Pyridoxal Phosphate. II. Benzene Analogs. 2-Formylphenoxyacetic Acids as Potential Antimetabolites of Pyridoxal Phosphate[1,2]

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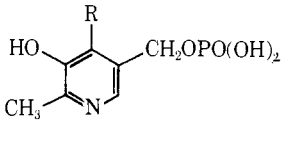
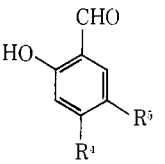
To determine if simple benzene compounds have potential as inhibitory analogs of pyridoxal phosphate (PPal), a series of substituted 2-formylphenoxyacetic acids has been synthesized and evaluated enzymically on tyrosine decarboxylase apoenzyme (TDC). Three halo acids, 4,6-dichloro-, 3,4,6-trichloro-, and 4,6-dibromo-2-formylphenoxyacetic acids, were the most inhibitory analogs, $([I]/[S])_{0.5}$ = 2000–13,000, and were competitive with PPal. The 2-formyl group and the unsubstituted oxyacetic acid function were both required for inhibitory effectiveness. 4-Nitrosalicylaldehyde and other simple salicylaldehydes were ineffective as inhibitors of TDC. It is suggested that the inhibitory compounds may bind to the PPal binding site *or* to the tyrosine binding site of TDC.

Olivard and Snell reported[3] that a pyridoxal phosphate (PPal,[4] **1**) dependent enzyme, alanine racemase from *Streptococcus faecalis*, could be inhibited competitively by 4-nitrosalicylaldehyde (**2a**), presumably because **2a** can adequately simulate Pal.[5] To use this observation as the basis for a general series of simple nonheterocyclic, PPal-specific enzyme inhibitors, some 4- and 5-substituted salicylaldehydes (**2**) were synthesized and evaluated enzymically using tyrosine decarboxylase (TDC) as the test enzyme. However, none of the compounds inhibited TDC.



1, R = CHO
20, R = CH_2OH

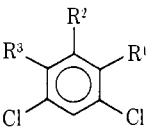


2a, $R^4 = NO_2$; R^5 = H
b, $R^4 = NMe_2$; R^5 = H
c, R^4 = COOMe; R^5 = H
d, R^4 = H; R^5 = COOMe
e, R^4 = H; R^5 = NHAc

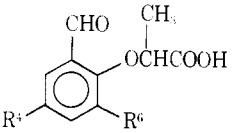
This result suggests that benzene analogs of PPal must possess an anionic side chain which can simulate, in enzyme-binding capacity, the phosphate monoester function of PPal.[6] 2-Formylphenoxyacetic acids are readily constructed benzene derivatives which possess the required anionic side chain and the desired 2-formyl group.[7] This paper describes the synthesis and enzymic evaluation on TDC of a series of substituted 2-formylphenoxyacetic acids (**3c–19**).

Chemistry.—Salicylaldehydes **2** were synthesized by standard reactions. The general synthesis of the substituted 2-formylphenoxyacetic acids is outlined in Scheme I. Alkylation of the sodium salts of the appropriate salicylaldehyde (a) with methyl bromoacetate in DMF smoothly gave the methyl phenoxyacetates (b) which were saponified to furnish the phenoxyacetic acids (c) in high over-all yield. Selected 2-formylphenoxyacetic acids were reduced to the 2-hydroxymethylphenoxyacetic acids (d). Data pertinent to these compounds are collected in Table I.

Two variants of **3c**, namely, 2-formyl-4,6-dichlorophenoxyacetamide (**3e**) and 2,4-dichloro-5-hydroxyphenoxyacetic acid (**3f**), and two branched phenoxypropionic acids, namely, 2-(4,6-dichloro-2-formylphenoxy)-propionic acid (**18**) and the 4,6-dibromo isomer (**19**), were synthesized by analogous methods. Nmr analysis of **18** and **19** showed conclusively that they were the 2-substituted propionic acids rather than the isomeric 3-substituted acids.

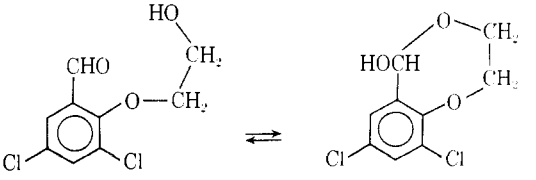


3e, $R^1 = OCH_2CONH_2$; R^2 = CHO; R^3 = H
f, $R^1 = OCH_2COOH$; R^2 = H; R^3 = OH



18, $R^4 = R^6$ = Cl
19, $R^4 = R^6$ = Br

Reaction of 3,5-dichlorosalicylaldehyde (**3a**) with ethylene oxide in dilute aqueous alkali gave a product formulated on the basis of spectroscopic data (see Experimental Section) as the hemiacetal (**3h**) of 2-(2-formyl-4,6-dichlorophenoxy)ethanol (**3g**). In DMSO solution hemiacetal **3h** is in equilibrium with the aldehyde **3g** (K_{eq} = *ca.* 2.5; hemiacetal/aldehyde).



3g ⇌ **3h**

Enzymic Evaluation.—The phenoxyacetic acids were evaluated as simulators of pyridoxal phosphate by determining their ability to inhibit the PPal-dependent enzyme tyrosine decarboxylase.

