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Ninhydrin and Ninhydrin Analogs. Syntheses and Applications

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Contents

1. INTRODUCTION

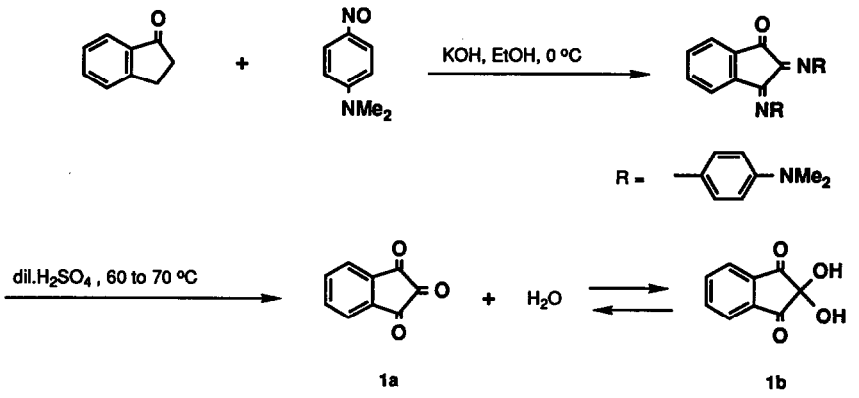
Since its discovery in 1910, ninhydrin (**1a**, 1,2,3-indanetrione or 1,2,3-triketohydrindene) has established itself as an important analytical tool in the fields of chemistry, biochemistry, and forensic science. Despite the widespread application of ninhydrin, most text-books, with few exceptions,[1] either do not list the compound or misrepresent its important reaction with amino groups. The purpose of this review is to initiate a renaissance of interest in this unique compound. This is particularly timely in view of the recent investigations of tricarbonyl compounds in synthesis,[2,3] and the occurrence of this moiety in a natural product such as FK-506, a potent immunosuppressor,[4] and rapamycin, an antifungal antibiotic.[5] The chemistry and early applications of ninhydrin, as well as up-to-date structural and mechanistic studies on Ruhemann's purple (diketohydrindylidenediketohydrindamine or DYDA), the colored compound which results from the reaction of ninhydrin with an amine functionality, will be discussed. Early syntheses of ninhydrin and novel synthetic approaches to ninhydrin analogs will be examined. Finally, the current and projected chemical,

biochemical, and forensic applications of ninhydrin and its analogs will be discussed. This review does not intend to be comprehensive, since there are a number of such reviews in the literature.[6-10]

2. HISTORY

2.1. Discovery

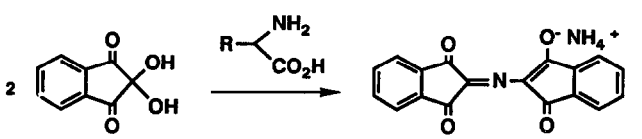
The discovery of ninhydrin was a chance occurrence which had a significant impact on the scientific community. Siegfried Ruhemann, a professor of chemistry at the University Chemical Laboratories at Cambridge University,[11] attempted to make a dicarbonyl compound by the reaction of 1–indanone with *p*–nitrosodimethylaniline, followed by subsequent hydrolysis of the imine (**Scheme 1**).[12] The desired 1,2-indanedione was not formed, but instead a triketone, 1,2,3-indanetrione (ninhydrin) was the final product.[12] Ninhydrin (**1a**) exists as its hydrate in the presence of water (**1b**).



1a 1b

Scheme 1

Perhaps it was also a coincidence that Ruhemann allowed this tricarbonyl compound to react with ammonia and amines.[13] The colored compounds that resulted stimulated Ruhemann's curiosity, and he quickly established the potential of **1a** as a qualitative and quantitative tool in the bioanalytical and bioorganic chemistry of amino acids, peptides, and proteins. In the thorough studies that followed,[14-17] Ruhemann laid down the foundations of ninhydrin chemistry and it is appropriate that the deep blue compound, which he accurately characterized[16] and studied, be known as Ruhemann's purple (**2**).



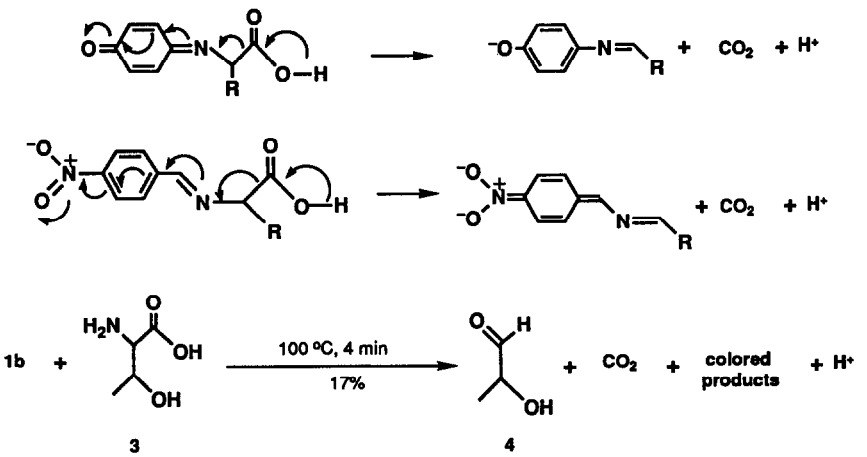
1b 2

2.2. The Mechanism of Formation for Ruhemann's Purple. Reactions of Ninhydrin with Amine Functionalities

Because of its significance in the detection and quantitative estimation of α–amino acids in peptide chemistry, Ruhemann's purple became the focus of numerous structural and mechanistic studies. The reaction of an amine functionality with ninhydrin has been studied for some 80 years. During this time, various mechanistic interpretations have been set forth. The formation of Ruhemann's purple involves three general steps: 1) the initial attack of the amine function on ninhydrin; 2) oxidation and reduction steps leading to intermediates along the pathway; and 3) formation of Ruhemann's purple from these intermediates.

2.2.1. Initial Attack. The initial attack on ninhydrin is shown either as a S_N2 displacement of one of the hydroxyl groups of ninhydrin hydrate by the amine function,[6,18] or as a Schiff's base condensation involving an amine attack on the central carbonyl of the anhydrous keto form.[19,20] Since the keto form of ninhydrin is in equilibrium with its hydrate, it is reasonable to assume that the reaction proceeds by nucleophilic attack on the carbonyl, especially since a Schiff's base is an intermediate in the mechanism.

When ninhydrin reacts with an α-amino acid, ammonia and carbon dioxide are produced, along with an aldehyde with one carbon less than the α-amino acid. This reaction is a special case of the more general Strecker degradation, a transformation in which a carbonyl group conjugated to another carbonyl or nitro group reacts with an α–amino acid to give carbon dioxide and an aldehyde after hydrolysis, (**Scheme 2**).[21] Inorganic oxidizing agents such as ozone, hydrogen peroxide, and silver oxide can also be used to initiate the reaction. The ease with which carbon dioxide is lost depends



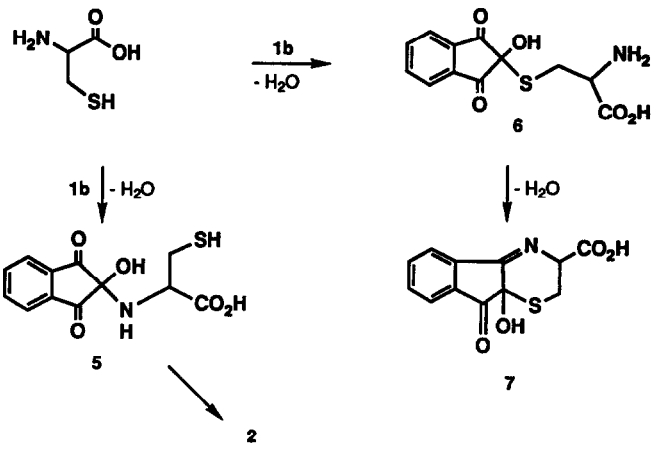
Scheme 2

on the ability of the group alpha to the carbonyl of the acid to accommodate the electron pair provided by the loss of carbon dioxide. The reaction has been used for the quantitative estimation of α-amino acids by measuring the amount of carbon dioxide evolved.[21] The ninhydrin reaction has also been used as an aldehyde synthesis, albeit a poor one (**Scheme 2**). Huff and Rudney synthesized lactaldehyde (**4**) by the reaction of ninhydrin with threonine (**3**).[22]

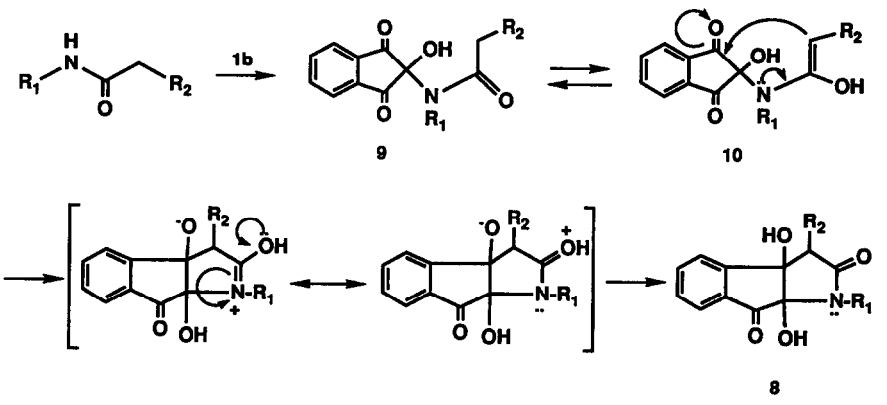
A mechanistic description of Ruhemann's purple formation has to accomodate the fact that α-amino acids react with ninhydrin faster than amines. Compounds that contain other nucleophilic groups close to the reacting amino function form cyclic intermediates that either are not converted to Ruhemann's purple or are converted only slowly. For example, the low color yield observed with aminothiols such as cysteine is due to competitive nucleophilic displacements of the amino and sulfhydryl groups to give products **5** and **6**, respectively (**Scheme 3**).[23] Product **5** can then react further to produce Ruhemann's purple, but product **6** cyclizes to **7**, which prevents further reaction of the amine functionality. Rapid cyclization also occurs with amino acids such as methionine, ornithine, and lysine, as well as with penta- and hexamethylenediamine.[23] These cyclizations are reversible, but the stability of the cyclized product is such that Ruhemann's purple formation is essentially suppressed.

N-Alkylacetamides react with ninhydrin to afford 1,3,3a,8a-tetrahydro-3a,8a-dihydroxy-1-alkylindeno[2,1-*b*]pyrrole-2,8-diones (**8**, **Scheme 4**).[24] This reaction is believed to involve a 2-*N*-alkylacetamido-2-hydroxyindan-1,3-dione (**9**) in equilibrium with its enamino tautomer (**10**). An intramolecular aldol condensation, followed by proton transfer affords product **8**, thereby preventing formation of Ruhemann's purple.

Other amino acids that do not give Ruhemann's purple or evolve ammonia are proline, hydroxyproline, and tryptophan. Cyclic secondary amines and aromatic amines also fail to give **2**, but form colored derivatives corresponding to one molecule of ninhydrin and one molecule of the amine. With aromatic amines, the electron shifts necessary for deamination are energetically unfavorable due to the loss of aromaticity [25]



Scheme 3

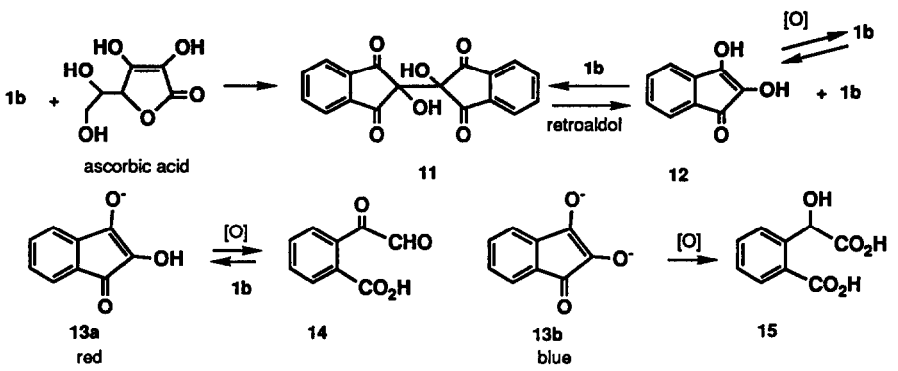


Scheme 4

2.2.2. Proposed Intermediates. A compound that has figured prominently in discussions of the mechanism of the ninhydrin reaction is hydrindantin (**11, Scheme 5**). Authentic samples of this compound may be prepared by reduction of ninhydrin hydrate with ascorbic acid. Retroaldol cleavage of hydrindantin gives 2,3-dihydroxy-1-indenone, the enol form of 2-hydroxy-1,3-indanedione (**12**) and ninhydrin, followed by the rapid air oxidation of compound **12** to ninhydrin. Product **12**, containing an enediol stabilized at the α-position, is a reductone and is expected to have strong reducing properties.[26] Therefore, the reaction of **12** and **1b** to form **11** is an oxidation-reduction reaction. Hydrindantin is sometimes postulated as an intermediate in the pathway to Ruhemann's purple, while in other cases its formation is considered a side reaction. Some of the facts agreed upon are: 1) hydrindantin (**11**) is formed when amino acids react with ninhydrin; 2) compound **11** also reacts with ammonium salts to give Ruhemann's purple; and 3) in dilute alkaline solution **11** gives a red color and in concentrated alkaline solution it gives a blue color.

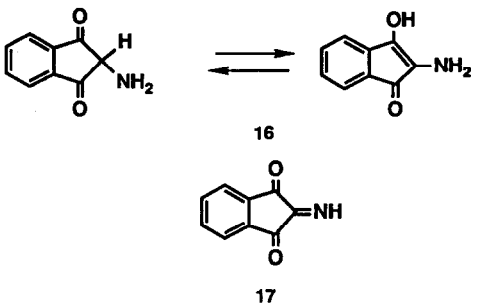
MacFadyen investigated this last observation.[27] The structure of the red-colored compound is believed to be due to the formation of the monovalent anion of the indanone-enediol (**13a**). The blue color is attributed to the divalent ion of this same compound (**13b**). Under anaerobic conditions, the two colors are interconvertible by acid-base titration. The

oxidation products for **13a** and **13b** are *o*-carboxyphenylglyoxal (**14**) and *o*-carboxymandelic acid (**15**), respectively (**Scheme 5**).



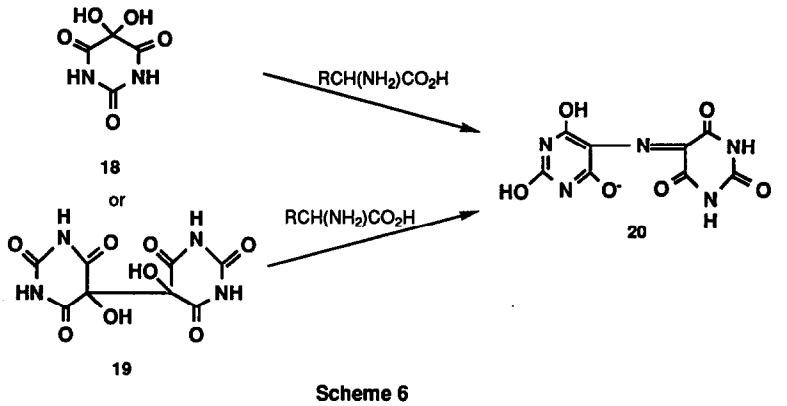
Scheme 5

Another intermediate of interest is 2-amino-1,3-indanedione (**16**). Under some reaction conditions, **16** can readily undergo oxidation to **17**. Mechanistic studies have suggested the intermediacy of **12** and **16**, proposing that the 2-amino derivative (**16**) reacts with ninhydrin hydrate to give Ruhemann's purple or is hydrolyzed to the 2-hydroxy derivative (**12**), which reacts with ninhydrin hydrate and produces hydrindantin (**11**). The conditions of the reaction are the determining factor in the reaction's outcome. It has also been proposed that Ruhemann's purple is actually formed from the reaction of 2-imino-1,3-indanedione (**17**) and 2-hydroxy-1,3-indanedione (**12**).

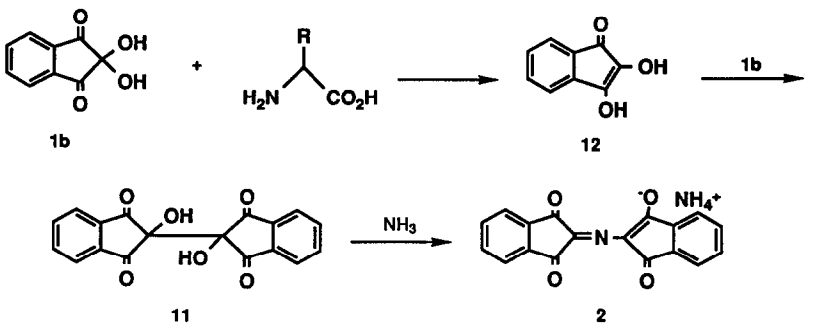


2.2.3. Stability of Ruhemann's Purple (2). The stability of Ruhemann's purple has been investigated by Friedman and Williams who observed the decomposition of **2** under certain reaction conditions.[23] Compound **2** is susceptible to hydrolysis, oxidation, and photolysis. Although it is relatively stable in neutral and basic solutions, the dark blue compound undergoes acid hydrolysis to produce ammonia and hydrindantin (**11**). The acid strength of **2** is comparable to sulfuric acid, and its pK_a is approximately zero. This estimation of the pK_a was based on the observation that the rate of hydrolysis of **2** increases with increasing acidity until it reaches a pH of zero, where it levels off. Given its pK_a, Ruhemann's purple is expected to exist entirely in its ionized salt form in the pH range of 4 to 14. The ionized form of **2** is stable to attack by hydroxide ion. However, strong acid medium will destroy electron delocalization and generate an electrophilic site at the imine function, thus leading to hydrolytic cleavage. Ruhemann's purple is hydrolyzed ten times more slowly in the presence of 80-90% DMSO than in water at room temperature. Oxidizing agents reduce the yield and stability of Ruhemann's purple by oxidizing the intermediates, thereby preventing the formation of the purple compound. Exposure to daylight, especially in the presence of oxygen, also destroys **2**.

2.2.4. Mechanistic Studies of Ruhemann's Purple Formation. As early as 1910, the structure and mechanism for the formation of Ruhemann's purple (**2**) were being investigated by Ruhemann,[12,13,15-17] who assigned a structure to the ammonium salt of the colored product on the basis of its resemblance to murexide (**20**), the product of alloxan (**18**) or alloxantin (**19**) and amino acids (**Scheme 6**).[15] Alloxantin (**19**) bears a striking similarity to hydrindantin (**11**). The mechanism postulated by Ruhemann (**Scheme 7**) failed to explain the details of the formation of 2-hydroxy-1,3-indanedione (**12**) and **2**. He observed that α-amino acids reacted with ninhydrin faster than amines, yet was unable to explain color formation from amines. This phenomenon was later explained by others and will be addressed.



