fer the view that after the initial loss of the $OH^-$ group, it is a series of transfers of protons (or $CH_3^+$ groups) to the catalyst with consequent formation of an olefin, followed by re-transfer of the proton from the catalyst onto the olefin. This preference is motivated by the consideration that the re-transfer should not occur at random, but preferentially onto that side of the double bond where electron density is highest. The number of probable isomers is thus cut down, and fits the experimental results closer. Addition of a proton from an acid catalyst to an olefin is by no means in contradiction with Whitmore's work.[7]

(7) Laughlin, Nash and Whitmore, THIS JOURNAL, **56**, 1395 (1934).

Table I shows that repeated or complex shifts are infrequent and occur only with the more acid catalysts; it also shows a marked tendency to reduce the amount of branching; methyl group shiftings from 2- to 3-position are pronounced, but rare from 3- to 4-position.


## Summary

Ten secondary and two tertiary alcohols have been dehydrated by means of agents of varied acidity, and at different temperatures. The ratios of the resulting olefins have been established. Results are tabulated and shown to be in good agreement with Whitmore's theory.


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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, THE JOHNS HOPKINS UNIVERSITY, SCHOOL OF MEDICINE]

# Porphyrexide and Porphyrindine Analogs Derived from 1-Hydroxyaminocyclohexyl Cyanide

BY CURT C. PORTER AND LESLIE HELLERMAN

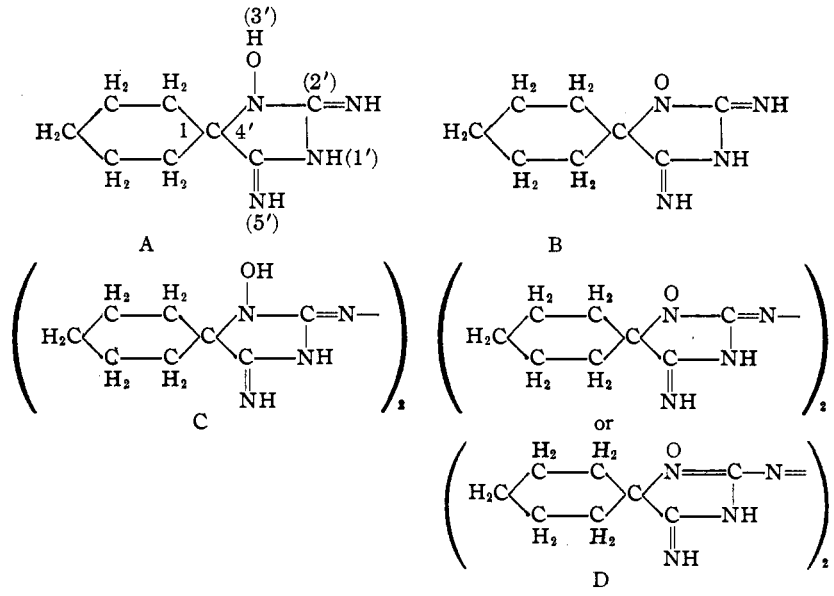
Porphyrexide and porphyrindine, synthesized by Piloty and his co-workers[1,2] some forty years ago, are substances of considerable general interest. They are also of some special utility in connection with certain analytical and structural problems relating to proteins that possess substituent sulfhydryl groups.[3,4] In this Laboratory, porphyrindine has been used advantageously in studies of so-called sulfhydryl-enzymes; for example, as one of the diagnostic reagents for the differentiation in the protein-enzyme, urease, of the more "accessible" and rapidly oxidized *a* sulfhydryl fraction from the *b* category that has appeared to be more directly associated with reversible inactivations of this enzyme.[5,6]

Our present purpose has been to test the generality of the Piloty synthesis and, if possible, to make available additional useful substances of this class. A spirocyclohexyl analog of porphyrexide, here described, has been found to be non-inactivating with respect to urease.[6] This observation gains in significance, in its bearing upon certain structural relationships in urease, when it is considered that the magnitude of the potential[7] of the half-reduced dye system, $E_0'$, at *p*H 7, was ascertained in this work to be 0.690 volt. This is approximately the apparent potential of the iodine:iodide system (in very dilute aqueous solution) under comparable conditions; it will be recalled that urease is inactivated readily in the presence[6] of iodine plus iodide-ion under conditions that do not permit a similar drastic action by porphyrindine.

