**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

T. L. HULLAR AND W. C. FRENCH

*Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14214*

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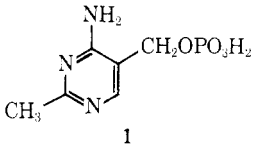
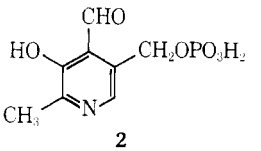
Several substituted 3-(4-amino- and 4-hydroxy-5-pyrimidyl)propionic acids (**3a–d**, **4a,c**, and **5a,c**) have been synthesized as analogs of toxopyrimidine phosphate and evaluated as inhibitors of pyridoxal phosphate mediated enzymic decarboxylation and transamination. None of the compounds showed 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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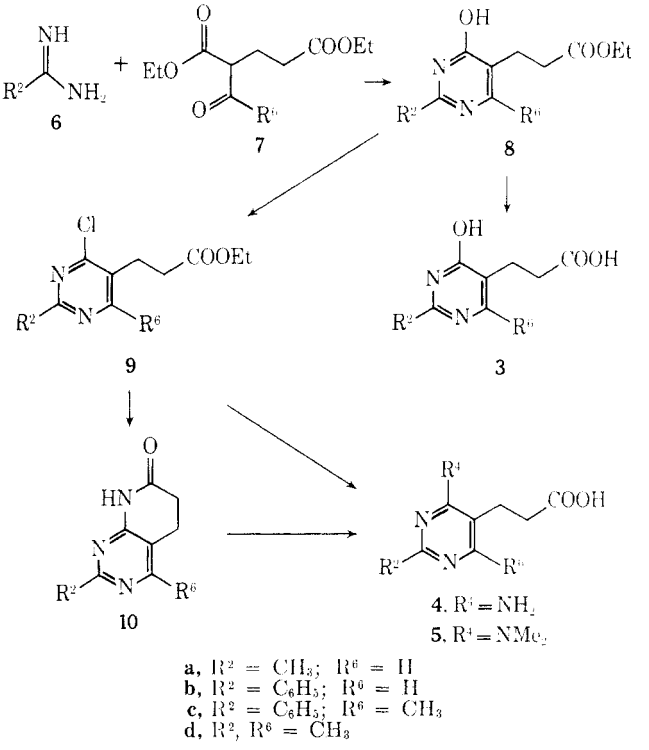
**Chemistry.**—The reaction sequences used for synthesis of **3–5** are shown in Scheme I and follow generally those used previously[4] to synthesize **4a** as a partial analog of thiamine. Methyl and phenyl groups were chosen as the $R^2$ and $R^6$ substituents because they would provide convenient loci for the placement of hydrophobic bonding and potential alkylating functions.[5]

SCHEME I



**a**, $R^2$ = $CH_3$; $R^6$ = H
**b**, $R^2$ = $C_6H_5$; $R^6$ = H
**c**, $R^2$ = $C_6H_5$; $R^6$ = $CH_3$
**d**, $R^2$, $R^6$ = $CH_3$

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).

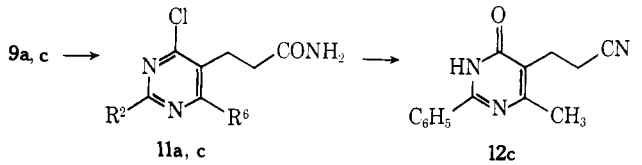
(4) J. Biggs and P. Sykes, *J. Chem. Soc.*, 1849 (1959).

(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.

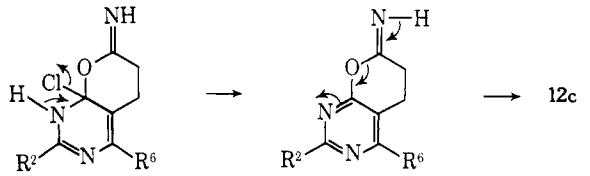
of at least five compounds resulted from which only a small amount of chloro amide (**11a**) could be isolated. In a convenient, alternative route the chloro esters **9** were subjected to ethanolic $NH_3$ at 110–150° to give smoothly the lactams **10**; alkaline hydrolysis of **10a,c** furnished the desired acids **4a,c**. Treatment of **9a,c** with alcoholic $Me_2NH$ gave, after alkaline hydrolysis, the dimethylamino acids **5a,c**.

