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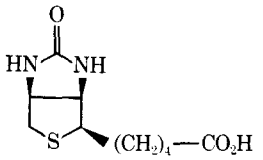
Synthesis of Azabiotin Analogs as Potential Cofactors for Biotin-Dependent Enzymes

HENRY C. WORMSER

Abstract □ As part of a program to synthesize azabiotin analogs and homologs as potential substitutes for the natural coenzyme, several 4- and 5-substituted derivatives of *cis*-hexahydropyrrolo-[3,4-*d*]imidazole-2-one have been prepared. Spectral data and certain side reactions in the synthetic scheme used point to the stereochemistry of the 4-substituted compounds.

Keyphrases □ Azabiotin analogs—synthesis □ Biotin-dependent enzymes—potential cofactor, azabiotin analogs □ NMR spectroscopy—structure □ UV spectrophotometry—structure □ IR spectrophotometry—structure □ Mass spectroscopy—structure

Biotin (I) is a cofactor required for several enzyme-catalyzed carboxylation reactions and, as such, plays a significant role in carbon dioxide fixation reactions. Although a wide variety of compounds closely related to the vitamin have been prepared, very few have been found to possess significant biochemical and growth-promoting activity in microorganisms and animals.



I

Recently (1–3), controversy has arisen about the significance of the sulfur atom of biotin with regard to interactions between the enzyme protein and the cofactor. NMR data have indicated that both the ureido oxygen and the sulfur atom should be considered as

Table I—NMR Spectra at 60 Mc. in $CDCl_3$ Using TMS Reference (Pyrrolidino Compounds)[a]

Compound	Data	*N*-Acetyl	Ester CH_2	Ester CH_3	N—H	5—CH_3	Miscellaneous
III	δ p.p.m.		4.17	1.28	6.60		C-2 and C-5
		—				—	4.25
	J, c.p.s.		q, 7.0	t, 7.0	s		s
XV	δ p.p.m.	—	4.26	1.41	6.02	1.22	C-2 4.61 (1H)
	J, c.p.s.		q, 7.0	t, 7.0	s	d, 6.0	C-5 4.25 (2H)
IV	δ p.p.m.	2.20	4.25	1.30	4.90		
				t, 7.1		—	
	J, c.p.s.	s	q, 7.0	1.33	d, 7.4		
				t, 7.1			
XVI	δ p.p.m.	2.19	4.22	1.30	5.54	2.18	C-5 4.25 (2H)
	J, c.p.s.	s	q, 7.0	m	s		
XVII	δ p.p.m.	2.37	4.43	1.36	7.95	2.20	C-2 7.81 (1H)
				t, 7.1			
	J, c.p.s.	s	q, 7.0	1.46	s	s	
				t, 7.1			
V	δ p.p.m.	1.94	4.20	1.25	7.03		
						—	
	J, c.p.s.	s	q, 7.0	t, 7.0	d, 7.0		
XVIII	δ p.p.m.	2.01	4.17	1.25	7.1	1.20	
	J, c.p.s.	s	q, 7.1	t, 7.2	d, 9.5	d, 6.0	
VI	δ p.p.m.	1.99	4.16	1.26	7.36		
	J, c.p.s.	s	q, 7.1	t, 7.0	d, 7.0	—	
XXI	δ p.p.m.	2.01	4.17	1.25	7.29	1.10	
	J, c.p.s.	s	q, 7.1	t, 7.1	d, 7.0	d, 6.0	

[a] s = singlet, d = doublet, m = multiplet, t = triplet, and q = quartet.

Table II—NMR Spectra at 60 Mc. in $CDCl_3$ Using TMS Reference (Pyrrolo[3,4-*d*]imidazolone Compounds)[a]

Compound	Data	3-Acetyl	Carbamate CH_2	Carbamate CH_3	N—H	4—CH_3
IX	δ p.p.m.	2.51	4.20	1.29	7.1	—
	J, c.p.s.	s	q, 7.0	t, 7.0	s	
XXIV	δ p.p.m.	2.51	4.16	1.28	6.92	1.05
	J, c.p.s.	s	q, 7.0	t, 7.0	s	d, 6.0
XXV	δ p.p.m.	—	4.16	1.28	6.77, s	1.28
	J, c.p.s.		q, 7.0	t, 7.0	6.54, s	d, 6.0

