Free Rad. Res. Comms., Vol. 10, No. 1-2, pp. 85-93
Reprints available directly from the publisher
Photocopying permitted by license only

STUDY OF THE MODE OF ACTION OF SOME NITRODIPHENYL ETHERS

DOMINIQUE PIEKARSKI, PIERRE POTIER and CHARLES GIANNOTTI*

Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette, France

Nitrosoderivatives of the nitrodiphenyl ether herbicides (nitrofen, bifenox) have been studied. UV irradiation in different organic solvents gives degradation products. In buffered aqueous media, in the presence of chloroplasts and spin traps such as DMPO, hydroxy and peroxy radicals have been characterized.

In organic media and in the presence of spin traps such as DMPO, PBN, 4-POBN, solvent radicals ($^{\cdot}CHCl_2$, $^{\cdot}CCl_3$, $^{\cdot}CH_2O$) have been formed.

Nitro-derivatives have been studied under UV irradiation and in the presence of tetramethylethylene (TME), alkenylhydroxylamines are formed which autoxidize in nitroxide radicals. The formation of the stable nitroxide radical occurs in the dark process after continuous irradiation. The intensity of the signal decreases strongly when a new irradiation is applied. Radical species, with analogous ESR spectral characteristics are formed on reaction with nitrodiphenyl ethers and fatty acids.

The reactivity of these herbicides in micellar media (SDS, Brij 35, and CTAB) has been investigated. The kinetics of formation of the ESR signal corresponding to the photoreduction of the nitrodiphenyl ether in the presence of TME behave differently in a micellar environment as compared to solution. The intensity of the formation of the nitroxide increases under irradiation and decreases in the dark; the rotational correlation time τ_c has been determined for each type of micelle.

Synthetic nitrosodiphenyl ether made by the reduction of nitrodiphenyl ether using hydrogen gas and PtO_2 as a catalyst gives the corresponding amine, which is oxidized with meta-chloroperbenzoic acid (m.CPBA). The nitrosodiphenyl ether in the presence of soja azolectin liposome containing a fluorescent probe has been analysed. When this synthetic nitrosodiphenyl ether is added to a medium containing soja azolectin liposomes and a carboxyfluorescein, fluorescent probe placed inside the liposomes, a rapid increase in the fluorescence of the medium is observed. The nitrosodiphenyl ether induce a break in the liposome membrane.

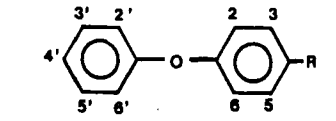
KEY WORDS: Nitrodiphenyl ethers, nitrosodiphenyl ethers, fatty acids, micelle, liposome.

INTRODUCTION

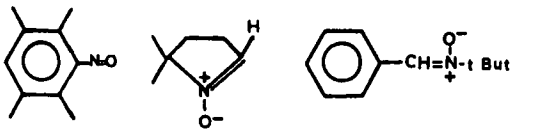
It is known that nitrodiphenyl ethers (NO_2DPE) (Fig. 1) require light activation, probably a light induced reduction, for herbicidal activity.[1-3] Free radicals are implicated in the NO_2-DPE phytotoxic action,[2-6] but the nature of the radicals involved remains unclear. Oxyfluorfen **1**, acifluorfen-methyl **2** and bifenox **3** are known to initiate lipid peroxidation in radical-mediated processes.[5-7] It has been reported[8] that nitroaryl compounds exhibit photoinduced radical intermediates as first observed on irradiation of nitrobenzene. Cowley *et al.*,[9] have suggested that solvent-adduct radicals are probably responsible for the long lived nitroxide radical signals in nitroarene-ether mixtures, while nitro radical anions and hydrogen-adduct radicals occur as transients in these systems.[10]

Knight[11] and Barlow *et al.*[12] found that nitrosophenyl derivatives and nitrosoalkanes undergo "ene-type" additions to double bonds yielding alkenylaryls and

*to whom correspondence should be addressed.



