[Contribution from the Carbohydrate Laboratory, Bureau of Chemistry, United States Department of Agriculture.]

# THE ROTATORY POWERS OF SOME NEW DERIVATIVES OF GENTIOBIOSE.

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Three disaccharides have the composition *glucose*<*glucose*<, namely maltose, cellose and gentiobiose. While the first two have been studied considerably and many derivatives of them have been prepared, the only compounds from gentiobiose that have ever been described are the phenylosazone[1] and the octacetate.[2] Peculiar interest attaches to the latter substance because Zemplen has shown that it may be prepared directly from powdered gentian root, thus opening a way for readily obtaining a crystalline derivative of this very rare sugar. Since we desired to search for an octacetate of gentiobiose that should be isomeric with the one discovered by Zemplen, we began the preparation of the latter substance. By the introduction of certain modifications in his procedure we found that the yield could be increased from his value of 1.2 g. per kilo of dried gentian root to 10 g. per kilo. Having obtained from this source a considerable supply of Zemplen's octacetate, we sought to prepare certain derivatives of it, the rotatory powers of which could be calculated in advance by comparison with the specific rotations of similar derivatives of other sugars. These experiments have yielded in pure crystalline condition the expected isomeric octacetate of gentiobiose, a heptacetyl methyl gentiobioside and a methyl gentiobioside. Before passing to the description of these preparations, the calculation of the rotatory powers of the three new derivatives will be presented.

## Calculation of the Rotatory Powers of Alpha Gentiobiose Octacetate, the Alpha and Beta Methyl Gentiobiosides and Their Heptacetates.

We have previously shown[3] that the α- and β-octacetates of maltose, of specific rotations +122 and +63, differ by 59°, those of lactose by 57° and those of cellose by 56°. The reason for the constancy of this difference has been shown to lie in the constant value of the rotatory power of that end asymmetric carbon atom which is common to these octacetyl sugars. Since the analogous α- and β-octacetates of gentiobiose would be expected to have similar structure, their specific rotations should also differ by this constant quantity, 57°. Zemplen found the specific rotation of his octacetate in chloroform to be —5.3, a value which we have duplicated, hence the rotation of its isomeric form is calculated to be 57° higher or lower than this value, according as Zemplen's acetate is considered to be the β- or α-modification. It is highly probable that it is the β-form

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[1] Bourquelot and Herissey, *Compt. rend.*, 132, 573 (1901).

[2] Zemplen, *Z. physiol. Chem.*, 85, 402 (1913).

[3] This Journal, 37, 1279 (1915).

because the method of its preparation by the action of acetic anhydride and sodium acetate on a sirup containing gentiobiose is a procedure that usually yields beta acetates of the sugars. Assuming such to be the case, the specific rotation of the expected isomeric α-octacetate of gentiobiose is calculated to be $(-5 + 57) = +52°$.

In a previous article[1] it was shown that the rotatory powers of the α- and β-forms of gentiobiose can be calculated from the rotations of sucrose, gentianose and the α- and β-forms of *d*-glucose. The values thus obtained were +39 and —11 for α- and β-gentiobiose, respectively. Let us now calculate the rotatory powers of the corresponding α- and β-methyl gentiobiosides by the method which has previously been explained in connection with the rotations of the methyl glucosides.[2] The molecular rotation of methyl gentiobioside is assumed to be the sum of the rotation of the gentiobiose chain (Gb) and the rotation of the asymmetric lactonyl carbon atom (B′) of the methyl glucosides. The molecular rotation of the gentiobiose (m. w. 342) chain (Gb) is $(39 - 11)342/2 = 4790$, and the value of B′ has been shown to be 18430.[3] Since the molecular weight of methyl gentiobioside is 356, the specific rotation in water of its α-modification is calculated to be $(\text{Gb} + \text{B}')/356 = +65°$ and that of its β-form to be $(\text{Gb} - \text{B}')/356 = -38°$.

In quite similar manner the rotations of the heptacetyl methyl gentiobiosides may be calculated.[4] The molecular rotation of the acetylated gentiobiose chain (Gb′) may be obtained from the previously mentioned specific rotations in chloroform of the α- and β-octacetates of gentiobiose (m. w. 678), to be $(52 - 5)678/2 = 15900$. The value of the rotation of the lactonyl carbon of the acetylated methyl glucosides (B″) has been found[5] to be 26900, hence the specific rotation of heptacetyl α-methyl gentiobioside (m. w. 650) in chloroform is calculated to be $(\text{Gb}' + \text{B}'')/650 = +66°$ and that of the β-form to be $(\text{Gb}' - \text{B}'')/650 = -17°$.

