Study of the Isolation and Stability of α-Trichloromethylbenzyl(*tert*-butyl)aminoxyl, the Trichloromethyl Radical Adduct of α-Phenyl-*tert*-butylnitrone (PBN)

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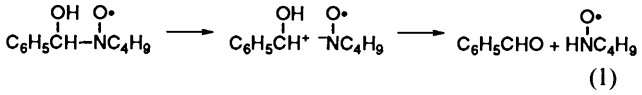
The aminoxyl produced from trapping trichloromethyl radicals by α-phenyl-*tert*-butylnitrone (PBN) † has been isolated. Isolation has been achieved from the metabolism of carbon tetrachloride by rat liver microsomal dispersions followed by extraction or by photolysis of bromo(trichloro)methane in benzene. Chromatographic techniques were used to purify the isolated aminoxyl. The stability of this spin adduct aminoxyl has been evaluated in various solvents and at different pH in buffer solutions. The intrinsic stability of this spin adduct is remarkable. Solutions of this aminoxyl survive in aromatic solvents almost unchanged for over 90 days. In aqueous media the spin adduct is stable for over 30 days. Light and low pH cause decay but the rates are moderate. The spin adduct is difficult to oxidize and slow to reduce. The outstanding stability of this alkyl spin adduct must be associated in some manner with the proximity of the trichloromethyl group.

The success of spin-trapping experiment depends critically on the stability of the spin adduct under the conditions of the immediate environment where the radical is trapped. Recent studies have shown that some spin adducts are surprisingly unstable in polar solvents or when in contact with active biological systems. Thus, the hydroxyl adduct of PBN disappears in aqueous buffer solutions by first-order kinetics in a matter of seconds [1] although this adduct is persistent in organic solvents such as benzene.[2] The hydroperoxyl adduct of PBN produced from hydrogen peroxide is similarly unstable in aqueous or polar solvents.[1,3] This feature seems to be a common characteristic for PBN-type spin traps and their hydroxyl radical adducts.[4]

In the case of the hydroxyl adduct of PBN, the mechanism of decay has been proposed to involve a polar transition state producing benzaldehyde and *tert*-butylhydroaminoxyl [reaction (1)].[1] Support for this mechanism is provided by the effect



(1)

of substituents on the rate of decay of the EPR spectrum of the spin adduct [3] and the fact that *tert*-butyl hydroaminoxyl is detected following the decay of the hydroxyl adduct of PBN.[1,4] It is assumed that this manner of decay largely depends on the polarity of the solvent or the polarity of the biological medium wherein the spin adduct finds itself.

However, the lifetime of the spin adduct will be influenced not only by the local environment of the medium but also by the structure of the spin trap. For example, the hydroxyl adduct of DMPO has a longer lifetime in polar solvents than does that of PBN.[5] Probably this is due to the cyclic structure of DMPO as the spin-trapping compound. It is also possible that the stability of the spin adduct will depend on the structure of the radical trapped and this feature will be explored in detail for one example in this paper.

Thus, we wish to distinguish a property of the spin adduct which will be named the 'intrinsic stability' of the spin adduct. Since the lifetime of the spin adduct may depend on the immediate environment in contact with the molecule, the intrinsic stability of the spin adduct will also depend on the solvent or biological medium of interest in the study. Ideally, the intrinsic stability is measurable using a first-order decay function in very dilute solutions in accordance with the assumption that unimolecular decomposition of the spin adduct is the decay mechanism. However, this may not be the case in every example. Thus, the loss of EPR signal as a function of time should be examined for linearity when the logarithm of the peak intensity is plotted as a function of time. At higher concentrations, either dimerization or disproportionation of the spin adduct is possible and second-order decay kinetics may apply. Even when a dilute solution of the spin adduct is examined, the actual mechanism of decomposition may be influenced by another solution component such as a water molecule, proton or hydroxide ion. Thus, the reaction could be second order overall.

We have begun a study of the intrinsic stabilities of spin adducts as a function of structure of spin traps and type of free radical trapped in various solvents and different biological systems of interest. The fate of the hydroxyl radical adduct of PBN has already been studied in detail.[1] Also, the decay of hydroperoxyl adducts of PBN has been followed.[3] Data is being collected for alkoxyl adducts of PBN,[6] and a study of alkyl adducts of PBN has been completed.[7] Here we report on the decay chemistry of the trichloromethyl adduct of PBN in organic solvents, in aqueous buffer at various pH and in the presence of light.

