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AN ELECTRON SPIN RESONANCE STUDY OF RADICALS FROM CHLORAMINE-T—2

SPIN TRAPPING OF PHOTOLYSIS PRODUCTS OF CHLORAMINE-T AT ALKALINE pH

JEFFREY C. EVANS,* SIMON K. JACKSON and CHRISTOPHER C. ROWLANDS
Chemistry Department, University College, P.O. Box 78, Cardiff CF1 1XL, U.K.

and

MARTIN D. BARRATT
Environmental Safety Laboratory, Unilever Research, Colworth House, Sharnbrook, Bedford, U.K.

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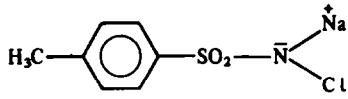
Abstract—Irradiation of chloramine-T at alkaline pH in the presence of the spin trap 2-methyl-2-nitroso-propane gave evidence for the trapping of several sulfur-centred radicals and a carbon-centred radical. Trapping experiments with 5,5-dimethyl-pyrrolidine-1-oxide gave evidence for the production of a nitrogen-centred radical and a carbon-centred radical. The spin trap α-phenyl-t-butyl-nitrone gave evidence for a nitrogen-centred radical, a sulfur-centred radical and the H-atom adduct of the spin trap. The identity of the trapped species was confirmed by irradiation of the following chemical analogues of chloramine-T as "model compounds" in alkaline solution; chloramine-B (sodium salt of N-chlorobenzene sulfonamide), *p*-toluenesulfonamide, *p*-toluenesulfonic acid, *p*-toluenesulfinic acid. To aid in the assignment of the radical adducts where mixtures of species occurred, computer simulation of the spectra was performed.

INTRODUCTION

It has been shown[1] that under acidic conditions chloramine-T (I) undergoes loss of a Cl atom to produce a N-centred chloramine-T free radical. This radical, is thought to be one of the species involved in the covalent



I

binding of chloramine-T to various soluble proteins at pH 6.5.[2] The observation that covalent binding of chloramine-T to protein could also be obtained at alkaline pH by irradiation with UV light[2] prompted a study of the radicals produced by the photolysis of chloramine-T at alkaline pH. To study the short-lived radicals produced in this system we have used the spin-trapping method; this technique employs diamagnetic molecules (spin traps) which react with free radicals to form stable nitroxide radicals which have spectral parameters characteristic of the adduct.[3] To help in the identification of the trapped radicals and to determine mechanisms by which they could be produced, compounds similar in structure to chloramine-T have been used as "model compounds".

EXPERIMENTAL

Materials. Chloramine-T (BDH), chloramine-B, *p*-toluenesulfonamide (Fluka), *p*-toluenesulfonic acid, *p*-toluenesulfinic acid, 4-aminobenzoic acid, 2-methyl-2-nitroso-propane (MNP), 5,5-dimethyl-l-pyrrolidine-l-oxide (DMPO), α-phenyl-t-butyl nitrone (PBN) (Aldrich) were used without further purification.

Procedure. ESR spectra were recorded on a Varian E3 spectrometer operating at 9.5 GHz (X-band). Samples were placed in the cavity in quartz or pyrex aqueous sample cells. The g-values were calculated using either diphenylpicryl-hydrazyl introduced into the sample cell in a small capillary tube, or using di-t-butyl nitroxide as a secondary standard.

Preparation of samples. The spin traps DMPO and PBN were made up in aqueous soln (0.01 M) and diluted as necessary. Aqueous solns of MNP were prepared by stirring in the dark for 24 hr at room temp. All solns of spin traps were degassed with N_2 and stored in the dark in the refrigerator.

Aqueous solns of chloramine-T and the various model compounds were made to a strength of 0.05 M and diluted if necessary.

Before mixing, solns for ESR spectroscopy were degassed with N_2 for a few minutes to remove dissolved O_2.

The pH of the solns was adjusted by the addition of 0.01 M HCl or 0.01 M NaOH prior to running the ESR spectra.

