Synthesis of 1-Hydroxy-L-proline and Related Cyclic *N*-Hydroxyamino Acids. Metabolic Disposition of ^{14}C-Labeled 1-Hydroxy-L-proline in Rodents†

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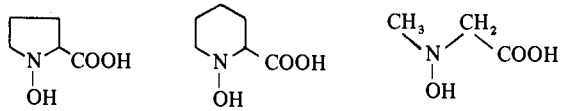
The L-, D-, and racemic forms of 1-hydroxyproline (1) as well as 1-hydroxy-DL-pipecolic acid (2) and *N*-hydroxysarcosine (3) were prepared from the respective amino acids by (a) cyanoethylation, (b) N-oxidation of 1-(2-cyanoethyl)amino acids, and (c) elimination of acrylonitrile in a Cope elimination. 2, 3, or the L form of 1 did not inhibit the growth of L-1210 lymphoid leukemia in BDF_1 mice, and L-1 did not inhibit the growth of *Escherichia coli* B or of *Phaseolus aureus* in culture. Uniformly ^{14}C-labeled 1-hydroxy-L-proline was extensively degraded in mice and rats, up to 60% of the radioactivity being excreted as radioactive CO_2-^{14}C in the expired breath within 24 hr after ip administration.

N-Hydroxy analogs of certain natural α-amino acids occur in bound form as hydroxamic acids in a variety of antibiotics and growth factors isolated from microbial sources.[1] Recently, N-hydroxylated peptides that are hydrolyzable to *N*-hydroxyamino acids have been found in human brain tumor, in virus-induced tumors of mouse spleen, and in carcinogen-induced tumors of the rat.[2] Hadacidin, *N*-formyl-*N*-hydroxyglycine, the simplest of the known naturally occurring hydroxamic acids,[3] is an antimetabolite of L-aspartic acid[4] with antitumor properties,[5] while 1-hydroxyaminocyclopentanecarboxylic acid, a *synthetic* α-hydroxyamino acid, is a potent growth inhibitor of Erlich ascites carcinoma cells *in vitro* and *in vivo*[6] and is active against L-1210 leukemia.[7]

A new synthetic procedure for preparing racemic α-hydroxyamino acids has been described, and some of the earlier synthetic methods have been reviewed.[8] None of these reactions are applicable to the synthesis of cyclic *N*-hydroxyamino acids or *N*-hydroxy-*sec*-amino acids of natural origin; *e.g.*, for the preparation of N-hydroxylated proline (1), pipecolic acid (2), or sarcosine (3).



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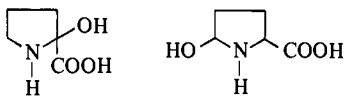
1-Hydroxyproline (1) represents the last of the three possible stable positional isomers of hydroxyproline and is therefore of intrinsic theoretical interest. All eight positional and diastereoisomers including optical antipodes associated with the 3- and 4-hydroxyprolines are known.[9] Some of these occur naturally in bound form in collagen,[10] in sponge[11] and in the antibiotic telomycin,[12] while *trans*-3-hydroxy-L-proline has been found in the free amino acid pool of the tropical legume, *Delonix regia*.[13] The 2- and 5-hydroxyprolines (4 and 5) are cyclic tautomers of α-keto-δ-



4 5

aminovaleric acid and glutamic acid-γ-semialdehyde, respectively, and would be expected to be unstable relative to the open-chain compounds or to their dehydrated products, Δ^1-pyrroline-2- or -5-carboxylic acid.[9]