## Preparation of the Beta Octacetate of Gentiobiose from Gentian Root.

Zemplen[6] first prepared this octacetate by acetylating the sirup which was obtained from an aqueous extraction of the powdered gentian root of commerce, but the yield was only 1.2 g. recrystallized acetate from a kilogram of the root. Later[7] he used an alcoholic gentian extract such as pharmacists keep in stock, with somewhat better results.

[1] Hudson, This Journal, 38, 1569 (1916).

[2] Hudson, *Ibid.*, 31, 66 (1909).

[3] *Ibid.*, 31, 70 (1909). In the present calculation the more recent values of Bourquelot (*J. pharm. Chem.*, [7] 14, 2 (1916)) are used, namely, $[\alpha]_D^{20}$ for α-methyl glucoside = +157.9° and for the β-isomer = —32.5°.

[4] Hudson and Sayre, *Ibid.*, 38, 1867 (1916).

[5] Hudson and Dale, *Ibid.*, 37, 1265 (1915).

[6] *Loc. cit.*

[7] *Ber.*, 48, 233 (1915).

After many experiments we were able to introduce into Zemplen's method of treating gentian root certain modifications which resulted in a largely increased yield of gentiobiose octacetate. However, it should be mentioned for the benefit of those who may use these directions that several of the numerous samples of gentian root which we tested, representing purchases from many dealers, gave unsatisfactory yields. It would be advisable, therefore, for such chemists to purchase powdered gentian root after testing samples from several sources.

Two hundred and fifty g. of powdered gentian root (*Gentiana lutea*) were mixed with 2000 cc. water at 30°, bakers' yeast added and the mixture allowed to ferment to completion which required two days, more yeast having been added on the morning of the second day. Lead subacetate solution (sp. gr. 1.25) was added (100 cc.), the mixture well shaken and filtered on a large Büchner funnel. The filtrate and wash water were treated with $H_2S$ gas until all lead was precipitated and the excess of $H_2S$ was blown out by an air current. It was then filtered on a Büchner funnel containing a mat of decolorizing carbon. The filtrate came through almost colorless. It was then evaporated with the aid of a good water-pump to a sirup as dry as possible, the temperature of the outside bath being 65°. This sirup was digested under a reflux condenser on a steam bath with 75 cc. absolute methyl alcohol during thirty minutes with frequent vigorous shaking. The mixture was then cooled rapidly to 20°, filtered, and the filtrate poured with stirring into such a volume of absolute alcohol (about 750 cc.) that an addition of more alcohol caused no further precipitation. After filtration the alcoholic solution was evaporated under reduced pressure to a sirup as dry as possible which was then acetylated by heating it on the steam bath with four times its weight of acetic anhydride and one-fourth its weight of anhydrous sodium acetate. On pouring the solution into water an insoluble phase separated and in the course of a few hours began to crystallize. After 24 hours the crystals were separated from the viscous mother liquor with 50% alcohol. The sirupy precipitate which has been mentioned as resulting from the addition of the methyl alcoholic solution to absolute alcohol was also acetylated and yielded a small amount of the crystalline octacetate, which was difficult to purify however. The crude octacetate was recrystallized first from 50% alcohol and then from absolute methyl alcohol, and the yield of pure gentiobiose octacetate averaged about 2.5 g. from 250 g. gentian root. In total about 425 g. of the octacetate were prepared. Its melting point was 192–3°, corrected, and its specific rotation in chloroform was to the left, $[\alpha]_D^{20} = -5.3°$, in concentrations of 6.3 and 12.9 g. per 100 cc. These data agree with Zemplen's observations.

