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USAMRICD-TR-96-02

The Chemistry of Perfluoroisobutylene
(PFIB) with Nitron and Nitroso Spin
Traps. An EPR/Spin Trapping Study

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May 1996

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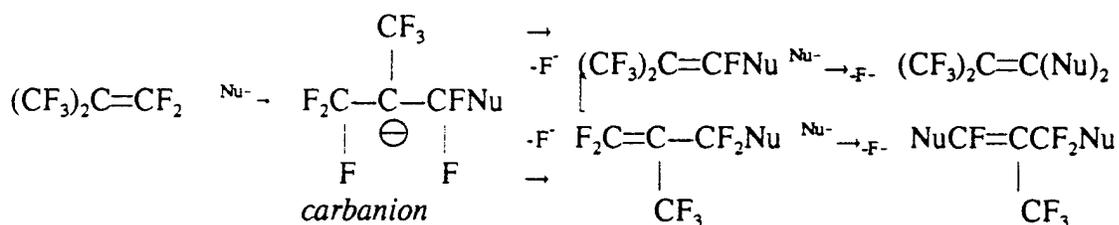
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REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
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1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE May 1996	3. REPORT TYPE AND DATES COVERED Technical Report	
4. TITLE AND SUBTITLE The Chemistry of Perfluoroisobutylene (PFIB) with Nitron and Nitroso Spin Traps. An EPR/Spin Trapping Study.			5. FUNDING NUMBERS TA - E, A 61102A 3M161102BS11	
6. AUTHOR(S) Carmen M. Arroyo				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) U.S. Army Medical Research Institute of Chemical Defense 3100 Ricketts Point Road Aberdeen Proving Ground, MD 21010-5425 ATTN: MCMR-UV-DA			8. PERFORMING ORGANIZATION REPORT NUMBER USAMRICD-TR-96-02	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research Institute of Chemical Defense 3100 Ricketts Point Road Aberdeen Proving Ground, MD 21010-5425 ATTN: MCMR-UV-RC			10. SPONSORING/MONITORING AGENCY REPORT NUMBER USAMRICD-TR-96-02	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Distribution A Approved for public release; distribution unlimited.			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) Applying EPR/Spin Trapping techniques, several reactive intermediate species were identified in the reaction of perfluoroisobutylene (PFIB) with nitron and nitroso spin trap agents: the carbon dioxide radical anion (CO ₂ ⁻), a carbonyl fluoride intermediate (COF), and vinyl carbanions of PFIB. The reaction of PFIB with N-t-butyl-α-phenylnitron (PBN) forms a dipolar ion which undergoes electron transfer reactions generating stable nitron spin adducts. Nitroso compounds reacted with carbanions derived from PFIB, which raises the possibility that electron transfer reactions of this type might account for the observed nitroxides. Our results suggest that PFIB undergoes some type of electron transfer reactions leading to several reactive intermediate species (RIS). The implications of these observations on pulmonary damage caused by inhalation of PFIB are discussed.				
14. SUBJECT TERMS PFIB, EPR Spectroscopy, Nitron and Nitroso Spin Trap Agents Carbonyl Fluoride, Carbon Dioxide Radical Anion, Nucleophilic Attack			15. NUMBER OF PAGES 17	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT	

INTRODUCTION

The chemistry of perfluoroisobutylene (PFIB) has been studied since 1953 (England and Krespan, 1966). This fluoro-olefin is of interest because of its high electrophilic properties. PFIB is produced by thermal decomposition of polytetrafluoroethylene (e.g., Teflon) and reacts with practically all known nucleophiles (Smith et al., 1982; Oberdorster et al., 1994). Overheating of insulating material generates fumes that may pose a serious health hazard to the respiratory tract in humans, resulting in so-called "polymer fume fever" with symptoms ranging from slight irritation to severe pulmonary edema (Oberdorster et al., 1994). The high electrophilicity of PFIB is a result of the strong electron-attracting effect of the fluorine atoms of the CF₃ groups and the possible conjugating with the C=C bond of the vinylic fluorine atoms. PFIB is a colorless, highly toxic gas, approximately ten times as toxic to the lungs as phosgene. It is possible that it exerts its effects by the depletion of intracellular nucleophiles (Lailey et al., 1991). Reactive nucleophiles occurring *in vivo* would include amines, thiols, and alcohols.

When PFIB reacts with charged nucleophiles (Nu⁻), a vinylic fluorine atom may be replaced. This process can be accompanied by bonding of the nucleophile to PFIB. In a number of cases, allylic substitution products are formed because of elimination of a fluoride anion from one of the CF₃ groups. The ratio of vinylic and allylic substitution products is determined by the nature of the entering nucleophile and by the reaction conditions (*Scheme 1*).



