material was dissolved in as small a volume of acetone as possible. Dry ethanol was added to effect a partial separation of the isomers. The precipitate which formed on standing was filtered and was found to be the almost pure *trans*-β-chlorothioether VII, yield 1.95 g., m.p. 186–190°. The solvent was evaporated from the filtrate leaving an oil.

The solid was dissolved in 200 ml. of glacial acetic acid, to which 20 ml. of 30% hydrogen peroxide was then added. The solution was heated for 0.5 hr. on a steam-bath and was then allowed to stand overnight. Addition of water formed a precipitate of 2.3 g. of *trans*-12-*p*-toluenesulfonyl-11-chloro-9,10-dihydro-9,10-ethanoanthracene (IX), m.p. 156–161°. Two recrystallizations from ethanol gave 1.73 g., m.p. and mixed m.p. with authentic material,[1] 160–162°.

The oil obtained above was treated similarly using 100 ml. of glacial acetic acid and 20 ml. of 30% hydrogen peroxide, yielding 1.06 g., m.p. 185–200°. Several recrystallizations from absolute ethanol gave 0.72 g. of white crystals of *cis*-12-*p*-toluenesulfonyl-11-chloro-9,10-dihydro-9,10-ethanoanthracene (X), m.p. 216–218°.

Anal. Calcd. for $C_{23}H_{19}ClO_2S$: C, 69.95; H, 4.85. Found: C, 70.13; H, 4.99.

The products from the treatment with alkali in ethanolic dioxane[14] of IX and X were isolated separately. Each of the samples of XI[1] melted at 176.5–177.5° and each did not depress the m.p. of the other sample.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, LOUISIANA STATE UNIVERSITY SCHOOL OF MEDICINE]

Two New Glucose Monoacetates, Apparently 6-O-Acetyl-α- and β-D-Glucose, and a Comparison of the Metabolism of Glucose, Acetylglucose and 6-O-Methylglucose[1]

BY RICHARD E. REEVES, ROLAND A. COULSON, THOMAS HERNANDEZ AND FLORINE A. BLOUIN

RECEIVED JUNE 21, 1957

Two new crystalline glucose monoacetates, apparently the α- and β-anomers of 6-O-acetyl-D-glucopyranose have been prepared. The mixed anomeric forms crystallize together under certain conditions and the properties of some of the mixtures are in agreement with those of a substance recently isolated from cultures of *Bacillus megaterium*. Injected acetylglucose is metabolized by the alligator almost as rapidly as is glucose while 6-O-methylglucose disappears from the blood at a much slower rate.

At the time this work was undertaken there appeared to be no record in the literature of a well-characterized mono- or diacetate of glucose. This is somewhat surprising in view of the great number of well characterized acetates of glucose derivatives. It was anticipated that the direct partial acetylation of glucose would yield a large number of isomers and that if a pure substance was isolated the definite establishment of its structure by chemical methods would be difficult because of the lability of the acetate group. Both difficulties were encountered. However, one pure monoacetate has been isolated repeatedly and a second substance, believed to be the anomer of the first, was obtained on two occasions.

Recently Duff, Webley and Farmer[2] have described as 6-O-acetylglucopyranose a crystalline glucose monoacetate, m.p. 133°, sp. rot. 48° (equil. H_2O). The physical properties of their substance agree closely with those observed by us for crystalline preparations thought to be mixtures of the α- and β-anomers of 6-O-acetylglucose. The chemical properties of their monoacetate are similar to those reported in the present investigation. Duff, *et al.*, obtained their substance as a metabolite from cultures of *Bacillus megaterium*. Evidence of the metabolic activity of our acetylglucose was obtained in experiments with the alligator.

Experimental

Preparation of Monoacetylglucoses.—Eighteen g. of powdered glucose was shaken with 40 ml. of pyridine and 5 ml. of acetic anhydride for one hour at room temperature. Another 5 ml. of acetic anhydride was then added and shaking was continued for 3 hours. Unreacted glucose (6.6 g.) was separated by filtration and the filtrate evaporated in *vacuo*, finally in a dish over sulfuric acid in a desiccator. The sirupy residue was dissolved in 15 ml. of methyl ethyl ketone and 10 ml. of alcohol and placed on a column 4 cm. in diameter containing 200 g. of Whatman cellulose powder. The column previously had been washed exhaustively with water, and then with 2 liters of methyl ethyl ketone–water azeotrope. At this stage a sample of the crude preparation spotted on paper and developed by the descending technique with *n*-butanol:alcohol:water (4:1:1) separated into three well resolved spots (aniline hydrogen phthalate spray reagent) of R_F 0.19, 0.45 and 0.72, respectively. The R_F of glucose in this system is 0.19.

