Action of Methylthiopyrimidine Experimental Herbicides as Diuron-Like Inhibitors of Photosynthesis

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Derivatives of 6-chloro-5-methylthiopyrimidines provide potent inhibitors of the photosynthetic electron flow, which act like Diuron on fluorescence induction kinetics and competitively displace it from its binding site. Structure-activity relationships show that, unlike triazines, activities of 2- or 4-alkylamino derivatives are restricted by steric hindrances. Decreases in inhibitory activities of these compounds observed in triazine-resistant chloroplasts are lower than decreases reported for triazines themselves.

Introduction

Most inhibitors of the photosynthetic electron flow, such as substituted ureas, triazines and other groups [1, 2] act at the reducing side of photosystem II, by preventing the electron transfer between the primary acceptor Q_A and the secondary acceptor Q_B [3]. These inhibitors, which can displace each other competitively from a site of affinity [4], possess a common structural pattern [5, 6]:

$$-NH-\underset{|}{C}=X, \text{ where } X = O, S \text{ or } N.$$

Among the quantitative structure-activity relations [7], two features emerge:

– Hydrophobicity, estimated by the octanol/water partition coefficient, plays a major role in inhibition [7, 8]. This can be ascribed to a partition in the membrane but also, in the case of substituted ureas, to an interaction between the N-linked hydrophobic bulky substituent and an hydrophobic niche within the site [8, 9].

– The role of N-linked unsubstituted hydrogens has also been pointed out [5, 10], although it is not a strict requisite for inhibitory activity [8].

Recently, a new class of experimental herbicides, the thiopyrimidines [11], has appeared. They act as Hill reaction inhibitors, although one of them was also an inhibitor of the mitochondrial electron flow [12]. Experiments reported here were performed to localize the site of inhibition of these compounds in the photosynthetic electron transfer chain and to study the influence of different substitutions on their inhibitory activity.

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Material and Methods

Triazine susceptible and resistant biotypes of *Chenopodium album* were furnished by Drs. Al Mouemar and Gasquez. The plants were grown in a greenhouse, with 16 h/day of additional light.

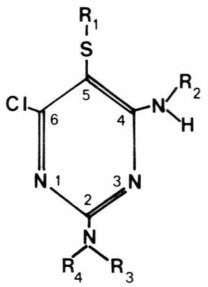
Chloroplasts were prepared as previously described [9]. Oxygen measurements were carried out with a Clark electrode, at 15 °C and at a 15 μg/ml chlorophyll concentration in a medium containing 0.4 M saccharose, 10 mM KCl, 2 mM $MgCl_2$, 50 mM tricine pH 7.8. For uncoupling, 4 mM NH_4Cl was added, and 2 mM K ferricyanide was used as the electron acceptor.

Fluorescence induction kinetics, under a broad-band blue excitation, was recorded at a 5 μg/ml chlorophyll concentration, in the same medium. Binding experiments were carried out according to Tischer and Strotmann [4]. Hydrophobicity was evaluated by HPLC, using a Dupont Zorbax ODS C_{18} reverse phase 15 cm column, operated at 200 bars, with MeOH/H_2O = 60/40 as the mobile phase. $k' = (t_r/t_o)$-1, where t_o and t_r are the retention time of solvent and solute respectively, was used as an index of the hydrophobicity of compounds assayed [13].

Results and Discussion

The I_{50} value of the 6-chloro-5-methylthiopyrimidine derivative (**I**) fell in the same range as that of atrazine or monuron [2], as shown in Table I. The most powerful term of this series was the 2-ethylamino-derivative (**III**), which appeared as active as diuron, since its I_{50} was 0.04 μM. These compounds (data not shown), increased the amplitude of the photochemical rise of the fluorescence induction kinetics, in the same way as diuron. The 2-ethyl-

Table I. Half inhibitory concentrations (I_{50}) for lettuce chloroplasts and HPLC hydrophobic index (k') of derivatives of 6-chloro-5-methylthio 2,4-diamino pyrimidine. Values for atrazine and monuron are given for comparison. Measurement of hydrophobic index is described in **Material and Methods.**



Nos of compounds	R_1	R_2	R_3	R_4	K' (index of hydrophobicity)	I_{50} [μM]
I	Me	H	H	H	1.1	0.5
II	Me	H	Me	H	1.9	0.07
III	Me	H	E_t	H	3.1	0.04
IV	Me	H	Pr	H	5.1	0.1
V	Me	H	i-Pr	H	5.8	20
VI	Me	Me	H	H	2.0	150
VII	Me	E_t	H	H	3.3	150
VIII	Me	E_t	E_t	H	10.6	3
IX	E_t	H	H	H	3.7	0.8
X	But	H	H	H	10.5	25
XI	−O−Me	H	E_t	H	0.6	0.9
XII	Me	H	Me	Me	3.7	>100
atrazine					3.2	0.2
diuron					4.4	0.05

