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ESR Spin-Trapping Study of the Radicals Produced in NOx/Olefin Reactions: A Mechanism for the Production of the Apparently Long-Lived Radicals in Gas-Phase Cigarette Smoke

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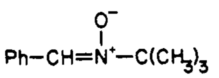
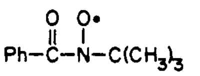
Abstract: Gas-phase cigarette smoke contains high concentrations of both oxygen- and carbon-centered free radicals. We have detected these radicals using several variations of the electron spin resonance (ESR) spin-trapping technique, including the use of spin traps in the solid state, to show that the radicals are trapped *directly* from the gas phase. These gas-phase radicals can still be trapped from gas-phase smoke that is more than 5 min old, a result that is clearly inconsistent with the highly reactive nature of oxygen- and carbon-centered radicals. To rationalize this apparent paradox, we hypothesize that free radicals are continuously produced and destroyed in cigarette smoke and exist in a *steady state*. We suggest that one mechanism by which radicals can be formed involves the slow oxidation of the relatively unreactive nitric oxide (which acts as a "radical reservoir") to the much more reactive nitrogen dioxide. Nitrogen dioxide can then react with a number of the species that are present in smoke to produce the radicals that we detect. As a model, we have studied the reactions of NO/air mixtures with unsaturated hydrocarbons. Isoprene is one of the most abundant species in smoke and is known to be very reactive toward NO_2; therefore, we have studied the nature of the radicals that can be spin trapped from gaseous mixtures of NO, isoprene, and air. We find that the NO/air/isoprene model system gives essentially the same types of radicals (oxygen and carbon centered) as does cigarette smoke. We have also studied the gas-phase reactions of NO_2 with several small olefins and 1,3-butadiene and find evidence for peroxyl radical intermediates. In solution, NO_2 reacts with isoprene much faster than it does with the spin-trap phenyl-*tert*-butylnitrone (PBN). We find that NO_2 oxidizes PBN to benzoyl *tert*-butyl nitroxide and propose a mechanism for this reaction.

Cigarette smoke contain high concentrations of two distinctly different populations of free radicals, one in the tar and one in gas-phase smoke.[2–6] Tar contains several types of paramagnetic species that are relatively stable and that can be detected directly by electron spin resonance (ESR) techniques.[4] The predominant signal is due to quinone and hydroquinone groups held in a tarry matrix.[4] Gas-phase cigarette smoke contains much shorter-lived radicals that are too reactive to be detected by ESR directly; however, these radicals can be studied by using ESR spin-trapping techniques.[5–7] The radicals that are detected from the gas phase of a standard 1R1 research cigarette, by using α-phenyl-*N*-*tert*-butylnitrone (PBN) as the spin trap, are primarily alkoxyl radicals with smaller amounts of carbon-centered radicals. In addition, an oxidation product of PBN, benzoyl *tert*-butyl nitroxide (PBNOx), also is observed.



$\mathrm{Ph{-}CH{=}\overset{O^-}{N^+}{-}C(CH_3)_3}$

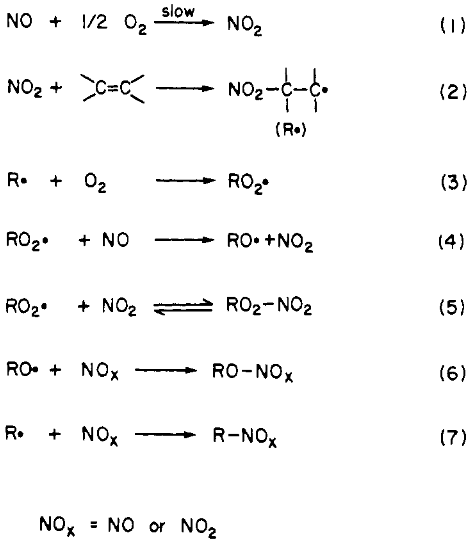
PBN



$\mathrm{Ph{-}\overset{O}{\overset{\|}{C}}{-}\overset{O\bullet}{N}{-}C(CH_3)_3}$

PBNOx

Scheme I



We have previously reported an unexpected and paradoxical property of the radicals in gas-phase cigarette smoke.[5,6] These radicals appear to have lifetimes in the gas phase of over 5 min, whether measured as a residence time in glass tubing in a flow system or in glass or plastic syringes in a static system.[5,6] Such lifetimes are clearly inconsistent with the nature of the species that we detect (oxygen- and carbon-centered radicals) which are very reactive and are known[8–10] to have lifetimes of much less than a second under the conditions that occur in gas-phase smoke. Therefore, the radicals that we observe could not be those that are formed in the flame during combustion. To resolve this paradox, we have suggested that radicals are *continuously produced* in gas-phase cigarette smoke as well as being *continuously destroyed* by the usual combination reactions that terminate radical reactions. In other words, we have proposed that the radicals in gas-phase smoke exist in a *steady state*, and we have suggested one mechanism that might occur in typical cigarette smoke.[5,7] This mechanism involves the slow oxidation of nitric oxide (NO), which is typically present at levels of 300 μg/cig[3] in undiluted smoke and is known to be unreactive, to nitrogen

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(1) On Sabbatical leave from the University of Tokyo, Tokyo, Japan.

(2) Tar is defined as the material that is held on a Cambridge filter, a standard glass fiber filter that retains 99.9% of the particles greater than 0.2 μm in diameter; the gas phase is that portion that penetrates the filter.[3]

(3) Guerin, M. R. In "Banbury Report: A Safe Cigarette?"; Gori, G. B., Bock, F. G., Eds.; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1980; p 191.

(4) Pryor, W. A.; Hales, B. J.; Premovic, P. I.; Church, D. F. *Science (Washington, D.C.)* **1983**, *220*, 425.

(5) Pryor, W. A.; Prier, D. G.; Church, D. F. *Environ. Health Perspect.* **1983**, *47*, 345.

(6) Pryor, W. A.; Terauchi, K.; Davis, W. H., Jr. *Environ. Health Perspect.* **1976**, *16*, 161.

(7) Pryor, W. A.; Tamura, M.; Dooley, M. M.; Premovic, P.; Church, D. F. In "Oxy Radicals and Their Scavenger Systems: Cellular and Molecular Aspects"; Cohen, G., Greenwald, R., Eds.; American Elsevier: New York, 1983; pp 185–192.

(8) Carlsson, D. V.; Ingold, K. U. *J. Am. Chem. Soc.* **1967**, *89*, 4885.