Scheme 1

In addition, the fluorine could be replaced by an addition-elimination mechanism, i.e., the nucleophile adds to the π-bond to form a relatively stable carbanion (Tedder and Walton, 1980).

However, because of its diversity of reactions and because PFIB is so reactive, it is possible that some of its toxicological mechanisms may be free radical mediated. Electron Paramagnetic Resonance (EPR)/Spin Trapping techniques have been successfully applied to determine whether short-lived free radicals are involved as reaction intermediates by scavenging the reactive radical to produce more stable nitroxide radicals. Therefore, first the reaction of PFIB and spin trapping agents must be understood prior to applying EPR/Spin

Trapping techniques to investigate possible free radical mechanisms in the interaction of PFIB with biological systems. In this report we describe the reaction of PFIB with nitron and nitroso spin traps.

EXPERIMENTAL PROCEDURES

Chemicals. PFIB was purchased from Flura Corporation, Newport, TN. 4-POBN, PBN and DBNBS were obtained from Sigma Chemical Co., St. Louis, MO. MNP was obtained from Aldrich Chemical Co., Milwaukee, WI. Dried benzene was acquired from Fisher Scientific, Pittsburgh, PA. Nitrogen gas was obtained from Matheson Co, Secaucus, NJ. All chemicals were commercial samples of high purity and used as supplied.

Aqueous solutions (0.1-0.2 mM) of 4-POBN and DBNBS were prepared and transferred to an EPR/flat cell glass apparatus tube as previously described (Arroyo and Kohno, 1991). Concentration-dependent studies were performed with PFIB in which the aqueous solutions of 4-POBN or DBNBS were reacted with 15 μL (24.1 ppm; 1 ppm = 8.2 mg/m³), 30 μL (48 ppm) and 60 μL (96.4 ppm) of PFIB. The PFIB concentration of 15 μL generated a weak EPR signal with poor resolution making the characterization and identification of the signal difficult. A clear EPR spectrum with defined signal intensity and better resolution was generated using 30 μL . Therefore, 30 μL was injected into the trap solutions contained in the EPR sealed-glass apparatus using gastight syringes from Supelco, Inc., Bellefonte, PA. Anaerobic conditions were achieved by bubbling N₂ through the aqueous solutions at a constant rate (0.1 ml/min) for approximately 15 min.

Aprotic solutions (0.1-0.2 mM) of PBN and MNP were also prepared and purged with N₂ for 10 min to remove oxygen, which causes EPR line broadening. These solutions, contained in the EPR sealed-glass apparatus, were exposed to 48 ppm PFIB and were analyzed by EPR.

EPR Spectra Measurements. EPR spectra were measured at room temperature using a Varian E-109 Century Series X-band EPR spectrometer which was equipped with a TM₁₁₀ microwave cavity and an E-102 microwave bridge. Spectrometer conditions are given in the Fig. legends.

Data analysis. EPR spectra were stored on a COMPAQ DESKPRO 386S computer. The hyperfine coupling constants were measured either with a spectrum simulation program written by P. Kuppusamy or directly from the spectrum. Each experiment was performed five times.

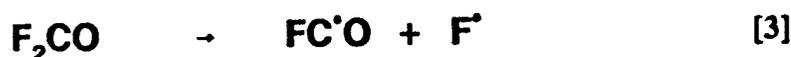
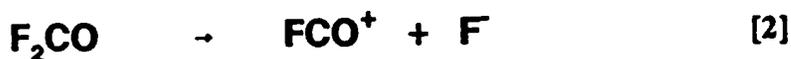
RESULTS

PFIB reacts rapidly with nitron and nitroso spin traps forming various spin adducts (Figs. 1-4). These results suggest that in solution it is not the PFIB itself that reacts directly with the spin traps. Instead, the spin adducts originate from spin trap reactions with highly reactive intermediates formed in the decomposition of the dissolved PFIB. It is known that PFIB

decomposes in aqueous solutions to form fluorophosgene (F_2CO), and furthermore, fluorophosgene ultimately decomposes leading to carbon dioxide radical anion ($CO_2^{\cdot -}$) and hydrogen fluoride (*equation 1*) (Riesz et al., 1985; Fawcett et al., 1962; Zeifman et al., 1984):



Like phosgene (Cl_2CO) (Arroyo et al., 1993), two initial dissociation mechanisms for fluorophosgene are conceivable. These mechanisms involve the heterolytic cleavage (*equation 2*) and/or the homolytic cleavage (*equation 3*) of a fluorine-carbon bond.



To identify reactive intermediates formed in the decomposition of PFIB, PFIB was dissolved in solutions containing nitron or nitroso spin traps. The reactions were carried out under protic (aqueous) and aprotic conditions.

