crude amine was dissolved in 100 ml. of water containing 17 g. of potassium dihydrogen phosphate and 2.4 g. of sodium nitrite. The resulting solution was heated 8 hr. on a steam bath. The product was taken up in ether and extracted with dilute hydrochloric acid to remove any unchanged amine. The neutral fraction was evaporated. Vapor phase chromatography showed three principal components in the approximate ratio 20:40:40. The two major components were separated by vapor phase chromatography on a 5-mg. scale. Infrared spectroscopy indicated that each of these compounds contained hydroxyl and carbonyl functions.

2-Bromomethyl-2,4,4-trimethyltetrahydropyran (V).—The acid II, 27.8 g., and 16 g. of red mercuric oxide were placed in 100 ml. of carbon tetrachloride and a solution of 24 g. of bromine in 40 ml. of carbon tetrachloride was added dropwise with stirring. The mixture was refluxed until no further carbon dioxide evolution was observed. This required about 1.5 hr. The bromide was recovered by distillation at 90–92° at 11 mm., $n^{25}D$ 1.4838, yield 13.7 g. The n.m.r. spectrum of V showed absorption at δ 3.8 (t, J = ~5 c.p.s.), 3.4 (s), 1.22–1.52 (m), and 1.02 (s) in the ratio 2:2:7:6.

Anal. Calcd. for $C_9H_{17}BrO$: C, 48.87; H, 7.75; Br, 36.13; O, 7.27. Found: C, 49.2; H, 7.6; Br, 36.0; O, 7.2.

3,3,5-Trimethyl-5-hexen-1-ol (VI).—The bromo compound V, 7.3 g., was treated with 4 g. of sodium in 100 ml. of ether for 1 hr. Excess sodium was destroyed with methanol and water, and the solution was acidified with acetic acid. The solution was extracted with ether. The ether solution was washed with water and dried over magnesium sulfate, and the ether was evaporated under vacuum. The resulting alcohol VI was distilled at 96° at 12 mm. and had $n^{23}D$ 1.4515.

Anal. Calcd. for $C_9H_{18}O$: C, 76.00; H, 12.75. Found: C, 76.1; H, 12.7.

The infrared spectrum was compatible with the structure proposed with sharp bands at 6.1 and 11.25 μ. The n.m.r. spectrum showed absorption at δ 4.81 (m), 4.67 (m), 3.62 (t, J = ~8 c.p.s.), 3.16 (s), 1.97 (s), 1.70 (s), 1.51 (t, J = ~8 c.p.s.), and 0.92 (s) in the ratio 1:1:2:1:2:3:2:6. The absorption at δ 3.16 was moved downfield by the addition of a trace of trifluoroacetic acid.

Alcohol VI was converted to the allophanate by treatment with cyanic acid in ether. The allophanate was recrystallized from benzene, m.p. 149.8–151.4°.

Anal. Calcd. for $C_{11}H_{20}H_2O_3$: C, 57.86; H, 8.83; N, 12.27. Found: C, 57.9; H, 8.5; N, 12.3.

3,3,5-Trimethyl-1-hexanol (VII).—The alcohol VI, 2 g., was saturated by hydrogenation over 1 g. of 5% rhodium on alumina in 50 ml. of acetic acid. The saturated alcohol VII was recovered by ether extraction after neutralization of the acetic acid. It was distilled at 56° at 0.5 mm., $n^{24}D$ 1.4352.

Anal. Calcd. for $C_9H_{20}O$: C, 74.93; H, 13.97. Found: C, 75.1; H, 13.7.

The infrared spectrum differed principally from that of VI by absence of the 6.1- and 11.25-μ absorptions. The n.m.r. spectrum showed absorption at δ 3.70 (t, J = ~7 c.p.s.), 2.97 (s), 1.52 (t overlying broad complex, J = ~7 c.p.s.), 1.16 (d), and 0.85–0.98 (3 peaks) in ratio 2:1:3:2:12. The absorption band at δ 2.97 was moved downfield by addition of a trace of trifluoroacetic acid.

The alcohol VII was converted to the allophanate by treatment with cyanic acid in ether. The allophanate was recrystallized from benzene and methanol, m.p. 152–153°.

Anal. Calcd. for $C_{11}H_{22}N_2O_3$: C, 57.36; H, 9.63; N, 12.16; O, 20.84. Found: C, 57.4; H, 9.4; N, 12.2; O, 21.1.

3,3,5-Trimethylhexanol.—An authentic sample of 3,3,5-trimethylhexanol was prepared *via* the published[9a] alkaline cleavage of isophorone to a mixture of 3,3,5-trimethyl-5-hexenoic acid and 3,3,5-trimethyl-4-hexenoic acid (56.6%). Recovery of this mixture rather than a single product was established by correspondence of vapor phase chromatographic determinations and isopropylidene determinations. This mixture was reduced by lithium aluminum hydride to a mixture of alcohols. The mixture of alcohols was hydrogenated in acetic acid with 5% rhodium on alumina to give a single saturated alcohol (95.5% pure by vapor phase chromatography), $n^{25}D$ 1.4332. This alcohol on reaction with cyanic acid gave an allophanate, m.p. 152–153°. The melting point of a mixture of this allophanate with the allophanate of VII was also 152–153°. Further, the infrared spectra of the two allophanates were identical.

