were devoid of these residue constituents (Table II). Protocatechuic, syringic, and gallic acids were detected in several legumes, essentially the same species that exhibited these acids in the soluble ester fraction. The presence of gallic acid in the hydrolyzed fractions of hulls was expected, due to the presence of hydrolyzable tannins in the hulls.

Pigeon pea and fababean contained the highest levels of total (esterified plus residue) phenolic acids in the hulls, followed by cowpea and lentil (Table II). In the case of pigeon pea and fababean, the hulls were estimated to contain 65 and 46%, respectively, of the total seed phenolics. In most other legumes, the hulls contributed only a minor fraction of total seed phenolic constituents. Thus, dehulling of legumes would be effective in reducing the phenolic acid contents only in the case of pigeon pea, fababean, and, possibly, lentil and mung bean.

The legume flours showed little variation in brightness with the L values being in the range of 88–92 (Table III). However, distinct yellow-green colors were exhibited by pigeon pea, lupine, and lentil. In contrast, the flour of navy bean was essentially white. Peak absorbance values for the methanol–acetone–water extracts of the legume flours were about 323 nm for mung bean, field pea, lentil, fababean, and pigeon pea while values of 319 nm were obtained for navy bean, lima bean, chickpea, and cowpea. Lupine gave a slightly higher λ_{max} of 327 nm and lentil gave a second peak at 347 nm.

Absorbance at λ_{max} of the legume flour extracts was quite low for field pea, fababean, and chickpea; intermediate values were obtained for mung bean, lentil, pigeon pea, and navy bean (Table III). Lima bean, cowpea, and lupine gave high absorbance values of 0.319, 0.431, and 0.777, respectively. These absorbance values gave nonsignificant correlation coefficients of +0.36 with the b values in Table III and +0.44 with total phenolic acids in the flours in Table I. It was evident that phenolic compounds were not major factors influencing the natural colors of the flours or of aqueous extracts at neutral pH. Under alkaline conditions (pH 9.5), all extracts exhibited significant increases in color intensity. Presumably this was due to oxidative changes in the soluble phenolic esters as well as flavanoid and quinone compounds and their interactions under alkaline conditions. When the alkaline extracts are in contact with the protein components of the legume or oilseed meals, brown and green protein isolates are often obtained (Sosulski and Bakal, 1969).

The extract from lentil was fractionated by TLC to demonstrate the presence of several colored constituents. Only three spots gave blue fluorescence under long-wave UV light, suggesting a phenolic nature. It appeared that a broader study than the present investigation of phenolic compounds would be required for identification of all major color-forming compounds in these legume flours.

Registry No. *trans*-Ferulic acid, 537-98-4; *trans*-*p*-coumaric acid, 501-98-4; syringic acid, 530-57-4; *p*-hydroxybenzoic acid, 99-96-7; protocatechuic acid, 99-50-3; gallic acid, 149-91-7.

LITERATURE CITED

Dabrowski, K. J.; Sosulski, F. W. *J. Agric. Food Chem.* **1984**, second paper of three in this issue.
Deshpande, S. S.; Sathe, S. K.; Cornforth, D.; Salunkhe, D. K. *Cereal Chem.* **1982**, *59*, 396.
Fan, T. Y.; Sosulski, F. W. *Can. Inst. Food Sci. Technol. J.* **1974**, *7*, 256.
Khan, M. A.; Jacobsen, I.; Eggum, B. O. *J. Sci. Food Agric.* **1979**, *30*, 395.
Krygier, K.; Sosulski, F. W.; Hogge, L. *J. Agric. Food Chem.* **1982a**, *30*, 330.
Krygier, K.; Sosulski, F. W.; Hogge, L. *J. Agric. Food Chem.* **1982b**, *30*, 334.
Ma, Y.; Bliss, F. A. *Crop Sci.* **1978**, *18*, 201.
Pierpoint, W. S. *Biochem. J.* **1969**, *112*, 609.
Price, M. L.; Hagerman, A. E.; Butler, L. G. *J. Agric. Food Chem.* **1980**, *28*, 459.
Reschke, A.; Herrmann, K. *Z. Lebensm.-Unters. -Forsch.* **1982**, *174*, 5.
Salunkhe, D. K.; Jadhav, S. J.; Kadam, S. S.; Chavan, J. K. *CRC Crit. Rev. Food Sci. Nutr.* **1982**, *17*, 277.
Schmidtlein, H.; Herrmann, K. *Z. Lebensm.-Unters. -Forsch.* **1975**, *159*, 213.
Sosulski, F. W.; Bakal, A. *Can. Inst. Food Technol. J.* **1969**, *2*, 28.
Sosulski, F. W.; Krygier, K.; Hogge, L. *J. Agric. Food Chem.* **1982**, *30*, 337.
Sosulski F. W.; Youngs, C. G. *J. Am. Oil Chem. Soc.* **1979**, *56*, 292.

Received for review March 17, 1983. Accepted August 26, 1983. The research project was supported by a grant from the Natural Sciences and Engineering Council of Canada.

Quantitative Structure–Activity Relationships in the Inhibition of Photosystem II in Chloroplasts by Phenylureas

Emil Kakkis, Vincent C. Palmire, Jr., Cynthia D. Strong, Walter Bertsch, Corwin Hansch,* and Ulrich Schirmer

Quantitative structure–activity relationships (QSAR) have been formulated for the inhibition of the Hill reaction in spinach chloroplasts by 1,1-dimethyl-3-(X-phenyl)ureas and 1-methyl-1-methoxy-3-(X-phenyl)ureas. These mathematical models are compared with other such QSAR for Hill reaction inhibitors. From these results it is clear that the molecular requirements for inhibitors are an active polarizable sp^2 nitrogen atom attached to a large lipophilic moiety. Although the lipophilic area in chloroplasts is shown to be very large and surprisingly free of steric effects, one significant steric effect caused by branching of substituents near the phenyl ring has been clearly estabished. Evidence is marshaled to show that the optimum lipophilic effect occurs with inhibitors having a log P of 5–6.

A natural route open to organic and physical organic chemists for the study of the interaction of organic compounds with macromolecules has grown out of the extrathermodynamic approach to rate and equilibrium studies pioneered by L. P. Hammett. The great success of Hammett's σ constants inspired Taft to develop the steric parameter E_s, which then was followed by the formulation of a hydrophobic constant (π or log P) for substituents (Hansch and Leo, 1979). With these parameters the

Department of Chemistry and Biology, Pomona College, Claremont, California 91711 (E.K., V.C.P., C.D.S., W.B., and C.H.), and BASF-Aktiengesellschaft, D-6700 Ludwigshafen, West Germany (U.S.).

0021-8561/84/1432-0133$01.50/0

various roles of electronic, steric, and hydrophobic effects of substituents on the course of biochemical reactions could be dissected via regression analysis (Hansch and Fujita, 1964; Hansch, 1969a, b, 1981; Martin, 1978). This approach to the study of substituent effects in vitro and in vivo biological reactions has come to be called quantitative structure–activity relationships (QSAR) and has reached the point where a journal is now devoted to this rapidly developing study ("Quantitative Structure-Activity Relationships", 1982). The subject of this report is a review of the application of this methodology to inhibitors of photosystem II (Hill reaction) and further study of very lipophilic ureas in the Hill reaction.

The use of physicochemical substituent constants and regression analysis for studying the mechanism of action of organic compounds with biological systems was initiated in the study of plant-growth regulators (Hansch et al., 1963). Shortly after the initial work, the method was applied to phenylureas inhibiting photosystem II (Hansch and Deutsch, 1966). Since then a variety of rather disparate studies have been published on the QSAR of inhibition of the Hill reaction (Hansch, 1969b; Brown et al., 1981). A summary of the QSAR obtained in these studies is given in equations 1–12.

isopropyl *N*-phenylcarbamates (Hansch, 1969b), $X\text{-}C_6H_4NHCOOCH(CH_3)_2$

$$pI_{50} = 0.71\ (\pm0.27)\log P + 0.65\ (\pm0.67)\sigma + 0.87\ (\pm0.77) \quad (1)$$
$$n = 9 \quad r = 0.948 \quad s = 0.186$$
$$pI_{50} = 0.81\ (\pm0.24)\log P + 0.58\ (\pm0.76) \quad (1a)$$
$$n = 9 \quad r = 0.947 \quad s = 0.173$$

ethyl *N*-phenylcarbamates (Hansch, 1969b), $X\text{-}C_6H_5NHCOOC_2H_5$

$$pI_{50} = 0.77\ (\pm0.46)\log P + 0.99\ (\pm1.2)\sigma + 1.34\ (\pm1.1) \quad (2)$$
$$n = 7 \quad r = 0.957 \quad s = 0.291$$
$$pI_{50} = 0.89\ (\pm0.25)\log P + 1.13\ (\pm0.70) \quad (2a)$$
$$n = 7 \quad r = 0.972 \quad s = 0.210$$

anilides of isobutyric acid (Hansch, 1969b), $X\text{-}C_6H_4NHCOCH(CH_3)_2$

$$pI_{50} = 1.23\ (\pm0.38)\log P + 1.74\ (\pm0.99) \quad (3)$$
$$n = 10 \quad r = 0.935 \quad s = 0.280$$
$$pI_{50} = 1.59\ (\pm0.27)\log P + 0.35\ (\pm0.75) \quad (3a)$$
$$n = 11 \quad r = 0.975 \quad s = 0.218$$

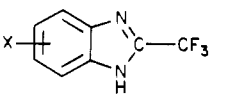
1-phenyl-3,3-dimethylureas (Hansch, 1969b), $X\text{-}C_6H_4NHCON(CH_3)_2$

$$pI_{50} = 1.08\ (\pm0.49)\log P + 1.93\ (\pm1.1)\sigma + 2.71\ (\pm0.71) \quad (4)$$
$$n = 12 \quad r = 0.929 \quad s = 0.457$$
$$pI_{50} = 1.17\ (\pm0.36)\log P + 0.61\ (\pm0.57)\sigma + 2.72\ (\pm0.56) \quad (4a)$$
$$n = 12 \quad r = 0.966 \quad s = 0.319$$

1-phenyl-3-methylureas (Seewald et al., 1978), $X\text{-}C_6H_4NHCONHCH_3$

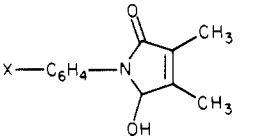
$$pI_{50} = 1.03\ (\pm0.19)\log P + 4.27\ (\pm0.28) \quad (5)$$
$$n = 15 \quad r = 0.957 \quad s = 0.189$$
$$pI_{50} = 0.96\ (\pm0.21)\log P + 4.10\ (\pm0.36) \quad (5a)$$
$$n = 15 \quad r = 0.941 \quad s = 0.221$$

benzimidazoles (Büchel and Draber, 1969)



