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# The Rearrangement of Sugar Acetates by Aluminum Chloride. Crystalline Celtrobiose and Some of its Derivatives[1]

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Although it is stated in well-known texts[2] that celtrobiose is *d*-glucosido-*d*-altrose, the evidence rests on the single observation[3] that after hydrolysis of acetochloroceltrobiose with 0.5 *N* hydrochloric acid for two and a half hours at 98° the solution is levorotatory. By analogy with neolactose (*d*-galactosido-*d*-altrose),[4] both in its preparation through the rearranging action of aluminum chloride, and in its behavior on hydrolysis, whereby a levorotatory solution also is obtained, such a formulation seemed probable. We now know that this negative rotation is due, not to *d*-altrose itself which rotates[4c,5] +32.6°, but to a non-reducing derivative, and that the rotation −98° originally ascribed to *d*-altrose[4b] refers to the equilibrium mixture of *d*-altrose and its anhydro form that results from sufficiently strong acid conditions. The present report, dealing with celtrobiose and some of its derivatives, proves conclusively that celtrobiose is *d*-glucosido-*d*-altrose.

Acetochloroceltrobiose has now been obtained in yields of 40–45% by the action of a mixture of aluminum chloride and phosphorus pentachloride on a chloroform solution of cellobiose octaacetate. From this acetochloro compound with acetic anhydride and sodium acetate the α-octaacetate was prepared; by converting the acetochloro derivative to the β-heptaacetate with silver carbonate and aqueous acetone, and then acetylating with acetic anhydride and pyridine the β-octaacetate was prepared. The rotations of these carefully purified substances are recorded in Table I, and from them values for the rotations of the end asymmetric carbon atoms ($A_{Ac}$ and $A_{Cl}$) have been obtained in the usual way.[6]

It is noted that the value for $A_{Ac}$ is in agreement with those obtained for cellobiose, lactose, and neolactose, and the two octaacetates may be assumed to represent a normal α,β-pair. The value for $A_{Cl}$ is much smaller than in the case of cellobiose and lactose, but agrees closely with that found for neolactose; it seems probable that this $A_{Cl}$ value is a characteristic of the acetochloro-*d*-altrose portion of the disaccharide derivative, and that the $A_{Cl}$ value for acetochloro-*d*-altrose itself will be of the same magnitude, namely, about 30,000.

Although aluminum chloride was originally suggested as being the reagent which causes the double epimerization at the second and third carbon atoms, the best yields of rearrangement products have been obtained by using a mixture of aluminum chloride and phosphorus pentachloride. On the other hand, the use of aluminum chloride alone is recommended for the preparation of the normal chlorination product; for example, acetochloroceltrobiose has been obtained in 90% yield by warming a chloroform solution of celtrobiose octaacetate with twice its weight of commercial aluminum chloride.

In our study of the α- and β-octaacetates of celtrobiose six different crystalline modifications were isolated. The α-octaacetate separates from alcohol in large prisms which melt at 130°, or in prisms which melt at 112°, then resolidify and melt at 130°; either form can be obtained at will. The β-octaacetate crystallizes from aqueous alcohol in prisms as a monohydrate melting at 87–93°, or as solvent-free needles melting at 114°; from ether it separates in fine needles melting at 103-105°. Here also any of the three forms is obtainable as desired. In addition to the pure α- and β-forms, a double compound is known, and serves to identify readily one octaacetate in the presence of the other. It crystallizes from ether in typical rectangular plates melting at 70° with evolution of gas; it has the composition 2α-octaacetate·1β-octaacetate·3ether, as shown by analysis, and rotation of +24.9° as compared with the value +25.0° calculated from the known rotations of the two octaacetates. This appears

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(2) *E. g.*, Tollens–Elsner, "Kurzes Handbuch der Kohlenhydrate," 4th edition, Johann Ambrosius Barth, Leipzig, 1935, p. 476; Abderhalden "Biochemisches Handlexikon," Vol. XIII, edited by Zemplén, Verlag von Julius Springer, Berlin, 1931, p. 606.

(3) Hudson, THIS JOURNAL, **48**, 2002 (1926).

(4) (a) Kunz and Hudson, *ibid.*, **48**, 1978 (1926); (b) Kunz and Hudson, *ibid.*, **48**, 2435 (1926); (c) Richtmyer and Hudson, *ibid.*, **57**, 1716 (1935).

(5) Throughout the article the rotations are specific rotations at 20° for sodium light.

(6) Hudson, THIS JOURNAL, **46**, 462 (1924).

