is offset by the good adherence to ideality, thus permitting high numbers of transfers to be applied.

In this system both chymotrypsinogen and bovine serum albumin had K values of infinity. By addition of varying amounts of 1-propanol to the system, useful K's could be obtained with both proteins. With bovine serum albumin in the system of ethanol, 1-propanol, ammonium sulfate solution (40 g. in 100 ml. of water), water with volume ratios of 1.1:0.9:2:2, a K value of 1.6 was obtained; however, on standing, the K value gradually decreased. This shift was prevented by substitution of 0.01 N sodium caprylate in place of water in the above system; sodium caprylate was originally discovered to be a stabilizing agent for serum albumins against heat or urea denaturation.[13] Preliminary results obtained in this Laboratory showed promise for the fractionation of serum albumins in this solvent system.

With chymotrypsinogen in the system containing ethanol, 1-propanol, ammonium sulfate solution and water with volume ratio of 1.5:0.5:2.5:1.5, a K value of 1 was also obtained. On standing, precipitation took place and the precipitate was found to be no longer soluble in water, an indication that the cause was denaturation rather than the solubility of the protein in the system. Substitution of ethyl cellosolve for ethanol did not prevent precipitation though this solvent was successfully used by Porter[15] for the partition chromatography of chymotrypsinogen.

While it is an often observed fact that most proteins are denatured readily by organic solvents at room temperature, we feel that it is worthwhile to search for agents that will modify the labile nature of proteins. Operationwise it is easier to carry out a fractionation at room temperature than at lower temperatures. Our attempt to find a stabilizer for chymotrypsinogen in the system described above has thus far been unsuccessful. Among the compounds tried have been caprylic acid, mandelic acid, glycine, cholesterol, sodium citrate, phosphates and β-phenylpropionic acid, a known reversible inhibitor for the chymotrypsins. Work along these lines is being continued.

Acknowledgments.—The technical assistance of Mrs. Judith O'Brien and Miss Gerty Walker is gratefully acknowledged. We also wish to thank Dr. Mayo Uziel for helpful discussions on ion-exchange chromatography.

NEW YORK 21, NEW YORK

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLUMBIA UNIVERSITY, COLLEGE OF PHYSICIANS AND SURGEONS]

A Comparison of the Rate of Mutarotation and O^{18} Exchange of Glucose[1]

BY D. RITTENBERG AND CHARLES GRAFF[2]

RECEIVED FEBRUARY 6, 1958

The effect of pH on the exchange reaction between the aldehyde oxygen of glucose and the oxygen of water has been measured. The rate is at a minimum at pH 4 and rises rapidly below pH 3 and above pH 6.

The exchange reaction between aldehydic oxygen atoms and the oxygen atom of water has long been known, and various aspects of this phenomenon have been reported. Cohn and Urey[3] demonstrated that acetone readily exchanges its oxygen with that of water, and Titani and Goto[4] have shown that glucose exchanges one-sixth of its oxygen, presumably the oxygen atom on carbon atom one, with the solvent, water. During the course of another investigation we observed that the rate of exchange between glucose-1-O^{18} and water was erratic when the pH was uncontrolled. Cohn and Urey had shown in the case of acetone that the exchange reaction is both acid and base catalyzed. Accordingly, we undertook a study of the effect of pH on the rate of the exchange reaction between glucose-1-O^{18} and water. We hoped, further, that a determination of the glucose–water exchange kinetics might permit the re-evaluation of existing theories as to the mechanism of glucose mutarotation.

Experimental

The rate of exchange reaction was followed in the system normal water–glucose-1-O^{18}.

Glucose-1-O^{18}.—Glucose-1-O^{18} was prepared by heating a solution of glucose containing 29.2 atom per cent. excess O^{18} water for 18 hours at 100°. The water was removed by distillation *in vacuo* and the sirupy residue further dried over fresh P_2O_5. The anhydrous sirup was dissolved in a minimum of normal water and alcohol added. Scratching of the cold solution induced crystallization after some hours. The crystalline glucose was dried and analyzed for O^{18}.[5] It contained 4.12 ± 0.03 atom per cent. excess; somewhat less than one-sixth of the isotope concentration of the water. Another sample of glucose was prepared by this procedure. It contained 3.51 ± 0.03 atom per cent. excess O^{18}.

