Photobleaching Activity of 2-(Phenylamino)methylidenecyclohexane-1,3-diones in Tobacco (*Nicotiana tabacum*) Cultured Cells

Jing-Ming Wang,[†] Tadao Asami,*[,‡] Fan-Sik Che,[§] Noboru Murofushi,[†] and Shigeo Yoshida[‡]

Department of Applied Biological Chemistry, The University of Tokyo, Bunkyo-Ku, Tokyo 113, Japan, The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako-shi, Saitama 351-01, Japan, and Nara Institute of Science and Technology (NAIST), Takayama 8916-5, Ikoma-shi, Nara 630-01, Japan

A series of phenylalkylidenecyclohexane-1,3-dione derivatives were designed and synthesized as vinylogous analogs of phthalimides, which are known photobleaching herbicides. The bleaching activity of synthesized compounds was assayed with photomixotrophic tobacco cultured cells under either light or dark conditions. Both the chlorophyll and the carotenoid contents of the cells treated with the cyclic diones decreased to almost zero within 24 h under the light condition but not under the dark condition. This rapid emergence of bleaching activity with light is one of the most distinctive actions of Protox-inhibiting herbicides; however, the cyclic dione did not inhibit protoporphyrinogen oxidase *in vitro*. Thus, we concluded that the cyclic diones possessed a different herbicidal mode of action from Protox-inhibiting herbicide.

Keywords: *Photobleaching herbicide; tobacco cells; chlorophyll biosynthesis*

Photobleaching herbicides, such as photosystem I disrupting herbicides, carotenoid biosynthesis inhibitors, and chlorophyll biosynthesis inhibitors, are widely used to protect crops. The physiological characteristic point of these herbicides is their requirement for photoirradiation of plants to exhibit their herbicidal activity. To design a new lead compound for photobleaching herbicides, we focused our attention on chlorophyll biosynthesis inhibitors because the physiological effects of these inhibitors have been well investigated. The primary site for the action of these herbicides is protoporphyrinogen oxidase (Protox) (Matringe et al., 1989a; Camadro et al., 1989, 1991; Nicolaus et al., 1993), which is the last enzyme that is common to the biosynthetic pathways of both heme and chlorophylls. Plants treated with these herbicides accumulate large amounts of protoporphyrin IX (proto IX), which readily generates radicals upon exposure to light and oxygen and induces membrane damage (Matringe et al., 1988; Sandmann et al., 1988). Although these herbicides belong to different chemical classes, such as phthalimides and diphenyl ethers, biochemical investigations have demonstrated that these herbicides all bind with Protox (Matringe et al., 1992; Versano et al., 1990), which suggests that these different classes of Protox inhibitors share some common structural features. The search for a common structural feature of known Protox inhibitors has revealed that they should contain at least two aromatic or alicyclic rings (Matringe et al., 1989b; Sato et al., 1987), which could be a key factor in discovering other novel inhibitors. X-ray crystallography of some Protox inhibitors has further revealed that the structures of such inhibitors mimic protoporphyrinogen IX (protogen IX) in that the two rings of the inhibitors correspond to the pyrrole rings of protogen IX (Nandihalli et al., 1992). On the basis of these findings, we designed new compounds which can be considered vinylogues of phthalimide herbicides, since some bioactive compounds retain their activity even after modification into vinylogues (McFadden et al., 1993). We named the series of compounds in this paper the "RWH series".

The sensitivity of photomixotrophic cultured cells to the stress of potential herbicides is expected to be intermediate between those of enzymes and whole plants. Since cultured cells retain essential cellular functions to keep growing, they may provide a convenient system for assaying phytotoxic action at the cellular level. In particular, cultured tobacco cells have been reported to be a good material for surveying new photosynthetic electron transport inhibitors and photobleaching herbicides because they are easy to handle and highly sensitive to herbicides (Kohno et al., 1994; Sato et al., 1991). In this study, we used photomixotrophic tobacco cells as a plant material to measure the photobleaching activity and *in vitro* Protox inhibitory activity of the synthesized chemicals.

RESULTS AND DISCUSSION

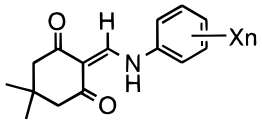
Structure−Activity Relationships in the RWH Series. There have been several reports that the chemical substituents on the aromatic ring play a significant role in the photobleaching activity of known Protox inhibitors. On the basis of these studies, we introduced various substituents onto the aromatic ring of phenylaminomethylidene-1,3-cyclohexanediones. The effects of the ring substituents of the anilide moiety on photobleaching activity are listed in Table 1. Compound **1**, which has no substituent on the phenyl ring, is used below as a standard of this activity for a discussion of structure−activity relationships.

(1) Effect of the Substituent on the Phenyl Ring Moiety of the RWH Series. The introduction of a chlorine atom (**3**), methoxyl group (**12**), or butyloxycarbonyl group (**19**) to the 4-position of the aromatic ring of compound (**1**) did not give a good result for an enhancement of its

* To whom correspondence should be addressed.
[†] The University of Tokyo.
[‡] RIKEN.
[§] NAIST.

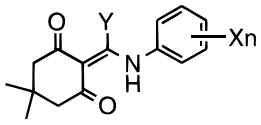
S0021-8561(96)00962-4 CCC: $14.00

Table 1. Effect of the Substituents on the Phenyl Moiety of Cyclohexanedione Derivatives on Bleaching Activity



compound no.	X	pI_{50}
1	H	4.1
2	2-Et	4.2
3	4-Cl	3.4
4	2,3-Cl_2	4.1
5	2,4-Cl_2	3.5
6	2,5-Cl_2	4.8
7	3,4-Cl_2	4.4
8	3,5-Cl_2	4.5
9	2,4,6-Cl_3	4.8
10	2,4,6-F_3	4.7
11	2,6-Cl_2-3-Me	5.0
12	4-methoxy	<3
13	2-methoxycarbonyl	4.6
14	2-ethyloxycarbonyl	4.7
15	2-*n*-butyloxycarbonyl	4.7
16	2-pentyloxycarbonyl	4.3
17	2-*n*-hexyloxycarbonyl	3.7
18	2-*n*-heptyloxycarbonyl	<4
19	4-*n*-butyloxycarbonyl	4.0
20	2-chloro-5-methoxycarbonyl	4.7
21	2-chloro-5-*n*-propyloxycarbonyl	5.8
22	2-chloro-5-(2-*n*-propenyloxycarbonyl)	4.8
23	5-chloro-2-*n*-propyloxycarbonyl	5.8
24	4-chloro-3-*n*-propyloxycarbonyl	4.2

Table 2. Effect of Structural Modification of the Alkylidene Moiety on Bleaching Activity



