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

Nucleosides of Disaccharides; Cellobiose and Maltose[1]

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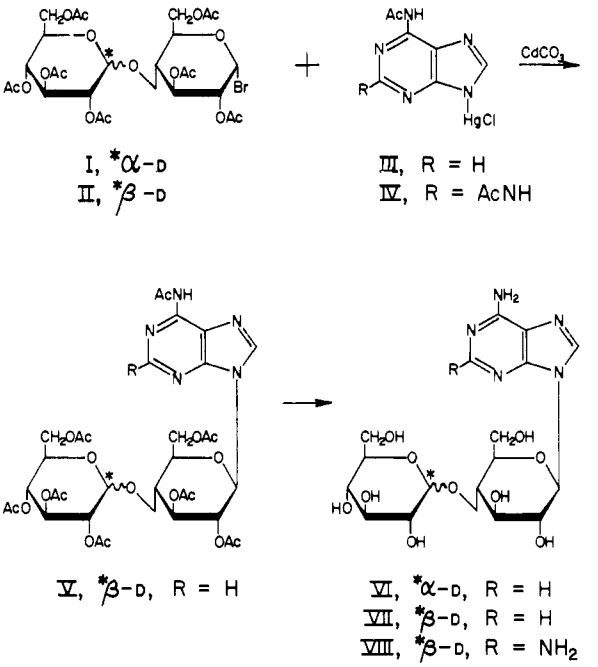
The disaccharide nucleosides 9-β-maltosyladenine (VI), 9-β-cellobiosyladenine (VII) and 2,6-diamino-9-β-cellobiosylpurine (VIII) have been synthesized in crystalline form and characterized.

We believe that 9-β-lactosyladenine and 2,6-diamino-lactosylpurine are the only examples of nucleosides containing a disaccharide which have been recorded.[2] We report herein the preparation and characterization in crystalline form of 9-β-maltosyladenine (VI), 9-β-cellobiosyladenine (VII) and 2,6-diamino-9-β-cellobiosylpurine (VIII). These substances were synthesized essentially according to the method of Davoll and Lowy[3] with the exception that a small amount of cadmium carbonate was added during the condensation to neutralize any acidity developing in the reaction mixture. The 6-acetamido-9-(hepta-*O*-acetyl-β-cellobiosyl)-purine was purified by column chromatography using Micro-Cel C[4,5] as the adsorbent. Methanolic solutions of ammonia, sodium methoxide and *n*-butylamine were used as deacetylating agents, preference being given to *n*-butylamine in the preparation of the unacetylated products. These compounds add maltose and cellobiose to the list of nucleosides and nucleoside derivatives containing disaccharides as sugar components.

Experimental

9-β-Maltosyladenine (VI).—A mixture of 12 g. of 6-acetamido-9-chloromercuripurine (III),[3,6] 12 g. of cadmium carbonate and 3 g. of Celite[4] in 400 ml. of xylene was dried by azeotropic distillation of 100 ml. of the solvent. To this suspension was added 20 g. of hepta-*O*-acetyl-α-maltosyl bromide[7] with stirring and the mixture was refluxed for 4 hr. After cooling, the product was precipitated with petroleum ether (b.p. 30–60°), filtered and extracted with 250 ml. of hot chloroform. The chloroform solution was washed successively with 30% potassium iodide and water, dried over sodium sulfate, and concentrated under reduced pressure to a sirup.

