flow of 0.5 l./min. Some product was lost due to aerosol entrainment by the nitrogen flow. The pyrolysate amounted to 28 g. which on fractionation through a spinning-band column yielded 17.7 g. (63%) of cycloheptatriene, b.p. 61° (125 mm.). The remainder of the pyrolysate was a mixture of unchanged 7,7-dichloronorcarane and two partially dehydrochlorinated materials, b.p. 72–75° (26 mm.). Characteristic infrared bands at 3050, 1640, and 1614 cm.$^{-1}$ suggest these materials are chlorocycloheptadienes. The identity of the cycloheptatriene was verified by infrared examination and by gas–liquid chromatography on a silicone fluid column. The product contained less than 2% toluene, probably much less.

The Synthesis of 2,3,6-Tri-*O*-methyl-D-galactose and Its Derivatives[1]

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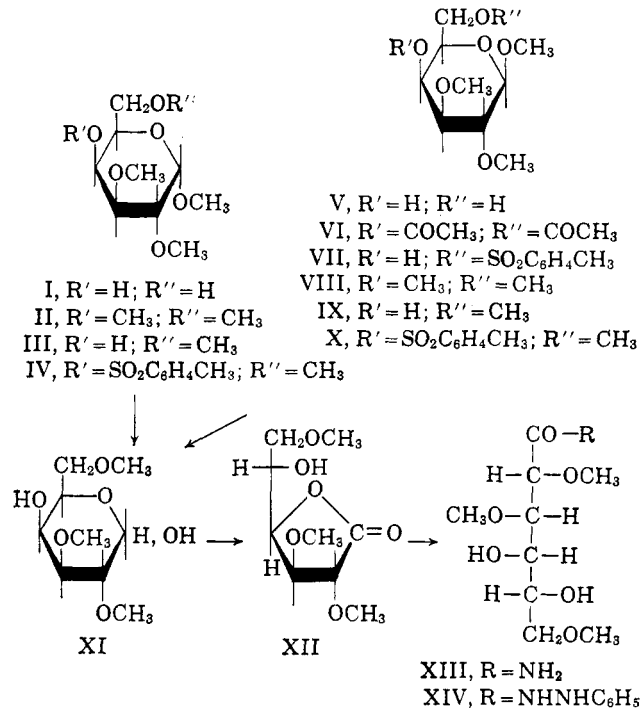
Application of the methylation procedure to the study of the chemical structure of glycoproteins[3,4] has shown the need for methylated derivatives of D-galactose as reference compounds, especially for the trimethyl ethers. Search of the literature to the present time (for review of literature to 1955, see ref. 5 and 6) showed that no synthesis of 2,3,6-tri-*O*-methyl-D-galactose (XI) had been devised, as far as we know. This sugar is known only by isolation from methylated polysaccharides. The present paper describes such a synthesis.

In the preparation of methyl 2-acetamido-2-deoxy-3,6-di-*O*-methyl-α-D-galactopyranoside,[7] it was found that the hydroxyl at C-4 of methyl 2-acetamido-2-deoxy-3-*O*-methyl-α-D-galactopyranoside was quite resistant to methylation. Such resistance may be ascribed to the axial configuration of the hydroxyl group and to the influence of the vicinal methoxyl group at C-3. In a similar fashion, sirupy methyl 2,3-di-*O*-methyl-α-D-galactopyranoside (I)[8,9] was treated with methyl iodide and silver oxide. The resulting sirupy mixture was fractionated by column chromatography on silica gel, giving 12% of sirupy methyl 2,3,4,6-tetra-*O*-methyl-α-D-galactopyranoside (II),[10,11] 7% of a mixture of II and III, 33% of sirupy methyl 2,3,6-tri-*O*-methyl-α-D-galactopyranoside (III), and 24% of a mixture of III and starting material I. The trimethylgalactoside III was characterized by a crystalline 4-tosylate IV. Hydrolysis with hydrochloric acid of III gave sirupy 2,3,6-tri-*O*-methyl-D-galactose (XI).[12,13] Attempts to prepare crystalline derivatives of XI with α-benzyl-α-phenyl-, toluene-*p*-sulfonyl-, *p*-bromophenyl-, and 2,4-dinitrophenylhydrazine, or with aniline, were not successful. Characterization was obtained by oxidation of XI into 2,3,6-tri-*O*-methyl-D-galactonic acid, characterized by the crystalline lactone XII,[12,14–17] amide XIII,[14,15] and phenylhydrazide XIV. The sirupy starting material I had been obtained from crystalline methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-galactopyranoside,[8,9,18] and characterized by the crystalline 4,6-dinitrate.[9]



