JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, January 2010, Vol. 55, No. 1
doi: 10.1111/j.1556-4029.2009.01231.x
Available online at: interscience.wiley.com

TECHNICAL NOTE

CRIMINALISTICS

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Ninhydrin Thiohemiketals: Basic Research Towards Improved Fingermark Detection Techniques Employing Nano-Technology*

ABSTRACT: In the first part of a comprehensive research project towards more efficient application of nano-technology to fingerprint visualization, we investigated the possibility of more selective binding of gold nanoparticles (NP) to fingerprint material. We synthesized derivatives of ninhydrin and 1,2-indanedione containing loosely bound thiol groups. In particular: thiohemiketals (THK) of ninhydrin, and thioketals of 1,2-indanedione were prepared and tested as potential fingerprint reagents. By reacting ninhydrin with various thiols we were able to produce a series of novel THK, bearing the SR group always at C2. Ninhydrin THK reacted with amino acids to produce the expected Ruhemann's purple, and they also developed latent fingermarks on paper in a similar manner to ninhydrin. Ketals and thioketals derived from 1,2-indanedione reacted neither with amino acids nor with latent fingermarks. In the second part of the research, the thiols which are formed on the ridges as byproducts of the reaction with amino acids will be tested for their potential as stabilizers for gold NP that will become covalently bound to the fingerprint ridges.

KEYWORDS: forensic science, fingermarks, ninhydrin, indanedione, hemiketals, thiohemiketals, isononyl ninhydrin

The introduction by Saunders of the multi-metal deposition (MMD) technique in 1989 (1) can be recognized as the first application of nano-technology to visualizing latent fingermarks. Gold nanoparticles (NP) (then named "colloidal gold") stabilized by citrate ions are selectively deposited on the fingerprint material. In second stage, the bound gold colloids serve as nucleation sites for metallic silver from a Physical Developer (PD) solution. The black silver which precipitates on the gold deposits allows the visualization of the latent marks as dark impressions on lighter background. (Silver PD, which preceded MMD, also contains nanometric size particles, but they may easily grow beyond these dimensions [2].) A few years later Schnetz and Margot modified Saunders' original conditions. They showed that smaller NP and hence better contrast are obtained at a lower pH (3,4). In a recent study Sametband and coworkers showed that gold NP which are stabilized by long chain thiols, can catalyze the reduction-precipitation of silver in nonaqueous solutions. Longer chain thiols (C18) were found to be more efficient in binding gold than short ones (5). Several other articles dealing with the high potential of nanotechnology in fingermarks enhancement have been published lately (6–12). In this study, we examined the possibility of forming thiol molecules on the latent ridges *in situ*, as a part of the fingermarks visualization process, with the intention of using them in a second step to facilitate binding the gold NP to the ridges. We aimed at preparing thiol-containing derivatives of known fingerprint reagents, ninhydrin (Fig. 1), and 1,2-indanedione (Fig. 2). The thiol groups should be loosely bound to the reaction center, so that they can come off upon reaction with the amino acids constituent of latent fingermarks (and lay on the fingerprint ridges). The most suitable derivatives which theoretically meet with this requirement are thiohemiketals (THK) of the two reagents (Figs. 3 and 4, respectively).

The THK are supposed to react with amino acids in a similar fashion to the unsubstituted reagents, producing the known Ruhemann's purple (from ninhydrin) or Jouillé's pink (from indanedione) and giving off, besides carbon dioxide and aldehyde, as in the common ninhydrin reaction, also two equivalents of thiol per amino acid (Fig. 5) ("Jouillé's pink": like Ruhemann's purple, which is named after the discoverer of the ninhydrin reaction with amino acids, we suggest naming the pink product which is obtained in the reaction between 1,2-indanedione and amino acids, *Joullié's pink*, in honor of Professor Madeleine M. Joullié, the discoverer of this reaction [13,14]).