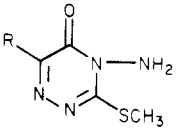
$$pI_{50} = 1.35\ (\pm0.23)\log P + 0.56\ (\pm0.80) \quad (6)$$
$$n = 25 \quad r = 0.930 \quad s = 0.424$$

N-arylpyrrolones (Brugnoni et al., 1979)



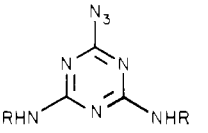
$$pI_{50} = 0.75\ (\pm0.17)\log P + 3.15\ (\pm0.39) \quad (7)$$
$$n = 32 \quad r = 0.852 \quad s = 0.328$$

1,2,4-triazinones (Draber et al., 1968)



$$pI_{50} = 0.86\ (\pm0.38)\log P + 4.84\ (\pm0.57) \quad (8)$$
$$n = 11 \quad r = 0.864 \quad s = 0.445$$

azidotriazines (Gabbott, 1969)



$$pI_{50} = 0.85\ (\pm0.28)\log P + 4.27\ (\pm0.70) \quad (9)$$
$$n = 17 \quad r = 0.859 \quad s = 0.272$$

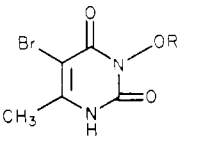
diphenyl ethers (van den Berg and Tipker, 1982), $X\text{-}C_6H_4OC_6H_4\text{-}4\text{-}NO_2$

$$pI_{50} = 0.46\ (\pm0.10)\log P + 2.30\ (\pm0.49) \quad (10)$$
$$n = 18 \quad r = 0.927 \quad s = 0.219$$

phenoxyphenyldimethylureas (van den Berg and Tipker, 1982), $X\text{-}C_6H_4OC_6H_4NHCON(CH_3)_2$

$$pI_{50} = 1.07\ (\pm0.36)\log P - 1.18\ (\pm0.71)\log(\beta\cdot10^{\log P} + 1) + 3.20\ (\pm1.0) \quad (11)$$
$$n = 14 \quad r = 0.935 \quad s = 0.246$$
$$\log P_0 = 4.60\ (\pm2.4) \quad \log\beta = -3.60$$

3-alkoxyuracils (Brown et al., 1981)



$$pI_{50} = 1.12\ (\pm0.10)\log P - 1.15\ (\pm0.21)\log(\beta\cdot10^{\log P} + 1) + 3.78\ (\pm0.18) \quad (12)$$
$$n = 23 \quad r = 0.991 \quad s = 0.261 \quad \log P_0 = 5.31$$
$$\log\beta = -3.76$$

In addition to the above QSAR, one for nitrophenols (Trebst and Draber, 1979) and another for benzotriazoles (Grieco et al., 1979) have been published. Since we do not have suitable log *P* values for the complex phenols we have omitted discussion of this data set. The benzotriazole equation is an extremely complex result correlating the activity of 422 compounds by using 16 variables and hence cannot easily be compared at this time with our simpler data sets.

In the above equations I_{50} is the molar concentration of inhibitor causing 50% inhibition in the Hill reaction, *n* is

Table I. Parameters Used in the Derivation of Equations 13-15 for Inhibition of Chloroplasts by X-$C_6H_4NHCON(CH_3)_2$

compd	X	obsd pI_{50}	calcd[a] pI_{50}	$\lvert\Delta pI_{50}\rvert$	log P[b]	BR
1	3-NO_2	4.61	5.09	0.48	1.46	0
2	3-CF_3	5.54	5.76	0.23	2.20	0
3	3-$COCH_3$	4.02	4.63	0.61	0.95	0
4	4-COC_6H_5	6.21	5.89	0.32	2.59	1.82
5	4-F	5.31	4.79	0.52	1.13	0
6	3,4-Cl_2	6.62	6.27	0.35	2.77	0
7	H	5.03	4.66	0.37	0.98	0
8	3-n-C_4H_9	6.41	6.69	0.25	3.23	0
9	3-$OCH_2CH_2C_6H_5$	6.84	6.80	0.04	3.39	0
10	4-c-C_6H_{11}	5.35	5.53	0.18	3.83	13.4
11	4-t-C_4H_9	5.68	5.89	0.21	3.10	5.65
12	4-$CH(CH_3)_2$	6.17	5.69	0.49	2.87	5.65
13	3-OH	3.76	4.23	0.47	0.51	0
14	3-NH_2	4.05	3.68	0.37	−0.10	0
15	3-$OCH_2C_6H_3$-2′,4′-Cl_2	7.14	7.28	0.14	4.10	0
16	3-Cl-4-$COOCH[CH_2C(CH_3)CH_3]_2$	7.43	7.29	0.14	5.15	1.82
17	4-$O(CH_2)_{11}CH_3$	7.24	7.27	0.03	6.93	0

[a] Calculated by using eq 17. [b] The log P values were all experimentally measured in the octanol/water system except 17, which was calculated (via eq 13).

Table II. Parameters Used in the Derivation of Equations 16-20 for Inhibition of Chloroplasts by X-$C_6H_4NHCON(OCH_3)CH_3$

compd	X	obsd pI_{50}	calcd[a] pI_{50}	$\lvert\Delta pI_{50}\rvert$	log P	BR
1	H	4.49	4.55	0.06	1.29[b]	0
2	4-NO_2	4.53	4.23	0.30	1.74[b]	1.82
3	4-NH_2	2.98	3.44	0.46	−0.16	0
4	4-$OCH_2C_6H_5$	6.07	6.44	0.36	3.11[b]	0
5	4-$OCH_2CH_2C_6H_4$-4′-Me	7.16	6.86	0.30	3.81[b]	0
6	3-t-C_4H_9	6.16	5.50	0.66	3.21[b]	0
7	3-F	5.58	4.74	0.84	1.68[b]	0
8	3-CN	3.66	4.25	0.60	1.34[b]	0
9	3-$OCH_2C_6H_5$	6.13	6.22	0.09	3.11[b]	0
10	3-CH_2OH	4.41	3.85	0.56	0.62[b]	0
11	3-OH	4.19	3.98	0.21	0.82[b]	0
12	3-NO_2	4.23	4.47	0.25	1.64[b]	0
13	3-CF_3	5.12	5.64	0.51	2.64[b]	0
14	4-$CH=C(CN)_2$	4.25	4.95	0.70	2.19[b]	0
15	4-NHC_2H_5	4.50	4.44	0.06	0.83[b]	0
16	4-$N(C_2H_5)_2$	3.93	4.36	0.43	2.23[b]	10.3
17	4-$COCH_3$	3.99	3.86	0.13	1.22[b]	1.82
18	4-Br	5.73	5.51	0.21	2.38[b]	0
19	4-F	5.02	4.69	0.34	1.46[b]	0
20	4-$COOC_2H_5$	4.24	4.94	0.69	2.24[b]	1.82
21	4-COC_6H_5	5.94	5.53	0.41	2.86[b]	1.82
22	4-$CH(CH_3)_2$	6.21	5.33	0.88	2.95[b]	5.65
23	4-$NHSO_2CH_3$	3.33	3.33	0.01	0.14[b]	0
24	4-c-C_6H_{11}	5.07	4.70	0.37	4.08[b]	13.4
25	4-OCH_2CH_2-c-C_6H_{11}	6.69	7.02	0.33	4.46[c]	0
26	4-$OCH(CH_3)CH_2$-c-C_6H_{11}	6.16	7.01	0.85	4.51[b]	0
27	3-$O(CH_2)_4OC_6H_5$	7.04	6.87	0.17	3.86[c]	0
28	4-$O(CH_2)_4OC_6H_5$	6.30	6.87	0.57	3.86[c]	0
29	3-OCH_2CO-adamantyl	6.64	6.61	0.03	3.79[c]	0
30	4-OCH_2CO-adamantyl	7.08	6.72	0.36	3.53[c]	0
31	3-Cl-4-OCH_2CO-adamantyl	7.16	6.79	0.37	4.48[c]	0
32	III (see the text)	3.55	4.29	0.74	3.24[c]	12.7
33	3-Cl-4-$OCH_2C_6H_5$	7.04	6.61	0.43	3.80[c]	0
34	4-OCH_2CH_2-α-naphthyl	6.81	7.07	0.26	4.81[c]	0
35	4-$(CH_2)_3C_6H_5$	6.76	6.99	0.23	4.67[c]	0
36	4-$(CH_2)_4C_6H_5$	7.06	7.05	0.01	5.15[c]	0
37	4-$(CH_2)_3C_6H_4$-4′-Cl	7.56	7.07	0.49	5.35[c]	0
38	4-$(CH_2)_3C_6H_4$-C_6H_5	7.19	7.18	0.01	6.54[c]	0

[a] Calculated using eq 22. [b] log P values experimentally determined. [c] log P values calculated via eq 14.

the number of compounds upon which the equation is based, r is the correlation coefficient, and s is the standard deviation from the regression. The figures in parentheses are for the construction of the 95% confidence limits. A number of the equations have been rederived to place them on a comparable log P basis and to establish confidence limits on all parameters. In those instances where experimental log P values were not available we have calculated them from log P of the parent compound by the addition of $\sum\pi$ for the substituents using π from the benzene system (Hansch and Leo, 1979). The parameter P is the octanol/water partition coefficient, which is our operational quantitative scale for hydrophobicity (Hansch and Leo, 1979). The Hammett constant (Hansch and Leo, 1979) σ has been used to assess the electronic effects of substituents on inhibitor potency.

Equations 1a–5a have been calculated by using log P values derived from the following regression equations:

dimethyphenylureas (Table I)

$$\log P_{measd} = 0.97\ (\pm 0.06)\ \log P_{calcd} + 0.52\ (\pm 0.25)\sigma + 0.23\ (\pm 0.15) \quad (13)$$

$$n = 16 \qquad r = 0.995 \qquad s = 0.149$$

methoxymethylphenylureas (Table II)

$$\log P_{measd} = 0.97\ (\pm 0.05)\ \log P + 0.70\ (\pm 0.14)\sigma + 0.19\ (\pm 0.12) \quad (14)$$

$$n = 25 \qquad r = 0.993 \qquad s = 0.152$$

Equations 13 and 14 were derived by using measured octanol/water partition coefficients. These equations are based on the work of Fujita et al. (1964), who showed that the major difference in log P or π values on substituted benzenes was due to the electronic effect of substituents on groups containing lone pair electrons attached directly to the benzene ring. The calculated log P values in eq 13 and 14 were obtained simply by adding π constants to log P for dimethylphenylurea (0.98) or to log P for methoxymethylurea (1.29). Such a simple approach does not take into account the electronic effect of the substituent on the urea moiety that affects the log P value.