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The Anomeric 9-(2-Amino-2-deoxy-D-glucopyranosyl)adenines[1,2]

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Condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide (I) with 6-acetamido-9-chloromercuripurine gives the crystalline blocked nucleoside derivative 6-acetamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]purine (Va) and also its α-D anomer (IIa). These products were *N*-deacetylated to the corresponding crystalline 6-amino derivatives Vb and IIb by way of the picrate salts Vc and IIc. Complete deblocking was achieved under mild conditions to give 9-(2-amino-2-deoxy-β-D-glucopyranosyl)adenine (VIa) and its α-D anomer (III), both in crystalline form. An ethyl thioglycoside (IVa) prepared from 2-deoxy-2-(2,4-dinitroanilino)-D-glucose is shown to be a pyranoside by conversion of its triacetate (IVb) into the known bromide I.

Several nucleosides of amino sugars exhibit antitumor or antibacterial properties,[3] and these observations have stimulated interest in the chemical synthesis of nucleoside derivatives of 2-amino-2-deoxy-D-glucose.[4-8] The synthetic nucleoside derivatives described have all been of the β-D configuration. The amino group of the sugar moiety in the purine nucleoside derivatives[4,5,7,8] has in each case been blocked by an acetyl or other group which could not be removed to yield the nucleo-

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(2) A preliminary report of part of this work has appeared: M. L. Wolfrom, H. G. Garg, and D. Horton, *Chem. Ind.* (London), 930 (1964).

(3) C. W. Waller, P. W. Fryth, B. L. Hutchings, and J. H. Williams, *J. Am. Chem. Soc.*, **75**, 2025 (1953); B. R. Baker, J. P. Joseph, and J. H. Williams, *ibid.*, **77**, 1 (1955); N. N. Gerber and H. A. Lechevalier, *J. Org. Chem.*, **27**, 1731 (1962); T. H. Haskell, A. Ryder, R. P. Frohardt, S. A. Fusari, Z. L. Jakubowski, and Q. R. Bartz, *J. Am. Chem. Soc.*, **80**, 743 (1958); T. H. Haskell, *ibid.*, **80**, 747 (1958).

(4) B. R. Baker, J. P. Joseph, R. E. Schaub, and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954).

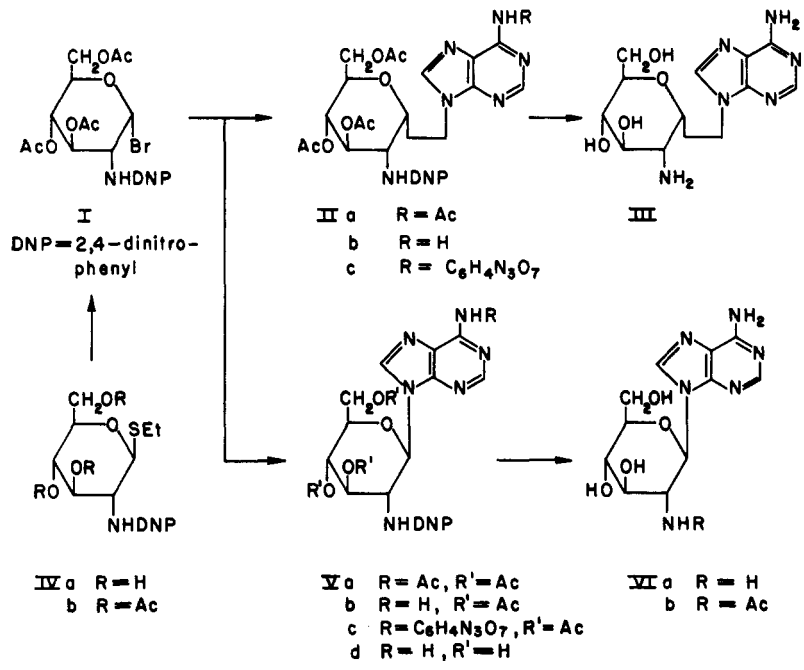
(5) T. Sugawa, Y. Kuwada, K. Imai, M. Morinaga, K. Kajiwara, and K. Tanaka, *Takeda Kenkyusho Nempo*, **20**, 7 (1961).

(6) C. L. Stevens and K. Nagarajan, *J. Med. Pharm. Chem.*, **5**, 1124 (1962).

(7) G. Schramm, H. Grötsch, and W. Pollmann, *Angew. Chem.*, **74**, 53 (1962); Farbwerke Höchst, A.-G., French Patent 1,324,011 (April 12, 1963); *Chem. Abstr.*, **59**, 11648d (1963).

(8) M. L. Wolfrom and R. Wurmb, Abstracts, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1964, p. 6D; *J. Org. Chem.*, in press.

SCHEME I



side of the free amino sugar; complete deblocking of pyrimidine nucleoside derivatives[6] has, however, been achieved. The present work describes the synthesis of blocked purine nucleoside derivatives of both anomeric configurations, and subsequent removal of all blocking groups under mild conditions, to yield 9-(2-amino-2-deoxy-α-D-glucopyranosyl)adenine (III) and its β-D anomer (VIa).