**The Piloty Synthesis.**—The original synthesis included a number of steps starting with the addition of the elements of hydrogen cyanide to acetoxime in the preparation of 1-hydroxyaminoisobutyronitrile. We have demonstrated[8] how this initial step may be accomplished reliably with the use of a phosphate–hydrocyanic acid buffer mixture and have found, in agreement with the experience of Kuhn and Franke, that the subsequent steps in the synthesis need occasion no difficulty, although the over-all yields may be low.[9] The essential steps in the building of a molecule of the porphyrexide type are well illustrated in the Experimental Part.

In exploratory tests with various oximes, we ascertained that our procedure for the hydrogen cyanide:ketoxime reaction gives excellent results when applied to the preparation of 1-hydroxyaminocyclohexyl cyanide from cyclohexanone oxime. This constitutes the initial step in the synthesis, recorded in the experimental part, that yields the cyclic N-hydroxy compounds, spiro-(1-cyclohexane-4')-2',5'-diimino-3'-hydroxyhydantoin A and the corresponding bis-hydrazino derivative C. These substances are oxidizable readily to compounds B and D, respectively. The oxidant, B, spiro-(1-cyclohexane-4')-2',5'-diimino-hydantoin-N-(3')-oxide, has been found to be noticeably more stable in aqueous solutions

(1) O. Piloty and V. Schwerin, *Ber.*, **34**, 1863, 1870, 2354 (1901)
(2) O. Piloty and W. Vogel, *ibid.*, **36**, 1283 (1903).
(3) R. Kuhn and P. Desnuelle, *Z. physiol. Chem.*, **251**, 14 (1938).
(4) J. P. Greenstein, *J. Biol. Chem.*, **125**, 501 (1938).
(5) L. Hellerman, in *Cold Spring Harbor Symposia on Quantitative Biology*, **7**, 165 (1939).
(6) L. Hellerman, F. P. Chinard and V. R. Deitz, *J. Biol. Chem.*, **147**, 443 (1943).
(7) Electrode potentials are referred to the normal hydrogen electrode. See W. M. Clark, "The Determination of Hydrogen Ions," Williams and Wilkins Co., Baltimore, Md., 1928.
(8) C. C. Porter and L. Hellerman, THIS JOURNAL, **61**, 754 (1939).
(9) The preparative details have been examined also by H. A. Lillevik, R. L. Hossfeld, H. V. Lindstrom, R. T. Arnold and R. A. Gortner, *J. Org. Chem.*, **7**, 164 (1942).




than porphyrexide. Oxidant D, in contrast to porphyrindine, proved to be only slightly soluble in water or in solutions buffered at *p*H 7; possibly it is a polymerized product. Pending additional information, the structure of D, in common with porphyrindine, evidently may be depicted as a "double" radical or, alternatively, as a kind of *bis*-nitrone.

The present work amply demonstrates the applicability of the Piloty synthesis to the preparation of a porphyrexide and a porphyrindine analog from 1-hydroxyaminocyclohexyl cyanide, and suggests that allied compounds may be prepared similarly with the use of suitable hydroxyaminonitriles. Slight modifications of the procedure necessitated in the present synthesis, are dealt with in the experimental section.

## Electrode Potentials

For the potentials at 18° of the half-reduced systems, porphyrexide:leuco-porphyrexide and porphyrindine:leuco-porphyrindine in phosphate buffer of *p*H 7, Kuhn and Franke[10] reported the values 0.725 and 0.565 volt, respectively. We titrated[11] porphyrindine with ascorbic acid in place of hydroquinone, experiencing steady and reproducible potentials under conditions similar to those cited in Table I and obtained, for the second-named system at *p*H 7, the value 0.553 volt.[12]

The reduction of porphyrexide involved a one-equivalent change, as would be anticipated for the conversion of this oxidant odd-molecule, to its stable reductant. The process may be schematized as $>NO + \epsilon + H^+ \rightleftarrows >NOH$. That the reduction of the analog (B) conforms satisfactorily to this pattern is indicated by the data of Table I. This system was studied over the *p*H range 1.93 to 8.63 (Table II).

