standard procedure. The methoxyl content obtained in one methylating operation increases with decreasing degree of polymerization. However, the highest methoxyl content still remains by about 1% below the theoretical value for trimethylcellulose.

2. This result is not changed by repeated remethylation. It is only on reacetylation followed by remethylation that the theoretical value is more closely approached. However, the preparations thus obtained are the derivatives of considerably degraded cellulose.

3. Acetylation of cellulose as a step preparatory to methylation is of no beneficial effect unless the acetate is dissolved in acetone.

4. The influence of the physical form in which the acetate is brought into contact with the methylating agents is also shown by the fact that acetates converted into finely divided precipitates yield a considerably higher methoxyl content than the same acetates used in their fibrous form or as a dry powder.

5. The rates of methylation and deacetylation are determined and are found to be initially slow, in spite of the fact that the acetate is in solution, the reason being that the strong sodium hydroxide solution does not mix sufficiently with the acetone solution. When the methoxyl content has reached a certain value, the product of reaction starts to precipitate and establishes contact with the alkali. This contact causes the rates of methylation and deacetylation to increase rapidly. However, after 44 to 45% methoxyl have been introduced the rate of methylation slows down considerably without the theoretical value of 45.57% being reached.

6. Methylation of cellulose and cellulose acetate dissolved in a quaternary ammonium base follows the normal course of cellulose reactions, since contact between the reactants exists from the start. But even in these cases the product of reactions precipitate after a certain methoxyl content has been reached, and the reaction comes practically to a standstill when the reaction product possesses about 43% methoxyl. The highest methoxyl content is obtained only at low temperature.

7. In the light of the gradual transition of the initially lyophilic cellulose into a lyophobic derivative, which is accompanied by a change from a homogeneous into a heterogeneous system, an attempt is made to explain the failure to introduce the theoretical quantity of methoxyl by the activity of cohesive forces which might consist either of hydrogen bonds or of covalences operative between remaining free hydroxyl groups of adjacent chain molecules.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

The Structure of the Cori Ester

BY M. L. WOLFROM AND D. E. PLETCHER

The naturally occurring hexose phosphates are of great biological interest. One of the most significant of these substances is the hexose monophosphate isolated from frog muscle in the form of its barium (amorphous) and brucine (crystalline) salts by Cori and Cori.[1] This hexose phosphate has been designated the Cori ester. Cori and Cori[2] demonstrated that this ester was the main product when glycogen underwent phosphorolysis with an enzyme (phosphorylase) isolated from rabbit muscle. The reversibility of this enzymic system to produce a polysaccharide was demonstrated by Schäffner and Specht[3] and by others,[4] using glycogen and phosphorylases isolated from yeast, muscle and liver.

In the vegetable world, Hanes[5] has demonstrated the widespread occurrence in leaves, roots, fruits and tubers, of a phosphorylase capable of converting starch to the Cori ester and of resynthesizing the latter to a polysaccharide by reversal of the enzymic reaction.

With other enzymic systems, sometimes termed phosphoglucomutases, the Cori ester, whether

(1) C. F. Cori and Gerty T. Cori, *Proc. Soc. Exptl. Biol. Med.*, **34**, 702 (1936).

(2) C. F. Cori and Gerty T. Cori, *ibid.*, **36**, 119 (1937).

(3) A. Schäffner and H. Specht, *Naturwissenschaften*, **26**, 494 (1938).

(4) C. F. Cori, G. Schmidt and Gerty T. Cori, *Science*, **89**, 464 (1939); *J. Biol. Chem.*, **129**, 629 (1939); W. Kiessling, *Biochem. Z.*, **302**, 50 (1939); P. Ostern, D. Herbert and E. Holmes, *Biochem. J.*, **33**, 1858 (1939).