(9) Carlsson, D. V.; Ingold, K. U. *J. Am. Chem. Soc.* **1967**, *89*, 4891.

(10) Batt, L.; Milne, R. T. *Int. J. Chem. Kinet.* **1976**, *8*, 59.

dioxide (NO_2), which is known to be quite reactive (Scheme I, eq 1). We suggest that the NO_2 then reacts with reactive components in smoke such as, for example, olefins, which are present in smoke at high levels and are known to react with NO_2 to give radicals.[11,12] The alkyl radicals resulting from this olefin–NO_2 reaction (Scheme I, eq 2) react rapidly with oxygen (Scheme I, eq 3) to give peroxyl radicals, which are then rapidly deoxygenated by NO (Scheme I, eq 4) to produce alkoxyl radicals. The last three reactions in Scheme I (eq 5–7) represent the possible termination reactions that "remove" alkyl, alkoxyl, and peroxyl radicals from the system and establish the steady-state condition. The mechanism outlined in Scheme I includes both of the radicals that we spin trap (alkyl and alkoxyl, eq 8 and 9) as well as peroxyl radicals which, as we will discuss, do not appear to be spin trapped from cigarette smoke (eq 10) even though they are certainly present.

$$R\bullet + PBN \longrightarrow R{-}PBN\bullet \qquad (8)$$

$$RO\bullet + PBN \longrightarrow RO{-}PBN\bullet \qquad (9)$$

$$RO_2\bullet + PBN \longrightarrow RO_2{-}PBN\bullet \qquad (10)$$

In this publication we present the results of experiments that are designed to test the mechanism shown in Scheme I. We have modeled gas-phase cigarette smoke by experiments in which NO is allowed to mix with air at ambient temperatures and then flow into a air stream containing isoprene; this gas mixture is then passed into a spin-trap solution. The radicals that are detected are very similar to those in gas-phase cigarette smoke, including both oxygen- and carbon-centered radicals.

When gas-phase radicals are passed into a solution and then spin trapped, it is possible that some or all of the radicals could arise from reactions that occur in solution. In experiments that more directly confirm the mechanism in Scheme I, we have passed smoke from 1R1 cigarettes over solid spin traps. We again observe the spin adducts of both oxygen- and carbon-centered radicals, just as in the solution spin-trapping experiments. This report represents the first time that a solid spin-trapping method has been used to study radicals that might be of interest in environmental toxicology, and we suggest that the technique may have wide usefulness.

Experimental Section

Materials and Equipment. α-Phenyl-*N*-*tert*-butylnitrone (PBN) from Kodak was used either without further purification or after recrystallization from hexane. Although commercial PBN often has weak paramagnetic signals due to alkoxyl spin adducts and PBNOx, these signals were always much weaker than the signals that we observe and using unpurified PBN did not affect our results. Benzene (Aldrich "Gold Label") was first washed with concentrated sulfuric acid and then distilled over anhydrous calcium chloride. The nitric oxide and isoprene used to make the synthetic smoke streams were purified as described below.

Research cigarettes (1R1) were obtained from the University of Kentucky, Tobacco and Health Research Institute, and stored in a freezer in sealed packages. They were conditioned by storing them in a desiccator at 0 °C over a saturated aqueous solution of ammonium sulfate for at least 7 days. After conditioning, a 1R1 cigarette weighs about 1.2 g.

Spectra were obtained by using either an IBM Model 100D ESR spectrometer equipped with an ASPECT 2000 data system and an ER 4111VT variable temperature unit or a Varian E-109A spectrometer. The microwave power was 20 mW, the modulation amplitude 0.02 mT, and the modulation frequency was 100 kHz. A 0.5-s time constant was used with 500-s scan times and a 5.0-mT scan range.

Procedure for Smoking Tobacco. Research cigarettes were smoked by using one of two different protocols. Figure 1 shows an apparatus in which a cigarette is mounted inside a 5-L three-necked flask; a nichrome ignition wire is used to light the cigarette. Air was pulled through the flask to support the combustion of the cigarette; a flow rate of 500 mL/min was maintained by means of a small suction pump at the outlet of the flow system. Alternatively, the cigarette was attached directly to the Cambridge filter and was allowed to burn in the laboratory atmosphere. The same results were obtained by either protocol. The smoke stream was pulled through the cigarette and through a glass-fiber Cambridge filter (3 × 25 mm diameter).[2,3] After filtering, the smoke was passed through a section of glass tubing, the length of which could be changed to allow aging of the smoke for varying periods of time. Finally, the smoke was drawn through the spin-trapping medium (see below). The tobacco cigarettes were consumed at a rate of about 0.1 g/min.

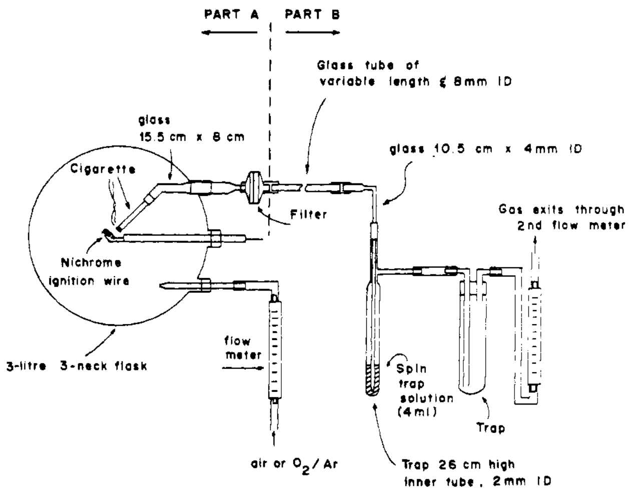

Figure 1. Apparatus for smoking cigarettes and spin trapping the gas-phase radicals.

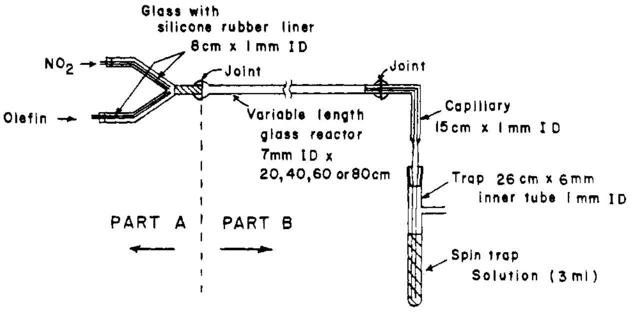

Figure 2. Details of the mixing system used to prepare NO_2/olefin mixtures and spin trap resulting gas-phase radicals.

