Heterocyclic Free Radicals. Part IV.[1] Some Reactions of Phenothiazine, Two Derived Radicals, and Phenothiazin-5-ium Ion

By **P. Hanson*** and **R. O. C. Norman**, Department of Chemistry, The University of York, Heslington, York YO1 5DD

The reactions brought about when phenothiazine radical cation or phenothiazinium ion in acetonitrile is treated with aqueous solutions of various pH-values have been studied by spectroscopic methods. The proposed structure of a green dye which is formed under various conditions by the oxidation of phenothiazine has been confirmed. Evidence has been obtained about the modes of interconversion of phenothiazine and its oxidised derivatives.

PHENOTHIAZINE (1) and many of its derivatives have long been known to undergo oxidation in single-electron steps,[2] but only more recently have the relationships between oxidised phenothiazines been studied. Thus, Billon [3] has investigated the electrochemical oxidation of phenothiazines using e.s.r. spectroscopy to detect free radicals; Shine and Mach [4] have studied, by absorption spectroscopy and e.s.r. spectroscopy, relationships between some of the oxidised forms of phenothiazine as part of a general study of sulphur heterocycles; and Tozer and Tuck,[5] using oxidative titration procedures and e.s.r., have elucidated stoicheiometric and kinetic relationships between various phenothiazine species, with particular reference to those of biological importance. In the main, these workers have used acidic media for their studies. In addition, the characteristics of phenothiazines as donors in forming charge-transfer complexes have been reported.[6]

Several groups of workers have noted the formation of an unidentified green compound from the oxidation of phenothiazine under various conditions. First, Cavanaugh,[7] using hydrogen peroxide in the presence of peroxidase as oxidant, in propylene glycol containing water, reported that the initial phase of oxidation is characterised by a dark-green colour which is rather unstable, more so above pH 5, and which is eventually replaced by a red product which was identified spectroscopically as the phenothiazinone (2). The conversion of the green into red material at higher pH-values was found to occur

---

[1] Part III, M. F. Chiu and B. C. Gilbert, preceding paper.

[2] L. Michaelis and S. Granick, *J. Amer. Chem. Soc.*, 1942, **64**, 1861, and references therein.

[3] J.-P. Billon, *Ann. Chim. (France)*, 1962, **7**, 183.

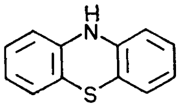
[4] H. J. Shine and E. E. Mach, *J. Org. Chem.*, 1965, **30**, 2130.

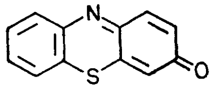
[5] T. N. Tozer and L. D. Tuck, *J. Pharm. Sci.*, 1965, **54**, 1169.

[6] R. Foster and P. Hanson, *Biochim. Biophys. Acta*, 1966, **112**, 482.

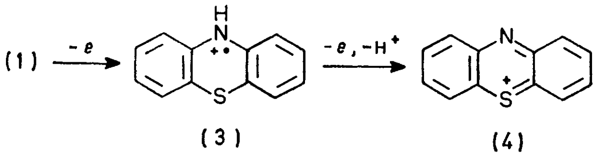
[7] D. J. Cavanaugh, *J. Amer. Chem. Soc.*, 1959, **81**, 2507.

in the absence of oxygen and not to involve hydrogen peroxide as oxidant; however, water is apparently essential to the system.


(1)


(2)

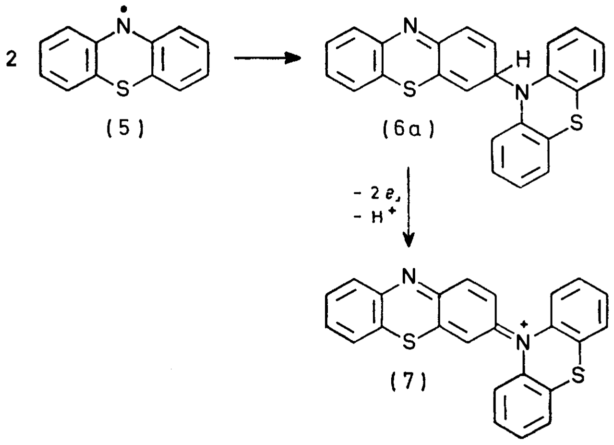
Secondly, Billon,[3] studying the anodic oxidation of phenothiazine, observed the green product in non-acidic conditions in the presence of water and showed that it is in the same oxidation level as phenothiazinium ion (4), *i.e.* +2 relative to phenothiazine. By examining the behaviour of various methylphenothiazines, he showed that the *N*- and 3- (or equivalent 7-) positions must be unsubstituted for analogous green products to be formed. He suggested that the green product is a 'Lewis complex' between water or hydroxide ion and the cation (4), involving the 3-position; the isolation of the phenothiazinone (2) on chromatography was attributed to rearrangement of the 'Lewis complex' to 3-hydroxyphenothiazine, which is known to be oxidised readily in air to give (2). In contrast, Billon observed that electrochemical oxidation in an acidic medium (perchloric acid in acetonitrile) occurs by two one-electron steps to give the phenothiazinium ion (4); the intermediate was thought to be the radical cation (3) on the basis of its low resolution e.s.r. spectrum.



(3) (4)

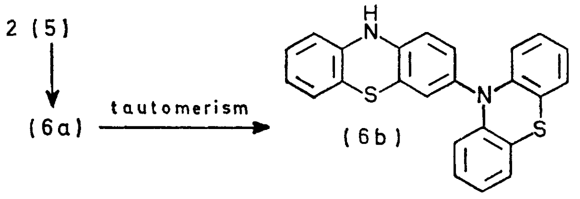
Finally, Foster and Hanson[6] observed that the green compound is formed when phenothiazine reacts with a powerful electron-acceptor, such as 2,3-dicyano-*p*-benzoquinone in non-acidic conditions (dry acetonitrile). It was characterised by its absorption bands at 450 and 620 nm, the latter being broad and somewhat variable in profile; the spectrum persisted long after transient paramagnetism had died away. Attempts to isolate the compound were unsuccessful; chromatography or the removal of the solvent resulted in the formation of the phenothiazinone (2). To account for their own and previous observations, Foster and Hanson suggested that the green compound contains the cation (7) and is formed by oxidative dimerisation of the hitherto unobserved neutral radical (5) (Scheme 1).