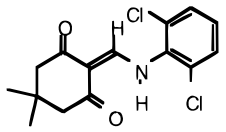
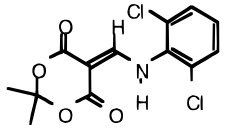
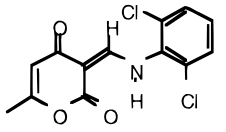
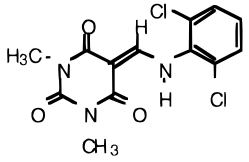
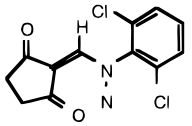
compound no.	Y	X	pI_{50}
25	CH_3	2,6-Cl_2	nd[a]
26	CH_3	2-F-4-Cl	nd
27	CH_3	2,6-Et_2	nd
28	H	2,6-Cl_2	4.8
29	H	2-F-4-Cl	4.2
30	H	2,6-Et_2	3.6

[a] nd, not detected.

photobleaching activity. This result suggests that a monosubstitution at the 4-position of the aromatic ring of compound **1** may have a negative effect on this activity regardless of the electronic character of the substituent. The introduction of two chlorine atoms increased photobleaching activity, except for compound **5**, and the introduction of three chlorine or fluorine atoms to the 2-, 4-, and 6-positions on the phenyl ring (**9, 10**) also increased this activity. Methoxy, ethoxy, butyloxy, and pentyloxycarbonyl derivatives (**13**−**16**), when substituted at position 2, are more active than **1**, whereas hexyloxy and heptyloxy derivatives (**17, 18**) are less active than **1**. This suggests that an alkoxyl group of a certain length is required for this activity: e.g., between an ethyl and a butyl group should be suitable. The most active compounds synthesized were **21** and **23**, in which one chlorine atom and one propylcarbonyl group were introduced into positions 2 and 5, and vice versa, of the phenyl ring.

(2) Effect of Modification of the Alkylidene Moiety of the RWH Series. Table 2 shows the role of alkylidene substitution on bleaching activity. The result indicates that the alkylidene moiety plays a significant role in producing the bleaching activity. While the methylidene derivatives (**28**−**30**) were active, the ethylidene derivatives (**25**−**27**) showed no activity.

Table 3. Effect of the Cyclic Dione Moiety on Bleaching Activity

	compound	pI_{50}
28		4.8
31		nd[a]
32		nd
33		nd
34		nd

[a] nd, not detected.

The cyclic 1,3-dione derivatives synthesized in this study can be considered vinylogous phthalimide derivatives, but their need for substituents on the phenyl group for exhibition of bleaching activity differs from phthalimide-type Protox inhibitors. Examination of the structure−activity relationships of phthalimide-type Protox inhibitors revealed the importance of both fluorine at position 2 and chlorine at position 4 on the phenyl ring (Wakabayashi, 1988). On the other hand, 1,3-cyclohexanedione derivative **29** which possesses such a 2-fluoro-4-chlorophenyl ring is less active than 3,4-dichlorophenyl (**7**) or 4-methoxyphenyl (**12**) derivatives, although in phthalimide herbicides 3,4-dichlorophenyl or 4-methoxyphenyl derivatives are far less active than the 2-fluoro-4-chlorophenyl derivative.

(3) Effect of Modification of the Cyclic Dione Moiety of the RWH Series. Table 3 shows the effect of the structure of the cyclic dione moiety on bleaching activity. Among the compounds listed, only the cyclohexanedione, **28**, shows this activity.

Our new chemicals consist of three parts; *i.e.*, phenyl, methylidene, and cyclic dione moieties. With regard to the phenyl and alkylidene parts, we have demonstrated their importance in photobleaching activity. Considering the structural variation in the phthalimide part of so-called phthalimide herbicides, new inhibitors might show this activity after structural modification of the cyclic dione part. Table 3 shows the activities of several cyclic dione derivatives. Although all of the compounds share some common chemical structure, the cyclic dione moieties are different. Among these compounds, only the cyclohexane-1,3-dione derivative **28** is active. Thus, we have shown that new inhibitors should have a 2-phenylaminomethylidene-5,5-dimethylcyclohexane-1,3-dione structure for the expression of bleaching acti-

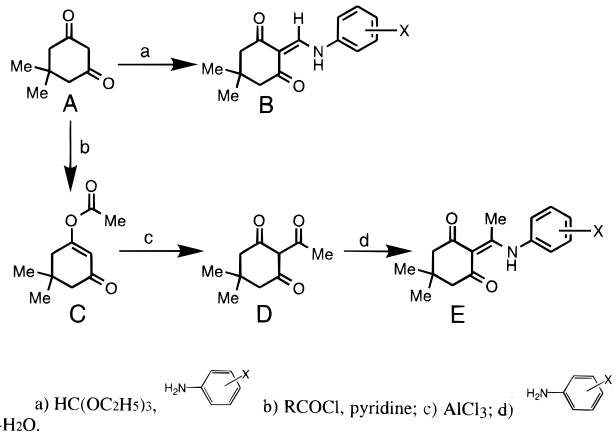


Figure 1. General synthetic route for phenylaminoalkylidene-1,3-cyclohexanedione analogues.

vity. Next, we studied the mode of action of cyclohexanedione derivatives by using compound **21**, the most potent inhibitor among the compounds synthesized.

Examination of the Mechanism of Action of Compound 21. The cyclic dione derivatives can be considered vinylogous phthalimide herbicides, but the structure−activity relationships of the substituents on the phenyl ring and the dione portion are different from those of phthalimide herbicides. There are several series of photobleaching herbicides which include a phenyl group, but their requirements for the substituents on the phenyl ring for potent activity show wide variety. Although the structure−activity relationships of the substituents on the phenyl ring and the dione portion of the cyclic dione derivatives are also different from those of phthalimide herbicides, the diones decreased the chlorophyll content of cultured cells under illumination, which is similar to the effects of photobleaching herbicides. On the basis of this finding, we analyzed the contents for both chlorophyll and carotenoids after treatment with the newly synthesized compounds under light or dark conditions to study the mechanism of action of these compounds using tobacco cells treated with compound **21** at a final concentration of 50 μM or S-23142 at 1 μM, respectively. In the light condition, the chlorophyll content of cells treated with compound **21** or S-23142 slightly decreased after 12 h. After 24 h, the tobacco cells showed almost no chlorophyll, whereas in the dark condition there was no change in the content of chlorophyll (Figure 2). Similar results were seen for carotenoids (Figure 3). These results indicate that (1) cyclic diones require illumination for the emergence of bleaching activity and (2) cyclic diones show a quick response, like other known photobleaching herbicides. On the basis of these results, we consider that cyclic diones most likely have a mode of action similar to those of photobleaching herbicides. To confirm this point, activity of **21** was enzymatically assayed.

Protox Assay with HPLC. Proto IX biosynthesized from ALA in chloroplasts was analyzed by HPLC, and the effect of Protox-inhibiting herbicides on the Proto IX biosynthesis was examined. Protox inhibitor S-23142 (1 μM) inhibits Proto IX synthesis from ALA at Protox in stromal extracts, while cyclic dione **21** (100 μM) has no effect on Proto IX synthesis (Figure 4). This result clearly indicates that the mode of action of **21** is different from that of Protox inhibitor in spite of its structural and physiological similarities to phthalimide herbicide.

