tion of the C-2 hydroxyl group in the favored conformation and the extent of such bonding. The first group of derivatives (**1**, **2**, and **3**), which show the 1-OH signal at low field (τ 4.5), have in their planar conformations (**1a**, **2a**, and **3a**) the H-2 atom bisecting the dihedral angle of the aryl residue and the 1-hydroxyl group and have the 2-hydroxyl group *trans* to the 1-hydroxyl group. Interaction between the C-1 and C-2 hydroxyl groups in this conformation is not possible. Maximum staggering of large groups along C-1–C-2 is achieved. In the third group of derivatives (**6** and **7**), where the 1-OH signals are at higher field (τ ~4.75), the 2-hydroxyl group is largely constrained to bisect the angle of the aryl group and the 1-hydroxyl group. In this orientation the 1-OH–N hydrogen bond may be weakened by *gauche* steric interference, by competitive hydrogen bonding of the 2-hydroxyl group with O-1 or with the aryl nitrogen atom, or by a combination of these effects.[24] In substance **4** the same situation exists as noted with **6** and **7** and the somewhat lower chemical shift (τ 4.67) of the 1-OH group may be due to greater conformational freedom because of the smaller side chain. In the case of **5**, the 1-OH proton is further deshielded (τ 4.63), probably reflecting the fact that there is less steric interference with hydrogen bonding in the rotamer **5b** which apparently makes a major contribution to the conformational population.

Further studies in this laboratory are in progress to furnish additional data on related systems.

(24) The unusually small $J_{1,2}$ values observed with **6** and **7** may result from distortion of the favored C-1–C-2 rotamer to give an H-1–H-2 dihedral angle larger than 60°.

Experimental Section

Preparation of Phenylosotriazoles.—Each of the derivatives **1–7** was prepared by standard methods[25] by way of the appropriate phenylosazones. L-*erythro*-Pentulose phenylosotriazole[26] (**1**) was obtained from L-arabinose, 6-deoxy-L-*lyxo*-hexulose phenylosotriazole[27] (**2**) was obtained from L-fucose, D-*lyxo*-hexulose phenylosotriazole[28] (**3**) was obtained from D-galactose, D-*threo*-pentulose phenylosotriazole[28] (**4**) was obtained from D-xylose, L-*xylo*-hexulose phenylosotriazole[28] (**5**) was obtained from L-sorbose, 6-deoxy-L-*arabino*-hexulose phenylosotriazole[27] (**6**) was obtained from L-rhamnose, and D-*arabino*-hexulose phenylosotriazole[29] (**7**) was obtained from D-glucose. Each product was recrystallized several times and all had melting points and specific rotations in good agreement with literature values.

Nmr Measurements.—Spectra were measured with a Varian HR-60 nmr spectrometer, at a temperature of approximately 32°. Tetramethylsilane (τ 10.00) was used as the internal standard and spectra were calibrated by the side-band technique. Chemical shifts and coupling constants are first-order values, as measured directly from spectral spacings. The measured spacings are believed to be accurate to ±0.15 Hz or better.

Samples were used as ~10% solutions in methyl sulfoxide-d_6. After each spectrum had been recorded, a trace of hydrogen chloride gas was introduced and the spectrum was recorded again. The addition of hydrogen chloride gas was effected by filling a fine-tipped capillary pipet with the vapor from the top of a bottle of concentrated hydrochloric acid and then bubbling this vapor through the prepared sample. The resulting concentration of hydrogen chloride was of the order of 0.0005 wt %.

Registry No.—**1**, 15476-35-4; **2**, 15476-31-0; **3**, 15476-32-1; **4**, 15476-33-2; **5**, 15476-34-3; **6**, 15476-07-0; **7**, 6341-06-6.

(25) N. K. Richtmyer, *Methods Carbohydrate Chem.*, **2**, 132 (1963).
(26) W. T. Haskins, R. M. Hann, and C. S. Hudson, *J. Am. Chem. Soc.*, **68**, 1766 (1946).
(27) W. T. Haskins, R. M. Hann, and C. S. Hudson, *ibid.*, **69**, 1461 (1947).
(28) W. T. Haskins, R. M. Hann, and C. S. Hudson, *ibid.*, **67**, 939 (1945).
(29) R. M. Hann and C. S. Hudson, *ibid.*, **66**, 735 (1944).

An Investigation of the Role of Dimethyl Acetals in the Formation of Methyl Glycosides[1]

