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SYNTHESIS OF α-ARYL *N*-ADAMANT-1-YL NITRONES AND USING THEM FOR SPIN TRAPPING OF HYDROXYL RADICALS

Cecília P. Sár,[a] Éva Hideg,[b] Imre Vass,[b] Kálmán Hideg[a]*

[a] *Institute of Organic and Medicinal Chemistry, University of Pécs, H-7643 Pécs, P.O. Box 99, Hungary*

[b] *Institute of Plant Biology, Biological Research Center, H-6701 Szeged, P.O. Box 521, Hungary*

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Abstract: α-Aryl *N*-adamant-1-yl nitrones were synthesized and evaluated with respect to the stability of the hydroxyl radical adduct. The polarity and water solubility of nitrones were altered with changing the α-aryl groups. Introduction of adamantane ring instead of *tert*-butyl group resulted in a reasonable good stability of hydroxyl radical adduct for biological measurements. © 1998 Elsevier Science Ltd. All rights reserved.

Introduction

All aerobic organisms are confronted with dangerous oxygen toxicity. Oxidative damage often imposes irreversible structural changes on biological membranes and impairs related functions.[1-3] Reactivity of free radicals - the cause of their toxicity - makes them difficult to detect and quantify. At present, electron paramagnetic (EPR) spectroscopy appears the only direct tool to overcome this difficulty.[4-6] Both cyclic and non-cyclic nitrones are used for spin trapping of the most toxic hydroxyl radical (HO').[7-10] The most frequently used cyclic nitrone is 5,5-dimethylpyrroline 1-oxide (DMPO),[11,12] non-cyclic ones are phenyl *N*-*tert*-butyl nitrone[13] (PBN) and 4-(pyridyl-*N*-oxide) *N*-*tert*-butyl nitrone[14] (4-POBN). The hydroxyl adduct of DMPO is fairly stable but the DMPO is rather hydrophilic to be detected for radicals in lipophilic systems.[15] The PBN and 4-POBN are more lipophilic but their hydroxyl radical adduct proved too prone for decomposition to sterically less hindered paramagnetic transient species.[14,16] Herein, we describe the synthesis and stability evaluation of some new PBN type non-cyclic nitrone spin traps. The goal of the synthesis was to increase the lipophilicity and stability with preserving the solubility of the nitrone. In our strategy we concentrated on the modification of *tert*-butyl side of the reagents. We have found that the easily available 1-aminoadamantane which was itself investigated as bioactive (antiviral, anticancer, anti-HIV) molecule[17,18] may be ideal to obtain more stable lipophilic new non-cyclic nitrones by replacing the *tert*-butyl group in a PBN type nitrone for a 1-adamantyl group.

* Fax: (36)(72) 325-731; E-mail: KHIDEG@ main.potc.hu

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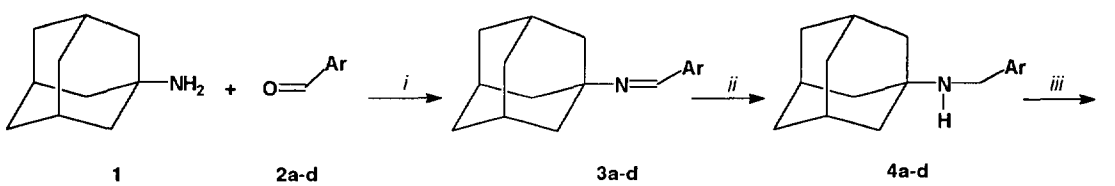
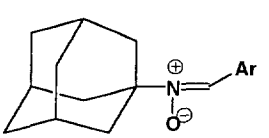
Chemistry

1-Adamantyl-amino Schiff's bases (**3a-d**) were obtained when 1-aminoadamantane (**1**) and the appropriate aromatic aldehyde (**2a-d**) were heated in the presence of toluenesulfonic acid catalyst in toluene for 2 h. Interestingly, the nitrones (**5a-d**) could not be obtained directly from Schiff's bases with aqueous H_2O_2, they decomposed back to amine and aldehyde. Therefore, the Schiff's bases (**3a-d**) were reduced in EtOH with $NaBH_4$ to secondary amines (**4a-d**). Nitrones **5a-d** were readily obtained from the oxidation of secondary amines in a Na_2WO_4 catalysed H_2O_2 oxidation in MeOH. In case of compound **4d** the oxidation did not taken place on pyridine nitrogen. However, compound **5d** could be oxidized to compound **7** with *m*-chloroperbenzoic acid (MCPBA) at room temperature (r.t.).



