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THE PROTON MAGNETIC RESONANCE SPECTRA AND TAUTOMERIC EQUILIBRIA OF ALDOSES IN DEUTERIUM OXIDE[1]

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ABSTRACT

The proton magnetic resonance spectra of D-xylose, D-lyxose, D-arabinose, D-ribose, D-glucose, D-mannose, and D-galactose were determined at 100 Mc.p.s. in deuterium oxide. The chemical shifts and structures of a number of ring protons in these compounds were determined either by spin-decoupling experiments or by synthesis of specifically deuterated compounds. The proton magnetic resonance parameters are shown to provide considerable information on the conformations and tautomeric equilibria for the sugars in aqueous solution. It is concluded that, for aldopyranoses in a chair conformation, the chemical shift of equatorial protons at a given position is virtually independent of configurational changes at other positions. However, an axial proton is shielded about 0.3 p.p.m. less by an axial hydroxyl group at a neighboring position than when the hydroxyl group is in an equatorial orientation. An axial hydroxyl group leads to deshielding of an opposing axial proton by about 0.35 p.p.m. By using the chemical shifts of the ring protons of β-D-xylopyranose and β-D-glucopyranose as reference point, the chemical shifts of protons in other pyranose structures could be anticipated to within a useful degree of accuracy.

Evidence was obtained that D-ribose and 2-deoxy-D-ribose exist in aqueous solution both in the pyranose and in the furanose forms. None of the other pentoses showed readily detectable amounts of the furanose forms at equilibrium. Although D-allose does not give readily detectable amounts of the furanose forms when at equilibrium in aqueous solutions, D-altrose does. D-Talose showed only two forms, one of which was the β-pyranose structure.

Lenz and Heeschen (1) examined the nuclear magnetic resonance (n.m.r.) spectra of D-glucose, D-mannose, 2-deoxy-D-glucose, and D-xylose with deuterium oxide as solvent. Only the signals of the anomeric protons were readily observed, and the spacings of these signals were used as coupling constants to estimate conformational features for these sugars in aqueous solution. The observed spacings were undoubtedly strongly influenced by virtual long-range coupling (2, 3) and, therefore, were not a direct measure of the coupling constants involved. Also, it is now evident (4, 5) that the relationship between the coupling constant and the dihedral angle defined by neighboring protons (the Karplus relationship) is subject to parameters other than the dihedral angle. Therefore, attempts to relate the spacings of the doublet signals for the anomeric protons of sugars to precise molecular geometry are without either a theoretical or an empirical basis. Although coupling constants between neighboring protons cannot be used to predict precise conformational features, their use in appropriate instances for establishing configurational features (6) remains unchallenged.

Use has also been made of chemical shifts for establishing the configurations of sugars

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and their derivatives. To our knowledge, there is no exception to the rule that an equatorial proton will produce its signal to lower field than a chemically similar but axial proton (7), when the compounds considered are epimeric and both in the same chair conformation, and when the protons considered are not at the α-position to a carbonyl group (8). However, as will be seen later, this rule may not apply, for example, to the anomeric forms of D-altropyranose in the C1-conformation.

It has long been recognized that important shielding effects can arise from positions other than the neighboring positions (3, 9, 10), so that any rationalization of chemical shift must take into consideration the total structure of the molecule. We have demonstrated (3) that the chemical shifts of the ring protons of acetylated sugars are strongly dependent on configurational changes at positions other than the neighboring positions. Also, it is clearly apparent that chemical shifts are subject to specific solvent effects which cannot, as yet, be predicted. Nevertheless, it proved possible to establish simple empirical rules for correlating chemical shift measured under standard conditions with the configurational and conformational properties of acetylated sugars (3), and the purpose of this communication is to report a similar success for free sugars dissolved in deuterium oxide. Furberg (11) has commented on the use of n.m.r. to establish points of configuration and on its application to reducing sugars dissolved in deuterium oxide. Both Lenz and Heeschen (1) and Hall (12) have commented on the diamagnetic anisotropy of the C—O bond and have rationalized the chemical shifts of axial anomeric protons in sugars (glucose and mannose) differing in the configuration of the 2-position. Lenz and Heeschen considered the chemical shifts of protons at positions other than the anomeric center, but had no experimental evidence to support the "intuitive" rationalizations. This effort was premature since, for example, it took no cognizance of the strong deshielding influence of an axial hydroxyl on an opposing axial proton (10). We have now determined the chemical shifts of a number of protons at positions other than the anomeric center for sugars in deuterium oxide by employing both double irradiation and specific deuteration techniques.

