cherry-red color was observed eventually. However, when both formaldehyde and ammonia were added, a dichroic effect similar to that of the glycine digestion solutions developed. Like the latter, the solution became red when made strongly alkaline, and blue when made strongly acid (caustic soda, hydrochloric acid).

Preliminary efforts to isolate the colored substance that seems from the experiment just described to be formed from ammonia, oxidized *p*-cresol, and the fragment of the glycine molecule were unsuccessful, but the important thing is that we have found at least a qualitative indication of the fate of the ammonia and *p*-cresol in question, whereas before there was only speculation.

There is a discrepancy in the literature regarding resorcinol as a possible inductor in the tyrosinase–glycine reaction, which should be mentioned. Robinson and McCance[4] state that it functions, although there is a long "induction period," and the deamination of glycine is far from complete. Pugh and Raper[2] and Happold and Raper[5] obtained completely negative results. The clue is that Robinson and McCance used a basidiomycete as the source of the enzyme; the other workers used meal-worms and potatoes, respectively. Since Gortner has shown[5] that tyrosinase not only does not oxidize *m*-dihydroxylic compounds, but also is hindered by them in its oxidation of other phenols, it follows that Robinson and McCance probably did not free their preparation from a laccase which functions with resorcinol,[5] and which is found in the fungus they used, whereas the other workers evidently had preparations free from this laccase. The delayed action and incomplete deamination observed by Robinson and McCance are just what one would expect from a preparation in which the laccase was only a contaminant.

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Determination of the Ionization Constant of Aci-Nitroethane

BY SAMUEL H. MARON[1] AND THEODORE SHEDLOVSKY

In a preceding paper[2] were reported kinetic measurements on the rate of isomerization of nitroethane from the nitro to the aci form. To explain the kinetics of the reverse process of isomerization from aci to nitro form, a knowledge of the ionization constant of aci-nitroethane, $CH_3CH{=}NOOH$, is essential. The only value available is that given by Junell,[3] 7×10^{-5}, and obtained from kinetic measurements at 0°. The importance of this constant for kinetic purposes lends interest to a direct determination by an electrometric method.

The determination involves the complication of the instability of the ions of the aci acid, which isomerize by mutual interaction to the nitro form. Any appreciable concentration of either hydrogen ion or aci ion leads to such a rapid rate of isomerization that the *p*H of the solution varies too much during the course of a *p*H measurement for the results to have significance. This difficulty can be obviated by measuring the *p*H of solutions where the concentration of both ions is very small, *i. e.*, solutions of the aci acid practically free of salt.

A solution of barium nitroethane, 0.06341 *N*, was prepared by dissolving a weighed quantity of nitroethane in water, adding an equivalent quantity of barium hydroxide, and allowing the solution to stand for several days. This stock solution was diluted then to the desired concentrations. In these salt solutions *nearly all* the acid was liberated by the addition of a definite quantity of either hydrochloric or sulfuric acid. The *p*H of the solution was determined with a sensitive glass electrode assembly, with which it was possible to make several measurements to 0.01 *p*H unit within one minute following the acid addition. The electrode[4] was calibrated with a potassium acid phthalate buffer.[5]

The results of three measurements at 23° are given in Table I. Line (1) gives the initial concentration of barium nitroethane, lines (2) and (3) the acid added and its final concentration, and (4) the observed *p*H, while (5), (6), and (7) give the concentrations of hydrogen ion, nitroethane ion, and the undissociated acid at equilibrium. The ionization constants, K_i,[6] calculated from

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(6) In the computation of K_i, which is on a concentration basis, it has been assumed that *p*H measures hydrogen-ion concentration. We are aware of the theoretical difficulties in such an assumption. It is, however, a sufficient approximation for our purpose.

these are given in the last line, and show satisfactory agreement over better than a three-fold concentration range.