The trichloromethyl radical was first detected by PBN in the

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† IUPAC recommended name: *N*-benzylidene-*tert*-butylamine *N*-oxide.

rat liver metabolism of carbon tetrachloride supplemented with NADPH as the reducing equivalent.[8] The assignment of this spin adduct was at first challenged on the reasonable basis that similar spectra could be obtained from some carbon-centred radicals.[9] This point was tested using 99 atom% carbon-13 labelled CCl_4 which gave a spectrum with an additional doublet hyperfine splitting (hfs) while all other splittings retained the same value as with carbon-12 CCl_4.[10] Since the carbon atom in the trichloromethyl adduct of PBN is in the β-position from the aminoxyl function, substantial β-carbon-13 splitting should be expected. Although the actual value of this splitting could not be predicted, the fact that an additional doublet is resolved *while all other hyperfine splittings remained the same* was proof that the original assignment was correct. This experiment has been confirmed by others.[11,12] Subsequent studies showed that PBN could be used in the rat directly following CCl_4 administration.[13] After a reasonable period of time (*e.g.*, 30–60 min) the liver is removed and the spin adduct isolated by extraction. This experiment has been called an *in vivo* experiment, *i.e.*, *in vivo* trapping followed by '*ex vivo*' detection.

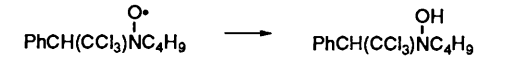
Another form of an *ex vivo* experiment is the use of rat hepatocytes. The trichloromethyl radical spin adducts can be detected by PBN when hepatocytes are exposed to CCl_4 followed by extraction.[11,14] In an *in-situ* experiment, this adduct was not directly detectable by EPR spectroscopy. Either the spin adduct is reduced in the biological system to the hydroxylamine, or broad lines are generated from the immobilization of the aminoxyl within the lipid environment wherein the trichloromethyl adduct is most soluble. A similar situation seems to exist in blood where a broad-lined spectrum has been assigned to the trichloromethyl radical adduct of PBN.[15] The detection of the trichloromethyl radical as a metabolite of CCl_4 has attracted the attention of many authors and this phenomenon has been extensively reviewed.[16–22]

An important advance in spin-trapping methodology was initiated when CCl_4 derived free radical adducts of PBN were detected in liver perfusate, biliary secretions and urine of the living rat.[23–26] In addition to the trichloromethyl radical adduct of PBN, two other adducts have been found: the carbon dioxide anion radical adduct (also verified by others[14,27]) and a glutathione–trichloromethyl adduct of unknown structure.[26] If the methylenic carbon-centred radical adduct and an oxygen-centred radical adduct of PBN is added to the list[28,29] a total of five different PBN adducts have now been recognized in the metabolism of carbon tetrachloride. However, different conditions (*in vitro*, *in vivo*, hepatocytes, perfused liver, biliary fluids and urine) give different radical signatures for each system. Before any progress can be made on the question of quantitation involving the actual number of free radicals trapped by the spin-trapping method, it is necessary to know something about the stability of the adducts in the systems under investigation. PBN was selected for further study because considerable spin trapping information is presently available on this spin trap and because some data has been collected on the pharmacokinetics of this compound.[30,31]

There are at least four different types of spin adduct product which can be envisioned in a reactive system generating trichloromethyl radicals. The first, of course, is the simple spin adduct [reaction (2)]. This spin adduct may be reduced to

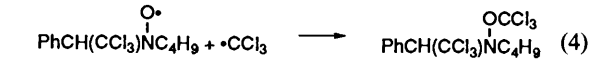
(2)

the hydroxylamine which is EPR-silent [reaction (3)].



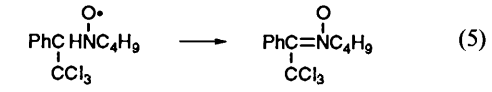
(3)

The hydroxylamine of the trichloromethyl adduct of PBN has been implicated before in the analysis of extracts from rat liver microsomal dispersions metabolizing CCl_4,[32] but in this paper the isolation of this species is described for the first time. Two more products containing the trichloromethyl moiety could be produced. One of these is the double adduct of PBN [reaction (4)]. Although this species has not been detected to



(4)

date, the equivalent product was detected by mass spectroscopy in the metabolism of halothane.[33,34] The other product is a new trichloromethyl substituted nitrone. If the trichloromethyl radical or some other oxidizing agent reacts with the spin adduct to abstract the β-hydrogen, a new nitrone would be produced which is also EPR-silent but retains the trichloromethyl group [reaction (5)].



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This type of compound has not been isolated in biological systems but MS evidence has been obtained for the presence of such products in chemical spin-trapping studies.[35] It is clear that a spin trapping experiment could produce a complex mixture of adducts all derived from the same radical. In this paper, we report data only on the isolation of the simple trichloromethyl radical spin adduct of PBN.

Experimental

Materials.—PBN was obtained from Sigma (St. Louis, MO). Bromo(trichloro)methane (Spectro Grade) was purchased from Kodak (Rochester, NY). Buffer solutions were obtained from Fisher. All solvents used were HPLC grade.