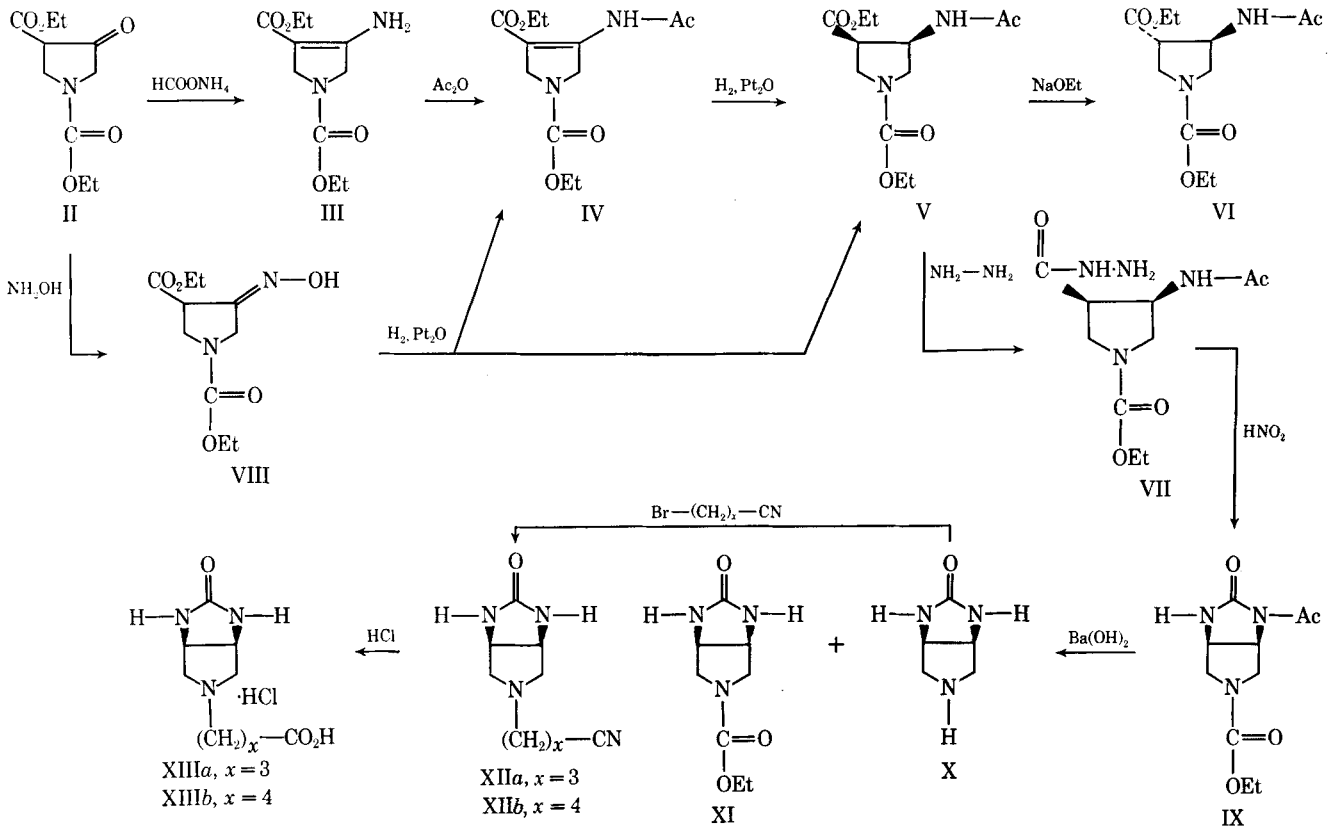
[a] s = singlet, d = doublet, m = multiplet, t = triplet, and q = quartet.

potential hydrogen-bond formers with polar groups on a protein to which biotin binds (3, 4) (Tables I and II).

