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HYDROGEN ATOM FORMATION BY ULTRASOUND IN D_2O SOLUTIONS OF NITRONE SPIN TRAPS

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To eludicate the mechanism of ultrasonically induced H atom formation in D_2O solutions of nitrone spin traps, α-phenyl-N-tert-butylnitrone (PBN) and α-(4-pyridyl-1-oxide)- N-tert-butylnitrone (POBN) were exposed to 50 kHz ultrasound in the presence of Ar, and the ratio of the ESR signal of the H-spin adduct to that of the D-spin adduct (H/D ratio) was examined. The H/D ratio increased with the concentration of the spin traps. The magnitude of the H/D ratios correlates with the hydrophobicity of the spin traps. The H/D ratios for the more hydrophobic spin trap, PBN, are larger than those for POBN at all concentrations. Xenon, which has a lower thermal conductivity than argon, was employed for creating higher final temperatures of the cavitation bubbles. For the less hydrophobic spin trap POBN the H/D ratio is lower for xenon than for argon. A similar result was found for PBN at lower concentrations. These results show that the H adducts of PBN and POBN are formed from the spin traps or their decomposition products by homolytic scission of C-H bonds due to pyrolysis.

KEY WORDS: PBN, POBN, Hydrogen atom formation, Ultrasound.

Abbreviations used: ESR, electron spin resonance; NMR, nuclear magnetic resonance; PAT, phenylazotriphenylmethane, PBN, α-phenyl-N-tert-butylnitrone; POBN, α-(4-pyridyl-1-oxide)-N-tert-butylnitrone; PYBN, 4-(N-methylpyridinium)-tert-butylnitrone.

INTRODUCTION

Nitrone spin traps have been used for identifying free radicals generated in "*in vivo*" systems by ionizing radiation[1], laser ablation[2] and ultrasound[3]. Hence a better understanding of the sonochemistry of nitrone spin traps is desirable.

In the last few years it has become clear that the sonochemistry of aqueous solution takes place in three regions. The first region is the gas phase of collapsing cavitation bubbles. The very high temperatures, several thousand degrees K, and high pressures in this region lead to the thermal dissociation of water vapour to give H atoms and OH radicals which have been detected by ESR and spin-trapping[4]. Volatile organic solutes also undergo pyrolysis reactions in this region. The second region is the interfacial region between the gas and the liquid where large temperature and pressure gradients exist[5,6]. Suslick *et al.*[7] have shown that in the sonolysis of organic liquids the temperature of the gaseous region is 5200 ± 650 K while that of the thin spherical shell surrounding it is ~ 1900 K. Recent studies of the sonolysis of aqueous solutions have shown that non-volatile solutes present at high concentrations in the interfacial

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regions undergo pyrolysis to yield radicals (typically methyl radicals) which are different from those produced by OH radical and H atom reactions.[8] Evidence for the occurrence of pyrolysis reactions in the interfacial regions of cavitation bubbles has been obtained by Henglein and Gutiérrez[9] who investigated carbon monoxide formation in the sonolysis of argon-saturated aqueous solutions of polymers (eg. polyethyleneglycol, polymethacrylic acid and polyvinylpyrrolidone). The third region is the bulk solution at ambient temperature where OH radicals and H atoms react with solutes to give products similar to those of radiation chemistry.[6,8]

Several years ago Makino *et al.*[4] observed an unexpectedly large yield of the H-atom spin adducts of POBN and PYBN in the 50 kHz sonolysis of argon-saturated 99.8% D_2O solutions. Only very tentative suggestions regarding this result could be made at that time.[4]

Recently we have obtained evidence for the formation of pyrolysis radicals in the sonolysis of nitrone spin traps in argon-saturated solutions.[10] Methyl, *tert*-butyl and phenyl radicals were shown to be generated by the thermal decomposition of PBN during the sonolysis of PBN solutions. However, the relative contributions of two pathways for the formation of H atoms could not be determined. The first is in the thermal dissociation of water vapour in the collapsing argon cavitation bubbles, the second is the homolytic scission of C–H bonds of the spin trap or of its decomposition products. In order to investigate the possibility that pyrolysis reactions of the hydrophobic spin traps in the interfacial region might contribute to the H-atom yield from the sonolysis of D_2O solution of non-deuterated spin traps, the effect of PBN and POBN concentrations on the H/D ratio (ESR signal of H-spin adducts/ESR signal of D-spin adduct) was examined. The present results suggest that relatively high yields of H atoms from spin traps are generated by pyrolysis of the spin traps due to cavitation, when argon saturated D_2O solutions of nitrone spin traps are sonicated.

