EPR and mass spectroscopic identification of radical adducts produced spontaneously from reactions of phosgene, chlorine gas and bromine with *C*-phenyl *N*-*tert*-butyl nitrone (PBN)

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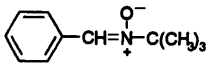
The toxic gas phosgene reacts with the spin trap *C*-phenyl *N*-*tert*-butyl nitrone‡ (PBN) to produce spontaneously *N*-*tert*-butyl-*N*-chloroacylaminoxyl as the main radical product identified by EPR and mass spectrometry. The reaction mechanisms for the formation of N-*tert*-butyl-*N*-chloroacylaminoxyl and other radicals are proposed. Reactions of chlorine gas and bromine with PBN have also been investigated. First α-chloro- or α-bromo-PBN is formed followed by chlorination or bromination of the aryl ring of these compounds, respectively.

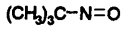
Introduction

The metabolism of CCl_4 *in vitro* and *in vivo* has been investigated intensively. In the study of the toxicity of CCl_4, phosgene (carbonic acid chloride, or carbonyl dichloride, $COCl_2$) was considered to be produced during the aerobic metabolism of CCl_4 to CO_2 in rat liver microsomes,[1] or by rat liver homogenate.[2] It was suggested that the trichloromethyl radical ($Cl_3C^{\cdot}$) derived from the metabolism of CCl_4 reacted with oxygen to form the $Cl_3COO^{\cdot}$ radical, which in further steps produced $COCl_2$ and CO_2.[3] Therefore the local oxygen concentration at the site of CCl_4 activation was considered to be of crucial importance in the subsequent formation of $COCl_2$. The pathway of $COCl_2$ formation proposed by Slater *et al.*[4] is shown in Scheme 1.

However all the steps shown in Scheme 1 are speculative. Only one intermediate shown in Scheme 1 has been definitely identified by EPR spectroscopy, namely the trichloromethyl radical. Supportive evidence comes from PBN spin trapping[5,6] and from inspection of mass spectral data of trichloromethyl adducts of unsaturated lipids.[7]



PBN

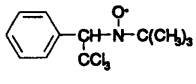


MNP

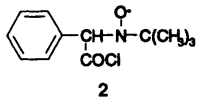
By assuming that trichloromethyl radicals react with oxygen to form trichloromethylperoxyl radicals, the relative reactivity of this radical has been evaluated by pulse radiolysis.[3,8] In a chemical system we have found chlorine atoms are produced when trichloromethyl radicals are exposed to oxygen implying that the trichloromethoxyl radicals are formed and that these alkoxyl radicals undergo β-scission to form phosgene and chlorine atoms as in eqn. (1).[9,10] In addition to the

$$Cl_3CO^{\cdot} \longrightarrow Cl^{\cdot} + Cl_2CO \qquad (1)$$

trichloromethyl radical adduct (**1**), the chloroacyl adduct of PBN (**2**) has also recently been detected by mass spectrometry (MS) from rat liver microsomal metabolism of CCl_4 or $BrCCl_3$ in the presence of a NADPH-generating system and PBN.[11]

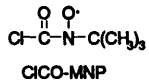


1



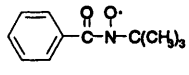
2

The chloroacyl adduct of MNP (**3**) has also been detected in the UV irradiation of dichromate in $CHCl_3$[12] and γ-radiolysis of CCl_4[13] in the presence of oxygen and 2-methyl-2-nitrosopropane (MNP). Presumably in both cases the trichloromethyl



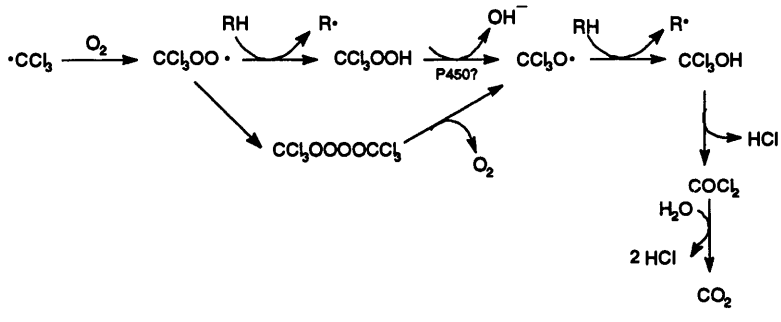
ClCO-MNP

3



PBNOX

4



Scheme 1

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‡ IUPAC name: *N*-benzylidene-*tert*-butylamine *N*-oxide.