In conclusion, we have synthesized novel compounds carrying a cyclohexane-1,3-dione moiety with a vinylogous moiety at the 2-position which cause photobleaching in photomixotrophic tobacco cells. Among the compounds synthesized, **21** was the most active, with a pI_{50} of 5.8 in our assay system. This activity is lower than that of the typical photobleaching herbicide acyfluorfen (pI_{50} = 8.1); however, the structures of the cyclic diones are new among photobleaching herbicides and their herbicidal mode of action is different from that of Protox inhibitor. These findings imply that the RWH series could be a good lead for new herbicides possessing new modes of action. Considering the structural and physiological features of the RWH series, there would be some possibilities that it affects chlorophyll biosynthesis. However, further studies on mode of action should reveal its physiological role in plant cell. Further studies on structure−activity relationships of the RWH series will also reveal more active structures for new herbicides.

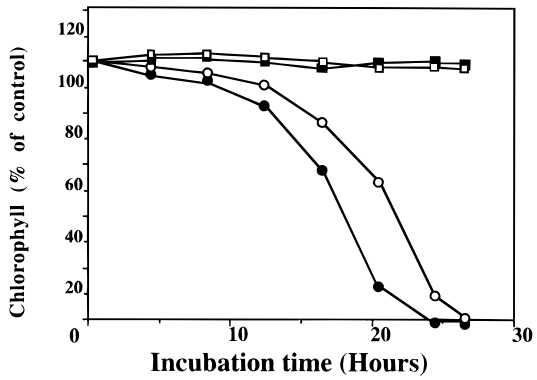


Figure 2. Effects of **21** analogue, S-23142, and Fluridone on the chlorophyll content of tobacco cells (*N. tabacum* cv. Samsun NN; cell line NI). Tobacco cells (6 days old) were placed into new culture medium containing 50 μM of **21** analogue (■, incubated in darkness; ●, incubated in light: 30 μE/m^{2}s), 100 μM Fluridone (□, incubated in light: 30 μE/m^{2}s), and 1 μM S-23142 (○, incubated in light: 30 μE/m^{2}s). The cell density was 1 mg fresh weight/mL of culture medium.

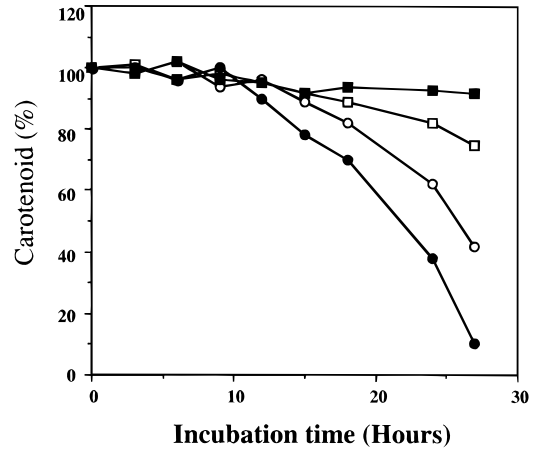


Figure 3. Effects of **21** analogue, S-23142, and Fluridone on the carotenoid content of tobacco cells. Tobacco cells (6 days old) were cultured in the presence of **21** analogue at a final concentration of 50 μM (■, incubated in darkness, ●, incubated in light: 30 μE/m^{2}s), 100 μM Fluridone (□, incubated in light: 30 μE/m^{2}s), and 1 μM S-23142 (○, incubated in light: 30 μE/m^{2}s). The cell density was 1 mg fresh weight/mL of culture medium.

MATERIALS AND METHODS

Cell Culture Methods. A photomixotrophic culture of tobacco (*Nicotiana tabacum* cv. Samsun NN; cell line NI) (Takeda et al., 1990) was maintained in modified Linsmaier

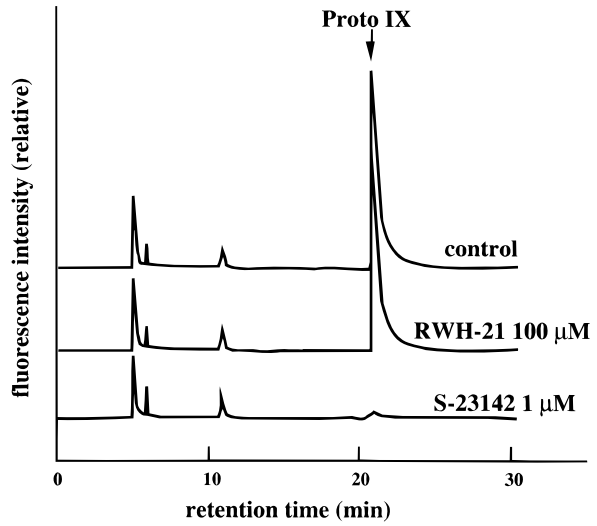


Figure 4. HPLC profiles of porphyrins synthesized by intact protoplasts. Poryphyrin in the reaction mixture were separated by reverse phase HPLC as described under Materials and Methods. This experiment was done three times with similar results.

and Skoog liquid medium with 3% (w/v) sucrose (Yamada et al., 1978). The cells were subcultured at 25 ± 1 °C with reciprocal shaking (100 rpm/min) under continuous light (ca. 30 μE/m^{2}s) for 6 days.

Determination of Chlorophyll Content and Bleaching Activity. The method used to determine chlorophyll content was based on that of Arnon et al. (1949). The tobacco cells collected by filtration were transferred into a test tube with 80% acetone solution (10 mL) and stored in a refrigerator (4 °C) overnight until all visible pigment had been extracted. The absorbance values at 645 and 663 nm were measured with a Beckman spectrophotometer (DU 640). The chlorophyll content was determined using Arnon's equation. The chlorophyll content in the treated tobacco cells was calculated as a percentage of that in the control. All of the measurements were performed in triplicate. The bleaching activity of all of the compounds was determined as the concentration that reduced the chlorophyll content by 50%. In tables, activity is expressed by the pI_{50} value, which means $-\log_{10}$ [the concentration (M) that reduced the chlorophyll content by 50%].

Carotenoid Determination. The method used to determine carotenoids was based on that of Lambert and Böger (Lambert et al., 1983) with slight modifications. The tobacco cells were collected by filtration and washed with distilled water twice. Ten milligrams (fresh weight) of tobacco cells were treated with 5 mL of 6% KOH in methanol (w/v) and incubated at 65 °C for 20 min until the pigment had been extracted. The methanol layer was then extracted with *n*-hexane−diethyl ether (9:1 v/v). The absorbance values at 445 nm were measured with a Beckman spectrophotometer (DU 640).