The starting point for the synthesis (see Scheme I) was 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide[9] (I), a stable synthetic intermediate which can conveniently be prepared in quantity,[10] and which has been shown[11] to yield glycosides of both anomeric configurations when treated with alcohols in the presence of a suitable acid acceptor. The electron-withdrawing 2,4-dinitroanilino group stabilizes the bromo sugar, and shows no apparent tendency to participate in glycoside-forming reactions; furthermore the 2,4-dinitrophenyl group can subsequently be removed under mild basic conditions.[9] Condensation of I with 6-acetamido-9-chloromercuripurine in refluxing xylene by the general procedure of Davoll and Lowy[12] gave a sirupy anomeric mixture of fully blocked nucleoside derivatives which was separable by preparative thin layer chromatography into the crystalline 6-acetamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl]purine (IIa), and the crystalline β-D anomer (Va), in 15 and 25% yields, respectively. The blocked nucleoside derivatives were *N*-deacetylated by boiling in methanolic picric acid[13] to give the picrate salts IIc and Vc of the corresponding 6-amino derivatives in analytically pure form. Treatment of the picrate salts with Dowex-1 (CO_3^{-2}) ion-exchange resin gave the crystalline anomeric 9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α- and -β-D-glucopyranosyl]adenines (IIb and Vb). All yields to this point were satisfactory. *O*-Deacetylation of the β-D-blocked nucleoside (Vb) with methanolic ammonia smoothly converted it into the crystalline 9-[2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]adenine (Vd).

Complete deblocking of 9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α- and -β-D-glucopyranosyl]-adenine (IIb and Vb) was achieved by treatment with Dowex-1 (OH^-) ion-exchange resin in warm aqueous acetone, to give 9-(2-amino-2-deoxy-α-D-glucopyranosyl)adenine (III) and its β-D anomer (VIa) in crystalline form, in yields of 30 and 25%, respectively. Although these yields are relatively low, it was nevertheless possible to obtain the anomeric deblocked nucleosides III and VIa in 2 and 3% yield, respectively, from readily available 2-amino-2-deoxy-D-glucose hydrochloride, if isolation at some of the intermediate stages was avoided.

The validity of the anomeric assignments made to III and VIa may reasonably be based on their specific rotations, +83 and −17°, respectively (in water), but a firm independent correlation was made by conversion of the β-D anomer VIa into the known[8] crystalline 9-(2-acetamido-2-deoxy-β-D-glucopyranosyl)adenine (VIb) by selective *N*-acetylation of the sugar moiety with acetic anhydride in water. The structure of the product (VIb) has been firmly established[8] by chemical and spectroscopic procedures. From this correlation it follows unambiguously that the blocked derivatives IIa-c belong to the α-D series, and the derivatives Va-d belong to the β-D series. In each anomeric pair of blocked derivatives the α-D anomer has a higher specific rotation than the β-D anomer, and for the pair of derivatives IIb and Vb it is shown (Figure 1) that this holds true over the wave-length range 700–450 mμ, indicating a qualitative agreement with the Hudson rules of rotation[14] in each pair of anomers. This agreement with the Hudson rules cannot be used alone as an unambiguous configurational proof for the pairs of derivatives IIa, Va, IIb, Vb, IIc, and Vc, since it has been shown[15] that in

(9) P. F. Lloyd and M. Stacey, *Tetrahedron*, **9**, 116 (1960).
(10) D. Horton and M. L. Wolfrom, *J. Org. Chem.*, **27**, 1794 (1962).
(11) P. F. Lloyd and G. P. Roberts, *J. Chem. Soc.*, 2962 (1963).
(12) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).
(13) J. R. Parikh, M. E. Wolff, and A. Burger, *ibid.*, **79**, 2778 (1957).
(14) C. S. Hudson, *ibid.*, **31**, 66 (1909); *Advan. Carbohydrate Chem.*, **3**, 15 (1948).
(15) D. Horton, *J. Org. Chem.*, **29**, 1776 (1964).

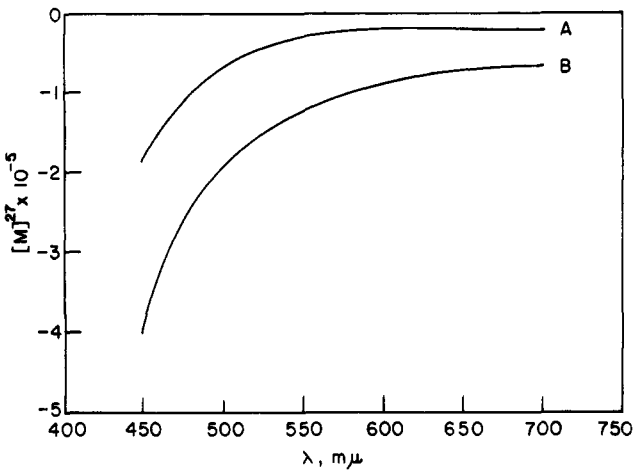


Figure 1.—Optical rotatory dispersion curves (in chloroform): A, 9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl]adenine (IIb); and B, 9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]adenine (Vb). See ref. 23 for details.

certain cases the 2-(2,4-dinitroanilino) group may cause reversal of the normal relative rotatory magnitudes of a pair of anomers; this has also been observed[16] with certain 2′-deoxynucleosides.

