Characterisation of 3,7-dibromophenothiazin-5-ium perbromide and its use for enhancing latent fingerprints

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Solutions were formulated by dissolving 3,7-dibromophenothiazin-5-ium perbromide in primary and secondary alcohols with pyridine as base. These solutions were used as dips for the enhancement of latent fingerprints. Treatment of 3,7-dibromophenothiazin-5-ium perbromide with cyclohexene or sodium sulfite gave 3,7-dibromophenothiazine.

Keywords: phenothiazine, perbromide, bromine, ninhydrin, fingerprint

The chemical enhancement of latent fingerprints is an important method of capturing marks that may be used to assign the identity of criminals handling stolen goods and money and who commit other crimes. Sweat contains small quantities of amino acids which can be reacted with a colour developer such as ninhydrin **1**,[1-7] 1,8-diazafluorenone (DFO) **2**,[8-10] indan-1,2-dione **3**[11] or alloxan **4**[12] to create a print that can be imaged and stored (Scheme 1).

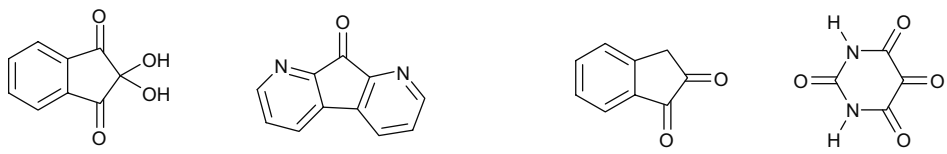
Ninhydrin **1** and alloxan **4** are the oldest colour developers for amino acids. Alloxan **4** was the earliest discovered by Strecker in 1862.[12] Reagents **1**, **2** and **4** have a similar elegant mechanism of action which involves stripping out a single nitrogen atom from the amino acid and incorporating it into a dye. The dyes are structurally similar. Development conditions for the reagents can involve heating at temperatures up to 100 °C. New, more sensitive reagents are desirable particularly those which react under milder development conditions enabling their use at crime scenes early in an investigation.

Results

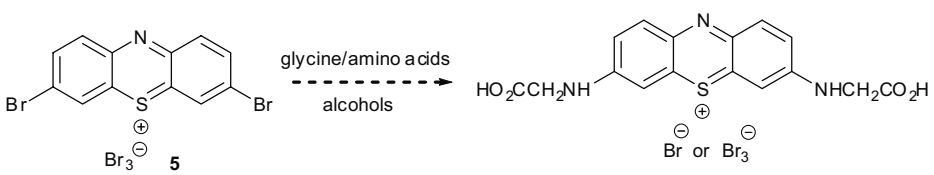
3,7-Dibromophenothiazin-5-ium salt

In this paper 3,7-dibromophenothiazin-5-ium perbromide **5** has been investigated as a colour former for latent fingerprints (Scheme 2). Condensation with two equivalents of an amino acid such as glycine might give coloured derivatives of methylene blue which should be tinctorially stronger than the starting reagent. The heterocyclic salt 3,7-dibromophenothiazin-5-ium perbromide **5** was first prepared by Kehrmann in 1916 by treatment of phenothiazine **6** with an excess of bromine in acetic acid (Scheme 3).[13,14] Treatment of salt **5** with dimethylamine in ethanol gave methylene blue **7**.[14] The methodology was reinvestigated in 1997 and exploited to make analogues of methylene blue.[15] However, the authors of the later investigation characterised the salt **5** as a bromide rather than the original perbromide.[13,14,16] This is unusual because bromide readily reacts with bromine to give perbromide and excess bromine is present (20 equiv.). Dilute solutions of tetrabutylammonium bromide (TBAB) react completely with solutions of bromine as determined by UV spectrophotometry.[17] Water soluble perbromides studied for their biocidal properties are also quite stable and are formed by mixing a bromide salt with 1 eq of bromine.[18]

