Chromatographic Adsorption. VI. Isomer Distribution and Mechanism of Formation of the Methyl Glycosides of D-Glucose and D-Galactose by the Fischer Method

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The formation of the four methyl D-glucosides from D-glucose and the four methyl D-galactosides from D-galactose, using a strongly acidic ion-exchange resin as catalyst, was followed by gas–liquid partition chromatography of their trimethylsilyl ethers. The final equilibrium mixtures in refluxing methanol contained approximately 73% methyl α-D-glucopyranoside and 27% methyl β-D-glucopyranoside and 60% methyl α-D-galactopyranoside, 17% methyl β-D-galactopyranoside, 5% methyl α-D-galactofuranoside, and 18% methyl β-D-galactofuranoside, respectively. Reaction mechanisms for the acid-catalyzed alcoholysis of hexoses, 6-deoxyhexoses, and pentoses are proposed.

The previous paper in this series[1] reported the change of isomer distribution with time for the Fischer reaction of methanol with D-mannose and with L-arabinose using a cation-exchange resin as catalyst and liquid chromatography on a starch column as the analytical method. This analytical procedure failed to separate the reaction products of the Fischer reactions of D-glucose or D-galactose. It has since been reported[2] that a separation of the methyl D-glucosides has been effected by gas–liquid partition chromatography of their *O*-trimethylsilyl derivatives on a Carbowax 6000 column at 140°. Preliminary work in this laboratory with a 25 ft × 1/8 in. copper column packed with 10% Carbowax 6000 on acid-washed Chromosorb W (80–100 mesh) did yield five peaks, as reported, but the order of elution appeared to be slightly different from that reported; the column rapidly deteriorated after a sample or two had been run through, possibly due to attack of the silylation mixture upon the stationary phase at the temperature required, 170°. Several other columns were tried and the most successful was a high efficiency 50 ft × 1/8 in. stainless steel column furnished by Analabs, Inc., which contained 4.8% OV-17 on 80–100 mesh Anakron H. This column showed no deterioration at the required temperature of 205° but failed to separate the α- and β-D-glucopyranosides. Since these were the only components of the mixtures that were not resolved and since they differ markedly in their optical activity, a polarimetric measurement upon each sample withdrawn from the reaction mixture, in addition to the gas chromatographic analysis, allowed calculation of the mole per cent of each component present.

In the case of the methyl D-galactosides a partial separation by gas chromatography of their trimethylsilyl ethers has been reported[2] and a complete analysis by use of two different columns demonstrated. We have found it possible to effect a complete separation of the trimethylsilyl ethers of α- and β-D-galactose and the four methyl D-galactosides using a 26 ft × 1/8 in. copper column containing 9% OV-1 on Chromosorb W-HP (80–100 mesh).

Experimental Section

Materials.—D-Glucose and D-galactose were Pfanstiehl CP materials of specific rotations +52.5 and +80.2°, respectively. Methyl α-D-glucopyranoside, of specific rotation +159.5°, was obtained from Corn Products Refining Co; and methyl β-D-glucopyranoside, of specific rotation −33.8°, from Mann Research Labs. Methyl α-D-galactopyranoside and methyl β-D-galactopyranoside of specific rotations +190 and 0°, respectively, were obtained as recrystallized crystalline products from a Fischer reaction. The methanol was reagent grade and the strongly acidic ion-exchange resin was Dowex-50W (X-8) 50–100 mesh, equilibrated with methanol as described previously.[1] Pyridine was obtained from Reilly Tar and Chemical Corp., Indianapolis, Ind., and was dried over sodium hydroxide pellets. Trimethylchlorosilane, hexamethyldisilazane, *O*-trimethylsilyl α-D-glucose, *O*-trimethylsilyl β-D-glucose, and *O*-trimethylsilyl α-D-galactose were all obtained from Pierce Chemical Co., Rockford, Ill.

Methyl Glycoside Formation.—Fischer glycosidation of D-glucose and D-galactose was carried out essentially as described previously.[1] Complete solution of 50 g of glucose in 195 g of methanol occurred in 20–30 min and of galactose in about 15 min, and equilibrium was reached in about 12 hr for glucose and 24 hr for galactose. Aliquots (0.1 ml) of the 1-ml pyridine-quenched samples were vacuum evaporated several times with dry pyridine to remove the methanol and the trimethylsilyl ethers were formed by addition of 1 ml of dry pyridine, 0.2 ml of hexamethyldisilazane, and 0.1 ml of trimethylchlorosilane according to the method of Sweeley, *et al.*[3]

For the polarimetric measurements 7-ml samples were withdrawn. The resin was allowed to settle in a 60° bath and weighed aliquots containing a few milligrams of $NaHCO_3$ were evaporated under vacuum at 50°. The residue was made up to a volume of 25.75 ml and the optical rotation determined at 22° in a 4-dm tube using a sodium lamp. Polarimeter readings and sample weights, respectively, follow: for D-glucose 0.5 hr, 4.40°, 4.43 g; 1 hr, 5.30°, 4.40 g; 2 hr, 6.40°, 4.39 g; 4 hr, 9.90°, 4.42 g; 8 hr, 13.01°, 4.45 g; 12 hr, 15.85°, 4.45 g; 24 hr, 24.07°, 6.73 g; 48 hr, 19.59°, 5.42 g and for D-galactose 1 hr, −0.72°, 4.38 g.

