Phloroglucinol Derivatives as Potent Photosystem II Inhibitors

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

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

Shigeo Yoshida, Nobutaka Takahashi, Hiroyuki Koike*, and Yorinao Inoue*
Chemical Regulation of Biomechanism Lab. and
* Solar Energy Research Group, The Institute of Physical and Chemical Research (RIKEN),
Wako-shi, Saitama 351-01, Japan

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Studies on structure/activity relationships of phloroglucinol derivatives that had been designed based on the structures of grandinol and homograndinol, potent photosystem II (PS II) inhibitors in *Eucalyptus grandis*, revealed that two electron-withdrawing groups which differ by their electron-withdrawing power on a phloroglucinol nucleus were essential for activity. A larger difference in the electron-withdrawing power between the two groups enhanced the activity, and 3-nitro-phloroglucinecarboxamides and the corresponding thioamides were the most active ones when they had proper lipophilic side chains. Their binding domain seems to overlap with those of DCMU and atrazine, whereas they may approach to the site in a similar manner to that of phenol type inhibitors. Accordingly, the phloroglucinol derivatives represent a new type of PS II inhibitors.

Introduction

New PS II inhibitors of high potency are useful tools for probing the topography of the binding niche on D 1 protein and experiments with such inhibitors will provide novel information of the interaction between the inhibitors and the binding niche. In particular, new PS II inhibitors which structurally differ from well-known inhibitors are better tools in such studies, since they may interact with the receptor site in a different manner from those of well-known inhibitors. Under these circumstances, isolation and identification of grandinol (**1**) and homograndinol (**2**) as potent PS II inhibitors in leaf extracts of *Eucalyptus grandis* provided a new basis for molecular design of PS II inhibitors, because of their unique structures (Fig. 1) and of their high potencies in PS II inhibition [1]. These two inhibitors seemed to fit the structural requirements for phenol type inhibitors proposed by Trebst *et al.* [2], however the formyl group, which should correspond to the strongly electron-withdrawing *para* substituent in their model, is not as strong as nitro group in its electron-withdrawing power. Therefore, we tried to find new active PS II inhibitors by modifying the structures of those natural inhibitors.

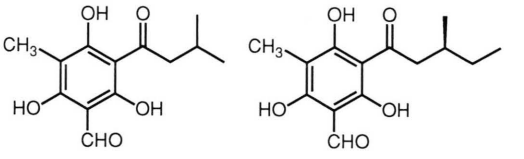

Fig. 1. Chemical structures of grandinol and homograndinol, natural PS II inhibitors in *Eucalyptus grandis*.

Materials and Methods

Preparation methods for phloroglucinol derivatives were described in [3–6]. 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. Thermoluminescence measurements were done as described in [8].

Reprint requests to Dr. K. Yoneyama.

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Results and Discussion

Structure/activity relationships of phloroglucinol derivatives

To clarify the structural features essential for PS II inhibition in phloroglucinol derivatives, structure/activity relationships of phlorophenone derivatives including grandinol and homograndinol were examined [9]. Simple phlorophenones themselves showed weak activities and the introduction of formyl group to these phlorophenones greatly enhanced the activity. Therefore, both of the acyl groups on a phloroglucinol nucleus seemed to be essential for high activity. The length or lipophilicity of the acyl group also affected the activity, the optimized chain length being around C_6 (**3**) in these compounds (Fig. 2).

When the formyl group was replaced with an acyl group, in this case propionyl group (**4**), in order to eliminate the difference of electron-withdrawing power between the two carbonyl functionalities, the activity dropped by a magnitude of one order. This clearly indicates that these two substituents should differ in electron-withdrawing power for high activity.