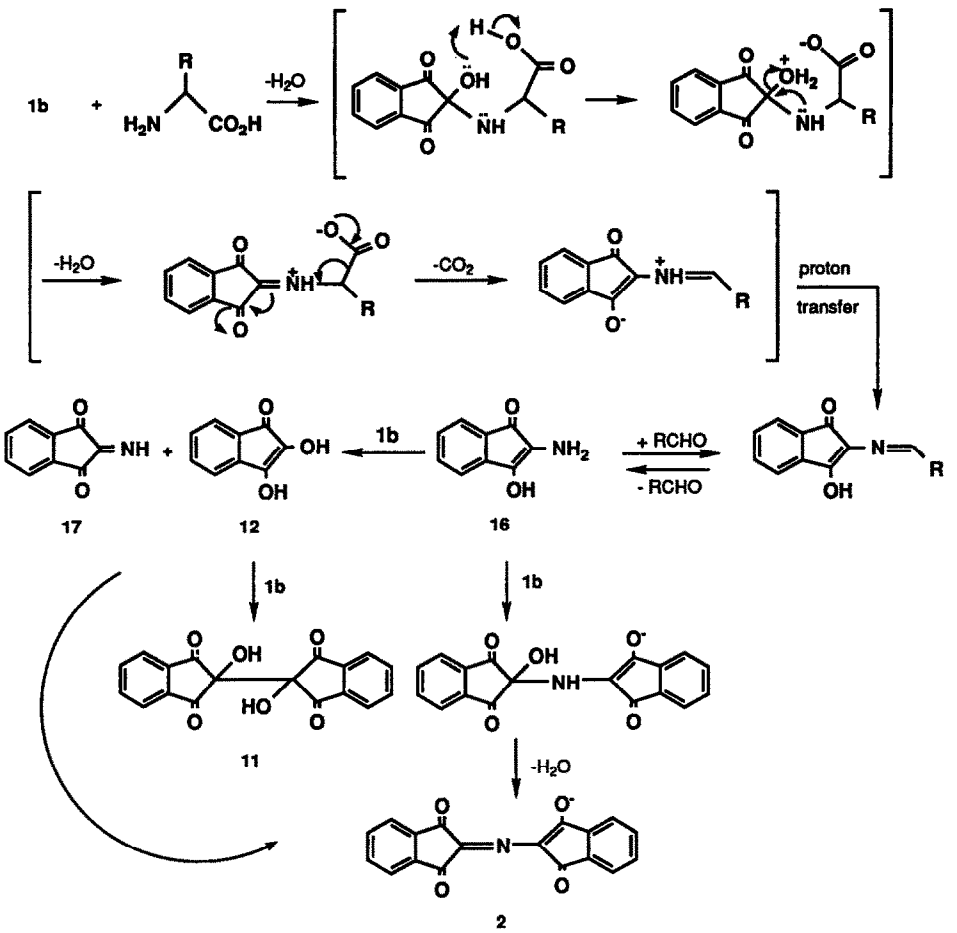
Scheme 6



Scheme 7

Although they agreed with Ruhemann's structural assignment for the colored compound, Harding and Warneford,[28] and Harding and MacLean[29] proposed a slightly modified mechanism of formation. Their interpretation explained how ammonium salts reacted with ninhydrin to produce a colored product,[28] but did not explain why amino acids reacted faster than amines, even though a common intermediate, ammonia, was postulated.[29] The first step of the proposed mechanism involved loss of ammonia from the amino acid to give an α-ketoaldehyde, which was subsequently oxidized to an α-keto acid. Such a reaction is unlikely as this transformation does not occur in the absence of ninhydrin. The source of carbon dioxide is more likely the amino acid rather than the α-ketoacid, since the former loses CO_2 more readily.

Moubasher and Schönberg suggested a mechanism based on the Strecker degradation (**Scheme 2**),[21,30] which was further elaborated by McCaldin,[6] and Johnson and McCaldin[31] to account for the formation of products **2** and **11** in the reaction of ninhydrin with amino acids, imino acids, and amines (**Scheme 8**). The formation of hydrindantin was shown as a side reaction that was not directly involved in the color-forming pathway. Johnson and McCaldin reasoned that since the 2-amino-1,3-indanedione (**16**) was a common intermediate in the formation of both **2** and **11**, excess ninhydrin in amino acid analysis would prevent formation of **11** by driving the equilibria toward product **2**,[31] which was found to be true. The proposed mechanism also explained why amino acids react faster than amines and ammonia, as the former could

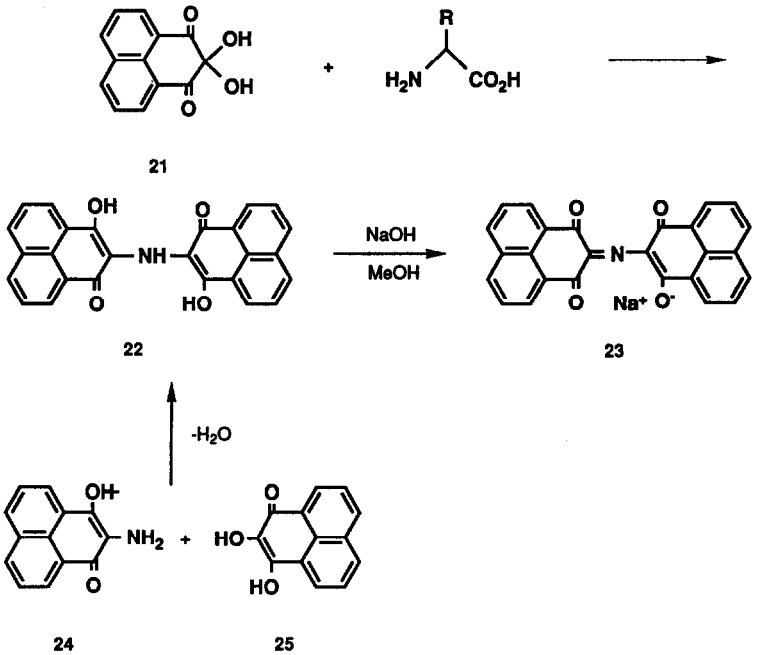


Scheme 8

lose carbon dioxide via the adjacent carboxylate, while the latter involved cleavage of a carbon-carbon or carbon-hydrogen bond. The appearance of the aldehyde, carbon dioxide, ammonia, hydrindantin, and Ruhemann's purple were all rationalized, and a common mechanistic route could be employed for amines, imino acids, and amino acids.

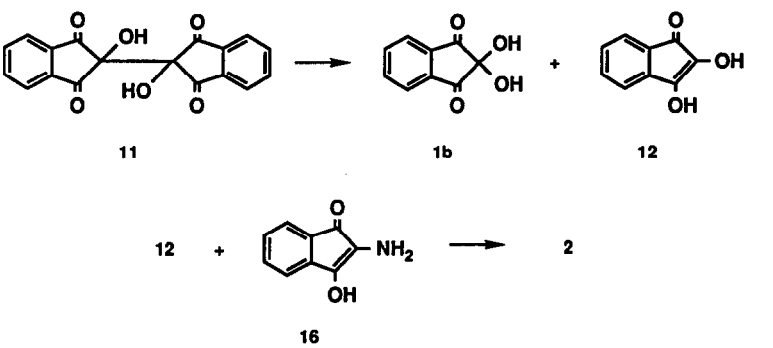
The critical nature of the hydrindantin concentration was also noted by others. MacFadyen and Fowler presented the most thorough study of the role of hydrindantin (**11**),[27] however, their mechanism for Ruhemann's purple formation included dimer **11** as an active participant in the color forming pathway. These authors observed that when hydrindantin reacted with α-amino acids the red color due to hydrindantin disappeared at a rate equal to the formation of Ruhemann's purple. It was postulated that the 2-hydroxy-1,3-indanedione (**12**) came from hydrolysis of hydrindantin (**11**) since one molecule of **12** was used for each molecule of product **2** formed.

Wittmann and co-workers examined the mechanism of the ninhydrin reaction using dihydrophenalenetrione as a model (**Scheme 9**).[32] They reported a direct reaction between two moles of 1,2,3-trioxo-2,3-dihydrophenalene (**21**) and one mole of an α-amino acid to form the corresponding bis(3-hydroxy-1-oxophenalen-2-yl)amine (**22**). This intermediate, on treatment with sodium hydroxide, was reported to form **23**, which is an analog of Ruhemann's purple. The reaction of 2-amino-3-hydroxy-1-oxophenalene (**24**) and 2,3-dihydroxy-1-oxophenalene (**25**) yielded **22**, which afforded **23** after treatment with sodium hydroxide in methanol. Based on their studies, Wittmann suggested a role for **11** in the formation of 2-hydroxy-1,3-indanedione (**12**).[32] As shown in **Scheme 10**, compound **12** could react with 2-



Scheme 9

amino-1,3-indanedione (**16**) to give the analog of **22**, which could form Ruhemann's purple (**2**) under the same conditions reported for **22**.



Scheme 10

Wittmann's mechanism has some drawbacks. Treatment of compound **22** with sodium hydroxide in methanol is shown to result in oxidation. Although a reaction between compound **12** and **16** might give a compound analogous to **22**, dehydrogenation seems unlikely under aqueous conditions. It seems more likely that intermediate **16** is oxidized to 2-imino-1,3-indanedione (**17**) by the ninhydrin present (**1b**). However, their conclusion that compounds **1b** and **12** come from **11** is reasonable.

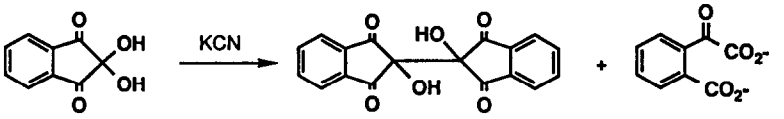
Lamothe and McCormick expanded on the mechanism of the reaction of amino acids with ninhydrin and explained the dependence of color formation on hydrindantin concentration,[33] which had already been noted by MacFayden and others. The behavior of the various intermediates was investigated by voltammetric techniques and rate constants were calculated by conventional kinetic methods and digital simulation programs. Lamothe and McCormick believed that the reaction between **1** and an amino acid first afforded the enolic form of 2-amino-1,3-indanedione (**16**) and an aldehyde

with one less carbon than the original amino acid. The rate of this reaction had already been shown to depend on the nature of the amino acid, with the rate determining step being the nucleophilic displacement of the hydroxyl group of ninhydrin hydrate by a nonprotonated amino group.[18] Lamothe and McCormick observed that the enolic form of 2-amino-1,3-indanedione (**16**) underwent hydrolysis to give 2-hydroxy-1,3-indanedione (**12**) and ammonia.[33] They suggested that compound **16** (-0.05 v) was unstable in a solution containing ninhydrin. An oxidation-reduction reaction should take place to give the corresponding 2-imino derivative **17** and **12**. 2-Amino-1,3-indanedione (**16**) is a better reducing agent than ascorbic acid (+0.2 v), which is known to reduce **1**. Compounds **17** and **12** would then react to form **2**, which was shown to be favored by a factor of seven over the hydrolysis of **17** when externally-added ninhydrin was present.

The factors that affect the stoichiometry of formation of Ruhemann's purple were considered in detail by Friedman and Williams and their proposed mechanism is shown in **Scheme 11**.[23] This mechanism is very similar to that of Lamothe and McCormick,[19] but does not incorporate the last step before the formation of Ruhemann's purple, where the 2-imino-1,3-indanedione (**17**) reacts with **12** to give the colored adduct. Furthermore, Friedman and Williams proposed an initial Schiff's base condensation rather than a nucleophilic displacement.[23] They showed that different amino acids react with ninhydrin at different rates and, since the reaction involves multiple steps, there are several stages at which the amino function might be diverted from forming Ruhemann's purple. However, the loss of carbon dioxide and the formation of the aldehyde are essentially irreversible.

In most analytical methods (pH ≥ 4, excess ninhydrin), the intermediate 2-amino-1,3-indanedione (**16**) could be rapidly trapped by excess ninhydrin to form Ruhemann's purple (**Scheme 11**). However, at low ninhydrin concentration, **16** could degenerate to ammonia and 2-hydroxy-1,3-indanedione (**12**) before being trapped by **1**. Low pH gave rise to fast acid-catalyzed hydrolysis of **2**. High pH and high hydrindantin concentration yielded Ruhemann's purple nearly quantitatively, while at low pH and low hydrindantin concentration the yield of **2** was greatly diminished. At high pH the amino group exists in its neutral form and can therefore act as a nucleophile. At low pH the protonated amine is no longer nucleophilic. Because all steps are reversible, excess **11** should drive the equilibrium towards **2**. Variable amounts of Ruhemann's purple are formed from the reaction of ammonia and ninhydrin, even in the absence of **11**.

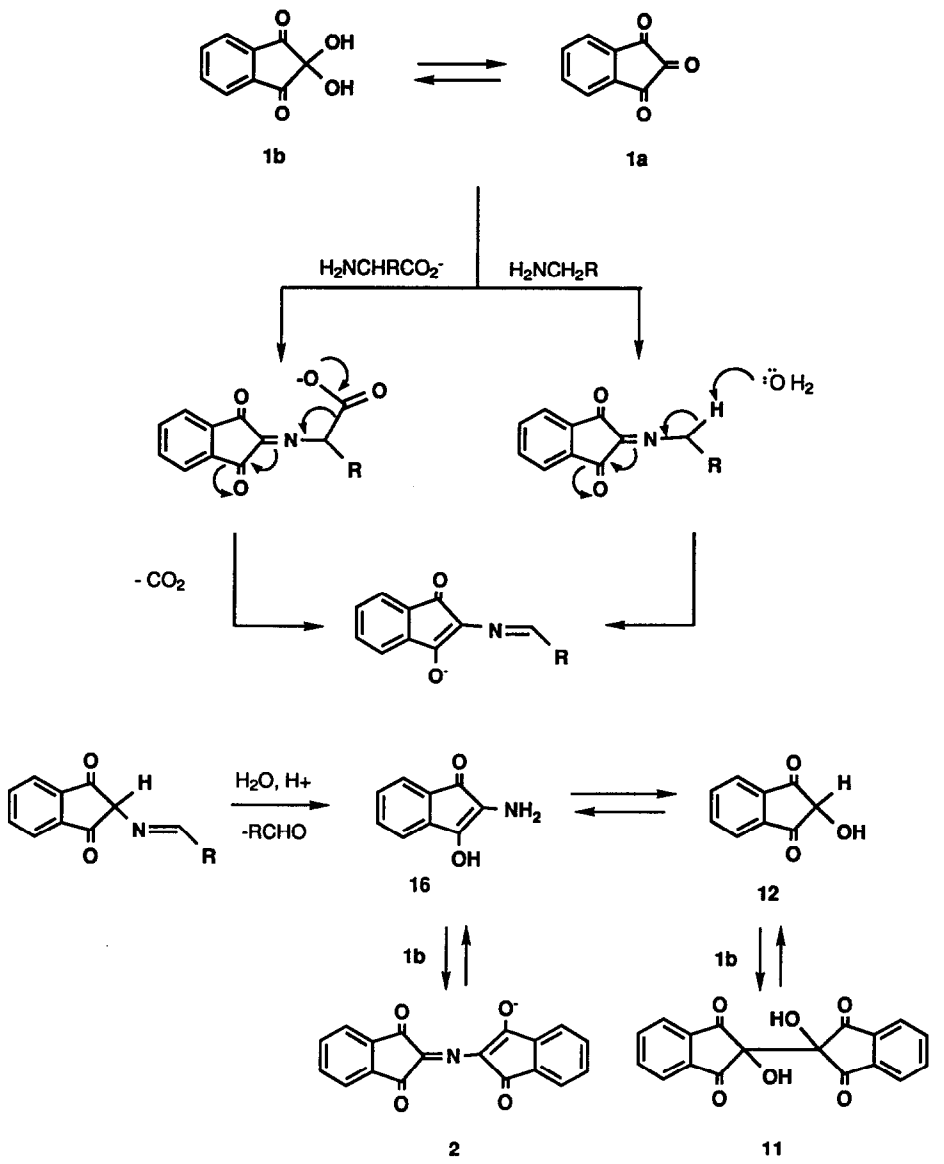
In the presence of nucleophiles such as cyanide ion, at pH levels below 6, ninhydrin disproportionates to give hydrindantin and phthalonic acid (**26**).[34]



1b **11** **26**

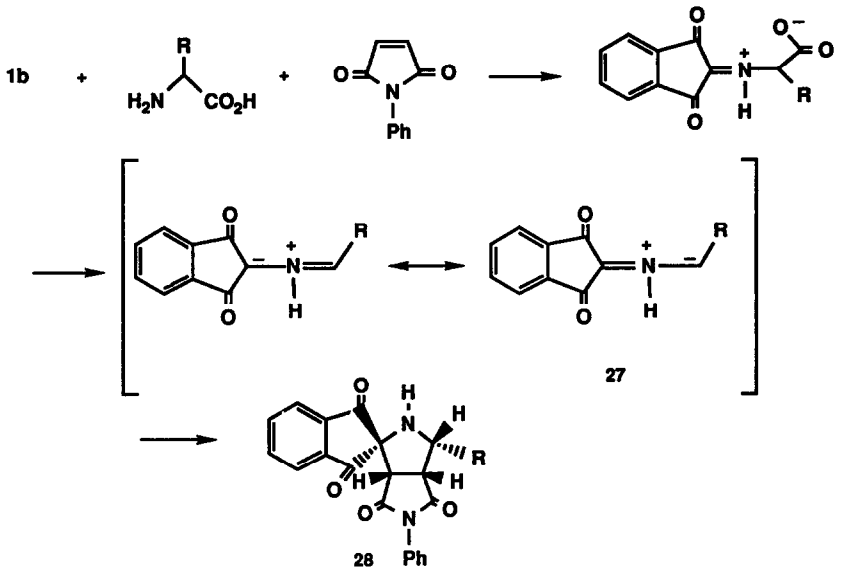
In 1978, Bottom[20] reviewed the possible mechanisms for the formation of Ruhemann's purple and concluded that the one postulated by Friedman and Williams[23] was the most consistent with the experimental findings. It may be difficult to discount the conclusions of Lamothe and McCormick, who suggest that 2-imino-1,3-indanedione and 2-hydroxy-1,3-indanedione are the actual reactants in Ruhemann's purple formation.[19] However, both pathways involve an intermediate 2-amino-1,3-indanedione (**16**). Free ammonia rather than compound **16** is shown in the mechanisms proposed by Ruhemann and in those found in most organic chemistry textbooks. Ninhydrin is assumed to react with an amino acid in its carboxylate form to give Ruhemann's purple. In the case of an amino acid, an imino acid, or an amine, a common aldimine is formed, which is hydrolyzed to the intermediate 2-amino derivative **16**. Compound **16** can then react with more ninhydrin to give Ruhemann's purple and/or be oxidized to **17**, which reacts further with **12**.

The involvement of a 1,3-dipolar species was invoked in the decarboxylative transamination of α-amino acids, and this reaction is directly relevant to the mechanism of the ninhydrin reaction with α-amino acids.[35-37] 1,3-Dipole formation is a prototropic process that involves a formal 1,3-hydrogen shift in "X=Y-ZH" systems where the central atom must possess a lone pair of electrons. This behavior is exhibited by many imines, hydrazones, and oximes, and can be demonstrated by trapping their 1,3-dipoles as cycloadducts. Imines of α-amino acids and their esters also provide good examples of this prototropic process.[35-40] The transamination proceeds in such a way that the intermediate imine undergoes decarboxylation via a zwitterionic form that generates the 1,3-dipole. The final location of the proton in the neutral imine product depends upon a kinetically-controlled proton transfer to the site of the dipole with the greatest electron density.



Scheme 11

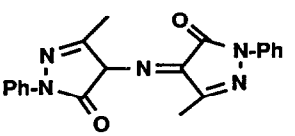
Reaction of ninhydrin with an α-amino acid, in the presence of *N*-phenylmaleimide, gave the corresponding cycloadduct (**28**, **Scheme 12**) with stereospecificity and in good isolated yield via an *endo* transition state.[34] Decarboxylative transamination produces the aza-allylic species **27**, which can undergo cycloaddition when an appropriate dipolarophile is present. Interestingly, when alternative carbonyl compounds are employed, more vigorous reaction conditions are necessary.



