Photosystem II Inhibition by Phloroglucinol Derivatives Having Both Phenol and Urea Functionalities

Ichiro Honda, Makoto Sibagaki
Life Science Research Lab., Japan Tobacco Inc., Midori-ku, Yokohama 227, Japan

Koichi Yoneyama, Yoshihiro Nakajima, Makoto Konnai
Weed Control Research Institute, Utsunomiya University, 350 Mine-machi, Utsunomiya 321, Japan

Nobutaka Takahashi, Shigeo Yoshida
Chemical Regulation of Biomechanisms Lab., The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-01, Japan

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Esters of 3-nitrophloroglucinecarboxylic acid were found to be active photosystem II (PS II) inhibitors, as were the amides, indicating that, in their structures, the substituted phloroglucinol nuclei themselves plays a major roles in PS II inhibition. Among the phenylureidoalkyl esters tested, 3,4-dichlorophenylurea compound having two amino hydrogen atoms showed high activities. Optimal activity was associated with compounds in which the oxygen atom and the urea structure in the ester side chain were connected *via* an ethylene group.

Introduction

Phloroglucinol derivatives such as grandinol and homograndinol, potent PS II inhibitors in *Eucalyptus grandis* [1], amides (**I**) and thioamides (**II**) of 3-nitrophloroglucinecarboxylic acid have so far been developed as the most active compounds (Fig. 1) [2, 3] in the studies on photosystem II (PS II) inhibition. The (thio)amides have a phloroglucinol nuclei and an NH group, so can be regarded as both phenol and urea/triazine type PS II inhibitors. Biochemical studies on their mode of inhibition have shown, moreover, that these compounds have characteristics of both types of inhibitors [4], suggesting that the amide group and the phloroglucinol nucleus (possibly the phenolic hydroxyl(s)), may interact with different amino acid residues of the D 1 protein.

In fact, since all N,N-disubstituted-3-nitrophloroglucinecarboxamides tested were weak inhibitors ($pI_{50} < 5.0$) [3], the free amino hydrogen atom would also be involved in the binding of the (thio)amides. However, phloroglucinol derivatives without the amide moiety, such as grandinol and homograndinol, are active PS II inhibitors [5, 6] and their modes of inhibition were similar to that of the (thio)amides [4]. This indicates that, in the structures of the (thio)amides, the phloroglucinol nuclei plays major role in the binding with the receptor site, and that the free hydrogen atom on the amide nitrogen seems to improve the fit, possibly by forming a hydrogen bond between the amino hydrogen atom and some amino acid residue in the binding niche. Accordingly, if a side chain attached to the phloroglucinol nucleus allows an optimal interaction with some specific amino acid

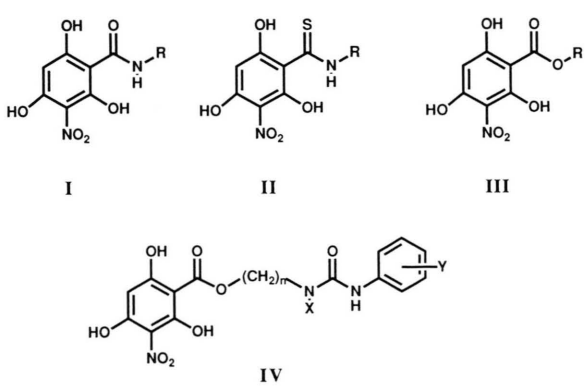

Fig. 1. Chemical structures of 3-nitrophloroglucinecarboxylic acid derivatives.

Reprint requests to Dr. Ichiro Honda, Faculty of Agriculture, Utsunomiya University, 350 Mine-machi, Utsunomiya 321, Japan (present address).
Fax: (+81) 286-35-4662.

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residues in the binding niche, such a compound may be expected to be an active PS II inhibitor.

Based on this idea, nitrophloroglucinol derivatives having a phenylurea structure in the side chain (**IV**) (Fig. 1) were synthesized, since phenylureas are potent PS II inhibitors and their structures seem to have a high affinity to the receptor site. In this study, the phenylurea moieties are connected to the phloroglucinol nuclei *via* alkoxycarbonyl groups, and thus the resulting compounds have ester instead of amide functions.

Materials and Methods

Chemicals

All melting points (m.p.) are uncorrected. ^{1}H NMR and IR spectra were obtained with a Bruker p-300 and JASCO FTIR-5000 spectrometer, respectively. Satisfactory analytical data were obtained for all the compounds.

Synthesis of 1-(2-hydroxyethyl)-3-phenylurea

In a 100 ml flask, 610 mg (10 mmol) of 2-hydroxyethylamine was dissolved in 50 ml of dichloromethane and was cooled in an ice bath. To the solution, 1.19 g (10 mmol) of phenyl isocyanate in 50 ml of dichloromethane was added slowly. After being stirred for 30 min, the precipitate was collected by filtration, and air-dried. M.p. 115–116 °C; ^{1}H NMR δ_H ($CDCl_3$/MeOH-d_4) ppm: 3.3 (3H, t, J = 6 Hz), 3.6 (3H, t, J = 6 Hz), 7.0–7.5 (5H).