Procedure for Preparing Gaseous Mixtures of NO_2 and Olefins. A stream of NO_2 (5–6 μM) and a stream of ethylene, propylene, isobutylene, or 1,3-butadiene (24–20 mM) were mixed in air, allowed to react, and passed through a PBN spin-trapping solution for 1000 s using the system shown in Figure 2.[13] Total flow rates of 32 mL/min were used.

Procedure for Preparing Gas Streams of NO and Isoprene. Mixtures of NO (13 μM) and isoprene (74 μM) in air were prepared by using the apparatus shown in Figure 3; mixing and spin trapping were carried out as in Figure 2, part B. Commercial NO (Matheson) was first passed through a 50% solution of sodium hydroxide to remove any traces of NO_2, after which the gas stream was passed through a cold trap at −78 °C. The NO was then metered into a stream of air through a needle valve. The NO in the gas stream was measured by allowing sufficient time for the NO to react with the oxygen that was present (in the absence of olefins) and then measuring the resulting NO_2 using the method of Nash.[14] A gas stream containing isoprene was prepared by passing air through liquid isoprene (Aldrich "Gold Label" that was not additionally purified), followed by metering this gas stream into the main gas stream through a needle valve. The concentration of the isoprene could be regulated by varying either the size of the orifice or the temperature of the liquid isoprene. The gas streams were then mixed together and allowed to flow down a tube of variable length before being bubbled through the spin-trapping solution. The total flow rate was adjusted to 500 mL/min.

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(11) Sprung, J. L.; Akimoto, H.; Pitts, J. N. *J. Am. Chem. Soc.* **1971**, *93*, 4358–4363.

(12) Pryor, W. A.; Lightsey, J. W. *Science* (*Washington, D.C.*) **1981**, *214*, 435–437.

(13) We have used μM to indicate, for example, μmol NO_2/L total gas.

(14) Nash, T. *J. Chem. Soc. A*, **1970**, 3023.

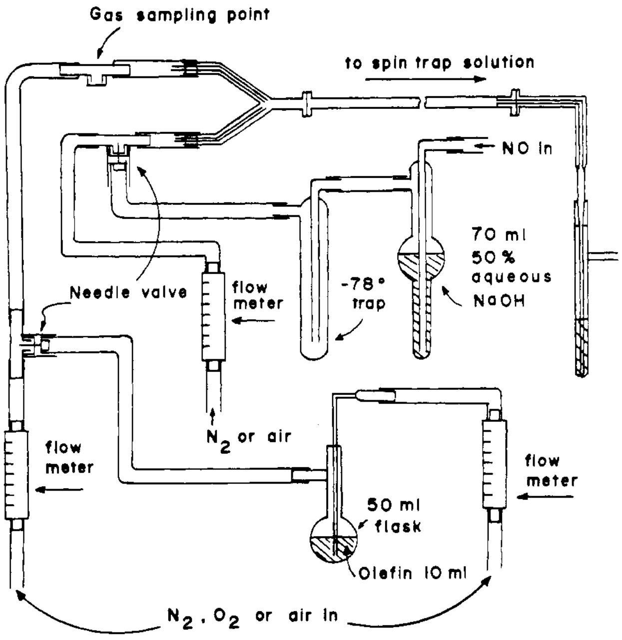

Figure 3. Apparatus for preparing and spin-trapping radicals from the NO/isoprene/air gas-phase mixtures (see text for detailed description).

Procedure for Spin Trapping at Room Temperature. The gas stream was passed through 4 mL of a solution of 0.1 M PBN in benzene or carbon tetrachloride. About 0.5 mL of this solution was then transferred to a standard cylindrical ESR tube and purged with a slow stream of nitrogen for about 10 min. The sample was stored at −78 °C until it was analyzed by ESR at room temperature.

Procedure for Spin Trapping with a PBN-Coated Glass Filter. Approximately 100 mg of PBN was placed on a Cambridge filter, and benzene was dripped onto the PBN until it all had been washed into the filter (about 3 mL of benzene was required). The filter was then dried in vacuo to leave a thin film of PBN on the filter fibers. The trap containing the spin-trapping solution shown in Figure 1 was replaced with a filter holder containing the PBN-impregnated filter, and smoke from ten 1R1 cigarettes was pulled through the PBN-coated filter. The filter was removed from the filter holder and eluted with 10 mL of benzene. The volume of the benzene solution was then reduced to 2 mL in vacuo, the solution was transferred to an ESR tube and degassed, and the ESR spectrum was obtained.

Procedure for Spin Trapping with PBN Adsorbed on Silica Gel. The PBN (300 mg) was dissolved in 5 mL of benzene and this solution mixed with 5 g of GC-grade (60–80 mesh) silica gel. The benzene was removed under vacuum to give 6% by weight of PBN adsorbed onto the silica gel. The treated silica gel was packed into a 6-mm i.d. glass tube to give a column of silica gel 50 mm long. The trap shown in Figure 1 was then replaced with this column, and the smoke from four 1R1 cigarettes was passed through the PBN-treated silica gel. The silica gel column was then removed, eluted with 5 mL of benzene, the volume of the benzene solution reduced to 1 mL, placed in an ESR tube, degassed, and the ESR spectrum obtained.

Analysis of Spectra. Di-*tert*-butyl nitroxide was used to calibrate the field. Since all of the spectra involved overlapping peaks, the second derivative spectra were computed and used to determine peak locations for calculation of hfsc. Where the spectra were particularly complex, fourth and sixth derivatives were used to measure hfsc more precisely.