Irradiation conditions. Irradiation of samples was carried out at room temp either outside or "*in situ*" in the cavity of the spectrometer, using a Bausch and Lomb 150 W Xe arc lamp. The light from the lamp was either unfiltered, or filtered when required using pyrex glass to cut off the shorter wavelength light.

Computer simulations. Computer simulations of ESR spectra were carried out using a North Star Horizon microcomputer. Where computer simulations were helpful in confirming the assignments of a mixture of spectra the simulations are shown beneath the original spectra.

RESULTS

Photolysis products trapped by 2-methyl-2-nitroso-propane (MNP)

When an aqueous solution of chloramine-T (pH 8.5) containing MNP was irradiated *in situ* in the cavity of the ESR spectrometer for 1 min, several signals were detected. These are shown in Fig. 1(a). This shows three triplets, one of which (labelled 1) had an N-splitting constant, $a_N = 1.716$ mT and $g = 2.0055$, which is often seen when irradiating solutions containing MNP, and can be identified as di-t-butyl-nitroxide (DTBN).[4] The

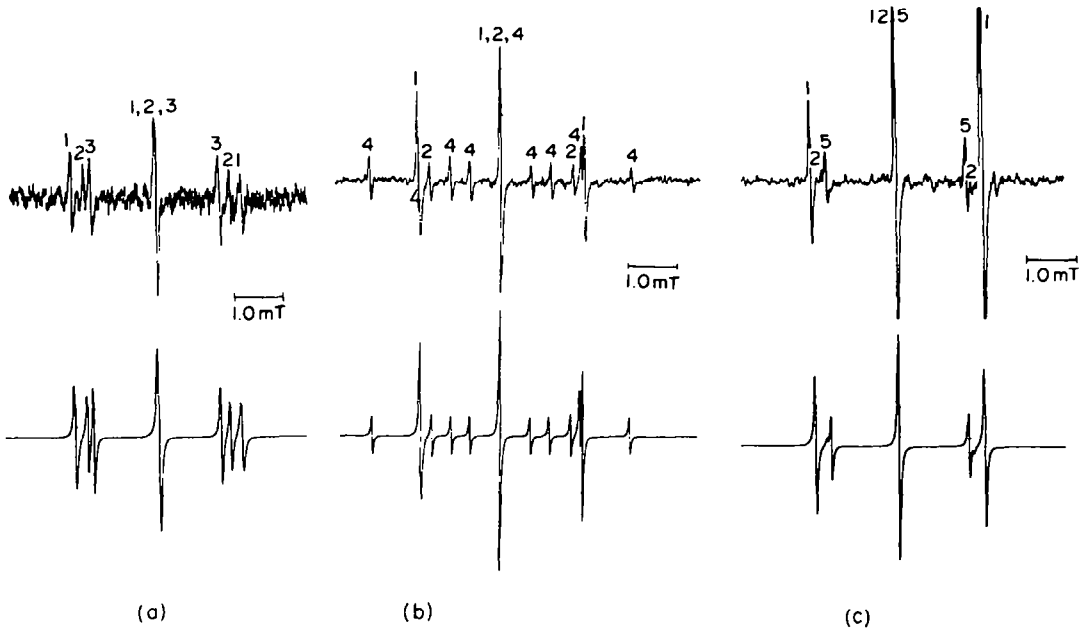


Fig. 1. (a) ESR spectrum of radical adducts 1, 2 and 3 obtained by irradiation of aqueous solutions of MNP/chloramine-T; together with its computer simulation. (b) ESR spectrum of radical adducts 1, 2 and 4 obtained by prolonged irradiation (15 min) of 1a; together with its computer simulation. (c) ESR spectrum of radical adducts of 1, 2 and 5 obtained by irradiation of *p*-toluenesulfonamide/MNP together with its computer simulation.

other signals (2,3 in Fig. 1(a)) were also triplets with *g*-values close to that of DTBN. If the same solution of chloramine-T (pH 8.5) was irradiated for a longer time (15 min) another signal appears in the spectrum. This is shown in Fig. 1(b). It can be seen that one of the triplets (labelled 3 in Fig. 1(a)) has disappeared, and a new adduct appeared (labelled 4) as a weak triplet of triplets with an intensity ratio of 1 : 2 : 1, suggesting hyperfine splitting from two equivalent H-atoms (a_N = 1.675 mT, a_H = 1.038 mT (2H)). This species is assigned to trapping the chloramine-T radical formed by loss of a H-atom from the *p*-methyl group.

