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.

PASADENA, CALIF. RECEIVED MARCH 13, 1939

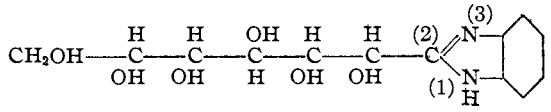
[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

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).

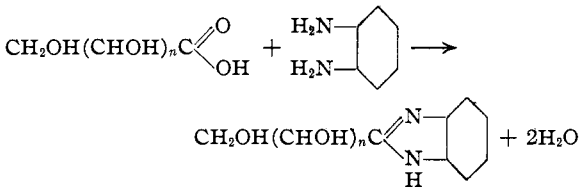
(9) Kuhn, *ibid.*, **67**, 904 (1934).

TABLE I

PROPERTIES OF [2]-SUBSTITUTED BENZIMIDAZOLES

Substituent group	$[\alpha]^{20}D$ N/1 HCl	M. p. (corr.), °C.	% yield once recryst.	Recryst. from Parts	Recryst. from % EtOH	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
D-Gluco-D-gulo-hepto[a]	+14.3	215 d.	74	25	50	52.31	6.09	9.40	52.47	6.18	9.57
D-Gluco-D-ido-hepto[a]	−27.6	192 d.	57	10	75	52.31	6.09	9.40	52.17	6.03	9.24
D-Manno-D-gala-hepto[a,c]	+49.5	241 d.	59	[b]		52.31	6.09	9.40	52.20	6.35	9.42
D-Gala-L-manno-hepto[a]	+18.5	218 d.	73	63	50	52.31	6.09	9.40	52.13	6.42	9.23
D-Gluco	+ 8.9	210 d.	52	5	50	53.72	6.02	10.45	53.91	6.20	10.53
D-Gulo	+16.7	201 d.	75	11	75	53.72	6.02	10.45	53.84	6.02	10.48
D-Manno	−23.7	224 d.	56	43	30	53.72	6.02	10.45	53.63	6.01	10.39
D-Galacto	+44.4	246 d.	62	50	50	53.72	6.02	10.45	53.95	6.13	10.39
D-Ido[c]	−19.2	154–156	30	5	95	53.72	6.02	10.45	53.65	6.19	10.48
D-Altro	−48.1	198 d.	60	40	75	53.72	6.02	10.45	53.86	6.09	10.48
D-Talo	−23.0	190–191	60	27	90	53.72	6.02	10.45	53.58	6.07	10.42
L-Mannomethylo[a]	+29.1	210 d.	41	6	75	57.12	6.40	11.11	57.25	6.41	11.13

[a] Concerning the nomenclature for these compounds, see Hudson, THIS JOURNAL, **60**, 1537 (1938). [b] This compound is insoluble in all common solvents. The hydrochloride was recrystallized from 11 parts 85% alcohol and then decomposed with ammonium hydroxide. [c] Require addition of 2 moles hydrochloric acid for preparation.



A number of these bases have been prepared and Table I gives a summary of their properties. These bases are relatively weak and therefore are not suitable for the resolution of weakly ionized acids. For example, 2-[D-*gluco*-D-*gulo*-*hepto*-hexahydroxyhexyl]-benzimidazole is useless for the resolution of racemic lactic acid, the free base precipitating from the aqueous solution upon concentration.

The rotations of the hydrochlorides of these bases, as given in column 2 of Table I, do not conform in any simple manner with the configurations.

Experimental

Resolution of Racemic Tartaric Acid.—Twenty grams of racemic tartaric acid[10] was dissolved in 150 ml. of hot water and 35.5 g. of 2-[D-*gluco*-D-*gulo*-*hepto*-hexahydroxyhexyl]-benzimidazole added with stirring. To the clear solution was added 50 ml. of ethanol and crystallization was readily induced by scratching. After standing a few hours at 5° the crystals were filtered and washed successively with cold 50, 75 and 95% ethanol. The acid L-tartrate salt thus obtained (28.8 g. or 100%) occurs as the dihydrate and may be recrystallized with negligible loss from 5 parts 25% ethanol if desired. Specific rotation[11] of the dihydrate is −0.5° (H_2O, l = 4, c = 0.84) and m. p. is 118–125° (corr.). The water of crystallization may be quantitatively removed at 78° *in vacuo*.

Anal. Calcd. for $C_{17}H_{24}O_{12}N_2 \cdot 2H_2O$: C, 42.12; H, 5.83; H_2O, 7.44. Found: C, 42.33; H, 5.70; H_2O, 7.49.

The L-tartaric acid was recovered by decomposing the acid salt with excess ammonium hydroxide and removing the base by filtration. The filtrate was made barely acid with acetic acid and lead L-tartrate was precipitated with lead acetate solution. The filtered and washed lead L-tartrate was suspended in water and the lead removed by precipitation with hydrogen sulfide. The clear filtrate was evaporated to a small volume and allowed to crystallize in a desiccator. Recovery of 96.5% of L-tartaric acid from the original acid salt was obtained; after one recrystallization from water, it showed a specific rotation of −14.2° (H_2O, l = 4, c = 4.05) and a m. p. of 168–170° (corr.), which are the known values for pure L-tartaric acid.

Preparation of [2] Substituted Benzimidazoles

2 - [D - gluco - D - gulo - hepto - Hexahydroxyhexyl]-benzimidazole.—The preparation of this benzimidazole will be taken as an example. All others were prepared in an analogous manner, except the two cases noted in Table I where 2 moles of hydrochloric acid were added during evaporation to facilitate ring closure.

