THE SYNTHESIS OF THE \( p\)-AMINOPHENOL \( \alpha\)-GLYCOSIDES OF MALTOSE, LACTOSE, CELLOBIOSE, AND GENTIOBIOSE

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The attempt to understand the phenomenon of type specificity exhibited by the polysaccharides derived from encapsulated microorganisms has led to a study of the rôle which simple carbohydrates of known chemical structure play in orienting the antigenic specificity of conjugated sugar-proteins. The immunological specificity of complex antigens composed of protein chemically combined with the diazophenol glycosides of monosaccharides appears to be related to differences in the molecular structure of the hexosides, irrespective of the protein to which they are attached. It has been found that the diazophenol glycosides of glucose and galactose, when coupled with protein, incite in the animal body the formation of antibodies specific for the particular carbohydrate radical in the antigenic complex (1). Although these hexosides are identical in structure save for the stereochemical arrangement of the hydrogen and hydroxyl on the 4th carbon atom, yet this change in molecular structure suffices to alter completely the immunological specificity of each glycoside. A similar relationship has been shown to exist in antigens prepared from the \( \alpha \) and \( \beta \)-diazophenol glycosides of glucose (2). Thus the study of the serological and antigenic properties of complex antigens prepared by conjugating proteins with derivatives of monosaccharides has been of value in ascertaining the relationship between biological specificity and chemical constitution of the simple carbohydrates.

The serological specificity of the complex bacterial polysaccharides, on the other hand, is probably governed not alone by differences in molecular configuration. The possibility of variations in intermolecular linkages and their influence in determining
specificity must likewise be taken into account. The attempt to
determine the influence of both inter- and intramolecular changes
on specificity might be made through a study of glycoside-protein
antigens containing disaccharide radicals, the constitutions of
which have been well established. Hence any specific differences
in the immunological properties of these disaccharide antigens
could be directly related to changes in chemical constitution, and a
further insight might be gained into the problem of the chemical
basis underlying the biological specificity of polysaccharides of
bacterial origin.

The p-aminophenol glycosides of maltose, lactose, cellobiose,
and gentiobiose have therefore been synthesized. When the
amino group of the aglucon is converted to its diazonium salt, the
resultant derivative can be combined with protein by means of the
chromophoric group \(-\text{N}==\text{N}\) to yield an artificial protein-
disaccharide antigen. A study of the immunological characteris-
tics of these derivatives will be presented elsewhere. The follow-
ing is an account of the synthesis and chemical properties of these
glycosides.

**EXPERIMENTAL.**

*Heptacetyl p-Nitrophenol P-Lactoside*—Heptacetyl nitrophenol
lactoside was prepared by the method of Glaser (3) by dissolving
100 gm. of acetobromo lactose (4, 5) in 200 cc. of acetone and
adding this to a solution of 39.8 gm. of p-nitrophenol and 11.58
gm. of NaOH in 400 cc. of water and 400 cc. of acetone. After
standing for 15 hours at room temperature, the acetone was
removed \(\text{in vacuo}\). A gummy cake formed in the bottom of the
flask. The supernatant liquid was poured off and discarded.
The cake was washed several times with ice water and finally
dissolved in 200 cc. of absolute methyl alcohol. On cooling,
crystals of heptacetyl p-nitrophenol lactoside separated from the
solution. The substance melted at 132–133\(^\circ\) (corrected).

\[
\left[\alpha\right]^n_D = -\frac{0.97 \times 100}{2 \times 1.369} = -35.4^\circ \text{ (in chloroform)}
\]

*Analysis*—4.300 mg. substance: 7.955 mg. CO\(_2\) and 2.070 mg. H\(_2\)O

C\(_{12}\)H\(_{35}\)O\(_{20}\)N. Calculated. C 50.71, H 5.19

Found. “ 50.45, “ 5.30
\[ [\alpha]_D^\text{25} = \frac{-1.41 \times 10^0}{2 \times 0.9510} = -74.2^\circ \text{ (in water)} \]

Subsequent crystallizations failed to change this specific rotation. The compound darkens at 250° and melts with decomposition at 258–260° (uncorrected).1

\[ [\alpha]_D^\text{25} = \frac{-0.52 \times 100}{2 \times 0.714} = -36.4^\circ \text{ (in water)} \]

The specific optical rotation did not change on subsequent crystallizations. The compound melted sharply at 233° (uncorrected) with decomposition.