When aqueous solutions of chloramine-T containing MNP were irradiated under more alkaline conditions (pH 11), this triplet of triplets was the only species observed.

When an aqueous solution of chloramine-B (Na-salt of N-chlorobenzene sulfonamide) containing MNP was irradiated at pH 8.5 the ESR spectrum obtained was identical to that produced from chloramine-T (Fig. 1(a)), indicating a similar route of radical production from chloramine-B and chloramine-T. However, as the pH of the solution was raised, no other species were detected.

When a basic solution of *p*-toluenesulfinic acid was irradiated in the presence of MNP, signals identical to adducts 2 and 3 seen with chloramine-T were detected in addition to DTBN. Irradiation of *p*-toluenesulfonic acid Na-salt (pH 8.5), in the presence of MNP shows a signal due to DTBN, and a signal identical to adduct 2 obtained on irradiating chloramine-T and MNP (Fig. 1(a)). When an aqueous solution of *p*-toluensulfonamide (pH 9) was irradiated with a solution of MNP, the spectrum shown in Fig. 1(c) was obtained. This shows a strong signal due to DTBN, which increased in strength as the spectrum was scanned, a weak signal identical to that of adduct **2** (Fig. 1(a)) and another triplet (a_N = 1.401 mT, g = 2.0056) labelled 5.

The use of computer simulation not only enables the verification of the experimentally obtained splitting constants but in the cases of Figs 1(a) and (b) allowed an estimation to be made of the relative concentration of the various species (Table 1).

Photolysis products trapped with 5,5-dimethyl-pyrollidine-1-oxide (DMPO)

When an aqueous alkaline solution of chloramine-T was irradiated in the presence of the spin trap DMPO initially the spectrum shown in Fig. 2(a), was obtained (species 6). This spectrum is consistent with the trapping of an N-centred radical (a_N = 1.587 mT, a_H = 1.813 mT, a_{N_β} = 0.238 mT). A very weak triplet was also observed between the main group of lines in

Table 1. Relative concentration used in computer simulation of Figs 1(a) and (b)

Species	Relative concentrations (%)
Fig. 1(a)	
1	38
2	28
3	34
Fig. 1(b)	
1	45
2	12
4	43