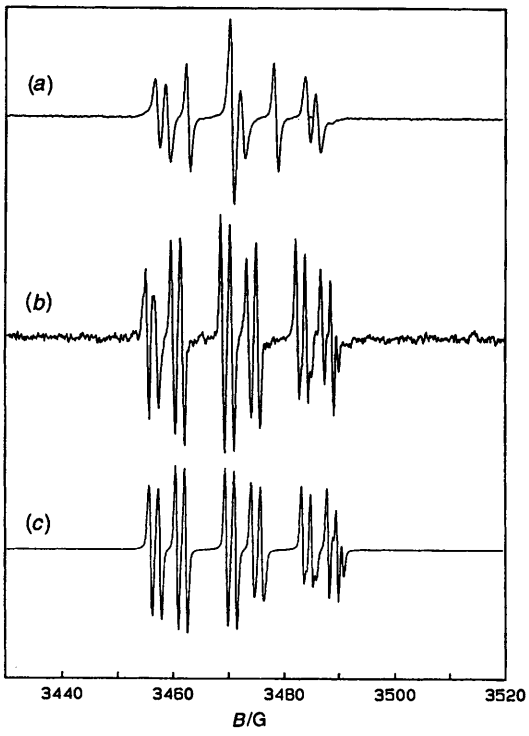


Fig. 1 EPR spectrum recorded from the reaction of a few bubbles of phosgene and (*a*) PBN; (*b*) [α-^{13}C,^{2}H$_9$]PBN in hexane. (*c*) Computer simulation of (*b*). The parameters used for this simulation are: PBN adduct **1**, a_N = 13.72 G, $a_{\beta H}$ = 1.70 G, $a_{\alpha\text{-}^{13}C}$ = 4.70 G, line width (1w) = 0.6 G; for PBN adduct **2**, a_N = 14.20 G, $a_{\beta H}$ = 1.70 G, $a_{\alpha\text{-}^{13}C}$ = 5.0, 1w = 0.6 G, ΔG = 0.20 G. The ratio of their intensity is 10:4.

radical is formed first which in the presence of oxygen produces the peroxyl radical. Two trichloromethylperoxyl radicals eventually become phosgene as shown in Scheme 1. However the mechanism of formation of chloroacyl radicals from phosgene is not known.

We have pointed out that the combination of peroxyl radicals need not necessarily be symmetrical[9] *i.e.* unsymmetrical peroxyl radicals could combine as well and this could lead to lipid alkoxyl radicals during CCl_4 enhanced lipid peroxidation as shown in eqn. (2).

$$Cl_3COO\text{–}OOL \longrightarrow Cl_3CO^{\bullet} + O_2 + {}^{\bullet}OL \qquad (2)$$

Since PBN is often used to study the metabolism of halocarbons of various structure,[14–23] and in addition prevents liver damage initiated by CCl_4,[24–25] it was of interest to see whether phosgene itself reacts with PBN. Phosgene is very toxic and is believed to be an end product in CCl_4 and $CHCl_3$ metabolism.[26,27] The protective action demonstrated by PBN might be accounted for in part by its reaction with phosgene. Both EPR and mass spectral techniques were used to investigate this question and the results are presented in this paper.

Results

EPR results

When 30 mM PBN is reacted with a few bubbles of phosgene gas in hexane, the initial radical products detected by EPR are benzoyl-*tert*-butylaminoxyl (PBNOX, **4**) and an O-centred radical adduct [spectrum shown in Fig. 1(*a*)].

The hyperfine splitting constant (hfsc) of PBNOX is a_N = 7.89 G. The hfscs of the O-centred radical adduct are a_N = 13.72 G, $a_{\beta H}$ = 1.70 G. The α-^{13}C hfsc of this oxygen-centred radical adduct obtained by using [α-^{13}C, ^{2}H$_9$]PBN[28] instead of PBN [Fig. 1(*b*)] is 4.70 G. A computer simulation of the

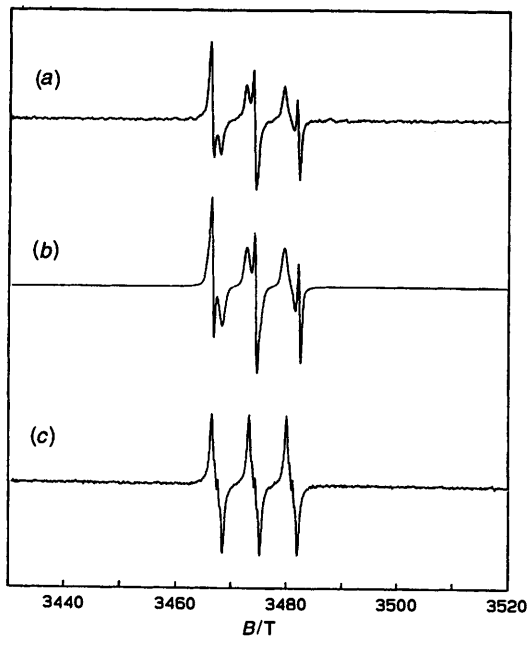


Fig. 2 EPR spectrum recorded from the reaction of a larger amount of phosgene and (*a*) PBN; (*c*) [α-^{13}C,^{2}H$_9$]PBN in hexane. (*b*) Computer simulation of (*a*). The parameters used for this simulation are: radical adduct **1**, a_N = 6.79 G, a^{35}_{Cl} = 0.60 G, 1w = 1.0 G; for radical **2**, a_N = 7.89 G, 1w = 0.5 G, ΔG = −0.50 G. The ratio of their intensity is 6:1.

