[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

The Chemistry of Vitamin B_6. VI. Pyridoxylamino Acids[1]

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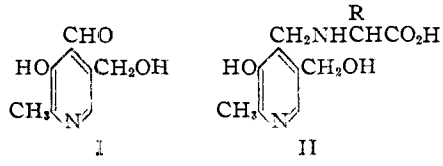
Pyridoxal, I, has been reductively coupled with amino acids and amino acid esters to give pyridoxylamino acids, II, and esters.

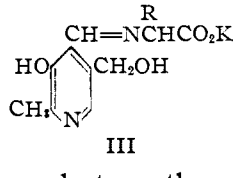


I II

When the work on the structure and synthesis of pyridoxal and pyridoxamine had been completed,[2] it became of interest to synthesize a series of pyridoxyl derivatives, II, of the natural α-amino acids and related compounds. It was believed that the availability of these compounds would expedite certain studies of the biological role of the vitamin B_6 group. Because of a mutual interest in this research, chemical and biological studies were carried out collaboratively, and the microbiological results have been detailed in an accompanying paper.[3] The pyridoxylamino acids (II) did not show significant vitamin or antivitamin activity against three diverse microorganisms[3]; some of the acids do appear to have limited vitamin activity for the mold *Neurospora sitophila*.[3] These pyridoxylamino acids have also been tested for vitamin B_6 activity in the rat, and it appears that some of them possess definite but limited activity. We are indebted to Dr. Gladys Emerson[4] of the Merck Institute for Therapeutic Research for the rat tests.

While these researches were in progress, considerable evidence concerning the function of vitamin B_6 in enzymatic transaminations appeared in the literature; this evidence is summarized with the microbiological data.[3]

Pyridoxal condensed readily with the potassium salts of the amino acids to give bright yellow solutions of the Schiff bases III (with three exceptions). The only Schiff base which was isolated

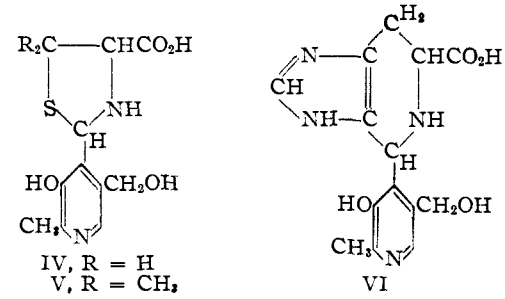


III

as a crystalline product was the potassium salt of N-pyridoxylidene-DL-alanine. All of the Schiff bases were hydrogenated over a platinum catalyst with the exception of N-pyridoxylidene-DL-methionine, which was hydrogenated over a palladium catalyst. In the later, more successful experiments (method A), the free pyridoxylamino acids were isolated as crystalline products. In the earlier experiments (methods C and D), most of the products were isolated as crystalline hydrochlorides. The hydrochlorides were found to be unstable both in solution and in the solid state. The condensation and hydrogenation reactions usually took place quantitatively. It was difficult to separate some of the pyridoxylamino acids from potassium chloride, because of their similar solubility properties, and in these cases the yields were lower.

Twenty amino acids, which are enumerated in Table I, were converted to the corresponding pyridoxylamino acids. A few esters (L-tyrosine butyl ester, DL-aspartic acid diethyl ester, DL-glutamic acid diethyl ester, DL-alanine ethyl ester) were condensed directly with pyridoxal in alcoholic solution to give bright yellow solutions of the Schiff bases. Hydrogenation over a platinum catalyst then gave the corresponding pyridoxylamine acid esters (method B).

Two amino acids, L-cysteine and L-penicillamine,[5] reacted with pyridoxal to give 4-thiazolidinecarboxylic acid derivatives, IV and V, respectively, similar to those compounds prepared[6,7] previously from cysteine. Although a fleeting yellow color was observed during the condensation reactions which indicated transitory formation of the Schiff bases, the colorless thiazolidines IV and V were the products isolated. These thiazolidines could not be hydrogenated under the conditions used for the hydrogenation of the Schiff bases.



IV, R = H
V, R = CH_3

VI

DL-Histidine reacted with pyridoxal to give a colorless condensation product, which resisted hydrogenation under similar conditions, and which is represented by structure VI, corresponding to

(1) Trivial names in the vitamin B_6 group have been extended to these derivatives of pyridoxine. The term "pyridoxylidene" is used to denote the 2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridylmethylene radical; the term "pyridoxyl" denotes the 2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridylmethyl radical. Paper presented at the Chicago meeting of the American Chemical Society, April 19, 1948.

