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Reagents for the Chemical Development of Latent Fingerprints: Scope and Limitations of Benzo[f]ninhydrin in Comparison to Ninhydrin

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ABSTRACT: Benzo[f]ninhydrin was compared to ninhydrin for fingerprint development on paper. Overall, the performance of ninhydrin on exhibits was slightly better than that of benzo[f]ninhydrin. The significant advantages of the benzo[f]ninhydrin over ninhydrin were the much stronger fluorescence it gave after treatment with zinc salts and a slightly quicker reaction under ambient conditions. This fluorescence is, however, similar to that obtained with other reagents, such as DFO or ninhydrin analogs. These advantages apparently are not sufficient to justify regular usage of benzo[f]ninhydrin, especially when one considers its low solubility and high cost.

KEYWORDS: forensic science, latent fingerprints, fingerprint development, benzo[f]ninhydrin, ninhydrin, ninhydrin analogs

Benzo[f]ninhydrin (I) was the first ninhydrin analog whose potential as a latent fingerprint developer was examined (1). However, no full-scale study to evaluate its capability has ever been carried out due to its high cost: US$ 51.45 per 100 mg (2). Lately, we have designed a synthetic protocol by which fair amounts of (I) can be obtained from relatively low-cost materials, with an overall yield of 26.5% from o-xylene (3) (internal report available on request). As a result, it became possible to carry out a thorough study of the potential of benzo[f]ninhydrin as a substitute or auxiliary to ninhydrin.

Benzo[f]ninhydrin, or its exact chemical name, 2,2-dihydroxy-1H-benzo[f]indene-1,3(2H)-dione (I), was synthesized for the first time by Meier and Lotter in 1957 (4), but they did not pay attention to its reaction with latent fingerprints. They did mention, though, that like ninhydrin, this triketone did react with amino acids to give a colored product. A few years later, Jones and Wife reported on the preparation of (I) as an intermediate in their investigations of o-quinonoid chemistry (5). In 1982, benzo[f]ninhydrin (I) was reported for the first time as a potential fingerprint reagent (1). One property of this compound which was particularly attractive to the fingerprint practitioner was the color it gave with amino acids and with latent fingerprints on paper. It was dark green, nearly black, which could mean higher contrast and better detectability than that of ninhydrin. Menzel and Almog studied in addition the visualization of latent prints that were first developed by benzo[f]ninhydrin and were then subjected to secondary treatment with zinc chloride. They found that such prints luminesced nicely upon excitation with a frequency-doubled Nd:YAG laser (6).

Subsequently, a few other groups started to explore benzo[f]ninhydrin as a fingerprint reagent. Warrener and his co-workers from the University of Canberra, reported an elegant, but small-scale, synthetic route to this compound and other ninhydrin analogs (7,8). They also compared colors and luminescence that were obtained by treating latent fingerprints with the new analogs followed by zinc or cadmium salts (9). Joullie, at the University of Pennsylvania, and her co-workers conducted a thorough research toward the systematic preparation of benzo[f]ninhydrin and similar compounds (10–14). The pathway they suggested to (I) was quite different from the previous routes (10). Attempts by this group to repeat their protocol on a medium size scale (a few grams), however, failed. One of the stages seemed to be quite tricky.

The modified synthesis that was lately designed (Fig. 1), (details of the synthesis are available on request) (3) enabled us the production of ca. 50 g of (I) in one batch and gave us the opportunity to explore this reagent without the fear of losing a few milligrams in each experiment.

Experimental

Various portions of the experiments were independently carried out by two separate research groups. The study was divided into three parts. First, solubility tests on the benzo[f]ninhydrin were performed. Next, a preliminary comparison was made between the benzo[f] and the regular ninhydrin reagent. Lastly, a comparison between the two was carried out on "real" exhibits.

