Aminopyrazolones: Novel Photosystem II Inhibitors

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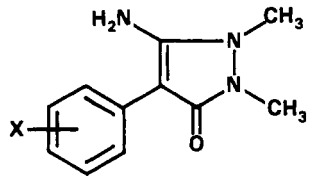
We have investigated the inhibition of photosynthetic electron flow by the aminopyrazolones, a novel class of photosystem II herbicides. The pattern of substitution in the aryl moiety of these molecules affects the level of inhibition of the Hill reaction. Hydrophobic substituents that are also electron withdrawing favor inhibition. Similarities in the pattern of structure–activity of the aminopyrazolones and the phenylureas indicate that both classes of compounds block electron flow at the acceptor site of photosystem II and also occupy common enzymic space on the 32-kDa herbicide binding protein.

INTRODUCTION

A large number of commercial herbicides act by inhibiting the flow of electrons on the acceptor side of photosystem II (PSII) (Fedtke, 1982). The herbicide binding site on PSII can accommodate diverse classes of molecules with an apparently low degree of structural limitation. Some well-known examples are the phenylureas (Hansch and Deutsch, 1966; Kakkis et al., 1984; Camilleri et al., 1987), anilides (Mitsutake et al., 1986), triazines (Gabbot, 1969; Mitsutake et al., 1986), uracils (Brown et al., 1981), cyanoacrylates (Huppatz and Phillips, 1987a,b), and phenylbiurets (Camilleri et al., 1988). Molecules from these classes of inhibitors are thought to bind to the 32-kDa protein (D_1) of the PSII reaction center, affecting the binding of the secondary plastoquinone electron acceptor Q_B, which can no longer participate in the photosynthetic electron relay system. It is currently thought (Fedtke, 1982) that the different chemical classes of PSII inhibitors occupy both distinct and overlapping binding sites. This explains the competitive interaction of the different classes of PSII inhibitors.

In the present study we report a new class of PSII inhibitors, namely the pyrazolones, which have the general structure



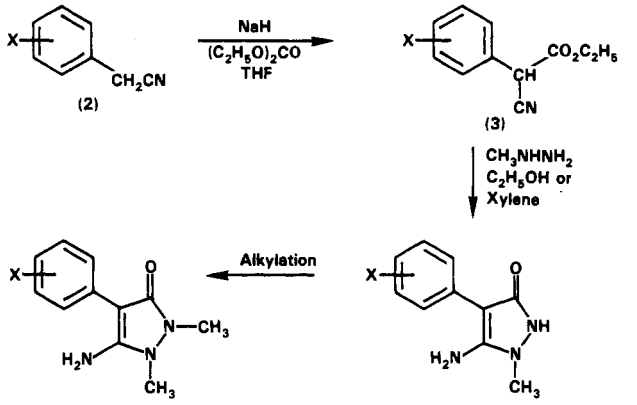
(1)

We have analyzed the effect of the substituent X (in the 3-, 4-, or 3,4-positions of the phenyl ring) on the inhibition of the Hill reaction. The results presented show that close similarities exist in the structure–activity requirements of this novel class of PSII inhibitors and the phenylureas. As in the latter case (Hansch and Deutsch, 1966) both the hydrophobic and the electronic nature of X affect the binding of the pyrazolones at the site of action.

EXPERIMENTAL PROCEDURES

Synthesis. The aminopyrazolones in Table I were synthesized according to the general route shown in Scheme I. The appropriate arylacetonitrile (2) was treated in tetrahydrofuran (THF) with diethyl carbonate in the presence of sodium hydride to provide the corresponding α-aryl-α-cyano ester (3). Treatment with methylhydrazine gave the monomethyl amino pyrazolone (4) exclusively as the 1-isomer. The second nitrogen was finally methylated via standard alkylation (using potassium carbonate as base) to provide the *N*,*N*-dimethylaminopyrazolones (1). Melting points and elemental analysis of the 11 compounds prepared for this study are given in Table I. All the compounds in this table were crystallized from methanol.

Scheme I



Hill Inhibition. The aminopyrazolones were assayed for inhibition of the Hill reaction by using thylakoid fragments isolated from pea chloroplasts (*Pisum sativum*). The procedure has been outlined in a previous paper (Camilleri et al., 1987).

RESULTS AND DISCUSSION

The Hill inhibition potency of the 11 aminopyrazolone derivatives in Table I was first related to the hydrophobicity contribution by the 3- and 4-substituents, X, on the phenyl moiety. Multiple regression analysis gave

$$pI_{50} = 0.94\ (\pm 0.32) \sum \pi + 4.42\ (\pm 0.23) \qquad (1)$$

$$n = 11,\ r = 0.88,\ s = 0.25$$

where pI_{50} is the logarithm of the molar concentration of compound that causes 50% inhibition of the Hill reaction, $\sum \pi$ is the sum of the Hansch hydrophobicity constants for substituents in the 3- and 4-positions (Hansch and Leo, 1979), n is the number of compounds, r is the correlation coefficient, and s is the standard deviation for the regression. The percentage variance in pI_{50} accounted for by the hydrophobic term $\sum \pi$ is 77%, indicating that the