1 The melting points of compounds reported are all corrected for temperatures below 220°. Unfortunately, we are not in possession of a calibrated thermometer for temperatures higher than this.
Heptacetyl p-Nitrophenol β-Maltoside—Heptacetyl p-nitrophenol maltoside was prepared by dissolving 50 gm. of acetobromo maltose (7, 8) in 100 cc. of acetone and adding this solution to a solution of 19.9 gm. of p-nitrophenol and 5.74 gm. of sodium hydroxide in 200 cc. of water and 200 cc. of acetone. After standing at room temperature for 5 hours, the acetone was removed in vacuo. The supernatant liquid was poured from a gum which settled to the bottom of the flask. This gum was washed several times with ice water and was finally dissolved in 100 cc. of hot absolute methyl alcohol. Crystals of the glycoside separated and were filtered from the solution. The compound was recrystallized from methyl alcohol. 11 gm. of pure heptacetyl p-nitrophenol maltoside were recovered. The compound melted at 175–176° (corrected).

\[
[a]_D^\infty = \frac{+0.98^\circ \times 100}{1.447 \times 2} = +33.8^\circ \text{ (in chloroform)}
\]

Analysis—5.185 mg. substance: 9.581 mg. CO₂ and 2.365 mg. H₂O
C₂₂H₁₄O₁₉N. Calculated. C 50.71, H 5.19
Found. " 50.40, " 5.10

p-Nitrophenol β-Maltoside—33 gm. of heptacetyl p-nitrophenol maltoside were deacetylated with barium methylete.

The barium was quantitatively removed by precipitation with sulfuric acid. The alcoholic solution of p-nitrophenol maltoside was concentrated to a syrup in vacuo and dissolved in 100 cc. of 95 per cent ethyl alcohol. On cooling, the glycoside crystallized from the solution; 17.0 gm. were recovered. After four recrystallizations from 95 per cent ethyl alcohol, the glycoside melted at 221° (uncorrected).

\[
[a]_D^\infty = \frac{+0.35^\circ \times 100}{4 \times 1.460} = +6.0^\circ \text{ (in methyl alcohol)}
\]

Subsequent crystallizations did not change this specific optical rotation.

Analysis—4.235 mg. substance: 7.220 mg. CO₂ and 2.120 mg. H₂O
C₁₆H₁₂O₁₂N. Calculated. C 46.63, H 5.44
Found. " 46.50, " 5.60

p-Aminophenol β-Maltoside—10 gm. of p-nitrophenol maltoside were catalytically reduced in methyl alcoholic solution, as pre-
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viously described. 7.5 gm. of p-aminophenol maltoside were recovered.

\[ [\alpha]_D^B = \frac{+1.53^\circ \times 100}{4 \times 1.083} = +35.3^\circ \text{ (in 50 per cent methyl alcohol)} \]

Subsequent crystallizations did not change the specific optical rotation. When warmed from 20° at the rate of 1° per second, the glycoside partly collapsed without completely melting at 40°, and then melted sharply at 91–92° (corrected).

\[ \text{Analysis—}4.300 \text{ mg. substance: } 7.815 \text{ mg. } \text{CO}_2 \text{ and } 2.420 \text{ mg. } \text{H}_2\text{O} \]
\[ \text{C}_{18}\text{H}_{21}\text{O}_{11}\text{N. Calculated. } \text{C } 49.87, \text{ H } 6.28 \]
\[ \text{Found. } \text{C } 49.57, \text{ H } 6.30 \]

Heptacetyl p-Nitrophenol β-Gentiobioside—11.8 gm. of acetobromo gentiobiose (9) were dissolved in 50 cc. of acetone and added to a solution of 4.7 gm. of p-nitrophenol and 1.36 gm. of NaOH in 100 cc. of water and 100 cc. of acetone. The mixture stood for 6 hours. The acetone was removed in vacuo, and the crystals of gentiobioside which separated from the solution were filtered and washed with water. The compound was recrystallized twice from a mixture of 30 per cent chloroform and 70 per cent methyl alcohol (by volume). 3.1 gm. of material were recovered. The compound melted sharply at 215–216° (corrected).

\[ [\alpha]_D^\beta = \frac{-0.925^\circ \times 100}{2 \times 0.977} = -47.35^\circ \text{ (in chloroform)} \]

\[ \text{Analysis—}4.880 \text{ mg. substance: } 9.100 \text{ mg. } \text{CO}_2 \text{ and } 2.270 \text{ mg. } \text{H}_2\text{O} \]
\[ \text{C}_{35}\text{H}_{39}\text{O}_{24}\text{N. Calculated. } \text{C } 50.71, \text{ H } 5.19 \]
\[ \text{Found. } \text{C } 50.85, \text{ H } 5.21 \]

p-Nitrophenol β-Gentiobioside—7.6 gm. of heptacetyl p-nitrophenol gentiobioside were suspended in 150 cc. of absolute methyl alcohol and deacetylated by the method described. 4.4 gm. of p-nitrophenol gentiobioside were recovered. After two recrystallizations from absolute alcohol the compound melted at 221–223° (uncorrected).

\[ [\alpha]_D^B = \frac{-1.86^\circ \times 100}{2 \times 0.884} = -105.2^\circ \text{ (in 50 per cent methyl alcohol)} \]
Subsequent crystallization did not change the specific optical rotation.