spectrum in Fig. 1(*b*) reveals the contribution of another spin adduct; its hfscs are a_N = 14.20 G, $a_{\beta H}$ = 1.70 G, $a_{\alpha\text{-}^{13}C}$ = 5.00 G. These values are also characteristic of an O-centred radical spin adduct. A 10:4 ratio of these two O-centred radical spin adducts was used to get the best fit for the simulated spectrum. The yield of PBNOX depends on the concentration of phosgene and O_2. Sometimes only very small amounts of PBNOX are detected.

When PBN reacts with larger amounts of phosgene, a different EPR spectrum is observed as shown in Fig. 2(*a*). This spectrum consists of two radicals: PBNOX and a radical with hfsc a_N = 6.79 G. Again the observation of PBNOX depends on the amount of O_2 involved in the reaction and the concentration of phosgene; sometimes no PBNOX was detected. These two radicals are the final radical products obtained in this reaction when larger amounts of phosgene are used. Because of the limitations associated with phosgene studies, extensive investigations into the oxygen effect could not be performed.

When [^{2}H$_{14}$]PBN or [α-^{13}C, ^{2}H$_9$]PBN[29] was used instead of PBN, the radical with hfsc a_N = 6.79 G shows further hyperfine splitting [Fig. 2(*c*)]. This radical could be assigned[12,13] to the chloroacyl-MNP adduct **3**. A small hfsc (I = 3/2) of 0.60 G is attributed to the β-^{35}Cl. Because no splitting from α-^{13}C was observed when PBN was replaced by [α-^{13}C, ^{2}H$_9$]PBN [Fig. 2(*c*)], the carbonyl group in the structure of this radical must originate from the phosgene molecule not from PBN. The spectra in Fig. 2 are quite stable in the reaction system and can survive for a few days. The computer simulation of spectrum 2(*a*) is in good agreement with the experimental result [see Fig. 2(*b*)]. The triplet of PBNOX becomes a triplet of doublets after using [α-^{13}C, ^{2}H$_9$]PBN; the hfsc of α-^{13}C in PBNOX is 4.80 G (spectrum not shown).

When benzene was used as a solvent, the same final radical products were observed by EPR spectroscopy as when hexane was used as a solvent. In addition to PBNOX and ClCO-MNP, sometimes EPR evidence showed formation of the chlorine atom adduct of PBN, **5**. The hfscs for the Cl-PBN observed are

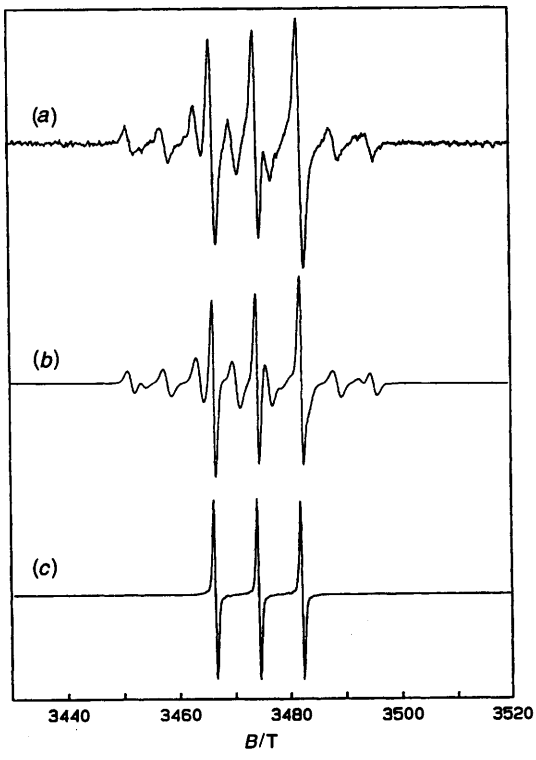
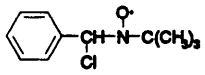


Fig. 3 (*a*) EPR spectrum obtained from the reaction of phosgene and PBN in benzene. (*b*) Computer simulation of (*a*). The parameters used for the simulation are: PBN adduct **1**, a_N = 12.31 G, a^{35}_{Cl} = 6.36 G, a^{37}_{Cl} = 5.00 G, $a_{\beta H}$ = 0.75, lw = 1.4 G; for radical **2**, a_N = 7.89 G, lw = 0.8 G, ΔG = 0.88G. The ratio of their intensity is 8:5. (*c*) EPR spectrum obtained from the reaction of Cl_2 and PBN in hexane.

a_N = 12.31 G, $a_{\beta^{35}Cl}$ = 6.36 G, $a_{\beta^{37}Cl}$ = 5.00 G, $a_{\beta H}$ = 0.75 G [Fig. 3(*a*)], which are very close to the literature values.[13,30] The EPR spectrum shown in Fig. 3(*a*) was recorded without degassing because the Cl-PBN radical adduct is very short-lived.



