Photosystem II Inhibition by Pyran-enamine Derivatives

Koichi Yoneyama, Yoshihiro Nakajima, Masaru Ogasawara, Hitoshi Kuramochi, and Makoto Konnai
Weed Science Center, Utsunomiya University, 350 Mine-machi, Utsunomiya 321, Japan

Hajime Iwamura and Fumihiko Sato
Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Kitashirakawa-Oiwake-cho, Kyoto 606, Japan

Katsunori Ichinose, Tadao Asami, Nobutaka Takahashi, and Shigeo Yoshida
The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-01, Japan

Z. Naturforsch. **48c**, 163–168 (1993); received November 3, 1992

Photosystem II, Electron Transport Inhibition, Pyran-enamine Derivatives

Through the studies on structure-activity relationships of 5-acyl-3-(1-aminoalkylidene)-4-hydroxy-2H-pyran-2,6(3H)-dione derivatives in photosystem II (PS II) inhibition, overall lipophilicity of the molecule was found to be a major determinant for the activity. In the substituted N-benzyl derivatives, not only the lipophilicity but also the electronic and steric characters of the substituents greatly affected the activity. Their mode of PS II inhibition seemed to be similar to that of DCMU, whereas pyran-enamine derivatives needed to be highly lipophilic to block the electron transport in thylakoid membranes, which in turn diminished the permeability through biomembranes.

Introduction

Various chemicals are known to inhibit photosynthetic electron flow in the photosystem II (PS II) by displacing plastoquinone Q_B from its site on the D1 protein [1, 2]. Among them, compounds having a carbonyl-conjugated enamine system have constituted a relatively new class of PS II inhibitors [3–12]. These are cyanoacrylates (**1** [3–5]), cyclohexane-diones (**AC, 2** [6, 7]), and pyran-enamines (**AP, 3** [8–10] and **PT, 4** [11, 12], Fig. 1). In particular, **AP** and **PT** were very similar in their structural requirements for inhibition [8, 11], however, their modes of inhibition seemed to be different from one another [9–11].

Structure-activity relationships in PS II inhibition by **PT** compounds showed that they required rather high lipophilicity to block electron flow in thylakoid membrane as compared to standard PS II herbicides [12]. Thus, **PT** compounds active *in vitro* were not herbicidally active presumably due to their high lipophilicities.

In order to reduce this effect, **PT** compounds were designed with a hydrophilic center in the lipophilic N-substituent, and their PS II inhibitory activities were examined in both chloroplasts and photoautotrophic cultured plant cells [13–15].

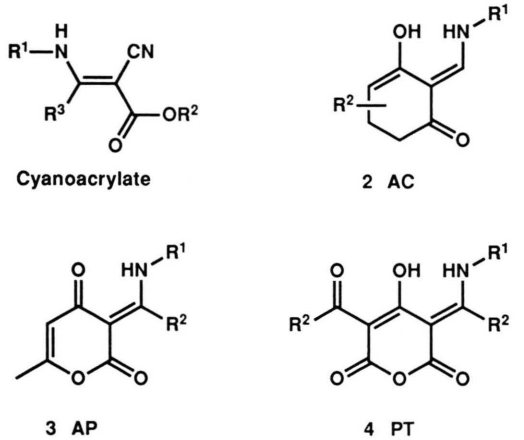


Fig. 1. Chemical structures of new PS II inhibitors having a carbonyl-conjugated enamine system. *Abbreviations:* **AC,** 2-(aminoalkylidene)-1,3-cyclohexanediones; **AP,** 3-(1-aminoalkylidene)-2H-pyran-2,4(3H)-diones; **PT,** 5-acyl-3-(1-aminoalkylidene)-4-hydroxy-2H-pyran-2,6(3H)-diones.

Materials and Methods

PS II inhibition in chloroplasts

Spinach (*Spinacia oleracea* L.) chloroplasts obtained in the usual way [16] were stored in liquid nitrogen. Photosynthetic activity was measured at pH 7.0 in 2 ml of the medium (50 mM HEPES,

Reprint requests to Dr. K. Yoneyama.

Verlag der Zeitschrift für Naturforschung, D-W-7400 Tübingen
0939–5075/93/0300–0163 $ 01.30/0

10 mM NaCl, 20 mM methylamine, 50 μM DCIP (2,6-dichlorophenolindophenol) and 0.5 μg/ml of chlorophyll). The photoreduction of DCIP was measured at 600 nm, the buffer for chlorophyll dilution being 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$ and 40 mM tricine (pH 7.8). The PS II inhibitory activities of the compounds are expressed by pI_{50} values, which indicate the negative logarithms of the concentration (M) of the compounds for 50% inhibition of the electron transport. The experiments were repeated at least three times, and the average values were reported. The range of the experimental error was within ±0.1.

