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A METHOD FOR THE IDENTIFICATION AND ESTIMATION OF THE 6-HYDROXYL GROUP IN GLUCOSE

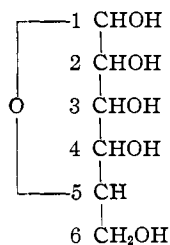
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It has long been known that one of the terminal hydroxyl groups of the glucose chain is sharply distinguished from the others, in that it is responsible for reducing action and for the formation of glucosides, oximes, etc. With regard to other groups the only one that may be said to be in any sense easily identified is that indexed as No. 4 in the accompanying formula, since substitution in this position prevents the formation of γ -glucosides and so, in the glucose series at least, inhibits condensation with acid methyl alcohol in the cold. The basis for this statement is the fact that of the four trimethylglucoses of the normal type only 2,3,6-trimethylglucose can exhibit this reaction. These statements definitely do not apply to the pentose series or possibly to fructose or to mannose.

Of the remaining hydroxyl groups in glucose No. 6 displays a number of special reactions, of which, however, none so far described serves as a reliable method for its identification and estimation. The first of these special reactions was described by Fischer,¹ who prepared a 1,6-dibromotriacetylglucose by acting on penta-acetylglucose with liquid hydrogen bromide. Soon afterward Purdie and Irvine² prepared a 2,3,4-trimethylmethylglucoside by methylating methylglucoside in presence of excess of methyl alcohol. Subsequently it was shown by one of us³ that when position 6 is substituted by the nitrate group or by bromine these substituents can be replaced by iodine by means of sodium iodide in acetone at 100°. A more definitely useful observation was subsequently made by Helferich and collaborators,⁴ viz.: that the triphenylmethyl group enters position 6 preferentially when triphenylmethyl chloride acts on glucose and on α - and β -methylglucoside. Work carried out more recently in this Laboratory appears to show, however, that the aromatic residue is capable of entering positions other than 6, and that in one or more of these (positions unknown) the subsequent removal of the triphenylmethyl residue is a matter of great difficulty. Finally Freudenberg⁵ showed that the toluenesulfonyl group is replaceable by iodine in the 6-position of galactose, but he does not seem to have extended the

¹ Fischer, *Ber.*, **35**, 836 (1902).² Purdie and Irvine, *J. Chem. Soc.*, **83**, 1028, 1037 (1903).³ Oldham, *ibid.*, **127**, 2840 (1925).⁴ Helferich, *Ann.*, **440**, 1 (1924); *Ber.*, **58**, 872 (1925).⁵ Freudenberg, *ibid.*, **60**, 1633 (1927).

observation beyond this point. It is obvious that none of these methods except the last could be used as a means of identifying quantitatively position 6 when unsubstituted.

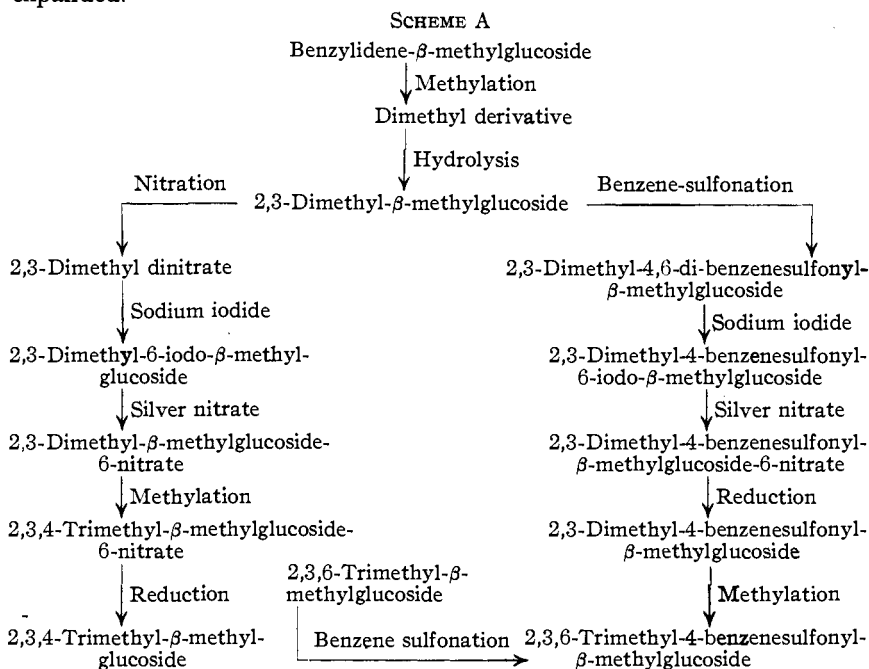
During the course of a research on starch carried out in this Laboratory by Irvine and Rutherford it became necessary to identify the components of a mixture of dimethylglucoses, and it was at first thought that the capacity of the nitrate group to be replaced by iodine was peculiar to position 6. In order to explore further the general reactions involved, experiments were begun on the crystalline dinitrate of 2,3-dimethyl- β -methylglucoside prepared as described in the experimental part. The reaction, however, followed an unexpected course, yielding besides the desired 6-iodo-2,3-dimethyl- β -methylglucoside-4-nitrate, an equal or in most cases a considerably larger quantity of 6-iodo-2,3-dimethyl- β -methylglucoside. The result is completely anomalous and it is noteworthy that no di-iodo compound was produced. In view of the behavior of the nitrate just described the corresponding benzenesulfonyl derivative was examined. The di-benzenesulfonate of 2,3-dimethyl- β -methylglucoside was found to be a crystalline substance in which one benzenesulfonyl group was replaced quantitatively by iodine in the course of heating for two hours with sodium iodide in acetone solution at 100°. It is important to limit the time of heating to two hours since prolonged treatment seems to lead also to the replacement of the 4-group and to other complications still under investigation. The first part of the research having shown that in the case of 2,3-dimethyl- β -methylglucoside di-benzenesulfonate, only the acyl group in position 6 is replaced by iodine under the conditions described, it was further extended to include the investigation of similar products obtained from the corresponding 2,3-diacetyl- β -methylglucoside, and also in a general manner to investigate as far as possible whether any other group than No. 6 shared this property and whether these observations applied only to the normal series or were equally valid for the γ -series and for anhydro compounds. In this extension of the work the *p*-toluenesulfonyl derivatives were employed as a matter of experimental convenience in place of the benzenesulfonates.

