alumina to give a hydroxy ester (XII), 1.9 g, a colorless oil: bp 119–120° (0.3 mm); ν_{max}^{film} 3495, 1730, and 1020 cm^{-1}.

Anal. Calcd for $C_{13}H_{24}O_5$: C, 59.98; H, 9.29. Found: C, 60.29; H, 9.30.

Hydrolysis of XII.—A solution of XII (1.7 g) in 80% AcOH (10 ml) was allowed to stand for 5 hr at room temperature. The solution was poured into ice-water and extracted with ether. The extract was washed (2 *N* Na_2CO_3, H_2O), dried (Na_2SO_4), and evaporated, leaving an oily residue (1.2 g). The residue was chromatographed on alumina to give a diol ester (710 mg), which was distilled at 99–100° (0.2 mm) to give a colorless oil (XIII), 700 mg, ν_{max}^{film} 3370 and 1725 cm^{-1}.

Anal. Calcd for $C_9H_{16}O_4$: C, 54.53; H, 9.15. Found: C, 54.32; H, 9.07.

Methyl 5-Methyltetrahydrofuran-2-acetate (XIV).—Toluene-*p*-sulfonyl chloride (780 mg, 1.3 equiv) was added to a solution of XIII (600 mg) in dry pyridine (4.0 ml) with stirring in an ice bath and left overnight at room temperature. The mixture was poured onto ice-water and extracted with ether. The extract was washed (2 *N* H_2SO_4, 2 *N* Na_2CO_3, H_2O), dried (Na_2SO_4), and evaporated, leaving an oily residue (506 mg). The residue was chromatographed on alumina to give XIV, a colorless oil: 170 mg; bp 80° (30 mm); ν_{max}^{film} 1738, 1200, 1168, and 1085 cm^{-1} (*Anal.* Calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.82; H, 8.99). This ester showed two peaks at retention times of 8.3 and 9.5 min in a ratio of 1:1 on the gas chromatogram,[10] and was separated into each compound by preparative gas chromatography, XIV having a peak at retention time of 8.3 min (colorless oil; $\nu_{max}^{CHCl_3}$ 1730, 1160, and 1079 cm^{-1}), XIV having a peak at retention time of 9.5 min (colorless oil; $\nu_{max}^{CHCl_3}$ 1730, 1158, 1070, and 1003 cm^{-1}).

5-Methyltetrahydrofuran-2-ethanol (VII).—A solution of XIV, retention time 9.5 min (15 mg), in dry ether (1 ml) was added to a suspension of $LiAlH_4$ (20 mg) in dry ether (1 ml) with stirring and stirring was continued for 3 hr at room temperature. To this mixture was added ether (3 ml) containing water and filtered. The ether solution was dried (Na_2SO_4) and evaporated, leaving an oily alcohol (7.5 mg), which was distilled at 100–105° (bath) (30 mm) to give VII, a colorless oil: $\nu_{max}^{CHCl_3}$ 3442, 1103, 1072, 1036, 929, 870, and 835 cm^{-1}; retention time[9] 4.4 or 13.5 min, which was identical with VII obtained from furanomycin by comparison with their infrared spectra and gas chromatographic retention times.

Anal. Calcd for $C_7H_{14}O_2$: C, 64.58; H, 10.84. Found: C, 64.41; H, 10.78.

XIV, retention time 8.3 min (21.2 mg), was reduced ($LiAlH_4$) under the same conditions to give 5-methyltetrahydrofuran-2-ethanol (VII) having a retention time[9] of 4.4 or 13.5 min; $\nu_{max}^{CHCl_3}$ 3440, 1063, 1030, 930, and 867 cm^{-1}.

(10) A column, 10 ft × ¼ in. consisting of 5% diethylene glycol succinate on Chromosorb W (45–60 mesh) was operated at 120° with a flow rate of 100 ml/min of He.

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Notes

Synthesis and Evaluation of the Local Anesthetic Activity of a Series of 2-Alkoxy-4-(ω-alkylaminoacylamino)benzoic Acid Esters[1,2]

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In a previous communication,[3] the synthesis and evaluation of the local anesthetic activity of a series of 4-(ω-alkylaminoacylamino)salicylic acid esters was reported. Compared to lidocaine, these compounds were generally more irritating, less toxic, and less active. Those compounds which had local anesthetic activity approaching that of lidocaine were extremely irritating. Einhorn and Oppenheimer[4] reported that nirvanine, methyl 5-diethylaminoacetamidosalicylate, which possessed strong local anesthetic activity and low toxicity was also extremely irritating.

