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

Semicarbazone and Oxime Acetates of Maltose and Cellobiose. *Aldehydo*-Cellobiose Octaacetate

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In previous work reported from this Laboratory we have shown that on acetylation of a sugar oxime or semicarbazone, either acetylated ring structures, or the acetylated open chain structure, or a mixture of both, is obtained. If an open chain structure is produced, methods have been devised for removing the nitrogen residue and obtaining the *aldehydo*-acetate.[1] In the case of the acetylated oximes, the open chain or *aldehydo* forms are readily detected by acetylated nitrile formation on heating and by the selective hydrolysis of the O-acetyl on the oxime group. These criteria are not applicable to the acetylated semicarbazones. With a hexose, a ring structure may be assumed if the product is a tetraacetate. If the substance is a semicarbazone pentaacetate of the ring structure, then the fifth acetyl group is attached to nitrogen and may be distinguished by the difference between the total acetyl and O-acetyl analyses.[2] The total acetyl value is determined by the method of Freudenberg and Harder[3] and the O-acetyl is determined by the Kunz[4] procedure, using phenolsulfonphthalein indicator.

In the work herein reported, the above-described procedures have been extended to maltose and cellobiose. Acetylation of the hitherto unknown crystalline maltose semicarbazone produced a crystalline octaacetate of the ring type. The rotations of these ring forms of acetylated sugar oximes and semicarbazones are very similar to those of the β-forms of the completely acetylated cyclic sugars and we have consequently distinguished them by the prefix β.[5] Acetylation of either the hydrated or anhydrous form of cellobiose semicarbazone likewise yielded the ring octaacetate as the sole crystalline product isolated. Mild acetylation produced the ring heptaacetate.

Cellobiose heptaacetate was oximated and acetylated with the production of the ring or β-cellobiose oxime nonaacetate. We would predict that this should be identical with the product isolated by Zemplén[6] as a by-product in the formation of cellobionic acid nitrile octaacetate.

Zemplén called his product cellobiose *anti*-oxime octaacetate although his published analytical values are in closer agreement with a nonaacetate. His recorded constants are: melting point, 165°; $(\alpha)_D$ −7° (chloroform). Those obtained by us for our compound are: melting point, 195–195.5°; $(\alpha)_D$ −8.5° (chloroform). The β-isomer would be expected to form in Zemplén's experiments by analogy with the behavior of glucose and galactose oximes under the vigorous nitrile forming conditions,[7] The low melting point recorded by Zemplén might be accounted for by the presence of some of the acetylated nitrile, which is frequently very difficult to separate.

When cellobiose oxime was acetylated at low temperatures an amorphous product was obtained which was essentially the *aldehydo*-cellobiose oxime nonaacetate. This fact was established by the formation of cellobionic acid nitrile octaacetate on heating and by its transformation in good yield into a crystalline oxime octaacetate. The latter was deoximated with nitrous acid to yield *aldehydo*-cellobiose octaacetate in the form of an analytically pure, colorless, amorphous powder that resisted crystallization. The substance was nitrogen-free and regenerated the crystalline oxime octaacetate on oximation.

At this point we wish to call attention to the differences in behavior of cellobiose semicarbazone, oxime and heptaacetate as compared to the glucose[1] semicarbazone, oxime and tetraacetate, the latter being analogous to the cellobiose heptaacetate. Thus, cellobiose semicarbazone yielded only the ring form on acetylation, while glucose semicarbazone gave predominantly the open chain form. Cellobiose oxime, on the other hand, produced the open chain acetate, while glucose oxime under the same conditions gave the ring

(1) M. L. Wolfrom, L. W. Georges and S. Soltzberg, THIS JOURNAL, **56**, 1794 (1934).

(2) M. L. Wolfrom, M. Konigsberg and S. Soltzberg, *ibid.*, **58**, 490 (1936).

(3) K. Freudenberg and M. Harder, *Ann.*, **433**, 230 (1923).

(4) A. Kunz and C. S. Hudson, THIS JOURNAL, **48**, 1982 (1926).

(5) M. L. Wolfrom and A. Thompson, *ibid.*, **53**, 625 (1931).