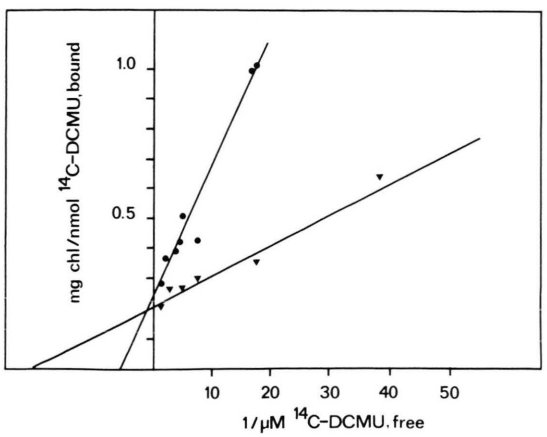


Fig. 1. Double reciprocal plots of [^{14}C]diuron binding in the presence (●) or absence (▼) of compound **III** (Table I), 0.2 μM.

amino-derivative (**III**) also competitively displaced [^{14}C]diuron in binding experiments, as checked by Lineweaver-Burk plots (Fig. 1): statistical analysis showed that intersections of regression lines with the y axis were not significantly different in the presence or absence of unlabeled compound **III**. This demonstrates [4] that 6-chloro-5-methylthiopyrimidines are diuron-like inhibitors, since they bind at the same site.

The 2-4-6-trichloro-derivative was totally inactive at 100 μM. Substitution of the chlorine in position 4 by an ethylamino-moiety resulted in a weak inhibitory activity (30% inhibition at 100 μM).

The I_{50} values of 6-chloro-5-methylthiopyrimidines substituted by alkylamino groups in positions 2 or 4 are listed in Table I. Upon alkyl substitution in R_3, the hydrophobicity evaluated by k', increased with the number of carbon atoms (**I** to **V**), as expected. This was related to an increase of inhibitory activity from **I** to **III,** but substitution by bulkier substituents revealed unfavorable; the activity was slightly lower for compound **IV** (propyl) and falled dramatically for compound **V** (isopropyl). This suggests that some steric hindrance was introduced by propyl and, at a greater extent, by isopropyl substituents. Introduction of a dimethylamino substituent in position 2 (**XII**) caused a more than 1000 fold loss of activity, compared to **II** or **III.** It cannot be concluded whether this results from the requirement of an unsubstituted NH [5, 10] at this position or from steric constraints of the site, revealed by compounds **IV** and **V.** A similar effect of N-dialkylation occurs also in triazines, as demonstrated by trietazine [2].

Conversely, and in accordance with observations of herbicidal properties [11], alkylation of nitrogen in position 4 (R_2) induced an important loss of activity (**VI, VII**) compared to the initial compound **I.** Comparison of homologous compounds **VI** *versus* **II** (methylamino-) and **VII** *versus* **III** (ethylamino) shows a 1500 fold and 3000 fold decrease respectively, whereas k' values were similar within each pair. Similary, substitution of the most active derivative **III** by an ethyl in R_2 (**VIII**) caused a 75 fold decrease, the residual activity being possibly ascribable to a very high k' value. There also, this decrease can be explained either by the necessity of a free NH_2 or by harsh steric constraints at the level of the 4-NH_2.

Replacement of the methyl by an ethyl on sulfur (R_1) caused a very slight decrease of activity (**I** *versus* **IX**). However, a butyl (**X**) was much more detrimen-

Table II. Half inhibitory concentrations in triazine susceptible and resistant chloroplasts from *Chenopodium album*, and R/S = I_{50}(R)/I_{50}(S). Compounds **I** to **V** are listed in Table I. Values for atrazine and diuron are also given for comparison.

Nos of compounds	R_3	K' (Index of hydrophobicity)	I_{50}(S) μM	I_{50}(R) μM	R/S
I	H	1.1	0.5	12	24
II	Me	1.9	0.07	8	114
III	Et	3.1	0.035	4	114
IV	Pr	5.1	0.08	10	125
V	i-Pr	5.8	10	150	15
atrazine		3.2	0.21	≈100	≈500
diuron		4.4	0.070	0.11	1.6

tal to activity, suggesting here also some steric hindrance. A methoxy substituent (**XI**) also reduced activity, which can be ascribed to a lower hydrophobicity, as shown, for example, by comparison of compounds **III** and **XI.**

The activity of 6-chloro-5-methylthiopyrimidines was decreased on triazine-resistant chloroplasts (Table II), at a somewhat lesser extent than that of triazines themselves. The greater increase of I_{50} values was found for the most active compounds **II, III, IV,** as shown by R/S ratios. This is consistent with the loss of a high-affinity site caused by triazine-resistance, while an unspecific inhibition remains. However, the steric hindrance introduced by the 2-isopropylamino in compound **V** was also present in R chloroplasts, which suggests that some steric constraints remain in the R-modified site. The residual activities in R chloroplasts were higher for compound **III** than for atrazine, but were not very different from those of methylthiotriazines (they were nearly the same for **III** and terbutryne [14].

A structural analogy can be found between 6-chloro-5-methylthiopyrimidines and chloro-triazines, by replacing the methylthio-substituted carbon in 5 by an aromatic ring nitrogen. Furthermore, the important losses of activity observed on triazine-resistant chloroplasts suggest that the sites of action of thiopyrimidines and triazines are very close. However, triazines do not exhibit the steric hindrances observed here for alkylamino substituents [1, 2] namely a depressing effect of propylamino and isopropylamino groups in 2, and the requirement of a free NH_2 in 4. Further comparisons between these two related series could lead to a better insight into their site of action.

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