Preparation and Purification of the Trichloromethyl PBN Adduct from Photolysis.—A 0.2 mol dm^{-3} bromo(trichloro)-methane–benzene solution containing 0.1 mol dm^{-3} PBN was irradiated with UV light (200 W Hg) for 15 min. The solution was applied to a TLC plate and chromatographed with benzene–hexane–pyridine (60:50:5) for 60 min. The first fluorescent zone was located with a hand-held long-wavelength UV light detector. After the chromatography was complete, the plate was dried, the fluorescent zone scraped off and the spin adduct extracted three times with benzene. This solution of trichloromethyl spin adduct in benzene was used as prepared. It should be noted that for EPR experiments the adduct was prepared in the laboratory not in the EPR cavity. Approximately 20 min transpired before the EPR spectrum was recorded.

EPR Analysis of the Trichloromethyl PBN Spin Adduct in Organic Solvents.—The adduct solution purified by TLC was divided into five portions and evaporated with nitrogen until dryness. The residue was redissolved in toluene (two portions), benzene, chloroform or hexane, degassed with nitrogen for 30 min and placed in an ST-EPR cavity. EPR spectra were immediately recorded with a Bruker ER-200 X-band spectrometer. The intensity of the EPR spectra was measured according to the formula, signal strength = peak height × $W_{\frac{1}{2}}$, where $W_{\frac{1}{2}}$ is the peak width at half peak height.

A pre-experiment showed that light affects the adduct lifetime. Therefore, one of the toluene portions was exposed to a sun lamp in the laboratory at a distance of 1 m. The other samples were wrapped with aluminium foil and kept at room temperature (25 °C). The EPR spectra were recorded at

Table 1 Trichloromethyl PBN spin adduct hyperfine splitting constants[a]

Solvent	pH	a_N	a_β^H
Hexane	—	13.82	1.68
Toluene	—	13.90	1.61
Benzene	—	13.97	1.66
Chloroform	—	14.24	1.86
3:2 CH_3CN–H_2O	5.0 (acetate)	14.57	2.22
3:2 CH_3CN–H_2O	6.0 (phosphate)	14.63	2.25
3:2 CH_3CN–H_2O	6.6 (phosphate)	14.60	2.30
3:2 CH_3CN–H_2O	7.0 (phosphate)	14.60	2.32
3:2 CH_3CN–H_2O	7.4 (phosphate)	14.61	2.26
3:2 CH_3CN–H_2O	7.8 (phosphate)	14.63	2.24

[a] At room temperature in Gauss; error ±0.5 G.

different time points. Before recording, the EPR spectrometer sensitivity was calibrated with a small crystalline sample of diphenylpicrylhydrazyl (DPPH).

EPR Analysis of the Trichloromethyl PBN Spin Adduct in Buffer Solution.—A pre-experiment showed that the trichloromethyl–PBN spin adduct is difficult to dissolve in water or buffer solution. Thus, the trichloromethyl–PBN adduct purified by TLC was dissolved in mixtures of different buffer solutions with acetonitrile (3:2 buffer–acetonitrile). The buffer solutions were varied as regards pH (5.0–7.8) and buffer species (pH 5.0 acetate buffer; pH 6.0, 6.6, 7.0, 7.4, 7.8 phosphate buffer). The concentration was 0.2 mol dm^{-3} in each case. The above buffer solutions were prepared at constant ionic strength (I = 0.2 mol dm^{-3}) adjusted with potassium chloride. The solution aliquots were withdrawn into Accupette pipettes and sealed with Critolseal.

The first group of solutions at different pH was exposed to the sun lamp at a distance of 1 m. The second group of solutions was wrapped with aluminium foil. The EPR spectra were recorded and the signal strength calculation was made as before with the organic solutions.

Trichloromethyl PBN Spin Adduct Chromatography by HPLC.—HPLC analyses were performed using a Hewlett Packard 1050 liquid chromatograph. An ODS Spheri-5 (4.6 mm × 22 cm) column was utilized and acetonitrile–water (70:30) containing 0.02 mol dm^{-3} phosphate buffer (pH 7.4) was selected as the mobile phase. A flow rate of 0.8 cm^3 min^{-1} and chart speed 1 cm min^{-1} was used. An electrochemical detector with a pyrolytic graphite working electrode at +0.7 V *vs.* Ag/AgCl reference electrode was connected for simultaneous monitoring.

To check which peak was the hydroxylamine, the toluene solutions tested by EPR were oxidized with potassium ferricyanide [$K_3Fe(CN)_6$] and bubbled with oxygen for 5 min. After degassing with nitrogen, the oxidized toluene solutions were checked by EPR.

Preparation and Purification of the Trichloromethyl PBN Adduct from Metabolism.—A microsomal dispersion was prepared from four fasted rats, washed with isotonic KCl, and divided into six round-bottom flasks each containing phosphate buffer (50 mmol dm^{-3}, pH 7.4), PBN (65 mmol dm^{-3}) and an NADPH-generating system,[8-12] in a volume of 20 cm^3 per flask. Carbon tetrachloride (CCl_4) was added (130 mmol dm^{-3}) to five flasks, the sixth flask serving as a control. Nitrogen was bubbled through the buffer solution prior to addition of microsomes and passed over the top of the flask prior to sealing the flask for incubation. After 2 h at 37 °C, the contents of the flasks were extracted into two volumes of benzene, dried and clarified by filtration through anhydrous sodium sulfate, and evaporated to dryness with nitrogen flow at room temperature. Total weight of PBN added to microsomes, 1.2 g; dry weight of organic extract approximately 1.2 g.

