Site of Action and Quantitative Structure-Activity Relationship of a Series of Herbicidal N-Aryl-Substituted 3,4-Dimethyl-2-hydroxy-5-oxo-2,5-dihydro-pyrrolones

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Z. Naturforsch. **34 c,** 1028 – 1031 (1979)

Pyrrolones, Site of Action, Structure-Activity Relations, Photosynthetic Electron Transport, Herbicidal Action

Substituted phenyl-hydroxy pyrrolone derivatives are potential herbicides and are found to be effective inhibitors of photosynthetic electron flow in chloroplasts. Their site of inhibition is localized at the acceptor side of photosystem II similar to DCMU. They have no effect on photophosphorylation. The correlation of photosynthetic acitivity to chemical structure is studied by comparing 36 derivatives. A clear dependence of inhibitory activity on lipophilicity is found. The dependence of herbicidal activity on chemical structure is more complex.

Introduction

Numerous commercial herbicides have been shown to be inhibitors of photosynthetic electron flow. In particular, the large herbicide groups of urea [1] and triazine [2, 3] derivatives block electron flow between the primary acceptor (Q) of photosystem II and the functional site of plastoquinone (see general reviews [4, 5]). Recently we have described a new group of potential herbicides, derivatives of hydroxy pyrrolones, which were shown to also affect photosynthesis [6].

We wish to describe the effect of some of these compounds on photosynthetic electron flow in chloroplasts and to correlate their biological function with chemical parameters.

Methods

For the measurement of photosynthetic activity, spinach chloroplasts were prepared and tested in a volume of 3 ml containing Hepes buffer of pH 8.0, 5 μM $MgCl_2$, 5 μg gramicidin and washed thylakoid membranes with 20 μg chlorophyll at 20 °C using 2.5×10^5 ergs/cm^2/sec red light. Oxygen uptake in methyl viologen (0.1 mM) reduction was measured with an oxygen electrode, ferricyanide (1.5 mM) reduction at 320 nm and DCPIP reduction at 600 nm. In cyclic photophosphorylation 3 nM ATP, 3 nM P^{32} (90.000 cpm) and 0.1 mM PMS were added to the chloroplasts.

Herbicidal activities were determined by a greenhouse test. Avena, Setaria and Lolium were treated with aqueous suspensions of the compounds at concentrations of 4 kg/ha. The herbicidal activity given in Table II is the arithmetic mean of the observations (9 = no activity, 1 = total kill) after postemergence application on the three weeds.

Results and Discussion

One of the compounds, dichlorophenyl-hydroxypyrrolone (dichloro-PHP) was selected as a representative of all derivatives to indicate the site of inhibition in the photosynthetic electron flow system by measuring a number of artificial acceptor and donor systems. Dichloro-PHP has an I_{50} value of 0.04 μM calculated for zero chlorophyll concentration, as the inhibitory concentration to obtain 50% inhibition (I_{50}) depends on the amount of chloroplast membranes in the reaction mixture (Fig. 1).

A higher concentration of dichloro-PHP completely inhibits the photoreduction of methylviologen, a photosystem I acceptor. This inhibition is reversed by adding an artificial electron acceptor system for photosystem I: TMPD/ascorbate (Table 1 a). TMPD alone is not effective as it is in bypassing an inhibition site (like the one of dibromothymoquinone) after the function of plastoquinone [7]. This places the inhibition site of dichloro-PHP before the function of plastoquinone. This is supported by the finding that ferricyanide reduction is inhibited by dichloro-PHP whether driven by both photosystem (no mediator) or driven by photosystem II only