The oxidant D was titrated in buffered water–dioxane solutions with ascorbic acid. Under conditions similar to those described in Table I, except that the *p*H number was 6.67, concordant data were obtained over the range 30 to 90% reduction; the value $E_0'$ was found to be 0.558 volt. The observed data fitted the electrode equation (Table I) *when n was taken as* 1. A similar result (at approximately *p*H 7) had been obtained with porphyrindine.[10] However, the structures of these

TABLE I

POTENTIOMETRIC TITRATION OF THE PORPHYREXIDE ANALOG *B* WITH ASCORBIC ACID AT 18°

In potassium phosphate buffer, $\mu = 0.1$, *p*H 7.22; total pigment concentration, $1.3 \times 10^{-4}$ *M*. For the expression $E_h = E_0' + \frac{0.0577}{n} \log \frac{[S_0]}{[S_r]}$, $n = 1$.

| Reduction, % | $E_h$, volt | $E_0'$, volt | Reduction, % | $E_h$, volt | $E_0'$, volt |
|---|---|---|---|---|---|
| 16.46 | 0.7190 | 0.6783 | 56.53 | 0.6726 | 0.6792 |
| 20.91 | .7125 | .6792 | 60.98 | .6680 | .6792 |
| 25.37 | .7064 | .6794 | 65.43 | .6631 | .6791 |
| 29.82 | .7008 | .6796 | 69.88 | .6580 | .6791 |
| 34.27 | .6956 | .6799 | 74.37 | .6524 | .6791 |
| 38.72 | .6908 | .6793 | 78.79 | .6461 | .6789 |
| 43.17 | .6861 | .6792 | 83.24 | .6387 | .6789 |
| 47.63 | .6816 | .6792 | 87.69 | .6295 | .6787 |
| 52.08 | .6770 | .6791 | 92.14 | .6172 | .6789 |

Average 0.6792

TABLE II

DEPENDENCE OF $E_0'$ UPON *p*H AT 18°

System (1), B:A (porphyrexide analog); System (2), D:C (porphyrindine analog); in water:dioxane (see text).

| Buffer[a] $\mu = 0.1$ | *p*H | System (1) $E_0'$, volt | System (2) $E_0'$, volt |
|---|---|---|---|
| HCl | 1.93 | 0.977 | |
| Citrate | 3.08 | .906 | |
| Acetate | 4.79 | .808 | |
| Phosphate | 6.49 | .714 | |
| Phosphate | 6.67 | | 0.558 |
| | 7.00 | .690[b] | .534[b] |
| Phosphate | 7.22 | .679 | |
| Phosphate | 7.43 | | .497 |
| Borate | 8.63 | .603 | |
| Borate | 8.88 | | ( .41) |

[a] Made up with potassium salts. [b] Obtained by interpolation.

(10) R. Kuhn and W. Franke, *Ber.*, **68**, 1528 (1935).

(11) The methods were similar to those described by W. M. Clark and co-workers, *Hygienic Lab. Bull.*, No. **151**, 13 (1928). See the Experimental Part.

(12) All titration data were analyzed by the method of L. J. Reed and J. Berkson, *J. Phys. Chem.*, **33**, 760 (1929).

substances suggest, and the analytical behavior of porphyrindine with reducing agents confirms, that their over-all reduction involves a two-equivalent change. It would be unprofitable to consider here a theoretical explanation of the electrode behavior of the porphyrindines with the few data in hand.

## Experimental Part

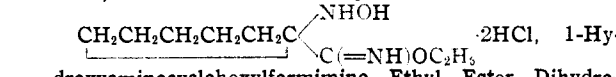
**1-Hydroxyaminocyclohexyl Cyanide.**—The preparation was conducted in a good hood. There was used apparatus similar to that described[8] for the synthesis of 1-hydroxyaminoisobutyronitrile. To an ice-cold mixture of 113 g. of cyclohexanone oxime, 476.8 g. of potassium dihydrogen phosphate ($KH_2PO_4$) and 190 ml. of water, there was added cautiously, with cooling and gentle agitation, a cold solution of 90.4 g. of sodium cyanide in 190 ml. of water. The mixture was shaken well. It was allowed to warm to room temperature (*ca.* 20°) and to stand for ten to fifteen hours.