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Since the decomposition of phosgene may produce chlorine gas, the reaction between chlorine gas and PBN was also investigated by EPR spectroscopy. When 30 mM PBN was reacted with a few bubbles of Cl_2 gas in hexane or in benzene, only the three lines due to PBNOX were observed by EPR spectroscopy. With a larger amount of Cl_2, EPR spectra still showed that PBNOX is the only radical product formed [see Fig. 3(*c*)]. This result excludes the possibility that the reaction of PBN and Cl_2 produces a detectable amount of COCl-MNP from some unknown precursors.

As an analogue to chlorine gas, bromine also reacts with PBN to produce PBNOX as the only detectable radical product (spectrum not shown).

MS results

MS was used to search for other products besides the radicals produced from PBN–phosgene reactions. The precursor ion spectrum of *m/z* 57 (*tert*-butyl ion, $C_4H_9^+$) recorded from the reaction system of PBN and phosgene in hexane [Fig. 4(*a*)] shows isotopic peaks at *m/z* 151 and 153, which are assigned to the protonated molecular ions [M + 1] of **3** [eqn. (3)]. When [$^2H_{14}$]PBN or [α-^{13}C,$^2H_9$]PBN were used instead of PBN, a shift of 9 daltons§ is observed in the parent ion spectrum of *m/z* 66 [deuteriated *tert*-butyl ion, $C_4D_9^+$, Fig. 4(*b*)], which confirms that the proposed structure contains a *tert*-butyl group.

§ 1 Da ≈ 1.66054 × 10^{-27} kg.

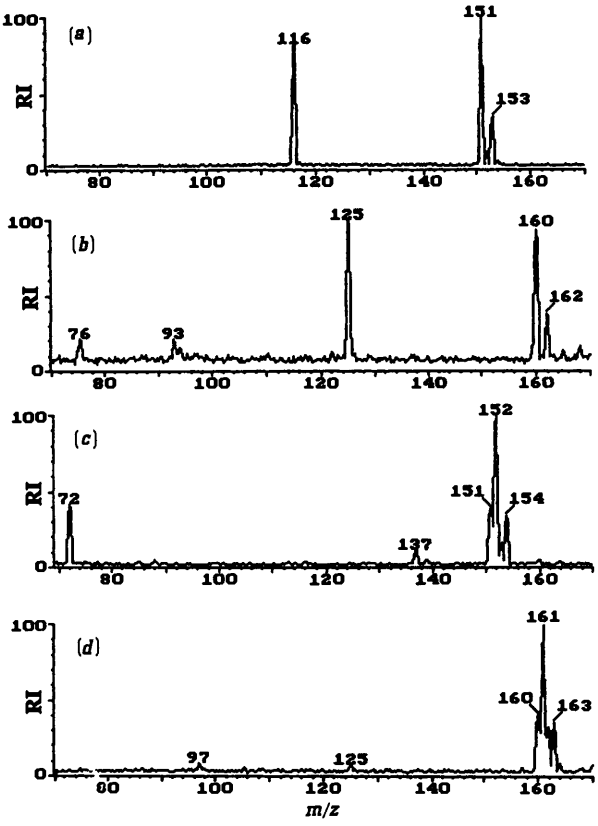
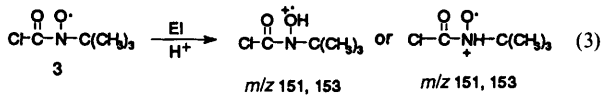
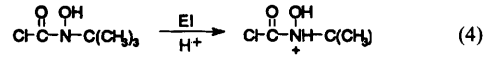


Fig. 4 (*a*) Parent ion spectrum of *m/z* 57 recorded from the reaction of phosgene and PBN in hexane. (*b*) Parent ion spectrum of *m/z* 66 recorded from the reaction of phosgene and [α-^{13}C,$^2H_{14}$]PBN in hexane. (*c*) Parent ion spectrum of *m/z* 57 recorded from the same sample of (*a*), but the reaction mixture was concentrated by removing some of solvent before MS measurement. (*d*) Parent ion spectrum of *m/z* 66 recorded from the same sample as (*b*), but the reaction mixture was concentrated by removing some of solvent before MS measurement.



