Inhibitors of Polyamine Biosynthesis VI: 2,5-Diamino-2-(cyanomethyl)pentanoic Acid, a Potential Irreversible Inhibitor of Ornithine Decarboxylase

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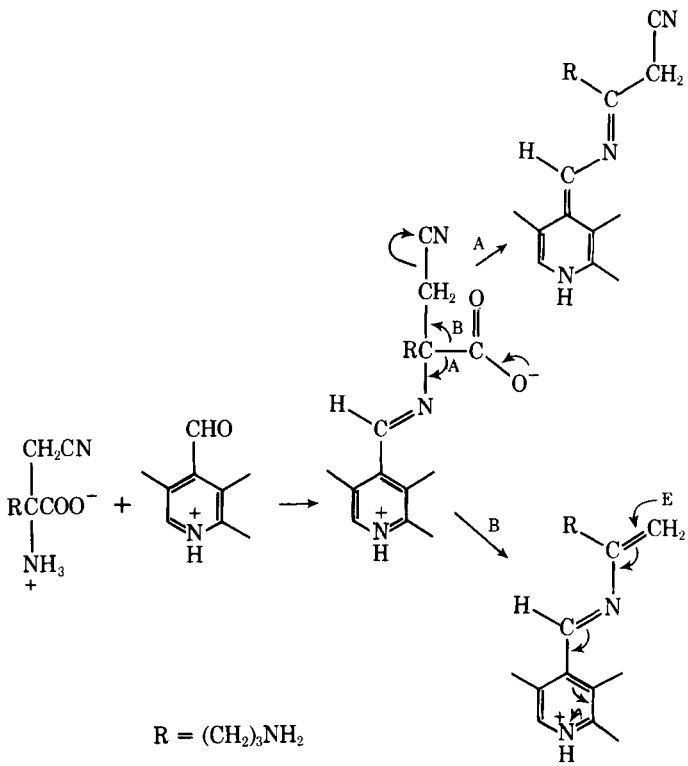
Abstract □ (±)-2,5-Diamino-2-(cyanomethyl)pentanoic acid was obtained by the reaction of chloroacetonitrile with the anion obtained by treatment of 3-(benzylideneamino)-2-piperidinone with sodium hydride, followed by hydrolysis in the presence of trifluoroacetic anhydride. The target compound was isolated as the monohydrochloride salt of the lactam. The compound was synthesized as a potential irreversible inhibitor of the enzyme L-ornithine decarboxylase by the mechanism generally known as suicide or K_{cat} inhibition. The synthesized compound produced no inhibition of the enzyme ornithine decarboxylase obtained from rat prostate gland. The inactivity of the target compound is attributed to the hydrophilicity of the cyanomethyl group.

Keyphrases □ (±)-2,5-Diamino-2-(cyanomethyl)pentanoic acid—synthesized, effect on ornithine decarboxylase activity evaluated □ Ornithine decarboxylase activity—effect of (±)-2,5-diamino-2-(cyanomethyl)pentanoic acid □ Pentanoic acid, substituted—synthesized, effect on ornithine decarboxylase activity evaluated □ Enzyme activity—ornithine decarboxylase, effect of (±)-2,5-diamino-2-(cyanomethyl)pentanoic acid □ Structure-activity relationships—(±)-2,5-diamino-2-(cyanomethyl)pentanoic acid, effect on ornithine decarboxylase activity evaluated

Previous reports (1–10) described the synthesis and evaluation of specific inhibitors of the enzyme L-ornithine decarboxylase. The present report describes the synthesis of 2,5-diamino-2-(cyanomethyl)pentanoic acid (VI) and its evaluation as a potential irreversible inhibitor of ornithine decarboxylase from rat prostate gland.

The rationale for the synthesis of VI was based on its potential to produce irreversible inhibition of ornithine decarboxylase, a pyridoxal phosphate enzyme, by the mechanism shown in Scheme I. This mechanism is similar to that proposed for the irreversible inhibition of collagen cross-linking enzymes by β-aminopropionitrile (11).