The column was developed with methyl ethyl ketone–water azeotrope using an automatic fraction collector to collect 15–20 ml. samples. Drops of the collected fractions were spotted on paper, sprayed with aniline hydrogen phthalate and heated to show which tubes contained reducing sugars. On the basis of these tests, tubes 1–8 were discarded and tubes 9–25 were pooled and evaporated yielding 3.36 g. of a sirup of R_F 0.72 in the butanol system. This sirup has failed to crystallize on standing for two years.

High-rotating Form.—Tubes 26–34 contained no reducing sugar and were discarded. Tubes 35–65 were pooled and evaporated yielding 1.57 g. of a sirup with R_F 0.45 in the butanol system. This sirup partially crystallized upon standing. The crystals were dissolved in a little acetic acid and one volume of ether was added. Upon standing this solution yielded 0.46 g. of needle-like crystals which were recrystallized from the same solvent mixture. After drying in high vacuum at 100° over P_2O_5, the m.p. of this substance was 150–152°, sp. rot.[3] 90° (2 min.) changing to 51° (*c*, 1.6; H_2O). *Anal.* Calcd. $C_8H_{14}O_7$ (222.19): C, 43.24; H, 6.35. Found: C, 43.42; H, 6.51.

Low-rotating Form.—On two occasions the tubes containing the monoacetate fraction were not pooled, but were worked up separately or in small groups. The tubes containing the faster-moving part of the fraction yielded the high-rotating substance described above and those containing the slower moving part of the monoacetate fraction yielded a crystalline substance of low optical rotation. Recrystallization of this substance from methanol and acetone gave clusters of crystals, m.p. 148–149°, sp. rot. 22° (2 min.) changing to 51° (*c* 1; H_2O). *Anal.* Calcd. $C_8H_{14}O_7$ (222.19); C, 43.24; H, 6.35. Found: C, 43.21; H, 6.22. We have been able to convert partially the high-rotating form to the low-rotating form by recrystallization from water and acetone, and the low-rotating form has been com-

(1) This work was supported, in part, by a grant from the Corn Industries Research Foundation.

(2) R. B. Duff, D. B. Webley and V. C. Farmer, *Biochem. J.*, **65**, 21P (1957).

(3) All specific rotations were determined at 25° using the sodium-D line.

pletely converted to the high-rotating form by recrystallization from acetic acid.

Evaporation of aqueous solutions of the monoacetates frequently has yielded preparations containing crystals of both forms. These preparations have had melting points in the region of 130 to 140°, and have exhibited intermediate optical rotations.

Comparison of the Periodate Oxidation of the New, High-rotating Glucose Monacetate with that of 6-O-Methylglucose.—Forty to fifty mg. samples of high-rotating glucose monoacetate and of 6-O-methylglucose were dissolved in sufficient 0.35 *M* sodium metaperiodate solution to provide 6 moles of periodate per mole of glucose. The monoacetate had consumed 3.77 moles of periodate in 15 min. and 3.93 moles in 150 min. At corresponding times the 6-methylglucose had consumed 3.90 and 3.94 moles of periodate, respectively. The solution of the acetate had formed 3.11 equivalents of acid per mole of glucose in 60 minutes while that of the methylglucose had formed exactly 3 equivalents.

Primary carbinol estimation[4] gave no formaldehydedimedon complex indicating the absence of a free primary carbinol grouping. A crystalline compound of m.p. 110° was obtained indicating that some fragment of the oxidized substance was capable of reacting with dimedon.

Comparison of the Metabolism of Glucose, Monoacetylglucose and 6-O-Methylglucose in the Alligator.—Due to the small amount of the glucose derivatives available for this work the experiments were restricted to the use of a single small (400 g.) alligator for each derivative. One alligator was injected intraperitoneally with 1 g. (5.55 mmoles) of glucose per kg. body weight; another with 5.55 mmoles of monoacetylglucose; and another with 5.55 mmoles of 6-O-methylglucose. The animals were kept at 28° and blood samples and urines were collected and analyzed for total reducing substances by the method of Folin–Wu.[5] Although this method tends to give abnormally high values when applied to mammalian blood, the alligator is singularly free from non-saccharide reducing substances and hence the results are quite reliable.

Results and Discussion

Structure of the Acetates.—The conclusion that the two new acetylglucoses are an anomeric pair rests upon the interconvertibility of the forms by recrystallization, the identity of the equilibrium rotations, the similarity of the mutarotation constants, and the agreement in 2A values calculated for glucose and acetylglucose. From the mutarotation curves for the acetylglucoses the calculated mutarotation constants ($k_1 + k_2$) are 0.0074 and 0.0068 (25°) for the high- and low-rotating forms, respectively. By extrapolation to zero time the initial rotations are calculated to be 91 and 21°, respectively. The difference between the molecular rotations of these substances gives a 2A value of 15,500 which is in close agreement with the value of 16,000 calculated by Hudson[6] for the α- and β-glucopyranose pair.