The crust of slightly brown, large hexagonal platelets that had formed was broken up with a glass rod, and the whole mass transferred to a large beaker. After the inorganic salts had settled, the crystalline crust was removed with a casserole and transferred to a Büchner funnel; the product was freed of mother liquor and washed on the filter with several portions of iced water; yield of crude material, 111 to 123 g.

Partial purification was effected by means of washing the crude product with about 70 ml. of *n*-butanol followed by several portions of ligroin. The compound crystallized from mixtures of ether and ligroin as glistening, white, slightly elongated, hexagonal platelets; decomposition point (bath preheated to 130°), 136° (cor.).

*Anal.* Calcd. for $C_7H_{12}N_2O$: N, 20.0. Found: N (Dumas), 19.8, 19.7.

The crude hydroxyaminonitrile, if reasonably light in color, was used in the next step.

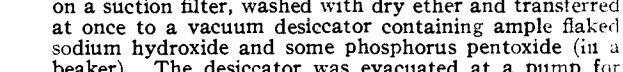


·2HCl, **1-Hydroxyaminocyclohexylformimino Ethyl Ester Dihydrochloride.**—Finely powdered hydroxyaminonitrile, 25 g., was covered with an excess of *anhydrous* ethanol, 50 ml., and 350 ml. of anhydrous ether in a 600-ml. Pyrex bottle. The suspension was cooled to 0° in ice, and *without manual or mechanical stirring*, dry hydrogen chloride gas was passed in to saturation. With its contents protected from moisture by use of a calcium chloride tube, the bottle was allowed to warm to approximately 20°, then tightly stoppered with a wired-on rubber stopper and allowed to stand at room temperature for fifteen hours. After the bottle and contents had been recooled to 0°, the stopper was removed and the bottom crust rapidly broken up with a glass rod. Prolonged exposure to the air was avoided. The bottle was closed again as before, and the suspension then was shaken for ten hours, after which it was allowed to stand in the cold room for twelve to twenty-four hours.

The dihydrochloride was collected as quickly as possible on a suction filter, washed with dry ether and transferred at once to a vacuum desiccator containing ample flaked sodium hydroxide and some phosphorus pentoxide (in a beaker). The desiccator was evacuated at a pump for some time, and the contents then allowed to stand *in vacuo* until free of excess hydrogen chloride; yield, 36 g. (63%); decomposition point, 184°.

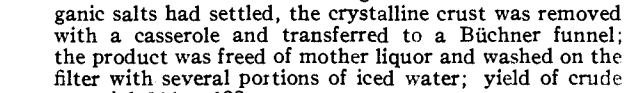
*Anal.* Calcd. for $C_9H_{20}N_2O_2Cl_2$: N, 10.8. Found: N (Kjeldahl), 10.7.

The product was used without further purification for the preparation of the hydroxyaminoamidine hydrochloride.



·HCl, **1-Hydroxyaminocyclohexylformamidine Hydrochloride.**—To 108.2 g. of the imino ester dihydrochloride, suspended in 300 ml. of anhydrous ethanol, suitably cooled with tap water, was added slowly 2.8 mol. equiv. of ammonia in an approximately 4 molar anhydrous ethanolic solution. The final volume was 600 ml. The container (a Pyrex bottle) was closed with a wired-in rubber stopper and the suspension shaken on a machine for four hours. After having stood in the cold room for six hours, the bottle was unstoppered and the mixture of hydroxyaminoamidine hydrochloride and ammonium chloride was collected on a Büchner funnel and dried in a vacuum over phosphorus pentoxide; weight of mixture, 82 g. Partial separation of the mixture into its components could be effected by repeated extraction of the product with methanol, addition of acetone to the extract, filtration, and recovery of amidine hydrochloride by concentration of the filtrate. The method was wasteful of solvents and did not make a particularly clear-cut separation. Since the subsequent oxidation by the addition of hypochlorite was carried out in the presence of an excess of acid, the danger of formation of nitrogen chlorides was minimized; hence, as a rule, no attempt at purification was made before proceeding with the following steps. A specimen of the purified hydroxyamidine hydrochloride was analyzed.

*Anal.* Calcd. for $C_7H_{16}N_3OCl$: N, 21.7. Found: N (Kjeldahl), 22.0.