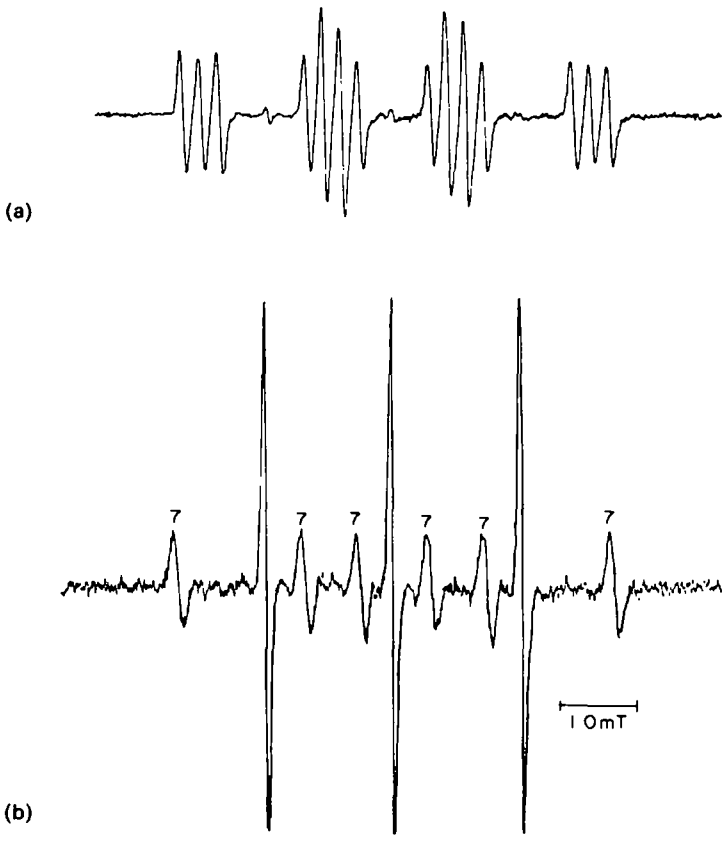


Fig. 2. (a) ESR spectrum of radical adduct 6 obtained by irradiation of chloramine-T/DMPO in aqueous solution. (b) ESR spectrum of radical adduct 7 together with breakdown product of spin trap obtained on prolonged irradiation of 2(a).

the spectrum, and this became more intense on longer irradiation. This species apparently arose from a breakdown product of the spin trap itself as the same species was formed after prolonged irradiation of a solution of the spin trap alone. If the solution of chloramine-T and DMPO (pH 8.5), which gave rise to the spectrum shown in Fig. 2(a) was irradiated for longer periods of time (15 min) another species (7) was observed as well as the strong triplet due to the breakdown of the spin trap. This is shown in Fig. 2(b).

This species (a_N = 1.638 mT, a_H = 2.35 mT) is characteristic of a C-centred radical adduct of DMPO. When chloramine-B was irradiated at pH 8.5 in the presence of DMPO, results identical to those with chloramine-T were obtained.

Irradiation of a solution containing *p*-toluenesulfinic acid and DMPO gave a mixture of species, one of which had the same splitting constant as the C-centred radical adduct produced from chloramine-T (7 Fig. 2(b)). The other species (a_N = 1.463, a_H = 1.650 mT) could be due to a sulfur-centred radical adduct of DMPO. When an aqueous alkaline solution of the Na-salt of *p*-toluenesulfonic acid, containing the spin trap DMPO was irradiated only the spectrum of the OH radical adduct of DMPO could be obtained ($a_N = a_H$ = 1.50 mT). If an alkaline solution of *p*-toluenesulfonamide was irradiated in the presence of DMPO, a species with the same splitting constants as the N-centred radical obtained from chloramine-T was observed.

Photolysis products trapped by α-phenyl-t-butyl-nitrone (PBN)

Irradiation of an aqueous alkaline solution of chloramine-T containing the spin trap PBN produced a spectrum which is identical to that produced when PBN is mixed with chloramine-T at acid pH in the absence of irradiation.[1] Irradiation of chloramine-B under the same conditions produced identical results. If a solution of chloramine-T (or chloramine-B) at alkaline pH containing PBN was irradiated for a longer time (15 min) the initial adduct decayed and was replaced by other adducts. These rapidly-changing signals were tentatively assigned to trapping of a H-atom (labelled 8) and a sulfur-centred radical (labelled 3) shown in Fig. 3.

The results of all the spin-trapping experiments are summarised in Table 2.

DISCUSSION

The results of the irradiation experiments give evidence for the production of a variety of radicals from chloramine-T at alkaline pH. This is interesting since at acid pH without irradiation chloramine-T gave N-centred radicals via a single route, i.e. homolytic cleavage of the N—Cl bond.[1]

The results of the irradiation studies with MNP point to the production of several S-centred radicals