Ninhydrin THK containing aliphatic or aromatic groups on the sulfur atoms could be readily synthesized by refluxing ninhydrin with the appropriate thiols. Even when large excess of the thiol was used, only one thiol group could be introduced, and only THK (Fig. 3) but not thioketals (Fig. 6) were obtained. In another experiment, the THK was isolated and further reacted with another equivalent of the thiol, but no second thiol could be noticed. This finding is in agreement with the report of Cockburn and Speakman, who were unable to introduce more than one phenylthio group into the ninhydrin molecule (15). The opposite occurred with indanedione: only doubly substituted derivatives (thioketals, Fig. 7) were obtained even when 1:1 equivalent amounts of both reactants were used. Furthermore, while thiols reacted with ninhydrin only at position 2, reaction with indanedione occurred preferably at position 1. Also in the reaction with alcohols instead of thiols, indanedione produced only ketals (Fig. 8), and no hemiketals could be isolated. But with alcohols, the reaction occurred at position 2 (Fig. 9). Neither the thioketals nor the ketals of indanedione produced any noticeable reaction with amino acids or with latent fingermarks on paper.

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*Financial support provided by the Casali Foundation, as a part of the nano-technology research program.

Received 6 Nov. 2008; and in revised form 7 Jan. 2009; accepted 11 Jan. 2009.

