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FREE RADICAL PRODUCTION DURING PHOTO-ASSISTED NADPH REDUCTION OF FOUR α-NITROARENOFURANS

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Generation of radical anions during NADPH reduction of four mutagenic and genotoxic α-nitroarenofurans was examined. ESR showed that free radicals were generated during reduction solely in the presence of light. Computer simulations of ESR spectra were in good agreement with the experimental ones.

KEY WORDS: Genotoxicity, α-nitro-arenofuran, free radicals, electron spin resonance, NADPH.

ABBREVIATIONS: ESR, electron spin resonance; NADPH, β-nicotinamide adenine dinucleotide phosphate, reduced form; DMSO, dimethyl sulfoxide; DMF, dimethylformamide

INTRODUCTION

Several studies have been devoted to the mutagenic and carcinogenic properties of nitroarenofurans.[1-3] Under aerobic conditions, the NO_2 group remains unchanged, and metabolism of the nitroarenofuran affords a nitroarenofuran dihydrodiol.[4] However, the genotoxic activity of nitro heterocyclic compounds is thought to require reduction of the nitro group.[5,6] The present study was designed to examine the relationship between the genotoxicity of four nitro-heterocyclic compounds (nitrated in the α-position relative to the oxygen atom)[1-3,7] and their ability to generate free radicals during reductive transformation. This was prompted by the observation of a radical anion derived from 7-methoxy-2-nitronaphtho[2,1-b]furan (4) on NMR,[8] and consideration of the particular route of activation and organospecificity of these genotoxic α-nitroarenofurans.[9-11]

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MATERIALS AND METHODS

Four genotoxic compounds with very different values of mutagenic activity,[1] synthesized in the Institut Curie (Section de Biologie, Service de Chimie, Paris) were studied: 2-nitrobenzofuran **1**,[12] 5-methoxy 2-nitrobenzofuran **2**[12,13], 2-nitronaphthol[2,1-b]furan **3**[14], and 7-methoxy-2-nitronaphto[2,1-b]furan (R7000) **4**[15,16] (see formulae in Table 1). The procedure used to generate radicals was a reduction of the nitro group by NADPH in the dark and also with exposure to light, under anaerobic conditions, in order to avoid quenching of radicals.

Nitroarenofuran derivatives (2 μmoles in 1.2 ml DMSO) and NADPH (1 mg in 0.1 ml H_2O) were introduced in the reactor and maintained at 37°C for 10 min in the absence of light under bubbling with oxygen-free argon. An aliquot was then rapidly transferred to an aqueous flat cell which was capped and placed in a Varian CSE 109 Spectrometer for the ESR measurements at 25°C. A first ESR spectrum was recorded, followed by a second one after exposure to daylight for 10 min.

The coupling constants (cf. Table 1) were juxtaposed to the spin density due to the electron attractor substituent according to the rules of alternation. The ESR spectra were also simulated on a Macintosh computer using a purpose-designed program written in Microsoft Quick Basic. We checked that the lineshapes were approximately Gaussian. The assignment of the lines was verified by this simulation.

RESULTS

In the dark, no spectrum was obtained for none of the four compounds studied.

2-nitro benzofuran **1**

After light exposure a partially resolved 16 line spectrum was observed (Figure 1b).

The stick diagram (Figure 1d) was constructed from a nitrogen triplet with a coupling constant $a_N = 9.53$ Gauss. The splitting of these 3 lines corresponds to three hydrogens with the following splitting constants: 6.35, 1.92, and 1.71 Gauss respectively.

Some contiguous sticks were not observed on the experimental spectrum. These lines are enclosed in brackets on Figure 1d. Thus 16 lines corresponding to three hydrogens of the molecule were observed. These 16 lines were resolved by further splitting of each line into 3 lines in a 1:2:1 intensity ratio. This 0.4 Gauss splitting was assigned to the last two hydrogens. Thus, interaction with the five hydrogens of the molecule could be observed. The computer simulation shown in Figure 1c, which is in good agreement with the experimental spectrum, confirms the assignment of the stick diagram; two doublets being assigned to hydrogens at positions 4 and 6, and a triplet to hydrogens at positions 5 and 7.

