Photosynthetic Electron Transport Inhibition by Phlorophenone Derivatives

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The structure/activity relationships of phlorophenone derivatives including grandinol and homograndinol, which are potent inhibitors of photosynthetic electron transport (PET) in *Eucalyptus grandis*, were examined. The results indicate that at least one acyl functionality on a phloroglucinol nucleus was essential for PET inhibition, and that the lipophilicity and/or length of the acyl group was the most prominent factor affecting the activity. In addition, the introduction of a formyl group to monoacyl-phloroglucinols remarkably enhanced the activity. The structural requirements for PET inhibition in these compounds were found to be very similar but not identical with those for the phenol type of inhibitors proposed by Trebst *et al.*

Compounds which interfere the photosynthetic electron transport (PET) at the reducing site of photosystem II (PSII) have constituted one of the most important group of herbicides.^{1~3)} Through extensive biochemical and physiological studies on their modes of action, those herbicides, inhibiting at the reducing site of PSII, have been divided into two chemically different classes, *i.e.*, the urea/triazine type and phenol type.^{4,5)} The latter type of compounds including hydroxybenzonitriles and nitrophenols are also potent uncouplers of mitochondrial oxidative phosphorylation, and thus it may be difficult to define which effect contributes more to their herbicidal action *in vivo*.⁵⁾ These two types

of PET inhibitors are different not only in chemistry but also in a number of functional aspects.^{6,7)} The structural requirements in the phenol type of PET inhibitors for the activity have been proposed by Trebst *et al.*⁶⁾

Recently, grandinol (1)⁸⁾ and homograndinol (2) were identified as PET inhibitors in leaf extracts of *Eucalyptus grandis*.⁹⁾ These compounds seem to be classified as the phenol type of PET inhibitors as they contain phloroglucinol nuclei. In their structures, although they do not have nitro or cyano groups, which are commonly found in the structures of typical phenol type of herbicides, as a potent electron-withdrawing function,⁶⁾ they were as active as the herbicides on the PET system

Fig. 1. Structures of Phlorophenone PET Inhibitors.

in isolated chloroplasts. This has prompted us to study on structure/activity relationships of the phlorophenone derivatives including grandinol and homograndinol.

Materials and Methods

Hill reaction assay. The compounds were assayed for their inhibition of the Hill reaction, using chloroplasts isolated from the leaves of *Spinacia oleracea* (cv. spinach). Photosynthetic activity was measured at pH 7.0 in 2 ml of the medium (50 mM HEPES, 10 mM NaCl, 20 mM methylamine, 50 μ M DCIP and 0.5 μ g/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 PET inhibitory activities of the compounds are listed in Tables I to IV as the pI_{50} values. These values indicate the negative logarithms of the concentration (M) of the compounds for 50% inhibition of PET.

Compounds. Melting points (mp) were not corrected. 1H -NMR spectra were obtained with JEOL MH-100 spectrometer using tetramethylsilane as an internal standard in $CDCl_3$. IR spectra were measured with a JASCO A-400 spectrometer, and mass spectra, including high resolution (HRMS) were measured with a JEOL DX 303 spectrometer.

Syntheses and physico-chemical properties of the compounds tested have already been reported in the preceding papers,^{9,10} except for the compounds having 3-methylvaleryl group (2, 4 and 6).

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Synthesis of homograndinol (2S). The established preparation method for grandinol (**1**)^{10,11} was applied for the synthesis of optically active **2** as shown in Scheme 1.

To a mixture of anhydrous phloroglucinol (**7**, 20 mmol) and anhydrous aluminum chloride ($AlCl_3$, 60 mmol) in 200 ml of nitrobenzene, *S*-3-methylvaleryl chloride (20 mmol) provided from *S*-(+)-2-methyl-1-butanol by four reaction steps was added slowly with efficient stirring under ice-cooling conditions. The reaction mixture was stirred for 60 hr at the ambient temperature and worked up as usual to give *S*-3-methylvalerophenone (**4**) in a 65% yield. To a mixture of **4** (10 mmol) and $AlCl_3$ (30 mmol) in nitrobenzene (100 ml), orthoethyl formate (50 mmol) was added dropwise at 0°C with efficient stirring. The formylation was completed within 30 min, and *S*-3'-formyl-3-methyl-valerophlorophenone (**6**) was then isolated by following the previous report¹¹ in a 53% yield. Finally, a mixture of **6** (5 mmol), potassium carbonate (20 mmol) and iodomethane (50 mmol) in 30% aqueous acetone was stirred at 50°C for 3 hr, and the product was purified with silica gel column chromatography to give the *S* isomer of **2** (45% yield), mp 153~155°C. IR $\nu_{max} cm^{-1}$ (Nujol): 3340(s), 1650 (s), 1630 (s), 1600 (s). 1H -NMR δ : 0.92 (3H, t), 0.95 (3H, d), 1.28 (2H, m), 2.05 (3H, s), 2.34 (1H, m), 2.88 (1h, dd), 3.15 (1H, dd), 10.1 (1H, s). HRMS: Found, 266.1169; Calcd. for $C_{14}H_{18}O_5$, 266.1156. The racemic mixture (**2**) had a melting point of 145~146°C.