	3	2'	4'	R	
1	OEt	Cl	Cl	NO_2	oxyfluorfen
2	COOMe	Cl	CF_3	NO_2	acifluorfen-methyl
3	COOMe	Cl		NO_2	bifenox
4		NO_2	CF_3	NO_2	fluorodifen
5		Cl	Cl	NO_2	nitrofen
6	COOMe	Cl	Cl	NH_2	
7	COOMe	Cl	Cl	OH	



8 9 10

FIGURE 1 List of different compounds used.

alkenylalkylhydroxylamines, respectively. These hydroxylamines autoxidize to stable alkenylaryl and alkenylarylnitroxide radicals. Such paramagnetic species have been characterized by electron spin resonance (ESR) spectroscopy.[13]

In this investigation, it is suggested that analogous reactions with membrane component models provide a possible mechanism for activation of nitroaryl herbicides. We first analysed the behaviour of the nitrodiphenyl ether dissolved in an organic solvent. We then considered several photochemical systems in which the reduction of the nitrocompounds to the nitrosoderivatives was a precursor to radical formation. Under irradiation NO_2DPE gives a nitrosodiphenyl ether derivative which reacts with olefins in homogeneous or micellar solutions. A biomembrane constituent model consisting of a soja lecitin liposome with a fluorescent probe in the liposome has been made. We have shown that in the presence of synthetic nitrosodiphenyl ether, the liposome membrane becomes very leaky.

RESULTS AND DISCUSSION

We have studied different nitrodiphenyl ether (NO_2DPE) herbicides such as bifenox **3**, fluorodifen **4** and nitrofen **5**; these compounds, dissolved in ethanol, absorb UV light λ_{max} = 283.4 nm (ε = 8870 M^{-1} l cm^{-1}), 290 (11900) and 228 (10200), respectively. The excitation of these molecules, dissolved in CH_2Cl_2 or isopropanol, by a Rayonet apparatus having 4 UV lamps emitting at 300 nm gives different products. Bifenox **3** irradiated over 200 h, gives a brown mixture which shows by thin layer analysis, several degradation products (acetone, hexane 1/5). After purification by

chromatography on silicagel (aetone, hexane 6/94), we can isolate two compounds corresponding to the reduction of **3**. The isolated compound **6** (9.3%) contains an NH_2 instead of an NO_2 functional group at position 4. The second isolated compound **7** (8.9%) contains a hydroxyl group at position 4. The photolysis of fluorodifen **4** or nitrofen **5** under the same conditions gives only degradation compounds. It is not possible to detect any major compounds by thin layer chromatography. Both because the quantum yield of formation of compounds **6** and **7** is very low and because numerous degradation products are formed, the irradiation time must be maintained for 200 h in order for there to be enough compound remaining for analysis.

In several organic solvents, after direct irradiation $\lambda < 320$ nm in the presence of nitrosodurene **8**, DMPO (5,5′-dimethylpyrroline-N-oxide) **9** or PBN (phenyl-N-t-butylnitrone) **10**, spin adducts of solvent free radicals $^{\cdot}CH\text{–}Cl_2$, $^{\cdot}CCl_3$, $^{\cdot}CH_2$◎) appear.

We then studied nitrodiphenyl ether in the presence of spinach chloroplasts and DMPO. A typical phosphate buffered solution of chloroplasts containing NO_2-DPE dissolved in DMF 10^{-4}M/l and DMPO 2.10^{-2}M/l is irradiated between 600–700 nm. Two kinds of signals were obtained, the first consisted of four lines 1.2.2.1 corresponding to a spin adduct of an $OH^{\cdot}$ free radical on DMPO. The second found in distilled water medium was a 12 line ESR signal with $g = 2.0065 \pm 0.0002$ $a_N = 14.2$ G, $a_{H\beta} = 11.22$ G and $a_{H\lambda} = 1.27 \pm 0.05$ G corresponding to the $O_2^{\bar{}}$ spin adduct trapped as $^{\cdot}OOH$ free radical on DMPO.[14–16] The formation of $O_2^{\bar{}}$ could come from an electron-transfer reaction between the chlorophyll excited state of the chloroplast and the triplet state of molecular oxygen. The formation of this signal is

FIGURE 2 ESR signal obtained after irradiation $\lambda > 320$ nm of bifenox (10^{-2}M:1) in the presence of TME (5×10^{-2}M:1) in ether solution.

