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°; ν_{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°; ν_{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°; ν_{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

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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

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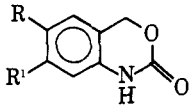
(2) (a) E. S. West, W. R. Todd, H. S. Mason, and J. T. Van Bruggen, "Textbook of Biochemistry," 4th ed, Macmillan, New York, N. Y., 1966, pp 1103–1104; (b) D. E. Moreland, *Ann. Rev. Plant Physiol.*, **18**, 365 (1967).

(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 (1966).

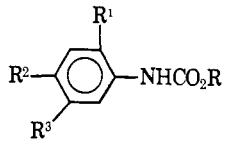
regression analyses. The hydrophobic bonding power of the N-phenyl substituent and not that of the molecule as a whole was the major factor involved in correlating activity of substituted N-phenylamides. However, 3,5-disubstituted N-phenylamides failed to show the predicted activity.

The present study represents an attempt to correlate the geometry of the carbamate group with the inhibition of the Hill reaction in a series of cyclic carbamates (**1a–c**). An effort was made to keep the electronic and



1a, R = H; R^1 = H
b, R = Cl; R^1 = H
c, R = H; R^1 = Cl

hydrophobic bonding effects constant so that the inhibition would be a function of the molecular geometry of the inhibitor. The corresponding linear carbamates (**2–7**) were also prepared so that a direct comparison could be made.



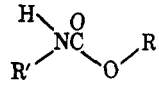
2, R = CH_3; R^1, R^2 = H; R^3 = Cl
3, R = CH_3; R^1, R^3 = H; R^2 = Cl
4, R = $PhCh_2$; R^1, R^2 = H; R^3 = Cl
5, R = $PhCH_2$; R^1, R^3 = H; R^2 = Cl
6, R, R^1 = CH_3; R^2 = H; R^3 = Cl
7, R, R^1 = CH_3; R^2 = Cl; R^3 = H

Chemistry.—For the synthetic approach to the cyclic carbamates **1**, the appropriate anthranilic acids were reduced to the corresponding aminobenzyl alcohols (**8**). Cyclization of **8** with $COCl_2$ gave the desired cyclic carbamates. The linear carbamates (**2–5**) were synthesized from the appropriate phenyl isocyanate and the corresponding alcohol. The *ortho*-substituted linear carbamates (**6**, **7**) were prepared from the substituted anilines and methyl chloroformate.

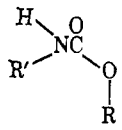
Biological Assays.—The molar concentration of the carbamate reducing the photolytic activity of the isolated chloroplasts to half its normal activity (I_{50}) was determined by previously described techniques,[5] except that ferricyanide reduction was followed by measuring the decrease in ferricyanide absorption at 420 mμ using a Klett colorimeter. All assays were performed in duplicate with chloroplasts obtained from spinach. Data are presented as the arithmetic averages of the individual determinations.

Results and Discussion

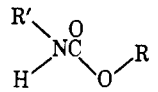
The carbamate group, $-O_2CN=$, is planar because of resonance and may exist in four possible conformations (A–D) depending on the position of the carbonyl group with respect to the imino hydrogen atom and the R group of the ester portion. Data concerning dipole moment measurements[6] indicate that the *cis* conformation with regard to the ester portion of the carbamate is the preferred conformation. In the case of amides, the *trans* form predominates with respect to the carbonyl group and the imino hydrogen atom.[7] Thus one can predict that the linear carbamate group possesses predominantly the *trans,cis* conformation (C). The geometry of the cyclic carbamate group involving medium-sized rings is such that only the *cis,trans* conformer B can exist.



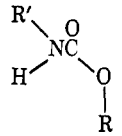
A, *cis,cis*



B, *cis,trans*



C, *trans,cis*



D, *trans,trans*

In an effort to assess the importance of conformational factors of the carbamate group during binding to the receptor, inhibitors containing a carbamate group with a fixed conformation were studied. These carbamates **1** exist solely in the *cis,trans* conformation. The electronic properties of the aromatic ring in the linear and cyclic carbamates appear to be similar based on the uv spectra. The linear carbamates were active inhibitors at 6×10^{-4} *M* (see Table II for the I_{50} values). The cyclic carbamates were inactive at all concentrations. The maximum concentration attainable was 3×10^{-3} *M* because of solubility limitations.