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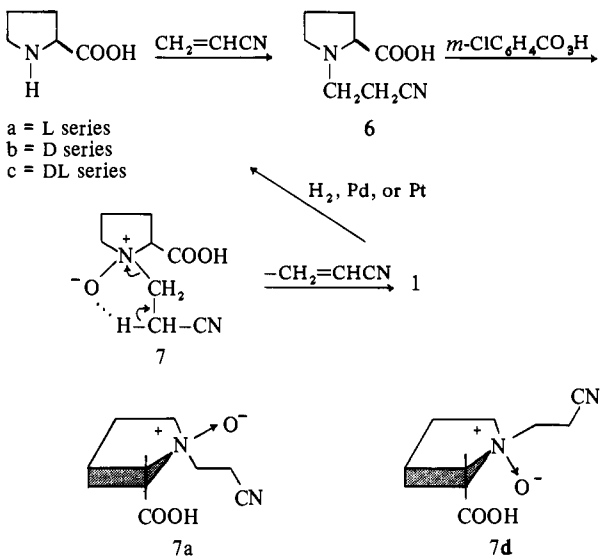
Scheme I



N-Hydroxyproline and its analogs and homologs are also of interest as potential antimetabolites of L-proline, L-pipecolic acid, and other natural amino acids.

Chemistry. We have developed a reaction sequence of general utility for the synthesis of cyclic *N*-hydroxyamino acids and *N*-hydroxy-*sec*-amino acids, exemplified here by the synthesis of 1-hydroxyproline (1) (Scheme I), as well as the *N*-hydroxy analogs of DL-pipecolic acid (2) and of sarcosine (3) (Table I).

The reactions are based on a similar route for the preparation of *N*-hydroxymorpholine and 1-hydroxypiperidine from morpholine and piperidine, respectively.[14] The presence of free carboxyl groups in the amino acids did not present serious obstacles and the use of carboxyl protective groups was unnecessary.

Cyanoethylation of L-proline gave 1-cyanoethyl-L-proline (6a) in 90–95% yields by modifying the procedure of McKinney, *et al.*,[15] who reported a yield of 17%. Oxidation of **6a** with *m*-chloroperoxybenzoic acid in MeOH gave the somewhat unstable 1-cyanoethyl-L-proline 1-oxide (7). Although two isomers (7a and **7d**) of this *N*-oxide were expected due to the generation of a new chiral center on nitrogen, only a single product which had a normal unionized carboxyl absorption in the infrared at 1700 cm^{-1} was isolated. Since the carboxyl proton of the *cis*-*N*-oxide **7d** would be expected to associate with the neighboring oxygen atom of the *N*-oxide group, and this would have been detectable in the in-

Table I. *N*-Hydroxyamino Acids and Their Precursors

Compound	Yield, %	Recrystn solvent	Mp, °C	$[\alpha]^{28}D$ (*c*)[a]	R_f[b]	Formula	Anal[c]
6a	90–95	Acetone	155–156[d]	−63.0 (2.28)		$C_8H_{12}N_2O_2$	C, H, N
6b	91	Acetone, or MeOH-Et_2O	156–157	+62.2 (2.00)		$C_8H_{12}N_2O_2$	C, H, N
6c	85	Acetone, or MeOH-Et_2O	163–164			$C_8H_{12}N_2O_2$	C, H, N
1-(2-Cyanoethyl)-DL-pipecolic acid	80–85	Acetone	165–167			$C_9H_{14}N_2O_2$	C, H, N
N-(2-Cyanoethyl)sarcosine	84	Acetone	131–132			$C_6H_{10}N_2O_2$	C, H, N
7a	85–89	MeOH-Et_2O	109–111 dec	+25.9 (2.00)	0.34	$C_8H_{12}N_2O_3$	C, H, N
7b	81	MeOH-Et_2O	109–110 dec	−24.1 (2.00)	0.34	$C_8H_{12}N_2O_3$	C, H, N
7c	68	MeOH-Et_2O	162–164 dec		0.25	$C_8H_{12}N_2O_3$[e]	C, H, N
1-(2-Cyanoethyl)-DL-pipecolic acid 1-oxide	71–74	MeOH-Et_2O	>105 dec		0.39	$C_9H_{14}N_2O_3$	C, H, N
N-(2-Cyanoethyl)sarcosine *N*-oxide	78–80	MeOH	101–102 dec		0.46[f]	$C_6H_{10}N_2O_3$	C, H, N
1a	45–55	MeOH, or MeOH-Et_2O	157–160 dec[g]	−79.6 (2.18)	0.43	$C_5H_9NO_3$	C, H, N
1b	38	Acetone	158–161 dec	+78.3 (2.22)	0.43	$C_5H_9NO_3$	C, H, N
1c	39	MeOH	151–155 dec		0.42	$C_5H_9NO_3$	C, H, N
2	79	Acetone-hexane	148–151 dec		0.50	$C_6H_{11}NO_3$	C, H, N
3	47–58	MeOH	128–130 dec		0.37[f]	$C_3H_7NO_3$	C, H, N

