

quantitatively shown by Johnson.³ Between 2.0 and 4.0 *F*, the rate of the reaction increases with increasing acid concentration, due, perhaps, to a kinetic salt effect, or perhaps the perchlorate ion concentration itself has a positive effect on the reaction due to ion pair formation.¹⁰ Attempts were made to study the reaction at lower acid concentrations, but the hydrolysis of the thallium(III) solutions interfered.¹¹

A direct comparison is afforded with Johnson's results by the run in 3.0 *F* HClO₄. The experimentally obtained value of *k* is 0.77. Using Johnson's formula,³ applicable at an ionic strength of 3, we get *k* = 0.74.

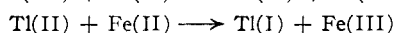
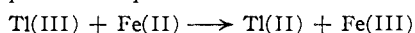
The results of some runs in the presence of certain cations are listed in Table II. The experiment in the presence of a small quantity of manganese was performed in order to see whether the presence of this ion could provide an alternative reaction path to the system. Shaffer¹² has shown manganese(II) to be a good catalyst for some reactions between two-electron reagents and one-electron reagents, as it can form oxidation states of 2, 3 and 4 in solution. No catalytic effect was found in this system. The other salts increased the rate, as was expected, in view of the results obtained at high acid concentrations. These results are, however, insufficient to draw any conclusions about the effect of ionic strength, perchlorate ion concentration or any specific ionic effect.

TABLE II
EFFECT OF SOME CATIONS ON THE RATE
b = 0.0113, *a* = 0.0201, [HClO₄] = 1.5 *F*

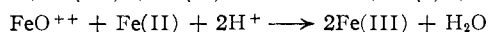
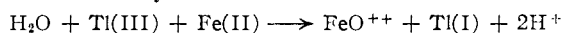
Run	Added salt and its concentration	<i>k</i>
13	None	0.67
21	0.50 <i>F</i> LiClO ₄	.81
22 ^a	.011 <i>F</i> Mn(ClO ₄) ₂	.68
23	.20 <i>F</i> Mg(ClO ₄) ₂	.83
24	.50 <i>F</i> NaClO ₄	.87

^a Acid concentration in this run 1.79 *F*.

One can account for the kinetics of the reaction by a simple two-step mechanism



or alternatively



where the first step in each case is the slow, rate-determining reaction. Since the specific rate is independent of the concentrations of the reactants (over the range studied) and also independent of the concentration of the products, no measurable equilibrium can be involved in the first step. On the basis of the kinetics no choice can be made between iron(IV) and thallium(II) as an intermediate. However, the path through thallium(II)

(10) Cf. A. R. Olsen and T. R. Simonsen, *J. Chem. Phys.*, **17**, 1167 (1949).

(11) Thallous hydroxide precipitates only slowly from solutions when the solubility product is not greatly exceeded. The solubility product of Tl(OH)₃ was found to be 8.8×10^{-44} by letting a small excess of Tl(OH)₃ precipitate from a 0.5 *F* perchloric acid solution. This value is in good agreement with the value 1.5×10^{-44} calculated by Sherrill and Haas (*THIS JOURNAL*, **58**, 952 (1936)).

(12) P. A. Shaffer, *ibid.*, **58**, 2169 (1933).

is preferred because an intermediate which can act as a reducing agent is necessary to explain the induced oxidation by oxygen.

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Improvements in the Preparation of Melibiose from Raffinose. A New Form of Melibiose

BY HEWITT G. FLETCHER, JR., AND HARRY W. DIEHL

RECEIVED JUNE 6, 1952

The preparation of the disaccharide melibiose by the fermentative hydrolysis of the trisaccharide raffinose was developed by Hudson and Harding¹⁻³ and has, in the past, uniformly yielded the well-known β-melibiose dihydrate melting at about 85 to 86°⁴ and mutarotating in water [α]²⁰_D +123.5° → +143.1° (anhydrous basis, *c* 4). Attempts to obtain melibiose in other modifications have failed although Loiseau⁵ reported that dehydration of β-melibiose dihydrate afforded a form, probably anorphous, which showed a slight levomutarotation.⁶