This suggestion is in accord with the observations described above. In particular, it reconciles the ostensibly contradictory observations that the green compound is in the same oxidation level relative to phenothiazine as phenothiazinium ion (+2) and yet gives phenothiazinone (2) (oxidation level, +4) without the requirement of an oxidant, for (7) would be expected readily to yield the phenothiazinone (2) and phenothiazine on hydrolysis. A structural precedent exists, for Musso[8] has prepared the phenoxazine analogue of (7). The formulation (7) for the green material has found acceptance,[9,10] but it has never been proved. Tsujino[11,12] has isolated 3,10′-biphenothiazinyl (6b) from mixtures of phenothiazine and various oxidants; again, the binuclear species is envisaged as arising from a dimerisation of (5). Further, Tsujino has observed a triplet species on dissolving his alleged (6b) in 98% sulphuric acid and has adduced spectroscopic evidence for the changes in Scheme 2 on dilution with water.[13]



SCHEME 1

In earlier parts of this series,[14,15] we reported and interpreted the e.s.r. spectra of the phenothiazine radicals (3) and (5), amongst others, and showed that when (3) is generated in acetonitrile, it is converted into (5) on addition of neutral or alkaline buffer. This observation is consistent with Foster and Hanson's[6] suggestions for the structure and mode of formation of the green compound (hereafter referred to as *G*), for it reconciles the observations that *G* is formed by the anodic oxidation of phenothiazine only in the presence of water[3] whereas it is formed by oxidation by 2,3-dicyano-*p*-benzoquinone and related acceptors in an anhydrous medium;[6] thus, under the former conditions, an added



---

[8] H. Musso, *Chem. Ber.*, 1959, **92**, 2862, 2873.

[9] C. Jackson and N. K. D. Patel, *Tetrahedron Letters*, 1967, 2255.

[10] C. Bodea and I. Silberg, *Adv. Heterocyclic Chem.*, 1968, **9**, 321.

[11] Y. Tsujino, *Tetrahedron Letters*, 1968, 4111.

[12] Y. Tsujino, *Tetrahedron Letters*, 1969, 763.

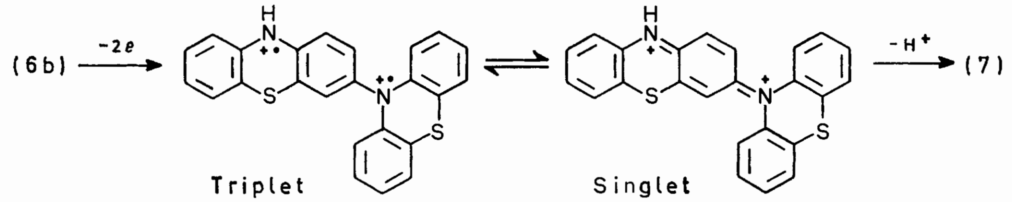
[13] Y. Tsujino, *Tetrahedron Letters*, 1968, 2545.

[14] B. C. Gilbert, P. Hanson, R. O. C. Norman, and B. T. Sutcliffe, *Chem. Comm.*, 1966, 161.

[15] M. F. Chiu, B. C. Gilbert, and P. Hanson, *J. Chem. Soc.* (*B*), 1970, 1700.

base (water) is evidently necessary to convert the protonated radical (3) into the neutral radical (5) before dimerisation can occur, whereas under the latter conditions the semiquinone radical-anion can act as the necessary base.

We now report the electronic spectral characteristics of the neutral radical (5) and evidence for its oxidative dimerisation to give *G*. We have also synthesised, unambiguously, 3,10′-biphenothiazinyl (6b) and found that, on oxidation, it gives a product which is identical in spectroscopic characteristics and chemical behaviour with *G*, proving that *G* contains the cation (7). Tsujino's results [12,13] are confirmed and a mechanism for conversion of phenothiazinium ion (4) into (7) is elucidated.



Scheme 2

RESULTS

Synthesis and Oxidation of 3,10′-*Biphenothiazinyl* (6b).—3,10′-Biphenothiazinyl was prepared as outlined in Scheme 3. The material is a colourless crystalline solid, the profile of whose u.v. spectrum in acetonitrile solution closely resembles that of phenothiazine itself; there is little shift in $\lambda_{max.}$ values but extinction coefficients are increased 1·7 fold. This is consistent with there being little conjugation through the internuclear bond. For convenience, spectroscopic data and oxidation levels (relative to phenothiazine itself) of species referred to in the text are collected in the Table.

Oxidation levels and electronic spectra in MeCN of phenothiazine and some derivatives

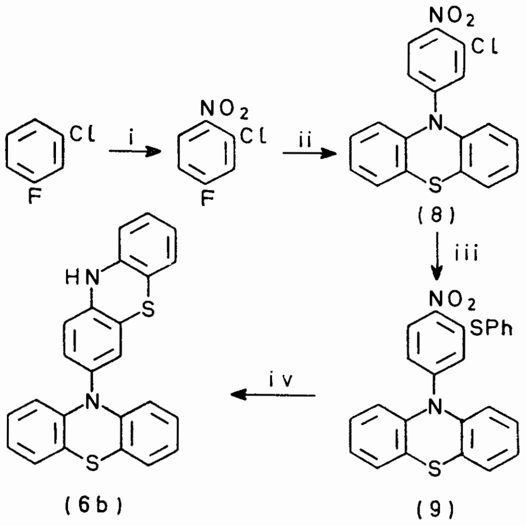
Substance	Text number	Oxidation level	$\lambda_{max.}$/nm (log ε/l mol^{-1} cm^{-1})
Phenothiazine	1	0	254 (4·73); 320 (3·71)
Phenothiazine radical-cation	3	1	271 (4·73); 437 (3·64); 516 (3·84)
Phenothiazinyl radical [a]	5	1	268 (—); 344 (3·75); 381 (4·00)
3,10′-Biphenothiazinyl	6b	1	258 (4·96); 318 (3·95)
Phenothiazinium ion	4	2	281 (4·75); 419 (4·10)
Phenothiazine 5-oxide	10	2	269 (4·14); 298 (3·86); 339 (3·72)
Binuclear cation	7	2	320 (4·40); 446 (4·18); 620 (4·33)
Phenothiazinone (2)	2	4	236 (4·31); 274 (4·23); 362 (3·96); 495 (3·90)

