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# Genipin—A Novel Fingerprint Reagent with Colorimetric and Fluorogenic Activity

**ABSTRACT:** Genipin, the hydrolytic product of geniposide, which is extracted from gardenia fruit, shows good potential as a fingerprint reagent. It develops latent fingerprints on paper as blue impressions with good contrast and resolution. Even very faint impressions that are barely visible in ambient light will fluoresce brightly upon illumination at ca. 590 nm and are best viewed with a barrier filter above 630 nm. Potential advantages of genipin are the combination of colorimetric and fluorogenic activity in one reagent as well as its being a safe and environmentally friendly natural product.

**KEYWORDS:** forensic science, fingerprint reagent, genipin, amino acid reagent, fluorogenic, colorimetric

In the early 1960s, Djerassy and his co-workers determined the structure of a natural product, $C_{11}H_{14}O_5$, which they named Genipin (Fig. 1). They described it as follows: "Genipin itself is colorless, but if brought in contact with the skin, it rapidly produces an indelible bluish-violet color" and later, "... in fact, this reaction also occurs very readily with amino acids" (1,2). A total synthesis of genipin was accomplished by Büchi's group in 1967 (3). In a recent article, Hahn and his co-workers evaluated the use of genipin for the development of amino acid stains on TLC plates and compared it with the performance of ninhydrin. The products of most amino acids with genipin had higher molecular adsorptivities than Ruhemann's purple. The genipin products were also more stable and the stains did not fade following the addition of certain metal salts (4). The structure of the blue pigments is still under investigation. It is clear though, that a number of colored compounds of polymeric nature are formed in this reaction (4–6).

Genipin's properties, which are described above, render it an obvious candidate as a fingerprint visualization reagent. We wish to report here the initial results of our study of genipin as a potential reagent for latent fingerprints, some 44 years after Djerassy reported its reaction with amino acids.

## Materials and Methods

Genipin was prepared by β-glucosidase hydrolysis of geniposide, which was extracted from the fruit of Gardenia jasminoides (4). Sequential natural fingerprints ranging from strong to weak ("depletion" prints), from two donors were deposited on strips of white, ground-wood free writing paper (copier paper). Each set of depleted prints was obtained by successive impressions of the same finger. Paper samples bearing one-day-old latent fingerprints were dipped for a few seconds in genipin solutions in HFE 7100 solvent

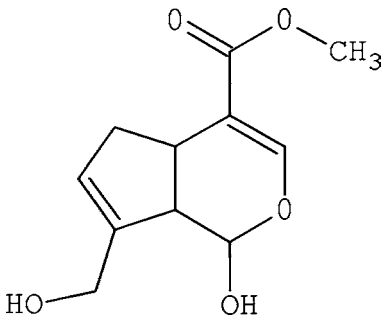


FIG. 1—*Genipin.*

(3M, UK), at three concentrations $10^{-4}$, $10^{-3}$ and $10^{-2}$ M, and developed for 10 min in a humidity chamber at 80°C, and 65% relative humidity, the optimal conditions for the ninhydrin method (7). The fingerprint impressions were examined under white light and also under fluorescence conditions with a Video Spectral Comparator VSC-1® (Foster & Freeman, UK, 8) using a Polilight® L-500 (Rofin, Australia, 9) as an external light source, between 350 and 590 nm.

Emission spectra in solution and on paper were recorded with a Perkin Elmer LS-50 Luminescence Spectrometer. For solution measurements, genipin and alanine in a 1:1 ratio were dissolved together in a 1:1 ethanol:water mixture. The solution was heated to 70°C and maintained at that temperature until the deep blue color remained unchanged. A few drops of the reaction mixture were diluted with water for spectra measurements in solution. No attempt was made to optimize the results at this stage.