The real value of this method lies in the further fact that the iodine can be replaced by the nitrate group on boiling in acetonitrile solution with silver nitrate. The process is sufficiently accurate to be used for the estimation of iodine and has a great advantage over other methods in that it involves no loss of material. The nitrate group may in turn be converted into the hydroxyl group by the method already described³ and thus ultimately any desired group may be introduced into position 6.

The work described in the present paper thus falls under three heads: (A) the products obtained from 2,3-dimethyl- β -methylglucoside; (B) the products obtained from the corresponding 2,3-diacetyl- β -methyl-

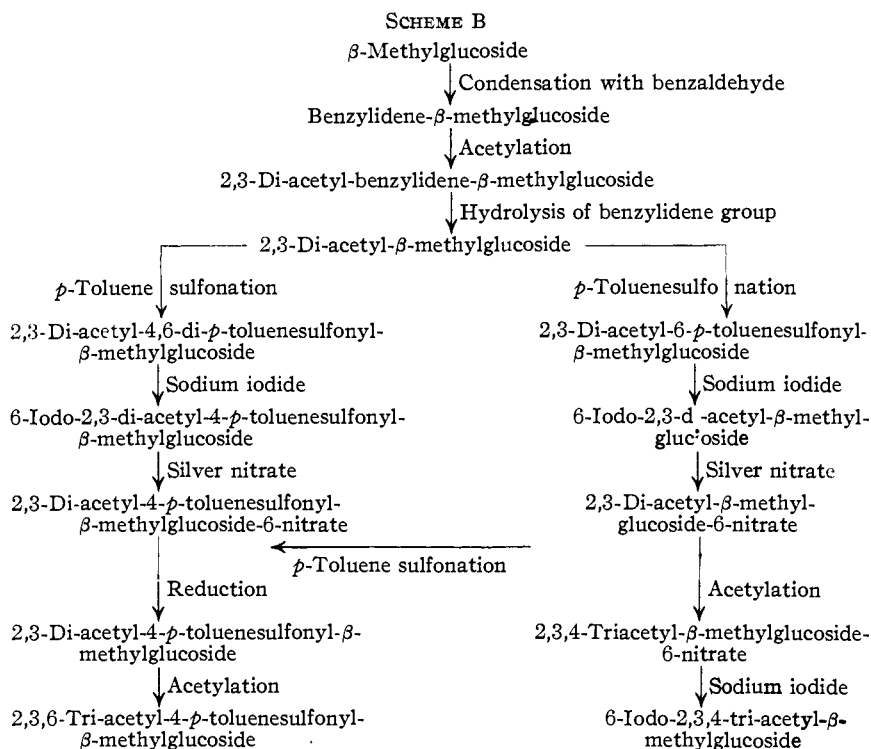
glucoside; (C) the general investigation which included the examination of a series of compounds prepared in order to study the special reactions of group No. 6.

The sections indexed A and B can best be represented schematically, notes being added where necessary, and inspection of the schemes will show that the synthetical possibilities of the sugar group have been greatly expanded.



It will be seen from the table that 2,3-dimethylglucose has been converted into 2,3,4- and 2,3,6-trimethylglucoses, respectively, and since 2,3-dimethylglucose has already been synthesized by Freudenberg⁵ from 3-monomethylglucose, and by the present authors from 2-monomethylglucose, the constitution of all this series of compounds would seem to be well established.

The 2,3-di-acetyl- β -methylglucoside-4,6-dinitrate was obtained in the same way as the corresponding dimethyl compound and is equally crystalline, but on treatment with sodium iodide the reaction is not so simple as in the methylated series owing to the migration of acetyl group or to the iodine being distributed between positions 4 and 6. This is unfortunate since otherwise the dinitrate would be the best source of 2,3-di-acetyl- β -methylglucoside-6-mononitrate, which is by far the most useful and interesting of all the compounds here described. The method by which this substance is actually obtained is described in detail in the experimental part



and it is our intention to use this compound as the starting material for a number of syntheses including that of 4-monomethylglucose and β-methyl-cellobioside [since synthesised by Helferich, *Ber.*, **64**, 2411 (1931)].

Unfortunately, owing to the fact that these compounds cannot be de-acetylated without at the same time removing the *p*-toluenesulfonyl group also, no direct link (except their common starting point) has been established between them and the methylated compounds just described. The consideration, however, of the following facts would seem to prove fairly conclusively that their constitution is similar.