TABLE I

| Derivative of celtrobiose | | α-Octaacetate | β-Octaacetate | α-Acetochloro |
|---|---|---|---|---|
| Molecular weight | | 678 | 678 | 655 |
| $[\alpha]^{20}D$ in $CHCl_3$ | | +48.0 | −13.0 | +64.2 |
| $[M]^{20}D$ | | +32,540 | −8810 | +42,050 |
| Rotation of end carbon | | $A_{Ac}$ = +20.700 | | $A_{Cl}$ = +30,200 |
| *A* for corresponding derivatives of | Cellobiose | 19,100[a] | | 37,600[b] |
| | Neolactose | 20,500[c] | | 30,900[c] |
| | Lactose | 19,800[d] | | 38,400[e] |

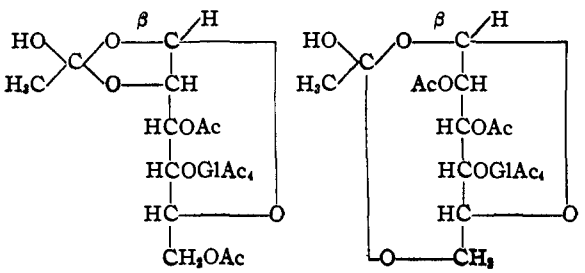
[a] Hudson and Johnson, THIS JOURNAL, 37, 1276 (1915). [b] Calculated from the rotation +71.7° for acetochlorocellobiose, found by Brauns, *ibid.*, 48, 2776 (1926). [c] Kunz and Hudson, ref. 4a. [d] Hudson and Johnson, THIS JOURNAL, 37, 1270 (1915). [e] Kunz and Hudson, *ibid.*, 47, 2052 (1925).

to be the first example of a double compound of two octaacetates, and indicates that considerable caution is required lest two isomers crystallizing together be mistaken for a pure chemical individual. Similar molecular compounds reported from this Laboratory include the novel modifications of methyl-*d*-xyloside,[7] lactose,[7] anhydrous *l*-rhamnose,[8] and the so-called neolactose "α"-heptaacetate.[4c] Also, Fischer's rhamnose β-triacetate rotating −19.4° was considered by him to represent a mixed compound.[9]

Exchange of the Cl atom in acetochloroceltrobiose by an OH group leads to the formation of celtrobiose β-heptaacetate which has been crystallized only in combination with one mole of ether; on a solvent-free basis the β-heptaacetate in chloroform rotates +3.9° changing to +15.1°. The α-heptaacetate crystallizes as a dietherate and also solvent-free; it mutarotates downward in chloroform from +22.3 to +15.1°.

Another heptaacetate of celtrobiose has been obtained, in very small yield, through the silver carbonate–aqueous acetone reaction with the chloro compound; it melts considerably higher than the other acetates, rotates +1.0° in chloroform without mutarotation, and on cautious acetylation with acetic anhydride and pyridine is transformed quantitatively to celtrobiose β-octaacetate. These properties, combined with the fact that the normal α- and β-heptaacetates are known, lead to the belief that this acetate may well have an ortho ester structure involving the 1,2- or even the 1,6-positions of the altrose molecule which are spatially much closer than the customary formula would indicate.

The previously known ortho esters of the sugar series in which one hydroxyl group remains unsubstituted appear to be limited to the heptaacetate of the ketone sugar turanose described by




Pacsu[10] and to a tetraacetate of glucose described by Helferich and Klein[11] as 1,2,3,6,(?)-tetraacetyl-β-*d*-glucose, but to which Haworth, Hirst and Teece[12] have assigned a 1,6-ortho ester structure. Reactions which were expected to prove an ortho ester linkage in these compounds were disappointing because derivatives of the normal form were usually obtained. Methylation with silver oxide and methyl iodide produced the normal acetylated β-glycoside in each case. Acetylation of the glucose tetraacetate did not lead to a new glucose pentaacetate but only to the well-known β-pentaacetate. In these reactions it has been assumed that a facile rearrangement from the ortho ester to the normal form has been brought about by the reagents employed. The lack of reactions suitable for proving an ortho ester structure together with the scarcity of our own extra heptaacetate make necessary a postponement of its further study.

Deacetylation of the hepta- or octaacetates with barium methylate solution resulted in crystallization of the parent disaccharide. Celtrobiose monohydrate, well-formed prisms from 75% alcohol, rotates +13.6° in water; although mutarotation was not observed the sugar is undoubtedly the β-form since on cautious acetylation it produced an 85% yield of celtrobiose β-octaacetate. The component hexoses liberated from celtrobiose by hydrolysis with dilute hydro-

(7) Hockett and Hudson, THIS JOURNAL, 53, 4454, 4455 (1931).
(8) Jackson and Hudson, forthcoming publication.
(9) Fischer, Bergmann and Rabe, *Ber.*, 53, 2362 (1920).
(10) Pacsu, THIS JOURNAL, 55, 2451 (1933).
(11) Helferich and Klein, *Ann.*, 450, 226 (1926); 455, 177 (1927).
(12) Haworth, Hirst and Teece, *J. Chem. Soc.*, 1408 (1930).

chloric acid at 100° had a rotation of −22.8°; the value −22.7° would be expected from an equilibrium mixture of *d*-glucose (+52.5°) and *d*-altrose (−98° in acid solution).[4c] The oxidation of celtrobiose with bromine water, followed by acid hydrolysis of the bionic acid, yielded crystalline *d*-glucose, and *d*-altronic acid which was isolated as the crystalline calcium salt. Since the biose linkage should not be affected by the aluminum chloride rearrangement of cellobiose octaacetate, these data prove conclusively that celtrobiose is 4-β-*d*-glucosido-*d*-altrose.