## Results and Discussion

The latent fingerprints treated with genipin developed as blue impressions with clear ridge detail (Fig. 2). Some of the depleted prints were too weak to be recorded under white light illumination. They could, however, be readily observed and recorded under fluorescence conditions: illumination at 590 nm and viewing with a barrier filter above 630 nm (Fig. 3). When a more concentrated solution of

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FIG. 2—*Blue-colored fingerprint developed with genipin on copier paper and recorded under white light illumination.*


FIG. 3—*Weak fingerprint developed with genipin on copier paper:* (a) *recorded under white light illumination,* (b) *recorded under fluorescence conditions.*

genipin, $10^{-2}$ M, was applied, even the fainter marks showed up as dark blue impressions, whereas concentration reduction to $10^{-4}$ M produced good results when viewed in the fluorescence mode described above even though the marks were not visible in white light.

The science of fingerprints owes one of its greatest leaps to the discovery of Oden and von Hofsten, in 1954, that ninhydrin can develop latent fingerprints on paper (10). It is somewhat surprising that 44 years had passed from the first discovery of the color forming reaction between ninhydrin and amino acids by Ruhemann (11) and the recommendation to use ninhydrin as a latent fingerprint reagent (10). Since then, the use of ninhydrin has become the most important method for chemical visualization of latent fingerprints on paper items (12,13). Other reagents have been developed over the years in an attempt to increase the sensitivity and maximize the yield of latent prints (12–15): 1,8-Diaza-9-fluorenone (DFO), which produces fluorescent impressions with the amino acid fraction of palmar sweat (16,17) and "Physical developer" (PD), that is particularly suitable for wet paper articles, since it reacts with the lipid fraction of the perspiration, which is not washed off by the water (18,19). 1,2-Indanedione, another fluorogenic amino acid reagent (20,21), has recently been adopted by several forensic science laboratories (22).

Despite the plethora of reagents, there is still a considerable number of potentially case solving latent prints that cannot be visualized by existing methods, either due to insufficient sensitivity or background interferences (23,24). This creates the need for more research towards advanced, more versatile reagents. Health hazards of ninhydrin must also be considered (25,26).