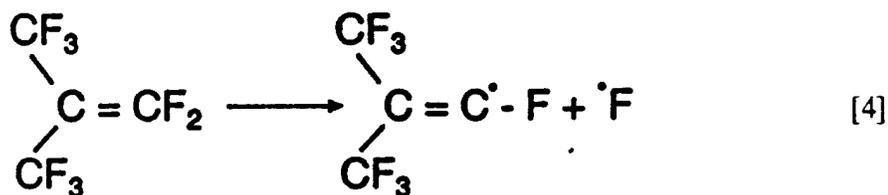
Reactions with nitron spin traps.

Addition of PFIB to an aerated aqueous solution containing the water soluble nitron spin trap 4-POBN yields the EPR spectrum shown in Fig. 1. This EPR spectrum consists of three overlapping spin adduct EPR spectra. One spin adduct yields an EPR spectrum consisting of a triplet of doublets indicated by the open circles (O). This spin adduct can be computer simulated using hyperfine coupling constants, $a_N = 1.550$ mT and $a_H^\beta = 0.3$ mT, which correspond to the previously characterized POBN- $CO_2^{\cdot -}$ spin adduct (Buettner, 1987). The formation of this spin adduct is also consistent with the dissociation products of PFIB (*equation 1*). The second spin adduct identified in Fig. 1A consists of a 1:2:2:1 quartet with hyperfine coupling constants, $a_N = a_H^\beta = 1.45$ mT, and corresponds to the t-butylaminoxyl radical ($H-N^{\cdot}(O)-tBu$) resulting from the hydrolysis or decomposition of 4-POBN (Buettner, 1987). The third spin adduct yields an EPR spectrum consisting of a triplet of doublets computer simulated using hyperfine coupling constants, $a_N = 1.525$ mT and $a_H^\beta = 0.250$ mT. This spin adduct has nitrogen and β -hydrogen couplings similar to those reported for the reaction of the phosgene-derived carbonyl chlorides and 4-POBN ($a_N = 1.58$ mT, $a_H^\beta = 0.26$ mT) (Arroyo et al., 1993). Therefore, its formation is attributed to the reaction (in a similar fashion as occurs with phosgene) of one of the carbonyl monofluoride-type species (*equations 2 and 3*) with 4-POBN. The addition of a carbonyl fluoride to 4-POBN produces a spin adduct

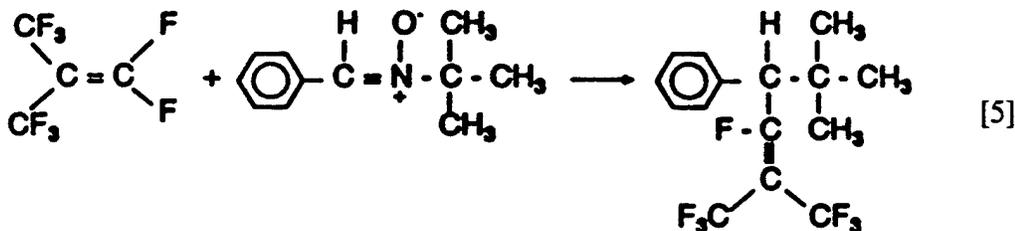
containing a β -hydrogen to interact with the nitroxide electron yielding a spin adduct EPR spectrum consisting of a triplet of doublets. The smaller nitrogen hyperfine coupling ($a_N = 1.525$ mT) for the 4-POBN-COF adduct when compared to the 4-POBN-COCl adduct ($a_N = 1.58$ mT) from phosgene is attributable to the larger electron withdrawing capability of the more electronegative fluorine. Fig. 1B shows the overall computer simulation which matches the experimental EPR spectrum in Fig. 1A.

This simulated EPR spectrum was obtained using a linewidth of 0.16 mT and the three spin adducts described to produce EPR simulation. Fig. 1C shows the EPR spectrum obtained in the absence of the 4-POBN spin trap corresponding to the CO_2^- radical anion ($g = 2.0008 \pm 0.0005$). This EPR spectrum was obtained using a closed EPR glass apparatus described earlier in the experimental procedures, and its g -value is consistent with previously reported g -values for CO_2^- (Ovenall and Whiffen, 1961).

