and eight-membered rings with rearrangement taking place to a considerable extent. The other experiments are at higher temperatures and in the presence of iron, conditions favoring dehydrogenation and giving toluene and *p*-xylene. In all of these cases the original compounds are saturated. Cycloöctene is dehydrogenated more easily than any of the saturated compounds mentioned probably because of a higher degree of adsorption on the catalyst.

Summary

1. An apparatus has been described for the catalytic study of the vapors of hydrocarbons in the gasoline range.

2. Catalytic dehydrogenation of cycloöctene with chromium oxide gives styrene, not cycloöctatetraene.

3. The eight-membered ring tends to form the six-membered ring at temperatures of 300° and over.

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[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 692]

The Magnetic Study of the Equilibrium between Ferrohemoglobin, Cyanide Ion, and Cyanide Ferrohemoglobin

BY FRED STITT[1] AND CHARLES D. CORYELL[2]

Cyanide ion combines readily with ferrihemoglobin to form the very stable compound ferrihemoglobin cyanide, containing one cyanide group per iron atom. This is characterized by an absorption spectrum showing a broad band with a maximum at about 5400 Å. The properties of a new stable compound of ferrohemoglobin with cyanide are reported in this paper.

In the course of an extensive investigation of the effects of various substances on the spectroscopic and magnetic properties of ferrihemoglobin and ferrohemoglobin, we noticed that on addition of sodium hydrosulfite ($Na_2S_2O_4$) to ferrihemoglobin cyanide solution at either *p*H 6 or 10 the spectrum changes immediately to one showing a double band, and then fades in about a minute into the ferrohemoglobin spectrum. This observation has been made also by Balthazard and Philippe[3] and by Anson and Mirsky.[4] If sufficient cyanide is added to the alkaline solution after the double-banded spectrum has faded, this spectrum reappears and then remains indefinitely. The same spectrum is obtained also by adding a large amount of cyanide to ferrohemoglobin solution alkaline enough to prevent the formation of hydrocyanic acid.

The spectrum of the cyanide compound has a fairly narrow band maximum at about 5610 Å., approximately 120 Å. wide, a minimum at about 5500 Å., and a second maximum (somewhat broader and slightly higher than the first) at about 5335 Å. This spectrum is closely similar to those of the previously recognized compounds of ferrohemoglobin with oxygen, carbon monoxide, nitric oxide,[5] and the alkyl isocyanides.[6] Each of these compounds contains one added molecule per iron atom; it is accordingly probable that the compound with cyanide ion whose existence is indicated by the spectrum is similar in composition, and contains one cyanide ion per iron atom. It is also probable that the cyanide ion is attached by a covalent bond directly to the iron atom, occupying the sixth coördination position about it, since similar covalent structures have been verified by magnetic measurements for the ferrohemoglobin compounds mentioned above[7] and for dicyanide hemochromogen[8] and ferrihemoglobin cyanide.[9] Evidence supporting this assumption is given in this paper.

Magnetic measurements of the equilibrium involved in the formation of cyanide ferrohemoglobin were made with the technique described in detail below. The first measurements were made

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(3) V. Balthazard and M. Philippe, *Ann. méd. légale*, **6**, 137 (1926).

(4) M. L. Anson and A. E. Mirsky, personal communication.

(5) F. Haurowitz, *Z. physiol. Chem.*, **138**, 68 (1924); D. L. Drabkin and J. H. Austin, *J. Biol. Chem.*, **112**, 51 (1935).

(6) O. Warburg, E. Negelein and W. Christian, *Biochem. Z.*, **214**, 26 (1929).

(7) (a) Oxyhemoglobin and carbonmonoxyhemoglobin, L. Pauling and C. D. Coryell, *Proc. Natl. Acad. Sci.*, **22**, 159 (1936); (b) nitric oxide hemoglobin, C. D. Coryell, L. Pauling and R. W. Dodson, to appear in *J. Phys. Chem.* (1939); (c) ethyl isocyanide ferrohemoglobin, L. Pauling and C. D. Russell, unpublished experiments.

(8) L. Pauling and C. D. Coryell, *Proc. Natl. Acad. Sci.*, **22**, 159 (1936).

(9) C. D. Coryell, F. Stitt and L. Pauling, THIS JOURNAL, **59**, 632 (1937).

by adding successive amounts of potassium cyanide solution to a ferrohemoglobin solution buffered at approximately *p*H 10.6 and determining the magnetic susceptibility at each step. The values obtained in this way for small cyanide concentrations agree closely with those given below, obtained by a different procedure; at larger cyanide concentrations the agreement is only approximate (to within about 1000×10^{-6} c. g. s. u.). It was observed that the suceptibility of the ferrohemoglobin-cyanide solutions changes with time, and that a fine precipitate appears, both effects being noted within a few minutes in the presence of large amounts of cyanide. (It seems not unlikely that the formation of hemochromogen might occur on denaturation of hemoglobin in solutions of this alkalinity, but the characteristic hemochromogen spectrum was not observed at any time.)