Table I. Analysis of the ESR Spectra Obtained by Trapping 1R1 Cigarette Smoke and NOx/Isoprene/Air Model Systems[a]

Trapping Conditions	a_N, mT	a_H, mT	%	radical
1R1 cigarettes				
PBN/benzene	1.36 (0.01)	0.19 (0.01)	67	RO–PBN
	1.44 (0.01)	0.20 (0.01)	30	R–PBN[b]
	0.80 (0.01)		3	PBNOx
PBN/CCl_4	1.38 (0.01)	0.18 (0.01)	51	RO–PBN
	1.45 (0.01)	0.33 (0.06)	14	R–PBN
	1.04[c]	*d*	35	*e*
PBN/glass filter	1.38 (0.01)	0.19 (0.01)	56	RO–PBN
	1.44 (0.03)	0.32 (0.03)	13	R–PBN
	1.05[c]	*d*	25	*e*
	0.80		6	PBNOx
PBN/silica gel	1.37 (0.01)	0.20 (0.01)	75	RO–PBN
	1.43 (0.01)	0.32 (0.01)	25	R–PBN
NO/isoprene/air				
PBN/benzene	1.38 (0.01)	0.21 (0.01)	42	RO–PBN
	1.42 (0.01)	0.21 (0.01)	55	R–PBN[b]
	0.79 (0.01)		3	PBNOx
NO_2/isoprene/air				
PBN/benzene	1.37[c]	0.20[c]	92	RO–PBN
	0.80		8	PBNOx

[a] See text for discussion of assignments; uncertainties in hfsc measurements are in parentheses. [b] Cyclohexadienyl adduct (see text); other R–PBN are alkyl adducts. [c] Not enough lines could be resolved to assign uncertainties to these values. [d] There are four unresolved lines due to long-range hydrogen splitting(s); a_H ca. 0.04–0.06 mT. [e] Possibly a vinyl nitroxide; see text.

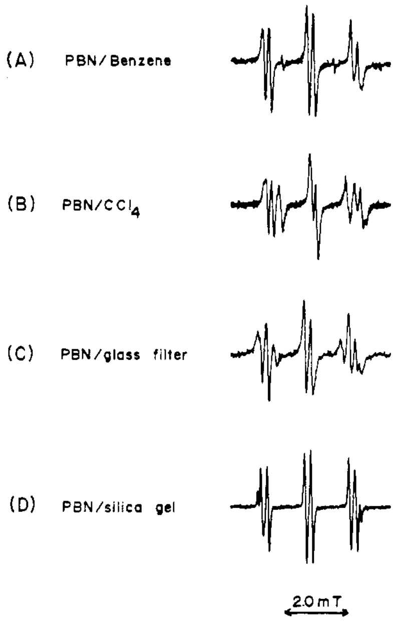

Figure 4. PBN spin adducts observed from spin-trapping 1R1 cigarette smoke using different protocols: (A) smoke from one cigarette bubbled through 0.1 M PBN in 4 mL of benzene; (B) smoke from one cigarette bubbled through 0.1 M PBN in 4 mL of carbon tetrachloride; (C) smoke from ten cigarettes passed through a Cambridge filter that had been coated with 100 mg of PBN and then eluted with benzene; (D) smoke from four cigarettes passed through a 6 × 50 mm column of 6% (w/w) PBN on silica gel and then eluted with benzene.

Results

Spin-Trapping Radicals from Cigarette Smoke. In our earlier spin-trapping studies of gas-phase cigarette smoke,[5-7] radicals appeared to be trapped when the smoke stream was bubbled through a solution of PBN in benzene or *tert*-butylbenzene (see Figure 4A and Table I). In this experimental protocol, the spin adducts that we observe could be formed by two mechanisms in addition to being formed by the direct trapping of radicals from the gas phase. In the first mechanism, the gas-phase radicals could react with the aromatic solvent to give the radicals that are then spin trapped. In addition to an alkoxyl spin adduct, trapping in an aromatic solvent gives a second adduct with a nitrogen hfsc (1.44 mT) characteristic of carbon-centered adducts. The hydrogen hfsc (0.20 mT) of this second adduct is too small to be due to an alkyl radical; rather, it is very similar to what has been reported for cyclohexadienyl spin adducts.[15] Homolytic additions of alkyl radicals to aromatic rings are well-known[16] and, of course, give cyclohexadienyl radicals. To probe whether the gas-phase radicals are (at least in part) reacting with aromatic solvents used in spin-trapping studies, we have carried out spin trapping studies in carbon tetrachloride; carbon tetrachloride is a solvent that can be regarded as being inert to most free radicals under the con-

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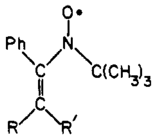
(15) Mao, S. W.; Kevan, L. *J. Phys. Chem.* **1976**, *80*, 2330.

(16) Perkins, M. J. In "Free Radicals"; Kochi, J., Ed.; Wiley: New York, 1973; Vol. II, pp 231–271.

ditions used here. The spectrum obtained from spin-trapping 1R1 cigarette smoke with PBN in carbon tetrachloride is shown in Figure 4B; the analysis of this spectrum (see Table I) shows a major contribution from an alkoxyl radical with smaller contributions from an alkyl radical spin adduct with a "normal" alkyl a_H of 0.33 mT (ref 17) and a species that gives a three-line spectrum with a_N = 1.04 mT. We suggest that this latter spin adduct is a vinyl nitroxide, **1**.[15] The broadening of the three lines in the spectrum for this species suggests that there are unresolved long-range hydrogen hfsc; analysis of the second derivative spectrum gives an approximate value for this splitting of about 0.40–0.06 mT. This splitting could result either from R or R′ (or both) being hydrogen or from coupling to aromatic hydrogens. The origin of this vinyl nitroxide species will be considered in more detail in the Discussion section below.



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The second mechanism by which radicals might appear to be formed in experiments where a gas stream is bubbled through a solution of spin trap is by solution-phase reactions of smoke components after they have dissolved in the solvent. Therefore, to prove that we have trapped radicals directly out of the gas phase, we have applied two variations of a solid-state spin-trapping technique to gas-phase cigarette smoke. Janzen et al.[18,19] have published two reports on the trapping of radicals from the gas phase using solid spin traps. In the first, they simply passed the gas-phase containing free radicals through a tube packed with powdered PBN. Spin adduct spectra were then observed either in the solid PBN directly or after dissolving the PBN in benzene. In the second protocol, the gas phase was passed through a tube coated with a thin layer of powdered PBN. The nitroxide spin adducts were sufficiently volatile to be vacuum transferred from the tube into a cold trap. They were then washed out of the trap with benzene, and the ESR spectrum of the benzene solution of the spin adducts was obtained.

We have used two protocols for solid spin trapping. In the first, a Cambridge glass-fiber filter was coated with PBN; alternatively, the PBN was adsorbed onto silica gel. Gas-phase cigarette smoke was then passed either through the PBN-coated filter or through a short column packed with the PBN/silica gel. Finally, the PBN spin adducts were dissolved off of the filter or eluted from the silica gel with benzene. The resulting spectra are shown in Figures 4, parts C and D. Both of these solid spin trapping methods give RO-PBN as the major spin adduct. The PBN-coated filter also gives both an alkyl spin adduct and **1** (the vinyl nitroxide discussed above), as well as a small amount of PbNOx. The PBN/silica gel gives an exceptionally clean spectrum, with only the spin adduct of an alkyl radical being observed in addition to the principal alkoxyl radical spin adduct.