Solubility Tests

The following benzo[f]ninhydrin solutions were prepared: 0.25 g benzo[f]ninhydrin was dissolved in 1 mL of acetic acid and 2, 5, or 10 mL of ethanol. When the benzo[f]ninhydrin totally dissolved, Freon 113 (CFC), Vertrel XF or HCFC 141B was added to complete to 100 mL. In addition, solutions were also prepared replacing the ethanol with 2,4 or 6 mL of THF.

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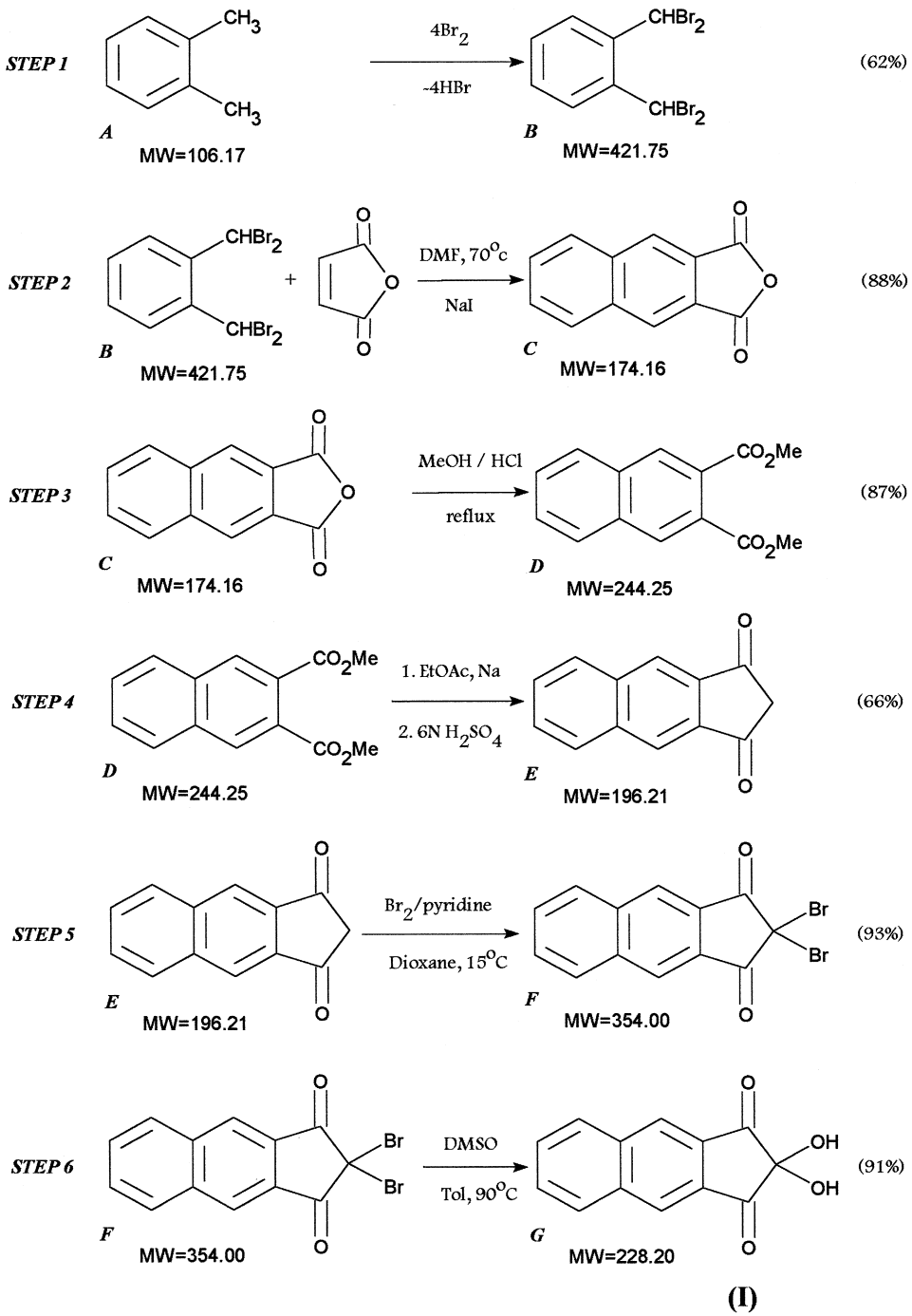

FIG. 1—*A medium scale synthesis of benzo[f]ninhydrin (I).*

Preliminary Comparison Between the Performance of Benzo[f]ninhydrin and Ninhydrin

The following working solutions of ninhydrin and benzo[f]ninhydrin were prepared: 0.25 g ninhydrin/benzo[f]ninhydrin, 1 mL acetic acid, 99 mL ethanol.

