

satisfactory analyses and was adequate for conversion to the next intermediate; yield, 73%; m.p. 161°.

Anal. Calcd. for $C_{14}H_{19}O_6N$: C, 56.56; H, 6.40; N, 4.71. Found: C, 56.45; H, 6.53; N, 4.54.

N-(*p*-Methoxybenzylidene)-tetra-O-acetyl-2-amino-2-deoxy- β -D-galactopyranose (II).—Eight grams of I was mixed in an ice bath with 27 ml. of ice-cold acetic anhydride and 48 ml. of anhydrous pyridine. The mixture was allowed to stand in ice with occasional shaking for 3 hr. and then for an additional 24 hr. at room temperature. The resulting solution was poured onto crushed ice and the tetraacetyl derivative allowed to crystallize for approximately 30 min. The crude crystals were washed with ice-water, recrystallized from methanol, and dried over phosphorus pentoxide *in vacuo*; yield, 75%; m.p., 187°; $[\alpha]^{25}_{578} +108.5$ (*c* 1, chloroform).

Anal. Calcd. for $C_{22}H_{27}O_{10}$: C, 56.53; H, 5.78; N, 3.00. Found: C, 56.84; H, 6.04; N, 2.98.

2-Aminotetra-O-acetyl-2-deoxy- β -D-galactopyranose (III).—Eight grams of II was dissolved in 400 ml. of acetone and the solution heated in water bath until boiling. Exactly 3.5 ml. of 5 *N* hydrochloric acid was added, resulting in the immediate formation of a white crystalline precipitate. The solution was refluxed for 15 min., cooled to below room temperature, and 500 ml. of ether added. After standing overnight at 4°, the precipitate was harvested, recrystallized from methanol, and dried over phosphorus pentoxide. The yield was essentially quantitative; m.p. 205° dec.; $[\alpha]^{25}_{578} +34.7$ (*c* 1.5, water).

Anal. Calcd. for $C_{14}H_{22}O_9NCl$: C, 43.80; H, 5.74; N, 3.65. Found: C, 43.75; H, 5.96; N, 3.59.

2-Acetamidotetra-O-acetyl-2-deoxy- β -D-galactopyranose (IV).—Five grams of III was mixed with 700 ml. of chloroform, 1.8 g. of anhydrous sodium acetate, and 27 ml. of water.⁷ After stirring until a homogeneous mixture was obtained, 20 ml. of acetic anhydride was added and the solution stirred for an additional 25 min. The chloroform layer was separated and dried over anhydrous magnesium sulfate. Solvent was removed *in vacuo*; crystalline material was obtained with heptane and recrystallized from ethanol-chloroform; yield, 90%; m.p. 240°; $[\alpha]^{25}_{578} +2.6$ (*c* 1, chloroform). Stacey reports this compound as melting at 235°; $[\alpha]^{20}_D +7.8$.

Anal. Calcd. for $C_{18}H_{23}O_{10}N$: C, 49.36; H, 5.91; N, 3.59. Found: C, 49.25; H, 5.98; N, 3.43.

Some crystalline material may appear at the chloroform-water interface and is also the β -pentaacetate.

2-Acetamido-2-deoxy-D-galactopyranose- α 1-Phosphate.—Phosphorylation of the fully acetylated amino sugar was carried out according to the procedure of MacDonal.⁸ After removal of lithium phosphate, the resulting solution was passed through a 2.5 \times 40 cm. column of Dowex 1 \times 8 200-400-mesh chloride form resin and the column washed extensively with water until no additional acetyl hexosamine was present in the effluent. The column was then eluted with a lithium chloride gradient consisting of 0.01 *M* of chloride, 0.003 *M* hydrochloric acid in a 1-l. mixing vessel and 0.2 *M* lithium chloride in the same concentration of hydrochloric acid in the reservoir. Column fractions were analyzed for acid-labile acetyl hexosamine,⁹ positive fractions combined and adjusted to pH 8 with lithium hydroxide. Solvent was removed *in vacuo* and the lithium salt of the acetyl hexosamine phosphate obtained by precipitation with methanol-acetone (1:9) and washing with the same solvent until the wash was chloride-free; yield, 20-35%.