The formation of an α,β anomeric mixture in the condensation reaction may be attributed to the nonparticipation of the 2-(2,4-dinitroanilino) group during ionization of I, permitting attack by the purine group from either side of a carboxonium ion intermediate. A 2-acetamido or 2-acetoxy substituent would participate in the ionization, to give a 1,2-cyclized closed-ion intermediate, and lead to exclusive formation of the 1,2-*trans* related product.[17] Anomeric mixtures of nucleoside derivatives have been obtained by use of nonparticipating C-2 substituents in the D-ribose[18] and D-arabinose[19] series. A synthesis of a 1,2-*cis*-nucleoside of 3-amino-3-deoxy-D-ribose, by C-2 configurational inversion from an α-D-*arabino* (1,2-*trans*) precursor has been reported.[20]

Treatment of 2-deoxy-2-(2,4-dinitroanilino)-D-glucose with ethanethiol and concentrated hydrochloric acid at room temperature gave a crystalline thioglycoside derivative which on acetylation gave a crystalline triacetate. When the latter was treated with bromine in an inert solvent it was converted into 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide (I). This conversion establishes that the thioglycoside and its triacetate are pyranosides (IVa and IVb, respectively) and provides a further example[21] of the conversion of 1-thioglycosides into 1-halogeno sugar derivatives by the action of halogen. The procedure is not the most satisfactory preparative route to the bromo sugar I since the yield in the reaction leading to the thioglycoside is low (14%); the reaction is accompanied by side reactions, one of which leads to the formation of 2,4-dinitroaniline. The thioglycoside IVa and its triacetate IVb are tentatively assigned the β-D configuration on account of their low specific rotations. Literature values[22] for the corresponding *O*-glycoside derivatives and their anomers are qualitatively in agreement with the Hudson rules[14] of isorotation.

Experimental[23]

6-Acetamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α- and -β-D-glucopyranosyl]purine (IIa and Va).—3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide (I, 4.5 g.)[9,10] was added to an azeotropically dried suspension of 6-acetamido-9-chloromercuripurine[24] (3.5 g.), cadmium carbonate (3.5 g.), and Celite[25] in xylene (200 ml.), and the whole was refluxed for 5 hr. with stirring. The mixture was diluted with *n*-hexane (250 ml.), left overnight at 0°, and filtered; the filter cake was extracted with hot chloroform (500 ml.). The combined filtrate and extracts were washed twice with 30% aqueous potassium iodide solution and twice with water; the dried (magnesium sulfate) solution was concentrated to a yellow glass, yield 4.2 g. (79%), which on thin layer chromatography with ethyl acetate as developer showed two principal yellow components, R_f 0.2 and 0.4. The crude product was resolved on 40 chromatoplates (200 × 200 × 1 mm.), and the two zones were removed and extracted with acetone. Evaporation of the extract from the slower zone (R_f 0.2) gave 6-acetamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl]-purine (IIa) as a chromatographically homogeneous yellow glass, yield 0.80 g. (15%). The product crystallized slowly from methanol: m.p. 185–188°; $[\alpha]^{19}D$ −39 ± 6° (*c* 0.9, chloroform); λ_{max}^{KBr} 5.65 (OAc), 6.15, 6.25, 6.80 (aryl C=C, purine) 6.60, 7.52 (NO_2), and 13.45 μ (substituted benzene); λ_{max}^{EtOH} 262 mμ (ε 23,200) and 341 mμ (ε 18,200); X-ray powder diffraction data[23] 11.63 (vw), 10.4 (vw), 7.76 (w), 7.14 (s, 2), 6.24 (w), 5.83 (w), 5.37 (vw), 4.87 (m, 3,3), 4.44 (m, 3,3), 4.08 (m), 3.77 (m), 3.38 (s, 1).

Anal. Calcd. for $C_{25}H_{26}N_8O_{12}$: C, 47.62; H, 4.15; N, 17.78. Found: C, 47.12; H, 4.70; N, 18.24.

Evaporation of the extract from the faster zone (R_f 0.4) from the chromatographic separation gave 6-acetamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]-purine (Va) as a chromatographically homogeneous yellow glass, yield 1.3 g. (25%). Crystallization from methanol gave a yellow solid: m.p. 255–257°; $[\alpha]^{22}D$ −179 ± 4° (*c* 0.2, chloroform); λ_{max}^{KBr} 5.70 (OAc), 6.12, 6.23, 6.80 (aryl C=C, purine), 6.57, 7.45 (NO_2), and 13.70 μ (substituted benzene); λ_{max}^{EtOH} 262 mμ (ε 14,500) and 342 mμ (ε 11,600).

Anal. Calcd. for $C_{25}H_{26}N_8O_{12}$: C, 47.62; H, 4.15; N, 17.78. Found: C, 47.37; H, 4.57; N, 17.12.

(16) J. J. Fox and I. Wempen, *Advan. Carbohydrate Chem.*, **14**, 340 (1959); R. U. Lemieux and M. Hoffer, *Can. J. Chem.*, **39**, 110 (1961); R. U. Lemieux, *ibid.*, **39**, 116 (1961).

(17) R. S. Tipson, *J. Biol. Chem.*, **130**, 55 (1939); B. R. Baker, in "The Chemistry and Biology of Purines," G. E. W. Wolstenholme and C. M. O'Connor, Ed., J. and A. Churchill Ltd., London, 1957, pp. 120–129.

(18) R. S. Wright, G. M. Tener, and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 2004 (1958).

(19) C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 3004 (1963).

(20) B. R. Baker and R. E. Schaub, *J. Am. Chem. Soc.*, **77**, 2396 (1955); compare E. J. Reist, A. Benitez, L. Goodman, B. R. Baker, and W. W. Lee, *J. Org. Chem.*, **25**, 3274 (1962).

