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Genipin, a Novel Fingerprint Reagent With Colorimetric and Fluorogenic Activity, Part II: Optimization, Scope and Limitations

ABSTRACT: Genipin, a hydrolytic product of geniposide extracted from gardenia fruit, was thoroughly studied as a potential fingerprint reagent, and optimal conditions for fingerprint development have been determined. Latent fingerprints on paper items that have been treated with a non-ink running formulation containing 0.17% of the reagent, showed up as both colored and fluorescent images. On brown wrapping paper and on papers with highly luminescent backgrounds, genipin developed more visible and clearer prints than did classical reagents such as ninhydrin or DFO. Another potential advantage of genipin is that it is totally harmless and an environmentally friendly reagent.

KEYWORDS: forensic science, fingerprint reagent, genipin, amino acid reagent, ninhydrin, DFO, 1,2-indanedione, fluorogenic, colorimetric

In a previous article we described the potential of genipin, a natural product produced from the extract of Gardenia fruit, as a fingerprint reagent for paper items (1). Fingerprints developed with genipin appear as blue impressions, which fluoresce upon illumination with ca. 590 nm light. We wish to report here the search for optimal development conditions with genipin, in regard to solvents, concentration, temperature, humidity, and warming regime. Other factors that have been studied are the possibility of sequential development with DFO and with ninhydrin, the performance comparison with these two reagents, and the generation of spectrofluorimetric data of genipin's products with several amino acids. We identified circumstances in which genipin could be advantageous over DFO and ninhydrin; for instance, on brown wrapping paper, or on documents written with fluorescent ink.

Materials and Methods

Genipin was purchased from Challenge Bioproducts Co., LTD (7 Alley 25, Lane 63, Tzu-Chiang St. 404 Taichung, Taiwan). Optimal procedures for ninhydrin (2) and DFO (0.025% solution in CFC113 containing methanol and acetic acid) were always used for comparison. After optimizing the working conditions with genipin, all further experiments were carried out under these conditions. Natural fingerprints from six donors (two males and four females) were deposited on different types of paper, as described below.

Solvents and Concentration

The following solvents were tried as initial solvents for genipin: methanol, ethanol, isopropanol, acetone, diethyl ether, ethyl acetate, acetonitrile, methyl ethyl ketone, dichloromethane and petrol ether. The effect of acetic acid on the genipin reaction was also tested. Optimum concentration of genipin was determined using ethanolic solutions of 5×10^{-2} to 5×10^{-4} M on serially depleted prints. HFE 7100 solvent (3M, UK) and petrol ether were used for dilution of the concentrated solution. Final composition of the genipin solution was determined taking into consideration solution stability, color and fluorescence intensity, paper background and ink spreading.

The pH Effect

Alanine solution (5×10^{-3} M, in 1:1 ethanol:water) and genipin solution (5×10^{-3} M in ethanol) were prepared at different pH (4.8; 5.5; 6.8; 8.5; 9.5) using acetic acid and triethylamine for pH modification, except for 6.8, the natural pH of genipin solution. Drops were pipetted on 5 types of paper: copier paper, newspaper, brown paper, notebook and white envelope and the color change was followed. In addition, solutions containing alanine and genipin were prepared at two extreme pH's: 1 (using hydrochloric acid) and 14 (using sodium hydroxide). The solutions were kept in the dark, at room temperature and the color change was followed.

Development Conditions

Paper strips of 3 different types (copier paper, notebook and brown paper) bearing alanine spots (2.5×10^{-3} M in 1:1 water:ethanol) were developed with genipin [working solution, (see Results), $0.17\% = 7 \times 10^{-3}$ M)]. The strips were processed in a humidity-controlled oven for various periods of time, at temperatures ranging from 60 to 90°C and relative humidity from 70 to 90%. Color intensity was compared by visual observation. Contrast and intensity curves were recorded with Mexameter MX 16® (3,4). Latent fingerprints on copier paper strips were also developed using genipin and processed with steam iron at 3 heat levels, and the color development was followed.