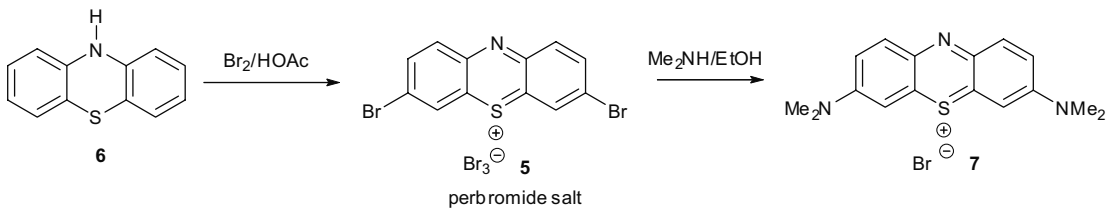
Since a number of perbromides are commercially available as brominating reagents we decided to see if the counterion in salt **5** could act as a source of bromine to leave bromide as the counterion. Salt **5** was stirred in acetonitrile with



Scheme 1 Some key reagents for developing latent fingerprints.



Scheme 2 Proposal for a colour developer based on 3,7-dibromophenothiazin-5-ium perbromide.



Scheme 3 Kehrmann's method of methylene blue synthesis.

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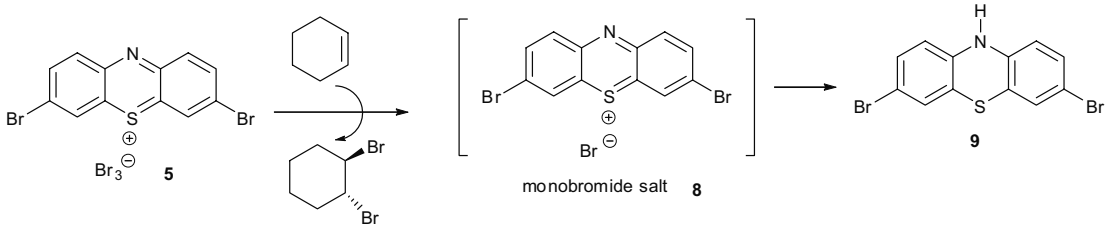


Scheme 4 Reduction of 3,7-dibromophenothiazin-5-ium perbromide **5**

an excess of cyclohexene for an arbitrary period of 16h (Scheme 4). The solution was filtered but there was no precipitate which a salt would have given. The solvent was therefore diluted with water which gave a pale green precipitate in 40% yield. This was collected by filtration, washed with water and methanol and dissolved in CH_2Cl_2. The solution was filtered through a pad of silica to remove a green impurity, concentrated *in vacuo*, and then dried under vacuum giving a yellow solid. It eluted on a TLC plate as a colourless spot with CH_2Cl_2 (R_f 0.9) showing that it was not a salt. Weak shadow spots were just in front and behind. NMR analysis in D_8THF gave satisfactory proton and carbon spectra. The proton spectrum showed a broad singlet at 6.4 ppm (2H), two doublets in the region 7.02–7.06 ppm (4H) and a flattened peak (1H) at 7.5–7.9 ppm. The carbon spectrum had the expected six resonances in the region 112–142 ppm (three CH carbons and three quaternary carbons determined by DEPT 135). The mass spectrum showed peaks at 511–519 ($C_{12}H_5NBr_4S$, 2%), 433–439 ($C_{12}H_6NBr_3S$, 7%), 355–359 ($C_{12}H_6NBr_2S$, 100%). An accurate mass was measured in all three ranges. The same product formed from salt **5** when it was stirred with sodium sulfite in water and ether. It was also present when a DCM solution of salt **5** was eluted on a silica plate with ether/light petrol 1 : 1. An X-ray crystal structure determination of the product produced by reduction with cyclohexene showed it to be 3,7-dibromophenothiazine **9** (Figure 1). This verifies the position of the bromines since bromination can also occur in the 1 and 9 positions of phenothiazine.[16,19-20] Both cyclohexene and sulfite were expected to reduce perbromide to bromide, but salt **5** also appears to be spontaneously reduced by these reagents. This result casts doubt on the claim by Kehrmann to have made phenothiazinium perbromide by brominating phenothiazine in acetic acid because only traces of phenothiazine were present after treatment of salt **5** with bisulfite or cyclohexene and Kehrmann's first paper reported the use of bisulfite in the work-up procedure.[13] Kehrmann's incorrectly assigned 'phenothiazinium perbromide' is probably 3,7-dibromophenothiazine **9**. Bisulfite reacts with bromine according to the equation:

$$Br_2 + 3NaHSO_3 = 2NaBr + NaHSO_4 + H_2O + 2SO_2$$

3,7-Dibromophenothiazin-5-ium perbromide **5** has sometimes been mistaken for phenothiazinium perbromide [21,22] as well as their being confusion over the counterion.[15-16] The presence of unoxidised 3,7-dibromophenothiazine **9** in the salt **5,** observed by TLC**,** suggests that the one minute reaction time may not be long enough for complete oxidation to occur, possibly due to HBr protonation of the amine, or that the salt slowly decomposes to this compound.