The inactivity of the cyclic carbamates may be the result of several factors besides the conformation of the inhibitor. The need for a free and sterically unhindered imino H has been demonstrated.[3b,5] The cyclic carbamates possess a CH_2 group *ortho* to NH which may result in reducing the accessibility of the imino group to a binding site on the receptor site on the receptor. In an effort to assess this effect, two linear carbamates **6** and **7** containing CH_3 *ortho* to NH were prepared. The I_{50} values for these CH_3-substituted compounds are similar to the I_{50} values of **2** and **3**. Therefore, the steric factor of the CH_2 group appears to be negligible.

A second factor which could conceivably play a role in the inactivity of the cyclic carbamates is that of metabolic inactivation. Oxidation of the CH_2 group is a possibility, since it is benzylic and activated by the inductive effect of the oxygen atom of the ester portion of the carbamate.[8]

In an attempt to evaluate this factor, two linear carbamates **4** and **5** containing a benzyl ester function were prepared and assayed. The I_{50} values (see Table II) for the benzyl carbamate **4** and the methyl carbamate **2** are very similar. A comparison of the benzyl carbamate **5** with the methyl carbamate **3** is difficult since the solubility properties of **5** do not allow an I_{50} value to be determined. A saturated test solution of **5**

(5) D. E. Moreland and K. L. Hill, *J. Agr. Food Chem.*, 7, 832 (1959).

(6) C. M. Lee and W. D. Kumler, *J. Am. Chem. Soc.*, **83**, 4596 (1961).

(7) (a) S. Mizushima, T. Simanouti, S. Nagakura, K. Kuratani, M. Tsuboi, H. Baba, and O. Fujiopa, *J. Am. Chem. Soc.*, **72**, 3490 (1950); (b) L. LaPlanche and M. Rogers, *ibid.*, **86**, 337 (1964).

(8) Professor C. Hansch, Department of Chemistry, Pomona College, Claremont, Calif., private communication, 1966.

produced a 35% inhibition. However, conclusions drawn from data obtained from saturated test solutions must be viewed with some uncertainty, since crystallization or a salting out of the inhibitor may take place in the reaction mixture.[3b] Therefore, the observed inhibition may have resulted from a lower concentration of an inhibitor. On the basis of the similar activities of carbamates **2** and **4** the factor of metabolic inactivation appears to be negligible.

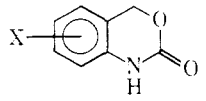
The geometry of the carbamate group as well as the shape of the over-all molecule is defined in the cyclic carbamates. In this case, the phenyl ring, the N atom, the ester oxygen atom, and the CO carbon atom are essentially coplanar. This condition is not required in the linear carbamate, and the carbamate group as well as the phenyl ring may assume a conformation which allows binding to the active site of the receptor. The present series of cyclic carbamates examines only one conformation of the four which might be involved during the binding of the carbamate group. Therefore, further studies must be performed with other specifically designed inhibitors in order to assess the role of the conformational factor in the inhibition of the Hill reaction.

Experimental Section[9]

4H-3,1-Benzoxazin-2-ones (Tables I and II).—To a cold solution (5–10°) of 0.1 mole of the appropriate *o*-aminobenzyl alcohol and 0.2 mole of TEA in 500 ml of C_6H_6 was added dropwise

TABLE I

PROPERTIES AND BIOLOGICAL ACTIVITY OF SUBSTITUTED 4H-3,1-BENZOXAZIN-2-ONES



No.	X	Mp, °C	Yield, %	Inhib of photolytic act.
1a	H	118–120[a,b]	50	None
1b	6-Cl	200–202[c]	50	None
1c	7-Cl	169–171[d]	65	None

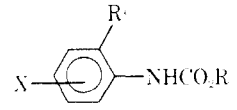
[a] Recrystallized from C_6H_6–petroleum ether (PE) (bp 60–75°). [b] H. Lindeman and W. Schulheis, *Ann.*, **464**, 237 (1928), gives mp 119°. [c] Recrystallized from $CHCl_3$–PE. [d] Recrystallized from $CHCl_3$.

with stirring, 86 ml of a solution of $COCl_2$ in C_6H_6 (12.5%, Matheson Coleman and Bell). The temperature was kept below 15°. The mixture was then stirred at room temperature for an additional 2 hr, then heated at reflux for 3.5 hr. The mixture was cooled and the precipitated solid was filtered off. The benzoxazine–TEA·HCl precipitate was washed (H_2O) to remove the TEA·HCl. The C_6H_6 filtrate was taken to dryness *in vacuo* and the remaining solid benzoxazine was combined with the H_2O-washed benzoxazine and recrystallized.