(2) Harris, Heyl and Folkers, THIS JOURNAL, **66**, 2088 (1944).

(3) Snell and Rabinowitz, *ibid.*, **70**, 3432 (1948).

(4) The details and results of these tests will be described later.

(5) Committee on Medical Research, O. S. R. D., and the Medical Research Council, London, *Science*, **102**, 627 (1945).

(6) Schubert, *J. Biol. Chem.*, **111**, 671 (1935); **114**, 341 (1936).

(7) Ratner and Clarke, THIS JOURNAL, **59**, 200 (1937).

TABLE I

PYRIDOXYLAMINO ACIDS PRODUCT AND REACTION DATA

Product N-pyridoxyl-	Reactants Amino acid or ester, g.	Pyridoxal, g.	Method	Yield, %	M. p. (dec.), °C.	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
DL-Phenylalanine	10.0	10.12	A	93	233–234	$C_{17}H_{20}N_2O_4$	64.54	64.40	6.37	6.49	8.86	8.85
DL-Alanine	4.27	8.00	A[a]	78	213–214	$C_{11}H_{16}N_2O_4$	54.99	54.82	6.71	6.69	11.66	11.72
L-Tyrosine	10.0	9.24	A[b]	96	242–250	$C_{17}H_{20}N_2O_5$[g]	61.43	61.15	6.07	6.34	8.43	8.50
Glycine	3.54	7.9	A	46[c]	228–229	$C_{10}H_{14}N_2O_4$[t]	53.09	53.29	6.24	6.25	12.39	12.28
DL-Norleucine	10.00	12.75	A	21[c]	220–221	$C_{14}H_{22}N_2O_4$	59.55	59.59	7.86	7.79	9.92	9.94
L-Leucine	4.23	5.4	A	7[c]	228–229	$C_{14}H_{22}N_2O_4$	59.55	59.53	7.86	7.71	9.92	10.10
DL-Leucine	10.00	12.74	A	64[c]	232–233	$C_{14}H_{22}N_2O_4$	59.55	59.53	7.86	7.85	9.92	9.69
DL-Isoleucine	10.00	12.74	A	16[c]	222–223	$C_{14}H_{22}N_2O_4$	59.55	59.69	7.86	8.08	9.92	9.81
DL-Valine	9.9	14.1	A	84[c]	245–246	$C_{13}H_{20}N_2O_4$	58.19	57.86	7.51	7.51	10.44	10.35
DL-Tryptophan	10.0	8.21	A	95	240–241	$C_{19}H_{21}N_3O_4$[u]	64.21	64.48	5.96	5.95	11.82	11.67
DL-Threonine	10.0	14.0	A	32[c]	239–240	$C_{12}H_{18}N_2O_5$	53.32	53.19	6.71	6.53	10.37	10.46
DL-Glutamic acid	5.00	5.05	A	13[c]	188–189	$C_{12}H_{18}N_2O_6$[t]	52.34	52.53	6.08	6.12	9.39	9.50
L-Glutamic acid	3.00	3.41	A[d,e]	31[c]	181–182	$C_{12}H_{18}N_2O_6$	52.34	52.21	6.08	6.02	9.39	9.28
DL-Methionine	2.62	2.93	A[f]	10[c]	217–218	$C_{13}H_{20}N_2O_4S$[t]	51.98	52.17	6.71	6.77	9.33	9.20
DL-Aspartic acid	3.00	3.77	A[d]	20	227–228	$C_{12}H_{16}N_2O_6$	50.70	50.72	5.67	5.54	9.86	9.81
L-Asparagine	3.00[g]	3.79[h]	A	21	209–210	$C_{12}H_{17}N_3O_6$	50.87	50.66	6.05	5.81	14.83	14.63
β-Alanine	1.00	1.87	A	61[c]	212–213	$C_{11}H_{16}N_2O_4$	54.99	54.89	6.71	6.50	11.66	11.54
L-Lysine	0.91[i]	0.83	A[j]		211–213	$C_{14}H_{23}N_3O_4$	56.55	56.29	7.80	8.16	14.13	13.82
L-Tyrosine butyl ester	3.8	2.7	B[j]	66	141–142	$C_{21}H_{28}N_2O_5$[v]	64.93	65.21	7.27	7.11	7.21	7.26
ε-N-Benzoyl-DL-lysine	1.17	0.83	C[k]	27	220–221	$C_{21}H_{27}N_3O_5$	62.82	63.04	6.78	6.93	10.47	10.43
DL-Serine	3.0	4.78	D[l,m]	56	217–218	$C_{11}H_{16}N_2O_6 \cdot H_2O$	48.17	48.53	6.61	6.62	10.22	10.34
				Hydrochlorides								
DL-Aspartic diethyl ester	6.1	5.4	B	78	168–169	$C_{16}H_{28}N_2O_6Cl_2$	46.49	46.74	6.34	6.39	6.78	6.48
DL-Glutamic diethyl ester	6.0	4.7	B[n,p]	79	155–156	$C_{17}H_{28}N_2O_6Cl_2$[p]	47.78	47.96	6.60	6.51	6.56	6.66
DL-Alanine ethyl ester	0.62	0.88	B[q]	30	180–181	$C_{13}H_{22}N_2O_4Cl_2$[p]	45.75	45.80	6.50	6.30	8.21	8.55
DL-Alanine	0.89	1.67	C	50[r]	202–203	$C_{11}H_{17}N_2O_4Cl$	47.74	47.64	6.19	5.87	10.13	10.14
L-Leucine	3.00	3.82	D	48	156–157	$C_{14}H_{23}N_2O_4Cl$	52.74	52.99	7.27	7.30	8.79	8.55