The decomposition of PFIB depends on the presence of oxygen and/or protons in the reaction medium. For instance, when PFIB is dissolved in a deaerated (N_2 saturated) aprotic (benzene) solution containing the nitron spin trap PBN, it generates an adduct which yields the EPR spectrum shown in Fig. 2A. PBN differs from 4-POBN in that it is less water soluble and does not contain the aminoxide group in the aromatic *para*-position to the *t*-butylnitron functional group. As shown in Fig. 2B, the spin adduct EPR spectrum (Fig. 2A) can be computer simulated (0.08 mT linewidth) as a triplet of doublets ($a_N = 1.10$ mT, $a_H^\beta = 0.25$ mT) with an additional γ -coupling $a^\gamma = 0.05$ mT. Two possible mechanisms which cannot be differentiated by the experimental results are conceivable to explain the EPR spectrum in Fig. 2A. One mechanism involves the defluorination of PFIB to form a carbon centered radical on PFIB and a fluorine radical (equation 4).



The other mechanism is the direct addition of the PFIB to the PBN nitron carbon and simultaneous defluorination of PFIB to form a fluoride radical (equation 5). The carbon centered PFIB radical (equation 4) will react with PBN in a direct spin trapping-type reaction to yield an identical adduct as shown in equation 5.

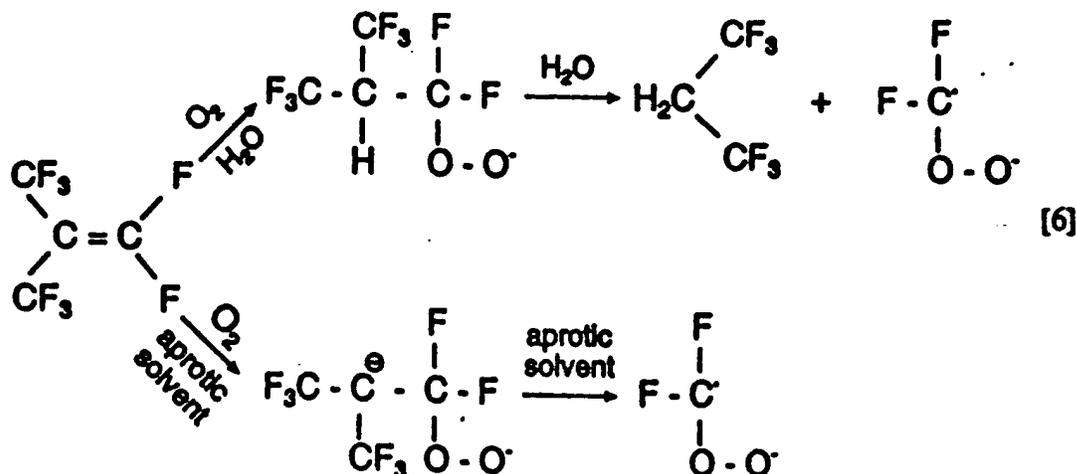


Fluorine has a nuclear spin, $I = 1/2$. Therefore, the interaction of a single fluorine nucleus with the unpaired nitroxide electron would cause each line in the triplet of doublets to be further split into doublets, yielding the overall triplet of quartets observed in Fig. 2A. The broad outer peaks in the EPR spectrum (Fig. 2A) can be explained as the PBN trapping of fluorine radicals yielding a spin adduct with a hyperfine coupling for the β -fluorine of 5.1 mT (Haire et al., 1988). This observation also supports the mechanisms described in equations 4 and 5.

Reactions with nitroso spin traps.

Dissolving PFIB in an aerated aqueous solution containing the nitroso spin trap DNBNS (Kaur et al., 1981) yields the spin adduct EPR spectrum shown in Fig. 3A. This EPR spectrum consists of a triplet of triplets with each triplet arranged in a 1:2:1 pattern indicating that the unpaired nitroxide electron is interacting with two equivalent nuclei with a nuclear spin, $I = 1/2$. The interaction of the unpaired nitroxide electron with two equivalent β fluorine nuclei ($I_F = 1/2$) would yield an EPR spectrum in which each line in the primary triplet is further split into a 1:2:1 triplet. Therefore, it is possible that a reactive intermediate is formed during the decomposition of PFIB and prior to the formation of fluorophosgene. Equation 6 shows a possible mechanism which would explain the observed EPR results in Fig. 3. Addition of the intermediate $F_2C^{\bullet}OO^{\bullet}$ to the DNBNS nitroso nitrogen would yield a spin adduct containing two β -fluorines. Such an intermediate could rapidly dehydrate, leaving a F_2COH functional group attached to the DNBNS nitroso nitrogen. In support of this type of adduct is the lack of γ -hydrogen splittings usually observed in DNBNS adducts. The electron withdrawing capability of the fluorines would minimize the delocalization of the unpaired nitroxide onto the aromatic ring, thus minimizing the interaction of the unpaired nitroxide electron with the nuclei of the aromatic protons. Fig. 3B shows the computer simulation that matches the EPR spectrum in Fig. 3A. This simulation was obtained using hyperfine coupling constants, $a_N = 1.365$ mT and $a_{F(2)}^{\beta} = 1.035$ mT, and a linewidth of 0.1 mT.