(a) The di-acetyl-β-methylglucoside mononitrate just described undoubtedly has its nitrate group in the 6-position since it can be acetylated quantitatively to the already known tri-acetyl-β-methylglucoside-6-mononitrate.³

(b) It is also converted nearly quantitatively into the *p*-toluenesulfonyl derivative which is identical with that obtained from 2,3-diacetyl-di-*p*-toluenesulfonyl-β-methylglucoside by the series of reactions outlined above.

(c) It is true that these facts leave the position of the *p*-toluenesulfonyl group open to some slight doubt, but on removal of the nitrate group, followed by acetylation, a tri-acetyl-mono-*p*-toluenesulfonyl-β-methyl-

glucoside is obtained having the same melting point as that described by Helferich,⁶ to which he has assigned position 4 for the *p*-toluenesulfonyl group. The allocation of this group can lie only between positions 4 and 2 since tri-acetyl-3-*p*-toluenesulfonyl and tri-acetyl-6-*p*-toluenesulfonyl- β -methylglucosides are both known and have different melting points. Tri-acetyl-2-*p*-toluenesulfonyl- β -methylglucoside is not known but it is exceedingly unlikely that the present compound should have that constitution.

The third and more general section of the research involves too many reactions to be represented schematically. In the first place the tetra-*p*-toluenesulfonyl derivative of β -methylglucoside was prepared as a crystalline compound in good yield and was converted almost quantitatively to a mono-iodo derivative, thus showing that only one group reacts under these conditions. Both these compounds owing to their extreme insolubility did not react fully with hydriodic acid, so that their methoxyl content could not be determined in the usual way. In consequence the iodo compound was converted in almost quantitative yield into the nitrate upon which an accurate methoxyl determination was carried out. In view of this and the fact that the iodine content of the iodo compound was almost the theoretical there can be little doubt concerning the composition of these substances.

In order to obtain further evidence that only position No. 6 is involved in this reaction 4,6-dimethyl-2,3-di-*p*-toluenesulfonyl α -methylglucoside (which has been recently prepared in this Laboratory by Mr. D. S. Mathers) was subjected to the sodium iodide reaction but was recovered quantitatively unchanged. In the same manner it was found that no iodine could be introduced under these conditions into the following compounds: tri-benzenesulfonyl-glucosan, 3-*p*-toluenesulfonyl-diacetoneglucose and the 2,3,6-trimethyl-5-*p*-toluenesulfonyl- β -methylglucoside obtained from 2,3,6-trimethylglucose as described below. All these facts taken together indicate that this reaction is strictly confined to the 6-position of glucose. The drawback to this method from the synthetical point of view is that no reliable process has yet been found for the subsequent removal of the *p*-toluenesulfonyl groups, but this is still under investigation and it is hoped that a means may yet be discovered.

Experimental

Section A.—It may be explained that β -glucosides were employed throughout since the initial difficulty of obtaining them is more than compensated for by their great crystallizing power.

2,3-Dimethyl- β -methylglucoside.—This compound was obtained from the corresponding benzylidene derivative by the method described by Freudenberg.⁷ The

⁶ Helferich, *Ann.*, **458**, 111 (1927).

⁷ Freudenberg, *Ber.*, **61**, 1750 (1928).

benzylidene residue was removed by the action of *N*/20 hydrochloric acid in acetone containing, in all, 10% of water by volume. The reaction was followed polarimetrically, the changes observed being as follows.

Time	α	$[\alpha]_D$
Initial	-3.28	-65.4
30 Minutes	-2.42	-50.7
70 Minutes	-1.51	-42.4
100 Minutes	-1.42	-39.5
150 Minutes	-1.43	-39.8

The last four values are calculated for change in concentration due to loss of benzylidene. When hydrolysis was complete the solution was neutralized with barium carbonate, filtered, and taken to dryness in the presence of a small quantity of barium carbonate to neutralize any benzoic acid formed. The residue was dried thoroughly at 100° in a vacuum, taken up in acetone, filtered, taken to dryness and distilled at 150° (0.3 mm.). The distillate was a colorless sirup having n_D 1.4690 and OCH₃, 40.6%; calcd. for C₉H₁₈O₆OCH₃, 41.8%. The yield was about 70–80%, there being always a fair residue on distillation.

Solvent	c	$[\alpha]_D$
Chloroform	4.100	-46.9
Acetone	3.296	-38.9
Water	3.198	-34.3
Methyl alcohol	3.405	-38.4

2,3-Dimethyl- β -methylglucoside-4,6-dinitrate.—In making this substance the best yield seems to be obtained on a small scale. Two typical preparations are given. In the first, 4.3 g. of substance was dissolved in 25 cc. of chloroform and the solution cooled in ice. To this was added a mixture of 45 cc. of chloroform and 30 cc. of fuming nitric acid also cooled in ice. The mixture was allowed to stand for two minutes in ice, poured into ice and water in a separating funnel, well shaken and the lower layer removed, washed with ammonium carbonate solution, dried and taken to dryness at 45°. The yield at this stage was quantitative. The product was then boiled with methyl alcohol and barium carbonate and subsequently methylated by silver oxide in the usual manner. This on isolation and treatment with ethyl alcohol gave 4.2 g. of crystals (m. p. 96–99°). The mother liquors were treated with aqueous alcoholic soda solution in ice for half an hour, the solution being thereafter flooded with water and extracted with chloroform. In this way 1.3 g. of sirup was recovered which on treatment successively with absolute alcohol and a mixture of this solvent with petroleum ether gave a further 0.5 g. of crystals, a total yield of 78% of the theoretical amount. The residue which was still largely crystalline had OCH₃, 32.2%; calcd. for dimethyl- β -methylglucoside-dinitrate, C₉H₁₆O₁₀N₂: OCH₃, 29.8%, and for dimethyl- β -methylglucoside-mononitrate, C₉H₁₇O₈N: 34.8%. In another experiment 1.5 g. of dimethyl- β -methylglucoside was treated for one minute in ice with 30% nitric acid in chloroform. The product was worked up as before, being taken to dryness, however, at 60°. On treatment with methyl alcohol, 1.3 g. of crystals was obtained in the first crop. The mother liquors were boiled with barium carbonate, giving on isolation 0.5 g. of sirup which on treatment with absolute alcohol gave about 0.3 g. of crystals and a residue of 0.1 g. which was still largely crystalline. The yield on this occasion is thus about 78%. The product can also be made by the direct action of 30% nitric acid in chloroform on the benzylidene compound, but