**1-Nitrosocyclohexylformamidine Hydrochloride.**—A stock solution of alkali hypochlorite was prepared by passing washed chlorine gas, generated from 59.3 g. of potassium permanganate and 300 ml. of concentrated hydrochloric acid, into a solution of 86.8 g. of sodium hydroxide in 470 ml. of water at 0°. The solution retained its strength upon storage in a dark bottle in the cold room for several months. The hypochlorite was assayed iodometrically, and excess alkali also was determined.

To a stirred, ice-cold solution of 78.9 g. of the hydroxyaminoamidine hydrochloride–ammonium chloride mixture referred to above [which contained presumably 62 g. (0.32 mole) of the organic hydrochloride] in 395 ml. of approximately 6 *M* hydrochloric acid, there was added slowly in an ice-cold solution 0.315 mole of sodium hypochlorite.[8] The solution used had assayed 0.00123 mole of sodium hypochlorite and 0.0019 mole of excess sodium hydroxide per ml.; accordingly there was required 256 ml.

After the chilled mixture had stood for thirty minutes, the nitrosoamidine hydrochloride was collected and washed with ice-cold water, yield of desiccator-dried product, 50.3 g. (82%). Although it was not necessary to purify the compound before proceeding to the next step, recrystallization could be accomplished by dissolving the product in slightly warm water; cooling the solution and adding cold concentrated hydrochloric acid. Small, white, square platelets were obtained; decomposition point, 155°.

*Anal.* Calcd. for $C_7H_{14}N_3OCl$: Cl, 18.5. Found: Cl (Volhard), 18.9.

**Compound A (Hydrate).**—Nitrosoamidine hydrochloride, 50.3 g. (0.26 mole) was added, all at once, to a solution of 14 g. (0.28 mole) of sodium cyanide in 225 ml. of water at 65°. The mixture was stirred, and solution took place almost immediately; the temperature rose to 85°. The solution was straw-colored.

The solution was cooled in ice, and acetone (ten volumes) was added slowly after which the mixture was allowed to stand in an ice-bath for some hours. If an oil came out, it was taken up in a minimum amount of water; acetone was added carefully, whereupon crystallization usually resulted. The yield was nearly quantitative. After several recrystallizations by this procedure, the reduced-porphyrexide analog A was obtained as clusters of light yellow, hexagonal prisms with non-planar ends; decomposition point (bath preheated to 220°), 235.5°.

The substance proved to be a monohydrate [Calcd. for $C_9H_{14}N_4O\cdot H_2O$, $H_2O$, 9.0. Found (cor. for ash): $H_2O$, 9.3].

*Anal.*[13] Calcd. for $C_8H_{16}N_4O_2$: C, 48.0; H, 8.1; N,

(13) Microanalyses by Dr. T. S. Ma, Chicago, Ill.

28.0. Found (cor. for ash, 4.0%): C, 47.4; H, 7.4; N, 27.8, 28.6.

**Compound B (Porphyrexide Analog).**—In a chilled solution of 1.1 g. of sodium hydroxide in 30 ml. of water, there was dissolved 5 g. of Compound A. The liquid was cooled in ice and a cold solution of 9 g. of potassium ferricyanide in 25 ml. of water was added all at once. After the mixture had been shaken thoroughly the precipitated product was collected by suction, on a sintered glass disk, and washed with small portions of ice-cold water until the washings were bright orange. Too much washing was avoided since the product was appreciably soluble in cold water. The yield of desiccator-dried product was 3 g.

The dye was fairly soluble in water, dioxane and chloroform; less soluble in ether; insoluble in xylene, cyclohexane, ligroin and petroleum ether. Recrystallization was wasteful of solvents, but could be accomplished by allowing the bright orange-red saturated solution of the dye in ether, to which there had been added an equal volume of purified petroleum ether, to stand for twenty-four hours in the cold room. About 250 ml. of ether was required to dissolve 0.3 g. of the crude dye. Under the conditions described, the dye crystallized in colonies of glistening, yellow-orange, very thin, friable, trapezoidal leaflets. In a melting point bath preheated to 150°, the crystals lost their orange color at 155° and decomposed more extensively at 177–180°. The oxidizing capacity of the substance was estimated both iodometrically and potentiometrically. The specimen analyzed by microcombustion contained a little inert material (3.5%), and decomposed violently when heated.

