Preparation of the labeled 2,3,4,6-tetra-O-benzoyl-D-glucoses. The labeled 1,2,3,4,6-penta-*O*-benzoyl-D-glucoses described were transformed into 2,3,4,6-tetra-*O*-benzoyl-D-glucosyl bromides following Ness, Fletcher, and Hudson,[23] and then by the method of Fischer and Noth[25] into 2,3,4,6-tetra-*O*-benzoyl-D-glucoses.

(a) *2,3,6-Tri-O-benzoyl-4-O-benzoyl-carbonyl-C*^{14}*-D-glucose.* M.p. 125–127°; $[\alpha]^{25}D$ +42.8°.

(b) *2,3,4-Trio-O-benzoyl-6-O-benzoyl-carbonyl-C*^{14}*-D-glucose.* M.p. 124–126°; $[\alpha]^{25}D$ +45.4°.

(c) *2,3-Di-O-benzoyl-4,6-di-O-benzoyl-carbonyl-C*^{14}*-D-glucose.* M.p. 129–131° $[\alpha]^{25}D$ +44.5°.

(d) *4,6-Di-O-benzoyl-2,3-di-O-benzoyl-carbonyl-C*^{14}*-D-glucose.* M.p. 127–129°. $[\alpha]^{25}D$ +48.5°.

(e) *2,6-Di-O-benzoyl-3,4-di-O-benzoyl-carbonyl-C*^{14}*-D-glucose.* M.p. 120–121°. $[\alpha]^{25}D$ +43.8°.

All labeled tetra-*O*-benzoyl-D-glucopyranoses were recrystallized from benzene-petroleum ether (b.p. 40–60°). When recrystallized from a petroleum fraction of b.p. 100–130°, substances with m.p. 117–120° and $[\alpha]^{25}D$ +72.5° were obtained. None of these products depressed the m.p. when mixed with 2,3,4,6-tetra-*O*-benzoyl-D-glucose m.p. 126–129°; $[\alpha]^{25}D$ + 44.2° prepared from pure 1,2,3,4,6-penta-*O*-benzoyl-β-D-glucose and recrystallized several times from benzene-petroleum ether (40–60°).

On ammonolysis, the labeled tetra-*O*-benzoyl-D-glucoses listed in Table III gave an average yield of II of 25.2% (25–26%).

(25) E. Fischer and H. Noth, *Ber.*, **51**, 321 (1918).

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AV. SIR A. FLEMING 1653
MARTÍNEZ (PCIA. BUENOS AIRES), ARGENTINA

[CONTRIBUTION FROM THE CANCER RESEARCH LABORATORY, DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, UNIVERSITY OF FLORIDA]

Methyl 3,4-Anhydro-β-D-galactopyranoside. I. Reduction[1,2]

MURIEL DAHLGARD, BARBARA H. CHASTAIN,[3] AND RU-JEN LEE HAN

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Upon reduction with lithium aluminum hydride methyl 3,4-anhydro-β-D-galactopyranoside (I) gave a 73% yield of methyl 3-deoxy-β-D-galactopyranoside (II) and a 5% yield of methyl 4-deoxy-β-D-glucopyranoside (VII). Catalytic reduction gave 24% of II and 30% of III. Hydrolysis of II to 3-deoxy-D-galactose followed by reduction with sodium borohydride gave 3-deoxy-D-galactitol. Hydrolysis of VII gave 4-deoxy-D-glucose which upon reduction gave 4-deoxy-D-glucitol (3-deoxy-L-galactitol). The enantiomorphic alcohols were characterized as their pentaacetates.

2-Deoxy-D-glucose[4–6] and 2-deoxy-D-galactose[6] have been shown to be potent glycolytic inhibitors of various tumor tissues. On the other hand, hexose analogs substituted at C-3 and C-6 do not possess this property.[6] We wished to prepare 4-deoxy-D-glucose, not only to determine if it inhibits tumor growth, but also to use as an intermediate in the preparation of possible antimetabolites of the pentose phosphate pathway in carbohydrate metabolism.[7]

The most direct route to 4-deoxy-D-glucose appeared to be through the reduction of methyl 3,4-anhydro-β-D-galactopyranoside (I). This oxide had been prepared by Müller *et al*[8] by conversion of 4-methanesulfonyl-β-D-glucose tetraacetate to methyl 4-methanesulfonyl-2,3,6-triacetyl-β-D-glucopyranoside, followed by reaction with one equivalent of sodium methoxide. Starting with β-glucose pentaacetate, we prepared methyl 2,3,6-tri-*O*-acetyl-β-D-glucopyranoside by the standard laboratory procedures. Reaction with excess methanesulfonyl chloride gave the 4-methanesulfonyl derivative, which was then converted into the oxide I using Müller's procedure.