**Analysis**—4.152 mg. substance: 7.100 mg. CO2 and 2.018 mg. H2O
C16H16O12N. Calculated. C 46.63, H 5.44
Found. " 46.65, " 5.44

*p*-Aminophenol β-Gentiobioside—4.3 gm. of optically pure *p*-nitrophenol gentiobioside were reduced catalytically at 50°. 3.3 gm. of a snow-white product were recovered from the mother liquor. When recrystallized from 90 per cent ethyl alcohol, the substance melted, with decomposition, at 237–238° (uncorrected).

\[
\frac{[\alpha]_D^2}{2 \times 0.914} = -79.8° \text{ (in water)}
\]

**Analysis**—4.755 mg. substance: 8.626 mg. CO2 and 2.825 mg. H2O
C18H21O11N. Calculated. C 49.85, H 6.28
Found. " 49.51, " 6.64

Heptacetyl *p*-Nitrophenol β-Cellobioside—Heptacetyl *p*-nitrophenol cellobioside was prepared by adding an acetone solution of 50 gm. of acetobromo cellobiose (8, 10) to a solution of 19.9 gm. of *p*-nitrophenol and 5.74 gm. of NaOH in 200 cc. of water and 200 cc. of acetone. After standing 5 hours at room temperature, the acetone was removed in vacuo. An equal volume of cold water was added, and the crystals of *p*-nitrophenol cellobioside filtered and washed with water until colorless. 20 gm. of glycoside were recovered. The compound when recrystallized from methyl alcohol melted at 234–235° (uncorrected).

\[
\frac{[\alpha]_D^2}{1.972 \times 2} = -42.6° \text{ (in chloroform)}
\]

**Analysis**—4.737 mg. substance: 8.736 mg. CO2 and 2.160 mg. H2O
C26H38O28N. Calculated. C 50.71, H 5.19
Found. " 50.29, " 5.10

*p*-Nitrophenol β-Cellobioside—20 gm. of heptacetyl nitrophenol cellobioside were deacetylated by the method previously described. After removing the barium quantitatively with sulfuric acid, the supernatant liquid was concentrated in vacuo. Crystals of *p*-nitrophenol cellobioside were separated by filtration, and re-
peatedly recrystallized from 80 per cent ethyl alcohol. 12.5 gm. of glycoside were recovered. The compound darkened at 245° and melted with decomposition at 255–256° (uncorrected).

\[ [\alpha]_D = \frac{-5.82 \times 100}{4 \times 1.710} = -85.1^\circ \text{ (in 40 per cent methyl alcohol)} \]

**Analysis**—4.820 mg. substance: 8.245 mg. CO₂ and 2.340 mg. H₂O  
C₁₈H₁₉O₁₁N. Calculated. C 46.63, H 5.44  
Found. C 46.66, H 5.43

**p-Aminophenol β-Cellobioside**—5.0 gm. of optically pure p-nitrophenol cellobioside were reduced catalytically in 90 per cent methyl alcohol. 3.8 gm. of p-aminophenol cellobioside were recovered.

\[ [\alpha]_D = \frac{-2.49 \times 100}{4 \times 1.176} = -52.9^\circ \text{ (in 50 per cent methyl alcohol)} \]

Subsequent recrystallizations failed to alter the optical rotation of this derivative. The compound had a peculiar melting point. When the temperature of the bath was raised 1° per second, the glycoside partly collapsed at 170°, but did not melt. When the temperature of the bath was further raised, the substance melted sharply at 245° (uncorrected) with decomposition.

**Analysis**—4.731 mg. substance: 8.620 mg. CO₂ and 2.680 mg. H₂O  
C₁₈H₁₉O₁₁N. Calculated. C 49.87, H 6.28  
Found. C 49.70, H 6.34

In conclusion it may be said that the glycosides described above are of the β variety. Aqueous solutions of the nitrophenol glycosides of gentiobiose, lactose, cellobiose, and maltose were treated with emulsin in acetate buffer at pH 4.8 and at 37°. In each instance a marked splitting of the glycoside by the enzyme occurred, whereas control experiments with heated enzyme, buffer at the same pH and glycoside of the same concentration, showed no liberation whatsoever of the aglucon.

**SUMMARY**

The synthesis of the p-aminophenol β-glycosides of gentiobiose, cellobiose, maltose, and lactose has been described.
BIBLIOGRAPHY

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