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Evaluation of Some Oxygen, Sulfur, and Selenium Substituted Ninhydrin Analogues, Nitrophenylninhydrin and Benzo[f]furoninhydrin

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ABSTRACT: Six ninhydrin analogues containing oxygen, sulfur, and selenium substituents at the C-5 position, 5-(4-nitrophenyl)ninhydrin, and benzo[f]furoninhydrin were evaluated as fingerprint development reagents. The analogues all showed good fingerprint color development but were not superior to ninhydrin in this respect. The benzo[f]furoninhydrin complex was strongly luminescent at room temperature following zinc complexation, while the remaining analogues required cooling to −196°C to produce optimum luminescence. The benzo[f]furo, nitrophenyl, and methyl selenide analogues showed the best potential as fingerprint reagents with the benzo[f]furo analogue comparing favorably with DFO.

KEYWORDS: forensic science, latent fingerprints, fingerprint development, ninhydrin analogues, amino acids, luminescence

Ninhydrin is the commonly used reagent for the development of latent fingerprints on porous surfaces. Ninhydrin reacts with the amino acid residues present in sweat secretions deposited by the fingers on the surface, resulting in a purple product called Ruhemann's Purple. Weak fingerprints developed with ninhydrin can be further enhanced by treating with a metal salt solution, MX_2 (M = Zn, Cd; X = Cl, NO_3) and the luminescence inspected under a suitably filtered light source (1) at liquid nitrogen temperature.

Since luminescent methods offer significant improvement in the sensitivity of fingerprint detection, much interest has been directed toward reagents with good luminescence properties and an extensive range of ninhydrin analogues has been synthesized and evaluated (2–5). Three of the potentially more useful analogues reported were 5-methoxyninhydrin (6), 5-methylthioninhydrin (7), and thieno[f]ninhydrin (8). These showed strong color development and good room temperature luminescence properties after metal complexation. Cooling to liquid nitrogen temperature, however, did enhance the luminescence. It would be advantageous if the metal complexation and low temperature conditions were unnecessary for luminescence enhancement.

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1,8-diaza-9-fluorenone (DFO) (9,10), although not a ninhydrin analogue, is widely recognized as the most sensitive of the amino-acid–specific reagents. It produces a product that is strongly luminescent at room temperature without metal complexation. However, the visible color development is relatively weak, and therefore, fingerprint visualization needs to be made exclusively through the luminescent mode. More recently, Almog et al. (11) and Hauze et al. (12) reported on a range of indandiones. The investigation of the optimum conditions and evaluation of 1,2-indanedione and 5,6-dimethoxy-1,2-indanedione was carried out by Roux et al. (13). This preliminary study confirmed that these indanediones may offer less expensive alternatives to DFO while still developing similar quality prints. They also produced a brightly luminescent product but with relatively poor color development.

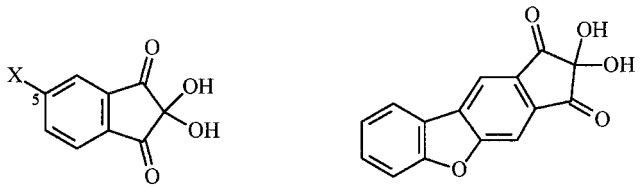
From an operational fingerprint perspective, it would be useful if the good luminescence properties of DFO or the indanediones could be combined with the strong color development of ninhydrin in a single reagent. Good quality prints could then be quickly identified or photographed and luminescence only used when enhancement of weak prints was required.

Our group has prepared a number of oxygen, sulfur and selenium analogues (14,15) aimed at influencing color, luminescence, and reagent solubility. It was considered that an extended planar and rigid framework incorporating oxygen or sulfur might offer enhanced luminescence properties over currently known ninhydrin analogues. With this in mind, benzofuro(2,3-f)-ninhydrin (benzo[f]furo) was produced as one of a range of analogues prepared by Taylor (14). An evaluation of these compounds was reported by Kobus et al. (16) and has been extended in the present study. The problem of poor reagent solubility was addressed by the incorporation of a lipophilic side chain in some five substituted ninhydrins. It was hoped that such analogues would be suitable for use in hydrocarbon solvents and other Freon replacements. The analogues evaluated are shown in Fig. 1.