The procedure used in these investigations was to dissolve the sugar in deuterium oxide and to determine the n.m.r. spectrum on the Varian A60 spectrometer with tetramethylsilane as the external standard. Normally, the course of the mutarotation was readily followed in the spectrometer. The 100 Mc.p.s. spectra of the solutions at equilibrium were measured. The line positions were established relative to calibration side bands and placed on the scale in parts per million from tetramethylsilane using the positions for the anomeric protons found in the A60 spectra. The spin-decoupling experiments were obtained as described by Johnson (13) with the Varian H.R. 100 spectrometer (10).

A freshly prepared solution of α-D-xylopyranose in deuterium oxide showed a sharp, one-proton doublet at 5.26 p.p.m. The rest of the spectrum was a fairly narrow band in the region 3.5–3.9 p.p.m. When it was allowed to stand the solution mutarotated to equilibrium and at 35° showed signals (Fig. 1A) for anomeric protons at 5.26 and 4.65 p.p.m. with spacings of 3.1 and 7.4 c.p.s.; these signals were assigned to the α- and β-anomers, respectively. Integration of these signals showed that the mixture contained 67% of the β-anomer.

Spin decoupling at 1.63 p.p.m. to higher field from the signal for H_1^α collapsed the doublet; therefore, the chemical shift for H_2^α is at 3.63 p.p.m. The quartet at 4.00 p.p.m. with spacings of 4.3 and 10.4 c.p.s. was assigned to the 5-proton in an equatorial orientation, H_{5e}^β, in view of its intensity and structure. The intensity of the quartet was equal to that of the signal for H_1^β and, as the small coupling constant can only arise from H_4^β to H_{5e}^β coupling, this signal must belong to H_{5e}^β, since the signal for H_4^β should be more complex. The assignment of the H_{5e} signal was confirmed by examination of the spectrum of the

