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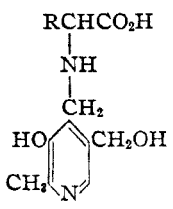
Chemistry of Vitamin B_6. VII. Pyridoxylidene- and Pyridoxylamines[1]

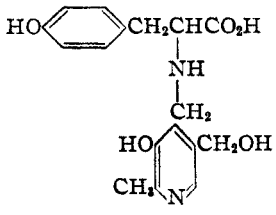
BY DOROTHEA HEYL, EILEEN LUZ, STANTON A. HARRIS AND KARL FOLKERS

Pyridoxylidene and pyridoxyl derivatives of certain amines, including several pressor amines, have been synthesized. These compounds, being derivatives of both pyridoxine and the pressor amines, are of interest to study biologically for vitamin B_6 activity and for pressor activity. A preliminary report on the synthesis and vitamin activity of some of these compounds has been made.[2]

The preparation of pyridoxyl derivatives of amino acids, I, as represented by N-pyridoxyltyrosine, II, has been described.[3] The pyridoxylideneamines, III, are Schiff bases, prepared by the condensation of pyridoxal with amines. The pyridoxylamines, IV, were prepared by catalytic reduction of the corresponding pyridoxylideneamines.



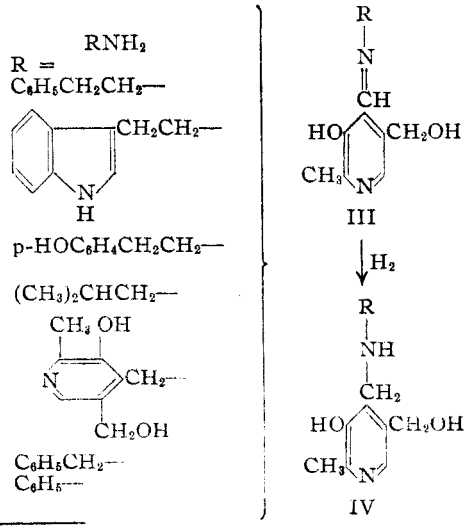
I



II

The amines listed in Tables I and II reacted with pyridoxal in methyl alcohol solution at room temperature to give the pyridoxylideneamines, III, which are yellow, crystalline compounds. These Schiff bases, with the exception of the two derived from aniline and pyridoxamine, were catalytically hydrogenated in methyl alcohol solution to give the pyridoxylamines, IV, which were isolated as colorless hydrochlorides.

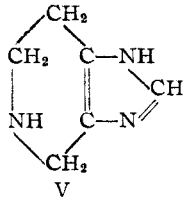
TABLE I



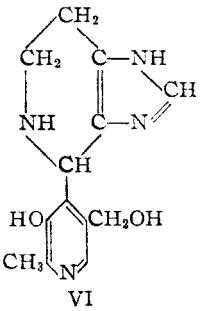
III

IV

Although pyridoxylidenehistamine was obtained as a bright yellow compound by the reaction of histamine with pyridoxal in alcoholic solution, an isomeric colorless product was obtained from these two reactants in aqueous–alcoholic solution. By analogy to a condensation product of histamine with formaldehyde,[4] V, this isomeric product, which does not react with hydrogen, is formulated as 4-(2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridyl)-1-imidazo[c]tetrahydropyridine, VI.



V



VI

We are greatly indebted to Dr. Gladys Emerson and Miss Elizabeth Wurtz of the Merck Institute for Therapeutic Research for tests on these new compounds for vitamin B_6 activity in rats. It has been found that pyridoxyltryptamine, pyridoxyl-β-phenylethylamine, pyridoxyltyramine and pyridoxylbenzylamine show activities that range between 50 and 100% of the activity of a molar equivalent of pyridoxine. It is interesting to note that these compounds show a considerably higher activity than do the corresponding pyridoxylamino acids.[3]

Experimental[5]

The pyridoxylideneamines are described in Table II; a typical experiment for the preparation of a pyridoxylideneamine is described under Procedure A for pyridoxylidene-β-phenylethylamine. The pyridoxylamines are described in Table III; a typical experiment for the preparation of a pyridoxylamine is described under Procedure B for pyridoxyl-β-phenylethylamine. Pyridoxylamines can be prepared directly without the isolation of the intermediate Schiff bases, as described under Procedure C for pyridoxyltyramine.

Procedure A. Pyridoxylidene-β-phenylethylamine.—Five grams of pyridoxal was added to a solution of 3.62 g. of β-phenylethylamine in 150 cc. of absolute methyl alco-

(1) 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.