To determine the rate of the exchange about 18 mg. of glucose-O^{18} (0.1 mM) was weighted into a tube shown in Fig. 1A and then about 90 mg. of a buffer solution (5 mM) carefully added. The tube was sealed off and kept in a thermostat for a known time after which it was cooled in Dry Ice–alcohol to stop the reaction. The frozen sample tube was transferred to a vacuum system in which the water from the glucose solution could be distilled to another tube similar to that shown in Fig. 1A; CO_2 was added to a pressure of 10 cm. (~0.02 mM), the tube sealed off and kept at 100° for 3 hours to equilibrate the O^{18} concentration in the CO_2 and the water. Separate tests showed equilibration to be complete in this period. The tube was transferred to

(1) This work was supported by contracts to Columbia University from the Office of Naval Research, Department of the Navy (ONR 26602), from the Atomic Energy Commisstion (AT (30-1) 1803) and from the National Institutes of Health (E-1461). Reproduction of this article in whole or in part is permitted for any purpose of the United States Government.

(2) Holder of a U.S.P.H.S. Medical Student Part Time Research Fellowship of the National Institutes of Health.

(3) M. Cohn and H. C. Urey, THIS JOURNAL, **60**, 679 (1938).

(4) Toshizo Titani and Kokiti Goto, *Proc. Imp. Acad. Tokyo*, **15**, 298 (1940); **16**, 398 (1940).

(5) D. Rittenberg and L. Ponticorvo, *J. App. Rad. and Isotopes*, **1** 208 (1956).

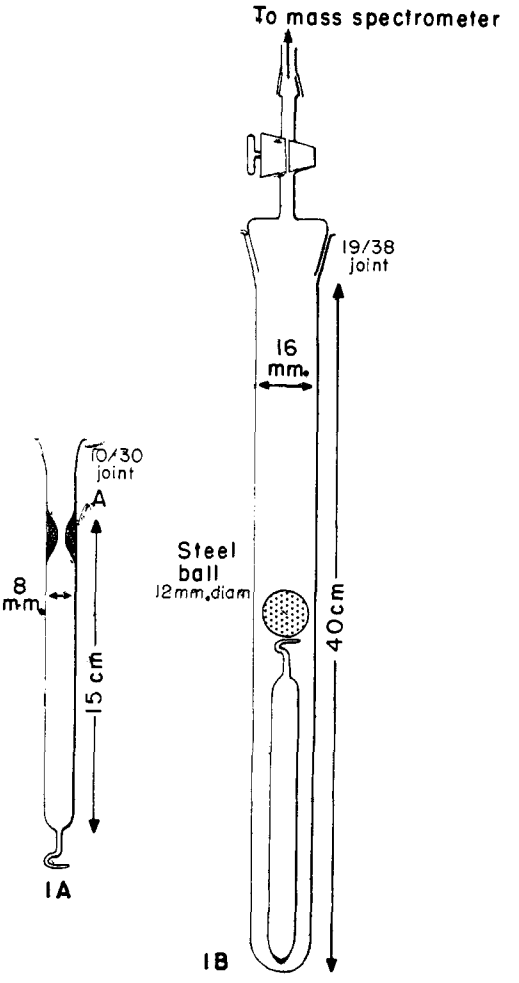

Fig. 1.—Apparatus for carrying out exchange reactions between glucose and water, water and CO_2 and transferring CO_2 to mass spectrometer.

the gas line of a mass spectrometer (Fig. 1B), opened and the O^{18} concentration of the CO_2 measured.

Calculation of Results.—We assume that the appearance of O^{18} in the water follows a unimolecular law

$$C = C_\infty(1-e^{-kt})$$

where

C = O^{18} concentration of the water at time t
C_∞ = O^{18} concentration at equilibrium
k is the rate constant
t is the time in hours

C_∞ can be calculated from the initial conditions

$$C_\infty = \frac{6aC_g}{a+b}$$

where

a = mM O^{18}-labeled glucose
b = mM water
C_g = atom per cent. excess O^{18} in glucose

The constant 6 enters the equation because only one of the six oxygen atoms of glucose contains excess O^{18} and exchanges with the oxygen of the water.

The value of t was always chosen so that C/C_∞ was approximately 1/2. Buffers were made up at 25°; no correction for the effect of temperature on the buffers was made.