An alternate route to the synthesis of 2,3,6-tri-*O*-methyl-D-galactose (XI) was also investigated, using the β-galactoside derivatives. From the crystalline methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-β-D-galactopyranoside,[18,19] sirupy methyl 2,3-di-*O*-methyl-β-D-galactopyranoside (V) was prepared. It was characterized by crystalline, low-melting 4,6-diacetate VI, and by the crystalline 6-monotosylate VII. Methylation of V with methyl iodide and silver oxide followed by fractionation on silica gel column gave 7% of crystalline methyl 2,3,4,6-tetra-*O*-methyl-β-D-galactopyranoside (VIII),[10,20] 63% of sirupy methyl 2,3,6-tri-*O*-methyl-β-D-galactopyranoside (IX), and 17% of starting material V. The trimethylgalactoside IX was characterized by crystalline 4-tosylate X. Hy-

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(2) To whom inquiries should be addressed.

(3) R. W. Jeanloz and E. H. Eylar, *Intern. Symp. Makromol. Chem., Wiesbaden, West Germany, 1959*, Sektion V., A 8.

(4) R. W. Jeanloz and A. M. Closse, *Federation Proc.*, **22**, 538 (1963).

(5) D. J. Bell, *Advan. Carbohydrate Chem.*, **6**, 11 (1951).

(6) G. G. Maher, *ibid.*, **10**, 273 (1955).

(7) D. K. Stearns, R. G. Naves, and R. W. Jeanloz, *J. Org. Chem.*, **26**, 901 (1961).

(8) G. J. Robertson and R. A. Lamb, *J. Chem. Soc.*, 1321 (1934).

(9) D. J. Bell and G. D. Greville, *ibid.*, 1136 (1955).

(10) D. J. Bell, *ibid.*, 1543 (1940).

(11) F. Micheel and O. Littmann, *Ann.*, **466**, 115 (1928).

(12) W. N. Haworth, H. Raistrick, and M. Stacey, *Biochem. J.*, **31**, 640 (1937).

(13) J. J. Connell, R. M. Hainsworth, E. L. Hirst, and J. K. N. Jones, *J. Chem. Soc.*, 1696 (1950).

(14) P. Andrews, L. Hough, and J. K. N. Jones, *ibid.*, 806 (1954).

(15) W. N. Haworth, H. Raistrick, and M. Stacey, *Biochem. J.*, **29**, 2668 (1935).

(16) W. N. Haworth, E. L. Hirst, and M. Stacey, *J. Chem. Soc.*, 2481 (1932).

(17) E. Pacsu, S. M. Trister, and J. W. Green, *J. Am. Chem. Soc.*, **61**, 2444 (1939).

(18) F. Reber and T. Reichstein, *Helv. Chim. Acta*, **28**, 1164 (1945).

(19) J. W. H. Oldham and D. J. Bell, *J. Am. Chem. Soc.*, **60**, 323 (1938).

(20) H. H. Schlubach and K. Moog, *Ber.*, **56**, 1957 (1923).

drolysis of IX gave the sirupy 2,3,6-tri-*O*-methyl-D-galactose (XI), identical with the product described above.

Experimental

Melting points were taken on a hot stage, equipped with a microscope, and correspond to "corrected melting point." Rotations were determined in semimicro- or micro- (for amounts smaller than 3 mg.) tubes with lengths of 100 or 200 mm., using a Rudolph photoelectric polarimeter attachment, Model 200; the chloroform used was analytical reagent grade and contained approximately 0.75% of ethanol. Chromatograms were made with the flowing method on Silica Gel Davison, from the Davison Co., Baltimore 3, Md. (grade 950, 60–200 mesh), which was used without pretreatment. When deactivation by contact with moist air occurred, reactivation was obtained by heating to 170–200° (manufacturer's instructions). The sequence of eluents was hexane, benzene or chloroform, ether, ethyl acetate, acetone, and methanol individually or in binary mixtures. The proportion of weight of substance to be adsorbed to weight of adsorbent was 1:50–100. The proportion of weight of substance in grams to volume of fraction of eluent in milliliters was 1:100. The ratio of diameter to length of the column was 1:20. Evaporations were carried out *in vacuo*, with an outside bath temperature kept below 45°. Amounts of volatile solvent smaller than 20 ml. were evaporated under a stream of dry nitrogen. The microanalyses were done by Dr. M. Manser, Zürich, Switzerland.

