[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

The Polymer-homologous Series of Oligosaccharides from Cellulose[1]

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A polymer-homologous series of crystalline α-D-acetates obtained from cellulose by acetolysis and chromatographic resolution has been extended to include the acetate of the heptasaccharide member. The parent sugars of the acetates from the tri- through the heptasaccharide were obtained, characterized, and compared with sugars of this series reported previously. The crystallinities of the members of both the unsubstituted sugar and the acetate series have been studied by photomicrography and X-ray powder diffraction. From the cellotetraose on the unsubstituted members exhibit the X-ray diagram characteristic of cellulose hydrate. The crystallinity decreases as the series is ascended but all members exhibit some degree of order. The incipient hydrolysis constants in 51% sulfuric acid at 30° were evaluated for cellopentaose and cellohexaose.

In a previous communication,[2] the literature on the cellulose oligosaccharides was reviewed and there was described the isolation and characterization of a polymer-homologous series of α-D-sugar acetates from cellulose, complete from α-D-glucopyranose pentaacetate to α-D-cellohexaose eicosaacetate, inclusive. Isolation was made from cellulose acetolyzates by chromatographic procedures[3,4] developed in this Laboratory. We report herein the extension of the series to include α-D-celloheptaose tricosaacetate. Air-dried cotton linters were subjected to the mild acetolysis conditions of Hess and Dziengel.[5] The resulting cellulose acetolyzates were resolved by chromatographic techniques on "Silene EF".[2] The lower members of the series have been previously well characterized.[2] Some variations in the zoning capacity of the adsorbent over that previously reported[2] was noted. Such deviations were found by Hoffman[6] to be very pronounced in different batches of this adsorbent. Hoffmann also found that "Silene EF" could be standardized with respect to its adsorptive powers toward a mixture of D-glucose and maltose by prewashing and treating with inorganic bases or salts. The adsorbent used in this work was the Hoffman-defined[6] "Silene EF" No. 5, whereas that used in the previous work[2] was not defined.

By rechromatography on the same adsorbent, pure α-D-cellopentaose heptadecaacetate, α-D-cellohexaose eicosaacetate and α-D-celloheptaose tricosaacetate were obtained. The properties of the former two compounds were in excellent agreement with those reported previously.[2] The properties of the last named compound (Table I) identified it as the next higher member of the series; additional evidence of its identity was provided by its position on the chromatogram and by a molecular weight estimation by the Rast method.

According to Freudenberg,[7] if the linkages in a polymer-homologous series are uniform, the plot of $[M]_n/n$ against $(n-1)/n$, where $[M]_n$ is the molecular rotation and n is the degree of polymerization, will yield a straight line where n is 2 or greater. This was substantiated by Dickey and Wolfrom[2] for the α-D-acetates of this series from cellobiose to cellohexaose, inclusive. Such a plot including α-D-celloheptaose tricosaacetate is given in Fig. 1. It was also observed that a straight line is formed in plotting the values for β-D-cellobiose octaacetate, β-D-cellotriose hendecaacetate, and the limiting value for cellulose triacetate. It is evident from these plots that the difference in rotatory power between the α-D- and β-D-anomers becomes increasingly small as the degree of polymerization is in-