Addition of PFIB to an aerated aprotic (benzene) solution containing the nitroso spin trap MNP yields a spin adduct EPR spectrum consisting of a 1:2:3:2:1 quintet (Fig. 4). This EPR spectrum could also originate from the $F_2C^{\bullet}OO^{\bullet}$ species (equation 6).



In this case, there are no solvent protons required for the dissociation of the PFIB. However, it is possible that the carbanion reacts with solvent molecules subsequently releasing the $F_2C^{\bullet}OO^{\bullet}$ species which reacts with MNP directly adding to the nitroso nitrogen. The EPR pattern in Fig. 4A suggests that the primary nitrogen and β -fluorine couplings are similar, if not identical. Therefore, the spectrum can be computer simulated using hyperfine coupling constants, $a_N = a_{F(2)}^{\beta} = 1.345 \text{ mT}$ (Fig. 4B).

These results suggest that PFIB decomposes when dissolved, forming various reactive intermediates initiated by the attack of dissolved oxygen to form a $F_2C^{\bullet}OO^{\bullet}$. In aqueous environments, this intermediate rapidly forms fluorophosgene which then decomposes ultimately yielding the carbon dioxide radical anion and hydrogen fluoride.

DISCUSSION

The ability of PFIB to enter into reactions with diverse nucleophiles distinguishes it from other highly hydrophobic gases. PFIB is an extremely powerful electrophile whose role as a toxicant is modulated by lung thiol levels (Lailey et al., 1989). The cellular thiol nucleophilic protectants, glutathione and cysteine, have different major roles. Glutathione is a cofactor in transport and transferase reactions, protein synthesis and in detoxication of reactive intermediates formed intracellularly. Initial studies on the mechanism of pulmonary injury by PFIB (Lailey et al., 1989; Makulova, 1965) showed that the amount of both non-protein thiol and glutathione in lung was reduced by 30% and 49%, respectively, in animals exposed to PFIB. Pretreatment with cysteine esters protected against toxicity and raised cysteine levels by 100%. These authors concluded that the role of glutathione as a co-factor in transferase and peroxidation reactions may not be important for protection against PFIB.

This study provides evidence that PFIB is reactive towards nucleophilic reagents to yield substitution and addition radical byproducts. Furthermore, PFIB undergoes nucleophilic reactions typical of fluoro-olefin, such as addition, and vinylic and allylic substitution. In the addition reactions, the nucleophilic catalysis acquires special significance, since it provides a possible mechanism for its toxicity. The toxicity of PFIB may be correlated with its susceptibility to nucleophilic attack (Zeifman et al., 1984) and the generation of reactive intermediate species. A striking correlation exists between fluoro-olefins and their toxicological properties: the toxicity of a halogenated olefin is directly proportional to the reactivity of that olefin with nucleophiles (Cook and Pierce, 1973). Therefore, it is probable that raising the overall levels of nucleophiles (thiols) increases the level of protection by neutralizing the incoming PFIB before it can damage cellular constituents. Further research is required to validate the scavenging role of lung nucleophiles in reducing the toxicity of PFIB.