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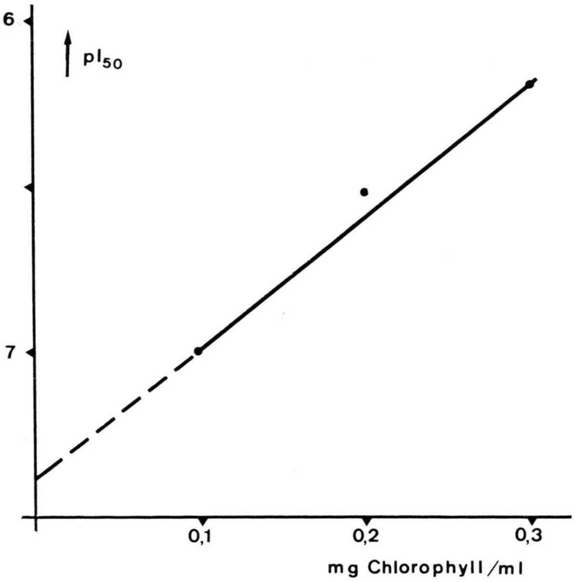


Fig. 1. Dependence of pI_{50} of *m,p*-dichloro-phenyl pyrrolone on chloroplast concentration (given as chlorophyll concentration).

(*i. e.* in the presence of an artificial lipophilic mediator, like *p*-phenylene-diamine [7]) (Table 1 b). Cyclic photophosphorylation, which is only photosystem I dependent, is not affected by even very high concentrations of dichloro-PHP. This then indicates an inhibition site before plastoquinone, but after photosystem II *i. e.* alike the inhibition of electron flow by urea and triazine derivatives. The new inhibitors have no effect on the ATP synthesizing system of the chloroplasts.

The inhibition site at the acceptor side of photosystem II of the hydroxy pyrrolone derivatives, identical to that of the urea herbicides, is not unexpected. As Fig. 2 is to indicate both groups contain the same chemical fragment ($\rangle N-\overset{|}{C}=$) recognized to be essential [4, 5] for inhibition of and for binding to the thylakoid membrane.

The Table II shows the effectiveness of a number of substituted phenyl-hydroxypyrrolones in inhibiting photosynthetic ferricyanide reduction and their herbicidal activity.

A quantitative structure-activity relationship (QSAR) by the Hansch approach [8] was sought

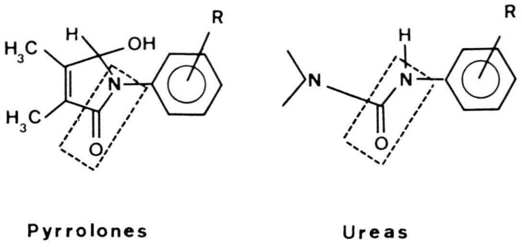


Fig. 2. Comparison of structures of pyrrolone and urea herbicides. A common chemical fragment essential for inhibition of photosynthetic electron flow is indicated.

Table 1. Influence of dichloro-PHP on photosynthetic reaction in isolated spinach chloroplasts thylakoid preparations.

Table 1a. Photoreduction of methylviologen (pseudocyclic e-flow, uncoupled by gramicidine).

	μ equivalents oxygen (2e) taken up	conclusions on the influence of the inhibitor
control	240	Hill reaction and TMPD bypass are inhibited, but photoreduction by photosystem I (TMPD ascorbate) is not
+ 0.3 μM dichloro-PHP	42	
+ 0.3 μM dichloro-PHP + 0.1 μM TMPD	30	
+ 0.3 μM dichloro-PHP + 0.1 μM TMPD + 20 μM ascorbate	276	

Table 1b. Photoreduction of K-ferricyanide + *p*-phenylene diamine (PD).

	e-flow mg Chlorophyll·h	ATP formation mg Chlorophyll·h	P/e_2	
control	216	240	1.1	Photoreductions by photosystem II are also inhibited
+ 1 μM dichloro-PHP	42			
+ 1 μM dichloro-PHP + 0.1 μM PD	36			

