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2-Substituted-3H-indol-3-one-1-oxides: Preparation and Radical Trapping Properties

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A series of 2-alkyl and 2-aryl substituted-3H-indol-3-one-1-oxides was prepared and evaluated for its radical trapping properties. Spin trapping and electron paramagnetic resonance experiments demonstrate the ability of these indolone-1-oxides to trap hetero- and carbon-centered radicals. The most stable spin adducts (lifetime of several hours) are obtained with 2-alkyl substituted nitrones, the 2-ethyl-5,6-dioxolo-3H-indolone-1-oxide, **5e** and the 2-secbutyl-3H-indolone-1-oxide, **5f**. These two nitrones are also sensitive to redox reactions in solution. Therefore this indolone-1-oxide series lacking a β-hydrogen atom gives rise to highly stable adducts with free radicals.

Keywords: Indolone-1-oxide; Nitrones; Free radicals; Spin trapping; EPR

Abbreviations: EPR, electron paramagnetic resonance; *tert*-BPN, *N*-*tert*-butylphenylnitrone; DMPO, 5,5-dimethyl-1pyrroline-*N*-oxide; HFS, hyperfine splitting

INTRODUCTION

2-Substituted-3H-indol-3-one-1-oxides, which are known as isatogens, were described more than 100 year ago.[1,2] Many studies were reported on this family of compounds until late in the seventies. New interest has been given to these products due to their biological activities against a range of bacteria,[3] mycobacteria[4] and fungi[5] but also because some of them are able to antagonize the relaxant response to adenosine-5-triphosphate in mammals.[6–8]

2,2′-pyridylisatogen tosylate (PIT) has been reported to be a selective antagonist of P2Y response[6,9] and has been shown to form highly stable adducts with free radicals.[10] The role of spin trapping and P2Y receptor antagonism in the neuroprotective effect of PIT and related compounds was recently discussed.[11] Other isatogens have been reported such as phenylisatogen.[12,13] We recently reported a convenient route for the synthesis of the 2-substituted-3H-indol-3-one-1-oxides.[14,15] Therefore, the present study was undertaken to compare the spin trapping properties of a series of indolone-1-oxides.

For this purpose we prepared indolone-1-oxides using procedures previously described[14–17] and evaluated their spin trapping properties by EPR.

MATERIALS AND METHODS

$Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$, Na_2SO_3, $KHPO_4$, $NaHPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}H_2O$, silica, silica gel 60 F-254 plates, DMF, Eppendorfs, borosilicate glass micropipettes for EPR measurements were purchased from VWR International (Strasbourg, France). Other chemicals were purchased from Sigma-Aldrich-Fluka Co (Saint Quentin Fallavier, France). Column chromatography was carried out using 200–400 mesh chromagel. Melting points were determined on an Electrothermal 9300 capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were

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recorded on an AC Bruker spectrometer at 200 and 400 MHz using $CDCl_3$ or DMSO-d_6 as solvent, chemical shifts (δ) are reported in ppm relative to tetramethylsilane (0 ppm) and the following multiplicity abbreviations were used: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; dd, double doublet; dt, double triplet. ^{13}C NMR spectra were recorded on a Bruker spectrometer at 75 and 100 MHz (Bruker, Wissembourg). Mass spectra were obtained on a MS-Nermag R10-10 spectrometer. IR spectra were recorded on a Perkin–Elmer PARAGON 1000 FT-IR spectrometer. UV-visible spectra were recorded on an Uvikon 931 Kontron spectrophotometer. EPR spectra were obtained at X-band at room temperature on a Bruker EMX-8/2.7 (9.86 GHz) equipped with a high-sensitivity cavity (4119/HS 0205) (Bruker, Wissembourg, France) for most of the experiments and on a Magnettech Miniscope MS100 (9.34 GHz) (Magnettech, Berlin, Germany) to compare the EPR signal intensities of the spin adducts.

Synthesis (Fig. 1)

General Synthetic Procedure for Alkene Compounds 3

To a solution of *ortho*-nitro benzaldehyde (10.7 mmol) in dichloromethane (140 ml), was added the appropriate phosphonium salt[18,19] (14.6 mmol), an aqueous NaOH (50%) solution (12.8 mmol) and tetrabutylammonium chloride (0.54 mol). The mixture was stirred at r.t. until complete disappearance of the starting product. It was then extracted by dichloromethane; the organic phase was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by column chromatography (SiO_2) to give a mixture of *E*/*Z*-diastereoisomers.

5-[(E/Z)-2-(4-ETHOXYPHENYL)ETHENYL]-6-NITRO-1,3-BENZODIOXOL, **3a**

Yield 82%. Rf 0.59 and 0.52 (cyclohexane/ethyl acetate, 70:30). IR (KBr) cm^{-1}: 2971, 1642, 1605, 1511, 1482, 1325, 1259, 1175, 1120, 1033, 928, 873, 844, 815. ^1H NMR ($CDCl_3$, 200 MHz): (*cis*) δ 1.36 (t, $J = 7.0$ Hz, 3H), 3.96 (q, $J = 7.0$ Hz, 2H), 6.03 (s, 2H), 6.59 (d, $J = 11.8$ Hz, 1H), 6.61 (s, 1H), 6.69 (d, $J = 8.6$ Hz, 2H), 6.70 (d, $J = 11.8$ Hz, 1H), 6.98 (d, $J = 8.6$ Hz, 2H), 7.60 (s, 1H). (*trans*) δ 1.40 (t, $J = 7.0$ Hz, 3H), 4.03 (q, $J = 7.0$ Hz, 2H), 6.08 (s, 2H), 6.86 (d, $J = 8.6$ Hz, 2H), 6.88 (d, $J = 16.5$ Hz, 1H), 7.08 (s, 1H), 7.42 (d, $J = 8.7$ Hz, 2H), 7.47 (s, 1H), 7.58 (d, $J = 16.5$ Hz, 1H). MS (EI): m/z 313 (M^+), 296 ($M^+ - 17$), 268 ($M^+ - 45$), 163, 152, 135, 121, 107, 77.

Compounds **3b**–**3f** have been fully characterized; these data are available for download from the journal website.

General Synthetic Procedure for Diketone Compound 4

To a solution of alkene **3** (4.8 mmol) in acetic anhydride (32 ml), was added (at 0–5°C with stirring) $KMnO_4$ (19.2 mmol) in five portions over a period of 20 min. After completion of the addition

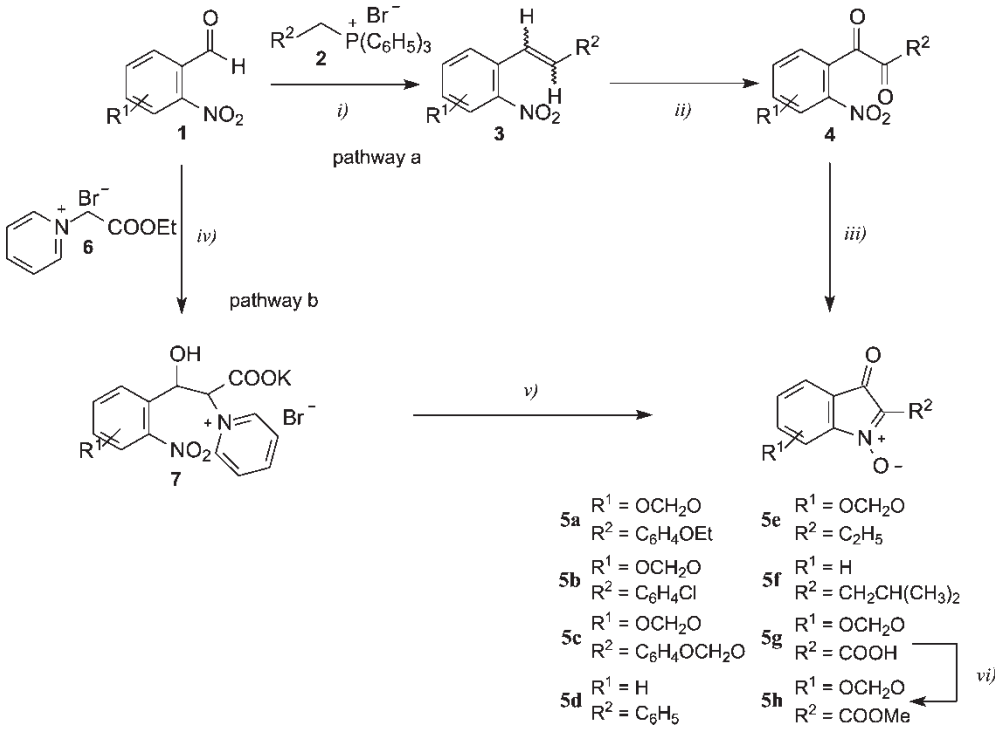


FIGURE 1 Synthesis of **5a**–**5h** nitrones. (i) 2/NaOH aq/CH_2Cl_2/$Bu_4N^+Cl^-$; (ii) $KMnO_4$, acetic anhydride; (iii) Zn/NH_4Cl aq/THF; (iv) 6/EtOH/KOH; (v) hν/acetic acid; (vi) MeI/DMF/K_2CO_3.

