TABLE II

EXTINCTION COEFFICIENTS AND BASE STRENGTH OF *p*-BROMOACETOPHENONE

Wave length, Å.	%, H_2SO_4 0	60.1	73.7	76.2	78.4	80.4	90.9	96.0
3700							100	130
3600			70	130	170	260	740	1250
3500			420	730	1200	1850	5200	6500
3400		80	1850	3300	4800	6600	14500	18000
3300	60	180	4600	7300	10000	12500	22000	24500
3200	100	490	7000	10600	13900	17000	23100	13800
3100	160	1150	9200	12200	14700	17000	19000	17900
3000	350	2700	10600	11700	12800	13800	12100	10000
2900	1600	5700	11300	11200	10700	10000	6100	4700
2800	4300	10000	11700	10200	8500	7000	2800	2000
2700	11000	15000	11300	8500	6400	4800	1200	860
2600	16700	14900	8300	6100	4400	2900	600	440
2500	13500	8800	4600	3200	2100	1450	360	305
2400	6600	4000	1700	1300	1050	840	700	980
$H°$		−4.33	−6.00	−6.30	−6.58	−6.88	−8.27	−8.88
					pK'			
pK'			−6.40 ±0.17	−6.38 ±0.08	−6.38 ±0.13	−6.45 ±0.10		

Average value of *pK'*, −6.40.

method of measurement. Nevertheless, it may be said with certainty that this is a reaction in which substituents have large effects, the best value of the reaction constant ρ being −2.56 (in terms of the basic ionization) with a "probable error" of 0.12. That is to say, the effect of a substituent upon the quantity *pK'* of acetophenone is approximately 2.56 times as great as its effect upon the *pK* of benzoic acid. This is in interesting contrast with the effects of substituents upon rate of the acid catalyzed bromination of acetophenone,[3] which undoubtedly proceeds by way of the same basic ionization, and for which the value of ρ is only −0.55.[2a]

(3) Nathan and Watson, *J. Chem. Soc.*, 217 (1933); Evans, Morgan and Watson, *ibid.*, 1167 (1935).

Summary

We have measured the ultraviolet absorption of solutions of *p*-methylacetophenone and of *p*-bromoacetophenone in various mixtures of sulfuric acid and water, and have derived therefrom values of the base strengths of these ketones. The effect of substituents upon the free energy of the basic ionization of acetophenone is of the order of −2.56 times the effect upon the acid ionization of benzoic acid.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

The Synthesis of Crystalline 6-[β-*d*-Glucosido]-α-*d*-mannose, the Epimer of Gentiobiose, and its Octaacetate[1]

