Tetrahedron Letters No.24, pp. 2255-2263, 1967. Pergamon Press Ltd. Printed in Great Britain.

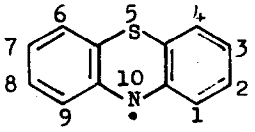
FREE RADICALS DERIVED FROM PHENOTHIAZINE

by C. Jackson and N.K.D. Patel

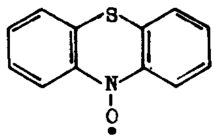
School of Pharmacy,
Sunderland Technical College,
Sunderland, England.

(Received 29 March 1967)

In studies of the antioxidant action of phenothiazine Murphy et al. have suggested the formation of free radicals by the homolytic fission of the N-H bond (1). Since then several workers have published electron spin resonance (e.s.r.) spectra of the free radicals from phenothiazine. In addition to the spectrum obtained for the cationic free radical (2) two further spectra of neutral free radicals from phenothiazine have been published which are identical with spectrum (1a) and spectrum (1c) recorded by us (3). Spectrum (1a) has been assigned to both the phenothiazinyl radical I (4) and to the phenothiazine nitroxide radical II (5). Spectrum (1c) has likewise been assigned to both of these radicals (6,7).



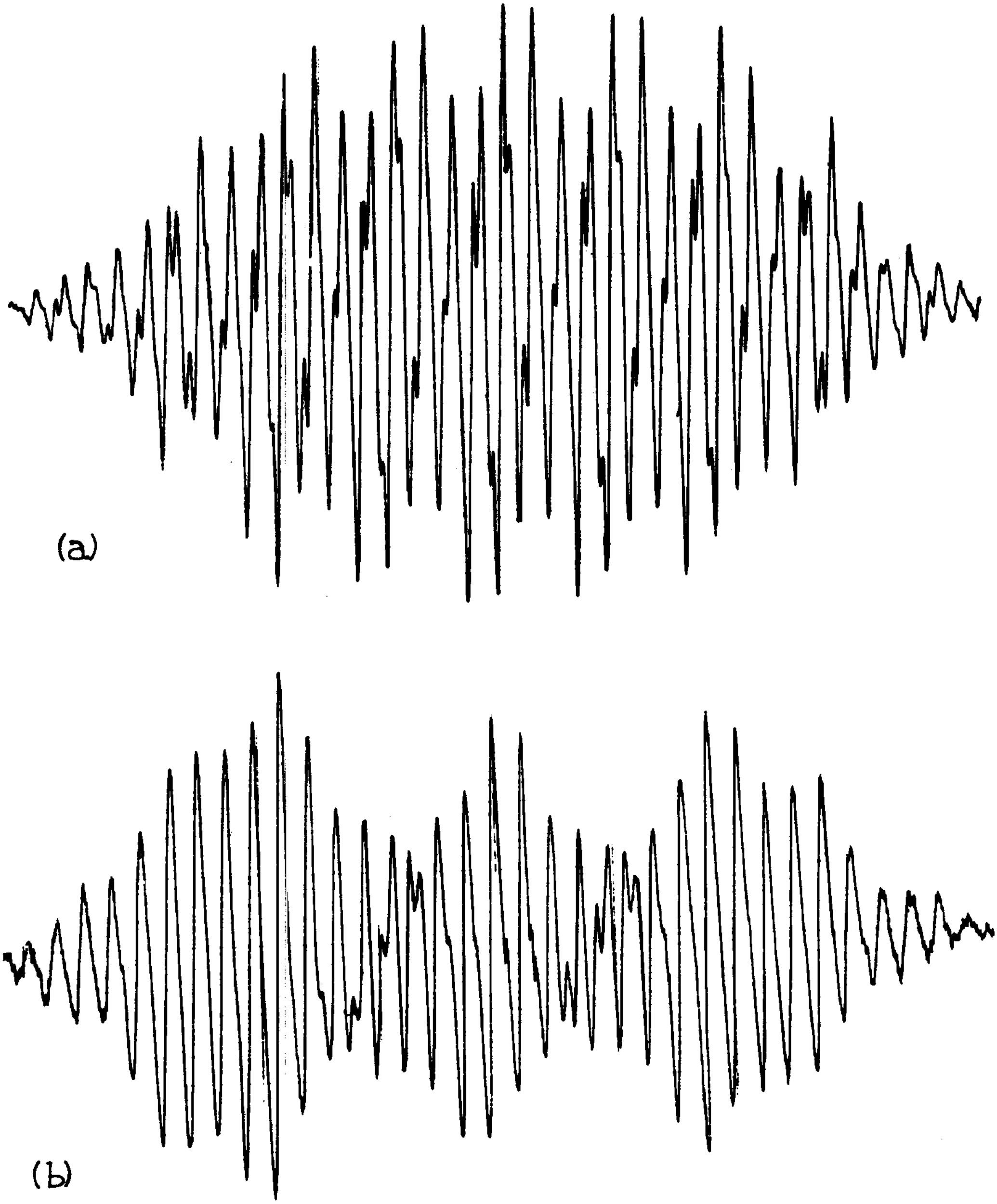
I



II

Spectra (1a) and (1b) are derived from the same free radical and are recorded in ethanol and benzene respectively. The different appearance arises from line broadening and small coupling constant changes due to solvent effects. We have carried out a series of oxidation studies on phenothiazine using a wide variety of oxidising agents. From a knowledge of the mode of action of these oxidising agents and by comparison of their reaction on diphenylamine and other amines, we conclude that spectra (1a) and (1b) arise from the phenothiazinyl radical I and that spectrum (1c)

FIG. 1.



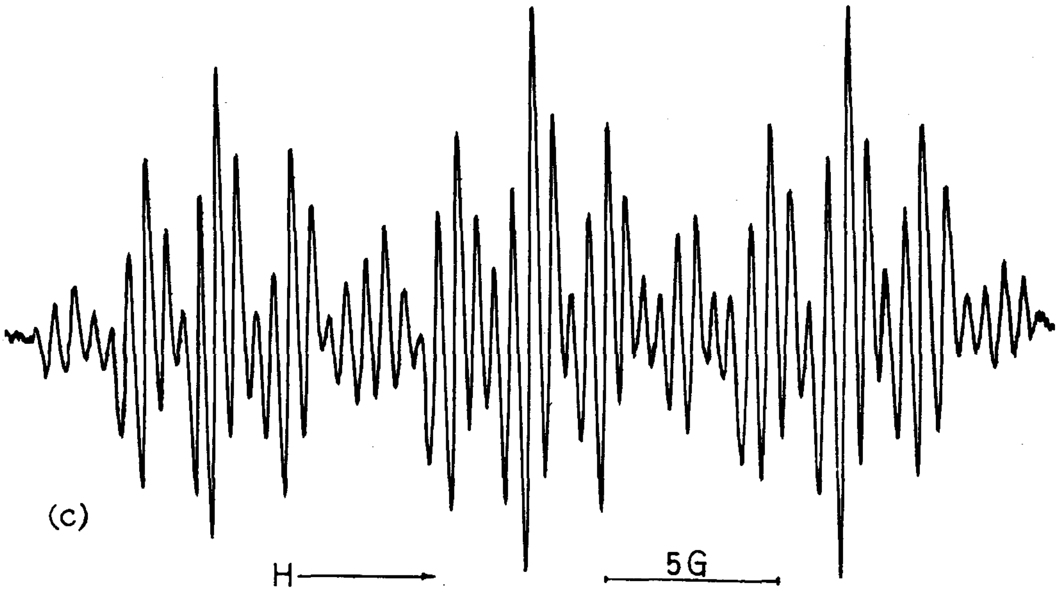
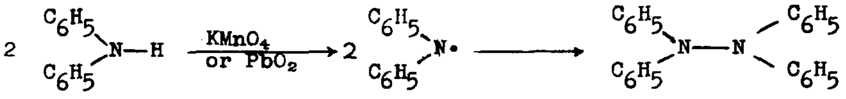


Fig. 1. (a) and (b) ESR spectra assigned to radical I in ethanol and benzene respectively.
(c) ESR spectrum assigned to radical II in ethanol.

is derived from the phenothiazine nitroxide radical II. Our evidence for these assignments is given in the following text. The coupling constants for these radicals in various solvents are shown in Table I.