*Anal.* Calcd. for $C_8H_{13}N_4O$: C, 53.0; H, 7.2; N, 30.9. Found (cor.): C, 53.0; H, 6.9; N, 31.0, 30.6.

**Compound C.**—A solution of 5 g. (0.0275 mole) of Compound A and 0.69 g. (0.0138 mole) of pure hydrazine hydrate in 20 ml. of 0.05 *M* sodium hydroxide was refluxed for two and one-half hours. The mixture was cooled to 0°, and the solid product collected and washed with cold water; yield, 1.3 g. (28%). Recrystallization was accomplished from a solution of the product in the minimum amount of dilute hydrochloric acid by the addition of cold, dilute alkali to cloudiness, followed by one volume of ethanol. The light yellow material that crystallized under these conditions as slightly S-shaped rods decomposed at 261°. The compound turned light blue after standing in air.

*Anal.* Calcd. for $C_{16}H_{26}N_8O_2$: C, 53.0; H, 7.2; N, 30.9. Found: C, 52.5; H, 6.8; N, 30.3.

**Compound D (Porphyrindine Analog).**—To an ice-cold solution containing 0.5 g. of the leuco compound and 0.11 g. of sodium hydroxide in 5 ml., there was added rapidly a cold solution of 0.9 g. of potassium ferricyanide in 5 ml. of water. The deep blue solid material that precipitated was collected on a sintered glass disk and washed thoroughly with cold water. The slight solubility of the dye permitted extensive washing; yield, 0.4 g.

The blue substance was soluble in acids and alkalies, yielding blue and purple-red solutions, respectively. A saturated solution of the dye in 1,4-dioxane possessed a deep blue color. The addition of two volumes of cyclohexane to the dioxane solution brought out the material as minute, glistening crystals.

When placed in a melting point bath preheated to 160°, decomposition did not occur until the temperature had reached 210° (decomposition point variable with temperature of preheated bath).

*Anal.* Calcd. for $C_{16}H_{24}N_8O_2$: C, 53.3; H, 6.7. Found: C, 53.0; H, 6.3.

**Potentiometric Titrations.**[11]—Titrations were conducted at 18.0°. In those experiments where the solubility of the compound under scrutiny permitted, a solution of the oxidant in water was added to ten volumes of potassium salt buffer, of which the ionic strength was 0.1, the solution was deaerated in pure nitrogen and then was titrated with 0.001 *M* ascorbic acid solution. The *p*H of the dye solution was determined by means of a glass electrode, before and after reduction, the *p*H being assumed to vary linearly between these extremes during the titration.

For the titration of Compound D, 5 ml. of a saturated solution of the pigment in dioxane was added to 50 ml. of buffer, and the titration with ascorbic acid carried out as usual. In a comparative test, oxidant Compound B (porphyrexide analog) was titrated in the presence of a comparable concentration of dioxane; there was no discrepancy between the potentials so obtained and those from the titrations in purely aqueous solutions.

## Summary

1. To test the generality of the Piloty synthesis, and to provide additional substances useful in the study of sulfhydryl-proteins and enzymes,[5,6] there have been synthesized compounds B and D, spirocyclohexyl-analogs of porphyrexide and porphyrindine, respectively. Compound B is appreciably more stable than porphyrexide in aqueous solutions. The porphyrindine analog, D, is only slightly soluble in water (or in buffers of *p*H 7).

2. The synthesis of these substances required the preparation of the following compounds, also undescribed hitherto: 1-hydroxyaminocyclohexyl cyanide, 1 - hydroxyaminocyclohexylformimino ethyl ester dihydrochloride, and the corresponding hydroxyaminoamidine hydrochloride and nitrosoamidine hydrochloride, as well as Compounds A and C from which the "radicals," B and D were derived by oxidation.

3. Determinations of the oxidation–reduction potentials of the new dyes indicated that the values, $E_0'$, for the half-reduced systems B:A and D:C at *p*H 7 and 18° are 0.690 and 0.534 volt, *i. e.*, 0.035 and 0.02 volt negative to the respective porphyrexide and porphyrindine systems.

BALTIMORE, MD. RECEIVED JULY 11, 1944