[a] In H_2O. [b] Tlc on silica gel HF_{254} with MeOH-HOAc (98:2). The spots corresponding to the *N*-oxides and the N-hydroxylated amino acids all gave red or red-orange colors when the chromatograms were sprayed with a 1–2% ethanolic soln of 2,3,5-triphenyltetrazolium chloride (TPT) followed by 1.0 *N* NaOH and then heated at 105° for 15 min. The same reagents can also be used in a spot test, *cf.* ref 26. [c] Analytical results obtained for these elements were within ±0.3% of the theoretical values. [d] Reported mp 137–140° (ref 15); the reported value for carbon analysis was low by 0.6%. [e] Analyzed as a monohydrate. [f] Silica gel F_{254}. [g] Nmr (D_2O, DSS internal std): δ 4.16 (t, 1, J = 7 Hz), 3.16 (q, 2, J = 7 Hz), ~2.1 (m, 4).

frared spectrum, the major product of this reaction must be the *trans*-*N*-oxide 7a.

When heated in boiling acetone, acrylonitrile was lost from 7a in a Cope elimination giving 1-hydroxy-L-proline (1a) in 50% yield. The chiral center α to the carboxyl group was unaffected in the three steps leading to 1a, as catalytic reduction of 1a gave L-proline of identical optical rotation as the starting material. In contrast to the *N*-oxide 7a, 1a was relatively stable in the solid form, and even aqueous solutions were stable at room temperatures. When heated in H_2O, 1a underwent an apparent disproportionation reaction,[16] proline being detected as one of the decomposition products.

For metabolic studies, a ^{14}C-labeled 1a was prepared *via* the above sequence (Scheme I) starting with L-proline-*U*-^{14}C. Partial degradation of the latter by ninhydrin decarboxylation showed that 19.7% of the total radioactivity of the molecule resided in the carboxyl carbon, as would be expected for a uniformly ^{14}C-labeled compound.

By an entirely analogous series of reactions the enantiomeric and racemic forms of 1a, as well as 1-hydroxy-DL-pipecolic acid (2) and *N*-hydroxysarcosine (3), were prepared (Table I).

Metabolic Disposition of ^{14}C-Labeled 1a. 1-Hydroxy-L-proline (1a) is relatively nontoxic to mice; *e.g.*, Swiss-Webster mice readily tolerated single intraperitoneal doses up to 500 mg/kg without observable toxic or lethal effects. This proline analog is extensively degraded in rodents (Table II). During 3.5 hr following intraperitoneal administration of ^{14}C-labeled 1a to a mouse, 20% of the dose appeared in the breath as CO_2-^{14}C, while only 14% of the dose appeared in the urine. In a separate, longer experiment (mouse 2), CO_2-^{14}C expiration *via* the breath accounted for 57% of the administered dose at the end of 25 hr, while the urine contained 3.8%.

The excretion of radioactivity in urine and feces is essentially complete at 24 hr; *e.g.*, urinary excretion of radioactivity between 24 and 48 hr after ^{14}C-labeled 1a accounted for only 0.13% of the dose (mouse 3, Table II), while fecal excretion of radioactivity was only 0.4% during this time.