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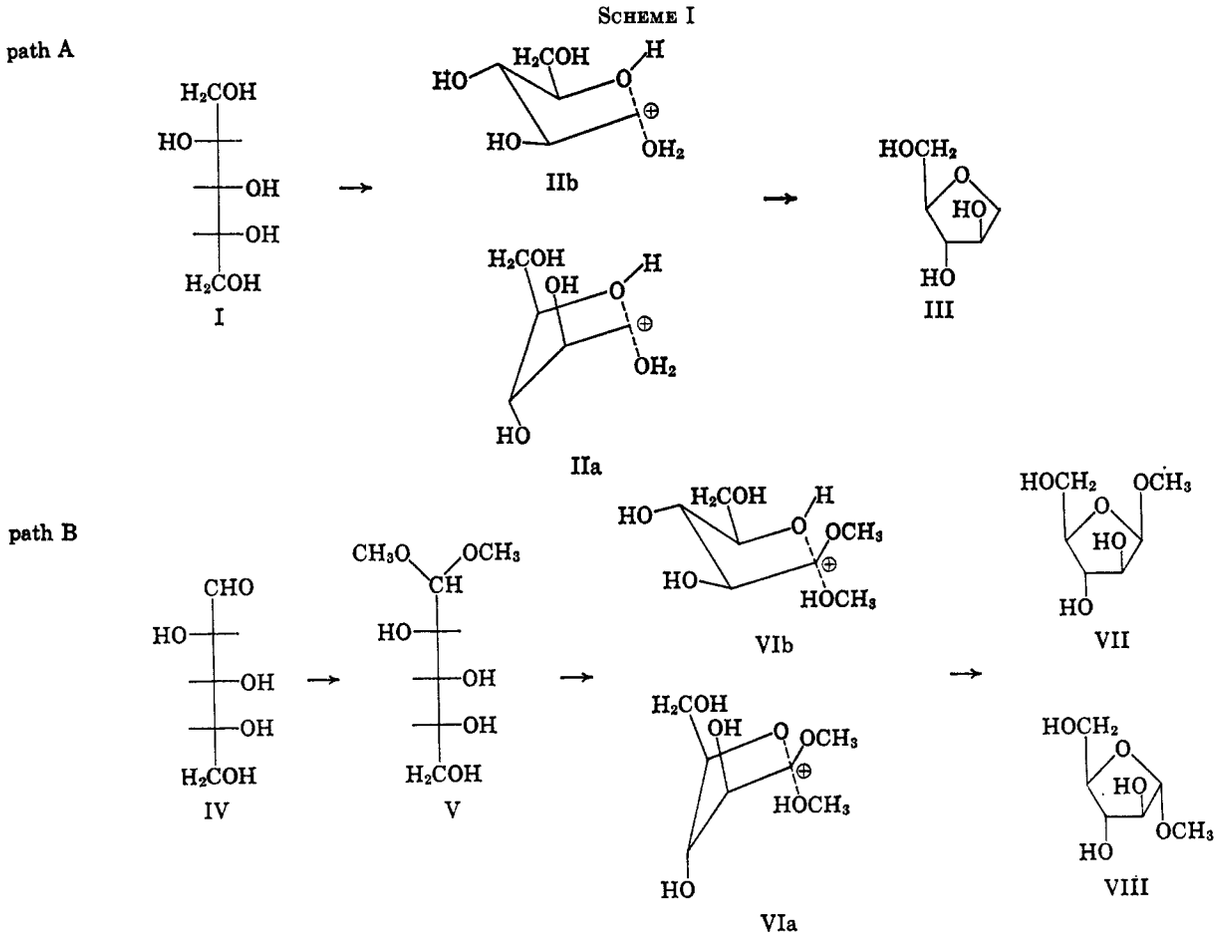
The effect of configuration on the rates of glycosidation of the pentoses has been studied and found to be similar to that observed in the acid-catalyzed dehydration of the pentitols, indicating that an acyclic intermediate may be involved in the glycosidation. The hypothesis that the dimethyl acetals are intermediates in the glycosidation reaction has been disproved and the possibility that the acyclic methyl hemiacetals are intermediates in the glycosidation process cannot be tested readily. Partial glycosidations of D-arabinose-1-C^{14} and D-galactose-1-C^{14} have been carried out and the respective dimethyl acetals isolated by chromatographic and by dilution techniques. An investigation of the kinetics of the glycosidations of D-arabinose, D-galactose, and their dimethyl acetals has permitted an estimation of the role of acetals as intermediates in glycosidation. A comparison of this theoretical variation of acetal concentration with time with that obtained experimentally has disproved Fischer's proposal that the dimethyl acetals of sugars are obligatory intermediates in the formation of glycosides.

The acyclic dimethyl acetals of sugars were proposed as intermediates in the formation of methyl glycosides by Fischer in one of his first papers on the preparation of methyl glycosides.[2a] Later Campbell and Link[2b] and Wolfrom and Waisbrot[3] examined the behavior of the dimethyl acetals of galactose and glucose under glycoside-forming conditions and showed that the acetals were rapidly converted to glycosides. The initial rapid reaction appeared to be the formation of furanosides which were slowly converted to pyranosides. The over-all process of glycoside formation also involves the initial formation of furanosides. This fact was clearly demonstrated by Bishop and Cooper,[4] who confirmed the earlier observations of Levene, *et al.*[5]

The effect of configuration on the rate of methyl glycoside formation was examined by Levene, *et al.*,[5] and

(1) Supported in part by a Public Health Service Grant (GM 11,963) and a Public Health Service Research Career Program Award (GM 24,808 to R. B.) from the Institute of General Medical Sciences.
(2) (a) E. Fischer, *Ber.*, **28**, 1145 (1895); (b) H. A. Campbell and K. P. Link, *J. Biol. Chem.*, **122**, 635 (1938).
(3) M. L. Wolfrom and S. W. Waisbrot, *J. Am. Chem. Soc.*, **61**, 1409 (1939).
(4) C. T. Bishop and F. P. Cooper, *Can. J. Chem.*, **41**, 2743 (1963).
(5) P. A. Levene, A. L. Raymond, and R. T. Dillon, *J. Biol. Chem.*, **95**, 969 (1952).

SCHEME I



by Bishop and Cooper.[4] The former workers observed that in the pentoses the rate of glycoside formation decreased through the series ribose, xylose, arabinose, and lyxose, although they did not draw attention to this observation in their conclusions. Bishop and Cooper, on the other hand, observed that the rate of glycoside formation decreased through the series ribose, xylose, and lyxose and predicted that arabinose should undergo glycosidation more readily than ribose. A similar effect of configuration on the rate of ring formation has been observed in the acid-catalyzed formation of anhydro alditols.[6] The rate of this dehydration, which leads primarily to the formation of tetrahydrofuran derivatives, *i.e.*, 1,4-anhydropentitols, decreases through the series ribitol, xylitol, arabinitol, and lyxitol.[7]

The similarities between the dehydration reaction and methyl glycoside formation led us to reexamine the effect of configuration on the rate of glycoside formation. We have found that the rate of methyl glycoside formation decreases through the series ribose, xylose, arabinose, and lyxose. Since the effect of configuration was found to be similar to that observed in the dehydration reaction, which probably proceeds through an acyclic transition state, the possibility that the former reaction might also proceed through an acyclic intermediate was thought to warrant further examination.

This manuscript describes experiments which provide a test for Fischer's hypothesis concerning the role of dimethyl acetals in the formation of methyl glycosides.[8] The possibility that methyl hemiacetals, which are acyclic, and which could serve as intermediates in methyl glycoside formation, has been considered but has not been tested.