(21) W. A. Bonner, *J. Am. Chem. Soc.*, **70**, 3491 (1948); F. Weygand, H. Ziemann, and H. J. Bestmann, *Ber.*, **91**, 2534 (1958); F. Weygand and H. Ziemann, *Ann.*, **657**, 179 (1962); M. L. Wolfrom and W. Groebke, *J. Org. Chem.*, **28**, 2986 (1963); M. L. Wolfrom, H. G. Garg, and D. Horton, *ibid.*, **28**, 2989 (1963).

(22) P. F. Lloyd and G. P. Roberts, *J. Chem. Soc.*, 2962 (1963).

(23) Melting points were determined with a Hershberg-type apparatus [A. Thompson and M. L. Wolfrom, *Methods Carbohydrate Chem.*, **1**, 517 (1962)]. Specific rotations were determined in a 2-dm. polarimeter tube. Optical rotatory dispersion spectra were measured with a Rudolph Model 260/655/850/810–614 recording photoelectric spectropolarimeter. Infrared spectra were measured with a Perkin-Elmer Infracord infrared spectrometer. Ultraviolet spectra were measured with a Bausch and Lomb Spectronic 505 spectrometer. Microanalyses were determined by W. N. Rond. X-ray powder diffraction data give interplanar spacings, Å., for Cu Kα radiation. Relative intensities were estimated visually: s, strong; m, moderate; w, weak; v, very. The first three strongest lines are numbered (1 = strongest); double numbers indicate approximately equal intensities. Thin layer chromatography was carried out by the ascending method with Desaga equipment using silica gel G (E. Merck, Darmstadt, Germany) activated at 110°. Unless otherwise indicated, the layer thickness was 0.25 mm.; colored compounds were visualized directly, nucleoside derivatives were visualized under ultraviolet light, and sulfuric acid was used to detect other components.

(24) B. R. Baker, K. Hewson, H. J. Thomas, and J. A. Johnson, Jr., *J. Org. Chem.*, **22**, 954 (1957).

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

9-[3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino-α-D-glucopyranosyl]adenine Picrate (IIc).—A solution of 6-acetamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl]purine (IIa, 0.8 g.) in methanol (20 ml.) was mixed with a solution of picric acid (1.0 g.) in methanol (100 ml.). The mixture was refluxed for 30 min. and allowed to cool slowly. The solid product which separated was filtered and washed with cold methanol: yield 0.6 g. (67%); m.p. 145–150° dec.; $[\alpha]^{21}D$ +57 ± 2° (*c* 0.8, acetone); λ_{max}^{KBr} 5.73 (OAc), 5.93, 6.20, 6.35, 6.65 (picrate, aryl C=C, purine), 6.50, 7.60 (NO_2), 13.40, and 14.00 μ (substituted benzene).

Anal. Calcd. for $C_{29}H_{27}N_{11}O_{18}$: C, 42.61; H, 3.33; N, 18.84. Found: C, 42.71; H, 3.66; N, 18.52.

9-[3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]adenine Picrate (Vc).—A solution of 6-acetamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]purine (Va, 1.0 g.) in methanol (100 ml.) was treated with picric acid by the procedure used in the preceding experiment. The crystalline product, yield 0.7 g. (55%), had m.p. 175–185° dec.; $[\alpha]^{21}D$ −91 ± 3° (*c* 0.4, acetone); λ_{max}^{KBr} 5.70 (OAc), 5.90, 6.15, 6.35, 6.45 (picrate, aryl C=C, purine), 6.60, 7.55 (NO_2), 13.40, and 14.05 μ (substituted benzene); X-ray powder diffraction data[23] 12.78 (m), 10.40 (m), 7.56 (m, 3), 7.08 (w), 6.42 (w), 5.95 (w), 5.13 (w), 4.77 (w), 4.13 (m, 2,2), 3.95 (m, 2,2), 3.65 (vw), and 3.21 (s, 1).

Anal. Calcd. for $C_{29}H_{27}N_{11}O_{18}$: C, 42.61; H, 3.33; N, 18.84. Found: C, 42.50; H, 3.48; N, 18.92.

9-[3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl]adenine (IIb).—The picrate salt IIc (0.8 g.) was dissolved in acetone (120 ml.), water (60 ml.) was added, and the solution was stirred with an excess of Dowex-1 (CO_3^{-2}) ion-exchange resin for a few minutes at 35°. The mixture was filtered, and the resin was washed with acetone until the washings were colorless. The filtrate and washings were evaporated and the residue was recrystallized from methanol: yield 0.25 g. (57%); m.p. 192–195° dec.; $[\alpha]^{19}D$ −14 ± 2° (*c* 0.3, chloroform); optical rotatory dispersion data, see Figure 1; λ_{max}^{KBr} 5.70 (OAc), 6.20, 6.29, 6.80 (aryl C=C, purine), 6.60, 7.50 (NO_2), and 13.40 μ (substituted benzene); λ_{max}^{EtOH} 264 mμ (ε 17,800) and 337 mμ (ε 17,300); X-ray powder diffraction data[23] 11.79 (vw), 9.03 (m), 8.35 (w), 7.20 (vs, 1), 5.95 (m), 5.57 (m), 5.19 (w), 4.87 (w), 4.55 (s, 3,3), 4.23 (m), 4.06 (s, 3,3), 3.38 (s, 2).