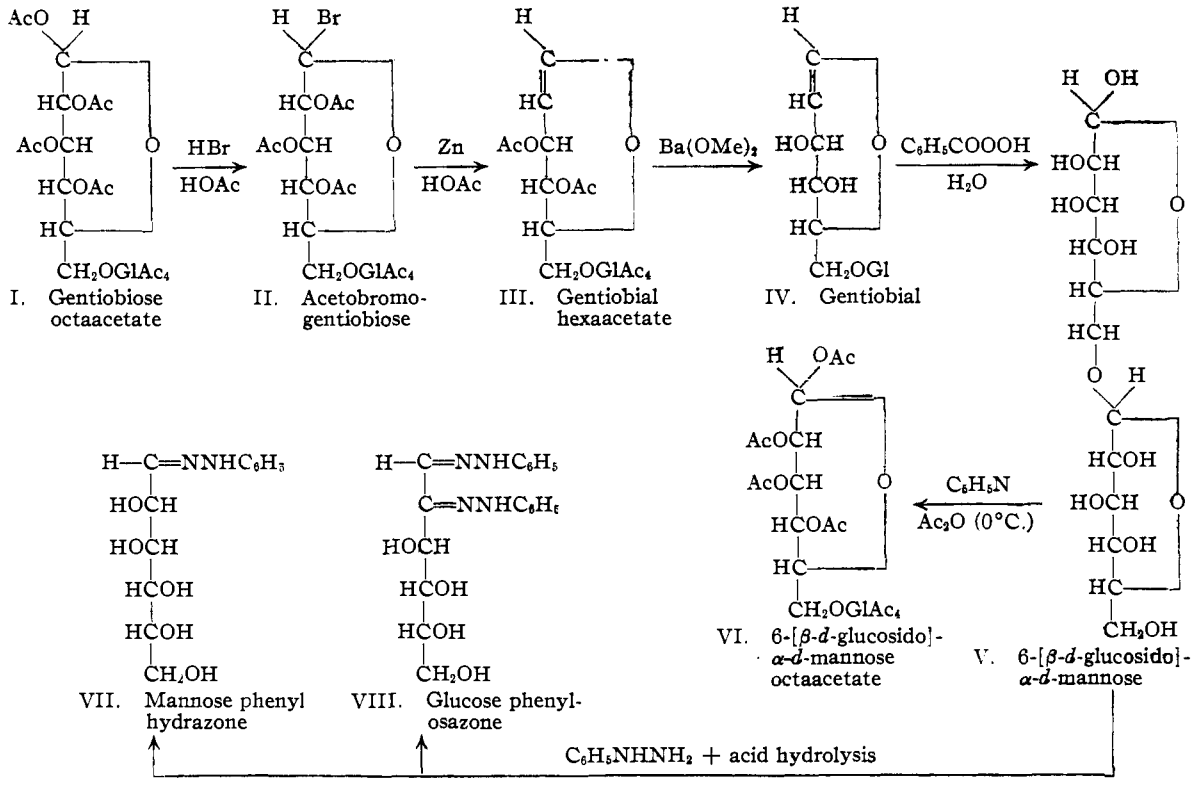
BY HYP J. DAUBEN, JR., AND WM. LLOYD EVANS

Although several epimeric pairs of disaccharides have been synthesized and their properties carefully studied, they have been exclusively of the 4-linked type. No 6-linked epimeric pair has been reported. Foreseeing the possible problems which would arise from such a synthesis, gentiobiose was epimerized by the glycal-oxidation method of Bergmann and Schotte[2] to give 6-glucosidomannose, *i. e.*, epigentiobiose. This afforded the first application of this method of synthesis to 6-linked disaccharides and at the same time offered an opportunity to observe whether the *cis* epimer (*cis* C_2-C_3 configuration) was formed in preponderance by this type of saccharide.[3]

(1) This paper is based upon a thesis submitted by Hyp J. Dauben, Jr., to the Graduate School of The Ohio State University in partial fulfilment of the requirements for the degree of Master of Science.

(2) Bergmann and Schotte, *Ber.*, **54**, 440 (1921).

(3) Levene and Tipson, *J. Biol. Chem.*, **93**, 631 (1931).



From a study of the optical rotational properties of this new disaccharide, it should be possible: (1) to compare the observed molecular rotational value with that calculated by the application of Hudson's rules; (2) to calculate the epimeric difference of a disaccharidic pair and to compare it with differences for similar pairs; (3) to correlate the behavior of this compound with other members of the mannose series. This disaccharide would also be a valuable reference compound for use in the study of the structure of polysaccharides which contain both mannose and glucose.

Experimental Part

Preparation of β-Gentiobiose Octaacetate (I).—This compound was prepared according to the method of Bourquelot, Hérissey and Coirre and of others,[4] which consisted essentially of the acetylation of gentiobiose, synthesized enzymatically from glucose. The material, after the original crystallization from ether, was recrystallized several times from hot, absolute methanol using Carboraffin for decolorization; m. p. 196° (corr.); $[\alpha]^{24}D$ −6.8° (*c*, 2.0; *l*, 2; $CHCl_3$).[5]

Anal. Calcd. for $C_{28}H_{38}O_{19}$: C, 49.6; H, 5.65. Found: C, 49.6; H, 5.71.