Preliminary experiments have shown that calcium *d*-altronate, obtainable through the aluminum chloride rearrangement of lactose or cellobiose octaacetates, is a convenient source for the preparation, by degradation, of *d*-ribose.

## Experimental

**α-Acetochloroceltrobiose.**—The modified procedure is as follows. To a solution of 50 g. of recrystallized cellobiose octaacetate[13] in 400 cc. of alcohol-free dry chloroform in a one-liter flask is added 100 g. of powdered commercial aluminum chloride and 50 g. of powdered phosphorus pentachloride. The flask is shaken for a few minutes to ensure thorough mixing, then heated for one hour in a bath at 60–63°. The granular mixture is decomposed carefully with ice and water, the resulting solution extracted with chloroform, and the chloroform extracts washed several times with water, dried with granular calcium chloride and concentrated *in vacuo* to a thin sirup. Dry ether is then added cautiously in order to obtain a small crop (*ca.* 10 g.) of acetochlorocellobiose which carries down most of the colloidal impurities derived from the cellulose. After a few minutes the solid is removed by filtration, and the mother liquor diluted to 500 cc. with dry ether. Crystallization is allowed to proceed overnight at room temperature and then for a week in the refrigerator. The mixture of acetochloro derivatives thus obtained is separated mechanically; partial disintegration of the crystals with a spatula allows the fine needles of acetochlorocellobiose to be floated away in ether suspension, leaving the heavier prismatic crystals of the celtrobiose compound. A small additional crop of the latter may be obtained by combining the two fractions of crude acetochlorocellobiose and shaking them with ether for several hours at room temperature; the more soluble celtrobiose derivative is dissolved, together with some of the cellobiose compound, and the two are separated by crystallization. The yield of crude acetochloroceltrobiose is 19–22 g. (40–45%). Two recrystallizations from chloroform–ether give a product, rotating about +61°, which is pure enough for subsequent transformations. However, thirteen recrystallizations brought the rotation to +63.9°, a figure which was not appreciably changed by eight additional recrystallizations, and the value +64.2°, obtained after the twenty-first recrystallization is accepted for the rotation of α-acetochloroceltrobiose in U. S. P. chloroform (*c*, 4). The m. p. is 141–142°, with no visible decomposition.

**α-Acetochloroceltrobiose from Celtrobiose α-Octaacetate.**—A solution of 25 g. of pure α-octaacetate (see below) in 200 cc. of alcohol-free dry chloroform was heated with 50 g. of powdered commercial aluminum chloride for two hours at 60–63°, then decomposed carefully with ice and water. The chloroform extracts, after being washed with water and dried with calcium chloride, were concentrated *in vacuo* to a thin sirup. Upon the addition of dry ether the solution deposited typical hexagonal plates of acetochloroceltrobiose in a yield of 21.6 g. (90%) plus a few centigrams of the acicular crystals of acetochlorocellobiose. After five recrystallizations the acetochloroceltrobiose melted at 141–142°, and rotated +64.2° in U. S. P. chloroform (*c*, 4); these values were unchanged by five additional recrystallizations.

**Celtrobiose α-Octaacetate.**—A mixture of 25 g. of acetochloroceltrobiose, 25 g. of fused sodium acetate and 200 cc. of acetic anhydride was heated on the steam-bath for two hours, then boiled gently for five minutes and poured into two liters of ice and water. The solution was neutralized by adding solid sodium bicarbonate, whereupon most of the octaacetate separated as a spongy, light brown mass. This was extracted with chloroform, and the chloroform solution washed with water, dried with granular calcium chloride, and concentrated *in vacuo*. The resulting sirup was dissolved in alcohol and the solution, inoculated with a crystal already obtained, deposited 22.1 g. of heavy clusters of prisms, melting at 127–129°. Recrystallized from three volumes of warm alcohol, it melted at 129–130° and rotated +48.0° in U. S. P. chloroform (*c*, 4); these values were unchanged by eight additional crystallizations.

A second modification of the α-octaacetate, melting at 112°, was obtained in a preliminary experiment; the same form appeared during recrystallizations when the 130° form was left in contact with its mother liquor, especially in a cool place, the original sharp-edged prisms assuming a "weather-beaten" appearance and showing the lower melting point. It was later discovered that either the 112° or the 130° modification could be obtained at will by seeding an alcoholic solution of the octaacetate with a crystal of the desired form. This second modification crystallizes in prisms which melt at 112°, then resolidify and melt again at 129–130°; it contains no solvent of crystallization, and its melting point was unchanged after four hours of heating at 100° *in vacuo*. After five recrystallizations it rotated +48.0° in U. S. P. chloroform (*c*, 4).

*Anal.* Calcd. for $C_{28}H_{38}O_{19}$: C, 49.54; H, 5.65; acetyl, 11.79 cc. of 0.1 *N* NaOH per 100 mg. Found: (130° form) C, 49.39; H, 5.64; acetyl, 11.83 cc.; (112° form) C, 49.31; H, 5.63; acetyl, 11.84 cc.