(6) G. Zemplén, *Ber.*, **59**, 1260 (1926).

(7) A. Wohl, *ibid.*, **26**, 730 (1893); M. L. Wolfrom and A. Thompson, THIS JOURNAL, **53**, 622 (1931); V. Deulofeu, M. L. Wolfrom, Pedro Cattaneo, C. C. Christman and L. W. Georges, *ibid.*, **55**, 3488 (1933).

form. Cellobiose heptaacetate, after oximation and acetylation, yielded the ring form, whereas glucose tetraacetate after the same treatment gave the *aldehydo* form. Thus the introduction of a glucose molecule in the fourth position completely reversed the behavior of the active glucose function with respect to the acetylation of the oximes and semicarbazones under the conditions employed.

Experimental

Maltose Semicarbazone.—Semicarbazide hydrochloride (9 g.) was rubbed to a paste with 6 g. of fused potassium acetate and heated on the water-bath with 75 cc. of absolute ethanol to extract the semicarbazide, the solution being filtered to remove potassium chloride. The warm ethanol extract was added to a warm solution of 25 g. of maltose in 50 cc. of water and the mixture allowed to cool to room temperature. The proportions of ethanol and water should be such that no sirupy phase forms at this point. Crystalline material appeared on standing. After several days, this material was removed by filtration; yield 12.5 g. Pure material was obtained by dissolving in a minimum of hot water and adding ethanol; m. p. 213° (dec.); $[\alpha]^{25}D$ +80° (*c*, 3.5; H_2O), initial value, changing slowly in the dextro direction. The substance crystallized in the anhydrous condition.

Anal. Calcd. for $C_{13}H_{25}O_{11}N_3$: N, 10.53. Found: N, 10.45.

β-Maltose Semicarbazone Octaacetate.—Powdered maltose semicarbazone (12 g.) and 96 cc. of pyridine were heated in a bath maintained at 60–65° and 48 cc. of acetic anhydride added. The reaction mixture was stirred mechanically for seven hours at this temperature to effect solution of the semicarbazone and was then allowed to stand overnight at room temperature. The solution was then poured into ice and water, a small amount of amorphous material removed by filtration and the filtrate extracted with chloroform. The chloroform extract was washed successively with 5% hydrochloric acid, sodium bicarbonate solution, and water dried, and the solvent removed under reduced pressure. The residue was crystallized from ethanol; yield 8.3 g.; m. p. 206–208° (dec.); $(\alpha)^{25}D$ +61° ($CHCl_3$). Pure material was obtained on further recrystallization from ethanol; m. p. 209–210° (dec.); $(\alpha)^{26}D$ +61° (*c*, 3; $CHCl_3$). The analytical data indicated that the compound was a maltose semicarbazone octaacetate with one N-acetyl group.

Anal. Calcd. for $C_{13}H_{17}O_4N_2(OCOCH_3)_7(NCOCH_3)$: total acetyl, 10.9 cc. of 0.1 *N* NaOH per 100 mg.; O-acetyl, 9.5 cc. Found: total acetyl, 11.0 cc.; O-acetyl, 9.5 cc.

β-Cellobiose Semicarbazone Heptaacetate.—Cellobiose semicarbazone dihydrate[8] (6.2 g.) was acetylated at room temperature with pyridine (48 cc.) and acetic anhydride (24 cc.) for eighteen hours with mechanical stirring. The reaction mixture was then poured into 150 cc. of ice and water and on standing at ice box temperature for several hours the liquid set to a semi-solid mass of crystals; yield 5.8 g.; m. p. 205–207°; $(\alpha)^{25}D$ −20° ($CHCl_3$). A further small amount of material (0.8 g.) of lower purity could be obtained by chloroform extraction of the mother liquor. Pure material was obtained on recrystallization from ethanol; m. p. 207–208°; $(\alpha)^{27}D$ −21° (*c*, 3; $CHCl_3$).

Anal. Calcd. for $C_{13}H_{18}O_4N_2(OCOCH_3)_7$: total acetyl, 10.1 cc. of 0.1 *N* NaOH per 100 mg.; O-acetyl, 10.1 cc. Found: total acetyl, 10.1 cc.; O-acetyl, 9.8 cc.