Methyl 2,3-Di-*O*-methyl-α-D-galactopyranoside (I).—In a mixture of 60 ml. of glacial acetic acid and 30 ml. of water, 13.2 g. of methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-galactopyranoside[8,9,18] was dissolved and heated on a steam bath for 45 min. The solution was then evaporated, and the residual benzaldehyde was removed by evaporation with toluene. This procedure was repeated on the residual sirup to ensure complete removal of benzaldehyde, yielding 9.4 g. of sirup, $[\alpha]^{22}D$ +151° (*c* 2.3, $CHCl_3$). Infrared analysis of this sirup indicated the presence of a carbonyl group, presumably due to acetyl groups incorporated during the removal of the benzaldehyde group. The sirup was therefore treated with 100 ml. of 0.03 *N* barium methylate in methanol, and the solution stood at 0° overnight. After neutralization with carbon dioxide, the solution was filtered and evaporated to give 9.1 g. (97%) of methyl 2,3-di-*O*-methyl-α-D-galactopyranoside (I) in sirupy form, free from carbonyl absorption by infrared analysis.

Methyl 2,3,6-Tri-*O*-methyl-α-D-galactopyranoside (III).—A solution of 4.60 g. of I was dissolved in 80 ml. of methyl iodide and treated with 7.8 g. of silver oxide. The mixture was shaken at room temperature for 18 hr., then filtered, and the precipitate was washed with chloroform and methanol. Evaporation of the combined filtrates yielded 5.1 g. of a fluid sirup which was dissolved in a mixture of benzene–ether, 2:1, and purified by chromatography on 260 g. of silica gel. The fractions were characterized by hydrolysis of an aliquot with 2 *N* sulfuric acid for 2 hr. on a boiling water bath, followed by neutralization and descending paper chromatography in the mixture butanol–ethanol–water, 4:1:5 (upper phase). The spots were made visible with the aniline phosphate reagent.[21]

Elution with benzene–ether, 2:1, gave 0.62 g. (12%) of sirupy methyl 2,3,4,6-tetra-*O*-methyl-α-D-galactopyranoside (II). Elution with benzene–ether, 1:1, gave 0.33 g. (7%) of a sirupy mixture composed of methyl 2,3,6-tri-*O*-methyl-α-D-galactopyranoside (III) and methyl 2,3,4,6-tetra-*O*-methyl-α-D-galactopyranoside (II). Further elution with the same mixture of solvents and with pure ether gave 1.66 g. (34%) of sirupy methyl 2,3,6-tri-*O*-methyl-α-D-galactopyranoside (III), $[\alpha]^{25}D$ +158° (*c* 0.8, $CHCl_3$). Finally, elution with ether, ethyl acetate, acetone, and methanol gave 1.21 g. (25%) of a sirupy mixture containing methyl 2,3,6-tri-*O*-methyl-α-D-galactopyranoside (III) and the starting material I.

Treatment of 81 mg. of III with *p*-toluenesulfonyl chloride in pyridine, followed by the usual work-up, yielded 21 mg. (15%) of **methyl 2,3,6-tri-*O*-methyl-4-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside (IV)** as prisms, melting at 94°, after recrystallization from a mixture of ether and hexane; $[\alpha]D$ +126° (*c* 0.3, $CHCl_3$).

Anal. Calcd. for $C_{17}H_{26}O_8S$: C, 52.30; H, 6.71; S, 8.20. Found: C, 52.33; H, 6.85; S, 8.29.

(21) J. L. Bryson and T. J. Mitchell, *Nature*, **167**, 864 (1951).

2,3,6-Tri-*O*-methyl-D-galactose (XI).—A solution of 1.54 g. of IV in 50 ml. of 1 *N* hydrochloric acid was refluxed for 6 hr. The cooled solution was neutralized with 2 *N* sodium hydroxide and evaporated to dryness. The residue was extracted with four 50-ml. portions of boiling ethyl acetate, and the extracts were evaporated to give a residue which, after treatment with charcoal in methanol solution, filtration, and evaporation of the filtrate, gave 1.43 g. (99%) of 2,3,6-tri-*O*-methyl-D-galactose (XI) as a colorless sirup; $[\alpha]^{25}D$ +95° (*c* 0.6, water).[22]

Anal. Calcd. for $C_9H_{18}O_6$: C, 48.64; H, 8.16; OCH_3, 41.85. Found: C, 48.12; H, 8.07; OCH_3, 41.25.

