Pyridine Ring Analogs of Iodotyrosine (1)

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Many structural analogs of iodotyrosine and diiodotyrosine, as well as of the iodinated thyronines, have appeared in the literature (2). Since all of these analogs contain the benzene ring, it seemed worthy to attempt the synthesis of iodotyrosine analogs containing the pyridine ring. Effective analogs of phenylalanine, tyrosine, and DOPA have been prepared by substituting the pyridine moiety for the benzene moiety of these compounds (3-5). Such successful substitutions in the preparation of iodotyrosine analogs would support the feasibility of the preparation of analogs of the thyronines in which one or both of the aromatic rings of these hormones were substituted by the pyridine ring. Also, due to the recent synthesis of various *N*-oxides of certain natural and synthetic nitrogen heterocycles, some of which have had striking biological activity (6-8), the synthesis of iodotyrosine analogs containing the pyridine *N*-oxide moiety was of interest. In this paper we wish to report the synthesis and structural proofs of the compounds β-(5-hydroxy-6-iodo-2-pyridyl 1-oxide)-DL-alanine (VII), and β-(5-hydroxy-6-iodo-2-pyridyl)-DL-alanine (XI), analogs of iodotyrosine.

The iodoamino acids, VII and XI, were synthesized through a sequence of reactions utilizing kojic acid (5-hydroxy-2-hydroxymethyl-4*H*-pyran-4-one) as the starting material, and the complete sequence of reactions is shown in Scheme I. For the preparation of VII the *N*-oxide function was introduced early in the reaction sequence; peracetic acid oxidation of 4-chloro-2-chloromethyl-5-methoxypyridine (II) afforded III in good yield. Condensation of III with sodio ethyl acetamidomalonate gave the key intermediate IV. Acid hydrolysis of IV followed by hydrogenolysis yielded β-(5-hydroxy-2-pyridyl 1-oxide)-DL-alanine (VI).

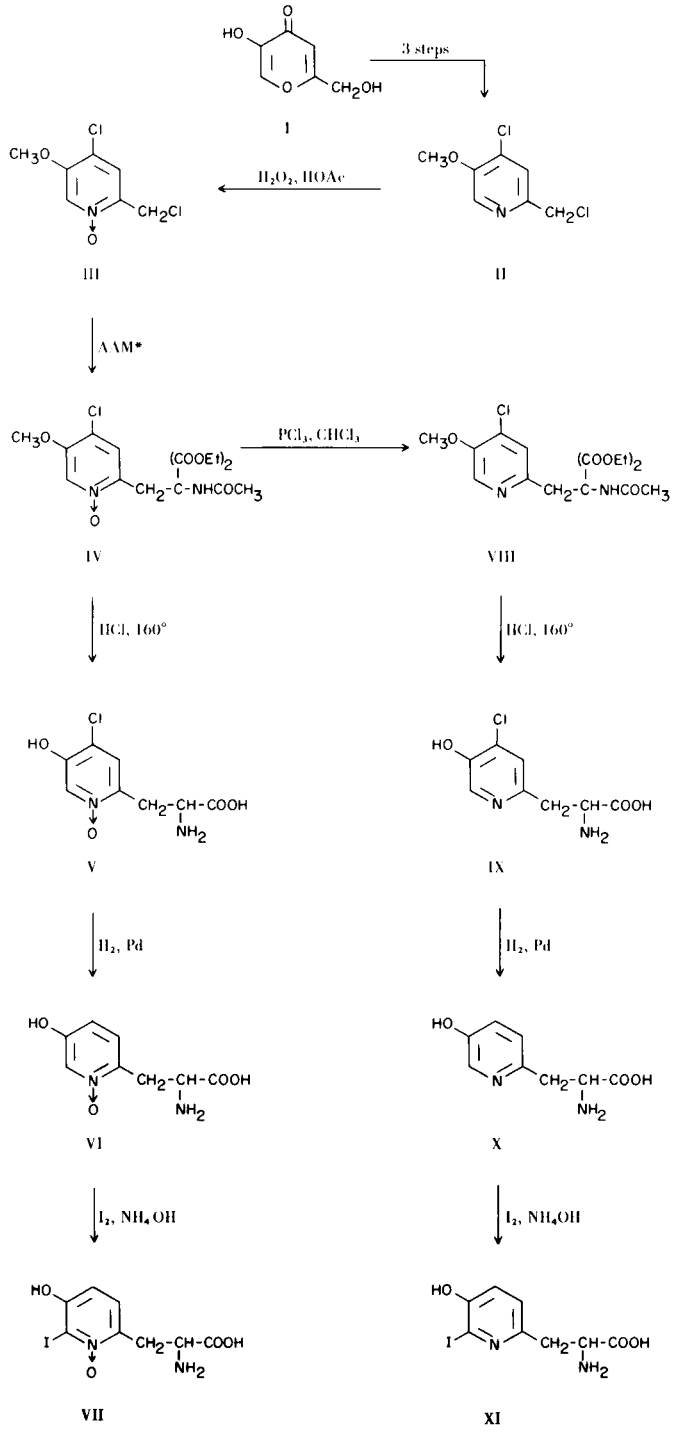
Removal of the *N*-oxide function for the preparation of XI was smoothly accomplished by treating a portion of IV with phosphorus trichloride in chloroform. The resulting compound, VIII, was also subjected to acid hydrolysis followed by hydrogenolysis to give β-(5-hydroxy-2-pyridyl)-DL-alanine (X), which has previously been prepared as the dihydrochloride salt (4).

