Assured Structural Identification of the Spin Adducts Generated from the Nitrone Spin Traps by ESR, MASS, and HPLC Analyses

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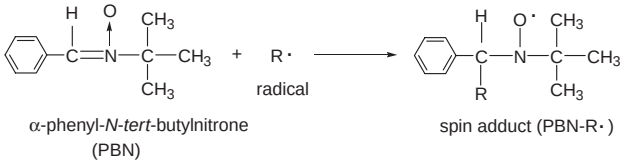
(Received July 22, 2005; CL-050951)

Structural identification of the spin adducts produced by α-phenyl-*N*-*tert*-butylnitrone (PBN) with short-lived carbon centered radicals (phenyl, *n*-butyl, and *tert*-butyl) can be carried out unambiguously by combining the data obtained with ESR, Mass, and HPLC analyses.

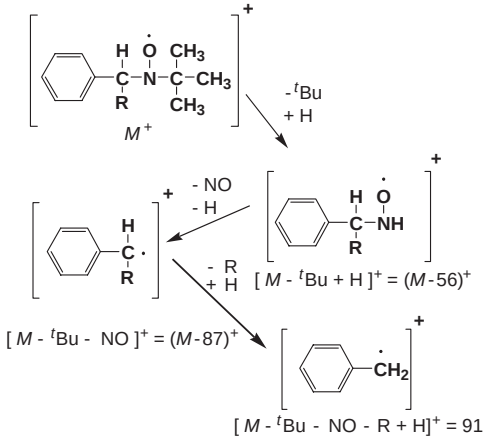
Spin trapping[1] is a useful method to capture short-lived reactive radicals and to convert them to the stable nitroxyl radicals suitable for the ESR analyses (Scheme 1).



Scheme 1. Spin trapping reaction.

However, it is rather difficult to identify the trapped radicals clearly by only measuring ESR spectra because of the scarce diversity of the hyperfine splitting constants (a_N and a_H^β) and *g*-factors of the spin adducts due to the structural change of the trapped radicals.[2] Among the nitrone spin traps, α-phenyl-*N*-*tert*-butylnitrone (PBN) is a very popular spin trap and its spin adducts (nitroxyl radicals) have the advantage of the stability. However, PBN-type nitrones are limited by their ability to discern different radicals.[3] Structural differences among phenyl, *n*-butyl, and *tert*-butyl radicals, for example, did not reflect on a_N and a_H^β values so much as shown in Table 1 from our ESR measurement (JEOL JES-FA200 spectrometer with 100 kHz modulation, power 4 mW, and modulation width 0.03 mT). Though the sterical bulkiness of the trapped radicals (R•) was evaluated with a steric substituent constant Ωs[4] (C_6H_5: 0.256, *n*-C_4H_9: 0.269, *t*-C_4H_9: 0.352, larger number indicates more bulky substituent), any correlation between a_H^β values and the steric bulkiness of the trapped radicals could not be observed. Judging from the a_N values of the three spin adducts, spin density on the nitroxyl nitrogen atom of each spin adduct is almost the same. Another appropriate structural information is necessary to identify the trapped radicals clearly. What kind of structural analyses are suitable to identify the trapped radicals for which the ESR spectra are available? In this work, the PBN spin adducts of phenyl, *n*-butyl, and *tert*-butyl radicals were prepared and purified by a reported synthetic method[5] in order to carry out the several analyses by use of spectroscopic and separation methods. Mass spectrum data and the retention time for the HPLC (high performance liquid chromatography) were shown to be useful to identify the trapped radicals clearly. The results were shown in detail below.

Electron impact (EI) mass spectroscopic analyses were carried out at first with a JEOL JFE-600 mass spectrometer with a direct inlet probe method on these three spin adducts (phenyl, *n*-butyl, and *tert*-butyl) after measuring ESR spectra to make sure that the spin adducts are genuine radical species. As shown in Table 1, the molecular ion peaks (M^+; m/z 234) for the *n*-butyl and *tert*-butyl spin adducts were observed with rather small intensity. The high-resolution mass spectrum measurement of the *tert*-butyl spin adduct has confirmed that the spin adduct has an exact mass number corresponding to the spin adduct $C_{15}H_{24}NO$ (234.18579, theoretical value 234.185789). The fragmentation pathway for the alkyl radical PBN spin adducts can be assumed to proceed as shown in Scheme 2 ($[M]^+ \rightarrow [M - {}^t\mathrm{Bu} + \mathrm{H}]^+ \rightarrow [M - {}^t\mathrm{Bu} - \mathrm{NO}]^+ \rightarrow [M - {}^t\mathrm{Bu} - \mathrm{NO} - \mathrm{R}]^+$). Due to the stability of the benzyl-type cation, $[C_6H_5 - \mathrm{CHR}]^+$ (m/z 147) and $[C_6H_5 - CH_2]^+$ (m/z 91) fragment ions have larger relative intensity (R.I.) values. The base peaks of the *n*-C_4H_9 and C_4H_9 PBN spin adducts are m/z 91 and m/z 147, respectively.



Scheme 2. Fragmentation pathway of the alkyl radical PBN spin adducts (PBN-R•).