On descending paper chromatogram Whatman No. 1 developed with the mixture butanol–ethanol–water, 4:1:5 (upper phase), the product showed *R*(2,3,4,6-tetra-*O*-methyl-D-galactose) 0.87 and *R*(2,3,4,6-tetra-*O*-methyl-D-glucose) 0.77. Under similar conditions the *R*(2,3,4,6-tetra-*O*-methyl-D-glucose) of D-galactose was 0.19; of 2,3-di-*O*-methyl-D-galactose, 0.59; and of 2,3,4,-6-tetra-*O*-methyl-D-galactose, 0.89.

2,3,6-Tri-*O*-methyl-D-galactono-1,4-lactone (XII).—A solution of 0.936 g. of XI in 30 ml. of water was shaken with 1.5 ml. of bromine at room temperature for 18 hr. The excess bromine was then removed under a nitrogen stream, and the resulting colorless solution was neutralized with silver oxide. The solution was filtered, and traces of silver ions were removed from the filtrate on a short Dowex-50 (H^+ form) resin column. The eluate was evaporated to yield 0.887 g. of sirup which rapidly crystallized. The mixture of lactone and acid was converted to the pure γ-lactone by repeatedly heating and evaporating with glacial acetic acid, to give 0.791 g. (86%) of crystals with a constant m.p. of 100–101°.[23] The product showed a very slow mutarotation in water from $[\alpha]^{22}D$ −29° (5 min.) to −18° (12 days, *c* 0.5).

Anal. Calcd. for $C_9H_{16}O_6$: C, 49.09; H, 7.32; OCH_3, 42.28. Found: C, 48.99; H, 7.26; OCH_3, 42.60.

2,3,6-Tri-*O*-methyl-D-galactonamide (XIII).—A solution of 108 mg. of XII in 1.0 ml. of methanol at 0° was saturated with ammonia and left at 0° for 1 hr., then at room temperature overnight. The solution was diluted with methanol, filtered, and evaporated. The residue was crystallized from acetone, to give 66 mg. (66%), m.p. 134–135°; $[\alpha]^{21}D$ +38° (*c* 0.5, $CHCl_3$).[24]

Anal. Calcd. for $C_9H_{19}NO_6$: C, 45.56; H, 8.07; N, 5.90. Found: C, 45.29; H, 8.01; N, 5.87.

2,3,6-Tri-*O*-methyl-D-galactonic Acid Phenylhydrazide (XIV).—A mixture of 140 mg. of XII and 73 mg. of phenylhydrazine was heated on a water bath for 30 min. The mixture was cooled and triturated with ethyl acetate. Fibrous crystals (76 mg., 36%) slowly separated, which were collected after the solution was allowed to stand overnight at 0°. After recrystallization from ethyl acetate, 38 mg. was obtained, m.p. 126–127°, $[\alpha]^{26}D$ +24° (*c* 1.1, CH_3OH).[25]

Anal. Calcd. for $C_{15}H_{24}N_2O_6$: C, 54.86; H, 7.37; N, 8.53. Found: C, 54.52; H, 7.78; N, 8.64.

Methyl 2,3-Di-*O*-methyl-β-D-galactopyranoside (V).—Methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-β-D-galactopyranoside[18,19] was treated with glacial acetic acid and sodium methylate in a similar fashion as the α-anomer I. The resulting sirup V,[9] $[\alpha]^{20}D$ −11° (*c* 0.12, $CHCl_3$), was obtained in quantitative yield.

Acetylation of V with acetic anhydride and pyridine in the usual manner gave a sirup, which crystallized slowly at very low temperature. Recrystallization from a mixture of ether and pentane at −20° gave **methyl 4,6-di-*O*-acetyl-2,3-di-*O*-methyl-β-D-galactopyranoside (VI),** m.p. 31–35°, $[\alpha]^{27}D$ −7° (*c* 2.7, $CHCl_3$). The product is hygroscopic and no satisfactory analytical results for C,H were obtained.[26]

Anal. Calcd. for $C_{13}H_{22}O_8$: C, 50.98; H, 7.53; OCH_3, 30.39; *O*-$COCH_3$, 28.10. Found: C, 49.41; H, 6.90; OCH_3, 30.75; *O*-$COCH_3$, 27.29.

Methyl 2,3-Di-*O*-methyl-6-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside (VII).[27]—To a solution of 455 mg. of V in 3 ml. of pyridine was added a solution of 427 mg. (1.08 moles) of *p*-toluenesulfonyl chloride in 3 ml. of ethylene dichloride. Both solutions had been precooled at 0°, and the mixture was left at room temperature

(22) Values of $[\alpha]D$ +87[12] and +80°[13] in water have been reported.

