

Spin Trapping of Radicals in Gas-Phase Cigarette Smoke

Alexander Halpern* and Josef Knieper

Institut für Chemie 1: Nuklearchemie,
Kernforschungsanlage Jülich GmbH, Postfach 1913,
D-5170 Jülich, FRG

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Spin trapping with *N*-*t*-butyl- α -phenyl nitron (PBN) and 5,5-dimethyl-1-pyrroline-oxide (DMPO) has been utilized to identify the radicals in the gas phase of cigarette smoke. ESR spectra reveal predominantly alkoxy radicals which are continuously formed in smoke, but not directly in the flame.

Introduction

Since the spin trapping technique was developed and utilized to determine radicals in the gas phase (see reviews [1–3]), it has become attractive as a method of detecting radicals in gas-phase tobacco smoke. A first study of this sort was reported by Bluhm *et al.* in 1971 [4], while previous studies were forced to rely on the measurements of smoke condensate at liquid nitrogen temperature [5, 6]. Menzel *et al.* [7] estimated the concentration of spin-trapped radicals in smoke, but the principal aim of this paper was to explore the utility of the dipolar broadening of ESR lines for quantification rather than to assign a structure of the trapped radicals. The laboratory of W. A. Pryor is presently the only research group carrying out systematic studies of radicals in tobacco smoke by spin trapping [8–11]. We decided to publish our own results at this stage which, in the main, present an independent confirmation of those by Pryor *et al.*, and in certain respects offer new evidence.

Experimental

The manual smoking apparatus (Fig. 1), designed to smoke simultaneously three cigarettes, was operated from a gas-tight syringe so that every 20 s a 25 ml puff of about 5 s duration (a total of 25 puffs per cigarette) was drawn through a cylindrical chamber (volume: 220 ml) with a paper filter (Schleicher & Schüll Nr. 589) on the porous glass support in the middle, and a 0.5 mm i.d. capillary, into a vessel containing 2 ml of a spin trap solution. The total length from the cigarette holder to the solu-

* Reprint requests to Dr. A. Halpern.

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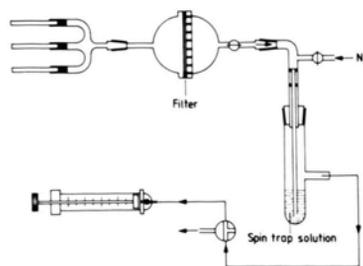


Fig. 1. Sketch of the manual smoking device.

tion was 550 mm. Commercial non-filtered cigarettes were used. The spin trap solution was then deoxygenated by flushing nitrogen for 3 min, and transferred under nitrogen atmosphere to a ESR tube which was tightly closed and placed in the cavity.

ESR spectra were recorded at room temperature with a Varian E-9 spectrometer (9.3 GHz) using 100 kHz modulation frequency, modulation amplitude of 0.1 G, microwave power of 10 mW, and a scan rate of 6.5 G/min. *N*-*t*-butyl- α -phenyl nitron (PBN) and 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), both supplied by Sigma, were used as spin traps; PBN was used without further purification, DMPO after filtration through charcoal.

Results and Discussion

In Fig. 2a the ESR spectrum produced in 0.05 M PBN in benzene by smoke from three cigarettes is shown, while Figs 2b and 2c show the spectra of the same sample recorded after 15 min and 90 min, respectively. The structure in these spectra suggests the presence of two nitroxides, the dominant one with the coupling constants $A^N = 13.63$ G and $A_{\beta}^H = 2.0$ G, and the other (marked with arrows) with $A^N = 10.25$ G and no visible proton splitting. The relative intensities of the lines characteristic of the two adducts change with the storage time in favor of the second adduct (simple triplet), but it is clearly seen that the first one (with $A^N = 13.63$ G) always prevails. These assignments agree well with those reported by Pryor *et al.* [8], who identified the major component as the alkoxy radical adduct, PBN-RO. This triplet of doublets is likely to represent the superposition of lines due to different radical adducts having similar hfsc's, of which the PBN-RO is the prevailing adduct, but it may also include the alkyl adduct, PBN-R. However, since (i) alkyl radicals are trapped much less efficiently than alkoxy radicals (e.g. the rate constants for reactions of $(\text{CH}_3)_3\text{C}\cdot$ and $(\text{CH}_3)_3\text{CO}\cdot$ with PBN are $1.0 \cdot 10^4 \text{ M}^{-1}\text{s}^{-1}$ [12] and $5.5 \cdot 10^6 \text{ M}^{-1}\text{s}^{-1}$ [13], respectively), and (ii) alkyl radicals are known to