In fact, when the heptanoyl group in **4** was replaced with a weakly electron-withdrawing amide group (**5**), the activity increased again [10]. In addition, the introduction of a much weaker electron-withdrawing thioamide group (**6**) further enhanced the activity [11]. On the other hand, replacement of the propionyl group in **4** with a strongly electron-withdrawing nitro group afforded different types of potent inhibitors [5]. In these nitro-phloroglucinol series (**7**–**9**) (Table III), activity increased with an increase in the difference in electron-withdrawing power between the two functionalities on the nuclei; nitro-ketone (**7**) < nitro-amide (**8**) < nitro-thioamide (**9**) [5, 6, 12]. The latter two compounds showed highly potent activities, and in particular some of nitro-amide and nitro-thioamide derivatives are 10-fold more active than DCMU [12]. Therefore, structure/activity relationships of these highly active nitro-amides and thioamides were precisely examined.

As well as other phloroglucinol derivatives [5, 9–11], inhibitory activity of nitro-amide and thioamide derivatives seemed to depend largely on the lipophilicity of the molecules as shown in Fig. 3 [6, 12]. This was confirmed by QSAR analysis [12].

The QSAR analysis of the nitro-amides and thioamides afforded Eqn. (1).

$$pI_{50} = -0.10\,\pi^2 + 1.18\,\pi - 0.35\,I(Ph) - 0.87\,I(Aral) + 0.36\,I(S) + 4.52 \quad (1)$$
$$n = 55,\ s = 0.37,\ r = 0.92$$

In this equation, π is the π value of the substituent on the amide nitrogen atom, and *I(Ph)* and *I(Aral)* are indicator variables that take one for N-phenyl and N-phenylalkyl derivatives, and zero for N-alkyl derivatives, respectively. *I(S)* is also a indicator variable that takes one for thioamides and zero for amides. The coefficients of these vari-

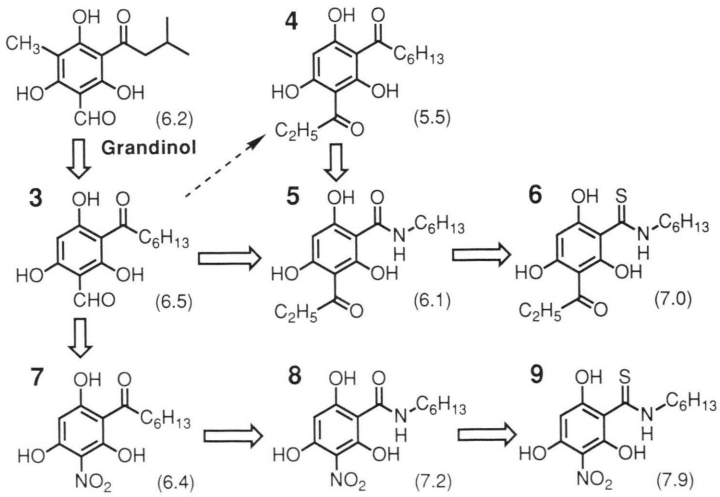


Fig. 2. Chemical structures of phloroglucinol derivatives and their PS II inhibitory activity. Figures in parentheses are pI_{50} values.

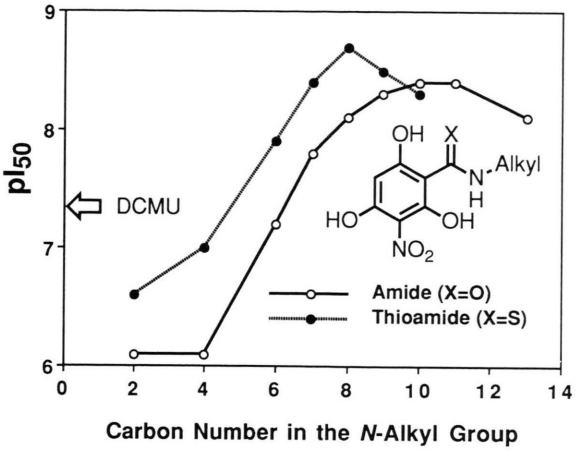
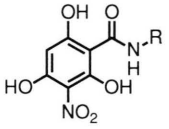


Fig. 3. PS II inhibitory activity of nitro-amide and thioamide derivatives carrying N-alkyl groups.

ables suggest that the introduction of phenyl or phenylalkyl groups into the amide moiety reduces the activity, and thioamides are more active than the corresponding amide derivatives.