Fluorescence Observation

Fluorescence curves of the products of genipin with several amino acids were recorded with a Cary Eclipse Fluorescence

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Spectrophotometer (5). For solution measurements, equal volumes of ethanolic genipin and aqueous amino acids (2.5×10^{-3} M each) were mixed together. The solutions were heated to 40°C and maintained at that temperature for 12 h. Solutions fluorescence was recorded under excitation at 590 nm.

Latent fingerprints developed with genipin were observed using Polilight® PL 500 lamp (6) as an excitation source, using the 590 nm band. Various cut-off observation filters have been tried. Observation was also studied with the Video Spectral Comparator VSC-1® (7), with Polilight® as an external light source.

Reaction with Compounds Containing Amino Groups other than Amino Acids

Genipin in ethanolic solution (5×10^{-3} M) was allowed to react in a test tube with aqueous solutions of ammonia, benzylamine and urea (10^{-3} M), all at 1:1 ratio. The test tubes were kept in the dark at room temperature and the color change was followed.

Comparison with Ninhydrin and DFO

Two sets of experiments have been carried out, reaction with amino acid stains and with latent fingerprints. Drops of alanine solutions at decreasing concentrations (from 5×10^{-1} to 9.8×10^{-7} M) were pipetted on copier paper strips and dried in air. The stains were developed with ninhydrin, DFO and genipin until no noticeable reaction, color or fluorescence could be observed.

Six individuals, 2 males and 4 females, as mentioned above, deposited their latent fingerprints on strips of paper, including various types of brown, colored and fluorescent paper. The strips were processed for latent fingerprints by the three reagents mentioned above. Prints' quality was compared in both modes, color and fluorescence and were assigned a score between 0 and 3, according to the quality of the prints: (0) no ridge details could be observed, (1) few ridge details were observed, (2) sufficient ridge details were observed to allow identification (minimum 9 points), (3) very good fingerprint quality (AFIS). For each reagent, the total number of identifiable prints (ranking 2 and 3) was counted and the average quality of the prints was calculated.

Sequential Development

Copier papers bearing latent fingerprints were developed sequentially by the following pairs: genipin following ninhydrin and *vice-versa*; genipin following DFO and *vice versa*; and the color changes were followed. The possibility of a color reaction between genipin and DFO without amino acid was also considered: two solutions, aqueous and organic (ethanol) containing 1:1 mixture of genipin:DFO (2.5×10^{-3} M) were allowed to react at room temperature in the dark and the color change was followed.

Results

Optimal Formulation and Development Conditions—Genipin dissolves in most of the solvents on the list except for the least polar: petrol ether, diethyl ether and dichloromethane. Optimal working solution was found to contain 0.17% of genipin. There is no need for higher concentration, as there is no further gain in sensitivity. Addition of acetic acid did not improve the sensitivity and caused yellow background discoloration. An optimal non ink-removing solution is prepared as follows: genipin (1.71 g) is dissolved in ethyl alcohol (57 mL) and ethyl acetate (86 mL) is added. The solution is diluted to 1000 mL with HFE 7100 solvent. If flame hazard is of no great concern, the latter can be replaced with the much less expensive petrol ether. The solution is stable at room temperature in the dark for at least one month. This solution has a pH 6.8. Under strong alkaline conditions (pH 14) yellow color develops in the solution (after 24 H it turned to brown-wine), while under strong acidic conditions (pH 1), no color change is observed. Large deviations from neutral pH to both sides reduce the sensitivity. In general, no significant difference was observed between the various types of paper, except for newspaper. In the latter, weak color and fluorescence were observed at basic pH only.

