neutral solution; 256 mμ (ε 5820), 287 mμ (ε 4490) and 296 mμ (ε 4490) in base.

Anal. Calcd. for $C_{12}H_{12}N_2O_3Cl_2$: C, 47.54; H, 3.98; N, 9.24; Cl, 23.39. Found: C, 47.81; H, 4.03; N, 9.34; Cl, 23.49.

1-(5-Deoxy-α-D-ribofuranosyl)-5,6-dichlorobenzimidazole (VII). Partition Chromatogram B.—The 2.0 g. of glass, which had been obtained above, was dissolved in 4 ml. of the lower and 4 ml. of the upper phase of the solvent system used in the previous chromatogram, and the solution was mixed with 8 g. of Celite. This mixture was packed on top of a column which had been prepared from 150 g. of Celite and 75 ml. of the lower phase of the solvent system. The column (61 cm. × 3 cm.) was washed with the upper phase and the ultraviolet absorption spectrum of the effluent was determined as before. The first 470 cc. of effluent was discarded. The following 895 ml. contained ultraviolet absorbing material which was isolated by evaporation of the solution *in vacuo*. The residual solid was triturated with a little ether and there was obtained 0.93 g. of mixed solids (m.p. 121–160°). Washing with hot ether left 0.84 g. of solids (m.p. 130–160°) and from the ether wash there was obtained 0.046 g. of material with m.p. 123–125° (*i.e.*, impure VI). The 0.84 g. of mixed solids was rechromatographed in *partition chromatogram C*. The material was dissolved in 5 ml. of the upper and 5 ml. of the lower phase of the solvent system heptane:ethyl acetate:methanol:water (3.5:2:3:2), and the liquid was mixed with 10 g. of Celite. This mixture was added to a column prepared as described above and the chromatogram was developed with the upper phase of the solvent system. Material with ultraviolet absorption was eluted after 840 ml. of the solvent had passed through the column and continued to come off throughout the subsequent 950 ml. The effluent containing this material was collected in 50-ml. fractions and each fraction was evaporated to dryness independently. Each residue was crystallized from a little ether and melting points were taken. This procedure showed that the first ten fractions contained mostly the β-anomer VI (m.p. 127–130°), the following three fractions contained mixed materials (m.p. 124–175°) and the last five fractions contained a substance with m.p. 173–183°. These last fractions were pooled and were recrystallized several times from chloroform to afford 0.3 g. (5% yield over-all from V) of a compound, m.p. 182–183°; $[\alpha]^{25}D$ +12.1° (*c* 0.99, in ethanol). In the ultraviolet, the material showed the following maxima: λ_{max} 253 mμ (ε 4000), 286 mμ (ε 6310) and 294 mμ (ε 6180) in acid; 257 mμ (ε 5340), 288 mμ (ε 4610) and 297 mμ (ε 4850) in neutral solution; 257 mμ ε 5460), 287 mμ (ε 4610) and 297 mμ (ε 4490) in base.

Anal. Calcd. for $C_{12}H_{12}N_2O_3Cl_2$: C, 47.54; H, 3.98; N, 9.24; Cl, 23.39. Found: C, 47.17; H, 4.00; N, 9.31; Cl, 23.81.

The combined mixed solids from fractions 11 through 13 weighed 0.45 g. (7% over-all yield from V).

Antiviral Tests

Influenza in Mice.—The PR8 strain of influenza virus was administered intranasally to 14 to 16 g. Swiss white albino mice under light ether anesthesia. DRB (III) was administered intraperitoneally (2.0 to 3.0 mg., suspended in 0.25 ml. of water with the aid of Tween-20[23]) 4 hours later and continued twice daily for 5 days. At the end of 13 days, the mortality rates of treated groups were compared to that of the control groups. Table I lists the results of five separate tests with decreasing doses of virus.

Poliomyelitis in Tissue Culture.—Washed test-tube cultures of a human epidermoid carcinoma, strain HeLa, were infected with Type I human poliomyelitis virus, Mahoney strain. One hour later, the media were replaced with maintenance media (Lactalbumen hydrolyzate plus 4% cow serum)[24] containing the compound to be tested in various dilutions. The cultures were incubated at 37° and were observed microscopically daily for 6 days. An identical set of cultures received the compound but no virus. A control group of non-treated and non-infected cells as well as a titration of the virus inoculum was carried along with each treated group. A negative result was manifested by a cytopathogenic degeneration in the treated group when no signs of toxicity were present in the group containing the compound and no virus. Table II lists the results of separate tests on the three compounds.