Chromatography of the Trimethylsilyl Ether Derivatives.—A Hewlett-Packard Model 5750 gas chromatograph with a FID was used with either the previously mentioned 50 ft × 1/8 in. OV-17 column for D-glucose or the 26 ft × 1/8 in. OV-1 column for D-galactose reaction mixtures. A Perkin-Elmer printing integrator was used to determine peak areas from the OV-17 column and a planimeter for the areas of the peaks from the OV-1 column, as these were eluted on the trailing edge of the solvent peak. In the latter case vacuum evaporation of the silylated samples and addition of 1 ml of *n*-hexane produced level baselines and yielded the same peak areas as before this treatment. Sample injections varied from 0.5–5 μl and electrometer attenuations from $10^2/4$ to $10^3/1$. Prepurified nitrogen at 40 psi and 25 ml/min was used for the OV-17 column and 50 psi and 20 ml/min for the OV-1 column. Relative detector constants for the pure materials available were found to be 1.00 ± 0.02 and were therefore assumed to be 1.00 for all components of the silylated mixtures. Reproducibility of the reaction conditions was found to be within about ±2 for the mole per cent of each component of the mixtures.

(1) D. F. Mowery, Jr., *J. Org. Chem.*, **26**, 3484 (1961).
(2) V. Smirnyagin, C. T. Bishop, and F. P. Cooper, *Can. J. Chem.*, **43**, 3109 (1965).
(3) C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Amer. Chem. Soc.*, **85**, 2497 (1963).

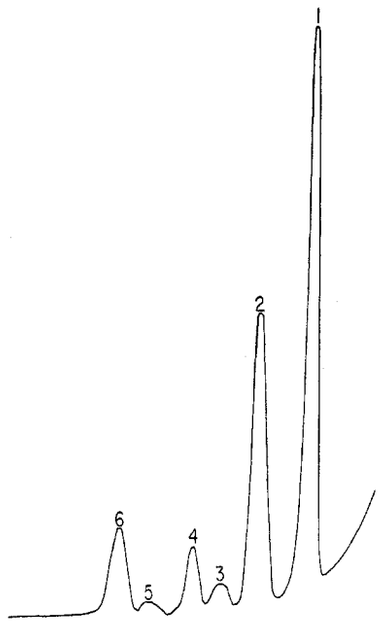


Figure 1.—Silylated 0.25-hr methyl D-galactoside reaction mixture chromatographed at 190° and 50 psi on a 26 ft × 1/8 in. copper column packed with 9% OV-1 on 80–100 mesh Chromosorb W-HP. Peak order and retention times (in min): 1, β-furanoside (35); 2, α-pyranoside (41); 3, β-pyranoside (46); 4, α-D-galactose (49): 5, α-furanoside (54); 6, β-D-galactose (58).

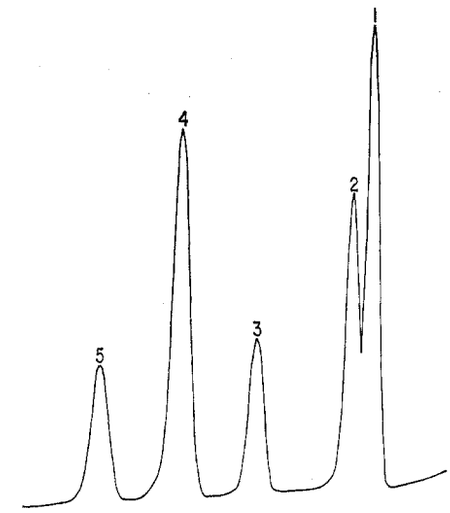


Figure 2.—Silylated 1-hr methyl D-glucoside reaction mixture chromatographed at 205° and 40 psi on a 50 ft × 1/8 in. stainless steel column packed with 4.8% OV-17 on 80–100 mesh Anakron H. Peak order and retention times (in min): 1, β-furanoside (46); 2, α-furanoside (49); 3, α-D-glucose (61); 4, α- and β-pyranosides (71); 5, β-D-glucose (82).