In an effort to obtain a 4-amino amide, **9a** was treated with liquid $NH_3$ at room temperature. No amino amide was isolable, but there was obtained lactam **10a** in 20% yield, the chloro amide **11a** in 16% yield, and starting material. Treatment of **9c** with $NH_4OH$–dioxane at room temperature gave the chloro amide **11c** in 84% yield.

Attempted sublimation of **11c** gave in quantitative yield the hydroxy nitrile **12c**, identical with **12c** formed




by independent synthesis from condensation of benzamidine with 2-(2-cyanoethyl)acetoacetate.[6] This facile reaction most likely occurs by nucleophilic attack of the amide oxygen on the 4 position to displace the 4-chloro group followed by decomposition of the intermediate imino ester.




**Enzymic Evaluation.**—The hydroxy (**3**) and the amino (**4a,c, 5a,c**) acids were tested[7] as inhibitors of the recombination of PPal with the apoenzyme of tyrosine decarboxylase (TDC) and aspartate aminotransferase (AAT). Only minimal inhibitory activity (10–15% inhibition at [I]/[S] = 100 (for AAT) and 10,000 (for TDC)) was found. These compounds are therefore not sufficiently analogous either to TXP (**1**) or PPal (**2**) to bind satisfactorily to the PPal site of TDC and AAT and thereby prevent recombination of PPal with apoenzyme.

## Experimental Section[8]

**Ethyl 3-[4-Hydroxy(2-methyl-, -2-phenyl-, -2-phenyl-6-methyl-, and -2,6-dimethyl)-5-pyrimidyl]propionates (8). A.**—A mixture of equimolar amounts of acetamidine hydrochloride, NaOEt, and diethyl 2-formyl-[4] (or 2-acetyl-) glutarate (**7**, $R^6$ = H, $CH_3$) in EtOH (*ca.* 500 ml/0.1 mol of **7**) was refluxed 20 hr, concentrated to 40 ml, and filtered. Addition of HBr gas or HCl gas gave crude **8a**·HBr, mp 206–210° (EtOH), in 49% yield and crude **8d**·HCl in 23% yield. The crude salts were dissolved in $H_2O$ (*ca.* 1 ml/g) and concentrated aqueous $K_2CO_3$ was added until $CO_2$ evolution ceased. Addition of saturated $(NH_4)_2SO_4$ (20 ml/g of **8a**·HBr or **8d**·HCl) precipitated the free bases. Recrystallization from $CHCl_3$–petroleum ether gave **8a** in 42% yield, mp 110–112° (lit.[4] mp 113°), and **8d** in 20% yield, mp 112–114°. *Anal.* ($C_{11}H_{16}N_2O_3$) C, H, N.

**B.**—A mixture of equimolar amounts of benzamidine hydrochloride, NaOEt, and diethyl 2-formyl-[4] (or 2-acetyl-) glutarate (**7**, $R^6$ = H, $CH_3$), in EtOH (100 ml/0.03 mol of **7**) was refluxed 12 hr, filtered, and concentrated to 25 ml/0.03 mol of **7** to give a gummy precipitate which was filtered off (for **8b**, the precipitate formed at 0° before concentration and was consequently removed at that point). Recrystallization of the precipitate from $CHCl_3$–$Et_2O$ gave **8b** in 54% yield, mp 145–146° [*Anal.* ($C_{15}H_{16}N_2O_3$) C, H, N], and **8c** in 38% yield, mp 145–146° [*Anal.* ($C_{16}H_{18}N_2O_3$) C, H, N].

**3-[4-Hydroxy(2-methyl-, -2-phenyl-, -2-phenyl-6-methyl-, and -2,6-dimethyl)-5-pyrimidyl]propionic Acids (3). A.**—A solution of ester **8a** (6–10 mmol) in 1 *N* KOH (50 ml) was refluxed 12 hr, neutralized, and concentrated to dryness. The solid was dissolved in $H_2O$ (20 ml) and EtOH (150 ml), then petroleum ether (50 ml) was added; the mixture was filtered, and the resulting solution was concentrated to give crude **3a** (*ca.* 86%). Crystallization from EtOH gave pure **3a** (54% yield), mp 210–211°. *Anal.* ($C_8H_{10}N_2O_3$) C, H, N.

**B.**—The alkaline hydrolysates (as above) of esters **8b** and **8c** were acidified to pH 3 to precipitate the crude acids **3b** and **3c** in *ca.* 85% yields. Recrystallization from EtOH or EtOH–petroleum ether gave **3b** in 68% yield, mp 233–234° [*Anal.* ($C_{13}H_{12}N_2O_2$) C, H, N], and **3c** in 75% yield, mp 219–220° [*Anal.* ($C_{14}H_{14}N_2O_3$) C, H, N]. Acids **3b** and **3c** readily sublimed *in vacuo* on heating.