Table II. Metabolic Disposition of Uniformly ^{14}C-Labeled 1a in Rodents

Radioactivity recovered in	% of administered dose at				
	3.5 hr Mouse 1	25 hr Mouse 2	24 hr[a] Mouse 3	48 hr[a] Mouse 3	24 hr Rat
CO_2	20.2	57.2	40.3	2.5	37.0
Urine	14.2[b]	3.8	8.2	0.13	18.9
Feces	*c*	*d*	4.8	0.40	*d*
Other				0.24 (blood)	2.5 (liver) 0.38 (kidney) 0.55 (heart, lung, testes, spleen)

[a] The CO_2-^{14}C excretion was monitored at 2, 4, 8, 15, 19, 26, 31, 37, 43, and 48 hr; the cumulative results are presented. [b] Carrier experiment (see Experimental Section) indicated that part of this radioactivity is due to unchanged 1a. [c] Not excreted. [d] Not counted.

Very little radioactivity amounting to 0.24% of the initial dose remained circulating in blood after 48 hr. The liver proteins isolated from this mouse were only slightly labeled (216 dpm/mg), as were the lung proteins (86 dpm/mg).

The rat likewise readily degraded 1a. After 24 hr, 37% of the radioactive dose was excreted in the breath as CO_2-^{14}C and 19% in the urine. The liver still retained 2.5% of the radioactivity, while the kidney had 0.38%, and heart, lungs, testicles, and spleen collectively accounted for 0.55% of the dose.

Discussion

The lack of toxicity of 1a in mice is undoubtedly a reflection of its rapid metabolic degradation as demonstrated in the above experiments. Although rat liver mitochondria contain an NADH-requiring hydroxylamine reductase that reduces hydroxylamine to ammonia, and hydroxamates to amides[17,18] and liver homogenates contain enzymes that (a) convert *N*-arylacethydroxamates and *N*-arylhydroxylamines to *N*-arylacetamides and arylamines,[19] respectively, and (b) reduces *N*-alkylhydroxylamines to *N*-alkylamines,[20] reduction of the *N*-hydroxy group does not appear to be the first

step in the metabolism of **1a**. The consequence of such a transformation would yield L-proline which could be incorporated into protein or metabolized by known pathways[21] to glutamate and arginine.

The extremely low level of labeling of liver proteins strengthens this conclusion. If the process, **1a** → L-proline → protein, had occurred, the liver proteins should contain considerably more than the calculated 0.22 μg of "proline" incorporated/mg of protein, on the basis of the specific radioactivity of the ^{14}C-labeled **1a** of 1.06×10^3 dpm/μg and assuming no dilution of radioactivity with endogenous proline pools. The observed low radioactivity of the liver proteins can be adequately explained by assuming a recycling of smaller carbon fragments into the *de novo* pathways of amino acid biosynthesis followed by protein incorporation.

The early production of 40-60% of radioactive CO_2 from the uniformly labeled carbon atoms of the molecule (each representing 20% of the total radioactivity)indicates that the pyrrolidine ring carbons are degraded to short-chain fragments and suggests a direct entry of **1a** into the tricarboxylic acid cycle—possibly *via* α-ketoglutarate. Such an entry would be provided if the first step in the catabolism of **1a** led to either Δ^1-pyrroline-2- or -5-carboxylic acid. Considerable additional work outside the scope of this paper will be required to elucidate the precise degradative pathway for **1a** in mammalian systems. The metabolic fate of the enantiomeric **1b** may shed additional light in this regard. Nevertheless, it appears established that **1a** is metabolically unstable in the mouse and rat and is rapidly degraded to endogenous products that are utilized for cellular energy production.

These results preclude any antimetabolic role for **1a** but do not rule out the possibility that suitably substituted **1a** might be degraded *in vivo* to lethal antimetabolites.