Discussion

Identification of Peaks in the Methyl D-Galactoside Mixtures.—Figure 1 shows the chromatogram produced by the 0.25-hr silylated methyl D-galactoside reaction sample. Peak identity for α- and β-D-galactose and methyl α- and β-D-galactopyranosides was established using authentic samples. The peaks produced by the two furanosides were distinguished by comparison of the observed molecular rotation of the reaction mixture at 1 hr, −900°, with that calculated assuming the first peak is β-furanoside and the fifth peak is α-furanoside, −500°. If the first peak is assumed to be α-furanoside and the fifth peak β-furanoside, then the calculated molecular rotation is +22,700, proving conclusively that the first peak must be β-furanoside and the fifth peak α-furanoside. The specific rotations used for these calculations[2] were +104, −113, +80, +192, and 0° for the α-furanoside, β-furanoside, D-galactose, α-pyranoside, and β-pyranoside, respectively.

Identification of Peaks in the Methyl D-Glucoside Mixtures.—Figure 2 shows the chromatogram produced by the 1-hr silylated methyl glucoside reaction sample. Peak identity for α- and β-D-glucose and methyl α- and β-D-glucopyranosides was established using authentic samples. Peaks produced by methyl α-D-glucofuranoside and methyl β-D-glucofuranoside were identified by comparison with a chromatogram of the same sample on Carbowax 6000, for which stationary phase the first and second peaks have been identified by Smirnyagin, Bishop, and Cooper[2] as methyl β-D-glucofuranoside and methyl α-D-glucofuranoside, respectively. Confirmation of this assignment comes from calculation of the molecular rotation of the reaction mixture at 0.5 hr as described below. The figure of 5640 is obtained, and, if the first peak is assumed to be β-furanoside and the second α-furanoside, a value of 11,700 is calculated for the molecular rotation of the unresolved α- and β-pyranosides. This yields figures of 8 and 9 mol %, respectively, for α- and β-pyranoside in the reaction mixture and a value of 0.9 for the ratio of α/β. If, on the other hand, the first peak is assumed to be α- and the second β-furanoside, a value of −3900 is obtained for the molecular rotation of the unresolved α- and β-pyranosides, which leads to values of 1 and 16 mol %, respectively, for α- and β-pyranoside and a value of 0.06 for the ratio of α/β. From the areas under peak 2 (α-pyranoside), peak 3 (β-pyranoside + α-D-glucose) and peak 4 (β-D-glucose) of the 0.25-hr sample chromatographed on the OV-1 column and the ratio of β- to α-D-glucose of 1.2 from the OV-17 column, the ratio of α-pyranoside/β-pyranoside may be calculated as 0.8. Since the α-pyranoside/β-pyranoside ratio increases as the reaction proceeds, it is evident that this ratio cannot have a value of 0.06 for the 0.5-hr sample; therefore the first peak must be β-furanoside and the second α-furanoside.

Calculation of Mole Percentages of the D-Glucopyranosides.—The mole per cent of α-pyranoside, $100 \cdot X_4 \cdot (R_4 - R_6)/(R_5 - R_6)$, and of β-pyranoside, $100 \cdot X_4 \cdot (R_5 - R_4)/(R_5 - R_6)$, was calculated from the observed polarimeter reading, a, of the given aliquot of weight, c, from the reaction mixture by means of the two relationships

$$R(\text{expt}) = (a \times 25.75 \times 180 \times 245)/(c \times 4 \times 50)$$

$$R_4 \times X_4 = \text{r(expt)} - X_1 \times R_1 - X_2 \times R_2 - X_3 \times R_3$$

where R represents molecular rotation and X mol fraction, obtained from the chromatogram, and 1 refers to α-furanoside (+118°), 2 to β-furanoside (−77°), 3 to D-glucose (+52.5°), 4 to the α- + β-pyranoside mixture, 5 to α-pyranoside (+158°) and 6 to β-pyranoside (−34°). The ratio of α/β glucopyranoside was also determined chromatographically in two cases using the OV-1 column. Retention times in minutes on this column at 200° follow: α- and β-furanosides (28), α-

pyranoside (38), α-D-glucose and β-pyranoside (42), and β-D-glucose (58).

Results.—Tables I and II show the variation with time of each component in the Fischer reactions of

TABLE I

MOLE PERCENTAGES DURING REACTION OF D-GALACTOSE

Time, hr	D-Galactose α	D-Galactose β	Furanosides α	Furanosides β	Pyranosides α	Pyranosides β
0[a]	32	68				
1/12	17	29		27	25	2
1/6	12	21	1	35	28	3
1/4	8	12	1	46	29	4
1/2	7	9	2	48	29	5
1	2	1	2	57	30	8
2	1	1	4	48	35	11
4	1		5	33	44	17
8			4	30	49	17
12			5	26	52	17
24			5	18	60	17
48			5	18	60	17

[a] After 0.5-hr reflux but before addition of ion-exchange resin.

