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Aminoninhydrins: Fingerprint Reagents with Direct Fluorogenic Activity—Preliminary Studies

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ABSTRACT: Four hitherto unknown amino derivatives of ninhydrin have been prepared and examined as potential fingerprint reagents. All gave intensive colors upon reaction with amino acids in solution and developed latent fingerprints on paper in a manner similar to ninhydrin. The 5-substituted derivatives, 5-aminoninhydrin and 5-dimethylaminoninhydrin, also exhibited direct fluorogenic activity with amino acids and with latent fingerprints. Addition of Group IIb metal salts had no marked effect on the fluorogenic process.

KEYWORDS: criminalistics, fingerprints, ninhydrins

Since the discovery of the excellent properties of 5-methoxyninhydrin (I) as a fluorogenic reagent for the visualization of latent fingerprints [*1–4*], it has been clear that one of the next candidates for preparation and study as a potential reagent should be 5-dimethylaminoninhydrin (II). In their 1988 paper on ninhydrin analogues as potential fingerprint reagents [*2*], Prof. R. Warrener and his co-workers challenged forensic scientists to synthesize two new compounds that relate to ninhydrin (III). These were naphthoninhydrin (IV) and 5-dimethylaminoninhydrin (5-DMAN, II). Although the authors of this paper have been unable, to date, to prepare the former, we were more successful in synthesizing the latter (5-DMAN, II), its positional isomer, 4-dimethylaminoninhydrin (4-DMAN, V), and the two primary aminoninhydrins, 5-aminoninhydrin (5-AN, VI) and 4-aminoninhydrin (4-AN, VII). We wish to report the first synthesis of ninhydrin derivatives bearing amino groups on the aromatic ring and the reactivity of the new compounds with amino acids in solution and with latent fingerprints on paper.

Materials and Methods

Synthesis of Aminoninhydrins

The preparation of the new ninhydrin derivatives is illustrated in Fig. 1. Nitration of 1-indanone (VIII), as described by Semple [*5*], gives a mixture of 4- and 6-nitroindan-1-

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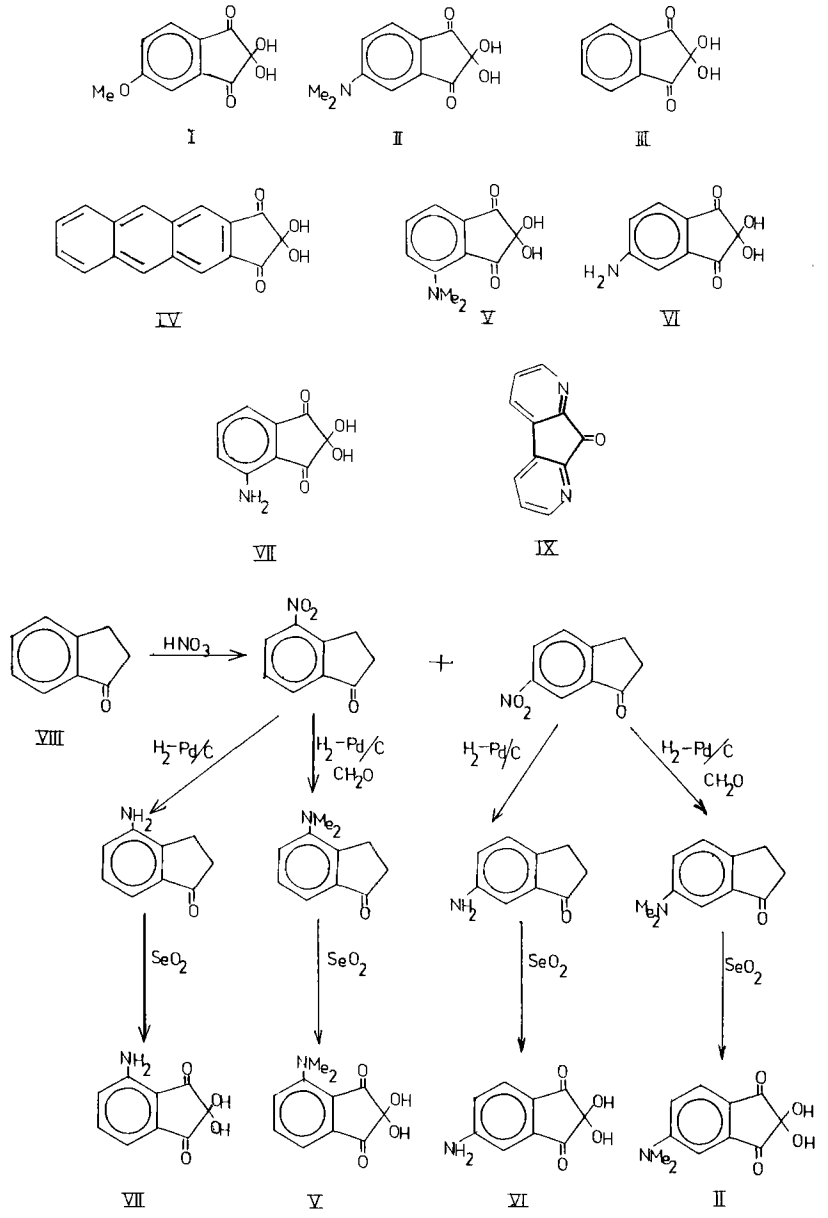