Complete deacetylation was effected by refluxing a solution of the sirup in 100 ml. of methanol containing 30 ml. of *n*-butylamine.[8,9] After 1 hr. a precipitate began to form and refluxing was continued for an additional 2 hr. The mixture was cooled overnight at 10°, filtered and the brown solid was washed with methanol; yield 7.7 g. The solid was dissolved in water, decolorized with carbon and concentrated under reduced pressure to a thin sirup. The sirup was triturated with three 60-ml. portions of hot 1-butanol, saturated with water, and the solution was allowed to stand at room temperature until crystallization as fine needles was effected. The crystals were separated by filtration, washed with methanol and recrystallized from aqueous ethanol; m.p. 223–225°, $[\alpha]^{25}D$ +82° (*c* 0.4, water); absorption spectra data[10]: $\lambda_{max}^{H_2O}$ 260 mμ; λ_{max}^{KBr} 3.05, 3.16 μ (NH, OH), 6.03, 6.20, 6.40, 6.80 μ (NH and purine ring), 6.05, 6.25, 6.60, 6.82 μ (C–O–C, C–OH); X-ray powder diffraction data[11]: 13.15w, 10.46w, 8.01vw, 7.44m, 7.19m, 6.49vw, 5.80w, 5.37w, 5.15w, 4.82vs(1), 4.64w, 4.56vw, 4.20m, 4.02m, 3.85s(2), 3.70 m, 3.64m, 3.45m(3).



Anal. Calcd. for $C_{17}H_{25}N_5O_{10}$: C, 44.44; H, 5.48; N, 15.25. Found: C, 44.57; H, 5.94; N, 15.23.

6-Acetamido-9-(hepta-*O*-acetyl-β-cellobiosyl)-purine (V).—A suspension of 6 g. of 6-acetamido-9-chloromercuripurine[6] (III), 6 g. of cadmium carbonate and 1 g. of Celite[4] in 400 ml. of xylene was dried by azeotropic distillation of 100 ml. of the solvent. Hepta-*O*-acetyl-α-cellobiosyl bromide[12,13] (II, 10.2 g.) was added and the mixture was refluxed with stirring for 2 hr. The hot mixture was filtered and the filtrate was evaporated under reduced pressure to a sirup. The sirup and filter cake were extracted with 250 ml. of hot chloroform. The chloroform extract was washed successively with 30% potassium iodide solution and water, dried over anhydrous sodium sulfate and concentrated under reduced pressure to a sirup.

A portion (1.2 g.) of this sirup was dissolved in 10 ml. of benzene and placed on a column (150 × 44 mm.) of Micro-

(1) Supported by Grant No. CY 3232 from the Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, Bethesda 14, Md.

(2) M. L. Wolfrom, P. McWain, F. Shafizadeh and A. Thompson, THIS JOURNAL, **81**, 6080 (1959).

(3) J. Davoll and B. A. Lowy, *ibid.*, **73**, 1650 (1951).

(4) A product of Johns-Manville Co., New York, N. Y.

(5) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

(6) J. J. Fox, N. Yung, Iris Wempen and Iris L. Doerr, *ibid.*, **79**, 5060 (1957); B. R. Baker, Kathleen Hewson, H. Jeanette Thomas and J. A. Johnson, Jr., *J. Org. Chem.*, **22**, 954 (1957).

(7) D. H. Brauns, THIS JOURNAL, **51**, 1820 (1929).

(8) L. Goldman, J. W. Marsico and R. B. Angier, *ibid.*, **78**, 4173 (1956).

(9) E. J. Reist and B. R. Baker, *J. Org. Chem.*, **23**, 1083 (1958).

(10) The ultraviolet absorption analyses were made on a Cary recording spectrophotometer, model 10, Applied Physics Corp., Pasadena, Calif. The infrared spectral data were obtained by an infrared recording spectrophotometer, model B, Baird Associates Inc., Cambridge, Mass. Structural assignments were made following W. B. Neely, *Advances in Carbohydrate Chem.*, **12**, 13 (1957), and B. R. Baker and Kathleen Hewson, *J. Org. Chem.*, **22**, 959 (1959), and other publications of B. R. Baker and co-workers.

(11) Interplanar spacing, Å., $CuK\alpha$ radiation. Relative intensity, estimated visually: s, strong; m, medium; w, weak; v, very. Parenthetic numerals indicate order of the three most intense lines; 1, most intense.

(12) R. Ditmar, *Monatsh.*, **23**, 865 (1902).

(13) C. S. Hudson and A. Kunz, THIS JOURNAL, **47**, 2052 (1925).