Acknowledgment.—We would like to thank Mr. Charles Pidacks for advice and help in carrying out the partition chromatograms and Mr. J. P. Joseph for the preparation of 5,6-dichlorobenzimidazole. We wish also to thank Dr. J. R. Vaughan for bringing this problem to our attention. Microanalyses were carried out by Mr. L. Brancone and staff and spectroscopic and polarimetric data were supplied by Mr. W. Fulmor and staff.

(23) Tween-20, a product of the Atlas Powder Company, is a wetting agent.

(24) J. L. Melnick, *Ann. N. Y. Acad. Sci.*, **61**, 754 (1955).

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

Synthesis of D-3,4-di-*O*-methyl-erythritol[1]

BY IRWIN J. GOLDSTEIN AND F. SMITH

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D-3,4-Di-*O*-methyl-erythritol has been prepared from methyl 5,6-di-*O*-methyl-D-glucofuranoside by oxidation with periodate followed by reduction and hydrolysis. It has been characterized as the bis-*p*-nitrobenzoate.

The successful use of periodic acid and its salts in synthetic and analytical organic chemistry for cleaving 1,2-glycols has been firmly established.[2-6]

This paper described its use for the synthesis of D-3,4-di-*O*-methyl-erythritol from methyl 5,6-di-*O*-methyl-D-glucofuranoside. The latter was obtained by the following series of reactions: D-glucose → 1,2;5,6-di-*O*-isopropylidene-D-glucofuranose → 3-*O*-benzyl-1,2;5,6-di-*O*-isopropylidene-glucofuranose → 3-*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose → 3-*O*-benzyl-5,6-di-*O*-methyl-1,2-*O*-isopropylidene-D-glucose → 5,6-di-*O*-methyl-1,2-*O*-isopropylidene-D-glucose → methyl 5,6-di-*O*-methyl-D-glucofuranoside. Oxidation of the latter with periodic acid at room temperature in the usual manner furnished a dialdehyde by cleavage between the hydroxyl groups at C_2 and C_3. Reduction of the dialdehyde with sodium borohydride gave the corresponding alcohol from which D-3,4-

(1) Paper No. 3583, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota.

(2) E. L. Jackson, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 341.

(3) J. R. Dyer, "Methods of Biochemical Analysis," Vol. III, Ed. by D. Glick, Interscience Pub., Inc., New York, N. Y., 1956, p. 111.

(4) J. C. Sowden, THIS JOURNAL, **72**, 808 (1950).

(5) J. C. Sowden, *ibid.*, **73**, 5496 (1951).

(6) G. W. Huffman, Bertha A. Lewis, F. Smith and D. R. Spriestersbach, *ibid.*, **77**, 4346 (1955).

di-*O*-methyl-erythritol was obtained by acid hydrolysis. The D-3,4-di-*O*-methyl-erythritol is readily characterized as its 1,2-bis-*p*-nitrobenzoate. This D-isomer of 3,4-di-*O*-methyl-erythritol proved to be the enantiomorph of the 3,4-di-*O*-methyl-erythritol previously obtained in periodate oxidation studies of methyl 6-*O*-trityl-α-D-glucopyranoside followed by reduction and methylation.[7] The structure of the D-3,4-di-*O*-methyl-erythritol was confirmed by the fact that it consumed approximately 1 molecular proportion of periodate with the liberation of formaldehyde which was recognized as the dimedone derivative.

Experimental

Unless otherwise stated, all concentrations were carried out *in vacuo* at temperatures not greater than 40° (bath temp.).

3-*O*-Benzyl-1,2-*O*-isopropylidene-D-glucofuranose.—A solution of 3-*O*-benzyl-1,2;5,6-di-*O*-isopropylidene-D-glucofuranose[8] (7 g.) in a mixture of glacial acetic acid (250 ml.) and water (60 ml.) was allowed to stand at room temperature until the optical rotation became constant (48 hr.). Removal of solvent yielded 3-*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose (6.7 g.) which had $[\alpha]^{23}D$ −44° in chloroform (*c* 1.4); lit. value[9] $[\alpha]^{15}D$ −46° ($CHCl_3$).

3-*O*-Benzyl-5,6-di-*O*-methyl-1,2-*O*-isopropylidene-D-glucofuranose.—The sirupy 3-*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose (6.6 g.) was methylated five times using silver oxide (15 g.) and methyl iodide (40 ml.) in the usual way giving 3-*O*-benzyl-5,6-di-*O*-methyl-1,2-*O*-isopropylidene-D-glucofuranose (6.1 g.) as a mobile yellow oil, b.p. (bath temp.) 145–150° (0.001 mm.), $n^{23}D$ 1.4932, $[\alpha]^{23}D$ −17° in acetone (*c* 1.3); lit. value $[\alpha]^{20}D$ −16° in acetone (*c* 1).[10] *Anal.* Calcd. for $C_{18}H_{26}O_6$: OCH_3, 18.6. Found: OCH_3, 18.7.