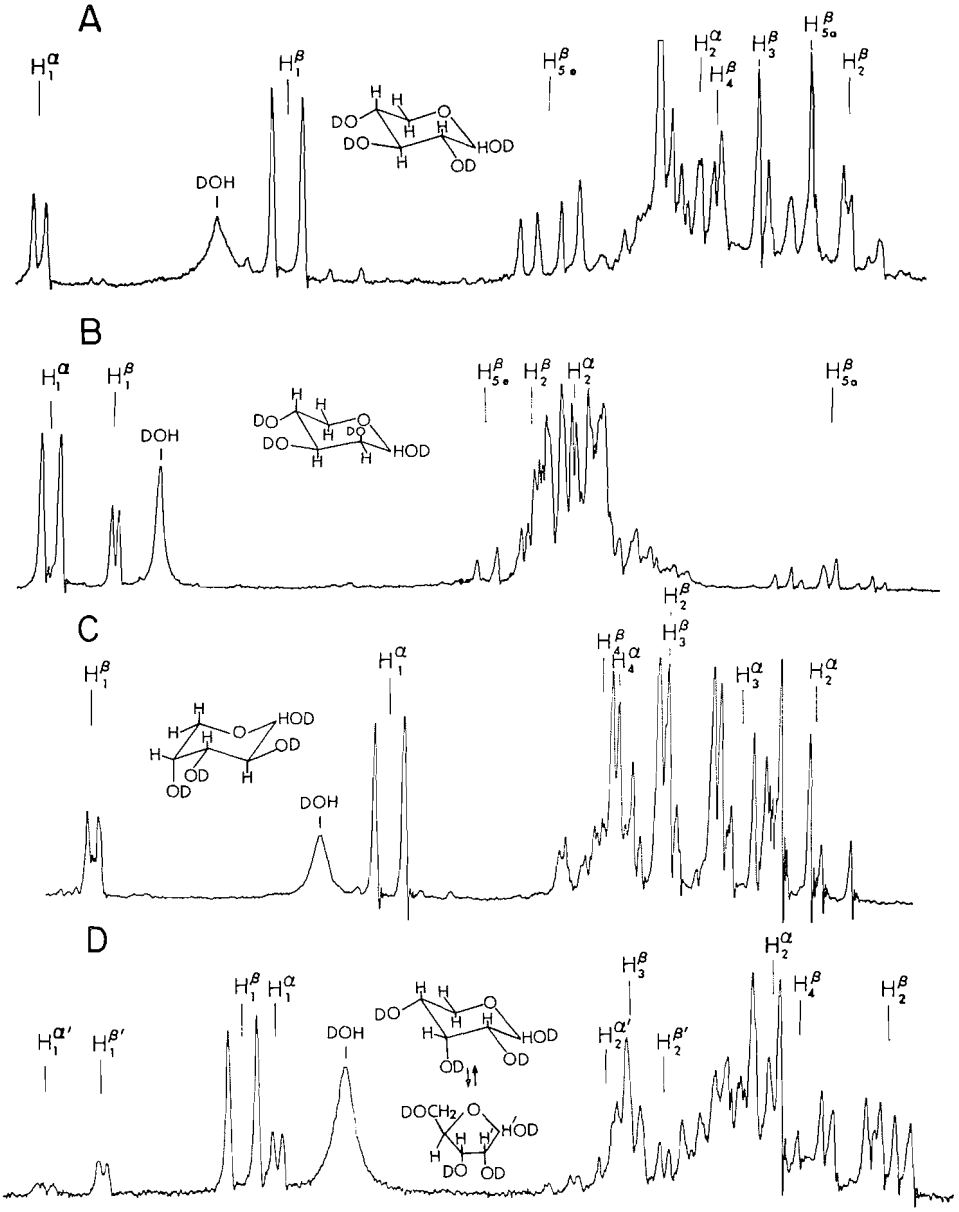

FIG. 1. Nuclear magnetic resonance spectra at 100 Mc.p.s. of aldopentoses at tautomeric equilibrium in deuterium oxide at 35°. LEGEND: A, D-xylose; B, D-lyxose; C, D-arabinose; D, D-ribose.

5-deuterio-D-xylose prepared by Lemieux and Howard (14). The quartet was now a doublet with an intensity corresponding to that expected from the composition of the compound. Irradiation at 42 c.p.s. to higher field than the signal for H^{β}_{5e} caused the quartet to collapse to a doublet with the larger spacing. Therefore, the signal for H^{β}_{4} is centered at about 3.58 p.p.m. The intensitites of the signals to higher field than 3.5 p.p.m. corresponded to three protons, based on the intensity of H^{β}_{1}. It was apparent, therefore, in view of the spectrum of the α-anomer, that H^{β}_{2}, H^{β}_{3}, and H^{β}_{5a} gave their signals in this region. Since irradiation

of H^{β}_{5e} caused extensive collapse of the signal at 3.36 p.p.m., it was most likely that the signal for H^{β}_{5a} was near this position. Irradiation of H^{β}_{1} caused the quartet with spacings of about 7.4 and 8.5 c.p.s. at 3.26 p.p.m. to collapse to a doublet. Therefore, the chemical shift of H^{β}_{2} is 3.26 p.p.m. and $J^{\beta}_{3,2} = 8.5$ c.p.s. The spectrum resulting from this double irradiation experiment showed clearly the triplets for H^{β}_{3} at 3.49 p.p.m. and for H^{β}_{5a} at 3.36 p.p.m. The spacings require $J^{\beta}_{3,4} = 8.5$ and $J^{\beta}_{4,5a} = 10$, and confirmed $J^{\beta}_{5a,5e} \simeq 10$ c.p.s.

The spacings observed in the spectrum for β-D-xylopyranose (see Table I) require that the compound exist in a near C1-chair conformation as anticipated from conformational analysis. All that can be said for the α-form is that the spacing from the H_1 signal agrees with that for a gauche interaction between H_1 and H_2. Also, as will be seen below, the chemical shift for H_2 is in the region expected for the compound in the chair conformation.