Results and Discussion

The pathway proposed for the dehydration of D-arabinitol (I) to its 1,4 anhydride (III) is shown in Scheme I, path A. In the dehydration of alditols it was shown that where the hydroxyl group at position 2 was *cis* to the hydroxymethyl group at position 4 in the product the reaction proceeds much less rapidly than when these groups were *trans* to each other.[6] In that study, it was deduced that an interaction could occur in the transition state between the hydroxyl at carbon 2 and the group being displaced from carbon 1 which interfered with the ability of the latter to leave. This interaction causes the conformations in which the hydroxyl at carbon 2 is equatorial to be unable to serve as transition states. For example, the dehydration of I was proposed to occur *via* conformer IIa rather than conformer IIb even though the nonbonded interactions between substituents at carbons

(6) B. G. Hudson and R. Barker, *J. Org. Chem.*, **32**, 3650 (1967).

(7) The rate constants for the dehydration of arabinitol and lyxitol were calculated from the proportion of products having these configurations in the dehydration of arabinitol, and the observed rates of dehydration of xylitol and ribitol were corrected for the fact that these pentitols are symmetrical.

(8) A preliminary report of this work was presented at the 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., April 1967, p 18C. During the preparation of this paper R. J. Ferrier and S. R. Hatton have informed us that they have examined the role of acyclic acetals in the alcoholysis of xylose and glucose. The results of their study agree with those presented here and have been submitted for publication to *Carbohydrate Research*.

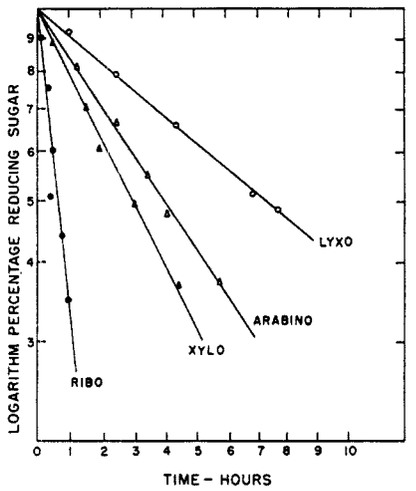


Figure 1.—The rates of acid-catalyzed glycosidation of the pentoses (0.2% sugar, 0.028 *N* sulfuric acid, 28 ± 0.1°).

2, 3, and 4 are significantly lower in the latter. In Scheme I, path B, the structures of D-arabinose (IV), D-arabinose dimethyl acetal (V), and the D-arabinofuranosides (VII and VIII) are shown. It is clear that an interaction similar to that described above could occur when a dimethyl acetal is converted to a glycoside by a displacement reaction (SN2). The leaving group, a protonated methoxyl group, in conformer VIb would be eclipsed with the hydroxyl at C-2 and would be susceptible to hydrogen bond formation with, or loss of a proton to, that group.

On the basis of this analysis the effect of configuration on the rate of methyl glycoside formation from the dimethyl acetals of the pentoses should be the same as that observed for the formation of 1,4 anhydrides from the pentitols.[6] Similarly, if the formation of methyl glycosides from reducing sugars proceeds through the dimethyl acetal (or methyl hemiacetal) the same relationship between configuration and rate might be expected. That the latter expectation is borne out is shown in Figure 1, in which the logarithm of the percentage of reducing sugar remaining is plotted *vs.* time. The pseudo-first-order rate constants and the relative rates of glycosidation of the pentoses are given in Table I. The rate constants and relative rates of dehydration of the corresponding pentitols are also given.

TABLE I
THE RATE CONSTANTS AND THE CALCULATED RELATIVE RATES OF THE ACID-CATALYZED GLYCOSIDATION OF THE PENTOSES AND ANHYDRIZATION OF THE PENTITOLS[a]

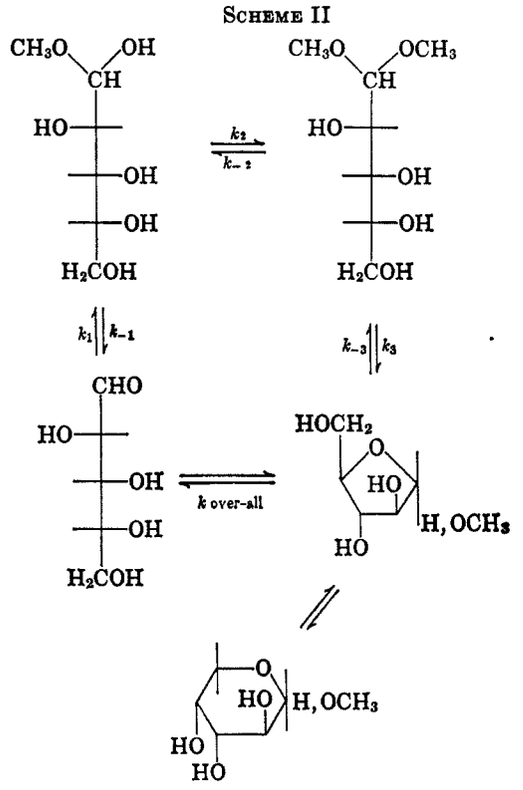
Configuration	Pentose $k\psi$ (±2%)	Rel rate	Pentitol $k\psi$ (±4%)	Rel rate
lyxo	2.6×10^{-5} sec^{-1}	1.0	1.2×10^{-5} sec^{-1}	1
arabino	5.0×10^{-5} sec^{-1}	1.9	1.18×10^{-4} sec^{-1}	10
xylo	6.4×10^{-5} sec^{-1}	2.5	3.5×10^{-4} sec^{-1}	29
ribo	3.2×10^{-4} sec^{-1}	12.4	6.0×10^{-4} sec^{-1}	50

[a] The rate constants are pseudo-first-order constants. The experiments were performed in 0.028 *N* methanolic sulfuric acid at 28 ± 0.1°. The rates of glycosidation of D-arabinose dimethyl acetal (1.66×10^{-3} sec^{-1}), D-galactose dimethyl acetal (2.3×10^{-3} sec^{-1}), and D-galactose (2.6×10^{-5} sec^{-1}) were determined under identical conditions.