Optimal development conditions were found to be as follows: The paper exhibit is immersed for a few seconds in the working solution, dried in air and processed for 15 min in a humidity controlled oven at 75–85°C and 80% relative humidity. Under these conditions best clarity and contrast are obtained in both, color and fluorescence modes. Color is best observed and photographed under white light. Optimal conditions for fluorescence observation are illumination at 590 nm and viewing through a 620 nm cut-off filter (Kodak gelatin red filter No. 92).

The color and fluorescence of genipin-developed prints were stable for at least 6 months. Processing the treated prints with steam iron instead of humidity oven gave much poorer results.

Reaction with Compounds that Contain Amino Groups other than Amino Acids—No color change was observed between genipin and urea during a two-week period. A blue color developed between ammonia and genipin within a few hours, and between benzylamine and genipin after several days.

Comparison with Ninhydrin and DFO—In the color mode, the sensitivity of genipin with alanine spots on copier paper was slightly lower (5×10^{-4} M) than that of ninhydrin (1.25×10^{-4} M). In the fluorescence mode, the sensitivity of DFO with alanine spots was higher (7.8×10^{-6} M) than that of genipin (1.25×10^{-4} M).

In the emission spectra in solution, large variations, up to 42 nm, in λ_{max} were noticed between the products of various amino acids with genipin (Fig. 1).

On half prints developed with genipin and ninhydrin (Fig. 2), in the color mode, no significant difference could be noticed. In the fluorescence mode however, DFO is more sensitive than genipin. Prints developed with DFO fluoresce more intensely than genipin prints. On some types of paper, however, genipin shows clear advantage over DFO. For instance, papers with strong background fluorescence or documents written with ink that contains fluorescent ingredients emitting between 500 and 600 nm (Fig. 3).

The average quality of the developed fingerprints as well as the total number of identifiable fingerprints on the various types of brown, colored and fluorescent paper, using ninhydrin, DFO and genipin (in both modes) is shown in Table 1. The best results were observed with genipin in the fluorescence mode.

Sequential Development—In neither of the sequences tried were new prints produced by the subsequent application of a second

TABLE 1—*Total number of identifiable prints and the average quality of the prints developed on different types of brown, colored and fluorescent papers, using ninhydrin, DFO and genipin.*

	Ninhydrin	DFO	Genipin Color Mode	Genipin Fluorescence Mode
# Identifiable prints	21	15	20	23
Average quality	2.29	1.36	2.11	2.43