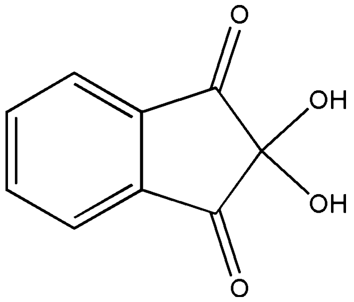


FIG. 1—*Ninhydrin.*

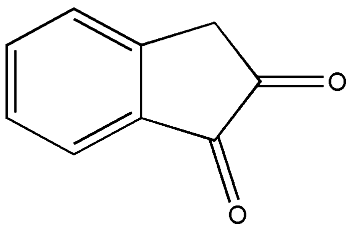


FIG. 2—*1,2-Indanedione.*

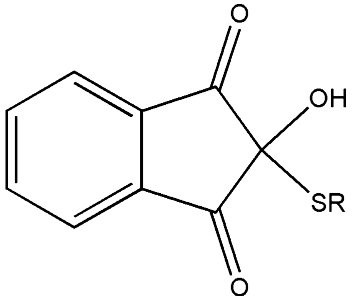


FIG. 3—*Thiohemiketal of ninhydrin (general structure).*

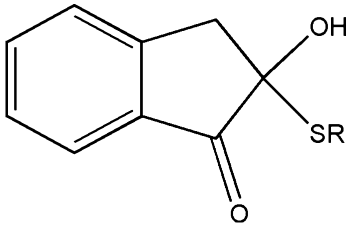


FIG. 4—*Thiohemiketal of 1,2-indanedione (general structure).*

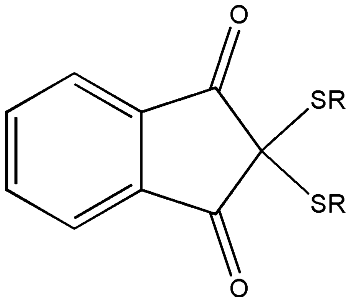


FIG. 6—*Thioketal of ninhydrin (general structure).*

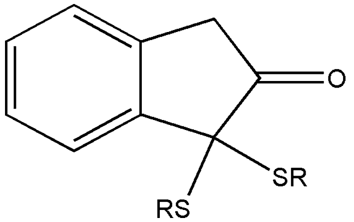


FIG. 7—*Thioketal of 1,2-indanedione (general structure).*

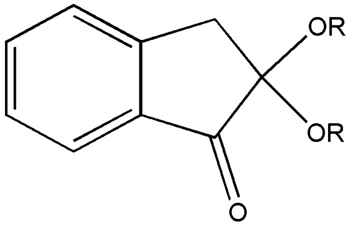


FIG. 8—*Ketal of 1,2-indanedione (general structure).*

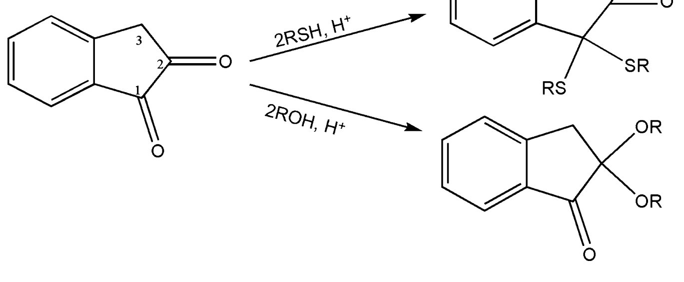


FIG. 9—*The reaction between 1,2-indanedione (2) and alcohols or thiols. Notice that while alcohols attack at C2, thiols generally attack at C1.*

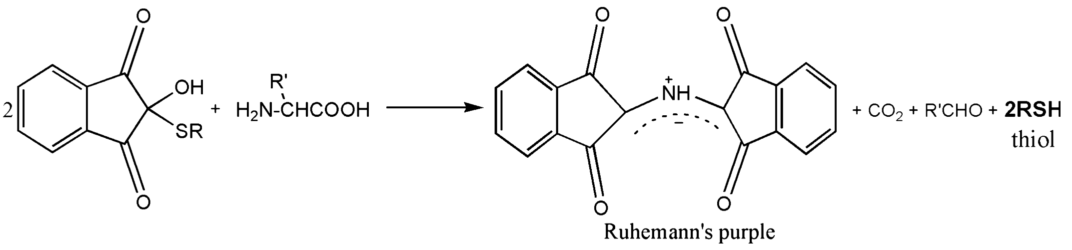
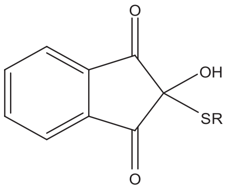
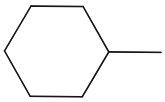
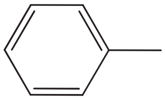
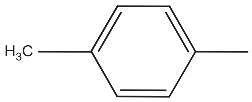


FIG. 5—*The assumed reaction between ninhydrin thiohemiketals (3) and amino acid. Notice the formation of two molecules of thiol (5) per reaction unit.*

TABLE 1—*Ninhydrin thiohemiketals that were prepared and tested in this work.*



Entry	R	Yield (%)	mp (°C)	Remarks
I	$(CH_3)_2CH$-	60	122.7–124.3	
II	$CH_3(CH_2)_{11}$-	79	72.0–73.0	Recrystallization from pet. ether
III	$CH_3(CH_2)_{17}$-	62	86.0–87.0	Recrystallization from pet. ether
IV		68	105.9–108.3	Containing some impurities. Confirmation by ^1H NMR
V		88	122.7–124.3	This compound has been reported previously (15)
VI		89	168.9–170.7	

Materials and Methods

Natural latent fingermarks on A4 paper were collected from four volunteers, three females and one male (first set of latents only. No depletion and no secretion-charged marks were used.) They were maintained at room temperature (RT) in a closed drawer and processed at ages from 1 h to 1 month by dipping in ethanolic solution (28 mmol, equivalent to 0.5% ninhydrin) of the new reagents, and air dried.

Synthesis of Ninhydrin Thiohemiketals (Generic Procedure for Compounds I–VI; Table 1)

Ninhydrin was dissolved in toluene and heated to 110°C under reflux with a Dean-Stark distillation system, until a green color appeared (*c.* 15 min.). Equivalent amounts of thiols were added and the solution heated for another 2 h. The solution was allowed to cool to RT. The white precipitate was collected by filtration and recrystallized if required.

In an attempt to convert a THK to thioketal, THK-C18 (III; Table 1) was reacted with another equivalent of thiol under reflux with a Dean-Stark distillation system, in presence of a catalytic amount of *p*-toluenesulfonic acid. No reaction occurred and only starting materials could be recovered.