Synthesis of 2-(3-phenylureido)ethyl 3-nitrophloroglucinecarboxylate (**1**)

To a mixture of 3-nitrophloroglucinecarboxylic acid (10 mmol) and 1-(2-hydroxyethyl)-3-phenylurea (10 mmol) in 50 ml of THF, dicyclohexylcarbodiimide (10 mmol) in 10 ml of THF was added at 0 °C. After being stirred for 30 min at room temperature, the precipitated urea was removed by filtration. The filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography to give **1** in a 60% yield. M.p. 125–127 °C; IR ν_{max} (KBr) cm^{-1}: 3390, 1660, 1600, 1560, 1320, 1230, 1160; ^{1}H NMR δ_H (DMSO-d_6) ppm: 3.7 (2H, d, t, J = 6 Hz, 6 Hz), 4.4 (2H, t, J = 6 Hz), 6.1 (1H, s), 6.3 (1H, t, J = 6 Hz), 6.8–7.4 (5H), 8.9 (1H, s).

$C_{16}H_{15}N_3O_8$

Calcd	C 50.93	H 4.01	O 11.14%,
Found	C 51.01	H 4.12	O 10.86%.

All the compounds tested in this study were prepared in a similar manner.

Compounds having 2–4 methylene groups between the oxygen atom and the phenylureido moiety were examined in this study. 1-Hydroxy-3-phenylurea and 1-hydroxymethyl-3-phenylurea derivatives could not be prepared due to the instability of intermediates.

PS II inhibition assay

PS II inhibitory activities of the compounds were determined by DCIP photoreduction method [7], and the compounds' activities are expressed as pI_{50} values which indicate the negative logarithms of the concentration (M) of the compounds to show 50% inhibition of electron transport.

Results and Discussion

Fig. 2 shows PS II inhibitory activities of straight chain alkyl derivative of both the amide and the ester (**III**) derivatives (Fig. 1) of 3-nitrophloroglucinecarboxylic acid. In general, the activity of the esters is 1/100 that of the corresponding amides and seems to depend largely on the lipophilicity of the side chain, as is the case in other phloroglucinol derivatives.

Although the methyl ester is a weak inhibitor (pI_{50} = 4.7), further lengthening of the alkyl chain

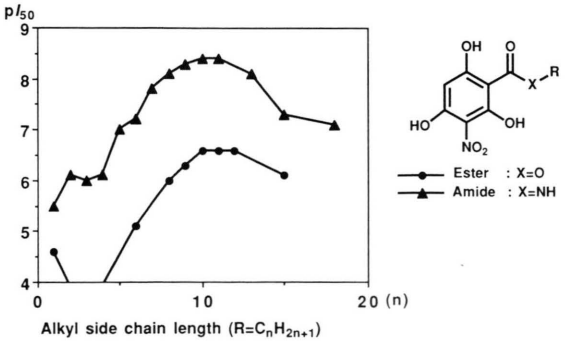


Fig. 2. PS II inhibition of amides (**I**) and esters (**III**) of 3-nitrophloroglucinecarboxylic acid in spinach chloroplasts. pI_{50} of ethyl and butyl esters were below 4.0.

produces a reduction in activity ($pI_{50} < 4.0$) until chain length reaches C_6. In the amides, also, the N-propyl derivative is less active than N-ethyl which is comparable to the N-butyl derivative. Such changes in activity can not be explained merely by increase in lipophilicity.

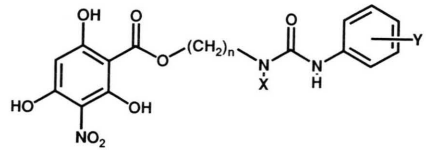
As reported earlier [2–4], the structural requirements for PS II inhibition in phloroglucinol derivatives were very similar to those proposed by Trebst *et al.* [8] for phenol type inhibitors. In the structures of nitrophloroglucinol derivatives, the nitro group should be regarded as the strongly electron-withdrawing substituent in their model. The ester or the amide group may be then be regarded as either the slightly electron-withdrawing group with strict steric requirements, or the lipophilic group without steric requirements in the model. Presumably in the esters, only the methyl group could conform to the strict steric requirements. C_2–C_4 alkyl groups may be too bulky to fit the steric requirements, and furthermore their lipophilicity may not be high enough to be considered as the lipophilic substituent. The rather unusual changes in activity of the amides carrying short N-alkyl groups can be interpreted in a similar manner. In fact, Trebst and Draber reported that the receptor site for phenol type PS II inhibitors could recognize one *ortho*-substituent from another and that such recognition of substituent would vary depending on the shape or bulkiness of the substituent [8].