**Celtrobiose β-Octaacetate.**—A mixture of 50 cc. of acetic anhydride and 125 cc. of pyridine, cooled to −10°, was added to 25 cc. of twice recrystallized celtrobiose β-heptaacetate monoetherate (see below), also at −10°. The solution was kept in the refrigerator overnight, then poured into ice water and extracted with chloroform. The chloroform solution was washed in succession with water, cold dilute hydrochloric acid until free from pyridine, water,

(13) Prepared in about 30% yield by the acetolysis of alpha sulfite, soda, or kraft paper pulp which had been furnished through the courtesy of Mr. Warren E. Emley of the Division of Organic and Fibrous Materials of the National Bureau of Standards.

aqueous sodium bicarbonate, and water, dried with granular calcium chloride, and the chloroform evaporated *in vacuo*. The thick sirup was dissolved in 75 cc. of warm alcohol and treated with a little activated carbon, filtered, and diluted with an equal volume of water. After being inoculated with a crystal obtained in a preliminary experiment the solution deposited 21.9 g. of β-octaacetate rotating −10°. This material was recrystallized once from dry ether, then four times from 50% alcohol; the rotation in U. S. P. chloroform (*c*, 6) was −13.0°, and was unchanged by four additional crystallizations from 50% alcohol.

The β-octaacetate, like the α-octaacetate, may crystallize in more than one form. Originally it separated from the aqueous alcohol solution in prisms melting at 87–93°; analyses showed it to be a monohydrate. Upon recrystallization from ether it crystallized in solvent-free needles melting at 103–105°. Recrystallization from 50% alcohol then produced a third modification, anhydrous needles melting at 113–114°. Any of the three forms can be obtained as desired, by suitable manipulation.

*Anal.* Calcd. for $C_{28}H_{38}O_{19}\cdot H_2O$: C, 48.25; H, 5.79; $H_2O$, 2.59. Found (87–93° prisms): C, 48.36; H, 5.85; $H_2O$ (4 hours in pistol at 100°), 2.49. Calcd. for $C_{28}H_{38}O_{19}$: C, 49.54; H, 5.65; acetyl, 11.79 cc. of 0.1 *N* NaOH per 100 mg. Found (103–105° needles): C, 49.30; H, 5.83; (113–114° needles): C, 49.36; H, 5.68; acetyl, 11.88 cc.

**The Double Octaacetate: 2α-Octaacetate·1β-Octaacetate·3($C_2H_5$)$_2$O.**—In the preparation of celtrobiose β-octaacetate just described the original alcoholic mother liquor was evaporated *in vacuo* to a thick sirup which was dissolved in the mother liquor from the crystallization from ether, and the solution concentrated. In addition to needles of β-octaacetate it deposited 1 g. of rectangular plates which, after one recrystallization from ether, melted with decomposition at 70°, and rotated +24.9° in U. S. P. chloroform (*c*, 4). From the known rotations of the α- and β-octaacetates the rotation calculated for a double octaacetate of celtrobiose having the composition 2α-octaacetate·1β-octaacetate·3($C_2H_5$)$_2$O is +25.0°. The compound was then synthesized by mixing pure α- and β-octaacetates in the proportions indicated and crystallizing from ether; the product formed in plates melting at 70° with evolution of gas, rotated +25.8° and lost the calculated amount of ether when heated for several days at 56° in a Fischer pistol; the ether-free compound melted over the range 70–85°.

*Anal.* Calcd. for $C_{28}H_{38}O_{19}\cdot C_4H_{10}O$: ether, 9.85%; acetyl, 10.63 cc. of 0.1 *N* NaOH per 100 mg. Found: ether, 9.80%; acetyl, 10.66 cc. For the ether-free compound, calcd. for $C_{28}H_{38}O_{19}$: C, 49.54; H, 5.65. Found: C, 49.30; H, 5.66.

**Celtrobiose β-Heptaacetate Monoetherate.**—The heptaacetates of celtrobiose were prepared by shaking 10 g. of acetochloroceltrobiose with 5 g. of silver carbonate in 190 cc. of acetone and 10 cc. of water for twelve hours. The silver salts were removed by filtration and the clear solution concentrated *in vacuo* at 25° to a sirup from which the crude heptaacetate could be obtained in nearly quantitative yield by adding ether and cooling. The product could not be purified by simple recrystallization, and from a mixture of acetone and ether there usually separated at the same time four crystalline forms melting, when pure, at 60° (dec.), 80° (dec.), 131, and 216°, respectively. However, the several heptaacetates were finally isolated and purified by the following procedure. The crude product was packed into the thimble of a Soxhlet apparatus and extracted with boiling anhydrous ether for two or three hours. The ethereal solution was set aside, and the undissolved residue, containing mostly 80° heptaacetate, was then extracted with fresh ether for six or eight hours; during this extraction the 80° compound crystallized slowly from the warm ether solution in the form of rectangular plates. By decanting the ether while still warm the plates could be separated from the more soluble α-forms which stayed in solution and from the tiny needles of the 216° compound which floated away with the ether. From 50 g. of acetochloroceltrobiose there was thus obtained 25 g. of plates which were purified by two additional crystallizations in the same manner. Analyses showed the substance to be a heptaacetate which contained one mole of ether of crystallization. The rotation of the monoetherate in U. S. P. chloroform (*c*, 10) was +3.5°, changing in the course of a week at 20° to +13.5°; calculated for the solvent-free heptaacetate these values become +3.9° changing to +15.1°. It could not be obtained crystalline in the absence of ether, and the ether of crystallization could be removed completely only by melting the substance at 80° *in vacuo;* during this process mutarotation took place and the glassy solid when dissolved in chloroform rotated +14.5° changing overnight to +15.0°.