MATERIALS AND METHODS

Chemicals

PBN, POBN and 2-octanol were purchased from Aldrich Chemical Company. D_2O (99.8 atom % D) was obtained from Sigma Chemical Company. PAT, a source of phenyl radicals by heating in D_2O at 80°C for 5 min, was generously provided by Dr. Louis A. Levy (National Institute of Environmental Health Science, Research Triangle Park, NC). Xenon gas (99.995%, Research Grade) was acquired from Matheson Gas Products, Inc.

Measurement of Partition Coefficient

As a measure of the hydrophobicity of PBN and POBN the partition coefficients of the spin traps were determined, $K = Co/Cw$, where Co is the concentration of the spin trap in 2-octanol and Cw is the concentration of the spin trap in water. The concentrations of the spin traps were measured spectrophotometrically.

NMR measurement of D content

The D content of the solution immediately after 10 min sonolysis of argon-saturated

PBN (50 mM) was measured by NMR on a Varian XL-200 NMR spectrometer using HPLC grade dioxane as the proton standard, and was found to be 99.7%.

Gamma-irradiation

Argon-saturated PBN and POBN solutions (20 mM) were gamma-irradiated to a dose of 2 × 10^3 Gy in a ^{60}Co gamma source at a dose rate of 30 Gy/min.

Sonication and Spin-trapping

A sample solution (4 ml) was placed into a specially designed Pyrex test tube with a side arm and clamped in the center of the sonication bath (Bransonic 1200, the frequency and input power being 50 kHz and 80.5 W respectively). The level of the liquid inside the tube was maintained to be the same as that of the water in the bath, in order to obtain sufficient reproducibility in the standing wave field. The temperature of the coupling water was 25°C. The sample solution was bubbled with argon or xenon gas through a fine needle attached to a Teflon tube at a flow rate of 100 ml/min for 5 min before and also during sonication.

Immediately after sonication, the sonicated sample was transferred into a quartz flat cell for ESR measurement. About 1 min after sonolysis, the ESR spectra were recorded on a Varian E-4 X-band spectrometer operating at 100 kHz modulation frequency and 9.5 GHz microwave power at 20 mW. The relative ESR signal intensity was calculated from the product of ESR peak-to-peak amplitude x the square of the peak to peak width x a correlation factor (1.5) for the total number of lines in the ESR spectrum and their multiplicity. For this calculation the lower field line of the H-adduct and the average of the three lowest field lines of the D-adduct were used.

RESULTS AND DISCUSSION

ESR Spectra of Sonicated PBN and POBN in D_2O)

The ESR spectrum of an argon saturated D_2O solution of 20 mM PBN after 10 min of sonolysis is shown in Figure 1 (a). The lines labeled H were analysed as a primary nitrogen triplet (a_N = 1.675 mT) further split by two secondary protons (a_H^β = 1.06 mT). These hyperfine coupling constants (hfccs) are in agreement with those reported previously for PBN-H[10]. The lines labeled D were analysed as a primary nitrogen triplet (a_N = 1.675 mT) further split by a secondary proton (a_H^β = 1.06 mT) and a secondary deuterium (a_D^β = 0.16 mT) which is consistent with the expected value $a_H^\beta/6.52$, the ratio of the gyromagnetic ratios of H and D. Hence this radical is attributed to the PBN-D adduct. Two other sets of lines labeled Ph and X were also observed. The lines labeled Ph were assigned to a primary nitrogen triplet (a_N = 1.605 mT) further split by a secondary hydrogen (a_H^β = 0.425 mT). These hfccs are very close to those of the PBN-phenyl radical adduct in water which was reported previously[11] and are almost identical to the value for the phenyl radical induced by thermolysis of PAT (a_N = 1.608 mT, a_H^β = 0.427 mT). The lines labeled X were analysed as a primary nitrogen triplet (a_N = 1.62 mT) further split by a secondary hydrogen (a_H^β = 0.32 mT). The PBN-X spin adduct seems to be produced by thermal decomposition of PBN[12], but has not been identified at present. The half life of the PBN-D and PBN-H were about 105 min, and 75 min, respectively, under our