Assignment of the position of the acetyl group on the glucose molecule is based upon the behavior of the high-rotating form upon oxidation with periodate. The consumption of four moles of periodate with liberation of 3 equivalents of acid, and no formaldehyde suggests that the substituent is located on position 6. On substantially similar evidence Duff, *et al.*,[2] concluded that their monoacetylglucose was substituted at the sixth position. In the present work the parallel behavior of the monoacetate and 6-O-methylglucose is considered to offer evidence in support of this conclusion. The evidence presented below that acetylglucose is metabolized more rapidly than methylglucose is not regarded as contradictory to the assignment of the acetyl group to position six because the metabolism of the monoacetate probably is preceded by its deacetylation.

Metabolism Studies.—In comparing the utilization of glucose, acetylglucose and 6-O-methylglucose the alligator was selected as the experimental animal of choice. The speed with which mammals utilize glucose, or excrete most "unnatural" substances imposes practical difficulties upon studies of this nature. On the other hand in a cold-blooded species the lower metabolic rate and lower renal filtration rate allows sufficient time for observation of the absorption and disappearance of the injected substance. Information on such facts as the rates of renal filtration and glucose metabolism of the alligator is available in the literature.[7,8]

The changes in reducing sugar levels in the blood following the injections of glucose and of the two glucose derivatives are shown in Fig. 1. The lowest curve in this figure illustrates the response of an animal to the handling incidental to the injection. All these curves have been adjusted by subtraction of the small amount of glucose (40 to 60 mg. %) initially present in the blood. In each instance the highest level of reducing sugar in the blood was reached before 12 hours. In the animals receiving glucose and acetylglucose, and in the uninjected control animal, the reducing sugar level dropped rapidly reaching an essentially normal value within two days. On the other hand, in the animal receiving methylglucose the blood level of reducing sugar remained elevated for the duration of the experiment.

The concentrations of reducing sugar in the urines following injection with glucose or the glucose derivatives are shown in Fig. 2. Reducing sugar disappeared from the urine very rapidly after injection of glucose or acetylglucose, but it persisted for approximately five days in the animal receiving methylglucose. The bar graph in the upper right corner of the figure records the per cent. of each injected substance recovered in the urine, based upon the presumption that the injected and excreted substances were identical. In the animal receiving methylglucose this substance and a trace of glucose were identified by paper chromatography in the urine 4 and 11 hours after injection. Methylglucose, but not glucose, was found in the 34 hour urine.

The similarity of the reducing sugar curves both in blood and urine following injection of glucose or acetylglucose indicates that the latter substance is metabolized almost as rapidly as is glucose. These experiments do not prove that the acetyl group is removed prior to the metabolism of the acetylated glucose; however, there are reasons for the presumption that this occurs. First, if glucose metabolism proceeds principally through the formation of the 6-phosphate the prior removal of the acetate would be required, provided that the structure on

(4) R. E. Reeves, THIS JOURNAL, **63**, 1476 (1941).

(5) O. Folin and H. Wu, *J. Biol. Chem.*, **41**, 367 (1920).

(6) C. S. Hudson, *Sci. Papers Bur. Standards*, **21**, 247 (1926), No. 533.

(7) R. A. Coulson and T. Hernandez, *Endocrinology*, **53**, 311 (1953).

(8) T. Hernandez and R. A. Coulson, *Federation Proc.*, **15**, 91 (1956).

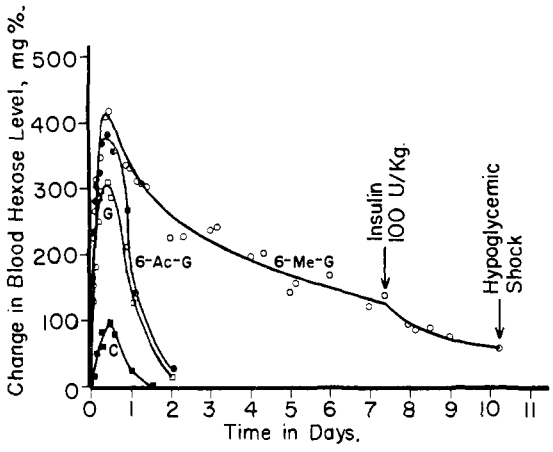

Fig. 1.—Change in blood reducing sugar levels in the alligator following (O, 6-Me-G) injection of 6-O-methylglucose; (●, 6-Ac-G) injection of monoacetylglucose; (□, G) injection of glucose; (■, C) handling comparable to that given the injected animals.