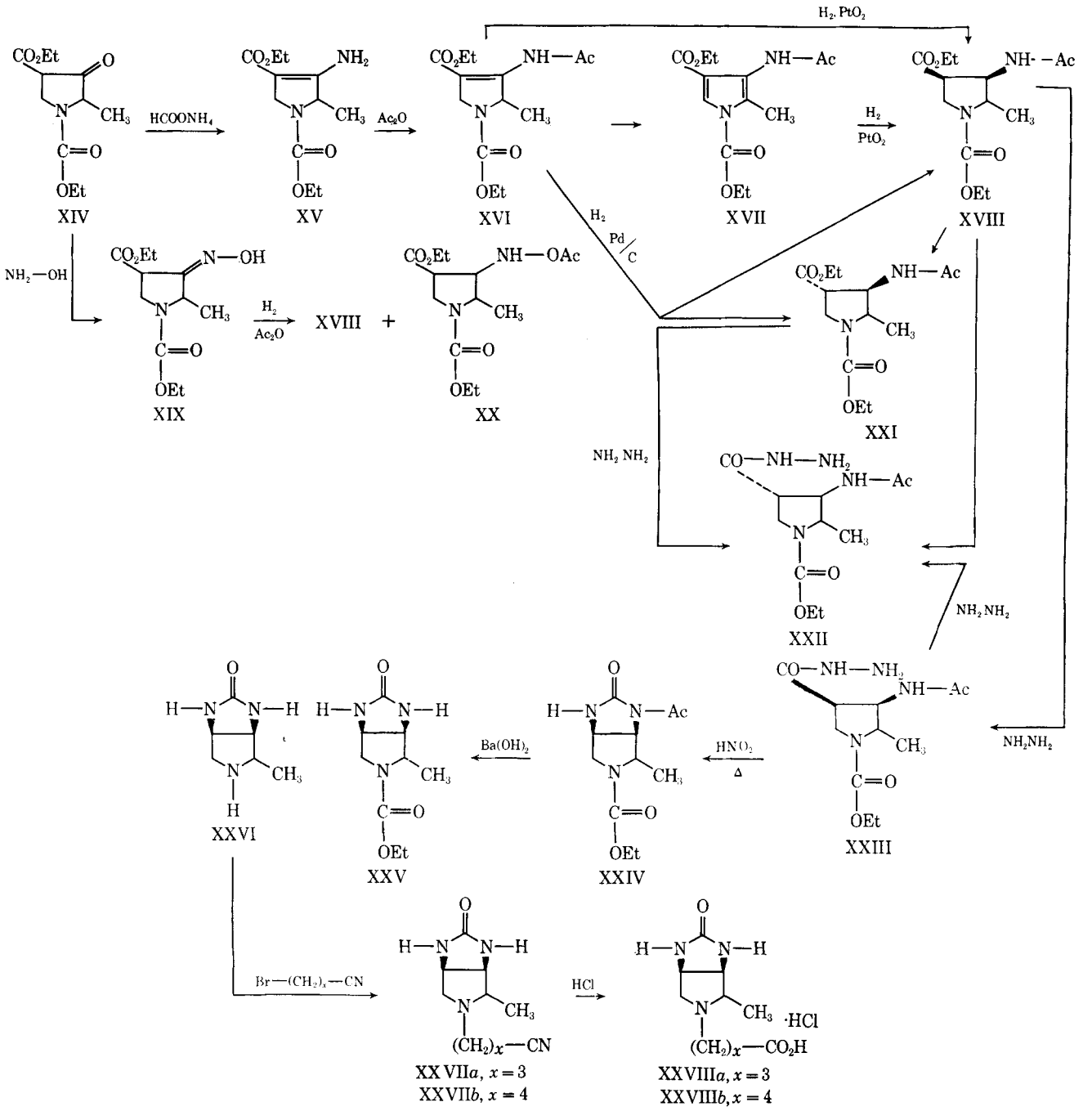
In a preceding paper (5), an approach to the pyrrolo[3,4-*d*]imidazole nucleus was presented. It was hoped that the method described would be applicable to the preparation of 4-substituted derivatives, ultimately leading to the synthesis of azabiotin. The method, in fact, offers an unequivocal synthetic route to the desired heterobicyclic system. A somewhat modified route had to be adopted to allow the introduction of a side chain on the carbon atom adjacent to the nitrogen of the pyrrolidine ring.

1,3-Dicarbethoxy-4-amino-3-pyrrolines (III and XV in Schemes I and II) had not been employed previously as intermediates for the synthesis of the pyrrolo[3,4-*d*]-imidazole system. Such compounds are readily formed by heating the extensively enolized 1,3-dicarbethoxy-4-pyrrolidones (II and XIV) with ammonium formate in ethanol solution (6, 7). The pyrrolidones were obtained by cyclizations of the Dieckmann type carried out as described previously (8). Initially, several attempts were made to obtain 1,3-dicarbethoxy-4-ureido-5-methyl-3-pyrroline *via* the condensation of urea with the corresponding pyrrolidone, but the starting materials were invariably recovered unchanged. This failure suggests that the occurrence of this type of reaction is hampered, on the one hand, by a high degree of crowding of the carbonyl group and, on the other, by the poor nucleophilic character of the attacking species. The aminopyrrolines were acetylated in good yields in the presence of acetic anhydride. *N*-Acetyl (IV) proved quite stable. However, XVI, on standing at room temperature, oxidized to the corresponding pyrrole (XVII).

Catalytic reduction with Adams' catalyst of IV, XVI, and XVII afforded stereospecifically in each case the corresponding *cis*-pyrrolidines. However, reduction of the pyrroline XVI with 10% palladium on charcoal gave a 2:1 mixture of *cis*-product XVIII and *trans*-product XXI. The *trans*-products, XXI and VI, were



Scheme I



Scheme II

independently obtained by base-catalyzed epimerization reactions. The conversion to XXI was rather facile, whereas the formation of VI required more drastic conditions. The acetamido pyrrolidines, V and XVIII, were also sought from oximes VIII and XIX, respectively, by catalytic hydrogenation in acetic anhydride. This latter method offered no advantage over the previously outlined procedure and, in both cases, led to the production of mixtures. Hydrazinolysis of ester XVIII under reflux condition afforded exclusively *trans*-hydrazide XXII, whereas milder conditions afforded only *cis*-product XXIII. This latter can be converted to hydrazide XXII by prolonged treatment with hydrazine hydrate under reflux condition or by the addition of sodium ethoxide in ethanol at room temperature. Hydrazide XXII can also be obtained in nearly quantitative yield from the *trans*-ester XXI and hydrazine hydrate in boiling ethanol. These rather facile epimerizations at carbon 3 are significant, because they point to a rather unstable situation arising from steric compression brought about by the *cis* relationships at C-3, C-4, and C-5 of the pyrrolidine ring system and, consequently, the need for relief from this strain. These findings are coupled with similar observations found in the literature (9, 10).