Derivatives of alkoxyaminobenzoates, *e.g.*, 2- and 3-alkoxy derivatives of diethylaminoethyl 4-aminobenzoate,[5] 2-alkoxy derivatives of procaine,[6,7] and dialkylaminoethyl esters of 2-, 5-, and 6-alkoxy-3-aminobenzoic acids,[8] have been reported to possess local anesthetic activity. In addition, Clinton and co-workers[9] reported local anesthetic activity in a number of dialkylaminoacylamino derivatives of some 2-alkoxybenzoic acid esters. These studies suggested that etherification of the derivatives, reported in the previous communication,[3] might result in local anesthetic agents devoid of the observed irritancy.

Chemistry.—Treating an ester of 2-alkoxy-4-aminobenzoic acid with chloroacetyl or 3-chloropropionyl chloride and subsequently heating the intermediate 4-(ω-chloroacylamino) derivative (*cf.* Table I) with excess amine in ethanol produced ethyl, *n*-butyl, and 2-diethylaminoethyl esters of 2-ethoxy- and 2-*n*-butoxy-4-(ω-alkylaminoacylamino)benzoates as the hydrochloride salts (*cf.* Tables II and III).

(1) This investigation was supported by a research grant from the Royal Hellenic Research Foundation.

(2) A preliminary report of this work has been presented at the 25th International Congress of Pharmaceutical Sciences, Prague, Czechoslovakia, Aug 24–27, 1965. This paper comprises a portion of a thesis presented by D. K. at the University of Athens.

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(4) A. Einhorn and M. Oppenheimer, *Ann. Chem.*, **311**, 154 (1900).

(5) J. Buchi, E. Stunzi, M. Fleury, R. Hirt, P. Labbart, and L. Ragaz, *Helv. Chim. Acta*, **34**, 1002 (1951).

(6) F. P. Luduena and J. D. Hoppe, *J. Pharmacol. Exptl. Therap.*, **104**, 40 (1952).

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(8) E. Epstein and M. Meyer, *J. Am. Chem. Soc.*, **77**, 4059 (1955).

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TABLE I

2-ALKOXY-4-(ω-CHLOROACYLAMINO)BENZOATES

R	R'	n	Yield, %	Mp, °C	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Chlorine, % Calcd	Chlorine, % Found	Nitrogen, % Calcd	Nitrogen, % Found
C_2H_5	C_2H_5	1	83	112–114[a]	$C_{13}H_{16}ClNO_4$								
C_2H_5	C_2H_5	2	94	121–123	$C_{14}H_{18}ClNO_4$	56.09	56.08	6.06	6.04	11.84	11.81	4.67	4.85
C_2H_5	n-C_4H_9	1	93	81–83[b]	$C_{15}H_{20}ClNO_4$								
C_2H_5	n-C_4H_9	2	99.5	92–93	$C_{16}H_{22}ClNO_4$	58.62	58.72	6.76	6.64	10.82	10.86	4.27	3.96
n-C_4H_9	n-C_4H_9	1	93.5	73–74	$C_{17}H_{24}ClNO_4$	59.74	59.51	7.07	7.04	10.38	10.50	4.10	4.28
n-C_4H_9	n-C_4H_9	2	99	91–93	$C_{18}H_{26}ClNO_4$	60.74	60.72	7.36	7.49	9.96	9.98	3.94	3.79
$CH_2CH_2N(C_2H_5)_2$	C_2H_5	1	95.5	150–152	$C_{17}H_{26}Cl_2N_2O_4$[c]	51.91	51.85	6.66	6.65	18.03	18.04	7.12	7.20
$CH_2CH_2N(C_2H_5)_2$	C_2H_5	2	95	133–135	$C_{18}H_{28}Cl_2N_2O_4$[c]	53.07	52.81	6.93	6.70	17.40	17.05	6.88	6.70

[a] Lit.[9] mp 112.8–113.8°. [b] Lit.[9] mp 81.4–83.4°. [c] Hydrochloride.