To establish a basis for comparison, pyridoxol phosphate (**20**), a well-known effective inhibitor of TDC,[6b] was found to have an $([I]/[S])_{0.5}$ = 87, a value in

(1) For paper I see T. L. Hullar, *J. Med. Chem.*, 12, 58 (1969).

(2) This study was supported generously by Grant AM-10234 from the U. S. Public Health Service.

(3) J. Olivard and E. E. Snell, *J. Biol. Chem.*, 213, 203 (1955).

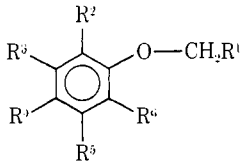
(4) Abbreviations used: AAT, aspartate aminotransferase; DMF, N,N-dimethylformamide; DMSO, dimethyl sulfoxide; PPal, pyridoxal phosphate; PPol, pyridoxol phosphate; TDC, tyrosine decarboxylase; THF, tetrahydrofuran.

(5) M. Ikawa and E. E. Snell, *J. Am. Chem. Soc.*, 76, 653 (1954).

(6) The necessity of the anionic phosphate ester to obtain good inhibitory PPal analogs is known: (a) E. E. Snell, *Vitamins Hormones*, 16, 78 (1958); (b) J. Hurwitz, *J. Biol. Chem.*, 205, 925 (1953); (c) J. Hurwitz, *ibid.*, 217, 513 (1955).

(7) The acidic group ($pK_a \sim 3$–4) is ionized at physiological and enzyme assay conditions, and its charge is about the same distance from the aromatic ring as the anion of the phosphate ester of PPal. The 2-formyl group may be capable of forming a Schiff base with the ε-amino group of the lysyl residue normally bound to PPal.

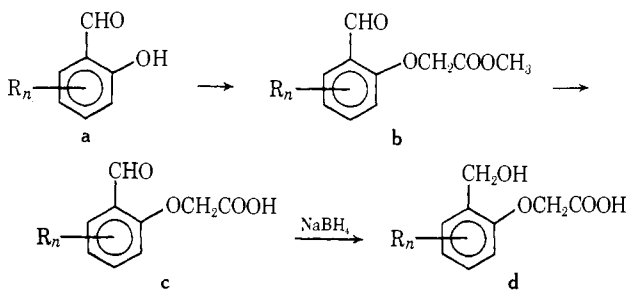
TABLE I
SUBSTITUTED 2-FORMYLPHENOXYACETIC ACIDS AND DERIVATIVES



					Methyl esters (b) R¹ = COOCH₃, R² = CHO[a]				Acids (c) R¹ = COOH, R² = CHO[a]				Alcohols (d) R¹ = COOH, R² = CH₂OH[a]				([I]/[S])₀.₅ for the 2-formyl
No.	R₃	R₄	R₅	R₆	Yield,[b] %	Mp, °C	Formula	Analyses	Yield, %	Mp, °C	Formula	Analyses	Yield, %	Mp, °C	Formula	Analyses	acids[c]
3	H	Cl	H	Cl	94	119–120	$C_{10}H_8Cl_2O_4$	C, H, Cl	84	168–169	$C_9H_6Cl_2O_4$	C, H, Cl	94	139–141[d]	$C_9H_6Cl_2O_4$	C, H, Cl	1,900 ± 350[e]
4	Cl	Cl	H	Cl	83	80–81	$C_{10}H_7Cl_3O_4$	C, H, Cl	97	147–148	$C_9H_5Cl_3O_4$	C, H, Cl					3,000 ± 440
5	H	H	H	Cl	87	76–77	$C_{10}H_9ClO_4$	C, H, Cl	94	138–139	$C_9H_7ClO_4$	C, H, Cl					38,000 ± 6300
6	H	Cl	H	H	96	69–70	$C_{10}H_9ClO_4$	C, H, Cl	90	173–174	$C_9H_7ClO_4$	C, H, Cl	95	140–143	$C_9H_9ClO_4$	C, H, Cl	[f]
7	H	Br	H	Br	93	129–130	$C_{10}H_8Br_2O_4$	C, H, Br	99	176–177	$C_9H_6Br_2O_4$	C, H, Br					13,000 ± 2400
8	H	H	H	Br	62	63–65	$C_{10}H_9BrO_4$	C, H, Br	74	148–149	$C_9H_7BrO_4$	C, H					48,000 ± 2600
9	H	Br	H	H	95	60–61	$C_{10}H_9BrO_4$	C, H, Br	96	174–176	$C_9H_7BrO_4$	C, H, Br					[f]
10	H	NO_2	H	H	72[g]	78–79	$C_{10}H_9NO_6$[h]		94[i]	190–192[j]			82	141–142[k]	$C_9H_9NO_5$	C, H, N	[f]
11	H	H	NO_2	H	87	102–104	$C_{10}H_9NO_6$	C, H, N	92	199–201	$C_9H_7NO_6$						[f]
12	H	CH_3	H	CH_3	77	30–31	$C_{12}H_{14}O_4$	C, H	97	126–128	$C_{11}H_{12}O_4$						[f]
13	H	H	H	CH_3	[l]				70[m]	90–91	$C_{10}H_{10}O_4$						[f]
14	H	CH_3	H	H	86	52–53	$C_{11}H_{12}O_4$	C, H	94	150–151[n]	$C_{10}H_{10}O_4$						[f]
15	H	OCH_3	H	H	84	73–74	$C_{11}H_{12}O_5$	C, H	87	157–159	$C_{10}H_{10}O_5$						[f]
16	H	H	H	H	80	55–56[o]			86	131–133[p]			71	119–121[k,q]			[f]
17	H	3,4-Benzo[r]			90	91–93[s]			89	176–177[s]							[f]