A silica gel column was prepared from 15 cm^3 silica gel, washed with 100 cm^3 mobile phase (hexane–benzene–pyridine 6:5:0.5), loaded with the extract and 2.5 cm^3 fractions collected. UV absorbance at 295 nm was used to monitor the eluent (PBN plus spin adduct) and the EPR signal or EC–HPLC response provided detection for the spin adduct alone. Intense UV absorbance was indicated beginning with fraction 7. The EPR signal was present in fractions 5 through 14 becoming most intense in fraction 7. Fractions 5 through 12 were pooled, evaporated to dryness (423 mg), and chromatography repeated except that 1 cm^3 fractions were collected for further analysis. EPR analysis indicated the presence of the adduct in fractions 7 through 28 with the most intense peak in fractions 11 and 12. Fraction 13 gave no EPR signal and an unusual HPLC profile. Therefore, this fraction was discarded. HPLC analysis indicated that PBN was essentially absent from all fractions prior to fraction 13 and was present beginning with fraction 14. A sample prepared from pooled fractions 8 through 12 contained the trichloromethyl radical spin adduct. Dry weight was approximately 1.1 mg or approximately 0.01% yield based on administered CCl_4. HPLC analysis of this sample indicated contamination of two components which were detectable by the UV but not by the EC detector.

Results

Hyperfine Splitting Constants.—The EPR spectrum obtained from the photolysis of bromo(trichloro)methane consists of a triplet of doublets. The hyperfine splitting constants (Hfsc) in various solvents are given in Table 1. The normal solvent effect is found, *i.e.*, both the nitrogen and β-hydrogen hfsc increase in magnitude with increase in polarity of solvent.[36] There is no noticeable effect of pH on the hfsc.

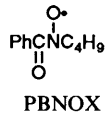
A method of obtaining only a triplet of doublets spectrum was developed. When carbon tetrachloride or bromo(trichloro)methane is photolysed directly in the EPR cavity or in an inert solvent, the chlorine atom adduct is detected first along with a triplet of doublets.[37-39] The bromine atom adduct of PBN is not known although with a different nitrone spin trap, bromine atoms can be detected.[40] The chlorine atom adduct of PBN is not stable and the signal disappears within a few minutes after photolysis is ceased [eqns. (6)–(8)].

$$BrCCl_3 + h\nu \longrightarrow Br^\bullet \text{ or } Cl^\bullet + {}^\bullet CCl_3 \quad (6)$$

$$Br^\bullet + PhCH{=}N(O)C_4H_9 \longrightarrow PhCH(Br)N(O^\bullet)C_4H_9 \text{ (not persistent)} \quad (7)$$

$$Cl^\bullet + PhCH{=}N(O)C_4H_9 \longrightarrow PhCH(Cl)N(O^\bullet)C_4H_9 \quad (8)$$

Depending on the amount of PBN used, another aminoxyl is detected in benzene solutions of halocarbons subjected to photolysis. This is the benzoyl(*tert*-butyl)aminoxyl with a 7.5–8.0 G triplet resulting from nitronyl carbon oxygenation. In the case of DMPO, this radical has been christened DMPOX.[41] In the PBN case, the analogous aminoxyl could be called PBNOX.[42]



PBNOX

When the ratio of PBN to the carbon tetrachloride or bromo(trichloro)methane is varied, the relative amounts of the

