Inasmuch as such a product combines the highly desirable physical and chemical characteristics of a long chain fatty acid and a phenol in one, extensive studies in related systems (mono- and di-basic, acyclic as well as isocyclic unsaturated acids and phenolic (thio-phenolic)[5] compounds are in progress.

(5) B. Whitman, Research Report, New York University, 1933.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

A New Preparation of Ninhydrin

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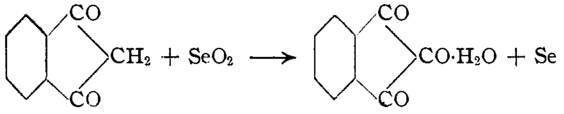
Ninhydrin, triketohydrindene hydrate, has been found to be an extremely valuable reagent for the detection of uncombined amino and carboxyl groups[2] in proteins, peptides and amino acids and in the diagnosis of pregnancy.[3] The only method of preparation, that by Ruhemann,[4] is laborious, expensive and requires starting materials not readily available.

In the present work it was found that diketohydrindene (I), prepared from diethyl phthalate and ethyl acetate according to the method mentioned by Wislicenus,[5] can be readily oxidized to ninhydrin (II) by means of selenious acid or selenium dioxide. The value of this selective oxidizing agent has been pointed out by Riley, Morley and Friend.[6]



I II

A thorough study of this oxidation reaction was made and the maximum yields obtainable were found to be 31–35%. Variations in the order of mixing the reagents, solvents, temperature and use of selenious acid instead of sublimed selenium dioxide have failed to increase the yield. The low yield is apparently due to a bimolecular product formed by the combination of two molecules of diketohydrindene or by the combination of one of the latter with one of triketohydrindene. Similar bimolecular products were formed by the action of hydrogen peroxide on 1,3-diketo-

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[1] Junior Fellow, The Textile Foundation. Published by permission of The Textile Foundation, Washington, D. C.

[2] Ruhemann, *J. Chem. Soc.*, **97**, 2025 (1910); Harding and MacLean, *J. Biol. Chem.*, **20**, 217 (1915); Herzfeld, *Biochem. Z.*, **59**, 249 (1914).

[3] Warfield, *J. Am. Med. Assoc.*, **62**, 436 (1914).

[4] Ruhemann, *J. Chem. Soc.*, **97**, 1438 (1910). The present quotation on ninhydrin is $25.00 per gram.

[5] Wislicenus, *Ann.*, **246**, 347 (1888).

[6] Riley, Morley and Friend, *J. Chem. Soc.*, 1875 (1932).

hydrindene.[4,7] This by-product does not cause any difficulty in the procedure and ninhydrin is easily isolated in the pure state.

Three other methods of preparation were studied. The action of nitrous acid on the diketohydrindene gave the β-oxime of ninhydrin in good yields. All attempts to hydrolyze this oxime failed. The condensation product between *p*-nitrosodimethylaniline and the diketohydrindene was also prepared and subjected to hydrolysis. No ninhydrin could be obtained from the hydrolysis mixture. An attempted oxidation with ceric sulfate gave only high molecular weight products and no ninhydrin.

Since the 1,3-diketohydrindene is readily prepared from inexpensive starting materials which are available commercially, the low yield in the oxidation step is not serious and ninhydrin can now be considered as a potentially cheap reagent.

Experimental

Sodium 1,3-Diketohydrindene-2-carboxylic Ester.—In a 2-liter round-bottomed flask fitted with an efficient reflux condenser and a dropping funnel were placed 500 g. of diethyl phthalate and 100 g. of powdered sodium. The flask was placed on a steam cone and a mixture of 490 g. of ethyl acetate and 10 g. of absolute ethyl alcohol was dropped in over a period of about ninety minutes. The material in the flask was refluxed gently during the addition of the ethyl acetate mixture. The heating was continued for six hours, the reaction mass cooled and 200 cc. of ether added. As much of the sodium salt as possible was poured on a filter and then washed with ether using as small a volume as possible. The total yield of the dry, yellow sodium salt was 385 g. (71% of the theoretical).