Scheme 12

2.3. Studies on the Structure of Ruhemann's Purple

In 1911, Ruhemann elucidated the structure of the violet compound **2**, he had obtained. He recognized that this product was the carbocyclic analog of known compounds such as purpuric acid, the unstable acid corresponding to murexide (**20, Scheme 6**), and rubazonic acid (**29**).[16] The structural assignment of **2** met with unquestioned acceptance



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throughout many decades. Ruhemann postulated that the ammonium cation associated with **2** was involved in its color. However, both MacFayden,[41] and Moore and Stein [42] showed that the color was intrinsically associated with the anion and that the sodium and potassium salts of **2** had identical molar absorption coefficients. In 1948, Moore and Stein reported the elemental analysis of Ruhemann's purple, which was in agreement with the original structure.[42] An infrared spectrum of **2** showed an absorption at 1670 cm^{-1}, which was consistent with a delocalized structure.[43]

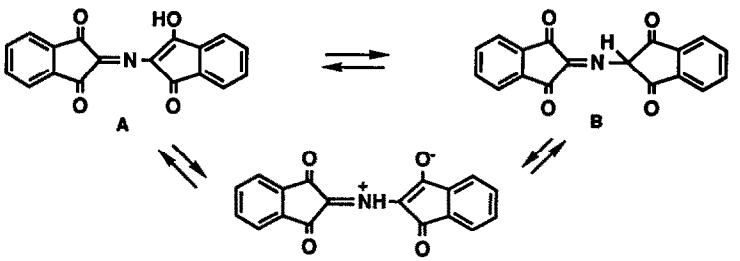
Friedman observed two resonances of equal intensity in the aromatic region of the proton nmr spectrum (DMSO-d_6) of compound **2**.[25] These signals were attributed to the aromatic protons of the two nonequivalent indanedione rings. The aryl groups are equivalent when the resonance delocalization of **2** is taken into account, therefore, these protons should exhibit one signal. It had been suggested that the two resonances might be due to protons *ortho* and *meta* to the five-membered rings, but this view was dismissed since these protons were shown to exhibit chemical shift equivalence.

Wigfield and co-workers decided to re-evaluate previous results and assigned the aromatic protons unequivocally.[44] Their studies involved multiple reactions of ninhydrin with glycine, followed by ^{1}H-nmr analysis of the adduct. The spectra obtained by Wigfield showed considerable variations and were not reproducible from one reaction to another.[44] In some instances the δ 7.65 signal was considerably more intense than the signal at δ 8.00. Sometimes the latter signal was not present. Other times an additional signal was observed at δ 6.50 with an intensity approximately 25% of the signal at δ 8.00. Both δ 6.50 and 8.00 signals diminished over time with the simultaneous appearance of signals at δ 8.07 and 7.70. The resonances at δ 6.50 and 7.70 disappeared upon addition of D_2O.

Wigfield rationalized that the signal at δ 7.65 must be the resonance corresponding to the aromatic protons of Ruhemann's purple.[44] The other signals at δ 6.50 and 8.00 were believed to be hydrindantin (**11**). This was confirmed by an independent examination of the ^{1}H-nmr of **11**. The disappearance of the signals at δ 6.50 and 8.00 is in agreement with the chemistry of hydrindantin, which undergoes retroaldol cleavage to ninhydrin and 2-hydroxy-1,3-indanedione (**12**) with subsequent air oxidation of **12** to **1**. An authentic sample of ninhydrin shows these same two signals at δ 8.07 and 7.70.

Wigfield also studied the ^{13}C-nmr of compound **2** and observed the expected 5-line pattern with the expected multiplicity patterns using off-resonance decoupled spectra.[44] Signals at 120.7 ppm and 132.8 ppm were assigned to the aromatic carbon atoms *ortho* and *meta* to the ring junction. The signal at 138.4 ppm was found to be solvent dependent. In DMSO, it was considerably more intense than a signal at 139.9 ppm, while in DMSO-d_6 the 138.4 ppm resonance was not observed. Wigfield assigned the signal at 138.4 ppm to the carbonyl carbons and the signal at 139.9 ppm to the ring junction carbons.

Under mild conditions the unstable parent acid **30** of Ruhemann's purple, which can exist as three tautomers, can be prepared. Wigfield and co-workers believed the ^{13}C-nmr data to be most consistent with tautomer **B**.[44] They attributed the symmetric 5-line pattern to any one of the tautomers (**A-C**), but interpreted the carbonyl signal at 181.7 ppm as support for the tetracarbonyl structure **B**. An upfield shift would have been expected for either **A** and **C** because of the the enolic nature of these tautomers. The infrared spectrum carbonyl absorption at 1680 cm^{-1} of the parent acid was also judged to be in agreement with structure **B**. It was assumed that the solubility of **30** ruled out the zwitterionic structure **C**.

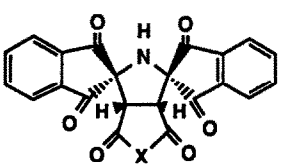


A **B** **C**

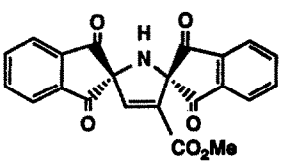
30

A more recent investigation of protonated Ruhemann's purple (**30**) did not corroborate Wigfield's assignment,[44] but rather showed the parent acid of **2** to be the stable *N*-protonated zwitterion (**C**).[34] The zwitterion assignment of protonated **2** was based on trapping experiments and X-ray analysis.[35] The ultraviolet spectrum of protonated Ruhemann's purple **30** [λ_{max} $CHCl_3$ 485 (ε 16,000)] was also in accord with a zwitterion. The cycloaddition reactions ($4\pi + 2\pi$) of **30** with dipolarophiles such as *N*-phenylmaleimide, maleic anhydride, and methyl propiolate produced the corresponding cycloadducts in yields of 89% (**31**), 75% (**32**), and 76% (**33**), respectively, lending support for the zwitterionic structure **C**.

In conclusion, Ruhemann's original structural assignment seemed essentially correct. By employing modern spectroscopic techniques, Grigg and co-workers have established that **30** is a zwitterion with the positive charge on nitrogen.[34] However, considering the tautomeric nature of **30**, one might suggest that **A-C** are equilibrating and are dependent on solvent and pH. The effects of these conditions were not considered by Wigfield[44] or Grigg.[35] Further investigations might elucidate the influence of solvent and pH on this tautomeric equilibrium.



(**31**) X = NPh
(**32**) X = O



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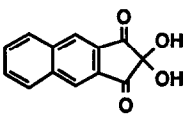
3. SYNTHESES OF NINHYDRIN AND ITS ANALOGS

3.1. Introduction

Ruhemann's synthesis of ninhydrin from 1-indanone and the subsequent discovery of the reaction of **1** with amino functions were the result of serendipity. Ruhemann and other investigators soon realized the potential of this reagent in the analysis of amino acids. The many applications of ninhydrin in chemistry, biochemistry, and its recent use in forensic science, stimulated numerous studies to find more efficient routes to the 2,2-dihydroxy-1,3-indanedione system.

Ninhydrin is the most commonly employed reagent for revealing latent fingerprints on paper and other porous surfaces, where it reacts with the amino acids of fingerprint deposits that are released by eccrine sweat glands. This technique although useful, is not without problems. The development of analogs with improved optical properties to meet the specific requirements of forensic applications was proposed. It was reasoned, *a priori*, that other triketones might react with amino acids as well or better to produce colored derivatives with the desired characteristics. Chemical modifications of ninhydrin were undertaken to produce derivatives better suited to forensic needs. The shortcomings of ninhydrin-developed fingerprints relate to problems with contrast and visualization. On dark surfaces (e. g. brown paper bags) or melamine-coated surfaces (e. g. bank notes, checks, or postal money orders), background coloration obscures the contrast between the background and the fingerprint, which prevents successful print retrieval. A solution to this problem is to treat the ninhydrin-developed fingerprints with group II metal salt solutions of zinc, cadmium, or mercury. This protocol changes the color of the print and, in some instances, makes it photoluminescent. However, the procedure is not applicable to paper surfaces with high background luminescence. Latent fingerprint detection can also be improved by using lasers, which serve to excite fingerprint fluorescence and are used in conjunction with metal salt treatment and chemical modifications of ninhydrin to enhance visualization. In 1985, Almog and Menzel reported that latent prints treated with benzo[*f*]ninhydrin (**34**) and sprayed with zinc chloride, exhibited greater detectability using a neodymium:yttrium aluminum garnet (Nd:YAG) laser than those treated with the parent compound.[45] Subsequent investigations have



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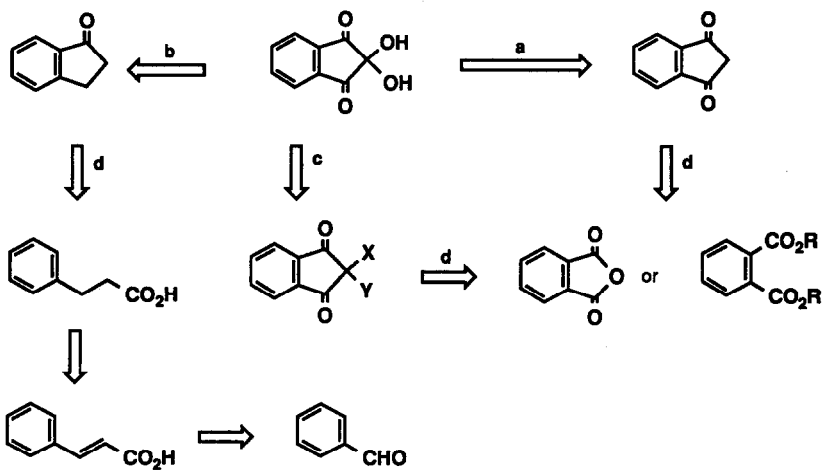
produced other ninhydrins with desirable fluorogenic properties that will be discussed in **Section 4.4.3.** Because of the usefulness of ninhydrin analogs and derivatives as reagents in the detection of latent fingerprints either before or after treatment with a metal salt solution and/or exposure to laser light, the synthesis of substituted ninhydrins is an area of practical importance. The early synthetic efforts, the problems encountered, and the more recent developments in the field will be discussed.

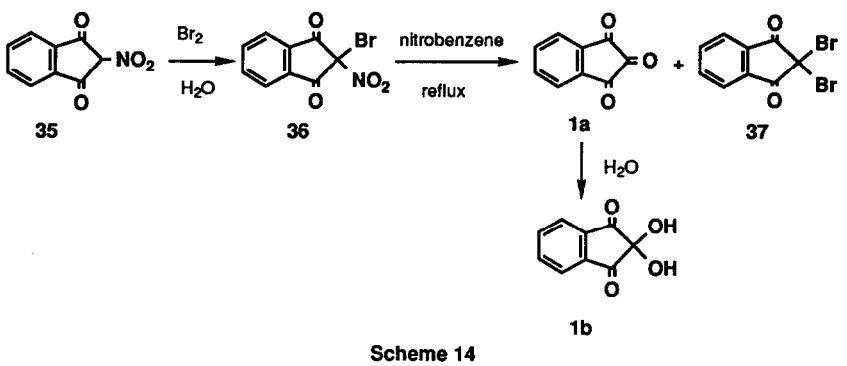
3.2. Retrosynthetic Analysis and Early Syntheses

Before chronologically reviewing the syntheses of ninhydrin and its analogs, the oxidation and cyclization methodologies employed will be discussed. Many of the syntheses share common features, as shown in the retrosynthetic analysis in **Scheme 13.** The majority of syntheses of ninhydrin and its analogs have involved the oxidation of the C-2 position of a 1,3-indanedione derivative as the final step (path a, **Scheme 13**), although a few recent investigations have chosen to oxidize the 1-indanone (path b), as Ruhemann did. Some of the early oxidation procedures employed were: 1) treatment with *p*-nitrosodimethylaniline, followed by hydrolysis;[12] 2) selenium dioxide oxidation in aqueous solution;[46,47] 3) diazotization, treatment with *tert*-butyl hypochlorite, and hydrolysis;[48] and 4) nitration and bromination of the 2-position with subsequent hydrolysis of the 2-bromo-2-nitro-1,3-dione.[49]

The first procedure was devised by Ruhemann (**Scheme 1**).[12] Both procedures 2 and 3 involve safety hazards as they utilize the toxic reagents selenium dioxide and tosyl azide, respectively, but some recent syntheses of ninhydrin analogs have utilized these methods.[50,51] The last protocol was introduced by Wanag and Lode in 1938 (**Scheme 14**), after attempts to oxidize the 2-position *via* hydrolysis of the 2-oximino group and oxidation using cerium sulfate failed.[49]



Scheme 13

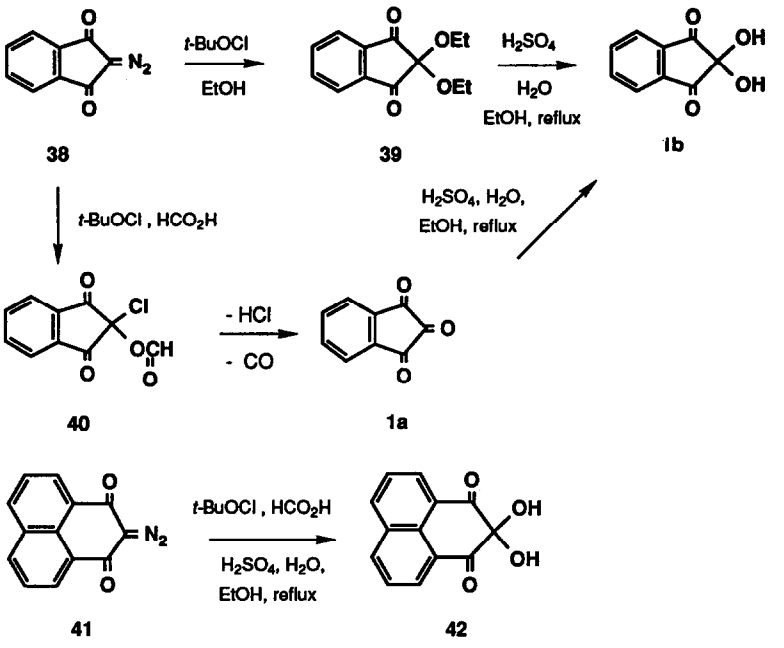


Scheme 14

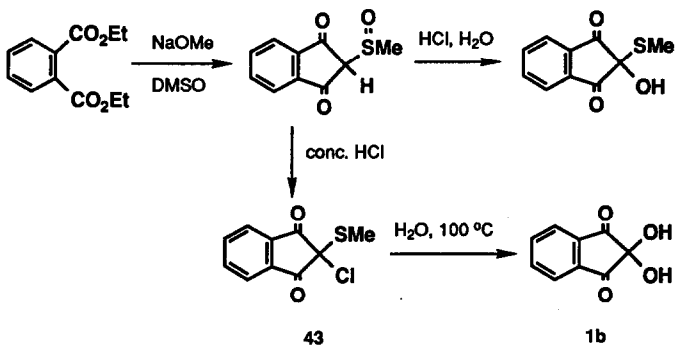
Nitration of 1,3-indanedione afforded 2-nitroindanedione (**35**), which was brominated to yield **36**. When **36** was heated in nitrobenzene at 210 °C, decomposition occurred to give equal parts of ninhydrin (**1a**) and 2,2-dibromo-1,3-indanedione (**37**). Recrystallization of ninhydrin from water afforded the hydrate. Although this synthesis involved several steps and produced equal parts of ninhydrin and derivative **37**, the procedure was simple and inexpensive, and the overall yield was only slightly lower than those obtained by other methods.[49] Subsequently, it was found that the 2,2-dibromoindanedione **37** could also be converted to ninhydrin in high yield (see **Section 3.3.1.**).

Procedure 3 was used by Regitz and Adolph to synthesize ninhydrin from 2-diazo-1,3-indanedione (**38, Scheme 15**).[52] 4,5-Benzo-1,2,3-indanetrione (benzo[*e*]ninhydrin) and 5,6-benzo-1,2,3-indanetrione (benzo[*f*]ninhydrin, **34**) were also prepared in both their anhydrous and hydrated forms from the corresponding 2-diazo derivatives.[52] When compound **38** was treated with *tert*-butyl hypochlorite in ethanol, 2,2-diethoxy-1,3-indanedione (**39**) was formed. When the reaction was run in formic acid, 2-chloro-2-formyloxy-1,3-indanedione (**40**) was obtained. Heating either product in mineral acid provided **1b** in good yield (86%). The same investigators also oxidized 1,3-dioxo-2-diazo-1,2-dihydrophenalene (**41**) to 1,2,3-trioxo-1,2-dihydrophenalene (**42**) utilizing this method.[53]

In some instances, cyclization of a precursor to give the fused five-membered ring dione gives an intermediate with the correct oxidation state at the 2-position, and then only functional group interconversion is needed to obtain the tricarbonyl function (path c, **Scheme 13**). The earliest synthesis involving such a transformation is that of Becker and Russell (**Scheme 16**).[54,55] Condensation of diethyl phthalate with dimethylsulfoxide in the presence of sodium methoxide gave 2-methylsulfinyl-1,3-indanedione, which underwent a facile Pummerer rearrangement with a variety of nucleophiles to yield α-substituted sulfides. For example, treatment with concentrated hydrochloric acid gave the



Scheme 15



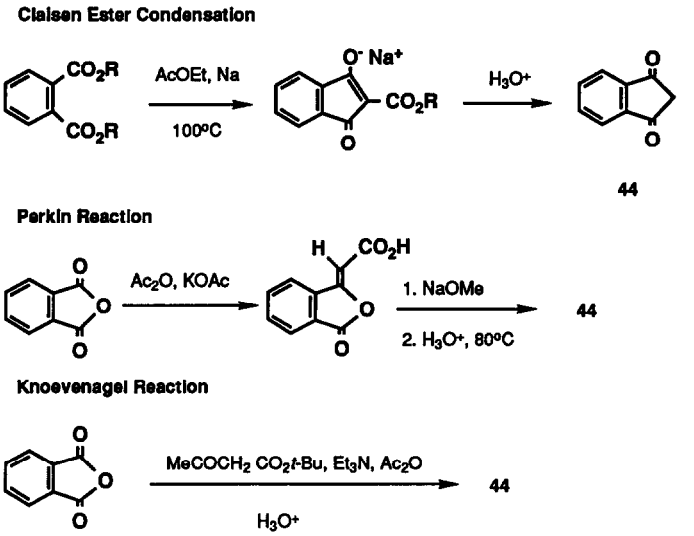
Scheme 16

corresponding 2-chloro-2-(methylthio)-1,3-indanedione (**43**), which yielded 2-hydroxy-2-(methylthio)-1,3-indanedione in dilute acid. The 2-hydroxy derivative could not be converted to **43** by treatment with concentrated hydrochloric acid, thereby supporting a direct rearrangement mechanism. Diketone **43** was quantitatively hydrolyzed to **1b** in boiling water.

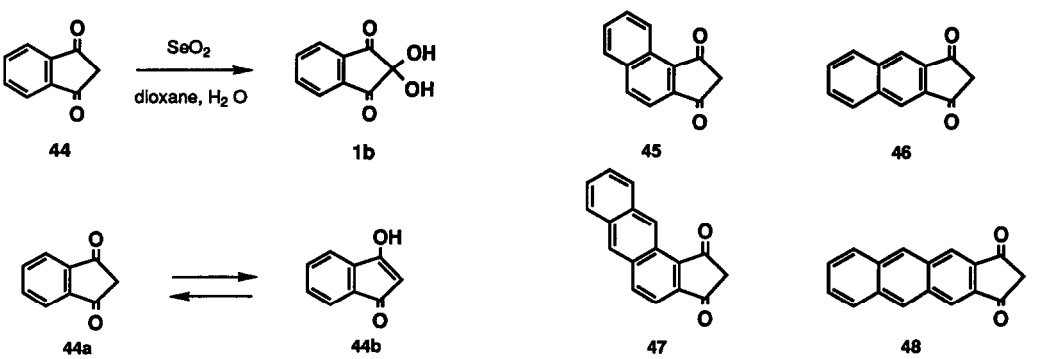
Cyclization precursors have typically involved an intact aromatic *ortho* diester or anhydride that can undergo ring closure to give the fused five-membered ring (path d, **Scheme 13**). Historically, three synthetic procedures (Claisen, Perkin, and Knoevenagel) have been utilized to obtain the fused ring system (**Scheme 17**).

