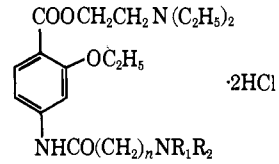
TABLE III
2-DIETHYLAMINOETHYL 2-ETHOXY-4-(ω-ALKYLAMINOACYLAMINO)BENZOATE DIHYDROCHLORIDES



No.	n	NR_1R_2	Yield,[a] %	Mp, °C	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Chlorine, % Calcd	Chlorine, % Found	Nitrogen, % Calcd	Nitrogen, % Found
19	1	Diethylamino	80	190 dec	$C_{21}H_{37}Cl_2N_3O_4$	54.06	54.18	8.00	8.07	15.21	15.36	9.01	9.18
				131–134	Picrate	52.08	51.84	6.15	6.12			13.50	13.60
20	1	Isopropylamino	87	195 dec	$C_{20}H_{35}Cl_2N_3O_4$	53.09	53.18	7.80	7.85	15.67	15.82	9.29	9.32
21	1	Piperidino	73	195 dec	$C_{22}H_{37}Cl_2N_3O_4$	55.24	55.44	7.79	7.74	14.82	14.91	8.78	8.56
				144–146	Picrate	52.99	52.82	6.03	6.05			13.25	13.36
22	1	Morpholino	77	183 dec	$C_{21}H_{35}Cl_2N_3O_5$	52.50	52.32	7.33	7.34	14.76	14.77	8.76	8.63
				152–154	Picrate	50.94	51.06	5.70	6.82			13.20	13.41
23	2	Diethylamino	78	167 dec	$C_{22}H_{39}Cl_2N_3O_4$	54.99	54.81	8.18	8.06	14.76	15.03	8.75	8.64
24	2	Isopropylamino	53	168 dec	$C_{21}H_{37}Cl_2N_3O_4$	54.06	53.86	8.00	7.76	15.21	15.13	9.01	8.72

[a] Yields of the salts are based on the starting chloroacylamino derivatives. All amino esters were oily.

the ester was collected by filtration and recrystallized from absolute ethanol. Yields and analytical data are given in Table I.

Alkyl 2-Alkoxy-4-(ω-alkylaminoacylamino)benzoates.—A suspension of the chloroamide (0.05 mole) in 200 ml of absolute ethanol was refluxed for 2 hr with an excess of the appropriate amine (0.15 mole). The ethanol was then distilled, the residue was treated with 50 ml of a saturated $NaHCO_3$ solution and 50 ml of water, and the separated aminoacylaniline was extracted with ether. The constants of the aminoacylanilines were prepared and their salts, after recrystallization from absolute ethanol or absolute ethanol–anhydrous ether, are given in Table II.

2-Diethylaminoethyl 2-Ethoxy-4-(ω-alkylaminoacylamino)benzoates.—2-Diethylaminoethyl 2-ethoxy-4-(ω-chloroacylamino)-benzoate hydrochloride (0.025 mole) was added in portions to a solution of the appropriate amine (0.125 mole) in 100 ml of anhydrous benzene, cooled in an ice–water bath. The mixture was left for 1 hr at room temperature and then refluxed for 4 hr. After distillation of the benzene, the residue was treated with 80 ml of a saturated $NaHCO_3$ solution and the separated aminoacylaniline was extracted with ether. The same products were obtained when the procedure was carried out using either absolute ethanol as solvent or in the absence of solvent. The **dihydrochlorides** of the aminoacylanilines were obtained and their analytical data, after recrystallization from absolute ethanol, are described in Table III.

Acknowledgments.—The authors are indebted to Arnar-Stone Laboratories, Inc., Mount Prospect, Ill., for carrying out the biological screening and to the Service Central de Microanalyse, Paris (France), for performing the microanalyses.