Biological Results. **1a** did not inhibit the growth of *Escherichia coli* B growing exponentially in a glucose-C medium up to a concentration of $1.0 \times 10^{-3} M$ or the growth of radicles of the mung bean, *Phaseolus aureus*[22] ($5.0 \times 10^{-4} M$). The *N*-hydroxyamino acids, **1a**, **2**, and **3**, and the cyanoethyl *N*-oxides, **7a**, 1-(2-cyanoethyl)-DL-pipecolic acid 1-oxide, and *N*-(2-cyanoethyl)sarcosine *N*-oxide, were inactive against L-1210 lymphoid leukemia in host BDF_1 mice when administered ip once daily in saline for 2 days—up to doses of 400 mg/kg. None of the compounds listed in Table I (the **b** and **c** series were not tested) exhibited significant antimalarial activity against *Plasmodium gallinaceum* in the standard mosquito test. *N*-Hydroxysarcosine, *N*-(2-cyanoethyl)-sarcosine *N*-oxide, and *N*-(2-cyanoethyl)sarcosine were tested in mice infected with *P. berghei*, but ($T - C$) survival times were <1 day.

Experimental Section‡

General Procedure for Preparing Cyclic or Secondary *N*-Hydroxyamino Acids (Table I). A. Cyanoethylation of Amino Acids. 1-(Cyanoethyl)-L-proline (6a). To a cooled (ice–salt bath) stirred soln of 16.2 g (0.25 mole) of KOH in 75 ml of H_2O was added 28.8 g (0.25 mole) of L-proline; when the latter was dissolved, acrylonitrile (14.6 g, 0.275 mole) was added dropwise over 30 min. Stirring was continued for 1 hr at ice-bath temp and an additional hr with the bath removed. The pH was then adjusted to 6.6 with 6 *N* HCl and the reaction mixture taken to incipient dryness on a rotating evaporator at bath temp <70°. The residue was transferred to a thimble of a soxhlet extractor and continuously extracted for 96 hr with 1500 ml of Me_2CO. When the Me_2CO extract was cooled in the refrigerator for 12 hr, **6a** was obtained, usually in two crops.

B. Oxidation of *N*-Cyanoethylamino Acids to Their *N*-Oxides. 1-(2-Cyanoethyl)-L-proline 1-Oxide (7a). To a cooled, stirred suspension of 16.8 g (0.10 mole) of **6a** in 300 ml of MeOH was added dropwise over 30 min a filtered solution of 21.6 g (0.10 mole) of *m*-chloroperoxybenzoic acid in 100 ml of MeOH. After 1 hr of additional stirring at 0–2°, the reaction mixture was diluted with 1500 ml of precooled anhyd Et_2O and placed in a refrigerator for 12 hr. The product **7a** which crystallized was collected, washed with Et_2O, dried for a short period in a vacuum dessicator, and stored at −5°. Storage at this temp is mandatory since the *N*-oxides slowly decompose at room temp.

C. Cope Rearrangement of *N*-Cyanoethylamino Acid *N*-Oxides to *N*-Hydroxyamino Acids. 1-Hydroxy-L-proline (1a). A stirred suspension of 10.1 g (0.055 mole) of **7a** in 1 l. of Me_2CO in an open flask was brought to a boil and the reaction mixture kept at the bp for 4 hr. The Me_2CO was allowed to boil away in order to entrain the acrylonitrile which formed, and fresh Me_2CO was added periodically to maintain the initial volume. The product (**1a**) which crystallized by refrigeration of the reaction mixture (12 hr) was collected and recrystallized (charcoal). Work-up of the mother liquor provided unchanged **7a** in crude form which could be recrystallized and recycled.

The optical rotation (Table I) of a dil soln of **1a** in H_2O did not change after standing at room temp for 7 days. When heated under reflux (N_2) until **1a** (or *N*-hydroxysarcosine) was no longer detectable (42 hr) with the TPT–NaOH reagent, one of the decomposition products formed was proline (or sarcosine) as determined by tlc. In 0.01 *M* phosphate buffer at room temp, pH 7.4, **1a** as well as *N*-hydroxysarcosine slowly decomposed; at the end of 50 days, the solutions gave only a faint test with the TPT–NaOH reagent.

