In continuing our chemical and immunological investigations on derivatives of glucuronic acid, it has become desirable to prepare azoproteins containing aldobionic acids. The latter were first found among the products of hydrolysis of the specific capsular polysaccharides of pathogenic microorganisms (1), and may be defined as disaccharides in which one of the sugar components is a uronic acid linked in glycosidic union to a hexose or pentose. Recently the chemical synthesis of two aldobionic acids has been achieved (2).

In the present investigation a method for the synthesis of aldobionides is outlined. Two aldobionic acids have been chosen for study, the aldobionic acid of gum acacia (3) 6-β-glucuronidosidogalactose, which we now propose to call acaciabiuronic acid, and cellobiuronic acid or 4-β-glucuronidosidoglucose. Cellobiuronic acid is obtained from the hydrolysis of the specific polysaccharide of pneumococcus Type III (4). When the carboxyl group of the aldobionic acid is protected by the formation of the methyl ester, the latter, on acetylation, yields a crystalline heptaacetate (5). Heptaacetylcellobiuronic acid methyl ester is readily converted to the 1-bromohexaacetyl derivative by treatment with acetic acid saturated with hydrogen bromide. When acetobromocellobiuronic acid methyl ester is condensed with methyl alcohol or with p-nitrobenzyl alcohol in the presence of silver oxide, the corresponding levorotatory crystalline glycoside is in each instance formed. Since cellobiuronic acid, like cellobiose, has a normal pyranose structure (4), the acetohalogen derivative of the former is believed
to have the same configuration. Acetobromocellobiuronic acid methyl ester is therefore designated as the \( \alpha \) compound according to the nomenclature of Hudson (6), and the levorotatory hexaacetylmethyl- and \( p \)-nitrobenzylglycosides of cellobiuronic acid methyl ester are assigned the \( \beta \) configuration.

The structural relationship of the acetyl and bromo derivatives of acaciabiuronic acid methyl ester is more complex than is that of the corresponding derivatives of cellobiuronic acid. Since acaciabiuronic acid is a compound having a glucuronosido linkage on carbon atom 6 of the hexose (7), one might anticipate that the aldobionic acid ester on acetylation would behave much as does the hexose galactose. The acetylation of acaciabiuronic acid methyl ester with pyridine and acetic anhydride yields two heptaacetates. The first is a crystalline compound melting at 203\(^\circ\) and having a rotation of \( [\alpha]_b = -17.5^\circ \) in chloroform. The second acetate is amorphous and may possibly be a mixture. The rotation of this substance is \( [\alpha]_b = +15.7^\circ \) (in CHCl\(_3\)). When the first acetate is warmed in acetic anhydride solution with zinc chloride, it is converted into a third crystalline heptaacetate melting at 195–197\(^\circ\), \( [\alpha]_b = +46.5^\circ \) (in CHCl\(_3\)). Since the difference in molecular rotation of the first and third acetates is 42,500\(^\circ\) (which is approximately the value for the difference in molecular rotation of the \( \alpha \) and \( \beta \)-octaacetates of disaccharides), these two acetates may tentatively be considered as an \( \alpha \) and \( \beta \) pair having the same ring structure.