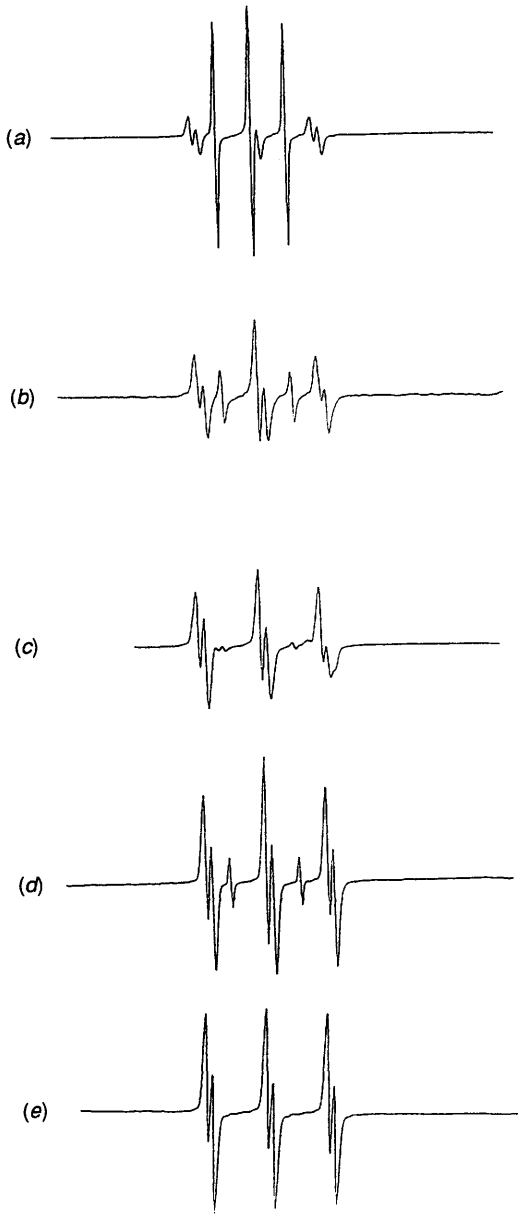


Fig. 1 EPR spectra obtained from photolysis of bromo(trichloro)-methane in benzene solutions of PBN: ratio of PBN:$BrCCl_3$ (*a*) 0.25; (*b*) 0.50; (*c*) 1.00; (*d*) 1.25; (e) 2.00

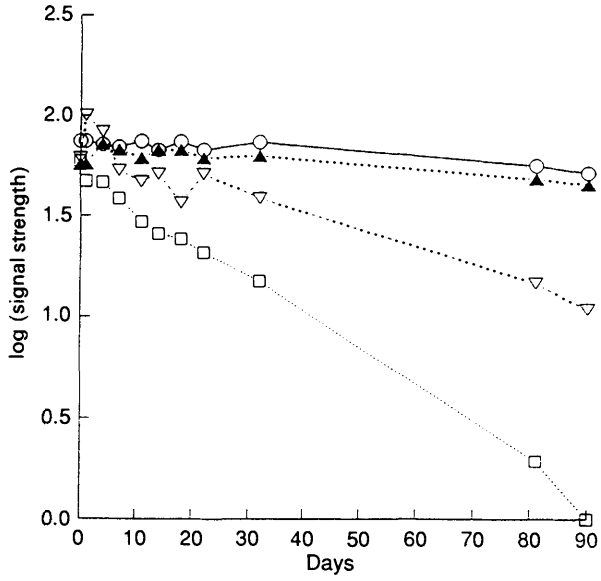


Fig. 2 Stability of the trichloromethyl PBN spin adduct EPR spectrum as a function of solvent in the absence of light: ▲, toluene; □, hexane; ▽, chloroform; ○, benzene

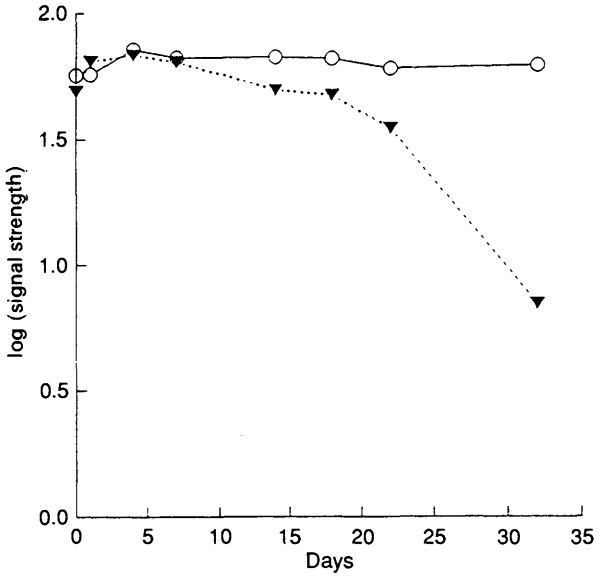


Fig. 3 Stability of the trichloromethyl PBN spin adduct EPR spectrum as a function of time exposed to light in a solution of toluene: ○, in toluene solution in the absence of light; ▼, in toluene solution with exposure to light

trichloromethyl adduct to PBNOX change (see Fig. 1). Also, the chlorine atom adduct is sometimes detected. Only at a ratio of 2:1 PBN–$BrCCl_3$ is the trichloromethyl adduct observed by itself. This ratio was used to prepare all the spectra of the trichloromethyl radical adducts of PBN.

Stability in Organic Solvents With or Without Light.—The persistence of the EPR spectra due to the trichloromethyl radical spin adduct of PBN is shown in Fig. 2 as a function of time in days. In benzene or toluene, the signal intensity diminishes only very slightly over the course of 90 days. For hexane and chloroform, some decay is observed. In Fig. 3 the plot of signal intensity as a function of time is shown when a toluene solution is exposed to light. The decay seems to be modest for about 20 days, after which an accelerated rate of disappearance is observed.

Stability in Aqueous Acetonitrile at Various pH With or Without Light.—In Fig. 4 are shown the decay rates of the EPR signal as a function of time and pH. At all pH, the trichloromethyl radical spin adduct is less stable in aqueous solutions than in organic solvents. In the pH range 7.8–6.6, the lifetimes are approximately the same. With increasing acidity, the decay is more rapid so that the adduct is detectable only for about 12 days at pH 5.0.