The concentrations of reducing sugars plotted in Figure 1 were obtained using the dinitrosalicylate procedure.[9] The method measures only reducing sugars, as was shown by assaying samples of D-arabinose in the presence of the dimethyl acetal, the furanosides and the pyranosides of D-arabinose in a similar fashion to samples taken from the glycosidation mixture. The accuracy of the method was further checked by comparing the data obtained with that obtained by gas chromatographic analysis of the reaction mixture.

The probable pathways involving acyclic intermediates for the formation of glycofuranosides from reducing sugars are represented in Scheme II. If the

SCHEME II



dimethyl acetal is involved in the process, it is probable that it is formed from the methyl hemiacetal. If each of the rate constants influenced the over-all process the kinetics would be complex and the question of whether the dimethyl acetal is an important intermediate would be difficult to answer. However, several factors which allow the proposed model to be simplified are: (a) The assays for reducing sugar measure both reducing sugar and methyl hemiacetal. (b) The conversions of reducing sugars to glycosides are all first order for over 80% of the reaction period. (c) The proportion of reducing sugar present at equilibrium is less than 1%. (d) The conversions of dimethyl acetals to glycosides can be studied independently and are also first order (at least in the case of the D-arabinose and D-galactose derivatives). At equilibrium these reactions contain less than 1% of the starting material. (e) The rate of conversion of the dimethyl acetals to glycosides is significantly faster than the rate of conversion of reducing sugar to glycosides. The ratio of rates is 33:1 in the case of D-arabinose and 88:1 in the case of D-galactose.

(9) K. H. Meyer, G. Noelting, and P. Bernfield, *Helv. Chim. Acta*, **31**, 103 (1948).

On the basis of the above observations we can make the following simplifications.

(1) The rate constants k_1 and k_{-1} can be neglected since reducing sugar and methyl hemiacetal are measured simultaneously and are considered as "reducing sugar" in obtaining a rate constant for the over-all reaction. If the methyl hemiacetal is involved in the reaction it must be formed rapidly and reversibly since there is no induction period or divergence from first-order kinetics observed in the over-all reaction.

(2) The conversion of dimethyl acetal to glycosides can be treated as a simple first-order process rather than an equilibrium since the proportion of starting material present at infinite time is very small.

(3) The over-all reaction can be treated as a series of two first-order processes to determine the least amount of dimethyl acetal which must be formed for it to serve as an obligatory intermediate, *i.e.*, k_{-2} can be neglected to compute the limiting case. This approach does not involve any assumptions since the value of k_3 (the rate constant of conversion of dimethyl acetal to glycosides) is known as is the value of $k_{\text{over-all}}$. The amount of dimethyl acetal necessary to produce the observed value of $k_{\text{over-all}}$ can then be calculated since at any time the rate of appearance of glycosides must equal k_3 times the concentration of dimethyl acetal at that time. The rate of appearance of glycosides also equals $k_{\text{over-all}}$ times the concentration of reducing sugar. Since $k_3 = 33k_{\text{over-all}}$ in the case of D-arabinofuranoside formation the proportion of dimethyl acetal must rise and account for at least 3 mole % of the carbohydrate very early in the reaction.

The rate constant $k_{\text{over-all}}$ is approximately equal to k_2 since this step in the reaction is rate limiting. It is possible therefore to calculate the variation which must occur in dimethyl acetal concentration with time if it serves as an intermediate in the formation of glycosides using the equation given by Frost and Pearson[10] (eq 1), where $[A_0]$ = the initial concentration of reducing sugar and [B] = the concentration of dimethyl acetal.

$$[B] = \frac{[A_0]k_2}{k_3 - k_2}(e^{-k_2 t} - e^{-k_3 t}) \quad (1)$$

Using the above assumptions the maximum proportion of D-arabinose dimethyl acetal (2.7%) which would be required if it were an obligatory intermediate was calculated and is shown in Figure 2. This concentration of dimethyl acetal might have been difficult to detect in earlier studies. For this reason it was decided to determine the acetal concentration in the reaction mixture at intervals by an isotope-dilution technique.[11]

The concentration of dimethyl acetal in the reaction mixture was calculated from the specific activity of the D-arabinose-1-C^{14} used in the glycosidation and the specific activity of the tetra-*O*-acetyl D-arabinose-1-C^{14} dimethyl acetal which was isolated after nonradioactive dimethyl acetal had been added to a neutralized sample from the reaction mixture and the whole acetylated. The results are presented in Table II and shown in Figure 2. The maximum proportion

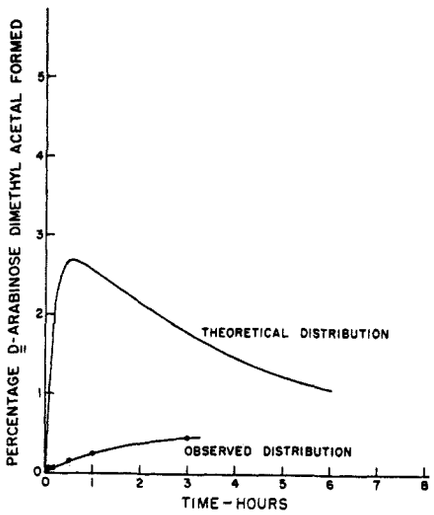


Figure 2.—A comparison of the theoretically derived distribution of D-arabinose dimethyl acetal assuming that it is an obligatory intermediate in glycosidation with the observed distribution obtained from the isotopic dilution experiment. The pseudo-first-order rate constants for D-arabinose and D-arabinase dimethyl acetal are 5.0×10^{-5} and 1.66×10^{-3} sec^{-1}, respectively. Rates were obtained using 0.28 *N* methanolic sulfuric acid at 28 ± 0.1°.