The complete $C_{12}H_7Br_2NS$ molecule is generated by crystallographic mirror symmetry, with the N–H group and S atom lying on the reflecting plane. The central ring has a distorted boat conformation and the overall molecule adopts a butterfly shape, with a dihedral angle of 32.84 (9)° between the aromatic ring planes. N1 and S1 deviate from the mean plane of C1–C6 by –0.044 (5) Å and 0.198 (4) Å, respectively and the C–S–C bond angle is 98.64 (17)°. The bond-angle sum at N1 is 348.3 (2)°, which is intermediate between the values expected for pure sp^3 (328.5°) and sp^2 (360°) hybridisations and the hydrogen atom adopts a quasi-equatorial[23] position. Otherwise, the geometrical parameters may be regarded as normal. In the crystal, the molecules interact by way of weak N–HLS^{ii} (ii = 1/2 + *x*, *y*, 3/2–*z*) hydrogen bonds [HLS = 2.80 (5) Å; N–HLS = 173 (4)°], leading to infinite C(5) chains propagating in [100]. There are no aromatic π–π stacking interactions. The only crystal structure containing the 3,7-dibromophenothiazine skeleton in the Cambridge Structural Database is that of $C_{14}H_{11}Br_2NS$[23], in which the dihedral angles between the aromatic rings in the two molecules in the asymmetric unit are 26.4 (2)° and 34.9 (2)°.

Scheme 5 proposes a second mechanism for the oxidation of phenothiazine **6** to salt **5**. Previously N-bromination was proposed followed by expulsion of the bromine.[15] A difficulty with this mechanism in acetic acid is the expected faster N-bromination of phenothiazine compared to that of 3,7-dibromophenothiazine **9** which might lead to salt formation earlier on in the reaction. However, *ipso* bromination of compound **9** would give compound **10** which could also expel bromine to give salt **5**. *Ipso* bromination occurs for example

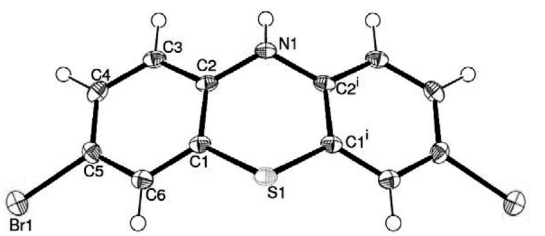
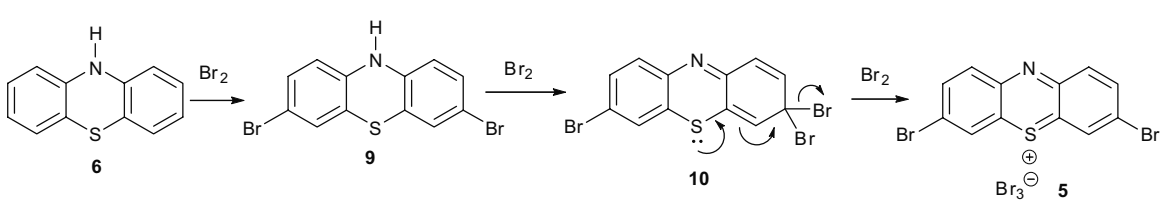
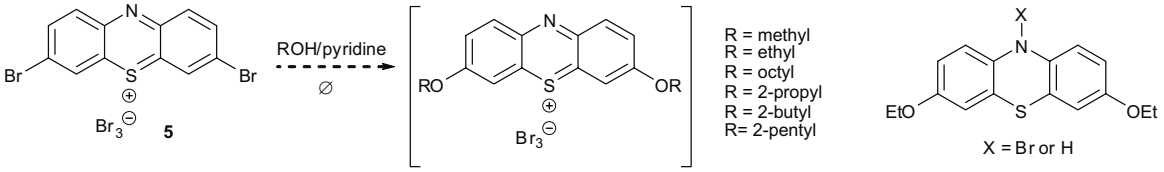


Fig. 1 The molecular structure of $C_{12}H_7Br_2NS$ **9** showing 50% displacement ellipsoids for the non-hydrogen atoms: symmetry code: (i) *x*, 1/2–*y*, *z*.