Synthesis of Indanedione Derivatives

In these reactions, the thiols served as both solvents and reagents. When they were used as reagents only, in toluene as solvent, no reaction occurred even in the presence of a catalyst. 1,2-Indanedione (0.5 g) was dissolved in desired thiol and a few drops of acetic acid were added. The solution was heated to 110°C for 24 h and allowed to cool to RT. The solution was washed with aqueous $NaHCO_3$ and the organic layer was dried (Na_2SO_4) and evaporated to dryness. The crude products were recrystallized or cleaned on a silica column.

The reaction of the new compounds with amino acids (alanine) and with latent fingermarks was compared with that of the unsubstituted reagents, ninhydrin and indanedione, and also with a known *hemi*ketal derivative of ninhydrin, isononyl ninhydrin or *INON* (Fig. 10), a ninhydrin hemiketal which, because of its solubility in petrol ether is operationally used as a fingerprint reagent for thermal paper (16). A sample of INON was kindly provided to us by Ms. Andrea Nobel of the Criminal Identification Unit of the BKA, Wiesbaden, Germany.

The THK and thioketals and their oxygen analogs have been characterized by elemental microanalyses, IR, ^1H NMR, and compounds I and VI (Table 1), also by X-ray crystallography (Fig. 11).

Results and Discussion

The new THK are presented in Table 1. Full synthetic details will appear elsewhere. The following observations have been made:

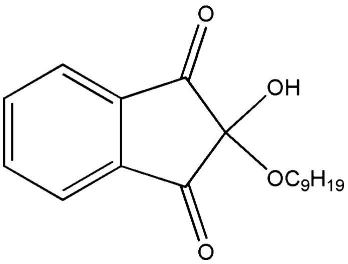
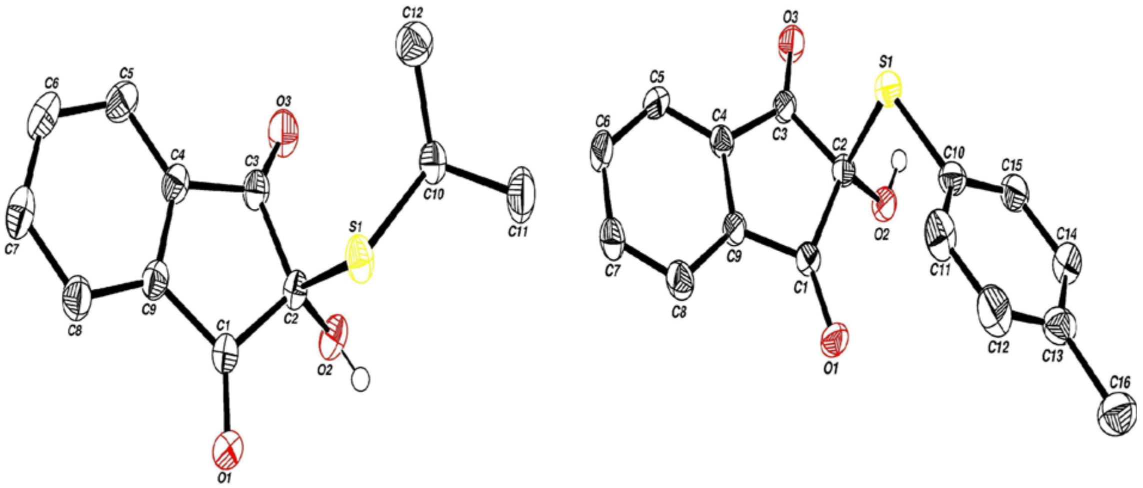


FIG. 10—*Isononyl ninhydrin or INON.*



I

VI

FIG. 11—*Crystallographic elucidation of the structures of ninhydrin thiohemiketals I and VI (Table 1).*