3-Methylvalerophlorophenone (4). mp 145°C; IR $\nu_{max} cm^{-1}$: 2900 (s), 1600 (s), 1450 (s); 1H -NMR δ : 1.07 (2 $\times$ CH_3 , d, $J = 7$ Hz), 1.93~2.29 (CH, m), 2.81 (CH_2 , d, $J \times$

Scheme 1. Preparation method for homograndinol.

7 Hz), 5.73 (2 × CH₃s); M⁺, 210.

3'-Formyl-3-methylvalerophlorophenone (**6**). mp 74°C; IR ν_{max} , cm⁻¹: 3350 (s), 1650 (s), 1630 (s), 1600 (s); ¹H-NMR δ : 0.96 (2 × CH₃, d, *J* = 7 Hz), 2.22 (CH, m), 2.97 (CH, d, *J* = 8 Hz), 5.84 (CH, s), 10.04 (CH, s); M⁺, 238.

Results and Discussion

Effect of substituents on PET inhibition

As grandinol and homograndinol are classified into acyl-formyl-methyl-phloroglucinol derivatives, it seemed logical to reveal which substituent (R¹, R² or R³ in Fig. 1) on a phloroglucinol nucleus would play a major role in exhibiting PET inhibitory activity.

As shown in Table I, the monoacyl-phloroglucinols (**3** and **4**) showed a weak activity, while phloroglucinol (**7**) itself was almost inactive. Accordingly, an acyl functionality on a phloroglucinol nucleus should be essential for PET inhibition. Furthermore, the introduction of a formyl group to the monoacyl-phloroglucinols clearly enhanced the activity, and such compounds without a methyl group on the nuclei (**5** and **6**) were as active as grandinol (**1**) and homograndinol (**2S**). These results indicate that, among the three substituents, the aromatic alkyl group

(R³) may affect the activity only to a small extent, while the other two (R¹ and R²) may have greater influence on the activity.

Although chiral recognition appears to be an important contributor to the affinity and activity of PSII inhibitors,^{12,13} the racemic mixture (**2**) was as active as the *S* isomer (**2S**), homograndinol. This may have been caused by the smallest difference in the bulkiness of the substituents on the asymmetric carbon atom; Gardner and Sanborn have reported that the differences in PET inhibitory activity of optically active triazine and urea derivatives were greatly reduced if the chiral substituents were methyl and ethyl rather than methyl and phenyl.¹²⁾

In general, the pI₅₀ values obtained by the dye method were higher than those obtained by the oxygen electrode method, mainly due to the lower chlorophyll concentration used in this study. Although the pI₅₀ values of grandinol and homograndinol have been reported to be 4.7 and 5.3 in the oxygen electrode system, and thus grandinol had been less active than homograndinol,⁹⁾ there was almost no difference of the activity between the two compounds with the dye method.

1) Effect of the carbon length of the acyl group

Table II shows the effect of the carbon length of the ketonic side chain on the PET inhibitory activity. The activity was enhanced by increasing the number of carbon atoms in the side chain, and reached a maximum with the hexanoyl group (**12**), which was almost as active as the phenol type of herbicide ioxynil (4-hydroxy-3,5-diiodobenzonitrile). This activity enhancement would be attributable to the increased lipophilicity of the side chain, similar effects having been observed for other PET inhibitors such as cyanoacrylates¹⁴⁾ and conjugated enamino compounds.^{15,16)} However, relatively short chain lengths would be preferable in the case of the phlorophenone derivatives as compared with the other PET inhibitors, in which optimized values were associated with C₁₀–C₁₂ alkyl chains. In addition, the 3-methylvaleryl derivative (**6**) was

Table I. PET INHIBITORY ACTIVITY OF PHLOROPHENONE DERIVATIVES

No.	R ¹	R ²	R ³	pI ₅₀
1	Isovaleryl	Formyl	Methyl	6.3
2	3-Methylvaleryl	Formyl	Methyl	6.2
2S (Homograndinol)				6.2
3	Isovaleryl	H	Methyl	5.2
4	3-Methylvaleryl	H	Methyl	5.2
5	Isovaleryl	Formyl	H	6.1
6	3-Methylvaleryl	Formyl	H	6.2
7	H	H	H	<4
	Ioxynil ^a			6.9
	DCMU ^b			7.3

^a 4-Hydroxy-3,5-diiodobenzonitrile.