Conversion of hydrazides VII and XXIII to their respective azides and Curtius rearrangement of these intermediates afforded the pyrrolo[3,4-*d*]imidazole derivatives IX and XXIV, respectively. Barium hydrolysis of these two compounds afforded two bicyclic moieties,

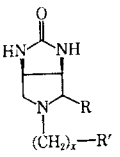


Table III—Azabiotin Analogs

Compound	R	x	R′	M.p.	Yield, %	Formula	Anal., % Calcd.	Found
XII*a*	H	3	CN[a]	127–130°	93	$C_9H_{14}N_4O \cdot HCl \cdot H_2O$	C, 43.46 H, 6.89 N, 22.52	C, 43.69 H, 6.20 N, 22.42
XIII*a*	H	3	CO_2H[a]	247–255° dec.	71	$C_9H_{15}N_3O_3 \cdot HCl$	C, 43.29 H, 6.46 N, 16.83	C, 43.06 H, 6.46 N, 16.80
XII*b*	H	4	CN	129–130°	77	$C_{10}H_{16}N_4O$	C, 57.65 H, 7.74 N, 26.90	C, 57.64 H, 7.67 N, 26.77
XIII*b*	H	4	CO_2H[a]	241–243° dec.	79	$C_{10}H_{17}N_3O_3 \cdot HCl$	C, 45.54 H, 6.88 N, 15.94	C, 45.55 H, 6.84 N, 15.89
XXVII*a*	CH_3	3	CN	174–175°	75	$C_{10}H_{16}N_4O$	C, 57.65 H, 7.74 N, 26.90	C, 57.59 H, 7.73 N, 26.95
XXVIII*a*	CH_3	3	CO_2H[a]	253–257° dec.	64	$C_{10}H_{17}N_3O_3 \cdot HCl$	C, 45.54 H, 6.88 N, 15.94	C, 45.59 H, 6.87 N, 15.90
XXVII*b*	CH_3	4	CN	201–202°	75	$C_{11}H_{18}N_4O$	C, 59.70 H, 8.20 N, 25.22	C, 59.68 H, 8.14 N, 25.15
XXVIII*b*	CH_3	4	CO_2H[a]	223–225° dec.	68	$C_{11}H_{19}N_3O_3 \cdot HCl \cdot H_2O$	C, 44.83 H, 7.70 N, 14.26	C, 44.83 H, 7.44 N, 14.17

[a] Hydrochloride salt.

which were functionalized at position 5 to yield the azabiotin analogs listed in Table III.

EXPERIMENTAL[1]

1,3-Dicarbethoxy-4-acetamido-3-pyrroline (IV)—A solution of 80.9 g. (0.35 mole) of 1,3-dicarbethoxy-4-amino-3-pyrroline (III) (11) in 250 ml. of acetic anhydride was refluxed for 5 hr. The excess acetic anhydride was removed under reduced pressure, and the crystalline product was collected to yield 89.4 g. (94%), m.p. 123–125°. Several recrystallizations from 95% ethanol gave colorless crystals of IV, m.p. 127–129°. UV spectrum showed $\lambda_{max.}^{EtOH}$ 274 mμ (ε 21,850). IR spectrum (mineral oil) showed bands at 3.01, 5.90, and 6.10 μ.

Anal.—Calcd. for $C_{12}H_{18}N_2O_5$: C, 53.31; H, 6.71; N, 10.37. Found: C, 53.31; H, 6.64; N, 10.33.

***cis*-1,3-Dicarbethoxy-4-acetamidopyrrolidine (V)**—A solution of 2.0 g. (7.4 mmoles) of IV in 100 ml. of 95% ethanol was hydrogenated in a Parr shaker over 0.2 g. (84.7%) of platinum oxide for 24 hr. Evaporation of the filtered solution under reduced pressure gave a thick, colorless, oily residue, which showed a single spot on TLC (20% methanol in ether). Treatment of the oily product with ether afforded a colorless crystalline material, 1.34 g. (66.5%), m.p. 123–125°. Two recrystallizations from ether gave the analytical sample, m.p. 127–129°. IR spectrum showed bands at 2.90, 3.00, 5.80, and 5.97 μ.