The values of $t_{1/2}$ for the reaction at 61° at various pH values is given in Table I. The calculated values of the rate constant are given in Fig. 2 and for comparison the rate

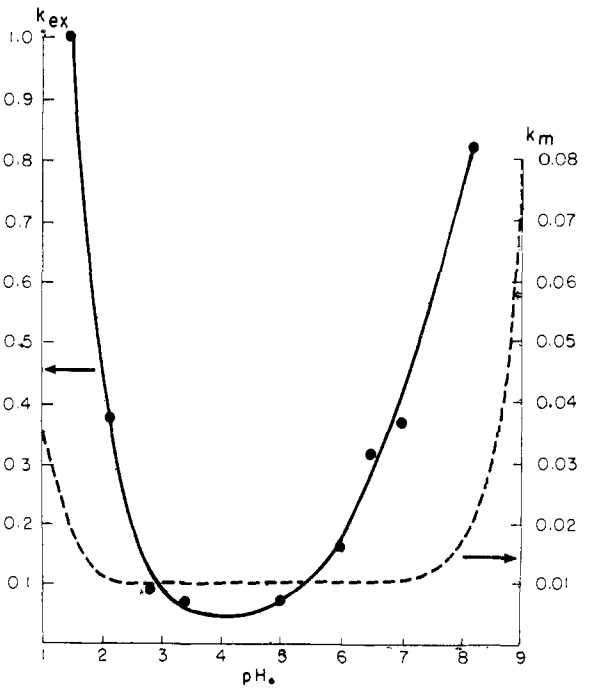

Fig. 2.—The effect of pH on the rate of the exchange reaction (k_{ex}) at 61° and for comparison the rate of mutarotation (k_m) at 20°.

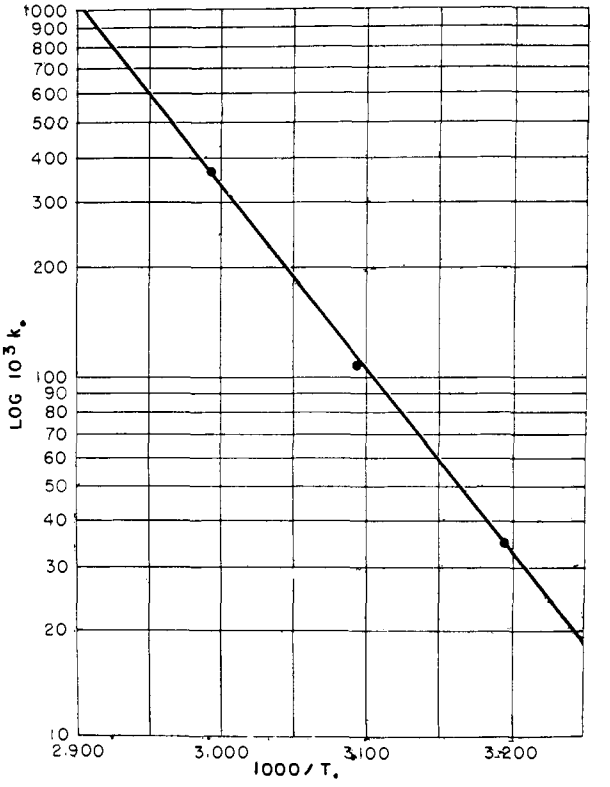

Fig. 3.—The variation of the rate of the exchange reaction with temperature at pH 7.

constants for mutarotation at 20°.[6] The variation of k with temperature at pH 7 is shown in Fig. 3. Every value of k is the average of at least two concordant results.

(6) Polarimetry, Saccharimetry and the Sugars by Frederick J. Bates and Associates, United States Government Printing Office, 1942, p. 452.

TABLE I

EFFECT OF *p*H ON THE RATE OF THE EXCHANGE REACTION AT 61°

*p*H	Buffer system	$t_{1/2}$, hr.
1.5	Oxalic acid	0.67
2.15	HCl–KCl	1.84
2.80	Phthalate	7.7
3.40	Acetate	10.0
5.0	Acetate	10.2
6.0	Phosphate	4.3
6.5	Phosphate	2.2
7.0	Phosphate	1.9
8.15	Barbiturate	0.83

Discussion

The effect of *p*H on the rate of the exchange reaction suggests that there are two independent reactions: one base catalyzed, the other acid. The rate of exchange is not linear with the hydrogen ion concentration in the acid range nor with the hydroxyl ion concentration in the alkaline region. The rate-limiting step for the exchange reaction is not the same as for mutarotation since it is less than one-thirtieth that calculated for the rate of mutarotation at 61°. At *p*H 7 the energy of activation of the exchange reaction calculated from the slope of the curve in Fig. 3 is 23,400 cal. while that for mutarotation is but 17,200 cal.[7] The kinetics as well as the different shapes of the rate *vs.* *p*H curves suggest that the rate-limiting steps of the mutarotation and the exchange are different. Our data are consistent with the hypothesis that the exchange takes place with the free aldehyde form of glucose.

Acknowledgment.—We are indebted to Dr. Israel Dostrovsky of the Weizmann Institute, Rehovoth, Israel, who supplied us with the O^{18}-labeled water and to Miss Laura Ponticorvo for her highly skilled assistance.