Recently we were furnished with a sample of material⁷ derived from raffinose by the Hudson-Harding procedure.¹⁻³ In sharp contrast to previous samples this melted at 179-181°.⁸ Elementary analysis and loss of weight at 100° *in vacuo* gave values corresponding to a disaccharide containing 0.85 mole water. Acetylation with acetic anhydride and sodium acetate afforded β-melibiose octaacetate in high yield. On an anhydrous basis the substance mutarotated [α]²⁰_D +166.0° → +142.3° (*c* 4) in water; the first-order reaction constant, 0.0090 (min., decimal logs) is close to that (0.0088) reported by Hudson and Yanovsky⁹ for melibiose. Recrystallization from aqueous alcohol gave the melibiose in crystalline form containing one or slightly less than one mole of water. From methanol the sugar was obtained in anhydrous form which, upon standing in the air, approached the monohydrate in composition. Regardless of state of hydration, the recrystallized material showed on an anhydrous basis the above rotational constants and was therefore pure melibiose.

Solutions of either β-melibiose or of the new form of melibiose could be induced to crystallize in either form by appropriate seeding. However, the new form, less soluble and a better crystallizer, was

(1) C. S. Hudson and T. S. Harding, *THIS JOURNAL*, **37**, 2734 (1915).

(2) T. S. Harding, *Sugar*, **25**, 514 (1923).

(3) F. J. Bates and Associates, "Polarimetry, Saccharimetry and the Sugars," U. S. Govt. Printing Office, Washington, D. C., 1942, p. 473.

(4) Melting points cited are corrected for stem exposure.

(5) D. Loiseau, *Z. Ver. deut. Zucker-Ind.*, **40**, 1050 (1903).

(6) The terms levomutarotation and dextrorotation are used here to denote rotatory changes in the negative and positive directions, respectively.

(7) We wish to thank Mr. Robert S. Black of the Pfanzstiehl Chemical Company for the donation of this sample.

(8) This value varied with the rate of heating; very slow heating afforded a value of 185-188°.

(9) C. S. Hudson and R. Yanovsky, *THIS JOURNAL*, **39**, 1013 (1917).

always obtained in greater yield than the β -melibiose dihydrate.

On the basis of the levomutarotation of this new form it may be assumed to be predominantly or wholly the hitherto unknown α -form of melibiose. In 1916 Hudson¹⁰ calculated from the rotation of raffinose that the initial rotation of α -melibiose in water ought to be $+175^\circ$; in the following year Hudson and Yanovsky⁹ measured the initial and final solubilities of β -melibiose and arrived at an initial rotational value of $+179^\circ$ for α -melibiose. These values, in remarkable agreement considering the wholly independent ways in which they were derived, are ten degrees higher than that shown by the new form of melibiose. Indeed, if Hudson's values were accepted, it would appear that the present substance is a mixture or molecular compound of the composition 4α -melibiose: 1β -melibiose. A similar combination of the anomeric forms of a free sugar was reported in the case of lactose by Hockett and Hudson.¹¹ Acetylation of the new form of melibiose with acetic anhydride in pyridine at 0° gave 10% of β -melibiose octaacetate but no other crystalline product.¹² Such evidence cannot, however, be regarded as decisive and until further evidence is adduced we suggest that the substance be named tentatively α -melibiose. Melibiose now becomes one of the relatively few sugars available in more than one crystalline anomeric form.

Kuhn¹³ has published the infrared absorption spectra of β -melibiose dihydrate. The infrared spectrum of α -melibiose monohydrate differs significantly from that of its anomer as may be seen by inspection of Fig. 1. X-Ray diffraction and optical crystallographic data are also given for α -melibiose monohydrate.

The low solubility and superior crystallizing quality of α -melibiose monohydrate as compared with β -melibiose dihydrate recommend it as the preferred form in which to isolate the sugar. In the course of this work the preparation of melibiose from raffinose has been greatly simplified by using ion-exchange resins and a yield of 88% of nearly pure α -melibiose has been isolated directly. The yield of β -melibiose dihydrate obtained by the older method³ is about 63%.