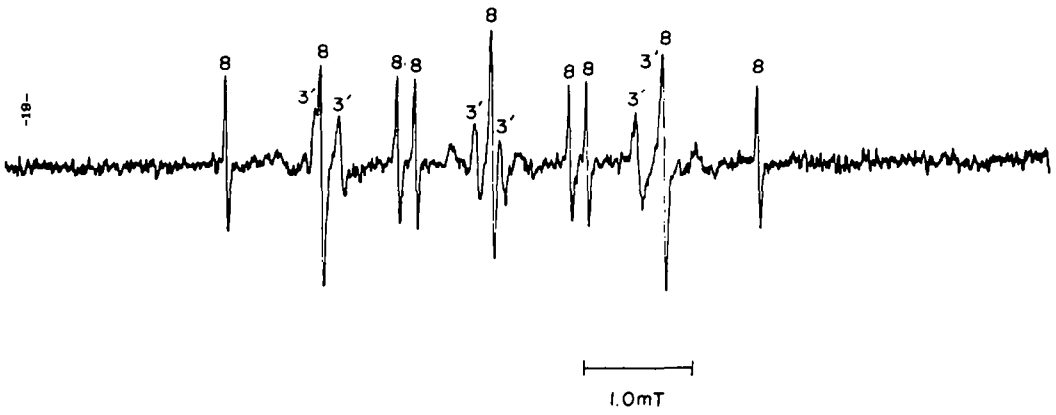


Fig. 3. ESR spectrum of radical adduct 8 and 3' obtained by irradiation of aqueous solution of chloramine-T/PBN.

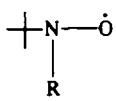
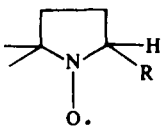
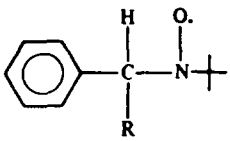
and a C-centred radical. The identity of these S-centred radicals can be determined from the fact that; their MNP-adducts lack hyperfine splitting on the nitrogen triplet, from the results of the photolysis of the various model compounds and from what is known about the aqueous photochemistry of organic S compounds.

Since the signal labelled 2 in Fig. 1(a) was also obtained on irradiation of toluenesulfinic acid and toluenesulfonic acid (Na-salt) with MNP, it might be reasonable to assign the structure of the radicals which gave rise to these adducts as the $SO_2^{\cdot-}$ radical anion. This is also a known photodegradation product of sulfanilamide[5] and it is probably formed via a similar mechanism in chloramine-T. When an alkaline solution of sodium dithionite was added to a solution of MNP, a spectrum identical to that due to adduct 2 was obtained.[6] Hence the radical has been shown to be $SO_2^{\cdot-}$.

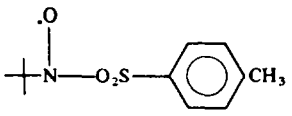
Since signal 3 can be generated by photolysis of *p*-toluenesulfinic acid, chloramine-B as well as chloramine-T in the presence of MNP, this suggests that the radical from which the adduct is formed in chloramine-T arises from S—N bond cleavage. The N hyperfine splitting constant of adduct 3 (a_N = 1.312 mT) (whose structure is shown below) lends weight to this assignment as this splitting is smaller than that from the other S adducts. This would be expected because of delocalization of the unpaired electron density onto the tolyl ring system.

Adduct 4 (Fig. 1(b)) has been assigned on the basis of known MNP adducts of similar structure[7] and also because it could not be produced from irradiation of chloramine-B in which the *p*-methyl group is absent. The hyperfine splitting constants for 4 (a_N = 1.675, $a_{H(2)}$ = 1.038 mT) are very similar to other adducts of MNP with an $\alpha(CH_2)$ group, e.g. benzyl adducts formed via irradiation of benzyl halides. A similar spectrum has been reported from the irradiation of L-phenylalanine

Table 2. Hyperfine coupling constants for the radical adducts obtained using MNP, DMPO, PBN with chloramine-T, respectively