Anal.—Calcd. for $C_{12}H_{20}N_2O_5$: C, 52.91; H, 7.40; N, 10.29. Found: C, 52.93; H, 7.36; N, 10.20.

[1] Melting points were determined on a Fisher-Johns melting-point stage and a Thomas-Hoover melting-point apparatus which had been calibrated with standard samples. UV absorption spectra were determined in 95% ethanol on a Beckman (model DK2A) recording spectrophotometer. IR absorption spectra were recorded in chloroform (unless otherwise specified) on a Beckman (model 8) recording spectrophotometer. NMR spectra were determined in deuterochloroform using TMS as reference standard on a Varian A-60A spectrometer. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. Mass spectra were determined on an Atlas CH-4 mass spectrometer with TO-4 ion source. TLC was carried out with silica gel G and silica gel $HF_{254+366}$ (Brinkmann Instruments).

Catalytic Reduction of 1,3-Dicarbethoxy-4-pyrrolidone Oxime (VIII)—A solution of 0.67 g. (2.7 mmoles) of 1,3-dicarbethoxy-4-pyrrolidone oxime (VIII) (12) in 50 ml. of acetic anhydride was hydrogenated at 3 atm. over 0.2 g. (84.7%) of platinum oxide for 24 hr. Evaporation of the excess acetic anhydride afforded a tan crystalline residue, 0.64 g., m.p. 112–115°. Several recrystallizations from ether gave colorless needles, m.p. 122–124°. TLC of this product (15% methanol in chloroform) showed two spots of nearly equal size. Preparative TLC on silica gel $HF_{254+366}$ using the same solvent system gave two crystalline compounds following extraction of the respective bands. The band with the higher R_f afforded a substance that was characterized as 1,3-dicarbethoxy-4-acetamido-3-pyrroline (IV), m.p. 126–129°; mixed melting point was not depressed upon admixture with an authentic sample. The band with the lower R_f gave *cis*-1,3-dicarbethoxy-4-acetamidopyrrolidine (V), also identical in all respects to the authentic sample prepared by a previous method.

***trans*-1,3-Dicarbethoxy-4-acetamidopyrrolidine (VI)**—A solution of V (0.20 g., 0.74 mmole) and freshly prepared sodium ethoxide (0.05 g., 0.74 mmole) in 15 ml. of absolute ethanol was refluxed for 15 hr. The alcohol was removed under reduced pressure, cold water was added to the residue, and the reaction product was extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. TLC of the residual oil (5% methanol in chloroform) showed two spots: a major spot and a smaller spot corresponding to the starting material (V). Preparative TLC on silica gel $HF_{254+366}$ afforded a colorless oil which crystallized on standing. Recrystallization from isopropyl ether gave 0.13 g. (65%) of colorless needles, m.p. 107–108°. IR spectrum showed bands at 2.91, 3.01, 5.81, and 6.00 μ.

Anal.—Calcd. for $C_{12}H_{20}N_2O_5$: C, 52.91; H, 7.40; N, 10.29. Found: C, 52.90; H, 7.31; N, 10.19.

***cis*-1-Carbethoxy-3-hydrazino-4-acetamidopyrrolidine (VII)**—A mixture of V (1.0 g., 3.7 mmoles) and hydrazine hydrate (3.0 ml.) was stirred at room temperature for 5 hr. Excess hydrazine hydrate was removed by distillation under reduced pressure, leaving a very viscous oil. Although the product was homogeneous on TLC (20% methanol in ethyl acetate), all attempts to crystallize it failed. IR spectrum (film) showed bands at 3.01, 5.97, and 6.05 μ.

3-Acetyl-5-carbethoxy-*cis*-hexahydropyrrolo[3,4-*d*]imidazole-2-one (IX)—*cis*-Hydrazide (VII) (0.60 g., 2.33 mmoles) was dissolved in 3 ml. of 2 *N* hydrochloric acid, and the cooled solution was treated dropwise with a cold solution of 0.15 g. (2.17 mmoles) of sodium nitrite in 2 ml. of water. The oily, yellow azide was extracted with five 10-ml. portions of ethyl acetate, and the combined extracts were dried over anhydrous sodium sulfate. The filtered solution was then refluxed on a steam bath for 2 hr., and the solvent was evaporated to dryness. On standing, the oily residue crystallized to give 0.46 g. (82%) of IX, m.p. 115–120°. Recrystallization from ether afforded colorless needles, m.p. 119–121°. IR spectrum showed bands at 2.91, 3.05, 5.80, and 5.99 μ.

Anal.—Calcd. for $C_{10}H_{15}N_3O_4$: C, 49.80; H, 6.27; N, 17.43. Found: C, 49.63; H, 6.26; N, 17.55.