5-methoxy-2-nitro benzofuran **2**

After light exposure a 38 line spectrum was observed (Figure 2b). On the left side, this spectrum was incompletely resolved with 26 lines corresponding to 34 calculated levels. As a first approximation, the stick diagram was determined from this experimental spectrum. The coupling constant corresponding to the nitrogen was

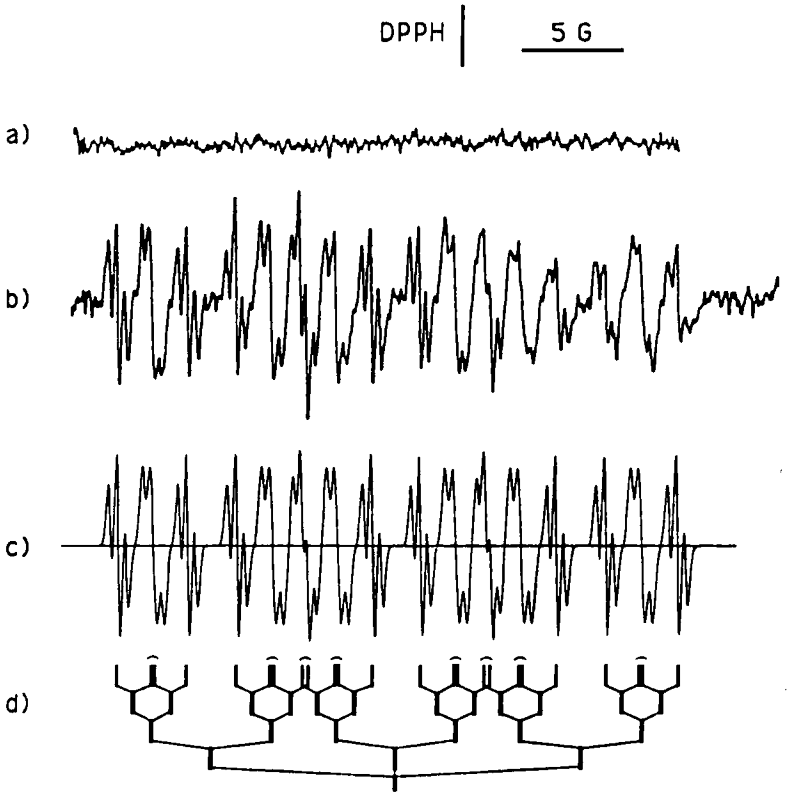


Figure 1 ESR spectra: 2-Nitro benzofuran 1, modulation amplitude 0.5 Gauss, microwave power 5 mW, time constant 0.128 s. (a) without light, (b) after light exposure. (c) Computer simulation. (d) Stick diagram $a_N = 9.53$ G, $a_H^3 = 6.35$ G, $a_H^6 = 1.92$ G, $a_H^1 = 1.71$ G.

$a_N = 9.54$ Gauss. Four doublets splitting of 6.08-2.12-1.55 and 0.3 Gauss were assigned for the four hydrogens of the molecule. Some neighbouring sticks (enclosed in brackets on Figure 2d) gave rise to an unresolved spectrum. The computer simulation in Figure 2c for the first half spectrum is in good agreement with the experimental spectrum, confirming the assignment of the stick diagram. On the right side, the spectrum exhibited twelve unresolved lines in five groups, which could be explained by the presence of two closely related radical species.

The triplet (two identical hydrogens in positions 5 and 7) observed for compound **1** was replaced by a doublet involving interaction with the single hydrogen at position 7.

2-nitro-naphtho[2,1-b]furan 3

After light exposure the spectrum (Figure 3b) consisted of a set of 10 multiplets. The three last unresolved multiplets probably indicated the presence of two closely related radical species. The stick diagram is shown in Figure 3d. The closely separated lines