Spin adduct	Trapped radical (R')	Adduct	Splitting constants of adduct (mT)	Figure
MNP				
	t-Bu	1	a_N = 1.716	1(a)
	SO_2	2	a_N = 1.487	1(a)
	$CH_3C_6H_4SO_2$	3	a_N = 1.312	1(a)
	$CH_2C_6H_4SO_2N(Cl)Na$	4	a_N = 1.675, $a_{H(2)}$ = 1.038	1(b)
	SO_2NH_2	5	a_N = 1.401	1(c)
DMPO				
	$CH_3C_6H_4SO_2NNa$	6	a_N = 1.587, a_H = 1.813, $a_{N(\beta)}$ = 0.238	2(a)
	$CH_3C_6H_4$	7	a_N = 1.638, a_H = 2.35	2(b)
PBN				
	$CH_3C_6H_4SO_2NNa$	6'	a_N = 1.558, a_H = 0.325, $a_{N(\beta)}$ = 0.163	Fig. 1(a) ref. 1
	H	8	a_N = 1.550, $a_{H(2)}$ = 0.875	3(a)
	$CH_3C_6H_4SO_2$	3'	a_N = 1.475, a_H = 0.225	3(a)

at alkaline pH (a_N = 1.66 mT, a_H = 1.06 mT (2H)).[9] This evidence supports the structure assigned to adduct 4.



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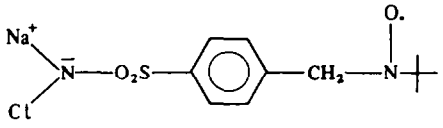
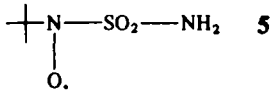
The other species obtained has been assigned to the SO_2^- adduct of DMPO since irradiation of aqueous alkaline sodium sulphite in the presence of DMPO



4

Irradiation of toluenesulfonamide produced another adduct (labelled 5 in Fig. 1(c)), with a_N = 1.40 mT. This species has been seen previously in the photolysis of sulfanilamide in the presence of MNP,[8] and assigned to the following adduct:



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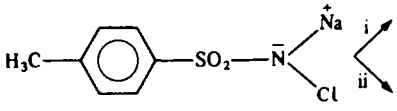
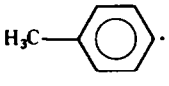
The corresponding radical was not observed on irradiation of chloramine-T, probably due to the fact that chloramine-T is chlorinated on the N atom.

The spectra from the irradiation of aqueous alkaline solutions of chloramine-T with DMPO suggest the presence of an N-centred and a C-centred radical. The N-centred adduct 6 has splitting constants (a_N = 1.587, a_H = 1.813, a_N = 0.238 mT) which are typical for DMPO adducts of this type.[10] Such an adduct could arise from chloramine-T via two different routes:



To try and distinguish between these two mechanisms, *p*-toluenesulfonamide was irradiated in the presence of DMPO at alkaline pH. This produced a species with identical splitting constants to those obtained with chloramine-T. If there was S—N bond scission in the sulfonamide (route i) then we would have trapped the $\dot{N}H_2$ radical. The splitting constants for the NH_2-adduct[11] however are different from those obtained here, and suggest that the N-centred radical produced by irradiation of both chloramine-T and *p*-toluenesulfonamide is generated, via route (ii). Prolonged irradiation with chloramine-T and DMPO generated a six-line spectrum (labelled 7, Fig. 2(b)) with splitting constants consistent with the formation of a C-centred adduct (7) (a_N = 1.638, a_H = 2.35 mT). Irradiation of *p*-toluenesulfinic acid and DMPO produces two species, one has been assigned to the DMPO adduct of radical 7, similar to that obtained on irradiating chloramine-T and DMPO.



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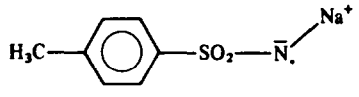
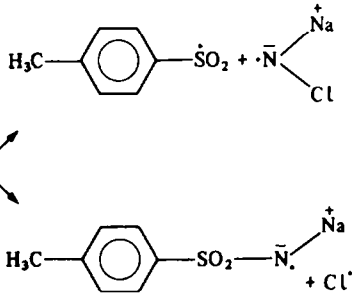
produced a spectrum with the identical splitting constants of a_N = 1.463, a_H = 1.650 mT. This has been assigned previously to the SO_2^- adduct of DMPO.[11]