FIGURE 1

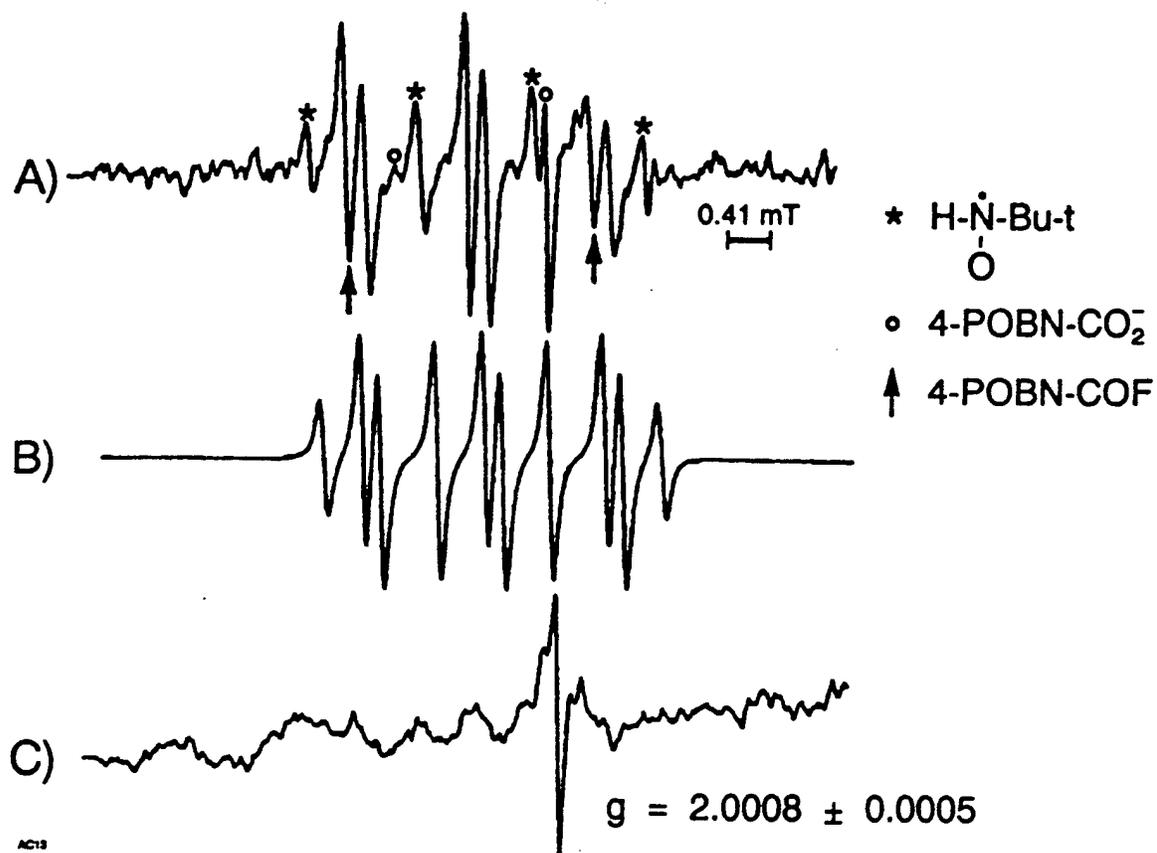


Fig. 1: Proposed mechanism for the reaction of PFIB with O-nucleophiles under aerobic conditions. A) POBN-adducts; the spectrometer conditions were 10 mW microwave power, 0.05 mT modulation amplitude, 2 s time constant, 2.5×10^5 receiver gain, and 0.27 mT/min scan rate. B) Computer simulation that best fit the experimental results. C) $\text{CO}_2\cdot^-$ radical; the spectrometer conditions were 10 mW microwave power, 0.1 mT modulation amplitude, 1 s time constant, 1.6×10^5 receiver gain, 1.25 mT/min scan rate.

FIGURE 2

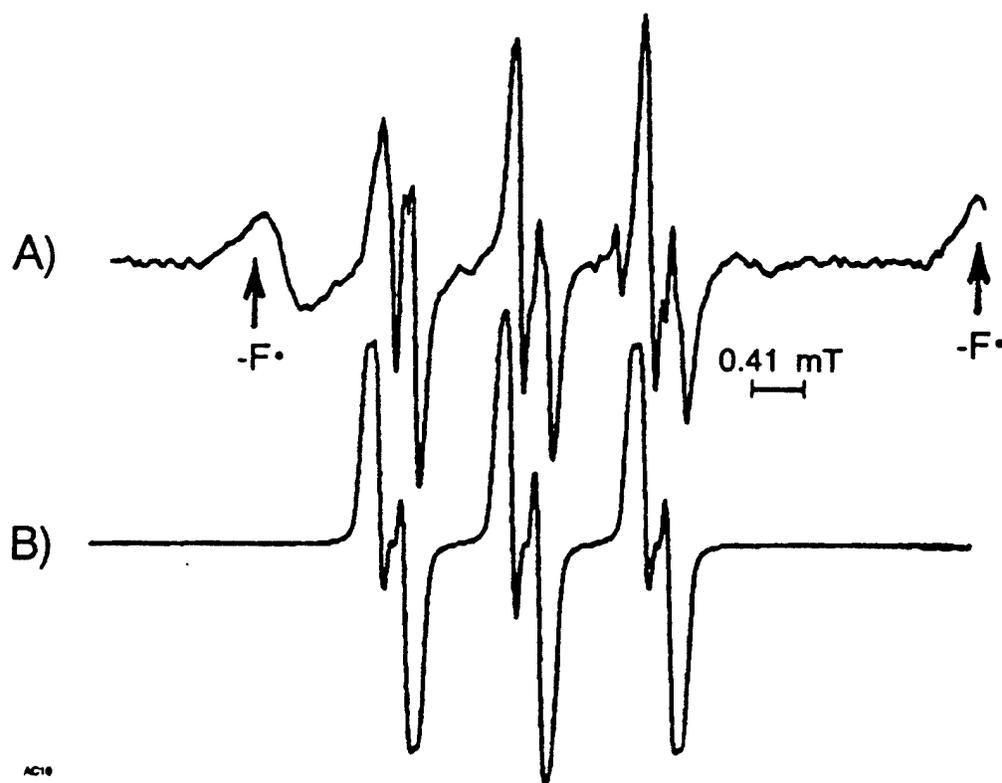


Fig. 2: Proposed mechanism for the reaction of PFIB with nitron PBN. A) EPR signal of the detected PBN-adducts. B) Computer simulation that best fitted the experimental results (A). Spectrometer conditions were 10 mW microwave power, 0.1 mT modulation amplitude, 2 sec time constant, 1.25×10^5 receiver gain, and 0.27 mT/min scan rate.