Anal. Calcd. for $C_8H_{14}O_9NPLi_2 \cdot 2H_2O$: C, 27.51; H, 5.16; N, 4.10; P, 8.8. Found for the galactosamine analog: C, 27.29; H, 5.25; N, 3.88; P, 8.4; $[\alpha]^{25}_{578} +197$ (*c* 0.7, water); reducing sugar and acetylhexosamine negative; ratio of acid labile acetyl hexosamine to acid-labile phosphorus was 0.89:1. Found for the glucosamine analog: C, 27.06; H, 5.01; N, 3.81; P, 8.6; $[\alpha]^{25}_{578} +144$ (*c* 0.88, water); reducing sugar and acetyl hexosamine negative; ratio of acid-labile acetyl hexosamine to acid-labile phosphorus was 0.82:1.

Equilibrium Constants for the Demetalation of Iron Porphyrins

ALSOPI H. CORWIN AND RANBIR SINGH

Chemical Laboratories of The Johns Hopkins University, Baltimore, Maryland

Received February 25, 1963

Conditions for the equilibration of magnesium with porphyrins and chlorins have been determined by Corwin and Wei.¹ Corwin and Bruck² reported that mesoheme appeared to equilibrate with ferrous chloride and mesoporphyrin in a solution containing acetic acid, a little water, and some salt. This led us to search for conditions suitable for measuring the equilibrium constants of metalation-demetalation reactions of porphyrins and hemins.

Method.—The method of study adopted was spectrophotometric. It was first learned that mesohemin and mesoporphyrin acetate could be distinguished easily by this method, since the hemin has a strong absorption at 632 $m\mu$ and the porphyrin salt at 594 $m\mu$. Using this method, the following preliminary observations were made in an attempt to arrive at suitable conditions for measurement of the equilibrium.

Preliminary Observations on Mesohemin.—(1) Mesohemin in glacial acetic acid was treated with mercury and heated at 114°. No porphyrin formation occurred.

(2) The solution was diluted with water sufficient to make fifty mole per cent. The mixture was heated at 100° for two hours. No porphyrin formation was observed.

(3) A solution was prepared with 2.595 mg. of mesohemin in 25 ml. of glacial acetic acid. A 10-ml. aliquot of this was diluted with 3.3 ml. of water, and the solution was saturated with sodium chloride. It was then treated with metallic mercury and heated to 105° for ninety minutes. It was estimated that 50% of the hemin was converted to porphyrin. This experiment established the need for halide ion in the equilibrium mixture.

(4) Mesohemin (ferric) in fifty mole per cent aqueous acetic acid did not equilibrate to porphyrin even in the presence of sodium chloride.³ The presence of a reducing agent (for example, mercury) was essential. In this dilute aqueous solution, the reducing agent did not change the spectrum but did permit the equilibration to proceed. This spectral stability indicates that no appreciable quantity of ferrous porphyrin is formed.

(5) The solubility of sodium chloride in glacial acetic acid is so low that even in the presence of sodium chloride and mercury and at elevated temperature, equilibration under these conditions was too slow to be measured conveniently.

(6) The concentration of chloride ion is critical for the position of the equilibrium as well as for the possibility of obtaining equilibrium.

(7) B. R. Baker, J. B. Joseph, and R. E. Schaub, *J. Org. Chem.*, **19**, 1786 (1954).

(8) M. Stacey, *J. Chem. Soc.*, 272 (1944).

(9) J. L. Reissig, J. L. Strominger, and L. F. Leloir, *J. Biol. Chem.*, **217**, 959 (1959).

(1) Porphyrin Studies XXV. Paper XXIV, A. H. Corwin and P. E. Wei, *J. Org. Chem.*, **27**, 4285 (1962). This work was supported by Public Health Service grant A-2877.

(2) A. H. Corwin and S. D. Bruck, *J. Am. Chem. Soc.*, **80**, 4737 (1958).

(3) H. Fischer, A. Treibs, and K. Zeile, *Z. Physiol. Chem.*, **196**, 1 (1931).

(7) Since oxygen would prevent the formation of ferrous porphyrin, a nitrogen atmosphere is required. In addition, mesohemin in glacial acetic acid, treated with mercury and saturated with sodium chloride, decomposes in the air when heated at 105°. The products are pale brown products.⁴ At room temperature this decomposition is extremely slow.

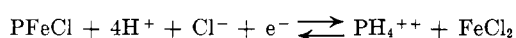
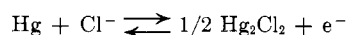
(8) The solution of mesohemin in acetic acid, glacial or diluted with water, can be stored for a number of days without appreciable change.