The first heptaacetate of acaciabiuronic acid methyl ester yields a dextrorotatory crystalline bromo derivative melting at 202\(^\circ\), \( [\alpha]_b = +194.7^\circ \) (in CHCl\(_3\)). When the latter is allowed to react in chloroform solution with silver acetate, instead of the parent levorotatory first acetate, a fourth crystalline heptaacetate melting at 110–112\(^\circ\) is obtained in excellent yields, \( [\alpha]_b = +92.1^\circ \) (in CHCl\(_3\)). When acetobromoacaciabiuronic acid methyl ester is shaken with methyl alcohol and silver oxide, a dextrorotatory hexaacetylmethylglycoside melting at 135\(^\circ\) is formed, \( [\alpha]_b = +86.4^\circ \) (in CHCl\(_3\)). This is not the same methylglycoside obtained by acetylatung the so called amorphous \( \beta \)-glycoside of Heidelberger and Kendall (3). The hexaacetate of the latter, which we have likewise prepared, is a levorotatory crystalline derivative melting at 140\(^\circ\), \( [\alpha]_b = -58.8^\circ \) (in CHCl\(_3\)). The
difference in molecular rotation of these two acetylated methyl-
glycosides (92,400°) is far greater than the value anticipated for the 
α- and β-methylglycosides of an acetylated disaccharide (about 
54,000° for the α- and β-methylglycosides of cellobiose heptaacetates 
(8)). It is apparent, therefore, that the dextro- and levomethyl-
glycosides of hexaacetylaceaibniuronic acid methyl ester are not 
true α and β isomeric pairs, but probably are glycosides with 
different ring structures. Although the two methylglycosides of 
the free aldobionic acid have not been prepared in a crystalline 
state, it is possible to measure the kinetics of hydrolysis of these 
derivatives in the manner outlined in the experimental procedure. 
Thus it is found that the first methylglycoside, obtained by 
deaetylation and saponification of the first, or dextrorotatory 
methylglycoside hexaacetate, hydrolyzes at the rate of a pyranoside. 
The second methylglycoside, obtained by deacetylation 
and saponification of the acetylated levorotatory methylglycoside 
ester hydrolyzes far more rapidly, or at the rate of a furanoside. 
The dextrorotatory methylglycoside of hexaacetylaceaibniuronic 
acid methyl ester may therefore be regarded as a pyranoside, 
whereas the corresponding levorotatory derivative, prepared by 
acetylation of Heidelberger and Kendall’s β-glycoside, appears to 
be a furanoside. An understanding of the configurational rela-
tionship of these two glycosides, as well as knowledge of the ring 
structures of the four heptaacetates, must await the outcome of 
further experimentation.

In the course of our studies on the synthetically prepared deriv-
atives of uronic acids we have been struck by the fact that a 
correlation seems to exist between the values for the molecular 
rotation of the saccharides and of the corresponding uronic acid 
derivatives. The values for the specific and molecular rotation of 
certain acetylated derivatives of glucose, cellobiose, and gentio-
biose have been tabulated and compared with those of the cor-
responding glucuronic, cellobiuronic, and gentiobiuronic acid 
methyl esters prepared in this laboratory.

From Table I it can be seen that the molecular rotations of the 
acetylated gluco- and glucuronopyranose derivatives, in which an 
acetyl, methoxyl, or halogen group has been substituted on the 
first or aldehydic carbon atom, differ only by a small and ap-
proximately constant amount. This same relationship holds
true for similar derivatives of the disaccharides cellobiose and gentiobiose and their corresponding aldobionic acid methyl esters.

**Table I**

Comparison of Molecular Rotations of Derivatives of d-Glucose and d-Glucuronic Acid

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular weight</th>
<th>[α] in chloroform</th>
<th>[M] for sugar</th>
<th>[M] for sugar derivative</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pentaacetylglucose</td>
<td>390</td>
<td>+101.6</td>
<td>+39,600</td>
<td></td>
</tr>
<tr>
<td>α-Tetraacetylgalacturonic acid methyl ester</td>
<td>376</td>
<td>+98.0</td>
<td>+36,800</td>
<td>-2,800</td>
</tr>
<tr>
<td>β-Pentaacetylglucose</td>
<td>390</td>
<td>+4.8</td>
<td>+1,870</td>
<td></td>
</tr>
<tr>
<td>β-Tetraacetylgalacturonic acid methyl ester</td>
<td>376</td>
<td>+8.7</td>
<td>+3,270</td>
<td>+1,400</td>
</tr>
<tr>
<td>α-Chlorotetraacetylglucose</td>
<td>367</td>
<td>+166.0</td>
<td>+60,900</td>
<td>-1,400</td>
</tr>
<tr>
<td>α-Chlorotriacetylgalacturonic acid methyl ester</td>
<td>353</td>
<td>+168.7</td>
<td>+59,500</td>
<td></td>
</tr>
<tr>
<td>α-Bromotetraacetylglucose</td>
<td>411</td>
<td>+198.0</td>
<td>+81,400</td>
<td>-2,800</td>
</tr>
<tr>
<td>α-Bromotriacetylgalacturonic acid methyl ester</td>
<td>397</td>
<td>+198.0</td>
<td>+78,600</td>
<td></td>
</tr>
<tr>
<td>Tetraacetyl-β-methylglucoside</td>
<td>362</td>
<td>-18.3</td>
<td>-6,620</td>
<td>-3,440</td>
</tr>
<tr>
<td>Triacetyl-β-methylglucoside of glucuronic acid methyl ester</td>
<td>348</td>
<td>-28.9</td>
<td>-10,060</td>
<td></td>
</tr>
</tbody>
</table>