TABLE II
THE VARIATION OF CONCENTRATION WITH TIME OF D-ARABINOSE DIMETHYL ACETAL IN THE GLYCOSIDATION OF D-ARABINOSE

Sample	Time	Amount of authentic D-arabinose dimethyl acetal added, mg	Specific activity[a] of diluted tetra-*O*-acetyl-D-arabinose dimethyl acetal, mcurie/mmole
1	1 min	101.5	2.25×10^{-6}
2	10 min	105.6	2.85×10^{-6}
3	30 min	100.6	5.6×10^{-6}
4	1 hr	101.7	8.65×10^{-6}
5	3 hr	103.2	1.95×10^{-5}
6	48 hr	103.1	1.70×10^{-5}

[a] Weighed samples from each of the final crops were combined. Five recrystallizations were performed without loss from the calculated mean specific activity (mean constant specific activity = 7.8×10^{-6} mcurie/mmole). The estimated maximum error in these values is ±5%.

of dimethyl acetal present in the reaction mixture is 0.55% after 3 hr. At 30 min the proportion of dimethyl acetal present was 0.16% of the total carbohydrate, approximately 6% of that proportion which would be required if the dimethyl acetal were an obligatory intermediate in the glycosidation process. The gradual increase in the proportion of dimethyl acetal during the reaction is consistent with the compound being in equilibrium with, or formed from, a product of the reaction rather than from one of the starting materials.

D-Arabinose and its dimethyl acetal were used because of the ease of preparation of the latter compound. Attempts were made to prepare the dimethyl acetals of D-ribose, D-lyxose, and D-xylose by transacetalation of the corresponding dithioacetals and an attempt was made to prepare the D-xylose derivative by the method described by Hudson, *et al.*[12] These attempts all gave complex mixtures from which it was not possible to isolate products having nmr

(10) A. A. Frost and R. J. Pearson, "Kinetics and Mechanism," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1961, p 166.

(11) T. T. Gorsuch, *Radiochem. Center Rev.*, **2**, 2 (1967).

(12) E. M. Montgomery, R. M. Hahn, and C. S. Hudson, *J. Am. Chem. Soc.*, **59**, 1124 (1937).

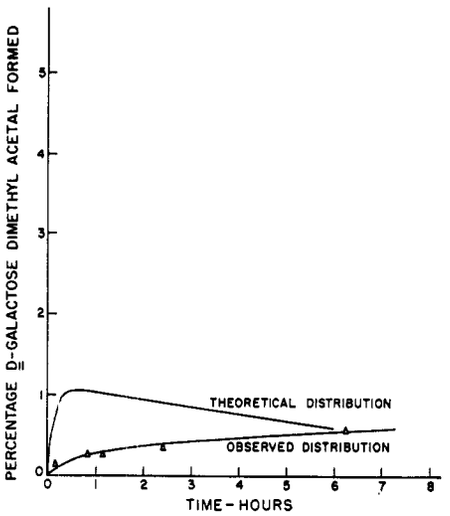


Figure 3.—A comparison of the theoretically derived distribution of D-galactose dimethyl acetal assuming that it is an obligatory intermediate in glycosidation with the observed distribution obtained from the isotopic dilution experiment. The pseudo-first-order rate constants for D-galactose dimethyl acetal are 2.6×10^{-5} and 2.3×10^{-3} sec^{-1}, respectively. Rates were obtained using 0.28 *N* methanolic sulfuric acid at 28 ± 0.1°.

spectra characteristic of other known dimethyl acetals. The reaction mixtures from the attempts to prepare D-ribose dimethyl acetal, when examined by paper and thin layer chromatography, were found to contain materials which had the properties of known ribofuranosides.

To establish that the findings with arabinose reflect the general characteristics of the glycosidation reaction, the conversion of D-galactose and D-galactose dimethyl acetal to glycosides was investigated. Using D-galactose-1-C^{14}, and nonradioactive D-galactose dimethyl acetal, an experiment similar to that described for D-arabinose was performed. The data obtained from the isotope dilution experiment are presented in Table III and the calculated and observed

TABLE III
THE VARIATION OF CONCENTRATION WITH TIME OF D-GALACTOSE DIMETHYL ACETAL IN THE GLYCOSIDATION OF D-GALACTOSE

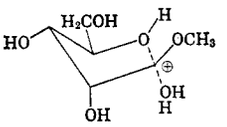
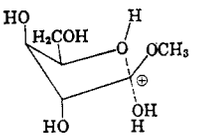
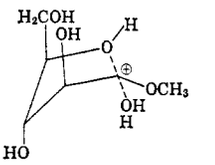
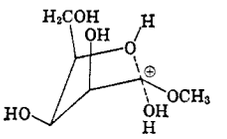
Sample	Time	Amount of authentic D-galactose dimethyl acetal added, mg	Specific activity[a] of diluted tetra-*O*-acetyl-D-galactose dimethyl acetal, mcurie/mmole
1	10 min	98.8	0.83×10^{-5}
2	50 min	97.5	1.2×10^{-5}
3	70 min	100.7	1.2×10^{-5}
4	2 hr,25 min	97.3	1.7×10^{-5}
5	6 hr,15 min	99.5	2.7×10^{-5}
6	22 hr	97.0	3.4×10^{-5}

[a] Weighed samples from each of the final crops were combined. Five recrystallizations were performed without loss from the calculated mean specific activity (mean constant specific activity = 1.7×10^{-5} mcurie/mmole). The estimated maximum error in these values is ±5%.

concentration–time curves for the dimethyl acetal are shown in Figure 3. Clearly the dimethyl acetal of D-galactose is not an important intermediate in the formation of methyl galactosides, in agreement with the findings with D-arabinose.