Spectrum (1a) is obtained from radicals produced by oxidising phenothiazine with silver oxide, lead dioxide, potassium permanganate and lead tetra-acetate. Both lead dioxide and potassium permanganate (8) oxidise diphenylamine to tetraphenylhydrazine which is formed by the dimerisation of diphenylamino radicals.



$$2\,(C_6H_5)_2N{-}H \xrightarrow[\text{or } PbO_2]{KMnO_4} 2\,(C_6H_5)_2N\cdot \longrightarrow (C_6H_5)_2N{-}N(C_6H_5)_2$$

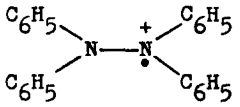
TABLE I

Coupling Constants of the ESR Spectra of Radicals I, II.

	Solvent	a^N	$a^H_{3,7}$	$a^H_{1,9}$	$a^H_{2,8}$	$a^H_{4,6}$
I Phenothiazinyl	Benzene	7.05	3.66	2.85	0.95*	0.95*
	Ethanol	7.20	3.60	2.61	0.98	0.72
II Phenothiazine nitroxide	Hexane	8.95	2.24	2.24	0.58	0.58
	Benzene	9.18	2.24	2.24	0.58	0.58
	Ethanol	9.63	2.27	2.27	0.58	0.58

* Incomplete resolution precludes the observation of possible differences in coupling constants of hydrogens at positions 2 and 4.

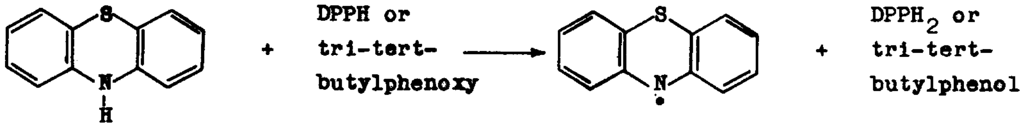
Oxidation of diphenylamine with lead tetra-acetate has not been reported to give tetraphenylhydrazine, but this is presumably formed since oxidation by this reagent in ethanol produces cationic radicals III with a five line e.s.r. spectrum of intensity ratio 1:2:3:2:1



III

By analogy we would expect these oxidants to produce the phenothiazinyl radicals (I) by loss of a hydrogen atom from the parent compound. Phenothiazinyl radicals are more stable than the diphenylamino radicals due to the rigid ring structure of the phenothiazine radical which sterically hinders dimerisation.

Spectra (1a) and (1b) were also produced from the free radicals formed during the reaction of phenothiazine with either 2,2-diphenyl-1-picrylhydrazyl (DPPH) or 2,4,6-tri-tertiarybutylphenoxy radicals. These substances are hydrogen abstraction reagents and their reduced forms, 2,2-diphenyl-1-picryl hydrazine ($DPPH_2$) and tritertiarybutylphenol were isolated from the reaction mixtures. These results can only be explained on the basis of the reactions postulated below.



As would be expected N-methylphenothiazine did not react with DPPH.

This would support the proposal that spectra (1a) and (1b) arise from the phenothiazinyl radicals. DPPH has been studied extensively in hydrogen abstraction reactions (9,10,11) and with diphenylamine (9) it gives tetraphenylhydrazine in 40% yield, suggesting diphenylamine radicals as intermediates.

Our evidence for the assignment of spectrum (1c) to the phenothiazine nitroxide radical follows mainly from irradiation studies using a mercury vapour lamp (12).

Irradiation of phenothiazine in benzene for 5 minutes did not produce a detectable concentration of free radicals. However, the reaction of phenothiazine with 2.5% v/v tertiary-butyl hydroperoxide in benzene either at room temperature or under irradiation conditions produced free radicals giving spectrum (1c). Coppinger and Swalen (13) have observed that several amines produced nitroxide radicals when treated with tertiary-butyl hydroperoxide. Phenothiazine, on irradiation in benzene containing 2.5% v/v di-tertiary-butyl peroxide, produced radicals giving spectrum (1b).

Pannell (14) has reported that irradiation of diphenylamine in undiluted di-tertiary-butyl peroxide yields the diphenylamino radical whereas irradiation in tertiary-butyl hydroperoxide produces the diphenyl nitroxide radical. This would appear to be consistent with our observations on phenothiazine described in the previous paragraph. However, Pannell's conclusions concerning the nature of the radical species were based on differences in the splitting constants of the two e.s.r. spectra. Our work on diphenylamine suggests that in both cases Pannell could have observed diphenyl nitroxide radicals and that the splitting constant differences reported by him were due to solvent effects rather than two different radical species. An authentic sample of diphenyl nitroxide (15) when dissolved in undiluted di-tertiary-butyl peroxide gave the same nitrogen splitting constant as Pannell has quoted for the diphenylamino radical.