The final series of measurements was made in the following way. To 20.0 ml. of ferrihemoglobin stock solution (*p*H 7) about $0.0118\,f$ in heme iron there was added with mechanical stirring 4.0 ml. of 0.86 *N* potassium hydroxide (making the *p*H about 10.6), 4.0 ml. of 2 molal potassium carbonate–bicarbonate buffer solution with *p*H 10.6, and the desired quantity of $5.69\,f$ potassium cyanide solution. Of this solution 25 ml. was placed in the magnetic susceptibility tube and reduced with weighed amounts of sodium hydrosulfite. Measurements were made as soon as possible to circumvent denaturation effects, and the observed forces (Δw in mg.) were extrapolated back to zero time from observations of the small but appreciable drift toward smaller values. The observed values were corrected, as is customary, for diamagnetism of the reagents determined in blank trials, and for dilution of the solution. A separate experiment was carried out for each cyanide concentration.

The results of the final series of measurements are given in Table I. Column 5 gives the values of Δw (extrapolated back to zero time when cyanide was present), which must be corrected for the Δw of the tube filled with water by the amount given in column 6. Column 7 gives the corrections for the diamagnetism of sodium hydrosulfite, values of 0.10 and 0.19 (corresponding to the use of 0.30- and 0.60-g. portions, respectively) are calculated from an observed force of 0.97 for 3.00 g. in 25.0 ml. of water. Columns 8, 9, and 10 give the corrections for diamagnetism of the potassium cyanide solution, the potassium carbonate–bicarbonate buffer, and the potassium hydroxide solution calculated from observations on solutions of closely the same concentration. The values *Rw* of column 11 are the sums of the Δw values of column 5 and the corrections of columns 6–10, and are multiplied by the dilution factor (values of column 3 divided by 20.0) to give the *Dw* values of column 12, which are the Δw values calculated for a hemoglobin solution of the same concentration as the stock solution corrected for tube blank and the diamagnetism of all

TABLE I

MAGNETIC SUSCEPTIBILITY OF IRON IN FERROHEMOGLOBIN–CYANIDE MIXTURES

20.0 ml. ferrihemoglobin solution taken

Prepn.	Ml. KCN 0.569 *f*	Total vol., ml.	(CN^-) *f*	Δw *t* = 0	Corrections to Δw: Tube	$Na_2S_2O_4$	KCN	Buffer	KOH	*Rw*	*Dw*	$10^6\,\chi_{molal}$	Observations
A	0	28.0	0	5.21	0.15	0.19	..	0.92	0.16	6.63	9.28	12,130	No pptn. in 30 min., Δw constant for 10 min.
B	0	28.0	0	5.42	.15	.10	..	.92	.16	6.75	9.45	12,210	No pptn. in 30 min., Δw constant for 10 min.
C	0	40.0	0	1.55	.15	.10	..	2.95[a]	..	4.74	9.50	12,300	Equal quantities of buffer and Hb^+ soln.; no pptn. in 30 min.
D	0.50	28.5	0.0998	4.67	.15	.10	0.06	0.90	.16	6.04	8.61	11,220	$HbCN^-$ spectrum visible pptn. noticed in 50 min., when Δw = 4.45
E	1.00	29.0	.196	4.00	.15	.19	.11	.89	.16	5.50	7.98	10,500	Δw = 3.62 in 60 min., pptn.
F	1.00	29.0	.196	3.84	.15	.10	.11	.89	.16	5.25	7.61	10,000	Some pptn. in 10 min.
G	1.50	29.5	.289	3.37	.15	.19	.17	.87	.15	4.90	7.23	9,600	Δw = 3.16 in 30 min.
H	2.00	30.0	.379	2.93	.15	.19	.22	.86	.15	4.50	6.75	9,000	Δw = 2.74 in 30 min. considerable pptn.
I	2.80	30.8	.517	2.48	.15	.19	.30	.84	.15	4.11	6.34	8,500	Δw = 2.05 in 37 min.
J	3.00	31.0	.550	1.72	.15	.19	.32	.83	.15	3.36	5.21	7,200	Nearly completely gelled in 30 min. Δw = 1.59
K	3.00	31.0	.550	1.42	.15	.10	.32	.83	.15	2.97	4.60	6,500	Gelled in 30 min.; Δw = 0.51; in 70 min. Δw = 0.09
L	5.00	33.0	.861	0.10	.15	.19	.50	.78	.12	1.84	3.04	4,600	No immediate ppt., some in 7 min. Gelled in 25 min., Δw = −0.36.