(23) Melting points from 97 to 101° have been reported.[12,14-17]

(24) Melting points of 129–130°[14] and 135°[15] have been reported.

(25) In ref. 6, a 2,3,6-tri-*O*-methyl-D-galactonic acid phenylhydrazide, m.p. 175°, $[\alpha]D$ −62.4°, is reported. From the reference quoted,[16] there is, however, no clear evidence that the compound described is a 2,3,6-trimethyl derivative of D-galactonic acid.

(26) This product was prepared by Mrs. D. A. Jeanloz.

(27) This product was prepared by Mrs. D. Baggett.

for 2 days. After addition of a trace of water and 50 ml. of chloroform, the organic layer was washed with ice-cold 1 *N* sulfuric acid, cold sodium bicarbonate, and water, and finally dried over calcium chloride. After evaporation, the pale yellow sirupy residue was dissolved in benzene and chromatographed on silica gel. Elution with a mixture of benzene–ether, 1:1, gave 437 mg. of crystalline fractions. Crystallization from a mixture of ethyl acetate and pentane afforded 388 mg. (50%), m.p. 105, $[\alpha]^{25}D$ −17° (*c* 1.11, $CHCl_3$).[28]

Anal. Calcd. for $C_{16}H_{24}O_8S$: C, 51.05; H, 6.43; S, 8.50; OCH_3, 24.73. Found: C, 51.19; H, 6.45; S, 8.45; OCH_3, 24.71.

Elution with a mixture of ether and ethyl acetate, 9:1, gave 124 mg. (27%) of crude starting material, identified by infrared spectra.

Methyl 2,3,6-Tri-*O*-methyl-β-D-galactopyranoside (IX).—A solution of 1.10 g. of V in 20 ml. of methyl iodide was treated with 1.63 g. of silver oxide, and the mixture was shaken at room temperature for 18 hr. The solution was then filtered, and the precipitate was washed with methanol. On evaporation, the filtrate yielded 1.144 g. of sirup, which was purified by chromatography on 50 g. of silica gel. Elution with a mixture of benzene and ether, 1:1, yielded 85 mg. (7%) of sirupy methyl 2,3,4,6-tetra-*O*-methyl-β-D-galactopyranoside (VIII), which crystallized on standing, and 720 mg. (62%) of methyl 2,3,6-tri-*O*-methyl-β-D-galactopyranoside (IX), as a colorless sirup, $[\alpha]^{20}D$ −16° (*c* 0.74, $CHCl_3$). Elution with acetone yielded 197 mg. (17%) of a mixture of IX and starting material.

Treatment of 61 mg. of IX with *p*-toluenesulfonyl chloride in pyridine at 70° for 2 hr., then at room temperature for 2 days with the usual work-up, yielded after recrystallization from benzene–pentane 29 mg. (27%) of **methyl 2,3,6-tri-*O*-methyl-4-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside (X)**, as needles, m.p. 131–132°, $[\alpha]^{21}D$ +15° (*c* 0.3, $CHCl_3$).

Anal. Calcd. for $C_{17}H_{26}O_8S$: C, 52.30; H, 6.71; S, 8.20. Found: C, 52.50; H, 6.28; S, 8.86.

Hydrolysis of IX with 1 *N* hydrochloric acid as described for III yielded a product with the same R_f and R(2,3,4,6-tetra-*O*-methyl-D-galactose) as the product XI described above.

(28) The synthesis of impure methyl 2,3-di-*O*-methyl-6-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside was reported by M. P. Khare, O. Schindler, and T. Reichstein [*Helv. Chim. Acta*, **45**, 1547 (1962)].

The Synthesis of Homoribose[1]

