Lawsone: a novel reagent for the detection of latent fingermarks on paper surfaces

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Lawsone (2-hydroxy-1,4-naphthoquinone) reacts with latent fingermark deposits on paper surfaces to yield purple-brown impressions of ridge details which are also photoluminescent; this compound represents the first in a completely new class of fingermark detection reagents.

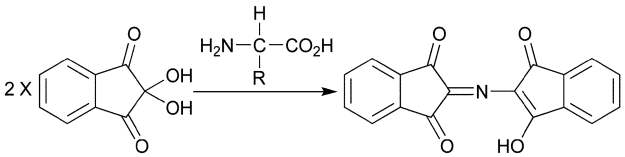
Fingermarks remain the most widely used and reliable means of personal identification and, hence, are extremely important to law enforcement as contact evidence.[1] A key element in the successful recovery of a latent (invisible) fingermark from a scene or object is detection; to this end, a range of physical and chemical methods are available for the visualisation of such fingermarks.[1,2]

Amino acids are a key component of the secretions that contribute to latent fingermarks[3] and are particularly important when porous surfaces, such as paper, are examined, as they tend to bond to paper fibres and are thus long-lived.[2] Ninhydrin (2,2-dihydroxy-1,3-indanedione) was the first amino acid sensitive reagent for developing fingermarks on porous surfaces and is still the most widely used. Ninhydrin reacts with amino acids to form a dark purple coloured compound known as Ruhemann's purple (Scheme 1).[2]

These treated impressions may be further enhanced by treatment with zinc and cadmium salts to produce complexes that exhibit photoluminescence when cooled to liquid nitrogen temperature (−196 °C) and excited with high intensity light sources.[2]

Since the early 1980s, a range of ninhydrin analogues have been synthesised and tested for their suitability as reagents for the detection of latent fingermarks. Despite the large body of work carried out, only two such reagents have found widespread operational use: 1,8-diazafluoren-9-one (DFO) and 1,2-indanedione[1,2,4] (Fig. 1). More recently, Almog and co-workers have suggested the natural product genipin as an operationally safer and environmentally benign fingermark reagent exhibiting both colour and photoluminescence.[5,6]

Traditionally genipin has been used as a fabric and skin dye, and this led us to investigate the potential of other natural products, which have been used in this fashion, as fingermark reagents. Henna, a natural product sourced from the leaves of *Lawsonia inermis*, in a similar manner to genipin, has been used as a skin and hair dye for millennia, with reports of its use dating back to 1400 BC.[7] Lawsone (2-hydroxy-1,4-naphthoquinone, Fig. 2) is the compound thought to be responsible for the staining properties of henna.[7] This compound was thus selected to be investigated for its ability to develop latent fingermarks on paper surfaces.



Scheme 1 Reaction of ninhydrin with amino acids.

Lawsone is a naphthoquinone, a group of compounds that are well known for their reactions with amino acids. 1,2-Naphthoquinone-4-sulfonate has been applied to the determination of amino acids through the formation of highly coloured compounds[8–10] and 1,2-naphthoquinone has been reported as yielding purple-brown pigments with cysteine and proteins.[11] Spectroscopic evidence indicates that the reaction of 1,2-naphthoquinone occurs as a result of the primary amine component of amino acids.[11] Intriguingly, a paper describing investigations into the reactivity of the phenyliodonium ylide of 2-hydroxy-1,4-naphthoquinone with amino compounds suggests the formation of indanedione-2-carboxamido compounds,[12] thus offering an alternative window on the reaction mechanism of 1,2-indanedione.[13] Naphthoquinones therefore represent a class of compounds of significant interest as potential fingermark detection reagents.

Latent fingermarks were collected on filter paper from a number of different volunteers. Volunteers were requested not to wash their hands immediately before collecting impressions; the fingers were not "charged" with additional secretions in any way. The filter paper strips were then dipped in the lawsone reagent solution, air dried and then heated, either in an oven or by direct heat using a laundry press. Initial experiments with lawsone in ethanolic solution yielded purple-brown developed marks, which also exhibited strong

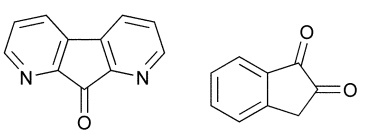


Fig. 1 Amino acid targeting reagents for the development of latent fingermarks.

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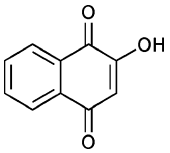


Fig. 2 Chemical structure of lawsone.

photoluminescence when illuminated with a forensic light source (Polilight PL500, Rofin, Australia) at 555 nm and viewed through red goggles (Fig. 3).