(3)

3



(4)

6

After the sample was concentrated under N_2 by removing some of solvent, the parent ion spectrum of *m/z* 57 shows two sets of isotopic peaks at *m/z* 151 and 153, and *m/z* 152 and 154, respectively [Fig. 4(*c*)]. The isotopic peaks at *m/z* 152 and 154 might be due to the protonated molecular ions [M′ + 1] of the hydroxylamine of the ClCO–MNP radical, **6** [eqn. (4)]. These two peaks also shift 9 daltons from *m/z* 152 and 154 to *m/z* 161 and 163 after using [α-^{13}C, $^2H_9$]PBN or [$^2H_{14}$]PBN [Fig. 4(*d*)].

Reactions of PBN and Cl_2 were also investigated by MS. Fig. 5(*a*) is the reconstructed total ion current (TIC) gas chromatogram recorded from the reaction system of PBN and Cl_2 in hexane. The peak at retention time *t* = 9.82 min is due to the unreacted PBN. The peak at *t* = 9.44 min is assigned to α-chloro-PBN, **7**, based on the observation of isotopic peaks at *m/z* 211 and 213 in its corresponding mass spectrum [Fig. 5(*b*)]. This assignment was supported by the [$^2H_{14}$]PBN experiments. When [$^2H_{14}$]PBN was used instead of PBN, isotopic peaks at *m/z* 211 and 213 shift 14 daltons to *m/z* 225 and 227 [Fig. 5(*e*)],

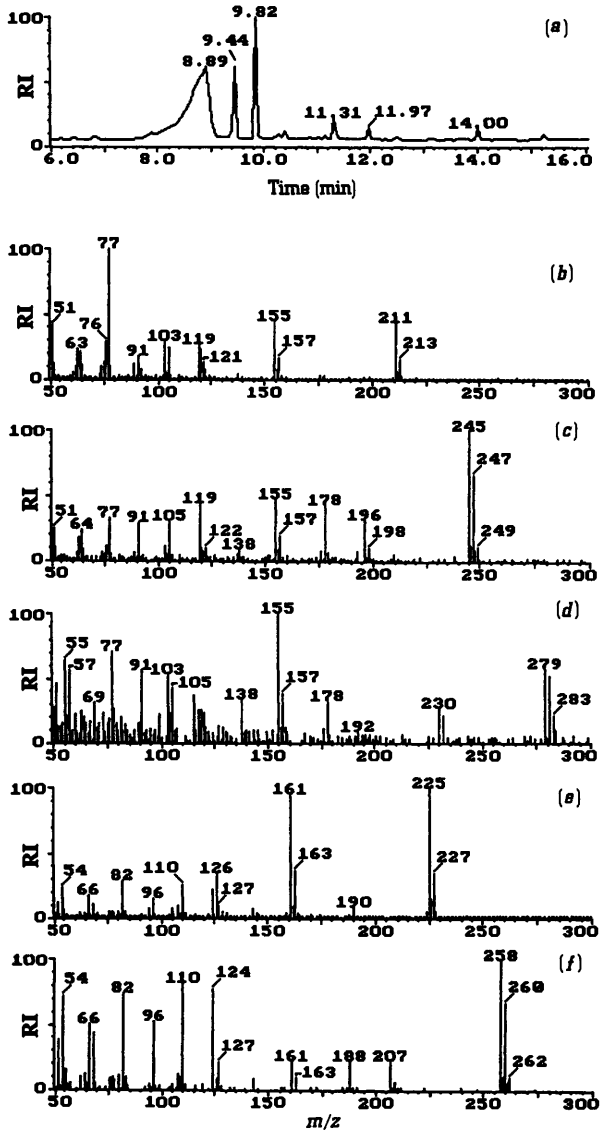
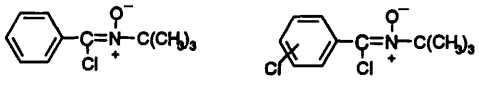


Fig. 5 (*a*) The reconstructed TIC gas chromatogram obtained from the reaction of Cl_2 and PBN in hexane. (*b*) The mass spectrum at retention time t = 9.44 min. (*c*) The mass spectrum at retention time t = 11.97 min. (*d*) The mass spectrum at retention time t = 14.00 min. (*e*) The mass spectrum at retention time t = 9.44 min when [$^2H_{14}$]PBN was used instead of PBN. (*f*) The mass spectrum at retention time t = 11.97 min when [$^2H_{14}$]PBN was used instead of PBN.

which indicates that the α-H of PBN was substituted by a chlorine atom, because substitution of a deuterium from either a deuteriated *tert*-butyl group or deuteriated phenyl group would cause a 13 daltons change.