*Anal.* Calcd. for $C_{26}H_{36}O_{18}\cdot C_4H_{10}O$: ether, 10.43%; acetyl, 9.85 cc. of 0.1 *N* NaOH per 100 mg. Found: ether, 10.19, 10.30%; acetyl 9.95 cc. For the ether-free compound, calcd. for $C_{26}H_{36}O_{18}$: C. 49.03: H, 5.70; acetyl, 11.00 cc. of 0.1 *N* NaOH per 100 mg. Found: C, 48.84; H, 5.70; acetyl, 10.99 cc.

**Celtrobiose α-Heptaacetate.**—The ethereal solutions left after isolation of the β-heptaacetate monoetherate as described above were united, concentrated and a mixture of crystalline heptaacetates obtained in which the α-form predominated. The material was placed in the Soxhlet thimble and extracted with ether; this time, after one or two hours of boiling, the α-heptaacetate began to separate in tiny crystals which united to form small granules. By filtering the warm solution most of the β-heptaacetate which had been extracted remained in the mother liquor. Five recrystallizations by this method were carried out, the final product melting at 130–131° and rotating in U. S. P. chloroform (*c*, 5) +22.3°, changing in the course of five days at 20° to +15.1°.

*Anal.* Calcd. for $C_{26}H_{36}O_{18}$: C, 49.03; H, 5.70; acetyl, 11.00 cc. of 0.1 *N* NaOH per 100 mg. Found: C, 48.83, 48.67; H, 5.83, 5.68; acetyl, 11.07 cc.

Celtrobiose α-heptaacetate may be converted to celtrobiose β-heptaacetate, or *vice versa*, by allowing a chloroform solution to mutarotate until equilibrium is reached, concentrating to a sirup, and isolating the mixture of heptaacetates with ether. Extraction in a Soxhlet with dry ether according to the procedures outlined above will then furnish the desired form.

**Celtrobiose α-Heptaacetate Dietherate.**—In the ordinary crystallization of the α-heptaacetate there is formed not the 130–131° compound just described but trans-

parent elongated prisms which melt at 60° with the evolution of gas, then resolidify, and melt again at 130–131°. Upon standing in the air, or more rapidly *in vacuo* below 60°, the prisms lose their transparency while retaining their crystalline structure and then show the higher melting point 130–131°. Analyses show the loss of two moles of ether of crystallization from each mole of the heptaacetate (calcd. for $C_{26}H_{36}O_{18}\cdot 2C_4H_{10}O$: ether, 18.89%. Found: ether, 18.79, 18.80%). Crystallization from a warm ethereal solution, as shown above, produces the solvent-free form, but left overnight in the presence of ether at 20°, or below, the granular heptaacetate disappears and beautiful prisms of the dietherate modification are deposited. Under certain intermediate conditions both forms thus are present at the same time.

**Celtrobiose β-Heptaacetate with Ortho Ester Structure.**—In addition to the α- and β-heptaacetates described above, there always appeared a small amount (5%) of another substance, melting at 216°, which also had the composition of a heptaacetate. It was obtained by the action of silver carbonate in aqueous acetone even from acetochloroceltrobiose which had been subjected to twenty-one recrystallizations, and from acetochloroceltrobiose recrystallized ten times after being prepared by the action of aluminum chloride on purest celtrobiose α-octaacetate. This new heptaacetate is very sparingly soluble in ether, and was recovered from the material left in the Soxhlet thimble after extraction of the more soluble α- and β-forms, or filtered from the ethereal solutions which were decanted during recrystallization of the 80° β-heptaacetate monoetherate. From a mixture of chloroform and ether it separated in small needles, melted at 216°, and after three recrystallizations showed the constant rotation of +1.0° in U. S. P. chloroform (using a 4-dm. tube; *c*, 1). Unlike the normal α- and β-heptaacetates of celtrobiose, which in chloroform solution mutarotate to the equilibrium value +15.1°, this β-heptaacetate showed no evidence of mutarotation on standing eight days. It reduces Fehling's solution readily at 65°. An alkoxyl determination was negative.

*Anal.* Calcd. for $C_{26}H_{36}O_{18}$: C, 49.03; H, 5.70; acetyl, 11.00 cc. of 0.1 *N* NaOH per 100 mg. Found: C, 48.81; H. 5.74; acetyl, 11.04 cc.

**Acetylation of the Heptaacetates.**—In order to relate the normal α- and β- and the ortho ester β-heptaacetates with the corresponding α- and β-octaacetates, a 1-g. sample of each of the five compounds was cooled to −10°, and to it was added a mixture of 5 cc. of acetic anhydride and 15 cc. of pyridine, also at −10°; the solution was kept at −10° for one hour, then at 4° overnight. The next morning the temperature was allowed to rise to 20°, the volume adjusted exactly to 25 cc., and the rotation observed. After twenty-four hours a second reading of the rotation showed no change in any case.