Six types of paper were used in this experiment: 1. White woodfree paper, 2. green envelope (used by the Israeli police), 3. brown wrapping paper, waxed on one side, 4. cardboard, 5. newspaper, and 6. Israeli 50 New Shekel currency.

The pH of each paper was tested. Merck pH paper was wetted with distilled water, and then left in contact with the paper for 10 min. The pH of the ninhydrin solution was also tested with Merck pH paper.

Two donors, one good and one average, placed both hands on six sheets of paper of each of the above types. Every sheet was divided in half vertically. One half was sprayed with ninhydrin and the other half with benzo[f]ninhydrin.

The papers were divided into three groups of four sheets of each kind, two from one donor, and two from the other. One group was developed in ambient conditions. The papers were placed in envelopes in a dark drawer until the fingerprints developed (an aver-

age of a few days). The second group was developed in a Sanyo-Gallenkamp Fingerprint Development Chamber, at a temperature of 80°C and 65% relative humidity (RH) (optimum ninhydrin developing conditions) (15) for 20 min. The third group was developed in an oven at 100°C for 20 min.

After developing the fingerprints and initial examination, the papers were divided in half horizontally and the bottom half was sprayed with $ZnCl_2$. The prints were then illuminated with a Rofin Polilight at wavelengths ranging from 350 nm to 555 nm, and viewed through orange and red barrier filters.

Comparison Between the Performance of Benzo[f]ninhydrin and Ninhydrin on Real Exhibits

For this comparison, used checks were processed for fingerprints using the following working solutions of ninhydrin and benzo[f]ninhydrin:

HFE-based Ninhydrin	BNF 6	
Ninhydrin 5 g	Benzo[f]ninhydrin	6 g
Ethanol 45 mL	Methanol	60 mL
Ethyl acetate 2 mL	Methyl acetate	60 mL
Acetic acid 5 mL	Acetic acid	30 mL
HFE7100 1 L	CFC113	850 mL

The checks were passed once through a shallow trough of the solutions, allowed to dry completely in the fume cupboard and then heated in a Sanyo Fingerprint Development Chamber (15).

Ninhydrin treated checks were processed at 80°C and 65% RH for 4 min (15). The efficiency of each reagent formulation was assessed by counting the number of fingerprints with 8 or more minutiae that developed over a two week period.

Results and Discussion

Solubility Tests

250 mg of benzo[f]ninhydrin (I) did not dissolve in 1 mL acetic acid and ethanol until the volume of ethanol was increased to 10 mL. Even then, it took about half an hour until it totally dissolved. The disadvantage of such a high ethanol concentration is that the prepared CFC solution caused the ink to run. This was true for Vertrel XF and 141B solutions as well.

THF proved to be a very good solvent for benzo[f]ninhydrin. 250 mg of benzo[f]ninhydrin dissolved immediately, even in as little as 2 mL of THF, but unfortunately, when CFC, Vertrel XF or 141B were added, it came out of solution. This phenomenon repeated itself even when the THF concentration was higher. Because of its high volatility, THF did not cause ink to run, even when benzo[f]ninhydrin was dissolved in 100% THF. It should be mentioned that the disadvantages of using THF as a solvent are its high flammability and toxicity.

Comparison with Ninhydrin Treated Fingerprints

The pH of the brown wrapping paper, cardboard, and newspaper was 4 while the white wood-free paper. Israel currency, and green envelope paper had a pH of 7. The pH of the ninhydrin solution was 3.