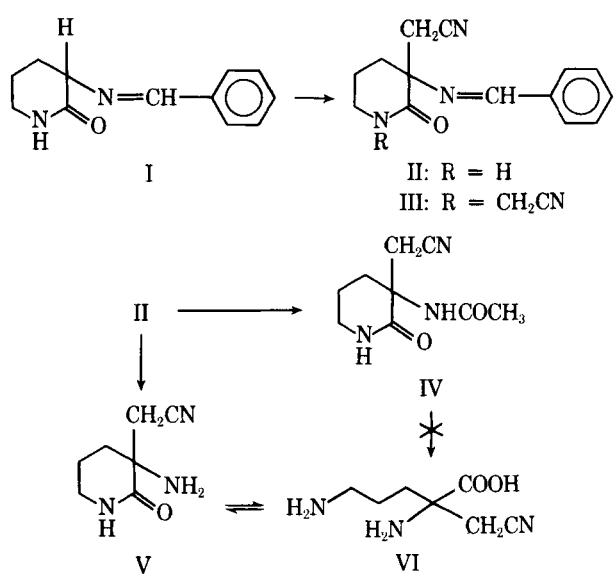
Scheme I

EXPERIMENTAL[1]

Melting points were determined in open capillary tubes and are uncorrected. NMR spectra were taken in deuterochloroform or deuterium oxide, with tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the internal standard, respectively. Mass spectral analyses were performed at 70 ev with a 200° chamber temperature.

3-(Benzylideneamino)-3-(cyanomethyl)-2-piperidinone (II) and 1-(Cyanomethyl) -3- (benzylideneamino) -3- (cyanomethyl)-2-piperidinone (III)—Sodium hydride (0.48 g, 0.01 mole of 50% oil dispersion) was added with stirring to dry tetrahydrofuran (50 ml). A solution of 3-benzylideneamino-2-piperidinone (I, Scheme II) (2.02 g, 0.01 mole) in tetrahydrofuran (80 ml) was added to the suspension. The reaction mixture was heated under reflux for 2 hr, and the reddish solution obtained was cooled to room temperature. Chloroacetonitrile (0.755 g, 0.01 mole) in tetrahydrofuran (10 ml) was added; the mixture was stirred overnight, whereupon its color changed to turbid yellow.

The mixture was filtered through diatomaceous earth[2], and the filtrate was concentrated under reduced pressure at 45°. The residue (2.9 g) was boiled with two separate 300-ml portions of dry benzene, and the combined benzene extract was filtered and concentrated under reduced pressure to 15 ml. The concentrated solution was immediately chromatographed on 15 silica gel plates (20 × 20 cm, 500 μm), using methylene chloride–acetone (8.4:1.6). Zones of R_f 0.3 (II) and 0.7 (III) were separately



Scheme II

[1] A Thomas-Hoover melting-point apparatus, a Perkin-Elmer 237 or Beckman IR-9 spectrophotometer, a Varian A-60 D NMR spectrometer, an AEI MS-30 mass spectrometer, and a Beckman LS-100C liquid scintillation counter were used. Elemental analysis was performed by MHW Laboratories, Garden City, Mich.

[2] Celite.

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scraped into a sintered-glass funnel, and each was eluted with a mixture of 5% methanol in ethyl acetate (400 ml). The solvent was removed *in vacuo* at 50°, and the oily residue obtained from the R_f 0.3 zone (II) was immediately utilized for the synthesis of V.

Analytical samples of II and III were prepared by rechromatography, and the purified samples were dried *in vacuo* for 48 hr. The yield of crude II was 1.1 g (45.8%); IR (neat): 3300, 2295, 1660, 1575, 750, and 695 cm^{-1}; NMR (deuterochloroform, tetramethylsilane): δ 2.0 (m, 4H), 2.84 (s, 2H), 3.3 (m, 2H), 6.72 (s, 1H), 7.25 and 7.6 (m, 5H), and 8.1 (s, 1H) ppm; high-resolution mass spectrum: 241.1233 (M^+) and 242.1310 (M + 1).