Table I shows that although all four of the spin-trapping protocols give somewhat different spin adduct mixtures, the basic features are the same, with the major spin adduct in all cases being that due to an alkoxyl radical. We have previously reported that the yield of radicals from one 1R1 cigarette is 10^{16} spins/cig, using PBN in benzene.[5] The yields obtained by using PBN in carbon tetrachloride and PBN on solid silica gel are about the same, whereas the yield obtained for PBN on a Cambridge filter is lower, probably because of the very short contact time of the smoke with the solid PBN.

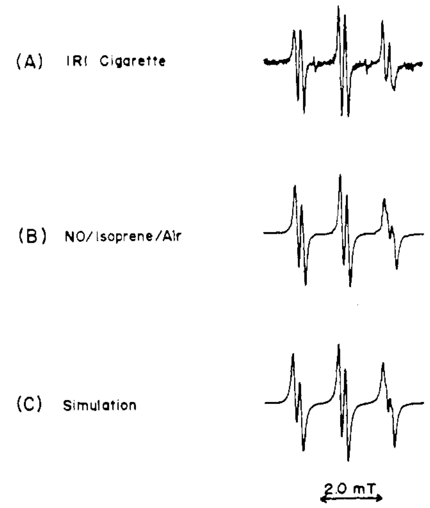

Figure 5. Comparison of the PBN spin adducts from 1R1 cigarette smoke with those from an NO/air/isoprene mixture: (A) spectrum from bubbling the smoke from one cigarette through 4 mL of 0.1 M PBN in benzene; (B) spectrum from bubbling 13 μM NO and 74 μM isoprene in air through 4 mL of 0.01 M PBN in benzene; (C) computer simulation of (B) using the following parameters: alkoxyl spin adduct with a_N = 1.365 mT, a_H = 0.200 mT, peak width = 0.1 mT, and modulation = 0.25; alkyl spin adduct with a_N = 1.440 mT, a_H = 0.200 mT, peak width = 0.1 mT, and modulation = 0.25. The ratio of RO• to R• is 1 to 0.8.

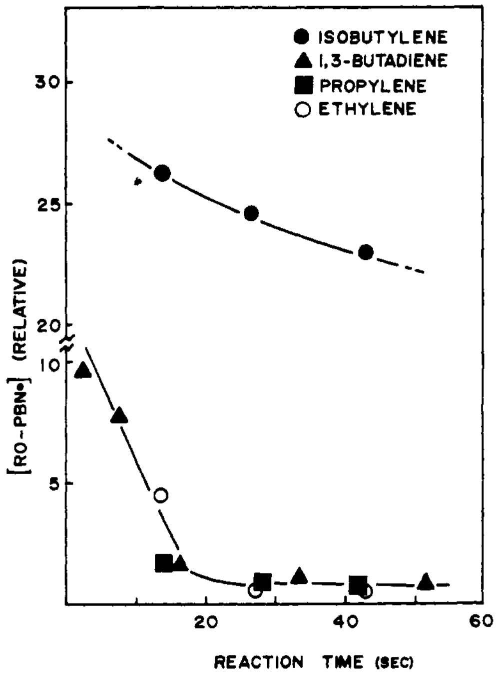

Figure 6. Relative PBN spin adduct concentrations (from peak heights) observed after various reaction times for gas-phase reaction mixtures of NO_2 and either 1,3-butadiene, isobutylene, propylene, or ethylene. In these experiments, the NO_2 concentration was 5–6 μM and the olefin concentration was 24–29 mM in air.

NO/Air/Isoprene Model for Gas-Phase Cigarette Smoke. As discussed above, the radicals that are detected from 1R1 cigarettes have much longer lifetimes in the gas phase than is consistent with their identification by spin trapping as reactive alkoxyl and carbon-centered radicals. For this reason, we have suggested a *steady-state model* for radical production in cigarette smoke.[5,7]

As described in the introduction, we propose Scheme I as a mechanism for the continuous production of radicals in cigarette smoke. Figure 5 compares the ESR spectra from spin trapping the radicals from 1R1 cigarette smoke with those from a gas mixture of 490 ppm NO and 3900 ppm isoprene in air, concentrations that represent a reasonable model for fresh, undiluted cigarette smoke.[3,13] The hfsc analysis of the different types of

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(17) (a) Janzen, E. G.; Blackburn, B. J. *J. Am. Chem. Soc.* **1969**, *91*, 4481–4490. (b) The spin adduct with a_H = 0.33 mT is clearly *not* due to the trapping of a trichloromethyl radical (resulting from chlorine abstraction from carbon tetrachloride solvent); this spin adduct has been shown to have a hydrogen hfsc of only 0.17 mT. Cf.: Kimura, K.; Inaki, Y.; Takemoto, K. *Makromol. Chem.* **1977**, *178*, 317.

(18) Janzen, E. G.; Gerlock, J. L. *Nature (London)* **1969**, *222*, 867.

(19) Janzen, E. G.; Lopp, I. G. *J. Phys. Chem.* **1972**, *76*, 2056.

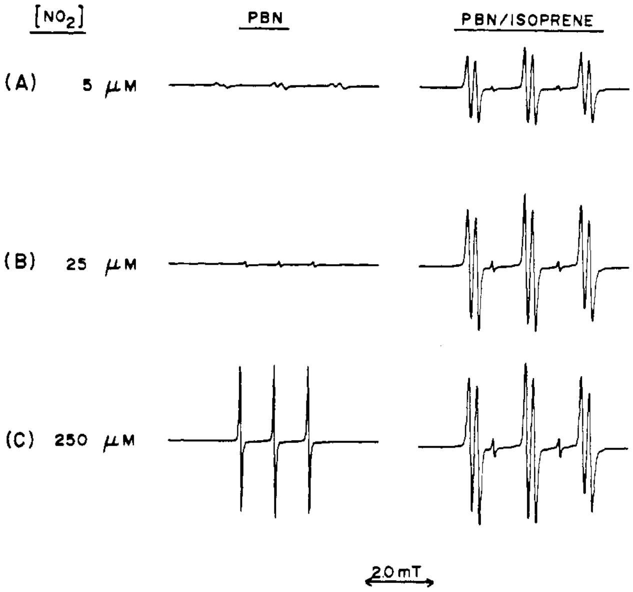


Figure 7. PBN spin adducts formed when hexane solutions of NO_2 are mixed with benzene solutions of either 0.1 M PBN alone or 0.1 M PBN + 0.025 M isoprene. The indicated concentration of NO_2 is that which would be obtained immediately after mixing.

spin adducts in these spectra are given in Table I. It can be seen that NO/air/isoprene system does indeed simulate many of the features of gas-phase cigarette smoke radicals.