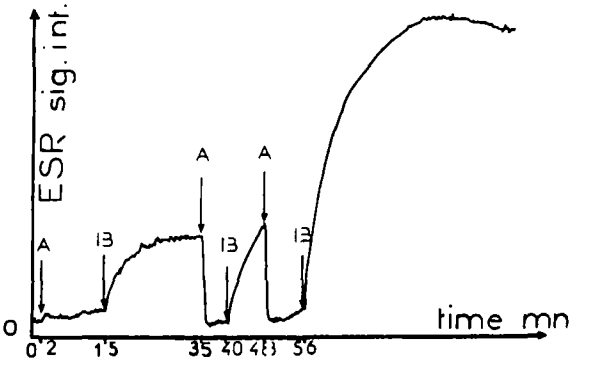
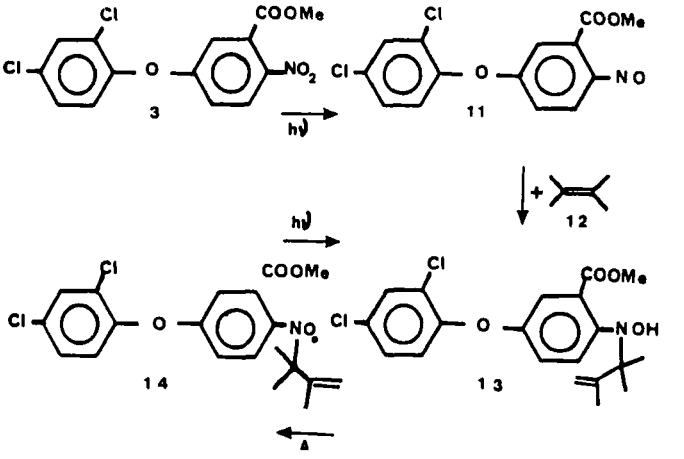


FIGURE 3 Kinetic of the triplet formation and decrease for bifenox (10^{-2}M/l) TME (5.10^{-2}M/l) in ether O → A, B → A dark, A → B light.

independant of the presence of any nitrodiphenyl ether and has been previously reported.[14,15]

We then studied the photolysis of compounds **3**, **4** and **5** in the presence of a very simple molecule: 2,3-dimethyl-2-butene or tetramethylethylene (TME) *12*.

Irradiation $\lambda < 330$ nm with a 1000 W/Xe–Hg lamp focused on an ESR cavity of nitrodiphenyl ether (bifenox or fluorodifen) 10^{-2}M/l and 5.10^{-2}M/l of TME dissolved in aerobic condition in ether, an ESR spectrum corresponding to a triplet of equal intensity is obtained (Figure 2). The splitting, $a_N = 11.48 \pm 0.05$G for the bifenox **3** and $a_N = 11.49 \pm 0.05$G for the fluorodifen **4**, are attributed to the hyperfine interaction of the unpaired electron with the ^{14}N nucleus (nuclear spin $I = 1$). One can obtain better resolution in the case of the bifenox **3** with an additional splitting. These values are well within the expected range for alkenylaryl-nitroxides.[11,13] The kinetics of apparition of this ESR triplet signal is peculiar. In the dark and under irradiation, small ESR signal appears. However when irradiation is stopped, the characteristic ESR triplet rapidly grows (its intensity being dependant on the time of the initial irradiation). The typical kinetics for this reaction is given in Figure 3. Bifenox **3**, nitrofen **5**, fluorodifen **4** in the presence of TME and in an ether solution give the same type of kinetics. Following Draper and Casida,[2,3] one can envisage the mechanism reported in scheme 1. The NO_2DPE **3** under irradiation gives



SCHEME 1 Mechanism of the formation of the hydroxylamine **13** and free radical **14**.

the nitrosodiphenyl either **11** which by "ene" addition with tetramethylethylene **12** forms the hydroxylamine **13**. This latter compound, being thermally unstable, equilibrates to the free radical **14**. Finally, the photosensitive compound **14** can revert back to the hydroxylamine **13**.