Isolation of Intact Chloroplasts. Spinach chloroplasts were isolated by the method of Mills and Joy (1980) with slight modifications. The leaves (50 g) were cut into small pieces, directly immersed in 150 mL of ice-cooled chloroplast extraction medium (330 mM sorbitol, 50 mM Tricine [*N*-tris-(hydroxymethyl)methylglycine]-KOH, pH 7.9, 2 mM EDTA, 1 mM $MgCl_2$, and 0.1% BSA), and homogenized for 5 s with a Polytron (PT 10/35; Kinematica, Basel, Switzerland). After filtration through four layers of gauze, 30 mL of the homogenate was poured into each 50 mL centrifuge tube and was underlayed with 14 mL of Percoll medium [40% (v/v) Percoll, 330 mM sorbitol, 50 mM Tricine, pH 7.9, and 0.1% BSA]. Chloroplasts were pelleted by centrifugation at 2500*g* for 1 min in an LC-121 centrifuge (Tomy Seiko Co., Ltd., Tokyo, Japan) equipped with a TS-7 swing-out rotor. The supernatant and then the Percoll layer were removed by aspiration, and the pellet was gently resuspended in appropriate medium with a small piece of nylon mesh attached to a glass rod. The entire isolation procedure could be accomplished in less than 5 min.

Protox Assay with HPLC. *Assay Using ALA.* Proto IX synthesis from ALA was measured using the procedure previously reported (Mito et al., 1991). The Protox activity of intact chloroplasts was assayed using ALA as the substrate. The intact chloroplasts were added to 500 mL of a reaction medium (4 mM ALA, 0.5 mM sucrose, 1 mM $MgCl_2$, 1 mM EDTA, 20 mM TES, 10 mM HEPES, pH 7.5, 4 mM glutathione, 0.6 mM NAD, 1.5 mM ATP, 2% BSA). The mixture was incubated for 3 h at 25 °C in darkness, and the reaction was stopped by addition of 1 mL of methanol. After centrifugation at 10000*g* for 5 min, amount of Proto IX was measured by HPLC. To measure the amount of Proto IX in the reaction mixture, a C8 reverse phase column (Senshu Pak, C8-2251-N, 6 mm × 250 mm, Senshu Science Co., Ltd., Tokyo, Japan) was used with a solvent system of 85% methanol containing 10 mM ammonium acetate (pH 6.0) with a flow rate of 1 mL/min. Proto IX detection was performed with a fluorescence detecter (F-1000, Hitachi Ltd., Tokyo, Japan) with excitation and emission wavelength settings at 420 and 630 nm, respectively. The amount of Proto IX was estimated using Chromatocorder-12 (Senshu Science Co., Ltd., Tokyo, Japan).

Synthesis of Phenylaminomethylidenecyclohexane-1,3-dione Derivatives. All melting points (mp) are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AC-300 Plus spectrometer. Chemical shifts are expressed in ppm downfield from TMS as an internal standard.

All of the phenylaminomethylidenecyclohexane-1,3-dione derivatives listed in Table 1 were prepared by the method described before (Zacharias et al., 1974). The phenylaminomethylidenecyclohexane-1,3-dione derivatives (**B**) were synthesized by the condensation of 1,3-cyclohexanedione homologues with triethyl formate and related anilines (Figure 1). The alkylidene moiety was modified as described previously (Asami et al., 1987). The condensation of 2-acetyl-1,3-diketones (**D**) with anilines gave ethylidene derivatives, as shown in Figure 1. All of the procedures gave good yields.

General Synthetic Procedure for 2-Phenylaminomethylidene-5,5-dimethylcyclohexane-1,3-dione (1). A 1.44 g (10 mmol) amount of 5,5-dimethylcyclohexane-1,3-dione (**A**) was added to 5 mL of triethyl formate and warmed to about 60 °C until all of the cyclohexane-1,3-dione was dissolved. A 0.93 g (10 mmol) amount of aniline was added with stirring. The mixture was warmed to 125−130 °C, and stirring was continued for 10 min. The reaction mixture was concentrated under a vacuum and washed twice with saturated aqueous sodium hydrogen carbonate. The residue was recrystallized with *n*-hexane−ethyl acetate twice to give 1.96 g of **1** (79%). Mp 136−139 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 2.45 (2H, s), 2.47 (2H, s), 7.18−7.46 (5H, m), 8.64 (1H, d, J = 13.8 Hz), 12.90 (1H, br). Anal. Calcd for (%) $C_{15}H_{17}NO_2$: C, 74.05; H, 7.04; N, 5.76; O, 13.15. Found: C, 74.11; H, 7.08; N, 5.78; O, 13.20.

Compounds **2**−**24** and **28**−**34** were prepared in the same manner as **1** using the corresponding aniline instead of the aniline.

2-(2-Ethylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (2). Mp 107−109 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 1.15 (3H, t, J = 7.5 Hz), 2.44 (2H, s), 2.46 (2H, q, J = 7.5 Hz), 2.48 (2H, s), 7.07−7.31 (4H, m), 8.17 (1H, d, J = 13.7 Hz), 12.47 (1H, br). Anal. Calcd for (%) $C_{17}H_{21}NO_2$: C, 75.25; H, 7.80; N, 5.16; O, 11.79. Found: C, 75.31; H, 7.96; N, 5.31; O, 11.88.

2-(4-Chlorophenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (3). Mp 192−195 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.09 (6H, s), 2.43 (2H, s), 2.47 (2H, s), 7.20 (2H, dd, J_1 = 6.9, J_2 = 2.1 Hz), 7.38 (2H, dd, J_1 = 6.9, J_2 = 2.1 Hz), 8.55 (1H, d, J = 13.4 Hz), 12.85 (1H, br). Anal. Calcd for (%) $C_{15}H_{16}NO_2Cl$: C, 64.87; H, 5.81; N, 5.04; O, 11.52; Cl, 12.76. Found: C, 64.84; H, 5.88; N, 5.11; O, 11.52; Cl, 12.77.

2-(2,3-Dichlorophenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (4). Mp 180−182 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.09 (6H, s), 2.44 (2H, s), 2.49 (2H, s), 7.24−7.38 (3H, m), 8.57 (1H, d, J = 13.1 Hz), 13.22 (1H, br).

Anal. Calcd for (%) $C_{15}H_{15}NO_2Cl_2$: C, 57.71; H, 4.84; N, 4.49; O, 10.25; Cl, 22.71. Found: C, 57.77; H, 4.83; N, 4.51; O, 10.37; Cl, 22.76.

2-(2,4-Dichlorophenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (5). Mp 185−186 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 2.45 (2H, s), 2.50 (2H, s), 7.34 (1H, J_1 = 2.5 Hz, J_2 = 9.1 Hz), 7.43 (1H, d, J = 8.9 Hz), 7.49 (1H, d, J = 2.2 Hz), 8.55 (1H, d, J = 13.1 Hz), 13.22 (1H, br). Anal. Calcd for (%) $C_{15}H_{15}NO_2Cl_2$: C, 57.71; H, 4.84; N, 4.49; O, 10.25; Cl, 22.71. Found: C, 57.75; H, 4.82; N, 4.71; O, 10.67; Cl, 21.76.