Materials and Methods

Preparation of Solutions

Ninhydrin (100 mg) was dissolved in absolute ethanol (0.4 mL). Freon was then added to dilute the solution to 20 mL. The ninhydrin analogue solutions (0.5% w/v) were prepared by dissolving the analogue (5 mg) in CH_2Cl_2 (1 mL). A few drops (0.1 mL) of methanol were added to the MeSe and benzo[f]furo analogues first to assist in dissolution; all other analogues were completely soluble in CH_2Cl_2. The DFO solution was prepared by dissolving DFO (2.5



X= MeSe, PhSe, BuS, PhS, $C_7H_{15}S$, $pMeOC_6H_4O$, NO_2Ph

Benzo[f]furo

FIG. 1—*Structures of the analogues studied.*

mg) in methanol (0.3 mL) and then diluting with Freon (10 mL). The formulations are a modification of the standard formulations such as reported by Margot et al. (1) in that acetic acid was omitted. The inclusion of acetic acid in the formulation did not influence the development of marks on the papers used in this study. It was therefore convenient not to add acetic acid, particularly when preparing the small volumes of stock solutions that were used for the analogues.

Fingerprints

Fingerprints were obtained from donors in the authors' laboratory. Latex gloves were worn for 15 to 20 min prior to laying down the print to facilitate palmar secretions. The gloves were then removed and the fingers rubbed together to obtain an even distribution of sweat and the fingerprints layed down on white A4/80 g Australian Copier Paper. Sequential prints ranging from strong to weak were deposited by making a series of prints using the same finger. When required, individual fingerprints were cut in half so that comparative tests could be performed on the same print to eliminate interdonor or interprint variation.

Visualization of the Fingerprints: Ninhydrin and Analogues

The fingerprints were developed by applying the solution to the prints with a pipette. After evaporation of the solvent, the prints were developed using one of four methods:

1. Ambient Conditions

The fingerprints were left in a dark cupboard to develop. Room temperature under ambient conditions was approximately 25°C with a humidity level between 20 to 25%.

2. Moisture

(a) *Humid atmosphere at room temperature*

A TLC tank (approximate volume of 1 L) containing a small amount of water (approximately 15 mL) was left covered for 30 min at room temperature. The pieces of paper containing the fingerprint were suspended in the tank and the lid replaced. Prints were left to develop for 30 min, 1 h, and 2 h, respectively, to determine the optimal duration of exposure required for each analogue.

(b) *Humid atmosphere in oven*

The fingerprints were placed over the edge of a jar or TLC tank (approximate volume of 1 L) containing a small amount of water (about 20 mL) and the lid was replaced. The tanks were placed in an oven at 50 or 70°C for 20 to 30 min during which time development of the prints occurred.

(c) *Humid atmosphere produced by steam*

Water was boiled in a Büchner flask capped with a rubber stopper. The fingerprints were then waved through the steam (produced via the side arm) for up to 30 s or until the prints developed.

DFO

Fingerprints treated with DFO were developed in a dry oven at 100°C for 20 min.

Photo Luminescence

Zinc Chloride Treatment—Fingerprints developed with ninhydrin and the analogues were treated with a $ZnCl_2$ solution (1). This was prepared by dissolving zinc chloride hexahydrate (4 g) in methanol (80 mL) and then diluting the solution with ethyl acetate (80 mL), acetic acid (10 mL), and Freon (to 1000 mL). The $ZnCl_2$ solution was applied using a pipette. The zinc complex developed under ambient conditions within a short time (0.5 to 1 min).

Luminescence Observation—Luminescence was generated using a xenon arc lamp (Polilight Ten, Rofin Australia) as the excitation source. The Polilight is fitted with a range of interference filters that allow selection of different wavelengths of excitation light. The luminescence emission was observed through interference filters that excluded the excitation light. The bandwidth of all the filters was 40 nm. The combination of excitation and emission filters used was as follows, (excitation wavelength/emission wavelength): 505/565 nm, 530/590 nm, and 555/610 nm. Luminescence was observed at room temperature (25°C) and at liquid nitrogen temperature (−196°C). For the latter observations, the pieces of paper bearing the fingerprints were secured to the base of a polystyrene tray with pins and sufficient liquid nitrogen poured into the tray to cover the surface of the paper.

