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Some Physicochemical Properties of 2-Methyl-2-nitrosopropane, Phenyl N-*tert*-Butyl Nitrone, 5,5-Dimethylpyrroline-N-oxide, and 2,5,5-Trimethylpyrroline-N-oxide and the Feasibility of Their Use as Spin Traps in Aqueous Solution

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The chemical and photochemical stabilities of 2-methyl-2-nitrosopropane (NtB), phenyl N-tert-butyl nitrone (PBN), 5,5-dimethylpyrroline-N-oxide (DMPO), and 2,5,5-trimethylpyrroline-N-oxide (TMPO) in aqueous solutions were examined and the feasibility of their use as spin traps was evaluated. NtB has a poor solubility in water. The dissociation of dimeric NtB to its active monomeric form is slow and is accompanied by complicated decomposition reactions even in the dark. PBN is subject to hydrolysis in strongly acidic solutions, the rate constant being 90 M^{-1} hr^{-1}. DMPO and TMPO were found to be stable to hydrolysis and fairly inert to photolysis. These results, together with other findings, led us to conclude that DMPO may be the first choice and PBN the second as a spin trap to be used in aqueous solutions.

Keywords——spin trapping; nitrone; phenyl N-*tert*-butyl nitrone; 5,5-dimethylpyrroline-N-oxide; 2,5,5-trimethylpyrroline-N-oxide; 2-methyl-2-nitrosopropane

The technique of spin trapping makes use of a compound (the spin trap) which reacts with a free radical to produce a relatively stable free radical (the spin adduct). It is a useful method to detect and often identify a free radical which itself cannot be observed by electron spin resonance (ESR) spectroscopy because of its short lifetime (hence too low concentration) or its very rapid spin relaxation (hence too broad spectral lines). The spin traps that have been most commonly employed are nitroso compounds and nitrones, which give rise to nitroxide radicals as a result of spin trapping. Although spin trapping of radicals in organic solvents is well-documented,1) application of the technique to aqueous systems is only at an early stage and appears to involve problems inherent in aqueous solutions, such as the solubility and stability of spin traps. In the present study we have examined some physicochemical properties and the utility as spin traps of a nitroso compound, 2-methyl-2-nitrosopropane (NtB)2) and three nitrones, phenyl N-*tert*-butyl nitrone (PBN), 5,5-dimethylpyrroline-N-oxide (DMPO), and 2,2,5-trimethylpyrroline-N-oxide (TMPO), in aqueous solutions. So far, no data are available on the fundamental properties of these spin trapping agents in aqueous solutions.