Fifty grams of D-gluco-D-gulo-heptonic lactone,[12] 26.6 g. of *o*-phenylenediamine (1:1.02 molecular ratio), and 50 ml. of water were heated on a steam-bath for seven hours with occasional addition of 25-ml. portions of water whenever the cake became dry. The resulting mass of crystals was treated with 100 ml. of ethanol and allowed to stand overnight. After filtering and washing with ethanol 57 g. of the crude product was obtained. One recrystallization from 25 parts of 50% ethanol yielded 53 g. (74%) of a product which gave a specific rotation of +14.3° (N/1 HCl, l = 1, c = 2.00) and a decomposition point of 215° (corr.), these constants remaining unchanged upon further recrystallization.

(10) The racemic acid was made from commercial tartaric acid according to the directions of Holleman, *Org. Syntheses*, **1**, 484 (1921).

(11) All specific rotations were taken at 20° with sodium light; l = length in dcm.; c = grams per 100 ml. of solution.

(12) This lactone is readily prepared from D-glucose by the modification of the Kiliani cyanohydrin synthesis described by Hudson, Hartley and Purves, THIS JOURNAL, **56**, 1248 (1934).

Summary[13]

1. The resolution of racemic tartaric acid with 2-[D-*gluco*-D-*gulo*-*hepto*-hexahydroxyhexyl]-benzimidazole is described. The over-all yield of L-tartaric acid is over 90%.

2. A new method of preparation of benzimidazoles substituted in the [2] position with sugar residues is described. This method is based on the reaction of aldonic acids (or lactones) with *o*-phenylenediamine. The properties of a number of these substituted benzimidazoles are described.

WASHINGTON, D. C. RECEIVED FEBRUARY 20, 1939

(13) Postscript added March 31, 1939. We have been informed by Professor Karl P. Link that he and his students have been studying the preparation of benzimidazoles from sugar acids with particular reference to the use of these derivatives for the identification of sugars.

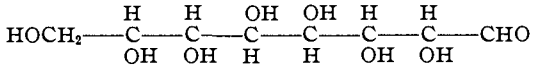
[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

Relations between Rotatory Power and Structure in the Sugar Group. XXXI. The Configuration of D-α,α-Mannooctose (D-Manno-L-manno-octose)[1]

BY RAYMOND M. HANN, W. DAYTON MACLAY, A. E. KNAUF AND C. S. HUDSON

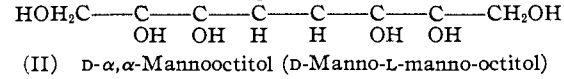
The establishment of the configuration of D-α,α-mannooctose (D-manno-L-manno-octose)[2] is of considerable importance since this octose is the precursor of D-α,α,α-mannononose, which was reported by Fischer[3] in 1890 to be fermentable by yeast. In his published volume of collected works[4] he stated that a repetition of his earlier work by Hagenbach indicated the formation of other products when hydrogen cyanide acted upon the mannooctose, and mentioned his intention of repeating the research, but there is no further record.

The accepted configuration of D-α,α-mannooctose (I) is based upon Peirce's[5] recognition of



(I) D-α,α-Mannooctose (D-Manno-L-manno-octose)

the configuration of the two D-mannoheptoses and his observation that the double lactone of D-α,α-mannooctaric (D-manno-L-manno-octaric) acid upon dissolving in sodium hydroxide gave a solution which showed no detectable rotation. This proof is an application of Fischer's classical procedure of determining the configurations of the aldoses through comparison of the rotational relationships of their reduction products, the sugar alcohols, or the corresponding dibasic acids. Unfortunately the rotation in water of many of the optically active alcohols is very small and recourse has been had to the substitution of saturated borax solution as a solvent to accentuate its value. Further, the low solubility of some of the higher alcohols prevents a reliable measurement; indeed Fischer prepared D-α,α-mannooctitol, but did not report its rotation, undoubtedly because of its very low solubility. The question to be decided is, whether the alcohol possesses optical activity. If it does not, it must be a substance that is inactive by internal compensation, like xylitol and dulcitol, and must therefore possess the configuration (II).



(II) D-α,α-Mannooctitol (D-Manno-L-manno-octitol)

Since this earlier work, methods have been developed which are of considerable aid in establishing configuration. The position of the hydrogen and hydroxyl groups about carbon two of the sugar chain may be assigned by utilization of the amide and the phenylhydrazide rules. It has also been shown that conversion of the sugar alcohols to their acetates offers a particular advantage in solving questions of configuration since the alcohol acetates in general are highly soluble in certain organic solvents and have rotations of considerable magnitude; thus Hockett and Hudson[6] found that D-arabitol, which requires borax to augment its rotation to a measurable value, upon acetylation yields a pentaacetate rotating +37.2° in chloroform.

It seemed desirable to supplement Peirce's proof

(1) Publication authorized by the Surgeon General, U. S. Public Health Service. No. XXX was published in THIS JOURNAL, **52**, 2534 (1930).

(2) Concerning this nomenclature see Hudson, *ibid.*, **60**, 1537 (1938).

(3) Fischer, *Ber.*, **23**, 2238 (1890).

(4) Fischer, "Untersuchungen über Kohlenhydrate und Fermente," **1**, 582 (1909).

(5) Peirce, *J. Biol. Chem.*, **23**, 327 (1915).

(6) Hockett and Hudson, THIS JOURNAL, **57**, 1753 (1935).