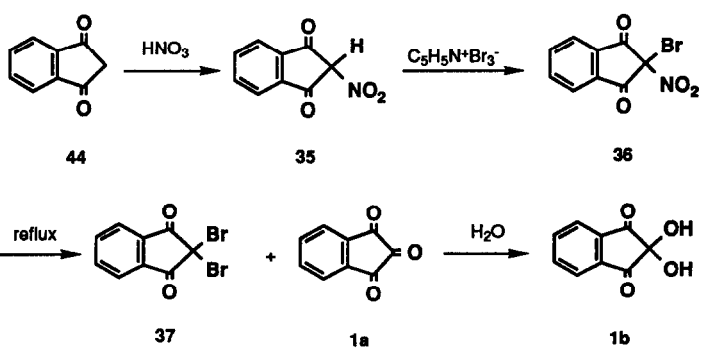
In 1933, Teeters and Shriner used the Claisen condensation to synthesize ninhydrin (**1b**).[46] The 1,3-indanedione was obtained in 70% yield, and was then oxidized with selenium dioxide to give ninhydrin in 25% overall yield. Fieser also used this cyclization method to prepare ninhydrin (**Scheme 18**).[56,57]

Although all 1,3-indanediones are shown in their keto form as **44a**, it should be kept in mind that these compounds are tautomeric, and in aprotic solvents they are predominantly enolized as in **44b**.

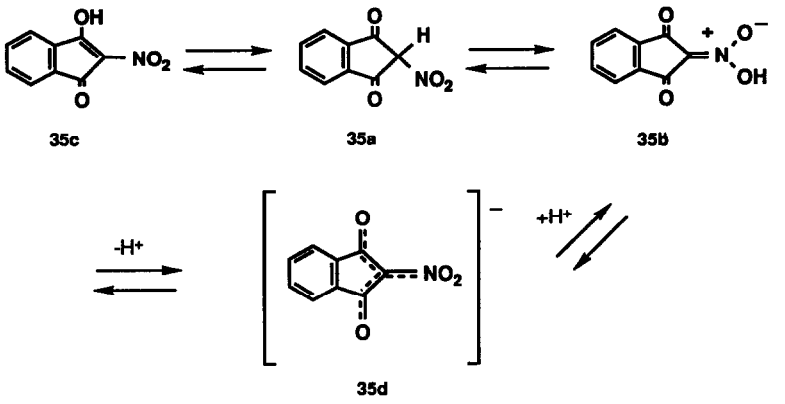


Scheme 17





Scheme 18



2-Nitro-1,3-indanedione (**35**) also exists in a tautomeric equilibrium. Bromine titration of **35** in several solvents showed that this compound existed predominantly in the nitronic acid form (**35b**) in polar solvents (water, ethanol, acetic acid). In benzene, and to a lesser extent in ether, the 2-nitro-1,3-indanedione is the major tautomer (**35a**). This solvent effect is opposite to that found in other α-nitroketones. The conjugate base (**35d**) is a delocalized species. The other enol form (**35c**) does not seem to be important in either protic or aprotic solvents.[58]

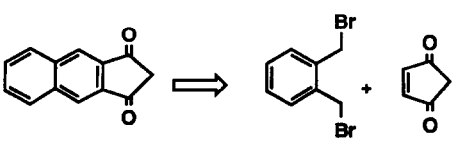
In 1957, Meier and Lotter synthesized 4,5- and 5,6-benzo-1,3-indanediones (**45** and **46**), as well as naphtho[2',3':4,5]- and naphtho[2',3':5,6]-1,3-indanediones (**47** and **48**) by this method.[47] The cyclization yields for the benzo-1,3-indanediones were similar to the yield obtained by Teeter and Shriner for **44**,[46] but were significantly lower for the naphtho-1,3-indanediones **47** and **48** (48% and 35% yields, respectively). Diones **45** and **46** were oxidized to the corresponding benzo[*e*]- and benzo[*f*]ninhydrins (53% and 23% yields, respectively) using selenium dioxide, but **47** and **48** could not be oxidized under the same conditions, quinones being the only products identified in these reactions.

The Perkin reaction was also used to prepare **44** as shown in **Scheme 17**. Dominguez used this procedure in an undergraduate laboratory synthesis of ninhydrin to give the 1,3-indanedione in 67% yield.[59] Oxidation with selenium dioxide afforded **1b** in 31% yield.

The third common method of cyclization was the Knoevenagel reaction (**Scheme 17**). This strategy has been the most utilized in the syntheses of ninhydrin and its analogs.

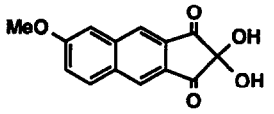
The methodologies illustrated in **Scheme 17** present synthetic problems when the target molecule is a substituted ninhydrin or a benzo[*f*]ninhydrin. The availability of starting materials is one concern. For example, 2,3-dimethylnaphthalene used to prepare the *ortho* diester precursor of benzo[*f*]ninhydrin is no longer commercially available. Another problem involves the altered reactivity of the aryl diester or anhydride when substituent groups are present on the aromatic ring. Both cyclization and oxidation may be affected by the presence of substituents, and it is important to recognize that a general synthesis for ninhydrin itself does not always constitute an equally applicable synthesis for an analog. Finally, the introduction of the desired substituents on the aryl ring at appropriate positions may be difficult.

These synthetic problems are especially acute for the benzo derivatives that require substituted 2,3-naphthalenedicarboxylates as precursors. Consequently, new ways to prepare the starting materials or totally novel strategies were needed to circumvent the difficulties inherent in the traditional methods. A new approach has utilized an intact five-membered ring and a Diels-Alder addition for annelation, followed by aromatization and oxidation to



benzo[*f*]ninhydrin. Joullié and co-workers have developed this procedure, optimized its conditions, and tested its general applicability.[60,61] This methodology was used in the syntheses of benzo[*f*]ninhydrin (**34**),[60] 6-methoxybenzo[*f*]ninhydrin (**49**) and thieno[*f*]ninhydrin (thieno[2',3':4,5]-1,2,3-indanetrione) (**50**).[61]

These syntheses will be discussed in **Section 3.3.4.**



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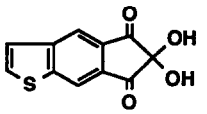

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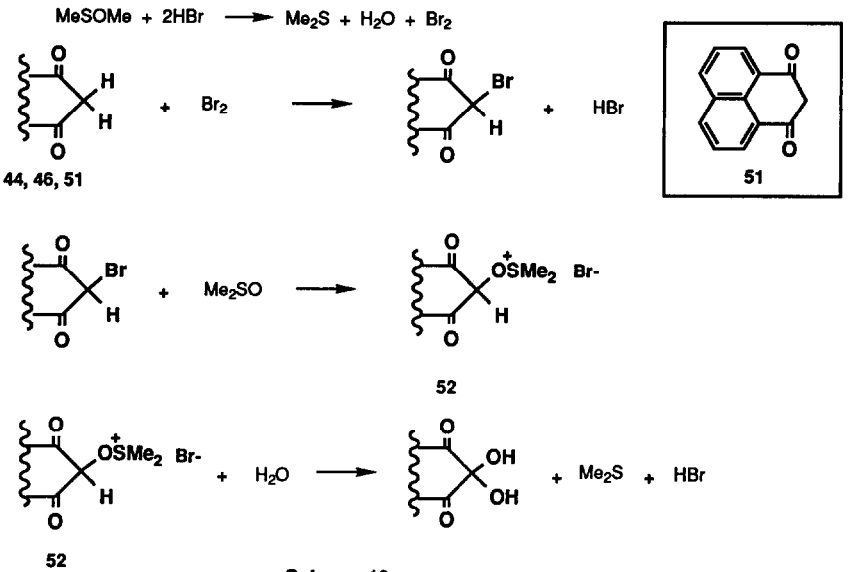
3.3. Other Approaches to Ninhydrin and Analogs

3.3.1. Syntheses Utilizing ortho-*Substituted Aromatics.* In search of selective methods to oxidize the C-2 position of 1,3-indanedione, Schipper and his colleagues employed the reaction of hydrobromic acid in dimethylsulfoxide to convert 1,3-indanedione (**44**), 1,3-benzo[*f*]indanedione (**46**), and 1,3-dioxo-1,2-dihydrophenalene (**51, Scheme 19**) to

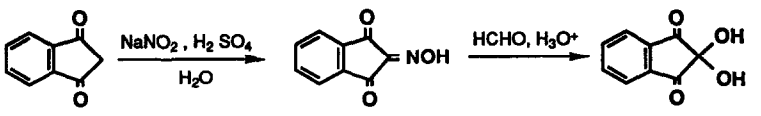


Scheme 19

the corresponding 2,2-dihydroxy products **1b, 34, and 21**, respectively.[62-64] This methodology achieved the oxidation of activated methyl and methylene groups to carbonyl groups. A molar excess (3-8 moles) of dimethyl sulfoxide was needed. The acids were hydrogen halides, preferably in anhydrous form, but concentrated aqueous solutions could be used. Hydrobromic acid was the hydrogen halide of choice. Only a small amount of acid was needed (0.05-0.5 mole is sufficient for each mole of compound to be oxidized). The mechanism was believed to involve the reaction of the acid with dimethyl sulfoxide to generate molecular bromine, which in turn reacted with the 1,3-dione. The resulting 2-bromo-1,3-dione then underwent nucleophilic displacement at the C-2 position to afford a dimethylalkoxysulfonium bromide (**52**), a postulated intermediate in the Kornblum oxidation.[65] Under the reaction conditions this compound collapsed to the triketone, dimethyl sulfide, and regenerated hydrogen bromide. Hydrolysis of the triketone provided an 80% yield of ninhydrin from the 1,3-indanedione. A variation of this method consisted of treating 1,3-indanedione with a molar excess of dimethyl sulfoxide in the presence of about 0.25 to 1.0 mole of bromine.

In order to develop a commercial preparation for ninhydrin, Wood patented a procedure involving the quantitative generation of 2-oximino-1,3-indanedione from **44** and sodium nitrite in aqueous sulfuric acid.[66] Although Wanag and Lode[49] had been unable to hydrolyze the β-oxime of ninhydrin, treatment of the 2-oximino-1,3-indanedione (**53**) with an aqueous solution of formaldehyde and mineral acid produced ninhydrin in over 90% yield.

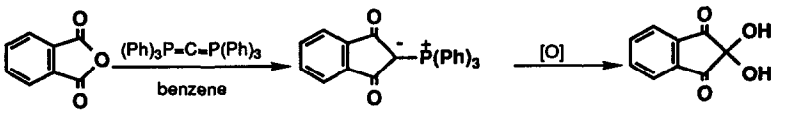
Jones and Wife[67] incorporated the cyclization method of Becker and Russell[54] (**Scheme 16**) in the synthesis of benzo[*f*]ninhydrin (**34**). Using the procedure of Meier and Lotter,[47] the desired 5,6-benzo-1,3-indanedione (**46**) could



44 53 1b

only be obtained in 18% yield. Condensation of dimethyl sulfoxide with dimethyl naphthalene-2,3-dicarboxylate, followed by Pummerer rearrangement to 2-chloro-2-methylthio-5,6-benzo-1,3-indanedione occurred in 49% yield. Hydrolysis in a boiling aqueous solution gave a 93% yield of benzo[f]ninhydrin (**34**).

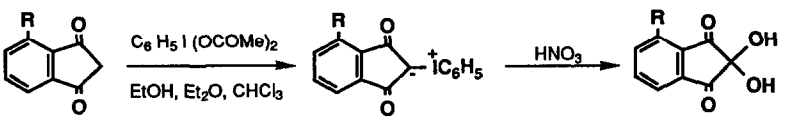
A novel approach to the 1,3-indanedione (**44**) involved the reaction of phthalic anhydride with hexaphenylcarbodiphosphorane in benzene to give the intermediate ylid, 2-triphenylphosphinyl-1,3-indanedione (**54**), in 60% yield .[68] Oxidation of **54** to ninhydrin using ozone in methylene chloride was reported to occur in 80% yield. Selenium dioxide and trifluoroacetic acid, or chromium trioxide in glacial acetic acid were also reported to be suitable oxidizing agents. The advantage of this procedure is that it involves a nonaqueous medium. Bestmann and Kloeters reported a high yield



54 1b

for this synthesis,[68] but attempts in our laboratory to prepare substituted ninhydrins from the corresponding substituted phthalic anhydrides have been unsuccessful.

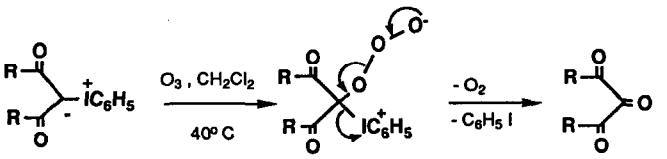
In their study of iodonium derivatives of β-diketones, Prikule and Neiland developed another method for the oxidation of 1,3-indanediones.[69] Addition of a solution of iodosobenzene diacetate, a stable, convenient oxidizing agent, to 1,3-indanedione afforded the corresponding phenyliodonium ylid **55** in good yield. On treatment with nitric acid, ylid



44 55 1b

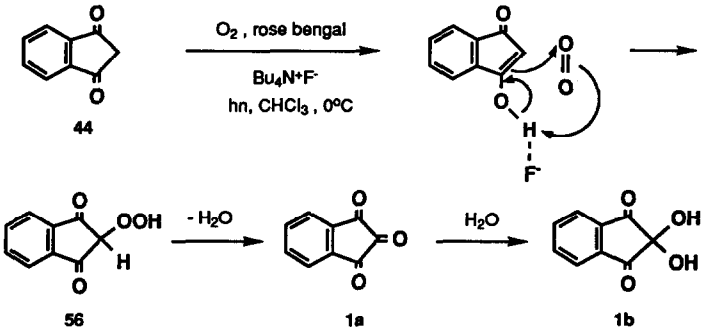
R = H
R = Cl, NO_2

55 was hydrolyzed to ninhydrin hydrate (64%, two steps), with the release of iodobenzene and nitrous acid. The 4-chloro- (38%) and 4-nitro- (64%) analogs were also prepared by this oxidation procedure, but the source of the 4-chloro- and 4-nitro-1,3-indanedione precursors was not disclosed. Schank and Lick also utilized a phenyliodonium ylid as an intermediate in the synthesis of open chain vicinal triketones.[70] After formation of the ylid, addition of ozone led to an intermediate, which underwent fragmentation to release oxygen, iodobenzene, and the desired triketone. This procedure has never been reported for the synthesis of ninhydrin or its analogs, but is likely to be applicable to such systems as long as no ozone-sensitive functional groups are present.



55

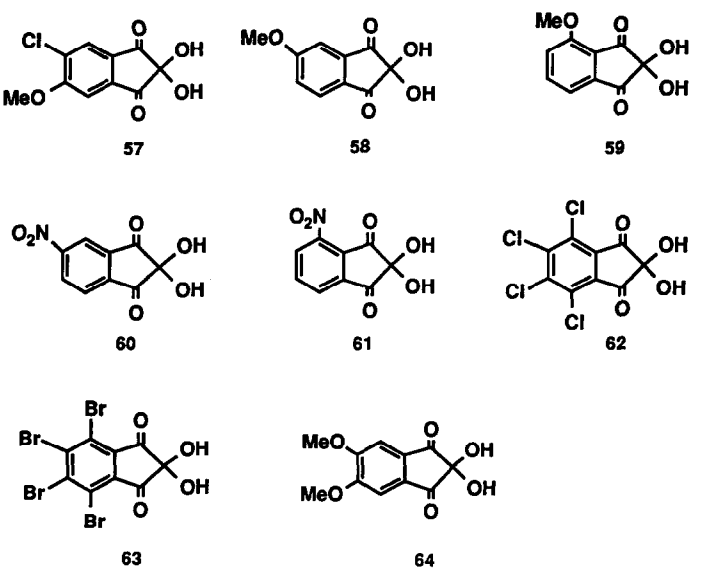
Wasserman and Pickett's study of fluoride-promoted photo-oxidations of enols led to another oxidation procedure (**Scheme 20**).[71] This procedure involved an initial ene-type reaction between singlet oxygen and the enol of 1,3-



Scheme 20

indanedione, followed by dehydration to the tricarbonyl compound. The reaction did not occur in the absence of tetrabutylammonium fluoride. This catalysis was attributed to a fluoride ion effect which has been reported to increase the nucleophilicity of enols in alkylation and condensation reactions. The electron density of the enol oxygen was increased via hydrogen bonding of the hydroxyl hydrogen with the fluoride ion. The hydroperoxide (**56**) that formed was subsequently hydrolyzed to ninhydrin hydrate (**1b**) in 75% overall yield.

In 1982, Almog and co-workers synthesized ninhydrin, benzo[*e*]ninhydrin, benzo[*f*]ninhydrin (**34**), and 5-chloro-6-methoxyninhydrin (**57**, **Scheme 21**)[51] by employing the method of Becker and Russell (**Scheme 16**).[54] Since the purpose of these investigations was to test these compounds for forensic use, no attempts were made to optimize yields.

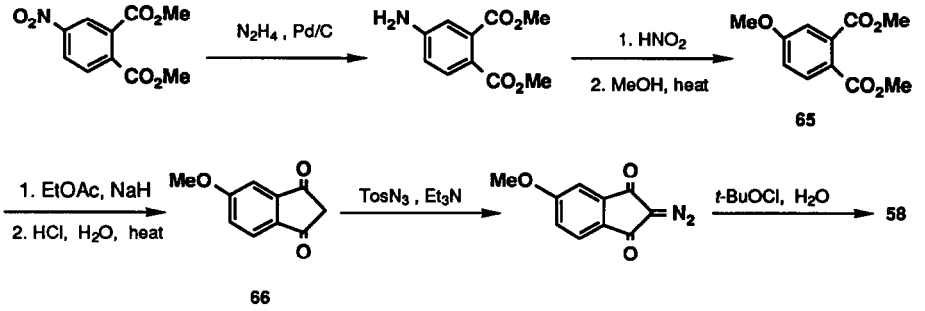


Scheme 21

The starting materials employed were dimethyl phthalate, dimethyl naphthalene-1,2-dicarboxylate, dimethyl naphthalene-2,3-dicarboxylate, and dimethyl 3-chloro-4-methoxyphthalate respectively, but the syntheses of these precursors were not described.