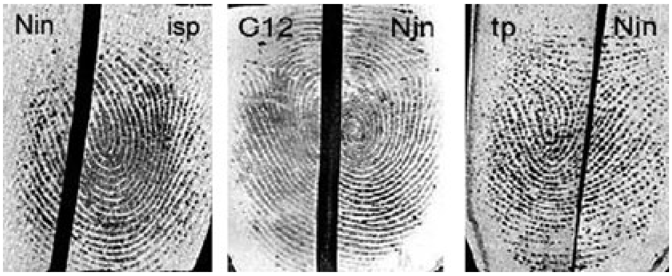


FIG. 12—*Latent fingermarks on A4 paper after development with ninhydrin (halves marked Nin) and ninhydrin THK I (isp), II (C12), and VI (tp).*

- Only ninhydrin THK react with amino acids and with latent fingermarks (Fig 12). The reaction can be followed visually by the formation of Ruhemann's purple. Ketals and thioketals derived from 1,2-indanedione are inactive.
- The reaction of ninhydrin THK with amino acids produces besides Ruhemann's purple also thiols whose presence, which is essential for the next stage of the study, was confirmed by gas chromatography–mass spectrometry analysis of the reaction mixture.
- At RT, long-chained aliphatic THK (compounds II and III; Table 1) react with amino acids in solution at a much slower rate than ninhydrin. The purple color does not reach the intensity of that of ninhydrin and it fades within days. Latent fingermarks treated by compounds II and III develop much slower than with ninhydrin. At RT, maximum intensity of processed prints may be reached after several weeks. The final color though, is of similar intensity to that obtained by ninhydrin.
- Iso-propyl and aromatic THK (compounds I, V, and VI; Table 1) react faster, nearly at the same rate as ninhydrin or INON. The color intensity of the Ruhemann's purple thus obtained is similar to that of ninhydrin and the color fading is much slower than that of aliphatic THK. At RT, latent fingermarks processed with these compounds develop at a similar rate to that of ninhydrin, and with a similar quality.
- In solution, Ruhemann's purple loses its color in the presence of excess thiol. One of the products of this reaction is the respective THK. Purple fingermarks on paper, obtained by treatment with ninhydrin, completely lose their color upon immersion in a dilute solution of thiol. A plausible mechanism for this reaction is suggested in Fig. 13.

It is postulated in the literature that the gold NP are attached to the fingerprint material by electrostatic attraction between the negatively charged gold colloid and the positively charged fingerprint material at low pH (17). Even under optimal conditions (pH ~2.7, particles not larger than a few nm, surfactants), this attraction seems to be rather weak as the preference of the NP to the fingerprint ridges is sometimes not very distinct and the contrast of the final result is often insufficient. The overall goal of this study was to enhance the preference of the gold NP to the fingermark ridges. Since gold NP can be stabilized by organic thiols, through strong covalent bonds, we assumed that if the fingerprint material could contain thiols, it would form with the gold stronger bonds than natural fingerprint material and thus improve their adherence to the ridges and therefore the contrast after the silver precipitation. Organic thiols are actually better stabilizers for gold NP than are the citrate anions, which are commonly used in aqueous solutions (1,5). Naturally occurring amino acids containing thiol groups, such as cysteine, which might serve this purpose, do not appear among the constituents of palmar sweat (18) so we had to devise a way to *selectively* add thiols to the fingerprint material. The chosen approach is based on the reaction between thiol-containing fingermark-reagents and latent fingerprints, in which thiols will be released as a byproduct of the reaction. Hemiketals that are derived from ninhydrin are known to react with amino acids in a similar manner to ninhydrin, forming Ruhemann's purple and two equivalents of alcohol per amino acid (16, Fig. 5). We assumed that THK of ninhydrin or of 1,2-indanedione (Figs. 3 and 4, respectively) would react similarly, but instead of producing two alcohol molecules they should produce two molecules of thiol per molecule of amino acid (Fig. 5). Being formed only on the ridges, the thiols may enhance the attraction between the fingerprint material and the

