Reaction of Cyanide with Triketohydrindane Hydrate (Ninhydrin)

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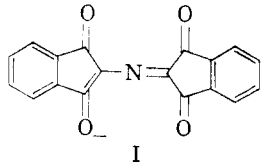
The purple color produced in the photometric ninhydrin procedures for the determination of amino acids is due to the anion of diketohydrindylidine-diketohydrindamine (I).[1,2] The diketohydrin-

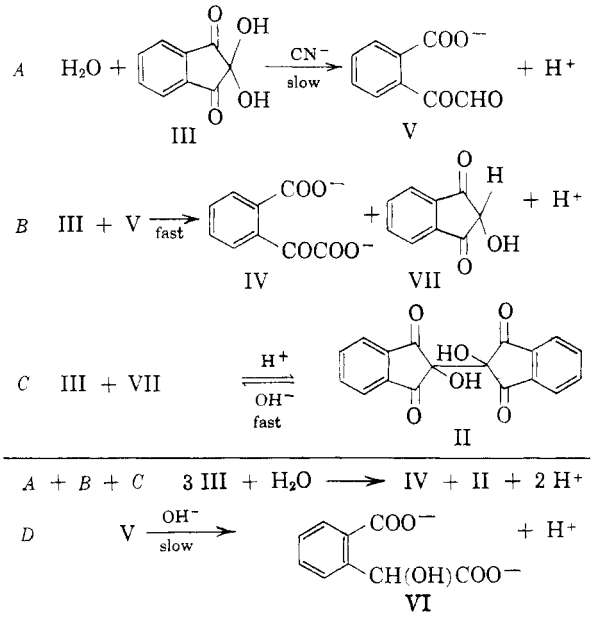


I

dylidinediketohydrindamine can arise from the reaction of an amino acid with either hydrindantin (II) or ninhydrin (III). In order to obtain I in a quantitative color yield a large excess of both II and III is required.[3] In practice the requisite proportions of hydrindantin and ninhydrin are assured either by preparing a solution of the two components[4] or by adding a small amount of a reducing agent, such as stannous chloride, to a solution of ninhydrin.[5] "In search of a more stable and soluble reducing agent", Troll and Cannan[3] observed that potassium cyanide led to the formation of hydrindantin when added to a solution of ninhydrin. Although the analytical procedures which have stemmed from this observation[3,6] are now widely used, the nature of the reaction whereby hydrindantin is produced from ninhydrin, under the influence of potassium cyanide, remains obscure. Some further observations on the reaction of cyanide with ninhydrin are reported here.

Initial experiments established that the addition of catalytic amounts of potassium cyanide to aqueous ninhydrin solutions resulted in the rapid precipitation of an amount of hydrindantin far in excess of that expected from the stoichiometric reduction of ninhydrin by cyanide. With trace amounts of cyanide, the release of protons in the reaction was followed at constant *p*H by means of an autotitrator.[7] At *p*H 6, 2 protons and 1 molecule of hydrindantin arose for each 3 molecules of ninhydrin consumed. In addition, phthalonic acid (IV) was identified as a product of the reaction, through its dianiline salt and by its reaction with phenylhydrazine to form 1-carboxy-3-phenyl-1-12, 3-napthyridone-4. When the reaction was carried out at *p*H values below 6, the base uptake, as directly measured on the autotitrator, was less than that observed at 6 because of the incomplete ionization of the carboxyl groups of phthalonic acid at the lower *p*H levels. Correction of the measured values by means of the titration curves of the reaction products indicated the same stoichiometry between *p*H 3 and *p*H 6. The yield of hydrindantin was the same at *p*H 4, 5, and 6. At *p*H values above 6 the per cent yield of protons increased while that of hydrindantin decreased.

If a great excess of KCN was employed (*p*H 6), no hydrindantin was formed and acidification of the reaction mixture precipitated *o*-carboxymandelic acid (VI). These results suggest the following reaction sequence.



The conversion of III to VI has previously been shown to be catalyzed by hydroxide ion[1] while C is known to be an equilibrium reaction.[2] The greater yield of protons at *p*H values above 6 is a consequence of the competition of reaction *D* with *B* as well as of the reversibility of reaction *C* with increasing *p*H.[2] At high cyanide concentrations reaction *B* is unable to compete with *A* and all the ninhydrin is converted to *o*-carboxyphenylglyoxal or *o*-carboxymandelic acid, thus, precluding the formation of phthalonic acid and 2-hydroxyhydrindanedion-1,3. In the sense of the overall reaction *A* + *B* + *C*, cyanide ion acts as a specific base catalyst. Thus, under condition of *p*H suitable for the OH^- catalysis of *A* the *o*-carboxyphenylglyoxal is consumed in the hydroxide-catalyzed internal Cannizzaro reaction *D*, thus, preventing reactions *B* and *C*.