(2) Heyl, Luz, Harris and Folkers, THIS JOURNAL, **70**, 1670 (1948).

(3) Heyl, Harris and Folkers, *ibid.*, **70**, 3429 (1948).

(4) Fränkel and Zeimer, *Biochem. Z.*, **110**, 234 (1920); Dale and Dudley, *J. Pharmacol.*, **18**, 104 (1921).

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

TABLE II

PREPARATION AND PROPERTIES OF PYRIDOXYLIDENEAMINES

(Prepared by Procedure A)

Product, Pyridoxylidene-	Reactants Amine, g.	Pyridoxal, g.	Solvent, cc.	Time of reaction, min.	Yield, %	M. p., °C.	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
β-phenylethylamine	3.62	5.00	150	30	86	101–102[a]	$C_{16}H_{18}N_2O_2$	71.08	71.12	6.71	7.01	10.37	10.34
tryptamine	1.00	1.04	40[b]	<5	73	160–161[c]	$C_{18}H_{19}N_3O_2$	69.88	69.81	6.19	6.18	13.58	13.46
tyramine	0.68	0.83	35	<5	90	168–169[d]	$C_{16}H_{18}N_2O_3$	67.11	67.24	6.34	6.34	9.79	10.05
isobutylamine	2.00	4.56	30[b]	60	45	67–68[e]	$C_{12}H_{18}N_2O_2$	64.84	64.89	8.16	8.05	12.61	12.77
pyridoxamine	3.31	3.30	400[b,f]	5 hr.	67	232–233[c]	$C_{16}H_{19}N_3O_4$	60.56	60.58	6.04	6.33	13.24	13.23
benzylamine	1.07	1.67	40[b]	<5	35	114–115[g]	$C_{16}H_{16}N_2O_2$	70.29	70.80	6.29	6.26	10.93	10.93
aniline	3 drops	0.1	10[b]	<5		178–179[h]	$C_{14}H_{14}N_2O_2$	69.40	69.49	5.82	5.61	11.57	11.36

[a] Crystallized from ether–petroleum ether and recrystallized twice from ethyl alcohol–ether–petroleum ether. [b] Ethyl alcohol was used instead of methyl alcohol. [c] Crystallized and recrystallized from ethyl alcohol. [d] Crystallized from ethyl alcohol, and recrystallized twice from ethyl alcohol and twice from ethyl alcohol–ether–petroleum ether. [e] Unreacted pyridoxal crystallized at once from ether–petroleum ether, and was collected on a filter. Pyridoxylideneisobutylamine then crystallized from the filtrate, and was not recrystallized. [f] The thick mixture was shaken mechanically. [g] Crystallized and recrystallized from ethyl alcohol–ether–petroleum ether. [h] The solution was heated to boiling during the reaction. Pyridoxylideneaniline crystallized on cooling and was not recrystallized.

TABLE III

PREPARATION AND PROPERTIES OF PYRIDOXYLAMINES

(Prepared by Procedure B)

Product hydrochloride of pyridoxyl-	Pyridoxylidene-amine, g.	Yield, %	M. p., (dec.), °C.	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
phenylethylamine	4.78	85	227–228[a]	$C_{16}H_{22}N_2O_2Cl_2$	55.65	55.89	6.42	6.15	8.12	8.25
tryptamine	1.00[b,c]	84	222–223[d]	$C_{18}H_{22}N_3O_2Cl$	62.15	61.86	6.38	6.57	12.08	12.28
tyramine	0.59[e]	66	234–235[f]	$C_{16}H_{22}N_2O_3Cl_2$	53.19	53.49	6.14	5.95	7.76	7.83
isobutylamine	2.66[b,c]	75	204–205	$C_{12}H_{21}N_2O_2Cl$	55.27	55.57	8.12	7.90	10.75	10.67
benzylamine	0.40[e,c]	54	219–220[g]	$C_{15}H_{20}N_2O_2Cl_2$	54.39	54.39	6.09	5.91	8.46	8.56

[a] Recrystallized twice from methyl alcohol–ether. [b] The filtrate was acidified only to *p*H 6. The monohydrochloride was isolated. [c] Ether was added to precipitate the crystals from the acidified alcoholic solution. [d] Recrystallized from ethyl alcohol–water. [e] The filtrate was concentrated to dryness and the residue dissolved in ethyl alcohol before acidification. [f] Recrystallized from water–ethyl alcohol–ether. [g] Recrystallized three times from ethyl alcohol–ether. A few drops of alcoholic hydrogen chloride were added during the last recrystallization.

hol. When the bright yellow solution had become clear at the end of a half hour of stirring, it was filtered and concentrated to dryness under reduced pressure. The residue was crystallized from ether–petroleum ether (b. p. 40–60°) to give 6.90 g. (86%) of pyridoxylidene-β-phenylethylamine, m. p. 101–102° (after two recrystallizations from ethyl alcohol–ether–petroleum ether).