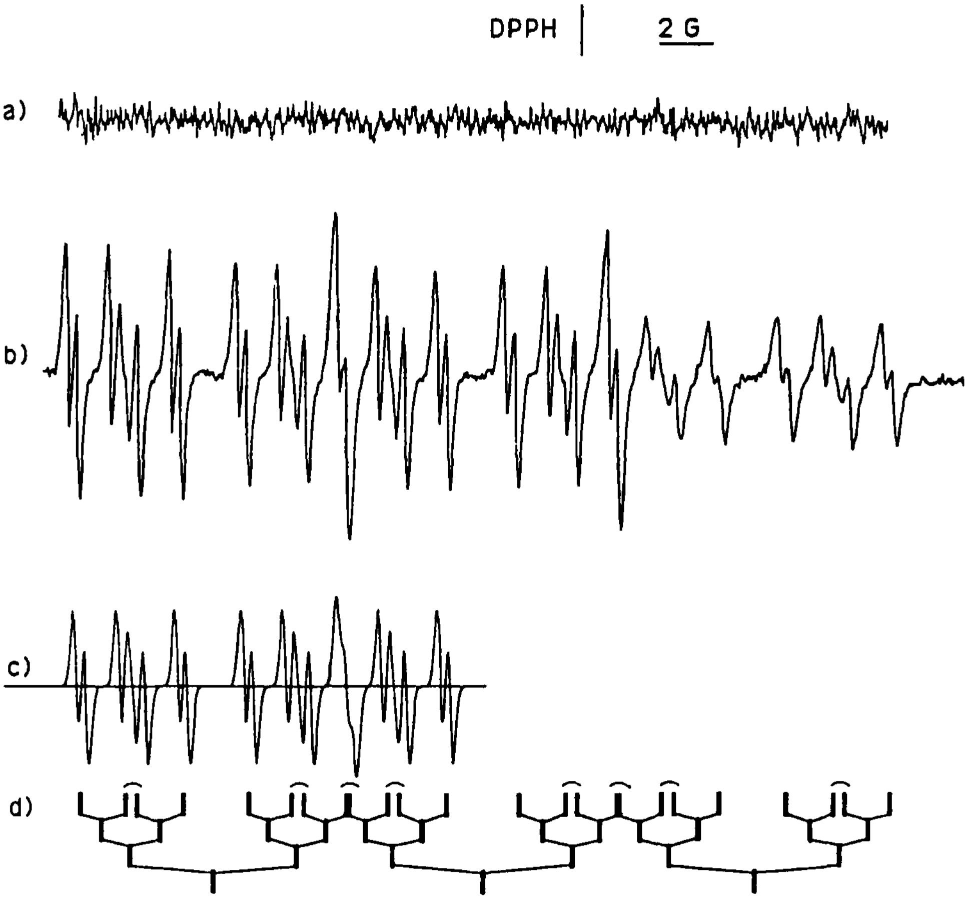


Figure 2 ESR spectra: 5-Methoxy-2-nitro benzofuran **2**, modulation amplitude 0.5 Gauss, microwave power 5 mW, time constant 0.128 s. (a) Without light, (b) after light exposure. (c) Computer simulation, (d) Stick diagram $a_N = 9.54$ G, $a_H^3 = 6.08$ Gm $a_H^6 = 2.12$ G, $a_H^1 = 1.55$ G.

in bracket are unresolved on the experimental spectrum where only ten multiplets could be observed.

The coupling constant could not be determined accurately due to the proximity of multiplets 4–5 and 8–9 and the unresolved multiplets. However, interaction of the nitrogen with the two hydrogens at positions 1 and 5 leads to a triplet (9.44 Gauss) and to two doublets (5.81 and 2.37 Gauss). The hydrogen at position 6 gave a doublet splitting of 0.6 Gauss. The four hydrogens at positions 4, 7, 8 and 9 may interact, and each line of the doublet from hydrogen 6 could be resolved following a further splitting into five lines with a 1:4:6:4:1 intensity ratio. This splitting of 0.3 Gauss was measured on the experimental spectrum. For the experimental spectrum, each multiplet was resolved into seven lines instead of five. To account for these features and the three unresolved multiplets, we assumed the presence of two closely related species with small differences in coupling constant and g-factor.