7 *m/z* **211, 213**

8 *m/z* **245, 247, 249**

The peak at t = 11.97 min is assigned to α-chloro *C*-chlorophenyl *N*-*tert*-butyl nitrone, **8**, based on the analysis of its mass spectrum [Fig. 5(*c*)]. The isotopic peaks at *m*/*z* 245, 247, 249 represent a molecular ion containing two chlorine atoms and is the appropriate molecular ion of product **8**. A 13 daltons change of the isotopic peaks from *m*/*z* 245, 247 and 249 to *m*/*z* 258, 260 and 262 [Fig. 5(*f*)] after using [$^2H_{14}$]PBN suggests a deuterium is substituted by a chlorine. It is thus concluded that a deuterium on the phenyl ring is replaced with a chlorine atom

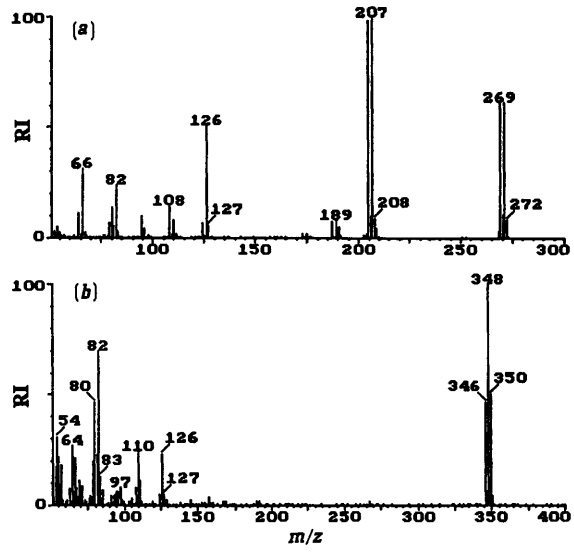
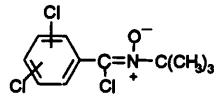


Fig. 6 The mass spectrum at retention time (*a*) t = 10.15 min; (*b*) t = 13.45 min obtained from the reaction of Br_2 and [$^2H_{14}$]PBN in hexane

based on analysis of the fragments in the mass spectrum. The position of the aryl chlorine is not known.

The formation of another ring substituted compound namely, α-chloro *C*,*C*-dichlorophenyl *N*-*tert*-butyl nitrone, **9**, was also observed at t = 14.00 min. The isotopic peaks at *m*/*z* 279, 281, 283 and 285 [Fig. 5(*d*)] are appropriate molecular ions of product **9**. The mass spectra show that two deuteriums



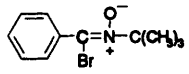
9

m/z **279, 281, 283, 285**

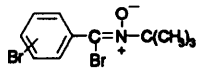
on the phenyl ring are replaced by two chlorine atoms for the formation of product **9** since a 12 daltons change of the isotopic peaks from *m*/*z* 279, 281, 283 and 285 to *m*/*z* 291, 293, 295 and 297 is observed after using [$^2H_{14}$]PBN. The positions of these aryl chlorines are not known.

The ion at *m*/*z* 193, which is the protonated molecular ion of PBNOX, was observed in the direct-probe parent ion spectrum of *m*/*z* 57 (spectrum not shown).

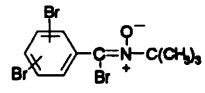
Similar to Cl_2, the mass spectrometry results show that Br_2 also reacts with PBN to produce α-bromo-PBN, **10**, α-bromo C-bromophenyl *N*-*tert*-butyl nitrone, **11** and α-bromo *C*,*C*-dibromophenyl *N*-*tert*-butyl nitrone, **12**. These assignments are supported by the [$^2H_{14}$]PBN experiments.



10 *m/z* **255, 257**



11 *m/z* **333, 335, 337**



12

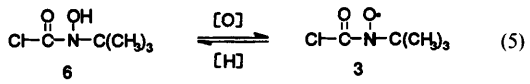
m/z **411, 413, 415, 417**

Fig. 6(*a*) is the mass spectrum at GC retention time t = 10.15 min. The one bromine-isotopic peaks (with 1 : 1 relative intensity) at *m*/*z* 269 and 271 are due to the molecular ions of α-bromo-[$^2H_{14}$]PBN, **10**. Fig. 6(*b*) is the mass spectrum at the

retention time t = 13.45 min. The observation of two bromine-isotopic peaks (with relative intensity about 1:2:1) at m/z 346, 348, 350 when [$^2H_{14}$]PBN was used indicates the formation of product **11**. The molecular ions of product **12** are observed in the direct-probe mass spectrum at m/z 423, 425, 427 and 429. The protonated molecular ion of PBNOX is also observed at m/z 193 in the direct-probe mass spectrum (data not shown) when [$^2H_{14}$]PBN was used.

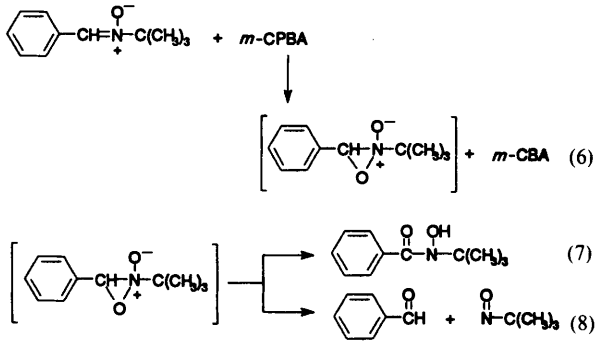
Discussion

The results in this paper show that a major product generated from phosgene and PBN and detected by EPR and mass spectrometry is the *N-tert*-butyl-*N*-chloroacylhydroxylamine and the corresponding aminoxyl radical as shown in eqn. (5).