The hypothesis that the dimethyl acetals are intermediates in the glycosidation reaction has been disproved and the possibility that the acyclic methyl hemiacetals are intermediates in the glycosidation process cannot be tested readily. However, the effect of configuration on the rate of the process strongly suggests the occurrence of an acyclic intermediate. If such an intermediate were involved, the interactions between the substituents at C-2, C-3, and C-4 and the methoxyl group at C-1 would determine the anomeric configuration of the initial product and it should be possible to predict what this configuration would be utilizing the "rule" that the hydroxyl group at C-2 must be axial in the transition state.[6] The conformations shown in Table IV are predicted for

TABLE IV
TRANSITION STATES FOR GLYCOSIDE FORMATION FROM PENTOSES

Pentose	Favored transition state	Furanoside: Predicted	Furanoside: Found
D-Ribose		β	β
D-Xylose		β	α + β
D-Arabinose		α	α + β
D-Lyxose		α	α

the transition states for glycoside formation from the pentoses. The least interaction between ring substituents and the methoxyl group at C-1 appears to be present in the conformers which would lead to the β-furanosides of D-ribose and D-xylose and to the α-furanosides of D-arabinose and D-lyxose. Bishop and Cooper[4] demonstrated that β-D-ribo- and α-D-lyxofuranosides are formed most readily; however, they also demonstrated that α- and β-D-xylofuranosides were found to be present in the ratio 1.8:1.0 after 15 min of reaction. We have found the same to be true for the arabinofuranosides. The α- and β-D-arabinofuranosides are present in the ratio 1.25:1.0 after 15 min of reaction. The tendency in the case of xylose and arabinose for glycosides to form which have a *cis* relationship between the aglycone and the hydroxyl at C-2 might be due to the fact that, in the transition states for these pentoses which have a *trans* arrangement between these groups, an interaction of the methoxyl group with an axial hydroxyl group at C-3 occurs. Repulsion between 1,3-*cis* substituents was previously proposed as important in determining the rates of anhydrization reactions.[6]

Experimental Section

Melting points are corrected.

Gas chromatography was performed with an Aerograph HY-F1 600-D equipped with a 5 ft × 1/8 in. column of 10% polyethylene glycol sebacate on Chromosorb Q using helium as the carrier gas. Liquid scintillation counting was carried out in a *p*-dioxane based scintillation fluid (Bray's solution),[13] using a Packard Tricarb Model 3950 liquid scintillation counter. Paper strips were counted on a Vanguard Autoscanner 880 with 1.3% butane–98.7% helium carrier gas. Constant temperature for kinetic experiments was maintained using a Haake Ultrathermostat NBS water bath. Absorbancies were measured in a Beckman DU with a Gilford attachment. Nuclear magnetic resonance spectra were obtained on a Varian A-60 spectrometer at ambient temperatures.

D-Arabinose diethyl mercaptal (IX) was prepared following Fischer's general procedure.[14] The product was recrystallized from ethanol–hexane (5:1) and had mp 127–128°, $[\alpha]^{25}D$ 0° (*c* 3.0, pyridine). Wolfrom, *et al.*,[15] reported mp 125–126° and $[\alpha]^{23}$ 0° (*c* 3.0, pyridine).

Tetra-*O*-acetyl-D-arabinose diethyl mercaptal (X) was prepared in 90% yield by acetylation of IX. The material was recrystallized from ether–hexane (3:1), mp 79–80°, $[\alpha]^{25}D$ +23° (*c* 3.0, $CHCl_3$). Wolfrom, *et al.*,[15] reported mp 80° and $[\alpha]^{23}D$ +30° (*c* 4.2, $CHCl_3$). The nmr spectrum of the product had a quadruplet at τ 7.3 and a triplet at 8.77 due to the $-S-CH_2-CH_3$ group, peaks at 7.9 due to the $-O-CO-CH_3$ groups, as well as multiplets between 5.7–6.25 and 4.1–5.1 due to the $-CH_2O-$ and $-CHO-$ groups.

Tetra-*O*-acetyl-D-arabinose dimethyl acetal XI was prepared in 20% yield from X by the method used by Wolfrom, *et al.*,[16] for the preparation of the corresponding galactose derivative. After three recrystallizations from ether–hexane (3:1) the material had mp 80.4°, $[\alpha]^{25}D$ +20° (*c* 3.0 $CHCl_3$). Hudson, *et al.*,[12] reported mp 80°, $[\alpha]^{25}D$ +21.8° (*c* 1.33, $CHCl_3$). The nmr spectrum showed peaks at τ 7.9 due to $-O-CO-CH_3$, peaks at 6.7 due to $-O-CH_3$, as well as multiplets between 5.5–6.0 and 4.3–5.0 due to $-CH_2O-$ and $-CHO-$ groups.

D-Arabinose dimethyl acetal (V) was obtained from XI by deacetylation with barium methoxide in methanol.[12] The product after two recrystallizations from methanol had mp 122.6° and $[\alpha]^{25}D$ −17.0 (*c* 3.0, water). Hudson, *et al.*,[12] reported mp 122° and $[\alpha]^{25}D$ −18.5° (in water).

D-Galactose dimethyl acetal was prepared from D-galactose as described by Wolfrom, *et al.*,[15] utilizing a similar series of reactions to those described from the preparation of V. The product after two recrystallizations from methanol had mp 122.6° and $[\alpha]^{25}D$ +16° (*c* 3.0, water). Wolfrom, *et al.*, reported mp 122–123° and $[\alpha]^{28}D$ +16° (*c* 2, water).

Methyl α-D-arabinofuranoside was prepared as described by Augestad and Berner.[17] Attempts to crystallize the compound from a variety of solvents failed. The glycoside obtained was of similar chromatographic mobility to that described by the above authors. The glycoside showed $[\alpha]^{25}D$ +123° (*c* 3.0, water) and the reported value is $[\alpha]D$ 119° (*c* 2.6, water).

Methyl β-D-arabinopyranoside was prepared as described by Hudson.[18] The product after two recrystallizations from methanol had mp 168° and $[\alpha]D$ −240° (*c* 3.0, water). Hudson reported mp 169° and $[\alpha]D$ −245° (*c* 7.2, water).