Compounds VI and X were iodinated in a manner analogous to that reported for the iodination of tyrosine (9). It was found however, that two mole equivalents of iodine to one mole equivalent of substrate was necessary for the complete mono-iodination (based on carbon, hydrogen, and nitrogen analyses) of VI and X to yield VII and XI, respectively. Schickh reported that iodination of 3-pyridinol yielded 2-iodo-3-pyridinol and 2,6-diiodo-3-pyridinol at elevated temperatures (10). Thus, it was anticipated that iodination of X would yield a monoiodo derivative with the iodine atom in the C_6-position of the pyridine ring. It is well documented that pyridine *N*-oxide nitrates readily at the C_4-position of the ring; little 2-nitropyridine *N*-oxide is isolated (11). Thus, due to an enhanced activation of the C_4-position for electrophilic substitutions in pyridine *N*-oxides, it was not certain whether iodination of VI would yield the 6-iodo or the 4-iodo derivative. The position of the iodine atom in compounds VII and XI was determined by nmr spectroscopy.

A symmetrical splitting pattern was observed for VII in the aromatic region of the nmr spectrum, doublets centered at δ 7.20 and δ 7.55. The absence of the low field resonance value as found in the nmr spectrum of V (δ 8.27) indicates that iodine occupies the α-position of VII. That iodine occupies the α-position in XI is indicated by a comparison of the nmr spectrum of XI with those of IX and X. Further confirmation of this assignment is made by a comparison with the nmr spectrum of 2-iodo-6-methyl-3-pyridinol (see experimental section).

Compounds VI, VII, and XI are presently being studied elsewhere in an exhaustive biological screening program. A preliminary biological study involving inhibition of bacterial growth was conducted in this laboratory. Compounds VI, VII, and XI completely inhibit the growth of *Escherichia coli* 9723 at concentrations of 20, 60, and 600 μg. per ml., respectively. Inhibitions by compounds VII and XI are reversed in an apparently competitive fashion by tyrosine. Paper chromatographic studies have indicated that no bacterial deiodination of the compounds occurs during the incubation period. It appears therefore, that the bulky iodine atom in these compounds does not interfere with their being recognized by tyrosine-utilizing enzymes of the test organism. Compound VI is also reversed by tyrosine, but the reversal is apparently not competitive. It was shown in an earlier study (6) that cells of *E. coli* catalyze a reduction of several pyridine *N*-oxides—reduction of the *N*-oxide function of compound VI by *E. coli* was confirmed in the present study. It is

SCHEME I



* Condensation of III with sodio ethyl acetamidomalonate.

probable that the enzymatic reduction product of the latter compound is actually the inhibitory agent, since supplements of certain noninhibitory pyridine *N*-oxides to the growth medium of *E. coli* prevent growth inhibition by compound VI (see reference 6). More detailed biological studies will be presented elsewhere in a future report.

EXPERIMENTAL

A Thomas-Hoover capillary melting point apparatus was employed for all melting point determinations, and the melting points reported are uncorrected. UV spectra were determined with a Beckman DBG recording spectrometer.

NMR Data.

Because of the low solubility of the substituted pyridylalanines (Scheme I) in water, especially the iodo amino acids, the hydrochloride salts of these compounds were prepared. The nmr spectra of the amino acid hydrochlorides were determined in deuterium oxide solution containing 1% sodium-2,2-dimethyl-2-silapentane-5-sulfonate as reference standard. The nmr spectrum of 2-iodo-6-methyl-3-pyridinol was obtained in DMSO-d_6 (TMS standard). The nmr data and proton assignments for the compounds studied are as follows:

	δ-value (ppm)		
Compound	H_α	H_β	H_γ
V	8.27 s	7.82 s	---
VII	---	7.55 d (a)	7.20 d (a)
IX	8.43 s	8.18 s	---
X	8.34 s	8.00 m	8.00 m
XI	---	7.54 s	7.54 s
2-Iodo-6-methyl-3-pyridinol	---	7.00 s	7.00 s

(a) J = 9 cps.