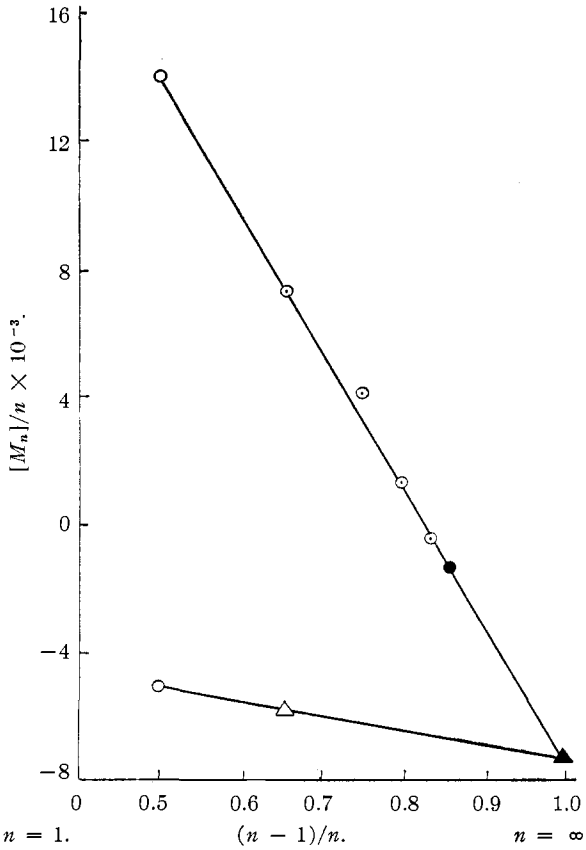

Fig. 1.—Relation between degree of polymerization, n, and molecular rotation, $[M]_n$(D line of sodium), for the sugar acetates from cellulose. Upper line, α-D-acetates; lower line, β-D-acetates. Data from: O, C. S. Hudson and J. M. Johnson, THIS JOURNAL, **37**, 1276 (1915); ⊙, E. E. Dickey and M. L. Wolfrom[2]; ●, this work; △, K. Hess and K. Dziengel[5]; ▲, extrapolated limiting value for cellulose triacetate.

(1) Presented October 19, 1951, before the Southwide Chemical Conference at Wilson Dam, Alabama.

(2) E. E. Dickey and M. L. Wolfrom, THIS JOURNAL, **71**, 825 (1949).

(3) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, *ibid.*, **67**, 527 (1945).

(4) L. W. Georges, R. S. Bower and M. L. Wolfrom, *ibid.*, **68**, 2169 (1946).

(5) K. Hess and K. Dziengel, *Ber.*, **68**, 1594 (1935).

(6) D. O. Hoffman, Ph.D. Dissertation, The Ohio State University, 1948.

(7) K. Freudenberg, "Tannin, Cellulose, Lignin," J. Springer, Berlin, 1933, p. 104.

TABLE I

PROPERTIES OF CELLULOSE OLIGOSACCHARIDES

D.p.	M.p., °C. (cor.)	$[\alpha]_D$ H_2O	°C.	n	Ref.	Incipient hydrolysis constant in 51% H_2SO_4 at 18° $k_{18} \times 10^4$	at 30° $k_{30} \times 10^4$	Ref.	α-D-Acetate M.p., °C. (cor.)	α-D-Acetate $[\alpha]^{20-25}D$, $CHCl_3$, $c < 5$	Ref.
1	146	+112° → +52.7°	20	4	a				112–113	+101.6°	g
	148–150	+103 → +52.7	20	4	a						
2	225	+14 → +34.6	20	8	c	1.07	6.9	f	229.5	+41	h
3	206–209 dec.	+35 → +21.6	26	4	b,c	0.64	4.5	f	223–224	+22.6	i,j
4	252–253 dec.	+8.4 → +16.5	23	3.4	b,d	0.51	3.7	f	230–234	+13.4	i
5	266–268 dec.	+8 → +11	30	4.1	b		3.5	b	240–241	+4.2	i
6	275–278 dec.	+10	30	1.2	b		3.2	b	252–255	−0.2	i
7	283–286 dec.	+7 ± 3	30	0.1	e				263–266	−4.4	h
∞							2.3	f	295	−22	k

[a] W. W. Pigman and R. M. Goepp, Jr., "Chemistry of the Carbohydrates," Academic Press, Inc., New York, N. Y., 1948. [b] This work; initial rotation values logarithmetrically extrapolated. [c] *Cf.* ref. 5, 8–10. [d] Ref. 10. [e] First-order k determined polarimetrically with time and extrapolated to initial time. [f] Ref. 14. [g] C. S. Hudson and J. K. Dale, THIS JOURNAL, **37**, 1264 (1915). [h] C. S. Hudson and J. M. Johnson, *ibid.*, **37**, 1276 (1915). [i] Ref. 2. [j] Ref. 5. [k] K. Hess, "Die Chemie der Zellulose und Ihrer Begleiter," Akademische Verlagsgesellschaft m.b.H., Leipzig, 1928, p. 512.

creased. When the molecular chain approaches infinite length the rotatory power of the molecule is not affected by the configuration of the terminal glycosidic acetyl group and in cellulose triacetate the chain has become sufficiently long that α- and β-anomers cannot be detected by the usual methods for determining rotatory power. By use of the graphs in Fig. 1, it is possible to predict the rotatory powers of compounds of these series that have not been isolated.