### Transformation of the Beta Octacetate to Its Alpha Isomer.

When 10.7 g. of β-gentiobiose octacetate were dissolved in 100 cc. acetic anhydride containing 2 g. $ZnCl_2$ and the solution heated on the steam bath the specific rotation changed gradually from —1.9° to the constant value of +48.0° in 40 minutes. The direction of this change is further indication that Zemplen's octacetate is the β-form and the magnitude of the change indicates that the equilibrium between the β- and α-form in acetic anhydride is far towards the α-form. When the transformed solution was poured into water, an insoluble phase separated and quickly crystallized. The product was recrystallized from 50% alcohol and the yield was 6.5 g. It was recrystallized from absolute methyl alcohol until the constancy of its specific rotation indicated that purity had been attained. Two recrystallizations were necessary. The pure substance melted at 188–9° corrected.

On combustion 0.1924 and 0.1904 g. substance yielded 0.3498 and 0.3442 g. $CO_2$, and 0.0954 and 0.1002 g. $H_2O$, corresponding to 49.58 and 49.33% C, and 5.55 and 5.65% H, respectively. Calculated for a gentiobiose octacetate, 49.54% C and 5.65% H.

An acetyl estimation made by boiling in a quartz flask with a reflux quartz condenser 0.5 g. substance with 100 cc. 0.25 *N* $H_2SO_4$ during 4 hours indicated 50.74% acetyl ($CH_3CO$), and a duplicate experiment in which the boiling lasted 6 hours gave 50.55% acetyl. The theoretical value for a gentiobiose octacetate is 50.74%.

The specific rotation was measured in pure chloroform. A solution containing 0.4731 g. substance to 25 cc. solution rotated to the right in a 20 cm. tube at 20° with sodium light +1.98°, circular, hence $[\alpha]_D^{20} = +52.3°$. In a second determination 0.8807 g. substance to 25 cc. rotated +3.69, hence $[\alpha]_D^{20} = +52.4°$. This rotation agrees within the limits of error with the value that has been calculated for α-gentiobiose octacetate, +52°, and there can be no doubt that the substance is the expected α-isomer, Zemplen's octacetate being thus proved to be the β-form.

### Preparation of Beta Heptacetyl Methyl Gentiobioside.

This compound was prepared in the usual way by transforming the β-gentiobiose octacetate with a solution of HBr in acetic acid[1] to the bromoheptacetyl derivative which was obtained as a sirup, and then transforming the latter in methyl alcohol with $Ag_2CO_3$ to β-heptacetyl methyl gentiobioside, which crystallized. As the yield is low the exact directions are recorded.

Ten g. of β-gentiobiose octacetate were dissolved in 20 cc. glacial acetic acid, 80 cc. of a saturated solution of HBr gas in glacial acetic acid were added and the mixture allowed to stand until the rotation became constant, which required somewhat less than one hour. About 100 cc. chloroform were then added and the mixture was poured into a separatory

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[1] Fischer, *Ber.*, 49, 584 (1916).

funnel containing much ice water and ice. The chloroform solution was washed again with ice water, then with sodium bicarbonate solution, and twice again with ice water. After drying it with $CaCl_2$ and filtering, the chloroform was evaporated under reduced pressure. The residue of sirupy acetobromogentiobiose could not be brought to crystallization. It was accordingly dissolved in absolute methyl alcohol and shaken with an excess of freshly prepared, dry silver carbonate until the solution was free from bromine, which required about three hours. The insoluble silver salts were filtered off and the filtrate evaporated in a partial vacuum to a sirup. A solution of this in a little absolute ether crystallized after 2 or 3 hours. The substance was recrystallized from ether, and then from 95% alcohol, and a yield of one gram was obtained. A larger quantity of it was carefully purified by recrystallization from 95% alcohol until the rotation reached a constant value, which required three crystallizations. The pure substance melted at 82°, corrected. The following data show that it is the fully acetylated derivative (heptacetate) of β-methyl gentiobioside.

On combustion 0.1680 and 0.1894 g. substance gave 0.0902 and 0.1013 g. $H_2O$, and 0.3065 and 0.3432 g. $CO_2$, corresponding to 6.01 and 5.98% H, and 49.76 and 49.42% C, respectively. Calculated for heptacetyl methyl gentiobioside, $C_{12}H_{14}O_{10}$-$(CH_3CO)_7(OCH_3)$: 5.89% H and 49.82% C.

Acetyl determinations were made by saponifying approximately 0.3 g. samples of the substance with 75 cc. 0.1 *N* sodium hydroxide during one or two hours' shaking at 0°.[1] Three experiments gave the values 46.47, 46.60 and 46.50% acetyl ($CH_3CO$) in comparison with the theoretical, 46.31%.