The study of the appropriate wavelength indicates that the range of excitiation is between 300–400 nm. Similar results are obtained when linoleic and linolenic acids are used instead of TME. In order to better mimic the natural environment, we decided to investigate the influence of more rigid media: namely the micelle and liposome.

For the micelle study, we used Brij 35, cetyltrimethylammonium bromide (CTAB) and sodium dodecysulfate (SDS). In these media, the nitrodiphenyl ether aggregates

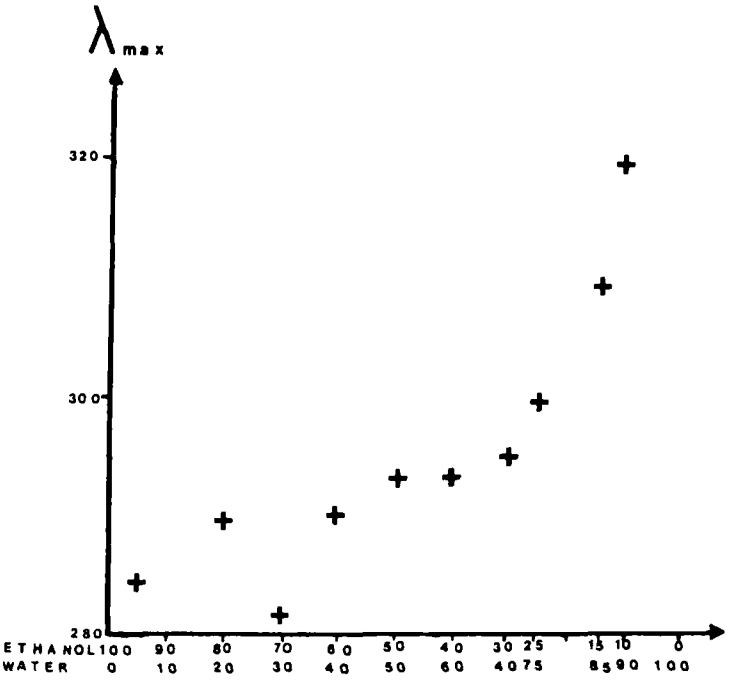


FIGURE 4 Evolution of λ_{max} of bifenox in various concentrations of water and ethanol.

(for bifenox 3, ethanol λ_{max} = 283, Brij 35 λ_{max} = 290, SDS λ_{max} = 299, CTAB λ_{max} = 320 nm). The evolution of the λ_{max} for the bifenox in various concentrations of H_2O and ethanol is given in Figure 4. We then studied in a micellar medium by ESR spectroscopy, the reaction between nitrodiphenylether and TME. The ESR spectrum obtained is not symmetric (Figure 5), the last line in the higher field is broadened. We can calculate using the Kivelson equation the rotational correlation time τ_c (τ_c is a measure of the degree of free rotation of the paramagnetic molecule in the studied medium). We have obtained the following values for the different miceles:

	τ_c
— SDS	$6.9\ 10^{-10}$
— Brij 35	$3.3\ 10^{-10}$
— CTAB	$8.1\ 10^{-10}$

FIGURE 5 ESR spectrum of bifenox (10^{-2}M/l) and TME (5.10^{-2}M/l) in Brij 35 micelle medium.

Analysing these results, one can say that with the SDS and CTAB (anionic and cationic detergent respectively) the free radical is more deeply embedded inside the bulk of the micelle than with the Brij 35 (neutral detergent). The value obtained with CTAB is not very different from that obtained with SDS. The former result has not been exploited because this particular diphenyl ether was not very soluble in the CTAB medium.

The kinetic behaviour of such systems corresponding to the apparition of the triplet ESR signal of the photolysis of NO_2DPE with TME in a micellar medium creates the apparition of a strong free radical signal which, under continuous photolysis reaches a steady state. When the light is removed, there is a rapid decay for both SDS and CTAB Figure 6. The kinetics of the ESR triplet formation corresponding to the TME adduct on NO_2DPE behave differently in micellar media than in homogeneous solution because in the former environment the free radical is stabilised inside the hydrophobic part of the micelle, a well known phenomenom.[17]