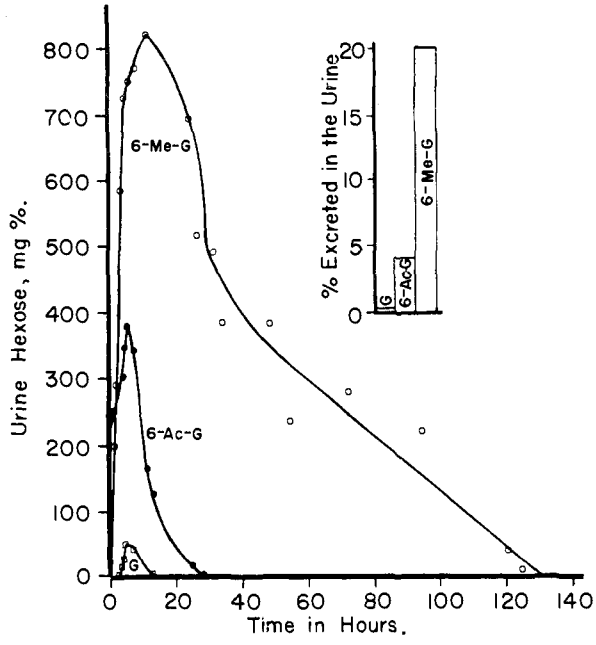

Fig. 2.—Urine reducing sugar levels in the alligator following injection of (O, 6-Me-G) 6-O-methylglucose; (●, 6-Ac-G) monoacetylglucose, (□, G) glucose.

the acetate is correctly represented by 6-O-acetylglucose. Secondly, methylglucose which bears a substituent of much greater chemical stability was metabolized at a much slower rate.

The observations on the animal receiving methylglucose present some interesting aspects in addition to the slow rate of disappearance of this substance from the blood and urine. It is noted that in this animal the blood sugar remained elevated long after reducing sugar ceased appearing in the urine. That this blood sugar was not entirely glucose was shown by that portion of the curve (Fig. 1) following the injection of a large amount of insulin, administered seven days after the injection of methylglucose. Insulin reduced the blood sugar level by an amount roughly equivalent to the normal glucose level in this animal, and hypoglycemic shock occurred three days later at a time when the total reducing sugar level in the blood was approximately 50 mg. % (calculated as glucose). Since hypoglycemic shock does not occur in the alligator until the blood glucose level is below 10 mg. %, it is concluded that the reducing sugar present in the blood in the later stages of the experiment was not principally glucose.

The non-glucose reducing sugar present in the blood from the 5th to the 8th day in an amount exceeding an estimated 100 mg. % was not being excreted in the urine. Further work will be required to show whether or not the circulating reducing sugar was largely 6-methylglucose.

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[CONTRIBUTION FROM THE FRUIT AND VEGETABLE CHEMISTRY LABORATORY, WESTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE]

Plant Polyphenols. II. The Benzylation of Ellagic Acid[1]

BY LEONARD JURD

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In strongly alkaline aqueous solution ellagic acid reacts with benzyl chloride to form the red quinoidal pigment, ellagorubin. Traces of pyridine or triethylamine inhibit the formation of ellagorubin and give both a colorless and a golden-yellow compound. The colorless compound has been identified as the hitherto unknown 5,5′-di-C-benzyl-tetra-O-benzyl-ellagic acid. The yellow pigment has a structure intermediate between that of ellagorubin and the colorless compound. One of its two rings is quinoidal as in ellagorubin while the other is aromatic as in the colorless compound.

The ellagitannins of the walnut pellicle produce a red pigment when treated briefly with alcoholic mineral acids.[2] The color reactions of this pigment in Robinson's tests[3] indicate that it is almost certainly not an anthocyanidin although a leucoanthocyanin giving rise to cyanidin with acids has been reported in the walnut seed coat.[4] Since ellagic acid and gallic acid are the only phenols which have been isolated by the complete acid or alkaline hydrolysis of the walnut tannins, it seems most likely that the pigment is derived from one or both of these compounds.

The constitution of ellagic acid (I) has been

(1) Financial support for this work was provided by the Diamond Walnut Growers, Inc.

(2) L. Jurd, THIS JOURNAL, **78**, 3445 (1956).

(3) G. M. Robinson and R. Robinson, *Biochem. J.*, **25**, 1687 (1931); **26**, 1647 (1932).

(4) E. C. Bate-Smith, *ibid.*, **58**, 122 (1954).