the yields are not so good. After one more recrystallization from absolute alcohol the product was obtained pure melting at 98–99° and crystallizing in large prisms insoluble in water and petroleum ether, sparingly soluble in ether and cold alcohols, soluble in other solvents.

Anal. Calcd. for $C_9H_{16}O_{10}N_2$: OCH_3 , 29.8; N, 8.97. Found: OCH_3 , 29.0; N, 8.97. The specific rotation was taken in chloroform when it showed $[\alpha]_D -20.8^\circ$ for $c = 3.112\%$; n_D 1.4668, approximately, for the superfused substance.

6-Iodo-2,3-dimethyl- β -methylglucoside.—The dinitrate obtained as above was treated with twice its weight of sodium iodide in acetone at 100° for fourteen to twenty hours. The solution colors deeply with iodine and the tube has to be opened with care as pressure develops. After taking the total solution to dryness, and treating the residue with a mixture of water and chloroform, sodium thiosulfate was added to remove iodine. Thereafter the liquid was extracted in all three times with chloroform and the extract taken to dryness. When thus obtained the product should have a refractive index greater than 1.50, otherwise it should be again put through the above reaction. The residue now consisted of a mixture of 6-iodo-2,3-dimethyl- β -methylglucoside-4-nitrate with a larger quantity of the corresponding 4-hydroxy compound. These components can be separated by extraction from benzene with water, the hydroxy compound passing into the latter, but since the former is of no interest and is usually contaminated with small quantities of the original dinitrate, it is better to convert the whole of the product into the hydroxy compound. For this purpose the total chloroform extract was taken to dryness and the residue treated with a mixture of iron dust and zinc dust in hot glacial acetic acid, this mixture of metals being more efficient than either of the two components. When no more nitrate could be detected by the diphenylamine reaction the mixture was filtered and the residue well washed with chloroform. The filtrate was poured into water and the water extracted three times with chloroform, the extract dried over potassium carbonate to remove acetic acid, and taken to dryness at 100°. The product crystallized but was too soluble in all solvents to be recrystallized. Three grams of starting material gave 2.75 g. of product, a yield of 89% of the theoretical amount. The substance crystallizes in prisms and melts at 52–55°, after previous softening at 50°. Calcd. for $C_9H_{17}O_5I$: OCH_3 , 28.0; I, 38.2. Found: OCH_3 , 27.4; I, 38.9. The specific rotation in chloroform was $[\alpha]_D -7.1^\circ$ for $c = 3.014\%$; n_D , 1.5130, on the superfused substance.

2,3-Dimethyl- β -methylglucoside-6-nitrate.—This was prepared from the corresponding iodo compound by boiling the latter with an equal weight of silver nitrate in acetonitrile for two hours. Thereafter the solvent was removed in a vacuum at 100°, the well-dried residue extracted with boiling benzene, the solvent decanted, and the solid residue washed several times with benzene. In order to estimate the iodine the silver residues were dried at 100° and thereafter boiled with strong nitric acid, the silver iodide being collected and weighed. On evaporation of the benzene extract the compound was obtained as a distillable sirup soluble in all solvents except petroleum ether. 0.9827 g. of the iodo compound gave 0.760 g. of product which on distillation at 140° (0.3 mm.) gave 0.685 g. of distillate, the yields being thus 95 and 87%, respectively.

Anal. Calcd. for $C_9H_{17}O_8N$: OCH_3 , 34.8; N, 5.24. Found: OCH_3 , 33.2; N, 5.18; n_D 1.4655.

The rotation in chloroform was $[\alpha]_D -27.7^\circ$ for $c = 3.422\%$.

Conversion of 2,3-Dimethyl- β -methylglucoside-6-nitrate into 2,3,4-Trimethyl- β -methylglucoside.—1.2 Grams of the nitrate derivative having n_D 1.4690 (obtained from 1.50 g. of the iodo compound which had not been purified by the treatment with iron dust described above) was methylated twice with silver oxide in the usual manner, giving 1.029 g. of product with n_D 1.4568. This crystallized on nucleation with 2,3,4-

trimethyl- β -methylglucoside-6-mononitrate, but for purposes of identification it was converted into the parent glucoside. On reduction with iron dust in the usual manner, 0.702 g. was obtained by extraction of the aqueous solution with chloroform and a further 0.069 g. by taking the aqueous portion to dryness. Both of these products crystallized hard and on recrystallization from petroleum ether showed m. p. 93–94° which was unaffected by admixture with an authentic sample of 2,3,4-trimethyl- β -methylglucoside. The yield is thus 72% of the theoretical amount, calculating from the starting material.