(9) Melting points, determined with a Thomas–Hoover capillary melting point apparatus, are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

TABLE II

PROPERTIES AND BIOLOGICAL ACTIVITY OF SUBSTITUTED ALKYL N-PHENYLCARBAMATES



No.	X	R	R′	Mp, °C	Yield, %	Inhib of photolytic act. I_{50}, *M*
2	3-Cl	CH_3	H	84–86[a,b]	43	5.2×10^{-5}
3	4-Cl	CH_3	H	115–117[a,c]	80	1.2×10^{-4}
4	3-Cl	$C_6H_5CH_2$	H	50–52[a,d]	51	6.0×10^{-5}
5	4-Cl	$C_6H_5CH_2$	H	110–112[a,e]	84	6.0×10^{-4}[g]
6	5-Cl	CH_3	CH_3	79–82[a]	15	6.0×10^{-3}
7	4-Cl	CH_3	CH_3	87–90[f]	25	8.5×10^{-4}

[a] Recrystallized from C_6H_6–PE. [b] M. M. deMonchy, *Rec. Trav. Chim.*, **53**, 141 (1934), gives mp 81°. [c] E. Dyer and K. McCormick, *J. Am. Chem. Soc.*, **68**, 986 (1946), gives mp 117°. [d] T. Mukaiyama and M. Iwanami, *ibid.*, **79**, 73 (1957), gives mp 45°. [e] Lit.[d] mp 110°. [f] Recrystallized from PE. [g] The test solution was saturated and a 35% inhibition resulted.

Substituted Alkyl N-Phenylcarbamates (2–5) (Table II).—A mixture of 0.1 mole of the appropriate phenyl isocyanate, 0.1 mole of the alcohol, and a trace amount of stannous stearate in 200 ml of dry C_6H_6 was heated at reflux for 4 hr. The cooled reaction mixture was filtered and the filtrate was washed (5% aqueous NaOH, H_2O), then dried ($MgSO_4$). The solvent was removed *in vacuo* to afford the desired carbamate, which was recrystallized.

Methyl N-2-Methyl-4-chlorophenylcarbamate (6).—To a cold (15°) solution of 7.0 g (0.05 mole) of 2-methyl-4-chloroaniline and 5.0 g (0.05 mole) of TEA in 100 ml of dry C_6H_6 was added dropwise 4.7 g (0.05 mole) of methyl chloroformate. The mixture was stirred at room temperature for 1 hr, then refluxed for 3 hr. It was cooled and the precipitated solid was removed by filtration. The filtrate was washed (H_2O) and dried ($MgSO_4$), and the solvent was removed *in vacuo*. The oily residue was crystallized from C_6H_6–PE to yield 2.5 g (25%) of **6**, mp 87–90°. *Anal.* ($C_9H_{10}ClNO_2$) C, H, N.

Methyl N-2-methyl-5-chlorophenylcarbamate (7) was prepared according to the procedure described for **6**. The crude carbamate was crystallized from PE to yield 1.5 g (15%) of **7**, mp 79–82°. *Anal.* ($C_9H_{10}ClNO_2$) C, H, N.

2-Amino-4-chlorobenzyl Alcohol (8b).—Reduction of 17.1 g (0.1 mole) of 4-chloroanthranilic acid with $LiAlH_4$ by the method of Nystrom and Brown[10] gave, after recrystallization from $CHCl_3$, 13.7 g (87%) of **8b**, mp 136–138°. *Anal.* (C_7H_8ClNO) C, H, N.

2-Amino-5-chlorobenzyl alcohol (8c) was prepared according to the procedure described for **8b**. The crude aminobenzyl alcohol was recrystallized from $CHCl_3$, to yield 12.1 g (77%) of **8c**, mp 107–109°. *Anal.* (C_7H_8ClNO) C, H, N.

Acknowledgment.—The author expresses his appreciation to Dr. Donald E. Moreland, U. S. Department of Agriculture, Agricultural Research Service, Crop Science Department, North Carolina State University, Raleigh, N. C., for the biological results and for many interesting discussions of this problem.

(10) R. R. Nystrom and W. G. Brown, *J. Am. Chem. Soc.*, **69**, 2548 (1947).