TABLE II

MOLE PERCENTAGES DURING REACTION OF D-GLUCOSE

Time, hr	D-Glucose α	D-Glucose β	Furanosides α	Furanosides β	Pyranosides α	Pyranosides β
0[a]	46	54				
1/12	31	35	13	18	3	
1/6	26	29	16	25	4	
1/4	19	21	20	31	4	5[b]
1/2	14	16	23	30	8	9[c]
1	11	13	20	26	15	15[c]
2	8	10	12	18	28	24[c]
4	4	4	5	8	46	33[c]
8	1	2	2	3	61	31[c]
12		1		1	71	27[c]
24					73	27[c]
48					73	27[b]

[a] After 0.5-hr reflux but before addition of ion-exchange resin. [b] Calculated from the α/β ratio obtained from the OV-1 column. [c] Calculated from polarimetric measurements.

D-galactose and of D-glucose, respectively. The composition of the equilibrium mixture for the D-glucose reaction falls within the limits of the 73 ± 5% methyl α-D-glucopyranoside and 27 ± 5% methyl β-D-glucopyranoside found by Capon, *et al.*,[4] for the methanesulfonic acid catalyzed reaction at 35°. Also, reaction of L-arabinose and analysis of silylated aliquots on the OV-17 column yielded essentially the same curves as obtained previously[1] using liquid chromatography. A comparison of the data of Table I with the isomer distribution curves of the homomorphous L-arabinose[1] and of the data of Table II with the curves of the homomorphous D-xylose[5] shows the expected similarities in the latter case but marked differences in the former case, in which it should be noted that the α and β designations are interchanged since the sugar is in the L series.

Proposed Mechanism for Methyl Glycoside Formation.—A satisfactory mechanism for methyl glycoside formation must be capable of explaining (a) the ratio of furanosides to pyranosides initially formed, (b) the ratio of α- to β-furanoside and α- to β-pyranoside initially formed, and (c) the fact that the same final equilibrium mixture of principally pyranosides is formed from any one of the methyl glycosides if subjected to Fischer reaction conditions.[1,5] It is immediately apparent that a and b are a result of kinetic control of the reaction whereas c results from thermodynamic control. The latter requires that all steps in the mechanism be reversible. Schemes I and II show a proposed mechanism applied to methyl D-galactoside and to methyl D-glucoside formations, respectively. These sugars differ from each other in the configuration of the C_4 hydroxyl group only; so their marked difference in the initial ratio of furanosides to pyranosides and α to β isomers must be due to this alone. The intermediate in the equilibration of the α and β forms of the sugars before addition of the ion-exchange resin is formulated classically as the aldehydo form (**2**) of the sugar and after addition of the resin the formation of this intermediate would be expected to be accelerated by protonation of the ring oxygen to give cation **5** in each case. In these cases the intermediate cannot be the resonance stabilized monocyclic carboxonium ion (**13**), shown below in the schemes, which would be produced by protonation of the anomeric hydroxyl group and loss of water, since this intermediate would react with methanol to give an immediate and rapid formation of methyl pyranosides rather than the furanosides actually obtained in the case of D-glucose.