* Values for the specific rotations of all sugar derivatives given in Tables I, II, and III were taken from Hudson (9). Values for the specific rotation of the uronic acid derivatives were taken from Papers IV to VII by Goebel (10, 2). Values for the galacturonic acid derivatives were taken from papers by Link and coworkers (11). The value for the specific rotation of acetobromogalactose in chloroform was furnished us through the courtesy of Dr. R. Stuart Tipson. This is in close agreement with the value for the molecular rotation of acetobromogalactose in chloroform as predicted by Hudson (12).

(Table II). It is apparent, therefore, that a change in molecular rotation, the value of which approximates a constant, accompanies the conversion of the terminal acetylated primary alcohol group.
(CH₂OAc) to the carboxy methyl group (COOMe). In all instances save one the change in rotation accompanying the transition from aldose to uronic acid derivative is toward a more negative value, and is about the same order of magnitude. The 2 carbon atoms adjacent to the terminal atom are those most likely to be

**Table II**

Comparison of Molecular Rotations of Derivatives of Cellobiose and Cellobiuronic Acid and of Gentiobiose and Gentiobiuronic Acid

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular weight</th>
<th>[α]ₜ in chloroform</th>
<th></th>
<th>MI₀ (alduronic acid derivative)</th>
<th>ΔM₀ (deoxy derivative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Octaacetylcellobiose</td>
<td>678</td>
<td>+41.0</td>
<td>+27,800</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Heptaacetylcellobiuronic acid methyl ester</td>
<td>664</td>
<td>+40.9</td>
<td>+27,200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Bromohexaacetylcellobiose</td>
<td>669</td>
<td>+96.0</td>
<td>+67,100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Bromohexaacetylcellobiuronic acid methyl ester</td>
<td>685</td>
<td>+99.4</td>
<td>+68,100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heptaacetyl-β-methylcellobioside</td>
<td>650</td>
<td>-25.4</td>
<td>-16,510</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexaacetyl-β-methylglycoside of cellobiuronic acid methyl ester</td>
<td>636</td>
<td>-27.1</td>
<td>-17,230</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Octaacetylgentiobiose</td>
<td>678</td>
<td>+52.4</td>
<td>+35,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Heptaacetylgentiobiuronic acid methyl ester</td>
<td>664</td>
<td>+48.4</td>
<td>+32,100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Octaacetylgentiobiose</td>
<td>678</td>
<td>-5.3</td>
<td>-3,590</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Heptaacetylgentiobiuronic acid methyl ester</td>
<td>664</td>
<td>-11.0</td>
<td>-7,300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See foot-note to Table I.

affected in their partial rotations by alterations in the grouping attached to the latter. Since the configurational relationship of the terminal and the 2 adjacent carbon atoms in glucose, gentiobiose, and cellobiose, as well as in their respective uronic acids, is identical, it is not surprising that the magnitude of the rotational
difference between the uronic acid and aldose derivatives is in each instance approximately the same.

In Table III the molecular rotations of certain acetylated galacto- and galacturonopyranose derivatives are compared. Here again it is obvious that the conversion of the acetylated primary alcohol to the carboxy methyl group is accompanied by a change in molecular rotation, the value of which is approximately constant, irrespective of the group attached to the aldehydic carbon atom. In this instance, however, the change is in a positive direction. Since the spatial relationship of the 2 carbon atoms adjacent to the 6th atom in galactose differs from that in glucose, one might expect that the difference in molecular rotation of the galacturonic acid and galactose derivatives would be of different magnitude than in the case of the corresponding derivatives of

