DERIVATIVES OF GLUCURONIC ACID

VII. THE SYNTHESIS OF ALDOBIONIC ACIDS*

BY ROLLIN D. HOTCHKISS AND WALThER F. GOEBEL

(From the Hospital of The Rockefeller Institute for Medical Research, New York)

(Received for publication, May 16, 1936)

In Paper VI of this series, the preparation of α-bromotriacetyl-glucuronic acid methyl ester was described (1). It was shown that when the latter derivative is condensed with alcohols in the presence of silver oxide, the corresponding β-glucuronides are formed. The suggestion was made that the successful preparation of acetobromoglucuronic ester should make possible the synthesis not only of glucuronides, but of aldobionic acids as well.

Aldobionic acids may be defined as disaccharides containing a uronic acid as one of the sugar components. These sugar acids were first found among the hydrolysis products of the type-specific polysaccharides of certain encapsulated bacteria (2). Aldobionic acids have since been obtained from a variety of plant gums. Thus, when gum acacia is hydrolyzed with dilute mineral acid, there is obtained an aldobionic acid, galactose glucuronide (3, 4). Challinor, Haworth, and Hirst (5) have shown this substance to have the structure of a galactopyranose-6-glucuronopyranoside and have suggested that the biose linkage possesses the β configuration. The present communication describes the chemical synthesis of this aldobionic acid, and of a second aldobionic acid, the heptaacetyl methyl ester of glucose-6-β-glucuronide.

By using a reaction similar to that employed by Freudenberg, Noe, and Knopf in synthesizing the disaccharide galactose-6-β-glucoside (6), it has been possible to prepare synthetically an aldobionic acid identical with that obtained from gum acacia. When

1,2,3,4-diacetonegalactose is condensed with α-bromotriacetyl-
glucuronic acid methyl ester in ether solution in the presence of
silver oxide, diacetonegalactose-6-β-triacylglucuronide methyl
ester is produced. Upon saponification with barium hydroxide
and subsequent removal of the acetone groups by boiling with
dilute sulfuric acid, the crystalline aldobionic acid is obtained.
The synthetic acid is identical in properties with the naturally
occurring aldobionic acid obtained from gum acacia, and the
melting point of a mixture of the two substances shows no depres-
sion. Unless inversion has occurred, the synthetic acid must
possess the β-biose configuration, inasmuch as the acetobromo
derivative of glucuronic acid from which it is prepared has been
found to yield only β-glucuronides (1). It is therefore possible
to confirm the suggestion of Challinor, Haworth, and Hirst that
the aldobionic acid of gum acacia has the β configuration, as shown
in Formula I.

Further confirmation of the identity of the synthetic and natural
aldobionic acids was afforded by the preparation of the correspond-
ing heptaacetyl methyl esters. In each case the aldobionic acid
was converted into the methyl ester by the action of diazomethane,
followed by acetylation with acetic anhydride and pyridine.
There was obtained, from both the synthetic and the natural
acids, the same crystalline heptaacetyl methyl ester, possessing
well defined and identical physical properties.

The synthesis of the aldobionic acid of gum acacia, already
shown by Challinor, Haworth, and Hirst to be a glucuronopyrano-
side, indicates that the acetobromo derivative of glucuronic acid
methyl ester itself is a pyranoside. This bromo derivative may
therefore be assigned the structure 1-(\(\alpha\))-bromo-2,3,4-triacetyl-
\(\alpha\)-glucuronic acid methyl ester.

Disaccharides have been synthesized by Helferich and his co-
workers by condensing 1,2,3,4-tetraacetyl-\(\beta\)-glucose with acetobromo sugars (7). The synthesis of the heptaacetyl methyl ester of the aldobionic acid, glucose-6-\(\beta\)-glucuronide, has been accompl-
ished by condensing 1,2,3,4-tetraacetyl-\(\beta\)-glucose with 1-bromo-
2,3,4-triacetylglucuronic acid methyl ester in chloroform solution in the presence of silver oxide. \(\beta\)-Heptaacetylglucose-6-\(\beta\)-glucu-
ronide methyl ester, represented by Formula II, is obtained as a

![Formula II](image)

crystalline substance with the specific rotation \([\alpha]_D = -11.0^\circ\). Inasmuch as the aldobionic acid glucose-6-\(\beta\)-glucuronide may be consid-
ered as derived from the disaccharide gentiobiose (glucose-6-
\(\beta\)-glucoside), the name \(\beta\)-heptaacetylgentiobiuronic acid methyl ester is suggested for the synthetic derivative.

The synthetic \(\beta\)-heptaacetate of gentiobiuronic acid methyl ester has been converted into its \(\alpha\) isomer by the action of zinc chloride in acetic anhydride solution. The difference in molecular rotation of the \(\alpha\) and \(\beta\) forms in chloroform is 39,500\(^\circ\), a value in close agreement with the known differences in molecular rotation of the \(\alpha\) and \(\beta\) isomers of sugar acetates.