Anal.—Calc. for $C_{14}H_{15}N_3O$: C, 69.73; H, 6.22; N, 17.42. Found: C, 69.89; H, 6.50; N, 16.93.

The yield of crude III was 0.6 g (21.4%); IR (neat): 2265, 2195, 1710, 1610, and 1591 cm^{-1}; NMR (deuterochloroform, tetramethylsilane): δ 4.25 (q, 2H, J = 14 Hz), 8.0 (s, 1H), 2.1 (m, 4H), 2.85 (s, 2H), 3.44 (m, 2H), 7.25, and 7.6 (m, 5H) ppm; mass spectrum: *m/e* 281, 241, 240, 213, 212, and 177 (base peak).

Anal.—Calc. for $C_{16}H_{16}N_4O$: C, 68.57; H, 5.70; N, 20.00. Found: C, 68.37; H, 5.89; N, 19.93.

3-Acetamido-3-(cyanomethyl)-2-piperidinone (IV)—A solution of II (0.12 g, 5 mmoles) in acetic acid (5.0 ml) was heated on a water bath at 60–70° for 30 min. Acetic anhydride (5.0 ml) was added, and the mixture was heated at 50–60° for 45 min. Water (10 ml) was added, and the solution was heated at 80–90° for 1 hr, cooled to room temperature, and extracted with methylene chloride (3 × 10 ml). The aqueous solution was evaporated to dryness under reduced pressure. The residue was triturated with dry ether, and the formed solid was filtered and recrystallized from absolute ethanol–ether (2:1) to afford IV (60 mg, 61% yield), mp 212–214°.

Anal.—Calc. for $C_9H_{13}N_3O_2$: C, 55.37; H, 6.71; N, 21.50. Found: C, 55.45; H, 6.55; N, 21.29.

3-Amino-3-(cyanomethyl)-2-piperidinone Hydrochloride (V)—A solution of II (0.12 g, 5 mmoles) in trifluoroacetic anhydride (5.0 ml) and chloroform (10 ml) was heated under reflux in an oil bath at 70–80° for 1 hr. The reaction mixture was cooled to room temperature and concentrated under vacuum. The residue was treated with dilute hydrochloric acid (10 ml), and the mixture was heated at 40–50° for 30 min, cooled to room temperature, and extracted with methylene chloride. The aqueous solution was evaporated to dryness under reduced pressure.

The formed solid was boiled with absolute ethanol (20 ml) and filtered while hot, and butyl ether (about 10 ml) was added until a slight turbidity was observed. The solution was stored at −20° for 7 days to afford 73.0 mg (76% yield) of V, mp 194–195°; IR (potassium bromide pellet): 3400, 3300, 2650, 3225, 2240, and 1680 cm^{-1}; NMR (deuterochloroform, tetramethylsilane): δ 1.95, 2.20 (d of d, 4H), 216 (m, 4H), 8.4 (s, 1H), and 8.9 (s, 2H) ppm; high-resolution mass spectrum: 153.0892, 152.0813, and 154.0973 emu.

Anal.—Calc. for $C_7H_{12}ClN_3O$: C, 44.32; H, 6.33; N, 22.16. Found: C, 44.33; H, 6.32; N, 22.51.

RESULTS AND DISCUSSION

The target compound I was obtained using the synthesis shown in Scheme II. Compound I was prepared following the procedure described by Abdel-Monem *et al.* (2). It was reacted with sodium hydride, followed by alkylation with chloroacetonitrile to provide the desired product (II) together with III. The ratio of II to III in the product was increased when equimolar molar amounts of the reactants were used.

1-(Cyanomethyl)-3-(benzylideneamino)-2-piperidinone, which would be obtained by *N*-alkylation of I, was not detected in the reaction products, indicating the much greater acidity of the C-3 proton relative to the lactam proton. Compound III is probably formed by the *N*-alkylation of the anion generated from II. The acidity of the lactam proton in II is greater than that in I because of the electron-withdrawing effect of the cyanomethyl substituent at the C-3 position.