PS II inhibition in cultured plant cells

The photoautotrophic (PA) cell cultures of liverwort (*Marchantia polymorpha* L.) were maintained in the light as described previously [13–15]. PA cells were used for assay on 5 days after inoculation when the cells were growing vigorously. PA cells were carefully collected by decantation, and an aliquot of 150 μl of the cells was transferred to a 96 welled plate in which each well contained 150 μl of sample solution. Each compound was dissolved in DMSO and the final concentration of DMSO in the assay medium was adjusted not to exceed 1%. These plates were kept in the light (*ca.* 150 μE/m^2/sec) for 10 min and then chlorophyll fluorescence was measured with a fluorescence multiwell plate scanner (Millipore CytoFluor 2300) where the chlorophyll fluorescence was excited at 420 ± 20 nm and measured at 690 ± 15 nm. Since chlorophyll fluorescence increases with an increase of PS II inhibition, PS II inhibitory activities of the compounds were calculated as follows: PS II inhibition (%) = ((fluorescence intensity in the presence of a sample) − (fluorescence intensity in the absence of inhibitor)) × 100/((fluorescence intensity in the presence of 10 μM DCMU) − (fluorescence intensity in the absence of inhibitor)). At least three replicates were employed for each data point. PS II inhibitory activities of the compounds were expressed as pI_{50} values.

Chemicals

The 5-acyl-3-(1-aminoalkylidene)-4-hydroxy-2H-pyran-2,6(3H)-dione (**PT**) derivatives were prepared as reported previously [11]. Structures of the synthesized compounds were confirmed by ^{1}H NMR and IR spectra, which were obtained with a Hitachi R 1200 and a SHIMADZU IR-400 spectrometer, respectively. Mass spectra were recorded on a Finnigan MAT INCOS 50 spectrometer. Satisfactory analytical data have been obtained for all the synthesized compounds.

Results and Discussion

Structure-activity relationships in PS II inhibition in chloroplasts

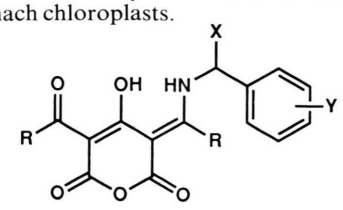
Since PS II inhibitory activity was detected in both mono- and dienamines but not in 3,5-diacyl-4-hydroxypyrans, an essential structural feature for PS II inhibition of the **PT** derivatives is the carbonyl-conjugated enamine system carrying a lipophilic N-substituent: the additional acyl group on the nucleus seems to enhance the activity [4]. Another important structural feature was the length of the alkylidene moiety. When this exceeded C_3, the activity dropped by 100-fold compared to the corresponding propylidene derivative. This indicated that the moiety was interacting with some constrained domain within the binding niche [4].

Quantitative structure-activity relationships (QSAR) analysis in PS II inhibition by **PT** derivatives indicated that the total π value or lipophilicity of the compounds was a major determinant for the activity as in the case for typical PS II inhibitors including phenylurea herbicides [17]. For example, the activity of the **PT** derivatives which have an N-alkyl rather than N-phenyl or N-benzyl moiety appeared to depend merely on the lipophilicity of the molecules [12].

Among the **PT** derivatives studied, N-phenyl derivatives were weak inhibitors. In contrast, N-benzyl, N-phenylpropyl and N-phenoxyethyl derivatives were all potent inhibitors, suggesting that the introduction of benzene ring into the N-substituent enhances the activity when the steric interaction between the benzene ring and the aminoalkylidene structure is minimized.

Similar steric interactions can be seen in the N-benzyl derivatives (Table I). In the N-benzyl derivatives, the introduction of a chlorine atom at the *para* position of the benzene ring resulted in significant enhancement of the activity. The 2-Cl derivatives were, however, less active than the unsubstituted ones [12]. Introduction of a methyl group to the benzylic position also decreased the activity when the resulting α-methylbenzyl deriva-

Table I. PS II inhibitory activities of N-benzyl derivatives in spinach chloroplasts.



R	X	Y	pI_{50}
Me	H	H	5.7
Me	H	2-Cl	5.2
Me	H	3-Cl	6.7
Me	H	4-Cl	7.2
Me	H	3,4-Cl_2	7.2
Et	H	H	6.3
Et	H	2-Cl	6.2
Et	H	3-Cl	7.6
Et	H	4-Cl	7.8
Et	H	3,4-Cl_2	8.1
(±) Me	Me	H	5.4
(*R*) Me	Me	H	4.3
(*S*) Me	Me	H	6.0
(±) Me	Me	3-Cl	7.3
DCMU*			7.3

* Positive control.

tives were assayed as enantiomeric mixtures. The *S* isomer was more active than the *R* isomer, and the effects of the substitution of the benzene ring seemed to be very similar to that in the benzyl derivative. Therefore, the *S* isomers of 3-Cl, 4-Cl and 3,4-Cl_2-α-methylbenzyl derivatives would be highly active PS II inhibitors.