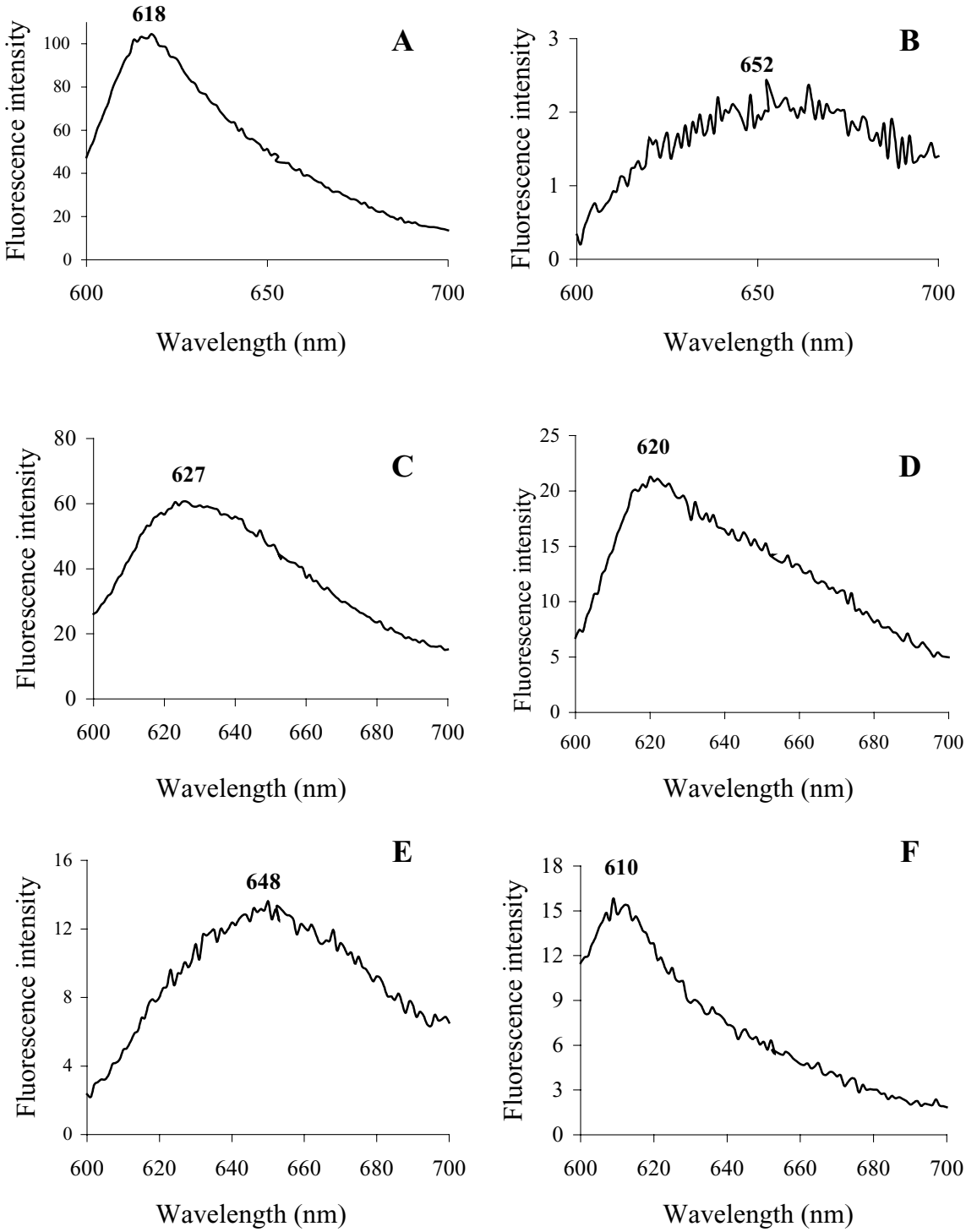

FIG. 1—*Emission spectra in solution of the reaction products between genipin and amino acids (excitation at 590 nm): (A) threonine, (B) alanine, (C) tryptophane, (D) serine, (E) isoleucine, (F) proline. The fluorescence intensities shown reflect the sensitivity of genipin toward the amino acids tried.*

reagent (genipin after ninhydrin and genipin after DFO (and vice-versa). No cross-reaction has been observed between DFO (which is an aromatic amine), and genipin.

Discussion

The reaction between genipin and amino acids is intriguing from both, chemical and forensic standpoints. The exact course of the reaction between genipin and latent fingerprint deposit is still unknown. One observation that emerges from our experiments is that the quality of genipin-developed prints is donor-dependent, very similarly to ninhydrin prints. Good "ninhydrin-donors" are also good "genipin-donors" and vice versa. Also, similarly to ninhydrin prints, no difference in quality could be observed between a few months old and fresh latents.

Spectrophotometric data reported earlier (9), as well as the fluorometric curves of this study (Fig. 1) however, indicate that the nature of the genipin reaction is different from that of ninhydrin.

