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SHORT COMMUNICATION

Irradiated Nifedipine—a Nitroso Spin Trap

Andrej Staško*
Department of Physical Chemistry, Faculty of Chemical Technology, Slovak Technical University, 81237 Bratislava, Slovak Republic

Vladimir Mišík
Institute of Experimental Pharmacology, Slovak Academy of Sciences, 84216 Bratislava, Slovak Republic

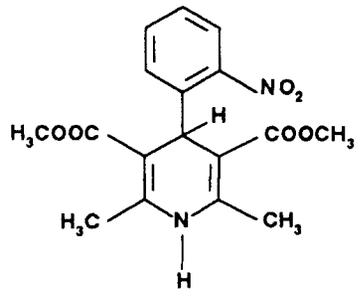
Ladislav Omelka
Department of Physical Chemistry, Faculty of Chemical Technology, Slovak Technical University, 81237 Bratislava, Slovak Republic

Irradiation of nifedipine yields a new nitroso spin trap which traps carbon-centred radicals and also forms relatively stable alkoxyl radical adducts. In addition, the reaction with unsaturated lipids yield highly stable covalent nitroxide radicals.

KEY WORDS ESR Nitroso spin trap Covalent nitroxide radicals Unsaturated lipids

INTRODUCTION

Previously we have reported[1] that nifedipine (**1**), a commonly used antihypertensive drug, is easily converted by irradiation into a nitroso compound (**2**) which has, in some respects, unusual spin trapping properties. In this work, we further analysed its spin trapping profile and investigated the formation of $2^{\bullet}$–X adducts in various redox-, photochemical- and thermally-initiated radical reactions.



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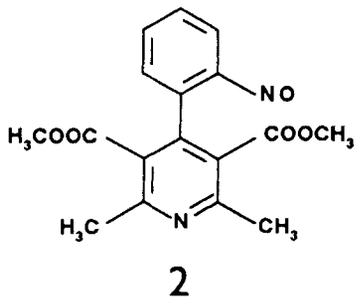

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* Author to whom correspondence should be addressed.

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EXPERIMENTAL

Preparation of 2

Exposure of **1** (5 mg ml^{-1} of ethanol) to sunlight or to a mercury lamp, selecting the region 300–580 nm, and subsequent recrystallization gives about a 70% yield of blue–green crystals of **2** (purity >99.9%, gas chromatography–mass spectrometry). Compound **2** is suitable for applications in most non-aqueous solvents, and for biological applications in aqueous systems it can be introduced in diheptanoylphosphatidylcholine vesicles.[2]

Photochemical experiments

The photochemical experiments were carried out directly in the cavity of a Bruker 200D ESR spectrometer equipped with an Aspect 2000 computer. The irradiation source was a 250 W medium-pressure mercury lamp (Applied Photophysics, UK) and a Pyrex filter was used to eliminate the absorption of radiation with $\lambda < 300$ nm.

RESULTS

Spin trapping

The spin trapping of phenyl radicals generated by oxidation of *N*-anilinophthalimide with $Pb(OAc)_4$ is demonstrated in Fig. 1(a). Similarly, Fig. 1(b) illustrates the formation of the $2^{\bullet}$–$C(CH_3)_2CN$ adduct in the pho-

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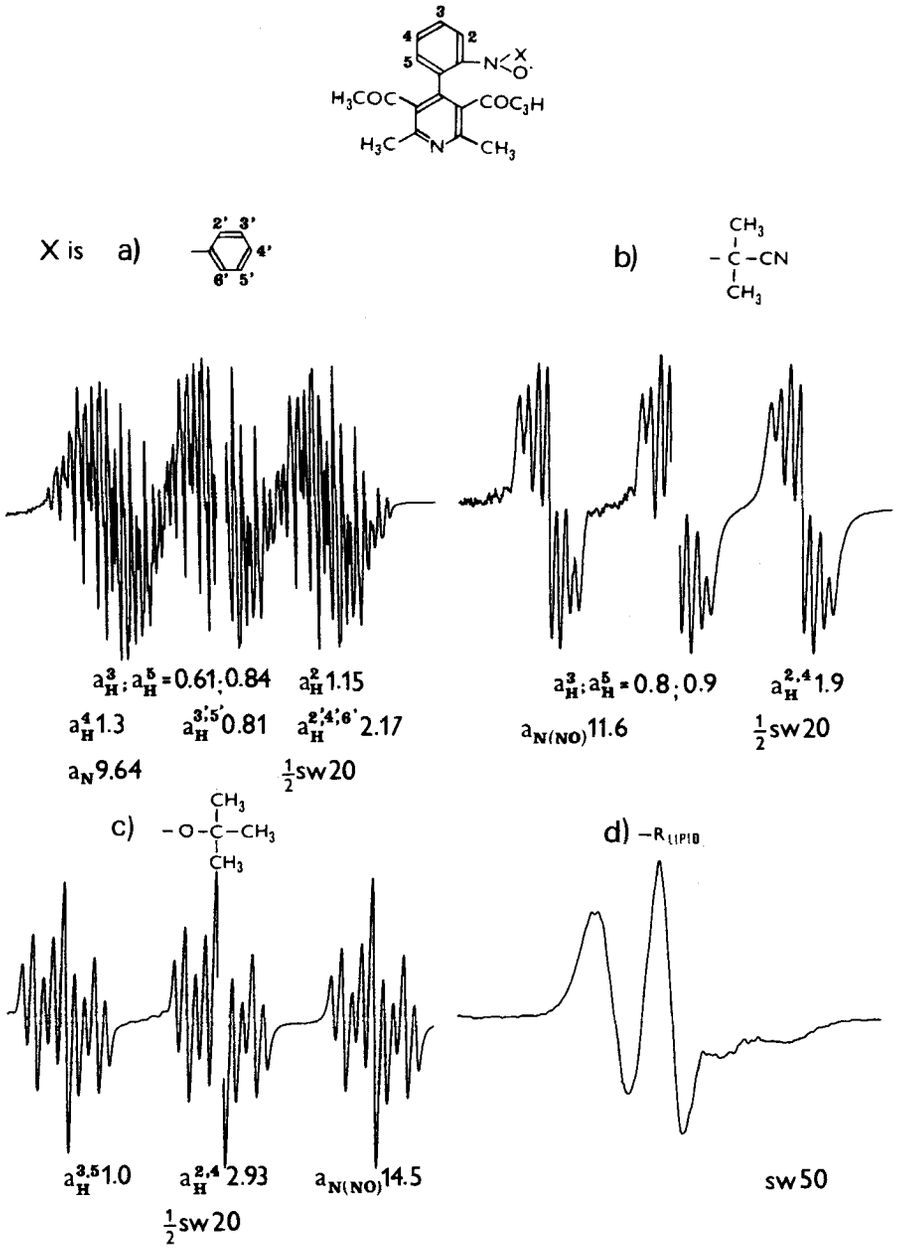