[a] See Scheme I. [b] Yields refer to initially isolated material corrected for the maximum possible mineral oil content. [c] For pyridoxol phosphate: $([I]/[S])_{0.5} = 87 \pm 15$. [d] Recrystallized from EtOH–H_2O. [e] Standard deviation. [f] 10–20% inhibition at [I]/[S] = 18,000. [g] Prepared by esterification, not alkylation (see Experimental Section). [h] Not analyzed. [i] Prepared by the method of W. Jacobs and M. Heidelberger, *J. Am. Chem. Soc.*, **39**, 2188 (1917). [j] Lit.[i] mp 190–192°. [k] Recrystallized from H_2O. [l] Ester not isolated but hydrolyzed directly. [m] Over-all yield from 3-methylsalicylaldehyde. [n] K. von Auwers [*Ann.*, **393**, 365 (1912)] reported mp 151°. [o] H. Cajar [*Ber.*, **31**, 2809 (1898)] reported mp 55–56°. [p] L. Katz, L. Karger, W. Schroeder, and M. Cohen [*J. Org. Chem.*, **18**, 1380 (1953)] reported mp 129°. [q] P. Biginelli [*Gazz. Chim. Ital.*, **21**, 257 (1891)] reported mp 120°. [r] From 2-hydroxy-1-naphthaldehyde and ethyl bromoacetate using the general procedure. [s] P. Emmott and R. Livingstone [*J. Chem. Soc.*, 3144 (1957)] reported mp 91–92° for the ethyl ester, and mp 176–177° for the acid.

SCHEME I



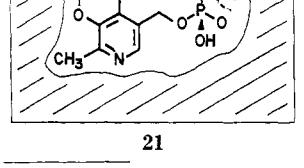
reasonable agreement with that obtained previously under different assay conditions.[6b] The inhibitory activity of the 2-formylphenoxyacetic acids is listed in Table I; acids **3c**, **4c**, **5c**, **7c**, and **8c** were competitive inhibitors, with respect to pyridoxal phosphate, as judged by the plotting method used (see Experimental Section).

The 2-formyl and the acetic acid functions must be present together to confer inhibition, for the 2-hydroxymethyl acid (**3d**), the 2-formyl amide (**3e**), the 2-formyl alcohol (**3g**, **h**), and the 3-hydroxy acid (**3f**) had only minimal inhibition on TDC (less than 10–15% at [I]/[S] = 18,000).

The branched phenoxypropionic acids **18** and **19** gave only 10–20% inhibition at [I]/[S] = 10,000. Thus, short α-alkyl substituents most likely cannot be used for either hydrophobic bonding or carriers for alkylating functions.[8]

The observed trend of inhibitory capacity is clearly that those acids with halogen substituents are better inhibitors than the corresponding methyl or methoxyl analogs (compare **3c**, **4c**, and **7c** to **12c**). The chloro- phenoxyacetic acids are better inhibitors than the bromo compounds (**3c** *vs.* **7c**, **5c** *vs.* **8c**). Electronically, the chloro and bromo groups are likely to exert similar effects on the *m*-CHO function[9] and hence on any Schiff bases which may be formed.[10] Sterically, the chloro group is somewhat smaller[11] than the bromo group and hence may be of a better size to fit the enzyme surface.

Because the inhibitory phenoxyacetic acids must possess a formyl group and the acidic side chain, it is possible that the CHO may form a Schiff base with the ε-amino group of the lysyl residue likely bound normally to PPal[12] and that the CH_2COOH may be interacting with the cationic region of the enzyme which normally binds the phosphate monoester of PPal (*cf.* **21** and **22**).



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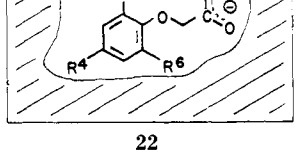

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(8) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley and Sons, Inc., New York, N. Y., 1967.

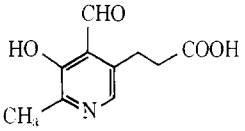
(9) The σ_m for Cl and Br are +0.37 and +0.39, respectively [H. H. Jaffe, *Chem. Rev.*, **53**, 191 (1953)].

(10) The rate of Schiff base formation may or may not show a marked dependency on the nature of the substituents. At neutral pH $\rho = 0.07$ for Schiff base formation showing that the reaction rate has little dependency on the σ constant of the substituents. In acid media, however, $\rho = +0.91$ showing that electron-withdrawing substituents markedly accelerate Schiff base formation [B. M. Anderson and W. P. Jencks, *J. Am. Chem. Soc.*, **82**, 1773 (1960)]. For TDC it is likely that the milieu is at least slightly acidic, suggesting that electron-withdrawing substituents such as halogen may promote more rapid Schiff base formation.

(11) The van der Waal's radii are 1.80 (Cl) and 1.95 Å (Br) [W. L. Koltun, *Biopolymers*, **3**, 665 (1965)].

(12) For a general survey of the evidence for Schiff bases formed between coenzymically active PPal and the ε-amino group of a lysyl residue, see I. Fasella, *Ann. Rev. Biochem.*, **36**, 185 (1967).