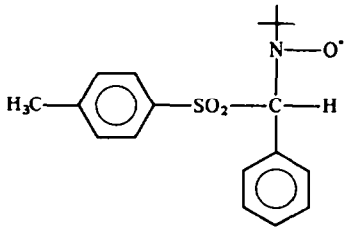
Irradiation of chloramine-T (pH 8.5) in the presence of PBN gave rise to a N-centred radical adduct 6′ (a_N = 1.58, a_H = 0.325, a_N = 0.163 mT). The same spectrum was also seen when chloramine-T was mixed with PBN at acid pH, without irradiation, see ref. 1, and therefore a similar radical must be produced in both cases. This radical was assigned the following structure:



and arrives via homolytic cleavage of the N—Cl bond. Evidently this bond is split both by UV irradiation and by the catalytic action of acid.



Further irradiation of chloramine-T/PBN systems (about 15 min) produced a variety of signals, which were difficult to assign. However the triplet of triplets which were in the ratio 1 : 2 : 1 with splittings (a_N = 1.55, $a_{H(2)}$ = 0.875 mT) can be tentatively assigned to the H-atom adduct of PBN, and this could be formed via loss of a H-atom from the *p*-methyl group of chloramine-T. The other species formed on continuous irradiation was a triplet of doublets (a_N = 1.475, a_H = 0.225 mT). This species could be assigned to a sulfur radical adduct of PBN, shown below:



CONCLUSIONS

These studies show conclusively that at alkaline pH, under the influence of UV light, chloramine-T reacts to form free radicals. The use of model compounds has provided direct evidence for the production of three S-centred radicals, two C-centred radicals and two N-centred radicals. These results are especially interesting in the light of the results from chloramine-T/protein binding experiments[2] which showed that on UV irradiation at alkaline pH chloramine-T could react with soluble proteins to form covalent conjugates similar to those produced at acid pH. These experiments suggest that the binding of chloramine-T to proteins occurs at least, in part, via free radical mechanisms, involving the radicals identified in this work. Since many of the radicals are reactive species, and sulfur radicals have been shown to be involved in numerous toxic effects,[12] it is postulated that under the influence of UV light, skin contact with chloramine-T could lead both to phototoxic and photoallergic responses, in addition to contact allergy already described.[13,14]

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REFERENCES

[1] J. C. Evans, S. K. Jackson, C. C. Rowlands and M. D. Barratt, *Tetrahedron* **41**, 5191 (1985).
[2] M. D. Barratt, S. K. Jackson, J. C. Evans and C. C. Rowlands, *Tetrahedron* (1985), submitted for publication.
[3] For a review see M. J. Perkins, *Adv. Phys. Org. Chem.* **17** (1981).
[4] T. Pfab, *Tetrahedron Lett.* 843 (1978).
[5] C. F. Chignell, B. Kalyanarraman, R. P. Mason and R. H. Sik, *Photochem. Photobiol.* **32**, 563 (1980).
[6] D. Mulvey and W. A. Waters, *J. Chem. Soc. Perkin Trans II* 772 (1974).
[7] Rustigi *et al. Int. J. Rad. Biol.* **31**, 415 (1977); **32**, 533 (1977).
[8] C. F. Chignell *et al., Photochem. Photobiol.* **32**, 563 (1980).
[9] Y. Lion, M. Kuwabara and P. Riesz, *Photochem. Photobiol.* **34**, 297 (1981).
[10] A. G. Motten and C. F. Chignell, *Photochem. Photobiol.* **37**, 17 (1983).
[11] C. F. Chignell *et al., Photochem. Photobiol.* **34**, 147 (1981).
[12] C. C. Harber and R. C. Baer, *J. Invest. Dermatol.* **58**, 327 (1972).
[13] J. H. Dijkman, P. H. Vooren and J. A. Kramps, *Int. Archs Allergy Appl. Immun.* **54**, 422 (1981).
[14] J. A. Kramps, A. W. van Toorenbergen, P. H. Vooren and J. H. Dijkman, *Int. Archs. Allergy Appl. Immun.* **54**, 428 (1981).