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## THE DEVELOPMENT OF LATENT FINGERPRINTS WITH NINHYDRIN

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For well over ten years, many of the federal, state, and city crime laboratories have used the ninhydrin technique for the development of latent fingerprints. During this period, numerous convictions were obtained as a result of latent fingerprints developed on documents using this technique. It is safe to state that many more prosecutions have resulted from fingerprint evidence after the introduction of ninhydrin than before.

### HISTORY OF NINHYDRIN

The earliest mention of 1,2,3-triketohydrindene hydrate, also known as 1,2,3-indantrione hydrate or ninhydrin, in the chemical literature was in 1910 by Ruhemann. He outlined the synthesis of ninhydrin and mentioned that the colorless prisms turned red at 125°C. (1) Ruhemann in England and Abderhalden and Schmidt in Germany subsequently reported that alpha amino acids, polypeptides, and proteins formed blue color products when reacted with ninhydrin. (2)(3) In 1913, Abderhalden and Schmidt commented: "Ninhydrin is a valuable reagent for the detection of non-biuret dialyzable amino acids. Various tissues, milk, urine, saliva, blood plasma, serum, lymph, cyst contents, fresh eggs, albumin, fresh and boiled meat, and sweat contain substances which dialyze and react with ninhydrin. The fact that sweat gives an intense reaction is of importance in carrying out the test. Caution must be taken that nothing is touched which later comes in contact with the reagent." (4)

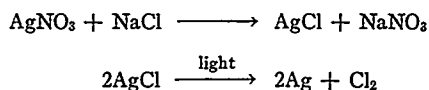
In the following years, ninhydrin found usage in various biochemical and medical test methods. Following the introduction of chromatographic techniques in the early 1940's, ninhydrin was routinely used to locate amino acids on chromatograms. In spite of the history of ninhydrin usage over the years and the oft repeated admonition

against touching chromatograms or other test material that was to be exposed to ninhydrin, a British patent was granted to Oden and von Hofsten in 1955 for the usage of ninhydrin to develop latent fingerprints. (5) Subsequently, American and German patents were also granted for the use of ninhydrin. Conway has eloquently discussed the questionable validity of these patent rights in his 1965 paper. (6) At the present time, ninhydrin is used rather widely in the United States and other countries. It is the purpose of this paper to illuminate the chemical reactions of ninhydrin and to present proven operational techniques that will be of practical value to others in the field.

### LATENT FINGERPRINT DEVELOPMENT

The perspiration exuded from the pores along the papillary ridges is composed of oils, fats, salts, proteinaceous residues, and water. Water comprises approximately 98% of the mixture. The fats and oils are the first constituents in perspiration to be dissipated after they have been deposited on a surface. Generally speaking, the fats and oils are not ordinarily detectable after approximately 72 hours. The well known iodine technique works well to illuminate the oleogenous residues. The iodine technique is physical in nature, rather than chemical. It provides brownish prints of a transitory nature. Fixation and/or photography of developed prints are a necessity.

The salts in the perspiration are detectable in latent fingerprints for several months usually. The chemical reaction of silver nitrate with salt is as follows:



In brief, the silver nitrate technique provides good prints but is complicated, time consuming and ruinous to documents because of the aqueous nature of the technique. Both the iodine technique and the silver nitrate technique are well covered in the literature. (7)(8)(9)(22)

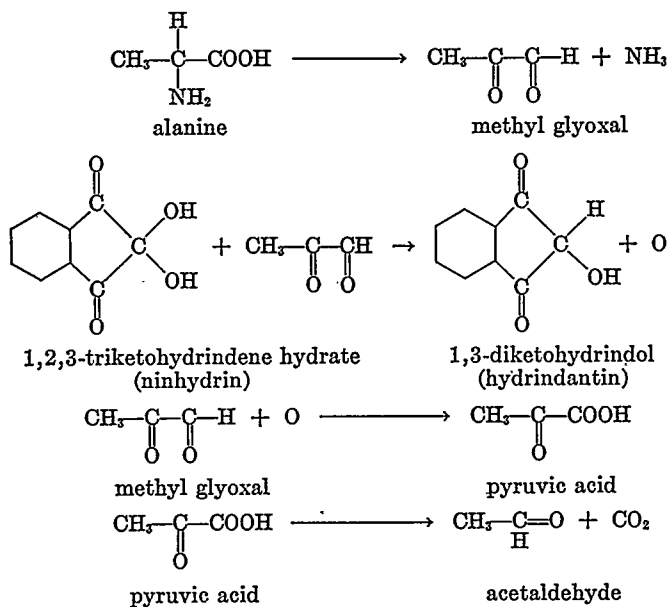
The third group of constituents in perspiration are the protinaceous residues. These protinaceous residues, usually in the form of alpha amino acids, persist for long periods of time and can be detected at long intervals, up to several years later, after their initial deposition on a document.