In methoxyl estimations 0.2270 and 0.2376 g. substance gave 0.0826 and 0.0851 g. AgI, respectively, corresponding to 4.89 and 4.73% ($OCH_3$), agreeing with the theoretical value, 4.77%.

The pure substance is levorotatory in chloroform. A solution in pure chloroform containing 2.5389 g. substance in 25 cc. solution rotated to the left in a 20 cm. tube at 20° with sodium light, —3.83°, circular, hence $[\alpha]_D^{20} = -18.8°$. In repetition 2.7014 g. substance per 25 cc. rotated —4.10, hence $[\alpha]_D^{20} = -18.9°$.

From the method of preparation of this heptacetyl methyl gentiobioside it is to be expected that the substance is the β-isomer. The close agreement between its rotation (—19°) and that which has previously been calculated for that isomer (—17°) leaves no doubt that the substance is indeed β-heptacetyl methyl gentiobioside.

## Preparation of Beta Methyl Gentiobiose.

Eight g. of β-heptacetyl methyl gentiobioside were saponified with a cold solution of barium hydroxide according to the directions that have been published in connection with the preparation of methyl fructoside from

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[1] Hudson and Brauns, THIS JOURNAL, 38, 1219 (1916).

its tetracetate.[1] The methyl gentiobioside crystallized readily and 2.95 g. were obtained after recrystallization from absolute alcohol until the rotation was constant. The pure substance melted at 98°, uncorrected. It does not reduce Fehling's solution.

On combustion 0.1675 and 0.1806 g. substance yielded 0.1044 and 0.1137 g. $H_2O$, and 0.2672 and 0.2883 g. $CO_2$, corresponding to 6.97 and 7.04% H, and 43.51 and 43.54% C, respectively. Theoretical for methyl gentiobioside, $C_{12}H_{21}O_{10}(OCH_3)$: 6.74% H and 43.82% C.

Methoxyl estimations with 0.2558 and 0.2380 g. substance gave 0.1738 and 0.1570 g. AgI, corresponding to 8.97 and 8.70% $OCH_3$, which agree with the theoretical value, 8.71%.

The compound is levorotatory in aqueous solution. A solution of 1.9779 g. of the pure substance in 25 cc. solution rotated to the left in a 2 cm. tube at 20° with sodium light, —5.73°, circular, hence $[\alpha]_D^{20} = -36.2°$. In repetition 2.2169 g. substance per 25 cc. rotated —6.36°, hence $[\alpha]_D^{20} = -35.9$, or the average value is —36.0°. This rotation agrees very well with the calculated value for β-methyl gentiobioside, —38°.

## Summary.

Zemplen's preparation of pure crystalline octacetyl gentiobiose from the acetylation of the sirup that may be readily obtained from commercial gentian root has opened a way for a study of several of the more important derivatives of this very rare disaccharide which is known to have the structure *glucose*<*glucose*< and is accordingly closely related to maltose and cellose. By introducing certain modifications in Zemplen's directions we have increased the yield of gentiobiose octacetate from 1.2 to 10 g. per kilo of dry gentian root, and have prepared a supply of 425 g. of the substance. By transforming it in acetic anhydride solution with $ZnCl_2$ an isomeric octacetate of gentiobiose has been crystallized, which proves to be the α-form, Zemplen's octacetate being the β-isomer. The β-octacetate has also been converted to β-heptacetyl methyl gentiobioside, and the latter to β-methyl gentiobioside, both substances crystallizing. It is possible to calculate the rotatory powers of these new derivatives from the rotations of other substances on the assumption of the principle of optical superposition, and it is found that the values so calculated agree satisfactorily with the values found by direct measurement.

| Substance. | Solvent. | Observed $[\alpha]_D^{20}$. | Calculated $[\alpha]_D^{20}$. |
|---|---|---|---|
| α-Gentiobiose octacetate......... | chloroform | +52° | +52° |
| β-Heptacetyl methyl gentiobioside | chloroform | —19° | —17° |
| β-Methyl gentiobioside.......... | water | —36° | —38° |

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[1] Hudson and Brauns, THIS JOURNAL, 38, 1220 (1916).