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1,2-Indanediones: New Reagents for Visualizing the Amino Acid Components of Latent Prints*

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ABSTRACT: A new class of reagents, 1,2-indanediones, was evaluated for latent print visualization on paper. Tests were carried out for fingerprints and amino acid stains. Selected 1,2-indanediones give fluorescent development superior to DFO. Zinc salt treatment further enhances the sensitivity. The new reagents can be obtained from inexpensive starting materials in a short reaction sequence.

KEYWORDS: forensic science, latent print development, 1,2-indanedione, DFO, room-temperature fluorescence, zinc salts

Since the serendipitous discovery of ninhydrin (1) and its reaction with amines and amino acids to afford a colored product (2), this reagent has earned a prominent position in forensic chemistry (3). Many synthetic strategies have been designed for the formation of the strained tricarbonyl ring (4), but few general, high-yielding routes to ninhydrin analogs have been found. In a continuation of our synthetic efforts to develop new approaches to ninhydrin analogs (5–14), we decided on a different oxidation strategy to introduce the third carbonyl group utilizing a 1,2-indanedione as a precursor rather than the commonly used 1,3-indanedione (4). During the course of these investigations, we noted that 6-methylthio-1,2-indanedione developed luminescent fingerprints. Although no colored prints were obtained, the ridges of the print fluoresced under an alternate light source (Volpi model Intralux 6000-1-F, green illumination < 530 nm, observation > 550 nm). This observation led us to examine the parent compound and several substituted 1,2-indanediones. All the compounds investigated (except the 6-nitro and 5-fluoro derivatives) produced fluorescent prints. Secondary treatment of the prints with zinc chloride further intensified the fluorescence. We now present the syntheses of these novel reagents and a preliminary evaluation of their application to latent print visualization.

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The 1,2-indanediones in Fig. 1 (1–10) were obtained in a synthetic sequence outlined in Fig. 2 through acidic hydrolysis of the corresponding 1,2-indanedione-2-oximes (11–20), which in turn can be obtained from 1-indanones, some of which are commercially available.

The 1-indanones (21–30) can also be synthesized from commercially available aldehydes as shown in Fig. 3. While there are many different conditions for the cyclization of 1-indanones and for introduction of the second carbonyl group, we have employed those that are simpler, more affordable and in a number of cases environmentally safe.

Although 1,2-indanediones and their syntheses are well documented in the literature (15,16), 6-methylthio-1,2-indanedione (2), 6-bromo-1,2-indanedione (5), 5-fluoro-1,2-indanedione (8) and the corresponding oximes have not been previously reported. 5-Chloro-1,2-indanedione (6) had also not been reported.

Experimental Procedures

Materials and Methods

All reagents were purchased from Aldrich except for *n*-butyl nitrite, which was synthesized according to a known procedure (17). Reagent-grade methanol and zinc chloride were purchased from Fisher Scientific. DFO was purchased from Lightning Powder. All 1,2-indanediones gave satisfactory analytical data.

Synthesis of 1,2-Indanediones

Generalized Procedure

To a solution of 1-indanone (10 mmol) in dimethoxyethane (8 to 16 mL), depending on the solubility of the indanone, concentrated aqueous HCl was added, so that $V_{\text{HCl}} = 1/3 V_{\text{dimethoxyethane}}$. After the solution was cooled to room temperature, freshly prepared *n*-butyl nitrite (17) (1.2 eq) was added dropwise with stirring to maintain the temperature of the reaction mixture below 30°C. Formation of a creamy white or a light yellow indan-1,2-dione-2-oxime precipitate was usually observed within 10 to 30 min after the *n*-butyl nitrite addition was completed. The reaction mixture was stirred for 4 to 8 h, and the precipitate (1,2-indanedione-2-oxime) was collected by suction filtration, washed with water (3 × 20 mL) and air-dried. The yield was typically 80 to 90%. Additional amounts of product may be obtained by dilution of the mother liquor with water.