Synthesis and Enzymological Activity of Some Pyridoxine Analogs[1a,b]

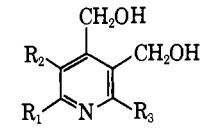
PAUL MELIUS AND DAVID L. MARSHALL[1c]

Department of Chemistry, Auburn University, Auburn, Alabama

Received May 12, 1967

Pyridoxine analogs, modified in the 2, 3, and 6 positions of the pyridine ring, were synthesized and examined as possible substrates for the enzyme pyridoxine dehydrogenase. A modification of an existing procedure[2] was used to synthesize the analogs listed in Table I. In the final step of the synthetic scheme, the pyridine dicarboxylic acid groups were reduced to hydroxymethyl groups with $NaBH_4$–$AlCl_3$ in diethylene glycol dimethyl ether.[3]

TABLE I
ACTIVITY OF ANALOGS



Compd	R_1	R_2	R_3	Concn, μmoles/ml	Act., mμmoles/10 min	K_m, M
Pyridoxine	CH_3	OH	H	10.0	2000	4.0×10^{-3}
I[a]	C_2H_5	OH	H	6.0	1900	2.1×10^{-3}
II	$CH(CH_3)_2$	OH	H	5.0	490	1.6×10^{-3}
III	CH_3	NH_2	Cl	15.0	0	...
IV	CH_3	OH	Cl	12.5	440	7.7×10^{-3}
V	CH_3	H	H	15.0	0	...
VI	CH_3	NH_2	H	15.0	0	...

[a] This compound was a gift from Dr. Stanton A. Harris of Merck Sharp and Dohme.

The ability of the analogs to replace pyridoxine was studied with the enzyme found in yeast which is responsible for the conversion of pyridoxine to pyridoxal.[4] The oxidation of pyridoxine and its analogs to pyridoxal compounds, as catalyzed by pyridoxine dehydrogenase, was assayed using the spectrophotometric method of Wada and Snell.[5] In this method the aldehyde formed is measured as the highly colored phenylhydrazone. The activity of the analogs is summarized in Table I. The importance of the 3-hydroxy group of pyridoxine in this metabolic reaction is demonstrated by the analogs in which the 3-hydroxy group has been replaced by hydrogen (V) or by an amino group (VI). These two structural analogs of pyridoxine had no activity under the conditions of the enzyme assay. Replacing the 2-methyl group of pyridoxine with an ethyl group (I) gave an analog which was nearly as active as pyridoxine. This is consistent

(1) (a) This work was supported by a National Defense Education Act fellowship. (b) A preliminary account of this work was presented at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 12–17, 1965. (c) Submitted by David L. Marshall to the faculty of Auburn University, 1966, in partial fullfillment of the requirements for the Ph.D. degree.

(2) H. M. Wuest, J. A. Bigot, Th. J. Delfoer, B. van der Wal, and J. P. Wibaut, *Rec. Trav. Chim.*, **78**, 226 (1959).

(3) R. K. Blackwood, G. B. Hess, C. E. Larrabee, and F. J. Pilgrim, *J. Am. Chem. Soc.*, **80**, 6244 (1958).

(4) Y. Morino and Y. Sakamoto, *J. Biochem.* (Tokyo), **48**, 733 (1960).

(5) H. Wada and E. E. Snell, *J. Biol. Chem.*, **236**, 2089 (1961).

with other studies of the metabolism of pyridoxine in which this analog was tested.[5,6] However, replacement of the 2-methyl group with the bulkier isopropyl group (II) or the addition of a chloro substituent at position 6 (IV) resulted in analogs with only one-fourth the activity of pyridoxine.

Experimental Section[7]

Synthesis. 2-Isopropyl-3-hydroxy-4,5-bis(hydroxymethyl)-pyridine (II).—The synthesis of this analog is illustrative of the method used for the other compounds. 3-Cyano-4-ethoxycarbonyl-6-isopropyl-2-pyridone was prepared by dissolving the sodium salt of ethyl isobutyropyruvate[8] (36 g, 0.174 mole) and cyanoacetamide (15 g, 0.18 mole) in 250 ml of absolute ethanol and refluxing for 2 hr. After cooling to room temperature, 35 ml of concentrated HCl made up to 210 ml with ice and water was slowly added with stirring. The product, after filtration and washing with ice water, amounted to 29 g (71%) and melted at 191–193°.

Anal. Calcd for $C_{12}H_{14}N_2O_3$: C, 61.5; H, 5.6; N, 12.0. Found: C, 61.6; H, 5.6; N, 11.9.