Reduction of I with lithium aluminum hydride gave a 73% yield of methyl 3-deoxy-β-D-galactopyranoside (II), together with 5% of methyl 4-deoxy-β-D-glucopyranoside (VII). On the other hand, catalytic reduction with freshly prepared Raney nickel resulted in the isolation of 24% of II and 30% of VII. When the catalyst was not prepared immediately before use, the main or only isolable product was II. The 3-deoxy derivative is the least soluble and more readily crystallized product, therefore, it is difficult to remove traces of II from methyl 4-deoxy-β-D-glucopyranoside. The presence of II is easily detected by the characteristic absorption bands at 780 cm.$^{-1}$ and 850 cm.$^{-1}$ in the infrared spectrum. These bands are absent in the spectrum of VII.

Compound II consumed no periodate ion, indicating the absence of adjacent hydroxyl groups, and it readily formed a benzylidene derivative

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(3) Present Address: Medical Div., Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn.

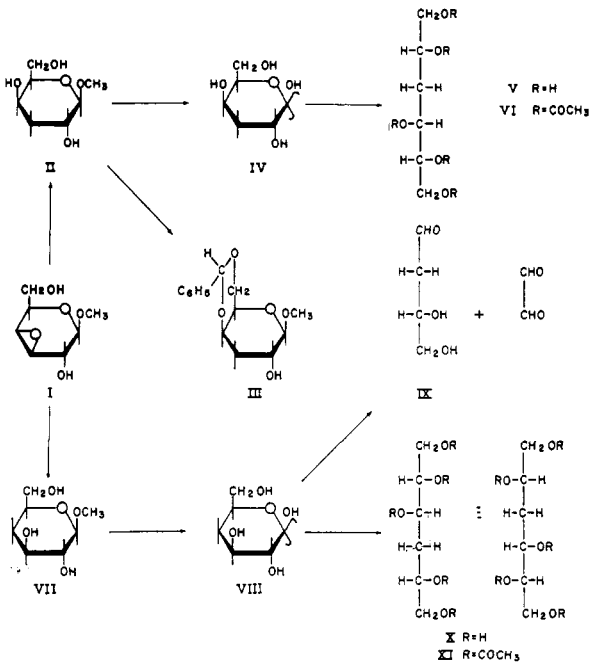
(4) G. E. Woodward and M. T. Hudson, *Cancer Res.*, **14**, 599 (1954).

(5) J. O. Ely, *J. Franklin Inst.*, **258**, 157 (1954).

(6) J. Laszlo, B. Landau, K. Wight, and D. Burk, *J. Nat. Cancer Inst.*, **21**, 475 (1958).

(7) M. Dahlgard and E. Kaufmann, *J. Org. Chem.*, **25**, 781 (1960).

(8) A. Müller, M. Moricz, and G. Verner, *Ber.*, **72B**, 745 (1939).



(III). Acid hydrolysis of II gave the known 3-deoxy-D-galactose (IV), as an oil having $[\alpha]^{28}D$ +4.2° in close agreement with $[\alpha]^{20}D$ +6.94 ±2° reported by Huber and Reichstein[9] and forming a crystalline 2,4-dinitrophenylhydrazone.

Compound VII consumed 1 mole of periodate ion, indicating the presence of a pair of adjacent hydroxyl groups. Acid hydrolysis of VII gave 4-deoxy-D-glucose (VIII) as an oil which solidified upon standing, m.p. 128–130°; $[\alpha]^{27}D$ +47.9° (equilibrium 2 hr.). Hedgley *et al.*[10] described the preparation of VIII from methyl 4-deoxy-α-D-glucopyranoside, m.p. 131–132°, from ethyl acetate, $[\alpha]D$ +44° → 60.3° (equilibrium 3.5 hr.).

Upon oxidation with 1 mole of periodate ion followed by hydrolysis, 4-deoxy-D-glucose should give 3,4-dihydroxybutyraldehyde (IX). When these reactions were carried out on compound VIII, a chromatogram on Whatman No. 1MM paper gave a spot with R_f 0.67, very close to the value of R_f 0.68 reported by Machell and Richards[11] for 3,4-dihydroxybutyraldehyde.

