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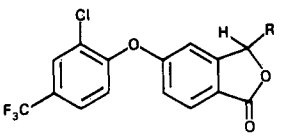
Some Novel Diphenyl Ether Herbicides with Peroxidizing Activity

Patrick Camilleri,* Karen Weaver, Michael T. Clark, John R. Bowyer, and Beverly J. Hallahan

5-[2-Chloro-4-(trifluoromethyl)phenoxy]phthalide and its 3-alkoxy derivatives are a new class of diphenyl ether herbicides with partitioning properties and symptoms of plant phytotoxicity similar to those shown by nitrodiphenyl ethers. At an applied concentration of 1 μM, they induce membrane lipid peroxidation on treated leaves at a rate similar to that seen with nitrodiphenyl ethers, with the 3-methoxyphthalide being the most active compound. Their redox properties preclude reduction by the photosynthetic electron-transport chain, nor do they significantly inhibit photosynthetic electron transport at herbicidally active concentrations. These compounds should prove useful in the identification of the primary mechanism of action of nitrodiphenyl ether and related herbicides.

It is now well established that nitrodiphenyl ether (NDPE) herbicides require both light and oxygen to elicit their activity on whole plants (Matsunaka, 1969; Orr and Hess, 1982; Kunert, 1984). However, the primary mode of interaction of these compounds, possibly at a receptor site within the chloroplast or the chloroplast envelope, is not understood. One of the hypotheses that has been proposed (Kunert and Böger, 1981; Lambert et al., 1984) is that the activity of NDPE's depends on the relative ease by which these compounds can be reduced by chloroplast photosystem I (PS I) in a way similar to that of paraquat, a well-known PS I electron acceptor. Such a mechanism would lead to the formation of a reactive anion radical that can transfer its electron to oxygen, leading to highly active oxygen species, such as H_2O_2 and $^{\bullet}OH$, which would peroxidize unsaturated lipid membranes. The occurrence of this mechanism has been questioned for a number of reasons. Thus, diuron treatment protects plants from paraquat toxicity but is much less effective in reversing or diminishing the phytotoxicity caused by NDPE's (Matsunaka, 1969; Orr and Hess, 1982; Ensminger and Hess, 1985). Moreover, recent electrochemical studies have shown that diphenyl ether compounds where the nitro group has been replaced by a chlorine atom cannot readily accept an electron to form an anion radical, as in the case of the nitro analogues (Ensminger et al., 1985). Despite these differences in electrochemical behavior, both the nitro and chloro compounds were found to be as effective on the green unicellular alga *Chlamydomonas eugametos* and in three weed species (*Xanthium pennsylvanicum*, *Abutilon theophrasti*, *Ipomoea*). Finally we have recently shown (Bowyer et al., 1987a) that a typical NDPE, namely 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitroacetophenone oxime *O*-(acetic acid methyl ester), which we called DPE I, causes rapid leaf wilting, membrane lipid peroxidation, and chloroplast destruction in barley mutants that are known to lack either PS I or PS II. As expected, these mutants were found to be resistant to paraquat action.

In this publication we introduce four novel diphenyl ether herbicides, which are derivatives of 5-[2-chloro-4-(trifluoromethyl)phenoxy]phthalide and show similar symptoms of phytotoxicity (rapid chlorosis and necrosis) on whole plants as NDPE's. The chemical structures of the four compounds we have studied are as follows:



I, R = H
II, R = OCH_3
III, R = OC_2H_5
IV, R = OC_3H_7

Like most conventional NDPE's these compounds contain a 2-chloro-4-(trifluoromethyl)phenoxy group. However, unlike NDPE's a phthalide ring replaces the nitro substituent. This structural property is of interest in the use of these compounds as important "tools" to help identify structure–activity features essential for the phytotoxicity of diphenyl ethers, in general.

MATERIALS AND METHODS

Chemicals. Phthalides I–IV (purity >95%) were synthesized (Clark and Gilmore, 1984) by the Organic Chemistry Department, Shell Research Centre, Sittingbourne.
Hill Inhibition: The procedure used for the preparation of the pea (*Pisum sativum*) thylakoid membranes and the measurement of photosynthetic electron transport have been outlined by us previously (Bowyer et al., 1987a).
Measurement of One-Electron Reduction Potential. The one-electron reduction potential of phthalide diphenyl ether II was measured from the equilibrium concentrations

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of the reduced and oxidized forms after pulse radiolysis (Wardman, 1976) in the presence of 1,1-butano-2,2′-bipyridinium dibromide (tetraquat). Deoxygenated solutions in propan-2-ol/5 mM aqueous phosphate buffer (1:3, v/v) containing 10–40 μM diphenyl ether and 30–90 μM 1,1′-butano-2,2′-bipyridinium dibromide (E_7^1 −635 mV in aqueous phosphate buffer (Anderson, 1976)) were irradiated with electron pulses as described by Wardman (1976). Rather low concentrations of redox compounds were used in these measurements owing to the low aqueous solubility of II.