Acknowledgments.—We wish to express our indebtedness to Miss Paula M. Parisius and Mr. Byron Baer for analytical determinations and to Mrs. Phyllis B. Smeltzer and Mrs. Iris J. Siewers for infrared absorption measurements. We also wish to thank Mr. William C. White of this Institute for X-ray diffraction data and Mr. Joseph F. Fahey of the U. S. Geological Survey for optical crystallographic data.

Experimental

α -Melibiose from Raffinose.—Raffinose pentahydrate (250 g.), dissolved in 2.5 l. of water, was treated with 50 g. of powdered calcium carbonate and 50 ml. of nutrient solu-

(10) C. S. Hudson, *This Journal*, **38**, 1566 (1916).

(11) R. C. Hockett and C. S. Hudson, *ibid.*, **53**, 4455 (1931).

(12) Up until the present, crystalline α -melibiose octaacetate has not been recorded in the literature.

(13) L. P. Kuhn, *Anal. Chem.*, **22**, 276 (1950).

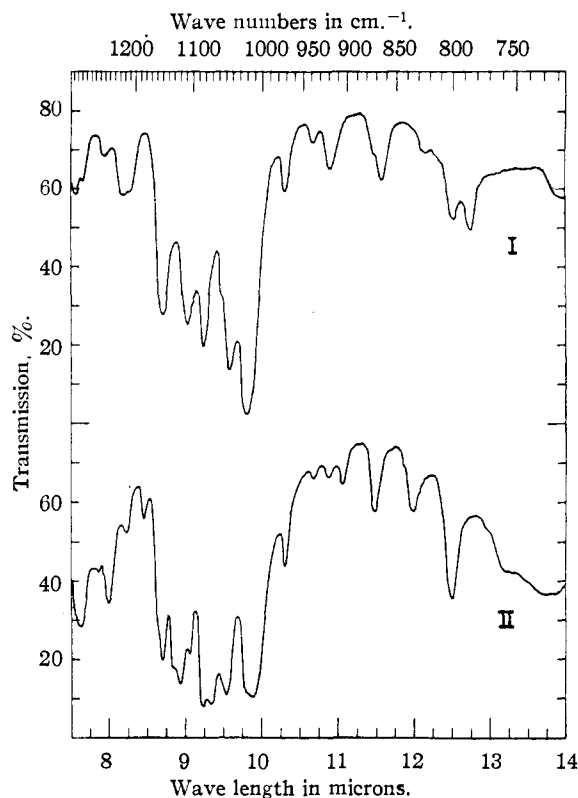


Fig. 1.—Infrared spectra of α -melibiose monohydrate (I) and β -melibiose dihydrate (II). The spectra were taken in Nujol mull with an automatic recording spectrophotometer (Perkin-Elmer, Model 21).

tion.¹⁴ One cake of baker's yeast was then added and the mixture held at 37° for 4 days. Insoluble matter was removed by filtration through a layer of 50 g. of decolorizing carbon covered by a layer of 15 g. of Filter-Cel on an 8-in. buchner funnel. The clear, colorless filtrate was deionized by passage through a column (5.5×100 cm.) of Duolite A-4¹⁵ and then through a column (5.5×100 cm.) of Amberlite IR-120.¹⁶ After concentration *in vacuo* (55° bath) to a stiff sirup the material was dissolved in 125 ml. of warm glacial acetic acid and seeded with α -melibiose. When crystallization appeared to have ceased at room temperature (24 hr.) the material was left at $+5^\circ$ for 24 hours and then filtered. Washed first with 25 ml. of glacial acetic acid and then with three 25-ml. batches of absolute alcohol the crystalline product amounted to 130 g. and melted at 183 – 184° . Analysis showed it to contain 4.62% of water corresponding to 0.92 mole of water per mole of melibiose. On standing at 0° the combined mother liquors and washings deposited 3.2 g. of similar material, raising the yield to 88%. In water the product showed $[\alpha]_D^{20} +141.9^\circ$ (c 0.94, anhydrous basis) at equilibrium and is therefore practically pure melibiose. Final purification may be made through recrystallization from aqueous alcohol, the product usually being obtained containing somewhat less than a whole mole of water which may readily be removed by heating *in vacuo* at 100° but is slowly regained on exposure to atmospheric moisture. On an anhydrous basis the substance shows in water $[\alpha]_D^{20} +166.0^\circ \rightarrow +142.3^\circ$ (c 4). Mutarotation data are given in Table I.