After this study in micellar media we investigated the reactivity of nitrodiphenyl ether towards a photosynthetic membrane model such as soja phosphatidylcholine azolectins which gave relatively stable, tight liposomes. In the mechanism of action of nitrodiphenyl ether the nitrosodiphenyl ether is supposed to be the active entity. We synthetized these derivatives in the following manner:

the NO_2DPE is reduced by platinium oxide and hydrogen (1):

$$R\text{-}NO_2 \xrightarrow[H_2]{PtO_2.\ EtOH} R\text{-}NH_2 \qquad (1)$$

and oxidised by action of meta-chloroperbenzoic acid (m. CPBA) (2):

$$R\text{-}NH_2 \xrightarrow[CH_2Cl_2.\ 0°C]{mCPBA} R\text{-}NO \qquad (2)$$

the synthetized nitrosodiphenyl ether has the same behaviour as the one made *in situ*

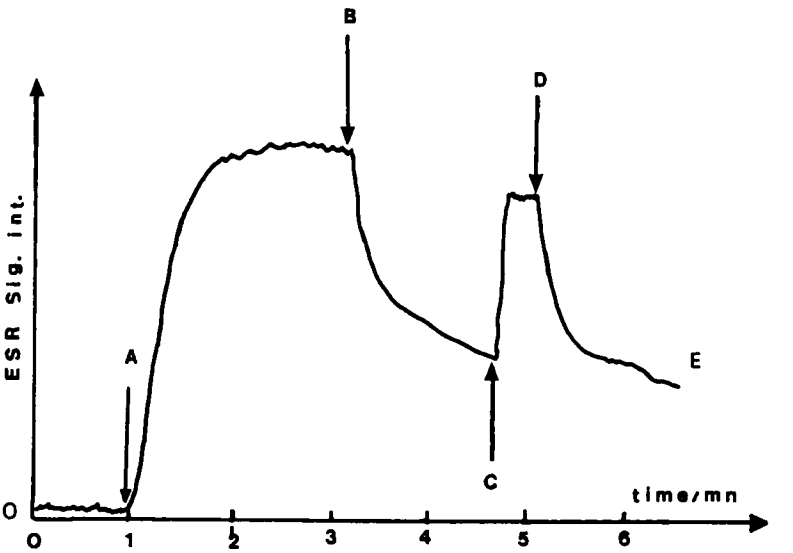


FIGURE 6 Kinetic of the triplet formation and decrease of the bifenox (10^{-2}M/l), TME (5.10^{-2}M/l) in SDS micellar medium. OA, BC, DE dark, AB, CD light.

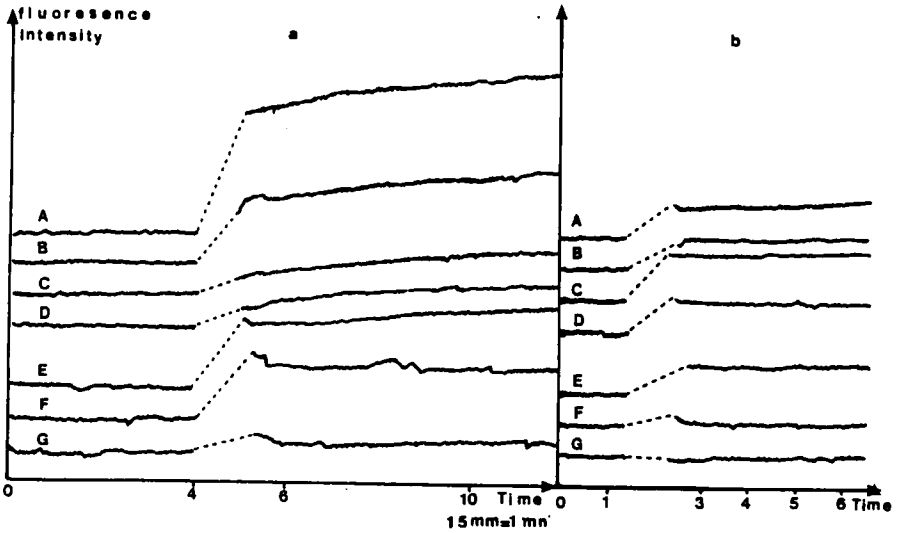


FIGURE 7a Kinetic at 520 nm (fluorescence emission) after addition of xμl of a solution containing 7 mg/ml of nitroso-bifenox in DMF. A: 100, B: 75, C: 50, D: 30, E: 20, F: 15, G: 10.