(9) In contrast, after treatment with mercury, mesohemin in dilute acetic acid decomposes quite rapidly in contact with air but in glacial acetic acid under the same conditions it is fairly stable.

(10) Mesohemin has peaks at 533 and 505 m μ . Dilution with water probably forms an aquo complex and destroys the peak at 533 m μ as well as that at 632 m μ so that the spectra in water become useless for analytical purposes.

(11) All four common halide ions, fluoride, chloride, bromide, and iodide, reverse the intensities of the peaks at 533 and 505 m μ in glacial acetic acid. Intensities of both peaks increase in the order F⁻ < Cl⁻ < Br⁻ < I⁻. The increase in intensity is accompanied by a shift toward higher wave lengths as the halide ion is changed.

These observations are consistent with the interpretation that no detectable amount of ferrous porphyrin is formed in the solution but that reduction by mercury to the ferrous form brings about the removal of iron from the porphyrin with the formation of ferrous chloride. Because of the large excess of mercury and chloride ion, the potential of the over-all system will be essentially that of a calomel half-cell. The relationships will be given by the following equations.



The over-all equilibrium constant would then be

$$K = \frac{[\text{PH}_4^{++}] [\text{FeCl}_2] [\text{Hg}_2\text{Cl}_2]^{1/2}}{[\text{PFeCl}] [\text{Cl}^-]^2 [\text{H}^+]^4}$$

Under the conditions of a single experiment, both calomel and hydrogen ion concentrations would be constant so that the observed equilibrium constant would be

$$K_0 = \frac{[\text{PH}_4^{++}] [\text{FeCl}_2]}{[\text{PFeCl}] [\text{Cl}^-]^2}$$

Equilibrium Constant of Mesohemin in Acetic Acid-Water, 79:1.—The value of the equilibrium constant was determined using 36.5×10^{-3} and 18.25×10^{-3} moles per liter of potassium chloride with 8.54×10^{-5} moles per liter of mesohemin. Each experiment was replicated four times and the standard deviation calculated by the method of Fisher. The ratio of acetic acid to water was 79:1 by volume. Values of K at the two concentrations were 0.122 ± 0.001 and 0.129 ± 0.003 , respectively.

Preliminary Observations with Tetramethyltetracarboxyhemins.—(1) Attempts to study 1,4,5,8-tetramethyl-2,3,6,7-tetracarboxyporphyrin and its

hemin were unfruitful due to their low solubility in acetic acid, even when hot.

(2) 1,3,5,7-Tetramethyl-2,4,6,8-tetracarboxyporphyrin (isomer I) and its hemin are almost completely insoluble in acetic acid containing one part in eighty of water.

(3) Glacial acetic acid is a suitable solvent for the porphyrin and its hemin but not for the sodium or potassium chloride necessary to bring about equilibration. Tetramethylammonium chloride was found to be sufficiently soluble in glacial acetic acid to secure equilibration.

(4) In pyridine solution in the presence of tetramethylammonium chloride and acetic acid (25 ml. of pyridine, 10 ml. of acetic acid), the porphyrin absorbs at 607 m μ whereas the corresponding hemin does not. This fact was used for the determination of the extent of reaction during equilibration.

Equilibrium Constants in Glacial Acetic Acid.—Using tetramethylammonium chloride at 0.432 and 0.576 moles per liter and the hemin at 3.082 and 2.773×10^{-5} moles per liter, the equilibrium constants for tetramethyltetracarboxyhemins (isomer I) were 0.00372 ± 0.00030 and 0.00355 ± 0.00036 , respectively. In the same solvent, using 0.01872 and 0.03744 moles per liter of tetramethylammonium chloride, mesohemin (8.897 and 13.44×10^{-5} moles per liter, respectively) gave constants of 0.1472 ± 0.0001 and 0.1529 ± 0.0001 , respectively.

Discussion

The solvent 79:1 acetic acid-water contains 3.86 moles per cent of water. The addition of this amount of water to glacial acetic acid causes an increase in stability of mesohemin by an average of 16.4%. This is the effect that would be caused by weakening of the bonding of ferrous chloride with incipient ionization.

The difference between the two sets of observations on mesohemin in glacial acetic acid is greater than the observational error. The direction of the difference is towards diminished stability with increasing concentration of chloride ion, the direction which would be expected for an order of dependence on concentration of chloride ion slightly higher than the second order which was assumed. This is the behavior to be expected of aggregates and suggests that in this solvent we are dealing with some association complexes of the ions rather than the completely dissociated species.