Recording the Fingerprints—Permanent records of the fingerprints were obtained as follows:

- *Nikon Colourpix 950 digital camera.*

This was used to obtain color images of the developed fingerprints.

- *Poliview Image Capture System (Rofin Australia).*

This was used in combination with the Polilight to capture luminescent images of fingerprints via a CCD camera and digital image recording. The Poliview is designed specifically for recording luminescent images and has a convenient sliding mechanism for interchanging emission filters.

Results and Discussion

Development of Fingerprints

Fingerprints treated with the analogues and stored under ambient conditions (20 to 25% relative humidity and 22 to 25°C) for up to ten days failed to show any significant development. The MeSe and NO_2Ph analogues did show some response, producing a weak pale purple print. Dry heat (oven up to 100°C) also failed to initiate fingerprint development. In comparison, ninhydrin-treated fingerprints developed strongly within 60 min under ambient conditions and continued to improve over 48 h.

Fingerprints exposed to the humid conditions following treatment with the analogues, however, showed rapid color development. The fingerprints that had failed to develop under ambient conditions after extended storage also developed rapidly when exposed to humidity. However, fingerprints treated with the MeSe analogue did not develop as strongly following storage as those exposed to humidity directly after treatment.

Under humid conditions, relatively high backgrounds were produced. A range of dilutions of the working solutions of the PhS and PhSe analogues (0.4, 0.2, 0.1% w/v) were made to determine the concentration for optimum image-to-background contrast. Although the weaker solutions produced lower background color, the intensity of the fingerprint development was also markedly reduced. The 0.5% w/v solution was therefore the most favorable and is similar to the standard ninhydrin formulation (1).

Fingerprints developed using the various humidity conditions showed similar image-to-background ratios. Exposure times of up to 30 s were required for development by the steam treatment compared to 20 to 30 min using the humid atmosphere in the oven method. The mildest humid condition was produced in the TLC tank at room temperature, but no advantage in the reduction of background color was achieved. Therefore the method of choice for the development of the fingerprints was using the steam treatment.

It is well known that humidity is important for the development of ninhydrin-treated prints and optimal conditions of 60 to 80% relative humidity are recommended (1), although development will also occur at lower humidity. The relative humidity at the time this work was done was about 20 to 25% and strong ninhydrin development did occur under ambient conditions. However, humidity appears to be far more critical for the development of prints treated with the analogues, requiring exposure to steam or confinement in a humid chamber. This suggests that humidity in the 80 to 100% range is required and that the fingerprints need continual exposure to the humid atmosphere while the print develops. A brief exposure to steam to humidify the paper was not sufficient to produce full development.

Fingerprints developed with the analogues using the Freon formulation (1) gave very similar results to the CH_2Cl_2 formulation on the paper surfaces used. Although it is well known that using dichloromethane as the solvent does cause running of ink during fingerprint development, it was convenient to use it in this study to aid in the dissolution of the analogues. A qualitative estimation of the solubility of some of the analogues in nonpolar solvents was carried out. It was found that the $C_7H_{15}S$ analogue had improved solubility in CH_2Cl_2 compared to the other analogues. This suggested that the introduction of an extended alkyl side chain could be a route to improving the solubility of ninhydrin analogues.

Comparison of the Analogues with Ninhydrin

1. *Visible Color*

To compare the development achieved using the ninhydrin analogues relative to ninhydrin, a series of sequential fingerprints was laid down. Each "finger" was cut in half with one half developed using ninhydrin and the other half using the analogues. Both were exposed to steam until the print was fully developed. The color and relative intensities of the fingerprints are given in Table 1 in decreasing sensitivity of the reagents for strong prints.

The higher sensitivity of ninhydrin compared to all the analogues was supported by examining the development of the weaker prints in each series. Except for the MeSe, *p*-$MeOC_6H_4O$ and NO_2Ph analogues, there was a rapid decline in the quality of the weaker prints developed with each analogue compared to ninhydrin. Moreover, the fingerprints treated with ninhydrin continued to improve after steam treatment when stored under ambient conditions. The fingerprints developed with the ninhydrin analogues and stored in a sealed plastic bag in the cupboard for around nine months showed no deterioration in the color and quality of the fingerprints. In the case of the benzo[f]furo analogue a noticeable increase in color development occurred. The final color and intensity of the print was comparable to that of ninhydrin.