[a] By extrapolation to zero time when derived by deprotonation of (3) with borate buffer (pH 9).

Oxidation of (6b). To study spectroscopically the oxidation of (6b) we chose as oxidant thallium(III) acetate sesquihydrate. This salt is a two-electron oxidant of which neither reduced nor oxidised forms has significant absorption in the region 275—700 nm, providing that solutions in acetonitrile contain a trace of acetic acid, typically two drops in 25 ml.

When oxidant was added to the colourless solution of (6b) the latter became green owing to absorptions with $\lambda_{max.}$ 446 nm and a broad maximum peaking between 610 and 630 nm. The spectrum exhibited an isosbestic region centred at 349 nm, absorption increasing on both sides of this. Thus, on oxidation of (6b), besides the appearance of the visible bands, the absorption initially due to (6b) ($\lambda_{max.}$ 318 nm) intensifies, developing into an absorption ($\lambda_{max.}$ 320 nm) of the oxidation product; we have, therefore, three absorptions in the region 300—700 nm which we may unambiguously assign to the two-electron oxidation product of (6b) which is doubtless the ion (7) (see later).

Rapid quantitative oxidation of (6b) was accomplished in the cuvette by addition of a few small crystals of the thallium salt directly to an acidified standard solution of (6b). With this procedure we estimated extinction coefficients for the green cation (7) (Table). We also showed that (6b) is instantaneously oxidised by phenothiazinium ion (4) in acetonitrile solution. In this instance the absorbance developed at 320 nm was greater, relative to that of the visible bands, than when the thallium salt was used as oxidant, for (4) is reduced to phenothiazine (1) when it oxidises (6b), and (1) also absorbs at 320 nm.



Scheme 3 Reagents: i, fuming HNO_3, <45°; ii, phenothiazine + NaH in Me_2SO; iii, PhS^-Na^+ in hot EtOH; iv, $(EtO)_3P$ in hot $PhBu^t$

We confirmed Tsujino's [12,13] results as follows. Impure material recovered from an oxidation of phenothiazine in dimethyl sulphoxide with iodine was found by t.l.c. to have a component identical in chromatographic behaviour with our unambiguously prepared (6b). Further, (6b) was shown to give a triplet paramagnetic species in solution in concentrated sulphuric acid the e.s.r. spectrum of which was in agreement with that published by Tsujino; it displayed two pairs of broad bands having a separation of 10·5 mT between the main pair, with line widths of *ca.* 3 mT. Superimposed upon the triplet e.s.r. spectrum was that of a phenothiazine radical which we have not resolved; indeed, it may have been due to a mixture of radicals, *i.e.* the two derivable by one-electron oxidations of each of the phenothiazine nuclei in (6b). (Presumably the geometric constraint upon rotation about the internuclear 3-10′-bond, which permits existence of a triplet and which is consistent with the lack of conjugation apparent from the u.v. spectrum of (6b) in acetonitrile, would also allow the existence of two separate radicals.)

Hydrolysis of (7).—When an acetonitrile solution of (7) was set aside over moist alumina for several hours, the solution became pink and its electronic spectrum was consistent with the presence of an equimolar mixture of phenothiazine and the phenothiazinone (2).

Spectroscopic Observations on the Phenothiazine Radical-cation (3) *and the Phenothiazinium Ion* (4).—In order to study the relationship between the various oxidised forms of phenothiazine, the radical cation (3) and the phenothiazinium ion (4), each as its perchlorate, were prepared, isolated as solids, and examined in acetonitrile solution. In carefully dried acetonitrile both salts are stable over a long period of time; only when moisture is introduced does reaction occur. It was found that various reactions may then occur simultaneously, but that, by the appropriate choice of conditions, each type of reaction could be made to predominate in turn so that the pattern of behaviour as a whole could be elucidated. The method adopted, therefore, was to add 0·05 ml (except where otherwise stated) of a solution of a given aqueous buffer to a solution of the phenothiazine derivative, in 3 ml anhydrous acetonitrile contained in a cuvette, and to record the changes in the electronic spectrum as a function of time. Although the pH-scale does not apply in these conditions, the pH of the buffer used provides a useful indication of the degree of basicity to which the phenothiazine species was subjected.

(a) *Behaviour of the Phenothiazine Radical-cation* (3).—(i) *Deprotonation.* The changes in the visible spectrum on the addition of aqueous borate buffer of pH 9 to acetonitrile containing 2 × 10^{-4}M-phenothiazine radical cation (3) are shown in Figure 1. The spectrum (*A*) of the radical cation ($\lambda_{max.}$ 437 and 516 nm) collapsed instantly and was replaced by one (*B*) with $\lambda_{max.}$ 344 and 381 nm. This faded slowly with time (27 min, in the spectrum shown, from highest to lowest absorbance, with a 3-min time interval between successive scans; downward arrows indicate absorbance decreasing with time), and the spectrum exhibited an isosbestic point at 331 nm. The corresponding changes in the u.v. region were followed by using a more dilute solution of the radical cation (3 × 10^{-5}M): the initial spectrum ($\lambda_{max.}$ 271 nm) was instantly replaced by an unstable one ($\lambda_{max.}$ 268 nm); this faded slowly to give a spectrum with the characteristics of that of a mixture of phenothiazine (1) and its 5-oxide (10).