β-Cellobiose Semicarbazone Octaacetate.—Further acetylation of β-cellobiose semicarbazone heptaacetate (0.5 g.) with pyridine (3 cc.) and acetic anhydride (1 cc.) for two days at 40° produced β-cellobiose semicarbazone octaacetate; m. p. 238–240° (dec.). This could also be prepared directly from cellobiose semicarbazone dihydrate (6 g.) according to the previously described acetylation procedure except that after the semicarbazone had dissolved in the acetylating mixture, the solution was kept at 40° for two days. No product was obtained on pouring into water but on extraction with chloroform in the usual manner, a crystalline product was obtained on the addition of ether to the concentrated chloroform solution; yield 3.1 g.; m. p. 215–220° (dec.). Pure material was obtained after several recrystallizations from ethanol; m. p. 240–241° (dec.); $(\alpha)^{25}D$ −26.5° (*c*, 3; $CHCl_3$). Acetylation of anhydrous semicarbazone with the same reagents maintained at 70–75° until solution was complete, produced the same octaacetate.

Anal. Calcd. for $C_{13}H_{17}O_4N_2(OCOCH_3)_7(NCOCH_3)$: N, 5.71; total acetyl, 10.9 cc. of 0.1 *N* NaOH per 100 mg.; O-acetyl, 9.5 cc. Found: N, 5.80; total acetyl, 10.8 cc.; O-acetyl, 9.5 cc.

β-Cellobiose Oxime Nonaacetate.—Cellobiose heptaacetate (10 g.)[9] was refluxed with two equivalents of free hydroxylamine in 35 cc. of ethanol on a water-bath for two hours. The sirup obtained on solvent removal was acetylated for two hours in an ice–salt bath with pyridine (50 cc.) and acetic anhydride (25 cc.). On pouring into water, a crystalline product (8 g.) was obtained which consisted essentially of a mixture of α-cellobiose octaacetate and the oxime nonaacetate. This mixture was heated with sufficient ethyl acetate to make a thin paste, filtered rapidly, and the residue washed with several portions of hot 95% ethanol. The crystals that separated from the filtrate were likewise filtered and washed with alcohol. The filtrate and washings were combined, concentrated to dryness, and the residue crystallized from a small amount of hot ethanol; yield 2.5 g.; m. p. 187–188°; $(\alpha)D$ +0.5° ($CHCl_3$). Pure material was obtained on repeated crystallization from 95% ethanol; m. p. 195–195.5°; $(\alpha)^{27}D$ −8.5° (*c*, 2.5; $CHCl_3$).

Anal. Calcd. for $C_{12}H_{14}O_3(OCOCH_3)_8(NCOCH_3)$: total acetyl, 12.25 cc. of 0.1 *N* NaOH per 100 mg.; O-acetyl, 10.9 cc. Found: total acetyl, 12.1 cc.; O-acetyl, 11.0 cc.

Unchanged material was recovered on heating the compound to incipient decomposition and also on refluxing for thirty minutes with a methanol solution of oxalic acid dihydrate. This behavior, together with the analytical data, shows that the compound was the oxime nonaacetate of cellobiose with one acetyl group attached to nitrogen. β-Glucose oxime hexaacetate also resists the hydrolytic ac-

(8) Maquenne and Goodwin, *Bull. soc. chim.*, [3] **31**, 1075 (1904).

(9) E. Fischer and G. Zemplén, *Ber.*, **43**, 2536 (1910).

tion of a methanol solution of oxalic acid and can be recovered unchanged under the above conditions.

Aldehydo-Cellobiose Oxime Octaacetate.—Cellobiose oxime (3 g.)[10] was acetylated with pyridine (24 cc.) and acetic anhydride (12 cc.) by stirring at 0° for two hours, followed by forty hours of standing at ice box temperature. The sirup ($(\alpha)_D$ +36°, $CHCl_3$) that was precipitated by pouring into ice and water was combined (total, 4.6 g.) with the sirupy material ($(\alpha)_D$ +35°, $CHCl_3$) obtained by chloroform extraction of the mother liquor and precipitated several times from benzene by means of heptane; $(\alpha)_D$ +37°, $CHCl_3$. The material resisted crystallization but produced crystalline cellobionic acid nitrile octaacetate when heated in the solid state to incipient decomposition.