Since the ester showed moderate PS II inhibitory activities, we introduced a phenylurea structure into the ester group so as to produce a better fit to the receptor site by forming additional hydrogen bonding(s) between the urea and some amino acid residue(s) in the binding niche.

In a preliminary experiment, PS II inhibitory activities of 1-(ω-hydroxyalkyl)-3-phenylureas were examined (data not shown). Only 1-(2-hydroxyethyl)-1-methyl-3-(3,4-dichlorophenyl)urea showed moderate activity ($pI_{50} = 6.1$) and the other ureas were inactive ($pI_{50} < 4.0$). These results were in good agreement with those reported for phenylurea PS II inhibitors [9].

PS II inhibitory activities of phenylureidoalkyl 3-nitrophloroglucinecarboxylate are listed in Table I. Considering the length of phenylureido substituent as that of C_7 straight alkyl chain, compound **1** ($pI_{50} = 5.5$) was less active than the corresponding alkyl ester derivative ($pI_{50} = 6.3$). This decrease in the activity may be attributable to the decreased lipophilicity brought about by the introduction of a hydrophilic urea moiety, since the activity of phloroglucinol derivatives depends largely on the lipophilicity of the substituents. Nevertheless, nearly 100-fold activity enhancement ($pI_{50} = 7.5$) was achieved by the introduction of two chlorine atoms into the phenylurea moiety (**2**), being more active than the corresponding straight alkyl ester. This activity enhancement can not be explained merely by the increased lipophilicity, and some specific interactions between the 3,4-dichlorophenyl structure and the receptor site seem to improve the affinity to the site.

Table I. PS II inhibitory activities of phenylureidoalkyl 3-nitrophloroglucinecarboxylate (**IV**) in spinach chloroplast.



No.	*n*	X	Y	pI_{50}
1	2	H	None	5.5
2	2	H	3,4-Cl_2	7.5
3	2	Me	3,4-Cl_2	6.6
4	3	H	3,4-Cl_2	6.2
5	4	H	3,4-Cl_2	6.4
DCMU*				7.2

* Positive control.

A free amino hydrogen atom may be needed for activity as in the case of the amide derivatives [4], because N-methylation decreased the activity to 1/10 (**3**) ($pI_{50} = 6.6$). However, the position of the amino hydrogen atom relative to the nuclei in the urea-phloroglucinol derivatives is different from that in the amide-phloroglucinol derivatives, and thus the corresponding hydrogen bond acceptors and amino acid residues of the D1 protein involved in the binding to the site would be different in these two types of phloroglucinol derivatives. PS II inhibitory activities of **4** ($pI_{50} = 6.2$) and **5** ($pI_{50} = 6.4$), which has 3 and 4 methylene groups respectively, were lower than the 2 methylene-analogue (**2**), indicating that the urea structure should be separated by 5 bonds from the phloroglucinol nucleus for an optimal activity.

Table II. R/S values of 3,4-dichlorophenylureidoethyl 3-nitrophloroglucinecarboxylate (**2**) in the chloroplasts from herbicide-resistant plants.

Plant compound No.	*Bra**1	Di22*2	Tob*3
		R/S*4	
2	1.2	0.8	1.0
DCMU	2.9	1093.0	24.0
Atrazine	1453.0	1.7	457.0
Ioxynil	0.5	0.9	0.9

Herbicide-resistant plant: *1 *Brassica napus* [10]; *2 cyanobacterium Di22 [11]; *3 tobacco [12]. *4 R/S value = I_{50}-resistant/I_{50}-susceptible.

To analyze the mode of action of the most active urea-phloroglucinol **2,** its inhibitory activities on PS II in thylakoids from several herbicide-resistant plants were examined (Table II). Since R/S values (I_{50}-resistant/I_{50}-susceptible) of **2** were very similar to that of the phenol type PS II inhibitor ioxynil, **2** can be classified as a phenol type inhibitor. These results suggest that the phloroglucinol nucleus of **2** would interact strongly with the binding domain for phenol type PS II inhibitors.

An amino acid residue His_{215} of the D1 protein is proposed as being involved in the binding of phenol type PS II inhibitor [13]. Recently, Oettmeier *et al.* have shown that azido-ioxynil bind to Val_{249} of the D1 protein [14]. Thus, the nuclei of the phloroglucinol PS II inhibitors would be oriented to His_{215} or Val_{249} and their side chains would interact with a lipophilic region, presumably in the parallel helix that lies on the top of the membrane. This lipophilic region in the parallel helix contains Tyr_{254} which has been shown to be involved in the phenylurea binding [15], and thus the 3,4-dichlorophenylurea moiety of **2** would also point towards to this region.

Further study is needed to clarify the actual binding site for the phloroglucinol derivatives including **2,** and biochemical study on their modes of action is now in progress.

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