2,3-Dimethyl-4,6-di-benzenesulfonyl- β -methylglucoside.—2.4 Grams of the dimethylmethylglucoside was treated with benzenesulfonyl chloride in pyridine for several days at the ordinary temperature. Thereafter the solid mass was treated with a mixture of benzene and water. When all was dissolved the water layer was removed and the benzene washed twice with strong hydrochloric acid and once with soda solution. It was then shaken with sodium sulfate and charcoal and taken to dryness at 100°. In this way 4.5 g. of product was obtained from the benzene and 0.226 g. of unchanged material from the first water washing. Only half of the product crystallized and this appears to be due to the fact that the benzenesulfonyl group does not easily enter position 4 when the 6-group is substituted by a large residue. As is shown below, the 6-nitrate-4-hydroxy compound reacts almost quantitatively with benzenesulfonyl chloride and is in fact the most convenient source of all these derivatives. The product consisted of slender prisms melting after two or three recrystallizations from absolute alcohol at 119–120°, insoluble in water and petroleum ether, sparingly soluble in ether and cold alcohols, soluble in other solvents.

Anal. Calcd. for $C_{21}H_{26}O_{10}S_2$: OCH_3 , 18.5. Found: OCH_3 , 18.4. The rotation in chloroform was $[\alpha]_D -21.0^\circ$ for $c = 5.171\%$.

6-Iodo-2,3-dimethyl-4-benzenesulfonyl- β -methylglucoside.—The best way of preparing this substance is from the compound just described by heating in acetone for two hours at 100° with an equal weight of sodium iodide. In this way 1.5 g. of starting material gave 1.4 g. of total product. This crystallized with difficulty from methyl alcohol containing 20% of water, the crystals being washed with methyl alcohol containing 40% of water. This is not a good crystallizing medium and the yield is poor but no superior method could be found. The product, when as pure as it could be obtained, consisted of prisms melting at 72–73°, soluble in all solvents except water and petroleum ether. Calcd. for $C_{15}H_{21}O_7SI$: OCH_3 , 19.7; I, 26.9. Found: OCH_3 , 19.6; I, 26.9. The rotation in chloroform was $[\alpha]_D -7.7^\circ$ for $c = 2.914\%$.

This product can also be obtained from the 6-iodo-4-hydroxy compound already described; 1.233 g. on treatment with excess of benzenesulfonyl chloride gave 1.6 g. of crude product (yield 90%). This gave 0.988 g. of crystals identical with those already described. The conversion of this substance to the corresponding nitrate is the most difficult example of this reaction yet encountered. On boiling for the usual period of two hours on one occasion the iodine content was found to be 23.2% and on another occasion only 24.2% after two consecutive treatments. As already stated the theoretical amount is 26.9%. It may be mentioned that the product from these estimations always contained iodine so that the low values quoted above cannot be due to an actual deficiency of iodine in the compound. On another occasion therefore the product was heated with silver nitrate overnight in acetonitrile at 100° when the theoretical value was obtained for the iodine content and the product contained no combined iodine. Considerable decomposition was caused, however, the yield of crude product being of the order 80%. Iodine is conveniently tested for in these compounds by heating with a little concentrated sulfuric acid, when the evolution of iodine can be easily observed.

2,3-Dimethyl-4-benzenesulfonyl- β -methylglucoside-6-nitrate.—This compound can

be prepared as already described but the most convenient method is by the action of benzenesulfonyl chloride in pyridine on the 6-nitrate-4-hydroxy compound already mentioned; 0.6 g. of this latter on the above treatment gave 0.053 g. of unchanged material and 0.8 g. of product from benzene. This latter gave 0.588 g. of crystals in the first crop and 0.229 g. in the second. The small residue largely crystallized on standing. The yield of crystalline material was thus of the order 90%. The product after one or two recrystallizations from absolute alcohol softened at 95° and melted at 96–97°. It consisted of prisms insoluble in water and petroleum ether, very slightly soluble in cold ethyl alcohol, rather more soluble in cold methyl alcohol and soluble in other solvents. Calcd. for $C_{18}H_{21}O_{10}SN$: OCH_3 , 22.8; N, 3.45. Found: OCH_3 , 22.6; N, 3.51. The rotation in chloroform was $[\alpha]_D -42.3^\circ$ for $c = 5.413\%$.

2,3-Dimethyl-4-benzenesulfonyl- β -methylglucoside.—The nitrate group was reduced out as already described, 2.048 g. of starting material giving 1.793 g. of crude product or 98% of the theoretical amount. The product was crystallized from butyl alcohol and washed with low-boiling petroleum ether when it consisted of short prisms soluble in all ordinary solvents except water and petroleum ether. It melted at 86–87°. Calcd. for $C_{18}H_{22}O_8S$: OCH_3 , 25.6. Found: OCH_3 , 24.2. The rotation in chloroform was $[\alpha]_D -42.6^\circ$ for $c = 2.498\%$.