Kinetics of Glycoside Formation. A. From the Reducing Sugars.—Solutions containing approximately 200 mg of pentose (or D-galactose) in 100 ml of dry methanol[19] were incubated at 28 ± 0.1°. To initiate the reaction a 4-ml aliquot was withdrawn from the solution and a 4-ml aliquot of 0.7 *N* sulfuric acid in methanol added with vigorous mixing. Samples (1 ml) were taken at intervals and were neutralized with 0.1 *N* sodium hydroxide. When all of the samples had been taken, the reducing sugar content was determined using the 3,5-dinitrosalicylate colorimetric procedure.[8] D-Arabinose was used as a standard for the determination and was found to give a linear response in the experimental range (5 μg–2 mg/ml). The rate of glycosidation of D-arabinose was also determined by gas chromatography of the acetates at 200°. Samples (5 ml) were withdrawn and neutralized and then concentrated to dryness in small conical flasks on a rotary evaporator at temperatures below 50°. The acetates were formed in the presence of the salt using acetic anhydride and pyridine and samples of these reaction mixtures were injected directly into the chromatography unit. The molar response of the detector to the acetates of the reducing sugar and the glycosides was shown to be the same using standards. The rate of glycosidation was obtained using the ratio of the area of the reducing sugar peak to the sum of the areas of reducing sugar and glycoside peaks as a measure of the amount of initial reducing sugar. The data obtained by this method was in excellent agreement with that obtained by the dinitrosalicylate method (Figure 1).

The rate of glycoside formation from D-galactose was also measured by gas chromatography using a column temperature of 210°.

B. From the Dimethyl Acetals.—The rate of conversion of D-arabinose and D-galactose dimethyl acetals to the glycosides were obtained by periodic gas chromatographic analyses of the reaction mixtures. Samples were neutralized and reduced in volume to dryness as previously described for reducing sugars. For D-arabinose dimethyl acetal the trimethylsilyl ethers were formed in the presence of the salt and samples of these reaction mixtures injected. An excellent separation of the glycosides from the acetal was obtained at 110°.

For D-galactose dimethyl acetal the acetates were used as described in section A.

The rate of conversion of D-arabinose dimethyl acetal was also measured by determining the periodate consumption of samples taken at intervals. The acetal consumes periodate more rapidly than do the furanosides. The difference between the relative rates of oxidation is sufficiently great that the amount of oxidant consumed in 10 min at pH 7.5 can be directly related to the amount of acetal present. Samples (25) of acetal (2 mg/ml) and of the glycosidation mixture (2 mg/ml) were pipetted directly into 0.002 *M* sodium metaperiodate and the consumption of oxidant was followed spectrophotometrically at 270 mμ.[20] The results obtained agreed with those obtained by gas chromatography.

Isotope Dilution Experiments. A. Demonstration of the Presence of D-Arabinose Dimethyl Acetal in the Glycosidation Reaction.—In a preliminary experiment 500 mg of twice-recrystallized D-arabinose[21] was mixed with approximately 0.05 mcurie of D-arabinose-1-C^{14},[22] specific activity 2.0 mcuries/mmole. After two recrystallizations the specific activity was 1.74×10^{-2} mcurie/mmole. A sample of this diluted D-arabinose-1-C^{14} (24.5 mg) was dissolved in methanol (12.5 ml) and the solution acidified with methanolic sulfuric acid (1.75 ml of 0.22 *N*). The solution was refluxed for 1 hr, cooled, neutralized with aqueous sodium hydroxide (0.1 *N*), and deionized by successive treatments with a strong base ion-exchange resin (Rexyn 201) and a weak acid ion-exchange resin (Rexyn RG 51).

A sample of the reaction mixture (0.8 of the total) was chromatographed on Whatman 1 using methyl ethyl ketone–water (92:8), solvent 1. The chromatogram was developed for 14 hr so that the solvent ran off the chromatogram. The distribution of radioactivity on the chromatogram was determined using a Vanguard Strip Scanner. Four main peaks of activity were detected. The slowest moving component had the same R_f value as D-arabinose. The two major components ($R_{arabinose}$ 6.6, $R_{arabinose}$ 11.4) had the same R_f values as the α- and β-methyl-D-arabinofuranosides. A minor component ($R_{arabinose}$ 4.1) had the same R_f value as the methyl-D-arabinopyranosides which do not separate well in this system. A fifth component, with $R_{arabinose}$ 5.3, was present in small amounts and was tentatively identified as D-arabinose dimethyl acetal. The $R_{arabinose}$ value of the authentic material is 5.3.

The band of activity corresponding to the D-arabinose dimethyl acetal was cut from the chromatogram and the acetal was eluted by successive washings with methanol and water. The eluate was reduced in volume and the residue was mixed with authentic crystalline D-arabinose dimethyl acetal (120 mg).

(13) G. A. Bray, *Anal. Biochem.*, **1**, 279 (1960).
(14) E. Fischer, *Ber.*, **27**, 673 (1894).
(15) M. L. Wolfrom, D. I. Weisblat, W. H. Zophy, and S. W. Waisbrot, *J. Am. Chem. Soc.*, **63**, 201 (1941).
(16) M. L. Wolfrom, L. J. Tanghe, R. W. George, and S. W. Waisbrot, *ibid.*, **60**, 132 (1938).
(17) I. Augestad and E. Berner, *Acta Chem. Scand.*, **8**, 251 (1954).
(18) C. S. Hudson, *J. Am. Chem. Soc.*, **47**, 265 (1925).
(19) Baker, Spectrophotometric Grade.
(20) R. Barker, *J. Org. Chem.*, **29**, 869 (1964).
(21) Pfanstiehl Chemical Co., Waukeegan, Ill.
(22) New England Nuclear, Boston, Mass.

The mixture was dissolved in methanol, filtered, and concentrated to dryness. The dry residue was taken up in pyridine (0.8 ml) and acetic anhydride (1.6 ml) was added dropwise with cooling. After 1 hr the acetate (179 mg, 85%) was isolated in the usual fashion. The acetate after recrystallization from ether–hexane (3:1) had a specific activity of 9.2×10^{-6} mcurie/mmole, which was not altered on further recrystallization from the same solvents.