**C.**—In attempted preparation of the 2,6-dimethyl acid **3d**, the hydrolysate (as above) of **8d** was acidified to pH 3 and concentrated to dryness, and the residue was triturated with EtOH. Concentration gave the acid as an amorphous, hygroscopic solid. An EtOH solution of the acid was neutralized to pH 7, concentrated, and triturated with EtOH. Upon concentration an amorphous hygroscopic solid was obtained [$\nu_{max}$ 1780, 1600, 1656, and 1115 $cm^{-1}$] which resisted clean crystallization.[9]

A solution of the hydroxy ester **8d** (1 mmol) in $H_2O$ (5 ml) containing KOH (1.2 mmol) was refluxed 3 hr and concentrated to dryness. The resulting K salt was recrystallized twice from EtOH to give the K salt of **3d** in 58% yield: mp >300°; $\nu_{max}$ 3000–2500, 1670, 1610, 1595, 1575, and 1400 $cm^{-1}$. *Anal.* ($C_9H_{11}KN_2O_3$) C, H, N.

**Ethyl 3-[4-Chloro(2-methyl-, -2-phenyl-, -2-phenyl-6-methyl-, and -2,6-dimethyl)-5-pyrimidyl]propionates (9).**—A solution of **8** (0.02–0.11 mol) in $POCl_3$ (50 ml) was refluxed 30 min and then concentrated to a syrup. The syrup was dissolved in a mixture of ice $H_2O$ (50 ml) and $Et_2O$ (75 ml), and concentrated aqueous $K_2CO_3$ was added with stirring until $CO_2$ evolution ceased. The aqueous layer was further extracted with $Et_2O$ (75 ml), and the combined $Et_2O$ solutions were concentrated to a syrup. Crystallization from petroleum ether ($Et_2O$ for **9c**) gave the crystalline chloro esters: **9a**[4] in 91% yield (mp 29–31°; $\nu_{max}$ 2960, 2910, 1740, 1575, 1525, and 1440 $cm^{-1}$) of sufficient purity for subsequent transformation, **9b** in 87% yield, mp 56–57° [*Anal.* ($C_{15}H_{15}ClN_2O_2$) C, H, Cl, N], and **9c** in 91% yield, mp 69–70° [*Anal.* ($C_{16}H_{17}ClN_2O_2$) C, H, N].

Chloro ester **9d** was liquid at room temperature and thus was distilled, bp 142° (0.65 Torr), to give 68% yield of **9d** of sufficient purity ($\nu^{neat}_{max}$ 2980, 2940, 1740, 1570, 1535, 1430, and 1200 $cm^{-1}$) for subsequent transformation.

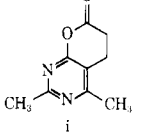
**7-Oxo(2-methyl-, -2-phenyl-, -2-phenyl-4-methyl-, and -2,4-dimethyl)-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidines (10). A.**—A solution of **9a–c** (2–5 mmol) in EtOH (40 ml) saturated with $NH_3$ at 0° was heated in a steel bomb at 140° for 2–3 days.[10]

(6) N. F. Albertson, *J. Am. Chem. Soc.*, **72**, 2594 (1950).

(7) T. L. Hullar, *J. Med. Chem.*, **12**, 58 (1969).

(8) Melting points were taken on a Fischer-Johns melting block and those below 220° are corrected. Ir spectra were determined in KBr disks, unless otherwise indicated, with a Perkin-Elmer Model 237 spectrophotometer; the absorptions were as expected. Uv spectra were taken with a Beckman DB-G spectrophotometer and were as expected. $Et_2O$ and $CHCl_3$ extracts were dried over $MgSO_4$. All solutions were concentrated by spin evaporation over a steam bath at reduced pressure (water aspirator) unless otherwise indicated. Petroleum ether had bp 30–60°. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(9) This compound is most likely lactone i. No evidence of such lactones was found in series **a–c**.