Comparison of the results obtained by e.s.r. spectroscopy [14,15] with those obtained by electronic spectroscopy shows that the unstable species obtained when the radical cation (3) in acetonitrile is treated with buffer of pH 9 is the neutral radical (5).

(ii) *Disproportionation.* The addition of 0·001 ml of aqueous acetate buffer of pH 4 to 3 ml acetonitrile containing 2·15 × 10^{-4}M-(3) brought about changes in the visible spectrum which are shown in Figure 2. The initial spectrum (*A*) again collapsed immediately and was replaced by that, (*C*), of the phenothiazinium ion (4) ($\lambda_{max.}$ 419 nm) together with phenothiazine (1) ($\lambda_{max.}$ 320 nm). The absorption due to (4) faded rapidly and was replaced, through isosbestic points at 435 and 347 nm, by the spectrum (*D*) of the dimeric species (7) [$\lambda_{max.}$ 320, 446, and *ca.* 620 nm, with additional absorption at 320 nm from (1)]; we ascribe the slight shoulder at *ca.* 380 nm on the absorption band due to (4) to the presence of a small amount of the neutral radical (5) arising by simultaneous deprotonation.

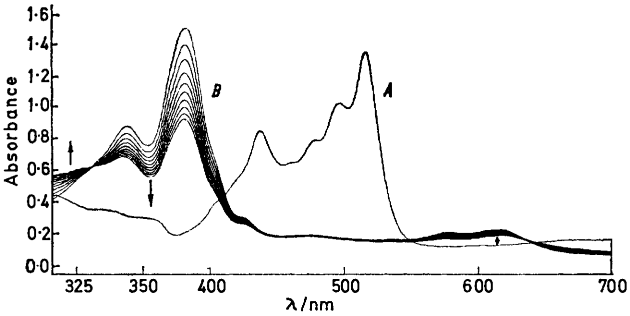


FIGURE 1 Changes in the visible spectrum on treatment of phenothiazine radical-cation (3) in acetonitrile with pH 9 buffer

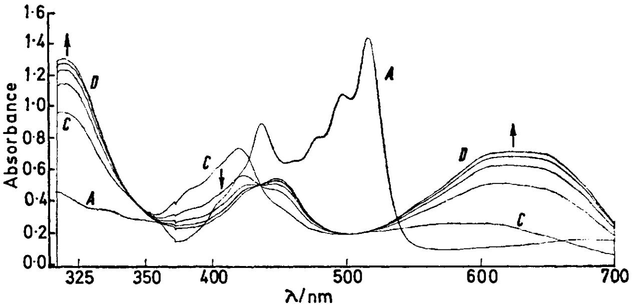


FIGURE 2 Changes in the visible spectrum on treatment of phenothiazine radical-cation (3) in acetonitrile with pH 4 buffer

When 0·025 ml of unbuffered water was used in place of acetate buffer, the initial spectrum (*A*) collapsed less rapidly and its disappearance could be followed in both the visible and u.v. regions. It was again replaced by a spectrum characteristic of a mixture of phenothiazine and phenothiazinium ion; the absorption due to the latter rapidly attained a maximum and thereafter decreased, the decrease being accompanied by the appearance of absorption due to (7). Throughout this time the absorption at 254 nm increased continuously, showing that phenothiazine was formed not only together with (4) by disproportionation but also by whatever process results in the destruction of (4).

(b) *Behaviour of the Phenothiazinium Ion* (4).—The observations reported below were made with an acetonitrile solution of phenothiazinium perchlorate which was initially 1·25 × 10^{-4}M for the recording of visible spectra and 3 × 10^{-5}M for those recorded in the u.v. region.

(i) *Action of unbuffered water.* This brought about the following changes in the visible spectrum during 20 min (Figure 3): absorbance due to the phenothiazinium ion

($\lambda_{\max.}$ 419 nm) faded; as it did so, absorption at 320 nm increased and a peak at 516 nm due to the radical cation (3) appeared, reached a low limiting value, and faded; as it faded, peaks at 320, 446, and *ca.* 620 nm, which we have shown to be due to (7), appeared and reached steady limiting values. The intermediate formation of (3) and subsequent formation of (7) could be followed in the u.v. region, for as the initial phenothiazinium absorption at 281 nm faded, there appeared transient absorption at 271 nm and continuously increasing absorption at 320 nm. The u.v. spectrum also showed absorption at 254 nm, ascribed to phenothiazine itself, increasing throughout the changes described. When the visible absorption of phenothiazinium ion had dropped to low values a shoulder at *ca.* 380 nm was apparent; we again attribute this to a low concentration of the neutral radical (5).

(ii) *Action of acetate buffer, pH* 4. In this case the intermediate formation of the radical cation (3) was suppressed; in both the visible and u.v. regions, the first absorption to supersede the fading phenothiazinium absorption was that of the neutral radical (5). It then faded while the absorption due to (7) developed to a final limiting value. No significance is attached to the fact that buffer of pH 4 yielded neutral radical (5) whereas unbuffered water yielded largely the corresponding radical cation (3), for nothing is known about the basicities of the aqueous buffer components in the final acetonitrile solution.