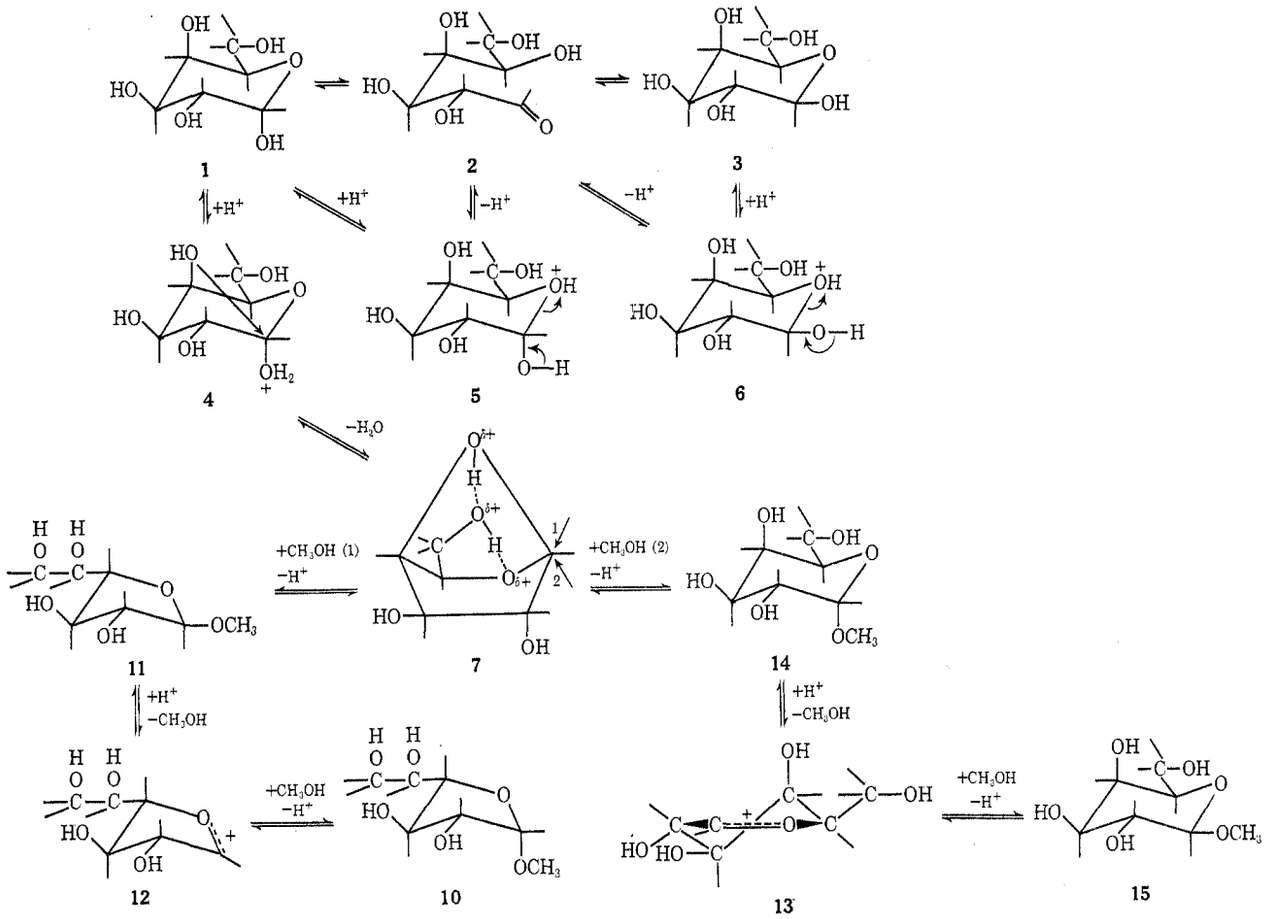
In the proposed mechanism for glycoside formation protonation of the anomeric hydroxyl group of α-D-galactose (**1**) in Scheme I or of β-D-glucose (**3**) in Scheme II is the first step. Water is then eliminated from the protonated form (**4**), in each case with anchimeric assistance from the C_4 hydroxyl group. In both cases a bicyclic cation (**7**) protonated on the C_4 ring oxygen is postulated.[4] In the case of D-galactose the C_6 hydroxyl group is in a position to hydrogen bond with the proton on the C_4 ring oxygen and at the same time form a hydrogen bond to the C_5 ring oxygen, thus producing a lower energy more stable cation in which the positive charge is distributed to three oxygen atoms. Attack of methanol upon this cation at the positions shown by the arrows 1 and 2 in Scheme I, followed by loss of a proton, would produce β-D-galactofuranoside (**11**) and α-D-galactopyranoside (**14**), respectively. The initial formation of β-furanoside and α-pyranoside in almost equal quantities suggests an equal distribution of positive charge on the C_4 and C_5 ring oxygen atoms. Assumption of this bicyclic cation stabilized by a double hydrogen bonding appears to explain the unusual isomer distribution found in the methyl D-galactoside formation at the 1/12-hr time. The 2% of β-D-galactopyranoside (**15**) may be accounted for by the anomerization of the α-pyranoside *via* the corresponding monocyclic carboxonium cation (**13**) proposed by other investigators.[5,6] If all steps in the mechanism are assumed reversible, the higher energy furanosides should gradually reform the bicyclic cation (**7**) and become converted into the lower energy pyranosides. In the case of methyl glucoside formation, shown in Scheme II, β-D-glucose (**3**) is protonated on the anomeric hydroxyl and this protonated form (**4**) loses water with anchimeric assistance from the C_4 hydroxyl. However, in this case the new oxygen

(4) B. Capon, G. W. Loveday, and W. G. Overend, *Chem. Ind. (London)*, 1537 (1962).

(5) C. T. Bishop and F. P. Cooper, *Can. J. Chem.*, **40**, 224 (1962).

(6) B. Capon, *Chem. Commun.*, **1**, 21 (1967).