Acetylation of the normal α-heptaacetate (m. p. 130–131°) gave a solution of specific rotation +33.3°, as compared with the rotation +38.3° for pure α-octaacetate and −18.6° for pure β-octaacetate in the same acetylating solvent (all rotations calculated on the basis of the amount of octaacetate present). The rotation +33.3° thus corresponds to a mixture containing 91% α-octaacetate and 9% β-octaacetate. By working up the acetylation mixture from 2.09 g. of α-heptaacetate in the usual way and crystallizing from ether there was isolated 1.84 g. of α-octaacetate and 0.20 g. of the 2α·1β-octaacetate trietherate described above. One recrystallization from alcohol yielded 1.58 g. of α-octaacetate with the correct melting point, and rotating in chloroform +47.4° as compared with +48.0° for the pure compound.

Acetylation of the β-heptaacetate monoetherate (m. p. 80° with decomposition), a method already used in the preparation of celtrobiose β-octaacetate, gave a solution of specific rotation −13.9°, corresponding to a mixture of 92% β-octaacetate and 8% α-octaacetate. How much of this α-octaacetate is derived from α-heptaacetate present as an impurity, and how much is due to mutarotation preceding the acetylation is difficult to estimate; a similar problem arises in considering the acetylation of the α-heptaacetate.

Acetylation of the β-heptaacetate with ortho ester structure (m. p. 216°) produced a solution having the rotation −18.3°, as compared with the value −18.6°, for the pure β-octaacetate; these figures are identical within the limits of error and represent a quantitative transformation to the β-octaacetate. When isolated in the usual way, the product from 2.01 g. of heptaacetate consisted of 2.05 g. of octaacetate (theoretical 2.14 g.). After recrystallization from 50% alcohol it was identified completely as the β-octaacetate by its melting point and mixed melting point of 113–114°, by its rotation in U. S. P. chloroform (−13.2° as compared with −13.0° for the known β-octaacetate), and by its characteristic change of melting point to 103–105° when crystallized from ether and back to 113–114° again on crystallization from 50% alcohol. In addition, a small sample was mixed with twice its weight of pure α-octaacetate and crystallized from ether; typical plates of the double octaacetate etherate were obtained, melting at 70° (dec.), having the rotation +24.9° (calcd. +25.0°) and losing the theoretical amount of ether at 65° *in vacuo* (calcd. 9.85%; found 9.81%).

**Celtrobiose Monohydrate.**—Deacetylation of any of the hepta- or octaacetates with barium methylate as catalyst in absolute methyl alcohol, followed by removal of the barium by adding the exact amount of dilute sulfuric acid required, led to the isolation of the parent disaccharide in practically quantitative yield. Celtrobiose crystallizes readily as a monohydrate from water, or better by adding three volumes of alcohol to its aqueous solution. The monohydrate is the stable form, and a dehydrated sample quickly reverts to the hydrate on exposure to moist air. It forms large well-shaped prisms which begin to soften at 133°, then slowly melt to a thick sirup which evolves gas at 148°. The rotation was unchanged by three recrystallizations, and the accepted value is +13.6° in water (*c*, 5). No mutarotation was observed in twenty-four hours, the first reading being taken five minutes after adding the water to dissolve the sugar.

*Anal.* Calcd. for $C_{12}H_{22}O_{11}\cdot H_2O$: C, 39.98; H, 6.72; $H_2O$, 5.00. Found: C, 39.93; H, 6.88; $H_2O$, 4.98 (six hours in pistol at 110°).

**Acetylation of Celtrobiose.**—Since celtrobiose shows no mutarotation, evidence for its designation as the α- or β-form was sought by acetylation studies. To this end a cooled mixture of 5 cc. of acetic anhydride and 15 cc. of

pyridine was added to one-half gram of the monohydrate. Most of the sugar dissolved when shaken for an hour at room temperature. After standing overnight the solution was diluted with pyridine to 25 cc. and found to rotate, in duplicate experiments, $-11.9°$ and $-11.4°$; this corresponds to a mixture containing 88% of the β-octaacetate and 12% of the α-octaacetate. When the solution was poured into ice water and examined in the usual way it yielded 85% of the theoretical amount of octaacetate as the β-form (identified by melting point, mixed melting point and rotation) and about 2% as the double octaacetate etherate.

**Hydrolysis of Celtrobiose.**—After 0.2594 g. of celtrobiose hydrate was heated with 8 cc. of 1 *N* hydrochloric acid for two and one-half hours at 100° and the solution diluted exactly to 10 cc. at 20°, it rotated $-22.8°$ and did not change when heated for another hour. This rotation is in agreement with the value $-22.7°$ calculated for an equimolecular mixture of *d*-glucose ($+52.5°$) and *d*-altrose ($-98°$) under the same conditions.