the mixture was stirred in a cooling bath for 2 h. Then the reaction was stopped by addition of ethyl acetate/cyclohexane (1:1) (32 ml) and an ice cold solution of sodium dithionite 10%. After stirring in the cooling bath for several minutes the mixture was extracted by dichloromethane, and the organic phase was washed by an aqueous NaOH solution, water and dried over anhydrous $MgSO_4$. It was then concentrated in an evaporator. The crude material obtained was purified by column chromatography (SiO_2).

1-(4-Ethoxyphenyl)-2-(6-nitro-1,3-benzodioxol-5-yl)-1,2 Ethanedione, **4a**

Yield 25%. Rf 0.33 (cyclohexane/ethyl acetate, 70:30). IR (KBr) cm^{-1}: 1690, 1649, 1598, 1565, 1514, 1483, 1426, 1327, 1265, 1171, 1140, 1073, 1031, 922, 865, 784, 756. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.46 (t, $J = 7.0$ Hz, 3H), 4.14 (q, $J = 7.0$ Hz, 2H), 6.23 (s, 2H), 6.98 (d, $J = 9.0$ Hz, 2H), 7.08 (s, 1H), 7.58 (s, 1H), 8.16 (d, $J = 9.0$ Hz, 2H). MS (EI): m/z 313 ($M^+ - 30$), 281, 194, 178, 166, 149, 121, 93, 65.

Compounds **4b–4f** have been fully characterized; these data are available for download from the journal website.

General Synthetic Procedure for Indolone-1-oxide Analogues 5

To a solution of **4** (0.59 mmol) in THF (10 ml) was added a 10% aqueous solution of NH_4Cl (11 ml) and Zn (2.50 mmol). After 20 min of stirring at r.t., the mixture was filtered and the two liquid phases separated. The organic phase was dried over Na_2SO_4 and concentrated under vacuum. The residue was dissolved in THF or dichloromethane (10 ml) and heated under reflux until complete disappearance of the hydroxylamine intermediate. After evaporation of the solvent the crude product was purified by column chromatography (SiO_2).

6-(4-Ethoxyphenyl)-7H-[1,3]dioxolo[4,5-f]indol-7-one 5 Oxide, **5a**

Yield 25%. Mp 169–170°C. Rf 0.58 (cyclohexane/ethyl acetate, 70:30). UV (EtOH) λ_{max} nm (ε): 298 (38 790). IR (KBr) cm^{-1}: 1708, 1684, 1593, 1529, 1499, 1475, 1387, 1366, 1311, 1291, 1266, 1182, 1072, 1030, 927, 819. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.42 (t, $J = 7.0$ Hz, 3H), 4.09 (q, $J = 7.0$ Hz, 2H), 6.12 (s, 2H), 6.96 (d, $J = 9.2$ Hz, 2H), 6.98 (s, 1H), 7.13 (s, 1H), 8.63 (d, $J = 9.2$ Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.8 (CH_3), 63.6 (CH_2), 97.3 (CH), 102.3 (CH), 103.1 (CH_2), 114.6 (CH × 2), 116.6 (C), 118.9 (C), 129.5 (CH × 2), 131.2 (C), 145.1 (C), 149.4 (C), 152.9 (C), 160.6 (C), 184.1 (C). MS (EI): m/z 311 (M^+), 283, 265, 190, 163, 149, 121, 119, 93, 77, 62. HRMS calculated for $C_{17}H_{13}NO_5$ 311.07937, found 311.07928.

6-(4-chlorophenyl)-7H-[1,3]dioxolo [4,5-f]indol-7-one 5 Oxide, **5b**

Yield 40%. Mp 210–211°C. Rf 0.61 (cyclohexane/ethyl acetate, 70:30). UV (EtOH) λ_{max} nm (ε): 298 (29 444). IR (KBr) cm^{-1}: 1707, 1584, 1521, 1465, 1370, 1301, 1071, 1035, 938, 831, 818. ^1H NMR ($CDCl_3$, 200 MHz): δ 6.11 (s, 2H), 6.98 (s, 1H), 7.11 (1s, 1H), 7.39 (d, $J = 8.9$ Hz, 2H), 8.55 (d, $J = 8.9$ Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 97.7 (CH), 102.5 (CH), 103.5 (CH_2), 116.8 (C), 124.8 (C), 128.8 (CH × 2), 129.1 (CH × 2), 131.7 (C), 136.5 (C), 146.0 (C), 150.1 (C), 153.2 (C), 186.0 (C). MS (EI) m/z 301 (M^+), 284, 139, 120, 62. HRMS calculated for $C_{15}H_8ClNO_4$ 301.01419, found 301.01409.

6-(1,3-Benzodioxol-5-yl)-7H-[1,3]dioxolo[4,5-f] indol-7-one 5 Oxide, **5c**

Yield 64%. Mp 202–204°C (dec.). Rf 0.55 (cyclohexane/ethyl acetate, 70:30). UV (EtOH) λ_{max} nm (ε): 301 (26 212). IR (KBr) cm^{-1}: 2915, 1703, 1472, 1302, 1259, 1189, 1104, 1033, 807. ^1H NMR ($CDCl_3$, 200 MHz): δ 5.99 (s, 2H), 6.10 (s, 2H), 6.88 (d, $J = 8.5$ Hz, 1H), 6.96 (s, 1H), 7.10 (s, 1H), 8.20 (d, $J = 1.4$ Hz, 1H), 8.28 (dd, $J = 8.5$ and 1.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 97.5 (CH), 101.7 (CH_2), 102.5 (CH), 103.3 (CH_2), 107.5 (CH), 108.8 (CH), 116.6 (C), 120.4 (C), 123.5 (CH), 131.3 (C), 145.0 (C), 147.8 (C), 149.5 (C), 149.6 (C), 153.1 (C), 186.2 (C). MS (FAB): m/z 312 ($M^+ + 1$), 295 ($M^+ - 16$), 149. HRMS (EI) calculated for $C_{16}H_9NO_6$ 311.04299, found 311.04321.

2-Phenyl-3H-indol-3-one 1-oxide, **5d**[20,21]

Yield 91%. Mp. 180°C, mp. lit. 186°C. Rf 0.75 (cyclohexane/ethyl acetate, 70:30). UV (EtOH) λ_{max} nm (ε): 285 (47 458). IR (KBr) cm^{-1}: 3064, 1719, 1700, 1596, 1521, 1470, 1382, 1311, 1179, 1071, 873, 755. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.74–7.45 (m, 7H), 8.67–8.62 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 114.8 (CH), 121.9 (CH), 122.8 (C), 125.9 (C), 127.8 (2 × CH), 128.5 (2 × CH), 130.6 (CH), 131.1 (CH), 133.0 (C), 134.7 (CH), 147.8 (C), 186.7 (C). MS (EI): m/z 223 (M^+), 206 ($M^+ - 17$), 179, 167, 105, 77, 76. HRMS calculated for $C_{14}H_9NO_2$ 223.06333, found 223.06350.