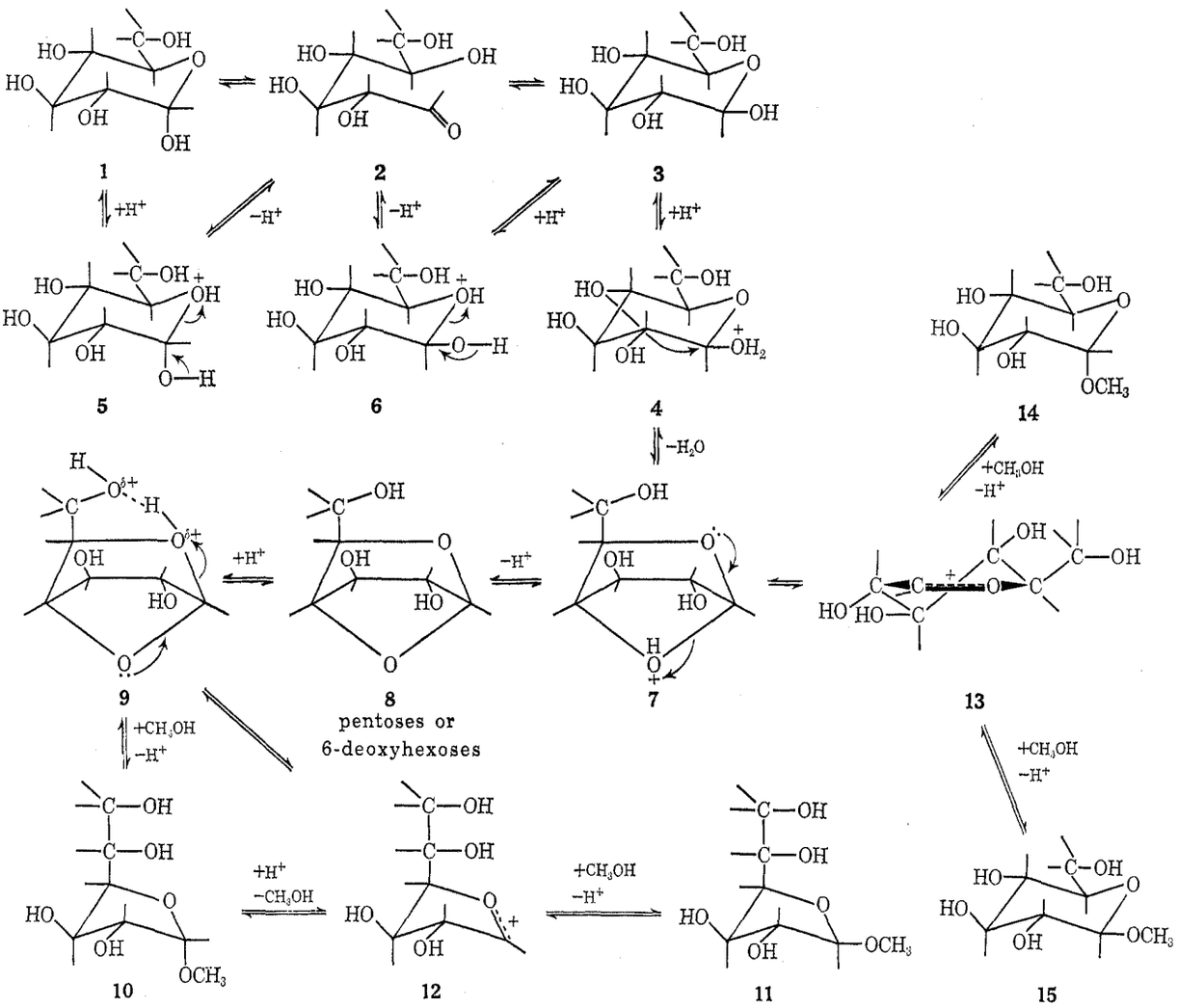
SCHEME I



bridge forms *below* the pyranose ring rather than *above* it so that the C_6 hydroxyl in the bicyclic cation (**7**) is not in a position to hydrogen bond and stabilize the protonated C_4 ring oxygen atom. A rapid equilibrium is assumed between this bicyclic cation (**7**) and the bicyclic cation protonated on the C_5 ring oxygen (**9**), which should be stabilized by hydrogen bonding with the C_6 hydroxyl. This equilibrium should favor the lower energy hydrogen-bonded bicyclic cation and thereby lead to a more rapid initial formation of furanosides than pyranosides. It is assumed that this stabilized bicyclic cation is attacked by methanol and forms α-D-glucofuranoside (**10**), which rapidly[4] equilibrates with β-D-glucofuranoside (**11**) *via* the monocyclic carboxonium cation (**12**). An alternative mechanism, probably the major one for pentoses and 6-deoxyhexoses, might be direct formation of the monocyclic carboxonium cation (**12**) by SN1 ring opening in the bicyclic cation (**9**). This appears less likely in the case of glucose since hydrogen bonding between the proton on the C_5 ring oxygen and the C_6 hydroxyl would reduce the positive charge on the ring oxygen and thereby reduce the tendency for opening of this ring. In the case of the formation of the glucopyranosides (**14** and **15**) from the unstabilized bicyclic cation (**7**), on the other hand, SN1 ring opening to form the monocyclic carboxonium cation (**13**) would be expected and must be assumed since otherwise the initial samples would show predominantly β-pyranoside (**15**) from attack by methanol on the bicyclic cation (**7**) followed by the much slower[4] β- to α-pyranoside anomerization. The very slightly faster rate of β-pyranoside (**15**) over α-pyranoside (**14**) formation during the first half hour of the reaction could be due to slightly greater blocking of attack by methanol on the monocyclic carboxonium cation (**13**) by the C_2 hydroxyl than by the C_6 hydroxyl group.