If **22** is indeed the case, the carboxylic acid analog (**23**) of PPal should inhibit more strongly than **3c** because the additional common structural features would be expected to increase binding. To test this hypothesis **23** was evaluated on TDC and found to possess only *minimal* inhibitory power, $([I]/[S])_{0.5} > 100{,}000$.[13] This shows that a carboxylic acid *closely*



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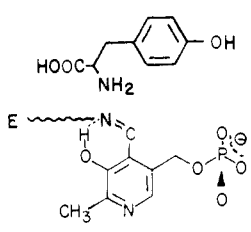
related to PPal cannot efficiently occupy the PPal binding site (**21**) and suggests, therefore, that a phenoxyacetic acid *cannot* directly occupy the PPal site (*e.g.*, **22**). In addition, preliminary studies show that the phenoxyacetic acids *do not* inhibit aspartate aminotransferase, whereas **23** does inhibit, $([I]/[S])_{0.5} = 12$.[13] This supports the view that the phenoxyacetic acids *may not* bind to the PPal site of TDC.

An alternative mode of inhibition is that the inhibitory phenoxyacetic acids are somewhat similar in general structural features to L-tyrosine and may be in competition with it as well as with PPal. Such L-tyrosine analogs could then possibly bind by adsorption to the L-tyrosine site (**24**) and undergo Schiff base formation (*e.g.*, **25**) with the ε-amino group of the lysyl residue normally bonded to the formyl group of PPal.[14]



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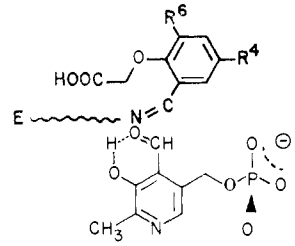

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Experimental Section

Melting points were taken on a Fisher-Johns or Mel-Temp apparatus and are corrected. Nmr spectra were recorded on a Varian A-60 instrument using the solvents indicated with TMS as internal reference. Ir spectra were recorded on a Perkin-Elmer 237 spectrophotometer and were as expected. All solutions were concentrated at reduced pressure at room temperature unless otherwise stated.[15]

4-Nitrosalicylaldehyde (2a) was synthesized more efficiently by the method[16] employing bromination of 2-methyl-5-nitrophenol followed by subjection of the pyridinium salt to the Kröhnke reaction[17] than by the H_2CrO_4 oxidation of 2-methyl-5-nitrophenol.[18,19]

4-Dimethylaminosalicylaldehyde (2b). A. 2-Benzoyloxy-4-N,N-dimethylaminobenzaldehyde.—Vilsmeier formylation of 3-benzoyloxy-N,N-dimethylaniline[20] under established conditions,[21] using ($ClCH_2$)$_2$ (10 ml/0.01 mole of amine) as cosolvent, gave the aldehyde in 90% yield, mp 113–114° ($CHCl_3$–isohexane). *Anal.* ($C_6H_{15}NO_3$) C, H, N.

B.—Alkaline hydrolysis of the aldehyde ester gave the salicylaldehyde **2b** in 94% yield, mp 79–80° (lit.[22] mp 79–80°).

C.—Attempted reduction by LAH of ethyl 4-aminosalicylate or 4-aminosalicylic acid gave only 3-aminophenol, the product of decarboxylation, in 57 and 30% yields, respectively. The product was identified conclusively by its melting point (116–118°), ir spectrum, and N-acetyl derivative, identical in melting point (140–143°) and ir spectrum with authentic 3-acetamidophenol.

4-Carbomethoxysalicylaldehyde (2c). A.—3-Hydroxybenzoic acid was treated with $CHCl_3$ in alkali under Reimer–Tiemann conditions to give, after isolation through the bisulfite addition product, 4-carboxysalicylaldehyde, mp 234° (lit.[23] mp 234°), in *ca.* 5% yield.

B.—A solution of 1.68 g of 4-carboxysalicylaldehyde in 50 ml of 10% MeOH–HCl was refluxed 14 hr. The mixture was cooled and then neutralized with solid $NaHCO_3$. After addition of H_2O and concentration the mixture was extracted ($CHCl_3$). The dried extract was concentrated and 20 ml of 50% HOAc was added to the residue. After heating on a steam bath for 1 hr 50 ml of H_2O was added to precipitate 1.55 g of **2c** (95%) as white needles, mp 131–133°. Vacuum sublimation of the needles gave pure **2c**, mp 134–135° in a sealed tube (Mel-Temp block). *Anal.* ($C_9H_8O_4$) C, H.

5-Carbomethoxysalicylaldehyde (2d). A.—5-Carboxysalicylaldehyde, mp 237–241° (lit.[21] mp 243–244°), was prepared in 20% yield from 4-carboxyphenol using the Reimer–Tiemann reaction and isolated by means of the bisulfite addition product.

B.—The 5-carbomethoxysalicylaldehyde (**2d**) was prepared as for the 4 isomer in 71% yield and was recrystallized from MeOH–H_2O to give white crystals, mp 80–81°. *Anal.* ($C_9H_8O_4$) C, H.