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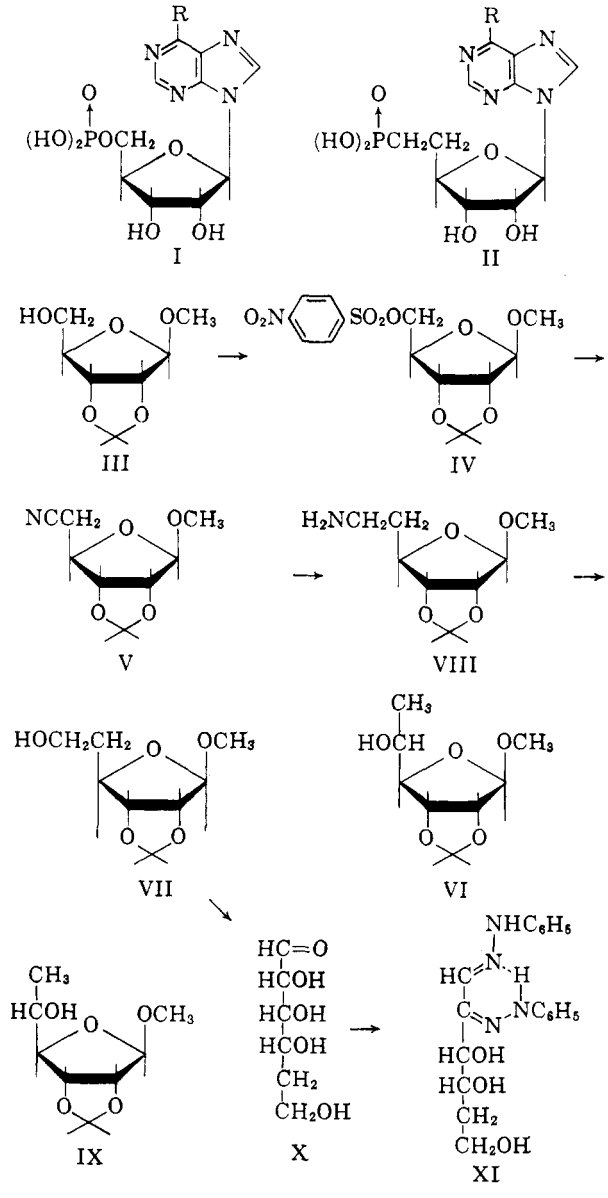
It has been established that many purines and ring analogs of purines are converted *in vivo* to their ribonucleotides (I) which inhibit purine biosynthesis (and presumably growth) by negative pseudo-feedback.[2] Exogenous ribonucleotides fail to inhibit certain resistant cell lines because they are split extracellularly to both ribonucleosides and purines prior to penetrating cell membranes.[3] Ribonucleotide analogs (II) derived from homoribose (5-deoxy-D-*ribo*-hexose), in which the P–O–C linkage is replaced by a P–CH_2–C bond, could not be split to nucleosides by phosphatases and might also be resistant to cleavage at the glycosyl linkage. To prepare compounds of this type, we undertook the synthesis of homoribose (X).



Ribose was converted by a known procedure to methyl 5-deoxy-5-iodo-2,3-*O*-isopropylidene-D-ribofuranoside[4] which on reaction with sodium cyanide in N,N-dimethylformamide was partially converted to methyl 5-cyano-5-deoxy-2,3-*O*-isopropylidene-D-ribofuranoside (V, α,β-mixture). The resulting mixture was separated by gas chromatography and the analytical sample of V (α,β-mixture) was obtained. Since this procedure was not satisfactory for the preparation of gram quantities of the sugar, the *p*-nitrobenzenesulfonyl derivative (IV) of methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (III)[5] was prepared for reaction with sodium cyanide in the manner described above. Although the yield of cyano sugar (V) from III was not high, it was readily separated by distillation from the rest of the reaction mixture and the preparation of gram quantities was easily accomplished.

(1) This work was supported by funds from the C. F. Kettering Foundation and from the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH-43-64-51.

(2) L. L. Bennett, Jr., L. Simpson, J. Golden, and T. L. Barker, *Cancer Res.*, **23**, 1574 (1963); L. L. Bennett, Jr., and D. Smithers, in press.

(3) J. A. Montgomery, F. M. Schabel, Jr., and H. E. Skipper, *Cancer Res.*, **22**, 504 (1962); J. A. Montgomery, G. J. Dixon, E. A. Dulmadge, H. J. Thomas, R. W. Brockman, and H. E. Skipper, *Nature*, **199**, 769 (1963).

(4) H. M. Kissman and B. R. Baker, *J. Am. Chem. Soc.*, **79**, 5534 (1957).

(5) Preliminary work leading to the preparation of V *via* methyl 5-deoxy-5-iodo-2,3-*O*-isopropylidene-D-ribofuranoside was carried out on a mixture of the α- and β-*O*-methyl glycosides. Later the pure methyl β-D-ribofuranoside[6] was converted to its isopropylidene derivative for the work that resulted in the complete synthesis of homoribose.

(6) R. Barker and H. G. Fletcher, Jr., *J. Org. Chem.*, **26**, 4605 (1961).