(5) C. S. Hanes, (a) *Proc. Roy. Soc.* (London), **B128**, 421 (1940); (b) **B129**, 174 (1940).

isolated from a plant or animal source, possesses the property of successive conversion to hexose-6-phosphate (Robison ester) and *d*-fructose-1,6-diphosphate (Harden–Young ester).[1,5]

Cori, Colowick and Cori,[6] working with the amorphous barium salt of the natural Cori ester, as purified through its brucine salt, demonstrated that the ester, on acid hydrolysis or on hydrolysis with a phosphatase obtained from intestinal mucosa, liberated a hydrolyzate of specific rotation +52.5° (the specific rotation of *d*-glucose), which was completely fermentable by yeast and gave what was described, without further characterization, as a typical glucosazone. The ester was also described as alkali-stable and very acid-labile. These same workers treated silver phosphate with α-acetobromo-*d*-glucose and obtained a product which on treatment with acid and then with base yielded a hexose monophosphate, isolated as its amorphous barium salt, which was described as identical in chemical and physiological properties with the natural Cori ester. On this basis, these workers concluded that the structure of the Cori ester was *d*-glucopyranose-1-phosphate.

One of the earliest[6a] attempts to prepare glucose-1-phosphate was that of Komatsu and Nodzu,[7] who treated glucose pentaacetate with phosphoryl chloride in the presence of barium hydroxide and at low temperature. Their product was an amorphous barium salt (spec. rot. +15°, D-line, water) of a hexose monophosphate which reduced Fehling solution only on prolonged boiling or after acid hydrolysis. This type of synthesis would not be expected to yield a pure α or β isomer. Zervas[8] treated α-acetobromo-*d*-glucose with silver dibenzyl phosphate and prepared what was stated to be the crystalline 2,3,4,6-O-tetraacetyl-*d*-glucose dibenzyl phosphate which was sensitive to both alkali and acid and possessed reducing properties. The free ester was then obtained by removing the benzyl groups by catalytic hydrogenation and this substance was also reported as being sensitive to alkali, but was apparently not obtained in the form of a crystalline derivative and no further characterization was given.

Although the work of Cori, Colowick and Cori[6] offers strong presumptive evidence that the Cori ester is *d*-glucopyranose-1-phosphate, it cannot be considered as a rigorous proof of structure and is rendered uncertain by the publication of Zervas.[8] The conclusions of Cori, Colowick and Cori[6] are based upon work which was not put upon a crystalline basis; the sugar component was not identified in the form of a crystalline derivative of definitive rotation; and the proof of structure based upon synthesis from α-acetobromo-*d*-glucose is rendered somewhat uncertain since the direct condensation product was subjected to further reaction procedures.

In the light of the above critique and of the great biological significance of the Cori ester, it was deemed pertinent to re-investigate its structure by other methods. In the work herein reported, we have repeated the synthetic preparation of the Cori ester and have based our investigations upon the well-crystallized dipotassium salt, first described, for the natural product, by Kiessling.[9] The sugar component of the ester was identified as *d*-glucose by the isolation from its hydrolyzate of crystalline *d*-glucose diethyl mercaptal pentaacetate of known melting point and rotation. The lack of Fehling reduction shown by the synthetic dipotassium salt was corroborative of the work of Cori and co-workers[6] rather than of Zervas.[8] Therefore carbon one is substituted. A molecular weight determination showed that the substance was a six carbon structure. The ring structure of the ester was determined by application of the excellent method of oxidation with sodium periodate, discovered by Malaprade[10] and established for the determination of the structure of sugar glycosides by Hudson and co-workers.[11] Under these conditions of oxidation, the dipotassium salt consumed two moles of periodic acid and produced one mole of formic acid, determined and identified. The absence of formaldehyde was demonstrated. This gives definite proof that the ring closure was on carbon five and that carbons two, three and four were free. It follows that carbon six must also be free since the fact that the substance was a dipotassium salt of a six carbon structure, allows no other alternative. Accordingly our work

(6) C. F. Cori, S. P. Colowick and Gerty T. Cori, *J. Biol. Chem.*, **121**, 465 (1937).

(6a) D. Amato, *Gazz. chim. ital.*, **1**, 56 (1871), records an amorphous disodium salt and a crystalline basic lead salt of a non-reducing glucose monophosphate obtained by the action of phosphoryl chloride upon helicin.