Hydrocarbon Formation in Herbicide-Treated French Bean Plants. French Beans (*Phaseolus vulgaris* cv. Prince) (14 day old) that had been grown under glasshouse conditions were used.

Aqueous Triton X100 solutions (0.01%, v/v) were prepared containing the phthalide herbicide I–IV at a concentration of 10^{-6} M, by dilution of a 10^{-2} M stock solution of each compound in dimethyl sulfoxide (DMSO; final DMSO concentration 0.01%). The two primary leaves of the French Bean plants were treated by completely immersing them in test solutions and then allowing them to drip-dry. Sufficient plants were treated for sampling up to 72 h in duplicate at daily intervals. French Bean plants were also treated with 0.01% Triton X100 and 0.01% DMSO solution containing no compound; these acted as control samples.

After treatment the plants were placed under continuous light at an intensity of 35 W m^{-2}, and soil irrigation was carried out daily. The rationale behind this light regime is that, at 35 W m^{-2}, progressive visible damage was induced by the herbicide at a rate similar to that seen in the glasshouse. It is assumed that, at least initially, the rate of hydrocarbon formation increases after application of the herbicide. In order to obtain a measure of the rate of ethane formation at specific time points, four treated leaves were detached and placed in a 25-cm^3 conical flask that was stoppered with a Suba seal stopper. These flasks were then exposed to light of intensity 330 W m^{-2} for 4 h. This high light intensity was used in an attempt to accelerate the rate of ethane formation at each time point so that a measurable amount of ethane could be rapidly accumulated. It is of course necessary to assume that at each time point the accelerated rate of ethane formation by the detached leaves is proportional to the rate of ethane formation by the intact plant at 35 W m^{-2}. A 1-cm^3 volume of headspace gas was analyzed after 4 h by gas chromatography (Hewlett-Packard flame ionization detector fitted to an HP 5710 A gas chromatograph), and the height of the ethane peak obtained was compared to that of a standard in order to measure the concentration of gas liberated by treated leaves.

After analysis the leaves were desiccated in a freeze-drier. For each treatment the dry weight of the tissue was used to calculate the amount of ethane formed per gram dry weight of plant material.

RESULTS AND DISCUSSION

Phthalides I–IV caused rapid light-dependent wilting and necrosis when sprayed onto a range of plants, suggesting that, like nitrodiphenyl ethers, they induce membrane lipid peroxidation.

The ability of phthalides I–IV to induce the peroxidation of unsaturated membrane lipids in French Bean plants was compared by measuring the amount of ethane generated by plants at fixed time intervals after the application of herbicide (Figure 1). Plants treated by the individual phthalides showed an initial sharp rise followed by a fall in ethane formation. This time course and extent of ethane

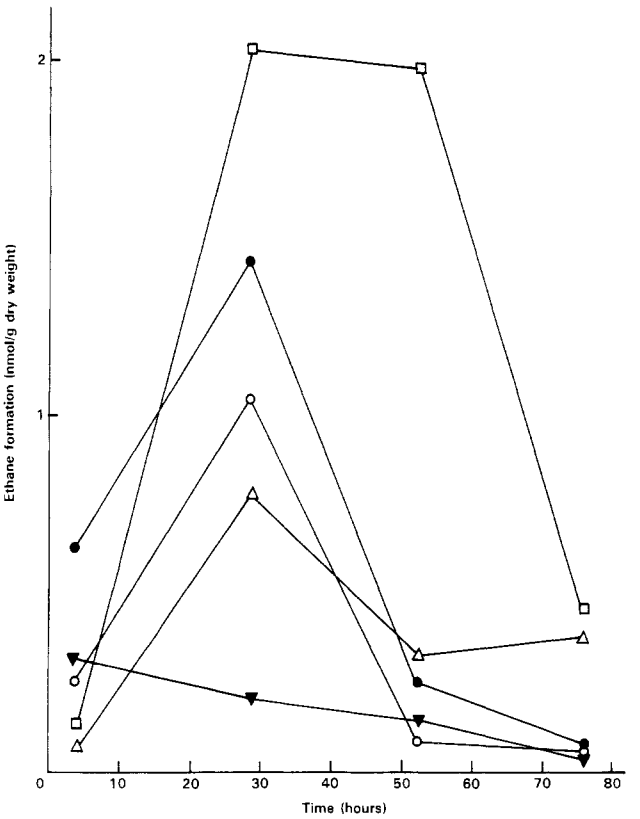

Figure 1. Production of ethane from French Bean leaves treated with 1 μM phthalide diphenyl ether. Compounds: I (○); II (□); III (●); IV (△). Control leaves were treated with 0.01% Triton X-100 solution (▼).

formation were similar to that found when French Beans were treated with NDPE's at similar concentrations (results not shown). The fall in ethane formation after longer periods of application was most probably due to completion of lipid peroxidation.

Methoxyphthalide II was the most active and persistent of the four DPE's tested. Replacing the methoxy group in the 3-position of the phthalide ring by a hydrogen atom or an ethoxy or propoxy group decreased activity considerably. The order of activity shown in Figure 1 was consistent with observations carried out on a number of broad leaf and grass species treated with these compounds in a primary screen bioassay. As in the case of NDPE's these phthalide diphenyl ethers were more toxic to broad leaf plant species than to grasses.