PS II inhibitory activity of the *para*-substituted N-benzyl derivatives was found to be correlated not only to lipophilicity but also to other characteristics of substituents on the benzene ring as shown in Eq. (1) which had been obtained by QSAR analysis of the N-benzyl derivatives.

$$pI_{50} = 1.63\ (\pm 0.65)\ \pi - 0.11\ (\pm 0.08)\ \pi^2 + 0.47\ (\pm 0.33)\ \sigma + 0.48\ (\pm 0.37)\ B_1bz + 1.67\ (\pm 1.32) \quad (1)$$

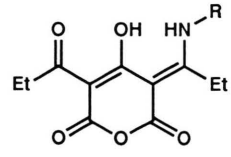
$n = 19 \qquad s = 0.22 \qquad r = 0.97$

In this equation, π is total π value of the substituents (R^1 and R^2) on the nucleus, σ is the Hammett σ constant. B_1bz is a STERIMOL steric parameter [18] which represents the minimum width in the CPK model of the *para*-substituent in the N-benzyl derivatives. The figures in parentheses are the 95% confidence intervals, *n* is the number of compounds analyzed, *s* is the standard deviation, and *r* is the multiple correlation coefficient.

In an equation obtained by QSAR analysis of **PT** compounds including N-phenyl and N-alkyl derivatives [12], coefficients with π and π^2 were very similar to those in Eq. (1), indicating that the contribution of lipophilicity to the activity enhancement is almost a constant level for all **PT** derivatives. Eq. (1) also suggests that electron-withdrawing substituents favor the activity, *i.e.*, cationic character of the nitrogen atom would be preferable if it serves as a hydrogen bond acceptor in the receptor binding. In addition, steric factor of the *para*-substituent seems to contribute to the activity enhancement to an extent similar to that for its electronic character. Within the set of compounds tested in this study, relatively bulkier *para*-substituent appears to be preferable for higher activity.

The optimal π values for **PT** compounds calculated by the equations are about 7.5 which is likely to be in a range too high to be herbicidal. Therefore, we synthesized **PT** compounds having an ether group in the lipophilic N-substituents to reduce compounds' lipophilicity, hopefully without loosing their intrinsic activity (Table II).

Table II. PS II inhibitory activities of **PT** derivatives having an ether structure in the N-substituent in spinach chloroplasts.



R	pI_{50}
Ethoxyheptyl	5.8
Propoxyhexyl	6.1
Butoxypentyl	6.0
Pentyloxybutyl	6.3
Hexyloxypropyl	6.4
Heptyloxyethyl	6.2
Decyl*	7.6
Phenoxyphenyl	7.3
Benzyloxyphenyl	7.3
Benzyloxybenzyl	8.0
Phenethyloxybenzyl	8.1
Phenylpropoxybenzyl	8.2

* Positive control.

All of the N-alkoxyalkyl derivatives were less active than the corresponding N-alkyl derivatives, and the position of the ether oxygen atom seemed not to affect the activity. However, since introduction of aryloxy groups into N-benzyl or N-phenyl derivatives afforded highly potent inhibitors, it seems possible to reduce lipophilicity by an introduction of proper hydrophilic groups into the N-substituent without loosing activity.

Structure-activity relationships in PS II inhibition in PA cells

Recently, Sato *et al.* reported that the assay using photoautotrophic (PA) cultured cells was well suited to the screening of potential PS II herbicides [15]. Since PA cells retain the chloroplasts, cell membranes and cell walls while lacking barriers represented by tissue organization like epidermis, they are more sensitive to potential PS II herbicides than whole plants, but being less sensitive than thylakoids.

When PS II inhibitory activity was examined using photoautotrophic (PA) cultured liverwort cells, all of the N-alkyl derivatives were not active even at the highest concentration tested (10^{-3} M), probably due to their lower solubilities in the test medium and lower permeabilities into membranes (data not shown). In contrast, the N-benzyl derivatives strongly inhibited PS II in PA cells and their inhibitory activities were well correlated with those obtained in isolated chloroplasts except for the 4-nitro derivative. In fact, a high correlation coefficient (r = 0.963) was obtained between pI_{50} values in chloroplasts and that in liverwort PA cells for all the other N-benzyl derivatives tested (Fig. 2).

Yanase *et al.* reported that water solubility of compounds was well correlated with compounds' systemicity, whereas correlation between systemicity and hydrophobicity (log K_{ow}) was moderate and less linear [19]. Thus, high activity of the 4-nitrobenzyl derivative in PA cells may be due to that the nitro group can form hydrogen bondings with water and thus increases water solubility.