After treating with $ZnCl_2$, the ninhydrin fingerprints fluoresced best when excited at a wavelength of 505 nm. The benzo[f]ninhydrin fingerprints fluoresced best when excited at a wavelength of 530 nm. In both cases, the fingerprints were viewed through an orange 549 nm cut-off filter.

The fingerprint developing results were rated 1–5, from low to high. The results are presented in Tables 1–3.

TABLE 1—*Developing in ambient conditions.*

	White Paper (pH = 7)				Brown Wrapping Paper (pH = 4)				Cardboard (pH = 4)				Envelope (pH = 7)				Newspaper (pH =4)				50 Shekels (pH = 7)			
	Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin	
Color	Good Donor Purple	Average Donor Purple	Good Donor Green	Average Donor Green Black	Good Donor Purple	Average Donor Purple	Good Donor Dark Green	Average Donor Green	Good Donor Purple	Average Donor Purple	Good Donor Dark Green	Average Donor Dark Green	Good Donor Dark Purple	Average Donor Purple	Good Donor Dark Green	Average Donor Dark Green	Good Donor Purple Gray	Average Donor Purple	Good Donor Green-	Average Donor Green	Good Donor Purple	Average Donor Purple	Good Donor Green	Average Donor Green
Develop	5	4	5	4	5	4	5	3	5	2	5	3	4	4	4	5	5	2	5	3	4	1	3	2
Quality	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	3	4	4	3	2	4	3
Contrast	5	4	5	4	5	3	5	3	4	2	4	3	5	4	5	4	4	1	4	1	4	4	4	4
Develop Rate																								
Fluorescence	++	++	+++	+++	–	–	++	+	–	–	+	+	–	–	++	+	–	–	–	+	–	–	–	–

TABLE 2—*Developing with heat and humidity.*

	White Paper				Brown Wrapping Paper				Cardboard				Envelope				Newspaper				50 Shekels			
	Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin	
Color	Good Donor Purple	Average Donor Purple	Good Donor Gray-Purple	Average Donor Dark Purple	Good Donor Purple	Average Donor Purple	Good Donor Brown	Average Donor Green	Good Donor Dark Purple	Average Donor Purple	Good Donor Brown	Average Donor Green	Good Donor Dark Purple	Average Donor Purple	Good Donor Gray-Green	Average Donor Green	Good Donor Purple	Average Donor Pink	Good Donor Gray-Brown	Average Donor Light Green	Good Donor Purple	Average Donor Purple	Good Donor Gray-Brown	Average Donor Green
Develop	5	3	5	4	4	3	4	3	5	3	5	3	5	4	5	5	5	2	5	3	1	1	2	2
Quality																								
Contrast	4	4	4	4	4	4	4	4	4	3	4	3	4	4	4	4	4	3	3	3	1	1	2	2
Fluorescence	++	++	++	+++	−	−	−	++	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−

TABLE 3—*Developing with heat and no humidity.*

	White Paper				Brown Wrapping Paper				Cardboard				Envelope				Newspaper				50 Shekels			
	Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin		Ninhydrin		Benzo[f] Ninhydrin	
Color	Good Donor Dark Purple	Average Donor Purple	Good Donor Green	Average Donor Green	Good Donor Purple	Average Donor Purple	Good Donor Green	Average Donor Green	Good Donor Purple	Average Donor Purple	Good Donor Green-Black	Average Donor Green	Good Donor Purple	Average Donor Purple	Good Donor Dark Green	Average Donor Green	Good Donor Gray-Purple	Average Donor Pink	Good Donor Gray-Green	Average Donor Light Green	Good Donor Purple	Average Donor Purple	Good Donor Brown	Average Donor Green
Develop	5	3	5	2	4	2	3	2	5	2	5	2	4	3	4	3	4	2	5	3	3	1	3	3
Quality																								
Contrast	4	4	4	4	4	3	4	3	4	2	4	2	4	4	4	4	4	2	4	2	2	2	2	3
Fluorescence	++	++	+++	+++	−	−	+	++	−	−	−	−	−	−	++	++	−	−	−	+	−	−	−	−

pH—Some of the papers tested were acidic and some were neutral. None of the papers tested were basic. As a rule, on acidic papers fingerprints developed more slowly and the impressions were fainter.