A freshly prepared solution of α-D-lyxopyranose showed a one-proton signal at 5.08 p.p.m. with a spacing of 4.2 c.p.s.; this signal was assigned to H^{α}_{1}. The rest of the spectrum was a rather narrow band in the region 3.7–4.1 p.p.m. On equilibration at 35°, a doublet appeared at 4.94 p.p.m. with a spacing of 1.5 c.p.s.; this signal was assigned to H^{β}_{1} (see Fig. 1B). An octet appeared that was centered at about 3.32 p.p.m. and had an intensity corresponding to that of the signal for H^{β}_{1}. This signal is provisionally assigned to H^{β}_{5a}. The signal collapsed to a much narrower complex multiplet on irradiation at 4.07 p.p.m. It seems most probable, in view of the chemical shift for H^{β}_{5e} of D-xylose, that the quartet centered at 4.09 p.p.m. is the signal for H^{β}_{5e}. The spacings suggest $J^{\beta}_{4,5e} \simeq 5$ c.p.s. and $J^{\beta}_{5a,5e} \simeq 10$ c.p.s. Irradiation at 3.90 p.p.m. collapsed the signal for H^{α}_{1} to a singlet, and it is concluded, therefore, that the chemical shift for $H^{\alpha}_{2} = 3.90$ p.p.m. Irradiation of H^{β}_{1} caused the quartet centered at 4.04 p.p.m. to collapse to a doublet. Therefore the chemical shift for $H^{\beta}_{2} = 4.04$ and $J^{\beta}_{2,3} \simeq 3$ c.p.s. Integration of the anomeric signals showed that the mixture contained 71% of the α-anomer at 35°.

A freshly prepared solution of β-D-arabinose in deuterium oxide gave a one-proton doublet signal at 5.34 p.p.m. with a spacing of 2.7 c.p.s.; this signal was assigned to H^{β}_{1}. The crystalline sugar is known by X-ray analysis (15) to have the pyranose ring in the 1C-conformation, wherein H_1 is in the equatorial orientation. On mutarotation, the solution produced a signal for H^{α}_{1} as a doublet with a spacing of 7.2 c.p.s. at 4.60 p.p.m. (see Fig. 1C). Although the DOH signal was reduced to an intensity of about that of H^{α}_{1} at equilibrium, no other signals for anomeric protons were observed. The shape of the signal for H^{β}_{1} showed the presence of second-order effects from virtual long-range coupling and, therefore, a small chemical shift for H^{β}_{2} and H^{β}_{3} (3). Irradiation at 3.93 p.p.m. collapsed the signal for H^{β}_{1}; therefore the signal for H^{β}_{2} is at this position. The signal for H^{α}_{1} is a sharp doublet and, therefore, H^{α}_{2} and H^{α}_{3} must be well shifted chemically. Double irradiation showed that H^{α}_{2} gave its signal at 3.58 p.p.m. The chemical shifts of the 3- and 4-protons in both the β- and α-forms were determined by examining the n.m.r. spectra of the 2,5,5-trideuterio derivatives. These compounds were prepared from D-glucurone as described in the Experimental section. The spectra measured at 60 Mc.p.s. are reproduced in Fig. 2B for both the freshly prepared solution of exchanged crystalline 2,5,5-trideuterio-β-D-arabinopyranose and the equilibrated solution. Figure 2A shows the corresponding spectra for ordinary D-arabinose. The two doublets for H^{β}_{3} and H^{β}_{4} seen on the spectrum of the freshly prepared solution of the deuterated compound must be at 3.93 and 4.07 p.p.m., respectively, in view of the strong second-order effects noted in the signal for H^{β}_{1} and the fact that the chemical shift for $H^{\beta}_{2} = 3.93$ p.p.m. The spacings of the signals require $J^{\beta}_{3,4} = 3.4$ c.p.s. Mutarotation caused the appearance of two new doublets at 3.75 and