Catalytic Reduction of 1a to L-Proline. A sample of **1a** (1.00 g, 7.63 mmoles) was hydrogenated in 50% aqueous MeOH with Pd black as catalyst in a Parr apparatus at 1.12 kg/cm². The reaction mixture, when monitored by tlc at 4 hr, showed the presence of both proline (ninhydrin test, R_f) and **1a** (TPT–NaOH test, R_f, see Table I). After 6 hr, **1a** was completely converted to proline. The product was recrystallized from abs EtOH–Et_2O to give 0.75 g (85%) of crystalline L-proline, $[\alpha]^{27}D$ −83.1° (*c* 1.26, H_2O).

A sample of **1a** reduced as above with a Pt catalyst (23 hr) gave 87% yield of L-proline, $[\alpha]^{27}D$ −84.5° (*c* 2.08, H_2O). The starting L-proline had $[\alpha]^{27}D$ −85.5° (*c* 2.45, H_2O).

Quantitative Ninhydrin Decarboxylation of L-Proline-*U*-^{14}C.[23] A solution containing 0.263 μg of L-proline-*U*-^{14}C (sp act. 219 mCi/mmole)§ was diluted with 11.5 mg (0.10 mmole) of unlabeled L-proline, and the mixture decarboxylated with 53 mg (0.30 mmole) of ninhydrin in 7.0 ml of glacial HOAc at room temp for 2 hr and at 70° for 1 hr. The evolved CO_2-^{14}C was trapped in 10 ml of 0.5 *M* methanolic Hyamine hydroxide and the latter counted. The results of triplicate analyses indicated that the carboxyl carbon contained 19.7% (±0.05) of the total radioactivity due to the carbon atoms of the molecule.

1-Hydroxy-L-proline-*U*-^{14}C. A solution containing 0.263 mg of L-proline-*U*-^{14}C (sp act. 219 mCi/mmole)§ in 5.0 ml of 0.01 *N* HCl was added to 115.1 mg (1.00 mmole) of unlabeled L-proline. The radioactive vial was rinsed with 1.0 ml of 1.0 *N* KOH and this rinse added to the proline. The soln was cooled (ice–salt bath) and acrylonitrile (100 μl, 1.50 mmoles) was added dropwise over 5 min. After work-up described in procedure A above, the crude cyanoethylproline was transferred by suspension in Me_2CO to a 10 × 50 mm soxhlet extraction thimble and continuously extracted with 50 ml of boiling Me_2CO until the residue was no longer radioactive (~5 days; the Me_2CO lost by evaporation must be replenished twice daily). The Me_2CO extract was evaporated to dryness, the solvent replaced with MeOH (5 ml) and to the cooled mixture was added a solution (filtered) of 215 mg (1.00 mmole) of *m*-chloroperoxybenzoic acid in 1.0 ml of MeOH over 15 min. After 1 hr of stirring, the clear MeOH solution was evaporated to dryness, 30 ml of Me_2CO added, and the Me_2CO gently boiled in an open flask for 4.5 hr, periodically replenishing the lost Me_2CO. Refrigeration overnight gave 63.5 mg of solids, which was recrystallized from MeOH to give 2 crops of ^{14}C-labeled **1a**, 41.0 mg and 5.0 mg; total overall yield, 35%. Both samples were homogeneous on tlc and gave single radioactive spots corresponding to **1a**.

Metabolic Disposition of ^{14}C-Labeled 1a. Uniformly ^{14}C-labeled **1a** dissolved in isotonic saline was adminsitered ip to male, Swiss-Webster mice weighing 37–39 g (dose: 135 mg/kg), or to a male Holtzman rat weighing 67 g (dose: 75 mg/kg). The specific radioactivity of the labeled **1a** (adjusted by diluting with nonradioactive

‡Melting points were taken on a Fisher-Johns mp apparatus and are corrected; optical rotations were determined in a Perkin-Elmer Model 141 polarimeter. Spectrophotometers used were: ir, Beckman IR-10; nmr, Varian A60A. Microanalyses by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Radioactive samples were counted in a Packard liquid scintillation spectrometer.

§New England Nuclear Corp., Boston, Mass., Lot No. 272-182C.