FIGURE 3

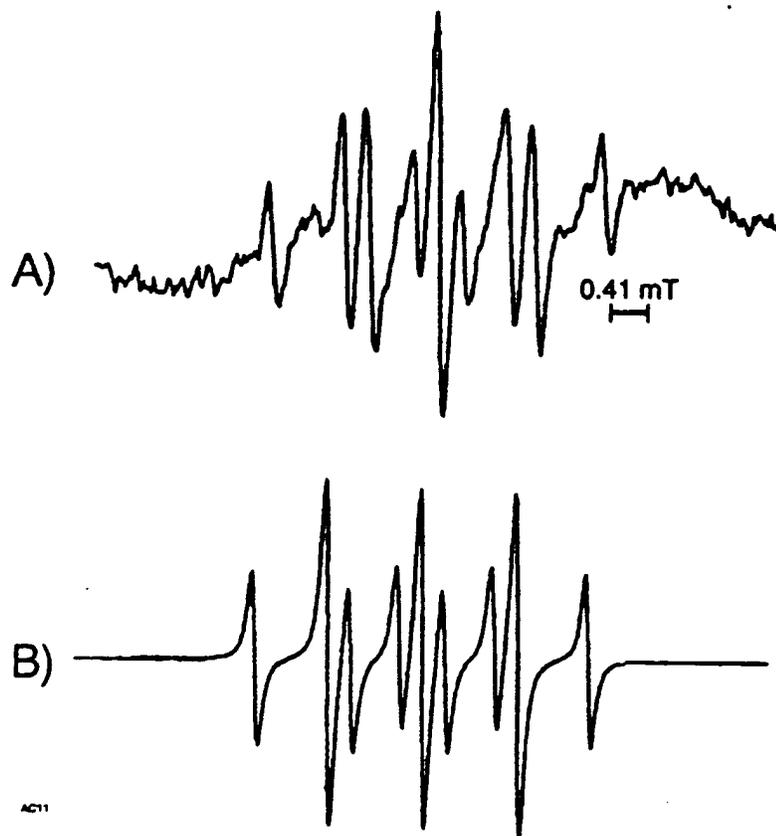


Fig. 3: Formation of DNBBS adduct when PFIB reacted with DNBBS. EPR signal of the observed DNBBS-adduct (A) including the computer simulation (B). Spectrometer conditions were 10 mW microwave power, 0.1 mT modulation amplitude, 1 s time constant, 1.25×10^5 receiver gain, and 1.25 mT/min scan rate.