**EXPERIMENTAL**

*Synthesis of 1,2,3,4-Diacetonegalactose-6-\(\beta\)-2,3,4-Triacetylglu-
curonide Methyl Ester*—To 4.4 gm. of diacetonegalactose (8), dissolved in 100 cc. of anhydrous ether, were added 3.5 gm. of dry silver oxide and 6.75 gm. of \(\alpha\)-bromotriacetylglucuronic acid methyl ester (1). The mixture was shaken until the solution no
longer contained the free bromine derivative. After filtering, the solution was evaporated in vacuo to a syrup. The latter was dissolved in 35 cc. of ethyl alcohol and treated with 10 cc. of water. After several hours 3.6 gm. of colorless needles were obtained, which on recrystallization from 50 per cent ethyl alcohol yielded 3.0 gm. of pure diacetonegalactose triacetylglucuronide methyl ester melting at 112.5–114° (uncorrected). The derivative is easily soluble in acetone, ether, methyl and ethyl alcohols, and chloroform.

\[ [\alpha]_D^N = -68.0° \text{ in chloroform (c = 2.0 per cent)} \]

*Analysis*—C_{12}H_{26}O_{14}(COOCH_3)(COCH_3)

Calculated. C 52.05, H 6.30, OCH_3 5.36, COCH_3 22.4


*Preparation of the Synthetic Aldobionic Acid, Galactose-6-β-Glucuronide*—2.5 gm. of diacetonegalactose triacetylglucuronide methyl ester, prepared as above, were dissolved in 100 cc. of acetone and treated with 42 cc. of 0.44 N (4.2 equivalents) of barium hydroxide solution. After the addition of 20 cc. of water, the solution was left at room temperature for 4 hours. The barium was precipitated with N sulfuric acid and the filtrate evaporated in vacuo to remove the acetone. The residue was taken up in 150 cc. of 0.02 N sulfuric acid and boiled for 12 hours. After cooling, the sulfuric acid was removed quantitatively by precipitation with barium hydroxide, followed by centrifugation to remove barium sulfate. The supernatant liquid was extracted once with ether to remove traces of condensation products of acetone and then concentrated in vacuo to a syrup. The syrup became crystalline on stirring with a small amount of water at 0°. A total of 1.5 gm. (92 per cent of the theoretical amount) of colorless needles of the aldobionic acid hydrate were recovered. The substance melted at 118–120° (uncorrected) with effervescence. A sample of aldobionic acid prepared from gum acacia melted at 116–119° (uncorrected). A mixture of this natural acid with the synthetic product melted likewise at 116–119°.

\[ [\alpha]_D^{20} = +9.4° \text{ in water (after 3 minutes), changing to a final value of } -7.3° \text{ within 2 hours (c = 1 per cent).} \]

The natural aldobionic acid hydrate gives an initial specific rotation of +10.5° (after 2 minutes) and a final value of -7.8° (3). The hydrate lost 2 moles
of water of crystallization on drying at 100° to constant weight. A weighed sample of the dried aldobionic acid, when analyzed by the method of Willstatter and Schudel (9), utilized the theoretical quantity of standard iodine solution. Thus, 0.574 gm. of aldobionic acid reduced 6.31 cc. of 0.05 N iodine solution (calculated, 6.45 cc.).

When the synthetic aldobionic acid is converted to the heptaacetyl methyl ester by the method described below, the purified derivative thus obtained melts at 200–201° (uncorrected). The latter resembles in appearance and solubility the same derivative obtained from the natural aldobionic acid. A mixture of the two substances melts at 200–201°. The identity of these derivatives seems, therefore, to be definitely established.

Preparation of the Methyl Ester of Galactose-6-β-Glucuronide—8.8 gm. of aldobionic acid hydrate, prepared from gum acacia, were dissolved in 300 cc. of absolute methyl alcohol and cooled to 0°. An ethereal solution of diazomethane, prepared from 18 gm. of N-nitrosomethylurea (10), was slowly added until a faint yellow color persisted. The solution was concentrated to small volume and placed in the ice box for several days. There were isolated 7.9 gm. of colorless needles. The derivative crystallizes from methyl alcohol with approximately 1 mole of solvent of crystallization. After several recrystallizations from methyl alcohol and drying to constant weight, the methyl aldobionate melted unsharply at 119° (uncorrected), with effervescence.

\[\alpha_0^\circ = -2.9^\circ \text{ in water (after 6 minutes), changing to a final value of } -9.1^\circ (c = 4.0 \text{ per cent}).\]

Analysis—C_{11}H_{14}O_{10}(COOCH_{3})
Calculated.  C 42.14, H 5.99, OCH_{3} 8.38
Found.  " 42.52, " 6.33, " 8.52