Organic Intermediates.

4-Chloro-2-chloromethyl-5-methoxypyridine (II).

2-Hydroxymethyl-5-methoxy-4*H*-pyran-4-one, m.p. 157-158°, was prepared by treatment of kojic acid with dimethyl sulfate in potassium hydroxide solution (12). The latter derivative was then allowed to react with concentrated ammonium hydroxide in a stainless steel bomb at 90° for two hours to form 2-hydroxymethyl-5-methoxy-4-pyridinol, m.p. 172-175° (13). Reaction of this compound with phosphorus oxychloride under reflux yielded II, m.p. 72-73° (4).

4-Chloro-2-chloromethyl-5-methoxypyridine 1-Oxide (III).

To a solution of 50 g. (0.260 mole) of 4-chloro-2-chloromethyl-5-methoxypyridine in 280 ml. of glacial acetic acid was added 50 ml. of a 30% aqueous hydrogen peroxide solution. The reaction mixture was heated with stirring in an oil bath at 70°. After three hours an additional 30 ml. of hydrogen peroxide was added and the reaction mixture was kept at 70° with stirring overnight. The solution was taken to dryness *in vacuo*, the residue extracted with acetone, decolorized with Norit A, and cooled to yield a white crystalline product. Recrystallization from acetone-ether yielded 20.0 g., m.p. 163-164°. Workup of the mother liquor gave another 12.3 g. resulting in an overall yield of 32.3 g. (60%).

Anal. Calcd. for $C_7H_7Cl_2NO_2$: C, 40.4; H, 3.4; N, 6.7.

Found: C, 40.5; H, 3.4; N, 6.4.

Ethyl 2-Acetamido-2-(4-chloro-5-methoxy-2-pyridylmethyl 1-Oxide)malonate (IV).

About 250 ml. of dry ethanol was collected in a 3-neck flask and was purged with dry nitrogen. Sodium, 2.17 g. (0.094 g.-atom), was dissolved in the ethanol and then 20.3 g. (0.094 mole) of ethyl acetamidomalonate was added. Finally, 19.5 g. (0.094 mole) of 4-chloro-2-chloromethyl-5-methoxypyridine 1-oxide was added and the reaction mixture was heated under reflux for about four hours (until the *p*H of an aliquot dissolved in distilled water had decreased to approximately 5). The solution was concentrated to about 75 ml. and then poured over ice. The resulting crystalline material was removed by filtration, washed with cold water, and dried to yield 19.5 g. Concentration and cooling of the filtrate yielded an additional 9.5 g. to give an overall yield of 79%. Recrystallization from ethanol-water afforded white needles, m.p. 156-158°; U.V. λ max (water) 269 mμ.

Anal. Calcd. for $C_{16}H_{21}ClN_2O_7$: C, 49.4; H, 5.4; N, 7.2. Found: C, 49.2; H, 5.5; N, 7.3.

β-(5-Hydroxy-2-pyridyl 1-Oxide)-DL-alanine (VI).

Compound IV, 4.0 g. (0.010 mole) was dissolved in 100 ml. of 25% hydrochloric acid in a 125 ml. flask, placed in a stainless steel bomb, and heated in an oven at 160° for four hours. The light yellow solution was then taken to dryness *in vacuo* and the residue dissolved in water (14). About 200 mg. of palladium black was added to the aqueous solution and the mixture was treated with hydrogen under three atmospheres of pressure for four hours. The catalyst was filtered off and the solution evaporated to dryness *in vacuo*. The solid was dissolved in water and again taken to dryness. The residue was redissolved in water and neutralized with Amberlite IR-45. The resin was removed by filtration and the filtrate concentrated to a smaller volume. Upon addition of acetone, followed by cooling, a light pink solid separated and was filtered off, washed with acetone, and dried to yield 1.1 g. (54%), m.p. 255-258° dec. A solution of the compound in water gave a positive ferric chloride test and showed only one spot on a paper chromatogram developed with ninhydrin reagent.

Anal. Calcd. for $C_8H_{10}N_2O_4$: C, 48.5; H, 5.1; N, 14.2. Found: C, 48.5; H, 5.0; N, 14.2.

β-(5-Hydroxy-6-iodo-2-pyridyl 1-Oxide)-DL-alanine (VII).

Compound VI, 0.396 g. (0.002 mole) was dissolved in 40 ml. of concentrated ammonium hydroxide, and 4 ml. of a 1 molar iodine solution was added at such a rate that the solution was decolorized between each successive addition of the iodine solution. The complete addition required about three hours, and the reaction mixture was left stirring for several hours. The solution was then taken to dryness *in vacuo*, and the residue was dissolved in a small volume of water. Acetone was added to the turbidity point, and the solution was stored in the refrigerator. A light tan solid subsequently separated and was filtered off, washed with acetone and ether, and dried to yield 0.3 g. (46%), m.p. 245-250° dec. The compound showed only a single ninhydrin-positive spot on a paper chromatogram.