This compound (137.5 g, 0.585 mole) was then nitrated with fuming HNO_3–Ac_2O as described by Wuest, *et al.*,[2] to give 121.6 g (74%) of 3-cyano-4-ethoxycarboxyl-5-nitro-6-isopropyl-2-pyridone melting at 253–254°.

Anal. Calcd for $C_{12}H_{13}N_3O_5$: C, 51.6; H, 4.7; N, 15.0. Found: C, 52.1; H, 4.8; N, 14.7.

The chlorinated compound, 2-isopropyl-3-nitro-4-ethoxycarbonyl-5-cyano-6-chloropyridine, was prepared by heating together the nitropyridone (123 g, 0.44 mole) and PCl_5 (104 g, 0.5 mole) at 120–130° for 2 hr. The liquid reaction mixture was cooled and the $POCl_3$ was removed *in vacuo*. The viscous residue was added to crushed ice and stirred until solidification was complete. Recrystallization from ethanol gave 97 g (74%) of product melting at 75–77°.

Anal. Calcd for $C_{12}H_{12}ClN_3O_4$: C, 48.4; H, 4.1; N, 14.1. Found: C, 48.9; H, 4.3; N, 13.5.

Reduction of the 3-nitro group to an amino group with iron powder and HCl[2] gave 2-isopropyl-3-amino-4-ethoxycarbonyl-5-cyano-6-chloropyridine (mp 167–170°).

Anal. Calcd for $C_{12}H_{14}ClN_3O_2$: C, 54.83; H, 5.27; N, 15.7. Found: C, 54.39; H, 5.53; N, 15.92.

Removal of the 6-chloro group by hydrogenolysis followed by acid hydrolysis of the ester and cyano groups as described by Wuest, *et al.*,[2] gave the dicarboxylic acid compound, 2-isopropyl-3-aminopyridine-4,5-dicarboxylic acid melting at 218°. Diazotization gave 2-isopropyl-3-hydroxypyridine-4,5-dicarboxylic acid (mp 230–235°). Reduction of this compound (1.35 g, 5.56 mmoles) with $NaBH_4$–$AlCl_3$ in diglyme according to Blackwood, *et al.*,[3] gave 0.6 g (46%) of II, mp 193–195° (lit.[9] mp 192°).

2-Methyl-3-amino-4,5-bis(hydroxymethyl)-6-chloropyridine (III).—The synthesis of this analog up to the aminochloro compound, 2-methyl-3-amino-4-ethoxycarbonyl-5-cyano-6-chloropyridine, was essentially as described previously.[2] Alkaline hydrolysis,[10] however, was used to obtain the dicarboxylic acid, 2-methyl-3-amino-6-chloropyridine-4,5-dicarboxylic acid. Reduction with $NaBH_4$–$AlCl_3$ in diglyme gave III, mp 217–219° (lit.[10] mp 220–222°).

2-Methyl-3-hydroxy-4,5-bis(hydroxymethyl)-6-chloropyridine (IV).—Analog III was diazotized by treating a 1-g solution in 2 *N* H_2SO_4 with 1 g of $NaNO_2$ in 5 ml of water. The temperature was maintained at 70–80° for 15 min. After cooling and neutralizing to pH 7 with NaOH, the solution was evaporated to dryness *in vacuo*. The residue was extracted with 50 ml of hot absolute ethanol and filtered. The filtrate was taken to dryness and the residue remaining was extracted with ether in a Soxhlet extractor. Reducing the volume of the ether extract to 5 ml followed by standing at room temperature for 2–3 hr resulted in the formation of crystals, which, after filtering and drying, amounted to 0.175 g (21%), mp 188–192° (lit.[3] mp 192–193°).