1 2a-d 3a-d 4a-d



5a-d

No	Ar
2a-5a	
2b-5b	NO_2
6	NH_2 (iv)
2c-5c	OH
2d-5d	N
7	$N^{\oplus}$–$O^{\ominus}$ (v)
8	$N^{\oplus}$–Me $I^{\ominus}$ (vi)

Scheme 1. Reagents: (*i*) toluene, (cat.) p-toluenesulfonic acid, reflux, 2h (74-85 %); (*ii*) $NaBH_4$ (2 equiv.), EtOH, -78 °C, 10 min then r.t., 30 min (80-87 %); (*iii*) H_2O_2 (30 % sol.), MeOH, r.t., 2 days (71-83 %); (*iv*) Fe powder (5 equiv.), AcOH, 50 °C for 10 min then r.t., 3 h (68 %); (*v*) MCPBA (2 equiv.), CH_2Cl_2, r.t., 2 h (72 %); (*vi*) MeI (2 equiv.), dry acetone, reflux, 1 h (64 %).

The increased bulkyness of carbones in the nitrone has made introduction of polar aryl groups more important because the **5a** compound is rather insoluble in aqueous media. The 4-nitrophenyl nitrone (**5b**) could be selectively reduced (**6**) when treated in acetic acid with iron powder without involvement of *N*-oxide function as it had been observed previously.[19] The more water soluble *N*-methyl-pyridinium compound (**8**) was obtained when **5d** was refluxed with in excess of methyl iodide in dry acetone.

Table 1: Physical and Spectroscopic Data of New Nitrones **5a-d, 6-8**

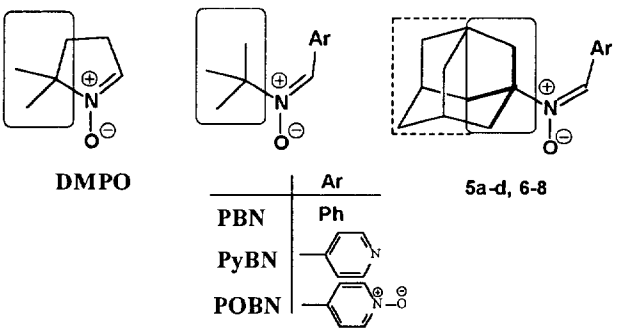
Compd.	Formula[a]	Mp (° C)	IR (nujol)	MS (m/z % rel. int.)
5a	$C_{17}H_{21}NO$ (255.36)	114-117	1570 (arom.), 1550 (C=N)	255 (M^+, 6), 239 (9), 182 (4), 135 (100)
5b	$C_{17}H_{20}N_2O_3$ (300.36)	210-211	1590 (arom.), 1550 (C=N), 1510 (NO_2)	300 (M^+, 2), 284 (4), 229 (4), 135 (100)
5c	$C_{17}H_{21}NO_2$ (271.36)	245-247	3200-2400 (OH), 1600 (arom.), 1570 (C=N)	271 (M^+, 3), 257 (18), 135 (32), 94 (100)
5d	$C_{16}H_{20}N_2O$ (256.35)	144-146	1590 (arom.), 1560 (C=N)	256 (M^+, 14), 240 (4), 213 (4), 135 (100)
6	$C_{17}H_{22}N_2O$ (270.37)	176-178	3450-3100 (NH_2), 1636 (NH_2 deform.), 1600 (arom.), 1570 (C=N)	270 (M^+, 13), 254 (22), 197 (14), 135 (100)
7	$C_{16}H_{20}N_2O_2$ (272.35)	222-225	1600 (arom.), 1550 (C=N)	272 (M^+, 1), 256 (9), 240 (2), 135 (100)
8	$C_{17}H_{23}N_2OI$ (398.29)	204-206	1640 (arom.), 1565 (C=N)	271 ($[M-I]^+$, 100)[b]

[a] Elemental analyses (C, H, N) were performed on a Carlo-Erba EA 1110 elemental analyser and the values were within 0.2 % of calculated theoretical values. The IR (Specord 85) spectra were in each case consistent with the assigned structure. Mass spectra were recorded on a VG TRIO-2 instrument in the EI mode (70 eV, direct inlet).

[b] Thermospray technique

Results and discussion

First representatives of new nitrone family demonstrated that the stability of the hydroxyl adducts of non-cyclic nitrones can be improved when the bulkyness is increased at the nitrogen of *N*-oxide (Scheme 2.). All nitrones investigated were readily soluble in EtOH. However, only the 4-nitrophenyl and pyridyl compounds proved soluble enough for spin trapping in aqueous media, a requirement for further biological studies. EPR spectra of the corresponding hydroxyl radical adducts are illustrated in Fig. 1. Most spectra resembled the 6-line EPR signal of PBN hydroxyl radical adduct with two hyperfine splittings due to nitrogen and hydrogen (Table 2).



Scheme 2.

Decay of hydroxyl radical adduct was followed as decrease of the height of the low field peak of the corresponding first derivative EPR. As it is shown in Fig. 2, this decay met well a monoexponential function for all traps. Hydroxyl adducts of the new traps exhibited significantly higher stability than that of PBN and some approached that of DMPO (Fig. 2, Table 2). due to the bulky lipophilic adamantane ring. The 4-pyridyl compounds, both **5d** and PyBN showed higher stability then their corresponding pyridin-*N*-oxides, **7** and POBN.