The intermediate 2-alkoxy-4-aminobenzoates were prepared from the corresponding esters of 4-nitrosalicylic acid by etherification with an alkyl halide in the presence of silver oxide[8] and subsequent reduction of the nitro group with Fe–HCl[10] (see Experimental Section). Attempts to etherify an ester of 4-aminosalicylic acid after blocking the amino group were either unsuccessful or inconvenient.[11] For instance, etherification of an ester of 4-acetaminosalicylic acid is performed in excellent yield, but hydrolysis of the acetamido compound requires drastic conditions which result in simultaneous deacetylation and saponification, giving poor yields of the corresponding 2-alkoxy-4-aminobenzoic acids.[12] On the other hand, etherification of the benzylidene derivative of ethyl 4-aminosalicylate in alkaline medium was easily accomplished with diethyl sulfate (see Experimental Section), but failed when dimethyl sulfate was used. The difference may be due to N-alkylation of the benzylidene derivative: alkylation of Schiff bases by methyl halides occurs readily, whereas the procedure is less satisfactory for the introduction of larger alkyl groups.[13]

2-Diethylaminoethyl 2-ethoxy-4-chloracetylaminobenzoate was obtained as a stable hydrochloride by treating the corresponding aniline with chloroacetyl chloride in acetic acid solution. Reaction of this intermediate with different amines to form the corresponding alkylaminoacetylamino derivatives was successful both in solvents, such as ethanol or benzene, and in the absence of solvent. Neither alcoholysis nor aminolysis of the diethylaminoethyl ester, which occurred with the 4-aminosalicylic acid analog,[3] was observed. This difference between the two series supports the view that these reactions are due to an intramolecular *o*-hydroxy catalysis.[14]

Pharmacology.—Local anesthetic activity and toxicity in mice were determined by methods previously described.[3] In contrast to the 4-aminosalicylic acid derivatives, the compounds of the 2-alkoxy-4-aminobenzoic acid series generally failed to show any significant local anesthetic activity. Of the compounds tested (**1–24** as the hydrochlorides, Tables II and III) only **8**, **13**, and **15** exhibited local anesthetic activity, equivalent to 8.6, 19.4, and 24% of lidocaine, respectively, calculated on a molar basis.

Experimental Section[15]

Alkyl 2-Alkoxy-4-aminobenzoates.—The preparation of ethyl 2-ethoxy- and 2-*n*-butoxy-4-aminobenzoates, by reduction of the corresponding ethyl 2-alkoxy-4-nitrobenzoates with iron powder and concentrated HCl in aqueous ethanol, has been described.[10]

***n*-Butyl 2-*n*-butoxy-4-aminobenzoate** was obtained by this procedure in 92% yield, mp 38–40°; **hydrochloride**, mp 80°.

Anal. Calcd for $C_{15}H_{24}ClNO_3$: C, 59.69; H, 8.02; Cl, 11.75; N, 4.64. Found: C, 59.23; H, 7.85; Cl, 11.50; N, 4.40.

Ethyl 2-ethoxy-4-aminobenzoate was also obtained by etherification of the benzylidene derivative of ethyl 4-aminosalicylate. A mixture of 10 g of ethyl 4-aminosalicylate and 6 g of benzaldehyde (equimolar quantities) was heated on a steam bath for 0.5 hr and then concentrated *in vacuo*. Half of the volume of a solution of 5.1 g of KOH in 8.5 ml of water was added to the viscous residue on a steam bath with stirring. The rest of the solution was added dropwise, simultaneously with 10.7 g of diethyl sulfate. The mixture, after standing overnight at room temperature, was extracted with ether and the ether evaporated. Dilute HCl (25 ml) and 25 ml of water were added to the viscous residue and the mixture was heated on the steam bath for 10 min. After extraction with ether, the aqueous layer was neutralized with 10% NaOH and made alkaline with K_2CO_3. The solid which separated was collected and dried, yielding 3.8 g (33%) of a colorless substance, mp and mmp 116–119° with a sample prepared by reduction (see above) (lit.[10] mp 120.7–121.8°).