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular weight</th>
<th>$[\alpha]_D$ in chloroform</th>
<th>$[\alpha]_D$</th>
<th>$[\alpha]_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-Pentaacetylgalactose.................</td>
<td>390</td>
<td>+107.0</td>
<td>+41,700</td>
<td>+12,000</td>
</tr>
<tr>
<td>$\alpha$-Tetraacetylgalacturonic acid methyl ester</td>
<td>376</td>
<td>+143.0</td>
<td>+53,700</td>
<td></td>
</tr>
<tr>
<td>Tetraacetyl $\alpha$-methylgalactoside........</td>
<td>362</td>
<td>+132.5</td>
<td>+48,000</td>
<td>+9,800</td>
</tr>
<tr>
<td>Triacetyl-$\alpha$-methylglycoside of galacturonic acid methyl ester........</td>
<td>348</td>
<td>+166.0</td>
<td>+57,800</td>
<td>+10,030</td>
</tr>
<tr>
<td>Tetraacetyl-$\beta$-methylgalactoside..........</td>
<td>362</td>
<td>-13.0</td>
<td>-4,710</td>
<td></td>
</tr>
<tr>
<td>Triacetyl-$\beta$-methylglycoside of galacturonic acid methyl ester........</td>
<td>348</td>
<td>+15.3</td>
<td>+5,320</td>
<td></td>
</tr>
<tr>
<td>$\alpha$-Bromotetraacetylgalactose............</td>
<td>411</td>
<td>+205.8</td>
<td>+84,600</td>
<td>+13,800</td>
</tr>
<tr>
<td>$\alpha$-Bromotriacetylgalacturonic acid methyl ester........</td>
<td>397</td>
<td>+248.0</td>
<td>+98,400</td>
<td></td>
</tr>
</tbody>
</table>

* See foot-note to Table I.
glucuronic acid and glucose. That this is true, and that the value is considerably greater, is seen from Table III.

Some years ago it was observed by Hudson (9) that an alteration in the 6th carbon atom of β-methylglucoside and β-methylmannoside from CH₂OH to CH₃, giving β-methylisorhamnoside and β-methylrhamnoside respectively, changes the molecular rotation by a small and nearly constant amount. This earlier observation is of interest, for it foreshadows the constant and small change in molecular rotation which the authors now find accompanies the change from CH₂OH to COOH in the glucose and galactose series. Whether this relationship will hold true for the various derivatives of the synthetic disaccharide 6-β-d-glucopyranosido-d-galactose (13) and its naturally occurring uronic acid, acaciabhirionic acid, must await the outcome of future work. However, the correlation of the rotations of the derivatives already studied is striking indeed. In conclusion it should be pointed out that the molecular rotations of the saccharides in question have been calculated from observations taken only at the D line. A study of the rotatory dispersion of these closely related compounds should be made in order to ascertain whether this relationship is true at other wave-lengths of light.

EXPERIMENTAL

α-Acetobromocellobhirionic Acid Methyl Ester—0.8 gm. of α-hepta-acetylcellobhirionic acid methyl ester (5) were dissolved in 5 cc. of chloroform and 7 cc. of acetic acid saturated with hydrobromic acid were added. After the solution had stood for 3 hours at room temperature, the solvent and hydrobromic acid were removed in vacuo (14). The residue was dissolved in toluene and the latter likewise removed by distillation in vacuo. This treatment was repeated twice more. The pale yellow, oily residue was dissolved in a small quantity of ether. Crystallization of the bromo derivative proceeded immediately. 0.52 gm. of a white crystalline substance was recovered. The derivative was recrystallized three times in all, first being dissolved in chloroform, followed by the addition of an equal volume of ether. After the third crystallization neither the melting point nor the specific rotation of the compound changed. 450 mg. of glistening needles were recovered. The substance crystallizes as rosettes of needles melting at 200°
(uncorrected) with decomposition. $[\alpha]_D^{24} = +99.4^\circ$ (CHCl$_3$, $c = 0.8$).

Analysis—C$_{25}$H$_{32}$O$_7$: Calculated, Br 11.7; found, Br 11.6

$\beta$-Methylglycoside of Hexaacetylcellobiuronic Acid Methyl Ester—1.1 gm. of acetobromocellobiuronic acid methyl ester were dissolved in 10 cc. of dry chloroform and 10 cc. of absolute methyl alcohol added. 0.37 gm. (2 moles) of silver oxide was added and the mixture shaken until the supernatant liquid gave no test for the bromine derivative (2½ hours). After the residual silver salts were filtered off and the filtrate concentrated in vacuo, the glycoside separated as needles. 765 mg. were recovered. The compound was recrystallized several times from methyl alcohol. The derivative melts at 200° (uncorrected). $[\alpha]_D^{33} = -27.2^\circ$ (CHCl$_3$, $c = 0.6$).