Lennard and co-workers[72] have synthesized several substituted ninhydrins. Among them are 5-methoxy- (**58**), 4-methoxy- (**59**), 5-nitro- (**60**), 4-nitro- (**61**), tetrachloro- (**62**), tetrabromo- (**63**), and 5,6-dimethoxyninhydrin (**64, Scheme 21**). Compounds **59-63** were prepared from the appropriately substituted 1,3-indanediones utilizing the methodology of Regitz and Adolph (**Scheme 15**).[48] 1,2,3-Trioxo-1,2-dihydrophenalene (**21**) and benzo[*f*]ninhydrin (**34**) were synthesized by the same method from the corresponding 1,3-diketones. The 2-diazo intermediate was generated by treating the appropriate 1,3-indanedione derivative with *p*-toluenesulfonyl azide in triethylamine. Compounds **58** and **64** were derived from the corresponding 1-indanones and were oxidized using selenium dioxide.[46]

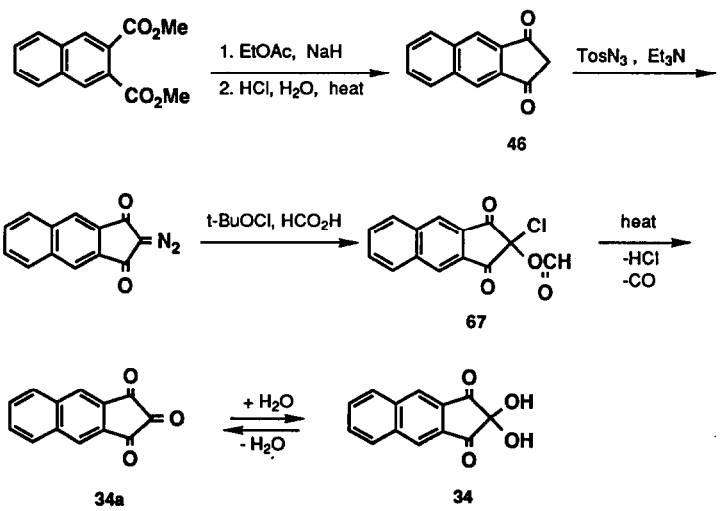
The same authors[72] reported an improved synthesis of 5-methoxyninhydrin (**58, Scheme 22**) and benzo[*f*]ninhydrin (**34, Scheme 23**). Dimethyl 4-nitrophthalate (**Scheme 22**) was reduced to the corresponding amine with hydrazine and



Scheme 22

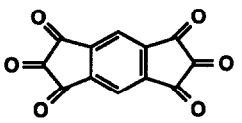
palladium on carbon. Treatment with nitrous acid afforded the corresponding diazonium salt, which underwent nucleophilic displacement when treated with hot methanol. The resulting dimethyl 5-methoxyphthalate (**65**) was cyclized to the corresponding 1,3-indanedione (**66**) via a Claisen condensation with ethyl acetate. The dione was then oxidized according to the method of Regitz and Adolph.[48]

Benzo[*f*]ninhydrin (**34**) was also synthesized by a Claisen condensation using dimethyl naphthalene-2,3-dicarboxylate and ethyl acetate (**Scheme 23**).[72] Reaction with tosyl azide in triethylamine, followed by reaction with *tert*-butyl hypochlorite in formic acid afforded the 2-chloro-2-formyloxy derivative **67**, which decomposed on heating



Scheme 23

to give the desired adduct with loss of carbon monoxide and hydrogen chloride. Unfortunately, yields for the syntheses of **58** and **34** were not given.[72] The same methodology[73] was also employed to prepare 1,2,3,5,6,7,-s-hydrindacenehexone (**68**) and 1,2,3,6,7,8-pyrenehexone (**69**) in 53% and 60% yields, respectively.[74]



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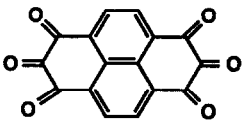
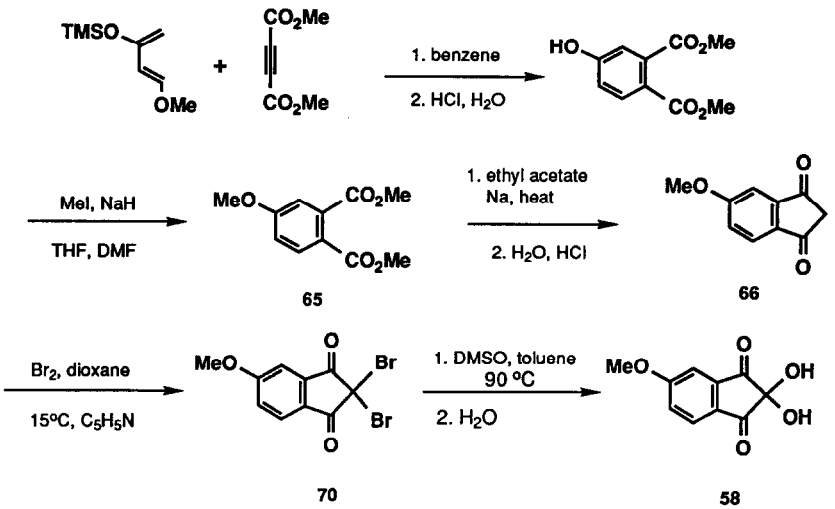


69

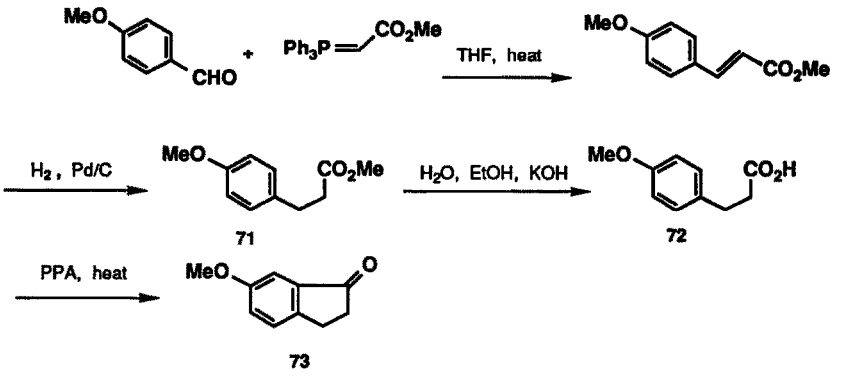
Joullié and Heffner[75] employed a different approach to the dimethyl 5-methoxyphthalate (**65**, **Scheme 24**) needed for cyclization *via* Claisen condensation, or other methods. Dimethyl 5-hydroxyphthalate was prepared by the Diels-Alder reaction of the Danishefsky diene and dimethyl acetylenedicarboxylate (75% yield).[76] The phenolic group was then methylated using sodium hydride and methyl iodide (80% yield). When the resulting dimethyl 5-methoxyphthalate (**65**) was cyclized using the Claisen condensation, only a 16% yield of the 5-methoxy-1,3-dione (**66**) was obtained. Although oxidation to the desired ninhydrin proceeded in 90% overall yield *via* the dibromide **70**, this approach was not satisfactory.[75]



Scheme 24

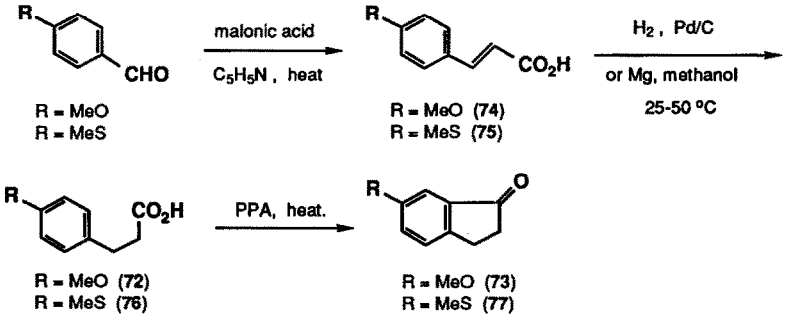
3.3.2. Syntheses Utilizing 1-Indanones. The majority of routes to ninhydrin and its analogs have involved cyclization to give the 1,3-indanedione intermediate (path a, **Scheme 13**). A different strategy involving cyclization to a 1-indanone derivative (path b, **Scheme 13**) has been found more suitable for the preparation of substituted ninhydrins.[75] For example, the Wittig reaction of *p*-methoxybenzaldehyde with methyl triphenylphosphoranilidene acetate gave an unsaturated ester that was subsequently reduced by catalytic hydrogenation to the corresponding methyl 3-(4′-methoxy)phenylpropanoate (**71**, **Scheme 25**). Hydrolysis to the acid **72**, followed by cyclization with polyphosphoric acid and heat, yielded 6-methoxy-1-indanone (**73**), which was subsequently oxidized to 5-methoxyninhydrin (**58**).[75]

An improved strategy employed the condensation of *p*-methoxybenzaldehyde with malonic acid (**Scheme 26**).[77] The condensation product (**74**) was then reduced at the double bond to provide **72**, which was cyclized by heating with polyphosphoric acid.



Scheme 25

A similar protocol was used to generate 6-(methylthio)-1-indanone (**77**, **Scheme 26**), the precursor to 5-(methylthio)ninhydrin (**81**), but in this case the reduction was carried out using magnesium in methanol.[78] Although indanone **73** was commercially available, an affordable, efficient method for its oxidation was still needed. Treatment of 1-indanone **73** with 2.1 equivalents of *N*-bromosuccinimide and AIBN in refluxing carbon tetrachloride, formed a 3,3-dibromo-1-indanone, which underwent loss of hydrogen bromide on treatment with triethylamine at 5 °C to yield 5-methoxy-3-bromoindenone (**78**) in 99% yield. Addition of a benzene solution of bromine and dimethylsulfoxide to enone **78** gave a 70% yield of **58**.[75] Treatment of indanone **77** under the same conditions afforded 4-(methylthio)-1-indenone (**78**), 3-bromo-4-(methylthio)-1-indenone (**79**), and 2,3-dibromo-4-(methylthio)-1-indenone (**80**) in 75% yield (12 : 65 : 23 ; **Scheme 27**). Attempts to suppress the formation of the two additional products were unsuccessful but both **79** and 2,3-dibromo-4-(methylthio)-1-indenone (**80**) could be converted to the desired target **81** by refluxing in benzene, followed by the addition of dimethyl sulfoxide and bromine (70% yield).



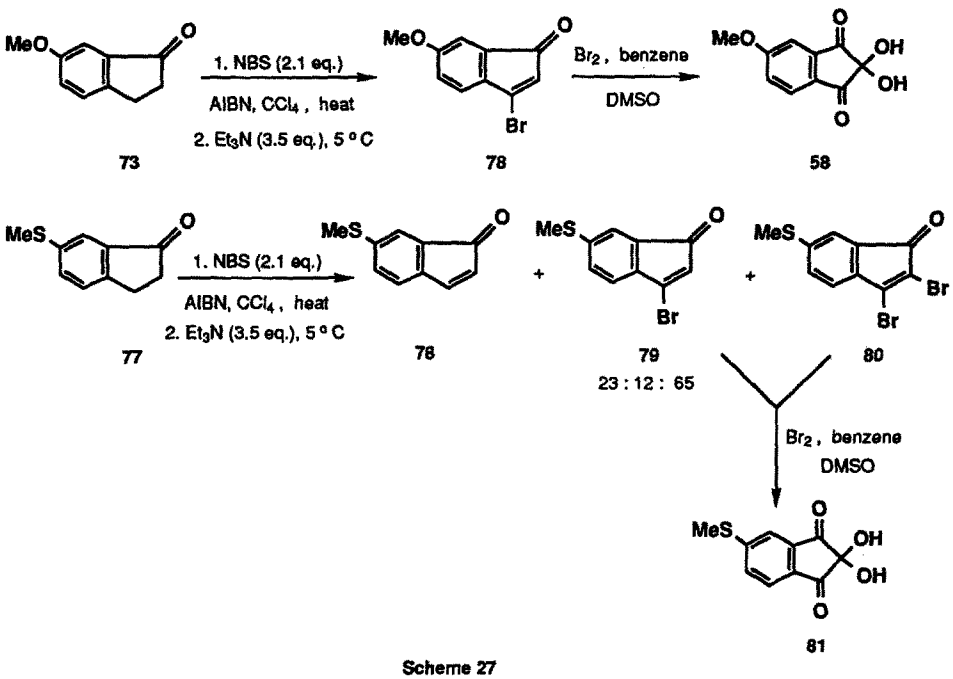
Scheme 26

This oxidation protocol and a plausible mechanism for the formation of the trione are illustrated in **Scheme 28**.

A similar methodology for the oxidation of indanones to the corresponding triketones was reported by Japanese investigators. Their protocol combined *N*-bromosuccinimide and dimethyl sulfoxide in a one-pot process. No yields were reported but they were stated to be good.[79,80]

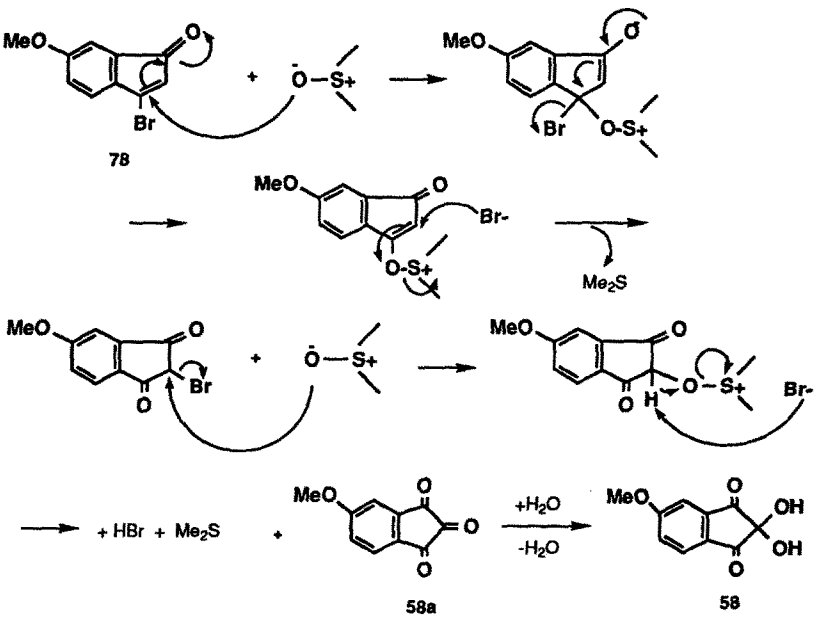
Lennard and co-workers achieved the synthesis of both ninhydrin **58** and 3,4-dimethoxyninhydrin *via* the selenium dioxide oxidation of the corresponding commercially available 1-indanones.[50] Oxidation of ninhydrin **58** from indanone **73** using selenium dioxide in refluxing dioxane has also been reported.[81] The 1-indanone has been prepared by cyclization of 3-(4′-methoxyphenyl)propanoyl chloride with anhydrous aluminum chloride in methylene chloride in 70% yield. The overall yield from the acid chloride was 28%.[82]

Kametani and co-workers were the first investigators to utilize Ruhemann's methodology to synthesize ninhydrin and two substituted ninhydrins (5,6-dimethoxyninhydrin and 6,7-methylenedioxyninhydrin in 37% and 11% overall



Scheme 27

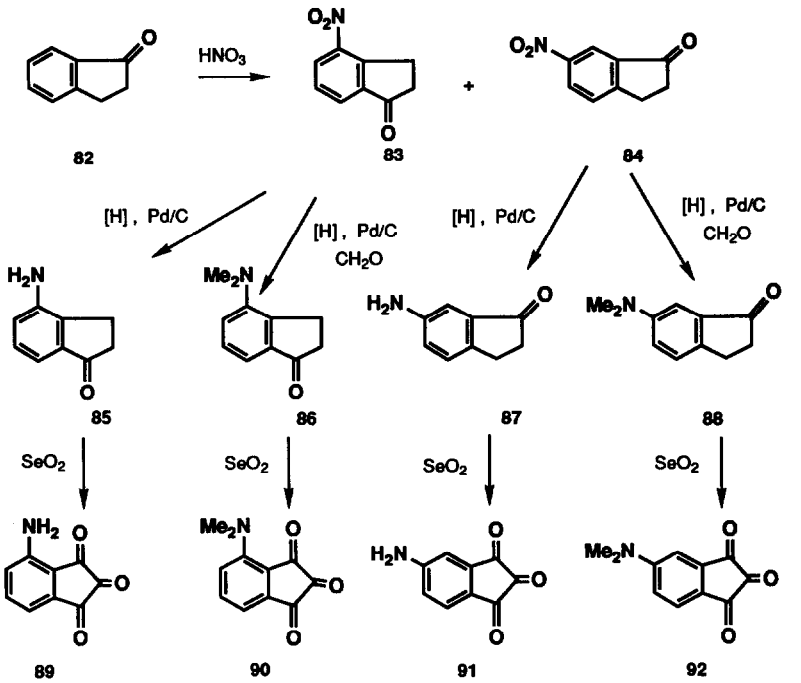
yields from the corresponding 1–indanones).[83] The synthesis of ninhydrin **58** from indanone **73** was also accomplished using this method. Reaction of the substituted 1-indanone with *p*-nitrosodimethylaniline in the presence of potassium hydroxide gave the Schiff base, which without isolation was hydrolyzed to give a 20% yield of **58** from indanone **73**.



Scheme 28

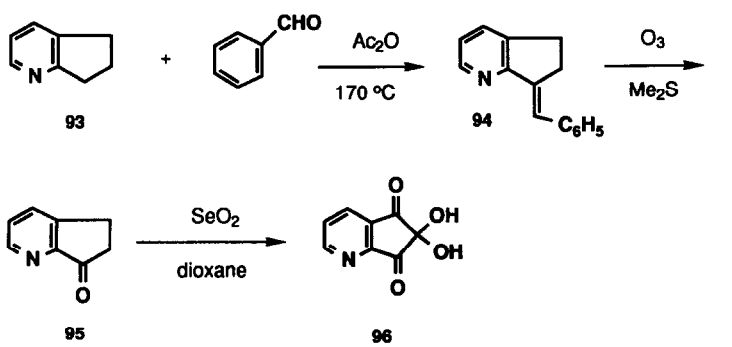
The same procedure was also used to synthesize the lipophilic 5-hexadecylninhydrin (12% overall from the corresponding indanone).[82]

The unsubstituted 1-indanone (**82**) has been used for the synthesis of 4-amino- (**89**), 4-dimethylamino- (**90**), 5-amino- (**91**), and 5-dimethylamino ninhydrin (**92, Scheme 29**).[84] Reaction of 1-indanone (**82**) with nitric acid gave a



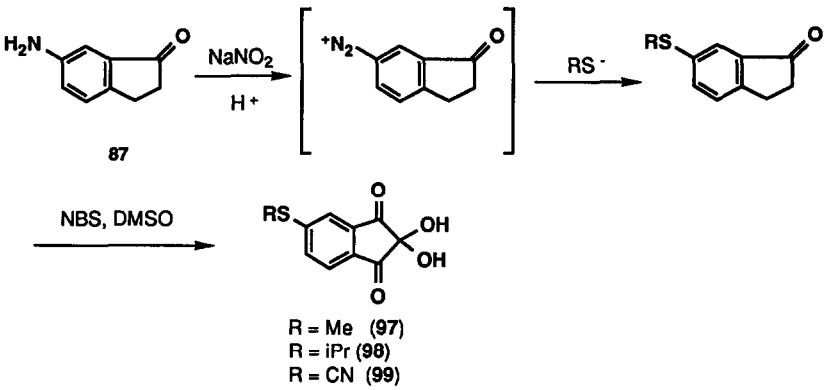
Scheme 29

mixture of 4-nitro- and 5-nitro-1-indanones (**83** and **84**), which were separated by flash column chromatography. The nitro groups were then reduced (10% Pd/C and aqueous sodium hyposulfite as the hydrogen source) to the amino derivatives(**85** and **87**) and, in the presence of formaldehyde, to the dimethylamino derivatives (**86** and **88**). Oxidation of the aminoindanones **85** and **87** and the dimethylaminoindanones **86** and **88** to the desired ninhydrin analogs **89**, **91**, **90**, and **92**, respectively was accomplished with selenium dioxide in dioxane at ambient temperature.[84]



Scheme 30

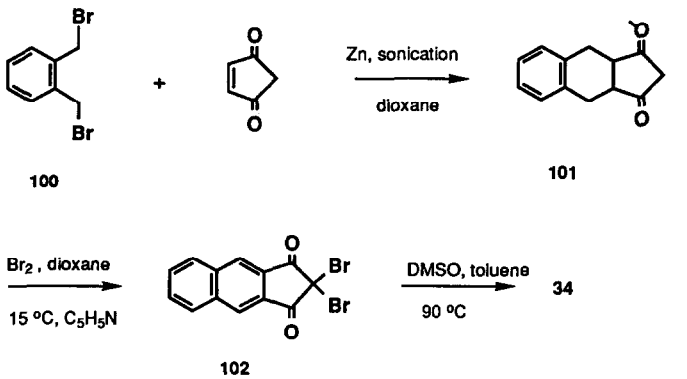
More recently, new ninhydrin derivatives (**97**, **98**, and **99**) were reported.[86] They were synthesized from the diazonium salt of indanone **87**, **Scheme 31**.