#### CHEMISTRY OF THE NINHYDRIN REACTION

Pankov was able to specifically detect aspartic acid, glutamic acid, serine, threonine, alanine, valine, and methionine in perspiration. (10) Other amino acids and protinaceous derivatives such as proline,

hydroxyproline, histidine, and indican may also be present. The total spectrum of possible protinaceous derivatives possible in perspiration is yet to be determined. Perspiration constituents vary from individual to individual, and there will be qualitative and quantitative differences in the types and proportions of oleogenous, inorganic, and protinaceous constituents. It has been established that not all proteins and products of protein degradation give the same color reactions with ninhydrin. (11) (12) This has been borne out by observations of developed fingerprints of different individuals.

The basic reaction of ninhydrin with the alpha amino acids and/or alpha amino acyl protinaceous constituents of perspiration is as follows, as originally predicated by Ruhemann, Abderhalden and Schmidt, and Harding and Warneford. (2)(3) (4)(13) Using alanine, a typical aliphatic alpha amino acid, the following reactions are predicated:



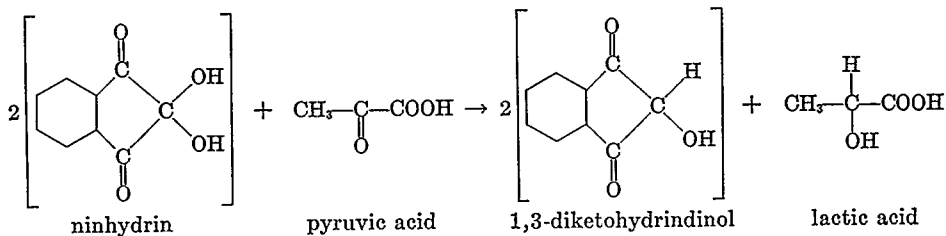
An alternate explanation of the oxidative deamination of alpha amino acids is as follows:



alanine



pyruvic acid

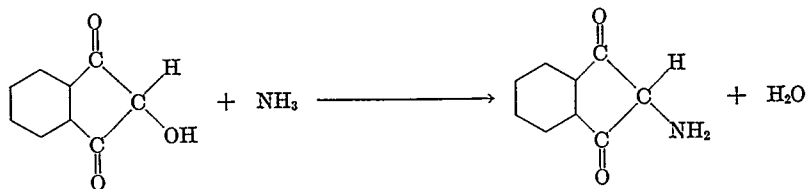


ninhydrin

pyruvic acid

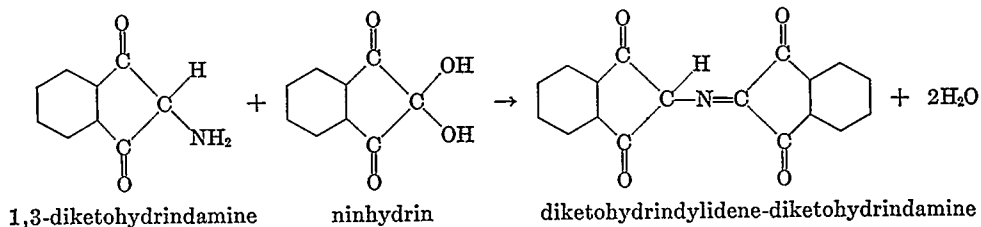
1,3-diketohydrindinol

lactic acid



1,3-diketohydrindinol

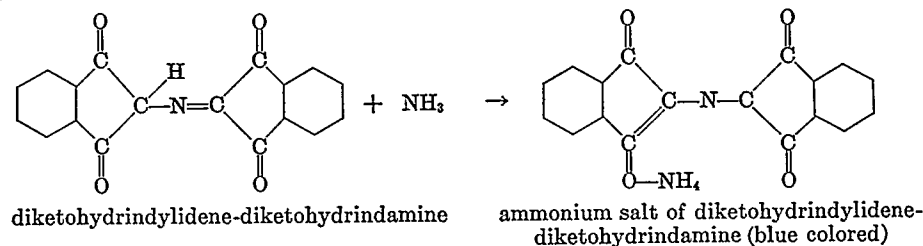
1,3-diketohydrindamine



1,3-diketohydrindamine

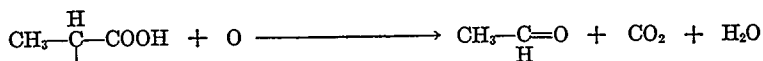
ninhydrin

diketohydrindylidene-diketohydrindamine



diketohydrindylidene-diketohydrindamine

ammonium salt of diketohydrindylidene-diketohydrindamine (blue colored)

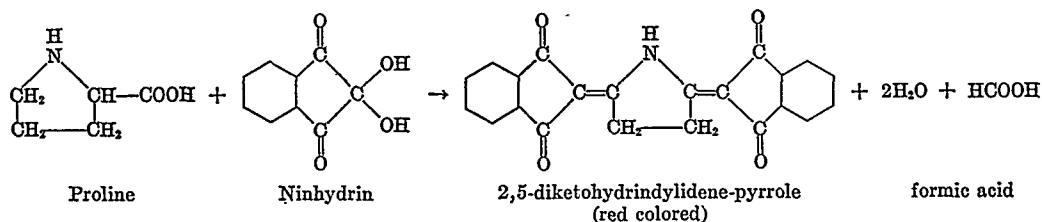


lactic acid

acetaldehyde

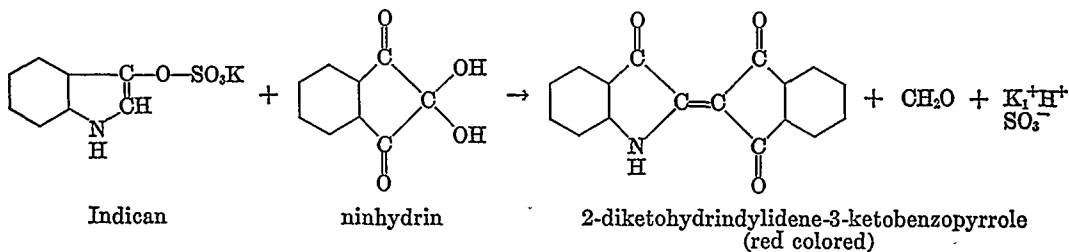
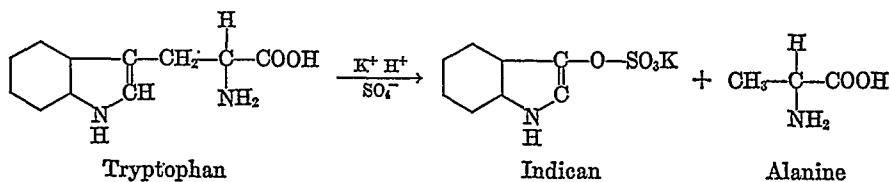
Heterocyclic monoamino acids such as proline, hydroxyproline, and histidine give red colored

reaction products with ninhydrin. (14)(15) The color reaction, in simplified form, is as follows:



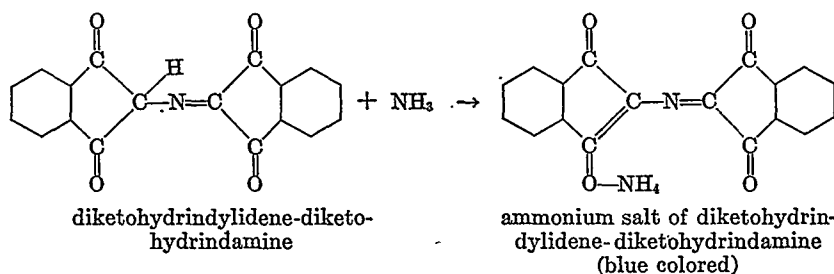
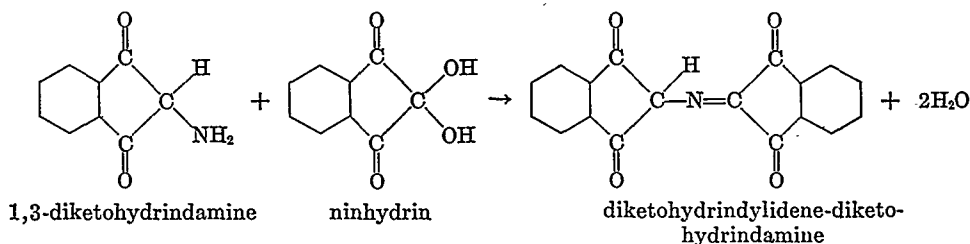
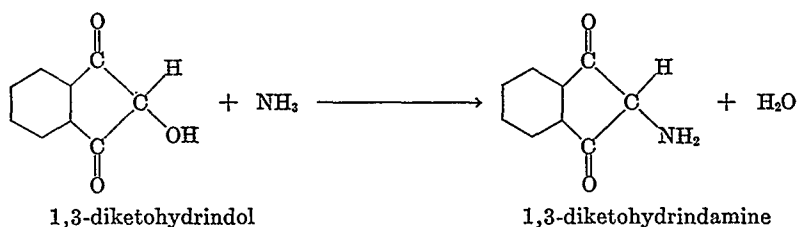
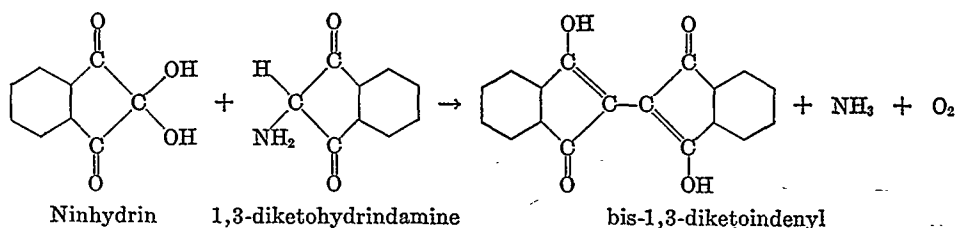
Tryptophan, another heterocyclic monoamino acid is broken down in the body for excretory purposes into substances including indican. Indican

reacts with ninhydrin to give a red colored substance. (16) Following is the reaction in simplified form:



The sum total of substances such as ammonium salt of diketohydrindylidene-diketohydrindamine, 2,5-diketohydrindylidene-pyrrole, and 2-diketohydrindylidene-3-ketobenzopyrrole give the coloration to the ninhydrin reaction. Additionally,

Moubacher, Ibrahim and Schoenberg (17)(18) have found violet colored bis-1,3-diketoindehyl in all developed ninhydrin colors. The following reaction is postulated:



#### MIXING NINHYDRIN SOLUTIONS

There are several approaches to the mixing of ninhydrin in solution. If the running of ink is no problem, a 0.5% solution of ninhydrin in acetone can be used. While much research has been directed to the subject of the ideal percentage of ninhydrin to be put into solution, the emphasis seems misplaced since there is always more ninhydrin available for reaction than amino acids on the documents. A 0.5% solution of ninhydrin in diethyl ether (medical ether) can also be used. While diethyl ether causes much less ink running than acetone, because of its high volatility, it presents a definite fire hazard especially if used in a spray.

If ink running poses a problem, as well it should in a document laboratory, there are two approaches

that can be used to avoid ruining good handwriting or typewriting evidence. On documents such as checks, the endorsement can be masked off with pieces of plastic held in place by spring clips. On documents where there is considerable amount of evidential writing to be preserved, non-polar solutions of ninhydrin should be used. It should be borne in mind that destroying existing evidence such as handwriting by using polar solvents in the hope of developing latent prints is an unwise course of action.

The dyestuffs in fluid inks and ballpen inks are, in the main, soluble in polar solvents such as methanol and acetone. Ninhydrin is not ordinarily soluble in non-polar solvents which do not cause ink running. By using an intermediate carrier, it has been possible to dis-

solve ninhydrin in a solvent of zero dipole moment, thereby achieving a ninhydrin solution which would not affect inks adversely. (19)

With the following procedure, 1000 cc of a non-polar solution of ninhydrin can be mixed. The directions are specific and should be followed step by step to achieve the desired ends.

7.5 grams of ninhydrin are dissolved in 40 cc of methanol. When the ninhydrin is completely dissolved, 960 cc of petroleum ether F (B.P. 30-60°C) is added and stirred for several minutes. The mixture is poured into a separatory funnel and allowed to stand for five to ten minutes. Two layers are formed; a small quantity of deep yellow liquid on the bottom, and a much larger quantity of less viscous pale yellow liquid on top. The deep yellow phase on the bottom is drawn off and discarded. The pale yellow upper phase is used to process documents. It is essential that none of the deep yellow fluid be allowed to come in contact with documents as this portion will cause inks to run. The pale yellow phase will not cause inks to run if properly prepared.