Analysis—C$_{25}$H$_{32}$O$_8$: Calculated. C 49.0, H 5.7, OCH$_3$ 9.7

Found. " 49.0, " 5.8, " 9.9

$\beta$-p-Nitrobenzylglycoside of Hexaacetylcellobiuronic Acid Methyl Ester—500 mg. of acetobromocellobiuronic acid methyl ester were dissolved in 5 cc. of anhydrous chloroform and 206 mg. of p-nitrobenzyl alcohol (2 moles) added. 252 mg. of silver oxide (3 moles) were placed in the flask and the latter shaken for 3½ hours, or until no more free bromine derivative could be detected in the supernatant liquid. After the silver salts were filtered off and the pale yellow filtrate concentrated in vacuo, the glycoside crystallized when the residue was dissolved in 3 cc. of methyl alcohol. The compound crystallizes as rosettes of pale yellow needles. 130 mg. of glycoside were recovered. After three crystallizations from methyl alcohol 80 mg. of pure glycoside were obtained. The substance melts at 199–200° (uncorrected). $[\alpha]_D^{23} = -41.7^\circ$ (CHCl$_3$, $c = 0.6$).

Analysis—C$_{25}$H$_{32}$O$_8$:N(COOC$_3$)$_3$

Calculated. C 50.7, H 5.2, OCH$_3$ 4.1

Found. " 50.4, " 5.1, " 4.1

First and Third Heptaacetates of Acaciabiuronic Acid Methyl Ester—10 gm. of acaciabiuronic acid methyl ester (2) were acetyl-
ated with 50 cc. of pyridine and 35 cc. of acetic anhydride at 0° for 18 hours. The mixture was poured with stirring into 1 1/2 liters of ice and water. The granular precipitate was filtered and washed. The moist precipitate was dissolved in chloroform and the solution extracted with dilute HCl to remove pyridine, and washed finally with water. After the chloroform solution was dried with sodium sulfate, the solvent was removed in vacuo and the residue dissolved in ethyl alcohol. Crystals of the first heptaacetate of acaciabiuronic acid methyl ester separated on standing. After 3 days at 0°, 5.8 gm. of crystalline product were obtained. After several recrystallizations from ethyl alcohol the product showed the same physical constants and analysis as previously reported (2).

The alcoholic filtrate from the first acetate failed to deposit crystals, even after many weeks of standing. The solvent was therefore removed in vacuo, and the residue dried for several weeks in a high vacuum. This substance, which may be a mixture, has been termed the third heptaacetate of acaciabiuronic acid methyl ester, and shows the following properties. \([\alpha]_D^{22} = +15.7°\) (CHCl₃, c = 1.0).

Analysis—C₁₁H₁₆O₁₉(COCH₃)₇(COOCH₃)
Calculated, OCH₃ 4.7; found, OCH₃ 4.6

Second Heptaacetate of Acaciabiuronic Acid Methyl Ester—1.12 gm. of the first acetate were dissolved in 20 cc. of acetic anhydride containing 2 gm. of freshly fused zinc chloride, and the mixture heated at 55°. After 20 minutes the rotation observed in a 1 dm. tube had changed from \(-1.48°\) to a constant value of \(+2.65°\). The mixture was slowly poured with stirring into 200 cc. of ice and water. After standing 1 hour at 0° the clear supernatant liquid was decanted from a pale yellow oil which had settled to the bottom of the container. The oil was dissolved in chloroform and extracted several times at 0° with sodium bicarbonate solution. The chloroform solution after drying was concentrated in vacuo and the residue dissolved in ethyl alcohol. After standing overnight, the solution deposited 300 mg. of a crystalline product. This was recrystallized several times from ethyl alcohol. The specific rotation did not change after the second crystallization. \([\alpha]_D^{22} = +46.5°\) (CHCl₃, c = 1).
The second heptacetate of acaciabiuronic acid methyl ester separates as a sparingly soluble crystalline product melting at 195-197°. The compound is less soluble in alcohol than is the first acetate from which it was derived. Since the difference in molecular rotation of the first and third heptacetates (42,500°) is approximately the same as that of α- and β-acetates of mono- and disaccharides, it seems justifiable to assume that the two compounds represent an α and β isomeric pair having the same ring structure, though it is not known whether these acetates are pyranose or furanose derivatives.