Procedure B. Pyridoxyl-β-phenylethylamine Dihydrochloride.—A bright yellow solution of 4.78 g. of pyridoxylidene-β-phenylethylamine in 125 cc. of absolute methyl alcohol was shaken with 0.1 g. of Adams platinum catalyst under 2–3 atmospheres of hydrogen until the theoretical amount of hydrogen had been absorbed and the solution had become colorless. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure to 30 cc., and alcoholic hydrogen chloride was added, with cooling in ice, until the solution was acid to congo red. Crystals of pyridoxyl-β-phenylethylamine dihydrochloride separated from the solution, and were obtained in a yield of 5.18 g. (85%). After two recrystallizations from methyl alcohol–ether, the crystals melted at 227–228° (dec.). The sample for analysis was dried at 100° (1 mm.) for two hours.

Procedure C. Pyridoxyltyramine Dihydrochloride.—In 100 cc. of absolute methyl alcohol, 4.15 g. of tyramine and 5.06 g. of pyridoxal were suspended. After five minutes, the practically clear, yellow solution was filtered, diluted with methyl alcohol to 150 cc., and shaken with 0.2 g. of Adams platinum catalyst under 2–3 atmospheres of hydrogen for one-half hour. After removal of the catalyst, the colorless solution was well cooled in an ice-bath, and alcoholic hydrogen chloride was added slowly until the solution was acid to congo red. The crystals of pyridoxyltyramine dihydrochloride, after being collected on a filter and washed with alcohol and then ether, were obtained in a yield of 9.6 g. (88%), m. p. 238–239° (dec.).

Anal. Calcd. for $C_{16}H_{22}N_2O_3Cl_2$: C, 53.19; H, 6.14; N, 7.76. Found: C, 53.35; H, 5.86; N, 7.59.

Pyridoxylhistamine Dihydrochloride (IV).—A suspension of 5.0 g. of histamine dihydrochloride and 5.0 g. of sodium bicarbonate in 150 cc. of absolute ethyl alcohol was boiled for thirty-five minutes. The inorganic material was collected on a filter, and the alcoholic solution treated with 4.5 g. of pyridoxal. The solution was stirred for an hour. The resulting thick, bright yellow precipitate was collected on a filter. Further crystalline material was obtained by chilling of the filtrate. The total yield of the condensation product was 2.93 g., which was reduced to 1.53 g. by recrystallization from alcohol; m. p. 240–241° (dec.). One gram of this pyridoxylidenehistamine[6] dissolved in 300 cc. of absolute methyl alcohol was hydrogenated under 2–3 atmospheres of hydrogen in the presence of 0.1 g. of Adams platinum catalyst. After removal of the catalyst, the filtrate was concentrated and then treated with an excess of alcoholic hydrogen chloride. Crude, crystalline pyridoxylhistamine dihydrochloride weighing 1.3 g. was obtained. After three recrystallizations from ethyl alcohol–water, the material was obtained in poor yield, and the melting point was 236–237° (dec.). The analytical sample was dried at 100° (1 mm.) for one and one-half hours.

Anal. Calcd. for $C_{13}H_{20}N_4O_2Cl_2$: C, 46.57; H, 6.01; N, 16.71. Found: C, 46.84; H, 6.10; N, 16.96.

4-(2-Methyl-3-hydroxy-5-hydroxymethyl-4-pyridyl)-1-imidazo[*c*]tetrahydropyridine (VI).—A solution of 1.68 g.

(6) This material was probably contaminated with the imidazole tetrahydropyridine derivative VI.

of potassium hydroxide in 5 cc. of water was added to a solution of 1.84 g. of histamine dihydrochloride in 5 cc. of water. The resulting solution was diluted with 100 cc. of ethyl alcohol, and 1.67 g. of pyridoxal was added. The initial bright yellow color gradually faded, and a thick, white precipitate appeared slowly. After a half hour, the mixture was cooled in ice. The white material was collected on a filter and washed with water until free of salt. It was then washed with alcohol and finally with ether; yield, 0.76 g. (29%); m. p. 252–253° (dec.). The analytical sample was dried at 100° (1 mm.) for four hours.

