.p. 194–196°; 4,5,5-trimethylproline (R = methyl), m.p. 226–228°.

4,5-Dimethylproline was prepared by catalytic hydrogenation of 2,3-dimethyl-Δ^1-pyrroline-5-carboxylic acid hydrochloride, the synthesis of which is described in part II, section F3 (81): 4,5-dimethylproline, m.p. 196.5–197.5°; hydrochloride, m.p. 131.5–133°.

5-Hydroxymethylproline together with 5-hydroxypipecolic acid was formed in the following way (291).

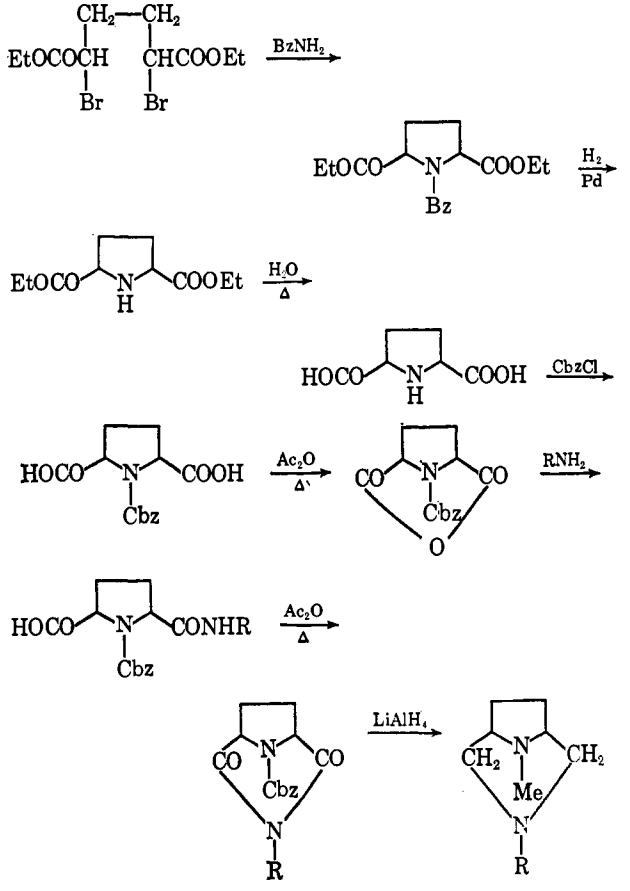


5-Allyl-N-methylproline methyl ester was obtained *via* cyanide cleavage of tropyl chloride (14).



3. 5-Carboxyproline Derivatives

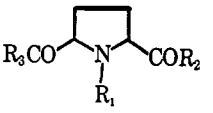
Derivatives of *cis*-5-carboxyproline (pyrrolidine 2,5-dicarboxylic acid) have been used extensively (49–52, 218) in the synthesis of 3,8-diazabicyclo[3.2.1]octanes, which is illustrated by the following synthesis (49).



(R = H, Me, Bu, Ph, or Bz)

During cyclization of amido esters the *trans* isomer of the starting material was sometimes isolated as a side product (52). Some of the numerous derivatives of pyrrolidine-2,5-dicarboxylic acid are shown in Table XVI.

TABLE XVI



DERIVATIVES OF PYRROLIDINE-2,5-DICARBOXYLIC ACID

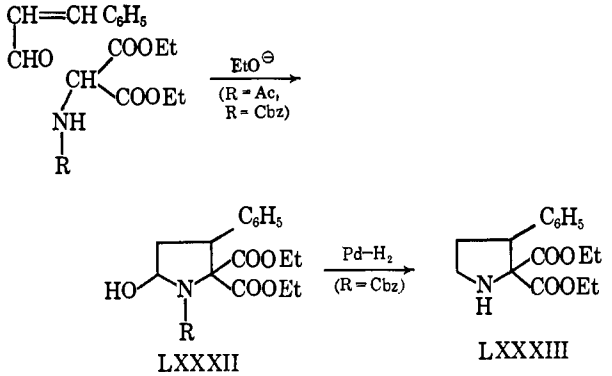
R_1	R_2	R_3	Isomer	M.p., °C.	Ref.
H	OH	OH	*cis*	260–261	49
H·HCl	OMe	OMe	*cis*	191–192	218
H·HCl	OEt	OEt	*cis*	135–137	51
H	NHBz	OH	*cis*	255–257	50
H	NHBz	OEt	*cis*	172–173	50
H	NHBz	OEt	*trans*	65–67	52
H·HCl	NHBz	OEt	*trans*	188–189	52
H	NHBz	NHBz	*cis*	181–182	52
H	NHBz	NHBz	*trans*	90–100	52
Cbz	OH	OH	*cis*	127–128	49
Cbz	Anhydride		*cis*	170–171	49
Bz·HCl	OEt	OEt	*cis*	123–125	49
Me	OH	OH	*cis*	270–273	218
Me	OMe	OMe	*cis*	34–36	218

D. ARYLPROLINES

1. 3-, 4-, and 5-Phenylprolines

3-Phenylproline was synthesized by three related procedures: (i) Michael condensation of diethyl acetamidomalonate and cinnamaldehyde gave a product LXXXII (R = Ac) which on treatment with tin and boiling hydrochloric acid gave a mixture of amino acids from which 3-phenylproline was isolated in 17% yield (156).