Preparation of Acetobromogentiobiose (II).—This was prepared from gentiobiose octaacetate by the method of Brauns.[6] The yield of white crystals obtained in the original crystallization was 57% of the theoretical. It had a m. p. of 135–136° (corr.) and was of sufficient purity for subsequent use.

Preparation of Gentiobial Hexaacetate (III).—Certain modifications of the general method of preparation of Bergmann and Freudenberg[7] gave increased yields. Acetobromogentiobiose (21.1 g.) was dissolved in 75% acetic acid (335 cc.) in a three-necked round-bottomed flask equipped with a mechanical stirrer and the solution was then cooled to 0° before adding zinc dust (36 g.) slowly with stirring to prevent the formation of lumps. The mixture was stirred at 0° for four hours, no chloroplatinic acid being used as a catalyst.[8] The excess zinc was filtered, washed with small portions of water, and the filtrate diluted with an equal volume of water. The solution was extracted twice with 450-cc. portions of chloroform, the chloroform extracts being washed with water, sodium bicarbonate solution, and water in that order. The chloroform solution was then dried with anhydrous sodium sulfate and decolorized with Carboraffin. The filtrate was concentrated first under reduced pressure at 40° to a thin sirup and finally to a thick sirup in a dry stream of air. An equal volume of anhydrous ether was added and the solution blown down slowly, crystallization taking place. After twelve hours' standing in the refrigerator, the cake

(4) Bourquelot, Hérissey and Coirre, *Compt. rend.*, **157**, 732 (1913); Zemplén, *Ber.*, **48**, 236 (1915); J. F. Leete, Ph.D. Dissertation, University of Greifswald, 1929.

(5) This notation has the meaning (concentration, g./100 ml. soln.; length of tube, dm.; solvent).

(6) Brauns, THIS JOURNAL, **49**, 3170 (1927).

(7) Bergmann and Freudenberg, *Ber.*, **62**, 2783 (1929).

(8) *Cf.* Haworth, Hirst, Streight, Thomas and Webb, *J. Chem. Soc.*, 2636 (1930); Felton and Freudenberg, THIS JOURNAL, **57**, 1637 (1935).

of crystals was filtered free of any sirup and washed with 25% methanol. The filtrate yielded another crop upon repetition of the crystallization process; yield 16.3 g., 96%; m. p. 121–122° (corr.); $[\alpha]^{18}D$ −7.4° (*c*, 1.1; *l*, 2; $CHCl_3$).

Preparation of Gentiobial (IV).—The barium methylate method of deacetylation of Weltzien and Singer[9] as used by Isbell[9] was employed with certain modifications. After the deacetylation was complete, the barium methylate was neutralized carefully with dilute sulfuric acid in the presence of phenolphthalein. The gelatinous barium sulfate was filtered through a layer of filtercel, the filtrate being treated with Carboraffin after which it was filtered again. The colorless solution was then concentrated to a small volume in a stream of dry air during which crystallization took place. After standing in the refrigerator for several days, the crop of small needles was filtered and washed with cold, absolute methanol. Upon repetition of the crystallization process, the mother liquor yielded a second small crop. After drying over phosphorus pentoxide, the total yield was 65% of the theoretical and had a m. p. of 191–192° (corr.).

6-Glucosido-α-mannose (V).—Gentiobial (4.5 g.) was dissolved in water (45 cc.) in a round-bottomed flask equipped with an efficient stirrer. When the aqueous solution had been cooled to 5°, perbenzoic acid[10] (3.6 g.) in ethyl acetate (45 cc.) was added in one portion and stirred at 10° for one hour, at 15° for two hours, and at 20° for two hours, at which time the aqueous layer no longer decolorized bromine water. The layers were carefully separated in a separatory funnel (the water layer containing the product) and the ethyl acetate layer extracted several times with small volumes of water. The combined aqueous extracts were extracted three times with chloroform to remove any benzoic acid. After treatment with Carboraffin at 50° for fifteen minutes, the extract was filtered through hardened filter paper. It was then concentrated under reduced pressure at 40°, or in a dry air stream to a thick, water-clear sirup. The sirup was dissolved in a small volume of 75% ethanol (20 cc.) and the resulting solution cooled to refrigerator temperature before adding absolute ethanol to incipient turbidity. On returning to the refrigerator, crystallization began soon thereafter (four hours). After several days the crystalline mass was filtered, washed with 95% ethanol and dried in the air. From the mother liquor two additional small crops could be obtained by concentration and subsequent solution in 80% methanol. When warmed gently, the crystalline material reduced Fehling's solution. It had a mildly bitter taste. It effloresced when placed over strong desiccants but was completely stable over soda-lime. This behavior was indicative of water of crystallization. The total yield (3.5 g.) was 68% of the theoretical on the basis of the gentiobial and 23% on the basis of the gentiobiose octaacetate used.