Cel C[4] and developed with 300 ml. of benzene–ethanol (50:1 by vol.). The extruded column was streaked with indicator (1% potassium permanganate in 10% sodium hydroxide[5]). The third zone (30 mm. from the column top) was sectioned, eluted with acetone and after concentration to a sirup, the product was separated from hot ethanol; yield 65 mg. of amorphous material, m.p. 119–123°, $[\alpha]^{25}D$ −21.5° (*c* 0.7, water); absorption spectra data[10]: λ_{max}^{EtOH} 268 mμ; λ_{max}^{KBr} 2.88, 2.97 μ (NH, OH), 5.75 μ (ester carbonyl), 6.10, 6.20, 6.86 μ (NH and purine ring), 8.15 μ (C–O–C of acetates).

Anal. Calcd. for $C_{33}H_{41}N_5O_{15}$: C, 49.81; H, 5.20; N, 8.80. Found: C, 49.36; H, 5.37; N, 8.52.

9-β-Cellobiosyladenine (VII).—Sirupy 6-acetamido-9-(hepta-*O*-acetyl-β-cellobiosyl)-purine (V, 10.6 g.) was dissolved at 0° in 150 ml. of methanol nearly saturated with ammonia at 0°. The solution was allowed to stand at this temperature for 21 hr. and was then concentrated under reduced pressure to a sirup, which was dissolved in 100 ml. of water and extracted thrice with 20-ml. portions of chloroform. The aqueous solution was evaporated under reduced pressure to a glassy solid; yield 3.6 g. (53%). This crude material was dissolved in 30 ml. of water and treated with 30 ml. of 10% picric acid. The solid yellow picrate that formed was separated by filtration, dissolved in water and the solution was stirred with Dowex-1 (carbonate form) until colorless. The filtered solution was concentrated to a thin sirup which was extracted with hot 1-butanol. After standing in an open beaker for 3 days at room temperature, prismatic crystals formed. The material was recrystallized from hot 1-butanol; yield 690 mg. (10.3%), dec. 304–307°, $[\alpha]^{26}D$ −11.4° (*c* 0.5, water); absorption spectra data[10]: $\lambda_{max}^{H_2O}$ 260 mμ; λ_{max}^{KBr} 2.87, 3.02 μ (OH, NH), 5.98 (H_2N—C=N) 6.18, 6.32, 6.75 μ (NH and purine ring), 9.30, 9.58, 9.75, 10.08 μ (C–O–C, C–OH); X-ray powder diffraction data[11]: 13.15vw, 10.53w, 8.31vw, 7.56w, 7.06m, 6.81w, 5.91vw, 5.55vw, 4.98w, 4.76m, 4.47s(3), 4.28m, 4.13s(2), 3.97m, 3.81w, 3.73w, 3.61m, 3.44vs(1), 3.26w, 3.18w, 3.00w.

Anal. Calcd. for $C_{17}H_{25}N_5O_{10}$: C, 44.44; H, 5.48; N, 15.25. Found: C, 44.42; H, 5.53; N, 15.57.

9-β-Cellobiosyladenine was also obtained from its acetate by deacetylation with *n*-butylamine according to the method of Reist and Baker[9]; yield 32%.