Color—The colored prints with ninhydrin were consistently purple, and the shade was dependent on the intensity of the reaction. The reaction product of benzo[f]ninhydrin had a broader variety of colors. The main factor affecting this was the method of developing. However the donors' quality (judged by their latent prints reaction with ninhydrin) had an effect as well. The most common color was green, which was the only color when the development was performed in ambient conditions. When there was a weak reaction, the shade was pale green and when the reaction was extremely intense (usually with prints from a good donor), the shade was nearly black. Developing with heat produced another color as well, brown. Sometimes the color was merely purple and on other occasions a mixture of green and purple. The purple product was more likely to appear on prints from a good donor or when the developing process involved humidity.

Development Quality—In most cases, there was no significant difference in quality between the fingerprints developed with benzo[f]ninhydrin and the fingerprints developed with ninhydrin. The advantage of benzo[f]ninhydrin was more pronounced with fingerprints of the "weaker" donor. Ninhydrin gave better results on paper that was only semi-porous such as the brown wrapping paper on the waxed side and the currency.

Contrast—There was no difference in contrast between the products of benzo[f]ninhydrin and those of ninhydrin. The green benzo[f]ninhydrin product was advantageous on the purple currency. This color advantage was not enough to improve the fingerprints, due to the strong background coloring.

Developing Rate—This was only relevant when the development was performed in ambient conditions. Benzo[f]ninhydrin developed fingerprints faster than ninhydrin. This difference was more noticeable on types of papers with a slower rate of developing, such as card board.

Fluorescence after Treatment with $ZnCl_2$—The fluorescence of benzo[f]ninhydrin developed prints was in most cases significantly stronger than the fluorescence of ninhydrin. The fingerprints fluoresced best on white paper and there was no fluorescence at all on the currency. Ninhydrin fingerprints on surfaces other than currency did not fluoresce. Benzo[f]ninhydrin fingerprints sometimes fluoresced and sometimes did not. There was no correlation between the developing conditions or the quality of the donor and the intensity of fluorescence.

Comparison Between the Performance of Benzo[f]ninhydrin and Ninhydrin on Real Exhibits—An operational trial between two non-CFC ninhydrin formulations showed that one based on HFE7100 was slightly more effective, when using the standard post treatment heat and humidification (15), than either the CFC formulation (15) or one based on HFC4310mee (16). It was, therefore, decided to use the HFE-based ninhydrin formulation against which to measure the effectiveness of benzo[f]ninhydrin.

The following considerations were taken into account when developing benzo[f]ninhydrin formulations for testing: 1. A solution with 6 g of benzo[f]ninhydrin in 1 liter is roughly equivalent in molarity to that of ninhydrin in the HFE-based formulation. 2. It was found that benzo[f]ninhydrin dissolved more effectively in methanol than ethanol. This helped to reduce the amount of polar solvents used in the formulations, thereby limiting the amount of ink running, which can reduce the contrast of developed fingerprints.

Benzo[f]ninhydrin formulations were initially evaluated against each other on a series of depleted, deposited fingerprints on photocopy paper and Basildon Bond blue writing paper. Fingerprints were then split down the middle, with one half being treated with each test formulation. Promising stable formulations were then used to treat small batches of bank checks with the most effective of these, formulation BNF 6, being used in the final check comparison. Longer heating times were used for