In conclusion, among the newly synthesized spin traps, the **5b**, **5d** and **7** nitrones are the most promising new lipophilic reagents for further spin trapping studies.

Table 2. Parameters of simulated spectra and half decay times of nitroxides from nitrones with HO˙ radical

Compound	a_N (mT)	a^{β}_H(mT)	half decay time (min)
5a	1.57	0.35	n.d.[a]
5b	1.60	-	4.4
5c	n.d.	-	n.d.
5d	1.52	0.24	9.8
6	1.61	-	n.d.[a]
7	1.53	0.24	5.1
8	1.54	-	3.0
PBN	1.58	0.35	1.5
POBN	1.56	0.29	5.9
PyBN	1.57	0.29	11.7
DMPO	1.47	1.47	10.9

[a] Half decay times were not detected because of the insufficient solubility of compounds in water.

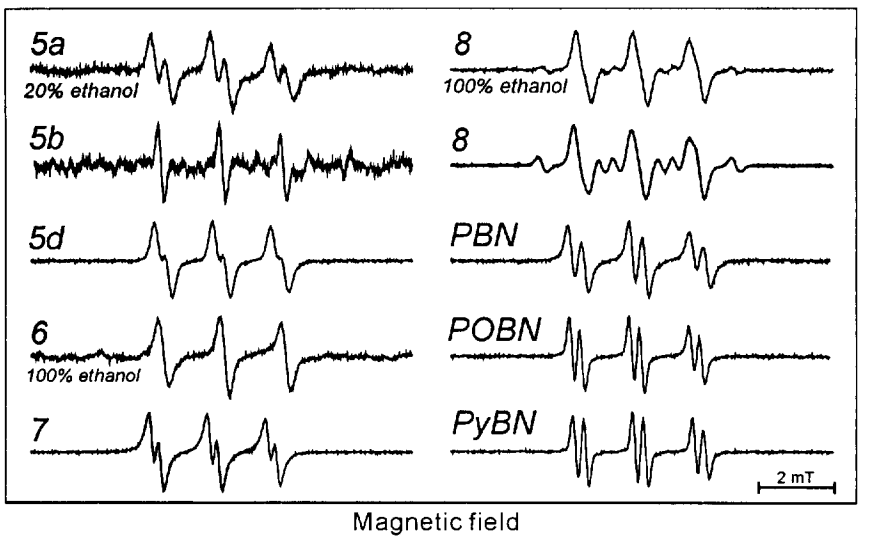


Figure 1. Reaction mixtures contained 0.5 mM H_2O_2 0.2 mM Fe(II) as ammonium ferrous sulfate and 10 mM spin trap and 5% ethanol unless indicated otherwise. Fenton's reaction was initiated by the addition of H_2O_2. After 10 s, 20μl was drained for EPR spectroscopy. EPR spectra were measured using Bruker ECS-106 X-band spectrometer. EPR parameters: 9.46 GHz microwave frequency, 16 mW microwave power, 100 kHz modulation frequency, 0.2 mT modulation amplitude, 8 x 10^3 receiver gain [except for **5a** and **6** (1.2 x 10^4) and for **5b** (2.5 x 10^4)] and 20 ms time constant. All spectra consist of 16 integrated traces acquired at room temperature.

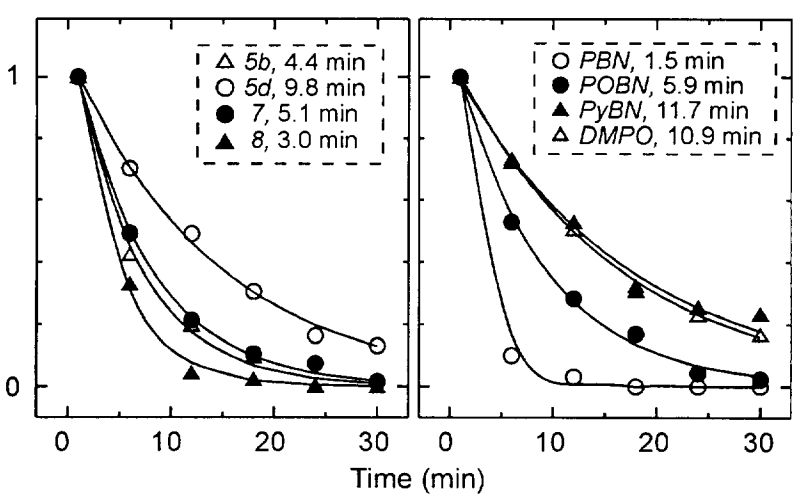


Figure 2. The amount of hydroxyl radical adduct from various spin traps. Decay was followed by monitoring the height of low field peak of the corresponding first derivative EPR spectrum.

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