Note that in eq 1–4, but not in eq 5, better correlations (compare standard deviations) are obtained by using the corrected log P values. Also in eq 1a and 2a the σ term is no longer significant at the $\alpha = 0.05$ level. In eq 4a the σ term is still significant, although confidence limits on its coefficient are very large. The σ term is probably artifactual.

There are really no significant differences between the parameters of eq 13 and 14; hence, we believe that these equations will be generally useful for obtaining better calculated log P values for aromatic amides, ureas, and carbamates.

In the case of eq 13 we did not measure log P for one congener [4-0$(CH_2)_{11}CH_3$]. This compound would be so difficult to measure that the measured value would be no better (maybe worse) than the calculated value.

With eq 14 there were more of these highly lipophilic compounds (13) for which experimental values of log P were not obtained.

The results of this survey of QSAR of photosystem II inhibitors confirms our first study (Hansch and Deutsch, 1966), which found the hydrophobic effect of substituents to be the dominant feature in the structure–activity relationship. In addition to the hydrophobic portion of the molecule, a bioactive functional group such as –NHCON-$(CH_3)_2$ must be present. In every instance the functional group contains a nitrogen atom (usually an amide) with considerable double-bond character.

Recent evidence (van den Berg and Tipker, 1982) suggests that the diphenyl ethers of eq 10 do not react at the same site as the other compounds upon which eq 1–11 are based. In fact, the different coefficient with log P in eq 10 suggests a different kind of hydrophobic effect is involved with the ethers. Hence, we shall not consider these substances further.

The coefficients with the 11 better log P terms have an average value of 1.04, suggesting that the inhibitors partition into the lipophilic portion of the chloroplast in much the same way they partition into octanol. In the above equations the intercepts provide a means for comparing the intrinsic activity of the various bioactive functions. The value of the intercept is determined by the sensitivity of the experimental system and the potency of the bioactive functional group of a given set of congeners. Assuming all of the above inhibitors are operating on the same active site and that *experimental conditions are the same*, the larger the intercept, the more potent the functional group. The I_{50} values do depend on the amount of chloroplasts used and this no doubt will vary from laboratory to laboratory. We would estimate that this could result in a difference of ±0.5 in intercepts from different laboratories on the basis of our experience with the action of inhibitors acting on bacterial cells (Hansch and Dunn, 1972). Comparing intercepts amounts to comparing systems whose log P and σ values are 0. Hence, since comparison is being made between isolipophilic congeners, differences in the intercepts reflect the stereoelectronic interaction of the bioactive functional group with its counterpart in the active site of photosystem II.

The relative potency of the inhibitors on an isolipophilic basis along with the intercepts from their QSAR are triazinones (4.84) > azidotriazines (4.27) > phenylmonomethylureas (4.10) > alkoxyuracils (3.78) > dimethyureas (3.20, 2.72) > arylpyrrolones (3.15) > ethyl carbamates (1.13) > isopropyl carbamates (0.58) > benzimidazoles (0.56) > isobutyranilides (0.35). This ordering gives some perspective but cannot be taken literally since different experimental conditions were employed by the various laboratories. The intercepts for the dimethylurea equations of 3.20 and 2.72 are lower than what we have found (3.77) in eq 17.

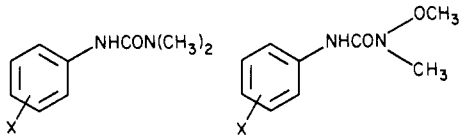
We find that there is a roughly even spread in the intrinsic activity from 0.35 to 4.27. Can we assert that all of these classes of inhibitors are acting at the same site in the same way? Although QSAR does not allow us to make such distinctions it does provide some clues about so-called nonspecific toxicity that is primarily the result of hydrophobic perturbation with little or no assistance from a functional group. The most nonspecific types of toxicity caused by alcohols and other simple neutral molecules yield equations linear in log P with intercepts from about 0 to 1.0 (Hansch and Dunn, 1972; Dunn and Hansch, 1974). These results strongly suggest that the benzimidazoles of eq 6 may be operating by a purely nonspecific mechanism such as one might expect from a set of ordinary alcohols, esters, ketones, etc. That is, one would expect almost any very lipophilic molecule to have an inhibitory effect on the Hill reaction. In fact, recent work by Yukimoto (1983) shows that simple lipophilic alcohols, acids, and halobenzenes are Hill reaction inhibitors.

A study of the above equations brings up a number of interesting questions about photosystem II inhibitors. Just how far does the linear relationship between pI_{50} and log P go? Would all of the different classes of inhibitors show a similar break in this linear relationship? Some equations have a term in σ, and some do not. What is the meaning of this? Since most of the work upon which eq 1–12 is based is with small substituents, we must find out the maximum size of groups that is still effective.

It has long been recognized that bioactivity cannot be increased indefinitely simply by making compounds more and more lipophilic. Sooner or later the linear relationship between log activity and log P or π breaks down (Hansch and Fujita, 1964; Hansch and Clayton, 1973; Kubinyi and Kehrhahn, 1978; Kubinyi, 1979). For only two of the above equations (11, 12) can this point (log P_0) be estimated. In formulating the QSAR for these two equations we have used the bilinear model (Kubinyi and Kehrhahn, 1978; Kubinyi, 1979), rather than the parabolic model [log P – (log $P)^2$ (Hansch and Leo, 1979; Hansch and Clayton,

1973)] since the former gives a better fit for the data. Since relatively few compounds with superoptimal lipophilic substituents were tested we cannot place narrow confidence limits on log P_0 for eq 11 (4.60 ± 2.4). Since the slope of the right-hand side of the bilinear model in eq 12 (1.12–1.15 = –0.03) is so close to zero we cannot place confidence limits on log P_0 of 5.31. Moreover, this is not an accuratte representation of reality; a careful plot of the data (Brown et al., 1981) shows a *sharp* break at log P of about 4. The bilinear relationship rounds between the two linear sections of the curve (which is what one normally observes in experimental data) and hence does not fit the data well. This yields an unrealistically high log P_0. However, these results do suggest a very large hydrophobic region with which inhibitors can react and that in all but two examples (eq 11 and 12) it should be possible to make more potent inhibitors by adding more hydrophobic moieties to the parent structures.

In attempting to obtain answers to the above questions we have prepared and tested in the Hill reaction two classes of inhibitors.



RESULTS

Phenyldimethylureas (I). From the data in Table I we have derived equations 15–17 for compounds of type I inhibiting the Hill reaction using measured log P values.

$$pI_{50} = 0.56\ (\pm 0.18)\ \log P + 4.24\ (\pm 0.58) \quad (15)$$

$n = 17 \quad r = 0.859 \quad s = 0.616 \quad F_{1,15} = 42.4$

$$pI_{50} = 0.88\ (\pm 0.37)\ \log P - 0.61\ (\pm 0.63)\ \log (\beta \cdot 10^{\log P} + 1) + 3.78\ (\pm 0.72) \quad (16)$$

$n = 17 \quad r = 0.897 \quad s = 0.571 \quad F_{2,13} = 2.26 \quad \log \beta = -2.91$

$$pI_{50} = 0.91\ (\pm 0.21)\ \log P - 1.08\ (\pm 0.58)\ \log (\beta \cdot 10^{\log P} + 1) - 0.12\ (\pm 0.07)\mathrm{BR} + 3.77\ (\pm 0.48) \quad (17)$$

$n = 17 \quad r = 0.952 \quad s = 0.414 \quad \log P_0 = 5.07 \quad \log \beta = -4.34 \quad F_{1,12} = 12.7$

The addition of a term in σ to any of the above equations did not produce a significant reduction in the variance. The stepwise development of eq 17 shows the relative importance of the different terms. Neither the addition of the BR term alone nor the bilinear log P term alone to eq 15 produces a significantly better equation; however, the addition of the two terms does yield a considerable improvement [$F_{1,12}\ \alpha(0.01) = 0.33$]. The log P values are experimentally determined except for one rather insoluble compound (17). Equation 18 has been derived by using calculated log P values (log P for the parent plus π for the substituents from the benzene solute system). Here, too, we find that the addition of a σ term does not improve the correlation.

$$pI_{50} = 1.18\ (\pm 0.29)\ \log P - 1.00\ (\pm 0.42)\ \log (\beta \cdot 10^{\log P} + 1) - 0.11\ (\pm 0.06)\mathrm{BR} + 3.80\ (\pm 0.42) \quad (18)$$

$n = 17 \quad r = 0.964 \quad s = 0.356 \quad \log \beta = -2.56$

Hence, there seems to be no evidence that in the case of spinach chloroplasts substituents show an electronic effect on inhibition. The coefficient with BR is essentially the same as for eq 17; however, the log P terms are slightly different and since the right-hand portion of the bilinear eq 18 is negative (1.18 – 1.00 = +0.18) log P_0 cannot be estimated. Although we can estimate log P_0 using eq 17, it is so high and we have so few log P values above 5 that we cannot place confidence limits on log P_0.

The BR term is a special steric parameter for 4-substituents. Attempts to use Taft's E_s parameter (Unger and Hansch, 1976) or Verloop's (1972, 1981) steric parameters were not as successful as BR in accounting for the unexpectedly low activity of congeners with a branch on the atom attached to the 4-position of the phenyl ring. Groups such as 4-$CH(CH_3)_2$ and 4-cyclohexyl all are less active than expected from log P alone. This lower activity could not be accounted for by any of the known electronic parameters (Hansch and Leo, 1979). A rough steric parameter was formulated for substituents of the type $-Y(R_1)R_2$ by assigning the smaller of the two R groups the value of its molar refractivity (Hansch and Leo, 1979). There are four such examples in Table I that are poorly fit unless this correction is employed. Although molar refractivity (MR) has often been used (Agin et al., 1965; Smith et al., 1982) in correlation work since its introduction (Pauling and Pressman, 1945), to our knowledge it has not been employed in this fashion before.

We interpret the negative coefficient with BR to mean that there is some kind of protuberance near the active site of photosystem II that makes a deleterious contact with branched groups attached to the phenyl ring. It is assumed that the substituent may orient itself so as to minimize this effect by placing the smaller of the two R groups in the unfavorable position.