(7) S. Komatsu and R. Nodzu, *Mem. Coll. Sci. Kyoto Imp. Univ.*, **7A**, No. 6, 377 (1924); *C. A.*, **19**, 2811 (1925).

(8) L. Zervas, *Naturwissenschaften*, **27**, 317 (1939).

(9) W. Kiessling, *Biochem. Z.*, **298**, 421 (1938).

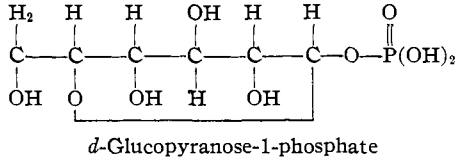
(10) L. Malaprade, *Bull. soc. chim.*, [4] **43**, 683 (1928); [5] **1**, 833 (1934).

(11) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 994 (1937); **62**, 958 (1940); R. M. Hann, W. D. Maclay and C. S. Hudson, *ibid.*, **61**, 2432 (1939).

verifies the structure of *d*-glucopyranose-1-phosphate assigned by Cori, Colowick and Cori.[6]

Since the α,β-isomer of the Cori ester is unknown, no definitive α,β-assignment may be made for the substance. Its rather high dextrorotation is indicative of an α-derivative on the basis of the Hudson nomenclature.[12] The reaction of an α-acetohalogen sugar with an alcohol in the presence of silver carbonate generally leads to a β-isomer[13] but in this case the reaction is between an α-acetohalogen sugar and the silver salt of an acid and is therefore not strictly analogous.

It is of interest to note the first and second dissociation constants for the free glucose-1-phosphoric acid as determined by Cori, Colowick and Cori,[6] $k_1 = 0.78 \times 10^{-1}$ and $k_2 = 0.74 \times 10^{-6}$, in comparison with those of orthophosphoric acid,[14] $k_1 = 1.0 \times 10^{-2}$ and $k_2 = 1.2 \times 10^{-7}$. Britton and Robinson[14] reported the third dissociation constant for orthophosphoric acid as $k_3 = 1.8 \times 10^{-12}$. From these results it is seen that the third hydroxyl in orthophosphoric acid possesses about the same order of acidity as a polyhydric alcohol.[15] Thus the Cori ester may be considered to resemble an acetal or glycosidic structure more closely than it does a true ester. An acetal structure is in accordance with its alkali-stability and it is to be noted that the phosphate groups of the Robison and Harden-Young esters are saponifiable by alkali.



d-Glucopyranose-1-phosphate

Experimental

Preparation of the Synthetic Dipotassium Salt Dihydrate of the Cori Ester.—α-Acetobromo-*d*-glucose (40 g.) in benzene solution was treated with silver phosphate as described by Cori, Colowick and Cori.[6] The silver salts were removed by filtration, the filtrate was treated with decolorizing charcoal (Darco), brought to the boiling point and again filtered through a prepared bed of decolorizing charcoal and Super-Cel (Johns-Manville), after which the filtrate was concentrated (30–40°) to a thick sirup under reduced pressure. When dried over phosphorus pentoxide in a vacuum desiccator, this material was a practically colorless glass that resisted crystallization; yield 35 g. Cori, Colowick and Cori[6] claim to have crystallized this intermediate but they cite no melting point or recrystallization.

The above intermediate product was partially hydrolyzed with hydrochloric acid in dilute methanol as described by Cori, Colowick and Cori[6] and the product isolated as the amorphous barium salt. The once reprecipitated and air-dried barium salt (5–8 g.) was dissolved in 100 cc. of warm water and treated with the equivalent amount of potassium sulfate (10%) solution. The precipitated barium sulfate was removed through a precoated filter and absolute ethanol was added to the filtrate to incipient turbidity. Crystallization ensued and was allowed to continue at room temperature with occasional additions of more absolute ethanol until a total of about 1.7 volumes of ethanol had been added. The mixture was then cooled, filtered, washed with dilute ethanol and recrystallized several times from water by the addition of an equal volume of ethanol; yield 2–6 g. (17–50% based upon acetobromo-*d*-glucose), spec. rot. +78° (20°, *c* 4, H_2O) at 5892.5 Å. and +90° at 5461 Å. The substance was very acid-labile but was stable to alkali. Thus, it showed no Fehling reduction on prolonged boiling but readily gave a reduction after treatment at room temperature for three minutes with 0.1 *N* hydrochloric acid.