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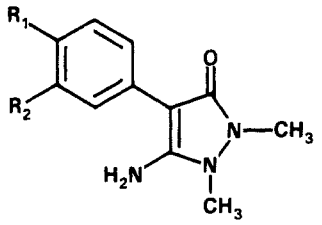
Table I. Melting Point and Elemental Analysis of the *N,N*-Dimethylaminopyrazolones



compd	R_1	R_2	mp range, °C	analysis, % found C	found H	found N	calcd C	calcd H	calcd N
5	H	H	150.5–151.0	64.9	6.7	20.9	65.0	6.4	20.7
6	H	CF_3	143.5–144.0	52.8	4.4	15.1	53.1	4.4	15.5
7	H	Cl	130.5–131.0	56.4	5.1	17.0	55.6	5.1	17.7
8	H	CH_3	166.0–166.5	65.8	6.8	19.2	66.4	6.9	19.3
9	H	OCH_3	146.0–146.5	61.5	6.5	17.3	61.8	6.4	18.0
10	Cl	Cl	244.0–244.5	48.0	4.0	15.2	48.5	4.0	15.5
11	CH_3	CH_3	179.0–180.0	66.0	7.3	17.5	67.5	7.4	18.2
12	F	H	167.0–167.5	58.9	5.5	18.3	59.7	5.4	19.0
13	Cl	H	164.0–165.0	54.9	5.1	17.3	55.6	5.1	17.7
14	CH_3	H	185.0–185.5	65.3	6.8	18.7	66.4	6.9	19.3
15	OCH_3	H	156.0–158.0	60.8	6.3	17.5	61.8	6.4	18.0

Table II. Hill Inhibition by Aminopyrazolones

compd	pI_{50} obsd	pI_{50} calcd (eq 1)	$\lvert\Delta\rvert$	pI_{50} calcd (eq 2)	$\lvert\Delta\rvert$
5	4.59	4.42	0.17	4.47	0.12
6	5.28	5.24	0.04	5.44	0.16
7	5.32	5.09	0.24	5.27	0.05
8	4.92	4.94	0.02	4.81	0.11
9	4.34	4.40	0.06	4.55	0.21
10	6.00	5.75	0.25	5.96	0.04
11	5.00	5.47	0.47	5.06	0.06
12	4.64	4.55	0.09	4.62	0.02
13	5.30	5.08	0.22	5.16	0.14
14	4.62	4.94	0.32	4.73	0.11
15	4.28	4.40	0.12	4.24	0.04

substituents X make considerable contact with a hydrophobic surface at the site of action. However, although eq 1 suggests that the potency of the aminopyrazolones as Hill inhibitors increases almost linearly with an increase in the hydrophobic nature of the substituent(s) X, the level of percentage variance tends to suggest that other factors, e.g., electronic, must be important in enhancing the binding of this class of molecule to the site of action.

Consideration of both the hydrophobic and the electronic nature of X yields

$$pI_{50} = 0.71\ (\pm 0.19) \sum \pi + 0.81\ (\pm 0.32) \sum \sigma + 4.47\ (\pm 0.12) \quad (2)$$

$$n = 11, r = 0.97, s = 0.13$$

where $\sum\sigma$ is the sum of the Hammett substitutent constants (Hansch and Leo, 1979). The percentage variance in pI_{50} as given by eq 2 has now increased to the 94% level. The improvement in the structure–activity correlation as represented by the regression eq 2 is also seen by the lower value in the standard deviation s and by the generally smaller differences between the observed and calculated pI_{50} values (Table II). The electronic parameter σ accounts for about 17% of the variance in the biochemical data. The positive coefficient with this parameter indicates that electron release by the substituent(s) X does not favor inhibition and, most probably, reduces binding at the active site.

Equation 2 is remarkably similar to a relationship derived by Hansch and Deutsch (1966) for the inhibition

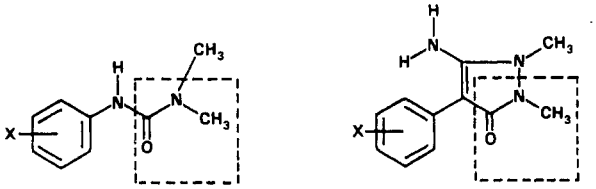


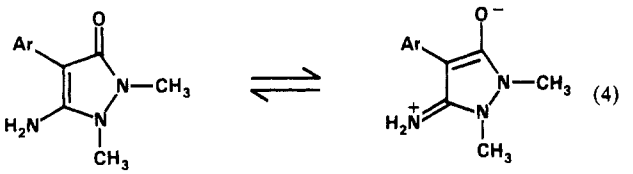
Figure 1. Some structural similarities between the aminopyrazolones and the phenylureas.

of the Hill reaction by a number of simple phenylureas:

$$pI_{50} = 1.29\pi + 0.54\sigma + 4.18 \quad (3)$$

$$n = 12, r = 0.94, s = 0.37$$

A comparison of eqs 2 and 3 shows that both the coefficients of π and σ and the level of activity of the unsubstituted compounds (π and σ equal to zero) are of similar magnitude for the two classes of molecules. The similarity in regression eqs 2 and 3 may be due to structural features (Figure 1) that are common in the two classes of PSII inhibitors. The $CON(CH_3)$ and the phenyl groups in both the aminopyrazolones and the phenylureas could be occupying common enzymic space. The site occupied by the *exo* NH_2 group in the pyrazolone structure may also be the same as that occupied by the NH group in the phenylureas. Electron density in this *exo* NH_2 group appears to be heavily delocalized into the pyrazolone ring (eq 4). In fact it has proved very difficult to functionalize this group.



(4)

The similarity in the structure–activity of the aminopyrazolones and the phenylureas tends to suggest that the former class of inhibitors belong to the urea/triazine family rather than the phenol family of PSII herbicides (Trebst, 1987). We intend to carry out further studies on this novel class of PSII inhibitors to confirm this and probe the binding environment at their site of action, most probably situated on the 32-kDa protein.

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