Preparation of the Heptaacetyl Methyl Ester of Galactose-6-β-Glucuronide—2.0 gm. of crystalline methyl aldobionate were dissolved in a cold mixture of 10 cc. of dry pyridine and 6 cc. of acetic anhydride and left at 25° for 2\frac{1}{2} hours. The reaction mixture was then poured into 250 cc. of ice water, 30 cc. of chloroform were added, and the suspension was cautiously treated at 0° with sodium hydroxide solution until just neutral. The mixture was extracted three times with chloroform; the extracts were dried and
concentrated in vacuo. The pyridine solution remaining was evaporated with absolute ethyl alcohol three times. The residual syrup was dissolved in 30 cc. of absolute ethyl alcohol and allowed to stand at room temperature for 24 to 48 hours until crystallization was complete. 1.4 gm. of acetyl ester melting at 198–200° were obtained. After recrystallization from absolute ethyl alcohol, the heptaacetylglucuronic acid methyl ester was obtained in the form of shining needles, melting sharply at 202–203° (uncorrected).

\[ [\alpha]_D^\text{B} = -17.5^\circ \text{ in chloroform (c = 3 per cent)} \]

**Analysis**—C\text{\textsubscript{11}}H\text{\textsubscript{19}}O\text{\textsubscript{10}}(COCH\text{\textsubscript{3}})(COOCH\text{\textsubscript{3}})

Calculated. C 48.8, H 5.46, OCH\text{\textsubscript{3}} 4.67, COCH\text{\textsubscript{3}} 45.3

Found. " 48.9, " 5.77, " 4.73, " 44.7

From the value of the specific rotation of the crystalline heptaacetate and from the fact that the amorphous material remaining in the filtrate possessed a higher specific rotation, it may be concluded that the crystalline substance is probably the \(\beta\)-heptaacetate.

**Preparation of \(\beta\)-Heptaacetylgentiobiuronic Acid Methyl Ester**—To 20 cc. of dry, alcohol-free chloroform were added 1.82 gm. of \(\alpha\)-bromotriaacetylgluconic acid methyl ester (1), 1.83 gm. of 1,2,3,4-tetraacetyl-\(\beta\)-glucose (7), and 0.85 gm. of dry silver oxide. The mixture was shaken with glass beads for 75 minutes, or until no free bromine compound remained in the solution. The latter was filtered and evaporated in vacuo. The syrup was dissolved by warming with 10 cc. of absolute methyl alcohol and the solution was allowed to cool slowly. 1.03 gm. of a crystalline product were recovered. Recrystallization from absolute methyl alcohol gave 0.84 gm. of pure \(\beta\)-heptaacetylgentiobiuronic acid methyl ester. The substance melted at 198–199° (uncorrected).

\[ [\alpha]_D^\text{B} = -11.0^\circ \text{ in chloroform (c = 1.0 per cent)}; [M]_D^\text{B} = -7310^\circ \]

**Analysis**—C\text{\textsubscript{11}}H\text{\textsubscript{19}}O\text{\textsubscript{10}}(COCH\text{\textsubscript{3}})(COOCH\text{\textsubscript{3}})

Calculated. C 48.8, H 5.46, OCH\text{\textsubscript{3}} 4.67, COCH\text{\textsubscript{3}} 45.3

Found. " 48.4, " 5.48, " 4.64, " 46.1

**Preparation of \(\alpha\)-Heptaacetylgentiobiuronic Acid Methyl Ester**—0.5 gm. of pure \(\beta\)-heptaacetylgentiobiuronic acid methyl ester was dissolved in 10 cc. of acetic anhydride solution containing 1 gm. of freshly fused zinc chloride. After 14 minutes heating at 50° the
optical rotation of the solution was practically constant. The solution was poured into ice water, extracted with chloroform, and neutralized at 0° with dilute sodium hydroxide solution. The chloroform solution was evaporated in vacuo, and the residue was recrystallized from absolute ethyl alcohol. 0.27 gm. of material was obtained. After several recrystallizations, the derivative melted at 201–202° (uncorrected).

\[ \alpha \] = +48.4° in chloroform (c = 0.9 per cent); [M] = +32,150°

Analysis—C₁₁H₁₁O₁₀(COCH₃)₇(COOCH₃)
Calculated. C 48.8, H 5.46, OCH₃ 4.67, COCH₃ 45.3
Found. " 48.8, " 5.58, " 4.62, " 45.4

The difference in the molecular rotation of the α- and β-heptaacetates is 39,500°.

The heptaacetyl glucose glucuronide methyl ester obtained from the aldobionic acid derived from the specific polysaccharide of Type III pneumococcus melts at 250° and shows the rotation \[ \alpha \] = +41.7° in chloroform (11). A mixture of this substance with the α-heptaacetyl methyl ester of gentiobiuronic acid shows a depression of the melting point, the mixture melting unsharply, beginning at 193°. The two aldobionic acid derivatives are therefore not identical.

**SUMMARY**

1. The aldobionic acid, galactose-6-β-glucuronide, has been synthesized and found to be identical with the aldobionic acid obtained from gum acacia.

2. A second aldobionic acid, gentiobiuronic acid, has likewise been synthesized and obtained in the form of its heptaacetyl methyl ester.

**BIBLIOGRAPHY**

DERIVATIVES OF GLUCURONIC ACID: VII. THE SYNTHESIS OF ALDOBIONIC ACIDS
Rollin D. Hotchkiss and Walther F. Goebel


Access the most updated version of this article at http://www.jbc.org/content/115/1/285.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/115/1/285.citation.full.html#ref-list-1