Alkyl 2-Alkoxy-4-(ω-chloroacylamino)benzoates.—Chloroacetyl or 3-chloropropionyl chloride (0.22 mole) was added dropwise, simultaneously with a solution of 8 g of sodium acetate in 30 ml of H_2O, to a cooled stirred solution of 0.2 mole of alkyl 2-alkoxy-4-aminobenzoate in about 150 ml of AcOH. Shortly after the addition, a precipitate formed and stirring was continued for 1 additional hr. Yields of crude products and analytical data, after crystallization from ethanol, are given in Table I.

2-Diethylaminoethyl 2-Ethoxy-4-(ω-chloroacylamino)benzoates.—Chloroacetyl or 3-chloropropionyl chloride (0.11 mole) was added dropwise, with stirring, to a solution of diethylaminoethyl 2-ethoxy-4-aminobenzoate[10] (0.1 mole) in a minimum volume of AcOH, cooled in an ice–water bath. Stirring was continued for 1 additional hr after addition was completed and the excess AcOH was evaporated *in vacuo*. The residue was extracted with anhydrous ether and the solid **hydrochloride** of

(10) R. O. Clinton, U. J. Salvador, S. C. Laskowski, and M. Wilson, *J. Am. Chem. Soc.*, **74**, 592 (1952).

(11) W. Hückel and K. Janecka, *Arch. Pharm.*, **284**, 341 (1951), state that the direct synthesis of 4-aminosalicylic acid ethers is not possible. The indirect route involves etherification of the 4-nitrosalicylic acid, followed by reduction of the nitro group.

(12) W. Grimme and H. Schmitz, *Chem. Ber.*, **87**, 179 (1954).

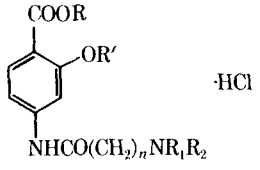
(13) E. H. Woodruff, J. P. Lambooy, and W. E. Burt, *J. Am. Chem. Soc.*, **62**, 922 (1940).

(14) G. Tsatsas, D. Kontonassios, and C. Sandris, *Tetrahedron Letters*, 783 (1966).

(15) Melting points of the intermediates were determined by the capillary tube method, those of the hydrochlorides by means of the Maquenne block. All melting point values are corrected.