2,6-Diamino-9-β-cellobiosylpurine (VIII).—A suspension of 2,6-diacetamido-9-chloromercuripurine[6] (IV, 6 g.), 12 g. of cadmium carbonate and 8 g. of Celite[4] in 400 ml. of xylene was dried by azeotropic distillation of 100 ml. of the solvent. Hepta-*O*-acetyl-α-cellobiosyl bromide (9 g.) was added and the mixture was refluxed for 5.5 hr. The suspension was filtered, the filtrate was concentrated under reduced pressure and the residue and the filter cake were extracted with 250 ml. of hot chloroform. The chloroform extract was washed successively with 30% potassium iodide, water, dried over sodium sulfate and concentrated to a sirup; yield 7.4 g. This material was dissolved in 120 ml. of 0.1 *N* sodium methoxide in methanol and refluxed for 2 hr. The mixture was cooled, neutralized with acetic acid and concentrated to dryness. The residue was dissolved in 150 ml. of water and extracted thrice with 100-ml. portions of chloroform. The aqueous solution was treated with 25 ml. of 10% methanolic picric acid and allowed to stand overnight at 10°. The yellow crystalline precipitate was separated by filtration, washed with a little cold water and redissolved in 400 ml. of warm water. The solution was stirred with Dowex-1 (carbonate form) until colorless. The aqueous solution was filtered and concentrated under reduced pressure to a sirup. The sirup was extracted with 1-butanol. Crystals deposited from the solution upon standing at room temperature in an open beaker for 7 days and were recrystallized from methanol; yield 830 mg. (13.6%), m.p. 232–235°, $[\alpha]^{25}D$ −22° (*c* 0.4, water); absorption spectra data[10]: $\lambda_{max}^{H_2O}$ 254, 276 mμ; λ_{max}^{KBr} 2.95, 3.05 μ (NH, OH) 6.10, 6.25, 6.82 μ (NH and purine ring), 8.95, 9.25, 9.40, 9.70μ (C–O–C, C–OH); X-ray powder diffraction data[11]: 14.74w, 7.38vw, 7.02s(3), 6.37w, 5.56vw, 4.91w, 4.74s(2), 4.38m, 4.22s, 3.95w, 3.81vs(1), 3.30vw, 3.24m, 3.15w.

Anal. Calcd. for $C_{17}H_{26}N_6O_{10}$: C, 43.03; H, 5.53; N, 17.72. Found: C, 43.11; H, 5.52; N, 17.54.

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[CONTRIBUTION FROM THE BIOCHEMICAL RESEARCH LABORATORY, THE DOW CHEMICAL CO.]

Optical Rotatory Dispersion Studies on Polysaccharides. II. Conformation of Partially Methylated Cellulose in Solution[1]

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Optical rotatory dispersion techniques have been used to examine solutions of methylcellulose. The complex dispersion exhibited by certain solutions demonstrated that aggregation of the polymer was taking place. This association of the polysaccharide was enhanced at elevated temperatures and prevented at temperatures below 10°. The formation of these aggregates was also shown to be concentration dependent.

The use of optical rotatory dispersion techniques for investigating the solution properties of polysaccharides is a rather recent innovation. We wish to follow up our preliminary studies on methylcellulose[2] in this paper. In the past few years there has been an intensive investigation of the rotatory dispersion curves of proteins and polypeptides.[3] These studies were an attempt to correlate dispersion curves with the helix–coil transitions occurring in protein denaturation.

(1) Presented in part at the Symposium on Solution Properties of Cellulose and Cellulose Derivatives, Am. Chem. Soc. Meeting, Cleveland, Ohio, April, 1960.

(2) W. B. Neely, *Nature*, **185**, 159 (1960).

(3) See for example (a) P. Doty and J. T. Yang, THIS JOURNAL, **78**, 498 (1956); (b) J. T. Yang and P. Doty, *ibid.*, **79**, 761 (1957); (c) B. Jirgensons, *Arch. Biochem. and Biophys.*, **74**, 57 (1958); **74**, 70 (1958); **78**, 227 (1958); **78**, 235 (1958); (d) E. R. Blout, "Optical Rotatory Dispersion: Applications to Organic Chemistry," C. Djerassi, ed., McGraw–Hill Book Co., Soc., New York, N. Y., 1960, Chapter 17.

Rotatory dispersion data are usually fitted by a modification of the single term Drude equation (1). Such modifications are used for the purpose

$$[\alpha] = \frac{k}{\lambda^2 - \lambda_c^2} \qquad (1)$$

k and λ_c are constants

λ = wave length at which measurement is made

$[\alpha]$ = specific rotation at given wave length

of obtaining linear plots of α against λ resulting in methods for evaluating the constants λ_c and k from slope-intercept relations. Yang and Doty[3b] have shown that to obtain λ_c with great precision, it is more advantageous to use the modification of the Drude equation shown in (2). This

$$\lambda^2[\alpha] = \lambda_c^2[\alpha] + k \qquad (2)$$

form of representing dispersion data will be used in the present investigation. The value of λ_c has