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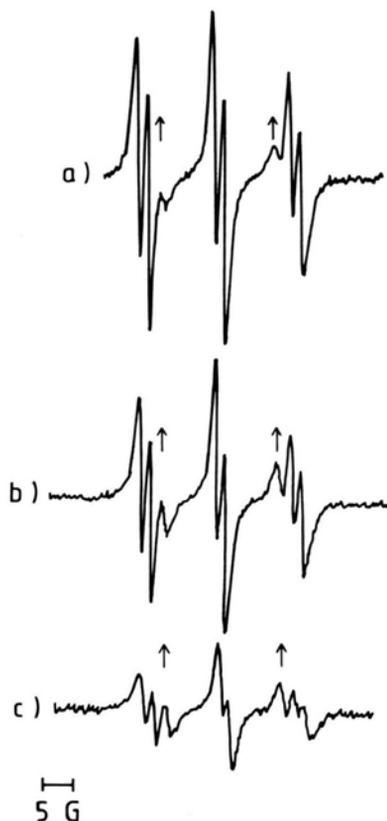


Fig. 2. a) The ESR spectrum from cigarette smoke in 0.05 M PBN in benzene; b) same sample, 15 min later; c) same sample, 90 min later.

react exothermically with oxygen to form alkylperoxy radicals [8, 11, 14], it can be foreseen that the contribution from PBN-R will be unimportant. The peroxy radicals can be trapped by PBN but the respective spin adducts are unstable toward light and are replaced by the alkoxy adducts [15].

As far as the 10.25 G triplet is concerned, our data do not allow to assign unambiguously the structure of the responsible spin adduct. It does not seem to result from the oxidation of PBN, since such a signal was not observed when we let a stream of O_2 , NO_2 or a mixture of NO and O_2 to pass through a 0.05 M PBN in benzene at room temperature. Recently, Pryor *et al.* [11] suggested that this spin adduct is a vinyl nitroxide.

In another series of experiments we employed DMPO, which is a convenient trapping agent for oxy-radicals, since the respective spectra exhibit the splitting due to β -hydrogens to be smaller by a factor of almost three than those from alkyl radical ad-

ducts, and an additional splitting by a γ -hydrogen [16]. The spectrum produced by smoke from three cigarettes in 0.05 M DMPO in benzene is shown in Fig. 3a. In this spectrum, one set of reasonably sharp lines can be distinguished which indicate a triplet of $A^N = 13.5$ G, further split into secondary doublet due to a β -proton, $A_{\beta}^H = 8.0$ G, and another doublet by a γ -proton, $A_{\gamma}^H = 2.2$ G. This hfs is consistent with the *t*-butoxy radical [16]. Moreover, a number of other lines which are superimposed and only partially resolved make the spectrum complex and its interpretation ambiguous, even though they bear witness to the presence of various radical species. Repeating ESR measurements after 40 min produced no change in the hfs pattern of the spectrum (Fig. 3b). Thus, we were not able to confirm the observation that aging of the sample yielded a spectrum significantly different from the initial one [8]. However, this contradiction does not influence the concordance of the basic conclusion from both studies that a mixture of several radical species, predominantly oxygen-centered, is obtained in the smoke, exemplified by the *t*-butoxy radical. It should be noted that we did not observe any ESR signal in methanolic or aqueous solutions of PBN, presumably because the alkoxy radicals are highly unstable in polar media [17, 18].