Light accelerates the decay of the trichloromethyl spin adduct in aqueous acetonitrile solutions at every pH. Although the signal is stable for almost 26 days in the pH range 7.8–6.0 (Fig. 5), in the presence of light significant decrease is noted during this time period. For the solution at pH 5.0, the same decay profile seems to be observed whether the spin adduct is exposed to light or not.

In HPLC solvents used for electrochemical detection, such as 60:40 acetonitrile–phosphate buffer, the stability of the trichloromethyl adduct is very good. No decay was found for at least 6 h.

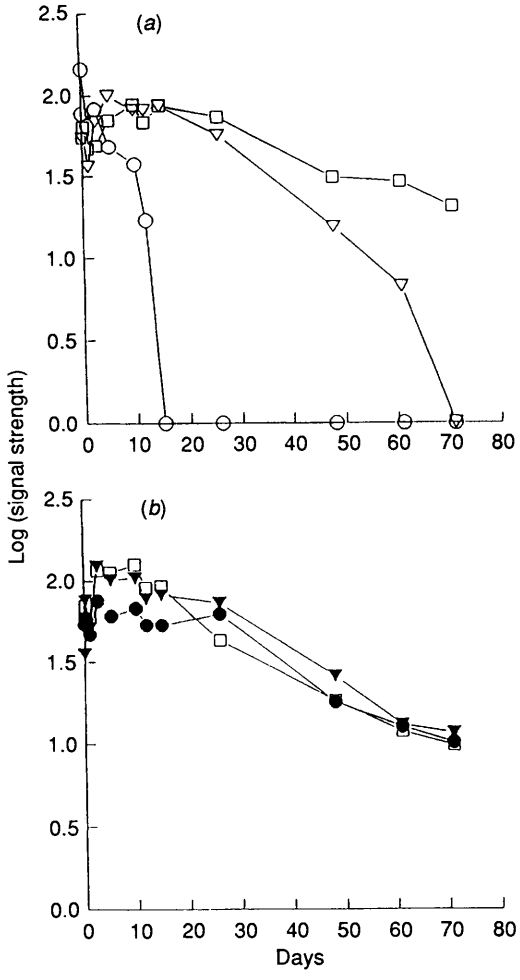


Fig. 4 Stability of the trichloromethyl PBN spin adduct EPR spectrum as a function of pH in the absence of light: (*a*) □, pH 6.6; ▽, pH 6.0; ○, pH 5.0; (*b*) □, pH 7.8; ▼, pH 7.4; ●, pH 7.0

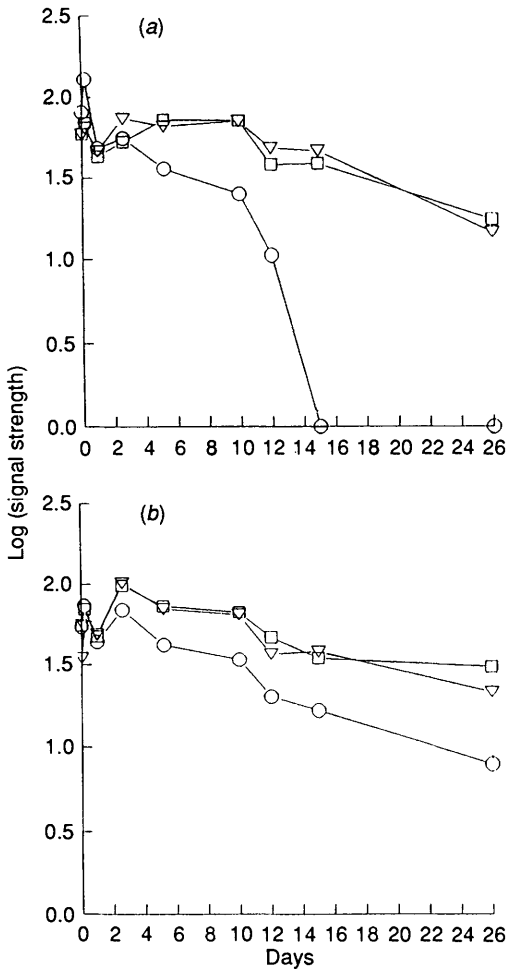


Fig. 5 Stability of the trichloromethyl PBN spin adduct EPR spectrum as a function of pH in the presence of light; note the different timescale as compared with Fig. 4: (*a*) □, pH 6.6; ▽, pH 6.0; ○, pH 5.0; (*b*) □, 7.8; ▽, pH 7.4; ○, pH 7.0

Stability to Mild Oxidizing Agents.—The trichloromethyl–PBN adduct appears to be stable to mild oxidizing agents. Bubbling oxygen through a solution of potassium ferricyanide or cupric sulfate in 60:40 acetonitrile–water containing the trichloromethyl–PBN adduct produced no noticeable change in the intensity of the EPR spectrum of the spin adduct. There was no effect on EPR signal strength when 30% hydrogen peroxide in water or solid lead dioxide was added to a benzene solution of the trichloromethyl PBN adduct. However, in acetonitrile–water, these reagents caused a slow decay of the EPR signal.