An interesting feature of the development of fingerprints using the PhS, PhSe, NO_2Ph, and benzo[f]furo analogues was the color variation that could be achieved. Short exposures to a humid atmosphere gave rise to blue/purple images, while longer exposure of the same print resulted in the bright purple/pink images characteristic of ninhydrin development. This result, seen only in the aryl and benzo[f]furo derivatives studied, suggests that the incorporation of water into the Ruhemann's Purple derivative influences the color of the image obtained. Recently, the investigation of benzo[f]ninhydrin as a fingerprint development reagent has been reported by Almog (17). A similar color variability was observed: a green/grey and purple product. Almog suggested that the green/grey product was not a final product, but a precursor of the Ruhemann's Purple analogue.

2. *Photo Luminescence*

2.1 *Before Metal Complexation*

Fingerprints developed with the analogues showed no luminescence at room temperature. Weak luminescence was observed for all the analogues at −196°C (liquid nitrogen) under 530/590 nm condition, with the benzo[f]furo showing stronger luminescence than the others. However, there was also a luminescent background evident and this together with the weak fingerprint luminescence resulted in no useful fingerprint ridge detail being visible.

2.2 *After Metal Complexation*

Fingerprints developed with all of the analogues reacted with $ZnCl_2$ to form complexes of a similar color to that produced by ninhydrin. However, the resultant luminescent properties varied widely between the different analogues.

TABLE 1—*Comparison of the strong fingerprint images developed with ninhydrin analogues by exposure to steam.*

Reagent	Color of Image	Intensity of Image	Color of Background	Rate of Development (Relative to Ninhydrin)
Ninhydrin	Bright purple	Strong	Pale pink	—
MeSe	Purple	Strong	Pale green/yellow	Slightly slower
NO_2Ph	Purple	Strong	Pale green/yellow	Slightly slower
Benzo[f]furo	Purple/pink	Strong	Pale green/yellow	Same
PhS	Purple	Strong	Pale yellow/grey	Same
p-$MeOC_6H_4O$	Dark pink	Medium	Pale cream	Same
PhSe	Purple/pink	Weak	Pale yellow/grey	Same
BuS	Purple/pink	Weak	Pale yellow/grey	Much slower
$C_7H_{15}S$	Pink	Weak	Pale pink	Much slower

TABLE 2—*Luminescence of fingerprints at room temperature after zinc treatment.*

Reagent	Luminescence of Image		Luminescence of Background	
	505/565 nm	530/590 nm	505/565 nm	530/590 nm
DFO	Strong	Strong	Weak	Weak
benzo[f]furo	Very strong	Very strong	Medium	Medium
NO2Ph	Medium	Medium	Medium	Medium
MeSe	Weak	Strong	Medium	Medium
Ninhydrin	Weak	Very weak	Weak	Weak
BuS	Weak	Medium	Medium	Medium
PhS	Weak	Weak	Strong	Strong
p-$MeOC_6H_4O$	Very weak	Very weak	Strong	Strong
$C_7H_{15}S$	…	…	Strong	Strong
PhSe	…	…	Strong	Strong

TABLE 3—*Luminescence of fingerprints at −196°C after zinc treatment.*

Reagent	Luminescence of Image		Luminescence of Background	
	505/565 nm	530/590 nm	505/565 nm	530/590 nm
DFO	Strong	Strong	Weak	Weak
Benzo[f]furo	Very strong	Very strong	Medium	Weak
NO_2Ph	Strong	Strong	Medium	Weak
MeSe	Strong	Strong	Medium	Medium/ weak
Ninhydrin	Strong	Weak	Weak	Weak
BuS	Strong	Medium	Medium	Medium
PhS	Weak	Medium	Strong	Strong
MeOPhO	Weak	Weak	Strong	Strong
$C_7H_{15}S$	…	…	Strong	Strong
PhSe	…	…	Strong	Strong

The luminescence observed at room temperature for the analogues is shown in Table 2. The benzo[f]furo analogue showed very strong luminescence at room temperature under both the 505/565 nm and 530/590 nm conditions and was clearly superior to the other analogues. Of the others, the MeSe and the NO_2Ph analogues showed good room temperature luminescence. The NO_2Ph analogue responded at both the 505/565 and 530/590 nm conditions, while the MeSe analogue only luminesced under the 530/590 nm conditions. The remaining analogues showed much weaker room temperature luminescence than the above three and would not be viable reagents under these conditions.