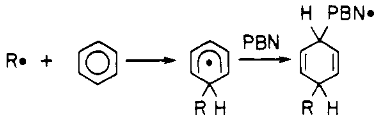
Spin-Trapping Study of NO_2/Air/Olefin and NO_2/Air/Butadiene Gas-Phase Mixtures. We have also studied mixtures of NO_2 and various olefins by the spin-trapping method. Figure 6 shows the relative yields of alkoxyl adducts when four different olefins were each allowed to react with NO_2 in the gas phase. Different path lengths were used in the apparatus shown in Figure 2 in order to vary the residence time in the gas phase before the gaseous mixture reached the spin trap solution. As Figure 6 shows, the yields of spin adducts fall off rapidly for all of the olefins except isobutylene; as we will suggest below, this is consistent with tertiary peroxyl radicals being produced from this olefin and primary or secondary peroxyl radicals from the other three.

Spin-Trapping Study of the Solution-Phase Reaction of NO_2 and Isoprene. While the solid-spin-trapping experiments clearly show that radicals are present in gas-phase smoke, these results do not preclude the possibility that radicals in the solution-phase trapping experiments are produced by reactions of NO_2 with reactive smoke components (such as isoprene) in the spin-trap solution. To study this possibility, we performed the following experiments. Hexane solutions of NO_2 were mixed with benzene solutions of either 0.1 M PBN alone or 0.1 M PBN plus isoprene (with the PBN/isoprene ratio equal to 5/1). Results obtained by using several concentrations of NO_2 are shown in Figure 7. These spectra show that while NO_2 reacts with PBN alone to give PBNOx, the major spin adduct observed when both PBN and isoprene are present is due to an alkoxyl radical (a_N = 1.35 mT; a_H = 0.19 mT). However, unlike the cigarette smoke or the NO/air/isoprene model system, no alkyl adducts are observed. Thus, it would appear that solution-phase reactions cannot account for the radicals that we spin trap from cigarette smoke or the model system.

Discussion

Spin-Trapping Studies of Cigarette Smoke. We have extended our earlier spin trapping studies of gas-phase cigarette smoke to include four different spin trapping protocols (see Figure 4 and Table I). All four protocols show that gas-phase cigarette smoke gives mainly an alkoxyl radical spin adduct. The results obtained by trapping with solid spin traps demonstrate that radicals can be trapped directly out of the gas phase. Moreover, the fact that both the solution-phase-trapping and solid-trapping methods give similar concentrations of spin adducts suggests that a major fraction of the radicals trapped in the solution-phase experiments are trapped from the gas phase and do not arise from solution-phase reactions.

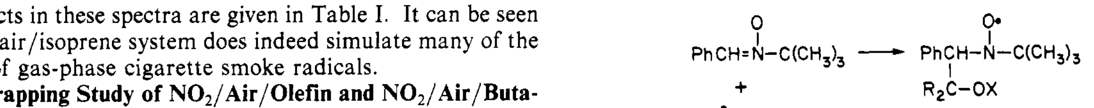
There is evidence for two different types of carbon-centered spin adducts, one with a_H = 0.20 mT from trapping in benzene and one with a_H = 0.32–0.33 mT from the other trapping protocols. We believe that this reflects the fact that benzene is susceptible to homolytic addition to give cyclohexadienyl radicals (eq 11).[16] The hydrogen hfsc that we observe in benzene has



(11)

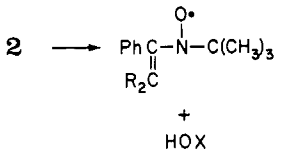
been observed for cyclohexadienyl adducts,[15] while the much larger hydrogen hfsc we observe by using the other protocols are more characteristic of alkyl–PBN spin adducts.[17]

We observe both an alkyl spin adduct with a_N about 1.44 mT and a species that we have suggested may be a vinyl nitroxide (**1**) with a_N ca. 1.04 mT. Interestingly, the sum of the contributions from the carbon-centered adduct and the vinyl nitroxide remains relatively constant, even though the spin-trapping protocol varies greatly, suggesting the possibility of a common precursor or that one of these spin adducts is being converted into the other. One possibility is that the carbon-centered radical has an α substituent (such as a nitrite or nitrate group) that is readily eliminated (see eq 12 and 13). The identity of this species can only be answered



$$\mathrm{PhCH{=}\overset{\overset{\displaystyle O}{|}}{N}{-}C(CH_3)_3 + R_2\dot{C}{-}OX \longrightarrow Ph\underset{\underset{\displaystyle R_2C{-}OX}{|}}{CH}{-}\overset{\overset{\displaystyle O\bullet}{|}}{N}{-}C(CH_3)_3} \qquad (12)$$

2



$$\mathbf{2} \longrightarrow \mathrm{Ph\underset{\underset{\displaystyle R_2C}{\|}}{C}{-}\overset{\overset{\displaystyle O\bullet}{|}}{N}{-}C(CH_3)_3 + HOX} \qquad (13)$$

unequivocally by isolating and determining the structure of this nitroxide.[20]

NO/Air/Isoprene Model System. Scheme I shows our hypothesis to explain the formation of radicals in cigarette smoke. In this mechanism, NO serves as the reservoir of radicals, slowly being oxidized to the more reactive NO_2. There are many substances that could react with NO_2 in smoke to produce radicals, including olefins, dienes, and aldehydes. However, isoprene is a particularly appropriate substrate for testing since it is one of the most prevalent compounds in smoke, typically occurring at concentrations of 400 μg/cig.[3]

As Figure 5 and Table I clearly show, the spin-trapping results with the NO/air/isoprene model system are very similar to those observed for cigarette smoke. Both alkoxyl and alkyl spin adducts are observed. The difference in the yields of alkyl spin adduct relative to alkoxyl adduct in the two systems cannot be explained, but cigarette smoke is so complex that it would be unreasonable to expect that the spin adduct ratios would be identical. We believe that our hypothesis that Scheme I can account for the radicals trapped from cigarette smoke is, therefore, correct. However, we must again caution that this mechanism is just one *possibility*; there may well be other as yet unidentified processes that also contribute to the spin adducts observed from smoke.