**Oxidation to Celtrobionic Acid and Subsequent Hydrolysis to *d*-Glucose and *d*-Altronic Acid.**—The method of Hudson and Isbell[14] was adopted. Thus, to 9.2 g. of benzoic acid and 11.8 g. of barium hydroxide octahydrate, combined by dissolving in 350 cc. of hot water, and cooled, was added 9 g. of the crystalline sugar and 2 cc. of bromine. The solution was kept in the dark, with occasional shaking, for forty-four hours. Excess bromine was expelled with a current of air; precipitated benzoic acid was removed by filtration; the barium ions were completely precipitated by adding a slight excess of sulfuric acid; bromide ions were removed by shaking the solution with 10 g. of silver carbonate; the filtered solution was freed from silver ions with hydrogen sulfide, and the excess hydrogen sulfide blown out with air. The dissolved benzoic acid was completely removed by extraction with chloroform and the solution concentrated to about 240 cc. No attempt was made to isolate the celtrobionic acid, but instead it was hydrolyzed by adding 6.7 cc. of concentrated sulfuric acid to make the solution about 1 normal, and refluxing gently for three hours. To the pale yellowish solution was added 38 g. of barium hydroxide octahydrate, the barium sulfate removed by filtration, and the slight excess of barium precipitated by titration with 0.5 *N* sulfuric acid, using sodium rhodizonate as an external indicator.[15]

The solution should now contain only *d*-glucose, and *d*-altronic acid and its lactone. The acid and lactone were neutralized by adding lime water until the solution would remain faintly alkaline to phenolphthalein for one hour. By concentrating to a small volume and adding alcohol there was obtained 4 g. of crystalline calcium *d*-altronate which was identified completely as described below.

The mother liquor was freed from the remaining calcium altronate by concentrating to a thin sirup and adding methyl alcohol, filtering the granular salts, and repeating the operations. The methyl alcoholic solution of glucose was then evaporated *in vacuo* to a very thick sirup which was extracted with 250 cc. of hot 95% alcohol; from this extract, upon concentrating and inoculating, was obtained 2.2 g. of fine needles. The product was identified as *d*-glucose through the melting point and mixed melting point 146–147°, through its phenylosazone with melting point and mixed melting point 208° (dec.), and finally by a study of its mutarotation. At 23° the initial rotation $+70.3°$ *in water (c, 5)* showed it to be a mixture of the α- and β-forms; the equilibrium rotation $+53.0°$ is in accord with the known value $+52.5°$ for *d*-glucose; and the mutarotation constant 0.0087 is in excellent agreement with the value 0.0085 for 23°, as calculated from the equation log $(k_1 + k_2) = 11.0198 - (3873/T)$ given by Hudson and Dale.[16]

**Identification of Calcium *d*-Altronate·3.5$H_2O$.**—The calcium altronate obtained above was recrystallized twice from water, and then found to be identical in its properties with a sample of calcium *d*-altronate prepared from neolactose by oxidation and hydrolysis in the same manner. The air-dried calcium salt contains three and one-half moles of water of crystallization[17] which it loses on heating one hour at 110° *in vacuo;* upon exposure to the moist air of the laboratory it quickly regains its original water content. This property of losing and regaining 3.5$H_2O$ as described can be used to distinguish calcium *d*-altronate (and presumably calcium *l*-altronate) from the known calcium salts of other aldohexonic acids except possibly gulonic.[18]

*Anal.* Calcd. for $(C_6H_{11}O_7)_2Ca·3.5H_2O$: $H_2O$, 12.78. Found: 12.79 for calcium altronate from celtrobiose, 12.78 for calcium altronate from neolactose.

The solubility of hydrated calcium *d*-altronate in water at 20° is very nearly 2 g. in 100 cc. Its rotation in water (*c*, 1.8; 4-dm. tube) was determined as $-2.36°$ from celtrobiose and $-2.35°$ from neolactose, as compared with $+2.08°$ for calcium *l*-altronate.[17c]

TABLE II

ROTATION OF CALCIUM *d*-ALTRONATE·3.5$H_2O$ IN 1 *N* HCl (*c*, 3)

| Time, min. | From celtrobiose | From neolactose | Steiger and Reichstein From *d*-ribose | Steiger and Reichstein From "levo-lactone" |
|---|---|---|---|---|
| 5 | +11.7 | +11.1 | | |
| 10 | 14.0 | 13.8 | +14.1 | +14.6 |
| 15 | 15.7 | 16.1 | | |
| 20 | 17.5 | 17.8 | | |
| 30 | 20.1 | 19.4 | | 19.8 |
| 45 | 22.4 | 21.7 | 22.3 | 22.3 |
| 60 | 23.2 | 23.6 | | |
| 90 | 24.3 | 24.4 | | |
| Constant | 24.8 | 24.9 | 24.7 | 24.3 |
| Final Rotation, Calculated as Lactone | | | | |
| | +34.4 | +34.5 | +34.2[a] | +33.7[a] |
| | | | 35.1[b] | |

[a] These values have been recalculated from the data given by Steiger and Reichstein.

[b] Levene and Jacobs, *Ber.*, **43**, 3142 (1910).

(14) Hudson and Isbell, *Bur. Standards J. Research*, **3**, 59 (1929).

(15) Giblin, *Analyst*, **58**, 752 (1933).

(16) Hudson and Dale, THIS JOURNAL, **39**, 320 (1917).