To the contrary, the phenyl spin adduct did not show a molecular ion peak. Its base peak (m/z 167) corresponds to $[M - {}^t\mathrm{Bu} - \mathrm{NO}]^+$ whose fragment can be derived by successive elimination of *tert*-butyl and NO group from the molecular ion of the phenyl spin adduct, and its fragmentation pattern is consistent with the formation of the PBN-phenyl spin adduct. The existence of the $[M + \mathrm{H}]^+$ ion for the PBN phenyl spin adduct could be confirmed by chemical ionization (mild ionization by using isobutane reaction gas) mass spectroscopic experiment. Thus, the aryl PBN spin adducts can be distinguished from the alkyl PBN spin adducts by checking whether molecular ion peak

Table 1. Characterization of the PBN spin adducts (PBN-R•) by several analytical methods

Analytical Methods	Spin Adducts (PBN-R•)		
	R• = C_6H_5•	R• = *n*-C_4H_9•	R• = *tert*-C_4H_9•
ESR			
a_N [mT]	1.48	1.49	1.49
a_H^{β} [mT]	0.216	0.284	0.221
Mass (E.I.)			
m/*e* (R.I.;%)[a)]	M^+: 254 (0.0)	M^+: 234 (1.7)	M^+: 234 (5.1)
		$[M - {}^t\text{Bu} + \text{H}]^+$: 178 (2.3)	$[M - {}^t\text{Bu} + \text{H}]^+$: 178 (8.8)
	$[M - {}^t\text{Bu} - \text{NO}]^+$: 167 (100)	$[M - {}^t\text{Bu} - \text{NO}]^+$: 147 (23.4)	$[M - {}^t\text{Bu} - \text{NO}]^+$: 147 (100)
		$[M - {}^t\text{Bu} - \text{NO} - {}^t\text{Bu} + \text{H}]^+$: 91 (100)	$[M - {}^t\text{Bu} - \text{NO} - {}^t\text{Bu} + \text{H}]^+$: 91 (94)
High Resolution Mass			M^+: 234.18579
			$C_{15}H_{24}NO$: calcd. value 234.185789
Mass (C.I.)			
m/*e* (R.I.;%)[a)]	$[M + \text{H}]^+$: 255 (26.7)		
	$[M - {}^t\text{Bu} - \text{NO}]^+$: 167 (100)		
HPLC			
R.T. [min.]	8.0	5.7	4.5

a) R.I. = relative intensity.

M^+ can be found, or not. The loss of the molecular ion peak M^+ in the phenyl spin adduct may be due to the less stability than the alkyl spin adducts against electron bombardment (70 eV) ionization process. Structural difference between *n*-butyl and *tert*-butyl PBN spin adducts can be discerned from the relative intensities (R.I.) of the fragment ions. More bulky *tert*-butyl group in PBN-spin adducts leads to the stabilization of the *t*-C_4H_9 spin adduct by blocking the attack of chemical species to the cationic center in the reaction chamber of the mass spectrometer by the steric hindrance effect than that of the *n*-C_4H_9 spin adduct. Therefore, relative intensities for parent ion M^+ and *m*/*z* 178 $[M - 56]^+$ fragment ion of the *tert*-butyl spin adduct did show larger numbers (M^+: 5.1, $[M - 56]^+$: 8.8) than those of the *n*-butyl spin adducts numbers (M^+: 1.7 $[M - 56]^+$: 2.3).

As shown above, the structural information obtained from the Mass spectrometric measurement on the molecular ion M^+ and fragmentation pattern, and the exact mass value by high resolution mass spectrometry is very useful to identify the spin adducts clearly. However, the separation process is necessary to isolate spin adducts efficiently for mass spectroscopic measurement. Though the PBN spin adducts are rather stable at an ambient temperature, their thermal stabilities are not enough for gas chromatographic (GC) separation experiments. Trimethylsilylation of the spin adducts has already been reported as a convenient method to identify spin adducts by using GC-MS experiment.[6] Though the molecular ion peak M^+ was not observed in the mass spectra of the trimethylsilylated aryl (R) PBN spin adducts [PBN(R) − $Si(CH_3)_3$: M^+] on the GC-MS measurement and it is not certain whether spin adducts can be trimethylsilylated completely, the characteristic fragment ion $[M - {}^t\text{Bu} - \text{H}]^+$ was found as an identifying fragmentation peak of the trimethylsilylated spin adducts. If HPLC separation of the PBN spin adducts is appropriately applicable, LC-Mass analyses can be considered as a very convenient and efficient analytical method to identify the spin adducts clearly. Therefore, we have carried out the HPLC experiment on PBN spin adducts in order to make sure whether spin adducts can be separated efficiently. Under the condition of *n*-hexane–chloroform 9:1 (V/V) eluent with 1.0 mL/min flow rate by using a Shimadzu LC-9A chromatograph with a Shiseido AG120 silicate column 25 cm × 4.6 mm (diameter), retention times for these three spin adducts in the HPLC experiments were shown in Table 1. The *tert*-butyl spin adduct has shorter retention time (4.5 min) than *n*-butyl spin adduct (5.7 min), and phenyl spin adduct has the longest retention time (8.0 min). HPLC separation is useful for distinguishing the structural change of these spin adducts. Thus, a combination of the separation process and the spectroscopic experiments such as ESR and Mass has been shown as a very useful tool to identify the spin adducts generated from the various spin trapping experiments without ambiguity. With regard to the detection limit of the spin adducts, up to about 10^{-7} mol dm^{-3} spin adduct solution is necessary for ESR and Mass (E.I.) experiments. An assured combined method by carrying out the ESR and Mass spectrometric analysis after appropriate separation process can be applicable not only to the field of organic chemistry, but also to the environmental analyses to detect and to quantify the toxic radical species in the environment.

Authors are grateful to Mr. Shinji Ishihara (Instrumental Analysis Center of Yokohama National University) for his technical assistance for measuring Mass spectra.

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Published on the web (Advance View) October 15, 2005; DOI 10.1246/cl.2005.1534