2-Methyl-4,5-bis(hydroxymethyl)pyridine (V). 3-Cyano-4-ethoxycarbonyl-6-methyl-2-pyridine[2] was chlorinated to give 2-chloro-3-cyano-4-ethoxycarbonyl-6-methylpyridine and then hydrogenated using Pd–$BaCO_3$ as previously described[10] to give 2-methyl-4-ethoxycarbonyl-5-cyanopyridine (mp 57–58°, lit.[10] mp 58°). Alkaline saponification of this compound with 15% NaOH for 16 hr at 120° gave, after acidification of the salt with 25% HCl, 2-methylpyridine-4,5-dicarboxylic acid (mp 249–250°, lit.[12] mp 249–251°). Reduction of this diacid with $NaBH_4$–$AlCl_3$ in diglyme gave V, mp 210–212° (lit.[3] mp 202–203°).

2-Methyl-3-amino-4,5-bis(hydroxymethyl)pyridine (VI). The procedure employed for the 2-isopropyl analog (II) was followed, using ethyl acetopyruvate in the initial condensation step in place of ethyl isobutyropyruvate. The final step, reduction of the 4,5-dicarboxylic acid with $NaBH_4$–$AlCl_3$, gave the expected product, mp 192–193° (lit.[3] mp 197°).

Enzymatic Assay Procedure. The enzyme was partially purified from baker's yeast essentially as described by Morino and Sakamoto.[4] The assay mixture contained, in a final volume of 2.5 ml, 2.5 μmoles of NADPH, 100 μmoles of Na_2CO_3–$NaHCO_3$ buffer (pH 9.0), and the appropriate amount of enzyme and substrate. The substrate concentration in each case was the amount required to give maximum activity. The substrate solution was added after the other components had incubated at 37° for 2 min. The reaction was stopped after 10 min by adding 0.3 ml of 100% (w/v) trichloroacetic acid. The precipitated protein was removed by filtration. Aliquots of the filtrate were added to 0.2 ml of phenylhydrazine solution[5] and the volume was adjusted to 4 ml. After heating at 60° for 20 min to develop the color of the phenylhydrazone, the absorbance at 410 mμ was measured in a Bausch and Lomb Spectronic 20 colorimeter.

(6) D. B. McCormick and E. E. Snell, *J. Biol. Chem.*, **236**, 2085 (1961).

(7) Melting points were determined on a Fischer-Johns apparatus and are uncorrected. Microanalyses were by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

(8) C. S. Marvel and E. E. Dreger in "Organic Syntheses," Coll. Vol. I, H. Gilman, Ed., John Wiley and Sons, Inc., New York, N. Y., 1946, p 238.

(9) H. Davoll and F. B. Kipping, *J. Chem. Soc.*, 1395 (1953).

(10) B. van der Wal, T. J. DeBoer, and H. O. Huisman, *Rec. Trav. Chim.*, **80**, 221 (1961).

(11) M. J. Reglier and R. C. Elderfield, *J. Org. Chem.*, **7**, 285 (1942).

(12) A. C. McLeod and F. S. Spring, *J. Chem. Soc.*, 2582 (1949).

(13) A. Ichiba, K. Michi, and S. Enoto, *Sci. Papers Inst. Phys. Chem. Res.* (Tokyo), **39**, 126 (1941).

Metabolism of 5-(3-Pyridyl)tetrazole

S. K. FIGDOR AND M. SCHACH VON WITTENAU

Department of Pharmacology, Chas. Pfizer & Co., Inc., Groton, Connecticut

Received August 7, 1967

In a recent publication Holland and Pereira described the synthesis and lipolysis inhibitory property of a group of heterocyclic tetrazoles.[1] One member of this group 5-(3-pyridyl)tetrazole (**1**) was shown to be considerably less active than nicotinic acid in an *in vitro* assay, but was equipotent to nicotinic acid when tested *in vivo* in the dog.[1] More striking, however, was the difference in the duration of pharmacological effect between **1** and nicotinic acid. In the dog nicotinic acid produced a fall in plasma free fatty acid which lasted approximately 1 hr, whereas the duration of decreased plasma free fatty acid caused by **1** lasted approximately 5 hr. Since nicotinic acid is known to be extensively metabolized, it was speculated that the improved duration of action of **1** over nicotinic acid may be attributable to greater metabolic stability of **1**. Results reported here indeed show that 5-(3-pyridyl)-

(1) G. F. Holland and J. N. Pereira, *J. Med. Chem.*, **10**, 129 (1967).