***cis*-Hexahydropyrrolo[3,4-*d*]imidazole-2-one (X)**—Bicyclic ureide (IX) (5.0 g., 20.8 mmoles) was hydrolyzed by the method previously described (5). Following workup, 2.49 g. (94%) of a colorless crystalline product was obtained, m.p. 210–214°. Recrystallization from methanol afforded X, m.p. 213–215°; mixed melting point was not depressed by admixture with an authentic sample obtained previously. In addition, 0.042 g. of 5-carbethoxyhexahydropyrrolo-[3,4-*d*]imidazole-2-one (XI) was obtained from the reaction mixture, m.p. 221–222°; mixed melting point was not depressed by admixture with an authentic sample.

1,3-Dicarbethoxy-4-amino-5-methyl-3-pyrroline (XV)—A solution of 121.7 g. (0.5 mole) of 1,3-dicarbethoxy-5-methyl-4-pyrrolidone (XIV) (13) and 78.6 g. (1.25 moles) of ammonium formate in 750 ml. of absolute ethanol was heated under reflux for 48 hr. Evaporation of the solvent under reduced pressure afforded a tan crystalline residue. The solid product was treated with 250 ml. of water to dissolve unreacted ammonium formate, and the reaction product was extracted with chloroform. The chloroform extract was washed with water and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded 118.4 g. of crystalline product, m.p. 126–127°. Recrystallization from 95% ethanol gave colorless crystals of XV, 112 g. (93%), m.p. 127–129°. UV spectrum showed $\lambda_{max.}^{EtOH}$ 274 mμ (ε 21,800). IR spectrum showed bands at 2.85, 2.96, 5.98, and 6.10 μ.

Anal.—Calcd. for $C_{11}H_{18}N_2O_4$: C, 54.52; H, 7.49; N, 11.56. Found: C, 54.63; H, 7.57; N, 11.54.

1,3-Dicarbethoxy-4-acetamido-5-methyl-3-pyrroline (XVI)—A solution of 112 g. (0.46 mole) of XV in 350 ml. of acetic anhydride was refluxed for 12 hr. The excess reagent was distilled under reduced pressure, and the remaining yellow oil crystallized from petroleum ether (b.p. 30–60°), 129.3 g. (98.4%), m.p. 69–70°. UV spectrum showed $\lambda_{max.}^{EtOH}$ 274 mμ (ε 14,050). IR spectrum showed bands at 3.01, 5.95, and 6.12 μ.

Anal.—Calcd. for $C_{13}H_{20}N_2O_5$: C, 54.91; H, 7.09; N, 9.85. Found: C, 54.77; H, 7.07; N, 9.81.

1,3-Dicarbethoxy-4-acetamido-5-methylpyrrole (XVII)—On standing at room temperature for several weeks, crystalline product XVI turned pale yellow and became quite sticky. TLC revealed two spots. A small portion of the mixture was separated by preparative TLC on silica gel $HF_{254+366}$ (15% methanol in ethyl acetate). The higher R_f band gave the starting material (XVI), m.p. 68–71°; mixed m.p. 68–71°. The lower R_f band gave product XVII, which crystallized from diethyl ether as colorless needles having m.p. 132–133°. This latter product gave no significant UV absorption at 274 mμ but showed absorption at 216 mμ. IR spectrum showed bands at 2.91, 5.70, 5.90, and 6.20 μ.

Anal.—Calcd. for $C_{13}H_{18}N_2O_5$: C, 55.31; H, 6.43; N, 9.92. Found: C, 55.24; H, 6.24; N, 9.81.

***cis*-1,3-Dicarbethoxy-4-acetamido-5-methylpyrrolidine (XVIII)**—*Method A*—A solution of XVI (3.0 g., 10.6 mmoles) in 100 ml. of 95% ethanol was hydrogenated in a Parr shaker over 0.3 g. (84.7%) of platinum oxide for 24 hr. Evaporation of the filtered solution gave a thick, colorless oil which showed a single spot on TLC (8% methanol in ethyl acetate). Crystallization from isopropyl ether afforded 2.87 g. (95%) of colorless crystals, m.p. 101–103°. The IR spectrum showed bands at 2.91, 5.82, and 5.99 μ.

Anal.—Calcd. for $C_{13}H_{22}N_2O_5$: C, 54.52; H, 7.75; N, 9.78. Found: C, 54.72; H, 7.77; N, 9.73.

Method B—A solution of XVII (1.0 g., 3.5 mmoles) in 50 ml. of 95% ethanol was hydrogenated as in Method A over 0.2 g. (84.7%) of platinum oxide. Following the usual workup, 0.94 g. (93%) of a crystalline product was obtained, m.p. 101–103°. The IR and TLC mobility of the product were identical to those obtained for the product of Method A.