6-Ethyl-7H-[1,3]-dioxolo[4,5-f]indol-7-one 5 Oxide, **5e**

Yield 35%. Mp 136°C. Rf 0.60 (cyclohexane/ethyl acetate, 70:30). UV (EtOH) λ_{max} nm (ε): 269 (30 035). IR (KBr) cm^{-1}: 3083, 3020, 2980, 2906, 1696, 1545, 1467, 1389, 1359, 1311, 1285, 1228, 1135, 1033, 929, 903, 825. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.18 (t, $J = 7.6$ Hz, 3H), 2.61 (q, $J = 7.6$ Hz, 2H), 6.11 (s, 2H), 6.95 (s, 1H), 7.08 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.9 (CH_3), 15.2 (CH_2), 97.5 (CH), 102.5 (CH), 103.2 (CH_2), 117.0 (C), 139.1 (C), 144.4 (C), 149.6 (C), 152.5 (C), 185.9 (C). MS (EI): m/z 219 (M^+), 202 ($M^+ - 17$), 174, 120, 62. HRMS calculated for $C_{11}H_9NO_4$ 219.05316, found 219.05324.

2-Isobutyl 3H-indol-3-one 1-oxide, **5f**

Yield 86%. Mp 101–102°C. Rf 0.68 (cyclohexane/ethyl acetate, 70:30). UV (EtOH) λ_{max} nm (ε): 246 (26 080). IR (KBr) cm^{-1}: 2960, 2913, 2875, 1700, 1601, 1530, 1459, 1431, 1374, 1162, 1077, 906, 765, 533. ^1H NMR ($CDCl_3$, 200 MHz): δ 0.95 (d, $J = 6.6$ Hz, 6H), 2.17 (m, 1H), 2.55 (d, $J = 7.3$ Hz, 2H), 7.54 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.8 ($CH_3 \times 2$), 26.8 (CH), 30.2 (CH_2), 113.9 (CH), 121.4 (CH), 123.1(C), 131.0 (CH), 134.4 (CH), 139.0 (C), 147.4 (C), 187.1 (C). MS (EI): m/z 203 (M^+), 186 ($M^+ - 17$), 161, 144, 116, 89, 76, 50, 43. HRMS calculated for $C_{12}H_{13}NO_2$ 203.09463, found 203.09455.

Synthesis of 7-oxo-7H-[1,3]-dioxo[4,5-f]indole-6-carboxylic Acid 5-oxide, 5g

To a solution of **6**[22] (3.0 g, 12.1 mmol) in ethanol was added **1** (2.6 g, 13.3 mmol) and an aqueous solution of KOH (50%) (2.6 ml). The mixture was then stirred for 4 h at r.t. The solid was filtered, washed with dichloromethane and dried at r.t. to give **7** (fully characterized: data available for download from the journal website), which was used in the next step without additional purification. The solid was then dissolved in a mixture of acetic acid/water (1:1) (120 ml) and exposed to sunlight for 7 days (6–8 h/day). The solvent was evaporated under reduced pressure and the crude product purified by column chromatography (SiO_2) (cyclohexane/ethyl acetate, 70:30).

Yield 25% from **6**. Mp 236–238°C. Rf 0.09 (cyclohexane/ethyl acetate, 70:30). UV (EtOH) λ_{max} nm (ε): 274 (31 138). IR (KBr) cm^{-1}: 3404, 2926, 1752, 1716, 1706, 1618, 1503, 1472, 1254, 1031. ^1H NMR ($CDCl_3$, 400 MHz): δ 6.19 (s, 2H), 7.31 (s, 1H), 7.50 (s, 1H), 10.27 (s, 1H). MS (EI) m/z 191 ($M^+ - CO_2$), 161, 135, 83, 53, 43. HRMS calculated for $C_9H_5NO_4$ ($M^+ - 44$) 191.02186, found 191.02179.

Synthesis of Methyl 7-oxo-7H-[1,3]-dioxolo [4,5-f]indole-6-carboxylate 5-oxide, 5h

To a solution of **5g** (0.330 g, 1.4 mmol) in DMF (2.0 ml), was added K_2CO_3 (0.240 g) and methyl iodide (0.54 ml, 8.7 mmol). The mixture was stirred at r.t. for 72 h, extracted with dichloromethane. The organic phase was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduce pressure. The crude product was purified by column chromatography (SiO_2) (cyclohexane/ethyl acetate, 70:30).

Yield 66%. Mp 214–215°C. Rf 0.13 (cyclohexane/ethyl acetate, 70:30). UV (EtOH) λ_{max} nm (ε): 277 (25 509). IR (KBr) cm^{-1}: 2920, 1739, 1711, 1612, 1498, 1474, 1379, 1337, 1239, 1088, 1026, 922, 803. ^1H NMR ($CDCl_3$, 200 MHz): δ 3.14 (s, 3H), 6.03 (s, 2H), 6.39 (s, 1H), 6.98 (s, 1H). MS (EI): m/z 205 ($M^+ - CO_2$), 177 (205 − CO), 163, 148, 49. HRMS calculated for $C_{10}H_7NO_4$ ($M^+ - 44$) 205.03751, found 205.03742.

The *n*-octanol/water partition coefficients were calculated with the program from Advanced Chemical Development Inc. (ACD/I-lab) which gives an estimation of log P based on structural considerations via a fragment-based approach (represented as clog P) (Table I).

Spin Trapping Experiments

Spin Trapping Experiments at 1–5 mM for Nitrones 5c–5f and 5h

$HO^{\cdot}$ was generated by Fenton's reagents (Fe^{2+}/H_2O_2: 100 μM/100 μM) in H_2O/DMF: 60/40 (v/v), 1 mM spin trap. $O_2^{\cdot -}$ was generated by a mixture of KO_2 /1,4,7,10,13,16-hexaoxacyclopentadecane (5×10^{-4} M) in DMSO in the presence of 5% water (v/v), 2 mM spin trap. $H_3C^{\cdot}$ was generated by Fenton's reagents (Fe^{2+}/H_2O_2: 100 μM/100 μM) in H_2O/DMSO: 40/60 (v/v), 1 mM spin trap. $CH_3{}^{\cdot}CHOH$ was generated by Fenton's reagents (Fe^{2+}/H_2O_2: 100 μM/100 μM) in H_2O/EtOH: 90/10 (v/v), 5 mM spin trap. $O_3S^{\cdot -}$ was generated by Fenton's reagents (Fe^{2+}/H_2O_2: 100 μM/100 μM) in a mixture of 60% of phosphate buffer (25 mM, pH = 7) (v/v), 20% DMF (v/v), 20% of an aqueous solution of Na_2SO_3 (270 mM) (v/v), 5 mM spin trap.

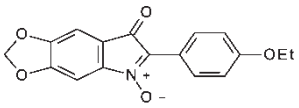
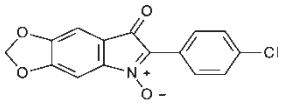
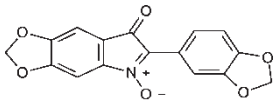
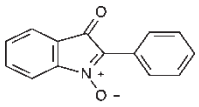
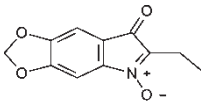
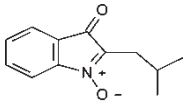
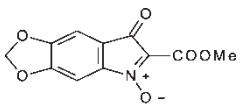
Spin Trapping Experiments at 0.2 mM for Nitrones 5e, 5a and 5b

$HO^{\cdot}$ was generated by Fenton's reagents (Fe^{2+}/H_2O_2: 100 μM/100 μM) in H_2O/DMF: 40/60 (v/v), 0.2 mM spin trap. $O_2^{\cdot -}$ was generated by a mixture of KO_2/1,4,7,10,13,16-hexaoxacyclopentadecane (5×10^{-4} M) in DMSO in the presence of 5% water (v/v), 1.5 mM spin trap. $H_3C^{\cdot}$ was generated by Fenton's reagents (Fe^{2+}/H_2O_2: 100 μM/100 μM) in H_2O/DMSO: 30/70 (v/v), 0.2 mM spin trap. $CH_3{}^{\cdot}CHOH$ was generated by Fenton's reagents (Fe^{2+}/H_2O_2: 100 μM/100 μM) in H_2O/DMF/EtOH: 10/60/30 (v/v), 0.2 mM spin trap. $O_3S^{\cdot -}$ was generated by Fenton's reagents (Fe^{2+}/H_2O_2: 100 μM/100 μM) in a mixture of 50% of phosphate buffer (25 mM, pH = 7) (v/v), 30% DMF (v/v), 20% of an aqueous solution of Na_2SO_3 (270 mM) (v/v) and 0.2 mM spin trap.