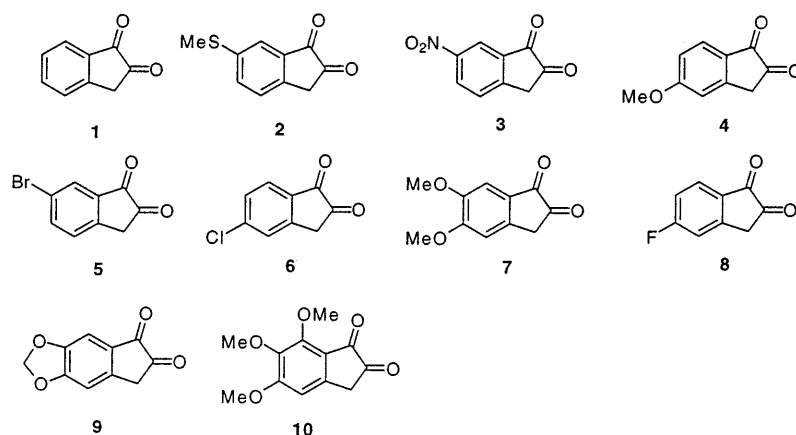


FIG. 1—Chemical structures of 1,2-indanediones.

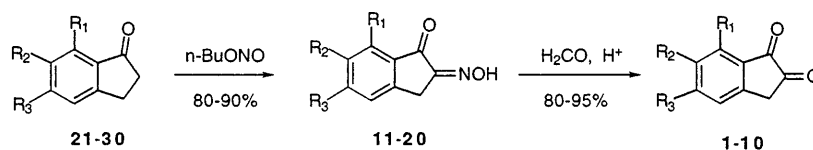


FIG. 2—Synthesis of 1,2-indanediones.

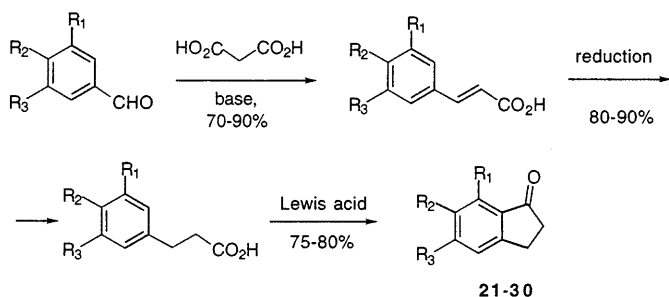


FIG. 3—Synthesis of the precursors for 1,2-indanediones.

The 1,2-indanedione-2-oxime (6 mmol) was suspended in a mixture of 36% aqueous formaldehyde (2 mL) and 37% aqueous HCl (4 mL), with vigorous stirring. Within 5 to 10 min the suspension thickened and the reaction color changed from light to dark yellow. The reaction mixture was allowed to stir for 4 to 8 h, and the precipitate (1,2-indanedione) was then collected by suction filtration, washed with water (3×20 mL) and air dried. The yield was typically 80 to 95%. Additional amounts of product may be obtained by diluting the mother liquor with water.

Application of Reagents

Methanolic solutions of the various 1,2-indanediones were prepared in 0.1% overall concentration by dissolving the 1,2-dione (0.05 g) in methanol (50 mL) and acetic acid (0.5 mL). Zinc chloride (0.25 g) was then added to the solution. In the case of the 5,6-dimethoxy-1,2-indanedione, the reagent was first dissolved in methylene chloride (2 mL) and diluted with methanol (50 mL).

The solutions were then applied with a capillary tube or brush to the paper to be developed. In the case of the amino acid spots, Whatman filter paper was soaked in a 0.25% solution of glycine

in water, and allowed to air dry over several days before application of the reagent. Steaming was effected with a steam iron placed about 1 to 2 cm from the surface for 20 to 30 s. Overexposure of the steaming process (>30 s) led to slight decomposition of the fluorescent product, but this problem was not observed with the solutions containing zinc. The conditions described are discussed in the next section.

In another study, the color and fluorescence data for the substituted 1,2-indanediones were obtained under different conditions (18). The substituted 1,2-indanediones were prepared as 1 mM solutions (1×10^{-3} mole/liter) and acidified with 1% v/v with glacial acetic acid. DFO, as a 1 mM methanolic solution, was acidified with 2% v/v with glacial acetic acid. Methanol solutions of glycine and zinc nitrate were 0.5 mM and 10 mM, respectively. Glycine, 1,2-indanedione reagent, and zinc nitrate were spotted sequentially on Whatman paper. Each spot was allowed to air dry before applying the next reagent. The spots were evaluated using a Hitachi model F-4500 fluorescence spectrophotometer. The results of this study are shown in Table 1.