5,6-Di-*O*-methyl-1,2-*O*-isopropylidene-D-glucofuranose.—To 3-*O*-benzyl-5,6-di-*O*-methyl-1,2-*O*-isopropylidene-D-glucofuranose (6.03 g.) was added sodium (8.5 g.). The flask was heated (steam-bath) under a reflux condenser while absolute ethanol was added over a period of 6 hr. until all the sodium had been dissolved.[11] After adding water (50 ml.) the reaction mixture was neutralized with acetic acid and distilled (steam-bath) to remove most of the ethanol. The residue was extracted with chloroform (five 50-ml. portions) and the dried ($MgSO_4$) extract concentrated and distilled to yield 5,6-di-*O*-methyl-1,2-*O*-isopropylidene-D-glucofuranose (4.0 g.), b.p. (bath temp.) 102–105° (0.035 mm.), $n^{25}D$ 1.4555, $[\alpha]^{21}D$ −11° in water (*c* 3.4); lit. value $[\alpha]^{30}D$ −13° in water (*c* 4.2).[12]

Methyl 5,6-Di-*O*-methyl-D-glucofuranoside.—A solution containing 5,6-di-*O*-methyl-1,2-*O*-isopropylidene-D-glucofuranose (1.5 g.) in 1.3% methanolic hydrogen chloride (25 ml.) was refluxed until its rotation rose to a constant value (15 hr. approx.). The reaction mixture was neutralized ($PbCO_3$), filtered and concentrated to a yellow sirup which was purified by extraction with ethanol and treatment of the ethanolic extract with charcoal. Removal of the solvent yielded methyl 5,6-di-*O*-methyl-D-glucofuranoside (1.4 g.) as a pale yellow sirup which reduced Fehling solution slightly. Distillation of a portion (0.8 g.) gave two fractions: (1) a colorless mobile liquid (0.32 g.), b.p. (bath temp.) 115–120° (0.001 mm.), $n^{27}D$ 1.4560; (2) a pale yellow liquid (0.2 g.), b.p. (bath temp.) 125–133° (0.001 mm.), $n^{25}D$ 1.4620. Since both fractions reduced Fehling solution, they were combined and boiled for 20 hr. with dry methanol (35 ml.) in the presence of a cation exchange resin (Amberlite IR 120)[13] to complete the glycoside formation. It is interesting to note the difficulty encountered in the removal of the 1,2-isopropylidene group from 5,6-di-*O*-methyl-1,2-isopropylidene-D-glucofuranose. Comment[14] also has been made upon the drastic conditions required to remove the 1,2-isopropylidene group from 3-*O*-benzyl-1,2-isopropylidene-5,6-di-*O*-methyl-D-glucofuranose.

After filtering the resin, the methanolic solution was concentrated in the presence of barium carbonate (100 mg.). The non-reducing, yellow sirup was distilled in the presence of barium carbonate giving: (1) 5,6-di-*O*-methyl-1,2-*O*-isopropylidene-D-glucofuranose (50 mg.), b.p. (bath temp.) 115–120° (0.001 mm.), $n^{25}D$ 1.4542; (2) methyl 5,6-di-*O*-methyl-D-glucofuranoside, a non-reducing colorless liquid (400 mg.), b.p. (bath temp.) 120–130° (0.001 mm.), $n^{25}D$ 1.4620, $[\alpha]^{23}D$ −3° in water (*c* 2.8). *Anal.* Calcd. for $C_9H_{18}O_6$: C, 48.6; H, 8.2; OCH_3, 41.9. Found: C, 48.9; H, 8.6; OCH_3, 41.8.

Oxidation of Methyl 5,6-Di-*O*-methyl-D-glucofuranoside with Periodic Acid.—To a solution of the sirupy glycoside (1.4 g.) in water (50 ml.) was added 0.1565 *N* periodic acid (50 ml.) and the volume adjusted to 250 ml. After 24 hr. at room temperature the optical rotation had risen to a constant value and titration revealed that 1.1 moles of periodic acid had been consumed. The reaction mixture was neutralized ($BaCO_3$), filtered and concentrated to dryness. Extraction with ether afforded the dialdehyde (1.2 g.), $[\alpha]^{25}D$ +16° in ethanol (*c* 25), $n^{24}D$ 1.4515.