TABLE II

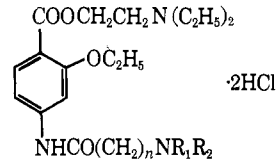
ALKYL 2-ALKOXY-4-(ω-ALKYLAMINOACYLAMINO)BENZOATE HYDROCHLORIDES



No.	R	R′	n	NR_1R_2	Free amine mp, °C[a]	Yield,[b] %	Mp, °C	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Chlorine, % Calcd	Chlorine, % Found	Nitrogen, % Calcd	Nitrogen, % Found
1	C_2H_5	C_2H_5	1	Diethylamino	Oil	53	170 dec[c]	$C_{17}H_{25}ClN_2O_4$	56.90	57.02	7.58	7.74	9.88	9.67	7.80	7.76
2	C_2H_5	C_2H_5	1	Piperidino	Oil	63.5	164 dec	$C_{18}H_{27}ClN_2O_4$	58.30	58.02	7.34	7.40	9.56	9.64	7.55	7.55
3	C_2H_5	C_2H_5	2	Diethylamino	Oil	61.5	125 dec	$C_{18}H_{29}ClN_2O_4$	57.98	57.85	7.84	7.64	9.51	9.43	7.51	7.54
4	C_2H_5	C_2H_5	2	Isopropylamino	Oil	60	198 dec	$C_{17}H_{25}ClN_2O_4$	56.90	56.87	7.58	7.36	9.88	9.77	7.80	7.64
							149	Picrate	50.09	49.93	5.30	5.26			12.70	12.90
5	C_2H_5	C_2H_5	2	Piperidino	58–60	43	183 dec	$C_{19}H_{29}ClN_2O_4$	59.29	58.67	7.59	7.53	9.21	8.72	7.28	6.92
6	C_2H_5	C_2H_5	2	Morpholino	Oil	75.5	215 dec	$C_{18}H_{27}ClN_2O_5$	55.88	55.83	7.03	6.90	9.16	8.95	7.24	7.32
7	C_2H_5	n-C_4H_9	1	Diethylamino	Oil	62	131 dec[d]	$C_{19}H_{31}ClN_2O_4$	58.96	58.96	8.07	8.04	9.19	8.91	7.24	7.32
							129–132	Picrate	51.80	51.96	5.74	5.94			12.09	12.31
8	C_2H_5	n-C_4H_9	1	Piperidino	Oil	63	160 dec	$C_{20}H_{31}ClN_2O_4$	60.22	60.24	7.83	7.88	8.89	8.72	7.02	7.24
9	C_2H_5	n-C_4H_9	2	Diethylamino	Oil	64.5	118	$C_{20}H_{33}ClN_2O_4$	59.91	59.85	8.30	8.42	8.84	8.96	6.99	6.85
							110–112	Picrate	52.61	52.82	5.94	5.84			11.80	11.62
10	C_2H_5	n-C_4H_9	2	Isopropylamino	Oil	63.5	156 dec	$C_{19}H_{31}ClN_2O_4$	58.96	59.06	8.07	8.09	9.19	9.28	7.24	7.35
							149–152	Picrate	51.80	51.72	5.74	5.76			12.09	12.18
11	C_2H_5	n-C_4H_9	2	Piperidino	64–67	82.5	211 dec	$C_{21}H_{33}ClN_2O_4$	61.08	61.19	8.05	8.16	8.59	8.73	6.78	6.85
							130–133	Picrate	53.55	52.93	5.83	5.73			11.56	11.61
12	C_2H_5	n-C_4H_9	2	Morpholino	77–79	85	215 dec	$C_{20}H_{31}ClN_2O_5$	57.89	57.75	7.53	7.55	8.54	8.62	6.75	6.58
							139–142	Picrate	51.39	51.22	5.47	5.51			11.53	11.81
13	n-C_4H_9	n-C_4H_9	1	Diethylamino	Oil	48.5	106	$C_{21}H_{35}ClN_2O_4$	60.79	60.49	8.50	8.45	8.54	8.41	6.75	6.96
							122–125	Picrate	53.37	53.38	6.13	5.95			11.53	11.63
14	n-C_4H_9	n-C_4H_9	1	Piperidino	37–40	79	161 dec	$C_{22}H_{35}ClN_2O_4$	61.89	61.79	8.26	8.37	8.30	8.19	6.56	6.60
							152–155	Picrate	54.28	54.30	6.02	6.15			11.30	11.55
15	n-C_4H_9	n-C_4H_9	2	Diethylamino	Oil	54	81	$C_{22}H_{37}ClN_2O_4$	61.59	61.48	8.69	8.72	8.27	8.35	6.53	6.62
16	n-C_4H_9	n-C_4H_9	2	Isopropylamino	Oil	71.5	158 dec	$C_{21}H_{35}ClN_2O_4$	60.79	60.58	8.50	8.42	8.54	8.22	6.75	6.58
							152–155	Picrate	53.37	53.45	6.13	6.09			11.53	11.66
17	n-C_4H_9	n-C_4H_9	2	Piperidino	50–54	88	191 dec	$C_{23}H_{37}ClN_2O_4$	62.64	62.58	8.46	8.32	8.04	8.14	6.35	6.24
							108–110	Picrate	54.96	54.83	6.20	6.43			11.07	10.97
18	n-C_4H_9	n-C_4H_9	2	Morpholino	73–75	64	202 dec	$C_{22}H_{35}ClN_2O_5$	59.65	59.67	7.96	7.85	8.00	7.89	6.32	6.15
							129–131	Picrate	52.91	52.68	5.86	5.71			11.02	10.99

[a] Melting points of free amines are given for nonpurified products. [b] Yields of the salts are based on the starting chloroacylamino derivatives. [c] Lit.[9] mp 171.7–172.5°. [d] Lit.[9] mp 133–134.4°.

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]

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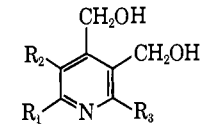
Pyridoxine analogs, modified in the 2, 3, and 6 positions of the pyridine ring, were synthesized and examined as possible substrates for the enzyme pyridoxine dehydrogenase. A modification of an existing procedure[2] was used to synthesize the analogs listed in Table I. In the final step of the synthetic 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

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