Results and Discussion

None of the 1,2-indanediones tested so far have developed fingerprints or amino acid spots with the intense color obtained with ninhydrin. The color obtained in most cases was a pale purple to pale pink depending on the analog or concentration of reagent, similar in many respects to the color obtained using DFO. Post-treatment of 1,2-indanedione treated amino acid spots with zinc salts generally changed the color of the spots from pale pink to a dark pink/pale purple color, depending on the concentration of reagent. Considerable enhancement of both color and fluorescence was observed with incorporation of zinc salts in the 1,2-indanedione formulation, the only drawback appearing to be a much reduced shelf life of the reagent, at least with the formulation used. Treat-

TABLE 1—Summary of fluorescence data for DFO and substituted 1,2-indanediones.*†

Reagents	$\lambda_{\text{excitation}}$	$\lambda_{\text{emission}}$	Intensity‡	Color§(initial)
DFO	565	580	59	pink
1	545	560	28	lt. pink
2	555	570	34	lt. orange
3	545	560	11	tan
4	550	565	73	lt. pink
5	550	560	77	lt. pink
6	550	565	65	lt. pink
7	560	575	139	pink
8	545	560	11	purple

* Taken in part from Ref 18.

† The substrate, Whatman filterpaper had a fluorescence intensity of approximately 13.

‡ Intensity refers to luminescence observed from zinc-treated glycine spots measured in arbitrary units. All samples, except DFO, were developed using a steam iron. DFO was developed with a dry iron only.

§ Color refers to the initial color observed prior to treatment with zinc nitrate.

ment with zinc salts also had the added benefit of increasing the longevity of the prints. This phenomenon was also observed with zinc-treated ninhydrin developed amino acids by a number of researchers (19,20). 1,2-Indanedione developed prints without zinc generally decomposed rapidly over a few days, losing both color and fluorescence. This effect was also observed with the zinc-treated prints, although decomposition generally took a number of weeks or months.

Solubility of the various 1,2-indanediones was generally higher than DFO. A 0.1 to 0.2% solution (more concentrated than those used for DFO) was found to offer a stable working solution. This high concentration produces a darker print color without background development. A small amount of acetic acid (1%) in the formulation improved the reaction on paper.

The development conditions giving best results were similar to those used for ninhydrin. For all of the diones examined, good results were obtained after steaming the treated spots (or prints) with a steam iron for 20 to 30 s. Air-dried prints generally took 4 to 8 h for full development and were generally less intense, in terms of both color and fluorescence, than those obtained by steaming.

Various experiments were performed to determine the practical applicability of the new potential reagents toward latent print detection. Results indicated that with the exceptions of the 5,6-dimethoxy (which gave a significantly higher fluorescence) and the 5-fluoro- and 6-nitro (which produced no fluorescent compounds), some of the 1,2-indanediones developed latent prints and amino acid spots on low background paper with comparable intensity to DFO developed prints and spots.

The best results were obtained from prints or spots developed with the 5,6-dimethoxy indanedione and treated with zinc salts. Before treatment with zinc salts, the fluorescence obtained was marginally better than DFO in terms of intensity. Post-treatment with zinc dramatically increased the intensity of the fluorescence, surpassing that of DFO at room temperature. This observation was emphasized with the experiments that examined the detection limits of each compound. Preliminary indications show that 5,6-dimethoxy 1,2-indanedione appears to have a slightly better detection limit than DFO. These results suggest that this reagent might reveal weak latent prints that were previously undetectable with either ninhydrin or DFO using similar fluorescence methods.

Conclusions

We have synthesized and evaluated a series of substituted 1,2-indanediones. Although many of these compounds were previously described in the literature, no reaction with amino acids was mentioned and no applications of 1,2-indanediones for latent print development were reported. Preliminary testing of the various 1,2-indanediones shows that they have considerable potential as latent print visualization reagents. While they produce a color less intense than ninhydrin, and in many cases more intense than DFO treated prints, the fluorescence observed at room temperature has been found in many cases to be superior to DFO. The conditions necessary for development of latent prints are very similar to those employed for ninhydrin and require only a steam iron. Viewing of the fluorescent products was achieved using equipment and techniques similar to those already employed for visualization of DFO and some ninhydrin treated prints. No cooling in liquid nitrogen was required for optimal fluorescence.

Economically the reagents will be less expensive to produce than DFO and, as they can be used in higher concentrations (0.1–0.2%) and in less harsh solvent formulations than used with DFO, they may compete with DFO in the visualization of latent prints.

Currently the mode of action and exact nature of the fluorescent products from the reaction with amino acids are unknown. Studies are underway to investigate the products of the reaction in an attempt to synthesize compounds that give a darker initial color. In addition, spot test and fingerprint studies are underway that involve mixing the 1,2-indanediones with both ninhydrin and zinc salts in an effort to produce a reaction product that maintains high fluorescence intensity and a darker color.

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