The product was recrystallized by dissolving in 75% ethanol and adding ethanol to turbidity. The crystals (microscopic triangular prisms) were air-dried and kept in a desiccator over soda-lime. Different crystalline habits of the α-monohydrate could be obtained from different solvents; prisms with triangular cross section from 80% ethanol, flat rectangular rods from 80% methanol, and rods with a square cross section from water.

Anal. Calcd. for $C_{12}H_{22}O_{11}\cdot H_2O$: C, 39.98; H, 6.72; H_2O (of hydration), 5.00. Found: C (micro), 40.23; H (micro), 7.11; H_2O, 5.18.

The anhydrous material, as prepared by dehydration in a drying pistol at the temperature of boiling toluene, was very hygroscopic and appeared amorphous under the microscope.

Anal. Calcd. for $C_{12}H_{22}O_{11}$: C, 42.08; H, 6.48. Found: C (semi-micro), 41.80; H (semi-micro), 6.40.

The anhydrous compound had a m. p. of 167.5–168.0° (corr.) in a closed tube. A similar m. p. could be obtained by heating the monohydrated material very slowly in an open tube. The m. p. of the monohydrate, which was 137.0–138.0° (corr.), could be obtained only when a closed tube was used.

Acid hydrolysis was used to determine the composition of the two constituents of the disaccharide. The method which was used by Brauns[11] for 4-glucosidomannose was employed, giving mannose phenylhydrazone (VII) which, after recrystallization from 60% ethanol, melted at 197° (corr.). A mixed melting point with an authentic sample gave no depression. The filtrate from the hydrazone yielded glucose phenylosazone (VIII), which after recrystallization from aqueous ethanol, had a m. p. of 208° (corr.). It likewise gave no depression in a mixed m. p. determination.

For the optical rotation measurements, 0.5069 g. of the powdered monohydrate was dissolved in 15.11 ml. of distilled water at 20° in a volumetric flask, mixed by shaking, and transferred to a 2-dm. polarimeter tube as rapidly as possible. Time was measured from the moment the first drop of water contacted the sugar. The specific rotation values, at 20° and calculated for the anhydrous sugar, were

Time, min.	$[\alpha]^{20}D$	$\frac{1}{t}\log\frac{r_0 - r_\infty}{r_t - r_\infty} = k_1 + k_2$
0.00	− 5.09°	...
2.58	− 5.65	0.017
4.83	− 6.12	.017
5.75	− 6.27	.017
6.50	− 6.43	.017
7.33	− 6.64	.018
8.67	− 6.98	.019
11.17	− 7.53	.020
14.58	− 8.16	.021
16.83	− 8.31	.020
20.50	− 8.78	.020
24.50	− 9.10	.020
28.00	− 9.33	.019
38.67	−10.04	.020
43.50	−10.19	.019
63.67	−10.67	.019
79.00	−10.82	.018
93.00	−10.90	.017
120.00	−10.98	.016
Final	−11.06	...
		Average 0.019

(9) Weltzien and Singer, *Ann.*, **443**, 104 (1925); Isbell, *Bur. Standards J. Research*, **5**, 1185 (1930).

(10) Braun, *Org. Syntheses*, **13**, 86 (1933).

(11) Brauns, THIS JOURNAL, **48**, 2787 (1926).