Mechanistic Interpretation of the NO/Air/Isoprene Spin-Trapping Results. The mechanism shown in Scheme I involves

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(20) We cannot at present rule out a nonradical (molecular) mechanism for the formation of the vinyl nitroxide. Such nonradical routes to nitroxides in other unrelated spin-trapping studies have been suggested before. See the discussion in: Pryor, W. A.; Govidan, C. K.; Church, D. F. *J. Am. Chem. Soc.* **1982**, *104*, 7563–7566.

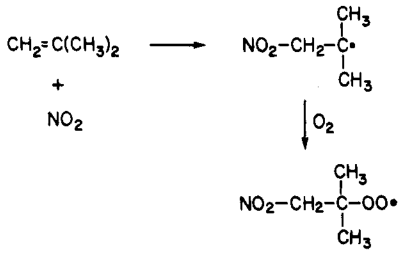
three different organic radical intermediates: alkyl, peroxyl, and alkoxyl radicals. However, we detect only alkyl and alkoxyl radicals by spin trapping. There are two possible explanations for our not observing peroxyl spin adducts in this study.

The first possible explanation is that peroxyl spin adducts are unstable under conditions similar to those used in this study and are known to decompose to give the corresponding alkoxyl spin adducts.[21] If this is the case in the present study, then the observed alkoxyl spin adducts may represent the trapping of either a mixture of alkoxyl and peroxyl radicals or the trapping of peroxyl radicals exclusively. The present data cannot distinguish the initial trapping of alkoxyl from the initial trapping of peroxyl radicals that subsequently rearrange to alkoxyl adducts.

The second possible explanation for not observing peroxyl spin adducts, and the one that we believe is correct, is that the concentration of peroxyl radicals in the gas phase is too low to allow them to be trapped in competition with alkoxyl or alkyl radicals. A recent study places the rate constant for the trapping of peroxyl radicals by PBN at 100–200 $M^{-1}\ s^{-1}$.[22] Alkoxyl radicals are trapped much more readily by PBN, with rate constants being near $1 \times 10^6\ M^{-1}\ s^{-1}$.[23] Thus, if the gas-phase concentration ratio of peroxyl to alkoxyl radicals is less than about 1000:1, then the ratio of alkoxyl to peroxyl spin adducts that we would observe would be greater than $1/1000 \times 10^6/200 = 5$. We believe the ratio of peroxyl to alkoxyl radicals is less than 1000:1, and the peroxyl spin adduct, therefore, may be too dilute to be observed even if it were stable. In order to estimate the gas-phase concentrations of the radicals both in cigarette smoke and in our NO/air/isoprene model system, we have begun to model the NO/air/isoprene reaction by computer simulation. Although many simulations of this type have been used in the study of smog, to our knowledge there are no reports on the application of this technique to cigarette smoke. Our initial calculations suggest that the peroxyl/alkoxyl concentration ratio is indeed no more than about 10–1000, depending on the rate constants used.[24] This relatively low peroxyl radical concentration appears to be due to the rapid deoxygenation of peroxyl radicals by NO (Scheme I, eq 4).[25]

NO_2–Olefin Experiments. Our spin-trapping results with the NO_2/olefin gas mixtures are also consistent with the mechanism shown in Scheme I (eq 2, 3, 5, and 7). Reaction 2, the addition of NO_2 to an olefin or diene, is known to be fast in the gas phase.[11] We have shown that low levels of NO_2 react with olefins in solution by allylic hydrogen abstraction,[12,26] and this process also would produce an alkyl radical. However, since we have no evidence for hydrogen abstraction by NO_2 in the gas phase, we have represented eq 2 as an addition reaction, consistent with the gas-phase data.[11]

Under our conditions, with the concentration of O_2 about tenfold higher than that of NO_2, the rate of reaction 3 should be faster than that of eq 7.[25] However, as discussed above, peroxyl spin adducts are detected. Our data do, however, provide evidence for the production of peroxyl radicals. Figure 6 indicates that a longer-lived radical precursor is produced in the gas phase when NO_2 is allowed to react with isobutylene relative to the other olefins shown. We interpret this as evidence that peroxyl radicals are produced, since isobutylene is the only olefin studied that would give a tertiary peroxyl radical (eq 14). Tertiary peroxyl radicals

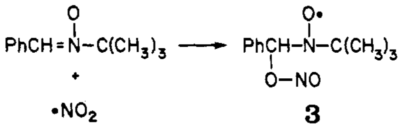


$$CH_2{=}C(CH_3)_2 + NO_2 \longrightarrow NO_2{-}CH_2{-}\dot{C}(CH_3)_2 \qquad (14a)$$

$$NO_2{-}CH_2{-}\dot{C}(CH_3)_2 \xrightarrow{O_2} NO_2{-}CH_2{-}C(CH_3)_2{-}OO\cdot \qquad (14b)$$

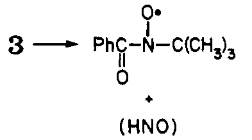
are known to undergo self-termination reactions at much slower rates than do primary or secondary peroxyl radicals,[27] and the self-combination of peroxyl radicals probably is the most important termination reaction in the system once the NO_2 has become depleted.

The solution-phase results with NO_2 and isoprene also are consistent with the proposed mechanism. Only alkoxyl spin adducts are observed, and the concentration of these adducts increases with increasing concentration of NO_2 (see Figure 7). In the absence of isoprene, NO_2 reacts with PBN to give PBNOx, as we had postulated before.[5] A mechanism for this oxidation can be suggested (eq 15 and 16), although it clearly is speculative and several alternatives could be devised.



$$PhCH{=}N(O){-}C(CH_3)_3 + \cdot NO_2 \longrightarrow PhCH(O{-}NO){-}N(O\cdot){-}C(CH_3)_3 \qquad (15)$$

3



$$\mathbf{3} \longrightarrow PhC({=}O){-}N(O\cdot){-}C(CH_3)_3 + (HNO) \qquad (16)$$