As a final proof of structure the two sugars IV and VIII were reduced to their corresponding deoxyhexitols. As the two deoxyhexitols should be enantiomorphs, they should exhibit the same melting points and the same degree of optical rotation, only in opposite directions. Upon reduction with sodium borohydride IV gave 3-deoxy-D-galactitol (V) as an oil with $[\alpha]^{25}D$ +21.2°, while VIII gave 4-deoxy-D-glucitol (3-deoxy-L-galactitol) (X) also as an oil, $[\alpha]^{25}D$ −19.5°. The corresponding pentaacetates VI and XI were obtained as solids, m.p. 84–85°, $[\alpha]^{28}D$ +34.0° and m.p. 85–86°, $[\alpha]^{27}D$ −34.5°, respectively.

Results of the biological testing of the compounds described in this paper will be reported elsewhere.

EXPERIMENTAL[12]

Methyl 4-O-methanesulfonyl-2,3,6-tri-O-acetyl-β-D-glucopyranoside. Methyl 2,3,6-tri-*O*-acetyl-β-D-glucopyranoside[13] (7.3 g.; 0.023 mole) dissolved in 25 ml. of dry pyridine with heating was treated in the cold with 7.8 g. (0.068 mole) of methanesulfonyl chloride, left overnight at room temperature, then poured over ice. After half an hour the oil which had separated crystallized readily upon rubbing with a stirring rod. The solid was dissolved in chloroform, treated with Norit, filtered, and evaporated to dryness. Treatment of the residue with ice water gave 5.5 g. (61%) of methyl 4-*O*-methanesulfonyl-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside m. 108–109° $[\alpha]^{26}D$ −41.4° (*c* 2.05; $CHCl_3$) in agreement with the literature value of m.p. 110–111°, $[\alpha]^{21}D$ −42.1°.[8]

Methyl 3,4-anhydro-β-D-galactopyranoside (I). The oxide was prepared by the method of Müller *et al.*[8] m.p. 157–158°, $[\alpha]^{25}D$ −121.8° (c 2.13; water). Lit. m. 158°, $[\alpha]^{21}D$ −119.4° (c 2.512; water).

Reduction of methyl 3,4-anhydro-β-D-galactopyranoside (I). (A) *With lithium aluminum hydride.* To a solution of lithium aluminum hydride (3.4 g.; 0.09 mole) in 100 ml. of anhydrous diethyl ether was added slowly a solution of I (5.2 g.; 0.03 mole) in 380 ml. of freshly distilled tetrahydrofuran. When the addition was completed (90 min.), another 0.5 g. of lithium aluminum hydride was added. No further evolution of hydrogen was observed. After stirring for 1 hr. the mixture was hydrolyzed with 300 ml. of water, and the precipitate of aluminum hydroxide removed by filtration. The filtrate was deionized by passing through columns of Amberlite IR-120 and Duolite A-4 ion exchange resins, then taken to dryness on an evaporator, the residue being treated several times with methanol and taken again to dryness, giving a theoretical yield of a white crystalline solid. Recrystallization from isopropanol gave a total of 3.8 g. (73%) of methyl 3-deoxy-β-D-galactopyranoside (II) m.p. 173–174° $[\alpha]^{25}D$ −69.4° (c 2.12; water). Compound II exhibited strong absorption in the infrared at 780 cm.$^{-1}$ and 850 cm.$^{-1}$ It consumed no periodate ion when treated with sodium metaperiodate according to the spectrophotometric method of Aspinall and Ferrier,[14] indicating no adjacent hydroxyl groups.

Anal. Calcd. for $C_7H_{14}O_5$: C, 47.20; H, 7.86. Found: C, 47.42; H, 8.17.

The isopropanol filtrate was evaporated to dryness, then extracted with chloroform (3 × 50 ml.), the chloroform extract was evaporated to dryness and the residue recrystallized from isopropanol: hexane (1:2) to give 232 mg. (5%) of methyl 4-deoxy-β-D-glucopyranoside (VII) m. 143°, $[\alpha]^{25}D$ −35.5° (*c* 1.270; water). Compound VII did not exhibit absorption bands at 780 cm.$^{-1}$ or 850 cm.$^{-1}$ It consumed one mole of periodate ion, indicating the presence of a pair of adjacent hydroxyl groups.

Anal. Calcd. for $C_7H_{14}O_5$: C, 47.20; H, 7.86. Found: C, 47.53; H, 8.24.

(9) H. Huber and T. Reichstein, *Helv. Chim. Acta.*, **31**, 1645 (1948).