FIGURE 4

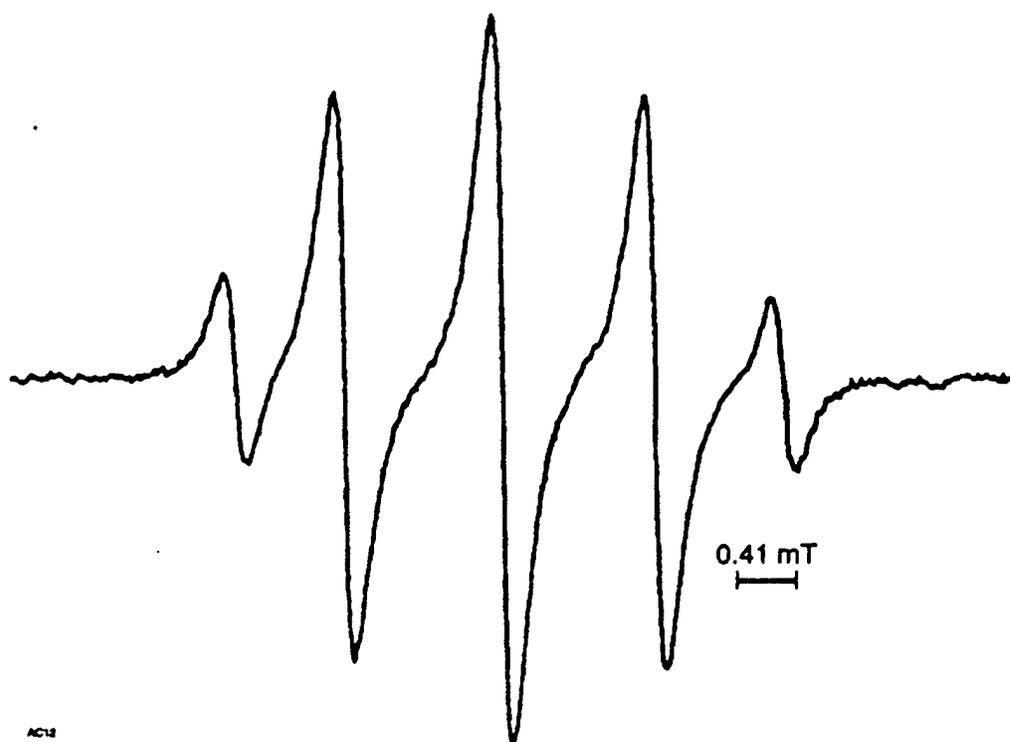


Fig. 4: Nitration of PFIB by the nitroso spin trap MNP. Spectrometer conditions were 10 mW microwave power, 0.1 mT modulation amplitude, 1 s time constant, 1.25×10^5 receiver gain, and 1.25 mT/min scan rate.

REFERENCES

- Arroyo CM and Kohno M. (1991) Difficulties encountered in the detection of nitric oxide (NO) by spin trapping techniques. A cautionary note. *Free Rad Res Commun* 14: 145-55.
- Arroyo CM, Feliciano F, Kolb DL, Keeler JR, Millette SR and Stotts RR. (1993) Autoionization reaction of phosgene (OCCl₂) studied by Electron Paramagnetic Resonance/Spin Trapping techniques. *J Biochem Toxicol*. 8: 107-10.
- Buettner GR. (1987) Spin Trapping: ESR Parameter of Spin Adducts. *Free Radical Biology and Medicine* 3: 259-303.
- Cook EW and Pierce JS. (1973) Toxicology of fluoro-olefins. *Nature* 242, 337-38.
- England DC and Krespan CG. (1966) Fluoroketenes. I. Bis(trifluoromethyl)ketene and Its Reactions with Fluoride Ion. *J Am Chem Soc* 88: 5582-87.
- Fawcett FS, Tullock CW and Coffman DD (1962). The chemistry of carbonyl fluoride. I. The fluorination of organic compounds. *J Am Chem Soc* 84: 4275-84.
- Haire LD, Krygsman PH, Janzen EG and Oehler UM. (1988) Correlation of radical structure with EPR spin adduct parameters: utility of the proton, carbon-13, and nitrogen-14 hyperfine splitting constant of aminoxyl adducts of PBN-nitonyl-13C for three-parameters scatter plots. *J Org Chem* 53: 4535-42.
- Kaur H, Leung KHW and Perkins MJ. (1981) A water-soluble, nitroso-aromatic spin-trap. *JCS Chem Comm* 1981: 142-3.
- Lailey AF, Leadbeater L, Maidment MP and Upshall DG. (1989) The mechanism of chemically-induced pulmonary oedema. Proc. 3rd Int. Symp. Protection Against Chemical Warfare Agents, 11-16 June, 1989, Sweden. pp. 153-161.
- Lailey AF, Hill L, Lawston IW, Stanton D and Upshall DG. (1991) Protection by cysteine esters against chemically induced pulmonary oedema. *Biochem. Pharmacol.* 42: PS47-54.
- Makulova ID. (1965) Clinical aspect of acute poisoning with perfluoroisobutylene. *Gig Tr Prof Zabol* 9: 20-3.
- Oberdorster G, Ferin J, Gelein R, Finkelstein J and Baggs R. (1994) Effects of PTFE fumes in the respiratory tract: A particle effect? Aerospace Medical Association 65th Annual Scientific Meeting 538: A52.
- Overall DW and Whiffen DH. (1961) Electron spin resonance and structure of the CO₂⁻ radical ion. *Molecular Physics* 4: 135-144.

Riesz P, Berdahl D and Christman CL. (1985) Free radical generation by ultrasound in aqueous and nonaqueous solutions. *Environ Health Perspective* 64: 233-52.

Smith LW, Gardner R and Kennedy GL. (1982) Short-Term Inhalation Toxicity of Perfluoroisobutylene. *Drug and Chemical Toxicology* 5: 295-303.

Tedder JM and Walton JC (1980) The importance of polarity and steric effects in determining the rate and orientation of free radical addition to olefins. *Tetrahedron* 36: 701-7.

Zeifman YB, Ter-Gabrielyan YG, Gambaryan NP and Knunyants IL. (1984) The chemistry of perfluoroisobutylene. *Uspekhi Khimii* 53: 431-61.

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