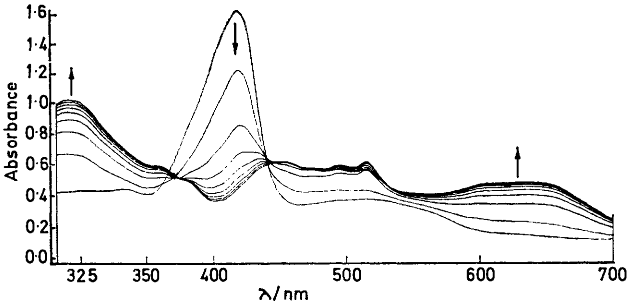


FIGURE 3 Changes in the visible spectrum on treatment of phenothiazinium ion (4) in acetonitrile with water

(iii) *Action of phosphate buffers, pH* 6 *and* 7. The pH 6 buffer caused the spectrum due to the phenothiazinium ion to fade rapidly; absorption due to the neutral radical (5) which resulted also faded; concurrently, there was appearance of absorption due to (7) and over a wide wavelength-range centred at 490—500 nm. The changes induced in the spectrum of phenothiazinium ion by pH 7 buffer were comparable; the main differences were that the phenothiazinium ion was shorter lived; the formation of (7) was less extensive whereas the broad absorption at *ca.* 490 nm became more prominent. The broad band at *ca.* 490 nm is identical in profile to that of a low concentration of the phenothiazinone (2) in the same conditions of basicity and we ascribe it to this species.

(iv) *Action of borate buffers, pH* 8 *and* 9. The absorption due to phenothiazinium ion had disappeared before the spectrum could be recorded. In its place there appeared that of phenothiazine 5-oxide (10) together with weak absorption due to a small concentration of the neutral radical (5), which disproportionated slowly (isosbestic point at 341 nm). The maximum concentration of (5) was not constant from one experiment to another and we ascribe the appearance of this species under these conditions to imperfect mixing, which produces local conditions [excess of (4)] similar to those obtained with buffer of higher acidity where neutral radical is also formed; in accord with this, the neutral radical was not detected when small amounts of phenothiazinium ion solution were added to large volumes of buffer to produce a final solution of comparable concentration.

(c) *Quantitative experiments.*—(i) *Rate of disproportionation.* The radical (5) was generated as in (a) (i) and the decrease in its absorption at 381 nm was monitored as a function of time; the reaction followed kinetics of the second order, with k 2·8 l mol^{-1} s^{-1} at 25°. The disproportionation of the radical cation (3) was too fast to be examined in this way when reaction was initiated with acetate buffer of pH 4 [see (a) (i) above] and, when reaction was initiated with unbuffered water, the kinetics were complex.

(ii) *Efficiency of conversion of* (4) *into* (7). This was determined by treating a standard solution of (4) in acetonitrile with pH 4 acetate buffer as in (b) (ii) and, when formation of (7) was complete, estimating its concentration from the absorption at 620 nm together with the extinction coefficient at this wavelength determined from a solution obtained from a known concentration of (6b) with thallium(III) acetate. With the assumption (see later) that $2(4) \longrightarrow (7) + H^+$, the conversion efficiency was found to be 43%. In a similar way, with a standard solution of the radical cation (3) which was assumed to yield (7) *via* disproportionation to (4) $[2(3) \longrightarrow (1) + (4) + H^+]$, the efficiency of conversion of (4) into (7) was found to be 78%. Finally, when a solution of (7) derived from (6b) with thallium(III) acetate was balanced in the spectrometer against one derived from (4), the difference in the visible region corresponded to the presence of a low concentration (*ca.* 10%) of the phenothiazinone (2) in the latter solution.