TABLE I

THE IONIZATION CONSTANT OF ACI-NITROETHANE AT 23°

(1)	Init. concn. Ba nitroethane	0.04039	0.02467	0.01268
(2)	Acid added	HCl	HCl	H_2SO_4
(3)	Concn. of added acid	0.03975	0.02429	0.01226
(4)	*p*H	3.01	3.10	3.28
(5)	Concn. H^+ ion $\times 10^4$	9.77	7.94	5.25
(6)	Concn. nitro ion $\times 10^3$	1.62	1.17	0.95
(7)	Concn. $CH_3CH{=}NOOH$	0.03877	0.02350	0.01173
(8)	$K_1 \times 10^6$ (av. 4.09 ± 0.10)	4.08	3.95	4.25

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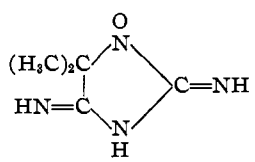
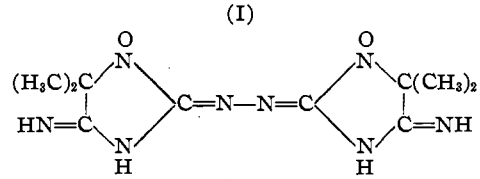
α-Hydroxylaminoisobutyronitrile—an Intermediate in the Synthesis of Porphyrexide and Porphyrindine

BY CURT C. PORTER AND LESLIE HELLERMAN

Kuhn and Franke[1] have shown that porphyrexide (I) and porphyrindine (II)[2,3] constitute, with their respective reductants, rapidly reversible oxidation-reduction systems, the characteristics of which may be measured potentiometrically.



(I)



(II)

Thermodynamically, they stand among the most powerfully oxidizing of the organic systems (E_0' at[4] *p*H 7 = +0.725 or +0.565, respectively).[1] The oxidants, which are unusually interesting "free radicals," have been employed recently for the estimation of certain mercaptans and of the sulfhydryl groups of certain proteins; as such, they have been used in the study of *protein denaturation*.[5,6]

The first step in the synthesis of these substances, involving the addition of hydrocyanic acid to acetoxime to give α-hydroxylaminoisobutyronitrile, requires the use of concentrated aqueous hydrocyanic acid and may be difficult to control. We have modified the procedure by substituting for liquid hydrocyanic acid a suitable cyanide–phosphate buffer. This device may have value also in certain other cases where concentrated hydrocyanic acid has been specified.

Preparation of α-Hydroxylaminoisobutyronitrile, $(H_3C)_2C(NHOH)CN$.—Powdered acetoxime, 94.9 g. (1.3 moles), and 626 g. (4.6 moles) of potassium dihydrogen phosphate (KH_2PO_4) are placed in a 2-liter round-bottomed flask with ground connections for a glass stopper (which preferably carries a stopcock); water, 260 cc., is added, and the mixture is placed in an ice-bath. *Subsequent operations are conducted in an excellently ventilated hood.* To the reaction mixture is added an ice-cold solution of 112.7 g. (2.3 moles) sodium cyanide dissolved in 280 cc. of water; the addition is made rather slowly while the reagents are kept well mixed. The mixture is allowed to warm to room temperature (about 20°) during which it is shaken occasionally. A yellow surface layer will have formed. The flask with contents is conveniently placed in a large closed vessel (desiccator) and allowed to stand (under the hood) at room temperature for about eighteen hours and no longer. The whole mixture is subjected to three ether extractions (total of 640 g. of purified ether) and from the extracts without preliminary drying the hydrocyanic acid and ether are removed in a stream of clean air. The residual aqueous suspension of crystals (which may be concentrated further, if necessary, in a vacuum desiccator) is ice-cooled, transferred to a cold suction filter, freed of mother liquor, and washed thrice with 2-cc. portions of ice water.

The crude product, consisting of nitrile and unchanged oxime, is dried in a vacuum desiccator over phosphorus pentoxide; then the oxime is completely removed by means of trituration with four or five 100-cc. portions of petroleum ether (b. p. 30–35°); oxime recoverable from the extracts amounts to 20–25 g. The α-hydroxylaminoisobutyronitrile is purified further, if required, by being washed with small portions of cold *n*-butanol, followed by recrystallization from ether and petroleum ether; yield 25 g.; m. p. 100°.

The procedure is reliable and convenient. Yields are not as large as those reported by Kuhn and Franke.[1] No difficulty is encountered in the subsequent steps of the porphyrexide synthesis, particularly if the ethanol, required as solvent in the preparation of the iminoester dihydrochloride (derived from nitrile) and of the related amidine hydrochloride, is properly dried.

For the oxidation of α-hydroxylaminoisobutyramidine hydrochloride to the corresponding nitroso compound, the calculated quantity of a standard solution of sodium hypochlorite may be substituted for chlorine gas. However, in order to prevent the formation of N-chloro derivatives, enough hydrochloric acid must be present in the reaction mixture to "cover" the amidine grouping and to generate chlorine *in situ*.

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