(7) Reference 6, p. 448.

NEW YORK 32, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, STANFORD UNIVERSITY]

Isomers of Tetra-*O*-acetyl-D-mannopyranose

BY WILLIAM A. BONNER

RECEIVED OCTOBER 14, 1957

When water and silver carbonate acted upon crystalline tetra-*O*-acetyl-α-D-mannopyranosyl chloride a levorotatory sirup resulted, from which two isomeric tetra-*O*-acetyl-D-mannopyranose derivatives could be crystallized: A, m.p. 124°, and B, m.p. 95°, respectively. When sirupy tetra-*O*-acetyl-α-D-mannopyranosyl bromide was similarly allowed to react isomers A and B were again obtained, accompanied by a third isomeric tetra-*O*-acetyl-D-mannose, C, m.p. 164°. Acetylation of isomer A with radioactive acetic anhydride and pyridine at −5° afforded an acetyl-labeled penta-*O*-acetyl-β-D-mannopyranose bearing 85% of its label at C-1, as indicated by its physical properties and the fact that it exchanged 85% of its radioactive acetyl groups under anomerizing conditions. Isomer A dextromutarotated rapidly in aqueous acetone, slowly in pyridine and rapidly in a pyridine–phenol mixture. Acetylation of isomer A with acetic anhydride in pyridine was markedly more rapid than its mutarotation in pyridine. Methylation of isomer A by several techniques afforded crystalline methyl tetra-*O*-acetyl-β-D-mannopyranoside. A study of the carbonyl absorption intensity of isomer A indicated four normal acetyl groups and no ortho-acid structure. These data are all explicable in terms of 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose as the structure of isomer A. Acetylation of isomer B with radioactive acetic anhydride and pyridine produced penta-*O*-acetyl-α-D-mannopyranose acetyl-labeled at C-1, characterized again by its physical properties, its carbonyl absorption intensity and its loss of all of its label under anomerizing conditions. The structure 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose is thus indicated for isomer B. Acetylation of isomer C with radioactive acetic anhydride yielded a labeled penta-*O*-acetyl-β-D-mannopyranose which *failed* to exchange its label under anomerizing conditions, a fact indicating that some hydroxyl group other than that at C-1 was free in this isomer. In aqueous acetone isomer C mutarotated to produce isomer B, a reaction clearly involving acetyl migration. Consideration of the known tetra-*O*-acetyl-D-mannopyranose derivatives in the literature, and of molecular models indicates that the structure of isomer C is best represented either by 1,3,4,6- or 1,2,4,6-tetra-*O*-acetyl-β-D-mannopyranose.

Introduction

Four tetraacetyl derivatives of mannose are described in the literature. Of these, only 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose has been particularly well characterized.[1,2] It is obtained by the usual detritylation of its 6-trityl precursor with a cold hydrogen bromide–acetic acid mixture. Having m.p. 135.5–136.5° and $[\alpha]^{20}D$ −22.5° ($CHCl_3$), its structure rests on: (a) its method of preparation, (b) its conversion[1] to penta-*O*-acetyl-β-D-mannopyranose with acetic anhydride, (c) its conversion to the corresponding 6-chlorohydrin with phosphoryl chloride,[1] (d) its lack of mutarotation[1] and (e) its conversion[2] to 1,6-linked di- and trisaccharide derivatives on reaction with poly-*O*-acetylglycopyranosyl halides.

A second tetra-*O*-acetyl-D-mannose has been described[3] by Micheel and Micheel. This material, obtained in very low yield on reaction of trimethylamine with tetra-*O*-acetyl-α-D-mannopyranosyl bromide, had m.p. 159–160° and $[\alpha]^{19}D$ −24.2° ($CHCl_3$). No further physical or chemical properties of this substance have been described, although the authors suggest 2,3,4,6-tetra-*O*-acetyl-D-mannopyranose as its possible structure.

In an investigation concerned with the ring structure of mannose acetates, Levene and Tipson report[4] a third tetra-*O*-acetyl-D-mannose. This substance, m.p. 93° and $[\alpha]^{27}D$ +26.3° ($CHCl_3$) resulted by action of excess silver carbonate and slightly over the calculated quantity of water on crystalline tetra-*O*-acetyl-α-D-mannopyranosyl bro-

(1) B. Helferich and J. F. Leete, *Ber.*, **62**, 1549 (1929).
(2) D. D. Reynolds and W. L. Evans, THIS JOURNAL, **62**, 66 (1940).
(3) F. Micheel and H. Micheel, *Ber.*, **63**, 386 (1930).
(4) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **90**, 89 (1931).