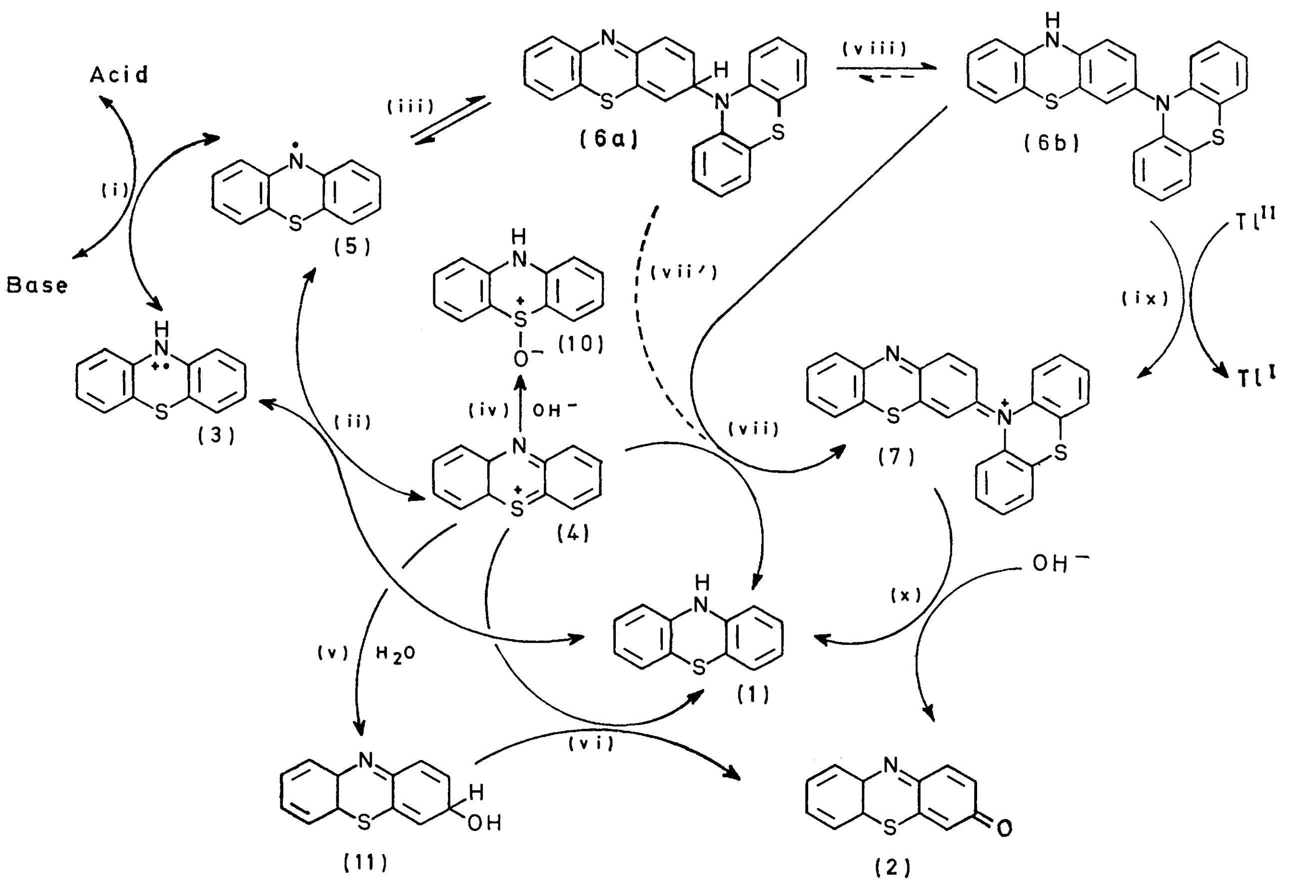
DISCUSSION

We believe that the results are accommodated by the reactions in Scheme 4; we justify these in the sequel.

Equilibrium (i) is the acid-base relationship between radical cation (3) and the neutral radical (5) which is established by the results in (a) (i) and the e.s.r. evidence previously reported.[14,15] Reaction (ix) describes the oxidation of the unambiguously prepared (6b) by thallium(III) acetate to give the green cation (7), and reaction (vii) describes the same oxidation with phenothiazinium ion (4) as oxidant, this being converted into phenothiazine (1) in the process [see the results for the oxidation of (6b)]. Reaction (x) represents the hydrolysis of the cation (7) which occurred in the presence of moist alumina. The last simple process, reaction (iv), is the hydration of the phenothiazinium ion to phenothiazine 5-oxide which we observed on treating an acetonitrile solution of the cation (4) with buffer of pH 8 or 9 [(b) (iv)]; this is formulated in Scheme 4 as a reaction with OH$^-$, and we discuss this subsequently.

We suggest that disproportionation occurs as in reaction (ii) on the following grounds. The radical cation (3) is stable in strongly acid solutions [2-5] and we have found it to be so in *dry* acetonitrile. We have also found that the neutral radical (5) disproportionates only slowly when generated in the presence of an alkaline buffer [see

(a) (i) and (c) (i)]. However, the disproportionation of the radical cation (3) is complete within seconds when its solution in acetonitrile is treated with acetate buffer of pH 4 [see (a) (ii)], and Tozer and Tuck reported that its rate of disproportionation in acid solutions of strengths below 2N increases with decrease in the acidity.[5] These facts together are consistent with the view that disproportionation is most likely to occur by reaction of the radical cation (3) with the neutral radical (5). Further, when the radical (5) is generated by deprotonation of its conjugate acid (3) with pH 9 buffer, so that disproportionation is relatively slow, the phenothiazinium ion (4) which results is immediately hydrated to the 5-oxide (10) with OH^- ion [reaction (iv); see (a) (i), (b) (iv)]. [The conversion of (10) into (4) is known to occur in highly acidic media.[3,4]]

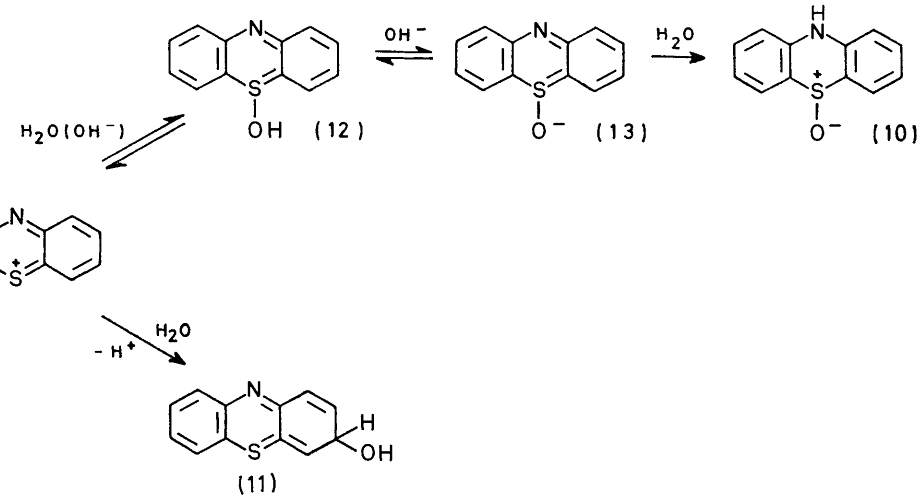


Scheme 4

Given that two molecules of the radical (5) do not readily disproportionate, it is reasonable to suggest that they dimerise [reaction (iii)]. The dimer (6a) is the tautomer of (6b), and Tsujino's result[11,12] confirms the occurrence of reaction (viii). However, this tautomeric change is not essential to the Scheme. Thus, we have shown that phenothiazinium ion (4) is capable of oxidising the dimer (6b) to the cation (7) and it is likely that it can also oxidise the dimer (6a); therefore, in the formation of the cation (7) from the radical cation (3) as in (a) (ii), it is possible that the dimer (6a) is oxidised rapidly after its formation from the radical (5) by phenothiazinium ion [reaction (vii′)], accounting for the isosbestic points between the absorptions of phenothiazinium ion and the cation (7) in this experiment. Our study of the dimer (6b) indicates that the equilibrium (viii) lies essentially completely in favour of this tautomer, for we have not observed monomeric species such as (5) arising from (6b).

We can summarise the dependence on the conditions of the reactions of the radical cation (3) as follows. Under the basic conditions of experiment (a) (i), the conversion of the radical cation (3) into its conjugate base (5) is very rapid and essentially complete; consequently, the likelihood of reaction between molecules of (5) and (3) is small and disproportionation is relatively slow; of the products from disproportionation, (1) and (4), the latter is immediately converted into the 5-oxide (10). In contrast, under the weakly acidic conditions of experiment (a) (ii), less rapid and complete deprotonation of the radical cation (3) favours disproportionation; the phenothiazinium ion which results is not so effectively removed as 5-oxide, allowing it to oxidise the dimer (6a) as the latter is formed from (5).