The twice-recrystallized tetra-*O*-acetyl-D-arabinose dimethyl acetal (20 mg) was deacetylated using barium methoxide. The D-arabinose dimethyl acetal so obtained was chromatographed on Whatman 3 M filter paper using solvent 1. The chromatogram was dried when the solvent had reached the leading edge of the paper and was redeveloped in solvent 1. Strips were cut from the sides of the chromatogram and the distribution of the D-arabinose dimethyl acetal determined by its reaction with periodate–benzidene spray.[23]

The remainder of the chromatogram was cut into ten 2.5-cm strips which were eluted successively with water and methanol, and then the eluates were reduced in volume. The radioactivity in each sample was determined. The radioactivity was centered at, and symmetrically distributed about, a position which corresponded in R_f to D-arabinose dimethyl acetal.

A sample of radioactive D-arabinose dimethyl acetal (12 mg) was dissolved in methanol (10 ml) and methanolic sulfuric acid added (1 ml, 0.7 *N*). The mixture was refluxed (18 hr), neutralized with sodium hydroxide, and deionized as described previously. The glycosidation mixture was chromatographed on Whatman 1 paper in solvent 1 and the distribution of radioactivity and sugar determined as described above. It was found that the D-arabinose dimethyl acetal-1-C^{14} had been converted into a mixture of the labeled glycosides as had been expected.

B. The Variation of Concentration with Time of D-Arabinose Dimethyl Acetal in the Glycosidation Reaction of D-Arabinose. Experiment 1.—Twice-recrystallized D-arabinose (1 g) was mixed with approximately 0.075 mcurie of D-arabinose-1-C^{14} (specific activity 2.0 mcuries/mmole). The mixture was recrystallized twice to give material having a determined specific activity of 1.26×10^{-2} mcurie/mmole.

The glycosidation of this D-arabinose-1-C^{14} was carried out under conditions used to obtain kinetic data (400 mg of D-arabinose-1-C^{14} in 200 ml of methanol). Samples (8 ml) were taken at intervals and were neutralized and deionized as described in A. The deionized samples were individually chromatographed on Whatman 1 using solvent 1 and the bands of activity located by scanning strips cut from the sides of the chromatogram. The bands having similar chromatographic mobility to D-arabinose dimethyl acetal were eluted, concentrated, and mixed with 50–60 mg of authentic D-arabinose dimethyl acetal. The samples were acetylated to facilitate handling and each recrystallized twice. Specific activities were determined after each crystallization. There was no significant loss in activity after the first recrystallization. The rate of reaction was determined by 3,5-dinitrosalicylate reduction using separate samples. The variation in concentration of dimethyl acetal with time was similar to that obtained as described below (experiment 2).

Experiment 2.—Twice-recrystallized D-arabinose (200 mg) was mixed with 0.10 mcurie of D-arabinose-1-C^{14} (specific activity 2.05 mcuries/mmole).[21] The mixture was recrystallized twice to give material having a specific activity of 7.0×10^{-2} mcurie/mmole.

The glycosidation was carried out under conditions used to obtain kinetic data (50 mg of D-arabinose-1-C^{14} in 25 ml of methanol). The reaction was sampled at intervals and the samples (2.5 ml) were neutralized with 0.1 *N* sodium hydroxide. Weighed amounts of authentic D-arabinose dimethyl acetal (100 mg) were mixed with the neutralized samples and were concentrated to dryness. The residues were acetylated in pyridine with acetic anhydride and worked up by destroying the excess acetylating reagent with chips of ice, adding methylene chloride, and extracting the solution with 2 *N* sulfuric acid, saturated sodium bicarbonate solution, and water. After drying over sodium sulfate the methylene chloride was removed *in vacuo* to give a crystalline acetate in quantitative yield. The acetates were recrystallized three times from ether–hexane (3:1). To ensure removal of radioactive reducing sugar and glycosides, each recrystallization was carried out with a large loss. Some change in activity was observed in some samples between the last two crystallizations. However, the small quantities involved made further recrystallization difficult and increased probable experimental error due to weighing and counting. To demonstrate that the results obtained were not due to minor contaminants of high specific activity, all of the samples were combined and the dimethyl acetal recrystallized five times. The specific activity remained constant at 7.8×10^{-6} mcurie/mmole after the second recrystallization. The specific activities of the separate samples ranged from 2.25×10^{-6} to 1.96×10^{-5} mcurie/mmole.

D. The Variation of Concentration with Time of D-Galactose Dimethyl Acetal in the Glycosidation Reaction of D-Galactose.—Twice recrystallized D-galactose (100 mg),[21] was mixed with approximately 0.05 mcurie of D-galactose-1-C^{14} (specific activity 3.06 mcuries/mmole).[22] The mixture was recrystallized twice to give material with a determined specific activity of 1.05×10^{-1} mcurie/mmole.

A sample of the diluted D-galactose-1-C^{14} (47.4 mg) was dissolved in methanol (25 ml) and the glycosidation was carried out under the conditions used to obtain kinetic data. The variation of dimethyl acetal concentration with time in the glycosidation reaction was determined as described in section B, experiment 2, for the D-arabinose derivative. Final specific activities are given in Table III and the variation of concentration with time is shown in Figure 3.

Registry No.—I, 488-82-4; IV, 10323-20-3; V, 15135-34-9; X, 5329-45-3; XI, 15135-36-1; D-lyxose, 1114-34-7; D-xylose, 133-56-2; D-ribose, 58-91-3; D-lyxitol, 488-83-5; D-xylitol, 7313-55-5; D-ribitol, 15135-41-8; D-galactose dimethyl acetal, 1824-95-9; D-galactose, 147-76-2.

(23) J. A. Cifonelli and F. Smith, *Anal. Chem.*, **26**, 1132 (1954).