A series of unsubstituted sugars (Table I) from cellotriose to celloheptaose, inclusive, were isolated from the acetates by deacetylation with catalytic amounts of sodium methoxide in anhydrous methanol. Cellotriose crystallized as the α-D-anomer, and cellotetraose and cellopentaose crystallized as the β-D-anomers. Due to their low solubilities and rotatory powers, the anomeric forms of cellohexaose and celloheptaose could not be determined with certainty. The extent of mutarotation decreases as the series is ascended (Table I) since the reducing end-group becomes a smaller contributor to the total rotation. Thus it is possible that mutarotation in the last two members of this series might be barely detectable under optimal conditions of observation.

The physical properties (Table I) of α-D-cellotriose compare favorably with those reported by: Willstätter and Zechmeister[8] (m.p. *ca.* 210°, +23°), Bertrand and Benoist[9] (m.p. 206–212°, +22.8° for "procellose"), Zechmeister and Tóth[10] (m.p. 238°, dec., +32 → +23°), and Hess and Dziengel[5] (m.p. 203–214°, +32 → +23°). The properties of our preparation of β-D-cellotetraose were in agreement with those (m.p. 251°, +11 → +17°) reported for cellotetraose by Zechmeister and Tóth.[10] Our β-D-cellopentaose exhibited properties strikingly similar to those of the "cellohexaose" reported by these authors. Staudinger and Leupold,[11] after making viscosity measurements on a specimen of the acetate of "cellohexaose" received from Professor Zechmeister, and determining the molecular weight of the acetate by the Rast method, stated that the results showed this compound to be a pentasaccharide rather than a hexasaccharide.

Incipient rates of hydrolysis have been employed in the characterization of the sugars of this series. Freudenberg, Kuhn and co-workers[12] observed the difference in the rates at which cellobiose and cellulose were hydrolyzed to D-glucose. Kuhn[13] attacked the problem mathematically and calculated incipient rate constants k_3 and k_4 for cellotriose and cellotetraose, respectively, in 51% sulfuric acid at 18 and 30°. These constants were determined experimentally[14] by polarimetric and iodimetric methods. The results (Table I) substantiated the theory. To these data are now added those (Table I) for k_5 and k_6 and the values fit well into the series.

The degree of crystallinity decreases as each series is ascended but a definite degree of order is present in each of these chromatographically purified substances. The crystallinity of the unsubstituted sugar series is greater than that of their acetates as the latter have much higher molecular weights. X-Ray powder diffraction data were obtained for the unsubstituted sugars. That of cellobiose has been recorded[15] and our data for cellotriose were in agreement with those reported by Trogus and Hess.[16] From cellotetraose on the main lines represented were those of cellulose hydrate[15,17] although other lines of low intensity were present. These lines are: 4.48 (very strong), 4.04 (very strong), 7.22 (strong), all expressed as interplanar distances in Å. The presence of cellulose hydrate as a contaminant in our specimens is definitely excluded by the chromatographic methods employed in their isolation. These oligosaccharides are built on the structure of cellulose and thus possess the same repeating unit. It is to be expected that they would show the properties of regenerated or solution-separated cellulose ("cellulose hydrate"). That the identity appears so early in the series, at the cellotetraose stage, is unexpected. Possibly equipment able to delineate the lines of

(8) R. Willstätter and L. Zechmeister, *Ber.*, **62**, 722 (1929).

(9) G. Bertrand and Mlle. S. Benoist, *Bull. soc. chim.*, [4] **33**, 1451 (1923).

(10) L. Zechmeister and G. Tóth, *Ber.*, **64**, 854 (1931).

(11) H. Staudinger and E. O. Leupold, *ibid.*, **67**, 479 (1934).

(12) K. Freudenberg, W. Kuhn, W. Dürr, F. Bolz and G. Steinbrunn, *ibid.*, **63**, 1510 (1930).