[a] Correction determined from Δw of equal parts of buffer and water.

reagents. The values of $\chi_{molal} \times 10^6$ are calculated by use of the equation

$$\chi_{molal} = \frac{(Dw - Dw_{COHb})}{(Dw_{Hb} - Dw_{COHb})} \times 12{,}290 \times 10^{-6} \text{ c. g. s. u.} \quad (1)$$

based on the revised value of $\chi_{molal} = 12{,}290 \times 10^{-6}$ c. g. s. u. for the susceptibility of the iron in ferrohemoglobin.[10] Values of Dw_{Hb} and Dw_{COHb} are, respectively, 9.49 and 0.84 for the ferrohemoglobin solution used in this investigation. The values of $\chi_{molal} \times 10^6$ represent, of course, an average value for the iron of the ferrohemoglobin and of the cyanide ferrohemoglobin formed.

Measurements on the first three preparations listed in Table I, particularly those on preparation *C* with equal volumes of buffer and hemoglobin solutions, indicate there are no appreciable specific effects of the high concentration of buffer or hydroxide on ferrohemoglobin, and provide a criterion of the precision of the measurements. (There was no observable difference between the absorption spectra of these solutions and those of ferrohemoglobin in more acid solutions.) On reference to Fig. 1, it is seen that the molal susceptibility decreases from the initial value approximately linearly with the cyanide concentration, according to the approximate empirical expression

$$\chi_{molal} \times 10^6 = 12{,}290 - 9000\ (CN^-) \quad (2)$$

The decrease in susceptibility provides support of the reasonable assumption that cyanide ferrohemoglobin, like the other ferrohemoglobin compounds, is diamagnetic. The assumption of diamagnetism permits the calculation of the amount of cyanide ferrohemoglobin present in the solutions: with cyanide concentration 0.86 *f* (the most concentrated solution studied) 64% of the ferrohemoglobin iron is combined with cyanide. In the preliminary experiments definite spectroscopic and magnetic evidence of the presence of the cyanide compound was obtained for solutions 0.05 *f* in cyanide, with only 4% of the ferrohemoglobin combined with cyanide.

If the dissociation of cyanide ferrohemoglobin could be represented as

$$HbCN^- \rightleftarrows Hb + CN^- \quad (3)$$

with Hb the symbol for the amount of ferrohemoglobin containing one heme, the expected dependence of susceptibility on cyanide concentration would have the form of curve A in Fig. 1. If the hemes are not independent but interact to the same extent as for the oxyhemoglobin equilibrium[7b] the form of the susceptibility curve would be that of curve B. The experimental values do not support either of these assumptions as to the nature of the equilibrium very well, but they seem to indicate that the interactions for cyanide ferrohemoglobin are smaller than those for oxyhemoglobin. There is no theoretical justification for the empirical equation (2).

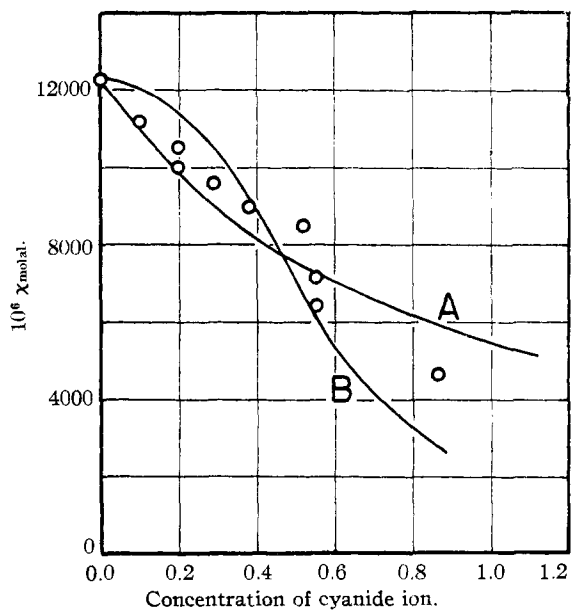

Fig. 1.

The equilibrium constant for reaction 3 would have the approximate value 1.0, since curve A, drawn to pass close to all of the points, corresponds to $K = 0.80$, whereas the five points for low cyanide concentrations lead to $K = 1.14$. Curve B corresponds to half saturation of hemoglobin with cyanide ion at a concentration of 0.55 *f*. No pronounced dependence on ionic strength is expected. Cyanide ferrohemoglobin is a much less stable compound than ferrihemoglobin cyanide, with dissociation constant[9] (to ferrihemoglobin ion and cyanide ion) about $10^{-7.44}$.

A number of substances (denatured globin, cyanide, pyridine, etc.) are known to form complexes with both ferriheme and ferroheme, but cyanide is the only substance now known to be capable of forming complexes with both native ferrohemoglobin and native ferrihemoglobin.