The photoluminescence was further investigated using a Cary fluorescence spectrophotometer (Varian, Mulgrave, Australia) with a fibre optic probe attachment (Varian, Mulgrave, Australia), and it was determined that the maximum intensity of luminescence occurred at around 640 nm with excitation at 590 nm. This is operationally significant as photoluminescence emission at longer wavelengths has the potential to improve detectability by avoiding native background luminescence.

It was found that using ethanol as a carrier solvent led to a significant discoloration of the paper substrate, resulting in a degradation of image quality. Ethanol would be unsuitable as a carrier solvent for operational use due to its polarity, which would cause problems with written document evidence due to ink diffusion through the paper. We investigated methyl nonafluoroisobutyl ether (HFE-7100) as the carrier solvent for the working solution; this solvent is used widely for fingermark reagents such as 1,2-indanedione.[2] In order to successfully dissolve lawsone, a co-solvent of higher polarity was required. Ethyl acetate (20%) was found to be the most successful of the solvents investigated. We also found that petroleum spirit 60–80 °C could substitute for HFE-7100 without a serious impact on the quality of the developed fingermarks.

Preliminary studies established that a concentration of 1 mg lawsone mL^{-1} gave the best compromise between contrast and background interference with excess concentrations leading to discoloration of the paper substrate. The formulation used for the remainder of the study was prepared in the following fashion: lawsone (50 mg; Sigma-Aldrich) was dissolved in ethyl acetate (10 mL; Univar) and the resulting solution was subsequently mixed with HFE-7100 (40 mL; 3 M Novec). Latent fingermark samples on filter paper were dipped in this solution, air dried and then developed with heat. Heating in an oven for 1 h at 140–170 °C produced more uniform development than heating with a laundry press, providing enhancement sufficient for complete visualisation of fingermark ridge details.

In order to verify that lawsone was reacting with the amino acid content of the latent fingermarks, solutions of amino acids in water (lysine, alanine, glycine, proline; all at 900 μg mL^{-1}) were dispensed (10 μL) onto filter paper and allowed to air dry before subsequent lawsone treatment. In common with the developed latent fingermarks, the amino acid spots, with the exception of proline, developed as purple-brown stains which were also photoluminescent. Fluorescence spectra of the developed amino acid spots and a developed latent fingermark are presented in Fig. 4. It is clear from the profiles that the photoluminescent characteristics of the product of the reaction

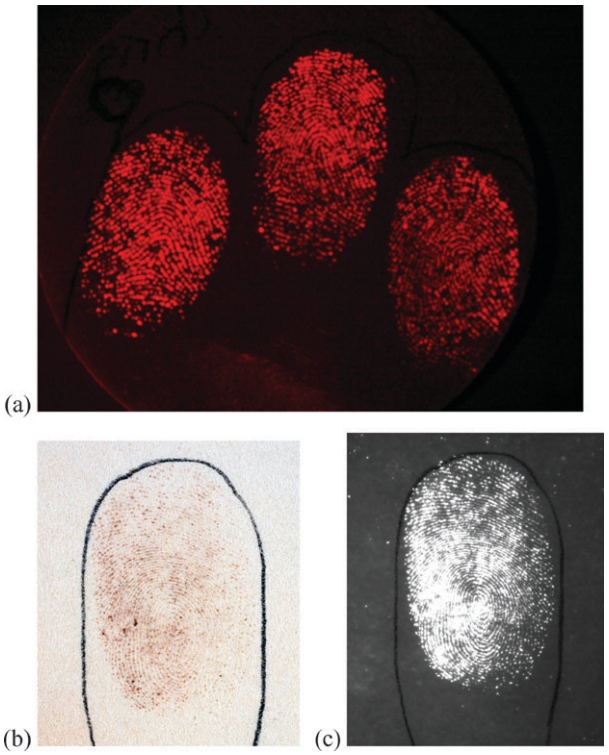


Fig. 3 Lawsone treated latent fingermarks. Images (a) and (b) were taken with a Pentax K10 digital SLR, 50 mm focal length, ISO 100. (a) Photoluminescence mode (excitation with a Polilight PL 500 at 590 nm and viewed through a Wratten NA29 filter, shutter speed 6.0 s, aperture f2.8). (b) Taken under white light (shutter speed 1/125 s, aperture f4). (c) Acquired using a Poliview digital imaging system (Rofin, Australia) with excitation at 590 nm, viewed through a 650 nm interference filter with a 1 s exposure time.