5-Acetamidosalicylaldehyde (2e). A. 5-Nitro-2-acetoxybenzal Diacetate.—A solution of 0.50 g of 5-nitrosalicylaldehyde in 50 ml of Ac_2O containing one drop of H_2SO_4 was kept 0.5 hr at room temperature and then poured into ice water. The precipitated product was filtered, dried, and recrystallized from EtOAc–petroleum ether to give 0.85 g (91%) of pure diacetate, mp 113–114°. *Anal.* ($C_{13}H_{12}NO_8$) C, H, N.

B. 5-Acetamido-2-acetoxybenzal Diacetate.—A solution of 3.11 g of 5-nitro-2-acetoxybenzal diacetate in 25 ml of THF was hydrogenated (Pt) at 3.16 kg/cm^2 for 0.5 hr. To the filtrate was added 2 ml of Ac_2O and one drop of H_2SO_4. Concentration gave a syrup which was poured into 75 ml of ice water. Recrystallization of the precipitated solid from C_6H_6–petroleum ether gave 2.25 g (70%) of pure product, mp 118–120°. *Anal.* ($C_{15}H_{15}NO_7$) N.

C.—The acetamido diacetate was hydrolyzed in alkali to give, after neutralization, a precipitate which was recrystallized from C_6H_6–petroleum ether to give salicylaldehyde **2e** in 89% yield, mp 154–155°. *Anal.* ($C_9H_9NO_3$) C, H, N.

3,5,6-Trichlorosalicylaldehyde (4a).—An attempt to prepare this compound using a literature method[25] for the preparation of 3,5-dichlorosalicylaldehyde failed due to the production of a bricklike precipitate which prevented further stirring during the AcOH addition. A slight modification, using diglyme as cosolvent, however, made this procedure usable. A three-neck flask was heated in an oil bath at 110°. 2,4,5-Trichlorophenol (13.16 g), hexamethylenetetramine (5 g), paraformaldehyde (5 g), and freshly distilled diglyme (10 ml) were then added and well mixed. Glacial HOAc (20 g) was added to this mixture over 1 hr at a bath temperature of 110°. The bath temperature was raised to 130° over 0.5 hr and then $H_2SO_4 \cdot H_2O$ (9.87 g) was added over 2 hr. The reaction mixture was heated an additional 0.5 hr at 130° and then poured onto 200 ml of H_2O. Steam distillation of this mixture afforded 2.49 g of crude product (mp 97–113°) in a distillate volume of about 2 l. This crude material was recrystallized twice from hot ligroin (high-boiling) to give 2.05 g (14%) of yellow needles, mp 113–116°. An analytical sample prepared by vacuum sublimation had mp 114–116°, ν_{max}^{KBr} 1660 cm^{-1} (aldehyde C=O). *Anal.* ($C_7H_3Cl_3O_2$) C, H, Cl.

(13) T. L. Hullar, to be published.

(14) Studies to demonstrate competition of **3c** with L-tyrosine were inconclusive.

(15) Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(16) A. A. Goldberg and H. A. Walker, *J. Chem. Soc.*, 2540 (1954).

(17) F. Kröhnke and H. Schmeiss, *Ber.*, **72**, 440 (1939).

(18) E. M. Bavin, R. J. W. Rees, J. M. Robson, M. Seiler, D. E. Seymours, and D. Suddaby, *J. Pharm. Pharmacol.*, **2**, 764 (1950).

(19) See also T. Nishimura, "Organic Syntheses," Coll. Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1963, p 713.

(20) F. Myenberg, *Ber.*, **29**, 501 (1896).

(21) E. Campaigne and W. L. Archer, ref 19, p 331.

(22) Joh. Rud. Geigy and Co., German Patent 105,103 (1898); cited by F. K. Beilstein's, "Handbuch der organischen Chemie," Vol. 14, 1931, p 234, and in *Chem. Zentr.*, 71 (1), 238 (1900).

(23) F. Tiemann and L. Landshoff, *Ber.*, **12**, 1334 (1879).

(24) K. Reimer and F. Tiemann, *ibid.*, **9**, 1268 (1876).

(25) Farbenfabriken Bayer-A.G., British Patent 794,885 (1958); *Chem. Abstr.*, **53**, 320e (1959).

Other Salicylaldehydes.—3-Chlorosalicylaldehyde[26] (**5a**), 3-bromosalicylaldehyde[27] (**8a**, prepared using the standard Reimer–Tiemann method[28]), 3,5-dimethylsalicylaldehyde[25] (**12a**), 3-methylsalicylaldehyde[29] (**13a**), and 5-methoxysalicylaldehyde[30] (**15a**) were prepared by the respective literature methods. The remaining salicylaldehydes were obtained from commercial sources.

Methyl-Substituted 2-Formylphenoxyacetates (3b–17b). A. General Procedure.—To a solution of 50 mmol of the starting salicylaldehyde in 50 ml of DMF contained in a water bath of ambient temperature was slowly added 2.25 g of approximately 53% NaH in mineral oil. The mixture was then well stirred until H_2 evolution ceased (generally 15–30 min was required), and 55 mmol of methyl bromoacetate (ethyl bromoacetate for **17b**) was added slowly. After stirring overnight at room temperature under a KOH drying tube, the mixture was poured slowly, with stirring, into about 400 ml of ice water. The precipitated product was collected by filtration, washed (H_2O), air dried, and then washed with petroleum ether (30–60°) to give material pure enough for further transformation. Analytical samples were prepared by vacuum sublimation. Data pertaining to the individual compounds prepared by this method are given in Table I.