Scheme 31

3.4. New Approaches to Benzo[f]ninhydrin and Analogs

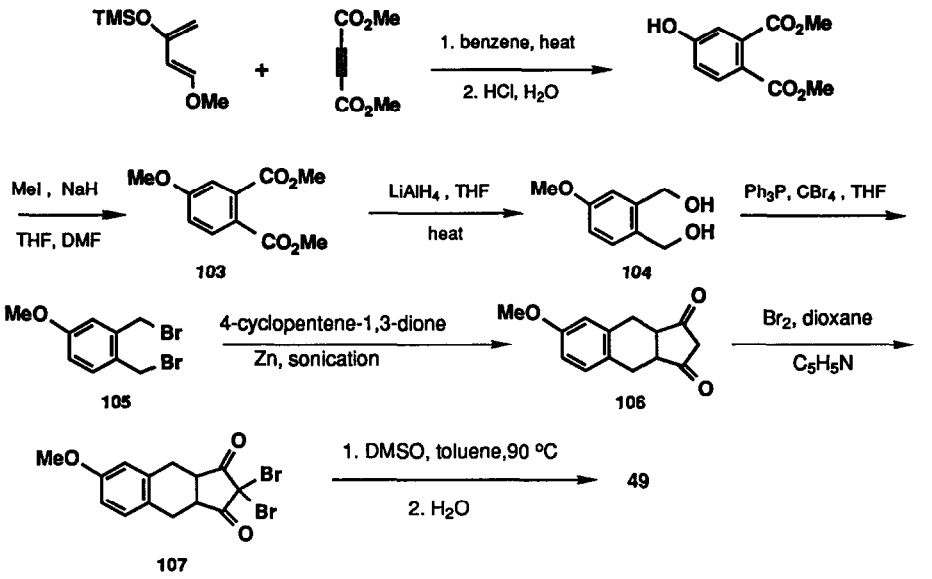
A new strategy for the synthesis of fused benzo-1,3-indanediones, very different from previous methods, was recently reported by Joullié *et al.* (**Scheme 32**).[60] Benzo[*f*]ninhydrin was synthesized via a Diels-Alder reaction between



Scheme 32

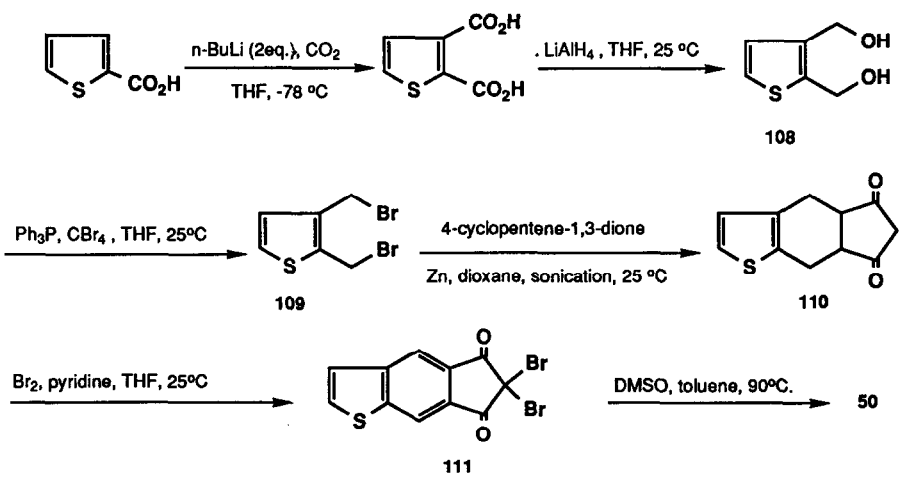
a transient *o*-xylylene and 4-cyclopentene-1,3-dione. Sonication of a dioxane solution of dibromide **100** at 25 °C, in the presence of activated zinc provided the *in situ* generation of the diene, *o*-xylylene, which was trapped by the dienophile to give the intermediate benzo[*f*]-4,9-tetrahydro-1,3-indanedione (**101**). Dione **101** was simultaneously aromatized and oxidized by exposure to bromine and light. 2,2-Dibromobenzo[*f*]-1,3-indanedione (**102**) was converted to benzo[*f*]ninhydrin (**34**) by the action of dimethylsulfoxide and toluene at 90 °C (**Scheme 32**).[60] This synthetic approach is most efficient (76 % yield for three steps) since it utilizes commercially available precursors, permits the introduction of substituents and is amenable to industrial-scale production.

Joullié and Heffner also used this protocol for the syntheses of 6-methoxybenzo[*f*]ninhydrin (**49**) and thieno[*f*]ninhydrin (**50**).[61] **Scheme 33** shows the synthesis of **49**. Dimethyl 3-hydroxyphthalate was obtained via a Diels-Alder reaction using the procedure of Danishefsky.[76] This product was methylated to give the diester (**103**). Reduction



Scheme 33

of **103** with lithium aluminum hydride gave the corresponding diol (**104**), which was converted to 4-methoxy-1,2-bis(bromomethyl)benzene (**105**), the precursor to compound **49**, with carbon tetrabromide and triphenylphosphine. The dibromide **105** was subjected to the same zinc-activated Diels-Alder conditions described for benzo[*f*]ninhydrin (**34**). A low yield of adduct (**106**) was obtained. Bromination of **106** provided dibromide **107**, which was converted to the target ninhydrin (**49**) under the same conditions as for **34**. The synthesis of 2,3-bis(bromomethyl)thiophene (**109**) and its conversion to ninhydrin **50** are shown in **Scheme 34**. Ninhydrin **50** was synthesized from diol **108** using the same methodology. The diol was obtained from the reduction of thiophene-2,3-dicarboxylic acid using the method of Chadwick.[87] Diol **108** was converted to the dibromide **109**, which underwent the Diels-Alder reaction in excellent yield to provide adduct **110**. This product was converted to the corresponding dibromide (**111**). Treatment of **111** with dimethyl sulfoxide as previously described gave the desired ninhydrin **50**.



Scheme 34

4. APPLICATIONS OF NINHYDRIN AND ANALOGS

4.1. Amino acid Analysis, Biochemical, and Chemical Applications

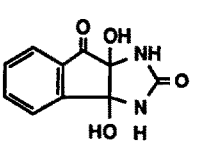
The earliest application of ninhydrin was based on its reaction with α-amino acids. Ruhemann observed that when ninhydrin reacted with an α-amino acid, a colored derivative was produced, which qualitatively confirmed the presence of an α-amino acid.[13] Three years after the discovery of ninhydrin, Abderhalden and Schmidt were also aware of the utility of **1** as an analytical reagent.[88]

Ninhydrin became widely used in biochemical and medical settings for the analysis of amino acids, both as a qualitative and quantitative tool. The amino acid content in biological fluids could, in principle, be determined by analyzing the amount of ammonia, color, aldehyde, or carbon dioxide formed. Analytical measurements of the aldehyde were complicated by various factors.[88-91] Glycine, proline, and hydroxyproline did not form aldehydes with ninhydrin.

In 1915, Harding and MacLean proposed a colorimetric method for the estimation of nitrogen in α-amino acids.[92] They observed that β-, γ-, and δ-amino acids gave only weak colors and that α-amino acids substituted at either the amino or carboxyl function did not react. Ruhemann's purple **2** was formed under conditions that produced a quantitative reaction between amino acids and ninhydrin. The content of free α-amino acids in solution could then be ascertained. This method was also used to determine the amount of amino acid α-nitrogen (in neutral solution) set free after protein hydrolysis.[92] With the exception of cysteine, for which the method could not give accurate results, it was possible to estimate the α-nitrogen content in ranges of 0.005 to 0.05 mg/cm^3. This colorimetric approach was later discarded because of its lack of specificity for individual amino acids.[28]

Van Slyke and co-workers investigated a quantitative procedure for the estimation of the α-nitrogen content in amino acids based on the emission of carbon dioxide.[93] Under suitable conditions, both primary and secondary α-amino acids gave off carbon dioxide quantitatively, and this gas could be measured in a Van Slyke-Neill manometer. This instrument was amenable to both micro- and macroanalytical scales. This technique gave rapid and precise measurements of the analytical purity of isolated amino acids and also of free amino acids encountered in protein digests or complex biological mixtures, including those containing free unconjugated carboxyl and primary or *N*-methylamino groups. The procedure could be used in combination with Van Slyke's nitrous acid method for amino nitrogen[94] to determine the amount of lysine plus hydroxylysine in a solution. The amount of aspartic acid in a solution could also be ascertained as this amino acid gave off two moles of carbon dioxide per mole of acid. Proteins did not have to be removed from blood plasma before analysis since they did not react appreciably under the given conditions. Likewise, urine samples could be analyzed without difficulty. Urea gave off carbon dioxide only very slowly, and when excess ninhydrin was present, this evolution was retarded even more, presumably due to the formation of a ninhydrin ureide (**112**), a urea derivative resulting from the combination of one molecule of ninhydrin with one molecule of urea.[95] This ureide was a stable, colorless, crystalline substance, which evolved very little CO_2.

Smakman and van Doorn reported a method for removing urea from dialysates, body fluids and the gastrointestinal tract of patients suffering from renal failure.[96] This procedure was based on the reaction of ninhydrin with urea to form derivative **112**. The same authors found that the binding was essentially irreversible and that the resulting complex was



112

nontoxic, nonpyrogenic, and stable under these conditions. The method was further improved and simplified by incorporating ninhydrin into an insoluble matrix.

With the advent of advanced chromatographic techniques, ninhydrin-based photometric methods began to dominate amino acid analysis. In 1948, Moore and Stein introduced a procedure which gave the concentration of various peptides and free α-amino acids in solution.[42] Although qualitative results were routine, quantitative data were still difficult to obtain as the color yield per microgram of amino acid decreased with reduced amino acid concentration.

Moore and Stein[42] found that the addition of a strong reducing agent such as stannous chloride resulted in higher yields of Ruhemann's purple by suppressing an oxidative side reaction. The stannous chloride effectively reduced excess ninhydrin *in situ* to provide hydrindantin (**11**), the reduced form of ninhydrin. Later experiments used **11** directly.[87] This protocol avoided the precipitation of tin salts and increased the effective concentration of hydrindantin to 0.3 per cent. The effects of variations in pH, temperature, heating time, and amounts of reagents were all studied. It was found that the percentage yield of colored product was independent of the initial amino acid concentration, and that less than quantitative yields could be attributed to oxidative side reactions. Reproducible results were possible for any given amino acid, but not all amino acids produced the same amount of color per mole, an observation already noted by Harding and MacLean.[92] The lack of specificity was a disadvantage in determinations made on unfractionated biological material. However, for experiments involving chromatographic analysis, amino acids and other components could first be separated on the column and then quantitative values for specific amino acids obtained.

A number of studies were attempted to further optimize quantitative amino acid analysis after chromatographic separation. Many of these dealt with the development of improved ninhydrin reagents. Others involved altering the conditions of the reaction or changing the mode of detection. In the early 80's, Wako Pure Chemical Industries, Ltd. patented a reagent containing thiols[98] such as 2-mercaptoethanol. The addition of the thiol provided a more stable ninhydrin reagent, which gave more intense peaks in amino acid analysis.

The same company also patented a series of new solvents for ninhydrin.[99,100] These were 1-alkoxy-2-alkanols, which could be used alone or in combination with dimethylsulfoxide to act as a replacement for the toxic methyl cellosolve. The new ninhydrin reagent containing 1-methoxy-2-propanol, DMSO, ninhydrin, $NaBH_4$, and an aqueous LiOAc buffer, was stable at low temperature and did not cause clogging in the automated analyzer.

Quantitative analysis of amino acids relies on a reduced ninhydrin reagent. Generally, hydrindantin (**11**) has either been added directly or generated *in situ* with excess ninhydrin and a reducing agent such as stannous chloride. In 1984, James suggested that titanous chloride be used to reduce ninhydrin in amino acid analysis.[101] The titanous chloride-reduced ninhydrin reagent was not quite as sensitive in its reaction with amino acids as was the reagent produced by direct addition of **11**, but it did exhibit greater stability. Hydrindantin is sensitive to light, atmospheric oxygen, and changes in pH and temperature. The titanous chloride-ninhydrin combination offered the advantages of being quicker and easier to prepare.

Hori and Kihara reported an electrolytic preparation of reduced ninhydrin reagent.[102] A nonreduced ninhydrin solution could be stored for long periods of time and in the presence of atmospheric oxygen. Electrolytic preparation of the reduced ninhydrin reagent was highly reproducible and gave a sensitive determination of amino acids eluted from the HPLC system. Both flow-through electrolysis and chemical reduction provided almost identical reduced ninhydrin reagents.

Some studies were undertaken to provide ninhydrin reagents, reaction conditions, and/or spectroscopic detection techniques that would be specific for a particular amino substrate. Aleksandrov *et al.* optimized the ninhydrin reaction conditions for valine and proline.[103] Their method was based on photometric detection after automatic chromatography, which obtained greater sensitivity by shortening the amino acid-ninhydrin reaction time to 0.2 minutes.

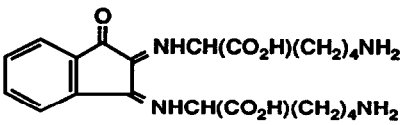
Esaki and co-workers[104] modified the ninhydrin colorimetric method of Gaitonde[105] to detect selenocysteine. Under their conditions, from 10 - 500 μM of selenocysteine could be determined.

The simultaneous detection of primary and secondary amino acids by liquid chromatography was accomplished using a modified ninhydrin reagent and UV detector.[106] The reagent contained ninhydrin, dimethylsulfoxide, a cellosolve solvent, and a lithium acetate buffer. A liquid chromatograph equipped with a UV detector separated the components of the reaction and detected both the primary and secondary amino acids using a single UV wavelength. A sample containing aspartic acid, threonine, serine, glutamic acid, and proline treated with this reagent provided a spectrum with five maxima when a 570 nm wavelength was employed.

In 1989, Molnár-Perl disclosed a spectrophotometric ninhydrin method for determining tryptophan in intact proteins.[107] After evaluating the second-order rate constants and molar absorptivity values, optimum conditions were established for the selective estimation of tryptophan, tryptophan residues in intact proteins, and indoles without the

interfering effects of tyrosine. The conditions allowed for reproducible molar absorptivity values with standard errors of ≤ 2.3% for any particular substrate.

The quantitative reaction of lysine with ninhydrin was investigated by Wang and Ji.[108] The reaction product, whose probable structure **113** is shown, was quantitatively evaluated using fluorometry (λ_{ex} = 380 nm, λ_{em} = 468 nm). The limit of detection was ≥ 0.005 ppm, and the recoveries were 92 to 95%.



113

Seracu studied the UV and visible absorption spectra of the complexes of amino acids with ninhydrin.[109,110] Usually amino acids were detected with post column ninhydrin derivatization using automatic analyzers at wavelengths of 570 nm for α-amino acids, 444 nm for imino acids, or an intermediate 520 nm for both. Seracu studied the absorption of numerous complexes between 275 nm and 700 nm and suggested that wavelengths of 290 nm and 444 nm be used and that the detector be modified to operate at these wavelengths

A new electrochromatoscanning method for the quantitative analysis of microvolume samples by paper chromatography was developed by Yoshimura and Okazaki.[111] This method used a voltammetric cell and chemical amplification by reaction of the amino acid solution with ninhydrin.

James suggested a replacement solvent for flushing the ninhydrin reagent through the flow lines of an automated amino acid analyzer in the shut-down phase of the instrument.[112] The modification involved replacing water with a solvent containing dimethylsulfoxide, water, and thiodiglycol. This new flushing solution allowed for a more rapid start-up phase when re-initializing the instrument, and some of the artifacts inherent in previous analyses could be eliminated.

To detect amino acids on thin-layer plates, Laskar and Basak developed a procedure that used fluorescein isothiocyanate to produce various and distinguishable colors for different amino acids.[113] The colors produced could be observed under visible and UV light. The heating procedure allowed for distinct colors inherent to the various amino acids to be observed with high sensitivity. Even at low detection limits (0.3 to 1.0 μg), different colors for the amino acids were observed under UV light (280 nm). Presumably, the amino acids first reacted with fluorescein isothiocyanate to give fluorescein thiocarbamyl derivatives, which then reacted with ninhydrin to produce the distinguishable colors.

The effects of various substances on the colorimetric amino acid-ninhydrin reaction were also investigated. D'Aniello and co-workers studied the effects of salts, acids, alkali, and buffer solutions on the color development of the Cd-ninhydrin-amino acid reaction.[114] Of the salt solutions examined, only cuprous and ferric ions interfered with color yield even at low concentrations. These studies established the need to separate amino acids from proteins that are complexed to metal ions when the ninhydrin method is used for amino acid analysis.

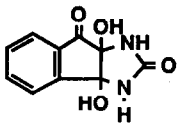
A different biochemical application was developed by Lenzen and co-workers in 1988.[115] Ninhydrin was used as an inhibitor of glucokinase. The study involved an examination of a number of compounds that might compete with glucose for the sugar-binding site of glucokinase. Potential inhibitors included compounds such as alloxan, other pyrimidine derivatives, and ninhydrin. The mechanism of inhibition was proposed to be an oxidation of the functionally essential mercapto groups of the enzyme. Presumably, the most reactive keto group of the inhibitor acted as a hydrogen acceptor.

4.2. Synthetic Applications

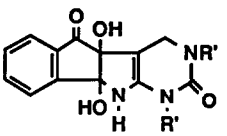
Although the reactivity of the central carbonyl of ninhydrin has long been recognized, this highly electrophilic moiety had not been utilized in synthesis. Recently, ninhydrin has found application in the total synthesis of fused and spirocyclic ring systems. The reactivity of the C-2 position in **1** allows for the formation of a number of carbon-heteroatom bonds. The synthesis of fused ring systems is illustrated in **Schemes 3** and **4** (**Section 2.2.1**). Grigg's studies showed that protonated Ruhemann's purple could undergo cyclization with appropriate dipolarophiles to give spirocyclic com-

pounds.[35] These 1,3-cycloadditions were reviewed in **Sections 2.2.3.** and **2.2.4.** The reader is referred to **28, Scheme 12** and cycloadducts **31-33.**

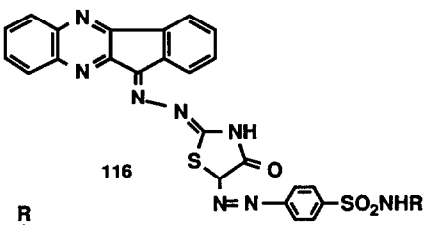
Other investigators have utilized the reactivity of ninhydrin to prepare fused heterocyclics, some of which are shown. Some of the products derived from ninhydrin were of medicinal importance. Chatterjie *et al.* prepared indenoimidazolediones (**114, 115**), via the reaction of ninhydrin with the appropriate monosubstituted ureas or 6-aminouracil derivatives, to examine the potential anticonvulsant effects of the addition of cyclic ureides in bioactive compounds.[116] Of the indenoimidazolediones synthesized, only the butylurea adduct did not show activity. The other derivatives showed anticonvulsant activity in mice against seizures induced by Metrazol. None however, showed activity against seizures induced by electroshock.