The luminescence observed at −196°C is shown in Table 3. Under these conditions no improvement in the luminescence for the benzo[f]furo analogue could be observed. However, the luminescence of the NO_2Ph, MeSe, and BuS analogues improved significantly. At this temperature, the MeSe analogue now showed strong luminescence under the 505/565 nm conditions as well as at 530/590 nm. Both the MeSe and the NO_2Ph analogues compared favorably with the benzo[f]furo analogue. The luminescence for the better performing analogues is shown in Figs. 2 and 3.

Ninhydrin itself shows poor luminescence at room temperature. At −196°C, strong luminescence is produced under the 505/565 nm conditions, while relatively poor luminescence is observed at the longer 530/590 nm conditions. This is in agreement with previously published results (18). None of the analogues offered improvement over ninhydrin under the conditions best suited to the luminescent enhancement of ninhydrin fingerprints (505/565 nm at −196°C).

The benzo[f]furo, MeSe, and NO_2Ph analogues were clearly superior to the other analogues and offered some advantages over ninhydrin in specific areas. In particular, cooling in liquid nitrogen was not required for the benzo[f]furo analogue, and the luminescence of all three analogues at the higher wavelength conditions (530/590 nm) may be useful in overcoming substrate interference.

2.3 *Background*

In comparison to ninhydrin, the analogues produced higher background luminescence. The worst-performing analogues (PhS, *p*-$MeOC_6H_4O$, $C_7H_{15}S$, and PhSe) produced the strongest background luminescence.

The concentration of the analogues in the working solution was similar to standard ninhydrin formulations. The effect of reducing the concentration of the three superior analogues was investigated.

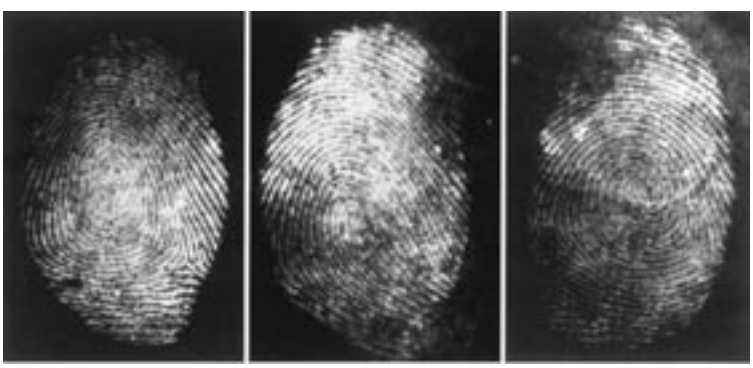


FIG. 2—*Luminescence of some ninhydrin analogues at −196°C at the 505/555 nm setting.*

FIG. 3—*Luminescence of some ninhydrin analogues at −196°C at the 530/610 nm setting.*

Significant reduction in the background was achieved at about a five times dilution. However, the sensitivity of the MeSe and NO_2Ph analogues was also reduced, resulting in weak fingerprint development and luminescence. The benzo[f]furo analogue, on the other hand, although now showing weak color development of the ridges, produced excellent fingerprint ridge luminescence. The considerable reduction in background luminescence contributed to the strong fingerprint image observed.

3. *Preliminary Comparison of the Benzo[f]furo Analogue with DFO*

Due to the excellent results obtained with the diluted benzo[f]-furo analogue, a preliminary comparison was made with DFO. The benzo[f]furo solution was prepared at the same concentration as standard DFO working solution (1). Fingerprints deposited on white bond paper and manilla card were cut in half, one half developed with DFO (dry oven 100°C 20 min) and the other with the benzo[f]furoninhydrin analogue by exposing to steam and leaving under ambient conditions for 24 h. The fingerprints were then treated with $ZnCl_2$.

At the concentration used, the benzo[f]furo analogue showed very weak color development. However, the fingerprint ridge luminescence was excellent and appeared superior to DFO under the 505/565, 530/590, and 555/610 nm conditions. Examples are shown in Figs. 4 and 5.