Scheme 5 A proposed mechanism of oxidation of phenothiazine with bromine.



Scheme 6 Proposed *in situ* formation of dialkoxyphenothiazin-5-ium salts with alcohols (see text for comments on the structure).

in the formation of tetrabromophenol.[24] In the authors' hands treatment of phenothiazine in acetic acid with 2 equivalents of bromine for 30 min gave compound **9** and two uncharacterised red compounds suggesting that other intermediates or side reactions may be involved.

The structure of salt **5** was clarified this way. However, purification by the literature method gave a product which still fumed when the vial was opened to the air. Further purification was achieved by stirring in acetonitrile for an arbitrary period of 2 h, filtration and washing with acetonitrile then ether. Its use for the development of latent fingerprints was then investigated.

Latent fingerprints

To formulate salt **5** a small quantity (~ 25–50 mg) was dissolved in different alcohols (100 mL) by heating with an excess of pyridine (0.5 mL). Without any pyridine dissolution was much slower suggesting that the base assisted the nucleophilic displacement of the bromine atoms by the alcohol. The solutions were initially dark green but over 2 days a fine precipitate settled leaving a red solution. Hence 3,7-dialkoxyphenothiazin-5-ium salts are proposed as intermediates generated *in situ* but have not been characterised any further (Scheme 6). The *in situ* generation of 3,7-diethoxyphenothiazin-5-ium bromide in ethanol from salt **5** has been studied previously.[15] It was reported as a cherry red salt but it was purified by chromatography on silica gel (Rf = 0.8 with $CHCl_3$). This is rather non-polar for a bromide salt so we propose that the compound is a neutral adduct (Scheme 6) with (X = Br) or the reduced form (X = H). It was prepared[15] by purging an ethanolic solution of salt **5** with nitrogen for 24 h which might drive off liberated bromine shifting the equilibrium structure of the counterion from perbromide to bromide. TLC of a methanolic solution of salt **5** after heating with a small quantity of pyridine and standing for 24 h, during which time the solution turned from green to red, showed a red product which eluted readily with ether but also much coloured dye on the baseline.

A strong fingerprint was prepared on a silica sheet by pressing a damp thumb onto it. This was dipped once into the dip solution then dried for up to 5–10 minutes in an oven at 100 °C. Fingerprints dipped into methanol, ethanol or octanol dips gave very poor images that had no detail of lines or ridges. Fingerprints dipped into isopropanol, 2-butanol, or 2-pentanol solutions of reagent **5** developed images displaying lines and ridges. This observation of the need for secondary over primary alcohols supports the view that the alcohols have reacted with salt **5**. 2-Butanol was the best solvent. The lines are pink or pale red and were observed in visible light. Heating a green methanol dip of salt **5** with a suspension of glycine for a few minutes gives a red solution (rather than the colour of methylene blue) which explains the colour of the developed fingerprints. The dips will develop fingerprints after many weeks.

The fingerprints showed good light stability without fading in ambient daylight after one week. The image quality is however reduced in some regions by pink blotches. The visibility of the fingerprint is enhanced by bleaching of the background reagent in the area of the fingerprint. The proposed intermediates generated by reacting secondary alcohols with salt **5**, rather than primary alcohols, will be less polar because the polar ether group is more hindered and less available to bind to a surface. This may allow more ready diffusion on a solid surface and enhance the surface reactivity. Chiral alcohols will give mixtures of racemic and meso products and so help to lower crystallinity and favour diffusion.

Summary

Latent fingerprints can be enhanced from a strong fingerprint using solutions prepared by dissolving 3,7-dibromophenothiazin-5-ium perbromide in secondary alcohols. The fingerprints show good light fastness. The image quality is however reduced by blotches. The reagent is a polar salt with a large surface area so will absorb strongly onto surfaces reducing its reactivity and preventing diffusion. The background colouration of the reagent is partly quenched in the fingerprint region so the enhancement of latent fingerprints is not solely due to displacement of alkoxy substituents from the proposed intermediate salts. Evidence is presented helping to characterise the 3,7-dibromophenothiazin-5-ium salt. Treatment with cyclohexene or sodium sulfite gives 3,7-dibromophenothiazine.