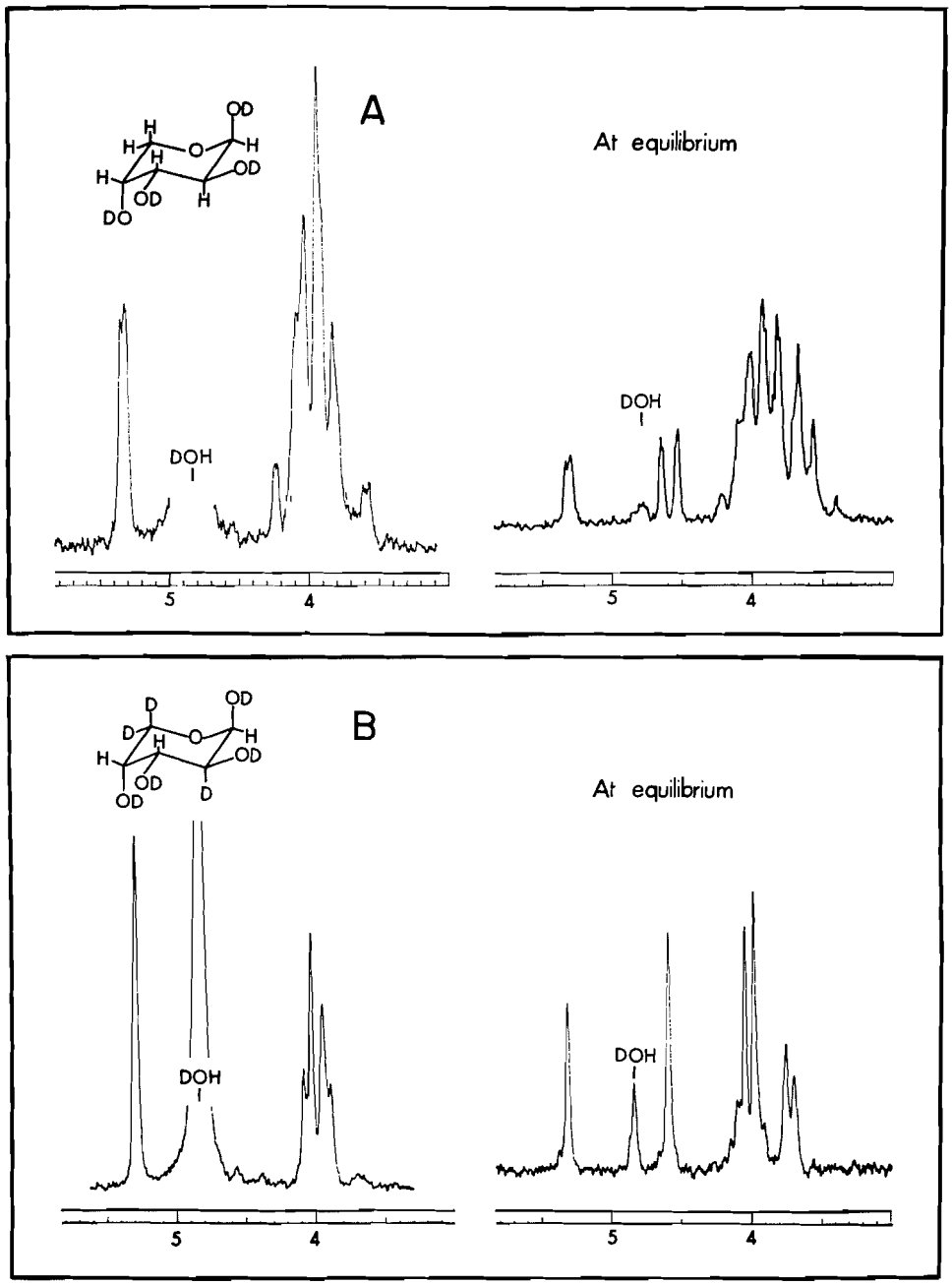

FIG. 2. Nuclear magnetic resonance spectra at 60 Mc.p.s. in deuterium oxide at 35°. LEGEND: A, β-D-arabinopyranose within a few minutes of dissolution and at tautomeric equilibrium; B, 2,5,5′-trideuterio-β-D-arabinopyranose within a few minutes of dissolution and at tautomeric equilibrium.

4.04 p.p.m. which are assigned to H_3^α and H_4^α, respectively. This assignment is based mainly on the rule that the anomerization from the β- to the α-form will lead to a greater shielding of the 3-proton. This effect is discussed in detail below. The spacings of the signals require $J_{3,4}^\alpha = 3.6$ c.p.s. The spectrum of this equilibrated solution substantiates the evidence discussed above that only the α- and β-forms of arabinopyranose are in appreciable concentration at 35°. Integration of the signals for the anomeric protons requires 63% of the α-anomer. The signal for H_2^α appears as a well-defined quartet at 3.58 p.p.m., from which it can be concluded that $J_{2,3}^\alpha = 9.9$ c.p.s. The coupling constants observed for $J_{1,2}$, $J_{2,3}$, and $J_{3,4}$ for the α-form are clearly in accord with the 1C-chair conformation for this compound in aqueous solution. It will be seen below that the observed chemical shifts also agree with this conclusion.