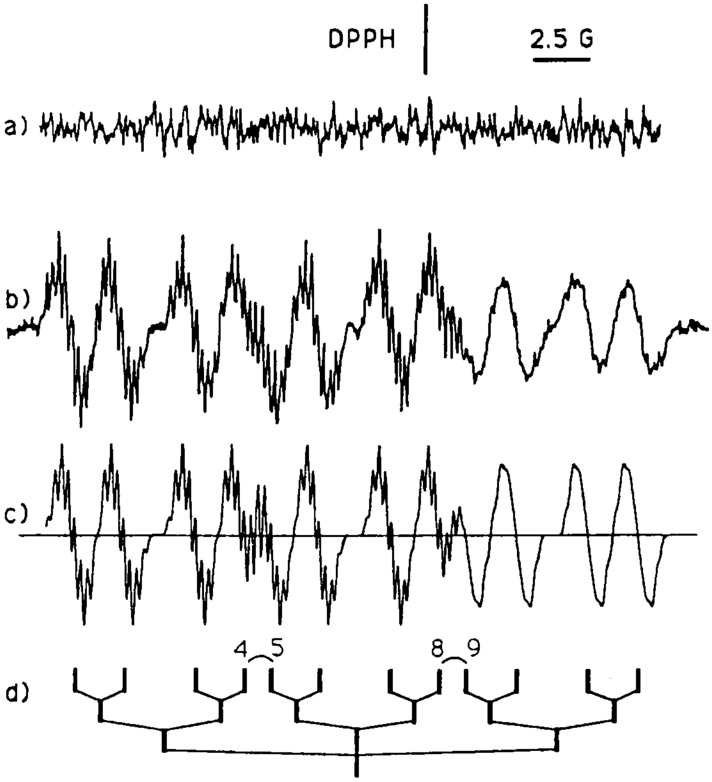


Figure 3 ESR spectra: 2-Nitro naphthofuran 3, modulation amplitude 0.125 Gauss, microwave power 5 mW, time constant 0.3 s. (a) Without light, (b) after light exposure. (c) Computer simulation. (d) Stick diagram $a_N = 9.44$ G, $a_H^1 = 5.81$ G, $a_H^5 = 2.37$ G.

After various computer simulations, a good agreement (Figure 3c) was obtained with the experimental spectrum with a shift of $\Delta g = 0.25$ Gauss between the spectra of the two species, the second having a value of $a_N = 9.54$ Gauss, only slightly different from that of the first species (9.44).

7-methoxy-2-nitro-naphtho[2,1-b]furan 4

After light exposure a spectrum with 10 multiplets (Figure 4b) was observed. The three last unresolved multiplets—as for the 2-nitro naphthofuran 3—suggest the presence of two closely related species. The close sticks 4–5 and 8–9 shown in brackets on the stick diagram (Figure 4d) led to an unresolved 10 multiplet spectrum. Examination of the stick diagram indicated a nitrogen triplet at 9.38 Gauss and two doublet splitting (5.82 and 2.44 Gauss) corresponding to the 1 and 5 hydrogens. The multiplets correspond to a triplet splitting of 0.4 Gauss produced by the hydrogens in positions 4 and 6.

The computer simulation was in good agreement with the experimental spectrum (Figure 4c).

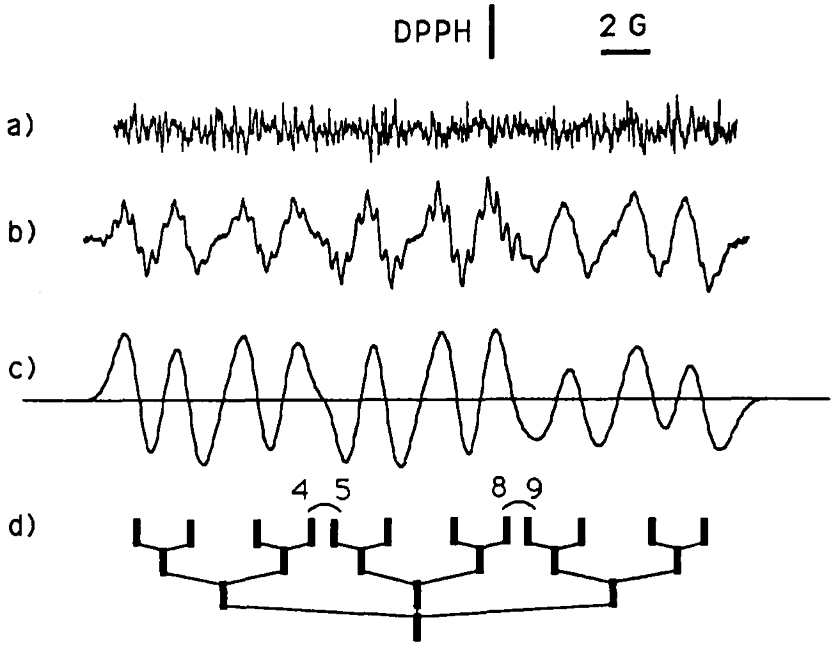


Figure 4 ESR spectra: 7-Methoxy-2-nitro naphthofuran 4 (R7000), modulation amplitude 0.5 Gauss, microwave power 5 mW, time constant 0.25 s. (a) Without light, (b) after light exposure. (c) Computer simulation without taking into account the interaction with hydrogens in 4 and 6 positions. (d) Stick diagram $a_N = 9.38$ G, $a_H^1 = 5.82$ G, $a_H^5 = 2.44$ G.