When this material was fractionated from water by the addition of ethanol in amounts insufficient to cause immediate complete precipitation, the rotations of the various fractions so obtained were as stated above. This was taken as a sufficient criterion of purity. Kiessling[9] recorded a specific rotation of +96° (5461 Å., H_2O) for the crystalline dipotassium salt prepared by the phosphorolysis of glycogen with a phosphorylase isolated from rabbit muscle and Hanes[5b] recorded a specific rotation of +78.5° (5892.5 Å., H_2O) for the crystalline dipotassium salt prepared by the phosphorolysis of starch with a phosphorylase isolated from potatoes. The latter author stated that the above rotatory value of +78 to +79° was also found by him for a preparation of the crystalline dipotassium salt isolated from muscle according to the procedure of Kiessling.[9]

Anal. Calcd. for $C_6H_{11}O_9PK_2 \cdot 2H_2O$: C, 19.34; H, 4.03; P, 8.33; H_2O, 9.68; apparent mol. wt. (complete salt dissociation basis), 124. Found: C, 19.12; H, 3.92; P, 8.38; H_2O, 9.25; mol. wt. (cryoscopic in water), 143.

Identification of the Sugar Component of the Cori Ester.—The synthetic dipotassium salt dihydrate of the Cori ester (1.00 g.) was hydrolyzed for ninety minutes at 60° with 10 cc. of 5% hydrochloric acid. The sirup obtained on concentrating the hydrolyzate under reduced pressure was dissolved in 2 cc. of concentrated hydrochloric acid (d. 1.19) and shaken at 0° for several hours with 1 cc. of ethyl mercaptan. Crystallization ensued on the addition of ice and the filtered product was washed with cold water and recrystallized from water containing a little pyridine and then from absolute ethanol; yield 0.12 g., m. p. 125° (mixed m. p. with *d*-glucose diethyl mercaptal unchanged). The product (0.12 g.) was acetylated for one day at room temperature with acetic anhydride (6 cc.) and pyridine (3 cc.) and the mixture obtained on pouring the solution into water (30 cc.) was extracted with chloroform. The product obtained on solvent removal from the washed

(12) C. S. Hudson, THIS JOURNAL, **31**, 66 (1909).

(13) See, however, P. A. Levene and M. L. Wolfrom, *J. Biol. Chem.*, **78**, 525 (1928).

(14) H. T. S. Britton and R. A. Robinson, *Trans. Faraday Soc.*, **28**, 531 (1932).

(15) L. Michaelis and P. Rona, *Biochem. Z.*, **49**, 232 (1913).

(successively with water, sodium bicarbonate and water) and dried extract was dissolved in a small amount of methanol and was crystallized by the addition of water to incipient turbidity; yield 0.20 g., m. p. 47° (mixed m. p. unchanged), spec. rot. +11° (23°, *c* 6, $CHCl_3$, 5892.5 Å.). Wolfrom[16] recorded for *d*-glucose diethyl mercaptal pentaacetate: m. p. 45–47°, spec. rot. +11° ($CHCl_3$, 5892.5 Å.).