This proposed mechanism for acid-catalyzed alcoholysis of carbohydrates would be expected to have three modifications depending on whether the C_4 and C_5 hydroxyl groups of a hexose are of (1) the same configuration or (2) opposite configurations or whether (3) the C_6 hydroxyl group is absent as in the case of pentoses and 6-deoxyhexoses. The *first* type should resemble D-glucose in initial furanoside/pyranoside and α/β ratios. D-Mannose, for example, when subjected to the same conditions as D-glucose and D-galactose, has been found[1] after $^1/_8$ hr to yield a mixture having a furanoside/pyranoside ratio of approximately 4.0, an α/β furanoside ratio of about 1.9 and an α/β pyranoside ratio of about 3.5. These ratios are to be compared with corresponding ratios after $^1/_{12}$ hr for D-glucose of about 10, 0.7, and 0.7 and for D-galactose of about 1.0, 0.0, and 8.3. In comparison with D-glucose the smaller furanoside/pyranoside ratio for D-mannose may be explained by inductive electron withdrawal through the solvent by the C_2 hydroxyl, which has opposite configurations in the mannose and glucose bicyclic intermediates (**8**) in Scheme II. This would decrease the basicity of the C_5 ring oxygen and increase that of the C_4 ring oxygen in D-mannose, with a resulting decrease in the furanoside/pyranoside ratio. The

SCHEME II



larger α/β furanoside ratio for D-mannose is undoubtedly caused by the slower anomerization of the α-D-mannofuranoside first formed to its equilibrium value of about 1.0 than of the α-D-glucofuranoside (**10**) to its equilibrium value of about 0.7. The larger initial α/β pyranoside ratio for D-mannose is probably due to an increased rate of α-pyranoside (**14**) and a decreased rate of β-pyranoside (**15**) formation caused by inverting the configuration of the C_2 hydroxyl group in the monocyclic carboxonium cation (**13**). The *second* type should resemble D-galactose, with a much smaller furanoside/pyranoside ratio and widely divergent α/β furanoside and pyranoside ratios. In the *third* type the C_6 hydroxyl is absent and therefore cannot hydrogen bond and stabilize the bicyclic cation intermediate. The mechanism would be expected to resemble that shown for D-glucose (Scheme II) except that the CH_2OH group would be replaced by either H or CH_3, eliminating the possibility of its hydrogen bonding with either protonated ring oxygen atom. Protonation on the C_5 ring oxygen (**9**) should be favored since inductive withdrawal of electrons from it by the C_3 hydroxyl group should be less than from the nearer C_4 ring oxygen. This should lead to predominantly furanosides *via* SN1 ring opening to produce the *furanose* monocyclic carboxonium cation (**12**). The bicyclic cation protonated on the less basic C_4 ring oxygen (**7**) would lead to smaller amounts of pyranosides *via* the *pyranose* monocyclic carboxonium cation (**13**). The actual structure of the bicyclic cation (**7**) would depend on the configuration of the C_4 hydroxyl, resembling in one case the D-glucose (Scheme II) and in the other the D-galactose (Scheme I) bicyclic cation. Since neither of these cations would be stabilized by internal hydrogen bonding, the less stable rings resulting would be expected to open to monocyclic carboxonium cations (**12** and **13**) before reacting with methanol. For the reaction of L-arabinose[1] [Scheme I with CH_2OH replaced by H and the bicyclic cation (**7**), protonated on the C_4 ring oxygen, assumed in equilibrium with the corresponding form protonated on the C_5 ring oxygen] the ratio of furanosides/pyranosides at $^1/_8$ hr is about 10 and of α/β furanosides and also α/β pyranosides about 0.9. The furanoside/pyranoside ratio could be due to the greater expected basicity and protonation of the C_5 ring oxygen as compared to the C_4 ring oxygen of the bicyclic intermediate because of reduced electron withdrawal by the more distant C_3 hydroxyl group. The α/β furanoside ratio of almost unity would result from an expected almost equal rate of attack by methanol upon both sides of the monocyclic carboxonium cation (**12**) in the preferred E_3 conformation with all three ring substituents equatorial. The α/β pyranoside

ratio of near unity would be expected if some SN2 attack by methanol on the bicyclic cation (7) to produce α-pyranoside (14) offsets a slight decrease in rate of formation of this isomer because of blocking of attack upon one side of the monocyclic carboxonium ion (13) by the C_2 hydroxyl group.

Registry No.—D-Galactose, 59-23-4; methyl α-D-galactofuranoside, 3795-67-3; methyl β-D-galactofuranoside, 1824-93-7; methyl α-D-galactopyranoside, 3396-99-4; methyl β-D-galactopyranoside, 1824-94-8; D-glucose, 50-99-7; methyl-α-D-glucofuranoside, 1824-88-0; methyl β-D-glucofuranoside, 1824-89-1; methyl α-D-glucopyranoside, 97-30-3; methyl β-D-glucopyranoside, 709-50-2.