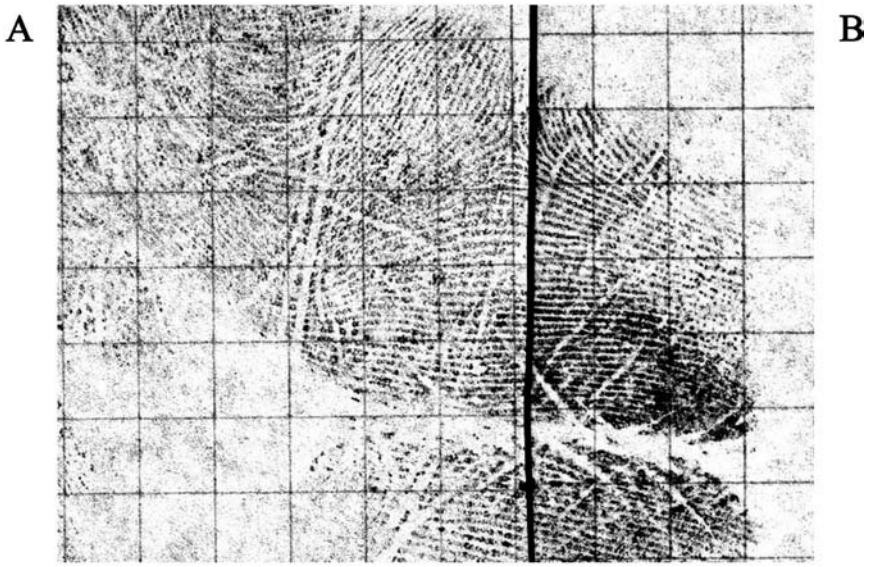

FIG. 2—*Half palm prints developed on a notebook paper with (A) genipin and (B) ninhydrin and recorded under white light.*