2-(2,5-Dichlorophenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (6). Mp 128−130 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 2.45 (2H, s), 2.50 (2H, s), 7.13 (1H, dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz), 7.38 (1H, d, J_2 = 2.2 Hz), 7.48 (1H, d, J_1 = 8.5 Hz), 8.54 (1H, d, J = 13.0 Hz), 13.11 (1H, br). Anal. Calcd for (%) $C_{15}H_{15}NO_2Cl_2$: C, 57.71; H, 4.84; N, 4.49; O, 10.25; Cl, 22.71. Found: C, 57.74; H, 4.86; N, 4.51; O, 10.22; Cl, 21.78.

2-(3,4-Dichlorophenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (7). Mp 175−177 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 2.44 (2H, s) 2.48 (2H, s), 7.18−7.38 (2H, m), 8.55 (1H, d, J = 13.0 Hz), 12.88 (1H, br). Anal. Calcd for (%) $C_{15}H_{15}NO_2Cl_2$: C, 57.71; H, 4.84; N, 4.49; O, 10.25; Cl, 22.71. Found: C, 57.68; H, 4.88; N, 4.55; O, 10.33; Cl, 21.82.

2-(3,5-Dichlorophenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (8). Mp 157−159 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 2.44 (2H, s), 2.48 (2H, s), 7.28−7.44 (2H, m), 8.55 (1H, d, J = 13.0 Hz), 12.88 (1H, br). Anal. Calcd for (%) $C_{15}H_{15}NO_2Cl_2$: C, 57.71; H, 4.84; N, 4.49; O, 10.25; Cl, 22.71. Found: C, 57.75; H, 4.66; N, 4.55; O, 10.42; Cl, 22.70.

2-(2,4,6-Trichlorophenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (9). Mp 139−141 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 2.43 (2H, s), 2.49 (2H, s), 7.43 (2H, s), 8.48 (1H, d, J = 13.0 Hz), 12.16 (1H, br). Anal. Calcd for (%) $C_{15}H_{14}NO_2Cl_3$: C, 51.97; H, 4.07; N, 4.04; O, 9.23; Cl, 30.68. Found: C, 51.90; H, 4.11; N, 4.12; O, 9.10; Cl, 30.81.

2-(2,4,6-Trifluorophenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (10). Mp 151−153 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 2.44 (2H, s), 2.49 (2H, s), 6.83 (2H, t, J = 8.3 Hz), 8.55 (1H, d, J = 13.1 Hz), 12.86 (1H, br). Anal. Calcd for (%) $C_{15}H_{14}NO_2F_3$: C, 60.61; H, 4.75; N, 4.71; O, 10.76; F, 19.17. Found: C, 60.35; H, 4.67; N, 4.64; O, 10.67; F, 19.30.

2-(2,6-Dichloro-3-methylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (11). Mp 90−92 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 2.40 (3H, s) 2.43 (2H, s), 2.49 (2H, s), 7.13 (1H, d, J = 8.3 Hz), 7.31 (1H, d, J = 8.3 Hz), 8.50 (1H, d, J = 13.2 Hz), 12.63 (1H, br). Anal. Calcd for (%) $C_{16}H_{17}NO_2Cl_2$: C, 58.91; H, 5.25; N, 4.29; O, 9.81; Cl, 21.47. Found: C, 59.14; H, 5.31; N, 4.15; O, 9.81; Cl, 21.70.

2-(4-Methoxyphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (12). Mp 101−102 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.09 (6H, s), 2.42 (2H, s), 2.47 (2H, s), 3.97 (3H, s), 6.95−6.98 (2H, m), 7.18−7.21 (1H, m), 7.40−7.43 (1H, m), 8.65 (1H, d, J = 14.0 Hz), 13.02 (1H, br). Anal. Calcd for (%) $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12; O, 17.56. Found: C, 70.22; H, 6.94; N, 5.08; O, 17.39.

2-(2-Methoxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (13). Mp 176−177 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.09 (6H, s), 2.45 (2H, s), 2.51 (2H, s), 4.04 (3H,s), 7.26−7.27 (1H, m), 7.61−7.63 (2H, m), 8.13 (1H, m), 8.70 (1H, d, J = 13.7 Hz), 13.12 (1H, br). Anal. Calcd for (%) $C_{17}H_{19}NO_4$: C, 67.76.; H, 6.36; N, 4.65; O, 21.24. Found: C, 67.68; H, 6.28; N, 4.52; O, 21.57.

2-(2-Ethoxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (14). Mp 156−157 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 1.42 (3H, t, J = 7.3 Hz), 2.45 (2H, s), 2.51 (2H, s), 4.52 (2H, q, J = 7.2 Hz), 7.24−7.28 (1H, m), 7.61−7.63 (2H, m), 8.12 (1H, m), 8.70 (1H, d, J = 13.7 Hz), 13.10 (1H, br). Anal. Calcd for (%) $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44; O, 20.29. Found: C, 68.42; H, 6.73; N, 4.48; O, 20.34.

2-(2-Butyloxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (15). Mp 128−129 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 0.92 (3H, t, J_2 = 6.9 Hz), 1.09 (6H, s), 1.34−1.45 (2H, m), 1.39−1.89 (2H, m), 2.44 (2H, s), 2.50 (2H, s), 4.44 (2H, t, J_1 = 7.4 Hz), 7.26 (2H, m), 7.60 (2H, m), 8.13 (1H, dd, J_1 = 8.3 Hz, J_2 = 1.1 Hz), 8.69 (1H, d, J = 13.8 Hz), 13.92 (1H, br). Anal. Calcd for (%) $C_{20}H_{25}NO_4$: C, 69.95; H, 7.34; N, 4.08; O, 18.64. Found: C, 70.02; H, 7.28; N, 4.12; O, 18.58.

2-(2-Pentyloxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (16). Mp 107−109 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 0.94 (3H, t, J = 7.4 Hz), 1.10 (6H, s), 1.36−1.41 (4H, m), 1.79 (2H, dd, J_1 = 6.9, J_2 = 7.4 Hz), 2.44 (2H, s), 2.50 (2H, s), 4.43 (2H, t, J = 6.9 Hz), 7.25 (1H, m), 7.59 (2H, m), 8.12 (1H, dd, J_1 = 8.3 Hz, J_2 = 1.1 Hz), 8.68 (1H, d, J = 13.8 Hz), 13.85 (1H, br). Anal. Calcd for (%) $C_{21}H_{27}NO_4$: C, 70.56; H, 7.61; N, 3.92; O, 17.90. Found: C, 70.42; H, 7.48; N, 3.99; O, 18.02.