DISCUSSION

As depicted in Figures 1–4 all the tested nitroarenofurans produced a radical compound with NADPH in the presence of daylight. It has been checked that no signal was obtained in the absence of either NADPH or light. The fact that the intensity of the ESR signal increases with the concentration of the electron donating agent (NADPH) is in good agreement with an electron exchange mechanism between 2-nitroarenofurans and the radical species.

The coupling constants correspond unambiguoulsy with the structure of the starting molecules and the triplets a_N indicate the existence of a nitrogen with an unpaired electron.

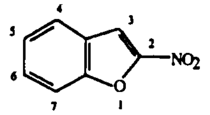
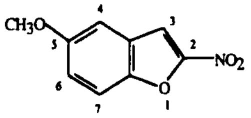
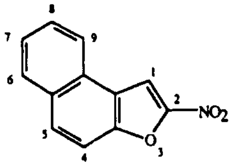
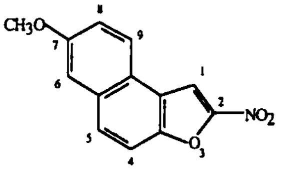
Although it seems difficult to differentiate between nitro or nitroso radical species since Wong *et al.*[17] and Cowley *et al.*[18] have shown the co-existence of such species with light irradiated nitrobenzene and nitrochlorobenzene. The increase of life time of the radical species for compounds 1–4 (5–60 min) compared with the very short life times (fraction of seconds) of Wong and Cowley species could result from the electron donating properties of NADPH. However, the ArNO·H structure could not be attributed to the radical species generated by compounds 1–4 since an a_H^{NH} doublet[17] was not observed in our experiments.

The formation and characterization of the radical anions obtained from nitro aromatic compounds has been the subject of several studies.[19–21] The spectra of these radicals are highly solvent-sensitive, due to the effect of hydrogen bonding with the NO_2^- group.[21] For example, the nitrogen coupling constant in the radical anion of nitrobenzene has been found to be 9.83 Gauss in DMF,[21] 10.32 in acetonitrile,[19] 13.87 in a mixture of 90% of water and 10% of DMF, and 14.12 in water as we have checked. The presence of two radical species is probably due to the two possible modes of solvation.

These results could be accounted for in terms of electroaffinity. From Koopmans theorem[22] for families of similar compounds, the electroaffinity is a function of the energy of the first unoccupied orbital of the molecule (LUMO: Lowest Unoccupied Molecular Orbital). In addition, the squares of the coefficients of the atomic orbitals of the LUMO, give a good approximation of the spin densities whose magnitudes are directly related to the coupling constants obtained from the ESR spectra. The spin densities were calculated by CNDO/S (Complete Neglect Differential Overlap/Spectra),[23] and the results are listed in Table 1. It can be seen that there is good correlation between the variations of the coupling constants and the calculated spin densities.

The formation of a radical anion is a step in the reductive transformation of these nitroarenofurans. This type of radical could be of importance in the metabolism of the highly mutagenic nitroarenofurans studied here. Thus the results of *in vitro* genotoxicity tests may be influenced by light.

Table 1

No	COMPOUNDS Formula	Hydrogen position	Coupling constants	Spin density
1		3	6.35	0.163
		4	1.71	0.028
		5	0.4	0.000
		6	1.92	0.028
		7	0.4	0.003
2		3	6.08	0.162
		4	1.55	0.028
		6	2.12	0.029
		7	0.3	0.003
3		1	5.81	0.156
		4	0.3	0.005
		5	2.37	0.045
		6	0.6	0.009
		7	0.3	0.000
		8	0.3	0.012
		9	0.3	0.001
4		1	5.82	0.156
		4	0.4	0.005
		5	2.44	0.046
		6	0.4	0.009
		8	≤0.3	0.012
		9	≤0.3	0.002

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