Stability to Reduction.—The trichloromethyl–PBN spin adduct is stable to ascorbate in acetonitrile–water solution for 1 h. No hydroxylamine is found by HPLC. When reduction was attempted using sodium dithionite or ferrous sulfate, no decrease in signal was found when the adduct was dissolved in benzene. However, in aqueous solutions of acetonitrile, some decrease in EPR signal could be detected.

When the trichloromethyl adduct of PBN was introduced into rat liver microsomes and NADPH added, the extract did not give rise to the EPR signal of the adduct. By HPLC, it could be determined that a new peak at very long retention time was produced using EC detection. This peak was assigned to the hydroxylamine on the basis of the fact that, when isolated, this fraction did not have an EPR signal, but produced the trichloromethyl spin adduct spectrum after oxidation with potassium ferricyanide and oxygen. The HPLC traces in Fig. 6 show the peaks due to only the spin adduct and the hydroxylamine by EC detection; PBN is not EC-active.

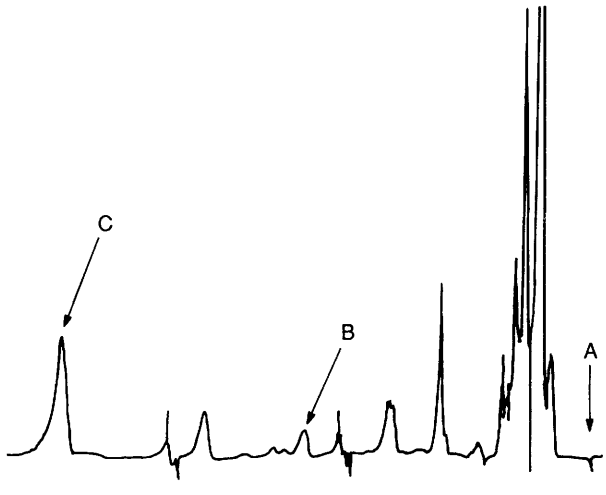


Fig. 6 HPLC trace from 60:40 acetonitrile–water containing 0.02 mol dm^{-3} phosphate buffer at pH 7.4 and extract from rat liver microsomes treated with the trichloromethyl adduct of PBN: A, start; B, trichloromethyl PBN adduct, t_R = 17.6–17.8 min; C, trichloromethyl PBN adduct hydroxylamine, t_R = 32.2–32.9 min

Discussion

Origin of EPR Spectra.—There are three published methods describing the production of the trichloromethyl spin adduct of PBN.

(*i*) Photolysis of carbon tetrachloride or bromotrichloromethane[43] [eqn. (9)].

$$CCl_4 \xrightarrow{h\nu} \cdot CCl_3 + Cl\cdot \qquad (9)$$

(*ii*) Electrolytic reduction of carbon tetrachloride or bromotrichloromethane[21] [eqn. (10)].

$$CCl_4 \xrightarrow{e(^-)} \cdot CCl_3 + Cl^- \qquad (10)$$

(*iii*) Rat liver metabolism of carbon tetrachloride or bromotrichloromethane[44] [eqn. (11)].

$$CCl_4 \xrightarrow{\text{Cy-P-450}} \cdot CCl_3 + Cl^- \qquad (11)$$

During the photolysis of CCl_4 the chlorine atom spin adduct is observed as expected. However, this spin adduct is not persistent in organic solvents. Decay is even faster in polar solvents such as water.[45] PBNOX is also observed when carbon tetrachloride is photolysed. This aminoxyl appears whether dioxygen is completely removed from the solvent or not. When oxygen is present, there is an appropriate explanation. Trichloromethyl radicals reacting with O_2 should produce peroxyl radicals[46] which, when trapped by PBN, give unstable spin adducts, eqn. (12).[34b]

$$Cl_3COO\cdot + PBN \longrightarrow PhC(\cdot O)(OOCCl_3)NHC_4H_9 \qquad (12)$$

At room temperature, the peroxyl spin adduct is expected to give PBNOX, eqn. (13). Trichloromethanol ($HOCCl_3$) will

$$PhC(\cdot O)(OOCCl_3)NHC_4H_9 \longrightarrow PhC(=O)NC_4H_9(\cdot O) + HOCCl_3 \qquad (13)$$

lead to phosgene (Cl_2CO) and HCl.