The diphenylamino radicals purported to be produced by Das et al (16) on heating tetraphenylhydrazine in xylene would also seem to be diphenyl nitroxide radicals, since heating tetraphenylhydrazine in xylene at 125°C in an oil bath for 30 seconds gave an e.s.r. spectrum exactly the same as that derived from the authentic diphenyl nitroxide in the same solvent. When tetraphenylhydrazine was heated in xylene solution, which had been thoroughly purged with nitrogen, no detectable concentration of free radicals was observed. Moreover, the g-value of 2.00549 quoted by Das et al.(16) for the diphenylamino radical is the same as that quoted by Fischer and Neugebauer (17) for the diphenyl nitroxide radical in xylene. Diphenyldihydrophenazine, which is the dismutation product of diphenylamino radicals is, however, formed on heating tetraphenylhydrazine (18) and it would therefore seem that although diphenylamino radicals are being produced in the above reactions, they are not being detected by e.s.r. because of their short life.

Irradiation of phenothiazine in ethanol produced radicals which gave specrum (1a) and not spectrum (1c) as observed by Shine et al.(7). The different results may be due to the different radiation sources used in the two experiments. In contrast to our assignments, Shine et al. have attributed spectrum (1c) to the phenothiazinyl radical on the basis that acidification of the irradiated solution produced phenothiazine cationic radicals, apparently by protonation of the neutral phenothiazinyl radicals. We do not regard this evidence as conclusive since it is possible to produce phenothiazine cationic radicals in detectable concentration by addition of very dilute sulphuric acid (1% v/v) to phenothiazine.

Para-nitroperbenzoic acid has been found to form nitroxide radicals from several amines (19). With phenothiazine in ethanolic solution para-nitroperbenzoic acid formed radicals which gave spectrum (1c) which again supports the proposition that phenothiazine nitroxide radicals give rise to spectrum (1c). The main product of the reaction was phenothiazine 5-dioxide which raises a question concerning the oxidation state of sulphur in the phenothiazine nitroxide radical.

During the study of charge transfer complexes in compounds of pharmaceutical interest we have observed that in ethanol, phenothiazine interacts with strong electron acceptors such as chloranil, bromanil and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) producing phenothiazinyl radicals together with rapidly decaying radicals from the electron acceptor molecules. The absorption curve of the phenothiazinyl radical in the visible region is shown in Fig. 2a; the intensity of absorption decreasing in sympathy with the free radical concentration. Foster and Hanson⑳have similarly observed the decay of a product formed from the reaction of phenothiazines with electron acceptors.

FIG. 2

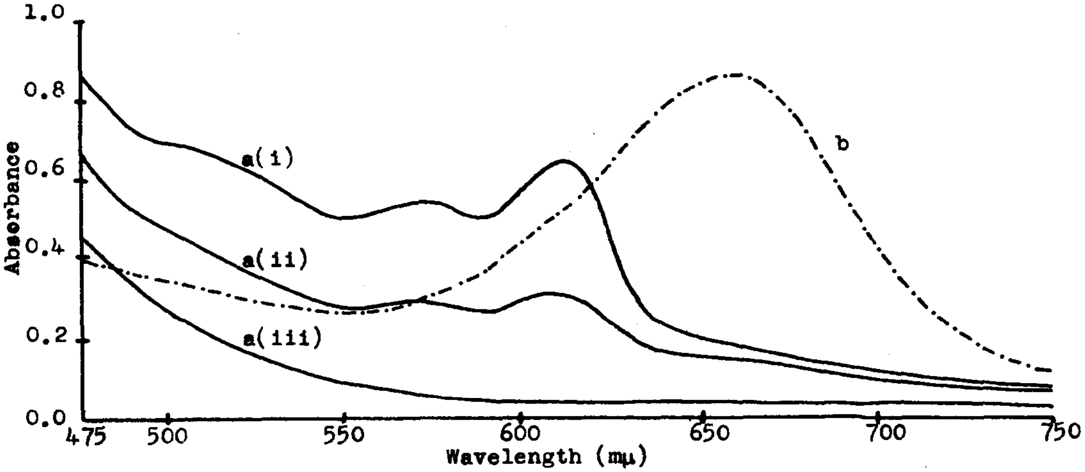
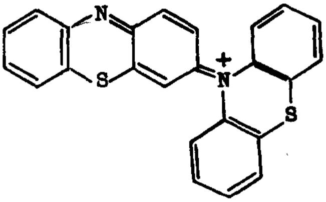