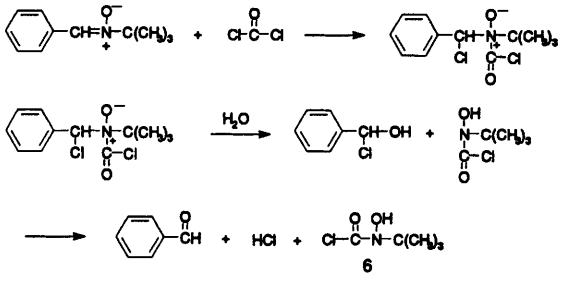
(5)

The acyl carbon comes from phosgene and not from the PBN nitronyl carbon (as shown from [α-^{13}C]PBN experiments). If this was the case, the reaction would resemble the reaction between PBN and *m*-chloroperbenzoic acid (*m*-CPBA) as shown in eqns. (6)–(8).[31]



(6) (7) (8)

The mechanism that is suggested is shown in Scheme 2. The nitronyl function in PBN is considered to be a nucleophile with bonding to the phosgene carbon coming from nitrogen. This adduct dissociates *via* an unknown mechanism (perhaps requiring water) to the hydroxylamine.

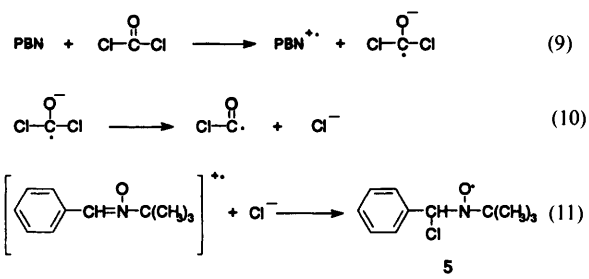


Scheme 2

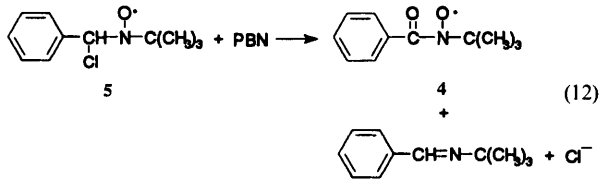
But radicals are formed simply by mixing the reagents without any apparent need for water and the EPR spectra depend on the presence of oxygen. In particular PBNOX is formed in addition to **3**. PBNOX is known to form from peroxyl spin adducts and from spin trapping chlorine atoms. Therefore the intermediacy of the chloroacyl radical suggests itself. Initial electron transfer oxidation of PBN could form the radical cation and the phosgene radical anion [eqns. (9)–(11)].[32]

However, the feasibility of this reaction depends on the relative redox potentials of the two components, namely PBN and phosgene.

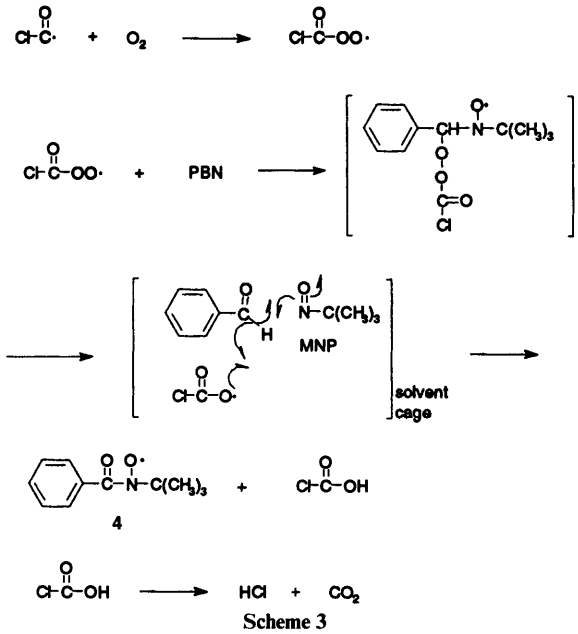
Since the chlorine atom adduct is sometimes detected this



interpretation is consistent with the results observed. Also PBNOX **4** is formed from the chlorine atom adduct of PBN in the presence of large amounts of PBN [eqn. (12)].[33]

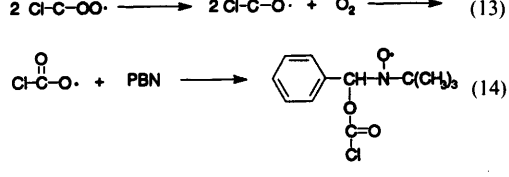


The influence of oxygen on the reaction could be exerted through peroxyl radical formation (see Scheme 3). The