(17) (a) Levene and Jacobs, in *Ber.*, **43**, 3142 (1910), reported 3.5 $H_2O$ for calcium *d*-altronate; (b) Austin and Humoller, THIS JOURNAL, **56**, 1152 (1934), reported 3.5$H_2O$ for calcium *l*-altronate; (c) Isbell, however, in *Bur. Standards J. Research*, **14**, 308 (1935), wrote calcium *l*-altronate·3$H_2O$.

(18) *Cf.* Fischer and Stahel, *Ber.*, **24**, 528 (1891).

Very convincing evidence of the identity of calcium altronate from celtrobiose with the calcium altronate from neolactose, as well as for their identity with samples of calcium altronate from *d*-ribose and from the 1,4-lactone of *d*-talomucic acid[19] is given in Table II. Here are recorded the changes in rotation which occur when calcium altronate is dissolved in 1 *N* hydrochloric acid and the liberated altronic acid undergoes lactone formation.

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## Summary

1. Acetochloroceltrobiose has been obtained in 40–45% yield by the action of a mixture of aluminum chloride and phosphorus pentachloride on cellobiose octaacetate in chloroform.

2. Both the $\alpha$- and the $\beta$-octaacetates of celtrobiose have been prepared. A double compound of the two acetates crystallizes with the composition 2$\alpha$-octaacetate·1$\beta$-octaacetate·3-ether.

3. Both the $\alpha$- and the $\beta$-heptaacetates of celtrobiose have been prepared. A third heptaacetate is believed to possess an ortho ester structure.

4. Celtrobiose monohydrate has been obtained in crystalline form. It has $[\alpha]^{20}D + 13.6°$, without mutarotation. Acetylation shows it to be the $\beta$-form.

5. Acid hydrolysis of celtrobiose indicates that the component hexoses are *d*-altrose and *d*-glucose. Oxidation to celtrobionic acid, followed by acid hydrolysis, yielded *d*-glucose, and *d*-altronic acid which was identified as calcium *d*-altronate·3.5$H_2O$. These data lead to the conclusion that celtrobiose is 4-$\beta$-*d*-glucosido-*d*-altrose.

(19) Steiger and Reichstein, *Helv. Chim. Acta*, **19**, 195 (1936).

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# The Asymmetric Oxidation of Sugars by Optically Active Alkaline Copper Solutions[1]

BY NELSON K. RICHTMYER AND C. S. HUDSON

In a recent paper dealing with neolactose and *d*-altrose[2] we had occasion to compare the reducing powers of the *d*- and *l*-forms of altrose toward the alkaline copper reagent of Shaffer and Hartmann.[3] The *d*-altrose was found to have only about 56% the reducing power of *l*-altrose; similarly, *d*-arabinose was found to have only 76% the reducing power of *l*-arabinose. The suggestion was made that, since the copper carbonate reagent contains an optically active substance, namely, *d*-tartaric acid. the *d*- and *l*-forms of the reducing sugars may behave differently in its presence.

A number of investigators[4] have compared the reducing powers of various sugars as determined by the methods of Hagedorn–Jensen, Folin–Wu, Shaffer–Hartmann, and others, but the behavior of a pair of antipodal sugars toward these reagents, and toward copper reagents containing other than the ordinary *d*-tartaric acid, has not been studied previously. The results of our experiments, in which *d*-glucose, *d*- and *l*-altrose, and *d*- and *l*-arabinose were compared by oxidation with the Hagedorn–Jensen–Hanes ferricyanide reagent, and with four modifications of the Shaffer–Hartmann–Somogyi Reagent 50 with 1 g. of potassium iodide, containing the *d*-, *l*-, racemic, and *meso* tartaric acids, respectively, are shown in Tables I and II.

The first three columns of figures in Table I show clearly that with the ferricyanide reagent, and with the alkaline copper reagents containing *meso* and racemic tartaric acids, the *d*- and *l*-forms of altrose have identical reducing powers, and the same is true of the *d*- and *l*-forms of arabinose, within the limits of experimental error. In the fourth and fifth columns, however, striking differences appear between *d*- and *l*-altrose in their behavior with the alkaline copper solutions containing *d*- and *l*-tartaric acids, respectively. Thus, *d*-altrose (1.2 mg.) requires 5.82 cc. of

(1) Publication authorized by the Surgeon General, U. S. Public Health Service.

(2) Richtmyer and Hudson, THIS JOURNAL, **57**, 1716 (1935).

(3) Shaffer and Hartmann, *J. Biol. Chem.*, **45**, 377 (1921).

(4) *E. g.*, Bertrand, *Bull. soc. chim.*, **35**, 1285 (1906); Thomas and Dutcher, THIS JOURNAL, **46**, 1662 (1924); Willaman and Davison, *J. Agric. Research*, **28**, 479 (1924); Greenwald, Samet and Gross, *J. Biol. Chem.*, **62**, 397 (1924); Rowe and Wiener, THIS JOURNAL, **47**, 1698 (1925); Pucher and Finch, *Proc. Soc. Exptl. Biol. Med.*, **23**, 468 (1926); Hawkins, *J. Biol. Chem.*, **84**, 79 (1929).