2-(2-Hexyloxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (17). Mp 108−109 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 0.91 (3H, t, J_2 = 6.9 Hz), 1.09 (6H, s), 1.33−1.43 (8H, m), 1.81 (2H, dd, J_1 = 7.4, J_2 = 6.9 Hz), 2.46 (2H, s), 2.52 (2H, s), 4.45 (2H, t, J = 6.8 Hz), 7.28 (1H, m), 7.62 (2H, m), 8.13 (1H, dd, J_1 = 8.5 Hz, J_2 = 1.2 Hz), 8.71 (1H, d, J = 13.0 Hz), 14.15 (1H, br). Anal. Calcd for (%) $C_{22}H_{29}NO_4$: C, 71.13; H, 7.88; N, 3.78; O, 17.28. Found: C, 71.22; H, 7.76; N, 3.88; O, 17.58.

2-(Heptyloxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (18). Mp 108−110 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 0.90 (3H, t, J = 6.7 Hz), 1.09 (6H, s), 1.22−1.40 (10H, m), 1.79 (2H, dd, J_1 = 7.4, J_2 = 6.9 Hz), 2.44 (2H, s), 2.50 (2H, s), 4.43 (2H, t, J = 6.8 Hz), 7.28 (1H, m), 7.62 (2H, m), 8.13 (1H, dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz), 8.69 (1H, d, J = 13.0 Hz), 13.95 (1H, br). Anal. Calcd for (%) $C_{23}H_{31}NO_4$: C, 71.66; H, 8.11; N, 3.63; O, 16.60. Found: C, 71.58; H, 7.98; N, 3.68; O, 16.48.

2-(4-Butyloxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (19). Mp 150−151 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 0.98 (3H, t, J = 7.3 Hz), 1.10 (6H, s), 1.44−1.49 (2H, m), 1.69−1.78 (2H, m), 2.44 (2H, s), 2.48 (2H, s), 4.32 (2H, t, J_1 = 6.6 Hz), 7.29 (2H, dd, J_1 = 1.9 Hz, J_2 = 6.9 Hz), 8.07 (2H, dd, J_1 = 1.9 Hz, J_2 = 6.9 Hz), 8.63 (1H, d, J = 13.4 Hz), 12.86 (1H, br). Anal. Calcd for (%) $C_{20}H_{25}NO_4$: C, 69.95; H, 7.34; N, 4.08; O, 18.64. Found: C, 69.89; H, 7.39; N, 4.03; O, 18.65.

2-(2-Chloro-5-methoxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (20). Mp 159−160 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.11 (6H, s), 2.46 (2H, s), 2.52 (2H, s), 3.96 (3H, s), 7.56 (1H, d, J = 8.4 Hz), 7.85 (1H, dd, J_1 = 8.4 Hz, J_2 = 1.9 Hz), 8.17 (1H, d, J = 1.9 Hz), 8.68 (1H, d, J = 13.0 Hz), 13.29 (1H, br). Anal. Calcd for (%) $C_{17}H_{19}NO_4Cl$: C, 60.63; H, 5.69; N, 4.16; O, 19.00; Cl, 10.53. Found: C, 60.42; H, 5.48; N, 4.08; O, 18.88; Cl, 10.44.

2-(2-Chloro-5-propyloxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (21). Mp 104−105 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.03 (3H, t, J = 7.4 Hz), 1.11 (6H, s), 1.82 (2H, tq, J_1 = 7.4 Hz, J_2 = 6.8 Hz), 2.46 (2H, s), 2.51 (2H, s), 4.32 (2H, t, J = 6.8 Hz), 7.53 (1H, d, J = 8.4 Hz), 7.83 (1H, dd, J_1 = 8.4 Hz, J_2 = 1.9 Hz), 8.13 (1H, d, J = 1.9 Hz), 8.68 (1H, d, J = 13.0 Hz), 13.29 (1H, br). Anal. Calcd for (%) $C_{19}H_{23}NO_4Cl$: C, 62.55; H, 6.35; N, 3.84; O, 17.54; Cl, 9.72. Found: C, 62.67; H, 6.52; N, 3.88; O, 17.43; Cl, 9.89.

2-(2-Chloro-5-propenyloxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (22). Mp 106−107 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 2.46 (2H, s), 2.51 (2H, s), 4.86 (2H, dt, J_1 = 5.9 Hz, J_2 = 1.5 Hz), 5.32 (1H, dd, J_1 = 10.4 Hz, J_2 = 1.5 Hz), 5.43 (1H, dd, J_1 = 17.6 Hz, J_2 = 1.6 Hz), 5.9−6.3 (1H, m), 7.55 (1H, d, J = 8.4 Hz), 7.85 (1H, dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz), 8.15 (1H, J = 1.8 Hz), 8.67 (1H, d, J = 13.0 Hz), 13.16 (1H, br). Anal. Calcd for (%) $C_{19}H_{21}NO_4Cl$: C, 62.90; H, 5.83; N, 3.86; O, 17.64; Cl, 9.77. Found: C, 62.72; H, 5.75; N, 3.92; O, 17.47; Cl, 10.00.

2-(5-Chloro-2-propyloxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (23). Mp 210−211 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.03 (3H, t, J = 7.4 Hz), 1.11 (6H, s), 1.82 (2H, tq, J_1 = 7.4 Hz, J_2 = 6.8 Hz),

2.45 (2H, s), 2.51 (2H, s), 4.34 (2H, t, $J = 6.8$ Hz), 7.52 (1H, d, $J = 8.3$ Hz), 7.82 (1H, dd, $J_1 = 8.4$ Hz, $J_2 = 2.0$ Hz), 8.13 (1H, d, $J = 2.0$ Hz), 8.15 (1H, $J = 1.9$ Hz), 8.64 (1H, d, $J = 13.1$ Hz), 13.10 (1H, br). Anal. Calcd for (%) $C_{19}H_{23}NO_4Cl$: C, 62.55; H, 6.35; N, 3.84; O, 17.54; Cl, 9.72. Found: C, 62.47; H, 6.28; N, 3.46; O, 17.66; Cl, 9.62.

2-(4-Chloro-3-propyloxycarbonylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (24). Mp 84−86 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.02 (3H, t, $J = 7.4$ Hz), 1.11 (6H, s), 1.82 (2H, tq, $J_1 = 7.4$ Hz, $J_2 = 6.8$ Hz), 2.46 (2H, s), 2.52 (2H, s), 4.35 (2H, t, $J = 6.8$ Hz), 7.53 (1H, d, $J = 8.4$ Hz), 7.86 (1H, dd, $J_1 = 8.4$ Hz, $J_2 = 1.8$ Hz), 8.12 (1H, d, $J = 1.8$ Hz), 8.68 (1H, d, $J = 13.0$ Hz), 13.31 (1H, br). Anal. Calcd for (%) $C_{19}H_{23}NO_4Cl$: C, 62.55; H, 6.35; N, 3.84; O, 17.54; Cl, 9.72. Found: C, 62.57; H, 6.12; N, 3.99; O, 17.68; Cl, 9.48.