Reduction of the Dialdehyde with Sodium Borohydride.[15]—To a solution of the dialdehyde (1.2 g.) in methanol (15 ml.) was added a solution of sodium borohydride (0.42 g.) in methanol (15 ml.). The vigorous reaction which ensued was moderated by cooling in ice-water. After 3.5 hr. the reaction mixture was neutralized with solid carbon dioxide and concentrated to dryness.

The product was separated from inorganic salts by two extractions with ethanol followed by two extractions with ether. Evaporation of the clear yellow solution yielded a glassy solid (1.45 g.). A solution of this in methanol was made slightly acid with glacial acetic acid and concentrated, methanol being added repeatedly during the concentration to remove borate. The mobile liquid (0.89 g.) thus obtained was distilled giving D-3,4-di-*O*-methyl-2-(α-methoxy-β-hydroxyethyl)-erythritol as a colorless sirup, b.p. (bath temp.) 130–140° (0.01 mm.), $n^{22}D$ 1.4515 and $[\alpha]^{24}D$ −4° in ethanol (*c* 1.8).

Bis-*p*-nitrobenzoate of 3,4-Di-*O*-methyl-2-(α-methoxy-β-hydroxyethyl)-erythritol.—To a solution of D-3,4-di-*O*-methyl-2-(α-methoxy-β-hydroxyethyl)-erythritol (35 mg.) in pyridine (3 ml.), *p*-nitrobenzoyl chloride (170 mg.) was added. After heating on an oil-bath at 90° for 30 min., the solution was cooled and poured into an ice-cold saturated sodium bicarbonate solution. The precipitate was filtered and washed. Recrystallization from acetone–ethanol yielded the bis-*p*-nitrobenzoate of D-3,4-di-*O*-methyl-2-(α-methoxy-β-hydroxyethyl)-erythritol (50 mg.) as flat needles, m.p. 133–134°, $[\alpha]^{25}D$ −13° in chloroform (*c* 0.8). *Anal.* Calcd. for $C_{23}H_{26}N_2O_{12}$: N, 5.4. Found: N, 5.3.

D-3,4-Di-*O*-methyl-erythritol.—A solution of D-3,4-di-*O*-methyl-2-(α-methoxy-β-hydroxyethyl)-erythritol (0.4 g.) in 50% aqueous ethanol (40 ml.) containing concentrated sulfuric acid (0.1 ml.) was refluxed for 4 hr.

Neutralization ($BaCO_3$) of the reaction mixture, filtration and concentration gave a yellow sirup which upon extraction with ethanol and distillation gave D-3,4-di-*O*-methyl-erythritol (0.236 g.), a colorless mobile liquid, b.p. (bath temp.) 111–116° (0.60 mm.), $n^{23}D$ 1.4485, $[\alpha]^{25}D$ −4° in ethanol (*c* 1). *Anal.* Calcd. for $C_6H_{14}O_4$: C, 48.0; H, 9.4; OCH_3, 41.3. Found: C, 47.9; H, 9.3; OCH_3, 45.2. The high methoxyl value is due to the fact that erythritol itself gives an apparent methoxyl value of 2–7%.

D-3,4-Di-*O*-methyl-erythritol 1,2-Bis-*p*-nitrobenzoate.—Treatment of D-3,4-di-*O*-methyl-erythritol (60 mg.) in pyridine (5 ml.) with *p*-nitrobenzoyl chloride (160 mg.) in the usual way yielded D-3,4-di-*O*-methyl-erythritol-1,2-bis-*p*-nitrobenzoate (120 mg.), m.p. 101.5°, $[\alpha]^{24}D$ −41.5° in chloroform (*c* 1) (after recrystallization from acetone–ethanol). *Anal.* Calcd. for $C_{20}H_{20}O_{10}N_2$: C, 53.6; H, 4.5; N, 6.3. Found: C, 53.8; H, 4.8; N, 6.5.

Oxidation of D-3,4-Di-*O*-methyl-erythritol with Periodic Acid.—A solution of 0.0288 *N* periodic acid (25 ml.) containing D-3,4-di-*O*-methyl-erythritol (48.2 mg.) was allowed to stand at room temperature until its rotation rose to a con-

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(8) N. Prentice, L. S. Cuendet and F. Smith, *ibid.*, **78**, 4439 (1956).

(9) A. S. Meyer and T. Reichstein, *Helv. Chim. Acta*, **29**, 152 (1946).

(10) K. Freudenberg and E. Plankenhorn, *Ber.*, **73**, 621 (1940).