Recrystallization of 1 g. of α -melibiose from a mixture of 0.35 ml. of water and 0.2 ml. of alcohol gave the monohydrate (0.41 g.) melting at 184 – 185° . The infrared absorp-

(14) One liter of this solution contained 100 g. of sodium nitrate, 8 g. of monobasic potassium phosphate and 6.6 g. of magnesium sulfate heptahydrate.

(15) A product of the Chemical Process Co., 901 Spring St., Redwood City, Calif.

(16) A product of the Resinous Products and Chemical Co., Washington Square, Philadelphia 5, Pa.

TABLE I

MUTAROTATION OF α -MELIBIOSE IN WATER AT 20° α -Melibiose (0.9798 g.) containing 3.64% water in 25 ml. of solution observed in a 4-dm. tube

<i>t</i> , min.	$[\alpha]^{20}_D$ (anhyd. basis)	$k_1 + k_2$ min., decimal logs
0	166.0 extrap.	...
1	165.6	0.0078
3	164.7	.0081
5	163.8	.0084
10	161.9	.0083
15	158.9	.0100
25	156.2	.0093
35	153.2	.0096
45	151.1	.0095
60	148.1	.0100
95	145.3	.0094
135	143.7	.0091
220	142.3	...
285	142.3	...

tion spectra of this substance and of β -melibiose dihydrate may be compared in Fig. 1. X-Ray diffraction data¹⁷: 8.96 (M), 7.70 (VW), 6.80 (W), 6.30 (S), 5.40 (VW), 5.13 (VS), 4.91 (VW), 4.63 (S), 4.41 (VS), 4.11 (S), 3.94 (M), 3.63 (VW), 3.46 (M), 3.35 (W), 3.27 (W), 3.19 (VW), 3.07 (M), 3.00 (W), 2.86 (W), 2.79 (M), 2.72 (VW), 2.58 (M), 2.51 (W), 2.47 (W), 2.31 (VW), 2.28 (VW), 2.22 (VW), 2.16-2.09 (W-B), 2.01-1.96 (W-B), 1.912 (VW), 1.868 (W), 1.797 (VW), 1.750 (VW).

Crystals of α -melibiose monohydrate, grown in a viscous aqueous sirup, were negative in sign and showed, n^{20}_D , $\alpha = 1.524$, $\beta = 1.542$, $\gamma = 1.559$, $2V = 86^\circ$ and birefringence = 0.035.

Anal. Calcd. for $C_{12}H_{22}O_{11} \cdot H_2O$: C, 40.00; H, 6.71; H_2O , 5.00. Found: C, 40.17; H, 6.70; loss of wt. *in vacuo* at 100°, 4.99.

Recrystallization of 1 g. of melibiose from 100 ml. of anhydrous methanol afforded 0.35 g. of essentially anhydrous α -melibiose (0.17% loss *in vacuo* at 100°). On exposure to air this approached a monohydrate in composition.

Acetylation of α -Melibiose.— α -Melibiose (2 g.) containing 4.2% water was acetylated in the usual fashion with acetic anhydride and sodium acetate. The crystalline product (3.2 g., 82%), recrystallized from 8 parts of absolute alcohol, showed $[\alpha]^{20}_D +104.3^\circ$ in chloroform (c 0.82) and melted at 177-178° either alone or in admixture with authentic β -melibiose octaacetate.

(17) Interplanar spacing, Å.; $CuK\alpha$ radiation (nickel filter); camera diameter 14.32 cm. Relative intensity estimated visually VS = very strong, S = strong, M = moderately strong, W = weak, VW = very weak and B designates broad, unresolved line or lines.