The initial rotation value was determined by extrapolation to zero time of the graph t *vs.* log $(r_t - r_\infty)$. Using this initial value of −5.09° and the final value of −11.06°, the individual mutarotation velocity coefficients were calculated for each observed value by the unimolecular law $\left(k_1 + k_2 = \frac{1}{t} \log \frac{r_0 - r_\infty}{r_t - r_\infty}\right)$.[12] Since this form of the sugar which is a sugar of the *d*-series mutarotates to a less positive value, it is the α-isomer according to the nomenclature of Hudson.[13] The mutarotation follows approximately the unimolecular course, its average velocity coefficient $(k_1 + k_2)$ being 0.019 at 20°. This value agrees better with that of mannose (0.019)[14] at the same temperature than with the value for gentiobiose (0.010)[15] at 22°.

6-Glucosido-α-mannose Octaacetate (VI).—Finely powdered 6-glucosido-α-mannose monohydrate (0.50 g.) was added to a cold (0°) mixture of pyridine (12 cc.) and acetic anhydride (14 cc.) in a small round-bottomed flask. The mixture was stirred mechanically at 0° for three hours, all the sugar dissolving in that time. It was then placed in the refrigerator for two days before adding it dropwise from a separatory funnel into a mechanically stirred ice-water mixture (450 cc.). The product separated out during the addition, but the entire solution was placed in the refrigerator overnight to complete crystallization. The white crystals were filtered and washed several times with water, giving a dry yield of 69% (0.65 g.). They were recrystallized by dissolving in hot absolute ethanol, treating with Carboraffin on a steam-bath, and filtering while hot, crystals forming in the filtrate. After standing in the refrigerator for twelve hours, the white micaceous plates (0.50 g., 53%) were filtered and dried in the air. Upon concentration the mother liquor yielded a small crop (0.09 g., 10%) of very fine needles.

Anal.[16] Calcd. for $C_{12}H_{14}O_{11}(COCH_3)_8$: acetyl, 11.79 ml. of 0.1 *N* NaOH per 100 mg. sample. Found: acetyl, 11.80 ml., 11.86 ml.

After several recrystallizations from absolute ethanol and subsequent drying in a drying pistol over phosphorus pentoxide at the temperature of boiling benzene, the first crop had a m. p. of 114° (corr.) and $[\alpha]^{21}D$ +26.0° (*c*, 0.6; *l*, 2; $CHCl_3$). It then crystallized in large octagonal plates, with the square or the diamond shape predominating, which tended to form radiating foliate masses.

The second crop, consisting of narrow needles with a rectangular cross section, was dried similarly and was found to have a m. p. of 142–143° (corr.) and $[\alpha]^{25}D$ +25.8° (*c*, 0.6; *l*, 2; $CHCl_3$). The plates could be converted into needles by solution in chloroform, then concentration to a sirup and subsequent dissolution in hot absolute ethanol. This behavior, in addition to the agreement of the specific rotations, seems to indicate that both crystalline forms are 6-glucosido-α-mannose octaacetate.

Discussion

It was of interest to observe whether this first application of the glycal-oxidation synthesis to a 6-linked disaccharide conformed with that of previous oxidations for other types of sugars. Levene[3] has found that if the C_3 hydroxyl group is not blocked, the hydroxyl group added to C_2 by oxidation with perbenzoic acid will go predominantly *cis* to that of C_3. This conclusion gains further support by the present oxidation of gentiobial, which formed 6-glucosidomannose (*cis* C_2-C_3 configuration) in predominance (68%) to gentiobiose (*trans* C_2-C_3 configuration).