2,3,6-Trimethyl-4-benzenesulfonyl- β -methylglucoside.—0.6 Gram of the last-mentioned compound was methylated twice with silver oxide and methyl iodide giving 0.635 g. of product. This on treatment with absolute alcohol gave 0.286 g. of material of m. p. 78–82°. The residue crystallized immediately on removal of the solvent, giving a further small crop of crystals. The yield is obviously poor but owing to its comparatively great solubility in alcohol, small amounts of impurities have a great effect in hindering the crystallization of this substance. After one further purification from absolute alcohol the product softened at 82° and melted at 83–84°. It consisted of small cubes soluble in all solvents except water and petroleum ether. Calcd. for $C_{18}H_{24}O_8S$: OCH_3 , 32.9. Found: OCH_3 , 31.8. The specific rotation in chloroform was $[\alpha]_D -35.6^\circ$ for $c = 4.104\%$. Owing to the difficulty of the removal of the benzenesulfonyl residue the substance just described could not be converted into 2,3,6-trimethyl- β -methylglucoside and it became necessary to synthesize the former from the latter. For this purpose 1.0 g. of 2,3,6-trimethyl- β -methylglucoside was treated with benzenesulfonyl chloride in the usual way, when 0.7 g. of pure product was obtained which proved to be identical in melting point and mixed melting point with the compound just described.

Section B

2,3-Di-acetyl-benzylidene- β -methylglucoside.—Benzylidene- β -methylglucoside was acetylated in the usual manner with acetic anhydride and sodium acetate, the mixture being poured into water and the crystals filtered off. In this way 20 g. of starting material gave 25 g. of crude product melting at 164–168°. After one or sometimes two recrystallizations from absolute alcohol the product melted sharply at 169–170°, 10.15 g. of crude crystals giving 9.7 g. of pure product. The compound crystallizes in fine needles insoluble in water and petroleum ether, sparingly soluble in cold alcohols and ether, soluble in other solvents. Calcd. for $C_{18}H_{22}O_8$: OCH_3 , 8.47; CH_3CO , 23.5. Found: OCH_3 , 8.47; CH_3CO , 23.9. The rotation in chloroform was $[\alpha]_D -95.2^\circ$ for $c = 5.214\%$.

2,3-Di-acetyl- β -methylglucoside.—The removal of the benzylidene group was carried out as described for the corresponding dimethyl derivative the only difference being that *N*/40 acid was used on this occasion. The reaction was followed polarimetrically, the following changes being observed.

Time	α	$[\alpha]_D$
Initial	-4.51	-84.4
90 minutes	-2.59	-65.3
190 minutes	-2.19	-55.2
250 minutes	-2.18	-54.9

The last three values are calculated for change in concentration due to loss of benzylidene. The product, isolated exactly as before but not distilled, consisted of a glass insoluble in ether and petroleum ether, almost insoluble in cold benzene and soluble in other solvents. It appears to be never quite free from traces of the benzylidene compound, since on dissolving in water a small quantity of this material is deposited. The analytical figures also are low. Calcd. for $C_{11}H_{18}O_8$: OCH_3 , 11.1; CH_3CO , 30.9. Found: OCH_3 , 10.8; CH_3CO , 28.5. The rotation in chloroform was $[\alpha]_D - 55.3^\circ$ for $c = 5.204\%$.

2,3-Di-acetyl-4,6-di-*p*-toluenesulfonyl- β -methylglucoside.—2.3 Grams of the di-acetyl compound was dissolved in a little hot benzene and treated with excess of pyridine and 4.0 g. (2.5 mols) of *p*-toluenesulfonyl chloride for several days at room temperature. The product was isolated in the manner already described, 1.2 g. crystallizing out of the benzene during isolation and 3.7 g. being recovered from the benzene. The latter portion, which was sirupy, was treated with sodium iodide in the usual manner to convert it into the iodo compound and also to remove any unchanged *p*-toluenesulfonyl chloride of which there is nearly always some present; 3.3 g. was recovered from this treatment which gave 1.8 g. of crystals, a total yield of about 60% of the theoretical amount. No further quantity could be obtained by re-treating the residue with *p*-toluenesulfonyl chloride. The di-*p*-toluenesulfonyl derivative was recrystallized from absolute alcohol and consisted of needles melting at 160–161°, insoluble in water and petroleum ether, sparingly so in cold alcohols, soluble in other solvents. Calcd. for $C_{26}H_{30}O_{12}S_2$: OCH_3 , 5.29. Found: OCH_3 , 5.37. The rotation in chloroform was $[\alpha]_D - 12.7^\circ$ for $c = 5.371\%$.

6-Iodo-2,3-di-acetyl-4-*p*-toluenesulfonyl- β -methylglucoside.—2.8 Grams of the compound just described of m. p. 157–160° was treated with sodium iodide in acetone in the usual manner, giving 2.6 g. of crude product and 2.4 g. of pure substance after one recrystallization from absolute alcohol, a yield of 92% of the theoretical amount. The product, which consisted of small prisms with the same solubilities as the substance last described, melted at 160–161° (mixed with the last, 140–150°) and showed $[\alpha]_D$ in chloroform -19.9° for $c = 4.364\%$. Calcd. for $C_{18}H_{23}O_9SI$: OCH_3 , 5.71; I, 23.3. Found: OCH_3 , 5.65; I, 23.0. An acetyl determination carried out in the cold gave CH_3CO , 23.8%. Allowing for the loss of one *p*-toluenesulfonyl and two acetyl groups (calculating the latter as acetyl) the required acetyl content is 23.8%.