(11) K. Freudenberg and E. Plankenhorn, *Ann.*, **536**, 257 (1938).

(12) M. R. Salmon and C. Powell, THIS JOURNAL, **61**, 3507 (1939).

(13) J. E. Cadotte, F. Smith and D. Spriestersbach, *ibid.*, **74**, 1501 (1952).

(14) C. T. Bishop and J. Schmorak, *Can. J. Chem.*, **34**, 845 (1956).

(15) F. Smith and J. Van Cleve, THIS JOURNAL, **77**, 3091 (1955).

stant value (12 hr. approx.). After 24 hr., titration of an aliquot revealed the consumption of 0.94 mole of periodic acid. An additional 24 hr. produced no further change in the periodate consumption.

The reaction mixture was neutralized ($BaCO_3$) and filtered. The filtrate was distilled at atmospheric pressure and the first 5 ml. of the distillate treated with a solution of dimedone (100 mg.) in ethanol (2 ml.). After standing several hours the white crystalline dimedone derivative of formaldehyde (10 mg.) was separated, m.p. and mixed m.p. 190° (after recrystallization from 50% ethanol).

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

Synthesis of D- and of L-α-*O*-Methylglycerol[1]

BY IRWIN J. GOLDSTEIN, J. K. HAMILTON AND F. SMITH

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The synthesis of D- and of L-α-*O*-methylglycerol is described.

During methylation studies on the reduced products of periodate oxidized pentosans, a substance believed to be an optically active α-*O*-methylglycerol was encountered. A literature search revealed that while the racemic modification of this substance has been known for many years[2,3] the preparation of either of its enantiomorphic forms has not been carried out, although an unsuccessful attempt to resolve the D,L-mixture by the use of optically active ketones (camphor and menthone) is reported.[2] α-*O*-Methylglycerol has been encountered but not characterized in studies on the glycosides of red algae (*Ceramium rubrum*).[4]

(1) Methyl 6-*O*-methyl-α-D-galactopyranoside
↓ Periodate oxidn.
D′-Methoxy-D-methoxymethyl-diglycolic aldehyde
↓ Reduction with H_2
D′-Methoxy-D-methoxymethyl-diethylene glycol
↓ Hydrolysis
D-α-*O*-Methylglycerol

(2) Methyl α-D-glucopyranoside
↓ Periodate oxidn.
D′-Methoxy-D-hydroxymethyl-diglycolic aldehyde
↓ Br_2 oxidn.
Strontium D′-methoxy-D-hydroxymethyl-diglycolate
↓ H^+, Ag_2O
Silver D′-methoxy-D-hydroxymethyl-diglycolate
↓ MeI + Ag_2O
D′-Methoxy-D-methoxymethyl-dimethyl-diglycolate
↓ $LiAlH_4$
D′-Methoxy-D-methoxymethyl-diethylene glycol
↓ Hydrolysis
D-α-*O*-Methylglycerol

This paper describes five different approaches to the preparation of optically active α-*O*-methylglycerol, two for the D- and three for the L-isomer; in each case the tedious process of resolution is avoided.

The D-isomer of α-*O*-methylglycerol was prepared by the two independent routes outlined.

The D-α-*O*-methylglycerol from the two experiments showed $[\alpha]^{21}D$ +5.9° and +5.4° (ethanol), respectively, and was characterized as the crystalline bis-*p*-nitrobenzoate. Its designation as the D-isomer follows the convention[5] which relates it to that form of glyceraldehyde into which the substance may be hypothetically oxidized without alteration or removal of substituents on the glycerol molecule.

By use of these procedures the configuration of each intermediate was known with certainty and hence it was possible to relate unambiguously the structure with sign of rotation, one of the purposes of this investigation. In addition, correlation with other species of known configuration was rendered unnecessary.

The three schemes employed for the synthesis of L-α-*O*-methylglycerol are shown.

The L-α-*O*-methylglycerol obtained by the three methods showed specific rotations in good agreement, numerically, with the values for the D-isomer.

Optically active 1,2-*O*-isopropylidene glycerol was used with great effectiveness by Baer and Fischer[6] for the preparation of optically active glycerides. These workers subjected the above compound to methylation to give 1,2-*O*-isopropylidene-L-3-*O*-methylglycerol from which L-α-*O*-methylglycerol was obtained by hydrolysis. The latter was not isolated and the authors, interested only in demonstrating the presence of adjacent hydroxyl groups, treated the hydrolyzed reaction mixture directly with periodic acid and reported a periodate consumption of one molecular proportion.

(1) Paper No. 3585, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul, Minn.

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