(13) W. Kuhn, *ibid.*, **63**, 1503 (1930).

(14) K. Freudenberg and G. Blomqvist, *ibid.*, **68**, 2070 (1935).

(15) K. Dziengel, C. Trogus and K. Hess, *ibid.*, **65**, 1454 (1932).

(16) C. Trogus and K. Hess, *ibid.*, **68**, 1605 (1935).

(17) F. Klages, *ibid.*, **64**, 1193 (1931).

longer spacing occurring in the center of the diagrams, might have yielded some differences.

Experimental

Chromatographic Resolution of the Cellulose Acetolyzate.—Air-dried cotton linters (45 g.) were subjected to acetolysis as described by Dickey and Wolfrom,[2] whose procedure was based upon that of Hess and Dziengel,[5] and the dried (at 40° and finally at 70°) product was stored in a vacuum desiccator over sodium hydroxide; yield 70–80 g.

Using the general procedure of Georges, Bower and Wolfrom[4] for the chromatographic separation of sugar acetates on Silene EF[18]–Celite[19] the cellulose acetolyzate from 45 g. of cotton linters was chromatographed in 1.5-g. portions by dissolving in 30 ml. of chloroform and placing this solution on a 215 × 45-mm. (i.d.) column of Silene EF–Celite (5:1 by wt.) and developing with 1400 ml. of benzene–ethanol (100:1 by vol.) to yield the following zones designated in mm. from the column top: A, 159–199; B, 93–142; C, 48–85; D, 15–42; top, 0–9. The alkaline potassium permanganate (1% in 2.5 *N* KOH) streak was made on the extruded column by spraying a fine stream along its length from a capillary pipet constructed by elongating the end of a medicine dropper. The streaks were scraped from the column, each zone was sectioned, suspended in enough acetone to make a mobile slurry, filtered through sintered glass, and the eluting solvent removed by evaporation under reduced pressure.

The material (1.33 g.) from the combined A zones was rechromatographed twice on the same adsorbent by developing with 300 ml. of benzene–ethanol (100:1 by vol.), the developing time being 12–14 hr.; yield 0.75 g. Pure α-D-**cellopentaose heptadecaacetate**[2] was obtained on three recrystallizations from 95% ethanol; yield 0.49 g., m.p. 240–243° (cor.), $[\alpha]^{28}D$ +4.7° (*c* 2.4, chloroform).

The material (1.74 g.) from the combined B zones was chromatographed as above, development being effected with 4000 ml. of benzene–ethanol (75:1 by vol.) requiring 14 hr. for each chromatogram; yield 0.64 g. Three recrystallizations from 95% ethanol yielded pure α-D-**cellohexaose eicosaacetate**[2]; yield 0.41 g., m.p. 252–254°, $[\alpha]^{30}D$ −0.23° (*c* 2.4, chloroform).

α-D-**Celloheptaose Tricosaacetate.**—The material (2.0 g.) from the combined C zones was rechromatographed four times as above, development being effected with 4000–4500 ml. of developer requiring 19 hr. for each chromatogram; yield 0.26 g. Pure α-D-celloheptaose tricosaacetate was obtained on three recrystallizations from 95% ethanol; yield 122 mg., m.p. 263–266°, $[\alpha]^{21}D$ −4.4° (*c* 1.7, chloroform).

Anal. Calcd. for $C_{88}H_{118}O_{59}$: C, 49.86; H, 5.61; mol. wt., 2120. Found: C, 49.73; H, 5.40; mol. wt. (Rast), 2080.

Deacetylation of the Sugar Acetates.—The α-D-acetates of the oligosaccharides were dissolved or suspended in 12 ml. of 0.2 *N* NaOMe and stirred mechanically at 25–30° for 1 hr. (1.5 hr. for α-D-cellohexaose eicosaacetate); the amounts of each employed were: 1.00 g. for the triose acetate, 1.00 g. for the tetraose acetate, 346 mg. for the pentaose acetate, and 258 mg. for the hexaose acetate. In the case of α-D-celloheptaose tricosaacetate, 47 mg. in 2 ml. of the reagent was shaken for 3 hr.

