Preparation of α-Tetracetyl Methyl Glucoside.—This substance was prepared by acetylating α-methylglucoside with acetic anhydride and sodium acetate, according to the directions of Koenigs and Knorr.[1] It was recrystallized from 95% alcohol until a constant specific rotation was obtained. It melted at 100–101° corrected.

Preparation of β-Tetracetyl Methylglucoside.—This compound has been prepared by Koenigs and Knorr[2] from β-bromoacetyl glucose and from β-acetonitroglucose, and Moll van Charante[3] has prepared it from β-methylglucoside. We followed Koenigs and Knorr's procedure slightly modified, using β-bromoacetyl glucose. Twenty-five g. of β-bromoacetyl glucose were dissolved in 200 cc. methyl alcohol. To this were added 12.5 g. silver nitrate dissolved in 200 cc. methyl alcohol and 50 cc. water. The filtrate from the silver bromide precipitate was treated with hydrogen sulfide and then shaken with barium carbonate. The filtrate from this was evaporated to a thick syrup on the steam bath under diminished pressure. On cooling, it crystallized to an almost solid mass. It was recrystallized from absolute methyl alcohol until the specific rotation was constant. The yield was 13.5 g., having a melting point of 104–105° (corr.).

THE ISOMERIC OCTACETATES OF LACTOSE.

By C. S. Hudson and J. M. Johnson.[4]

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Schutzenberger and Naudin[5] acetylated lactose by boiling it with acetic anhydride but did not obtain a crystalline product. Herzfeld[6] crystallized lactose octacetate by acetylating the sugar with acetic anhydride and anhydrous sodium acetate (the Liebermann[7] method), and found the substance to melt at 86°, but did not learn its rotatory power. Schmöger[8] prepared the octacetate by this method, reported a higher melting point, 95–100°, and observed a specific rotation of —3.5° in chloroform. Fischer and Armstrong[9] found this octacetate to melt at 106°,

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[1] *Ber.*, **34**, 970 (1901).

[2] *Loc. cit.*, 969 and 977.

[3] *Loc. cit.*

[4] Contribution from the Carbohydrate Laboratory, Bureau of Chemistry, United States Department of Agriculture. A preliminary notice was read at the Cincinnati meeting of the American Chemical Society, April, 1914. The authors desire to express their appreciation of the valuable services of Mr. A. S. Eastman, who has repeated during the summer of 1914 the preparation of the new octacetyl lactose. The data of the present article are partly taken from his work.

[5] *Bull. soc. chim.*, II, **12**, 208 (1869).

[6] *Ber.*, **13**, 265 (1880).

[7] *Ibid.*, **11**, 1619 (1878).

[8] *Ibid.*, **25**, 1452 (1892).

[9] *Ibid.*, **35**, 841 (1902).

regarded it as a mixture of two isomers, but were unable to separate it into its assumed components.

We attempted to prepare crystalline lactose octacetate by the methods which have been described by these different authors, and also by the method which Kremann[1] has used, but the products did not crystallize. Finally, a method was devised by which the octacetate may be readily prepared in crystals.

Four hundred cc. of acetic anhydride were heated to 100° in a flask of several liters' capacity and 25 g. of anhydrous sodium acetate were added. The mixture was heated nearly to the boiling point of the solution and there was added to it, a little at a time, 100 g. of commercial milk sugar, which consists principally of the monohydrated alpha form of lactose. After a small amount of the sugar had been added, a vigorous reaction began, accompanied by boiling of the acetic anhydride. The gas flame under the flask was then turned off and the addition of the sugar continued, a small amount at a time, until all was in solution, the reaction furnishing enough heat to keep the acetylation going to completion. After all the lactose had dissolved, the solution was boiled for ten minutes and poured into a large volume of cold water. The insoluble viscous mass which precipitated was stirred with the water, allowed to settle, the water poured off and fresh water added from time to time. After standing over night under water, the mass solidified and was broken up with a rod. It was then filtered on a Buchner funnel, washed well with water and dried in the air. The product was ground, and extracted with warm ether by decantation until all ether-soluble material was removed. The residue, which was by far the larger portion of the product, was then dried from all ether and dissolved in hot 95% alcohol. On cooling and stirring frequently, a spontaneous crystallization was invariably obtained. The compound may also be recrystallized from chloroform by the addition of alcohol or ether, or from benzene by adding ether. The recrystallization from alcohol was repeated until a constant value for the specific rotation was obtained, and the yield of the purified product was about 55% of the theoretical.