1,3-Diketohydrindene.—To a 3-liter beaker containing 1200–1500 cc. of hot water was added 100 g. of the sodium salt. The solution was cooled to 70° and with vigorous agitation the sodium salt was decomposed with 100 cc. of sulfuric acid solution (3 parts of concentrated sulfuric acid and 1 part of water). The mixture was cooled in an ice-bath to 15° and filtered. The product when dry weighed 59 g. (99% of the theoretical). The total diketohydrindene obtained from 500 g. of diethyl phthalate was 236 g. of a pale yellow solid; m. p. 127–129°. Recrystallization from dioxane–benzene mixture by addition of petroleum ether yielded long glistening needles, m. p. 130–131°, which checked the value given by Wislicenus.[5]

Ninhydrin.—In a 2-liter three-necked flask fitted with a reflux condenser and mechanical stirrer was placed 55 g. of sublimed selenium dioxide dissolved in 1200 cc. of dioxane and 25 cc. of water. The stirrer was started and the solution heated to approximately 60–70°. The flame was withdrawn, 73 g. of crude diketohydrindene was added and the resulting mixture refluxed for six hours. A solid separated during this period and was filtered off while the mixture was still hot. The filtrate was transferred to a distilling flask and three-fourths of the dioxane distilled. Between 400 and 500 cc. of water was added and the solution boiled to coagulate the tarry precipitate, which was then removed by filtration. The filtrate was concentrated by distillation to approximately 250 cc. and filtered. The filtrate was boiled with 1 g. of norite, filtered again, concentrated to 125 cc. and allowed to stand at room temperature. The crude ninhydrin which crystallized was filtered, the mother liquor concentrated and a second crop of crystals obtained; total yield of crude, 36–38 g.

The impure ninhydrin was contaminated with a trace of selenious acid which acted

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(7) Kaufmann, *Ber.*, **30**, 382 (1897).

as a bleaching agent and prevented the formation of the characteristic blue color reaction with α-amino acids. It was purified by recrystallization from hot water with the aid of norite. Long colorless prisms of ninhydrin were obtained by crystallization at room temperature. The yield of the pure ninhydrin was 28–31 g. (31–35% of the theoretical). The product gave none of the customary tests for selenium and gave the characteristic color reactions with α-amino acids. It lost water of hydration and turned red between 125–130° and finally melted with decomposition at 241–243°.

Anal. Calcd. for $C_9H_4O_3 \cdot H_2O$: C, 60.67; H, 3.37. Found: C, 60.83; H, 3.64.

The condensation product with *o*-phenylenediamine was prepared as a derivative and found to melt at 219–220°, which checked the value reported by Ruhemann.[4]

β-Oxime of Triketohydrindene.—In a 400-cc. beaker equipped with a stirrer and ice-bath was placed 5 g. of diketohydrindene dissolved in 200 cc. of dilute sodium hydroxide. After the addition of 2.5 g. of sodium nitrite the mixture was cooled to 0°. Hydrochloric acid was added dropwise until the solution was distinctly acid whereupon the ice-bath was removed and the mixture stirred for thirty minutes at room temperature. The solution was filtered and 5.9 g. (100% of the theoretical) of yellow amorphous powder was obtained. The oxime was recrystallized from acetic acid and formed yellowish green plates; m. p. 200–201° (decomp.).

Anal. Calcd. for $C_9H_5NO_3$: N, 8.00. Found: N, 7.91 (Dumas).

Hydrolysis.—The oxime was refluxed for varying periods of time with concentrations of sulfuric acid ranging from 5 to 35% but in each case the oxime was recovered almost quantitatively. No ninhydrin could be obtained from the hydrolysates.

Condensation of Diketohydrindene with *p*-Nitrosodimethylaniline.—In an 800-cc. beaker was dissolved 10 g. of diketohydrindene in 400 cc. of 1% sodium hydroxide. An additional 2 g. of sodium hydroxide was added with 21 g. of *p*-nitrosodimethylaniline. This mixture was heated to boiling and allowed to stand overnight. The reaction mixture was filtered and the condensed product washed with dilute sodium hydroxide.

Hydrolysis.—The black precipitate formed in the above condensation was suspended in 300 cc. of 10% sulfuric acid solution and refluxed for four hours. This solution was then cooled and continuously extracted with ether for eight hours. Evaporation of the ether left only a trace of tarry material from which no ninhydrin could be obtained.

Ceric Sulfate Oxidation.—The oxidation of 3.7 g. of diketohydrindene by a 0.1 *N* aqueous solution of ceric sulfate was carried out. A complex, green, high molecular weight compound was formed. No ninhydrin could be obtained.

Summary

Ninhydrin can be readily prepared from 1,3-diketohydrindene by selective oxidation with selenium dioxide.

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