The empirical method of calculation of the rotation of sugars of Hudson[17] affords a means of predicting the rotations of 6-glucosidomannose and its derivatives. If it is assumed that the epimeric difference of the molecular rotation between β-gentiobiose and β-epigentiobiose (6-glucosido-β-mannose) has the same value as that between β-glucose and β-mannose, then by the relation

$$[M]_{\beta\text{-gentiobiose}} - [M]_{\beta\text{-epigentiobiose}} = [M]_{\beta\text{-glucose}} - [M]_{\beta\text{-mannose}}$$

it is possible to calculate the molecular rotation of β-epigentiobiose from the known values of the molecular rotations of β-gentiobiose, β-glucose, and β-mannose.[18]

$$\begin{aligned}[M]_{\beta\text{-epigentiobiose}} &= [M]_{\beta\text{-gentiobiose}} - [M]_{\beta\text{-glucose}} + [M]_{\beta\text{-mannose}} \\ &= (-8.4)(342) - (+19)(180) + (-17)(180) \\ &= -9350 \\ [\alpha]_{\beta\text{-epigentiobiose}} &= -27°\end{aligned}$$

Then, to obtain the rotation of α-epigentiobiose, it is only necessary to assume that the difference of molecular rotation between its α- and β-forms is the same as for the forms of mannose, namely

$$[M]_{\alpha\text{-epigentiobiose}} - [M]_{\beta\text{-epigentiobiose}} = [M]_{\alpha\text{-mannose}} - [M]_{\beta\text{-mannose}}$$

$$\begin{aligned}[M]_{\alpha\text{-epigentiobiose}} &= [M]_{\beta\text{-epigentiobiose}} + [M]_{\alpha\text{-mannose}} - [M]_{\beta\text{-mannose}} \\ &= (-9350) + (+30)(180) - (-17)(180) \\ &= -890 \\ [\alpha]_{\alpha\text{-epigentiobiose}} &= -2.6°\end{aligned}$$

(12) An average mutarotation velocity coefficient of 0.019 was also obtained by the method of calculation whereby the first observed value is regarded as the initial value and its time as the zero time value. In this way the individual velocity coefficients can be calculated without knowledge of the true initial rotation value.

(13) Hudson, THIS JOURNAL, **31**, 66 (1909).

(14) Hudson and Sawyer, *ibid.*, **39**, 470 (1917).

(15) The mutarotation velocity coefficient was calculated from the data of Bourquelot and Hérissey, *J. pharm. chim.*, [6] **16**, 418 (1902), for β-gentiobiose. Their initial rotation value (−8.42°) was determined by extrapolation to zero time of the graph t *vs.* log $(r_t - r_\infty)$. Using this initial rotation value and a final value of +9.82°, an average velocity coefficient of 0.010 for β-gentiobiose at 22° was obtained.

(16) Kunz and Hudson, THIS JOURNAL, **48**, 1978 (1926).

(17) Hudson, *ibid.*, **38**, 1567 (1916).

(18) All values used in the calculations are those of Hudson as given in the Bureau of Standards Reprint No. 533, "Relations between Rotatory Power and Structure in the Sugar Group," Government Printing Office, Washington, D. C.

In a similar manner the molecular rotations of the α-octaacetate and the β-octaacetate of epigentiobiose may be calculated.

$$[M]_{\substack{\alpha\text{-epigentiobiose}\\ \text{octaacetate}}} = [M]_{\substack{\alpha\text{-gentiobiose}\\ \text{octaacetate}}} - [M]_{\substack{\alpha\text{-glucose}\\ \text{pentaacetate}}} + [M]_{\substack{\alpha\text{-mannose}\\ \text{pentaacetate}}}$$

$$= (+52.4)(678) - (+101.6)(390) + (+55.0)(390)$$

$$= +17{,}400$$

$$[\alpha]_{\substack{\alpha\text{-epigentiobiose}\\ \text{octaacetate}}} = +25.7°$$

$$[M]_{\substack{\beta\text{-epigentiobiose}\\ \text{octaacetate}}} = [M]_{\substack{\beta\text{-gentiobiose}\\ \text{octaacetate}}} - [M]_{\substack{\beta\text{-glucose}\\ \text{pentaacetate}}} + [M]_{\substack{\beta\text{-mannose}\\ \text{pentaacetate}}}$$

$$= (-5.3)(678) - (+3.8)(390) + (-25.2)(390)$$

$$= -14{,}900$$

$$[\alpha]_{\substack{\beta\text{-epigentiobiose}\\ \text{octaacetate}}} = -22.0°$$