FIGURE 7b Kinetic at 520 nm (fluorescence emission) after addition of xμl of DMF. A: 100, B: 75, C: 50, D: 30, E: 20, F: 15, G: 10.

by photolysis. The ether reacts with **12** in a homogeneous solution giving an analogous of the previously mentioned triplet ESR spectrum (Figure 2) of irradiation of NO_2DPE and **12**.

To test the reactivity of the synthetized nitrosodiphenyl ether, we constructed a system consisting of a soja phosphatidylcholine liposome containing within its water pool the 6-carboxyfluorescein in high concentration (such that the 6-carboxyfluorescein is not fluorescent). A certain amount of nitrosoderivative dissolved in DMF, added in the surrounding water, creates leaks and holes in the membrane of the liposomes (the solvent used for dissolving the nitrosodiphenyl ether creates no great disturbance to the system). The 6-carboxyfluorescein migrates to the surrounding water leading to an increase in fluorescence (as a result of its weak concentration) (Figure 7a,b).

MATERIALS AND METHODS

The ESR measurements were carried out using an ESR 0.1 mm quartz cell for aqueous solutions or in a 4 mm cylindrical quartz tube for ether solutions. They were placed in diffuse room light or in the dark and degassed by bubbling with argon 10–20 mn if necessary. The cell or the tube was then introduced into an ER-400X-RL cavity of a Bruker ER.420 spectrometer equipped with BHN 12 and B 16 accessories for magnetic field calibration and frequency measurements respectively. The samples were irradiated in the cavity with a Hanovia 977 B 0070 1000 W Hg–Xe, or Xe arc lamps in a Model LH 15 1 H Schoeffel lamp housing following the case. The light was

focused through quartz lenses and filtered through a Corning or MTO glass filter, following the experiment.

Continuous photolysis of argon degassed solutions have been carried out using a Rayonet apparatus equipped with 4 UV Hg quartz tubes emitting at 300 nm. The evolution of the reaction was followed by thin layer chromatography on silicagel using Schleicher et Schuell plates F 1500/LS-254. The chromatographic separations were performed using Schleicher et Schuell plates (1 mm thickness) for preparative t.l.c.

Nmr spectra were determined for solutions in deuteriochloroform with $SiMe_4$ as internal standard on Varian T-60 and Bruker AC200 instruments. Mass spectra were recorded with an AES MS9 or MS50 apparatus. IR spectra were recorded on a Perkin-Elmer 257 instrument. Fluorescence spectra were recorded on Jobin Yvon 3 apparatus.

All solvents used were distilled. The spin traps used in these experiments were the 5,5'-dimethyl-1-pyrroline 1-oxide (DMPO) and the phenyl-ter-butyl-nitrone (PBN), both from Aldrich. DMPO was purified under vacuum distillation (75°C/0.4 mm/Hg). PBN was recristallised in hexane (MP = 73°C). The chloroplasts were prepared from spinach leaves.[18] The tetramethylethylene (TME), linoleic, and linolenic acids (Fluka), Triton X-100 (Merck), Cetyltrimethylammonium bromide (CTAB) and Brij 35 (Janssen) were used as received. The 6-carboxyfluorescein (Eastman Kodak) was purified by recristallisation in absolute alcohol. Buffered solutions were prepared from 4-morpholinopropane sulfonic acid (MOPS) pH = 7.4 from Prolabo. The L-α phosphatidylcholine (Sigma) was purified in the following manner: 10 g of L-α phosphatidylcholine was dissolved in 20 ml of a 1/1 CH_2Cl_2/MeOH mixture; the solution was magnetically stirred for a few minutes, filtered and 100 ml of acetone was added. A yellow visquous precipitate was obtained; which was washed with acetone and dissolved in petroleum ether; the solution was kept under argon in the dark at 4°C.