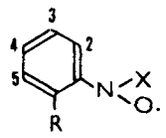
Figure 1. Experimental (lower field) and simulated [higher field half; (a), (b) and (c) only] EPR spectra observed in spin trapping with **2** in benzene solutions: (a) *N*-anilinophthalimide + $Pb(OAc)_4$; (b) photolysis of azobisisobutyronitrile; (c) photolysis of tBuOOtBu; (d) covalent nitroxide radical formation with dioleoyl-L-α-phosphatidylcholine dispersed in water. Splitting constants (a_H, a_N) and sweep widths (SW) are expressed in 10^{-1} mT.

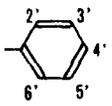
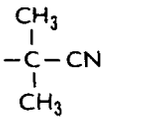
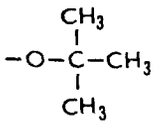
tolysis of azobisisobutyronitrile in solution. The yields of alkoxyl adducts were suprisingly high in comparison with other nitroso spin traps. Figure 1(c) illustrates the formation of the **2**'–OtBu adduct observed on photolysis of tBuOOtBu. The *g*-values obtained [$g = 2.0057$ for Fig. 1(a) and (b) and 2.0049 for Fig. 1(c)] correspond well with those found for other nitroso spin traps.[3] In numerous experiments the trapping of thiyl, peroxyl, hydroxyl and phenoxyl radicals was mostly unsuccessful as only low yields of the respective spin adducts were formed. Frequently, in photochemical applications of **2**, superimposed spectra were observed owing to the formation of the hydronitroxide radical **2**'–H and other unidentified nitroxides.

Covalent nitroxide labelling of unsaturated lipids

In addition to its spin trapping properties, **2** also possesses another interesting property that may be useful in biochemical and biophysical applications. It reacts, in a pseudo-Diels–Alder reaction, with unsaturated lipids, forming covalent nitroxide radicals which are highly stable[1] [an example of the immobilized nitroxide radical signal is given in Fig. 1(d)]. This appears to be a suitable procedure for covalent nitroxide radical labelling in systems containing unsaturated lipids such as liposomes, low-density lipoproteins, tissue homogenate cells and cell components.[1,2] The nitroxide radical labelling using **2** is superior compared with other stan-

Table 1. Splitting constants of radical adducts 2'–X



Radical X added	a_N(NO)	Splitting constant ($\times 10^{-1}$ mT) a_H^{2}	a_H^{4}	$a_H^{3,5}$	$a_H^{2',4',6'}$	$a_H^{3',5'}$
	9.64	1.15	1.30	0.61 0.84	2.17	0.81
	11.60	1.90	1.90	0.80 0.90		
	14.50	2.93	2.93	1.00		

dard nitroso spin traps.[2] These covalent labelling experiments can be conveniently performed without the prior preparation of **2**, by simply adding nifedipine to the biological material and subsequent *in situ* irradiation[1] with sunlight or a 300–580 nm irradiation source.

CONCLUSION

Compound **2** is a nitroso spin trap that can be easily prepared in gram quantities from inexpensive nifedipine. It is suitable for trapping carbon-centred radicals and also forms relatively stable alkoxyl radical adducts in high yields. A partial disadvantage is the relatively complex hyperfine structure in the spectra of its adducts. It may be especially useful in various biochemical and biophysical applications for covalent nitroxide radical labelling of biological materials containing unsaturated lipids for subsequent EPR monitoring.

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