B. **Methyl 2-Formyl-4-nitrophenoxyacetate (10b).**—A solution of 0.450 g (2 mmol) of 2-formyl-4-nitrophenoxyacetic acid[31] in 10 ml of MeOH containing 5 capillary drops of $MeSO_3H$ was refluxed 7 hr. After concentration of the solution the residue was partitioned between C_6H_6 and H_2O. The C_6H_6 layer was washed (H_2O), dried ($MgSO_4$), and concentrated to give 0.520 g of a white solid, mp 53–56°. The single C=O band (at 1750 cm^{-1}) suggested that the aldehyde function had been converted to a dimethyl acetal.[32] A portion of this material (0.270 g) in 2.5 ml of 90% HCOOH was kept at room temperature for 0.5 hr. The solution was then poured into 40 ml of ice water, stirred 15 min, and then filtered to give 0.180 g (72% yield) of white solid, mp 78–79°.

To a stirred solution of 1.67 g (0.01 mol) of 5-nitrosalicylaldehyde in 10 ml of DMSO was added 0.594 g (0.11 mol) of NaOMe followed by 1.68 g (0.011 mol) of methyl bromoacetate. After stirring for 15 min at room temperature, the mixture was heated on a steam bath for 2 hr. The cooled mixture was partitioned between C_6H_6 and H_2O. The C_6H_6 layer was washed with H_2O, dried ($MgSO_4$), and then concentrated to give 0.900 g (38%) of **10b**, mp 76–78°, ir spectrum identical with that of **10b** prepared above.

Substituted 2-Formylphenoxyacetic Acids (3c–17c). A. General Method.—A mixture of 25 mmol of the ester, 20 ml of MeOH, and 30 ml of 1 *N* KOH was stirred at room temperature for 4–6 hr. The solution was then concentrated and, when necessary, H_2O was added to the residue to dissolve any precipitated solid. After washing with 50 ml of Et_2O the basic solution was acidified with 2 *N* HCl. After refrigeration overnight the precipitated product was filtered and air dried. Samples for analysis and enzyme assay were prepared by recrystallization from aqueous EtOH. Data pertinent to these products are given in Table I.

B.—Alkylation of salicylaldehyde (**16a**) with chloroacetic acid in aqueous NaOH as described[33] gave, in our hands, a product difficult to purify. In consequence **16a** was alkylated with methyl bromoacetate in MeOH–MeONa and then saponified to give **16c**.

Substituted 2-Hydroxymethylphenoxyacetic Acids (3d, 6d, 10d, and 16d). General Method.—To a solution of 0.01 mol of the aldehyde in 15 ml of 1 *N* NaOH was added 0.200 g of $NaBH_4$ in small portions. After the solution was stirred at room temperature for about 1 hr, the product was precipitated by cautious addition of 2 *N* HCl and then collected on a filter. Pertinent data for the individual compounds are listed in Table I.

2-Formyl-4,6-dichlorophenoxyacetamide (3e).—Crude **3e** (mp 138–142°), prepared in 77% yield by the general method described for the synthesis of the methyl 2-formylphenoxyacetates, using iodoacetamide as the alkylating agent, was recrystallized twice from aqueous EtOH to give pure **3e**: mp 140–143°. *Anal.* ($C_9H_7Cl_2N_2O_3$) C, H, Cl, N.

2,4-Dichloro-5-hydroxyphenoxyacetic Acid (3f).—A solution of 3.58 g (20 mmol) of 4,6-dichlororesorcinol in 15 ml of DMF was maintained in a room-temperature water bath while 0.90 g of 53% NaH in mineral oil was slowly added. The mixture was stirred until gas evolution had ceased and then 3.37 g (22 mmol) of methyl bromoacetate was added. After being stirred overnight, the mixture was diluted with 100 ml of H_2O and extracted with 75 ml of $CHCl_3$. The $CHCl_3$ layer was washed (50 ml of 1 *N* KOH, 25 ml of H_2O). The combined aqueous washings were allowed to stand at room temperature for 2 hr and then acidified with concentrated HCl. Filtration gave 1.42 g (30%) of tan product, mp 167–171°. Decolorization and recrystallization from hot H_2O gave white needles: mp 172–173° (lit.[34] mp 175.5°); ν_{max}^{KBr} 3600–2400, 1710, and 1660 cm^{-1}.

2-(2-Formyl-4,6-dichlorophenoxy)ethanol (3g) and Its Hemiacetal (3h).—A mixture of 3.82 g (20 mmol) of 3,5-dichlorosalicylaldehyde, 15 ml of ethylene oxide, 5 ml of H_2O, and 0.1 ml of 1 *N* KOH was heated in a stainless steel bomb at 70° for 48 hr. The bomb was cooled and the contents was poured into 70 ml of ice water containing 20 ml of 1 *N* KOH.[35] The precipitated product was filtered off, washed well with H_2O, and air dried to give 3.45 g (74%) of white needles, mp 127–129°. *Anal.* ($C_7H_8Cl_2O_3$) C, H, Cl.

This crystalline product is formulated as the 1,3-dioxepane **3h** rather than the acylic aldehyde **3g** on the basis of ir spectral studies. In KBr pellet, the product showed *no* C=O absorption. In THF, however, the product showed absorption at 1695 cm^{-1} (CHO). Both solid and solution spectra showed OH stretching absorption at *ca.* 3360 and *ca.* 3300 cm^{-1}, respectively. These data indicate that this material exists exclusively as the seven-membered ring hemiacetal (**3h**) in the solid state, whereas a mixture of free aldehyde and hemiacetal exists in solution.