FIG. 1—*A scheme of the synthetic route to aminoninhydrins.*

ones, which were resolved by flash column chromatography [6].[3] Reduction of each over 10% palladium/carbon using aqueous sodium hypophosphite as a hydrogen source gave the corresponding amines. When the reduction was accomplished in the presence of formaldehyde, the corresponding dimethylamino derivatives were isolated [5,7].

[3]Flash column chromatography is a purification technique which differs from classical column chromatography in the use of smaller particle-size silica and low air or nitrogen pressure. This facilitates rapid flow of solvent through the column [6].

Oxidation of each of the aminoindanones to the corresponding ninhydrins was accomplished with selenium dioxide in dioxane at room temperature. Each ninhydrin derivative was isolated by flash column chromatography, followed by preparative layer chromatography. For the full details of synthesis, see Ref *8*.

Spectral Data

Absorption spectra in solution were recorded on a GBC ultraviolet/vis 911 spectrophotometer (GBC Scientific Equipment, PTY Ltd., Victoria, Australia). Two light sources were used for luminescence excitation: the Quaser 30 xenon arc (Mason-Vactron, London), emitting light between 375 and 585 nm and Omniprint 1000 indium vapor arc (Omnichrome, California), emitting between 400 and 570 nm.

Fingerprint Samples

Latent fingerprint samples of various individuals were collected on groundwood-free white paper. Prior to development, the samples were kept in the dark, at room temperature, for periods ranging from one day to two years.

Reaction with Alanine

Alanine was chosen as a model for amino acids. In a test tube, a few milligrams of the substituted ninhydrin (II and V–VII), were dissolved in ethanol containing 1% glacial acetic acid, alanine was added in slight excess, and the solution was boiled on a steam bath until the color remained unchanged (2 to 5 min). The solution was cooled to room temperature and diluted with ethanol, and the visible spectrum was recorded.

Reaction with Latent Fingerprints on Paper

Ethanolic solutions, 0.1% of the substituted ninhydrins, containing 1% glacial acetic acid, were applied to the latent prints by gently swabbing the paper with a cotton-wool swab soaked in the solution. The prints were allowed to develop at room temperature, in the dark.

Results

All four aminoninhydrins reacted with alanine to give colored products (Table 1). All four compounds developed latent fingerprints on paper (the marks developed with 4-aminoninhydrin were very weak).

Two of the new compounds, 5-dimethylaminoninhydrin (5-DMAN, II) and, particularly, 5-aminoninhydrin (5-AN, VI), exhibited direct fluorogenic activity with latent fingerprints, yielding luminescent images when excited with appropriate light sources such as an argon ion laser or a filtered xenon arc lamp. Treatment with zinc or cadmium salts had little effect on the color of the prints and did not significantly enhance the fluorescence, not even after the samples had been cooled by liquid nitrogen.

The colors of the reaction products of the 4-aminoninhydrin derivatives with alanine as well as the colors of the developed prints were quite different from those obtained with most other ninhydrins [*2,9*], as were the absorption spectra in solution (Table 1). Smaller differences were observed in the corresponding color and spectra of the 5-aminoninhydrin derivatives.