This reduced activity does not occur with bulky or branched substituents in the 3-position nor does it occur when branching is removed from the phenyl ring by two or more atoms. Although BR is an admittedly crude parameter it should be helpful in studying this restricted region of photosystem II.

Phenylmethoxymethylureas (II). From the data in Table II we have derived eq. 19–22 for the action of the methoxyureas II in the Hill reaction using measured log P values for 25 congeners and log P calculated via eq 14 for the other 13.

$$pI_{50} = 0.71\ (\pm 0.15)\ \log P + 3.47\ (\pm 0.50) \quad (19)$$

$n = 38 \quad r = 0.845 \quad s = 0.721 \quad F_{1,36} = 90.1$

$$pI_{50} = 0.73\ (\pm 0.12)\ \log P - 0.12\ (\pm 0.06)\mathrm{BR} + 3.61(\pm 0.42) \quad (20)$$

$n = 38 \quad r = 0.902 \quad s = 0.592 \quad F_{1,35} = 18.4$

$$pI_{50} = 0.96\ (\pm 0.19)\ \log P - 0.88\ (\pm 0.55)\ \log (\beta \cdot 10^{\log P} + 1) - 0.14\ (\pm 0.05)\mathrm{BR} + 3.22\ (\pm 0.44) \quad (21)$$

$n = 38 \quad r = 0.927 \quad s = 0.530 \quad \log \beta = -3.91 \quad F_{1,33} = 10.6$

$$pI_{50} = 1.05\ (\pm 0.20)\ \log P - 0.96\ (\pm 0.49)\ \log (\beta \cdot 10^{\log P} + 1) - 0.17\ (\pm 0.05)\mathrm{BR} - 0.61\ (\pm 0.46)\sigma + 3.20\ (\pm 0.44) \quad (22)$$

$n = 38 \quad r = 0.940 \quad s = 0.490 \quad \log \beta = -3.46 \quad F_{1,32} = 6.55$

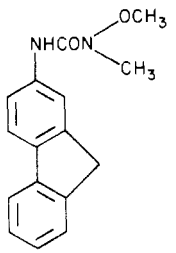
The stepwise development of eq 22 shows that all equations are highly significant [$F_{1,30}\ \alpha(0.01) = 7.56$; $F_{1,30}\ \alpha(0.001) = 13.3$].

Neither eq 21 nor 22 can be used to calculate log P_0 since in each case the right-hand side of the bilinear curve has a positive slope. Inspection of the data in Table II suggest that log P_0 must lie in the region of 5–6. We have not been able to sharply define log P_0 for either eq 17 or eq 22 but both sets of results suggest log P_0 in the region 5–6. Because of the great difficulty in working with such insoluble compounds it may not be possible to accurately define log P_0.

For both eq 17 and eq 22 we have found that replacing the bilinear log P terms with the log P – (log P)^2 combination results in a *slightly* poorer correlation in terms of the standard deviation. A definite advantage of the bilinear model is that the coefficient with the log P term can be compared directly with log P terms of the simple linear equation while this is not possible with the parabolic model.

An interesting aspect of eq 22 is its term in σ with a negative coefficient. Although this term is not important, it is the last term to enter eq 22 and accounts for only 2% of the variance in pI_{50}, it is significant at the level of α = 0.025. Since we do not find such a term for the dimethylphenylureas or with eq 1a, 2a, or 3a, we tend to view this term as being an artifact. It is not an artifact of the collinearity since r^2 for σ vs. log P is 0.05 and for σ vs. BR it is 0.05. The collinearity between the variables of interest for eq 17 is similar. In any case its importance is so low that it is of almost no value in the design of new herbicides. However, see eq 23.

The agreement between the BR terms in eq 17 and 22 is reasonably good considering that there are only four congeners supporting this term in eq 17. The eight branched side chains in congeners of eq 22 support this parameter more firmly. In the calculation of BR for symmetrical substituents such as cyclohexyl and phenyl we have used $^1/_2$MR of the complete substituent. For esters or ketones we have used MR for 0. For the fluorenyl analogue



it was assumed that the CH_2 unit between the two rings would orient away from the restricted region of the active site so that BR of $^1/_2$MR for phenyl was used.

Note that a branching factor has not been assigned to **23** (4-$NHSO_2CH_3$). Since this substance is well fit without it, we assume the large sulfur atom holds its attached oxygen atoms sufficiently far from the steric factor in the receptor site so as to avoid a bad contact.

The reasonably good fit of 3- or 4-$O(CH_2)_4OC_6H_5$ groups indicates that hydrophobic space is extremely large and extends for a distance of 8–10 carbon atoms from the phenyl ring, assuming the side chain conformations to be in the extended form. Hydrophobic space must be rather unconstrained since the bulky adamantyl group is a well fit as the planar ring compounds.

A fourth check on log P_0 comes from the recently reported eq 23 from researchers at Sumitomo in Japan (Takemoto et al., 1982).

$$\text{p}I_{50} = 1.32\pi - 0.14\pi^2 - 0.79\sigma + 4.57 \qquad (23)$$

$$n = 36 \quad r = 0.937 \quad s = 0.425 \quad \pi_0 = 4.71$$

Equation 23 correlates inhibition of chloroplasts by compounds of type II. It is based on 36 congeners containing the following substituents: H, F, Cl, Br, I, CF_3, CH_3, C_2H_5, C_3H_7, OCH_3, OC_2H_5, CN, NO_2, and $-O(CH_2)nC_6H_5$, n = 1, 2, 3, 4, 6, and 8. The results used to derive eq 23 were obtained by using radish chloroplasts. Unfortunately, since the data from which eq 23 was derived have not been reported, we cannot make a satisfactory comparison with our results. They have used the parabolic model, which may explain why their coefficient with π is considerably above 1. Since they did not report confidence limits on their parameters we cannot tell how important their σ term is. Note that the coefficient with σ is negative and about the same size as that of eq 22. This is especially interesting since the substituents upon which eq 22 and 23 are based are rather different. The Japanese set does not contain any bulky groups such as adamantyl nor does it contain any with branching on the α-carbon except for NO_2.

By substituting π = log P – 1.29 into eq 23 and then taking the partial derivative, we calculate log P_0 to be 6. Since confidence limits cannot be placed on this figure we cannot be sure that it is really outside the range estimated by eq 17 and 22.

Activity reaches a maximum at about pI_{50} of 7. This could be the point where the inhibitors begin to extend beyond lipophilic space or it could be the point where stoichiometric binding is occurring. Such lipophilic inhibitors may be so potent that one is simply titrating the active sites. Getting a firm figure on log P_0 will be difficult because such lipophilic compounds are so insoluble that they are on the edge of the limit of testing. Whether in fact these substances possess the calculated log P values is also a question that will be difficult to answer because of the great difficulty in measuring log P of >5.5.

Several points are not well fit by eq 22. One of the most unexpected is 3-F, which is about 7 times more active than eq 22 predicts. The 3-fluoromonomethylurea was tested by Seewald et al. (1678) and found to be more active than eq 5 predicts. Exactly what this high activity is to be attributed to is not clear. The steric and hydrophobic effect of F are so small that they can be discounted completely, and the normal electronic effect characterized by σ appears to be of little importance. The strong inductive effect may induce a dipole of ideal character for interaction with a dipole in the active site. This would have to involve very specific features since neither the 3-NO_2 or 3-CN congeners are badly fit.

One might take advantage of this point by placing an F in position 3 and a highly lipophilic group in position 4.

Attempts to test a 4-SO_2NH_2 analog were unsuccessful. Its activity was so low (as expected for the negative $\pi_{SO_2NH_2}$) and its solubility so poor that an I_{50} could not be established.

DISCUSSION

Systematic studies of the inhibition by phenylureas of the light reactions of photosynthesis began in the 1950s with the work of Wessels and van der Veen (1956) and Bishop (1958). These investigators showed that DCMU (diuron), 3-(3,4-dichlorophenyl)-1,1-dimethylurea, was reversibly absorbed onto an active site essential for photosynthesis and that the stoichiometry of binding was about 1 DCMU/100 chlorophyll molecules. Later studies (Tischer and Strotmann, 1977) suggest that about 1/300–500 is a more likely figure. The reversible nature of the binding demonstrated by Wessels and van der Veen was later confirmed by Izawa and Good (1965). Although Wessels and van der Veen (1956) suggested that both the

lipophilic and electronic properties of the substituents are important in the inhibition process, our results as well as others (Good, 1961) show that the lipophilic character of the substituents is of overwhelming importance in the amide-type inhibitor.

Two aspects of inhibitors have attracted the attention of researchers: the structure–activity relationships, the results of which are summarized in eq 1–23, and the nature and location of the site where action occurs. Major studies by Joliot (1965), Forbush and Kok (1968), Bouges-Bocquet (1973), and Velthuys and Amesz (1974) mainly using inhibitor and fluorescence studies of electron transport showed that phenylureas and triazines both work at the level of photosystem II in the thylakoid membrane of the chloroplasts. By comparing the similar fluorescence due to the back reactions of electrons between dithionite-reduced and DCMU-inhibited electron transport chains in thylakoids, Velthuys and Amesz (1974) suggested that DCMU worked between the primary electron acceptor of photosystem II, a quinone-like molecule called X-320, and B, the secondary quinone acceptor that donates its electron to the plastoquinone pool. They suggested that DCMU makes B harder to reduce by some uncertain spatial changes that affect B's midpoint potential.

Recent studies by Renger et al. (1976), Oettmeier et al. (1980), Gardner (1981), Mattoo et al. (1981), and Arntzen et al. (1979), using trypsin digestion and photoaffinity labeling, have determined that DCMU binds to a 32K dalton protein "shield" on the outer surface of the thylakoid membrane in photosystem II. Renger (1976) first proposed the protein shield theory to explain the fact that trypsin treatment of thylakoid membranes eliminated the inhibitory effect of DCMU on oxygen evolution if ferricyanide were supplied as an electron acceptor [see also Tischer and Strotmann (1977) and Trebst (1979)]. In addition, the effect of CO_2, which normally enhances electron transport (Govindjee and van Rensen 1978; Siggel et al., 1977), was lost on trypsin treatment (Govindjee and van Rensen, 1978). This protein shield appears to make up a large proportion of the proteins in the thylakoids (Mattoo et al., 1981).