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Octahydropyrrocoline: Resolution and Configuration

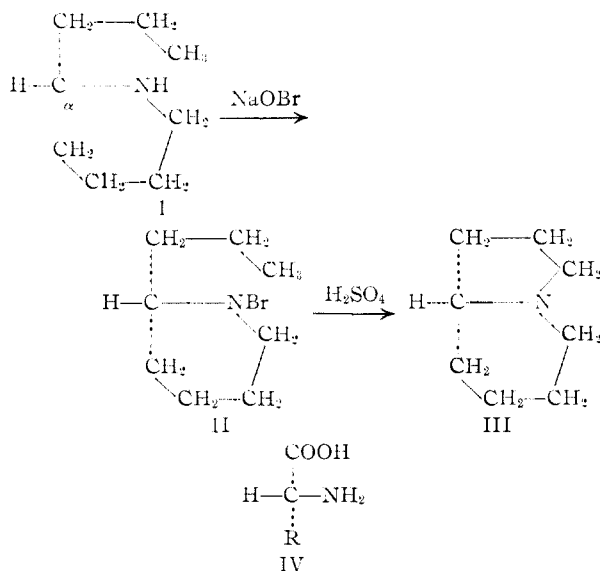
By NELSON J. LEONARD AND WILLIAM J. MIDDLETON

RECEIVED JULY 7, 1952

The establishment of the stereochemical configuration for (–)-octahydropyrrocoline (III)¹ is important because of the occurrence of this nucleus or moiety in many of the papilionaceous and solanaceous alkaloids. Such establishment is possible if we can be assured that the conversion by Lell-

(1) Alternative names: (–)-6-coniceae, piperolidine and 1-azabicyclo[4.3.0]nonane.

mann² of the hemlock alkaloid (+)-coniine (I), to the N-bromo compound (II) and thence by sulfuric acid to (–)-octahydropyrrocoline proceeds



with retention of configuration at the asymmetric carbon. The changes involved in the conversion would not be expected to result in inversion at the α -carbon, but it seemed advisable to determine whether any appreciable racemization had occurred. The rotation reported by Lellmann² for the active octahydropyrrocoline obtained from (+)-coniine was -7.8° . We have resolved synthetic octahydropyrrocoline by means of dibenzoyl-D-tartaric acid and have obtained active octahydropyrrocoline of constant maximum rotation, $[\alpha]^{27}_D -7.89^\circ$. It is thus apparent that the Lellmann product was of optical purity and identical configuration with (+)-coniine. Since (+)-coniine has been related to D(+)-pipercolinic acid,³⁻⁵ it follows that levorotatory octahydropyrrocoline has the D-configuration, wherein the designation "D" acquires meaning when represented by the projection formula III (*cf.* IV).

Experimental

Mono-(–)-octahydropyrrocoline Dibenzoyl-D-tartrate.—A solution of 112.8 g. (0.3 mole) of dibenzoyl-D-tartaric acid monohydrate⁶ in 400 ml. of methanol was mixed with 37.5 g. (0.3 mole) of synthetic octahydropyrrocoline.⁷ The white crystalline mass which formed a few minutes after mixing was removed by filtration and washed with cold methanol. This material was recrystallized from 4 liters of methanol. After five additional recrystallizations from methanol, 33.8 g. of large colorless prisms was obtained, m.p. 163-164° with decomposition; $[\alpha]^{27}_D -75.8^\circ$ (c 0.600, methanol).

Anal. Calcd. for $C_{26}H_{29}NO_8$: C, 64.58; H, 6.05; N, 2.89. Found: C, 64.29; H, 6.16; N, 2.93.

(–)-Octahydropyrrocoline (III).—A slurry of 31.4 g. of mono-(–)-octahydropyrrocoline dibenzoyl-D-tartrate and 200 ml. of 3 N hydrochloric acid was shaken for 30 minutes. The oil which separated solidified on addition of a few crys-

(2) E. Lellmann, *Ber.*, **23**, 2141 (1890).

(3) "The Alkaloids. Chemistry and Physiology," edited by R. H. F. Manske and H. L. Holmes, Academic Press, Inc., New York, N. Y., Vol. 1, 1950, pp. 215, 217, 225, 226.

(4) W. Leitlie, *Ber.*, **65**, 927 (1932).

(5) A. Neuberg, *Advances in Protein Chemistry*, **4**, 297 (1948).

(6) C. L. Butler and L. H. Cretcher, *This Journal*, **55**, 2605 (1933).

(7) V. Bockelheide and S. Rothschild, *ibid.*, **70**, 864 (1948).