The observation of major importance is the 41.3-fold decrease in the equilibrium constant caused by the substitution of four carboxy groups for four groups which are essentially alkyl groups. The equilibrium constants are determined by two factors, the relative oxidation-reduction potentials of the hemin-heme systems and the bond strengths of the Fe-N bonds in the hemes. Substitution of carboxy groups for alkyl groups will increase the oxidation-reduction potential of the hemin-heme system. Since the potential of the equilibrating system is held constant by calomel, this will increase the proportion of ferrous complex and should, therefore, favor demetalation. The actual finding is a 41.3-fold change toward stability. This is consistent with the interpretation that the bonds in the hemins measured are essentially covalent.⁶ It also is

(4) W. S. Caughey and A. H. Corwin, *J. Am. Chem. Soc.*, **77**, 1511 (1955).

(5) R. A. Fisher, "Statistical Methods for Research Workers," 13th Ed., Oliver and Boyd, Edinburgh, 1958.

(6) A. H. Corwin and M. H. Melville, *J. Am. Chem. Soc.*, **77**, 2755 (1955).

consistent with the expected diminution in the basicity of the carbethoxylated porphyrin.

Experimental

Mesohemin chloride was prepared by the method of Corwin and Erdman.⁷

1,4,5,8-Tetramethyl-2,3,6,7-tetracarboethoxyporphyrin was prepared by the method of Corwin and Andrews.⁸

1,3,5,7-Tetramethyl-2,4,6,8-tetracarboethoxyporphyrin was prepared by the method of Kleinspehn.⁹

Hemin.—Introduction of iron by the method used for mesohemin is not applicable in this case because of the greater difficulty of securing the reaction. Air oxidation of the ferrous acetate is complete before there is appreciable conversion to the hemin. The reaction will proceed with an excess of ferrous acetate and some ferric ions by refluxing under an atmosphere of nitrogen for 2 hr. The addition of sodium chloride and subsequent air oxidation stabilizes the hemin.

Anal. Calcd. for $C_{36}H_{36}O_8N_4FeCl$: C, 58.12; H, 4.88. Found: C, 58.14; H, 4.77.

Spectrophotometric Determinations.—The determinations on mesoheme in acetic acid–water, 79:1 by volume, were made on a Cary Model XI. All others were made on a Beckman DK 2. The amounts of mesohemin and mesoporphyrin at the equilibrium point were determined in acetic acid without the addition of pyridine. With the tetramethyltetracarboethoxyporphyrin, 10 ml. of the glacial acetic acid solution was added to 25 ml. of pyridine to obtain sufficient solubility for measurement.

Equilibration.—A known weight of the hemin under study was added to a measured volume of solvent containing the desired salt in each of several small test tubes. A drop (0.05 ml.) of mercury was added to each of the tubes. These were then sealed under nitrogen to eliminate air. The contents were shaken vigorously for 15 min. The reaction mixtures in these sealed tubes were heated at 108° in an electronically controlled oil bath for reaction at various time intervals from 0.5 hr. to 3 hr. by 0.5-hr. increments. To determine the length of time required for equilibration, two tubes were withdrawn after each interval and were immediately immersed in an ice bath to freeze the reaction. The tubes were warmed to room temperature, opened, and mixed with 25 ml. of pyridine for measurement.

Acknowledgment.—The authors wish to acknowledge the assistance of David Whitten and Earl Baker in the interpretation of the experiments.

(7) A. H. Corwin and J. G. Erdman, *J. Am. Chem. Soc.*, **68**, 2473 (1946).

(8) J. S. Andrews, A. H. Corwin, and A. G. Sharp, *ibid.*, **72**, 491 (1950).

(9) G. G. Kleinspehn, personal communication; See G. G. Kleinspehn, A. E. Briod, and W. S. Caughey, Abstracts, 135th National Meeting of the American Chemical Society, Boston, Mass., 1959, p. 350.