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(10) These conditions are undoubtedly more vigorous than necessary because action of methanolic ammonia on **9a** for only 7 hr at 90–100° gave **10a** in 51% yield.[4]

The solution was cooled to 0° to give **10b** and **10c** in 77 and 92% yields; for **10a** the solution was concentrated to 15 ml to give **10a** in 44% yield. Recrystallization of **10a–c** from EtOH gave pure samples: **10a**, mp 252–254° (lit.[4] mp 256°) [*Anal.* ($C_8H_9N_3O$) C, H, N], **10b**, mp 267–269° [*Anal* ($C_{13}H_{11}N_3O$) C, H, N], and **10c**, mp 190–191° [*Anal.* ($C_{14}H_{13}N_3O$) C, H, N].

The amination of the 2,6-dimethylchloro ester **9d** as above gave upon concentration a gummy residue which was dissolved in EtOH (1 ml/mmol of **9d**). Addition of $Et_2O$ (25 ml/mmol) gave a precipitate which was discarded. HCl gas was passed into the filtrate to give **10d**·HCl (72% yield) which was recrystallized from EtOH–$Et_2O$; mp >300°; $\nu_{max}$ 3100, 2980, 2800–2300, 1900, 1740, 1640, 1610, 1490, and 1210 $cm^{-1}$. *Anal.* ($C_9H_{12}ClN_3O$) C, H, Cl, N. When the above reaction was conducted at room temperature, only starting material was obtained.

**B.**—A solution of **9a** (1.63 g) in liquid $NH_3$ (15 ml) was kept in a steel bomb at room temperature for 40 hr. The $NH_3$ was evaporated to give a solid which was triturated with $CHCl_3$ (40 ml). The $CHCl_3$ solution was concentrated to a semisolid which showed ir absorption for ester, amide, and lactam carbonyls. Crystallization of the semisolid from EtOH (30 ml) furnished lactam **10a** (0.227 g, 20%), mp 250–252°. The solids from the mother liquors were crystallized from $CHCl_3$–petroleum ether (10:40 ml) to give the chloro amide, **3-(4-chloro-2-methyl-5-pyrimidyl)propionamide** (**11a**): 0.244 g, 16%; mp 134°, solidifies, then melts at 180–196°; $\nu_{max}$ 3410, 3190, 1650, 1615, 1575, and 1525 $cm^{-1}$. *Anal.* ($C_8H_{10}ClN_3O$) H, N; C: calcd, 48.1; found, 48.6; Cl: calcd, 17.8; found, 15.4. The remaining mother liquors furnished **9a** (0.30 g).

**C.**—In an effort to use previously adopted conditions[4] for the direct preparation of amino acid **4a**, a solution of **9a** (0.229 g) in concentrated $NH_4OH$ (50 ml) was stirred at room temperature for 25 hr and then concentrated to 1–2 ml. EtOH (7 ml) was added and solution was cooled to 0°, but no solid was obtained. Concentration to dryness and recrystallization from EtOH (3 ml) gave chloro amide **11a** (0.023 g). Tlc of the remainder showed **11a** together with at least four additional products. Further separation of the complex mixture was not attempted.

**3-[4-Amino(2-methyl- and -2-phenyl-6-methyl)-5-pyrimidyl]-propionic Acid (4a,c) Hydrochlorides.**—A solution of lactam **10a,c** (1 mmol) in 0.15 *N* KOH (20 ml) was refluxed 6 hr, acidified to pH 7, and concentrated to dryness. The residue was triturated with hot EtOH (three 25-ml portions), and the combined EtOH solutions were concentrated to 20 ml. $Et_2O$ (80 ml) was added, and HCl gas was added to precipitate the crude salts. The salts were recrystallized from EtOH or EtOH–$Et_2O$ to give **4a** in 75% yield: mp >300°; $\nu_{max}$ 3250, 3100, 2800–2500, 1670, 1645, 1610, 1550, 1400, and 1280 $cm^{-1}$ [*Anal.* ($C_8H_{11}ClN_3O_2$) C, H, Cl; N: calcd, 19.3; found, 18.0]; and **4c** in 61% yield: mp 187–189°[11] [*Anal.* ($C_{14}H_{16}ClN_3O_2$) C, H, Cl, N].

**3-[4-Dimethylamino(2-methyl- and -2-phenyl-6-methyl)-5-pyrimidyl]propionic Acid (5a,c) Hydrochlorides.**—A solution of chloro ester **9a,c** (3 mmol) in 50% EtOH–$Me_2NH$ (40 ml) was heated at 110–115° for 1–5 days and then concentrated to a syrup. A solution of the syrup in 0.2 *N* KOH (25 ml) was refluxed 24 hr, acidified to pH 3, and concentrated to dryness. The residue was triturated with two 15-ml portions of EtOH ($CHCl_3$ for **5c**) and the EtOH solution was diluted with $Et_2O$ (30 ml). Ethereal HCl was added to give crude **5a** and **5c** in 56 and 82% yields, respectively. Recrystallization from EtOH or EtOH–$Et_2O$ gave **5a**, mp 190–192° [*Anal.* ($C_{10}H_{16}ClN_3O_2$) C, H, Cl, N], and **5c**, mp 222–224° [*Anal.* ($C_{16}H_{20}ClN_3O_2$) C, H, Cl, N].