Anal. Calcd. for $C_{13}H_{16}N_4O_2$: C, 59.98; H, 6.20; N, 21.53. Found: C, 60.14; H, 6.10; N, 21.39.

Summary

Pyridoxal has been condensed with several amines to form Schiff bases. Most of these pyridoxylideneamines have been hydrogenated, yielding the pyridoxylamines: pyridoxyltyramine, pyridoxyltryptamine, pyridoxyl-β-phenylethylamine, pyridoxylhistamine, pyridoxylbenzylamine and pyridoxylisobutylamine. These compounds show an activity nearly as great as that of pyridoxine in rats.

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[CONTRIBUTION FROM HERCULES EXPERIMENT STATION, HERCULES POWDER COMPANY]

Resin Acids. V. The Composition of the Gum Oleoresin Acids of Pinus Palustris

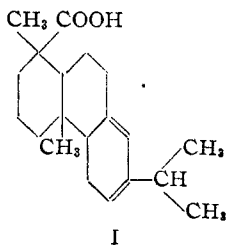
BY GEORGE C. HARRIS

For more than a century an extensive literature has dealt with the acids of gum oleoresin but only a limited number of valid results have been obtained. The reasons for this are that resin acids are unstable substances that easily change and are isomerized on treatment with heat and strong acids, and possess a great tendency to oxidize even with atmospheric oxygen. Especially variable are the primary acids present in unmodified oleoresins.

Because of their capacity for forming mixed crystals, the separation of mixtures of them into pure constituents by simple fractional crystallization often has been very difficult. It is, therefore, not surprising that many investigators working with oleoresins of different sources and using different separation methods obtained acids with different constants and gave them ever different names. Besides fractional crystallization, the only other technique available heretofore was the formation of insoluble sodium salts whereby levopimaric and dextropimaric acids[1] were isolated. A large number of "sapinic" (abietic-type) acids, which do not form crystalline salts have been reported as pure isomers obtained by fractional crystallization. Some of these are α-alepic, α-sapinic, Dupont's pineic,[2] Suzuki's densipimaric,[3] and Vocke's sapinic acid.[4] All of these were eventually shown to be mixtures.[5]

We have been more successful in the isolation of pure resin acids by applying and further developing a new technique,[6] the amine salt method for the separation of resin acids from non-resin acid material and for the isolation of pure resin acids. With the aid of this technique and others reported in the literature, it has been possible to determine fully, for the first time, the composition of the resin acids fraction of the oleoresin from *Pinus palustris*. In the following, the pertinent material reported previously in papers of this series will be brought together with new work in describing the composition of the primary resin acids.

For this investigation, as well as for the isolation of resin acids, reported earlier, a large batch of oleoresin was collected from the "longleaf" pine, *Pinus palustris*, and stored at 0–3° in the dark, out of contact with air. The acidic material was first separated from the turpentine as cyclohexylamine salts.[7] The regeneration of the acids from the amine salts was carried out using a weak acid, boric acid, at temperatures of 50° or below in order to retain the primary resin acids unaltered. The isolation of 15% of levopimaric acid, I, from this mixture of acids using butanolamine has been described.[7] However, it was not concluded to be



I

the total amount of levopimaric acid in the mixture because in the isolation of resin acids with the amine salt technique it was found that the desired separation was obtained but not always in quantitative fashion. To determine this value, a Diels–Alder addition reaction modified by Fleck and Palkin[8] was used whereby the resin acids in dry *n*-

(1) These words have purposely been written as one word since the compounds are not steroisomers as the prefixes *levo-* and *dextro-* would imply.

(2) G. Dupont, *Bull. soc. chim.*, **35**, 1207 (1923).

(3) S. Suzuki, *Chem. Zentr.*, **96**, I, 2383 (1925); **106**, II, 234 (1935).

(4) F. Vocke, *Ann.*, **508**, 11 (1933).

(5) G. Dupont, *Bull. soc. chim.*, **29**, 718 (1921). L. Ruzicka, Fr. Balas and Fr. Vilim, *Helv. Chim. Acta*, **7**, 458 (1924). K. Kraft, *Ann.*, **520**, 133 (1935). K. Kraft, *ibid.*, **524**, 1 (1936).

(6) Fr. Balas, *Časopis Československen Lékárnictva*, **7**, 320 (1927).

(7) G. C. Harris and T. F. Sanderson, "Resin Acids. I," THIS JOURNAL, **70**, 334 (1948).

(8) E. E. Fleck and S. Palkin, *Ind. Eng. Chem., Anal. Ed.*, **14**, 146 (1942).