Another possibility is that the oxidized form of PBN, namely the PBN radical cation may react with the oxygen atom of the imine oxide in PBN [eqns. (14) and (15)].

$$Cl\cdot + PhCH{=}N(O)C_4H_9 \longrightarrow PhCH(\cdot O)-N^+-C_4H_9 + Cl^- \qquad (14)$$

$$PhCH{=}N(O)C_4H_9 + PhCH(\cdot O)-N^+-C_4H_9 \longrightarrow PhC(H)(O)-N-C_4H_9 + PhCH(\cdot O)-N^+-C_4H_9 \longrightarrow PhC(=O)-N-C_4H_9 + PhCH{=}N(O)C_4H_9 \qquad (15)$$

In the complete absence of dioxygen or water in a non-polar solvent another explanation is required. PBN can serve as the oxygenation agent *via* the 'Kornblum reaction'.[47] Benzyl halides react quantitatively with compounds such as Me_2SO or pyridine *N*-oxides to give benzaldehyde, dimethyl sulfide or pyridine and the mineral acid, eqn. (16). Since PBN can be considered an imine *N*-oxide and the chlorine atom adduct is certainly a reactive benzyl halide (since the neighbouring aminoxyl function will enhance nucleophilic displacement

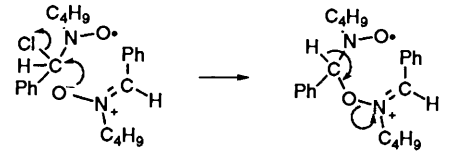
$$CH_3S(O)CH_3 + PhCH_2Br \longrightarrow PhCHO + CH_3SCH_3 + HBr \qquad (16)$$

reactions analogous to the case of α-halocarbonyl functions) we propose the reaction (17) to account for the production of

$$PhC(\cdot O)(Cl)NC_4H_9 + PhCH{=}N(O)C_4H_9 \longrightarrow PhC(=O)NC_4H_9(\cdot O) + PhCH{=}NC_4H_9 + HCl \qquad (17)$$

PBNOX during the photolysis of CCl_4 in dry oxygen-free benzene.

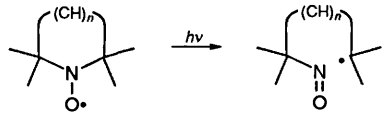
The proposed intermediate suggested arises as shown in reaction (18). Other mechanisms for PBNOX production have been suggested.[48]



Stability of the Trichloromethyl–PBN Spin Adduct.—The trichloromethyl adduct of PBN is clearly a very stable spin adduct in various solvents. Why the stability in some non-polar solvents such as hexane is less than in benzene is not known. Perhaps solubility is a factor. In hexane, molecular clusters may form which could decompose faster than monomers. A predimerization step has been proposed for the decomposition of certain aminoxyls[49] which decay by second-order kinetics. It is known that spin adduct signals decay when extracts are evaporated down to very small amounts or to 'dryness'.[50] This phenomenon may be related to a second-order dimerization decay mechanism. In water, the disappearance of the trichloromethyl–PBN adduct is still very slow although faster than in organic solvents. The mechanism of decay is not known although homolytic cleavage or hydrolysis by nucleophilic displacement is possible. Since the rate is more rapid at lower pH, proton-assisted decomposition might be involved [reaction (19)].

$$PhCH(CCl_3)NC_4H_9(\cdot O) \rightleftharpoons PhCH(CCl_3)NC_4H_9(O^-) + H^+ \rightleftharpoons PhCH(CCl_3)NC_4H_9(OH) \longrightarrow PhCH(CCl_3) + NC_4H_9(OH) \xrightarrow{H_2O} PhCH(OH)CCl_3 + H^+ + HNC_4H_9(\cdot O) \qquad (19)$$

Light is probably the most detrimental factor in the stability of the trichloromethyl–PBN spin adduct. Whether this is a characteristic of all spin adducts in general or a feature of the trichloromethyl–PBN spin adduct alone is not known. Solvents such as benzene or toluene may protect spin adducts from UV absorption. The photolysis of spin labels is believed to involve α-cleavage reactions of aminoxyl[51,52] [eqn. (20)]. In the



cyclic aminoxyl, some thermal return would be expected to provide back some unchanged aminoxyl, but in the PBN-type

spin adducts photolytic cleavage may be irreversible [eqn. (21)].

$$PhCH(CCl_3)\overset{O\bullet}{\underset{|}{N}}C_4H_9 \xrightarrow{h\nu} Ph\dot{C}H(CCl_3) + \overset{O}{\underset{||}{N}}C_4H_9 \quad (21)$$

Redox Stability of the Trichloromethyl PBN Spin Adduct.— The trichloromethyl PBN adduct seems to be very resistant to oxidation. Since the β-hydrogen is both tertiary and benzylic, it might be expected to be vulnerable to oxidation. The trichloromethyl substituted nitrone mentioned earlier should be formed. However, no evidence for this type of oxidation has been found to date.

Reduction is also not observed with chemical reducing agents. The fact that ascorbate does not kill the EPR signal of the trichloromethyl PBN spin adduct makes this aminoxyl unique within this family of molecules. This puzzling result is interesting and obviously not easy to explain. The electron-attracting ability of the trichloromethyl group should make the aminoxyl function easier to reduce compared with other alkyl adducts of PBN but just the opposite case is found. The only other feature to which this stability can be attributed is steric hindrance. The bulky trichloromethyl group may prevent close approach of the reducing molecule and thus stabilize the aminoxyl towards reduction.

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