Experimental

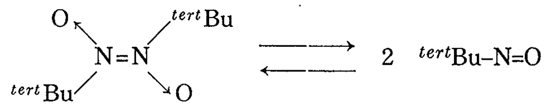
NtB was synthesized by the oxidation of *tert*-butylamine with Na_2WO_4–H_2O_2 following the procedure of Stowell.3) Monomeric NtB was distilled from the reaction mixture as a blue liquid (bp 55°), which changed into a dimeric form (colorless crystals, mp *ca.* 70°). The NMR spectrum of a CCl_4 solution of the crystals showed no peak other than at δ 1.23 and δ 1.53, which have been assigned to the monomeric and dimeric forms of NtB, respectively.4) PBN was synthesized by the condensation of benzaldehyde and *tert*-butylhydroxylamine, which was prepared by the reduction with zinc powder of 2-methyl-2-nitropropane.5) PBN was purified by recrystallization from petr. ether. Its purity was checked by comparison of its melting point and UV spectrum with the literature data6) and by elemental analysis and NMR spectroscopy. mp 74° (lit6) 76°). *Anal.* Calcd for $C_{11}H_{15}NO$: C, 74.54; H, 8.53; N, 7.90. Found: C, 74.38; H, 8.64; N, 7.89. UV $\lambda_{\max}^{\text{ethanol}}$ nm (ε): 285 (17800; lit6) 16700), 225 (6800). NMR (3% solution in CCl_4) δ: 1.54 (9H, s, *tert*-butyl), 7.22—7.34 (4H, m), 8.06—8.18 (2H, m). DMPO was synthesized according to Bonnett *et al.*7) by the dehydrative cyclization of 4-methyl-4-nitro-1-pentanal, which was prepared by the addition of 2-nitropropane to acrolein.8) TMPO was synthesized similarly from methylvinylketone instead of acrolein.9) DMPO and TMPO thus prepared were purified by distillation under reduced pressure (bp 61° at 1 Torr and 71° at 2 Torr, respectively) and obtained as colorless, highly hygroscopic liquids, which crystallized upon storage in a freezer. They were identified and the purity checked by NMR and IR spectroscopy or by elemental analysis of the picrates. DMPO—UV $\lambda_{\max}^{\text{ethanol}}$ nm (ε): 235 (8900; lit.7) 7700). IR cm^{-1}: $\nu_{C=N}$ 1577 (liquid film). NMR (3% solution in CCl_4) δ: 1.32 (6H, s, CH_3), 2.06 (2H, t, $J=3$ Hz, C_4–H), 2.54 (2H, t ($J=3$ Hz)–d ($J=7$ Hz), C_3–H), 6.42 (1H, t, $J=7$ Hz, C_2–H). TMPO—IR cm^{-1}: $\nu_{C=N}$ 1603 (liquid film), NMR (2% solution in CCl_4) δ: 1.26 (6H, s, $-C(CH_3)_2-$), 1.89 (3H, t, $J=1.5$ Hz, CH_3), 1.91 (2H, t, $J=7$ Hz, C_4–H), 2.53 (2H, q ($J=1.5$ Hz)–t ($J=7$ Hz), C_3–H). Picrate of TMPO—mp 100° (lit3.) 98°). *Anal.* Calcd for $C_{13}H_{16}N_4O_8$: C, 43.82; H, 4.53; N, 15.72. Found: C, 44.04; H, 4.47; N, 15.81.

UV spectra were obtained with a Hitachi EPS-3T spectrometer and NMR with JEOL PS-100 and FX-100 spectrometers. EPR measurements were carried out with a JEOL PE-1X (X band) spectrometer with 100 KHz field modulation. The light source for UV irradiation of samples was a 500W ultra-high pressure mercury lamp (Ushio Electric Co.).

Results

Dissociation Equilibrium of NtB

NtB exists as a dimer in the solid state and partially dissociates in solution to its monomeric form, which is active as a spin trap. We attempted to determine the dissociation rate and equilibrium constant.



(1)

Aqueous solutions of NtB (0.4 mM as a monomer) were kept at 30° in the dark and UV spectra are taken at appropriate intervals. At the initial stage the peak absorbance at 280 nm due to the dimeric NtB decreases with time with an isosbestic point at 240 nm. After 24 hr, however, there was no isosbestic point and a new peak appeared at 225 nm. This indicates that side reactions occur in addition to the dissociation reaction (1).

The dissociation reaction was also investigated by NMR spectroscopy. About 5 mg of NtB was added to 2 ml of heavy water. It was vigorously shaken for 1 hr in the dark, then filtered. To 1 ml of the filtrate in an NMR sample tube, 1 μl of dioxane (internal standard for concentration determination) and a samll amount of sodium 3-(trimethylsilyl)propanesulfonate (DSS) (internal standard for chemical shift) were added. The solution was kept at 30° in the dark and subjected to NMR measurements at appropriate intervals. The NMR spectrum at 2 hr after dissolution consisted of two peaks at δ 1.26 and δ 1.57, due to monomeric and dimeric forms of NtB, respectively. The sum of the intensity of the two signals compared with that of dioxane led us to conclude that the concentration of NtB in D_2O shaken for 1 hr at room temperature (about 20°) is 12 mM, expressed in terms of monomer concentration. While the signal intensity of the dimer decreased with time (the half-life was about 24 hr), the monomer peak did not increase to an extent fully corresponding to the decrease in the dimer peak, but new peaks appeared at δ 1.24 and δ 1.54. At later stages, other new peaks appared at δ 1.42 and δ 1.68. The time courses of these signals are shown in Fig. 1. Although we did not attempt to assign these new peaks, the peak at δ 1.24 was found to coincide with that of *tert*-butyl alcohol. The ratio of dimer to monomer concentration never became constant, indicating that the dissociation of the dimer to monomer at 30° is slow compared with the rate of the undefined decomposition reactions.