[a] Potassium chloride precipitated immediately on addition of alcoholic hydrogen chloride, and was removed at once by filtration. Chloride-free N-pyridoxyl-DL-alanine crystallized slowly from the filtrate. The water washing was omitted. [b] Potassium hydroxide beyond the theoretical amount was added until the tyrosine disappeared. [c] The reduction in yield was due to loss during the washing with water. The condensation and reduction steps took place in quantitative yields. [d] Alcoholic hydrogen chloride was added until a thick precipitate appeared; the solution was more acid than *p*H 6. [e] Additional amounts of crude material were obtained by addition of ether to the alcoholic filtrate. The fractions were combined and washed with water. [f] The catalyst was 14 g. of 5% palladium on Darco. [g] Obtained from General Biochemicals, Inc. [h] Several hours were required for the pyridoxal to react and be dissolved. [i] Lysine monohydrochloride was used, and only enough potassium hydroxide to neutralize the hydrochloride. The crude product was dissolved in hot water, treated with Darco, filtered, and precipitated by the addition of ethyl alcohol. The resulting material was then collected on a filter and washed with water as described in Method A. [j] The free amine crystallized from the concentrated alcoholic filtrate after the addition of water. It was recrystallized three times from water (including one decolorization with Darco). [k] The Schiff base solution was clear in a half hour. For hydrogenation it was concentrated to dryness under reduced pressure, and the residue dissolved in 50 cc. of absolute ethyl alcohol. After hydrogenation, the filtrate was concentrated to 20 cc. Potassium chloride, which precipitated from the alcoholic solution on neutralization, was removed by filtration. Halogen-free crystals of α-N-pyridoxyl-ε-N-benzoyl-DL-lysine were obtained by addition of ether to the filtrate. This material was recrystallized twice from dilute ethyl alcohol. [l] The condensation was carried out in methyl alcohol, and this solution diluted to 125 cc. for the hydrogenation. [m] The precipitate resulting from addition of alcoholic hydrogen chloride and containing both organic and inorganic material was filtered and dissolved in water; the solution was adjusted to *p*H 6 with hydrochloric acid. N-Pyridoxyl-DL-serine crystallized with one mole of water. It was not recrystallized. [n] The filtrate, after removal of the catalyst, was concentrated to a small volume, and was treated with an excess of alcoholic hydrogen chloride. [p] The product was recrystallized only once, from ethyl alcohol–ether containing a little hydrogen chloride. [q] After concentration of the filtrate after hydrogenation, the residue was dissolved in water and extracted four times with ether. The ethereal solution was washed with water, dried over anhydrous sodium sulfate, concentrated, and treated with an excess of alcoholic hydrogen chloride. The resulting dihydrochloride was recrystallized three times from ethyl alcohol–ether. [r] This yield is based on the hydrogenation step. See Method C. [s] Hygroscopic. [t] Dried at 130°. [u] Dried at 150°. [v] Dried at 61°.

the reaction product of L-histidine with formaldehyde.[8]

Experimental[9]

Method A is exemplified by the preparation of N-pyridoxyl-DL-phenylalanine; the free pyridoxylamino acid is the product of this preferred procedure. In method B, an ester of the amino acid was used, and the product was the dihydrochloride of the pyridoxylamino acid ester. Methods C and D gave the hydrochlorides of pyridoxylamino acids. Typical experiments for methods B, C and D are also described in detail. The significant experimental data for all the pyridoxylamino acids and esters are summarized in Table I.