The failure to observe PBNOx when solutions of NO_2 and isoprene are mixed indicates that the rate constant for the reaction of NO_2 with PBN is at least 100 times smaller than is the rate constant for the reaction of NO_2 with isoprene. The rate constant for the latter process in the gas phase is 550 $M^{-1}\ s^{-1}$.[11] In our experiments, the ratio of concentrations of isoprene to PBN is $(3 \times 10^{-3})/0.1$, or 3×10^{-2}. Therefore, the rate constant for the reaction of NO_2 with PBN is less than $((3 \times 10^{-2})550)/100 = 0.1\ M^{-1}\ s^{-1}$. Thus, eq 15 and 16 are very slow processes by spin-trapping standards, since typical rate constants for spin trapping by PBN are greater than $10^4\ M^{-1}\ s^{-1}$.[23]

Conclusions

Using the solid spin trap method, we have demonstrated that gas-phase cigarette smoke contains significant concentrations of both alkoxyl and alkyl free radicals. These radicals are too short lived to have been formed in the flame and then trapped. We have demonstrated that they could be *continually* produced in the gas phase by the oxidation of NO to NO_2, followed by the reaction of NO_2 with an olefin to ultimately give alkyl, peroxyl, and alkoxyl radicals. This process has been modeled using a flow system consisting of mixtures of NO and isoprene in air at room temperature. While this NO/air/olefin mechanism may not be the only one by which the apparently long-lived radicals in smoke are produced, the similarities between the radicals spin trapped in cigarette smoke and in the model system suggest it is an important route.

The radicals that we detect in gas-phase cigarette smoke are sufficiently reactive to explain some of the known biological effects of smoke.[4-7,28,29] Cigarette smoke has been reported to initiate

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the autoxidation of polyunsaturated fatty acids (PUFA) in pulmonary macrophages,[30,31] an effect that could be due to NO_2, peroxyl, alkoxyl, and/or alkyl radicals. Nitrogen dioxide is known to add to olefinic bonds, to abstract allylic hydrogen atoms, and to initiate PUFA autoxidation.[11,12,26] Oxy radicals and alkyl radicals also can both add to unsaturated bonds and abstract allylic hydrogen atoms.[32] Autoxidation of PUFA leads to the production of malondialdehyde and other compounds that are reactive in the thiobarbituric acid (TBA) test, and malondialdehyde is known to be mutagenic.[33-35] In addition, PUFA autoxidation produces other aldehydes, particularly 4-hydroxy-2-nonenal, that are strongly cytotoxic.[36,37] Unknown factors in smoke inactivate thiol-dependent enzymes, and we have suggested that NO and NO_2 are the compounds responsible for this.[38] Peroxyl radicals can epoxidize unsaturated compounds, producing electrophilic epoxides that may be mutagenic or carcinogenic.[39,40] And finally, the oxidation of α-1-proteinase inhibitor (a1PI) is thought to be involved in the etiology of smoker's emphysema, and peroxyl radicals and other radicals in smoke may be able to affect this oxidation.[41,42] We have recently shown that our NO/air/isoprene system, like gas-phase cigarette smoke, rapidly inactivates a1PI.[43]

Acknowledgment. This work was supported by the National Institutes of Health, Grant HL-25820, and by the Council on Tobacco Research.

Registry No. PBN, 3376-24-7; PBNOx, 35822-90-3; NO, 10102-43-9; NO_2, 10102-44-0; isoprene, 78-79-5; 1,3-butadiene, 106-99-0; isobutylene, 115-11-7; propylene, 115-07-1; ethylene, 74-85-1.

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Photoelectron Spectra and Molecular Orbital Calculations on Bis(cyclopentadienyldicarbonylchromium, -molybdenum, and -tungsten): Nature of the Bonding of Linear Semibridging Carbonyls

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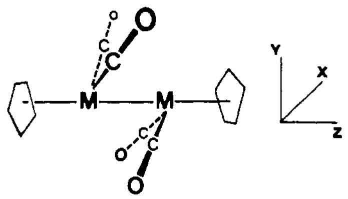
Contribution from the Department of Chemistry, Texas A&M University, College Station, Texas 77843. *Received November 14, 1983*

Abstract: The gas-phase ultraviolet photoelectron spectra are reported for the compounds $[(\eta^5\text{-}C_5H_5)M(CO)_2]_2$, where M = Cr, Mo, W. The spectra are compared to Fenske–Hall molecular orbital calculations on the chromium and molybdenum species. The 15-electron fragment, $(\eta^5\text{-}C_5H_5)M(CO)_2$, requires three additional electrons to satisfy the 18-electron rule. The fragments dimerize and the dimer can be described as a metal-to-metal triple bond with linear semibridging carbonyls. The spectra for all the dimers are similar in general appearance. The spectrum of the chromium species, however, has ionizations from the $15b_u$ and $14a_g$ orbitals that appear as distinct peaks at lower ionization energy than they do in the spectra of the molybdenum and tungsten species. These changes are a result of the nonlinear Cp–M–M framework in the chromium dimers. *The calculations suggest that the linear semibridging carbonyls are π acceptors, not π donors.* They bend over the $M{\equiv}M$ bond in order to accept electrons from the metal–metal π bonds, but they remain linear to avoid destroying this bond. Thus, they join with the two metals to form multicenter, two-electron bonds.

Recent work shows that the species $[(\eta^5\text{-}C_5H_5)M(CO)_2]_2$, **1**, has a rich chemistry,[1-5] The molybdenum dimer reacts readily with soft nucleophiles, like phosphines, breaking the triple bond and displacing the semibridging carbonyls. Electrophiles, like iodine and hydrochloric acid, also add to the triple bond. Depending on the temperature, the iodine forms either an iodo-bridged or terminal-bound species. Mixed metal clusters such as $(\eta^5\text{-}C_5H_5)Mo(CO)_3Co(CO)_4$ and $(\eta^5\text{-}C_5H_5)(CO)_3MoMn(CO)_5$ have been formed. Other complexes formed from the molybdenum dimer include $(\eta^5\text{-}C_5H_5)_2Mo_2(CO)_4(NCNMe)_2$, $(\eta^5\text{-}C_5H_5)_2Mo_2(CO)_4(RCCR')$, and $(\eta^5\text{-}C_5H_5)_2Mo_2(CO)_4$(allene).



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The fragment $CpM(CO)_2$ (Cp = $\eta^5\text{-}C_5H_5$) is a 15-electron fragment and needs three additional electrons to satisfy the 18-electron rule. Recent X-ray studies have revealed unusual structures for the series $[CpM(CO)_2]_2$ where M = Cr, Mo.[6,7]

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