Stability of the Nitrones in Solution

The EPR spectra of nitrones **5a–5f** and **5h** were recorded in partially deaerated or non-deaerated solutions DMF/Water (30/70, v/v), DMSO/Water (95/5, v/v) to evaluate their sensitivity to oxygen.

TABLE I Structures of indolone-1-oxides **5a**–**5f** and **5h**, c log *P* and EPR hyperfine splitting constants of the spin adducts (G) with *g* values = 2.0054 to 2.0056 ± 0.0002 (DPPH as reference)

	c log *P**	$^{\cdot}OH$	$O_2^{\cdot-}$	$SO_3^{\cdot-}$	$^{\cdot}CH_3$	CH_3CHOH
 **5a**	2.96	$A_N = 9.7$ $A_{H7} = 2.5$	$A_N = 9.5$ $A_{H7} = 2.8$	$A_N = 9.6$ $A_{H7} = 2.5$	$A_N = 9.8$ $A_{H7} = 2.7$	$A_N = 9.6$ $A_{H7} = 2.7$
 **5b**	3.02	$A_N = 9.6$ $A_{H7} = 2.5$	$A_N = 9.5$ $A_{H7} = 2.7$	$A_N = 9.6$ $A_{H7} = 2.6$	$A_N = 9.7$ $A_{H7} = 2.6$	$A_N = 9.7$ $A_{H7} = 2.6$
 **5c**	1.67	NS	$A_N = 9.4$ $A_{H7} = 2.7$	$A_N = 9.7$ $A_{H7} = 2.5$	$A_N = 9.7$ $A_{H7} = 2.6$	NS
 **5d**	3.28	$A_N = 9.9$ $A_{H7,5} = 3.3$ $A_{H6,4} = 0.9$	$A_N = 9.4$ $A_{H7,5} = 3.1$ $A_{H6,4} = 1.0$	$A_N = 9.7$ $A_{H7,5} = 2.9$ $A_{H6,4} = 1.0$	$A_N = 9.6$ $A_N = 9.58^{\dagger}$ $A_{H7,5} = 3.2$ $A_{H7,5} = 3.15^{\dagger}$ $A_{H6,4} = 1.0$ $A_{H6,4} = 1.05^{\dagger}$	$A_N = 10.1$ $A_{H7,5} = 3.4$ $A_{H6,4} = 0.9$
 **5e**	1.04	$A_N = 9.9$ $A_{H7} = 2.6$	$A_N = 9.4$ $A_{H7} = 2.6$	$A_N = 9.7$ $A_{H7} = 2.6$	$A_N = 9.8$ $A_{H7} = 2.6$	$A_N = 10.1$ $A_{H7} = 2.6$
 **5f**	2.94	$A_N = 10.0$ $A_{H7,5} = 3.3$ $A_{H6,4} = 0.9$	$A_N = 9.4$ $A_{H7,5} = 3.1$ $A_{H6,4} = 1.0$	$A_N = 9.6$ $A_{H7,5} = 2.9$ $A_{H6,4} = 1.0$	$A_N = 9.6$ $A_{H7,5} = 3.2$ $A_{H6,4} = 0.9$	$A_N = 10.0$ $A_{H7,5} = 3.4$ $A_{H6,4} = 0.5$
 **5h**	0.57	NS	NS	NS	NS	NS

[*] log *P* calculated with the ACD/I-Lab program, clog *P*(*t* − BPN) = 3.09, clog *P*(**5g**) = 0.01. [†] Ref. [13]. NS, no signal. **5g**, 7-oxo-7H-[1,3]-dioxo[4,5-f]indole-6-carboxylic acid 5-oxide does not trap any radical.

The partially deaerated solutions were prepared by bubbling with nitrogen for 5 min.

Stability of the Hydroxyle Radical Spin Adducts

The EPR spectra intensity of **5e**-OH and **5f**-OH spin adducts were recorded over approximately 240 min. Solutions were prepared as above in "Spin trapping experiment" for **5e** and **5f** (3 mM). Partial deaeration of the solvents was obtained by bubbling N_2 for 5 min.

EPR Scanning Conditions

EPR spectra were obtained at X-band at room temperature on a Bruker EMX-8/2.7 (9.86 GHz) equipped with a high-sensitivity cavity (4119/HS 0205) (Bruker, Wissembourg, France) for most of the experiments and on a Magnettech Miniscope MS100 (9.34 GHz) (Magnettech, Berlin, Germany) to compare the EPR signal intensities of the spin adducts. A flat quartz cell FZK160-5 × 0.3 (Magnettech) or 100 μl borosilicate glass micro-pipettes were used. Processing of EPR data and spectrum computer simulation were performed using WINEPR and SIMFONIA programs (Bruker). Typical Bruker scanning parameters were: scan rate, 0.6 G/s; scan number, 1; modulation amplitude, 1 G; modulation frequency, 100 kHz, microwave power, 20 mW; time constant, 40.96 ms. Typical Magnettech scanning parameters were: scan rate, 2 G/s; scan number, 1; modulation amplitude, 1 G; modulation frequency, 100 kHz, microwave power, 14 mW; time constant, 100 ms.

RESULTS

Synthesis

Two different pathways starting with *ortho*-nitrobenzaldehyde derivatives (**1**) were used for the preparation of the 2-substituted indolone-1-oxide (**5**) (Fig. 1). The indolone-1-oxides **5g** and **5h** were synthesized by a previously reported procedure[16,17] (pathway b), which involved a condensation reaction of *ortho*-nitropiperonal with 1-(ethoxycarbonylmethyl)-pyridinium bromide (**6**) in the presence of KOH. Under these strong basic conditions, the ester function was not stable and was hydrolyzed giving the corresponding potassium carboxylate salt **7**. An aqueous acetic acid solution of this salt was then exposed to light for 7 days to give **5g** with a 25% yield. Esterification of **5g** by the action of MeI led to **5h** in good yields (Fig. 1). In contrast, attempts to synthesize the 2-aryl indolone-1-oxide **5c** using this methodology were not successful. In fact, the yield of the cyclization step was very poor (<10%).

Compounds **5a**–**5f** were therefore synthesized by our new approach[14,15] reported recently (pathway a) For the first time this method allowed the synthesis of 2-alkyl indolone-1-oxide by an intramolecular reductive cyclization strategy. The procedure consists of preparing diketone **4** as a key intermediate via direct oxidation of the corresponding alkene **3**. The latter is easily obtained from an olefination reaction between the corresponding *ortho*-nitro benzaldehyde derivatives (**1**) and the appropriate phosphonium salt **2** in the presence of NaOH as base (Fig. 1). The oxidation of alkene **3** by the action of $KMnO_4$ in acetic anhydride leads to diketone **4.** Finally, cyclization of diketone **4** in the presence of Zn/NH_4Cl afforded indolone-1-oxides **5a**–**5f**.

Stability of Nitrones 5a–5f and 5h in Solution

When dissolved in a pure solvent or a mixture of solvents, nitrones **5e** and **5f** exhibit EPR signals without any radical generated. Nitrone **5e** is the most sensitive, notably in a DMF/H_2O mixture (30/70, v/v) (Fig. 2B), in pure DMF (continuous line) or DMSO (dotted line) (Fig. 2C). When solutions were partially deaerated (continuous line) by bubbling nitrogen through the pure solvents before preparing the final mixture, the intensity of the nitroxyle adduct of nitrone **5e** decreased by 42% (Fig. 2D). Powered samples of nitrones **5a**–**5h** do not present EPR signals. These results underline the sensitivity of nitrones **5e** and **5f** to oxygen and their reactivity in solvents.