Anal. Calcd. for $C_8H_9IN_2O_4$: C, 29.6; H, 2.8; N, 8.7. Found: C, 29.5; H, 2.9; N, 8.7.

Ethyl 2-Acetamido-2-(4-chloro-5-methoxy-2-pyridylmethyl)malonate (VIII).

Compound IV, 10.15 g. (0.026 mole), was dissolved in 100 ml. of chloroform, and 10 ml. of phosphorus trichloride was added. The reaction mixture was heated under reflux for one hour, and at the end of this period the dark solution was evaporated to near dryness *in vacuo*. Ice was added to the residue to decompose any residual phosphorus trichloride. Finally, water was added to the cooled solution, and it was heated for a few minutes. Filtration removed some insoluble material and the filtrate was neutralized in the cold by the addition of 30% sodium hydroxide solution. The resulting precipitate was recovered by filtration, washed with cold water, and dried over phosphorus pentoxide to yield 8.5 g. (87%), m.p. 152-153° (Lit. (4), m.p. 150-151°); U.V. λ max (water) 281 mμ (Lit. (4), λ max (water) 282 mμ).

β-(4-Chloro-5-hydroxy-2-pyridyl)-DL-alanine (IX).

Compound IX was obtained as previously described (4) except that 25% hydrochloric acid was employed, and the neutralization was conducted with Amberlite IR-45. From 4.5 g. (0.012 mole) of VIII, 0.52 g. (20%) of IX was obtained, m.p. 260-262° dec.

Anal. Calcd. for $C_8H_9ClN_2O_3 \cdot 2H_2O$: C, 38.1; H, 5.2; N, 11.1. Found: C, 38.2; H, 5.1; N, 11.2.

β-(5-Hydroxy-2-pyridyl)-DL-alanine (X).

Hydrogenolysis of compound IX was accomplished as previously described (4), with the exception that the reaction mixture was neutralized with Amberlite IR-45. Crystallization from water yielded X (60%), m.p. 240-241° dec.; U.V. λ max (water, *p*H 3) 285 mμ, λ max (water, *p*H 12) 306 mμ (Lit. (4), λ max (water, *p*H 3) 288-289 mμ, λ max (water *p*H 12) 303 mμ.

Anal. Calcd. for $C_8H_{10}N_2O_3$: H, 5.5; N, 15.4. Found: H, 5.4; N, 15.1 (15).

β-(5-Hydroxy-6-iodo-2-pyridyl)-DL-alanine (XI).

Compound X, 0.091 g. (0.0005 mole), was dissolved in 10 ml. of concentrated ammonium hydroxide solution. One ml. (0.001 mole) of an aqueous 1 molar iodine solution was then added with stirring over a period of about one hour. After the addition was completed, the reaction mixture was evaporated to dryness *in vacuo* and then dissolved in water. Acetone was added to the turbidity point and the dark solution was stored in the refrigerator. A dark material came out of solution and was removed by filtration. Acetone was added to the filtrate, and after standing in the cold, more of the dark, ninhydrin-negative material separated out of solution. This solid was removed by filtration, and acetone was added to the now light colored filtrate. After cooling for several days, a light tan solid was obtained (ninhydrin-positive). The solid weighed 0.050 g. (32%), m.p. 194-195° dec.

Anal. Calcd. for $C_8H_9IN_2O_3$: C, 31.2; H, 2.9; N, 9.1. Found: C, 30.9; H, 3.1; N, 9.0.

2-Iodo-6-methyl-3-pyridinol.

6-Methyl-3-pyridinol, 5.45 g. (0.050 mole) was reacted with iodine in a manner analogous to the iodination of 3-pyridinol reported by Schickh (10). The isolated 2-iodo-6-methyl-3-pyridinol weighed 6.8 g. (58%), m.p. 179-183° dec.

Anal. Calcd. for C_6H_6INO: C, 30.7; H, 2.6; N, 6.0. Found: C, 30.9; H, 2.6; N, 5.8.

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(14) Compound V was not usually isolated; however, in one instance the compound was isolated for use in nmr spectral studies. The product melted at 222-225° dec., and the carbon, hydrogen, and nitrogen analysis was in agreement with the molecular formula $C_8H_9ClN_2O_4 \cdot \frac{1}{2} H_2O$.

(15) An acceptable carbon analysis could not be obtained. The compound is considered pure, and its structure certain, on the basis of paper chromatography, U.V. spectrum, nmr spectrum, and the microbiological inhibition index which is identical to the previously reported β-(5-hydroxy-2-pyridyl)-DL-alanine dihydrochloride. See reference (4).

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