Full-color formation following the treatment of latent fingerprints with these aminoninhydrins took about seven days at room temperature. In contrast, identifiable prints

TABLE 1—*Solution spectral data: colors and absorption maxima before and after metal salt treatment.*

Compound	Product with Alanine, Color and λmax	Color and λmax After Treatment with		
		Zinc Chloride	Cadmium Iodide	Mercury Bromide
5-DMAN (II)	blue, 335, 423, 582	purple, 341, 561	purple, 340, 566	blue, 340, 593
4-DMAN (IV)	yellow, 335, 401, 571[a]	pink, 328, 398, 531[a]	pink, 332, 400, 531[a]	yellow, 330, 401, 543[a]
5-AN (V)	blue, 329, 407,[b] 565	pink, 328, 542	pink, 329, 546	purple, 327, 558
4-AN (VI)	brown-purple, 386, 570	orange, 384, 507	pink-orange, 383, 513	pink, 380, 533
Ninhydrin (II)[c]	blue, 408, 588	yellow, 486	pink, 410, 502	pink-purple, 415, 520
5-Methoxyninhydrin (I)[c]	blue, 407, 588	orange, 497	pink, 407, 507	purple, 414, 531

[a]A very low peak.
[b]Shoulder.
[c]Data are from this work. Slightly different data have been reported by Warrener and coworkers [*2*].

could be observed with ninhydrin (III) after one day and with 5-methoxyninhydrin (I) after two to three days under the same conditions. Color formation after room temperature reaction with alanine in solution was also much slower for the aminoninhydrins relative to ninhydrin or 5-methoxyninhydrin. Of the aminoninhydrins, 4-aminoninhydrin (VII) was the slowest to react.

Discussion

There were a number of reasons to make and study aminoninhydrins as potential reagents for amino acids and for latent fingerprints. The dimethylamino group, $-NMe_2$, and the amino group, $-NH_2$, contain unshared pairs of electrons and hence are among the strongest electron-donating groups in organic compounds [*10,11*]. After the discovery that 5-methoxyninhydrin (I), a ninhydrin derivative with a good electron-donating substituent (the methoxy group), showed certain advantages over ninhydrin as a superior fingerprint reagent [*1–4*], it became tempting to try and prepare ninhydrin derivatives with even stronger electron donors, such as amino and dimethylamino groups.

These groups should also facilitate certain chemical manipulations, such as salt formation, quaternization, solubility in dilute acids, and conversion to other derivatives (for example, via diazonium salts). These factors, along with chemical curiosity, have brought us to develop a synthetic pathway for aminoninhydrins. After numerous attempts to prepare these compounds by ring-closure of substituted phthalic acid derivatives (Fig. 2) had failed, a quite successful route starting with compounds already containing the 5-membered ring was tried. We found that the suitable amino-1-indanones can be oxidized to the corresponding ninhydrins by selenium dioxide in dioxane (Fig. 1). The aminoindanones themselves were obtained by reduction of the corresponding nitroindanones [*5,7*].

Perhaps the most interesting feature of aminoninhydrins is their direct fluorogenic reaction with amino acids. It is well known that dialkylamino groups enhance fluorescence efficiency [*12*], but we feel that, at this point, it is too early to speculate on the exact reasons for the direct fluorogenic reaction of II and VI with amino acids. In this respect, 5-amino- and 5-dimethylaminoninhydrin resemble the newly reported fluorogenic fingerprint reagent, diazafluorenone (DFO) (IX), which has a chemical structure analogous to that of ninhydrin [*13*].

The increased electron-donating nature of the 5-amino derivatives does not seem to have a great effect on the color or spectra of the reaction products with alanine. In contrast, the relatively large spectral shifts observed with the 4-amino derivatives may be due to steric interference to the molecular planarity [*9*]. Also, the possibility of a slightly different reaction mechanism cannot be ruled out [*14*].[4]

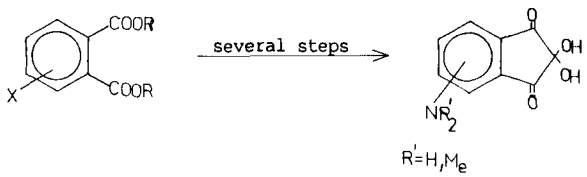
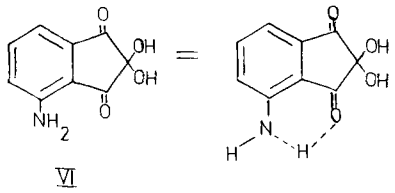


FIG. 2—*A schematic approach that failed, for the preparation of aminoninhydrins from phthalate precursors. The substituents X were amino groups or potential precursors for amino groups such as chloro or nitro.*

[4]Professor Ronald Grigg and his co-workers have recently shown that phenalene trione, a ninhydrin analogue, reacts with amino acids via a different mechanism than ninhydrin, despite the formal similarity of the two reagents [*14*].