Spin Trapping Experiments

Spin Trapping Properties

Spin trapping properties were investigated against oxygen- and carbon-centered radicals. Hyperfine splitting (HFS) constants of indolone-1-oxide nitrones, **5e**, **5f** and **5h**, for the different spin adducts are reported in Table I. Atom numbering is given in Fig. 3. The previously reported[13] spin trapping properties of nitrone **5d** with the methyl radical have been introduced in Table I for comparison. The EPR signal intensities are evaluated by subtracting from each EPR signal intensity in the spin trapping experiment, the intensity of the corresponding self-oxidation spin adduct. The spin adduct signal intensities were the highest with nitrones **5e** and **5f** whatever the radical trapped. EPR spectra of nitrones **5e** and **5f** clearly illustrate the influence of substituent R^1 on the EPR spectral features and comparisons are made in Fig. 4 (oxygen-centered radicals) and Fig. 5 (carbon-centered radicals). Simulated spectra are also given in Figs. 4 and 5. Because of the lower

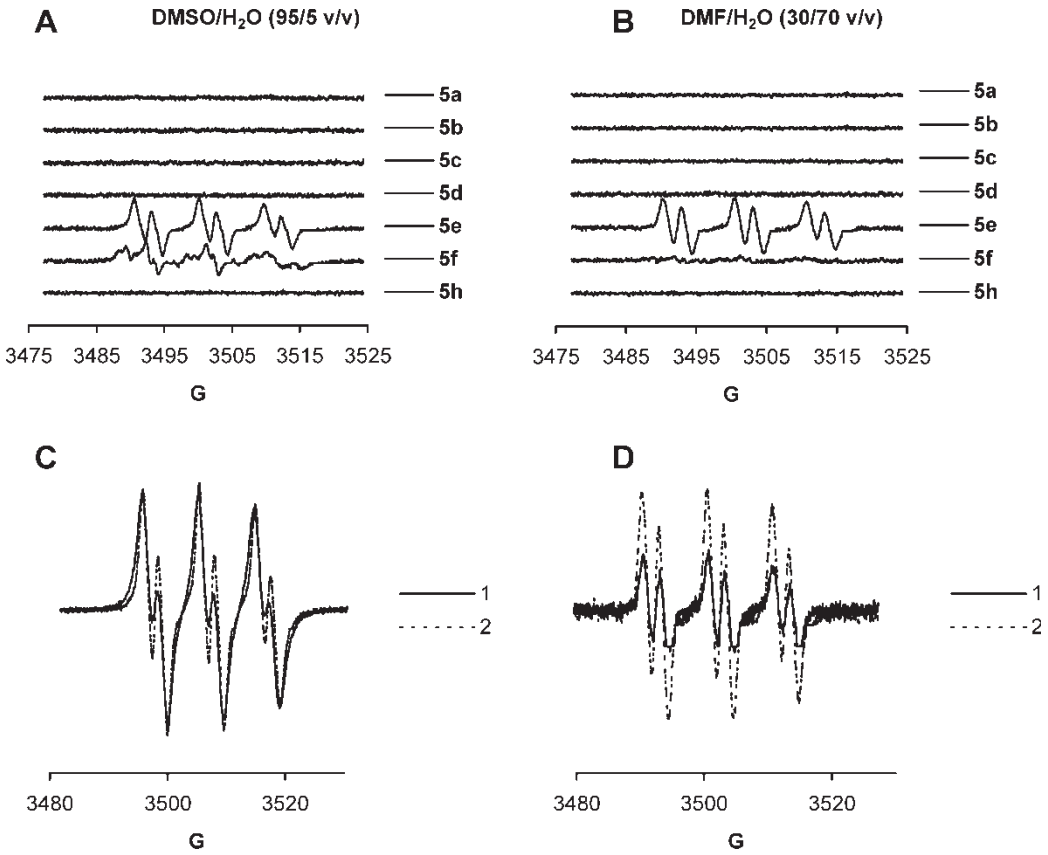


FIGURE 2 EPR signal detection of the adducts of nitrones **5a**–**5f** and **5h** in solution in the absence of any radical generating system. (A) **5a**–**5f** and **5h**, 2 mM, DMSO/H_2O (95/5, v/v); (B) **5a**–**5f** and **5h**, 1 mM, DMF/H_2O (30/70, v/v); (C) **5e**, 2 mM, 1—DMF, 2—DMSO; (D) **5e**, 3 mM, 1—DMF/H_2O (30/70, v/v) deaerated, 2—DMF/H_20 (30/70, v/v) non-deaerated.

solubility of nitrones **5a** and **5b** in aqueous solutions, these two nitrones were tested at 0.2 mM and compared to nitrone **5e** (Fig. 7). Then, Fig. 6 compares the EPR signal intensity of the spin adducts with nitrones **5c**–**5f** (1–5 mM) and Fig. 7 compares the EPR signal intensity of the spin adducts with nitrones **5e**, **5a** and **5b** (0.2 mM). In the same conditions, **5h**, DMPO and *tert*-BPN gave no EPR signal, except *tert*-BPN at 1 mM with the methyl radical.

Stability of the Spin Adducts 5e-OH and 5f-OH

Figure 8 describes the time course of the EPR signal intensity of **5e**-OH and **5f**-OH spin adducts over a 240-min period showing their high stability.

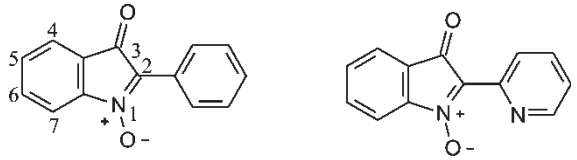


FIGURE 3 Structures of 2-phenyl indolone-1-oxide 5d (left) and 2,2′-pyridyl indolone-1-oxide PiT (right) and atom numbering.

DISCUSSION

Spin Trapping Properties

Stability of Nitrones 5e and 5f in Solution

In the absence of any free radical generated, nitrones **5e** and **5f** give an EPR signal characteristic of an oxygen-centered radical adduct. In the same conditions, no EPR signal is observed for nitrones **5a**–**5d**. It is well known that nitrones are highly reactive compounds which can be reduced or oxidized into a variety of products. Interconversion between nitrones, epoxides, hydroxylamines and nitroxides have been reported.[23–27] Our results indicate that **5e** and **5f** could undergo an epoxidation reaction in aqueous solution when exposed to oxygen and light. The cyclic intermediate could therefore be hydrolyzed to hydroxylamine then oxidized into nitroxide. Another possibility is the obtention of nitroxide compounds by reaction of one molecule of nitrone with one another, followed by air oxidation. The mixed nitrone/nitroxide product obtained can be the cause of the signal obtained.[28–30] When the solutions were partially deaerated, the EPR signal intensity decreased (Fig. 2D). In the following experiments with

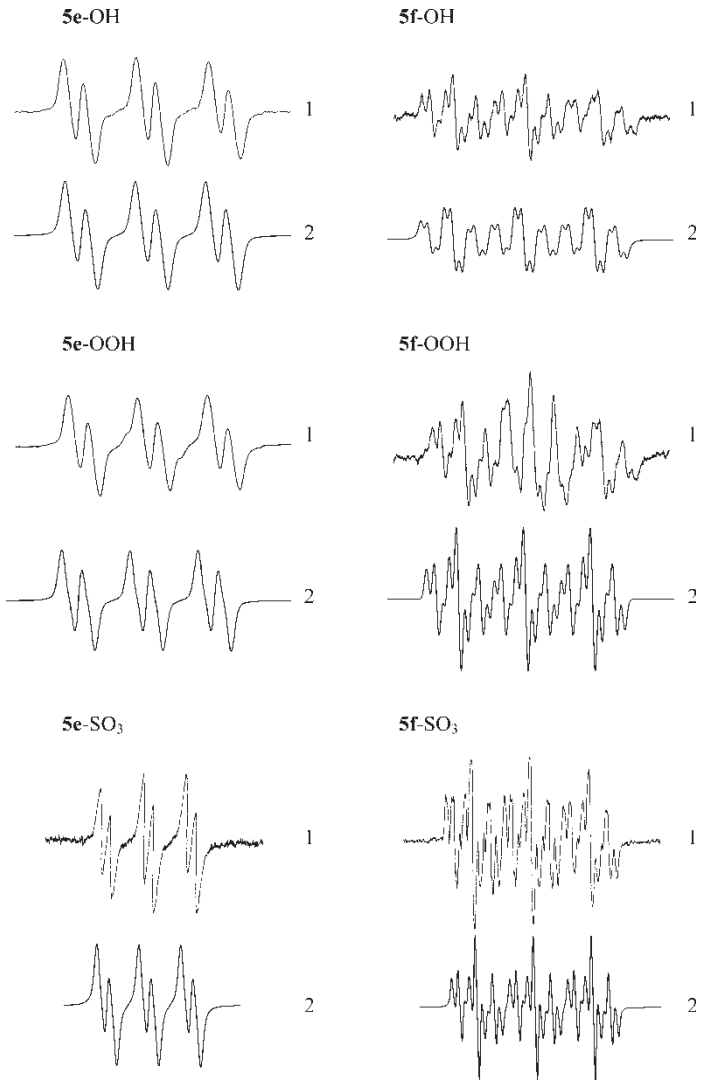


FIGURE 4 EPR spectra of the spin adducts with nitrones **5e** and **5f** (1–5 mM) with oxygen-centered radicals. 1—experimental and 2—simulated spectra.