(10) E. J. Hedgley, O. Mérész, W. G. Overend, and R. Rennie, *Chem. & Ind.*, 938 (1960).

(11) G. Machell and G. N. Richards, *J. Chem. Soc.*, 1924 (1960).

(12) Melting points are uncorrected. Microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Rotations were determined with a Keston standard polarimeter. Descending technique was used for all paper chromatograms.

(13) P. A. Levene, and A. L. Raymond, *J. Biol. Chem.*, **97**, 771 (1932).

(14) G. O. Aspinall and R. J. Ferrier, *Chem. & Ind.*, 1216 (1957).

(B) *Catalytic hydrogenation.* Using the procedure described by Prins[15] the oxide I (20 g.) was reduced at 1500 p.s.i. and 100–110° in ethanol solvent, using Raney nickel catalyst freshly prepared from 20 g. of nickel-aluminum alloy. The crude product (13.8 g.) was extracted with boiling chloroform (7 × 100 ml.) leaving a residue of 3.3 g. (24%). Recrystallization of the residue from isopropanol (125 ml.) gave 1.4 g. (10%) of methyl 3-deoxy-β-D-galactopyranoside m. 173° and showing no depression in melting point when admixed with compound II. Evaporation of the chloroform extract followed by recrystallization of the residue from isopropanol-hexane gave 4.0 g. (30%) of methyl 4-deoxy-β-D-glucopyranoside m.p. 127–130° which upon recrystallization from isopropanol m.p. 145–146°, $[\alpha]^{25}D$ −35.0° (*c* 2.0; water) and showed no depression when admixed with compound VII.

Methyl 4,6-benzylidene-3-deoxy-β-D-galactopyranoside (III). To 2.0 g. of II in 10 ml. of benzaldehyde was added 3.0 g. of pulverized anhydrous zinc chloride. After shaking for 0.5 hr. the solution had become warm and viscous; it was left overnight at room temperature. After trituration with pentane to remove unchanged benzaldehyde, the residue was washed several times with cold water, then it was taken up in chloroform. Upon addition of anhydrous sodium sulfate to the chloroform solution a precipitate formed which dissolved readily upon heating. The sodium sulfate was removed from the hot solution by filtration and the filtrate upon cooling deposited 1.5 g. (48%) of III, m.p. 199–201°. Analytical sample recrystallized from chloroform m.p. 211°, $[\alpha]^{23}D$ −90.3°.

Anal. Calcd. for $C_{14}H_{18}O_5$: C, 63.14; H, 6.81. Found: C, 62.85; H, 6.90.

3-Deoxy-D-galactose (IV). Hydrolysis of II (4.0 g.) in 0.1*N* sulfuric acid (196 ml.) by heating on the steam bath to constant rotation (5.25 hr.), followed by neutralization with barium carbonate, filtration and evaporation to constant weight at <45°, final traces of water being removed by co-distillation with benzene, gave a theoretical yield of IV as an oil with $[\alpha]^{26}D$ +2.9° (*c*, 1.948; water). Chromatography on Whatman 1MM paper using *n*-butanol–acetic acid–water (4:1:1), descending method, gave a single spot upon development with silver nitrate and sodium hydroxide with R_{xylose} 1.33. Because the material failed to crystallize it was purified by chromatography on Whatman 3MM paper using the same solvent system. Development with silver nitrate and sodium hydroxide showed one main spot and three smaller spots. The area of the main spot was eluted with water and the water removed by evaporation at <45°. The residue was taken up in methanol, filtered through Celite No. 535 and again evaporated to dryness, leaving an oil having $[\alpha]^{28}D$ +4.2° (*c* 4.3; water). Huber and Reichstein[9] reported $[\alpha]^{20}D$ +6.94 ±2° for 3-deoxy-D-galactose.

3-Deoxy-D-galactose 2,4-dinitrophenylhydrazone. IV, when treated with 2,4-dinitrophenylhydrazine in ethanol according to the procedure of Lloyd and Doherty[16] gave a theoretical yield of crude product which upon recrystallization from ethyl acetate gave a yellow crystalline solid m.p. 129–130°, $[\alpha]^{25}D$ −15.1° (*c*, 1.0; methanol).

Anal. Calcd. for $C_{12}H_{16}N_4O_8$: C, 41.86; H, 4.65; N, 16.28. Found: C, 41.91; H, 4.85; N, 16.37.