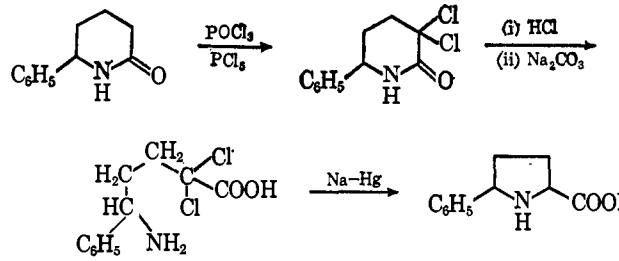
(ii) Condensation of diethyl aminomalonate, or, better, (iii) diethyl benzyloxycarbonylaminomalonate with cinnamaldehyde followed by catalytic hydrogenation gave LXXXIII (hydrochloride, m.p. 155°) which upon hydrolysis gave 3-phenylproline (55, 156): racemic 3-phenylproline, m.p. 275–277° (dec.); *p*-toluenesulfonyl derivative, m.p. 185–188°. An automatic amino acid analysis of 3-phenylproline (from method iii) indicated the presence of two diastereoisomers of 72 and 28% abundance.



4-Phenylproline, m.p. 265° (dec.), was synthesized by the standard route (part IV, section C1) from 5-phenyl-2-piperidone (250). N-Benzoyl-4-phenylproline, m.p. 151–152° (dec.), has been synthesized by an independent route (256).

5-Phenylproline was prepared by two methods: (i) by catalytic hydrogenation of 2-phenyl-Δ^1-pyrroline-5-carboxylic acid hydrochloride, the synthesis of which is described in part II, section F4 (81) (5-phenyl-

proline, m.p. 213–214°; hydrochloride, m.p. 115–117°) and (ii) by a variation of the 3-chloro-2-piperidone route (63)



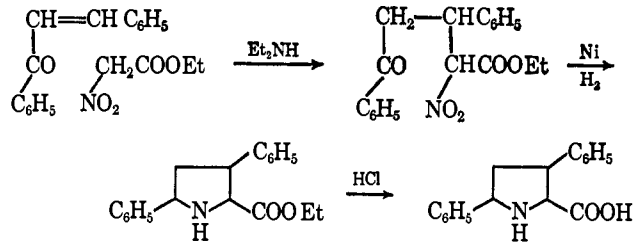
The product was separated by crystallization into two racemic diastereoisomers (distinguishable by vapor phase chromatography of their N-acetyl amyl esters), m.p. 215–217° (dec.) and 233–234° (dec.), respectively.

2. *Substituted 5-Phenylprolines*

A number of substituted 5-phenylprolines were prepared (83) by catalytic hydrogenation of the corresponding 2-aryl-Δ^1-pyrroline-5-carboxylic acid hydrochlorides, the synthesis of which is described in part II, section F4. Their structures and melting points are shown in Table III.

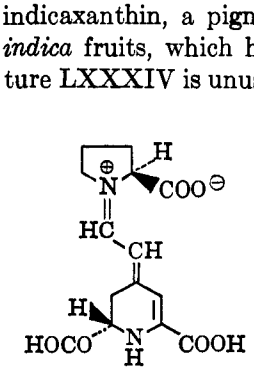
3. *3,5-Diphenylproline*

3,5-Diphenylproline (picrate, m.p. 184–185°) and its ethyl ester (picrate, m.p. 128–129°) were synthesized as follows (61).

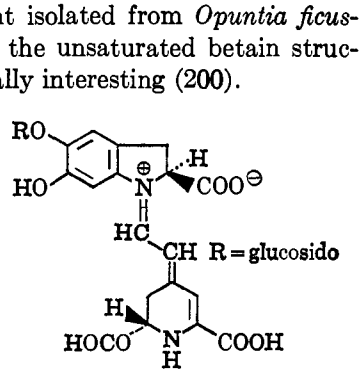


E. MISCELLANEOUS

The scope of this review has not included derivatives (as distinct from analogs) of proline itself; thus N-substituted prolines and the naturally occurring betaine stachydrine have not been reviewed here. However, indicaxanthin, a pigment isolated from *Opuntia ficus-indica* fruits, which has the unsaturated betain structure LXXXIV is unusually interesting (200).