The striking feature of the results obtained with solutions of phenothiazinium ion (4) is the intervention of radicals; both (3) and (5) appear in low concentration when unbuffered water is added to the solution of (4) [see (b) (i)], and (5) is observed with buffered solutions [see (b) (ii) and (iii)]. These radicals are in an oxidation level below phenothiazinium ion, indicating the occurrence of a one-electron reduction, but we suggest that it is improbable that water is the reducing agent, for the following reason. For equal concentrations of (3) and (4) in 0·1M-perchloric acid in acetonitrile, the potential is 0·69 V relative to Ag/Ag$^+$ (0·01M) in acetonitrile; the value varies with acidity and water content, the oxidised level being favoured by increasing water content and decreasing acidity.[3] The reference electrode is at 0·29 V relative to the saturated calomel electrode (aqueous),[16] which has a standard electrode potential of 0·24 V.[17] Thus, the redox potential for the couple (4)/(3) is 1·22 V on the standard scale, whereas the couple Ce^{IV}/Ce^{III}, with a much higher redox potential (*ca.* 1·6 V, depending on the associated acid) fails to oxidise water.[18]



Scheme 5

We suggest instead that the reducing agent is the hydroxy-derivative (11), or its phenolic tautomer, formed by reaction (v). We note, first, that Billon's redox data[3] show that the latter compound should reduce phenothiazinium ion (4) essentially completely to yield the phenothiazinone (2) and phenothiazine (1), as in reaction (vi). Secondly, the phenothiazinone (2) was detected in the experiments in which acid or neutral buffer was used. The fact that the yield obtained with acetate buffer (pH 4) (*ca.* 10%) was not comparable with that of the main product (7) is not exceptional, for once phenothiazine is formed in the presence of phenothiazinium ion, a path is open to the cation (7) which no longer involves the incursion of (11) and the occurrence of reaction (vi); thus, the path is (1) + (4) $\longrightarrow$ 2(5) + H$^+$, 2(5) $\longrightarrow$ (6a), (6a) + (4) $\longrightarrow$ (7) + (1), giving overall 2(4) $\longrightarrow$ (7) + H$^+$, so that the suggested reducing agent (11) is needed only as an initiator and the actual yield of (2) will depend on the competition for phenothiazinium ion between water [reaction (v)] and phenothiazine [reverse of reaction (ii)]. Finally, the suggestion can account for the different efficiencies of conversion of (4) into (7) according to whether the reactant is (4) (43%) or (3), from which both (1) and (4) arise, (78%), since in the former case, unlike the latter, some of the reactant is utilised in forming the reducing agent whereas in the latter case the radical precursor for formation of the dimer (6a) and thence of (7) is present in high concentration at the beginning of the reaction.

The postulate in the foregoing paragraph that phenothiazinium ion reacts with water at carbon to give (11) deserves comment since reaction at sulphur to yield the 5-oxide (10) is the only significant process when alkaline buffer is used. We first note that, in the experiments in which reaction at carbon is thought to occur, reaction at sulphur may nevertheless be predominant; thus, under these conditions comparatively little phenothiazinone (2) is formed and, from experiment (c) (ii), we can estimate that as much as 35% of the phenothiazinium ion may have afforded the 5-oxide (10), this escaping detection because it does not absorb in the visible region and its u.v. absorption could not be diagnosed amongst those from products (1), (2), and (7).

A possible reason for the difference in behaviour of phenothiazinium ion towards water according to the buffer used is as follows. Reaction with nucleophilic oxygen is faster at sulphur than at carbon; however, in basic solution the resulting intermediate (12) (Scheme 5) may be rapidly converted into the 5-oxide (10) whereas in neutral or acidic solution it may exist longer in equilibrium with phenothiazinium ion, allowing the alternative, less readily reversible reaction at carbon to occur. This argument requires that the tautomer (12) of the 5-oxide has moderate stability, and the following

---

[16] J.-P. Billon, *J. Electroanalytical Chem.*, 1959—60, **1**, 486.

[17] *Handbook of Chem. and Phys.*, 46th edn., 1965—6, table D-76.

[18] W. H. Richardson, in 'Oxidation in Organic Chemistry,' Part A, ed. K. B. Wiberg, Academic Press, 1965.

experiment provides evidence that this is so. The conjugate base (13) of the 5-oxide was generated from the 5-oxide with an excess of sodium hydride in dimethyl sulphoxide. When the resulting yellow solution was treated with a small amount of water, the absorption due to the anion (13) ($\lambda_{max.}$ 310, 333, and 366 nm) disappeared immediately and was replaced by a spectrum with $\lambda_{max.}$ 278, 307, and 347 nm; this changed during *ca.* 1 h to the spectrum of the 5-oxide ($\lambda_{max.}$ 276, 304, and 340 nm, slightly displaced from the values in acetonitrile; see Table). We infer that the anion (13) is protonated faster at oxygen than at nitrogen, the intervening spectrum being that of (12).

EXPERIMENTAL

Synthesis of 3,10′-*Biphenothiazinyl* (6b).—2-*Chloro*-4-*fluoronitrobenzene*. 3-Chlorofluorobenzene (26 g) was added, dropwise, to well-stirred fuming nitric acid (150 ml) at a rate which maintained the temperature at 40—45°. The mixture was stirred a further 1 h and then poured into ice–water. The lemon-yellow oily solid which precipitated was sucked free of occluded oil (isomeric chlorofluoronitrobenzenes) and washed until acid-free with water, giving 2-chloro-4-fluoronitrobenzene (45%) (from aqueous ethanol at 0°) as lemon-yellow needles, m.p. 35—37° (lit.,[19] 37°).