5e and **5f**, the EPR signal due to reactions in the solution with oxygen without any free radical generated was always recorded and subtracted from the EPR signals of the following experiments where radicals were chemically generated.

Spin Trapping Properties and EPR Spectral Features

The indolone-1-oxide nitrones exhibit EPR-detectable spin adducts, except the 2-carboxymethyl and 2-carboxy substituted ones, **5h** and **5g**. The introduction of the methylenedioxy bridge into the aromatic ring of the indolone-1-oxide moiety (substituent R^1) leads to a simplification of the EPR spectra as can be seen in Figs. 4 and 5 comparing nitrones **5e** and **5f** and to higher intensities of each resulting peak. The lack of hydrogen in 7- and 8-positions suppresses the possibility of two magnetic coupling. However, unlike usual nitrones (i.e. *tert*-BPN, DMPO), the indolone-1-oxide nitrones prepared do not have a hydrogen atom on the 2-carbon of the nitrone moiety. This leads to an extreme simplification of the EPR spectrum from which the nature of the radical trapped cannot be directly identified. As seen in Table I, the HFS constants do not vary very much between nitrones **5a**–**5f** and are comparable to those previously reported by Berti *et al.* for **5d**-CH_3 spin adduct.[13]

EPR Signal Intensities

Taking into account the two concentration ranges tested (1–5 and 0.2 mM) and the different radicals generated, 2-ethyl-indolone-1-oxide nitrone, **5e** appears as the best spin trap in this series followed by the 2-*sec*-butyl substituted one **5f** (Figs. 6 and 7). Then, 2-alkyl indolone-1-oxides appear to be better spin traps than 2-aryl substituted ones. The strong differences observed between **5e** and **5f** may be due to a steric effect of the *sec*-butyl compared to the ethyl

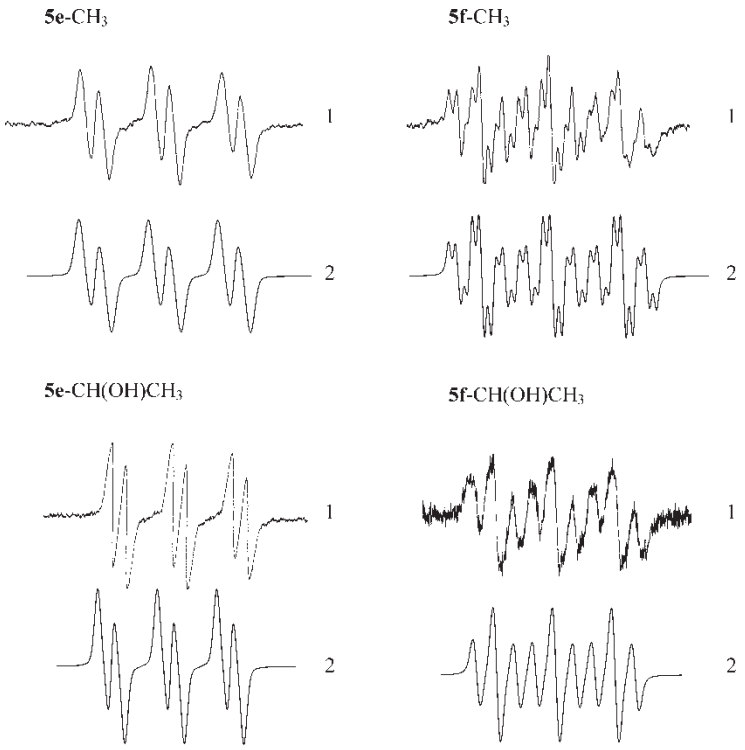


FIGURE 5 EPR spectra of the carbon-centered spin adducts of nitrones **5e** and **5f** (1–5 mM). 1—experimental and 2—simulated spectra.

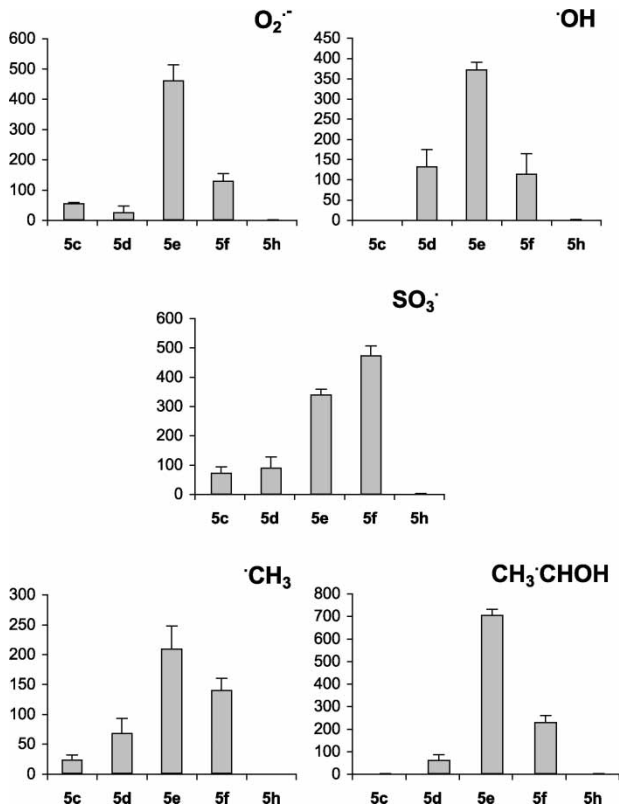


FIGURE 6 Comparison of the EPR signal intensities of the spin adducts with nitrones **5c**–**5f** and **5h** (1–5 mM).

group. In the same way, aromatic groups on the 2-position could decrease the yield of the nucleophilic addition on the N═C double bond or decrease the stability of the final spin adduct. In conclusion, the substituent on this 2-position, representing the salient feature of the chemical structures, has the strongest effect on the spin trapping ability of these nitrones.

Stability of the Spin Adducts

The spin adducts of **5f** and **5e** are stable for several hours. Illustration is given with the **5e**-OH and **5f**-OH adducts for which the intensity reaches a maximum of 20 min after mixing the different reactants (Fig. 8). Even after more than 2 h, over half (60%) of the maximum intensity obtained is still measured. Figure 8 also shows that the signal intensity does not differ very much between deaerated and non-deaerated starting solutions. These results show the great stability of the spin adducts **5e**-OH, **5f**-OH, knowing that after 45 s no signal remains with *tert*-BPN[31] and that the half-life of DMPO-OH spin adduct is less than 2 h.[32] These indolone-1-oxide nitrones are characterized by the lack of a β-hydrogen atom. Most of the nitrone spin traps in current use contain a β-hydrogen and nitroxides formed from these traps are susceptible to hydrogen abstraction or disproportionation.[33–35] In contrast, nitrones lacking a β-hydrogen, as previously shown for the 2,5,5-trimethyl-1pyrroline-*N*-oxide (TMPO), form