Acetobromoacaciabiuronic Acid Methyl Ester—This compound was prepared from the first heptaacetate of acaciabiuronic acid methyl ester exactly as was the acetobromo compound of cellobiuronic acid methyl ester. The derivative is obtained in excellent yields and crystallizes from a mixture of chloroform and ether as glistening rhombs melting at 201-202° (uncorrected). \([\alpha]_D^{22} = +194.7°\) (CHCl₃, \(c = 1\)).

Analysis—C₁₁H₁₆O₁₁Br. Calculated, Br 11.7; found, Br 11.6

Fourth Heptaacetate of Acaciabiuronic Acid Methyl Ester—1.0 gm. of acetobromoacaciabiuronic acid methyl ester exactly as was the acetobromo compound of cellobiuronic acid methyl ester. The derivative is obtained in excellent yields and crystallizes from a mixture of chloroform and ether as glistening rhombs melting at 201-202° (uncorrected). \([\alpha]_D^{22} = +92.1°\) (CHCl₃, \(c = 0.7\)).

Analysis—C₁₁H₁₆O₁₈(COCH₃)₇(COOCH₃)
Calculated. C 48.7, H 5.5, OCH₃ 4.7
Found. " 48.7, " 5.7, " 4.6
First Methylglycoside of Hexaacetylacaciobiuronic Acid Methyl Ester—1 gm. of acetobromoacaciobiuronic acid methyl ester was dissolved in a mixture of 5 cc. of chloroform and 10 cc. of absolute methyl alcohol. The mixture was shaken for 3 hours with 0.5 gm. of silver oxide, or until the solution gave no test for the soluble bromine derivative. After filtration and concentration of the solvent in vacuo, the glycoside crystallized on the addition of ether. 0.6 gm. of glycoside was recovered. On subsequent crystallization the derivative was obtained as beautiful prismatic needles melting at 134.5° (uncorrected). \([\alpha]_D^{24} = +86.4° (\text{CHCl}_3, c = 1.1)\).

Analysis—C_{15}H_{29}O_{15}(\text{OCH}_3)(\text{COOCH}_3)

Calculated. C 49.0, H 5.7, OCH\(_3\) 9.8
Found. “ 49.0, “ 5.8, “ 9.9

On account of its high dextrorotation the above glycoside is believed to be an \(\alpha\) derivative.

Second Methylglycoside of Hexaacetylacaciobiuronic Acid Methyl Ester—2 gm. of anhydrous acaciobiuronic acid were dissolved in 50 cc. of methyl alcohol containing 0.5 per cent HCl and allowed to stand at room temperature for 24 hours. The amorphous glycoside was isolated in the manner described by Heidelberger and Kendall (3). The substance was acetylated with 15 cc. of pyridine and 10 cc. of acetic anhydride at 0° in the usual manner. After the reaction mixture was poured into ice water, the granular precipitate which formed was separated by filtration and dried in the air for several days. When the substance was dissolved in methyl alcohol, crystals of the glycoside separated and 1.4 gm. were recovered. The compound was recrystallized four times from methyl alcohol. The product thus obtained melted at 140° (uncorrected).\(^1\) \([\alpha]_D^{23} = -58.8° (\text{CHCl}_3, c = 0.9)\).

Analysis—C_{23}H_{36}O_{14}(\text{OCH}_3)(\text{COOCH}_3)

Calculated. C 49.0, H 5.7, OCH\(_3\) 9.8
Found. “ 49.0, “ 5.7, “ 9.8

The difference in molecular rotation of the first and second methylglycosides of hexaacetylacaciobiuronic acid is 92,400°.

\(^1\) Since this investigation was begun, the preparation of this same derivative of acaciobiuronic acid has likewise been described by Levene and Tipson (15).
Since this value is considerably greater than that shown by the $\alpha$ and $\beta$ acetylated methylglycosides of hexoses and disaccharides (about 54,000$^\circ$), it appears that the two derivatives in question are not a true $\alpha$ and $\beta$ pair having the same ring structure.

In order to ascertain this point the kinetics of hydrolysis of the two glycosides were measured in the following manner, after saponification of the acetyl and ester groupings of the acetylated derivatives.