However, the introduction of phenyl or phenylalkyl groups does not always reduce the activity (Table I). For example, N-phenylpropyl (**13**) and N-phenylbutyl (**14**) derivatives show strong activities, indicating that in those compounds the benzene ring should be a correct orientation for optimal interaction with the receptor site, and this preferred orientation can be allowed when the alkyl chain between the benzene ring and the amide group exceeds C_3. Furthermore, highly potent activity of N-(4-phenoxy)phenyl derivatives (**15–17,**

Table I. Inhibitory activities of nitro-amide derivatives on Hill reaction in spinach thylakoids.



Inhibitors	R	pI_{50}
10	Phenyl	6.3
11	Benzyl	5.5
12	2-Phenethyl	5.3
13	3-Phenylpropyl	6.8
14	4-Phenylbutyl	7.4
15	4-Phenoxyphenyl	8.0
16	4-(4-Cl-Phenoxy)phenyl	8.2
17	4-(2,4-Cl_2-Phenoxy)phenyl	8.1

$pI_{50} \geq 8.0$, Table I) cannot be explained merely by the increased lipophilicity.

The three hydroxyl groups on the nucleus are also seen to be important structural features for activity, since the corresponding dihydroxy (resorcinol) and monohydroxy (phenol) analogs were less active [11].

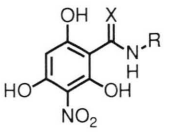
Modes of inhibition

The phloroglucinol derivatives seem to be classifiable as phenol type inhibitors as they contain the characteristic nucleus and in addition structural features essential for activity are similar to those for phenol type inhibitors proposed by Trebst *et al.* [2]. However, some of them, in particular most active nitro-amide and thioamide derivatives, contain an amide moiety and so these compounds can be seen from their chemical structures to have features of both urea/triazine type and phenol type inhibitors. Then the mode of action of phloroglucinol derivatives was investigated by pI_{50} comparison test between atrazine resistant (mutant) and susceptible (wild type) chloroplasts, and by means of thermoluminescence (TL) measurement.

In the experiments using chloroplasts from atrazine resistant and susceptible *Brassica napus*, pI_{50} values for nitro-amide and thioamide compounds carrying an N-heptyl group (**22, 23**) were determined and their ratio (I_{50} resistant/I_{50} susceptible) were compared in Table II. As already pointed out by several authors [13], atrazine resistant *B. napus* chloroplasts get more sensitive to the classical phenol type inhibitors. It is a conventional measure for classification of new PS II inhibitors. Both nitro-amide (**22**) and thioamide (**23**) compounds showed resistance factors less than 1.0, which is smaller than that of DCMU and close to those of ioxynil and dinoseb, typical phenol type inhibitors (Table II). Similar resistance factors were also obtained for the phloroglucinol derivatives tested in Table III (data not shown). The results indicate that all the phloroglucinol derivatives could be classified as phenol type inhibitors, as was predicted by their chemical structures.

We recently introduced a new technique, thermoluminescence (TL), to classify PS II inhibitors. The peak temperatures in the presence of phenol type inhibitors are much lower than those of urea/triazine type inhibitors [8]. Comparison of TL

Table II. Resistance factors (I_{50} resistant/I_{50} sensitive) observed in the Hill reaction in thylakoids isolated from atrazine resistant *Brassica napus* to PS II inhibitors including N-heptyl-3-nitrophloroglucinecarboxamide (**23**) and the corresponding thioamide (**24**).



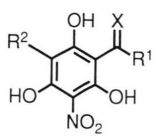
Inhibitors	R	X	Resistance factor
23	Heptyl	O	0.4
24	Heptyl	S	0.8
Dinoseb			0.2
Ioxynil			0.4
DCMU			3.7
Simetryne			>300
Atrazine			>1000

peak temperatures is, therefore, another new measure for the classification of PS II inhibitors.

The results of TL analyses done with a set of compounds listed in Table III indicate that all of the phloroglucinol inhibitors tested are classified as úrea/triazine type PS II inhibitors except for the nitrophlorophenone derivative containing a propyl group on the nucleus (**18**).