2,3-Di-acetyl-4-*p*-toluenesulfonyl- β -methylglucoside-6-nitrate.—1.001 Grams of the iodo compound was treated with silver nitrate in acetonitrile in the usual manner and worked up as already described. On crystallization from absolute alcohol, 0.756 g. was obtained in the first crop and 0.031 g. in the second, giving a total yield of crude crystals of 89% of the theoretical amount. The product was contaminated with silver and accordingly, after mixing with similar material from other preparations, it was dissolved in chloroform and filtered several times through charcoal. The solvent was removed and the residue crystallized twice from absolute alcohol. As so obtained it consisted of needles of much the same solubilities as the two last-mentioned compounds, melting at 128–129°. Calcd. for $C_{18}H_{23}O_{12}SN$: OCH_3 , 6.49; N, 2.93; CH_3CO (calculated in the same way as the last), 27.0. Found: OCH_3 , 6.40; N, 2.81; CH_3CO , 27.7. The rotation in chloroform was $[\alpha]_D - 32.9^\circ$ for $c = 5.138\%$.

2,3-Di-acetyl-4-*p*-toluenesulfonyl- β -methylglucoside.—The nitrate was removed in

the usual manner, the product being isolated in benzene by washing with water and alkali; 1.248 g. of starting material gave a quantitative yield of crude product. This on recrystallization from absolute alcohol gave 0.943 g. in the first crop of m. p. 164–166° and 0.111 g. in the second crop of m. p. 156–161°, the total yield of crystalline material being 93%. On recrystallization from absolute alcohol it was obtained pure, melting at 165–166°, consisting of small cubes with the same solubilities as the three compounds just described. Calcd. for $C_{18}H_{24}O_{10}S$: OCH_3 , 7.17; CH_3CO (calculated as before), 29.9. Found: OCH_3 , 7.48; CH_3CO , 30.5. The rotation in chloroform was $[\alpha]_D -55.4^\circ$ for $c = 5.174\%$.

2,3,6-Triacetyl-4-*p*-toluenesulfonyl- β -methylglucoside.—The hydroxy compound just described was acetylated in the usual manner with acetic anhydride and sodium acetate, the mixture being poured into water and the crystalline product isolated by filtration. In this way, 1.063 g. of starting material gave 1.133 g. of crude product that melted at 110–114°, the yield being 97%. After several recrystallizations from absolute alcohol, the substance melted at 116–117° after previous softening at 115°. The melting point is indefinite but could not be improved though Helferich⁶ gives the melting point of his compound which is doubtless the same as 118°. The compound crystallizes in slender prisms with much the same solubilities as the previous compounds though considerably more soluble in cold alcohols. Calcd. for $C_{20}H_{26}O_{11}S$: OCH_3 , 6.54; CH_3CO (calculated as before), 36.2. Found: OCH_3 , 6.55; CH_3CO , 36.8. The rotation in chloroform was $[\alpha]_D -43.8^\circ$ for $c = 5.481\%$.

2,3-Di-acetyl- β -methylglucoside-6-nitrate.—As already stated this material was first prepared from the dinitrate but the yield is too small for the method to be of much practical value. The following process was therefore adopted, which depends on the greater reactivity of the 6-hydroxyl group: 11.6 g. of diacetylmethylglucoside was treated with one molecular proportion of *p*-toluenesulfonyl chloride in pyridine for several days, giving 13.0 g. of total product and 2.6 g. of unaltered material. The product was then treated successively with sodium iodide and silver nitrate in the manner already described. The material so obtained was extracted about thirty times from benzene with water until the water washings were no longer optically active. The combined water washings were then extracted five or six times with chloroform and the latter removed in a vacuum. In this way 4.8 g. was obtained which on recrystallization from butyl alcohol gave 3.0 g. in the first crop and 0.4 g. in the second. The sirupy residue weighed 1.3 g. Calcd. for $C_{11}H_{17}O_{10}N$: OCH_3 , 9.59. Found: OCH_3 , 9.0. In view of its solubilities and composition the nature of this material is somewhat puzzling. The benzene residue consisted of a mixture of the 6-hydroxy- and 6-nitrate-4-*p*-toluenesulfonyl derivatives described above. The product after one further recrystallization from butyl alcohol consisted of stout prisms insoluble in petroleum ether, sparingly soluble in cold benzene and soluble in other solvents, and melted at 134–136°. Calcd. for $C_{11}H_{17}O_{10}N$: OCH_3 , 9.59; N, 4.33; CH_3CO (for two acetyl groups), 26.6. Found: OCH_3 , 9.56; N, 4.28; CH_3CO , 26.7. The rotation in chloroform was $[\alpha]_D -29.9^\circ$ for $c = 3.770\%$. On acetylation 1.7 g. of starting material gave after two recrystallizations from absolute alcohol 1.6 g. of product melting at 132–134°. The melting point of tri-acetyl- β -methylglucoside-6-mononitrate was given⁸ as 133–134°. Further confirmation was obtained by converting the above 6-nitrate into the corresponding iodo compound. This when recrystallized from rectified spirit melted at 114–115°, a value identical with the melting point of 6-iodo-triacetylmethylglucoside, and showing no depression when mixed with an authentic specimen of the latter.

On treating 2,3-di-acetyl- β -methylglucoside-6-mononitrate with *p*-toluenesulfonyl chloride in the usual manner, 1.8 g. of starting material gave 2.0 g. of product (a yield

of 77%) melting when crude at 127–129° and showing no alteration when mixed with the 2,3-di-acetyl-4-*p*-toluenesulfonyl-6-nitrate described above, thus finally proving the constitution of 2,3-di-acetyl- β -methylglucoside-6-mononitrate.