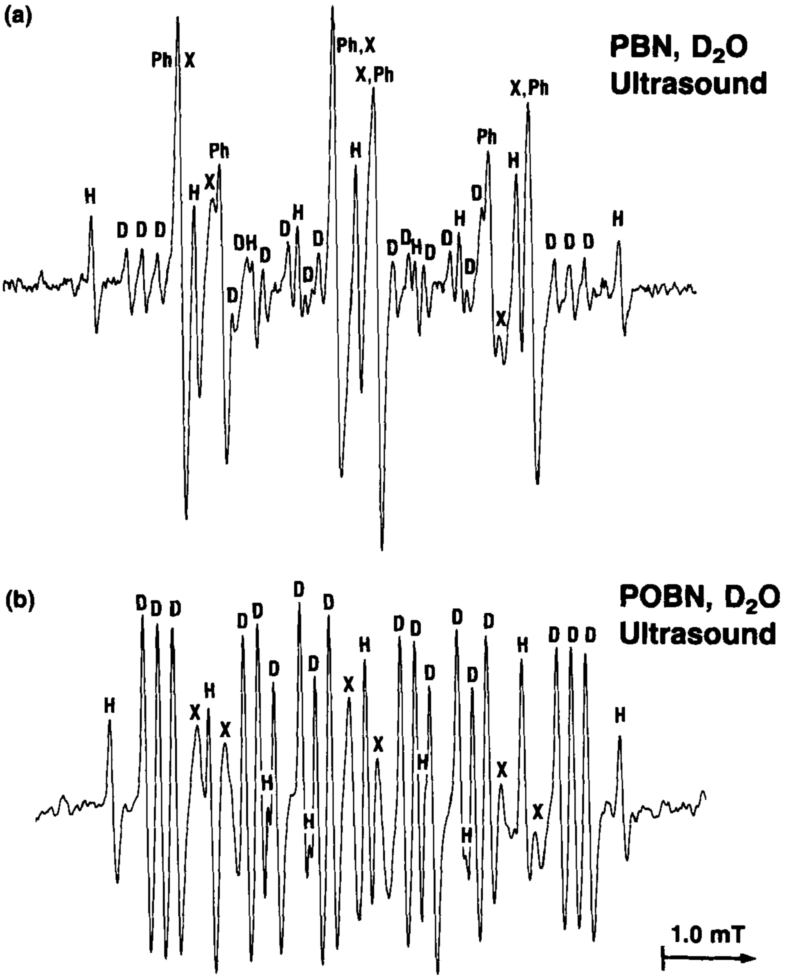

FIGURE 1 ESR spectra of spin adducts after sonication for 10 min of argon-saturated D_2O solution containing (a) 20 mM PBN; (b) 20 mM POBN.

TABLE I
Hyperfine coupling constants (mT) of the spin adducts trapped by PBN and POBN in D_2O after 10 min sonication in the presence of Ar.

Spin Trap	Untrapped Radical	a_N	a^{β}_H	a^{β}_D
PBN	H	1.675	1.06 (2H)	
	D	1.675	1.06	0.16
	Ph	1.605	0.425	
	X	1.62	0.32	
POBN	H	1.64	1.01 (2H)	
	D	1.63	1.04	0.15
	X	1.56	0.28	

conditions in which 10 mM PBN in D_2O was sonicated. Table I lists the hfccs of the radicals trapped by PBN and POBN.

The ESR spectrum displayed in Figure 1 (b) was obtained from a D_2O solution containing POBN (20 mM) which was sonicated for 10 min in the presence of Ar. The lines labeled H were analysed as a primary nitrogen triplet (a_N = 1.64 mT) further split by two secondary protons (a_H^β = 1.01 mT). The lines labeled D were analysed as a primary nitrogen triplet (a_N = 1.63 mT) further split by a secondary proton (a_H^β = 1.04 mT) and a secondary deuterium (a_D^β = 0.15 mT). These hfccs are almost the same as those reported previously by Makino *et al.*[4] Another set of lines labeled X was analysed as a primary nitrogen triplet (a_N = 1.56 mT) further split by a secondary hydrogen (a_H^β = 0.28 mT). At present this spin adduct has not been identified. The half lives of POBN-D and POBN-H were about 30 min and 20 min, respectively, under our conditions in which 10 mM POBN in D_2O was sonicated.

No significant H adducts of POBN and PBN were observed in gamma-irradiated argon-saturated D_2O solutions of these spin traps at 2×10^3 Gy. This observation is in accord with the previous results for PYBN.[4] These results indicate that there is no significant yield of H atoms arising from radical attack on the spin traps.