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The rapidity of the overall reaction $A + B + C$ led us to investigate its kinetics. Under conditions of constant total cyanide concentration, the rate of proton release, corrected for the extent of ionization of the products, increased with increasing pH. Since, in the *p*H range of these experiments the rate of solvolysis of ninhydrin is negligible, the major catalytic species is established to be CN^-. The reaction was found to follow *pseudo* first order kinetics at *p*H 3.0. Deviations from first order plots were apparent at *p*H 4.0 and continually increased to *p*H 7.0. Part of this deviation was traced to the loss of cyanide from the reaction mixture. The value of the apparent second order rate constant at *p*H 3.0 and 30° (in 0.2 N aqueous KCl) was found to be approximately 30 l mol^{-1} min^{-1} giving a value of k_c for CN^- of the order of magnitude 10^7 l mol^{-1} min^{-1}. Reliable initial rate constants could not be obtained above *p*H 4.0, though at this pH the approximate initial rate gave a value of k_c in agreement with that determined at *p*H 3.0.

EXPERIMENTAL

Cyanide in catalytic concentration. With constant mechanical stirring, 0.5 ml. of a 0.01*N* KCN solution (0.005 mmole) was added to 50 ml. water containing 0.5 g. (2.8 mmole) of ninhydrin. The solution was contained in a 100 ml. beaker equipped for automatic maintenance of pH by addition of standard NaOH using an automatic buret and recorder. The reaction was allowed to go to completion at pH 6.0 (approximately 40 min. at room temperature) when 2.1 protons per 3.0 molecules of ninhydrin were released (105% of theory) as measured by base uptake.

The reaction solution was acidified with HCl and the hydrindantin collected, washed with water, and dried *in vacuo* over P_2O_5 (0.29 g.; 97% based on the production of one molecule of II per 3 molecules of III), m.p. 249° dec. (lit. 249° dec.).[1] The filtrate and washings were taken to dryness *in vacuo*, the residue taken up in 4 ml. of 1*N* HCl and filtered to remove a small quantity of II (10 mg.).

The identification of phthalonic acid in the filtrate was carried out by the formation of its dianiline salt and 1-carboxy-3-phenyl-2,3-naphthyridone-4.

(*a*) The filtrate was saturated with aniline and heated on a steam bath for 30 min. After cooling, the black residue was collected, washed with CCl_4 and the tan product recrystallized from a mixture of ethanol and chloroform by addition of carbon tetrachloride to yield colorless needles (20 mg.), m.p. 164°. The dianiline salt of phthalonic acid is reported to melt at 165°.[8]

(*b*) The filtrate was saturated with NH_3, taken to dryness *in vacuo*, the residue taken up in ethanol and collected. After being washed free of color, the salt was dissolved in 2 ml. concentrated HCl and taken to dryness and the residue dissolved in 2 ml. water. The aqueous solution was saturated with phenylhydrazine hydrochloride, heated on the steam bath for 30 min., and chilled; the resulting orange crystals were collected and recrystallized from ethanol and water to yield 60 mg. of 1-carboxy-3-phenyl-2,3-naphthyridone-4, m.p. 219° (lit. 214°).[9]

High cyanide concentration. A warm solution of 0.5 g. (2.8 mmole) of ninhydrin in 15 ml. water was added dropwise with constant mechanical stirring to 25 ml. of an aqueous solution containing 1 g. of sodium acetate dihydrate, 1 g. of KCN (0.0156 mole) and about 18 ml. of 0.1*N* HC (pH 6.0). When this addition was completed, the solution was immediately adjusted to *p*H 2.0 by addition of 6*N* HCl. The resulting white flocculent precipitate was collected, washed with water, and dried over P_2O_5 *in vacuo;* yield 0.20 g. (40%) of *o*-carboxymandelic acid lactone, m.p. 153–154° (lit. 152–153°).

Kinetics. The rate of proton release was determined from the record obtained with a Radiometer TTT 1a Titrator equipped for graphical recording of base uptake. The reaction was carried out in 0.2*N* KCl at 30 ± 0.1° at various *p*H values. At low *p*H the reaction was followed to at least 80% completion and above *p*H 4 to 20 or 30% completion.

The maximum proton release at completion was known from the initial stoichiometry studies. Using these values as α_∞ the graphical data obtained were replotted as $\ln \alpha_\infty/\alpha_\infty - \alpha$ *vs. t.* The first order constants were obtained as the slopes of the best straight lines. Where marked deviations from the first order kinetics occurred, the rate for the initial 5% or less of reaction was estimated.

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Concerning a Preparation of Tryptamine[1]

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Although numerous methods for the synthesis of tryptamine have been reported, there existed none which fully satisfied the authors as a practical source, even though in this laboratory the gramine method[2] was observed to be a dependable alternate route. The Upjohn method[3] for the preparation of 5-benzyloxy tryptamine and several other substituted tryptamines appeared to be desirable even though little investigation into the preparation of the parent compound was reported. The last step of Woodward's 6-methoxy tryptamine synthesis[4] further prompted use of this method for the preparation of tryptamine.

The preparations of 3-indoleglyoxylyl chloride and 3-indoleglyoxylamide (I) which have been previously reported[3,5] have been carried out in this laboratory in 99 and 96 per cent yields respectively in an over-all time of three to four hours. The only

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