The acetylated product obtained from 12 g. of cellobiose oxime as described above, was refluxed for thirty minutes with a solution of 13 g. of oxalic acid dihydrate in 160 cc. of methanol. The material obtained after solvent removal was dissolved in water, extracted with chloroform, the extract washed with an aqueous solution of sodium bicarbonate, dried, and the solvent removed. The residue was obtained crystalline from ether; yield 10.4 g.; m. p. 150–151°. Pure material was obtained by dissolving in chloroform and adding ether; m. p. 154–155°; $(\alpha)^{28}_D$ +30° (*c*, 3; $CHCl_3$).

Anal. Calcd. for $C_{12}H_{15}O_3N(OCOCH_3)_8$: total acetyl, 11.5 cc. of 0.1 *N* NaOH per 100 mg.; O-acetyl, 11.5 cc. Found: total acetyl, 11.6 cc.; O-acetyl, 11.6 cc.

De-oximation of *Aldehydo*-Cellobiose Oxime Octaacetate.—*Aldehydo*-cellobiose oxime octaacetate (2 g.) was dissolved in 60 cc. of glacial acetic acid and a solution of 20 g. of sodium nitrite in 60 cc. of water was dropped in during one hour with slow stirring. The stirring was continued for an additional hour with the rate greatly increased during the last ten minutes. The reaction mixture was poured into ice and water, extracted with chloroform, the extract washed with an aqueous solution of sodium bicarbonate, dried, and the solvent removed under reduced pressure. The product so obtained was purified three times by precipitation from methanol by the addition of water; $(\alpha)_D$ +17.1° (*c*, 3.6; alcohol-free $CHCl_3$). This procedure was repeated four more times; $(\alpha)_D$ +17.7° (*c*, 3.4; $CHCl_3$). The substance was obtained as a snow-white, amorphous solid that resisted crystallization but was nitrogen-free. It reduced Fehling's solution and reacted with hydroxylamine to produce the original crystalline *aldehydo*-cellobiose oxime octaacetate.

Anal. Calcd. for $C_{12}H_{14}O_{11}(COCH_3)_8$: acetyl, 11.8 cc. of 0.1 *N* NaOH per 100 mg. Found: acetyl, 12.1 cc.

(10) P. A. Levene and M. L. Wolfrom, *J. Biol. Chem.*, **77**, 677 (1928).

Summary

1. Maltose semicarbazone has been synthesized. This compound on acetylation yields a crystalline octaacetate of ring structure.

2. Mild acetylation of cellobiose semicarbazone produces crystalline cellobiose semicarbazone heptaacetate. Evidence is presented to show that it is the ring form. More vigorous acetylation yields the crystalline octaacetate which also has the ring form.

3. A crystalline cellobiose oxime nonaacetate has been synthesized and evidence is presented to show that it has a ring structure.

4. *Aldehydo*-Cellobiose oxime octaacetate has been synthesized in crystalline form from cellobiose oxime. Evidence is presented to show that it has the true oxime structure.

5. Cellobiose oxime octaacetate has been de-oximated to give an amorphous *aldehydo*-cellobiose octaacetate which re-forms the original oxime on treatment with hydroxylamine.

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Pyridinium Vanadate

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In the course of some work on vanadium, crystals were obtained which we have identified as a new compound, of formula $(C_5H_6N)_3V_5O_{14}\cdot H_2O$.

Preparation

1. The compound can be prepared by double decomposition between ammonium metavanadate and pyridine hydrochloride. A 7% water solution of the metavanadate is prepared by saturating at the boiling point (filtering, if necessary), and to the cooled solution is added one-fifth of its volume of a cool 2:1 mixture of pyridine and concentrated hydrochloric acid. Addition of an equal volume of alcohol, and cooling, result in nearly complete precipitation of the crystalline compound.[1] The precipitate is collected on a Büchner funnel and washed with a pyridine–water mixture (1:1), 95% alcohol and, finally, ether.

(1) The cool solution sometimes remains supersaturated for remarkably long times, but crystallization is readily initiated by the usual methods.