^b 3-(3,4-Dichlorophenyl)-1,1-dimethylurea.

Table II. EFFECT OF THE CARBON LENGTH OF THE KETONIC SIDE CHAIN

No.	R ¹	pI ₅₀
8	Acetyl	4.3
9	Propionyl	5.1
10	Butyryl	5.9
5	Isovaleryl	6.1
11	Valeryl	6.3
6	3-Methylvaleryl	6.2
12	Hexanoyl	6.6
13	Heptanoyl	6.5
	Ioxynil ^a	6.9
	DCMU ^b	7.3

^a 4-Hydroxy-3,5-diiodobenzonitrile.^b 3-(3,4-Dichlorophenyl)-1,1-dimethylurea.

as active as the valeryl derivatives (**11**), indicating that branching in the ketonic side chain may have little effect on the PET inhibitory activity as discussed before.

2) Effect of the alkyl group on PET inhibition

As the data in Table I suggest, the aromatic methyl group of grandinol and homograndinol may be regarded as a non-influential moiety for the activity. To examine this, the PET inhibitory activity of compounds with an alkyl group on the nucleus was tested.

Table III shows the activity of the compounds with a varying alkyl group (R³) on the nuclei (**1**, **14**~**16**), the activity dropping by a magnitude of one order when R³ was longer than C₁, and then remaining at the constant level over the range C₂ to C₄. These results indicate that this part of the molecule may be affected by a slightly steric region of the receptor site, and compounds **1** and **5** would fit the region in more specific manner than the others would do.

3) Effect of a formyl group on PET inhibition

According to the model proposed by Trebst *et al.*,⁶⁾ a phenol needs two electron withdraw-

Table III. EFFECT OF AN ALKYL GROUP ON PET INHIBITION

No.	R ³	pI ₅₀
5	H	6.1
1	Methyl	6.2
14	Ethyl	5.3
15	Propyl	5.3
16	Butyl	5.1

ing groups for PET inhibition, and one of them located *para* to the hydroxyl should be a strongly electron withdrawing group, while the other (*ortho* to the hydroxyl) should be a slightly electron withdrawing group with strict steric requirements. In the structures of the phlorophenone derivatives, the formyl and ketone groups would be recognized as the *para* and *ortho* substituents in the model, respectively. The third substituent (*ortho* to the hydroxyl), a lipophilic group without steric requirements, might correspond to the alkyl group on the nuclei. This model seems to fit the structural requirements of grandinol, homograndinol, and their analogs discussed here. However, a formyl group is not as strong in its electron withdrawing potency as nitro or cyano groups and, in addition, steric requirements for the alkyl groups on the nuclei were observed as shown in Table III. These results suggest that the precise structural requirements of the phlorophenone derivatives should involve some difference from those of the phenol type of PET inhibitors.

As shown in Table IV, when the formyl group was replaced with an acyl group (**17**~**19**) in order to eliminate the difference of electron withdrawing potency between the two carbonyl substituents, the activity dropped by a magnitude of one order (*cf.* Table I).

Consequently, it is suggested that these two substituents should be different in electron withdrawing potency for high activity. The high activity of nitrophlorophenone deriva-

Table IV. PET INHIBITORY ACTIVITY OF DIKETO-PHLOROGLUCINOL DERIVATIVES

No.	R ¹	R ²	pI ₅₀
17	Propionyl	Propionyl	4.7
18	Valeryl	Propionyl	4.9
19	Valeryl	Valeryl	4.9

tives¹⁷⁾ is thus explained by the greater difference of electron withdrawing potency between the two substituents.

Through these studies, the structural requirements of the phlorophenone derivatives for PET inhibition have emerged; two electron withdrawing groups on a phloroglucinol nucleus are essential for activity, and these groups should be different by their electron withdrawing potencies. Furthermore, because of the symmetrical features of the phloroglucinol nucleus, it may be possible to obtain active PET inhibitors by replacing the formyl group in the structure of **1** with a weaker electron withdrawing group than the ketone group. In these compounds, the ketone group should be regarded as the substituent *para* to the hydroxyl in Trebst's model. Studies based on this idea are now progressing.

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