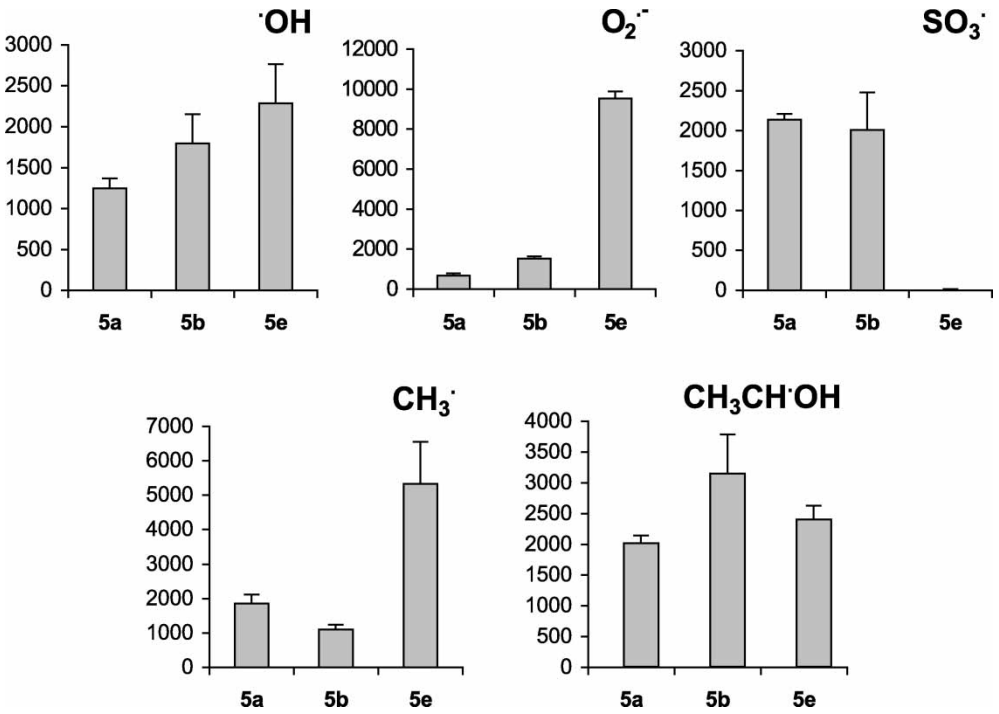


FIGURE 7 Comparison of the EPR signal intensities of the spin adducts with nitrones **5a**, **5b** and **5e** (0.2 mM).

more stable adducts.[36] The lack of a β-hydrogen in our nitrone series explains the very high stability of the nitrone adducts.

Concentration and Spin Trapping

Because of their low solubility in aqueous solutions, these nitrones **5a**–**5f** and **5g** were studied at low concentrations (1–5 and 0.2 mM); their EPR signal intensities (with only one scan) were strong or sufficient for spin trapping applications compared to *tert*-BPN and DMPO for which no EPR signals were detected in this concentration range except for the *tert*-BPN-CH_3 adduct. The low concentrations used decrease the probability of disproportionation reactions between spin adducts and may partly explain the longer half-lives of the spin adducts.

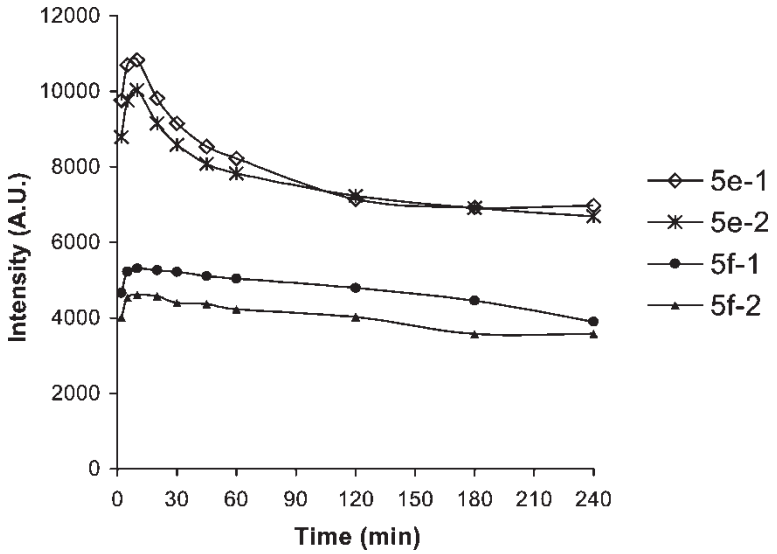


FIGURE 8 Time course of the EPR signal intensity: **5e**-OH, 1—non-deaerated solution and 2—deaerated solution; **5f**-OH spin adducts, 1—non-deaerated solution and 2—deaerated solution.

CONCLUSION

2-Substituted-3H-indol-3-one-1-oxides have been prepared and compared for their spin trapping properties. Spin trapping experiments demonstrate the ability of these nitrones to trap oxygen- and carbon-centered radicals. These compounds lacking a β-hydrogen present very good spin trapping properties except the ones with a carboxy- or carboxymethyl 2-substituent. The lack of a β-hydrogen leading to only A_N values which do not vary greatly between different trapped radicals limits the usefulness of these nitrones as spin traps for analytical purposes. However, there exists a trade-off between the ease of identification of the trapped radical species (using DMPO for instance) and spin adduct stability. The high stability of spin adducts of this indolone-1-oxide series associated with their strong lipophilicity and low working concentration make them possible candidates to trap free radicals in biological systems where hydrophilic spin traps such as DMPO have failed. Chemical and enzymatic control reactions have to be carried out to complete the identification of the trapped radical.

Acknowledgements

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SUPPLEMENTARY DATA

5-[(*E*/*Z*)-2-(4-chlorophenyl)ethenyl]-6-nitro-1,3-benzodioxol, **3b**

Yield 87%. Rf 0.64 and 0.54 (cyclohexane/ethyl acetate, 70:30). IR (KBr) cm^{-1}: 1613, 1519, 1499, 1486, 1434, 1323, 1262, 1090, 1028, 930, 894, 815. ^1H NMR ($CDCl_3$, 200 MHz): (*cis*) δ 6.06 (s, 2H), 6.53 (s, 1H), 6.62 (d, $J = 12.0$ Hz, 1H), 6.85 (d, $J = 12.0$ Hz, 1H), 6.99 (d, $J = 8.5$ Hz, 2H), 7.15 (d, $J = 8.5$ Hz, 2H), 7.61 (s, 1H). (*trans*) δ 6.11 (s, 2H), 6.87 (d, $J = 16.0$ Hz, 1H), 7.07 (s, 1H), 7,31 (d, $J = 8.5$ Hz, 2H), 7.42, (d, $J = 8.5$ Hz, 2H), 7.49 (s, 1H), 7.60 (d, $J = 16.0$ Hz, 1H). MS (EI): m/z 303 (M^+), 286 ($M^+ - 17$), 258 ($M^+ - 45$), 163, 139, 135, 125, 111, 86, 75, 63, 49.

5-[(*E*/*Z*)-2-(1,3 Benzodioxol-5-yl)ethenyl]-6-nitro-1,3-benzodioxol, **3c**

Yield 96%. Rf 0.43 (cyclohexane/ethyl acetate, 70:30). IR (KBr) cm^{-1}: 2906, 1609, 1520, 1500, 1485, 1443, 1326, 1256, 1241, 1033, 929, 811. ^1H NMR ($CDCl_3$, 400 MHz): (*cis*) δ 5.91 (s, 2H), 6.08 (s, 2H), 6.53 (d, $J = 1.7$ Hz, 1H), 6.59 (d, $J = 12.5$ Hz, 1H), 6.62 (dd, $J = 8.1$ and 1.6 Hz, 1H), 6.64 (s, 1H), 6.67 (d, $J = 8.0$ Hz, 1H), 6.72 (d, $J = 11.9$ Hz, 1H), 7.59 (s, 1H). (*trans*) δ 5.96 (s, 2H), 6.09 (s, 2H), 6.78 (d, $J = 8.0$ Hz, 1H), 6.85 (d, $J = 16.0$ Hz, 1H), 6.93 (dd, $J = 8.0$ and 1.8 Hz, 1H), 7.03 (d, $J = 1.8$ Hz, 1H), 7.06 (s, 1H), 7.44 (d, $J = 16.0$ Hz, 1H), 7.48 (s, 1H). MS (EI): m/z 313 (M^+), 296 ($M^+ - 17$), 268 ($M^+ - 45$), 163, 152, 149, 135, 121, 107, 77.