**3-[4-Chloro(2-methyl- and -6-methyl-2-phenyl)-5-pyrimidyl]-propionamides (11a,c).**—The synthesis of **11a** is described above under **10**, section B. For **11c**, a solution of **9c** (5 mmol) in concentrated $NH_4OH$–dioxane (40:40 ml) was stirred 1 day at room temperature and then concentrated to 15 ml to give crystalline **11c** in 84% yield, mp 164–165° and partially resolidifies.[12] *Anal.* ($C_{14}H_{14}ClN_3O$) C, H, Cl, N.

**3-(4-Hydroxy-2-phenyl-6-methyl-5-pyrimidyl)propionitrile (12c).** **A.**—A solution of 8 mmol each of benzamidine hydrochloride, NaOMe, and ethyl 2-(2-cyanoethyl)propionitrile[6] in EtOH (20 ml) was refluxed 26 hr and then cooled at 0° to furnish a crystalline mass. The crystals were washed ($H_2O$, EtOH, petroleum ether) to give pure **12c**, mp 238–239° (17% yield). *Anal.* ($C_{14}H_{13}N_3O$) C, H, N.

**B.**—Heating **11c** *in vacuo* (230° at <0.2 Torr) sublimed **12c** in quantitative yield, mp 230–234°, ir identical with **12c** prepared in A.

**Enzymic Evaluation.**—The enzymic methods used for evaluation of **3**, **4a,c**, and **5a,c** as inhibitors of TDC and AAT have been described.[7]

**Acknowledgment.**—The technical assistance of Mrs. K. Morris is acknowledged.

(11) This melting point is that of lactam **10c** and the ir spectrum of the melt was virtually identical with that of **10c**.

(12) The ir spectrum of the melt shows it to contain both chloro amide and hydroxy nitrile (**12c**).

# Conformational Aspects of Carbamates in the Inhibition of the Hill Reaction

MARVIN R. BOOTS[1]

*Agricultural Chemical Division, Kansas City Laboratory, Gulf Research and Development Company, Merriam, Kansas 66202*

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A series of linear and cyclic carbamates (substituted 4H-3,1-benzoxazin-2-ones) was prepared and investigated as inhibitors of the Hill reaction in isolated chloroplasts. These were chosen to investigate the conformational requirements of the carbamate group during binding to the receptor. The cyclic compounds were inactive while the linear carbamates exhibited activity. These results are discussed in terms of the conformational preference of the carbamate group, over-all molecular geometry, metabolic inactivation, and steric factors.

The Hill Reaction (photochemical activity) of isolated chloroplasts involves the oxidation of water to molecular oxygen with concurrent reduction of a suitable electron acceptor.[2a] The discovery of herbicidal activity of compounds containing the CONHPh moiety, ureas, carbamates, and amides, and their ability to inhibit the Hill reaction in isolated chloroplasts has stimulated numerous investigations[2b] to correlate a variety of physiochemical properties of the inhibitor such as the acidity[3a] and the hydrogen-bonding power[3b] of the NHR group with bioactivity.

An attempt[4] was made to overcome the difficulty of relating only one parameter with activity by including electronic and steric effects as well as hydrophobic bonding properties in the correlation. This was accomplished by using substituent constants and

(1) School of Pharmacy, Medical College of Virginia, Health Sciences Division of Virginia Commonwealth University, Richmond, Va. 23219.

(2) (a) E. S. West, W. R. Todd, H. S. Mason, and J. T. Van Bruggen, "Textbook of Biochemistry," 4th ed, Macmillan, New York, N. Y., 1966, pp 1103–1104; (b) D. E. Moreland, *Ann. Rev. Plant Physiol.*, **18**, 365 (1967).

(3) (a) N. D. Camper and D. E. Moreland, *Biochim. Biophys. Acta*, **94**, 383 (1965); (b) N. E. Good, *Plant Physiol.*, **36**, 788 (1961).

(4) C. Hansch and E. W. Deutsch, *Biochim. Biophys. Acta*, **112**, 381 (10966).