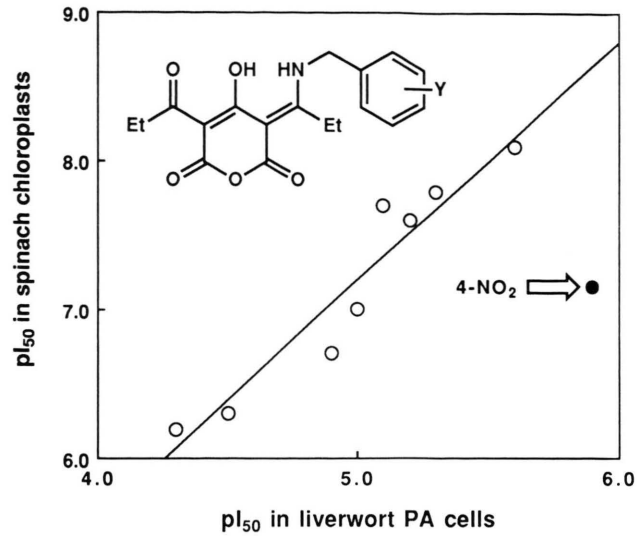


Fig. 2. PS II inhibitory activity of N-benzyl derivatives in spinach chloroplasts and in photoautotrophic cultured liverwort cells.

Consequently, to obtain *in vivo* active **PT** compounds, chemical modifications are needed to reduce lipophilicity and/or increase water solubility. In addition, the lipophilic N-substituent containing an aromatic ring structure seems to be preferable for high activity.

Acknowledgements

The authors acknowledge Dr. D. Yanase (Otsuka Chemical Co., Ltd.), Dr. H. Koike (Himeji Institute of Technology) and Dr. Y. Inoue (RIKEN) for their valuable advice on the bioassays.

[1] L. Mets and A. Thiel, in: Target Sites of Herbicide Action (P. Böger and G. Sandmann, eds.), pp. 1–24, CRC Press, Boca Raton, Florida 1989.
[2] J. R. Bowyer, P. Camilleri, and W. F. J. Vermaas, in: Herbicides (N. R. Baker and M. P. Percival, eds.), pp. 27–85, Elsevier Science Publishers, Amsterdam 1991.
[3] J. N. Phillips and J. L. Huppatz, Agric. Biol. Chem. **48,** 51–54 (1984).
[4] J. N. Phillips and J. L. Huppatz, Z. Naturforsch. **42c,** 670–673 (1987).
[5] H. G. McFadden and J. N. Phillips, Z. Naturforsch. **45c,** 196–202 (1990).
[6] T. Asami, N. Takahashi, and S. Yoshida, Z. Naturforsch. **41c,** 751–757 (1986).
[7] T. Asami, N. Takahashi, and S. Yoshida, Agric. Biol. Chem. **51,** 205–210 (1987).
[8] T. Asami, N. Takahashi, and S. Yoshida, Agric. Biol. Chem. **51,** 2775–2780 (1987).
[9] T. Asami, H. Koike, Y. Inoue, N. Takahashi, and S. Yoshida, Z. Naturforsch. **43c,** 857–861 (1986).
[10] H. Koike, T. Asami, S. Yoshida, N. Takahashi, and Y. Inoue, Z. Naturforsch. **44c,** 271–279 (1986).
[11] K. Yoneyama, M. Konnai, T. Asami, N. Takahashi, and S. Yoshida, Z. Naturforsch. **45c,** 1127–1132 (1990).
[12] K. Yoneyama, Y. Nakajima, M. Konnai, H. Iwamura, T. Asami, N. Takahashi, and S. Yoshida, Pestic. Biochem. Physiol. **41,** 288–295 (1991).
[13] Y. Yamada and F. Sato, Plant Cell Physiol. **19,** 291–299 (1978).
[14] F. Sato, S. Takeda, and Y. Yamada, Plant Cell Reports **6,** 401–404 (1987).
[15] F. Sato, Y. Yamada, S. S. Kwak, K. Ichinose, M. Kishida, N. Takahashi, and S. Yoshida, Z. Naturforsch. **46c,** 563–568 (1991).
[16] S. Katoh, in: Method in Photosynthesis Research (S. Katoh, S. Miyaji, and Y. Murata, eds.), pp. 251–253, Kyoritsu Suppan Inc., Tokyo 1981.
[17] E. Kakkis, V. C. Palmire, Jr., C. D. Strong, W. Bertsch, C. Hansch, and U. Schirmer, J. Agric. Food Chem. **32,** 133–144 (1984).
[18] A. Verloop, W. Hoogenstraaten, and J. Tipker, in: Drug Design (E. J. Ariëns, ed.), **Vol. VII,** pp. 165–207, Academic Press, New York 1976.
[19] D. Yanase and A. Andoh, Pestic. Biochem. Physiol. **44,** 60–67 (1992).