In line with the requirement of both light and oxygen for herbicidal activity by NDPE's, it has been reported that several NDPE's interfere with photosynthetic electron transport either as electron acceptors in the same way as the bipyridinium herbicides (e.g., paraquat and diquat) (Kunert and Böger, 1981; Lambert et al., 1984) or as inhibitors of PS II (e.g., diuron or atrazine) (Fedtke, 1982). Following pulse radiolysis of an anaerobic solution containing tetraquat and phthalide diphenyl ether II, no electron transfer from tetraquat to II was observed, indicating that the one-electron reduction potential of II is more negative than −700 mV. This is outside the range usually attributed to PS I electron-acceptor herbicides and, unlike the PS I acceptor paraquat, II did not stimulate oxygen uptake when added to illuminated thylakoids (not shown).

Figure 2 shows the effects of I and II on photosynthetic electron transport from water to paraquat with pea chloroplast thylakoids; I_{50} values were measured as 22 and 6 μM, respectively, considerably higher than the concen-

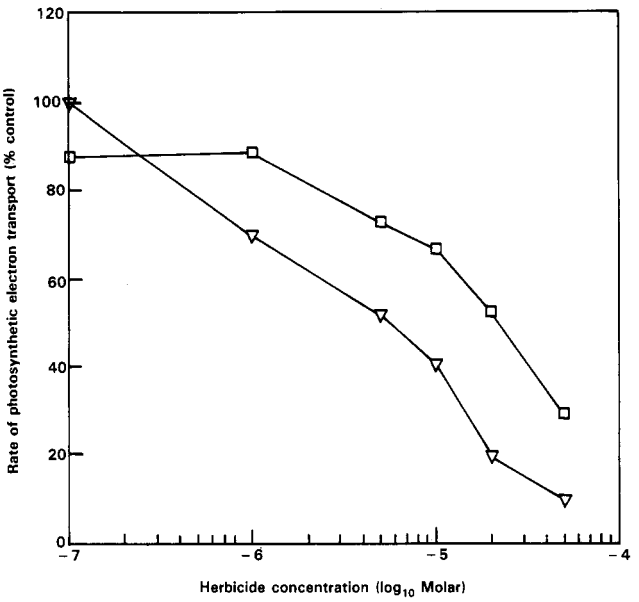

Figure 2. Effect of phthalide diphenyl ether compounds I (□) and II (▽) on photosynthetic electron transport from water to paraquat (0.1 mM) in isolated pea thylakoids.

tration needed to elicit rapid lipid peroxidation (1 μM). Their I_{50} values are of the same order of magnitude as those reported (Fedtke, 1982) for some well-known NDPE's such as nitrofen (I_{50} = 40 μM) and bifenox (I_{50} = 16 μM). The inhibition of photosynthetic electron transport by these diphenyl ethers is at least 1 order of magnitude lower than that of the commercial PS II herbicides, diuron ($I_{50} \approx 0.1$ μM) and atrazine ($I_{50} \approx 0.3$ μM) (Mitsutake et al., 1986). Moreover, the 1-octanol/water partition coefficients (P) of these PS II compounds have been reported to be around 600 (Mitsutake et al., 1986). On the other hand, diphenyl ethers I and II have a much higher partition coefficient of about 1.6×10^4, again of the same order of magnitude as that measured by us for nitrofen ($P \approx 5 \times 10^4$) and bifenox ($P \approx 1.4 \times 10^4$). Furthermore, unlike PS II inhibitors, which act over a relatively long period (about 5–10 days), phthalide diphenyl ethers are fast-acting (1–2 days) contact herbicides, most effective on foliar application. Thus, although most diphenyl ethers can act as inhibitors of photosynthetic electron transport in isolated chloroplast thylakoids, this type of activity is not expected to play an important role in determining the phytotoxicity of these compounds.

The discovery of phthalide diphenyl ethers has provided us with tools that should prove useful in identifying the structure–activity requirements of diphenyl ether herbicides and in probing the mode of action of these compounds. For example, we have now shown that the site of action of these compounds is highly stereospecific (Camilleri, P., Gray, A., Weaver, K. Bowyer, J. R., manuscript in preparation) providing evidence for the likely involvement of a specific protein-binding process in the mode of action of diphenyl ethers. We have also shown that in the green alga *Scenedesmus obliquus* diuron provides protection not only from peroxidizing nitrodiphenyl ether herbicides but also from a phthalide diphenyl ether (Bowyer et al., 1987b). The result with nitrodiphenyl ethers contrasts with that found (Orr and Hess, 1982; Bowyer et al., 1987a) in higher plants and was taken to indicate a role for PS I in reducing the herbicide to a radical anion (Kunert and Böger, 1981). The result with the phthalide diphenyl ether shows that the requirement for photosynthetic electron transport in *S. obliquus* is not to reduce the compound, since its redox properties preclude this. These results will be reported in more detail elsewhere.

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