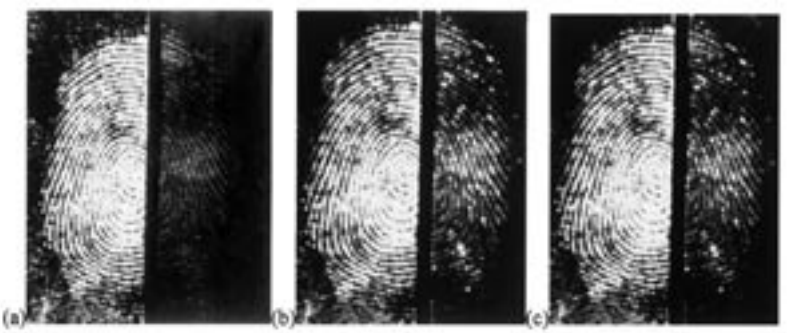


FIG. 4—*Fingerprints developed using benzol[f]furoninhydrin/DFO at* (a) *505/555 nm;* (b) *530/590 nm, and* (c) *555/610 nm at room temperature on manilla folders.*

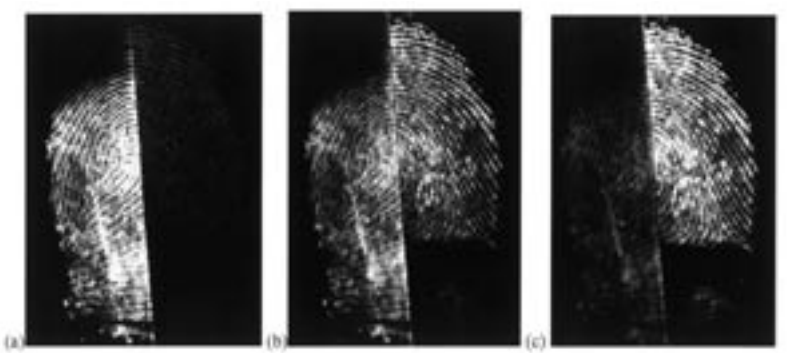


FIG. 5—*Fingerprints developed using benzo[f]furoninhydrin/DFO at* (a) *505/555 nm;* (b) *530/590 nm, and* (c) *555/610 nm at room temperature on white paper.*

Conclusions

It may be considered that it was not appropriate to use Freon as the carrier solvent in the study since it is a prohibited reagent and is therefore no longer used in operational fingerprint reagent formulations. The study, however, was aimed at looking at the range of analogues with a view to establishing their potential as fingerprint reagents. Since Freon has a proven history as a carrier solvent and only very small volumes of working solutions were used, it was considered that Freon formulations would provide a meaningful preliminary comparison of the analogues with each other and with ninhydrin and DFO.

Useful fingerprint development was obtained with all the analogues. The best performing analogues were the benzo[f]furo, MeSe, and NO_2Ph ninhydrin analogues. An interesting feature of the development of fingerprints with the analogues was the need for much higher humidity than required for ninhydrin. None offered any advantage over ninhydrin in terms of visible color development.

While the addition of the C_7H_{15} chain to the C-5 substituted sulfur analogue improved its solubility, the analogue performed poorly as a luminescent reagent and was not investigated further. However, the incorporation of lipophilic chains could be useful for influencing solubility of reagents where the synthetic chemistry is achievable and luminescence is not compromised.

The better-performing analogues showed good luminescence following zinc complexation. While the MeSe and NO_2Ph analogues required cooling in liquid nitrogen, the benzo[f]furo analogue showed excellent room temperature luminescence and gave results comparable to DFO. The results of this preliminary study have given clear indications that the benzo[f]furo analogue is the most promising, and a more detailed evaluation of this compound is warranted including performance in Freon-free formulations.

It is known that rigidity and planarity of the molecule is an important factor in luminescence (19), and this is the likely reason why fused ring structures such as DFO and the benzo[f]furo/Zn complex show good luminescence. The benzo[f]furo analogue itself may not turn out to be a viable operational reagent but the results do suggest that fused ring compounds of this type may be worth exploration. This together with the interesting results that have been obtained with the indandiones (11,12) suggests that further pursuit of C-5 substituted ninhydrin analogues may not be justified.

Acknowledgments

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