Summary

Spectroscopic and magnetic evidence of the formation of a compound of ferrohemoglobin with cyanide has been obtained and the equilibrium

(10) D. S. Taylor and C. D. Coryell, THIS JOURNAL, **60**, 1177 (1938).

has been studied. The stability of the compound is much less than that of ferrihemoglobin cyanide; about one-half of the ferrohemoglobin is in the form of cyanide ferrohemoglobin in 0.8 *f* cyanide solution. It is concluded that cyanide ferrohemoglobin is diamagnetic, with essentially covalent octahedral coördination about the iron atoms, its structure thus being similar to that of oxyhemoglobin and carbonmonoxyhemoglobin.

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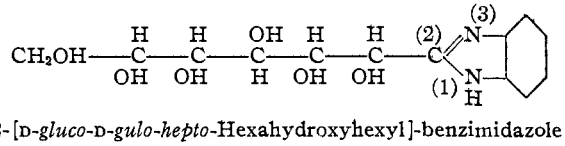
Improvements in the Preparation of L-Tartaric Acid from Racemic Tartaric Acid through Resolution by a Substituted Benzimidazole Base[1]

BY W. T. HASKINS AND C. S. HUDSON

The classical example of the resolution of racemic tartaric acid is the familiar mechanical separation of the mirror-image hemihedral crystals of sodium ammonium tartrate by Pasteur. Later Pasteur observed that *Penicillium glaucum* would consume ammonium D-tartrate[2] from a solution of the racemate, thus effecting a preparation of ammonium L-tartrate. The third method of Pasteur was resolution by salt formation with cinchonine. The latter is the only method of practical significance and still remains the usual procedure for the preparation of L-tartaric acid.[3] However, the procedure is tedious and the yield (41% of the theoretical)[4] leaves much room for improvement.

Benzimidazoles substituted in the [2] position with optically active sugar residues offer a considerable number of optically active bases, some of which may be prepared at relatively small expense. A good example, we find, is 2-[D-*gluco*-D-*gulo*-*hepto*-hexahydroxyhexyl]-benzimidazole (I).



2-[D-*gluco*-D-*gulo*-*hepto*-Hexahydroxyhexyl]-benzimidazole

This base forms a readily crystallizable acid salt with L-tartaric acid, whereas the corresponding D-salt does not crystallize under any conditions yet investigated. Attempts to force crystallization of the latter by addition of ethanol to a water solution result in the precipitation of the free base. Hence, a practically quantitative separation of the racemic acid may be obtained in one crystallization, since the L-salt is only slightly soluble in dilute ethanol.

The preparation of benzimidazoles substituted in the [2] position by sugar residues was first reported by Griess and Harrow[5] and was later studied by several other investigators.[6–9]

Their method consisted in the evaporation of an aqueous solution of *o*-phenylenediamine and an aldose, whereupon in most cases the desired product crystallized from the sirupy residue. The yields were very poor and in many cases involved by-products of the quinoxaline type.

The reaction evidently consists in an oxidation of the aldose by the diamine in two ways, leading to the formation of an osone and an aldonic acid, respectively; the osone forms quinoxaline derivatives with the diamine and the acid forms the substituted benzimidazole. It would seem that a preferable method would be the interaction of the pure aldonic acid (or its lactone) with *o*-phenylenediamine and experiment shows that such is the case. The reaction of these substances in equivalent quantities proceeds smoothly and produces the [2] substituted benzimidazole in good yields. In some cases it was found necessary to add two moles of hydrochloric acid to cause elimination of the second molecule of water, effecting the ring closure.

(1) Publication authorized by the Surgeon General, U. S. Public Health Service.

(2) Throughout the article the symbols D and L refer to the established configurations: D-tartaric is COOH·C(OH)H·C(OH)H·COOH and L-tartaric is COOH·C(H)OH·C(H)OH·COOH. The D form is the one commonly present in grapes; it is dextrorotatory.

(3) A recent article states that L-tartaric acid occurs naturally to the extent of 6% in the fruit and leaves of *Bauhinia reticulata*, a native tree of the French Sudan. Rabaté and Gourévitch, *J. pharm. chim.*, **28**, 386 (1938).

(4) Unpublished detail of the procedure followed by N. K. Richtmyer, THIS JOURNAL, **58**, 2543 (1936).

(5) Griess and Harrow, *Ber.*, **20**, 281, 2205, 3111 (1887).

(6) Hinsberg, *ibid.*, **20**, 495 (1887); **26**, 3092 (1893).

(7) Schilling, *ibid.*, **34**, 905 (1901).

(8) Ohle, *ibid.*, **67**, 155 (1934).

(9) Kuhn, *ibid.*, **67**, 904 (1934).