LXXXIV



LXXXV

Indicaxanthin, orange crystals, has a m.p. 160–162° (dec.), $[\alpha]^{20}D$ +394°, λ_{max} (H_2O) 260, 305, and 485 mμ (log ϵ 3.73, 3.19, and 4.63, respectively). Fusion of indicaxanthin with alkali gave DL-proline and 4-methylpiperidine-2,6-dicarboxylic acid; acid hydrolysis afforded L-proline. The configuration at the other asymmetric center was established by hydrogen peroxide oxidation to L-aspartic acid. The structure was supported by n.m.r. data (200).

Indicaxanthin has recently been partially synthesized (294) by base exchange of betanin (LXXXV) (151, 219, 292, 293) with L-proline.

NOTES ADDED IN PROOF.—(i) *3,4-Dehydroproline.* A detailed study of 3,4-dehydroproline and its derivatives has been made (Johnson, L. F., Robertson, A. V., Simpson, W. R. J., and Witkop, B., *Australian J. Chem.*, in press). The dicarbinolamide XX from the reaction of N-carbobenzyloxy-3,4-dehydroprolinamide with N-bromosuccinimide was given the *cis* configuration. Osmium tetroxide oxidation of its O,O′-diacetate gave a glycol which was shown by n.m.r. to possess a plane of symmetry.

(ii) *4-Hydroxyproline.* A conference on the clinical implications of hydroxyproline and collagen metabolism has been reported (*Ann. Internal Med.*, **63** (1965)).

(iii) *3,4-Dihydroxyproline* (215). Attempts to prepare a lactone from 2,3-*cis*-3,4-*trans*-dihydroxyproline analogous to that of 4-allohydroxyproline were made *via* the N,O,O′-tri-*p*-toluenesulfonyl derivative; instead, N-*p*-toluenesulfonylpyrroles were obtained by elimination. In this isomeric series, N,O,O′-tri-*p*-toluenesulfonyl-L-proline has m.p. 203°; methyl ester, m.p. 138°; *t*-butyl ester, m.p. 158°.

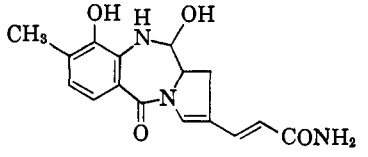
(iv) *4-Fluoroproline.* An independent synthesis of *cis*- and *trans*-4-fluoroprolines has appeared (Hayden, J. W., and Burgstahler, A. W., 1st Midwest Regional American Chemical Society Meeting, Kansas City, Nov. 1965; *Science*, in press). In mice, only the *cis*-4-fluoro-L-proline caused growth inhibition and degenerative changes in the liver and is considered an antimetabolite to proline. In *Streptomyces antibioticus* both *cis*- and *trans*-4-fluoroprolines were as readily incorporated into actinomycin as proline (Katz, E., private communication).

(v) *4-Chloro- and 4-bromoprolines* (12). Further physical properties are given: *trans*-4-chloro-L-proline, $[\alpha]D$ −55.6° (*c* 2.0, H_2O); *cis*-4-chloro-L-proline, m.p. 219°, $[\alpha]D$ −29.5° (*c* 1.5, H_2O); *trans*-4-bromo-L-proline, $[\alpha]D$ −38.2° (*c* 2.0, H_2O); *cis*-4-bromo-L-proline, $[\alpha]D$ −17.5° (*c* 2.5, H_2O).

(vi) *3-Methylproline.* A new synthesis of racemic *cis*- and *trans*-3-methylprolines depends upon hydrogenation and subsequent hydrolysis of *cis*- and *trans*-N-acetyl-4,5-dehydro-3-methylproline ethyl (or methyl) ester; the latter intermediates have also been converted to derivatives of alloisoleucine and isoleucine,

respectively (114). *cis*-3-Methyl-L-proline has been identified as one of the hydrolysis products of bottromycin A (Nakamura, S., Chikaike, T., Karasawa, K., Tanada, N., Yonehara, H., and Umezawa, H., *J. Antibiotics*, **A18**, 47 (1965); Nakamura, S., Chikaike, T., Yonehara, H., and Umezawa, H., *Chem. Pharm. Bull. Japan*, **13**, 599 (1965)), the complete structure of which has been reported (Nakamura, S., Chikaike, T., Yonehara, H., and Umezawa, H., *J. Antibiotics*, **A18**, 60 (1965)). Bottromycin B possesses the same structure with L-proline in place of *cis*-3-methylproline.

(vii) The structure of *anthramycin* has recently been elucidated (Leimgruber, W., Batcho, A. D., and Schenker, F., private communication); a proline analog may be involved as a biosynthetic precursor for the



N-acyl-Δ^2-pyrroline moiety. The other fragment, 3-hydroxy-4-methylanthranilic acid, is also implicated as a precursor of actinomycins (Weissbach, H., Redfield, B. G., Beaven, V., and Katz, E., *J. Biol. Chem.*, **240**, 4377 (1965)).

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