Recent photoaffinity labeling studies (Khanna et al., 1981; Pfister et al., 1979; Pfister and Arntzen, 1979), using photoactive triazines, identified a 32–34K dalton protein as the specific binding site for photosystem II inhibitors. Miller and Cushman (1979), using freeze–fracture etching techniques and electron microscopy, presented evidence that photosystem II is the larger particle of two particles present on the thylakoid membrane. Mutants lacking only photosystem II activity also lacked the large particles. The protein shield is probably a component of the 140-Å particle seen by Miller and Cushman. Tischer and Strotmann (1977), using data from radioactive triazine binding studies, showed that triazines and phenylureas compete for the same binding sites. Pfister and Arntzen (1979) followed this work with tests on triazine-resistant mutant weeds and discovered that these mutants were still susceptible to DCMU but have somewhat reduced susceptibility to other photosystem II inhibitors. They conclude that there appears to be overlapping regions recognized by Tischer and Strotmann in competitive assays. The proximity of these domains in the same protein shield is further supported by Pallett and Dodge (1979), who found that trypsinated chloroplasts simultaneously lost sensitivity to phenylureas, uracils, and triazinones.

The significance of DCMU inhibition was greatly enhanced upon the discovery of the possible allosteric changes of the protein shield as evidenced by the work of Giaquinta et al. (1973), Khanna et al. (1981), and Mattoo et al. (1981). Giaquinta et al. showed that the quantity of diazonium benzenesulfonate bound to thylakoid surface proteins changed when the thylakoids were illuminated. Kahnna et al. (1981) studied the effect of CO_2 depletion and DCMU on electron transport and found that the I_{50} of DCMU did not change although the total quantity of DCMU bound decreased, which suggests that CO_2 enhances DCMU binding allosterically. The CO_2 depletion results in a total inactivation of part of the total number of photosystems rather than a partial inactivation of all photosystem II centers. Thus, the binding of CO_2 to its site of action may determine whether a given photosystem is on or off (Stemmler, 1978). It was proposed that CO_2 was an allosteric modulator of the protein shield that allosterically regulates electron flow from X-320, the primary electron acceptor, to B, the secondary acceptor, and DCMU therefore forces an allosteric change that shuts off electron transport (Stemmler, 1978).

We would expect that a conformational change in a surface membrane produced by the partitioning of a lipophilic compound into the protein or into the membrane holding the protein would change the exposure of some of its residues, resulting in a different cleavage pattern on trypsin digestion.

For Hill reaction inhibitors two different reproducible patterns were found for the trypsinated protein, one with and one without DCMU (Matto et al., 1981). Another important finding was that trypsin digestion patterns of the 32K dalton protein were different for incubation in the light and in the dark, which suggests that this protein may also undergo light-activated conformational changes. Thus, recent work points to the existence of fine-tuning control mechanisms in photosynthetic electron transport that have gone unsuspected.

This mechanism of affecting electron transport reminds one of some recently proposed ideas for the mechanism of action of general anesthetics (Briggs, 1981). It has been observed that anesthetics such as ether, halothane, and barbiburates have two structural requirements for potency—a lipophilic region and rather nonspecific hydrogen-bonding character (Hansch et al., 1968, 1975). These features appear to work in concert on a lipid–protein membrane to disrupt nerve transmission in a relatively nonspecific way. By nonspecific we mean that a large number of rather loosely defined compounds containing these features will work. It is interesting that the amide linkage appears to be very effective in both the inhibition of nerves and photosystem II. Both mechanisms appear to be allosterically operative.

The QSAR of eq 1–23 clearly establish two structural features required for inhibitors of photosystem II: an intrinsically active functional group such as a methylurea or triazine unit and a large hydrophobic moiety.

Only one specific steric effect has been established: that of branching of substituents in the para position of the phenyl ring of the urea-type inhibitor.

Since the hydrophobic region is so large and apparently undemanding sterically, it seems likely that many kinds of more or less nonspecific lipophilic organic compounds could be taken up and that such molecules could cause conformational changes in the so-called protein shield. In an attempt to classify (Hansch and Dunn, 1972; Dunn and Hansch, 1974) the specificity of organic compounds by the intercepts of correlation equations, it was found that the most nonspecific QSAR had intercepts of about 0 ± 0.5. Membrane perturbations such as the change in resistance of a black lipid membrane by ROH gave an intercept of

−0.51, and the disaggregation of lipid coated 0.3-mm glass beads had the same intercept. The 50% inhibition of oxygen consumption by alcohols of red cells had an intercept of 0.11, and the I_{100} for frog heart intercept was found to be 0.13. These intercepts came from simple linear log P equations with slope near 1.

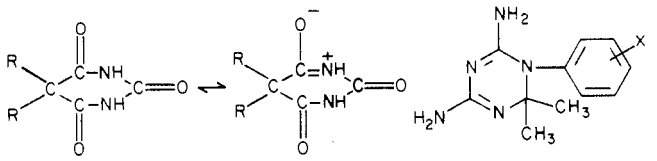
Several of the photosystem QSAR are quite similar to these so-called nonspecific QSAR. The benzimidazoles (eq 6 with an intercept of 0.56) and the anilides (eq 3 with intercept of 0.35) are certainly in this class. The isopropyl carbamates with an intercept of 0.58 might also be in this class, although in this case the branched isopropyl groups may be producing a depressing steric effect.

The ethyl carbamates (eq 2a, intercept = 1.13) may be showing some small degree of specificity. For comparison there are examples of the hemolysis of red cells (Hansch and Dunn, 1972) by alcohols or other simple *neutral* organic compounds with intercepts of 1.5. Given the ease of producing conformational changes in the protein shield, it is not surprising that the anilides (eq 3) show low activity. In fact, the very lipopholic diphenyl ethers (eq 10) do not have a very large intercept and show other signs that they do not conform to the interaction pattern of other typical Hill reaction inhibitors. Lacking a proper polar moiety to aid in the orientation of the inhibitor in the lipid pool of the chloroplast for maximum effect, they may operate by purely nonspecific hydrophobic binding. From the above discussion it seems likely that almost any molecule binding in the lipid site of photosystem II could bring about conformational changes in the protein shield. Thus, QSAR analysis can in the early stages of a structure–activity study assist in making an *estimate* of the type of inhibitor under study.

Specificity becomes clear with the phenylureas that have intercepts in the 3–4 range. One needs the QSAR to assess specificity; simply having a high pI_{50} value for a single compound is not enough. Since lipophilicity increases potency so greatly one must make comparison on an isolipophilic basis.

Some time ago it was pointed out (Hansch, 1969b) that the one structural feature all Hill reaction inhibitors appeared to possess was a polarizable >C=N– moiety, which was postulated to interact with a peptide moiety. The QSAR for eq 1–11 show that the most specific inhibitors contain this π bond system.

Since the double requirement of an easily polarizable π bond and hydrophobic moieties was postulated to be the essential ingredient for photosystem II inhibitors, the idea has been more closely examined by others and found to hold (Hansch and Deutsch, 1966; Büchel and Draber, 1969; Gabbott, 1969; van Assche and Carles, 1982). Such a polarizable double bond is a necessary but not sufficient condition. For example, it occurred to us that barbiturates IV have a urea moiety as well as the necessary hydro-



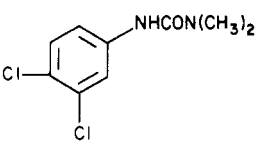
IV V

phobicity. However, testing barbiturates with log P values in the range 1.42–1.65, we were unable to detect Hill reaction activity in this class of compounds. Possibly more lipophilic congeners would show activity.

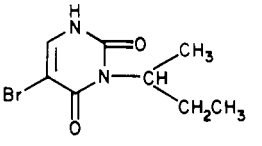
On the other hand we found, in the present study, that derivatives of V are quite potent inhibitors when X is highly lipophilic.

DESIGN OF HERBICIDES

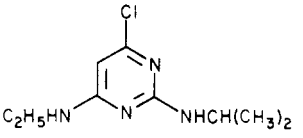
Of course one of the reasons so much work has been done on photosystem II inhibitors is that some of these compounds are important herbicides. However, it has long been recognized that simply being active in the Hill reaction is not enough to ensure that a compound will show good herbicidal activity. The ability of a compound to penetrate plant tissue as well as its stability to light, air, and metabolism are also important factors. Since penetration is so dependent on log P it seemed of interest to compare the hydrophobicities of some of the most successful commercial herbicides that inhibit photosystem II:



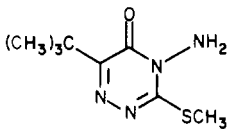
diuron, log P = 2.68
(Briggs, 1981)



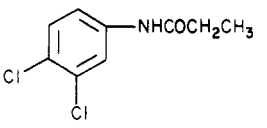
bromacil, log P = 2.02
(Metcalf and Lu, 1978)



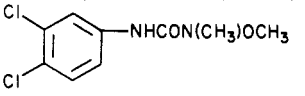
atrazine, log P = 2.60



metribuzin, log P = 1.70
(Draber et al., 1968)



propanil, log P = 3.50
(calculated value)

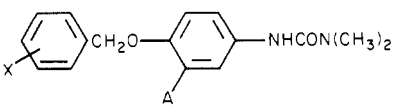


linuron, log P = 2.76
(Briggs, 1981)

The mean value for the above log P is 2.5. The spread in values is rather narrow considering that different basic structures are involved and that so many factors (including most of synthesis) must be considered in making the final selection of a particular congener for market.

Recently Brown et al. (1981) have derived an interesting structure–activity relationship for the alkoxyuracil inhibitors acting on plants that is bilinear with respect to log P. They used −log T_{90} for an end point, and under these conditions one can estimate log P_0 from their plot as being about 1. Since the actual test values were not reported we cannot treat the data statistically. Their log P_0 for whole plants is about 3 units less than their log P_0 for the Hill reaction.

Recent work by Cross et al. (1983) sheds more light on this problem. Equation 24 comes from their study of ureas V acting on whole plants (wild mustard). Equation 24 is derived from an equation of Cross et al. (1983), which was formulated in terms of π rather than log P. Using log P values instead of π gives eq 24.