2-(2,6-Dichlorophenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (28). Mp 135−138 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 2.43 (2H, s), 2.50 (2H, s), 7.18 (1H, dd, $J_1 = 7.7$ Hz, $J_2 = 8.5$ Hz), 7.41 (2H, d, $J = 8.2$ Hz), 8.55 (1H, d, $J = 13.1$ Hz), 12.65 (1H, br). Anal. Calcd for (%) $C_{15}H_{15}NO_2Cl_2$: C, 57.71; H, 4.84; N, 4.49; O, 10.25; Cl, 22.71. Found: C, 57.75; H, 4.82; N, 4.71; O, 10.47; Cl, 22.72.

2-(2-Fluoro-4-chlorophenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (29). Mp 173−176 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.09 (6H, s), 2.43 (2H, s), 2.47 (2H, s), 7.21−7.37 (3H, m), 8.53 (d, 1H, $J = 13.2$ Hz), 12.98 (1H, br). Anal. Calcd for (%) $C_{15}H_{15}NO_2ClF$: C, 60.92; H, 5.11; N, 4.74; O, 10.82; Cl, 11.99; F, 6.42. Found: C, 60.77; H, 5.10; N, 4.73; O, 10.88; Cl, 11.92; F, 6.35.

2-(2,6-Diethylphenyl)aminomethylidene-5,5-dimethylcyclohexane-1,3-dione (30). Mp 115−119 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 1.20 (6H, t, $J = 7.6$ Hz), 2.40 (2H, s), 2.48 (2H, s), 2.62 (4H, q, $J = 7.6$ Hz), 7.16−7.29 (3H, m), 8.13 (d, 1H, $J = 13.8$ Hz), 12.47 (1H, br). Anal. Calcd for (%) $C_{19}H_{25}NO_2$: C, 76.64; H, 8.68; N, 4.47; O, 10.21. Found: C, 76.42; H, 8.67; N, 4.54; O, 9.98.

5-(2,6-Dichlorophenyl)aminomethylidene-2,2-dimethyl-1,3-dioxane-4,6-dione (31). Mp 128−130 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.77 (6H, s), 7.15 (1H, dd, $J_1 = J_2 = 7.6$ Hz), 7.54 (2H, d, $J = 8.2$ Hz), 8.62 (1H, d, $J = 13.1$ Hz), 11.05 (1H, br). Anal. Calcd for (%) $C_{13}H_{11}NO_4C1_2$: C, 49.39; H, 3.51; N, 4.43; O, 20.24; Cl, 22.43. Found: C, 49.53; H, 3.49; N, 4.39; O, 13.88; Cl, 22.09.

6-Methyl-3-(2,6-dichlorophenyl)aminomethylidene-2H,-3H-pyran-2,4-dione (32). Mp 171−175 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 2.19 (3H, s), 5.79 (1H, s), 7.19 (1H, dd, $J_1 = J_2 = 7.6$ Hz), 7.52 (2H, d, $J = 8.2$ Hz), 8.70 (0.7H, d, $J = 13.1$ Hz), 8.77 (0.3H, d, $J = 13.1$ Hz), 13.46 (1H, br). Anal. Calcd for (%) $C_{13}H_9NO_3C1_2$: C, 52.38; H, 3.04; N, 4.7; O, 16.1; Cl, 23.78. Found: C, 52.43; H, 3.09; N, 4.75; O, 16.21; Cl, 23.78.

5-(2,6-Dichlorophenyl)aminomethylidene-1,3-dimethyl-2,4,6-pyrimidinetrione (33). Mp 203−207 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 3.37 (3H, s), 3.39 (3H, s), 7.13 (1H, dd, $J_1 = J_2 = 7.6$ Hz), 7.50 (2H, d, $J = 8.2$ Hz), 8.66 (1H, d, $J = 17$ Hz), 11.85 (1H, br). Anal. Calcd for (%) $C_{13}H_{11}N_3O_3Cl_2$: C, 47.58; H, 3.38; N, 12.8; O, 14.63; Cl, 20.61. Found: C, 47.46; H, 3.36; N, 12.77; O, 14.45; Cl, 20.46.

2-(2,6-Dichlorophenyl)aminomethylidenecyclopentane-1,3-dione (34). Mp 145−147 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 2.59−2.72 (4H, m), 7.15 (1H, dd, $J_1 = J_2 = 7.6$ Hz), 7.78 (2H, d, $J = 8.2$ Hz), 8.17 (1H, br). Anal. Calcd for (%) $C_{11}H_7NO_3Cl_2$: C,48.56; H, 2.59; N, 5.15; O, 17.64; Cl, 26.06. Found: C, 48.78; H, 2.44; N, 5.16; O, 17.53; Cl, 26.17.

General Synthetic Procedure for 3-Acyloxy-2-cyclohexenones (C). Acyl chloride (11 mmol) was added to a stirred solution of cyclohexane-1,3-dione (**A**, 10 mmol) and pyridine (0.79 g, 10 mmol) in dry chloroform (50 mL). The mixture was stirred at room temperature for 1 h, and then washed with water, diluted hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and water. The organic phase was dried with anhydrous Na_2SO_4 and evaporated to dryness *in vacuo* to give the pure enol ester (**B**), which was submitted to the isomerization reaction without further purification.

General Synthetic Procedure for 2-Acyl-1,3-cyclohexanediones (D). The enol ester (**B**, 10 mmol) was added to a stirred suspension of anhydrous aluminum chloride (20 mmol) in dry 1,2-dichloroethane (50 mL). The mixture was stirred for 1 h at room temperature and then poured into a mixture of ice (20 g) and concentrated hydrochloric acid (20 g). The organic phase was washed with water, dried with anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was dissolved in a minimum quantity of ether, treated with concentrated hydrochloric acid, and extracted with ether. The ether solution was dried with anhydrous sodium sulfate and concentrated *in vacuo*, and the residue was recrystallized from hexane.

General Synthetic Procedure for 2-(2,6-Dichlorophenylamino)ethylidene-5,5-dimethylcyclohexane-1,3-dione (25). 2-Acyl-1,3-cyclohexanedione (**D**, 10 mmol) was dissolved in toluene (100 mL), and 2,6-dichloroaniline (10 mmol) was added. After the reaction mixture had been refluxed for 12 h, the toluene was removed by concentration *in vacuo*. The residue was washed with saturated aqueous sodium hydrogen carbonate and extracted with ethyl acetate. The organic phase was dried with anhydrous Na_2SO_4. After removal of the solvent, the residue was chromatographed on silica gel by eluting with hexane−ethyl acetate (7:3) and ethyl acetate. Concentration of the ethyl acetate eluate under reduced pressure followed by recrystallization of residue from ethanol−H_2O gave 124 mg (4%) of **(25)**. Mp 134−136 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 2.37 (3H, s), 2.45 (2H, s), 2.49 (2H, s), 7.18 (1H, dd, $J_1 = J_2 = 7.6$ Hz), 7.41 (2H, d, $J = 8.2$ Hz), 15.01 (1H, br). Anal. Calcd for (%) $C_{16}H_{17}NO_2Cl_2$: C, 58.91; H, 5.25; N, 4.29; O, 9.81; Cl, 21.74. Found: C, 58.78; H, 5.22; N, 4.32; O, 9.89; Cl, 21.76.