5-[(*E*/*Z*)-2-(4-phenyl)ethenyl]-6-nitro-1,3-phenyl, **3d**

Yield 96%. Rf 0.78 and 0.73 (cyclohexane/ethyl acetate, 70:30). IR (KBr) cm^{-1}: 3056, 3028, 1627, 1604, 1571, 1519, 1495, 1443, 1344, 1302, 1142, 1076, 962, 920, 854, 783, 755, 698. ^1H NMR ($CDCl_3$, 200 MHz): δ 6.75 (d, $J = 12.0$ Hz, 1H, *cis*), 6.89 (d, $J = 12.0$ Hz, 1H, *cis*), 7.08–8.13 (m, H arom + H alkene, *trans*). MS (EI): m/z 225 (M^+), 208, 180, 178, 176, 165, 152, 139, 119, 105, 92, 91, 77, 63, 51, 39.

5-[(*E*/*Z*)-2-(4-ethyl)ethenyl]-6-nitro-1,3-benzodioxol, **3e**

Yield 78%. Rf 0.55 (cyclohexane/ethyl acetate, 70:30). IR (KBr) cm^{-1}: 2957, 2885, 1607, 1519, 1503, 1477, 1327, 1254, 1036, 933, 876, 813. ^1H NMR ($CDCl_3$, 200 MHz): δ 0.89 (t, $J = 7.5$ Hz, 3H), 0.99 (t, $J = 7.5$ Hz, 3H), 1.96 (m, 2H), 2.15 (m, 2H), 5.60 (dt, $J = 11.5$ and 7.5 Hz, 1H *cis*), 5.97 (2H, s), 6.00 (s, 2H), 6.02 (dt, $J = 15.6$ and 6.5 Hz, 1H *trans*), 6.49 (d, $J = 11.4$ Hz, 1H *cis*), 6.57 (1H, s), 6.74 (d, $J = 15.2$ Hz, 1H *trans*), 6.79 (s, 1H), 7.26 (s, 1H), 7.39 (1H, s). MS (EI): m/z 221 (M^+), 174, 164, 146, 136, 115, 106, 91, 79, 77, 65, 63, 57.

5-[(*E*/*Z*)-2-(4-*sec*-butyl)ethenyl]-6-nitro-1,3-benzodioxol, **3f**

Yield 96%. Rf 0.76 (cyclohexane/ethyl acetate, 70:30). IR (KBr) cm^{-1}: 2951, 2923, 2866, 1601, 1568, 1521, 1464, 1346, 1294, 1162, 1143, 963, 854, 784, 755, 736, 699. ^1H NMR ($CDCl_3$, 200 MHz): δ 0.80 (d, $J = 6.6$ Hz, 6H), 0.91 (d, $J = 6.6$ Hz, 6H), 1.60 (m, 1H), 1.75 (m, 1H), 1.94 (t, $J = 8.6$ Hz, 2H), 2.13 (t, $J = 8.2$ Hz, 2H), 5.79 (dt, $J = 11.6$ and 7.5 Hz, 1H *cis*), 6.13 (dt, $J = 15.6$ and 7.3 Hz, 1H *trans*), 6.69 (d, $J = 11.6$ Hz, 1H *cis*), 6.77 (d, $J = 15.6$ Hz, 1H *trans*), 7.21–7.55 (m, 2H arom), 7.81 (dd, $J = 8.1$ and 1.4 Hz), 7.93 (dd, $J = 8.1$ and 1.4 Hz). MS (EI): m/z 206 ($M^+ + 1$), 205, 146, 132, 121, 120, 116, 115, 92, 77, 57, 41.

1-(4-Chlorophenyl)-2-(6-nitro-1,3-benzodioxol-5-yl)-1,2 Ethanedione, **4b**

Yield 50%. Rf 0.51 (cyclohexane/ethyl acetate, 70:30). IR (KBr) cm^{-1}: 1691, 1673, 1583, 1506, 1483, 1424, 1325, 1266, 1148, 1094, 1030, 927, 881, 872, 858, 817, 781. ^1H NMR ($CDCl_3$, 200 MHz): δ 6.21 (2H, s), 7.06 (s, 1H), 7.48 (d, $J = 8.4$ Hz, 2H), 7.56 (s, 1H), 8.09 (d, $J = 8.4$ Hz, 2H). MS (EI): m/z 333 (M^+), 303 ($M^+ - 30$), 194, 178, 139, 120, 111, 75.

1-(1,3-Benzodioxol-5-yl)-2-(6-nitro-1,3-benzodioxol-5-yl)-1,2 Ethane Dione, **4c**

Yield 50%. Rf 0.36 (cyclohexane/ethyl acetate, 70:30). IR (KBr) cm^{-1}: 2906, 1690, 1664, 1602, 1519, 1503, 1488, 1441, 1431, 1327, 1265, 1109, 1031, 927. ^1H NMR ($CDCl_3$, 200 MHz): δ 6.13 (s, 2H), 6.28 (s, 2H), 6.98 (d, $J = 8.3$ Hz), 7.13 (s, 1H), 7.63 (s, 1H),

7.64 (d, $J = 2.0$ Hz), 7.93 (dd, $J = 8.3$ and 2.0 Hz). MS (EI): m/z 343 (M^+), 314, 194, 179, 149, 121, 84.

1-(4-Phenyl)-2-(6-nitro-1,3-benzodioxol-5-yl)-1,2 Ethanedione, **4d**

Yield 60%. Rf 0.33 (cyclohexane/ethyl acetate, 70:30). IR (KBr) cm^{-1}: 3064, 1786, 1691, 1672, 1592, 1573, 1521, 1445, 1341, 1256, 1204, 1181, 1043, 1029, 977, 845, 803, 788, 765, 736, 713, 689, 637. 1 H NMR ($CDCl_3$, 200 MHz): δ 7.48–7.90 (m, 6H), 8.25 (m, 3H). MS (EI): m/z 255 (M^+), 105, 77.

1-(4-Ethyl)-2-(6-nitro-1,3-benzodioxol-5-yl)-1,2 Ethanedione, **4e**

Yield 50%. Mp 81–82°C. Rf 0.41 (cyclohexane/ethyl acetate, 70:30). IR (KBr) cm^{-1}: 2978, 2916, 1706, 1607, 1524, 1509, 1483, 1426, 1368, 1332, 1275, 1114, 1036, 927, 886, 873, 811. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.12 (t, $J = 7.2$ Hz, 3H), 2.98 (q, $J = 7.2$ Hz, 2H), 6.20 (s, 2H), 6.89 (s, 1H), 7.56 (s, 1H). MS (CI): m/z 286 ($M^+ + N_2H_7$), 269 ($M^+ + NH_4^+$), 251 ($M^+ + H^+$), 195.

1-(4-*sec*butyl)-2-(6-nitro-1,3-benzodioxol-5-yl)-1,2 Ethanedione, **4f**

Yield 66%. Rf 0.63 (cyclohexane/ethyl acetate, 70:30). IR (KBr) cm^{-1}: 2960, 2932, 2866, 1710, 1573, 1530, 1464, 1393, 1341, 1275, 1237, 1148, 902, 854, 788, 755, 703, 615. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.93 (d, $J = 6.7$ Hz, 6H), 2.17 (m, 1H), 2.83 (d, $J = 6.8$ Hz, 2H), 7.50 (dd, $J = 7.2$ and 1.7 Hz, 1H), 7.70 (m, 2H), 8.11 (dd, $J = 7.9$ and 1.3 Hz, 1H). MS (EI): m/z 219 ($M^+ - 16$); 151, 121, 85, 76, 57, 41.

Potassium Bromide-3-hydroxy-3-(2-nitrophenyl)-2-pyridinium-1-ylpropanoate, **7**

Mp 300°C. Rf 0.40 (CH_2Cl_2/MeOH, 60:40). IR (KBr) cm^{-1}: 3419, 3075, 2928, 1641, 1528, 1483, 1362, 1322, 1258, 1069, 1030, 1035, 915, 890, 815, 728, 708. ^{1}H NMR (DMSO d_6, 200 MHz): δ 4.91 (s, 1H), 5.29 (d, 1H, $J = 2.8$ Hz), 6.12 (s, 2H), 6.34 (s, 1H), 6.36 (s, 1H), 6.38 (d, 1H, $J = 2.8$ Hz), 7.97 (t, 2H, $J = 6.9$ Hz), 8.51 (m, 1H), 8.83 (d, 2H, $J = 5.6$ Hz). MS (FAB): m/z 371 ($M^+ - 80$), 333, 289, 192.