The NMR spectrum of a solution kept at 50° for 2 hr showed peaks at δ 1.26 (monomeric NtB, 31% intensity relative to the sum), δ 1.56 (dimeric NtB, 13%), δ 1.24 (30%), δ 1.42 (5%), and δ 1.54 (21%). Warming the solution resulted in higher monomer concentrations because not only the dissociation rate but also the solubility and dissolution rate are apparently

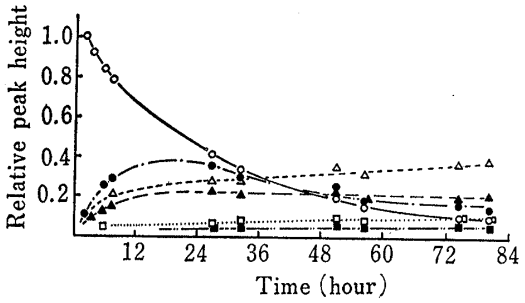


Fig. 1. Time Courses of Peak Heights of the NMR Signals for a D_2O Solution of NtB at 30°

○, δ 1.56 (dimeric NtB); ●, δ 1.26 (monomeric NtB); △, δ 1.54; ▲, δ 1.24 (^{tert}BuOH); □, δ 1.68: ■, δ 1.42.

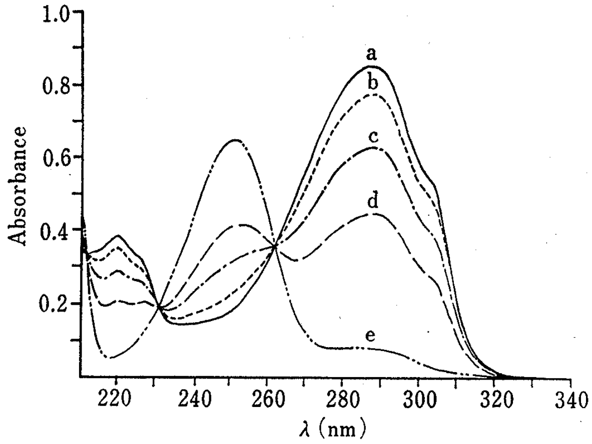


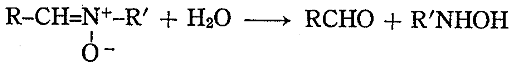
Fig. 2. UV Spectra of 1 mM PBN Solution at pH 2.94 and at 20° as a Function of Time

a, 5 min; b, 1 hr; C, 3 hr; d, 7.3 hr; e, 48 hr.

increased at higher temperatures. However, it is still difficult to determine the monomer concentration, since complicated decomposition reactions occur within 2 hr at 50°. Further difficulty arises because monomeric NtB is very volatile, to such an extent that it is readily lost when the solution is deoxygenated by nitrogen gas bubbling.

Hydrolysis of Nitrones

Since the nitrone is a Schiff base it is in general subject to hydrolysis into an aldehyde and a hydroxylamine.



$$\text{R-CH=}\underset{\displaystyle \text{O}^-}{\underset{|}{\text{N}^+}}\text{-R}' + \text{H}_2\text{O} \longrightarrow \text{RCHO} + \text{R}'\text{NHOH}$$

A slow rate of this hydrolysis is essential if a compound is to be suitable for use as a spin trap in aqueous solution.