Verification of this conclusion was supplied by nmr studies of the material in DMSO-d_6 solution at room temperature and at 60°. Nmr absorption at room temperature was δ 5.90, 5.96 (HOC*H*O in different magnetic environments due to ring inversion), 7.54–8.14 (multiplet, 2 H, aromatic), and 10.57 (s, OC*H*). At 60° the δ 3.5 peak became very diffuse and the 5.96 and 5.90 peaks collapsed, due to time averaging, to a singlet at 5.93. As required, the combined integrals of aldehyde and methine protons gave an integral ratio of 1. The ratio of aldehyde and methine protons was approximately the same (about 0.4:1.0) at room temperature and at 60°, thus indicating the aldehyde $\rightleftarrows$ hemiacetal equilibrium to be about 2.5 at room temperature and 60°.

2-Formylphenoxyacetamide (16e).—The procedure used for synthesis of the methyl phenoxyacetates was employed using iodoacetamide as the alkylating agent to give **16e** in 47% yield, mp 129–130°. *Anal.* ($C_9H_9NO_3$) C, H, N.

2-(4,6-Dichloro-2-formylphenoxy)propionic Acid (18).—The general procedure described above for the preparation of methyl phenoxyacetates but employing ethyl α-bromopropionate was used to prepare ethyl 2-(4,6-dichloro-2-formylphenoxy)propionate in 82% yield, mp 60–61°. *Anal.* ($C_{12}H_{12}Cl_2O_4$) C, H, Cl. This ester was saponified using the general procedure described above for synthesis of the phenoxyacetic acids to give a crude product which was readily recrystallized from EtOAc–petroleum ether to give acid **18** in 74% yield; mp 134–135°; nmr absorption (in CD_3COCD_3) at δ 1.76 (d, 3 H, J = 7 Hz, $CH_3CHCOOH$), 5.17 (q, 2 H, J = 7 Hz, $CH_3CHCOOH$), 7.75, 7.77 (d, 2 H, aromatic *H*), and 10.48, 10.66 (2 s, 2 H, COO*H* and C*H*O). *Anal.* ($C_{10}H_8Cl_2O_4$) C, H, Cl.

2-(4,6-Dibromo-2-formylphenoxy)propionic Acid (19).—The corresponding ethyl ester, **ethyl 2-(4,6-dibromo-2-formylphen-**

(26) W. Davies and L. Rubenstein, *J. Chem. Soc.*, **123**, 2850 (1923).

(27) J. Müller, *Ber.*, **42**, 3695 (1909).

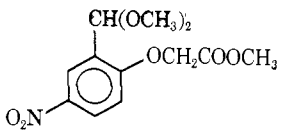
(28) R. J. Smith and W. H. Read, *Ann. Appl. Biol.*, **49**, 233 (1961).

(29) F. Tiemann and C. Schotten, *Ber.*, **11**, 767 (1878).

(30) L. Rubenstein, *J. Chem. Soc.*, **127**, 1998 (1925).

(31) W. Jacobs and M. Heidelberger, *J. Am. Chem. Soc.*, **39**, 2188 (1917).

(32) Sublimation of this product gave a compound mp 67–68°, whose elemental analysis (C, 51.68; H, 5.30; N, 5.37) was reasonably in accord with the calculated values (C, 50.52; H, 5.30; N, 4.91) expected for the dimethyl acetal (i).



i

(33) L. Katz, L. Karger, W. Schroeder, and M. Cohen, *J. Org. Chem.*, **18**, 1380 (1953).

(34) J. Moszew and J. Wojiechowski, *Roczniki Chem.*, **28**, 445 (1954); *Chem. Abstr.*, **49**, 13148*d* (1955).

(35) Reaction appeared incomplete as suggested by a yellow phenoxide color and residual ethylene oxide. A longer heating period may therefore give a higher yield.

oxy)propionate, was prepared as above for **18** in 79% yield, mp 96–97°. *Anal.* ($C_{12}H_{12}Br_2O_4$) C, H, Br.

The acid **19** was prepared as above for **18** in 74% yield; mp 161–162°; nmr absorption (in CD_3COCD_3) at δ 1.77 (d, 3 H, J = 7 Hz, $CH_3CHCOOH$), 5.22 (q, 2 H, J = 7 Hz, CH_3CHCO-OH), 7.97 (d, 1 H, J = 1 Hz, an aromatic *H*), 8.13 (d, 1 H, J = 1 Hz, an aromatic *H*), and 10.66 (s, 1 H, *CHO*). *Anal.* ($C_{10}H_8Br_2O_4$) C, H, Br.

Enzymic Evaluation.—The methods used for enzymic evaluation of the compounds on commercially available TDC apoenzyme (*Streptococcus faecalis*) have been described.[1] The inhibitors were ineffective unless incubated 15–45 min with the enzyme. Incubation of TDC with **3c** for 15–30 min prior to addition of PPal showed only a slightly significant increase in inhibition. Using simultaneous incubation of PPal and inhibitor with TDC, maximum effectiveness of inhibitors resulted from 15–30 min of incubation, and a 30-min incubation was therefore routinely used.