Compounds II and III were obtained separately from the mixture of products by preparative TLC, and their identity was confirmed by NMR spectroscopy. The NMR spectrum of III in deuterated chloroform was characterized by the absence of the lactam NH proton, which appeared as a broad singlet at δ 6.72 ppm in the spectrum of II. It also displayed an *AB* quartet centered at δ 4.25 ppm (J = 14 Hz), integrating for two protons, and was assigned to the *N*-cyanomethylene protons. This quartet was absent in the spectrum of II. The two protons of the cyanomethylene group on C-3 appeared as a sharp singlet at δ 2.84 ppm in the spectra of II and III.

Attempts to cleave the benzylidene group by the action of aqueous hydrochloric acid on II to provide the target compound VI were unsuccessful and resulted in the formation of polymers. It was rationalized that the cleavage of the benzylidene group in the presence of an acylating agent might prevent the formation of polymeric products. Indeed, heating II with a mixture of acetic anhydride and acetic acid, followed by the addition of dilute hydrochloric acid, provided IV. Attempts to cleave the acetamido group without the hydrolysis of the cyanomethyl group were not successful. The cleavage of the benzylidene group was also achieved by heating II with trifluoroacetic anhydride under reflux, followed by the addition of dilute hydrochloric acid. However, crystallization of the crude product provided only lactam V and not the expected VI dihydrochloride.

Golankiewicz and Wiewiorowski (12) reported that 3-amino-2-piperidinone was present in equilibrium with 2,5-diaminopentanoic acid in aqueous solutions over pH 3–13 and that the equilibrium was more shifted toward the amino acid at higher pH values. These findings suggest that V will be present in equilibrium with VI in aqueous solutions at pH 7.4. Accordingly, V was used for further biological testing.

Inhibition by V of the enzymatic decarboxylation of L-ornithine by ornithine decarboxylase was measured using extracts of rat prostate glands. The procedures for the tissue extract preparation, enzyme purification, and enzymatic activity assay were described previously (1). In the presence of 2.0×10^{-6} *M* pyridoxal phosphate, the ornithine decarboxylase activity obtained from rat prostate glands had an apparent K_m for L-ornithine of 4.7×10^{-5} *M* and V_{max} value of 0.22 nmole of carbon dioxide/mg of wet tissue/hr. Addition of V in concentrations varying from 1.0×10^{-6} to 1.0×10^{-3} *M* did not suppress the production of ^{14}C-carbon dioxide. Under the same conditions, addition of α-methyl-(±)-ornithine monohydrochloride (1) in concentrations varying from 8.7×10^{-6} to 2.7×10^{-4} *M* significantly suppressed the production of ^{14}C-carbon dioxide. The K_i for α-methyl-(±)-ornithine monohydrochloride was calculated to be 2.0×10^{-5} *M*.

The lack of inhibitory action of V on the enzymatic decarboxylation of L-ornithine was unexpected. The steric bulk of the cyanomethyl group is similar to that of the ethyl group, and replacement of the α-hydrogen in (±)-ornithine with an ethyl group resulted in a compound that retained the necessary features for binding to the active site of the enzyme (2). Therefore, the lack of activity of V could not be attributed to steric interference of the cyanomethyl substituent with binding to the enzyme active site.

In the majority of the previously studied inhibitors of ornithine decarboxylase, the α-hydrogen of the substrate L-ornithine was replaced by an electron-donating hydrophobic alkyl or benzyl group. In VI, the replacement of the α-hydrogen of ornithine with a cyanomethyl group was expected to have opposite effects since this group is hydrophilic and produces a slight electron-withdrawing inductive effect. The inactivity of Compound VI underscores the essentiality of a lipophilic substituent at the α-position of the substrate for enzyme binding. The possible contribution of electronic factors to the inactivity of V could not be ruled out.

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