3-Deoxy-D-galactitol (V). 3-Deoxy-D-galactose (III) as an oil obtained from the hydrolysis of 2.1 g. of methyl 3-deoxy-β-D-galactopyranoside (II) was dissolved in 40 ml. of water and treated with a solution of sodium borohydride (1 g.) in 30 ml. of water.[17] Hydrogen was evolved. After standing at room temperature for 2 hr., the solution was acidified with 50% acetic acid, then passed through columns of Amberlite IR-120 and Duolite A-4 ion exchange resins. Evaporation to dryness with benzene gave a theoretical yield of 3-deoxy-D-galactitol (V) as an oil with $[\alpha]^{25}D$ +21.2° (*c* 2.35; water).

3-Deoxy-D-galactitol pentaacetate (VI). Acetylation of V with excess acetic anhydride in pyridine in the usual manner gave VI as large crystals from ethyl acetate–hexane, m.p. 84–85°, $[\alpha]^{28}D$ +34.0° (*c* 2.0; methanol). Analysis indicated that the product contained 1 mole of ethyl acetate of crystallization.

Anal. Calcd. for $C_{20}H_{32}O_{12}$: C, 51.72; H, 6.94. Found: C, 51.78; H, 6.59.

4-Deoxy-D-glucose (VIII). Hydrolysis of VII (1.0 g.) with 0.2*N* sulfuric acid by heating on the steam bath to constant rotation (3 hr.) followed by neutralization with barium carbonate, filtration and evaporization to dryness gave 0.8 g. (88%) of VIII as a light yellow glass, having $[\alpha]^{27}D$ +47.9° (*c* 1.84; water; equilibrium 2 hr.). Upon standing overnight the material solidified to a white crystalline solid, m.p. 128–130°, R_{xylose} 1.13 (*n*-butyl alcohol–acetic acid–water; 4:1:1; 23 hr.). No suitable recrystallization solvent has yet been found for this deoxy sugar. The preparation of V from methyl 3,4-anhydro-α-D-galactopyranoside, m.p. 131–132° from ethyl acetate, $[\alpha]D$ +44° → 60.3° (equilibrium after 3.5 hr.) (*c* 2.4; water), has been reported recently.[10]

Oxidation of VIII *with sodium periodate.* Following the procedure described by Lee[18] for periodate oxidation of deoxyhexoses, VIII (0.10 g.; 6 × 10^{-4} mole) was dissolved in 6 ml. of 0.1*M* sodium metaperiodate and the solution was left in the dark for 0.5 hr. After neutralization with barium hydroxide and filtration, the filtrate was heated to 50° for 5 min., cooled, and extracted with chloroform. A chromatogram of the water layer on Whatman No. 1MM paper using *n*-butyl alcohol-pyridine–water (6:44:3) (12 hr.) showed a spot when sprayed with silver nitrate and sodium hydroxide with R_f 0.67 in good agreement with R_f 0.68 reported for 3,4-dihydroxybutyraldehyde (IX) by Machell and Richards.[11]

4-Deoxy-D-glucitol (*3-deoxy-L-galactitol*) (X). To a solution of VIII (0.39 g.) in 11 ml. of water was added 0.2 g. of sodium borohydride in 5 ml. of water. After standing for 2 hr. at room temperature, the reaction mixture was acidified with 50% acetic acid, deionized with Amberlite IR-120 and Duolite A-4 ion exchange resins, and evaporated to dryness with benzene to give a theoretical yield of X as an oil with $[\alpha]^{27}D$ −19.5° (*c* 2.24; water).

4-Deoxy-D-glucitol pentaacetate (*3-deoxy-L-galactitol pentaacetate* (XI). Reaction of X with excess acetic anhydride in pyridine gave 4-deoxy-D-glucitol pentaacetate (XI) as a white crystalline material from ethyl acetate–hexane, m.p. 85–86°; $[\alpha]^{27}D$ −34.5° (c 0.44; methanol). As in the case of 3-deoxy-D-galactitol, the analysis showed the presence of 1 mole of ethyl acetate of crystallization.

Anal. Calcd. for $C_{20}H_{32}O_{12}$: C, 51.72; H, 6.94. Found: C, 51.78; H, 6.59.

GAINESVILLE, FLA.

(15) D. A. Prins, *J. Am. Chem. Soc.*, **70**, 3955 (1948).

(16) E. A. Lloyd and D. G. Doherty, *J. Am. Chem. Soc.*, **74**, 4214 (1952).

(17) M. Abdel-Aker, J. K. Hamilton, and F. Smith, *J. Am. Chem. Soc.*, **73**, 4691 (1951).

(18) J. B. Lee, *J. Chem. Soc.*, 1474 (1960).