***trans*-1,3-Dicarbethoxy-4-acetamido-5-methylpyrrolidine (XXI)**—*Method A*—A solution of XVI (0.3 g., 1.06 mmole) in 50 ml. of 95% ethanol was hydrogenated over 0.1 g. of 10% palladium on charcoal for 12 hr. Evaporation of the filtered solution gave a colorless oil which showed two spots on TLC (10% methanol in ethyl acetate). Preparative TLC on silica gel $HF_{254+366}$ afforded 0.19 g. of product XVIII, m.p. 100–102°; mixed melting point was not depressed by admixture with Compound XVIII obtained previously. The lower R_f band gave 0.09 g. of product XXI, which crystallized from isopropyl ether as colorless needles, m.p. 128–129°. IR spectrum showed bands at 2.90, 3.00, 5.80, and 5.95 μ.

Anal.—Calcd. for $C_{13}H_{22}N_2O_5$: C, 54.52; H, 7.75; N, 9.78. Found: C, 54.66; H, 7.74; N, 9.80.

Method B—A solution of XVIII (0.10 g., 0.35 mmole) and freshly prepared sodium ethoxide (0.005 g., 0.07 mmole) in 5 ml. of absolute ethanol was stirred at room temperature for 12 hr. The solvent was removed under reduced pressure, ice was added to the residue, and the product was extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The tan solid residue (0.082 g., 82%) was recrystallized from isopropyl ether, affording colorless needles, m.p. 128–129°; mixed melting point was not depressed by admixture with the product obtained under Method A.

1,3-Dicarbethoxy-5-methyl-4-pyrrolidone Oxime (XIX)—A mixture of hydroxylamine hydrochloride (3.15 g., 0.045 mole) and sodium acetate trihydrate (6.30 g., 0.046 mole) in 10 ml. of 90% methanol was triturated in a glass mortar. The white suspension was filtered through diatomaceous earth,[2] and the clear filtrate was added to XIV (4.86 g., 0.02 mole). The solution, which immediately became turbid, was stirred at room temperature for 2 hr., followed by a period of heating at 60° until the reaction mixture failed to give a positive ferric chloride test. Following evaporation of the methanol, the product was dissolved in chloroform, washed with water, and dried over anhydrous sodium sulfate. Evaporation of the chloroform gave a colorless, viscous oil (5.01 g., 97%) which resisted crystallization. IR spectrum showed bands at 2.79, 3.02, 5.79, and 5.94 μ.

Catalytic Reduction of Oxime (XIX)—A solution of oxime (XIX) (4.0 g., 15.5 mmoles) in 75 ml. of acetic anhydride was hydrogenated over 0.2 g. (84.7%) of platinum oxide at 3 atm. for 48 hr. Evaporation of the filtered solution *in vacuo* gave a thick, yellow, oily residue which showed three spots on TLC. The mixture was separated by preparative TLC on silica gel $HF_{254+366}$ (5% methanol in chloroform). The higher R_f band gave 2.4 g. of the starting material (XIX) as an oil (superimposable IR spectra). The intermediate R_f band gave 0.78 g. of a crystalline product (XX), m.p. 186–187°. IR spectrum (mineral oil) showed bands at 3.02, 5.70, and 5.95 μ. An analytical sample was obtained by recrystallization from ethyl acetate, m.p. 188–190°.[3]

Anal.—Calcd. for $C_{13}H_{22}N_2O_6$: C, 51.66; H, 7.34; N, 9.27. Found: C, 51.60; H, 7.27; N, 9.35.

The lower R_f band gave 0.64 g. of *cis*-ester (XVIII), m.p. 101–102°; mixed melting point was not depressed by admixture with the compound obtained previously.

***trans*-1-Carbethoxy-3-hydrazino-4-acetamido-5-methylpyrrolidine (XXII)**—A solution of 0.14 g. (0.5 mmole) of XVIII in 3 ml. of absolute ethanol was treated with 0.2 ml. of hydrazine hydrate, and the mixture was refluxed for 5 hr. Evaporation of the solvent afforded 0.13 g. of crystalline product, m.p. 195–204°. Recrystallization from ethanol–ether mixture gave 0.11 g. (79.4%) of colorless crystals. The analytical sample had m.p. 211–212°. IR spectrum (mineral oil) showed bands at 3.04, 3.19. 5.96, and 6.05 μ. Mass spectrum (70 ev.) gave a molecular ion at *m/e* 272.

Anal.—Calcd. for $C_{11}H_{20}N_4O_4$: C, 48.52; H, 7.40; N, 20.57. Found: C, 48.60; H, 7.40; N, 20.58.

***cis*-1-Carbethoxy-3-hydrazino-4-acetamido-5-methylpyrrolidine (XXIII)**—A suspension of 1.99 g. (6.95 mmoles) of XVIII in 6 ml. of hydrazine hydrate was stirred at room temperature for 12 hr. The white suspension was filtered, affording 1.17 g. of a micro-

[2] Celite, Johns-Manville, New York, N. Y.