Method A. N-Pyridoxyl-DL-phenylalanine.—Ten grams of DL-phenylalanine was suspended in 250 cc. of absolute methyl alcohol, and 3.90 g. of potassium hydroxide (an amount equivalent to the acid present) was added. The suspension was stirred until the acid had dissolved; then 10.12 g. of pyridoxal was added. The dark yellow solution was clear in less than three minutes. It was filtered, diluted to 300 cc. with absolute methyl alcohol, and shaken under 2–3 atmospheres of hydrogen with 0.3 g. of Adams platinum catalyst. The theoretical

(8) Wellisch, *Biochem. Z.*, **49**, 173 (1913).

(9) We are indebted to Mr. Richard Boos and his associates for the microanalyses.

amount of hydrogen was absorbed in seven minutes. After removal of the catalyst by filtration, the colorless solution, cooled in an ice-bath, was treated with alcoholic hydrogen chloride until the *p*H was about 6, *i. e.*, disappearance of any green color on moist Alkacid Test Paper (Fisher Scientific Company). After further cooling, the thick precipitate was collected on a filter and washed with methyl alcohol followed by ether. The N-pyridoxyl-DL-phenylalanine was thoroughly washed with ice-water, first in suspension, then on a filter until the filtrate was free of potassium chloride. The product was washed with alcohol and then with ether, and finally was dried to constant weight in a vacuum desiccator; yield, 17.8 g. (93%); m. p. 233–234° (dec.). The analytical sample was further dried at 100° (1 mm.) to constant weight.

Method B. N-Pyridoxyl-DL-aspartic Acid Diethyl Ester Dihydrochloride.—A solution of 5.4 g. of pyridoxal and 6.1 g. of DL-aspartic acid diethyl ester in 200 cc. of absolute ethyl alcohol was shaken with 0.15 g. of Adams platinum catalyst under 2–3 atmospheres of hydrogen. The hydrogenation required a little over an hour. After removal of the catalyst by filtration, the filtrate was concentrated to dryness under diminished pressure to remove the water formed during the reaction. The residue, dissolved in 100 cc. of absolute alcohol, was treated with an excess of alcoholic hydrogen chloride, and ether was added to precipitate crystals of N-pyridoxyl-DL-aspartic acid diethyl ester dihydrochloride; the yield was 10.3 g. (78%). After two recrystallizations from alcohol–ether containing a little hydrogen chloride, the melting point was 168–169° (dec.). The material was dried at 25° (1 mm.) before analysis.

Method C. N-Pyridoxyl-DL-alanine Hydrochloride.—A concentrated solution of potassium hydroxide was added slowly to a suspension of 0.89 g. of DL-alanine in 10 cc. of water, until the solution became clear. After removal of the water by freeze drying, the residue was dissolved in 30 cc. of absolute ethyl alcohol; treatment with 1.67 g. of pyridoxal resulted in a bright yellow mixture, which was shaken frequently during two hours. The yellow crystalline potassium salt of the Schiff base, pyridoxylidene-DL-alanine, was collected on a filter and washed with ethyl alcohol. Part of this material (1.35 g.) in 125 cc. of absolute methyl alcohol was shaken with 0.1 g. of Adams platinum catalyst under 2–3 atmospheres of hydrogen. The resulting colorless solution, after removal of the catalyst, was concentrated to dryness under reduced pressure, dissolved in absolute ethyl alcohol, and treated with alcoholic hydrogen chloride until the solution was acid to congo red. The N-pyridoxyl-DL-alanine hydrochloride, which crystallized, was filtered and recrystallized from ethyl alcohol; yield, 0.68 g. (50%, based on 1.35 g. of the potassium salt of N-pyridoxylidene-DL-alanine); m. p. 202–203°.