The agreement of the calculated and the observed values is shown in the table

	$[M]_{Calcd.}$	$[M]_{Obsd.}$	$[\alpha]_{Calcd.}$	$[\alpha]_{Obsd.}$
6-Glucosido-α-mannose	− 890	−1740	− 2.6	− 5.1
6-Glucosido-β-mannose	− 9350	...	−27	...
6-Glucosido-α-mannose-octaacetate	+17,400	+17,600	+25.7	+26.0
6-Glucosido-β-mannose-octaacetate	−14,900	...	−22.0	...

The agreement of the calculated and observed values is sufficiently close to conclude that 6-glucosido-α-mannose and its octaacetate possess a ring structure similar to that found in α-mannose. This is to be expected since Hirst and Woolvin[19] have shown that the glycal-oxidation synthesis involves no ring shifting.

Knowing the rotational values for 6-glucosido-mannose and for gentiobiose, the epimeric difference of these two disaccharides may be calculated. It is to be recalled, as Isbell[20] has pointed out, that correlation exists separately for α-mannose derivatives and for β-mannose derivatives.

	$[\alpha]_D$	Molecular epimeric diff.
α-Glucose	+113	+14,900
α-Mannose	+ 30	
α-Gentiobiose	+ 31	+12,300
6-Glucosido-α-mannose	− 5.1	
α-Glucose pentaacetate	+101.6	+18,100
α-Mannose pentaacetate	+ 55	
α-Gentiobiose octaacetate	+ 52.4	+17,700
6-Gl.-α-mannose octaacetate	+ 26.0	

Similar respective values are to be found for the epimeric differences of cellobiose and 4-glucosidomannose and of lactose and 4-galactosidomannose.

(19) Hirst and Woolvin, *J. Chem. Soc.*, 1131 (1931).

(20) Isbell, *Bur. Standards J. Research*, 5, 1179 (1930).

It was discovered some time after this work had been begun that Nishida and Hashima,[21] had isolated a disaccharide which contained mannose and glucose from the acetolysis products of the polysaccharide konjacmannan. Their deacetylated crystalline disaccharide had a m. p. of 150–160° and $[\alpha]^{20}D$ +10.5 (H_2O). They assumed it to be 6-mannosidoglucose on the basis that it was a cleavage product of the trisaccharide (6-mannosido-6-mannosidoglucose) which they had isolated and whose structure they had proved. A comparison of the properties of their disaccharide with those of 6-glucosidomannose shows that they did not have the latter. It is more probable now that they had 6-mannosidoglucose, as they assumed.

The authors are indebted to Dr. J. D. Park for the supply of gentiobiose octaacetate which was used in this work. They also wish to express their appreciation for the coöperation which they have received from Drs. C. B. Purves and R. C. Hockett of the Massachusetts Institute of Technology in making it possible to carry out a few of the optical rotations included in this work.

Summary

1. A new disaccharide, 6-[β-*d*-glucosido]-α-*d*-mannose, epigentiobiose, has been prepared in the crystalline state by the oxidation of gentiobial with perbenzoic acid. The specific rotation of the α-isomer has been measured and the mutarotation velocity coefficient determined.

2. Crystalline 6-[β-*d*-glucosido]-α-*d*-mannose octaacetate has been prepared and its physical properties determined.

3. An improved method of preparation of gentiobial hexaacetate from acetobromogentiobiose is given.

4. The molecular rotations of the alpha and beta forms of the free sugar and of its octaacetate derivatives have been calculated by the application of the isorotation rules of Hudson. The calculated values were found to be in good agreement with the observed values.

5. The epimeric difference as determined from 6-glucosidomannose and gentiobiose is given and its significance is discussed. The optical behavior of this new disaccharide is correlated with that of certain members of the mannose series.

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(21) Nishida and Hashima, *J. Dept. Agr. Kyushu Imp. Univ.*, 2, 277 (1930); *C. A.*, 25, 498 (1931).