VI, A = Cl or H

$$\log (1/C) = 0.48\ (\pm 0.30) \log P - 0.86\ (\pm 0.34) \log (\beta \cdot 10^{\log P} + 1) - 5.10\ (\pm 1.8) E_R + 0.87\ (\pm 0.52) \quad (24)$$

$$n = 21 \quad r = 0.937 \quad s = 0.335 \quad \log P_0 = 2.10 \quad \log \beta = -1.98$$

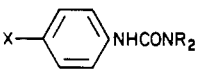
The free radical parameter E_R applies only to X. In eq

24, C is the molar concentration of urea killing 85% of the mustard plants and E_R is an electronic parameter for substituent effects on free radical reactions. The value of log P_0 in eq 24 is about 3 log units lower than the log P_0 values we have found for ureas in the Hill reaction. Hence, if one uses the Hill reaction to design the most potent inhibitors and moves to the study of whole plants, the results are bound to be poor. For example, an analogue of VI with log P = 5 and E_R = 0 would by eq 24 have a calculated log $(1/C)$ on whole plants of only 0.68 (log L mol^{-1} ha^{-1}) whereas a compound with log P of 2.1 would have a log $(1/C)$ value of 2 (i.e., 20 times more active). Because of the nature of the relationship expressed by eq 17 and 22, compounds with even greater values of log P than 5 would also show near maximum activity on photosystem II, but they would be extremely poor as herbicides on whole plants. An example from the data of Cross et al. (1983) is a urea with log P of 6.76. Its predicted log $(1/C)$ (eq 24) is −0.20 and its observed value if −0.24.

It is of course highly significant that the average log P for successful photosynthetic herbicides is 2.5 and log P_0 from eq 24 is 2.1. In line with this is the observation by Briggs et al. (1982) that log P_0 for the translocation of neutral compounds to barley shoots following uptake by roots centers is near 2. They note this to be true of other plant systems as well.

Armed with this information, plus the ability to estimate log P values of compounds before they are made and knowledge of the importance of the amide type linkage for specific interaction with photosystem II, one is able to go hunting for new herbicides in a somewhat more systematic way. The time has come when one can peruse a large data base of compounds, such as that of Chemical Abstracts Service, for novel structures containing the essential unsaturated nitrogen. The novel structures could be modified so that their calculated log P fell in the range of 2–3 if their activity in the Hill reaction was above 6.

Another point in the design of the photosynthetic inhibitor type is that one should try to maximize the hydrophobic interaction between herbicide and the very large hydrophobic area of the receptor. Simply increasing hydrophobic area of the herbicide fails by running up against the barrier of log P_0 in the whole plants. This could be offset by the introduction of groups with large negative π values in appropriate locations. Compound VII is a general suggestion.



VII

The long line in VII represents hydrophobicity (e.g., alkyl chains, arylalkyl chains, etc.). The variable substituents X and R could be used to modulate overall log P to hold it in the range 2–3. The group X would have to be placed far enough from the phenyl ring so that it falls outside of hydrophobic space in the receptor; otherwise, an unfavorable interaction would occur. One's first thought might be to consider charged groups for X and R. We believe, however, that noncharged groups with very negative π values would be easier to work with, at least initially. The behavior of charged groups in living systems is still poorly understood. However, properly balanced charged groups should work too. It is well-known that the negatively charged auxins are well transported in plant systems.

This approach to herbicide design is not without its pitfalls. There is still relatively little work of the type reported by Briggs et al. (1982), Brown et al. (1981), and Cross et al. (1983) on log P_0 in whole plants. The problems of metabolism and stability in the field are by no means simple and of course we know little about the molecular determinants of selective toxicity. It seems safe to say that QSAR will be of help in understanding these problems, but it means that some careful time-consuming science will have to be done. Some progress on selectivity is being made via QSAR in other living systems (Coats et al., 1981; Selassie et al., 1982) as well as in plants (Fujinami et al., 1974).

Resistance in plants to herbicides is also a difficult problem that is attracting more attention (Böger, 1981). Selassie et al. (1982) and Coats et al. (1981) have shown that QSAR can increase our understanding of resistance to drugs. Iwamura and Fujita (1982) have recently presented an interesting review of the use of QSAR in pesticide studies in Japan.

It is of interest to compare log P_0 of 2+ for herbicides with log P_0 for the nonspecific activity of general anesthetics that also have a log P_0 of 2+ (Hansch, 1981; Glave and Hansch, 1972). Some of the most potent widely used newer general anesthetics have log P values as follows: ethrane 2.10, methoxyflurane 2.21, and halothane 2.30. The most potent barbiturates also have log P values near 2 (Hansch, 1981). Two of the most widely used tranquilizers have similar log P values: librium 2.44 and valium 2.82. This is not to suggest that photosystem II inhibitors and CNS have the same mechanism of action. It suggests that optimum lipophilicity in the random walk of drugs, which act at a large lipophilic site, in plants and animals may be similar.

EXPERIMENTAL SECTION

Hill Reaction. Chloroplasts were isolated from fresh, washed, deveined, spinach leaves (100 g) that were homogenized with brief bursts in an ice-cold Waring blender with 400 mL of ice-cold grinding buffer consisting of 0.45 M sucrose, 0.001 M NaCl, 0.001 M $MgCl_2$, and 0.02 M Tricine at pH 7.4. The resulting homogenate was filtered through eight layers of cheesecloth and the filtrate divided among cold centrifuge tubes. The tubes were spun for 5 min at 6000g at 0.0–0.4 °C. The pellets were then resuspended in ice-cold grinding buffer and respun for 5 min at 6000g at 0.0–0.4 °C. These pellets were finally resuspended in a minimum of ice-cold medium of 0.15 M sucrose, 0.001 M NaCl, 0.001 M $MgCl_2$, and 0.02 M Tricine at pH 7. About 10–15 mL of medium was required. The low osmotic pressure of the resuspending medium serves to gently break the chloroplasts and expose the thylakoid membranes. This stock was kept on ice for the duration of the experiment. The chlorophyll content was determined by the method of Arnon (1949).

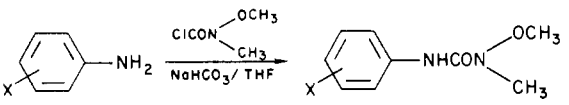
Our Hill reaction cocktail consisted of a reaction buffer (0.15 M sucrose, 0.001 M NaCl, 0.001 M $MgCl_2$, 0.02 M Tricine, pH 8.0), chloroplasts, potassium ferricyanide, and the appropriate inhibitor. Chloroplast stock was added in quantities of 20–60 μL depending on the stock concentration so that 60 μg of chlorophyll was present in the 3-mL reaction mixture. Ferricyanide was added as 15 μL of a 3.3×10^{-2} M stock, resulting in an initial concentration of 1.7×10^{-4} M. Inhibitors were added in quantities of 25–400 μL in distilled water or ethanol–water for the more insoluble ones. We and others (Franks and Lieb, 1982) have found that ethanol when kept in concentrations of less than 5% has no significant effect on the Hill reaction. We found that rates were slightly affected by ethanol concentrations of about 10%.

Reaction buffer was used in quantities necessary to result in a 3.0-mL reaction mixture. The quantities ranged from 2.65 to 2.95 mL of buffer. In all cases buffering was

sufficient to maintain the pH within 0.1 of 8.0. Recent evidence shows that DCMU inhibition is not dependent on pH 7.0 and 8.0 (Draber et al., 1968).

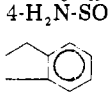
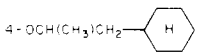
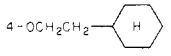
When an individual Hill reaction was run, the appropriate quantity of reaction buffer at room temperature was added to a 3-mL quartz cuvette with a Teflon-coated stir bar. Inhibitor in the appropriate quantity was added, the chloroplast stock solution was added, and the cuvette was inverted once for mixing by using a parafilm cover. The cuvette was then immediately placed in an Aminco DW-20 UV/VIS spectrophotometer with a magnetic stirrer and side illumination. The photomultiplier tube was protected from exciting light emission by a blue Corning CS4-96 filter. The recorder was started and zeroed at this time. It records the difference in absorption between 420 nm, a ferricyanide peak, and 375 nm, an isosbestic point estimated here for ferri- and ferrocyanide. Ferricyanide is injected after 1 min. The ferricyanide reduction was measured in the dark for an additional minute before the exciting light was turned on. The side illumination was filtered by a Corning CS2-64 red filter, which transmits the longer wavelengths of light necessary to excite the photoreaction center. The reduction of the ferricyanide was followed for 4–6 min. The I_{50} values for the 50% inhibition were calculated from a plot of the log of the inhibitor concentration vs. the percentage inhibition of the Hill reaction rate relative to that of the control.

Synthesis of Inhibitors. *Procedure I.* *N*-Methoxy-



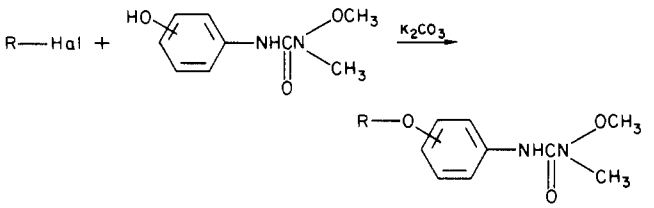
II

N-methylcarbamyl chloride (0.11 mol) was added dropwise to a mixture of 0.1 mol of X-substituted aniline, 0.15 mol of $NaHCO_3$ and 200 mL of THF at 20 °C and the mixture stirred overnight. After the solution has been filtered and concentrated, the residue was stirred with petroleum ether and white crystals separated.

X	melting point, °C	yield, %	footnote
4-NO_2	147–148	61	*a*
3-CN	93–95	50	*b*
3-CH_2OH	82–84	97	
3-NO_2	122–123	98	*b*
4-CH=C(CN)CN	227–230	12	*c*
4-$COCH_3$	120–121	77	
4-F	84–86	80	*d*
4-$COOC_2H_5$	73–75	79	
4-COC_6H_5	113–115	91	
4-c-C_6H_{11}	129–130	76	*e*
4-H_2N-SO_2-	220–223	75	*f*
	144–145	98	
	54–57	76	*b*
	108–109	68	*d*

[a] After recrystallization from 2-propanol. [b] After recrystallization from toluene. [c] After recrystallization from toluene/acetonitrile (1/1). [d] After recrystallization from cyclohexane. [e] After recrystallization from toluene/cyclohexane (1/1). [f] After being stirred overnight the precipitate was washed with water and dried.

Procedure II. A mixture of 0.1 mol of R-Hal, 0.1 mol of (hydroxyphenyl)urea, and 0.2 mol of K_2CO_3 was stirred

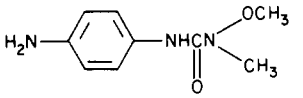


and refluxed in 200 mL of 2-butanone for 40 h. After filtration the solution was concentrated and the residue recrystallized.