1a) ranged from 2.00×10^5 dpm/mg to 1.06×10^6 dpm/mg. The animals were housed in all glass metabolism cages which allowed for the separate collection of urine and feces, and expiratory CO_2-^{14}C in 10 *N* NaOH. For the experiment which lasted 48 hr, the mouse was transferred to a clean metabolism cage at the end of 24 hr. Urine and CO_2-^{14}C (as Na_2CO_3-^{14}C) were counted in Diotol;[24] feces and blood were combusted in an O_2 flask, and the CO_2-^{14}C absorbed in 1 *M* Hyamine hydroxide and counted. Organs were homogenized in 9 vols of H_2O and aliquots were counted as a thixotropic gel suspension.[25] Proteins were precipitated by addition of an equal vol of 10% TCA, heated at 90° for 15 min, and the protein precipitates collected, washed successively with 5% TCA, boiling 95% EtOH-Et_2O (3:1), and Me_2CO and dried.

The 3.5-hr urine sample from mouse 1 was examined as follows: 5.0 mg each of unlabeled 1a and carrier L-proline were added, mixed thoroughly, and the urine charged on a column of Bio-Rad AG2-X10 (200–400 mesh, ^-OH) anion-exchange resin. The column was eluted successively with 20 ml of H_2O (fractions 1 and 2, 15% of the urinary radioactivity), 35 ml of 1 *N* HOAc (fractions 3–5, 30%) and 20 ml of 2 *N* NaOH (35%) for a recovery of 80%. The carriers were eluted with the HOAc. Fractions 1 and 3 were concentrated and chromatographed on silica gel HF_{254} with MeOH-HOAc-H_2O (46:1:3), and contiguous sections of the plate (0.5 cm) were scraped and counted. Two unidentified radioactive peaks were detected in fraction 1, while fraction 3 contained one major radioactive peak coincident with carrier 1a (TPT-NaOH test), and two minor peaks, one of which with an R_f close to that of carrier proline.

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Synthesis and Certain Pharmacological Properties of Lysine-vasopressinoic Acid Methylamide and Lysine-vasopressinoic Acid Dimethylamide†

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Lysine-vasopressinoic acid methylamide and lysine-vasopressinoic acid dimethylamide, analogs in which Me groups formally replace the amide hydrogens of the glycinamide residue of lysine-vasopressin, have been prepd by solid-phase peptide synthesis, and certain pharmacological properties of these neurohypophyseal hormone analogs have been detd. Lysine-vasopressinoic acid methylamide exhibits approx 1.5 units/mg of rat pressor activity, 0.4 unit/mg of oxytocic activity, and less than 0.1 unit/mg of avian vasodepressor (AVD) activity. Lysine-vasopressinoic acid dimethylamide exhibits approx 0.06 unit/mg of rat pressor, 0.4 unit/mg of oxytocic, and less than 0.1 unit/mg of AVD activity. Lysine-vasopressin possesses approx 266 units/mg of rat pressor, 7 units/mg of oxytocic, and 50 units/mg of AVD activity.

In connection with conformational studies on lysine-vasopressin (LVP), the methylamide and dimethylamide of lysine-vasopressinoic acid were desired (Figure 1). A suitable precursor of both LVP analogs, Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly resin (I), was synthesized by the general procedure of the Merrifield solid-phase method[1-3] as applied by Meienhofer and Sano[4] to the synthesis of the corresponding 1-*N*-tosyl polypeptide resin, with modifications described in the Experimental Section. Cleavage of the protected polypeptide resin I with $MeNH_2$ and Me_2NH[5,6] gave the methylamide and dimethylamide, respectively, of the protected nonapeptide. Removal of the protecting groups with Na in boiling NH_3[7] followed by air oxidn of the resulting disulfhydryl compds in dil aqueous soln yielded lysine-vasopressinoic acid methyl- and dimethylamide. Both analogs were purified by ion-exchange chromatog on IRC-50 resin.[8]

Lysine-vasopressinoic acid methylamide possesses approx 1.5 units/mg of rat pressor activity, 0.4 unit/mg of oxytocic activity, and less than 0.1 unit/mg of avian vasodepressor

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