A freshly prepared solution of β-D-ribose in deuterium oxide showed rapid mutarotation, and it was not possible to obtain a spectrum of the crystalline material in a virtually unchanged condition. The mutarotation at 35° gave rise to a solution the n.m.r. spectrum (Fig. 1D) of which had four readily discernible signals of total intensity one at 5.42, 5.30, 4.99, and 4.91 p.p.m. The relative intensities were 6:18:56:20. When the sample was heated to 80°, the signal for DOH moved upfield from 4.75 p.p.m. to 4.3 p.p.m. No further signals for anomeric protons were observed and the integration showed that the total of the intensities for anomeric protons was one-fifth of those of the other protons in the molecule. It was therefore clearly apparent that only four of the tautomeric forms for D-ribose are at appreciable concentrations in water in the temperature range 35–80°. Only the position of the signal for the high-field doublet changed appreciably from 4.91 to 4.83 p.p.m. when the solution was heated to 80°. The relative intensities of the signals changed to 11:19:47:23.

The doublet at 4.99 p.p.m. with a spacing of 6.4 c.p.s. was evidently from H_1^β of the pyranose form. Irradiation at 3.60 p.p.m. caused collapse of the H_1^β signal and therefore corresponds to the chemical shift for H_2^β. Irradiation of H_2^β collapsed the signal for H_3^β at 4.13 p.p.m. This signal was also collapsed by irradiation at 3.79 p.p.m., the signal for H_4^β. It was apparent, therefore, that $J_{2,3}^\beta = 3.1$ c.p.s. and $J_{3,4}^\beta = 3$ c.p.s. The quartet signal for H_4^β suggests that $J_{4,5}^\beta \simeq 10$ c.p.s. The coupling interactions for the vicinal protons of β-D-ribopyranose leave no doubt that the compound has a C1-chair conformation. The axial 3-hydroxyl group probably has caused some distortion, which has decreased the dihedral angle defined by H_1 and H_2.

The n.m.r. spectrum of 5-*O*-methyl-D-ribose was determined to provide a basis for the assignment of the signals for the anomeric proton. Unfortunately, at the time the compound was available, it was not possible to apply heat to move the DOH sample upfield. Nevertheless, the DOH signal was reduced to a very low level, and only two signals for anomeric protons were observed at 5.40 and 5.51 p.p.m. with spacings of 1.5 and 3 c.p.s., respectively. The signals for the two protons at the 5-position in this compound occurred as a band centered at about 3.75 p.p.m. with an intensity double that of the total intensity of the two signals for anomeric protons. Also, a band centered at about 4.2 p.p.m. had an intensity of three, as expected for the total signals for H_2, H_3, and H_4 protons. Only two methoxy-group signals were observed at 60 Mc.p.s. It seems probable, therefore, that 5-*O*-methyl-D-ribose does not exist to any appreciable extent in more than two tautomeric forms (either the two furanose forms or a furanose form and the open-chain form). In view of the equilibrium between the methyl D-ribosides in 1% methanolic hydrogen chloride at 35° and the apparent absence of dimethyl acetal (16), it is most probable that the signals at 5.40 and 5.51 p.p.m. correspond to those of the anomeric protons for the anomeric D-ribofuranoses. The ratio of the intensities of these signals was 2.6:1,