References

1. Matsunaka, S. Acceptor of light energy in photoactivation of diphenyl ether herbicides, *J. Agr. Food Chem.*, **17**, 171–175, (1969).
2. Draper, W.M. and Casida, E. Diphenyl ether herbicides: mutagenic metabolites and products of nitrofen, *J. Agric. Food Chem.*, **31**, 227–231, (1983).
3. Draper, W.M. and Casida, J.E. Diphenyl ether herbicides and related compounds: structure activity relationships as bacterial mutagens, *J. Agric. Food Chem.*, **31**, 1201–1207, (1983).
4. Orr, G.L. and Hess, F.D. Mechanism of Action of the diphenyl ether herbicide acifluorfen-methyl in excised cucumber (cacumis sativus L.) cotyledons, *Plant Physiol.*, **69**, 502–507, (1982).
5. Lambert, R., Kroneck, P.M.H. and Böger, P. Radical formation and peroxidative activity of phytotoxic diphenyl ethers, *Z. Naturforsch.*, **39c**, 486–491, (1984).
6. Draper, W.M. and Casida, J.E. Nitroxide radical adducts of nitrodiphenyl ether herbicides and other nitroaryl pesticides with unsaturated cellular lipids, *J. Agric. Food Chem.*, **33**, 103–108, (1985).
7. Lambert, R., Sandman, G. and Böger, P. Correlation between structure and phytotoxic activities of nitrodiphenyl ethers, *Pest. Biochem. Physiol.*, **19**, 309–320, (1983).
8. Lagercrantz, C. and Yhland, M. Photoinduced electron spin resonance in solutions of some electron-donor-acceptor complexes, *Acta Chem. Scand.*, **16**, 1043–1045, (1962).
9. Cowley, D.J. and Sutcliffe, L.H. A reinterpretation of the photo-induced free radicals produced from nitrobenzenes in tetrahydrofuran, *J. Chem. Soc. Chem. Commun.*, 201–202, (1968).
10. Davies, L.P., Wilkes, J.S., Pugh, H.L. and Dorey, R.C. Photochemical decomposition of 2,4,6-trinitrotoluene and related compounds in ether, *J. Phys. Chem.*, **85**, 3505–3509, (1981).
11. Knight, G.T. N-phenyl-N-(1,1,2-trimethyl prop-2-enyl) hydroxylamine and its nitroxide, *J. Chem. Soc. D*, 1016–1017, (1970).
12. Barlow, H.G., Hazeldine, R.N. and Murray, K.W. Polyfluoroalkyl derivatives of nitrogen. Part 49.

Ene reactions of trifluoronitrosomethane: formation of N-trifluoromethyl-hydroxylamines, *J. Chem. Soc. Perkin I*, 1960–1964, (1980).
13. Sullivan, A.B. Electron spin resonance studies of a stable arylnitroso-olefin adduct free radical, *J. Org. Chem.*, **31**, 2811–2817, (1966).
14. Harbour, J.R., Chow, V. and Bolton, J.R. An electron spin resonance study of the spin adducts of $^{\cdot}OH$ and $HO_2^{\cdot}$ radicals with nitrones in the ultra violet photolysis of aqueous hydrogen peroxide solutions, *Can. J. Chem.*, **52**, 3549–3553, (1974).
15. Harbour, J.R. and Bolton, J.R. Superoxide formation in spinach chloroplasts: electron spin resonance detection by spin trapping, *Biochem. Biophys. Res. Commun.*, **64**, 803–807, (1975).
16. Harbour, J.R. and Bolton, J.R. The involvement of the hydroxyl radical in the destructive photooxidation of chlorophylls in vivo and in vitro, *Photochem. Photobiol.*, **28**, 231–234, (1978).
17. Bernas, A., Grand, D., Hautecloque, S. and Giannotti, C. Interfacial electrical potential in micelles, influence on N-N, N′-N′ tetramethylbenzidine cation decay, *J. Phys. Chem.*, **90**, 6189–6194, (1986).
18. Briantais, J.M., Vernotte, C., Olive, J. and Wollman, F.A. Kinetics of cation-induced changes of photosystem II fluorescence and of lateral distribution of the two photosystems in the thylakoid membranes of pea chloroplast, *Biochim. Biophys. Acta*, **766**, 1–8, (1984).

Accepted by Prof. E.G. Janzen