Effect of Concentration of Spin Traps on H-adduct Formation

In order to study the effect of PBN or POBN concentration on the H/D ratio (ESR signal of H adduct/ESR signal of D adduct), D_2O solutions of PBN and POBN were sonicated for 10 min in the presence of Ar and the H/D ratios were calculated from the relative yields of PBN-H, PBN-D, and of POBN-H and POBN-D (Figure 2). The H/D ratio increased with the concentration of PBN reaching a value of 1.15 ± 0.30 at a concentration of 20 mM and also increased with the concentration of POBN in the region from 2 to 200 mM POBN. The differences of the H/D ratios for PBN and POBN correlate with the hydrophobicity of the spin trap. The 2-octanol/water partition coefficients of PBN and POBN were found to be 10.6 ± 0.6 and 0.12 ± 0.02, respectively.

The observed relative yields of H and D atoms in the present sonolysis experiments are independent of the spin trapping efficiencies of PBN and POBN but depend on the isotope effect, k_H/k_D, for addition of H and D to the N=C double bond to form nitroxide radicals. A survey of theoretical and observed values of analogous isotope effects indicates that k_H/k_D can be expected to be close to 1.0 ± 0.2. The processes considered include the gas phase reactions of H and D atoms with H_2 and D_2 in the temperature range 300–1000 K[13] and with normal and deuterated formaldehyde at 600 K.

Thus for

$$H + H_2CO \xrightarrow{K_H} H_2 + HCO$$

$$D + H_2CO \xrightarrow{K_D} HD + HCO$$

at 600°C, k_H/k_D = 1.1 (observed)[14] and 0.88 (calculated)[15,16].

A conceivable mechanism for the formation of H atoms in the sonolysis of D_2O solutions of PBN might be H-atom abstraction from PBN by OD or D to give HDO or HD respectively, followed by thermal dissociation of HDO or HD in the gas phase of the cavitation bubbles. However, any HD formed would be continuously removed by the high rate of argon bubbling (100 ml/min). Since the OD radical concentrations

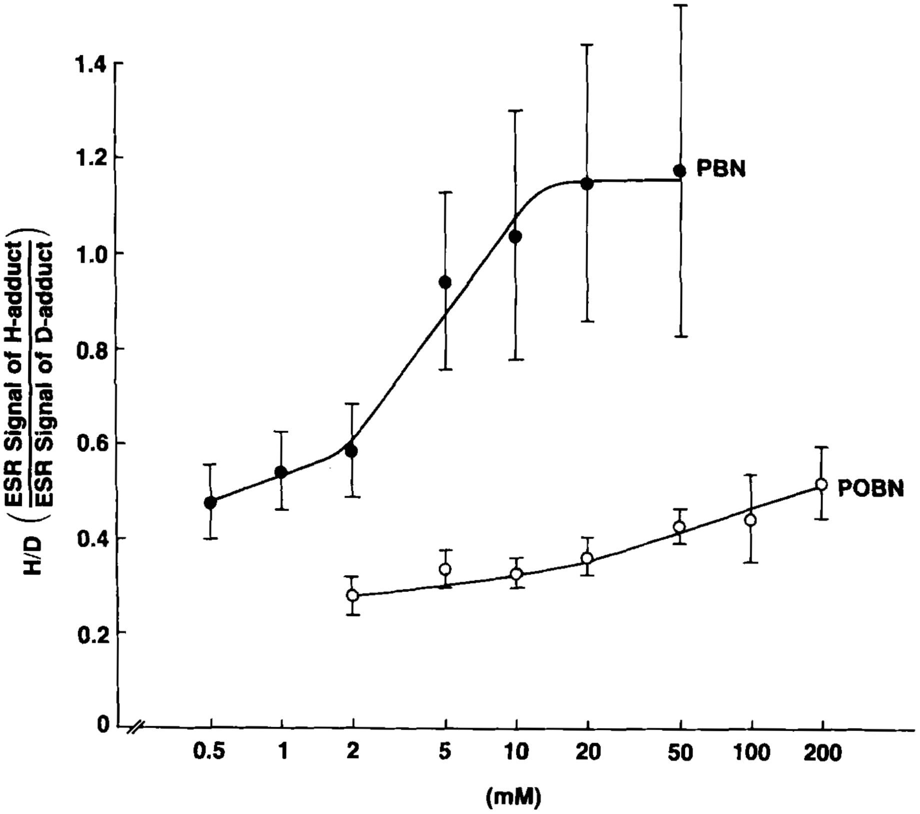

FIGURE 2 Effect of PBN and POBN concentration on the ratio of H-adduct/D-adduct calculated from their relative ESR signals.

formed are in the micromolar concentration range, it follows from Henry's law that the vapor pressure of HDO in the argon bubbles is negligible compared to the D_2O vapor pressure. Indeed it was shown that addition of 1–5% H_2O to D_2O solution of 20 mM PBN did not lead to a significant change in the H/D ratio of the spin adducts after sonolysis.