Experimental

IR spectra were recorded on an ATI Mattson FTIR spectrometer using KBr discs. UV spectra were recorded using a Perkin-Elmer Lambda 25 UV-VIS spectrometer with CH_2Cl_2 as the solvent. ^1H and ^{13}C NMR spectra were recorded at 400 MHz and 100.5 MHz respectively using a Varian 400 spectrometer. ^{13}C NMR DEPT 135 was used to distinguish quaternary carbons from CH carbons. Low resolution mass spectra were obtained at the University of Wales, Swansea using EI, CI and ES ionisation methods. Accurate mass spectra were determined at the University of Wales, Swansea using ES ionisation methods. Melting points were determined on a Kofler hot-stage microscope. 3,7-Dibromophenothiazin-5-ium perbromide **5** was prepared following the literature method with one modification.[15] Purification was achieved by stirring the red salt in acetonitrile for 2 h then filtration and washing with acetonitrile then ether. Solutions for the enhancement of latent fingerprints are described in the text with full details.

3,7-Dibromophenothiazine (**9**): Intensity data for a pale green prism of **1** were collected on a Nonius Kappa CCD diffractometer (MoKα radiation, λ = 0.71073 Å) at T = 120 K. The structure was solved by direct methods with SHELXS-97 and refined using SHELXL-97. The N-bound hydrogen atom was located in a difference map and its position was freely refined; the C-bound hydrogen atoms were geometrically placed and refined as riding. Crystal data: $C_{12}H_7Br_2NS$, M_r = 357.07, orthorhombic, *Pnma* (No. 62), Z = 4, a = 7.7968 (2) Å, b = 24.3333 (6) Å, c = 5.9341 (1) Å, V = 1125.83 (4) Å^3, $F(000)$ = 688, ρ = 2.107 g cm^{-3}, μ = 7.35 mm^{-1}, min., max. Δρ = –0.52, + 0.46 e Å^{-3}, $R(F)$ = 0.029 (1160 reflections), $wR(F^2)$ = 0.069 (1324 reflections). CCDC 732813

3,7-Dibromo-10H-phenothiazine (**9**): Compound **5** (330 mg, 0.554 mmol^{-1}) was stirred in acetonitrile with an excess (1 mL) of cyclohexene for 16h. The reaction was diluted with water forming a precipitate. This was collected by filtration, washed with water then methanol. The green solid was dissolved in CH_2Cl_2 and filtered through a pad of flash silica, concentrated *in vacuo* and dried over calcium chloride to give the *title compound* (95 mg, 40%) as a yellow solid m.p. 210–212 °C (lit. 206–209 °C)[19] (Found: C, 40.5; H, 1.9; N,

3.8. $C_{12}H_7NSBr_2$ requires C, 40.4; H, 2.0; N, 3.9%) λ_{max}(DCM)/nm 260 (log ε 4.4) and 320 (3.8) ν_{max}(KBr)/cm^{-1} 1457vs, 1386 s, 1291 s, 1237 s, 1089w, 1081w, 1037w, 881vs, 809vs, 750w, 732w, 674w, 649w, 585vs and 514 s; ^{1}H NMR (400 MHz; D_8THF) 6.40 (2H, s) 7.04 (2H, d, *J* 2.03), and 7.05 (2H, d, *J* 2.03) and 7.50-7.90(1H, s, br); ^{13}C NMR (100.5 MHz; D_8THF) 113.8(q), 115.6, 120.0(q), 128.9, 130.5 and 141.7(q); *m/z* 511/513/515/517/519 (M^+, 2%) Found: 510.6871 $C_{12}H_5NBr_4S$ requires 510.6871; 433/435/437/439 (M^+, 7) Found: 432.7762 $C_{12}H_6NBr_3S$ requires 432.7766; 355/357/359 (M^+, 100) Found: 354.8660 $C_{12}H_6NBr_2S$ requires 354.8661 and 276/278 (M^+ – Br, 20).

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