Anal. Calcd. for $C_{23}H_{24}N_8O_{11}$: C, 46.94; H, 4.11; N, 19.04. Found: C, 46.63; H, 4.25; N, 19.15.

9-[3,4,6-Tri-*O*-acetyl-2-deoxy-(2,4-dinitroanilino)-β-D-glucopyranosyl]adenine (Vb).—The picrate salt Vc (0.50 g.) was treated with Dowex-1 (CO_3^{-2}) resin exactly as in the preceding experiment to give the free base Vb: yield 0.25 g. (70%); m.p. 254–256° dec.; $[\alpha]^{22}D$ −187 ± 2° (*c* 0.6, chloroform); optical rotatory dispersion data see Figure 1; λ_{max}^{KBr} 5.73 (OAc), 6.15, 6.30, 6.79 (aryl C=C, purine), 6.58, 7.48 (NO_2), and 13.40 μ (substituted benzene); λ_{max}^{EtOH} 265 mμ (ε 13,250) and 341 mμ (ε 12,800); X-ray powder diffraction data[23] 13.39 (m), 11.95 (m), 8.04 (s, 2), 6.51 (w), 5.40 (m, 3), 4.93 (m), 4.48 (vs, 1), 4.23 (m), 3.87 (w), 3.62 (w), 3.53 (w), and 3.33 (vw).

Anal. Calcd. for $C_{23}H_{24}N_8O_{11}$: C, 46.94; H, 4.11; N, 19.04. Found: C, 46.98; H, 4.32; N, 18.82.

9-[2-Deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]adenine (Vd).—Dry ammonia gas was passed for 30 min. through a solution of 9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]adenine (Vb, 0.40 g.) in methanol (150 ml.) at 0°. After 2 hr. at room temperature the solution was evaporated and the residue was crystallized from hot ethanol: yield 0.20 g. (50%); m.p. 208–215° dec.; $[\alpha]^{22}D$ −240 ± 2° (*c* 0.6, chloroform); λ_{max}^{KBr} 3.00 (OH), 6.05, 6.15, 6.25, 6.80 (aryl C=C, purine), 6.56, 7.55 (NO_2), and 13.40 μ (substituted benzene); λ_{max}^{EtOH} 265 mμ (ε 12,700) and 347 mμ (ε 12,400).

Anal. Calcd. for $C_{17}H_{18}N_8O_8$: C, 44.16; H, 3.92; N, 24.24. Found: C, 44.00; H, 4.46; N, 23.99.

9-(2-Amino-2-deoxy-α-D-glucopyranosyl)adenine (III).—A solution of 9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl]adenine (IIb, 1.0 g.) in acetone (80 ml.) and water (20 ml.) was stirred with an excess of Dowex-1 (OH^-) ion-exchange resin at 45–50° until it became colorless. The resin was filtered and washed with hot methanol (500 ml.). The filtrate and washings were evaporated and the crystalline residue was recrystallized from aqueous methanol: yield 0.15 g. (30%), m.p. 238–240° dec. Further recrystallization gave a pure product: m.p. 242–244° dec.; $[\alpha]^{22}D$ +83 ± 6° (*c* 0.2, water); λ_{max}^{KBr} 2.95, 3.05 (OH, NH), 5.90, 6.15, 6.30, and 6.75 μ (NH, purine); $\lambda_{max}^{H_2O}$ 262 mμ (ε 19,600); X-ray powder diffraction data[23] 9.21 (s, 1), 6.81 (m), 5.91 (w), 5.61 (m, 3,3), 5.31 (m), 5.10 (m, 3,3), 4.29 (m), 4.08 (w), 3.95 (w), 3.69 (w), 3.47 (s, 2), and 3.10 (w).

Anal. Calcd. for $C_{11}H_{16}N_6O_4$: C, 44.60; H, 5.40; N, 28.38. Found: C, 44.33; H, 5.66; N, 28.24.

9-(2-Amino-2-deoxy-β-D-glucopyranosyl)adenine (VIa).—Treatment of 9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]adenine (Vb, 1.0 g.) with Dowex-1 (OH^-) resin as in the preceding preparation gave the unblocked nucleoside VIa: yield 0.12 g. (25%); m.p. 186–188° dec.; $[\alpha]^{23}D$ −17 ± 2° (*c* 0.2, water); λ_{max}^{KBr} 2.95 (OH), 6.0, 6.18, 6.32, and 6.80 μ (NH, purine); $\lambda_{max}^{H_2O}$ 261 mμ (ε 15,300); X-ray powder diffraction data[23] 9.61 (s, 2,2), 7.08 (vs, 1,1), 5.37 (vs, 1,1), 4.98 (vw), 4.85 (w), 4.31 (vw), 3.80 (w), 3.65 (s, 3), 3.43 (s, 2,2), 3.27 (s, 2,2), 3.08 (vw), and 2.94 (vw).

Anal. Calcd. for $C_{11}H_{16}N_6O_4$: C, 44.60; H, 5.40; N, 28.38. Found: C, 44.37; H, 5.91; N, 28.22.