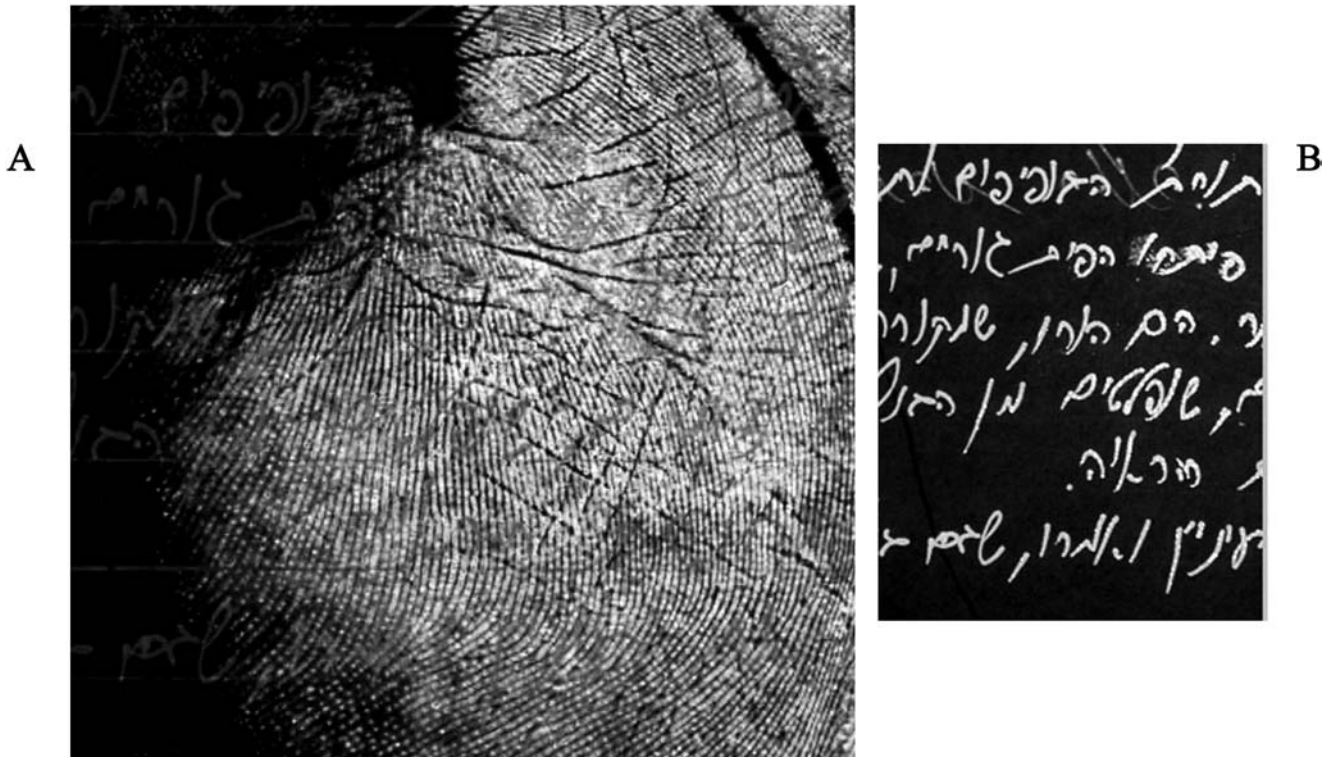

FIG. 3—*(A) palm print developed with genipin on a document written with ink containing fluorescent ingredients and recorded under fluorescence conditions (excitation at 590 nm, cut-off Kodak red filter No. 92); (B) same document observed at 505–530 nm (DFO conditions).*

Unlike the reaction of ninhydrin with amino acids, which produces virtually the same product—Ruhemann's purple—for all amino acids, the genipin reaction produces a slightly different product for each amino acid. In this respect it resembles 1,2-indanedione (8) more than ninhydrin or DFO. It seems that the individual residue, R, which is unique to each amino acid, $RCH(NH_2)COOH$, is still present in the blue products and hence, genipin is more sensitive to some amino acids, e.g., tryptophane, than to others, depending on their R (Fig. 1). An analytical study of the chemical reaction is underway. Urea, which is one of the nitrogeneous components of palmar sweat, apparently does not react in the same fashion as amino acids, but ammonia does.

As for the forensic aspect, the genipin project is a part of a comprehensive search for novel fingerprint techniques. There are several reasons for the constant search for new fingerprint reagents and formulations. Of particular importance are the following five:

a. Insufficient sensitivity. Many latent prints still escape detection: in a comprehensive experiment carried out by DIFS using DFO followed by ninhydrin, on used bank cheques, all of which must bear latent fingerprints, only 21% yielded identifiable prints.

b. Problematic surfaces. It is hard or nearly impossible to visualize latent prints on surfaces such as human skin, spent cartridge cases, fabrics, and certain types of paper.
c. Speed and simplicity. Fieldwork requires in most cases quick and simple visualization processes.
d. Environmental and safety considerations.
e. Cost.

There is no single reagent that meets with all these requirements. On the contrary, the multitude of fingerprint reagents indicates that no one is perfect. This statement applies also to genipin. Besides disadvantages such as cost, availability and lack of experience, it does show three potential advantages over the more traditional reagents, ninhydrin and DFO:

a. The combination of color and fluorescence in a single reaction. This would allow a two-stage fingerprint visualization, color at the field-unit level, and fluorescence, in a more sophisticated laboratory. Viewing the fluorescence does not require a secondary treatment.
b. The longer excitation and emission wavelength of genipin compared to DFO. This may improve the signal-to-noise ratio on papers that show a natural fluorescence in the 500–600 nm domain, e.g., brown wrapping paper, or documents written with inks containing fluorescent ingredients. On such papers, the performance of DFO is unsatisfactory.
c. Safety. The experience acquired with genipin as a food colorant and natural medication (9), and more recently as a building material for tissue engineering (10) supports its use as a safe, environmentally friendly fingerprint reagent. This is particularly important in view of the newly reported data on ninhydrin's potential health hazards (11,12).

We assume that since genipin is being extensively tried also for medicinal purposes—as a wound-healing accelerant (13,14)—it will become much more available and at a lower cost in the not-too-far future. It is also possible that along with better understanding of the chemical nature of the reaction between genipin and amino acids, it will be possible to design even better reagents of similar structure.

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