**Kinetics of Hydrolysis of First and Second Methylglycosides of Acaciabiuronic Acid**—0.0671 gm. of the dextrorotatory or first methylglycoside of hexaacetylacaciabiuronic acid methyl ester was dissolved in 10 cc. of 0.1 N NaOH. At the end of 24 hours 10 cc. of 0.1 N HCl were added and the solution diluted to 50 cc. after the introduction of sufficient standard acid to make the final solution 0.2 N with respect to HCl. The solution was boiled under a reflux and reducing sugars determined on duplicate 2 cc. samples at appropriate intervals by the Hanes modification of the Hagedorn-Jensen method (16). The results are as follows:

<table>
<thead>
<tr>
<th>Time, min.</th>
<th>0</th>
<th>4</th>
<th>15</th>
<th>30</th>
<th>77</th>
<th>138</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 N thiosulfate used, cc.</td>
<td>0</td>
<td>0.07</td>
<td>0.29</td>
<td>0.60</td>
<td>1.27</td>
<td>1.63</td>
<td>1.84</td>
</tr>
</tbody>
</table>

From the results the velocity constant was calculated by the method of Guggenheim (17); $K = 63.5 \times 10^{-5}$ min.$^{-1}$ for 0.01 N HCl. In order to correct this value for the disaccharide cleavage which occurs during hydrolysis of the aldobionide, a solution of pure acaciabiuronic acid was hydrolyzed under identical conditions, and the increase in reducing sugars determined. When this correction, found to be small, was applied to the above data, the corrected velocity constant was $K = 53.0 \times 10^{-5}$ min.$^{-1}$.

The velocity constant for the hydrolysis of the second methylglycoside of acaciabiuronic acid obtained by saponification of the levorotatory hexaacetyl methyl ester derivative was likewise determined. 0.0630 gm. of the levorotatory hexaacetilymethylglycoside ester was saponified and the solution neutralized as in the previous experiment. The final concentration of HCl was adjusted to 0.0196 N, at a volume of 50 cc. The hydrolysis and reducing sugar determinations were performed as above. Because
the rate of hydrolysis of the glycoside was very rapid, it was unnecessary to correct for the slight cleavage of the disaccharide.

<table>
<thead>
<tr>
<th>Time, min.</th>
<th>15</th>
<th>32</th>
<th>62</th>
<th>130</th>
<th>204</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 N thiosulfate used, cc.</td>
<td>0.39</td>
<td>0.71</td>
<td>1.09</td>
<td>1.62</td>
<td>1.73</td>
</tr>
</tbody>
</table>

From the results given above, the velocity constant $K$, calculated for 0.01 N HCl, is $716 \times 10^{-5}$ min.$^{-1}$. When recalculated on the basis of natural logarithms, the values found by Haworth and Hirst (18) for pyranosides in 0.01 N acid at 100° range from $9 \times 10^{-5}$ to $70 \times 10^{-5}$ min.$^{-1}$. The corresponding constants for furanosides range from $600 \times 10^{-5}$ to $11,000 \times 10^{-5}$ min.$^{-1}$. The results of the above experiments are regarded as evidence that the galactose portion of the acaciabiuronic acid molecule has, in the dextrorotatory glycoside, a pyranose ring, and in the levorotatory derivative a furanose structure.

It should be pointed out that neither the crystalline first nor the second hexaacetylmethylglycoside of acaciabiuronic acid methyl ester is an orthoacetate, since the acetyl groups of both glycosides are completely and quantitatively removed on alkaline hydrolysis.

If one calculates from the data of Heidelberger and Kendall (3) the velocity constant for the hydrolysis of their amorphous $\alpha$- and $\beta$-methylglycosides of acaciabiuronic acid, the values $K = 903 \times 10^{-5}$ and $734 \times 10^{-5}$ min.$^{-1}$ respectively for 0.01 N HCl are obtained. Since these constants are of the order of magnitude of those found for the hydrolysis rate of methylfuranosides, it appears, therefore, that the interpretation of these experimental results given by the authors in regard to the pyranose structure of the glycosides in question should be revised.

In conclusion the authors wish to express their thanks to Dr. Max Bergmann and Dr. Alexandre Rothen for their generous advice.

**SUMMARY**

1. The preparation of the acetobromo derivatives of cellobiuronic and acaciabiuronic acid methyl esters is described.
2. The isolation and properties of the heptaacetates of acacia-
biuronic acid methyl ester are outlined.
3. The synthesis of several aldobionides is described.
4. It has been shown that a relationship between the molecular
rotations of acetylated derivatives of certain aldoses and their
uronic acids exists.

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DERIVATIVES OF GLUCURONIC ACID:
IX. THE SYNTHESIS OF
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Walther F. Goebel and Richard E. Reeves


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