respectively. The signals are assigned to the β- and α-furanose forms, respectively, since, in general, derivatives of β-ribofuranose have $J_{1,2}$ of about 1 c.p.s. as compared to 4–5 c.p.s. for the α-anomers. Also, it can be expected that the 1,2-*trans*-β configuration will provide the more stable anomer in aqueous solution. For these reasons, the signals at 5.30 and 5.42 p.p.m. in the spectrum of D-ribose are assigned to the anomeric protons of the β- and α-furanose forms, respectively. Bishop and Cooper (16) found that the methyl ribosides at equilibrium contained 5.2% α-furanoside, 17.4% β-furanoside, 11.6% α-pyranose, and 65.8% β-pyranosides. The close correspondence between these values and those now estimated for the mutarotation of ribose at 35°, namely, 6% α-furanose, 18% β-furanose, and 56% β-pyranose, supports the above assignments and suggests that the signal with a spacing of 2.1 c.p.s. at 4.91 p.p.m. arose from the α-D-pyranose form which was present to an extent of 20%. The observed coupling of H_1 with H_2 for the open-chain form would be expected to be the weighted average of the coupling constants for the staggered conformations and, on this basis, to have a value substantially greater than 3 c.p.s. On this basis, the signal at 4.91 p.p.m. cannot have arisen from the open-chain form. This argument can also be applied to the signals at 5.30 and 5.42 p.p.m. There seems to be no alternative, therefore, to the conclusion that the signal at 4.91 p.p.m. arose from the α-D-ribopyranose form. The rather well defined doublet for this signal would be in keeping with the expectation of a substantial chemical shift between H_2 and H_3. It is noteworthy that the signal assigned to the anomeric proton of the α-furanose (H_1^{α}, Fig. 1D) seems to be too complex and, therefore, part of this signal may have arisen from the 1-proton of the hydrated open-chain form. On the other hand, the complexity of the signal may be caused by either long-range coupling or virtual long-range coupling, or both. Certainly, the indications are that the open-chain form is present at much lower concentrations than the 8.5% suggested by the results of a polarographic investigation (17).

Inspection of the chemical shifts for the ring protons of the pentopyranoses shows that there exists a good correlation of the shifts with the configuration of the sugar. This correlation is most readily appreciated by using the chemical shifts for β-D-xylopyranose as reference point and adjusting these shifts according to the following empirical rules. (*i*) If the proton under consideration is in an equatorial orientation, add 0.60 p.p.m. (3, 10). (*ii*) If the proton under consideration is in an axial orientation, (*a*) add 0.30 p.p.m. for each neighboring axial hydroxyl group and (*b*) add 0.35 p.p.m. for each axial hydroxyl group which is in opposition to the axial proton.

The results of these calculations are presented in Table I for the protons of known chemical shift. It is seen that the agreement is reasonably good, if one considers the inaccuracies in determining the chemical shifts by spin decoupling (at least $\pm$0.05 p.p.m.) and the fact that at least some of the rings must be distorted appreciably from the perfect chair form. Certainly, the agreement is sufficiently good to support the conformations assigned above and to indicate that the empirical rules deal with the main configurational effects on chemical shift in the n.m.r. spectra of the sugars. The main discrepancies are in the values for the chemical shifts of the anomeric protons for α-D-lyxopyranose, β-D-arabinopyranose, and α-D-ribopyranose, compounds which, in chair conformations, have two of the four hydroxyl groups in an axial orientation.

As was seen above, H_1 for α-D-ribopyranose gives its signal at 4.91 p.p.m., which is much to higher field than that, 5.25 p.p.m., expected for the compound in the C1-conformation. On the other hand, this chemical shift is in good agreement with that, 4.95 p.p.m., expected for the compound in the 1C-conformation. The chemical shift for H_2 agrees with that expected for the compound in either of the chair conformations. Thus, as

is also concluded by Rudrum and Shaw (18), the indications are that α-D-ribopyranose strongly prefers the 1C-conformation.

α-D-Lyxopyranose has an anomalously high value for $J_{1,2} = 4.2$ c.p.s. when compared to that, 1.7 c.p.s., for the configurationally related α-D-mannopyranose. Also, the chemical shifts for H_1 calculated for α-D-lyxopyranose in either the 1C- or C1-conformations are in poor agreement (see Table I) with the experimental value. However, the n.m.r. parameters are well rationalized by assuming that the compound exists in about equal amounts in the two chair conformations. Thus, by using the spacings for the signals of H_1 in β-D-ribopyranose and α-D-mannopyranose as those to be expected for α-D-lyxopyranose in the 1C- and C1-conformations, respectively, a value for $J_{1,2} = 0.5(6.4 + 1.7) = 4.1$ c.p.s. is obtained. The chemical shift for H_1 would be expected, on this basis, to be $0.5(5.25 + 4.99) = 5.12$ p.p.m., which agrees well with the experimental value of 5.08 p.p.m. Also, on this basis, the chemical shift for H_2 is expected to be 3.89 p.p.m., in good agreement with the observed value of 3.90 p.p.m. Thus, it can be concluded that crystalline α-lyxose is in the pyranose ring form and exists in both chair forms in about equal amounts when dissolved in water. This conclusion was also reached by Rudrum and Shaw (18).