Acknowledgment.—Mrs. B. Ritter and Mrs. K. Morris conducted the enzymic assays and Miss P. Lando synthesized compounds **8b**, **8c**, **18**, and **19**. Their assistance and the financial support tendered to P. L. by the National Science Foundation Summer Research Participation Program, administered by Roswell Park Memorial Institute, Buffalo, N. Y., is gratefully acknowledged.

Pyridoxal Phosphate. III. Pyrimidine Analogs. 3-(Substituted 5-Pyrimidyl)propionic Acids as Potential Inhibitory Analogs of Pyridoxal Phosphate

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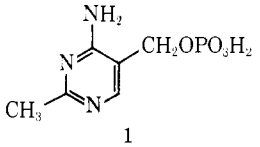
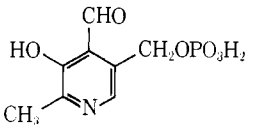
Several substituted 3-(4-amino- and 4-hydroxy-5-pyrimidyl)propionic acids (**3a–d**, **4a,c**, and **5a,c**) have been synthesized as analogs of toxopyrimidine phosphate and evaluated as inhibitors of pyridoxal phosphate mediated enzymic decarboxylation and transamination. None of the compounds showed significant inhibitory capacity. The amino acids (**4a,c**) were synthesized through the 2-methyl- and 2-phenyl-4-methyl-7-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidines (**10a,c**). The chloro amide 3-(4-chloro-6-methyl-2-phenyl-5-pyrimidyl)propionamide (**11c**) gave the hydroxy nitrile 3-(4-hydroxy-6-methyl-2-phenyl-5-pyrimidyl)propionitrile (**12c**) when heated *in vacuo*.

Toxopyrimidine phosphate[2] (**1**, TXP) and analogs[3] are potent inhibitors of pyridoxal phosphate (**2**, PPal) dependent enzymes. The previous paper[1a] in this series reported that certain substituted 2-formylphenoxyacetic acids are inhibitory toward the PPal-dependent enzyme tyrosine decarboxylase. In an effort to combine the apparent binding contributions of the pyrimidyl moiety of **1** and the carboxylic acid function of the phenoxyacetic acids, a series of 3-(substituted 5-pyrimidyl)propionic acids (**3–5**) have been synthesized and enzymically evaluated as inhibitory analogs of PPal.



1



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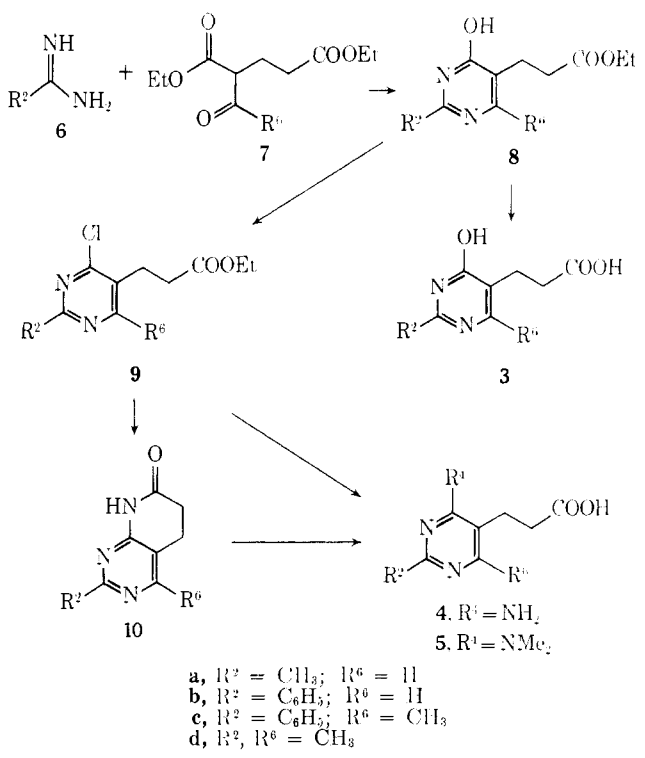
Chemistry.—The reaction sequences used for synthesis of **3–5** are shown in Scheme I and follow generally those used previously[4] to synthesize **4a** as a partial analog of thiamine. Methyl and phenyl groups were chosen as the R^2 and R^6 substituents because they would provide convenient loci for the placement of hydrophobic bonding and potential alkylating functions.[5]

SCHEME I



Amino and dimethylamino groups were selected as the 4-substituents because of their efficacy in the toxopyrimidine series.[3]

The action of concentrated NH_4OH on the chloro ester **9a**[4] failed to give amino acid directly; a mixture

(1) (a) For paper II, see T. L. Hullar and D. L. Failla, *J. Med. Chem.*, 12, 420 (1969). (b) This research was generously supported by Grant AM-10234 from the National Institutes of Health, U. S. Public Health Service.

(2) (a) K. Makino and M. Koike, *Enzymologia*, 17, 157 (1954); (b) see, however, B. G. Haughton and H. K. King, *Biochem. J.*, 70, 660 (1958).

(3) E. Seidler and A. Schellenberger, *Z. Physiol. Chem.*, **346**, 148 (1966).

(4) J. Biggs and P. Sykes, *J. Chem. Soc.*, 1849 (1959).

(5) For a full discussion of the strategy in locating positions for such groupings see B. R. Baker, "Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley and Sons, Inc., New York, N. Y., 1967.