At the end of the reaction time, the solution was made slightly acidic with acetic acid, filtered through sintered glass, and the precipitate washed on the funnel with four 5-ml. portions of cold methanol. The precipitate was then dissolved in water and passed through ion exchange columns[20] to remove, not always successfully, ionic contaminants. The solution was followed on the columns by 400–500 ml. of distilled water. The solutions of the sugars α-D-cellotriose to β-D-cellopentaose, inclusive, were concentrated to 5 ml. under reduced pressure and precipitated by adding ethanol to incipient turbidity. Crystallization was effected by standing for 24 hr. at 5–10°. The crystallization of cellohexaose and celloheptaose differed only in that the initial solutions were concentrated to 10 ml. The solids were collected and recrystallized from saturated aqueous solutions with ethanol until further recrystallization did not change their melting point, one to three recrystallizations being necessary. The sugars were then thoroughly dried under reduced pressure at the temperature of boiling ethanol; yields: 408 mg. of triose, 347 mg. of tetraose, 135 mg. of pentaose, 98 mg. of hexaose, 20 mg. of heptaose. The properties of the members of this series are given in Table I.

α-D-**Cellotriose** was sweet, was very soluble in water, slightly soluble in methanol and was insoluble in ethanol.

β-D-**Cellotetraose** was slightly sweet, was soluble in water and insoluble in methanol and ethanol. It dissolved slowly in 7–8 parts of warm water and crystallized readily on the addition of 6 volumes of absolute ethanol; X-ray powder diffraction data[21,22]: 7.16–60, 4.53–100, 4.03–100, 3.34–20, 3.00–15, 2.80–15, 2.63–50, 2.35–50, 2.21–60, 1.91–10, 1.84–10, 1.69–10.

β-D-**Cellopentaose** was very slightly sweet, was much less soluble in water (100 mg. was slowly soluble in 2.5 ml. of warm water) than the lower members of the series and was insoluble in methanol and ethanol; X-ray power diffraction data: 7.18–90, 4.41–100, 4.02–100, 3.38–20, 2.98–20, 2.81–20, 2.57–40, 2.35–40, 2.20–50, 2.02–10, 1.92–10, 1.85–10, 1.69–10, 1.45–10.

Anal. Calcd. for $C_{30}H_{52}O_{26}$: C, 43.48; H, 6.32. Found (after correction for an ash content of 1.4%): C, 42.8; H, 6.62.

Cellohexaose was a white powdery solid. It was very slightly soluble in water and was insoluble in methanol and ethanol. An amount of 100 mg. dissolved slowly in 10 ml. of warm water and readily separated on the addition of small amounts of absolute ethanol; X-ray powder diffraction data: 7.14–75, 4.43–100, 3.98–100, 3.36–10, 2.59–30, 2.35–30, 2.20–50, 1.68–10.

Anal. Calcd. for $C_{36}H_{62}O_{31}$: C, 43.63; H, 6.31. Found: C, 44.19; H, 6.69.

Celloheptaose was a white powdery solid that was very slightly soluble in water and was insoluble in methanol and ethanol. An amount of 26 mg. dissolved slowly in 25 ml. of warm water and separated on the addition of a small amount of absolute ethanol; X-ray powder diffraction data: 7.21–60, 4.45–100, 4.04–90, 3.38–10, 3.03–10, 2.83–10, 2.53–20, 2.35–10, 2.22–20, 1.91–10. It could not be obtained entirely free of ash.

Anal. Calcd. for $C_{42}H_{72}O_{36}$: C, 43.75; H, 6.30. Found (after correction for an ash content of 0.84%): C, 43.54; H, 6.33.

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(18) A synthetic calcium acid silicate manufactured by the Columbia-Southern Chemical Corp., Barberton, Ohio.

(19) No. 535, a siliceous filter-aid manufactured by Johns-Manville Co., New York, N. Y.

(20) Amberlite IR-120 (cation resin), manufactured by the Rohm and Haas Co., Resinous Products Div., Philadelphia, Pennsylvania. Duolite A4 (anion resin), manufactured by the Chemical Process Co., Redwood City, California.

(21) First number, interplanar distance in Å., CuKα radiation, effective diameter of camera 57.3 Å.

(22) Second number, visually estimated relative intensity as percentage of that of strongest line.