114 R = C_6H_5
R = $CH_2CH{=}CH_2$
R = n-C_4H_9



115 R' = H, CH_3



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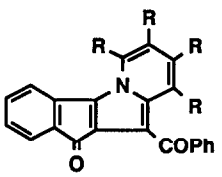

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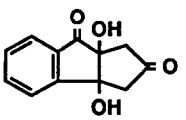

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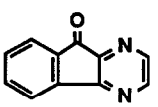

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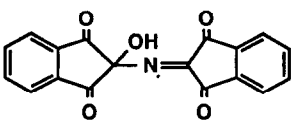

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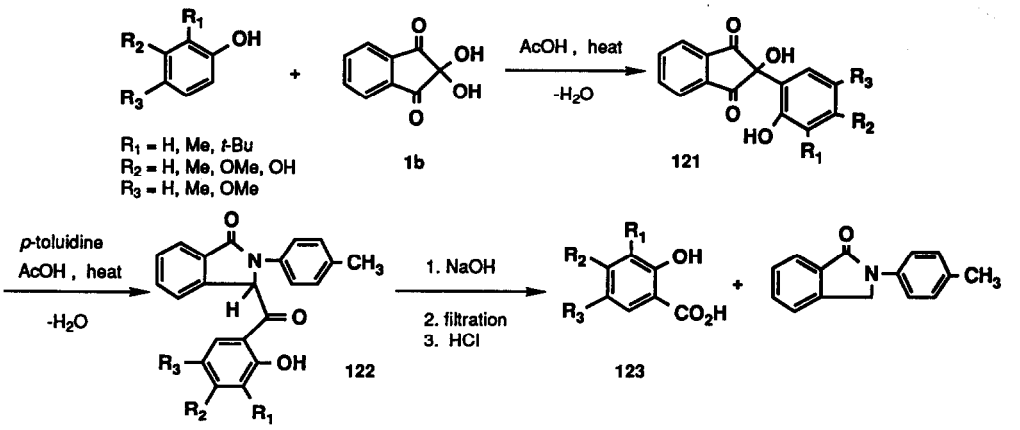
A group of potential fungicides that have been prepared from ninhydrin are the thiazolidinediones and their azo derivatives **116**.[117] 11*H*-indeno[1,2-*b*]quinoxalin-11-one thiosemicarbazone (from ninhydrin) was cyclized with α-chloroacetic acid, followed by treatment with diazotized alkyl sulfanilamides. One of the compounds (R = 4-pyrimidinyl) was found to be effective against *Urvularia lunata* at 360 μg/ml. The other derivatives had less fungicidal activity.

Carotti and co-workers reported a one-step synthesis of an indenoindolizine nucleus from 2-hydroxy-2-(2-oxo-2-phenyl)ethyl-1,3-indanedione.[118] Treatment of the indanedione with *p*-toluenesulfonyl chloride in pyridine gave the desired pyridopyrroloindenone, 11-benzoyl-10*H*-indeno[2,1-*b*]-indolizine-10-one, (**117**) in 90% yield. The structural assignment of **117** was confirmed by an X-ray analysis and proton NMR data. An example of a fused-ring carbon compound prepared from ninhydrin was reported by Usmani and Ismail.[119] The dihydroxycyclopentindenedione (**118**) was prepared by the cycloaddition of ninhydrin and acetone in the presence of concentrated HCl. Still another group of compounds prepared from ninhydrin were the pyrazinoindene derivatives **119**.[120] These were prepared by reaction of ninhydrin with 1,2-ethylenediamine. Indamine derivatives **120** were obtained by reaction of ninhydrin with sulfonyl urea.[121]

Substituted salicylic acids were prepared by heating ninhydrin with an appropriately substituted phenol in acetic acid to give addition product **121** selectively and in high yield (**Scheme 35**).[122] Further heating of **122** with *p*-methylaniline in acetic acid gave **123** which, in turn, was quantitatively hydrolyzed in 2N sodium hydroxide to afford the substituted salicylic acids **124**. This procedure was superior to the commonly used Kolbe-Schmitt synthesis in that it did not require high pressure or high temperature, the product could be isolated in high purity, and the yields were typically better than those obtained in the Kolbe-Schmitt reaction.



Scheme 35

A number of substituted 2-nitroindan-1,3-diones were tested for their antiallergic activity.[123] The parent unsubstituted compound showed an activity very close to that of disodium cromoglycate, a drug used in the treatment of bronchial asthma. Most substituents either had no effect on the activity or decreased it. Only compounds with substituents at the C-5 and C-6 positions showed a marked increase in activity.

4.3. Forensic Use in Latent Fingerprint Identification

In 1954, Odén and von Hoffsten, two Swedish scientists, recognized ninhydrin as a latent fingerprint reagent.[124] Their original formulation consisted of a ninhydrin solution in either acetone or diethyl ether, along with a small amount of acetic acid. Early studies by a number of investigators sought to develop conditions that would lead to maximum detection efficiency. Changes in concentration, solvent, temperature, heating time, pH, application method, and humidity were examined.

Various solvents were investigated (acetone, diethyl ether, ethanol, isopropanol, petroleum ether, and acetone-water mixtures). Petroleum ether showed particular promise as it did not cause ink to smear or dissolve.[125] However, all of the aforementioned solvents were flammable and therefore a hazard to forensic science laboratories.

In 1974, Morris and Goode introduced a new solvent which circumvented the hazards of volatile solvents.[126] This solvent, 1,1,2-trifluorotrichloroethane (fluorisol or freon 113) was a nontoxic, nonflammable, and practically odorless liquid that also did not dissolve ink or cause smearing. However, it was four times as expensive as other common solvents and required lengthy preparation time.

In 1984, Tighe introduced Freon-Plus Two, an improved reagent which required only ten minutes for its preparation.[127] Tighe also noted that technical grade freon was just as efficient as the pure grade freon used by Morris and Goode, but at a fraction of the cost. Due to its competitive price, and nontoxic, nonvolatile nature, Freon-Plus Two is the ninhydrin reagent of choice today, and it is known as non-flammable ninhydrin reagent or NFN.

The formation of Ruhemann's purple was known to be dependent on pH and humidity, and complete development was known to require heating.[128] The optimal pH had been determined to be approximately 5.0. This pH could be maintained by addition of acetic acid to the ninhydrin reagent, and this protocol was particularly effective for detection

on alkaline paper surfaces.[51] In the absence of moisture, ninhydrin treatment was ineffective. Room temperature procedures, although requiring more time, provided improved contrast between background and latent print.

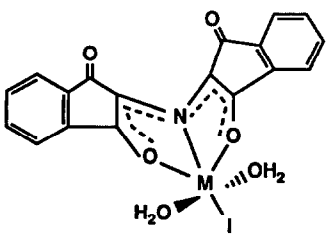
Though a variety of development conditions were explored, most methods were not sufficiently sensitive.[129] This limitation was largely overcome beginning in the late 1970's with the introduction of laser detection of latent fingerprints. The technique was based on detecting a small signal (photo-luminescence) rather than relying on the small difference between two large values (absorbance and reflectance), thus the method had higher sensitivity.[130] In 1977, Dalrymple and co-workers reported that latent fingerprints had intrinsic luminescent properties.[131,132] Natural fingerprint fluorescence however, was very weak, and only a very strong and well-filtered lamp could produce sufficient detail. Furthermore, the yellow-green prints lost their fluorescent property with time. In 1981, German noticed that ninhydrin-treated latent prints illuminated with laser light showed greater resolution than those illuminated with ordinary light.[133] In 1982, Herod and Menzel studied the laser detection of ninhydrin-developed latent fingerprints.[134] They found that well-developed ninhydrin prints that were visible in ordinary light by their distinctive purple-blue color were weakly fluorescent when illuminated with blue-green or near ultraviolet (330-360-nm) argon laser light. They displayed only weak fluorescence in the red and near-infrared regions under yellow or orange (570-590 nm) dye laser light.

The same authors further improved upon the ninhydrin/laser method by exposing ninhydrin developed latent fingerprints to a metal salt before illumination.[135] They were interested in eliminating the need for the additional dye-laser and in increasing fluorescent sensitivity. After spraying ninhydrin-developed fingerprints with zinc chloride, an orange print particularly suited to the 488 nm line of the argon laser was observed. This complex absorbed at approximately 485 nm and emitted at 560 nm. A well-developed latent print now showed strong luminescence under argon laser illumination. Fingerprints showed enhanced contrast and ridge detail, and normally unobservable prints could be identified. Nonporous surfaces were suitable substrates. The $ZnCl_2$ post-ninhydrin treatment together with laser examination had important and practical advantages. A latent fingerprint could first be developed by a conventional ninhydrin method, and if prints were still undetectable, $ZnCl_2$ treatment and laser examination could follow.[135]

Sensitivity enhancement had also been observed when ninhydrin-treated latent fingerprints were pre-treated with enzymes and coordinated to group IIb metal salts in post-treatment. Trypsin was the most commonly employed enzyme, although pronase was also used. Pre-treatment with the enzyme led to enhanced detectability of relatively fresh fingerprints, but showed only marginal improvement for older prints. The mechanism of action of the enzyme was not ascertained. Possibly, the enzyme acted as a dusting powder or produced amino acids by the hydrolysis of proteins present in the fingerprint.[136,137]

Warrener and co-workers also studied complexes between group IIb metals and Ruhemann's purple.[138] Complex formation between any of the group IIb metals and the ninhydrin-developed fingerprint required the presence of water. For complexes between zinc, cadmium, or mercury and Ruhemann's purple, atmospheric humidity was usually all that was necessary. Cadmium and mercury complexes were unaffected by excess water, but the zinc complex was unstable under such conditions. These authors proposed the use of cadmium instead of zinc in the fluorogenic stage of the process.[138] They advocated that cadmium complexes suffered less from the ninhydrin development conditions of heat and moisture, and that if the complexes were cooled with liquid nitrogen (77K) and examined with a xenon arc lamp, improved sensitivity over Herod and Menzel's ninhydrin/$ZnCl_2$/argon laser procedure[134,135] could be obtained.

Lennard *et al.* investigated the structures of complexes formed between **2** and group IIb metal salts.[139] An X-ray crystal structure of the cadmium-Ruhemann's purple complex (prepared from CdI_2) showed that **2** formed a 1:1 complex with the metal. Compound **2** acted as a tridentate ligand, and the metal was also bound to one iodide ion and two water molecules to form a distorted octahedral coordination complex (**124**). As water was an intrinsic part of the complex, it



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seemed only reasonable that it be present for complex formation to occur.

The coplanarity of the two indanedione rings was believed to be responsible for fluorescence. Because zinc and cadmium had similar chemical properties, the zinc-Ruhemann's purple complex was expected to have an analogous octahedral structure. The stronger fluorescence of the zinc complex was suggestive of more nearly coplanar indanedione rings. However, the higher atomic number of cadmium necessarily implied greater spin-orbit coupling, and hence faster intersystem crossing which tended to quench ligand fluorescence.[140]

A number of metal ions were studied in conjunction with complex formation.[140] Complexes of Ruhemann's purple with Cu^{2+} and Mn^{2+} showed no fluorescence. This observation was expected as these metals had unpaired electrons which could spin-orbit couple to the ligand system (2) and quench fluorescence. The open shell d-π interactions in Ni^{2+} complexes also quenched fluorescence. A La^{3+} complex formed weak orange fingerprint fluorescence and this suggested a coplanar indanedione ring system and a closed shell configuration. The weak color was attributed to the ion's high atomic number which produced spin-orbit coupling.

Successively weaker sensitivity and greater deviation from coplanarity were observed in the series of group IIb metals, Zn, Cd, and Hg. The complex between Ruhemann's purple and zinc absorbed at about 490 nm, very close to the 488 nm line of the argon-ion laser, and therefore the ninhydrin/$ZnCl_2$/argon laser procedure yielded impressive sensitivity and became routine in many forensic labs.

Argon lasers largely remained the primary light source in fingerprint detection, although other light sources were employed. Warrener and his co-workers used the xenon arc lamp equipped with a filter. At low temperatures this lamp could match the enhanced sensitivity of the argon laser. Other light sources included Watkin's indium lamp,[141,142] Kent's "Quaser,"[143,144] and Warrener's "Unilite," an advanced version of his original xenon arc lamp.[145,146] These light sources showed greater efficiency when samples were cooled to liquid nitrogen temperatures.

In the early 1980's, the copper-vapor laser (CVL), originally designed for use in isotope separation, found application in fingerprint studies. This laser is pulsed in contrast to the continuous wave of the argon laser and delivers lines at 510 and 578 nm. Ninhydrin-developed prints treated with $ZnCl_2$ did not respond well to the CVL as the shortest line of this laser was 510 nm, 20 nm above the absorbance of the ninhydrin complex (490 nm). However, it was found that fingerprints developed with either of the analogs, 5-methoxyninhydrin or benzo[f]ninhydrin and treated with $ZnCl_2$ showed strong fluorescence. These complexes had absorption maxima at 500 and 530 nm, respectively.[81]

5-Methoxyninhydrin and benzo[*f*]ninhydrin showed operational advantages over ninhydrin.[81] They developed latent fingerprints with equal or better sensitivity, and when treated with Zn(II) or Cd(II), proved useful in observing prints on difficult surfaces, such as manilla and yellow envelopes, where background luminescence was a problem. Fingerprints treated with 5-methoxyninhydrin were purple in color and had absorption maxima at 410 nm and 579 nm. When treated with zinc(II) they became orange and showed an excitation wavelength of 505 nm and an emission wavelength of 544 nm. Cadmium(II) complexes excited and emitted at 520 nm and 585 nm, respectively, and were red in color.[51] In general, electron-donating substituents gave enhanced fluorescence and caused the fluorescence to be shifted to longer wavelengths. The enhanced fluorescence was attributed not only to the coplanarity of the 1,3-indanedione moieties, but more importantly to the intramolecular charge transfer process. Electron-withdrawing groups on the aromatic ring reduced fluorescence efficiency and caused a large shift to longer wavelengths.[72,140] Halogens also decrease fluorescence. Alkyl and sulfonate groups have negligible effects on fluorescence.[147]

Analogs with more extended conjugation were expected to absorb light at longer wavelengths. Indeed, benzo[*f*]ninhydrin reacted with the amino acids in latent fingerprints to give a dark green-black compound with absorbance maxima at 435 nm and 628 nm. The room light development of a latent print with benzo[*f*]ninhydrin was superior to ninhydrin and substituted ninhydrin analogs because of the large red-shift in color from the normally observed purple hue. The dark green-black compound stood out against background coloration. When treated with $ZnCl_2$ a red complex formed which absorbed at 530 nm and emitted at 590 nm. The analogous cadmium(II) compound was purple (λ_{ex} 550 nm, λ_{em} 635 nm). Both complexes showed strong room temperature luminescence and in many cases the problems of high background luminescence inherent in a number of surfaces were reduced. These surfaces absorbed between 440 nm and 500 nm and emitted between 550 nm and 650 nm.[51]

Unfortunately, surfaces treated with benzo[*f*]ninhydrin/$ZnCl_2$ generally gave higher background coloration compared with ninhydrin. The shelf life of the reagent also appeared to be substantially less, as a freshly prepared solution was needed to affect good development. Moreover, benzo[*f*]ninhydrin was less soluble and required a higher concentration of methanol, which may lead to increased ink-running and dissolving.[51]

In spite of such drawbacks, the intensity and shift of the benzo[*f*]ninhydrin, when treated with group IIb metals, has made it an important forensic reagent. About the same time that the CVL was being developed, the frequency-doubled neodymium:yttrium aluminum garnet (Nd:YAG) laser became available.[45] These pulsed systems are portable and are operated at 532 nm, a wavelength particularly suited to the 530 nm absorption maximum seen in fingerprints developed with benzo[*f*]ninhydrin/$ZnCl_2$. Although these lasers are not as easily maneuverable as portable Ar-laser systems, their sensitivity is much greater. Fingerprints developed with benzo[*f*]ninhydrin/$ZnCl_2$ and exposed to the 532 nm line of a frequency-doubled Nd:YAG laser showed orange fluorescence regardless of the strength of the latent print. In contrast, a fingerprint subjected to a ninhydrin/$ZnCl_2$/Ar-laser system exhibited a greenish-yellow fluorescence with weak prints but an orange fluorescence with strong prints.

A number of other ninhydrin analogs have been tested for their potential in the detection of latent fingerprints. A very recent study involved the synthesis and evaluation of a number of amino substituted ninhydrin derivatives **89-92**(**Scheme 29**).[84] Both 5-aminoninhydrin (**91**) and 5-dimethylaminoninhydrin (**92**) showed unaided fluorogenic activity with latent fingerprints and exhibited luminescence when excited with an argon-ion laser or a filtered xenon arc lamp. When treated with zinc(II) or cadmium(II) salts little color change was seen and luminescence was not further enhanced. These analogs **91** and **92** reacted with latent fingerprints at a significantly slower rate than ninhydrin, presumably because the electron deficiency of the carbonyl at the 2-position was diminished by the electron-donating effect of the amino substituent. Compounds **91** and **92** showed particular potential as fingerprint reagents because their fluorogenicity occurred in a different spectral region than that of background surfaces. For compounds **91** and **92**, excitation was at approximately 570 nm and emission occurred at over 590 nm. More recently, sulfur-containing analogs such as **50** and **81** have shown considerable promise.[75,86]

Another method which has been discussed as a means of circumventing the problem of interfering background fluorescence was complexation with europium to produce an organo-rare earth complex.[148] The intent of developing such a complex was to induce rare earth luminescence which would have a longer lifetime than background fluorescence, and could be observed via time-resolved imaging. For fingerprints treated with ninhydrin and $EuCl_3$-6 H_2O, adequate contrast between latent print and background luminescence could only be obtained under dye laser illumination at 579 nm and visual observation through a red filter that transmitted at wavelengths longer than 600 nm. The principal emission was at 615 nm. The analogous benzo[*f*]ninhydrin system was also investigated in order to ascertain whether resonance or near-resonance ligand to rare earth energy transfer was partially responsible for the luminescence. If this energy transfer was involved, then benzo[*f*]ninhydrin should give a more intense luminescence. This was found to be the case. This analog has better ligand-rare earth spectral overlap and energy transfer efficiency. Even more efficient ligand-rare-earth energy overlap may be possible by careful design of analogs.

5. TOXICITY OF NINHYDRIN.

In 1957, Breton and co-workers reported the results of their investigations on the toxicity of ninhydrin in mice.[149] Although previous investigations with different animals had shown ninhydrin to be lethal, these studies did not elucidate the mechanism of action. Since alloxan had been found to induce diabetes, it was reasoned that ninhydrin might also be diabetagenic because of its structural similarity to alloxan. Transient hyperglycemia, glycosuria, and some pancreatic lesions had been observed with ninhydrin. These results were only qualitative.

In an effort to understand the cause of the toxicity, Breton and co-workers attempted to counterbalance the noxious effects of **1** with various substances.[149] They chose thiol-containing compounds that could react at the 2-position (cysteine, BAL, etc.), isonicotinic acid hydrazide, a compound known to react easily with carbonyl functions, and various antihemorrhagic agents which either modified capillary permeability (ascorbic acid, antihistamines, etc.) or affected blood coagulation (synthetic vitamin K analogs). The vitamin K analogs and the antihistamines slowed the toxic effects, but none of the other compounds were beneficial.

Breton's studies involved the intraperitoneal injection of 250 white mice having an average weight of 27.5 g with a solution of ninhydrin in water at physiological pH.[149] The ID_{50} value after 24 h was 78 ± 5 mg/kg. Symptoms varied with the injected dose. With a dose concentration of 250 mg/kg, mice died within 5 to 10 minutes of injection. Doses of approximately 100 mg/kg produced depression, vasodilation, and transient paralysis of the mice's hind legs. The vascular problems varied from simple vasodilation to abdominal and pulmonary hemorrhagies.