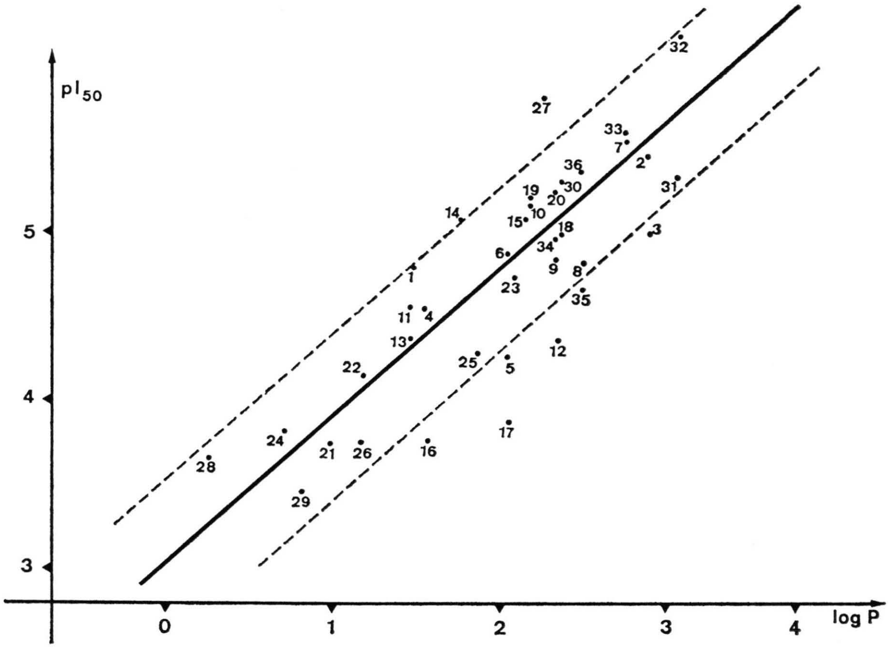


Fig. 3. Dependence of **pI**$_{50}$ on log *P* (octanol/water partition coefficient).

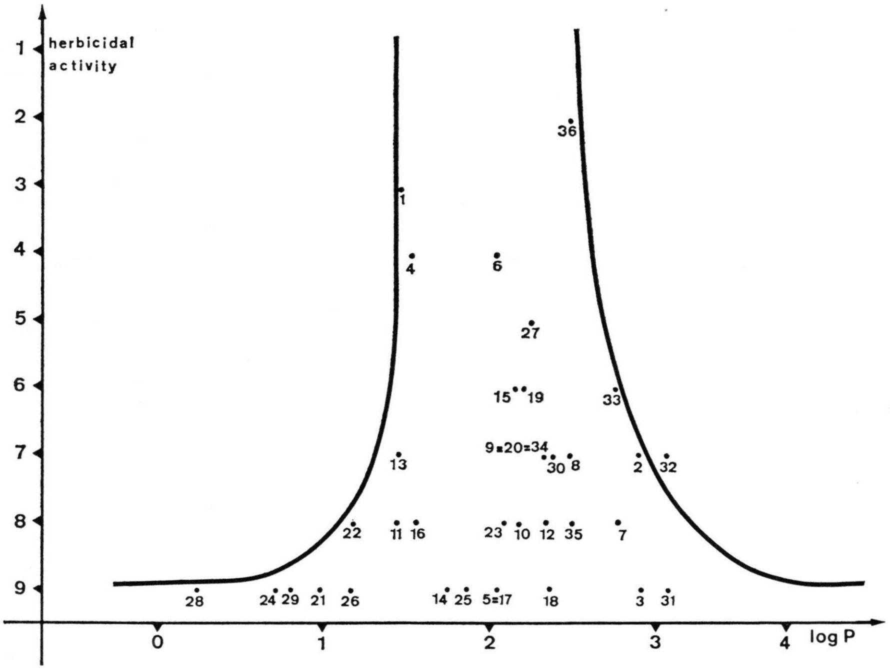


Fig. 4. Herbicidal activity vs. log *P*, average of 3 weeds, see text.

for the Hill reaction data. Lipophilicity of the molecules was expressed by their octanol/water partition coefficient, log *P* and (log *P*)^2. Most log *P*-values were calculated by the fragment method, but were based on measurements of some key derivatives. The other parameters tested in multiple regression analysis, using program BMDP2R, were

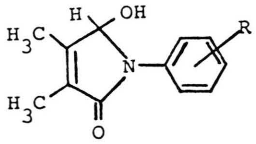
σ_o, σ_m, σ_p = Hammett constants of aromatic substituents R

$E_{s,o}$, $E_{s,m}$, $E_{s,p}$ = Taft steric constants of R

I_o, I_m, I_p, I_{mp}, I_{mm} = indicator variables [9]

(values 0 or 1) for the substitution pattern at the phenyl ring.