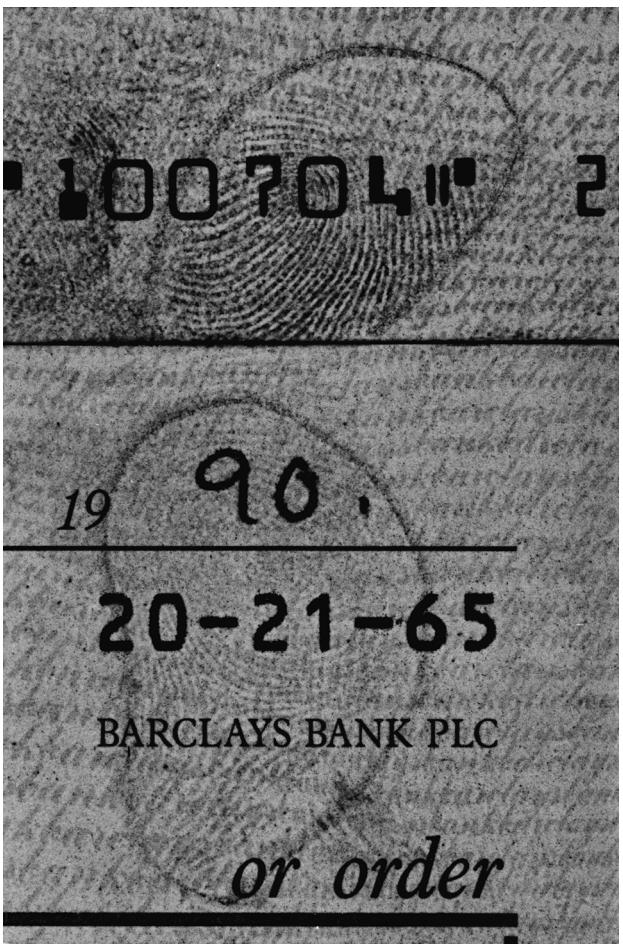

FIG. 2—*A typical matched comparison. Ninhydrin (top) vs. benzo[f]ninhydrin (bottom).*

TABLE 4—*The results from the check comparison.*

	No Fingerprints		Positive Checks		Positive Cases	
Days Since Treatment	Nin	BNF	Nin	BNF	Nin	BNF
0	97	60	38	31	17	16
7	111	66	41	33	19	16
14	117	72	43	35	20	16

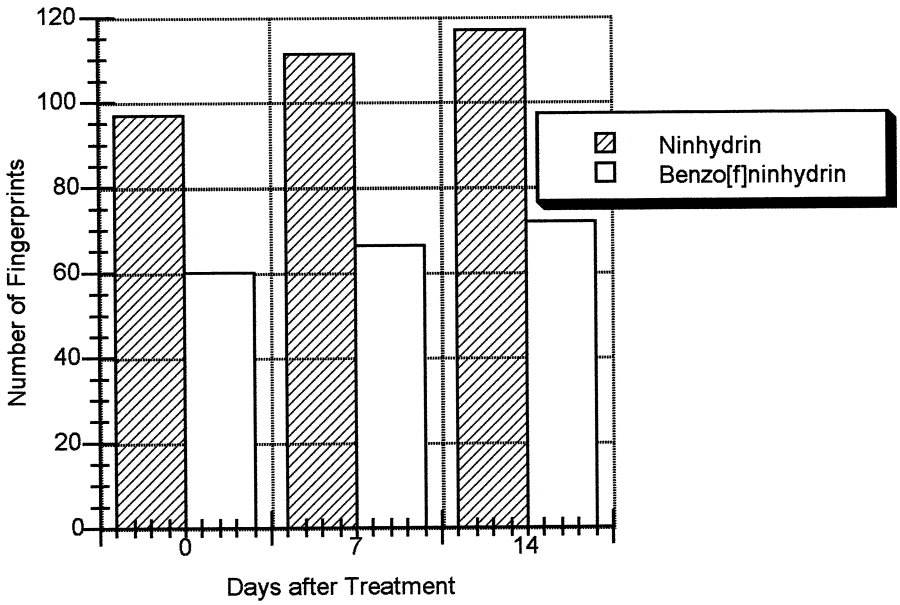

FIG. 3—*Number of fingerprints developed on checks with each reagent over time.*

checks treated with benzo[f]ninhydrin as the reaction was observed to be slower than that of ninhydrin with the same surface. Samples treated with benzo[f]ninhydrin, heated at 80°C and humidified at 65% RH for 20 min gave more fingerprints and greater development than those heated for 10 min. Samples heated for 30 min under the same conditions showed only minor increases in detail over those heated for 20 min, and accordingly 20 min was chosen for the main experiment.