When the ninhydrin solution was injected intravenously it was found to be less toxic. Mice injected intravenously with 100 mg/kg doses survived for several weeks after noticeable weight loss. Direct injection into the blood stream presumably had reduced toxicity because of the breakdown of ninhydrin into less toxic substances on contact with proteins.

6. SUMMARY

Some interesting features of the chemistry of ninhydrin have been described. The mechanism of formation of Ruhemann's purple was examined by numerous investigators, and although great strides were made in understanding the reactions of ninhydrin with amino functions, some unanswered questions remain.

The structure of Ruhemann's purple was well investigated, but further studies of the tautomeric equilibrium of the protonated form would be enlightening.

Synthetic endeavors resulted in more efficient and novel methodologies for the production of ninhydrin and its analogs. Ninhydrin analogs have not yet been exploited but recent investigations of these compounds show that they hold considerable promise.

Ninhydrin is an important analytical tool. The improvements made in amino acid analysis are impressive. Ninhydrin's synthetic and medical applications have not been extensively investigated and deserve some attention.

The importance of ninhydrin in forensic science illustrates the wide range of possible uses for this compound and its analogs. Although there are many methods for visualizing latent and contaminated fingerprints, chemical enhancement of latent fingerprints is still the most practical and affordable technique used in forensic laboratories. Advances in laser science and post-ninhydrin treatment with metals have provided the most successful technical developments in latent fingerprint detection.

The chemistry of ninhydrin is exciting, and it is hoped that this review will revive interest in this unique tricarbonyl compound.

We would like to thank Mssrs. John Piper and Arthur Raykemper for stimulating our interest in ninhydrins and for their support. We also want to acknowledge the help and valuable suggestions of Drs. Anthony Cantu and Joseph Almog.

8. REFERENCES

(1) Noller, C. C. *Chemistry of Organic Compounds*; 3rd ed.; W. B. Saunders Co.: Philadelphia, 1965; pp 931-933.
(2) Wasserman, H. H.; Fukuyama, J.; Murugesan, N.; van Duzer, J.; Lombardo, L.; Rotello, V.; McCarthy, K.; *J. Am. Chem. Soc.* **1989**, *111*, 371.
(3) Wasserman, H. H.; Kelly, T. A. *Tetrahedron Lett.* **1989**, *30*, 7117.
(4) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M. *J. Am. Chem. Soc.* **1987**, *109*, 5031.
(5) Baker, H.; Sidorowicz, A.; Sehgal, S.N.; Vezina, C. *J. Antibiotics* **1978**, *31*, 539.
(6) McCaldin, D. *J. Chem. Rev.* **1960**, *60*, 39.
(7) Yuferov, V. P. *Usp. Biol. Khim.* **1971**, *12*, 62.
(8) Oshkaya, V. *P. Chem. Abstr.* **1974**, *81*, 91246w.
(9) Rubin, M. *Chem. Rev.* **1975**, *75*, 177.
(10) Schonberg, A.; Singer, E. *Tetrahedron* **1978**, *34*, 1285.
(11) West, R. *J. Chem. Ed.* **1965**, *42*, 386.
(12) Ruhemann, S. *Trans. Chem. Soc.* **1910**, *97*, 1438.
(13) Ruhemann, S. *Trans. Chem. Soc.* **1910**, *97*, 2025.
(14) Ruhemann, S. *Trans. Chem. Soc.* **1911**, *99*, 1306.
(15) Ruhemann, S. *Trans. Chem. Soc.* **1911**, *99*, 792.
(16) Ruhemann, S. *Trans. Chem. Soc.* **1911**, *99*, 1486.
(17) Ruhemann, S. *Trans. Chem. Soc.* **1912**, *101*, 780.
(18) Friedman, M.; Siegel, C. W. *Biochemistry* **1966**, *5*, 478.
(19) Lamothe, P. J.; McCormick, P. G. *Anal. Chem.* **1972**, *44* ,.821.
(20) Bottom, C. B.; Hanna, S. S.; Siehr, D. *J. Biochem. Ed.* **1978**, *6*, 4.
(21) Schonberg, A.; Moubacher, R. *Chem. Rev.* **1952**, *50*, 261.
(22) Huff, E.; Rudney, H. *J. Biol. Chem.* **1959**, *234*, 1060.
(23) Friedman, M.; Williams, L. D. *Bioorg. Chem.* **1974**, *3*, 267.
(24) Crooks, P. A. *Chemistry and Industry* **1975**, 176.
(25) Friedman, M. *Can. J. Chem.* **1967**, *45*, 2271.
(26) Schank, K. *Synthesis* **1972**, 176.
(27) MacFadyen, D. A.; Fowler, N. *J. Biol. Chem.* **1950**, *186*, 13.
(28) Harding, V. J.; Warneford, F. H. S. *J. Biol. Chem.* **1916**, *24*, 319.
(29) Harding, V. J.; MacLean, R. M. *J. Biol. Chem.* **1916**, *24*, 337.
(30) Moubasher, R.; Ibrahim, M. *J. Chem. Soc.* **1948**, 702.
(31) Johnson, A. W.; McCaldin, D. J. *J. Chem. Soc.* **1958**, 817.
(32) Wittmann, H.; Muller, A. K.; Ziegler, E. *Monatsh. Chem.* **1969**, *100*, 497.
(33) Lamothe, P. J.; McCormick, P. G. *Anal. Chem.* **1973**, *45*, 1906.
(34) Bruice, T. C.; Richards, F. M. *J. Org. Chem.* **1958**, *23*, 145.
(35) Grigg, R.; Malone, J. F.; Mongkolaussavaratana, T.; Thianpatanagul, S. *J. Chem. Soc., Chem. Commun.* **1986**, 421.
(36) Grigg, R.; Gunaratne, H. Q. N. *Tetrahedron Lett.* **1983**, *24*, 4457.
(37) Grigg, R.; Gunaratne, H. Q. N.; Sridharan, V.; Thianpatanagul, S. *Tetrahedron Lett.* **1983**, *24*, 4363.
(38) Grigg, R.; Thianpatanagul, S. *J. Chem. Soc., Chem. Commun.* **1984**, 180.
(39) Grigg, R.; Moustafa, F. A.; Sridharan, V.; Thianpatanagul, S. *J. Chem. Soc., Chem. Commun.* **1984**, 182.
(40) Grigg, R.; Sridharan, V.; Thianpatanagul, S. *J. Chem. Soc. Perkin Trans. I* **1986**, 669.
(41) MacFayden, D. A. *J. Biol. Chem.* **1950**, *186*, 1.
(42) Moore, S.; Stein, W. H. *J. Biol. Chem.* **1948**, *176*, 367.
(43) Tominaga, N. *Bull. Chem. Soc. Jpn.* **1962**, *35*, 851.
(44) Wigfield, D. C.; Buchanan, G. W.; Croteau, S. M. *Can. J. Chem.* **1980**, *58*, 201.
(45) Menzel, E. R.; Almog, J. *J. Forensic Sci.* **1985**, *30*, 371.

(46) Teeters, W. O.; Shriner, R. L. *J. Am. Chem. Soc.* **1933**, *55*, 3026.
(47) Meier, R.; Lotter, H. G. *Chem. Ber.* **1957**, *90*, 222.
(48) Regitz, M.; Adolph, H.-D. *Chem. Ber.* **1968**, *101*, 3604.
(49) Wanag, G.; Lode, A. *Ber.* **1938**, *71*, 1267.
(50) Lennard, C. J.; Margot, P. A.; Stoilovic, M.; Warrener, R. N. *J. Forensic Sci.* **1986**, *26*, 323.
(51) Almog, J.; Hirshfeld, A.; Klug, J. T. Y. *J. Forensic Sci.***1982**, 27, 912.
(52) Regitz, M.; Adolph, H. G. *Chem. Ber.* **1968**, *101*, 3604.
(53) Regitz, M.; Adolph, H. G *Liebigs Ann. Chem.* **1969**, *723*, 47.
(54) Becker, H.-D.; Russell, G. A. *J. Org. Chem.* **1963**, *28*, 1896.
(55) Becker, H.-D. *J. Org. Chem.* **1964**, *29*, 1358.
(56) Fieser, L. F. *Experiments in Organic Chemistry*, 3rd ed.; D.C. Heath and Co Boston, **1957**; pp 123-145 .
(57) Fieser, L. F. *Organic Experiments*; D.C. Heath and Co.: Boston, **1964**; pp 141-145 .
(58) Wanag, G.; Bungs, J. *Chem. Ber.* **1942**, *75B*, 987.
(59) Dominguez, X. A. *J. Chem. Educ.* **1953**, *30*, 624.
(60) Joullié, M. M.; Heffner, R. J.; Safaryn, J. E. *Tetrahedron Lett.* **1987**, *28*, 6539.
(61) Joullié, M. M. ; Heffner, R. J. *Synth. Commun., in press.*
(62) Schipper, E.; Cinnamon, M.; Rascher, L.; Chiang, Y. H.; Oroshnik, W. *Tetrahedron Lett.* **1968**, *59*, 6201.
(63) Schipper, E. *U.S. Patent 3,385,894* **1968**.
(64) Schipper, E.; Cinnamon, J. M. *U.S. Patent 3,345,064* **1969**.
(65) Kornblum, N.; Powers, J. W.; Anderson, G. J.; Jones, W. J.; Larson, H. 0.; Levand, 0.; Weaver, W. M. *J. Am. Chem. Soc.* **1957**, *79*, 6562.
(66) Wood, L. L. *U.S. Patent 3,419,616* **1968**.
(67) Jones, D. W.; Wife, R. L. *J. Chem. Soc. Perkins I* **1972**, *3*, 1888.
(68) Bestmann, H. J.; Kloeters, W. *Tetrahedron Lett.* **1978**, *36*, 3343.
(69) Prikule, D. E.; Neiland, O. Y. *Zh. Org. Kim.* **1981**, *17*, 2119.
(70) Schank, K.; Lick, C. *Synthesis* **1983**, 392.
(71) Wasserman, H. H.; Pickett, J. E. *J. Am. Chem. Soc.* **1982**, *104*, 4695.
(72) Lennard, C. J.; Margot, P. A.; Stoilovic, M.; Warrener, R. N. *Forensic Sci. Soc.* **1988**, 3.
(73) Regitz, M. *Synthesis* **1972**, 351.
(74) Gleiter, R.; Schang, P. *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 715
(75) Heffner, R. J., Ph. D. Dissertation, University of Pennsylvania, 1991.
(76) Danishefsky, S.; Kitahara, T.; Yan, C. F.; Morris, J. *J. Am. Chem. Soc.* **1979**, *101*, 6996.
(77) Allen, C. F. H.; Van Allen, J. *Org. Syn. Coll. Vol.* 3, **1955**, 783.
(78) Youn, I. K.; Yon, G. H.; Pak, C.S. *Tetrahedron Lett.* **1986**, *21*, 2449.
(79) Tatsugi, J.; Okumara, S.; Izawa, Y. *Bull Chem. Soc. Jpn.* **1986**, *59*, 3311.
(80) Tatsugi, J.; Izawa, Y. *J. Chem. Research (S)* **1988**, 356.
(81) Almog, J.; Hirshfeld, A. *J. Forensic Sci.* **1988**, *33*, 1027.
(82) Pounds, C. A., *personal communication.*
(83) Kametani, T.; Hibino, S.; Takano, S. *J. Chem. Soc. Perkin Trans. I* **1972**, 391.
(84) Almog, J.; Hirshfeld, A.; Frank, A.; Sterling, J.; Leonov, D. *J. Forensic Sci.* **1991**, *in press.*
(85) Thummel, R. P.; Lefoulon, F.; Cantu, D.; Mahadevan, R. *J. Org. Chem.* **1984**, *49*, 2208.
(86) Almog, J.; Hirshfeld, A.; Frank, A.; Grant, H.; Harel, Z.; Ittah, Y. *J. Forensic Sci.***1991**, *submitted.*
(87) Chadwick, D. J.; Plant, A. *Tetrahedron Lett.* **1987**, *28*, 6085.
(88) Abderhalden, E.; Schmidt, H. S. *Hoppe Seyler's Zeit. Physiol. Chem.* **1913**, *85*, 143.
(89) Abderhalden, R. *Z. Physiol. Chem.* **1938**, *252*, 81.
(90) Virtanen, A. I.; Laine, T. *Nature* **1938**, *142*, 754.
(91) Virtanen, A. I.; Laine, T.; Toivonen, T. *Z. Physiol. Chem.* **1944**, *266*, 193.
(92) Harding, V. J.; MacLean, R. M. *J. Biol. Chem.* **1915**, *23*, 217.
(93) Van Slyke, D. D.; Dillon, R. T.; MacFadyen, D. A.; Hamilton, P. *J. Biol. Chem.* **1941**, *141*, 627.
(94) Van Slyke, D. D. *J. Biol. Chem.* **1929**, *83*, 425.
(95) Van Slyke, D. D.; Hamilton, P. B. *J. Biol. Chem.* **1943**, *150*, 471.

(96) Smakman, R.; van Doorn, A. W. *J. Clin. Nephrol.* **1986**, *26*, s58.
(97) Moore, S.; Stein, W. H. *J. Biol. Chem.* **1954**, *211*, 907.
(98) Wako Pure Chemical Industries, Ltd. *Jpn. Kokai Tokkyo Koho JP 57,206,858 [Chem. Abstr. 82,206,858]* **1982**.
(99) Yamanishi, K.; Hanada, T. *Jpn. Kokai Tokkyo Koho JP 61,107,160 [Chem. Abstr. 86,107,160]* **1986**.
(100) Yamanishi, K.; Hanada, T. *Jpn. Kokai Tokkyo Koho JP 62 98,261 [Chem. Abstr. 87,98,261]* **1987**.
(101) James, L. B. *J. Chromatogr.* **1984**, *284*, 97.
(102) Hori, T.; Kihara, S. *Fresenius' Z. Anal. Chem.* **1988**, *330*, 627.
(103) Aleksandrov, M. L.; Belen'kii, B. G.; Komarov, N. N.; Mal'tsev, V. G.; Melas, V.; Sudareva, N. N. *Zh. Prikl. Khim.* **1983**, *56*, 2097.
(104) Esaki, N.; Soda, K. *Methods Enzymol.* **1987**, *143*, 148.
(105) Gaitonde, M. K. *J. Biochem.* **1967**, *104*, 627.
(106) Murakita, H.; Fujiwara, M. *Jpn. Kokai Tokkyo Koho JP 6215,465 [Chem. Abstr. 87,15,465]* **1987**.
(107) Molnár-Perl, I.; Pintér-Szakács, M. *Anal. Biochem.* **1989**, *177*, 16.
(108) Wang, H.; Ji, H. *Gaodeng Xuexiao Huaxue Xuebao* **1989**, *9*, 732.
(109) Seracu, D. I. *Anal. Lett.* **1987**, *20*, 1417.
(110) Seracu, D. I. *Rev. Chim.* **1988**, *39*, 54.
(111) Yoshimura, T.; Okazaki, S. *Chromatographia* **1988**, *25*, 781.
(112) James, L. B. *J. Chromatogr.* **1987**, *391*, 269.
(113) Laskar, S.; Basak, B. *J. Chromatogr.* **1988**, *436*, 431.
(114) D'Aniello, A.; D'Onofrio, G.; Pischetola, M.; Strazzullo, L. *Anal. Biochem.* **1985**, *144*, 610.
(115) Lenzen, S.; Brand, F. H.; Panten, U. *Br. J. Pharmacol.* **1988**, *95*, 851.
(116) Chatterjie, N.; Opoku-Ofori, P.; Alexander, G. *J. Res. Commun. Chem. Pathol. Pharmacol.* **1985**, *47*, 297.
(117) Rao, V. R. *Curr. Sci.* **1984**, *53*, 799.
(118) Carotti, A.; Casini, G.; Gavuzzo, E.; Mazza, F. *Heterocycles* **1985**, *23*, 1659.
(119) Usmani, J. N.; Ismail, S. M. *Pak. J. Sci. Ind. Res.* **1987**, *30*, 89.
(120) Morrica, P.; Ferreri, C. *Rend. Accad. Sci. Fis. Mat., Naples* **1983**, *50*, 353.
(121) Morrica, P.; D'Ambrosio, C.; Behar, I. *Rend. Accad. Sci. Fis. Mat., Naples* **1983**, *50*, 349.
(122) Schmitt, G.; Dinh An, N.; Poupelin, J. P.; Vebrel, J.; Laude, B. *Synthesis* **1984**, 758.
(123) Buckle, D. R.; Morgan N. J.; Ross, J. W.; Smith, H.; Spicer, B. A. *J. Med. Chem.* **1973**, *16*, 1334.
(124) Oden, S.; von Hofsten, B. *Nature* **1954**, *173*, 449.
(125) Crown, D. A. *Journal of Criminal Law, Criminology and Police Science* **1969**, *60*, 258.
(126) Morris, J. R.; Goode, G. C. *Police Research Bulletin* **1974**, *24*, 45.
(127) Tighe, D. J. *Identification News* **1984**, *34*, 3.
(128) Almog, J. In *Advances in Fingerprint Technology*, Lee, H.; Gaensslen, R. E., Eds., Elsevier Science, N. Y. **1991**, in press.
(129) Pounds, C. A. in *Developments in Fingerprint Visualisation*; Springer-Verlag, Berlin, **1988**; pp 91-119.
(130) Menzel, E. R. *Anal. Chem.* **1989**, *61*, 557A.
(131) Dalrymple, B. E.; Duff, J. M.; Menzel, E. R. *J. Forensic Sci.* **1977**, *22*, 106.
(132) Dalrymple, B. E. *J. Forensic Sci.* **1979**, *24*, 586.
(133) German E. R. *Identification News*, **1981**, *31*,3.
(134) Herod, D. W.; Menzel, E. R. *J. Forensic Sci.* **1982**, *27*, 200.
(135) Herod, D. W.; Menzel, E. R. *J. Forensic Sci.* **1982**, *27*, 513.
(136) Menzel, E. R.; Everse, J.; Everse, K. E.; Sinor, T. W.; Burt, J. A. *J. Forensic Sci.* **1984**, *29*, 99.
(137) Everse, K. E.; Menzel, E. R. *J. Forensic Sci.* **1986**, *31*, 446.
(138) Stoilovic, M.; Kobus, H. J.; Margot, P. A.; Warrener, R. N. *J. Forensic Sci.* **1986**, *31*, 432.
(139) Lennard, C. J.; Margot, P. A.; Sterns, M.; Warrener, R. N. *J. Forensic Sci.* **1987**, *32*, 597.
(140) Menzel, E. R.; Bartsch, R. A.; Hallman, J. L. *J. Forensic Sci.* **1990**, *1*, 25.
(141) Watkin, J. E. *Proceedings of the International Fingerprint Conference organized by the Scientific Research and Development Branch,* **November, 1984**.
(142) Watkin, J. E. *Proceedings of the International Forensic Symposium on Latent Fingerprints, FBI Academy,* **July, 1987**.

(143) Kent, T. *Proceedings of the International Fingerprint Conference organized by the Scientific Research and Development Branch*, **November, 1984.**
(144) Kent, T. *International Symposium on Latent Fingerprints, FBI Academy*, **July, 1987.**
(145) Stoilovic, M.; Kobus, H. J.; Margot, P. A.; Warrener, R. N. *Proceedings of the 9th Australian International Meeting of Forensic Sciences*, **February, 1986.**
(146) Stoilovic, M. *A poster presented at the International Forensic Symposium on Latent Fingerprints, FBI Academy*, **July, 1987.**
(147) Seitz, W. R. *Fluorescence Derivatization CRC Critical Review in Anal. Chem.* **1980**, *8*, 367-445.
(148) Menzel, E. R.; Mitchell, K. E. *J. Forensic Sci.* **1990**, *1*, 35.
(149) Breton, J. C.; Schmitt, H.; Neuzil, E. *Societé de Biologie de Dakar* **1957**, 719.