Table 2. Inhibitory effect of substituted phenyl-hydroxy pyrrolones.



No	R	pI_{50}	Herbicidal activity
1	H	4.80	3
2	*m*-Cl, *p*-Cl	5.46	7
3	*m*-Cl, *m*-Cl	5.00	9
4	*p*-F	4.57	4
5	*p*-CH_3	4.26	9
6	*m*-CH_3	4.87	4
7	*m*-Cl, *p*-CH_3	5.53	8
8	*m*-CH_3, *p*-CH_3	4.80	7
9	*p*-Br	4.83	7
10	*p*-Cl	5.17	8
11	*m*-OCH_3	4.57	8
12	*m*-CF_3	4.35	8
13	*p*-OCH_3	4.37	7
14	*m*-NO_2, *p*-CH_3	5.08	9
15	*m*-Cl, *p*-OCH_3	5.07	6
16	*o*-F	3.57	8
17	*m*-COOH, *p*-Cl	3.89	9
18	*m*-CF_3	4.96	9
19	*m*-Cl	5.20	6
20	*m*-Br	5.25	7
21	*m*-OH	3.75	9
22	*m*-NO_2	4.14	8
23	*m*-SCH_3	4.72	8
24	*m*-$OCONHCH_3$	3.82	9
25	*m*-SH	4.29	9
26	*m*-COOH	3.74	9
27	*m*-$OCONHC(CH_3)_3$	5.80	5
28	*m*-NH_2	3.65	9
29	*m*-NH_2, *p*-CH_3	3.46	9
30	*m*-CF_3, *p*-OCH_3	5.30	7
31	*m*-CF_3, *p*-Cl	5.31	9
32	*m*-Cl, *p*-CF_3	6.18	7
33	*m*-CF_3, *p*-OC_2H_5	5.60	6
34	*m*-Cl, *p*-F	4.99	7
35	*m*-CF_3, *p*-F	4.63	8
36	*m*-C_2H_5	5.36	2

The following "best equation" was found

$$pI_{50} = 3.10 + 0.87(\pm 0.22)\log P - 0.96 \times (\pm 0.88) I_o - 0.22(\pm 0.30) I_p \quad (1)$$
$$n = 36 \quad s = 0.418 \quad r = 0.835$$
$$F = 25.3$$

(in parenthesis are the 95%-confidence limits of the regression coefficients).

The dependence of the pI_{50} on $\log P$ is clear and dominant. *Para-* and *ortho*-effects decreasing the activity are recognizable but are only little significant.

Fig. 3 shows the dependence of pI_{50} on $\log P$. The solid line represents the one parameter regression equation

$$pI_{50} = 3.02 + 0.83(\pm 0.22)\log P$$
$$n = 36 \quad s = 0.443 \quad r = 0.800 \quad (2)$$
$$F = 62.3$$

the dashed lines indicate the respective standard deviation *s*.

Fig. 4 is a plot of herbicidal activity *vs.* lipophilicity. Contrary to the inhibition of photosynthesis, no clear dependency on $\log P$ exists which could be expressed in a simple mathematical form. One can deduce from this figure, however, that for high activity the lipophilicity has to be near an optimum value of about $\log P = 2$ which seems to be a compromise between an enhancing effect of lipophilicity on inhibition at the active center and a negative effect on transport. As is apparent, optimum lipophilicity is not a sufficient condition for herbicidal activity. Other influences which have not yet been investigated, certainly play an important role.

Acknowledgement

We would like to thank Mr. K. Jäkel and Mr. C. Marletaz for their skillful experimental work.

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