A Phenylsotriazole Rule for the Determination of the Configuration of Monosaccharides

H. EL KHADEM

Faculty of Science, University of Alexandria, Alexandria, Egypt,
United Arab Republic

Received March 8, 1963

During the past two decades a sufficient number of monosaccharide phenylsotriazoles have been prepared to enable a correlation between their configuration and the sign of their optical rotation. This may be stated in the form of a "Phenylsotriazole Rule" as follows: "Whenever the hydroxyl group on C-3 of a monosaccharide is on the right in the conventional projection

TABLE I
SPECIFIC ROTATION OF KNOWN MONOSACCHARIDE
PHENYLOSOTRIAZOLES

Parent monosaccharide	Sign of OH —on C-3 on C-4—		[α] _D	Solvent ^a	Ref.
D-Arabinose	+	+	+23.1	W	^b
			+26.0	P	^b
L-Arabinose	—	—	—22.8	W	^b
			—25.8	P	^b
D-Xylose	—	+	—32.3	W	^c
L-Xylose	+	—	+32.5	W	^d
6-Deoxy-D-glucose	—	+	—67.5	E	^d
6-Deoxy-L-mannose	+	—	+101.5	P	^e
			+67.5	E	^d
			+49.4	W	^e
L-Fucose	+	+	+20.0	W	^e
D-Altrose	+	+	+28.0	P	^c
D-Glucose	—	+	—81.6	P	^f
L-Glucose	+	—	+82.0	P	^g
D-Galactose	—	—	—30.6	P	^c
			—13.3	C	^c
L-Sorbose	—	+	—46.7	P	^c
5-Desoxy-L-sorbose	—	+	—38.5	M	^h
D-Altroheptulose	—	+	—71.5	P	^{i,j}
			—71.0	P	^k
D-Glucoheptulose	+	—	+46.9	P	ⁱ
D-Mannoheptulose	—	—	—27.5	P	ⁱ
			—27.3	P	^k
D-Galaheptulose	+	—	+80.3	P	ⁱ
D-Guloheptulose	+	+	+17.6	P	^l
L-Guloheptulose	—	—	—18.3	W	^{..}
			—15.9	P	^{..}
D-Idoheptulose	—	+	—44.9	P	^m
D-Erythro-L-manno- octose	+	—	+77.3	P	ⁿ

^a W = water, E = ethanol, C = chloroform, M = methanol, P = pyridine. ^b W. T. Haskins, R. M. Hann, and C. S. Hudson, *J. Am. Chem. Soc.*, **68**, 1766 (1946). ^c **67**, 939 (1945). ^d E. Hardegger and H. El Khadem, *Helv. Chim. Acta*, **30**, 900 (1947). ^e W. T. Haskins, R. M. Hann, and C. S. Hudson, *J. Am. Chem. Soc.*, **69**, 1461 (1947). ^f R. M. Hann and C. S. Hudson, *ibid.*, **66**, 735 (1944). ^g F. A. Kuehl, E. H. Flynn, F. W. Holly, and K. Folkers, *ibid.*, **69**, 3032 (1947). ^h P. Regna, *ibid.*, **69**, 246 (1947). ⁱ W. T. Haskins, R. M. Hann, and C. S. Hudson, *ibid.*, **69**, 1050 (1947). ^j D. A. Rosenfels, N. K. Richtmyer, and C. S. Hudson, *ibid.*, **73**, 4907 (1951). ^k V. Ettel and J. Liebster, *Collection Czech. Chem. Commun.*, **14**, 80 (1949). ^l L. C. Stewart, N. K. Richtmyer, and C. S. Hudson, *J. Am. Chem. Soc.*, **74**, 2206 (1952). ^m J. W. Pratt, N. K. Richtmyer, and C. S. Hudson, *ibid.*, **74**, 2210 (1952). ⁿ J. V. Karabinos, R. M. Hann, and C. S. Hudson, *ibid.*, **75**, 4320, 4324 (1953).

formula, the rotation of the derived phenylsotriazole is positive, and, conversely, when the hydroxyl group is on the left the rotation of the phenylsotriazole is negative."

In Table I are recorded the specific rotations of the known monosaccharide phenylsotriazoles; these are all in agreement with this rule. The rule should, therefore, find application in the determination of the configuration of monosaccharides, in conjunction with the benzimidazoles rule¹ which gives the configuration of the hydroxyl group on C-2. Here as with benzimidazoles, when the hydroxyl group alpha to the heterocyclic ring is to the right, the compound is dextrorotatory and, when this hydroxyl group has a *cis* relationship with the following one, the rotation tends to be small.

(1) N. K. Richtmyer and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 1612 (1942).