The hydrolysis of PBN was followed by UV spectroscopy at various pH values and at 20°. A 1 mM aqueous solution of PBN gave a UV spectrum with a maximum at 288 nm (see trace a in Fig. 2). At pH 7.2—11.8 no change in the spectrum was observed within 3 days, indicating that no hydrolysis had occurred. The spectra for solutions of lower pH values underwent changes as exemplified in Fig. 2, and finally coincided with that of a benzaldehyde solution (trace e in Fig. 2). A semilogarithmic plot of PBN concentration (calculated from the absorbance) against time gave a straight line for the experiments at pH 4.96, 2.94 and 1.98. The reaction was found to be first order with respect to PBN and proton concentration, and the rate constant was determined to be 90.0 M^{-1} hr^{-1}.

The UV spectra of DMPO ($\lambda_{\max}$=235 nm) and TMPO ($\lambda_{\max}$=224 nm) did not show any change within 20 hr in the pH range of 1.0—12.0. Therefore, it can be concluded that practically no hydrolysis of these cyclic nitrones occurs.

Photochemical Stability

In situ photolysis-ESR experiments[10)] were carried out to examine the photochemical stability of the spin traps. UV-irradiation of NtB solution gave rise to a very intense triplet signal (a^N=17.2G) due to the di-*tert*-butylnitroxide radical. Photolysis of NtB is known[1)] to produce the *tert*-butyl radical, which is trapped by NtB to give di-*tert*-butylnitroxide. PBN in an aqueous solution gave no ESR signals upon photoirradiation, although it has been reported to give radicals in hydrogen-donating solvents.[1b)] Photoirradiation of a DMPO solution gave a very weak spectrum consisting of a quartet with lines in an intensity ratio of 1 : 2 : 2 : 1 and a splitting of 14.90±0.04G. This spectrum coincides with that of a hydroxyl radical adduct of DMPO.[10)] TMPO gave a weak triplet signal with a splitting of 15.84±0.04G. It is difficult to assign the observed signal since quite a large number of spin adduct radicals derived from TMPO has been found to show very similar ESR spectra with splitting constants of around 15.5G (data not shown).

Discussion

The utility of a spin trap depends on the properties of the trapping agent itself on one hand and of the spin adduct derived from it on the other. The spin trap should be fairly soluble and inert to chemical and photochemical reactions other than the radical addition reaction under study. The spin adduct should be stable enough to be observable by ESR, and the ESR spectrum of the spin adduct should depend on the nature of the trapped radical. NtB has a poor solubility in water. The dissociation of dimeric NtB to the active monomer is slow and is accompanied by complicated decomposition reactions even in the dark (the problem is especially severe in the light). These are serious disadvantages which make NtB unsuitable for use as a spin trap in aqueous solutions. It is particularly unsuitable for kinetic experiments where the concentration of the spin trap must be known. If NtB is used, however, it is desirable to warm the solution (up to 50°) and to avoid bubbling gas through the solution. The three nitrones are fairly stable in aqueous solutions. Using the rate constant of $90\ \mathrm{M}^{-1}\ \mathrm{hr}^{-1}$ for hydrolysis of PBN, we calculated the half-life of PBN to be 6 hr at pH 3. This may be the lower limit of pH at which PBN is usable as a spin trap. It is quite stable to UV-irradiation. No hydrolytic reaction was observed with DMPO and TMPO, and although DMPO and TMPO gave rise to a weak ESR signal upon UV-irradiation it is so weak that it does not constitute a serious problem.[10)]

The spin adduct derived from TMPO is quite insensitive to the nature of the trapped radical. It gives an ESR signal with little variation for different radicals trapped. The ESR spectra of spin adducts of PBN are known[1)] to show slight but significant changes in a^{H}_{β} depending on the structure of the trapped radical. DMPO produces spin adducts most sensitive to the nature of the trapped radical. A very large change in a^{H}_{β} and a significant change in a^{N} have been observed upon changing the structure of the trapped radical.[10)]

In conclusion, DMPO is the preferred choice and PBN is the second choice for use as a spin trap in aqueous solutions.

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