[3] Compound XX was characterized as an *O*-acetyl pyrrolidinohydroxylamine derivative, since the reduction of oximes to amines presumably involves the intermediacy of hydroxylamines [G. Vavon and Krajcinovic, *Bull. Soc. Chem. France*, **43**, 231(1928)].

crystalline substance, m.p. 146–152°. Evaporation of the excess hydrazine hydrate *in vacuo* afforded an additional 0.34 g. of product for an overall yield of 79.8%. Recrystallization from ethyl acetate gave 1.24 g. of a crystalline colorless product, m.p. 159–160°. IR spectrum (mineral oil) showed bands at 3.02, 5.85, 5.95, and 6.05 μ. Mass spectrum (70 ev.) gave a molecular ion at *m/e* 272.

Anal.—Calcd. for $C_{11}H_{20}N_4O_4$: C, 48.52; H, 7.40; N, 20.57. Found: C, 48.65; H, 7.46; N, 20.44.

3-Acetyl-4-methyl-5-carbethoxy-*cis*-hexahydropyrrolo[3,4-*d*]imidazole-2-one (XXIV)—One gram (3.67 mmoles) of hydrazide XXIII dissolved in 4 ml of 2 *N* hydrochloric acid and cooled to −5° was treated dropwise with a cold solution of 0.5 g. (7.2 mmoles) of sodium nitrite in 2 ml. of water. The white precipitate which formed was filtered, m.p. 100–104° dec. The azide was dissolved in 75 ml. of ethyl acetate and heated to reflux on a steam bath for 2 hr. Evaporation of the solvent afforded a yellowish oil which crystallized on standing, 0.62 g. (66%), m.p. 101–105°. Recrystallization from isopropyl ether gave XXIV, m.p. 108–110°. IR spectrum showed bands at 2.89, 3.02, 5.75, and 5.95 μ. Mass spectrum (70 ev.) gave a molecular ion at *m/e* 255.

Anal.—Calcd. for $C_{11}H_{17}N_3O_4$: C, 51.75; H, 6.71; N, 16.46. Found: C, 51.80; H, 6.81; N, 16.59.

4-Methyl-5-carbethoxy-*cis*-hexahydropyrrolo[3,4-*d*]imidazole-2-one (XXV) and 4-Methyl-*cis*-hexahydropyrrolo[3,4-*d*]imidazole-2-one (XXVI)—A mixture of 0.60 g. (2.35 mmoles) of XXIV and 3.0 g. (9.51 mmoles) of barium hydroxide octahydrate in 30 ml. of 50% aqueous methanol was refluxed for 12 hr. The creamy suspension was filtered while hot, and the filtrate was evaporated to dryness *in vacuo*. The tan residue was chromatographed over 50 g. of basic alumina, eluting successively with chloroform and 5, 10, 25, and 50% methanol in chloroform. The first three fractions (250 ml. each) afforded 0.40 g. (80%) of a crystalline product, m.p. 145–146°. Recrystallization from ethyl acetate gave an analytical sample, m.p. 145–146°. IR spectrum showed bands at 2.89, 3.09, and 5.82–5.98 (w) μ. Mass spectrum (70 ev.) gave a molecular ion at *m/e* 213.

Anal.—Calcd. for $C_9H_{15}N_3O_3$: C, 50.70; H, 7.09; N, 19.70. Found: C, 50.66; H, 6.99; N, 19.58.

The last two fractions (150 ml. each) gave a white product (0.06 g., 16%), m.p. 206–210°. Recrystallization from ethyl acetate gave XXVI, m.p. 209–210°. IR (mineral oil) showed bands at 3.10 and 5.90 μ. Mass spectrum (70 ev.) gave a molecular ion at *m/e* 141.

Anal.—Calcd. for $C_6H_{11}N_3O$: C, 51.03; H, 7.85; N, 29.76. Found: C, 51.11; H, 7.84; N, 29.69.

***N*-Alkylation of Pyrrolo[3,4-*d*]imidazolones (X and XXVI)**—One part of the secondary amine was refluxed for 12 hr. in the presence of a threefold excess of a nitriloalkyl bromide in the presence of anhydrous potassium carbonate in absolute ethanol. The salts were filtered, and the filtrate was evaporated to dryness. Recrystallization of the tertiary amines (XII*a*, XII*b*, XXVII*a*, and XXVII*b*) was achieved using ethyl acetate.

Hydrolysis to Amino Acid Hydrochlorides (XIII*a*, XIII*b*, XXVIII*a*, and XXVIII*b*)—The respective alkylated pyrrolo[3,4-*d*]imidazolone products were heated at 100° for 8 hr. in the presence of concentrated hydrochloric acid. The reaction mixture was evaporated to dryness under reduced pressure, and the crystalline residue was recrystallized from ethanol or ethanol–ether mixtures.

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