Section C

Tetra-*p*-toluenesulfonyl- β -methylglucoside.—Two grams of β -methylglucoside was treated with excess of *p*-toluenesulfonyl chloride in the usual manner for about a week. The hard mass was thereafter rubbed up with a mixture of alcohol and glacial acetic acid in the cold, the crystals filtered off and washed with more alcohol. In this way 8.9 g. of material was obtained. This was then boiled with enough glacial acetic acid to make a fairly fluid system, but not enough to dissolve the whole of the material. On cooling and filtering, 6.9 g. of product was obtained of m. p. 171–176°, a yield of about 82%. Only 0.3 g. of a stiff sirup was isolated from the various washings. The product after crystallization from glacial acetic acid consisted of very minute prisms melting with decomposition at 177–178°. It was fairly soluble in hot glacial acetic acid, but sparingly soluble or insoluble in all cold solvents. Owing to its insolubility in hydriodic acid the methoxyl content could not be determined. The specific rotation in chloroform was $[\alpha]_D -6.6^\circ$ for $c = 3.170\%$.

6-Iodo-2,3,4-tri-*p*-toluenesulfonyl- β -methylglucoside.—One and one-half grams of the compound described above on treatment with sodium iodide in the usual manner gave 1.3 g. of crude product, a yield of about 93%. This on recrystallization from much glacial acetic acid gave 1.1 g. of pure product consisting of long fine prisms melting at 211–212° and even more insoluble than the last compound, being very sparingly soluble in hot glacial acetic acid. It may be mentioned that a large proportion of this substance crystallizes out from the acetone on cooling after the treatment with sodium iodide described above, and may be isolated by filtering and washing the residue with water and alcohol. As in the case of the compound last described it was not possible to determine methoxyl. The iodine was, however, determined in the usual way by conversion into the nitrate derivative. Calcd. for $C_{28}H_{31}O_{11}S_3I$: I, 16.57. Found: I, 16.49. The specific rotation in chloroform was -1.2° for $c = 3.269\%$.

2,3,4-Tri-*p*-toluenesulfonyl- β -methylglucoside-6-mononitrate.—Owing to the extreme insolubility of the iodo compound the treatment with silver nitrate had to be slightly modified; 1.564 g. of the iodo compound was boiled with 3.0 g. of silver nitrate in acetonitrile for four hours, the product being thereafter isolated in the usual manner, when 1.372 g. of total product was obtained. This was crystallized from glacial acetic acid giving 1.163 g. in the first crop and 0.077 g. in a second crop. The yield of crystalline material was thus about 86% of that theoretically possible. The product consisted of minute prisms, sintering at 154°, melting at 166–168° and having rather greater solubilities than the two preceding substances. This is shown by the fact that the compound gives an accurate value for the methoxyl content. Calcd. for $C_{28}H_{31}O_{14}S_3N$: OCH₃, 4.42; N, 1.99. Found: OCH₃, 4.43; N, 1.74. The specific rotation in chloroform was -21.1° for $c = 5.638\%$. Since this compound was made only for the sake of getting an accurate value for the methoxyl content, it has not been further examined.

2,3,4-Tri-*p*-toluenesulfonyl- β -methylglucoside-6-mononitrate.—For reasons stated in the introduction the action of sodium nitrate on this and the following compounds was investigated. The present compound was prepared some years ago in this Laboratory by the action of benzene sulfonyl chloride on β -methylglucoside in the usual manner, the yield being good. The product was crystallized from glacial acetic acid in short prisms which sintered at 146° and melted at 166–168°. Since sulfur estimations in this series are not very satisfactory the compound was submitted to the action of acetylating agents, from which

it was recovered quantitatively unchanged, a result which showed it to be fully substituted, and that the constitution assigned to it was correct. On treatment with sodium iodide in the usual manner it was recovered quantitatively unchanged.

2,3,6 - Trimethyl - 5 - *p*-toluenesulfonyl - γ - methylglucoside.—2,3,6 - Trimethyl - γ - methylglucoside prepared from 2,3,6-trimethylglucose was treated with one molecular proportion of *p*-toluenesulfonyl chloride and the product isolated in the customary manner. The sirup so obtained was treated with sodium iodide in acetone but no trace of iodine could be found in the resulting product. The authors wish to express their thanks to Mr. C. Miller for carrying out this portion of the work.

As already stated the action of sodium iodide in acetone was also tried on the *p*-toluenesulfonyl derivative of glucosediacetone which was prepared in the manner already described by Freudenberg⁶ and on 4,6-dimethyl-2,3-di-*p*-toluenesulfonyl- α -methylglucoside which has been recently prepared in this Laboratory by Mr. D. S. Mathers, who kindly gave us a specimen, but in both cases the compounds were recovered quantitatively unchanged.

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Summary

1. Position 6 in the glucose chain can be identified (when not already substituted) by conversion of the sugar into the corresponding glucoside, substituting the vacant hydroxyl groups by *p*-toluenesulfonyl residues, and heating the product with sodium iodide in acetone at 100°.

2. Under these conditions the *p*-toluenesulfonyl substituent in position 6 is replaced by iodine quantitatively.

3. The iodo compound can be quantitatively estimated for iodine by boiling with silver nitrate in acetonitrile. This yields the corresponding nitrate, which can be subsequently converted into the corresponding hydroxy compound, thereby affording an accurate identification and estimation of the 6-hydroxy group in glucose.

4. Evidence has been collected to show that these properties are strictly confined to the 6 position of glucose.

5. A number of partially substituted glucose derivatives have been prepared for the first time by means of these reactions.

6. The ease with which the various compounds crystallize enables the reactions to be carried out on the small scale and the synthetical application of the results is being explored in the laboratory.

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