Effect of Xe on the H/D ratio

In order to create different final temperatures of collapsing cavitation bubbles, D_2O solutions saturated with Xe were sonicated. It has been shown that Xe gas creates the highest sonoluminescence intensity,[17,18] and the highest yield of OH radicals,[19] due to increasing final temperatures of the collapsing cavitation bubbles with decreasing thermal conductivity. Higher yields of the ESR signal of the H and D adducts in Xe-saturated in D_2O solution due to higher final temperature of cavitation bubbles were observed (Table 2).

The D atoms generated in the sonolysis experiments are formed by the thermal dissociation of D_2O vapor in the collapsing argon bubbles.

$$D_2O + Ar \rightarrow D + OD + Ar$$

TABLE II
Comparison of relative ESR signal of H and D adducts, and H/D ratios obtained from sonicated PBN and POBN aqueous solutions in the presence of Ar of Xe.

Spin Trap (Concentration)	Gas	ESR Signal H adducts	(relative units) D adducts	H/D Ratio[1] (n)[2]
PBN (0.5 mM)	Ar	10.8 ± 1.8[3]	23.2 ± 5.7	0.48 ± 0.08 (5)
	Xe	14.2 ± 0.8	41.3 ± 2.9	0.34 ± 0.02 (3)
PBN (20 mM)	Ar	49.0 ± 10.0	46.6 ± 18.1	1.15 ± 0.30 (8)
	Xe	194.0 ± 8.6	165.8 ± 33.6	1.20 ± 0.20 (3)
POBN (2 mM)	Ar	15.2 ± 3.7	52.9 ± 8.0	0.28 ± 0.04 (3)
	Xe	33.0 ± 7.8	151.4 ± 19.2	0.22 ± 0.03 (3)
POBN (20 mM)	Ar	64.0 ± 3.8	177.7 ± 18.5	0.36 ± 0.03 (5)
	Xe	112.7 ± 9.5	433.9 ± 49.5	0.26 ± 0.01 (3)
POBN (200 mM)	Ar	85.3 ± 9.1	167.3 ± 20.5	0.51 ± 0.08 (3)
	Xe	147.3 ± 5.0	440.3 ± 3.5	0.34 ± 0.02 (3)

1) H/D ratio was calculated from the equation, ESR signal of H adduct/ESR signal of D adduct, for each experiment.
2) Number of experiments.
3) Mean ± standard deviation.

The energy of activation for the corresponding reaction for H_2O is 114 kcal.[20] Since the isotopic purity of the D_2O was 99.7% (see experimental), the only possible source of H atoms is the spin trap. These H atoms could originate from the C–H bond adjacent to the double bond of PBN thus forming a resonance stabilized radical. Alternatively they could be formed from the *tert*-butyl group with a favourable statistical factor or they could arise from a pyrolysis radical (*tert*-butyl or methyl) generated from PBN. Since for all these pathways the bond-dissociation energies of the corresponding C–H bonds are considerably lower than that of the -O-D bond in D_2O, the energies of activation for the processes forming H atoms will also be lower. An increase in the temperature of the collapsing gas bubbles and of the surrounding interfacial regions when the rare gas is changed from argon to xenon would be expected to lead to a lower H/D spin adduct ratio. This result was observed for the less hydrophobic spin trap POBN and for PBN at lower concentrations (see Table II). However at higher PBN concentrations no statistically significant difference of the H/D ratio for Xe and Ar-saturated solutions was observed.

The vapour pressures of non-ionic organic compounds of molecular weight and composition similar to that of PBN are near one atmosphere at 240 ± 20°C[20]. If the mole fraction of PBN in the interfacial region is large because of the high hydrophobicity of PBN, the possibility of homolytic C–H bond scission in the gas phase should be considered in view of the high temperature of the interfacial region. If such a process goes to completion and is limited only by the total amount of PBN transported into the gas phase, the yield of H atoms would be independent of the rate constant for decomposition.

Combining the present results on the effects of rare gases on radical yields with our previous sonolysis studies[8,10], we have found the following generalization to hold. If R_U is the sonolytic radical yield of the radical formed directly by a reaction or indirectly by a series of reactions with the higher energy of activation, and R_L is the radical yield produced by processes with the lower energy of activation, then the ratio R_U/R_L increases with increasing temperature of the collapsing cavitation bubbles ($Xe > Kr > Ar > Ne > He$) and decreasing thermal conductivity of the rare gas.

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