The rotatory power of this octacetate in chloroform solution is to the left, and our measurements (see Table I) on the carefully purified material show that the concentration influences the specific rotation appreciably. Schmöger's value comes within the range of our results, but the concentration of his solution is not recorded.

The melting phenomena which are exhibited by the substance are peculiar, as is to be expected from the fact that observers have obtained melting points as far apart as 86° (Herzfeld) and 106° (Fischer and Armstrong). In our first observations on the carefully purified and dried

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[1] *Monatsh.*, **23**, 483 (1902).

octacetate, the microscopic crystals appeared to melt from 95° to 99°, but it was noticed that the mass kept a white appearance to 104°. This behavior was understood when the crystals were observed under magnification during the process of melting, because it was then seen that each crystal distinctly melted at about 90°, but the melt is so viscous at that temperature that the drops do not coalesce, or only very slowly. When they do unite, as the temperature rises toward 100°, air bubbles are included in the melt and give it a white appearance which vanishes as the viscous liquid becomes more fluid at or about 104°. We regard this lactose octacetate, therefore, as melting at about 90°, and fairly sharply, considering that the melt is very viscous and the heat transfer slow in consequence.

For analysis, the material was dried in a vacuum oven at 70° to constant weight. 0.6792 and 0.5513 g. subs. gave 1.2241 and 0.9951 g. CO_2 and 0.3454 and 0.2808 g. H_2O. Found: 49.16% and 49.23% C; 5.70% and 5.71% H; calc. for $C_{12}H_{14}O_{11}(CH_3CO)_8$: 49.54% C and 5.65% H.

The acetyl percentage was measured by boiling 0.5 g. samples with 50 cc. of *N*/3 sulfuric acid under a reflux condenser for three hours, and titrating with 0.1 *N* sodium hydroxide solution, using phenolphthalein as indicator. The reliability of the method was tested in numerous blank determinations, both with and without the addition of lactose. By varying the time of hydrolysis, the latter was shown to be complete in three hours. Using 0.5 g. samples, the percentages of acetyl (CH_3CO) found in two experiments were 50.75 and 50.71 while the theory requires 50.74%.

Preparation of an Isomeric Crystalline Octacetate of Lactose.—When lactose octacetate of m. p. 90° was dissolved in glacial acetic acid containing a small amount of zinc chloride, there was observed at 20° a slow change of rotation day by day to the right. The speed of this change could be hastened by heating the solution on the steam bath. A similar change of rotation towards the right was observed when acetic anhydride containing zinc chloride was used as the solvent, and in this case a new crystalline product was isolated by the following procedure:

Fifty grams of lactose octacetate (m. p. 90°) and 5 g. of fused zinc chloride were dissolved by warming in 250 cc. acetic anhydride, and then heated thirty minutes on the steam bath. The mixture was cooled, poured into a large volume of cold water and allowed to stand under the water over night. The hard mass was filtered off, ground in a mortar, dried in the air, and dissolved in ether. With an occasional scratching of the glass vessel the ether solution was brought to crystallization after a day or so at 20°. These crystals were then recrystallized from 95% alcohol, at first with some difficulty, but after several recrystallizations with ease. A yield of 56% was obtained. A compound differing from the original octacetyl lactose in many physical properties was thus prepared. It melts sharply at 152°, consists of fine felted needles, is more soluble in ether and in alcohol than the parent substance and, like the latter, is very soluble in chloroform, benzene and ethyl acetate, difficultly soluble in water and almost insoluble in petroleum ether. Determination of the acetyl value, elementary analysis, and the regeneration of lactose showed this compound to be an octacetyl lactose. Milk sugar was regenerated from it by pouring cold alcoholic potash on a suspension of the substance in alcohol, filtering off the lactose, recrystallizing, and identifying the sugar by its cupric reducing power, its specific rotation, its solubilities and its failure to ferment with yeast.

0.3202 and 0.4732 g. subs. gave 0.5810 and 0.8573 g. CO_2, and 0.1626 and 0.2372 g. H_2O. Found: 49.49% and 49.41% C; 5.69% and 5.62% H.

Calc. for $C_{12}H_{14}O_{11}(CH_3CO)_8$: 49.54% C and 5.65% H.