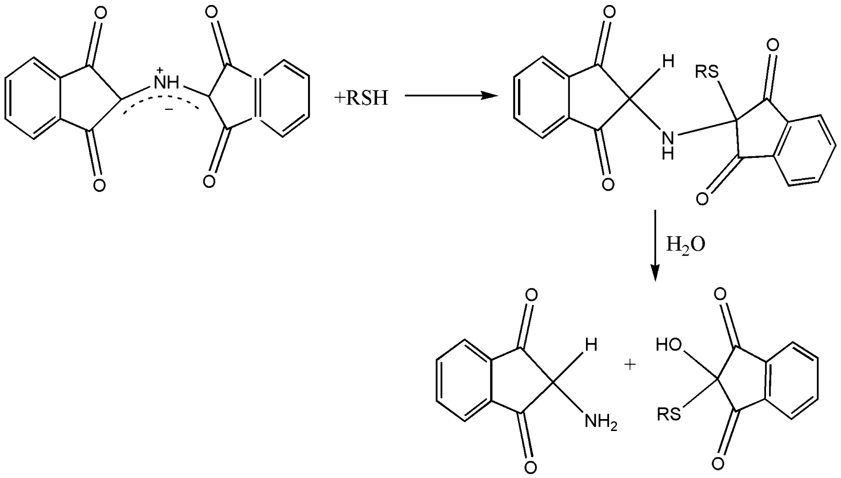


FIG. 13—*Proposed mechanism for the reaction between Ruhemann's purple and thiol.*

gold NP thus improving the contrast and the overall result. It is noteworthy that the THK themselves are not expected to interact with the gold and hence there will be no need to rinse the articles to remove excess reagent. Thus, by not rinsing, the formed thiol, RSH, remains on the fingerprint ridges. Gold NP will be stabilized by thiols only where the latter are formed: at the reaction site with amino acids, namely only on the fingerprint material. We assume that the water-insoluble thiols will not be swept away by the aqueous environment of the gold NP.

There were two consecutive goals to achieve in this study: synthesize the hitherto unknown THK of ninhydrin and indanedione, and examine whether they react with amino acids and with latent fingermarks by forming Ruhemann's purple and releasing thiols. While we were unable to prepare THK of indanedione, we successfully synthesized several THK of ninhydrin and found them quite reactive with amino acids and with latent fingermarks on paper. It is noteworthy that a few years ago, Wilkinson reported the formation of indanedione hemiketal in methanolic solution, but she did not isolate or characterize the pure product (19). In a more recent work, Wallace-Kunkel raised a question whether the hemiketal is actually formed under these conditions (20). (We reported lately our observation that alcohols attack 1,2-indanedione at C2 whereas thiols prefer attacking at C1 [21,22]. We assume that the difference between the two has to do with the nature of the nucleophile: "hard" nucleophiles such as alcohols prefer the "harder" carbonyl with the higher positive charge [C2], while "soft" nucleophiles such as thiols prefer the "softer" carbonyl [23].)

In future research, latent fingermarks that have been developed by ninhydrin THK will be treated by the MMD technique. We assume that even weak fingerprints, which contain only minute amounts of amino acids and do not produce sufficiently strong marks with ninhydrin may be enhanced by this technique. We assume that the contrast of the eventually formed silver prints will be stronger than that achieved by the standard MMD procedure, because of the strong (covalent) bonds that will be formed between the thiols on the ridges and the gold NP.

Conclusions

- Ninhydrin THK constitute a new class of amino acids and latent fingermark reagents.
- Ninhydrin THK develop latent fingermarks on paper in a similar manner to ninhydrin, and with a similar sensitivity.
- A potential advantage of the THK is that their reaction with amino acids is accompanied with the formation of thiols. The latter may contribute to the more selective binding of gold NP to the fingerprint material.

Acknowledgments

The authors thank Dr. Michal Elad, Head of the Latent Fingerprint Lab and Supt. Uri Yaalon, Head of the Photography Lab, DIFS, Israel Police, for their interest and assistance.

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