R	mp, °C	yield, %	purification
4-CH_2CO-adamantyl	120–121	51	*a*
3-Cl-4-CH_2CO-adamantyl	158–160	94	*b*
3-CH_2CO-adamantyl	90–92	44	*c*
4-$O(CH_2)_4O$-C_6H_5	104–106	85	*b*
3-$O(CH_2)_4$-O-C_6H_5	72–74	85	*b*

[a] Column chromatography over silica gel with toluene. [b] Washing with petroleum ether. [c] Recrystallization from cyclohexane.

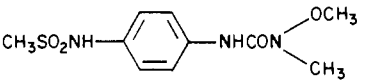
Special Compounds. A mixture of 162 g of *N*-meth-



A

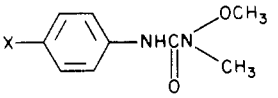
oxy-*N*-methyl-*N'*-(4-nitrophenyl)urea (see Procedure I), 8 g of palladium charcoal (5%), and 2000 mL of THF was hydrogenated under 1.1 bar of hydrogen pressure at 20–25 °C until 51.5 L of hydrogen was absorbed. After filtration and concentration the residue was chromatographed over silica gel with toluene. A total of 80 g (57% yield) of white crystals of melting point 76–78 °C was obtained.

A total of 10.7 g of CH_3SO_2Cl was added dropwise to a



B

mixture of 15 g of A, 8.4 g of $NaHCO_3$, and 100 mL of THF at 20 °C, and the mixture was stirred overnight. The precipitate was isolated and washed with water. A total of 14.3 g (68% yield) of white crystals of melting point 181–183 °C was obtained. A mixture of 30 g of A, 23.3 g



C, X = NHC_2H_5
D, X = $N(C_2H_5)_2$

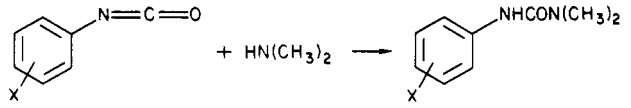
of triethylamine, 35.6 g of $(C_2H_5O)_2SO_2$, and 200 mL acetonitrile was refluxed for 5 h. The mixture was then poured into a mixture of H_2O/NaOH/ether. After separation, the organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was chromatographed over silica gel with toluene. After evaporation of the first fractions pure D (3 g, 7.8% yield) of melting point 108–110 °C was obtained. By evaporation of the following fractions pure C (10 g, 29.2% yield) of melting point 93–95 °C was obtained.

The details on the synthesis of the congeners containing 4-$CH_2CH_2CH_2C_6H_5$, 4-$(CH_2)_4C_6H_5$, 4-$(CH_2)_3C_6H_4$-4'-Cl, and 4-$(CH_2)_3C_6H_4$-C_6H_5 are contained in European Patent

41 145 (Schirmer et al., 1981).

The following derivatives of II are known compounds; H, 23838-30-4; 3-OH, 4849-46-1; 3-NO_2, 7159-98-0; 3-NH_2, 39938-79-9; 3-CF_3, 2164-17-2; 3-$OCH_2CH_2C_6H_5$, 70859-35-7; 3-$COCH_3$, 42865-65-6; 4-COC_6H_5, 61706-06-7; 4-$C(CH_3)_3$, 32745-69-0; 4-F, 332-33-2; 4-$CH(CH_3)_2$, 34123-59-6; 3,4-Cl_2, 330-54-1.

Congeners I were prepared according to the reaction



N,N-Dimethylamine (0.055 M) in 20 mL of toluene was added dropwise to a well-stirred solution of 0.05 M X-substituted phenylisocyanate in 30 mL of toluene at 0 °C. After the mixture was stirred overnight, the white precipitate was removed by filtration, washed with cold toluene, and dried.

X	mp, °C	yield, %
3-$COCH_3$	138-140	95
3-*n*-C_4H_9	99-100	86
3-$OCH_2C_6H_3$-2',4'-Cl_2	136-138	85
4-c-C_6H_{11}	184-186	88
3-Cl-4-$COOCH[CH_2CH(CH_3)_2]_2$	114-116	77
4-$O(CH_2)_{11}CH_3$	89-90	89

One derivative [4-$O(CH_2)_{11}CH_3$] was prepared according to the above procedure for the methoxymethylphenylureas except that $ClCON(CH_3)_2$ was employed.

The Chemical Abstracts Service numbers for the known derivative II are as follows: H, 1576-17-6; 4-$OCH_2C_6H_5$, 65033-07-0; 3-$C(Me)_3$, 70477-14-4; 4-$OCH_2CH_2C_6H_4$-3'-CH_3, 68358-79-2; 3-F, 28170-26-5; 3-$OCH_2C_6H_5$, 74109-81-2; 3-OH, 30087-17-3; 3-CF_3, 838-89-1; 4-Br, 3060-89-7; 4-$CH(CH_3)_2$, 34861-40-0; 3-Cl, 4-$OCH_2C_6H_5$, 25998-87-2; 4-OH, 20680-06-2.

Substituent Constants. The substituent constants in Tables I and II were taken from our recent compilation (Hansch and Leo, 1979) or calculated according to additivity principles (Hansch and Leo, 1979). In the calculation of log *P* for the compounds in Table II the measured value of 1.29 for the parent compound was added to the π constant from the benzene system (Hansch and Leo, 1979). In calculating π for the cyclohexyl containing substituents, we have used a revised value of 2.79 for the cyclohexyl moiety.

ACKNOWLEDGMENT

We thank P. Y. C. Jow for assistance in measuring partition coefficients and we are indebted to A. Leo for helpful discussions about partition coefficients. Cynthia D. Selassie also provided valuable technical assistance.

Registry No. I (X = 3-NO_2), 7159-98-0; I (X = 3-CF_3), 2164-17-2; I (X = 3-$COCH_3$), 42865-65-6; I (X = 4-COC_6H_5), 61706-06-7; I (X = 4-F), 332-33-2; I (X = 3,4-Cl_2), 330-54-1; I (X = H), 101-42-8; I (X = 3-C_4H_9), 88132-40-5; I (X = $OCH_2CH_2C_6H_5$), 70859-35-7; I (X = 4-C_6H_{11}), 88132-41-6; I (X = 4-*t*-C_4H_9), 32745-69-0; I (X = 4-$CH(CH_3)_2$), 34123-59-6; I (X = 3-OH), 4849-46-1; I (X = 3-NH_2), 39938-79-9; I (X = 3-$OCH_2C_6H_3$-2',4'-Cl_2), 88132-42-7; I (X = 3-Cl-4-$COOCH[CH_2C(CH_3)CH_3]_2$, 88132-43-8; I (X = 4-$O(CH_2)_{11}CH_3$), 88132-44-9; II (X = H), 1576-17-6; II (X = 4-NO_2), 88132-15-4; II (X = 4-NH_2), 88132-16-5; II (X = 4-$OCH_2C_6H_5$), 65033-07-0; II (X = 4-$OCH_2CH_2C_6H_4$-4'-Me), 68358-79-2; II (X = 3-*t*-C_4H_9), 70477-14-4; II (X = 3-F), 28170-26-5; II (X = 3-CN), 88132-17-6; II (X = 3-$OCH_2C_6H_5$), 74109-81-2; II (X = 3-CH_2OH), 88132-18-7; II (X = 3-OH), 30087-17-3; II (X = 3-NO_2), 88132-19-8; II (X = 3-CF_3), 838-89-1; II (X = 4-$CH{=}C(CN)_2$), 88132-20-1; II (X = 4-NHC_2H_5), 88132-21-2; II (X = 4-$N(C_2H_5)_2$, 88132-22-3; II (X = 4-$COCH_3$), 88132-23-4; II (X = 4-Br), 3060-89-7; II (X = 4-F), 88132-24-5; II (X = 4-$COOC_2H_5$), 88132-25-6; II (X = 4-COC_6H_5), 88132-26-7; II (X = 4-$CH(CH_3)_2$), 34861-40-0; II (X = 4-$NHSO_2CH_3$), 88132-27-8; II (X = 4-C_6H_{11}), 88132-28-9; II (X = 4-OCH_2CH_2-C_6H_{11}), 76253-20-8; II (X = 4-$OCH(CH_3)CH_2C_6H_{11}$), 88132-29-0; II (X = 3-$O(CH_2)_4OC_6H_5$), 88132-30-3; II (X = 4-$O(CH_2)_4OC_6H_5$), 88132-31-4; II (X = 3-OCH_2CO-Ad), 88132-32-5; II (X = 4-OCH_2CO-Ad), 88132-33-6; II (X = 3-Cl-4-OCH_2CO-Ad), 88132-34-7; II (X = 3-Cl-4-$OCH_2C_6H_5$), 25998-87-2; II (X = 4-OCH_2CH_2-α-$C_{10}H_7$), 88132-36-9; II (X = 4-$(CH_2)_3C_6H_5$), 81362-51-8; II (X = 4-$(CH_2)_4C_6H_5$), 88132-37-0; II (X = 4-$(CH_2)_3C_6H_4$-4'-Cl, 88132-38-1; II (X = 4-$(CH_2)_3C_6H_4$-C_6H_5, 88132-39-2; II (X = 4-SO_2-NH_2, 88132-45-0; III, 88132-35-8; *N*-methoxy-*N*-methylcarbamyl chloride, 30289-28-2; 4-nitroaniline, 100-01-6; 3-cyanoaniline, 2237-30-1; 3-(hydroxymethyl)aniline, 1877-77-6; 3-nitroaniline, 99-09-2; 4-(2,2-dicyanoethenyl)aniline, 17082-32-5; 4-acetylaniline, 99-92-3; 4-fluoroaniline, 371-40-4; ethyl 4-aminobenzoate, 94-09-7; 4-aminobenzophenone, 1137-41-3; 4-cyclohexylaniline, 6373-50-8; 4-sulfamoylaniline, 63-74-1; *N*-methyl-*N*-methoxy-*N'*-2-fluorenylurea, 153-78-6; 4-[(2-cyclohexyl-1-methylethyl)oxy]aniline, 88132-46-1; 4-[(2-cyclohexylethyl)oxy]aniline, 76253-34-4.