The acetyl percentage, estimated as previously described, showed 50.84% and 50.66% CH_3CO; calc.: 50.74%.

This isomeric octacetyl lactose is also formed in small amount along with the earlier discovered octacetate during the acetylation of lactose with acetic anhydride and anhydrous sodium acetate. We obtained a small quantity of the new isomer in crystals from the ether extraction which has been mentioned in connection with the description of the preparation of octacetyl lactose of m. p. 90°.

Determinations of the specific rotation of the new isomeric octacetyl lactose are recorded in Table I and show that the substance is strongly dextrorotatory, distinguishing it from the earlier discovered lactose octacetate which is levorotatory.

TABLE I.—THE SPECIFIC ROTATIONS OF THE TWO LACTOSE OCTACETATES.

	Conc. g. per 100 cc. solution.	Length of tube.	Reading.	$[\alpha]_D^{20°}$	Conc. g. per 100 cc. solution.	Length of tube.	Reading.	$[\alpha]_D^{20°}$
	Old isomer or β-octacetate. M. p. 90°.				New isomer or α-octacetate. M. p. 152°			
Solvent.		Dm.	Degrees.	Degrees.		Dm.	Degrees.	Degrees.
Chloroform[1]....	10.3644	4	— 1.95 Ang.	— 4.70	7.8560	4	+16.85 Ang.	+53.62
Chloroform[1]....	10.6512	2	— 2.69 V.[2]	— 4.37	10.1592	2	+31.44 V.	+53.54
Chloroform[1]....	10.7344	2	— 2.62 V.	— 4.22	10.3746	4	+22.05 Ang.	+53.13
Chloroform[1]....	10.8448	2	— 2.80 V.	— 4.47	10.6016	2	+11.51 Ang.	+54.28
Chloroform[1]....	19.4414	4	— 2.75 Ang.	— 3.54	20.3340	4	+44.55 Ang.	+54.77
Benzene.......	5.72	4	— 5.90 Ang.	—25.78	10.66	4	+35.2 V.	+28.6
Benzene.......	10.49	4	— 9.87 Ang.	—23.52	10.3044	2	+ 5.90 Ang.	+28.6
Benzene.......	9.8874	4	—27.31 V.	—23.90	10.57	4	+12.08 Ang.	+28.57
Benzene.......					5.8456	2	+ 8.43 V.	+24.95
Benzene.......					5.21	4	+ 5.17 Ang.	+24.81
Benzene.......					4.7518	4	+ 4.54 Ang.	+23.89
Glacial acetic acid.........	10.3014	4	No rotation[3]	....	9.8868	2	+11.85 Ang.	+59.93
					10.36	4	+71.7 V.	+59.87
					6.0932	2	+ 7.40 Ang.	+60.72
50% acetic acid	10.0	2	No rotation	....	4.9356	2	+ 6.06 Ang.	+61.40

[1] The chloroform used was chloroformum purificatum, U. S. P.

[2] 1° Ventzke = 0.346° Angular.

[3] None could be observed and the limit of detection was somewhat less than 0.05° V.

The Equilibrium between the Two Isomeric Octacetates.—Two and one-half grams of the older isomer were dissolved in glacial acetic acid containing zinc chloride, the solution was made up to 50 cc. and allowed to stand at 20° during five months. There was a slow change in the rotation as read in a 200 mm. tube with a Ventzke instrument from +0.7° to +15.3°. A corresponding solution of the new isomer changed in rotation during the same time from +17.6° to +15.2°. Equilibrium was thus reached between the two forms in glacial acetic acid under the specified conditions when about 86% of the new isomer was present.

Five grams of the older isomer and 0.5 g. $ZnCl_2$ when made up to 50 cc. with acetic anhydride at 20° gave a specific rotation of +4.3°. This solution was heated at 100° for 30 minutes, cooled to 20° and the specific rotation was found to be +51.9°, and the value was not changed by a further heating for ten minutes. Five grams of the new isomer were treated in the same manner and the specific rotation was found to change from +64.0° to +52.8°, at which value it became constant. The point of equilibrium at 100° is then about +52°, which shows that about 81% of the new isomer is present in the equilibrium mixture in acetic anhydride.