9-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)adenine[26] (VIb).—A solution of 9-(2-amino-2-deoxy-β-D-glucopyranosyl)adenine (VIa, 10 mg.) in water (0.1 ml.) was treated with acetic anhydride (0.05 ml.). After 45 min. at room temperature the solution was evaporated and the crystalline residue was found to have a mobility on thin layer plates coated with Avirin microcrystalline cellulose[27] identical with that of authentic[8] VIb and different from that of starting material (VIa), in the solvent systems 4:1:5 1-butanol–ethanol–water (upper phase) and 5:5:1:3 pyridine–ethyl acetate–acetic acid–water.[28] The entire product was purified by chromatography on an Avirin plate with the first solvent system, the product zone was excised and the product was extracted with water. The solution was concentrated to 0.05 ml., acetone (1 ml.) was added, and the solution was refrigerated to give crystalline VIb. The product had m.p. 236° dec., undepressed on admixture with authentic material,[8] and gave an X-ray powder diffraction pattern identical with that of authentic material prepared by a different route.[8]

Ethyl 2-Deoxy-2-(2,4-dinitroanilino)-1-thio-β-D-glucopyranoside (IVa).—A mixture of 2-deoxy-2-(2,4-dinitroanilino)-D-glucose[29] (5.0 g.), concentrated hydrochloric acid (20 ml.), and ethanethiol (10 ml.) was stirred for 24 hr. at room temperature. A small quantity of crystalline material was filtered from the solution, yield 20 mg., m.p. 178–180°, indistinguishable by mixture melting point and infrared spectrum from 2,4-dinitroaniline. The filtrate was extracted with chloroform (300 ml.) and then ether (300 ml.) and the combined extracts were evaporated to give a crystalline product, yield 0.80 g. (14%). Recrystallization from ethanol gave fine yellow needles: m.p. 204–206°; $[\alpha]^{18}D$ −90 ± 3° (*c* 0.2, methanol); λ_{max}^{KBr} 2.90 (OH, NH), 6.17, 6.31, 6.62 (aryl C=C), 6.60, 7.54 (NO_2), 7.80 (SEt), 13.46, and 13.84 μ (substituted benzene); λ_{max}^{EtOH} 265 mμ (ε 7500), and 349 mμ (ε 10,000); X-ray powder diffraction data[23] 15.78 (m), 11.48 (w), 11.19 (s, 3), 7.63 (vs, 1), 6.42 (m), 5.72 (m), 5.22 (w), 4.82 (w), 4.70 (vw), 4.51 (s, 2), 4.33 (vw), and 4.08 (w).

Anal. Calcd. for $C_{14}H_{19}N_3O_8S$: C, 43.17; H, 4.91; N, 10.08; S, 8.23. Found: C, 43.35; H, 5.05; N, 10.45; S, 8.37.

At longer reaction times the amount of 2,4 dinitroaniline isolated was increased.

Ethyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-1-thio-β-D-glucopyranoside (IVb).—Ethyl 2-deoxy-2-(2,4-dinitroanilino)-1-thio-β-D-glucopyranoside (IVb, 0.30 g.) was treated with pyridine (5 ml.) and acetic anhydride (5 ml.); the mixture was stored for 24 hr. at room temperature with occasional shaking, then poured into ice and water (50 ml.). The crystalline precipitate was filtered and recrystallized from hot ethanol as yellow needles: yield 0.35 g. (90%); m.p. 195–196°; $[\alpha]^{19}D$ −53 ± 1.5° (*c* 0.4, chloroform); λ_{max}^{KBr} 3.05 (NH), 5.74 (OAc), 6.13, 6.30, 6.70 (aryl C=C), 6.53, 7.52 (NO_2), 7.89 (SEt), 13.46, and 13.79 μ (substituted benzene); λ_{max}^{EtOH} 267 mμ (ε 6300) and 343 mμ (ε 9200); X-ray powder diffraction data[23] 11.63 (vs, 1), 10.28 (m), 8.67 (m), 5.54 (vw), 5.28 (m, 3,3), 4.98 (s, 2,2), 4.70 (s, 2,2), 4.40 (w), 4.08 (m), 3.88 (m), 3.75 (m, 3,3), and 3.41 (vw).

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(26) This experiment was performed by Dr. R. Wurmb.

(27) (a) M. L. Wolfrom, D. L. Patin, and R. M. de Lederkremer, *Chem. Ind.* (London), 1065 (1964); *J. Chromatography*, in press; (b) Avirin = Avicel, technical grade.

(28) F. G. Fischer and H. J. Nebel, *Z. Physiol. Chem.*, **302**, 10 (1955).

(29) K. H. Meyer and D. E. Schwartz, *Helv. Chim. Acta*, **33**, 1651 (1950).

Anal. Calcd. for $C_{20}H_{25}N_3O_{11}S$: C, 46.60; H, 4.88; N, 8.15; S, 6.21. Found: C, 46.12; H, 5.19; N, 8.47; S, 6.45.

Conversion of IVa into 3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl Bromide (I).—A solution of ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-1-thio-β-D-glucopyranoside (IVb, 50 mg.) in methylene chloride (5 ml.) was treated at room temperature with a slight excess of bromine in methylene chloride. The solution was concentrated after 5 min. and the crystalline material which separated was filtered and washed with anhydrous ether, yield 40 mg. (80%), m.p. 162–164°, identical by mixture melting point and infrared spectrum with an authentic sample[9] of I.