between primary amino acids and lawsone are similar to that in lawsone developed fingermarks, thus confirming that the lawsone reagent is non-specifically targeting primary amino acids in the latent fingermark deposit. This is a key requirement of latent fingermark reagents, as the amino acid content of natural secretions is highly variable from one subject to another.[3]

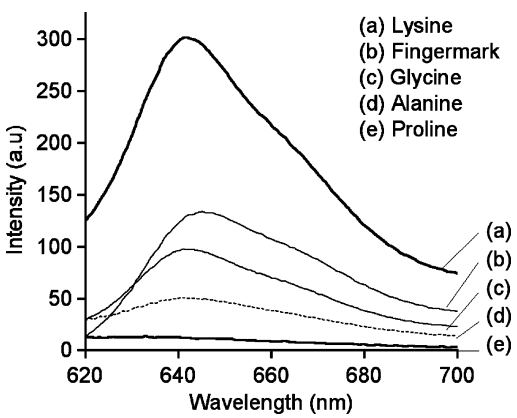
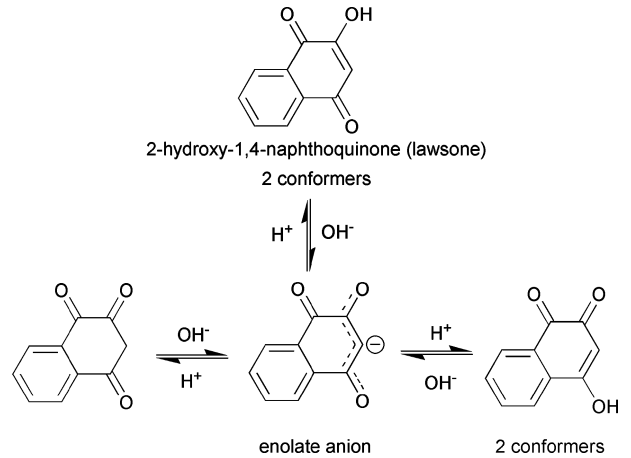
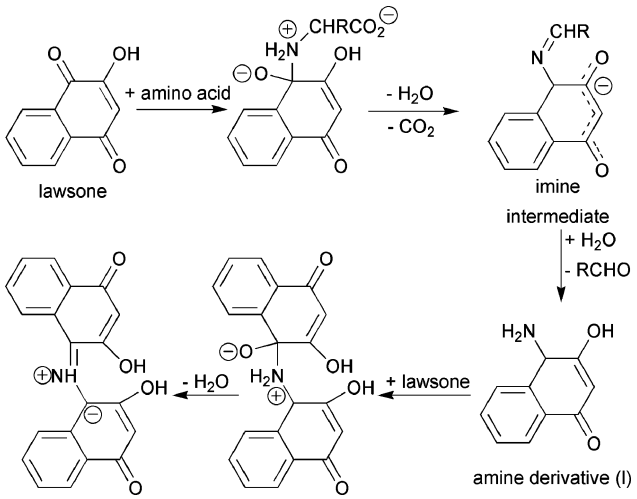


Fig. 4 Fluorescence spectra of a lawsone developed latent fingermark and of selected amino acids on filter paper (excitation wavelength 590 nm).



Scheme 2 Lawsone and isomers.



Scheme 3 Proposed reaction pathway and product for the reaction of lawsone with primary amino acids.

Lawsone can undergo keto–enol tautomerisation as shown in Scheme 2. Spartan '04[14] *ab initio* calculations using HF/6 31G* theory indicate that the 1-position of all 6 species/conformers is most likely to react with an incoming nucleophile.

Based on the known reactivity of hydroxyquinones[15] and ninhydrin,[16] we postulate that lawsone undergoes a Strecker degradation[17] at the 1-position to form the amine intermediate (**I**), as shown in Scheme 3. The Strecker degradation requires a primary amino acid,[17] which would explain why proline does not form the fluorescent product. The amine (**I**), is a nucleophilic base which can react with a second lawsone molecule to reduce the ketone at the 1′-position[18] to form the final product, in which the negative charge can be delocalised over the extended π-system.

Further research is required to investigate the reaction mechanism involved and optimise the development conditions for this fingermark detection reagent. In addition, there is the potential that lawsone could find wider application as a fluorogenic reagent for amino acids; however, additional studies are required to determine optimum reaction conditions in solution and sensitivity *versus* existing reagents.

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