10-(3-*chloro*-4-*nitrophenyl*)*phenothiazine* (8).—Phenothiazine (17 g) was dissolved in dimethyl sulphoxide (100 ml) which had been freshly distilled from calcium hydride. Sodium hydride (50% mineral-oil suspension, 4·5 g) was added and the mixture, protected from moisture, was stirred until evolution of hydrogen ceased. To the resulting deep orange solution of 10-sodiophenothiazine was added, in a single charge, 2-chloro-4-fluoronitrobenzene (15 g); the mixture was stirred for 1·5 h at 65—70°. The mixture was poured into water (1·5 l) to give a thick yellow precipitate which was washed with water, dried in a vacuum desiccator, and recrystallised from ethanol with carbon treatment to yield 10-(3-*chloro*-4-*nitrophenyl*)*phenothiazine* (8) (75%) which, after two further recrystallisations from ethanol, yielded yellow needles, m.p. 160—161·5° (Found: C, 60·7; H, 3·5; Cl, 10·0; N, 7·8; S, 8·9. $C_{18}H_{11}ClN_2O_2S$ requires C, 60·9; H, 3·1; Cl, 10·0; N, 7·9; S, 9·0%).

2-*Nitro*-5-(*phenothiazin*-10-*yl*)*phenyl Phenyl Sulphide* (9).—Sodium (0·7 g) was dissolved in ethanol (250 ml) and benzenethiol (3·5 g) was added to it. To the solution was added 10-(3-chloro-4-nitrophenyl)phenothiazine (8) (10·5 g) and the suspension was heated under reflux for 7 h, during which it cleared to give a red solution. The mixture when poured into water precipitated the intensely yellow sulphide; it was coagulated on addition of acid, separated by filtration, dried in air, and purified by column chromatography (alumina–chloroform) to yield 2-*nitro*-5-(*phenothiazin*-10-*yl*)*phenyl phenyl sulphide* (9) (55%) (from ethanol–chloroform), m.p. 130—131° (Found: C, 67·2; H, 3·9; N, 6·5. $C_{24}H_{16}N_2O_2S_2$ requires C, 67·3; H, 3·8; N, 6·5%).

3,10′-*Biphenothiazinyl* (6b).—2-Nitro-5-(phenothiazin-10-yl)phenyl phenyl sulphide (9) (4·3 g) was heated under reflux for 10 h with triethyl phosphite (6 g) in t-butylbenzene (75 ml). Volatile material was distilled out of the brown mixture (1 mmHg, 100°) and the tarry residue was purified (by column chromatography [silica; benzene–cyclohexane 1 : 1)] to yield white amorphous 3,10′-biphenothiazinyl (6b) (40%), m.p. 195—197° (from cyclohexane) (lit.,[11] 198—199°); the compound was homogeneous by t.l.c. [silica; benzene–cyclohexane (1 : 1) 15 cm development], with R_F 0·29, visualised by exposing the plate to iodine vapour; *m/e* 396 (*M*) and 198 ($C_{12}H_8NS^+$), with minor peaks corresponding to loss of S and H_2S, twice, from the molecular ion; u.v. data in the Table (Found: C, 72·5; H, 4·2; N, 6·9. Calc. for $C_{24}H_{16}N_2S_2$: C, 72·7; H, 4·1; N, 7·1%).

Materials for Spectroscopic Measurements.—Acetonitrile (B.D.H.), purified by the method of Forcier and Olver,[20] was transparent to below 200 nm. It was stored over type 4A molecular sieve. Phenothiazine was recrystallised twice from benzene, with carbon treatment, and twice from cyclohexane. Phenothiazine 5-oxide, prepared by the method of Gilman and Nelson,[21] had m.p. 243—245° (decomp.) (lit.,[21] 242° with decomp.). Phenothiazine radical-cation perchlorate had m.p. 175° (decomp.) as previously reported.[3] Phenothiazinium perchlorate was also prepared following Billon; [3] since the material decomposes on heating, without melting, the suitability of each preparation was determined by comparison of its electronic spectrum in acetonitrile with that of an analytical sample (Found: C, 48·6; H, 2·6; Cl, 12·0; N, 5·2; S, 10·7. Calc. for $C_{12}H_8ClNO_4S$: C, 48·4; H, 2·7; Cl, 11·9; N, 4·7; S, 10·8%). The phenothiazinone (2) made by the method of Olivier and Combé,[22] had m.p. 164—165° (lit.,[22] 165°). 3,10′-Biphenothiazinyl was prepared as described earlier; material of analytical purity was used for spectroscopic measurements. Thallium(III) acetate sesquihydrate (Emanuel) was used as supplied, stabilised in acetonitrile solution with AnalaR acetic acid.

Spectra.—U.v. and visible absorption spectra were measured on a Unicam SP 800 spectrophotometer. Extinction coefficients were determined with either a Cary 14 or a Unicam SP 500 spectrophotometer. E.s.r. spectra, for which the authors are indebted to Dr. B. C. Gilbert and Mr. M. Trenwith, were recorded on a Varian E-3 spectrometer.

We are grateful to Professor J. I. G. Cadogan for helpful discussion of the use of triethyl phosphite in the formation of phenothiazines from *o*-nitrodiaryl sulphides, and to Professor H. J. Shine for kindly reading the manuscript.

[2/2076 *Received, 4th September*, 1972]

---

[19] G. C. Finger, M. J. Gortatowski, R. H. Shiley, and R. H. White, *J. Amer. Chem. Soc.*, 1959, **81**, 94.

[20] G. A. Forcier and J. W. Olver, *Analyt. Chem.*, 1965, **37**, 1447.

[21] H. Gilman and R. D. Nelson, *J. Amer. Chem. Soc.*, 1953, **75**, 5422.

[22] S. C. J. Olivier and W. P. Combé, *Rec. Trav. chim.*, 1950, **69**, 526.