Its exact nature has not been determined; however, the following data are noted. Petroleum ether F and methanol are totally miscible. When ninhydrin is dissolved in methanol, this miscibility does not obtain. It is noted that the deep yellow phase is greater in quantity than the original 40 cc of methanol and ninhydrin. Experimental data indicates that a 4:96 proportion of methanol to petroleum ether in mixing the solution results in a pale yellow phase which does not cause ink running. A 25:75 mixture will produce a top layer which will dissolve inks after they have been immersed for 30 seconds.

The pale yellow phase is not a stable solution. Ninhydrin begins to crystallize out after 24 hours. Choate, Stanghor, and Somerford (20) have reported that ninhydrin oxidizes at a steady rate in air and that "this oxidation proceeds an appreciable extent before discoloration of the stock solution appears." As a consequence, it is recommended that solutions be mixed fresh, if at all possible, and that stored solutions be used within a week at most.

#### APPLICATION OF NINHYDRIN

Ninhydrin can be applied to documents by spraying, dipping, or by brushing. Spraying is advantageous when using the masking technique

to cover handwriting evidence. When used to process a mass of documents, it is more time consuming. Additionally, petroleum ether F in an aerosol mist produces an involuntary cough reflex necessitating the use of a fuming hood. It has been the experience of the author that dipping or immersion is the most effective way of delivering maximum ninhydrin to the document surface and the fastest method to use when there are numerous documents to be processed. The documents are dipped momentarily in a pan containing the ninhydrin solution, then the solution is allowed to run off the document. Drying is accomplished in a matter of 10-20 seconds with a petroleum ether mixture, if there is a flow of air past the documents. In the case of large objects such as cardboard boxes, brushing or spraying is recommended.

Since some papers have a gelatinous sizing, it would be wise to test the corner of a document if it is felt that the entire surface may react with ninhydrin.

#### DEVELOPMENT OF LATENT FINGERPRINTS

Latent fingerprints developed with ninhydrin appear anywhere from ten minutes to several weeks after processing. Heat can be used to accelerate the reaction. On controlled tests with known equivalent material it was found that using a pressing iron, infrared lamps, and air curing at room temperatures all produced the same results when examined after one week. Use of a constant temperature oven, with a temperature below 100°C for short periods of time, is recommended by several workers to speed up the reaction. (21) Temperatures over 100°C will cause background reactions which will obscure latent prints and may destroy some of the amino acids. For routine work, where there are no special time limitations, air curing at room temperatures gives the best results with the least background interference. After the documents are dry, they can be stored either in the open or in envelopes. Documents should be checked for fingerprint development at periodic intervals such as 24 hours, one week, and two weeks. While it is theoretically possible for identifiable prints to develop after a month or so, the greatest majority of prints develop within one week or so at room temperatures. Some workers have found that storing processed documents in an atmosphere with 70% humidity aids in development. Bowls of water in enclosed cabinets have been effective. (21) While it has been suggested that ninhydrin works

better on old prints than on new, research by Choate, Stanghor, and Somerford has demonstrated that this is not the case. (20)

#### PROBLEMS IN THE USAGE OF NINHYDRIN

Prints developed with ninhydrin are not permanent. Fading will start to occur as soon as one month after optimum development. It is good practice to photograph all developed prints having identification potential. In certain cases it may also be desirable to photograph documents prior to processing for latent fingerprints. Occasionally, ninhydrin solutions will fail to give full development of latent fingerprints. The reasons for this phenomenon are yet to be determined. Should a ninhydrin solution work less than satisfactory it should be discarded and a fresh solution used. Reprocessing of a document, under ordinary circumstances, will not damage a document or the quality of the developed fingerprints.

It should be remembered that not all individuals excrete sufficient perspiration through the papillary ridges to leave identifiable latent fingerprints. The use of ninhydrin on a document does not guarantee the development of latent fingerprints even though it may be known that a certain individual has handled a document.

Should it be necessary to employ several latent fingerprint techniques, ninhydrin can be used after iodine fuming and prior to processing with silver nitrate. In using ninhydrin, gloves are recommended to protect the hands from staining. When using flammable solvents, a fuming hood is advisable.

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