Method D. Pyridoxyl-L-leucine Hydrochloride.—Three grams of L-leucine and 1.28 g. of potassium hydroxide were shaken together in 125 cc. of absolute methyl alcohol until the solution was clear. Condensation took place quickly on the addition of 3.82 g. of pyridoxal. The solution was shaken with 0.15 g. of Adams platinum catalyst under 2–3 atmospheres of hydrogen. After removal of the catalyst, the filtrate was concentrated to dryness under reduced pressure; the residue was dissolved in absolute ethyl alcohol and was acidified with alcoholic hydrogen chloride. The inorganic precipitate which appeared at once was removed by filtration. N-Pyridoxyl-L-leucine hydrochloride was precipitated from the filtrate by addition of ether, and after one recrystallization from ethyl alcohol–ether, it weighed 3.5 g. (48%); m. p. 156–157° (dec.). The sample for analysis was dried at 61° (1 mm.).

2-(2-Methyl-3-hydroxy-5-hydroxymethyl-4-pyridyl)-4-thiazolidinecarboxylic Acid (IV).—To a solution of 1.21 g. of L-cysteine and 0.56 g. of potassium hydroxide in 30 cc. of 80% ethyl alcohol was added 1.67 g. of pyridoxal and 60 cc. of 50% alcohol. The slightly turbid solution was filtered. Addition of ether caused slow precipitation of the potassium salt of the condensation product. The salt was collected on a filter. It was then dissolved in water and acidified to *p*H 6 with 6 *N* hydrochloric acid. Needles of 2-(2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridyl)-4-thiazolidenecarboxylic acid crystallized in a yield of 1.16 g. (43%). After one recrystallization from ethyl alcohol, the product melted at 149–150° (dec.).

Anal. Calcd. for $C_{11}H_{14}N_2O_4S$: C, 48.88; H, 5.22; N, 10.37. Found: C, 49.18; H, 5.14; N, 10.21.

2-(2-Methyl-3-hydroxy-5-hydroxymethyl-4-pyridyl)-5,5-dimethyl-4-thiazolidinecarboxylic Acid (V).—A solution of 0.25 g. of potassium hydroxide in 7 cc. of water was added to a solution of 0.34 g. of L-penicillamine hydrochloride in 40 cc. of ethyl alcohol. The addition of 0.38 g. of pyridoxal produced a bright yellow colored solution. When the pyridoxal had all reacted, a small amount of insoluble material was removed by filtration. Acidification of the solution (to *p*H 6) caused the crystallization of 2-(2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridyl)-5,-5-dimethyl-4-thiazolidinecarboxylic acid, which was washed successively with water, alcohol and ether; the material melted at 190–191° (dec.); yield, 0.4 g. (73%).

Anal. Calcd. for $C_{13}H_{18}N_2O_4S$: C, 52.33; H, 6.08; N, 9.39. Found: C, 52.51; H, 6.26; N, 9.20.

4-(2-Methyl-3-hydroxy-5-hydroxymethyl-4-pyridyl)-1-imidazo[c]tetrahydropyridine-6-carboxylic Acid (VI).—To a suspension of 1.91 g. of L-histidine hydrochloride in 10 cc. of water was added 1.29 g. of potassium hydroxide dissolved in 8 cc. of water. Addition of 1.67 g. of pyridoxal to the clear solution produced a bright orange color, which immediately began to fade. The solution was diluted with 150 cc. of ethyl alcohol; after two hours the white solid material was collected on a filter and washed well with ethyl alcohol followed by ether. An aqueous solution of the material, cooled in ice, was treated with 6 *N* hydrochloric acid until the solution showed a *p*H of 4. The small amount of material that did not dissolve was removed by filtration, and the filtrate was cooled in ice. The thick white precipitate which separated was collected on a filter and washed well with water, followed by ethyl alcohol and then ether. The crystalline 4-(2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridyl)-1-imidazo[c]tetrahydropyridine-6-carboxylic acid, m. p. 207–208° (dec.), weighed 0.67 g. (22%).

Anal. Calcd. for $C_{14}H_{16}N_4O_4$: C, 55.25; H, 5.30; N, 18.41. Found: C, 54.76; H, 5.42; N, 18.10.

Summary

Twenty amino acids and four esters of amino acids have been reductively coupled with pyridoxal to form pyridoxylamino acids. These compounds have limited vitamin B_6 activity in rats and no activity for several bacteria and yeast.

L-Cysteine and L-penicillamine yielded thiazolidine derivatives, and L-histidine formed an imidazotetrahydropyridine derivative, upon reaction with pyridoxal.

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