Products from the Ortho Ester Form of Acetylated Maltose[1]

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Hot acetylation of β-maltose with sodium acetate and acetic anhydride leads to the formation of 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose and its anomer in addition to the principal product, β-maltose octaacetate. The acid-catalyzed chloroform mutarotation of β-maltose heptaacetate is complex and involves its anomer as well as 3,6,2′,3′,4′,6′-hexa-*O*-acetyl-β-maltose. The latter compound is formed by the successive action on β-maltose octaacetate of acetic anhydride–hydrogen bromide and warm aqueous sodium acetate with the anomeric forms of 2,3,4,2′,3′,4′,6′-maltose heptaacetate being produced in addition. These findings are explained on the basis of the intermediate formation of a 1,2-orthoacetate structure.

In previous investigations in this laboratory, the readily crystallizable 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-maltose (hereinafter designated β-maltose heptaacetate) had been isolated, first from the acetolysis products of amylopectin[2] and then from acetylated acid-reverted mixtures from maltose and D-glucose.[3] This anomalous result warranted further investigation and in particular we wished to determine whether this partially acetylated maltose structure was a normal product, hitherto undetected, of the acetylation of maltose with hot acetic anhydride and sodium acetate. Such an acetylation mixture was accordingly investigated by column chromatographic methods as piloted by thin layer techniques. β-Maltose heptaacetate was indeed found among the acetylation products and in 0.3% yield. Furthermore, these sensitive techniques showed the presence of two other substances, in small amount, in addition to β-maltose octaacetate, the major product (85% yield). One of these substances was identified as the hitherto unknown anomer of 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose or α-maltose heptaacetate. Its rotation in chloroform was found to be +128°, in excellent agreement with the value +131° calculated for it by Hudson and Sayre.[4] Confirmation of the anomeric nature of this substance was found by establishing it as a mutarotation product of the common β-D form. A partially acetylated aldose having its C-1 hydroxyl free would be expected to mutarotate and indeed Hudson and Sayre reported that a chloroform solution of β-maltose heptaacetate changed in rotation from $[\alpha]^{20}D$ +68 → +110° in 5 weeks. As sugar mutarotation is known to be subject to general acid–base catalysis, we found that this equilibrium could be attained in 5–10 hr. by acid catalysis[5]; Hudson and Sayre[4] had found the same on the addition of a trace of ammonia. An analysis of the equilibrated mixture by thin layer chromatographic methods showed two components besides the predominant starting material. Extrusive silicate column chromatography then served to isolate the products in crystalline form. One of these was the α-maltose heptaacetate and the other was a crystalline hexaacetate, m.p. 163–165°, $[\alpha]^{20}D$ +83 → +92° (chloroform). Both of these acetates produced β-maltose octaacetate on further acetylation under mild conditions (acetic anhydride and pyridine at room temperature).

The formation of maltose heptaacetate on acetylation of maltose can be rationalized by postulating an ortho ester intermediate. The ortho ester cation V, formed by attack (IV → V) of the neighboring *trans*-acetoxy group upon the glycosidic hydroxyl, can react with acetate ion to give the orthoacetate VI. On processing the reaction mixture with acidic water, as is customarily done in sodium acetate acetylations, the ortho ester VI would be hydrolyzed to maltose heptaacetate (IV, VIII). This anomerizable heptaacetate favors its β-D form (IV), as visually estimated by thin layer chromatography of the equilibrated mixture. Both anomers (IV and VIII) were, however, isolated.

The low yields of ortho ester reaction products from the sodium acetate acetylation of β-maltose is reasonable since the elevated reaction temperature does not favor ortho ester formation, as has been pointed out by Isbell and Frush[6] for the methyl orthoacetate form of acetylated D-mannose.

Ortho ester derivatives of maltose are known,[7] all of which have been obtained by further reaction of a hepta-*O*-acetylmaltosyl chloride of ortho ester structure discovered by Freudenberg and Ivers.[8] Freudenberg and co-workers[9] reported the isolation of a substance that may have been VI by reaction of the sensitive orthoacetyl chloride with silver acetate but the amorphous product was not characterized. If the orthoacetate VI is a product of the sodium acetate acetylation of maltose and leads to the formation of β-maltose heptaacetate on further processing with water, then maltose heptaacetate should not be formed if the acidic (acetic acid) water treatment be omitted. In

(1) Preliminary communication: Abstracts, 145th National Meeting of the American Chemical Society, New York, N. Y., Sept. 1963, p. 4D.

(2) M. L. Wolfrom, J. T. Tyree, T. T. Galkowski, and A. N. O'Neill *J. Am. Chem. Soc.*, **73**, 4927 (1951).

(3) M. L. Wolfrom, A. Thompson, and R. H. Moore, *Cereal Chem.*, **40**, 182 (1963).

(4) C. S. Hudson and R. Sayre, *J. Am. Chem. Soc.*, **38**, 1867 (1916).

(5) *Cf.* C. E. Ballou, S. Roseman, and K. P. Link, *ibid.*, **73**, 1140 (1951).

(6) H. S. Isbell and H. L. Frush, *J. Res. Natl. Bur. Std.*, **43A**, 161 (1949)

(7) E. Pacsu, *Advan. Carbohydrate Chem.*, **1**, 80 (1945).

(8) K. Freudenberg and O. Ivers, *Ber.*, **55**, 929 (1922).

(9) K. Freudenberg, H. v. Hochstetter, and H. Engels, *ibid.*, **58**, 666 (1925).