Compounds **26** and **27** were prepared in the same manner as **25** by using the corresponding anilines instead of the 2,6-dichloroaniline.

2-(2-Fluoro-4-chlorophenylamino)ethylidene-5,5-dimethylcyclohexane-1,3-dione (26). Mp 189−192 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 2.40 (3H, s), 2.45 (2H, s), 2.49 (2H, s), 7.11−7.65 (3H, m), 14.94 (1H, br). Anal. Calcd for (%) $C_{16}H_{17}NO_2ClF$: C, 62.04; H, 5.53; N, 4.52; O, 10.33; Cl, 11.44; F, 6.13. Found: C, 62.05; H, 5.59; N, 4.53; O, 10.20; Cl, 11.33; F, 6.25.

2-(2,6-Diethylphenylamino)ethylidene-5,5-dimethylcyclohexane-1,3-dione (27). Mp 81−83 °C. ^{1}H-NMR ($CDCl_3$/TMS, ppm): δ 1.10 (6H, s), 1.15 (6H, t, $J = 7.6$ Hz), 2.43 (2H, s), 2.48 (2H, s), 2.61 (4H, q, $J = 7.6$ Hz), 7.15 (1H, dd, $J_1 = J_2 = 7.6$ Hz), 7.28 (2H, d, $J = 8.2$ Hz), 8.43 (1H, d, $J = 13.8$ Hz),-12.66 (1H, br). Anal. Calcd for (%) $C_{20}H_{27}NO_2$: C, 76.64; H, 8.68; N, 4.47; O, 10.21. Found: C, 76.42; H, 8.67; N, 4.54; O, 9.98.

ABBREVIATIONS USED

Protox, protoporphyrinogen oxidase; Protogen IX, protoporphyrinogen IX; Proto IX, protoporphyrin IX; RWH, a name for a series of compounds reported.

LITERATURE CITED

Arnon, D. I. Copper enzymes in isolated chloroplasts. Phenoloxidases in *Beta vulgaris*. *Plant Physiol.* **1949**, *24*, 1−15.

Asami, T.; Takahashi, N.; Yoshida, S. Synthesis of conjugated enamino compounds inhibiting photosynthetic electron transport. *Agric. Biol. Chem.* **1987**, *51*, 205−210.

Camadro, J. M.; Matringe, M.; Scalla, R.; Labbe, R. Kinetic studies on protoporphyrinogen oxidase inhibition by diphenyl ether herbicides. *Biochem. J.* **1991**, *227*, 17−21.

Kohno, H.; Katsuyama, N.; Watanabe, H.; Wakabayashi, K.; Böger, P. Callus system to assay herbicidal peroxidation: Effects of peroxidizing herbicides on habituated tobacco *Nicotiana glutinosa*, green cell cultures. *Plant Tissue Cult. Lett.* **1994**, *11*, 49−54.

Lanbert, R,; Sandmann, G.; Böger, P. Inhibition of carotenogenesis by substituted diphenyl ethers of the *m*-phenoxybenzamide type. *Pestic. Biochem. Physiol.* **1983**, *20*, 183−187.

Matringe, M.; Scalla, R. Studies on the mode of action of acifluorfen-methyl in nonchlorophyllous soybean cells. *Plant Physiol.* **1988**, *86*, 619−622.

Matringe, M.; Camadro, J. M.; Labbe, P.; Scalla, R. Protoporphyrinogen oxidase as a molecular target for diphenyl ether herbicides. *Biochem. J.* **1989a**, *260*, 231−235.

Matringe, M.; Camadro, J. M.; Labbe, P.; Scalla, R. Protoporphyrinogen oxidase inhibition by three peroxidizing herbicides: Oxadazon, LS 82−556 and M&B 39279. *FEBS Lett.* **1989b**, *245*, 35−38.

Matringe, M.; Mornet, R.; Scalla, R. Characterization of [^{3}H]-acifluorfen binding to purified Pea etioplasts and evidence that protoporphyrinogen oxidase specifically binds acifluorfen. *Eur. J. Biochem.* **1992**, *209*, 861−868.

McFadden G. H.; Huppatz, J. L. Vinylogous sulfonylureas: A new class of acetohydroxy acid synthase inhibitors incorporating bridging moiety. *Z. Naturforsch.* **1993**, *48c*, 356−363.

Mito, N.; Sato, R.; Miyakado, M.; Oshio, H; Tanaka, S. *In vitro* mode of action of *N*-phenylimide photobleaching herbicides. *Pestic. Biochem. Physiol.* **1991**, *40*, 128−135.

Nandihalli, U. B.; Duke, M. V.; Duke, S. O. Relationship between molecular proporties and biological activities of *O*-phenylpyrrolidino- and piperidinocarbamate herbicides. *J. Agric. Food. Chem.* **1992**, *40*, 1993−2000.

Nicolaus, B.; Sandmann, G.; Böger, P. Molecular aspects of herbicide action on protoporphyrinogen oxidase. *Z. Naturforsch.* **1993**, *48c*, 326−333.

Sandmann, G.; Böger, P. Accumulation of proporphyrin IX in the presence of peroxidizing herbicide. *Z. Naturforsch.* **1988**, *43c*, 699−704.

Sato, F.; Yamada, Y.; Kwak, S.; Ichinose, K.; Kishida, M.; Takahashi, N.; Yoshida, S. Photoautotrophic cultured plant cells: A novel system to survey new photosynthetic electron transport inhibitors. *Z. Naturforsch.* **1991**, *46c*, 563−568.

Sato, R.; Nagano, E.; Oshio, H.; Kamoshita, K. Diphenyl ether-like physiological and biochemical actions of S-23142, a novel *N*-phenyl imide herbicide. *Pestic. Biochem. Physiol.* **1987**, *28*, 194−200.

Takeda, S.; Sato, F.; Ida, K.; Yamada, Y. Characterization of polypeptides that accumulate in cultured *Nicotiana tabacum* cells. *Plant Cell Physiol.* **1990**, *31* (2), 215−221.

Varsano, R.; Matringe, M.; Magnin, N.; Mornet, R.; Scalla, R. Competitive interaction of three peroxidizing herbicides with the binding of [^{3}H]acifluorfen to corn etioplast membranes. *FEBS Lett.* **1990**, *272*, 106−108.

Wakabayashi, K. Molecular design of cyclic imide herbicides using biorational approaches. *J. Pestic. Sci.* **1988**, *13*, 337−361.

Yamada, Y.; Sato, F. The photoautotrophic culture of chlorophyllous cells. *Plant Cell Physiol.* **1978**, *19*, 691−699.

Zacharias, G.; Wolfbeis, O. S.; Junek, H. Über Anilinomethylenverbindungen der Cyclohexandione. *Monatsh. Chem.* **1974**, *105* (6), 1283−1291.

Received for review December 16, 1996. Accepted April 11, 1997.[⊗]

JF960962I

[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1997.