These aminoninhydrins also differ from other ninhydrins in the rate of color formation after reaction with amino acids (or latent fingerprints). The slower observed rate could be due to a reduction in the electron deficiency and, hence, reactivity of the ninhydrin 2-carbonyl group because of the electron-donating effect of the amino substituent. This effect is further accentuated in the case of 4-aminoninhydrin (VII), as the possibility of



VI

FIG. 3—*A possible electron delocalization via hydrogen bonding in 4-aminoninhydrin.*

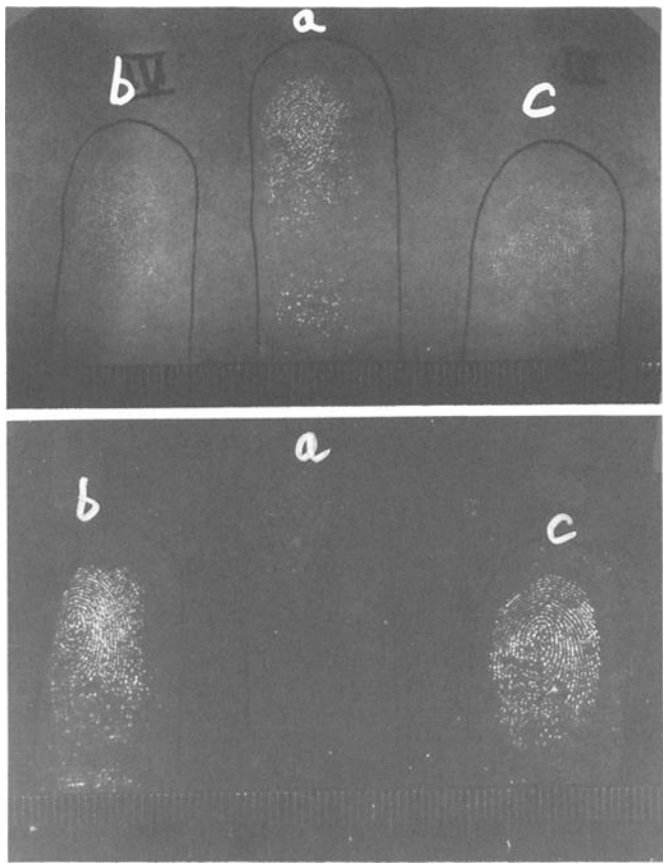


FIG. 4—*The potential of 5-aminoninhydrin (VI) as a fluorogenic reagent on surfaces with high background luminescence: Visualization of two-year-old prints on white paper by 5-methoxyninhydrin (I), followed by zinc chloride* (Finger a), *and by 5-aminoninhydrin (VI) without metal salt treatment* (Fingers b and c). (Top) *Optimal conditions for 5-methoxyninhydrin visualization—illumination by Omniprint, under 525 nm; observation through an orange filter* (cutoff at 549 nm); (bottom) *same fingers, illuminated around 570 nm; observation through a red filter (cutoff at 593 nm).*

hydrogen bonding with the 1-carbonyl leads to even greater electron delocalization (Fig. 3).

The direct fluorogenic effect of 5-aminoninhydrins (II and VI) on latent fingerprints and, particularly, the spectral region where it occurs (excitation around 570 nm and emission over 590 nm) suggests that the new compounds are potentially useful fingerprint reagents for highly fluorescent background surfaces (surfaces that absorb in the region between 400 and 500 nm and emit in the 550 to 650-nm range [*2*]), which precludes the use of ninhydrin or even 5-methoxyninhydrin (I). The red-shifted characteristics of prints developed with 5-aminoninhydrin (VI) may alleviate background luminescence problems, as demonstrated in Fig. 4.

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