Preparation of Bromoacetyl Lactose from the New Lactose Octacetate.—Five grams of lactose octacetate of specific rotation —4° were dissolved in 7 cc. warm acetic anhydride, the mixture cooled, 7 cc. acetic acid saturated with hydrobromic acid gas were added, and the solution kept at room temperature until its polariscopic reading became constant, which required about forty minutes. The solution was then poured into ice water and treated according to Fischer's[1] directions for the preparation of heptacetyl bromolactose. We obtained 3 g. of recrystallized substance of m. p. 139–141°, and specific rotation +105° in chloroform, which was evidently identical with Fischer's preparation which melted at 141–142° and had a specific rotation in chloroform of +104.9°. When the new lactose octacetate of specific rotation +53° was treated by the same procedure, there were obtained 2 g. of recrystallized substance melting at 139–141° and having a specific rotation of +104° in chloroform solution. From the two lactose octacetates, therefore, the same heptacetyl bromolactose was obtained.

Molecular Weights of the Isomeric Octacetates in Benzene.—The freezing temperatures of solutions of the new isomeric octacetate containing per hundred grams of benzene 1.34, 1.58, and 3.38 g. substance, respectively, were found to be 0.099°, 0.113,° and 0.242° below that of the solvent, indicating on an average the molecular weight of 692, in comparison with the theoretical value 678. The freezing-point depressions of solutions of the earlier discovered octacetate gave the value 611.

The Structural Formulas and Nomenclature of the Isomeric Oct-

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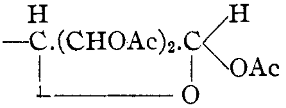
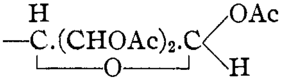
[1] *Ber.*, 43, 2530 (1910).

acetates.—The existence of isomeric pairs of the fully acetylated derivatives of all the aldose and ketose sugars, corresponding to the well-known alpha and beta forms of glucose pentacetate and of cellose octacetate, respectively, is to be expected. The isomerism of the two lactose octacetates presumably depends upon the space arrangement about the terminal asymmetric carbon atom, which may take the two forms



$$\underbrace{-\overset{\text{H}}{\text{C}}.(\text{CHOAc})_2.\text{C}}_{\text{O}}\begin{smallmatrix}\text{H}\\ \text{OAc}\end{smallmatrix}$$

and



$$\underbrace{-\overset{\text{H}}{\text{C}}.(\text{CHOAc})_2.\text{C}}_{\text{O}}\begin{smallmatrix}\text{OAc}\\ \text{H}\end{smallmatrix}$$

On this view, the two lactose octacetates are the derivatives, respectively, of the well-known alpha and beta forms of lactose, and the octacetate of stronger dextro rotation, which is the new isomer, is to be named in accordance with the naming of the forms of lactose, the alpha form, the older isomeric octacetate being the beta form. This naming of the octacetates is supported by the following comparison of their rotatory powers with those of the alpha and beta forms of glucose pentacetate, since the α- and β-pentacetates have been correlated with the alpha and beta forms of glucose by the experiments of Behrend and Roth. Writing the molecular rotations[1] of α- and β-glucose pentacetates as (A + B) and (—A + B), respectively, the molecular rotations of the corresponding α- and β-octacetates of lactose may be written (A + B′) and (—A + B′), respectively. It follows that the difference of the molecular rotations of the first pair of substances (2A) is equal to that of the second pair, a conclusion which may be tested experimentally from the data recorded in Table II.

The differences of the molecular rotations of the α- and β-glucose pentacetates in several solvents are quoted from the foregoing article by Hudson and Dale, and those of the α- and β-lactose octacetates are obtained from the averaged values of Table I, multiplied by 678, the molecular weight of the octacetate.

TABLE II.

	Difference of the molecular rotations of the alpha and beta forms.			
			Acetic acid.	
Substance.	Chloroform.	Benzene.	99.5%.	50%.
Glucose pentacetate	38,100	36,700	40,800	41,000
Lactose octacetate	39,400	34,300	40,600	41,600

The numbers agree closely in the two strengths of acetic acid and also the agreement in chloroform solution is a satisfactory one, since the difference between the two values corresponds to less than 2.° in specific rotation. The values in benzene solution are farther apart, and confirm Hudson and Dale's conclusion that benzene is not a suitable solvent for such comparisons.

WASHINGTON, D. C.

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[1] For the meaning of this formulation, see THIS JOURNAL, 31, 66 (1909).