All of nitro-amide (**8, 19, 20**) and thioamide compounds (**9, 21**) should be classified as urea/triazine type as their TL glow peak temperatures are very close to those of DCMU (+6 °C) and atrazine (+2 °C). In general, TL glow peak temperatures of thioamides (**9, 21**) are lower than those of amide analogs (**8, 20**), and the compounds carrying a longer N-alkyl group (C_8) have higher TL glow peak temperatures. These results suggest that the difference in electron-withdrawing power between the two groups on the nuclei affects not only electronic character of the nucleus but also the precise orientation of the molecule in the niche, which may also be influenced by the lipophilicity of the N-substituent.

Table III. Inhibitory activities on Hill reaction in spinach thylakoids and thermoluminescence (TL) glow peak temperatures of nitro-amide and thioamide derivatives, and standard PS II inhibitors.



Inhibitors	R^1	R^2	X	pI_{50}	TL (°C)
7	Hexyl	H	O	6.3	+4
8	Hexylamino	H	O	7.2	+4
9	Hexylamino	H	S	7.9	−1
18	Hexyl	nPr	O	6.4	−10
19	Hexylamino	Et	O	6.7	+4
20	Octylamino	H	O	8.1	+6
21	Octylamino	H	S	8.7	+4
DCMU				7.3	+6
Atrazine				6.6	+2
Ioxynil				7.0	−7

Introduction of a propyl group into the nuclei of nitrophlorophenones did not affect the activity [5]. However, in the case of nitro-amide derivatives, introduction of an ethyl group, smaller than propyl group, clearly reduced the activity (**8** *vs.* **19**). These results indicate that the 5-position of the nucleus is much more sterically hindered in nitro-amide derivatives than in nitrophlorophenones.

The TL glow peak temperature of nitrophlorophenone derivative (**7**) was +4 °C, which is close to those of **8, 19** and **21,** however, that of its analog containing a propyl group on the nucleus (**18**) was −10 °C. This suggests that the compound **18** should bind to the niche in a different manner from those of other compounds. Effects of the ketonic side chain on activity were also different between these two types of nitrophlorophenone derivatives. As well as other phloroglucinol derivatives, inhibitory activity of the nitrophlorophenone derivatives carrying an aromatic alkyl group, *e.g.* **18,** depended on the side chain length or lipophilicity of the molecules, but activity of nitrophlorophenone derivatives without the aromatic alkyl group, *e.g.* **7,** remained rather constant irrespective of the change in the side chain length [5]. This can be explained in terms of different orientation of the molecules in the niche, *i.e.*, ketonic side chain in the former compounds should correspond to the slightly electron-withdrawing group with strict steric requirement of Trebst's model, but in the latter compounds it should be recognized as the lipophilic group without strict steric requirement [3].

In the present study, we estimated the mode of action of phloroglucinol derivatives by two different measures. However, an opposite results were obtained: They were classified as phenol type by

resistance factor test (Table II), while most of them were classified as urea/triazine type by thermoluminescence measurement (Table III). The resistance factor test, the comparison of I_{50} between resistant and susceptible chloroplasts, represents the affinity of the inhibitor to the binding site(s). On the other hand, TL peak temperature measurement implies the change in the redox potential in D 1 protein. Thus, the binding affinity (I_{50}) to the site does not always correlate to the redox potential change of Q_A^- which results in the change of TL peak temperature.

Based on these, the apparent discrepancy could be rationalized as follows: The phloroglucinol derivatives approach to the binding niche in a similar manner to those of phenol type inhibitors, some dynamic change of the binding niche may be involved during this process [14, 15], which reflects I_{50} value. After sitting on the site, they interact with amino acids of D 1 protein in a similar manner to those of urea/triazine type inhibitors, which reflects peak temperature of thermoluminescence. Only the compound **18** would interact with the amino acid specific to the classic phenol type inhibitors.

Accordingly it is concluded that the phloroglucinol derivatives represent a new type of PS II inhibitor and hoped that further study with the compounds will provide novel information on the binding niche in D 1 protein.

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