The Synthesis of 4-β-D-Ribofuranosyl-*as*-triazin-3(4*H*)-one 1-Oxide, a Potential Uridine Antagonist[1]

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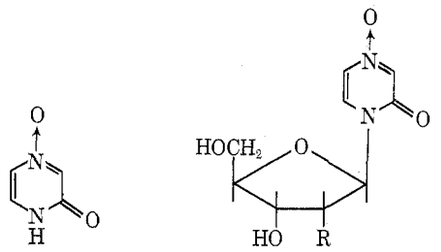
4-β-D-Ribofuranosyl-*as*-triazin-3(4*H*)-one 1-oxide (**8**), a structural analog of uridine, has been prepared by the reaction of 3-methoxy-*as*-triazine 1-oxide (**3**) with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**5**) followed by debenzoylation with $NaOCH_3$. Both proton and carbon-13 nmr were used to assign the site of nitrogen ribosylation, the first such reported application to a six-membered heterocyclic system. An unusual deoxygenation of the *N*-oxide function of 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-*as*-triazin-3(4*H*)-one 1-oxide (**6**) with ethanolic ammonia resulted in the formation of 4-β-D-ribofuranosyl-*as*-triazin-3(4*H*)-one (**10**). Reduction of the *as*-triazine ring of **6** was found to occur to yield 2,5-dihydro-4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-*as*-triazin-3(4*H*)-one (**11**). Small coupling constants for the anomeric protons of **6** and **8** were found to change to larger values on reduction of the aglycon portion of the molecule. pK_a measurements on *as*-triazin-3(4*H*)-one 1-oxide (**7**) and on the reduced product 2,5-dihydro-*as*-triazin-3(4*H*)-one (**15**) point out the unusual character of the *as*-triazin-3(4*H*)-one 1-oxide ring system.

Emimycin, an antibiotic isolated[2] from Streptomyces No. 2020-I, has been shown to be 2(1*H*)-pyrazinone 4-oxide (**1**).[3] The antibacterial activity of **1** is reversed by uracil, uridine, and 2′-deoxyuridine.[4] The syntheses of 1-β-D-ribofuranosylemimycin (**2**) and 1-β-D-2′-deoxyribofuranosylemimycin (**2a**) have recently been



1

2, R = OH
2a, R = H

reported.[5,6] The increased potency of **2a** over that of emimycin as a bacteriocidal agent illustrates the desirability of studying related nucleoside derivatives.

The present work describes the syntheses of *as*-triazin-3(4*H*)-one 1-oxide (**7**) (3-azaemimycin) and of the corresponding uridine analog 4-β-D-ribofuranosyl-*as*-triazin-3(4*H*)-one 1-oxide (**8**) (Scheme I). The synthesis of 3-methoxy-*as*-triazine 1-oxide (**3**) by oxidation of 3-methoxy-*as*-triazine (**4**) with perbenzoic acid has been reported in 15% yield.[7] Utilizing *m*-chloroperbenzoic acid[8] in refluxing benzene, the yield of **3** was increased to 30%. Treatment of **3** with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**5**) in acetonitrile yielded a single nucleoside product, 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-*as*-triazin-3(4*H*)-one 1-oxide (**6**) plus small amounts of another product which was identified as *as*-triazin-3(4*H*)-one 1-oxide (**7**) on the basis of pmr, mass spectra, and elemental analysis. The formation of **7** can be explained by the hydrolysis of 3-methoxy-*as*-triazine 1-oxide (**3**) by residual HBr and/or acetic acid, which are difficult to remove completely in the preparation of halogenose **5**. Addition of dilute methanolic HCl to an acetonitrile solution of **3** resulted in the formation of **7**.

Treatment of **6** with sodium methoxide removed the benzoyl blocking groups to give the desired uridine analog 4-β-D-ribofuranosyl-*as*-triazin-3(4*H*)-one 1-oxide (**8**). The assignment of the β-glycosidic configuration of **6** and **8** was based on the very small coupling constant of the anomeric proton observed in the pmr spectrum of **8** (*vide infra*).

Reductive removal of the *N*-oxide function was accomplished by hydrogenation of **8** in the presence of a 5% palladium-on-charcoal catalyst, but simultaneous reduction of the triazine ring was also observed. Unexpectedly, the formation of the nucleoside 4-β-D-ribofuranosyl-*as*-triazin-3(4*H*)-one (**10**) was found to occur upon treatment of the blocked nucleoside **6** with alcoholic ammonia. This deoxygenation of the *N*-oxide function of **6** with ethanolic ammonia at room temperature was indeed surprising, since only one analogous reaction could be found in the literature,[9] and in this example more vigorous conditions, heating in liquid NH_3 at 150°, resulted in the formation of 4,4′-dichloro-3,3′-dipicolyl from the corresponding di-*N*-oxide. Nevertheless, because both **6** and **8** were found to be completely stable even in refluxing EtOH, it

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