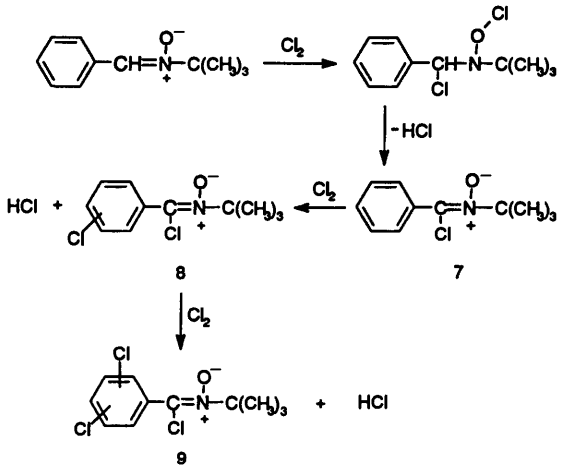
Scheme 3

chloroacylperoxyl radical should be trapped and the spin adduct would produce PBNOX through cleavage reactions. The interesting chloroacyloxyl radical could also be formed. Whether this radical has a lifetime long enough to be spin trapped is not known. Decarboxylation is expected to be very fast producing chlorine atoms and carbon dioxide. If the lifetime is long enough the oxygen-centred radical spin adduct detected might originate in this way [eqns. (13) and (14)].



(13) (14)

A puzzling point is the lack of the chloroacyl adduct of PBN in this study at least as evaluated by EPR spectroscopy. In a rat liver microsomal metabolism of $BrCCl_3$ or CCl_4 we have



Scheme 4

reported MS data showing that the chloroacyl radical is trapped by PBN but no EPR spectrum could be assigned to that spin adduct at the time.[11]

The first step of the reaction between PBN and Cl_2 is considered to be a simple addition reaction, followed by the loss of a molecule of hydrogen chloride to form the α-chloro-PBN (Scheme 4). Chlorine gas then reacts with α-chloro-PBN to form ring substituted products **8** and **9**. It is known that aromatic compounds can be chlorinated by treatment of chlorine in the presence of a catalyst, most often iron, and for active substrates, including amines, phenols, naphthalene and polyalkylbenzenes such as mesitylene and isodurene, no catalyst is needed.[34] Because the two ring substituted compounds are the chlorinated products of α-chloro-PBN and no ring chlorinated compounds of PBN were detectable by MS, the phenyl ring of α-chloro-PBN may be more active than PBN in the electrophilic reaction with chlorine.[35]

The reaction mechanism of PBN and bromine is considered to be the same as that of PBN and chlorine gas.

Conclusions

PBN reacts with phosgene to produce the chloroacyl-MNP and PBNOX as the main products. Perhaps liver tissue damage caused by CCl_4 or $CHCl_3$ *in vitro* or *in vivo* is prevented by PBN, in part, by removal of the very toxic metabolite phosgene. The extent to which PBN protects liver tissue by trapping free radicals and/or by reacting with toxic substances like phosgene in a molecular reaction is not known.

Experimental

Materials

PBN, [$^2H_{14}$]PBN, [α-^{13}C,$^2H_9$]PBN were purchased from OMRF Spin Trap Source (825 N. E. 13th. Street, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104). Phosgene was purchased from Matheson. It is a colourless, extremely poisonous liquid and gas under pressure.[36] Phosgene may be fatal if inhaled. Store and use with adequate ventilation. Do not breathe the gas. Liquid and gas are dangerous to eyes, skin and lungs. It may cause delayed lung damage. Cl_2 was produced by thermolysis of $CuCl_2$. Br_2 was purchased from Aldrich.

Reaction

Phosgene gas or Cl_2 was bubbled through a very thin tube into a 30 mM PBN solution in hexane or benzene. 100 mM Br_2 was mixed with 30 mM PBN in hexane at room temperature. After 0.5 h, the reaction mixture was bubbled with nitrogen gas to remove the unreacted Br_2.

EPR measurements

EPR spectra were measured with a Bruker ER300 spectrometer with 100 kHz field modulation. Round quartz sample cells with 3.5 mm id were employed.

Mass spectrometric analysis

EI mass spectra were obtained with a VG-Fisons Quattro triple stage quadrupole mass spectrometer. A direct insertion probe was used. Source temperature was 180 °C. The electron energy employed was 70 eV. The probe temperature was about 40–50 °C. Argon gas was used for the collision induced dissociation (CID). The collision energy was 25 ev. GC–MS spectra were recorded with a Hewlett-Packard 5890 series II GC interfaced directly with the VG-Fisons Quattro triple stage quadrupole mass spectrometer. Chromatographic separation was carried out with a Quadrex Corporation 0.25 mm × 30 m methyl 5% phenyl fused silica column with temperature programmed from 55 to 220 °C at 12 °C min^{-1}. Helium was the carrier gas.

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