Oxidation of the Dipotassium Salt Dihydrate of the Cori Ester with Sodium Periodate.—The oxidation technique used was essentially that described by Hudson and co-workers.[11] The crystalline dipotassium salt dihydrate of the synthetic Cori ester (0.3723 g., 1 mole) and 10 cc. of 0.2832 molar sodium metaperiodate ($NaIO_4$, 2.8 moles) were mixed and brought to a volume of 100 cc. of solution. Polarimetric readings were taken on the solution; aliquot portions (5 cc.) were titrated with 0.1 *N* sodium hydroxide, using methyl red indicator; and aliquot portions (5 cc.) were analyzed for excess sodium metaperiodate according to the procedure[17] for the determination of periodate in the presence of iodate. For the acidity determination, it was necessary to correct for the constant alkalinity of the dipotassium salt. This was accomplished by titrating against 0.1 *N* sodium hydroxide (methyl red indicator) like volumes of a dilute formic acid solution with and without the addition of a weighed amount of the dipotassium salt. For the 5-cc. aliquot portion used above, the correction was found to be +0.40 cc. of 0.1 *N* sodium hydroxide. The data obtained are tabulated in Table I.

Formaldehyde was shown to be absent in the reaction mixture by non-formation of a precipitate with Dimedon (5,5-dimethylcyclohexanedione-1,3).[18] Addition of known formaldehyde to the reaction mixture gave a crystalline precipitate with Dimedon. Formic acid was shown to be present in the oxidation mixture by reduction to formaldehyde and identification of the latter by means of the milk test.[19] To 2–3 cc. of the dipotassium salt oxidation solution was added 0.5 g. of magnesium ribbon and 6 cc. of 25% hydrochloric acid. This treatment reduced any formic acid present to formaldehyde and also destroyed the iodate and excess periodate which interfered with the test. Free iodine was liberated and was driven out by boiling gently. Upon adding 2 cc. of fresh milk and 7 cc. of a dilute ferric chloride solution and boiling, an intense violet color was obtained. By performing the same test on blanks and on known samples, it was shown that this test was applicable under the conditions present in the oxidation mixture.

TABLE I

OXIDATION OF THE DIPOTASSIUM SALT DIHYDRATE OF THE CORI ESTER (0.0010 MOLAR) WITH SODIUM PERIODATE (0.0028 MOLAR) AT 25°

Time, min.	α (obsd.), 4-dm.	Required for 5.02 cc. aliquots: As_2O_3, 0.0942 *N*, cc.	Moles $NaIO_4$ consumed	NaOH, 0.0933 *N*, cc.[a]	Moles formic acid
5	+1.01°				
10	0.89				
25	.72				
90	.56				
100	.56	0.95	1.9	0.53	1.0
200	.56	1.64[b]	2.1	1.08[b]	1.0
275	.55	0.72	2.1	0.58	1.1
490	.53	.71	2.1	0.57	1.0
24 hrs.	.53	.68	2.2	0.59	1.1

[a] Includes correction of +0.40 cc. for the constant alkalinity of the dipotassium salt. [b] 10.04 cc. aliquots.

We are indebted to Mr. H. A. Davis for assistance rendered in preparing the material used in this investigation.

Summary

1. The synthetic Cori ester has been characterized as the crystalline dihydrated dipotassium salt.

2. Crystalline *d*-glucose diethyl mercaptal pentaacetate was obtained from the sugar component of the dipotassium salt of the Cori ester.

3. Oxidation of the crystalline dipotassium salt of the Cori ester with sodium periodate consumed two moles of periodic acid and produced one mole of formic acid and no formaldehyde.

4. The apparent molecular weight of the dipotassium salt was determined and found to be normal.

5. The above results, together with the non-reducing properties of the salt, corroborate the *d*-glucopyranose-1-phosphate structure for the Cori ester assigned to it by Cori and co-workers.

COLUMBUS, OHIO RECEIVED JANUARY 8, 1941

(16) M. L. Wolfrom, THIS JOURNAL, **51**, 2188 (1929).

(17) Treadwell and Hall, "Analytical Chemistry," Vol. II, John Wiley and Sons, New York, N. Y., 8th ed., 1935, p. 616.

(18) D. Vorländer, *Z. anal. Chem.*, **77**, 241 (1929).

(19) Klein, "Handbuch der Pflanzenanalyse," J. Springer, Vienna, 1932, Vol. II, p. 376.