It is pertinent to close with a comment on the origin of radicals trapped from cigarette smoke. The radicals in question are not expected to have lifetimes long enough to allow them to travel a substantial distance of 550 mm to the spin trap solution, including through a filter. Therefore, one has to as-

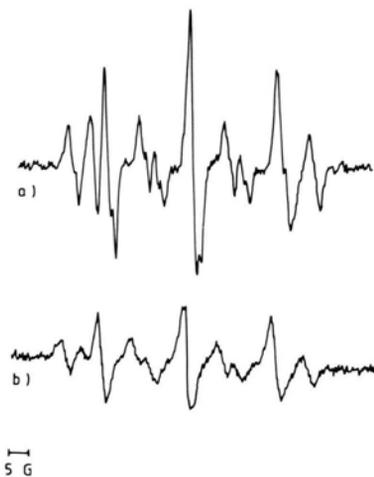


Fig. 3. a) The ESR spectrum from cigarette smoke in 0.05 M DMPO in benzene; b) same sample, 40 min later.

sume that a radical-forming process is in operation in the smoke stream, in which radicals are continuously formed as the smoke proceeds toward the solution during the entire course of smoking. Pryor *et al.* [9, 11] provided evidence that this process depends on the slow oxidation of NO to NO₂, followed by reactions of NO₂ with smoke components such a ole-

fins or dienes to give alkyl radicals R· which will be then oxidized to alkylperoxy radicals ROO· and eventually converted to alkoxy radicals RO·. Therefore, the radicals observed by the spin trapping, although initiated by the combustion process, are not those formed directly in the flame. A similar situation occurs in human smoking.

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- [1] M. J. Perkins, *Adv. Phys. Org. Chem.* **17**, 1 (1980).
[2] E. G. Janzen, in W. A. Pryor (ed.): *Free Radicals in Biology*, Vol. IV, Academic Press, New York 1980.
[3] E. G. Janzen, in W. R. Ware (ed.): *Creation and detection of the excited states*, Vol. IV, M. Dekker, New York 1976.
[4] A. L. Bluhm, J. Weinstein, and J. A. Sousa, *Nature* **229**, 500 (1971).
[5] M. J. Lyons, J. F. Gibson, and D. J. E. Ingram, *Nature* **181**, 1004 (1958).
[6] W. F. Forbes, J. C. Robinson, and G. F. Wright, *Can. J. Biochem.* **45**, 1087 (1967).
[7] E. R. Menzel, W. R. Vincent, and J. R. Wasson, *J. Magn. Reson.* **21**, 321 (1976).
[8] W. A. Pryor, K. Terauchi, and W. H. Davis, *Environm. Health Persp.* **16**, 161 (1976).
[9] W. A. Pryor, D. G. Prier, and D. F. Church, *Environm. Health Persp.* **47**, 345 (1983).
[10] W. A. Pryor, M. Tamura, M. M. Dooley, P. Premovic, B. J. Hales, and D. F. Church, in R. A. Greenwald, G. Cohen (eds): *Oxy Radicals and their Scavenger Systems*, Vol. II, 185, Elsevier, Amsterdam 1983.
[11] W. A. Pryor, M. Tamura, and D. F. Church, *J. Am. Chem. Soc.* **106**, 5073 (1984).
[12] T. Doba, T. Ichikawa, and H. Yoshida, *Bull. Chem. Soc. Jpn.* **50**, 3158 (1977).
[13] E. G. Janzen and C. A. Evans, *J. Am. Chem. Soc.* **95**, 8205 (1973).
[14] J. A. Howard, in J. E. Kochi (ed.): *Free Radicals*, Vol. II, J. Wiley, New York 1973.
[15] E. Niki, S. Yokoi, J. Tsuchiya, and Y. Kamiya, *J. Am. Chem. Soc.* **105**, 1498 (1983).
[16] E. G. Janzen and J. I. Ping Liu, *J. Magn. Reson.* **9**, 510 (1973).
[17] C. Walling and P. J. Wagner, *J. Am. Chem. Soc.* **86**, 3368 (1964).
[18] W. Bors, D. Tait, C. Michel, M. Saran, and M. Erben-Russ, *Israel J. Chem.* **24**, 17 (1984).