Fig. 2. Absorption spectra of (a) phenothiazine with DPPH in benzene showing bands due to the phenothiazinyl radicals decaying with time (i → iii).
(b) blue compound in acetone.

A number of workers (20,21) have observed the formation of a green solution in the oxidation reactions of phenothiazine. The compound giving the green solution has never been isolated, but Foster and Hanson (20) have suggested IV as a possible structure for this compound.



IV

From the reaction of DPPH with phenothiazine in acetone we have isolated a blue product which is responsible for the green colour in the reaction mixture. The visible absorption curve of this product is shown in Fig. 2(b) (λ_{max} at 660 mμ). It is possible that the blue compound is a decay product of the phenothiazinyl radical and further work is being carried out to elucidate its structure. The conjugated system in this blue compound probably resembles that of methylene blue (λ_{max} in acetone at 655 mμ) and structure IV suggested by Foster and Hanson (20) seems plausible.

Acknowledgements

We should like to thank Science Research Council for the grant to purchase the E.S.R. spectrometer. One of us (N.K.D.P.) is grateful to Sunderland Corporation for financial assistance.

REFERENCES

(1) C.M. Murphy, H. Ravner and N.L. Smith, Ind. Eng. Chem. **42**, 2479 (1950)

(2) J. Billion, G. Cauquis and J. Combrisson, J. Chim. Phys. France, 374 (1964)

(3) ESR spectra were recorded on Hilger and Watts Microspin spectrometer. The samples were examined at room temperature under nitrogen.

(4) B.C. Gilbert, P. Hanson, R.O.C. Norman and B.T. Sutcliffe, *Chem. Comm.* **6**, 161 (1966)

(5) J.C. Baird and J.R. Thomas, *J. Chem. Phys*. **35**, 1507 (1961)

(6) A.L. Buchachenko, *Stable Radicals*, Consultants Bureau, Plenum Press, N.Y. (1965)

(7) H.S. Shine, C. Veneziani and E.E. Mach, *J. Org. Chem.* **31**, 3395 (1966)

(8) H. Wieland and S. Gambarjan, *Ber*. **39**, 1499 (1906)

(9) J.E. Hazell and K.E. Russell, *Canad. J. Chem*. **36**, 1729 (1958)

(10) E.A. Braude, A.G. Brook and R.P. Linstead, *J. Chem. Soc*. 3574 (1954)

(11) A.G. Brook, R.J. Anderson and J. Tissot Van Patot, *Canad. J. Chem.* **36**, 159 (1958)

(12) Hanovia Medium pressure mercury arc lamp with major emissions at 5461, 4358 and 3660 mμ was used for U.V. irradiation.

(13) G.M. Coppinger and J.D. Swalen, *J. Am. Chem. Soc*. **83**, 4900 (1961)

(14) J. Pannell, *Mol. Phys*. **5**, 291 (1962)

(15) K. Tokumaru, H. Sakuragi and O. Simamura, *Tetrahedron Letters*, **52**, 3945 (1964)

(16) M.R. Das, A.V. Patankar and B. Venkataraman, *Proc. Indian Acad. Sci.* **53A**, 273 (1961)

(17) Von Peter H.H. Fischer and F.A. Neugebauer, *Z. Naturforch*. **19a**, 1514 (1964)

(18) H. Wieland, *Ann*. **381**, 200 (1911)

(19) G. Chapelet-Letourneux, H. Lemaire and A. Rassat, *Bull. Chem. Soc. France*, **11**, 3283 (1965)

(20) R. Foster and P. Hanson, *Biochim. Biophys. Acta*, **112**, 482 (1966)

(21) J. Cavanaugh, *J. Am. Chem. Soc*. **81**, 2507 (1959)