It was found that when benzo[f]ninhydrin reacts with fingerprints on bank checks, the result was generally a gray fingerprint, not unlike the color of fingerprints developed using physical developer. There were, however, rare occasions when the color of developed fingerprints was dark magenta or gray-green. The gray colored fingerprints developed with benzo[f]ninhydrin were often of lower contrast than the pink/purple of ninhydrin developed fingerprints making them more difficult to see and photograph.

Figure 2 shows a typical matched comparison from the same case where the differences in contrast may be seen between ninhydrin (top) and benzo[f]ninhydrin (bottom).

Table 4 shows the results from the check comparison.

The graph (Fig. 3) shows the number of fingerprints developed with each reagent over a two week period.

It can clearly be seen that in this comparison the ninhydrin formulation is markedly superior in every aspect; numbers of identifiable fingerprints, checks on which identifiable fingerprints were developed and the number of cases on which there were identifiable fingerprints. However, it must be taken into account that the ninhydrin formulation and treatment regime have been fully optimized, which is not so with benzo[f]ninhydrin.

Zinc toning to make a fluorescent product was not considered in this part of the experiment, because the extra treatment stage makes it less practical for day to day use in Police Service Laboratories than DFO (17).

As stated before, a wide range of colors was observed when using the benzo[f]ninhydrin. This, along with the fact that under certain conditions, a purple color similar to that of Ruhemann's purple is observed, hints possibly that the green/gray product is not a final product, similar to the Ruhemann's purple analog, but its precursor. This hypothesis is further supported by the observation that when the dark green product of I with amino acids in aqueous solution is shaken with chloroform, all the color is transferred to the organic layer, which turns purple (18).

Benzo[f]ninhydrin did not behave uniformly after $ZnCl_2$ treatment. The fingerprints sometimes fluoresced and sometimes absorbed. This may also indicate the presence of two species; the Ruhemann's purple and the precursor. It could, however, also indicate the possible formation of two different complexes (19). More studies must be done in order to establish whether or not this hypothesis is correct.

In spite of the initial assumption, that elongating the conjugated system of ninhydrin should bring about both a more intense color and color change (1), recent theoretical calculations showed that there is no reason that the Ruhemann's purple analog of benzo[f]ninhydrin should be dark green. The color differences should not be so great. The reason for this is that the main electron transitions take place on the central N and two attached carbon atoms of the Ruhemann's purple and are not much affected by the more distant groupings (20).

Slight differences in results were obtained by the two research groups. These can most probably be attributed to differences in formulae used, developing conditions, and types of paper examined. None the less, the same conclusions can be reached.

Conclusion

Benzo[f]ninhydrin has several slight advantages over ninhydrin. The first is the color that differs from the ninhydrin purple and is more visible on pink and purple surfaces. The second is the slightly faster rate of development on problematic surfaces, such as cardboard. Another, and perhaps the key advantage is the more pronounced fluorescence after treatment with $ZnCl_2$.

Ninhydrin's advantages are low cost and better solubility. It also developed overall, more fingerprints. As for fluorescence, there are other ninhydrin analogs (8–10), and obviously also DFO, that ex-

ceed benzo[f]ninhydrin in this respect. Benzo[f]ninhydrin's slight advantages might warrant small amounts being stocked in fingerprint development labs to expand its capabilities to deal with special cases.

From a forensic point of view, this seems to be the end of the story for benzo[f]ninhydrin as a practical fingerprint reagent. Nevertheless, the effort in this research was not in vain. It pioneered the exploration of other ninhydrin analogs and related compounds by a great many groups, the crowning achievements of which were the development of DFO and the recent discovery of the potential of indanedione.

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