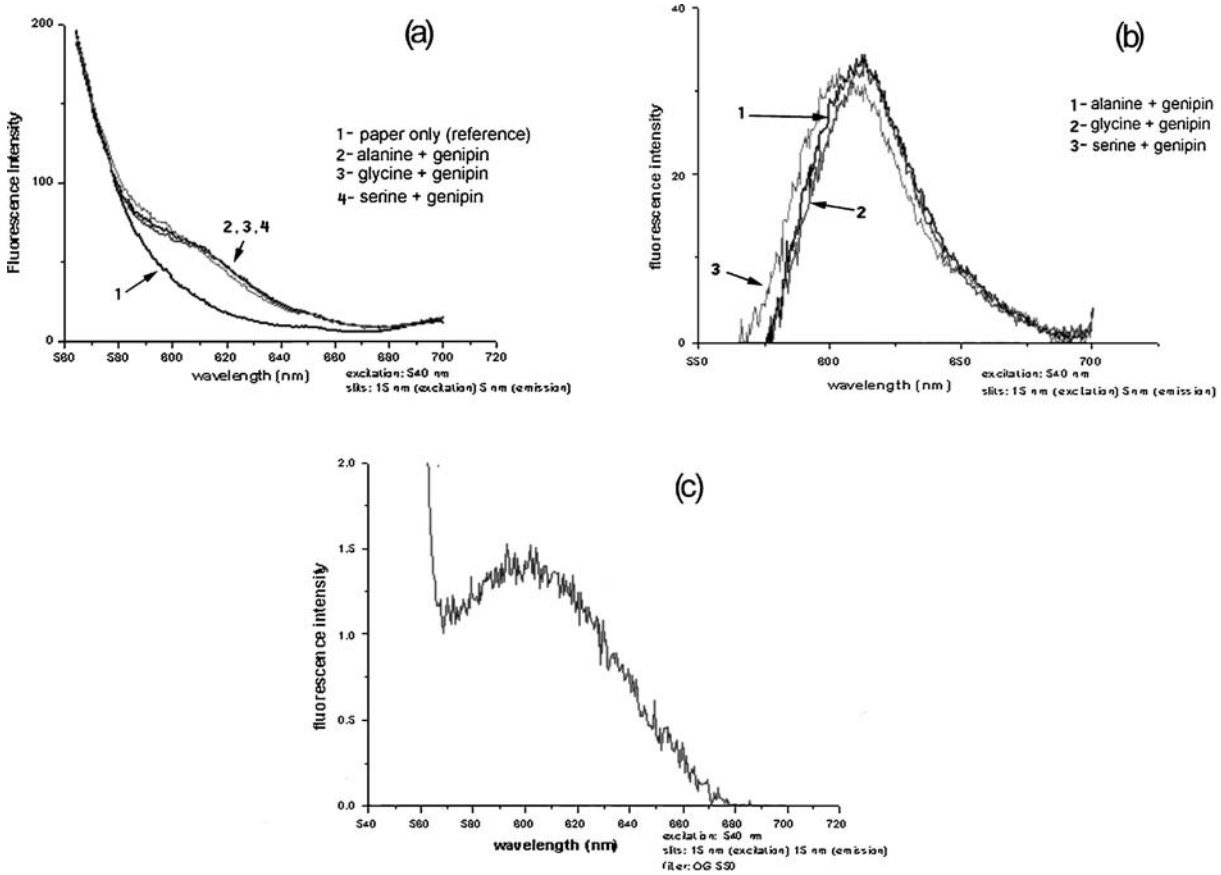


FIG. 4—*Emission spectra from the genipin reaction with amino acids (excitation at 540 nm):* (a) *emission of the blue stains on white paper,* (b) *emission of stains after subtraction of paper background, and* (c) *emission spectrum in solution (EtOH:$H_2O$).*

The reaction of an ideal reagent with a fingerprint should produce a product that is both colored and fluorescent. Color observation does not require specific instrumentation, and can be performed even at the field level. While fluorescent imaging requires more equipment, the results generally demonstrate higher sensitivity (27). Genipin appears to meet these expectations. Our initial experiments show that the blue impressions that are formed upon reaction with latent fingerprints are comparable in color intensity to ninhydrin prints. The reaction is simple, and can be carried out even in a modestly equipped laboratory. The potential of this reagent, however, stems from its fluorogenic reaction, which, to the best of our knowledge, has never been disclosed. Regular excitation in fingerprint visulaization is in the blue or blue-green domain (490–515 nm) and the prints are observed beyond 520 nm (7,12,28,29). With genipin-developed prints, the excitation-emission domain is shifted towards longer wavelengths (Fig. 4). This is an advantage, since there is less background fluorescence in the longer wavelength region. Fluorescence subtraction shows that the blue product has a clear fluorescence peak, with maximum emission at ca. 610 nm (wavelength varies slightly with the amino acid), a domain in which the paper self-fluorescence is relatively small (Fig. 4). Although the structure of the fluorescent product is still unknown, it is clear that its formation requires water besides amino acid and genipin. When the development process was conducted in total dryness, or when the reaction was carried out in non-aqueous solution (dry THF, 45 min reflux) neither a blue product nor a fluorescent one was obtained.

Because the gardenia blue pigment is used as a traditional medication, a food additive and a natural food and fabric colorant, genipin-based formulations can be considered safe and less hazardous than ninhydrin.

## Conclusion

Genipin shows great potential as a fingerprint developer on paper. Following genipin treatment, latent fingerprints appear as dark blue, easily recorded impressions. Even very weak prints can be visualized in the fluorescence mode, which is an advantage over ninhydrin.

Even at this stage, before any optimization of the development process, genipin can be considered a potential alternative to ninhydrin. A full-scale study of the scope and limitations of this reaction, as well as its economic aspects are underway.

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