LITERATURE CITED

Agin, D.; Hersh, L.; Holtzman, D. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *53*, 952.
Arnon, D. I. *Plant Physiol.* **1949**, *24*, 1.
Arntzen, C. J.; Ditto, C. L.; Brewer, P. E. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 278.
Bishop, N. I. *Biochim. Biophys. Acta* **1958**, *27*, 205.
Böger, P. *Z. Pflanzenkr. Pflanzenschutz* **1981**, *29*, 1.
Bouges-Bocquet, B. *Biochim. Biophys. Acta* **1973**, *314*, 250.
Briggs, G. G. *J. Agric. Food Chem.* **1981**, *29*, 1050.
Briggs, G. G.; Bromilow, R. H.; Evans, A. A. *Pestic. Sci.* **1982**, *13*, 495.
Brown, B. T.; Phillips, J. N.; Rattigan, B. M. *J. Agric. Food Chem.* **1981**, *29*, 719.
Brugnoni, G. P.; Moser, P.; Trebst, A. *Z. Naturforsch., C: Biosci.* **1979**, *34C*, 1028.
Büchel, K. H.; Draber, W. *Prog. Photosynthesis Res., Proc. Int. Congr., [1st], 1968* **1969**, *3*, 1777.
Coats, E. A.; Genther, C. S.; Dietrich, S. W.; Guo, Z. R.; Hansch, C. *J. Med. Chem.* **1981**, *24*, 1422.
Cross, B.; Hoffman, P. P.; Santora, G. T.; Spatz, D. M.; Templeton, A. R. *J. Agric. Food Chem.* **1983**, *31*, 260.
Draber, W.; Dickoré, K.; Büchel, K. H.; Trebst, A.; Pistorius, E. *Naturwissenshaften* **1968**, *55*, 446.
Dunn, W. J., III; Hansch, C. *Chem.-Biol. Interact.* **1974**, *9*, 75.
Forbush, B.; Kok, B. *Biochim. Biophys. Acta* **1968**, *162*, 243.
Franks, N. P.; Lieb, W. R. *Nature (London)* **1982**, *300*, 487.
Fujinami, A.; Mine, A.; Fujita, T. *Agric. Biol. Chem.* **1974**, *38*, 1399.
Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175.
Gabbott, P. A. *Prog. Photosynthesis Res.* **1969**, *3*, 1712.
Gardner, G. *Science (Washington, D.C.)* **1981**, *211*, 937.
Giaquinta, R. T.; Dilley, R. A.; Anderson, B. J. *Biochem. Biophys. Res. Commun.* **1973**, *53*, 1410.
Glave, W. R.; Hansch, C. *J. Pharm. Sci.* **1972**, *61*, 589.
Good, N. E. *Plant Physiol.* **1961**, *36*, 788.
Govindjee; van Rensen, J. J. S. *Biochim. Biophys. Acta* **1978**, *505*, 183.
Grieco, C.; Silipo, C.; Vittoria, A. *Farmaco, Ed. Sci.* **1979**, *34*, 79.
Hansch, C. *Acc. Chem. Res.* **1969a**, *2*, 232.
Hansch, C. *Prog. Photosynth. Res., Proc. Int. Congr., [1st], 1968* **1969b**, *3*, 1685.
Hansch, C. *Drug Dev. Res.* **1981**, *1*, 267.
Hansch, C.; Clayton, J. M. *J. Pharm. Sci.* **1973**, *62*, 1.
Hansch, C.; Deutsch, E. W. *Biochim. Biophys. Acta* **1966**, *112*, 381.
Hansch, C.; Dunn, W. J., III *J. Pharm. Sci.* **1972**, *61*, 1.
Hansch, C.; Fujita, T. *J. Am. Chem. Soc.* **1964**, *86*, 1616.
Hansch, C.; Leo, A. "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley-Interscience: New York, 1979.
Hansch, C.; Muir, R. M.; Fujita, T.; Maloney, P. P.; Geiger, F.; Streich, M. *J. Am. Chem. Soc.* **1963**, *85*, 2817.
Hansch, C.; Steward, A. R.; Anderson, S. M.; Bentley, D. *J. Med. Chem.* **1968**, *11*, 1.

Hansch, C.; Vittoria, A.; Silipo, C.; Jow, P. Y. C. *J. Med. Chem.* **1975**, *18*, 546.
Iwamura, H.; Fujita, T. *J. Pestic. Sci.* **1982**, *7*, 289.
Izawa, S.; Good, N. E. *Biochim. Biophys. Acta* **1965**, *102*, 20.
Joliot, P. *Biochim. Biophys. Acta* **1965**, *102*, 135.
Khanna, R.; Pfister, K.; Keresztes, A.; van Rensen, J. J. S.; Govindjee *Biochim. Biophys. Acta* **1981**, *634*, 105.
Kubinyi, H. *Drug. Res.* **1979**, *23*, 97.
Kubinyi, H.; Kehrhahn, O. H. *Arzneim.-Forsch.* **1978**, *28*, 598.
Martin, Y. C. "Quantitative Drug Design"; Marcel Dekker: New York, 1978.
Mattoo, A. K.; Pick, U.; Hoffman-Falk, H.; Edelman, M. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 1572.
Metcalf, R.; Lu, P., Department of Entomology, University of Illinois, Urbana, IL, personal communication, 1978.
Miller, K. R.; Cushman, R. A. *Biochim. Biophys. Acta* **1979**, *546*, 481.
Oettmeier, W.; Masson, K.; Johanningmeier, U. *FEBS Lett.* **1980**, *118*, 267.
Pallett, K.; Dodge, A. D. *Pestic. Sci.* **1979**, *10*, 216.
Pauling, L.; Pressman, D. *J. Am. Chem. Soc.* **1945**, *67*, 1003.
Pfister, K.; Arntzen, C. J. *Z. Naturforsch., C: Biosci.* **1979**, *34C*, 996.
Pfister, K.; Radosevich, S.; Arntzen, C. J. *Plant Physiol.* **1979**, *64*, 995.
"Quantitative Structure-Activity Relationships"; Verlag Chemie: Weinheim, Federal Republic of Germany, 1982.
Renger, G. *Biochim. Biophys. Acta* **1976**, *440*, 287.
Schirmer, U.; Rohr, W.; Würzer, R. European Patent 41 145, Dec 9, 1981.
Seewald, I.; Michel, H. J.; Klepel, M.; Held, P.; Ohmann, E.; Barth, A.; Metzger, U. In "Quantitative Structure-Activity Analysis"; Akademie-Verlag: Berlin, 1978; p 77.
Selassie, C. D.; Li, R. L.; Hansch, C.; Khwaja, T. A.; Dias, C. B. *J. Med. Chem.* **1982**, *25*, 518.
Siggel, U.; Khanna, R.; Renger, G.; Govindjee *Biochim. Biophys. Acta* **1977**, *462*, 196.
Smith, R. N.; Hansch, C.; Kim, K. H.; Omiya, B.; Fukumura, G.; Selassie, C. D.; Jow, P. Y. C.; Blaney, J. M.; Langridge, R. *Arch. Biochem. Biophys.* **1982**, *215*, 319.
Stemmler, A. In "Photosynthetic Oxygen Evolution"; Metzner, H., Ed.; Academic Press: New York, 1978; p 283.
Takemoto, I.; Yoshida, R.; Sumida, S.; Kamoshita, K., Abstracts of the 5th International Congress of Pesticide Chemistry, Kyoto, Japan, Aug 1982, 11d–13.
Tischer, W.; Strotmann, H. *Biochim. Biophys. Acta* **1977**, *460*, 113.
Trebst, A. *Z. Naturforsch., C: Biosci.* **1979**, *34C*, 986.
Trebst, A.; Draber, W. "Advances in Pesticide Science"; Greissbüller, A., Ed.; Pergamon Press: Oxford, 1979; Part 2, p 223.
Unger, S. H.; Hansch, C. *Prog. Phys. Org. Chem.* **1976**, *12*, 91.
van Assche, C. J.; Carles, P. M. *ACS Symp. Ser.* **1982**, *No. 181*, 1.
van den Berg, G.; Tipker, J. *Pestic. Sci.* **1982**, *13*, 29.
Velthuys, B. R.; Amesz, J. *Biochim. Biophys. Acta* **1974**, *333*, 85.
Verloop, A. In "Drug Design"; Ariëns, E. J., Ed.; Academic Press: New York, 1972; Vol. III, p 133.
Verloop, A. *Philos. Trans. R. Soc. London, Ser. B* **1981**, *295*, 45.
Wessels, J. S. C.; van der Veen, R. *Biochim. Biophys. Acta* **1956**, *19*, 548.
Yukimoto, M. *J. Pestic. Sci.* **1983**, *8*, 63.

Received for review June 14, 1983. Accepted October 18, 1983. This research was supported in part by Grant GM-30362 from the National Institutes of Health.

Chemical Characterization and Functionality Assessment of Oat Protein Fractions

Ching-Yung Ma* and Venktesh R. Harwalkar

Protein fractions (albumins, globulins, prolamins, and glutelins) were prepared from oat seeds (variety Sentinel). Column chromatography on Sephacryl S-200 revealed that the four solubility fractions had unique polypeptide compositions and there was little cross contamination among the fractions. Isoelectric focusing on polyacrylamide gels resolved the fractions into a large number of bands covering a wide pH range. Differential scanning calorimetric studies showed that albumins and globulins had an endothermic peak at 87 and 110 °C, respectively, while prolamins and glutelins had no thermal response. Some functional properties of the solubility fractions were determined to assess the potential use of oat proteins as a food ingredient. Some fractions had high emulsifying, fat-binding, and water hydration capacities, and the albumins also had excellent foaming properties.

Oats provide a potential source of low-cost proteins with good nutritional value (Hischke et al., 1968) but are not used extensively for human consumption in the form of processed food. This could partly be due to insufficient information on the physicochemical and functional properties of oat proteins. Protein concentrates and isolates have been prepared from oats (Bell et al., 1978; Cluskey et al., 1973, 1978; Wu and Stringfellow, 1973; Youngs, 1974; Ma, 1983a,b) and were found to have some good functionality, suggesting potential use in foods (Ma, 1983a,b). Protein fractions have also been isolated from oats by the Osborne fractionation scheme, and their chemical and amino acid compositions were determined (Wu et al., 1972; Draper, 1973; Pernollet et al., 1982). Oat globulins and prolamins (avenine) have been partially purified and characterized (Peterson, 1978; Kim et al., 1978; Brinegar and Peterson, 1982), while the other two solubility classes, albumins and glutelins, have not been extensively studied. There is a complete lack of information on the functionality of these protein fractions from oats.

In this work, the four solubility fractions from oat groats will be characterized by chromatography, isoelectric focusing, and differential scanning calorimetry to provide information on the polypeptide composition, charge heterogeneity, and conformation of the oat proteins. Such data are needed for comparative studies of cereal proteins that have received recent emphasis (Miflin et al., 1983). Some technological properties of cereals, such as bread-making properties of wheat and brewing quality of barley, are profoundly influenced by the storage proteins. A

Food Research Institute, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6.

0021-8561/84/1432-0144$01.50/0