The values for $J_{1,2}$ and $J_{3,4}$ determined for β-D-arabinopyranose do not distinguish between the 1C- and C1-conformations for this compound. It is seen in Table I that the observed chemical shifts for protons 1 to 4 are in as good agreement with those calculated on the basis of the 1C-conformation as with those calculated on the basis of the C1-conformation. In fact, the agreement, especially for H_3 and H_4, is not at all good. Furthermore, the signal for H_1 is at a rather anomalously low field and, therefore, the n.m.r. data would not have sufficed to establish the pyranose ring form. Since it is known (15) that the compound is in the pyranose form, perhaps the conformations are rather seriously distorted from the chair forms. A knowledge of $J_{2,3}$ would shed light on the problem of the chair–chair equilibrium for this compound, but this was not available since the 2- and 3-protons are not appreciably shifted chemically. This lack of chemical shift is to be expected for the compound in chair forms, since these protons are in very similar environments in both chair conformations. Rudrum and Shaw (18) assigned the 1C-conformation to the compound in aqueous solution, but this conclusion must obviously be held in reservation.

It is of interest to note that, as was to be expected, the configurational effects on chemical shift are much simpler for the free sugars than for their acetylated derivatives (3). A consideration of a molecular model of an acetoxy group in axial orientation on a six-membered ring and the anisotropy of the carbonyl group of the acetoxy group (9) makes evident the fact that the carbonyl group should strongly deshield the geminal proton but tend to shield equatorial protons at the neighboring and next to neighboring positions. Thus, whereas an axial hydroxyl group has no appreciable effect on the chemical shifts of these protons, an axial acetoxy group leads, in both cases (3), to a shielding of about 0.2 p.p.m. relative to that when the acetoxy group is in an equatorial orientation.

As seen in Table II, the chemical shifts for the 1- and 2-protons of β-D-glucopyranose are in close agreement with those found for β-D-xylopyranose. Therefore, the introduction of the hydroxymethyl group has little effect on these chemical shifts. By using the observed values for β-glucose as reference point and the foregoing empirical rules, the chemical shifts of protons in the other hexoses were calculated; the results are given in Table II. It is seen that the agreement is quite good and the trends excellent. That is, for example, the same chemical shift for the 2-hydrogens of the anomeric mannopyranoses was predicted. Also, although the absolute chemical shifts for the 2-hydrogens of the α- and

TABLE I

Nuclear magnetic resonance parameters for pentopyranoses in deuterium oxide

	Xylose		Lyxose		Arabinose		Ribose	
	α	β	α	β	β	α	α	β
Chemical shifts in p.p.m. from tetramethylsilane (external)*								
H_1	5.26	4.65	5.08	4.94	5.34	4.60	4.91	4.99
	[5.25]		[5.00],† [5.25]‡	[4.95]	[5.25],† [5.30]‡	[4.65]	[4.95],† [5.25]‡,	[5.00]
H_2	3.63	3.26	3.90	4.04	3.93	3.58	3.85	3.60
	[3.56]		[3.91],† [3.86]‡	[3.86]	[3.91],† [3.86]‡	[3.61]	[3.86]†, [3.86]‡	[3.56]
H_3	—	3.49	—	—	3.93	3.75	—	4.13
					[4.14],† [4.09]‡	[3.79]		[4.09]
H_4	—	3.58	—	—	4.07	4.04	—	3.79
					[4.18],† [4.23]‡	[4.18]		[3.88]
H_{5a}	—	3.36	—	3.32	—	—	—	—
				[3.36]				
H_{5e}	—	4.00	—	4.09	—	—	—	—
				[4.00]				
Observed spacings (approximate coupling constants) in c.p.s.								
H_1–H_2	3.1	7.4	4.2	1.5	2.7	7.2	2.1	6.4
H_2–H_3	—	~8.5	—	3	—	9.9	—	3.1
H_3–H_4	—	~8.5	—	—	3.4	3.6	—	~3
H_4–H_{5a}	—	~10	—	—	—	—	—	~10
H_3–H_{5e}	—	4.3	—	~5	—	—	—	—
H_{5a}–H_{5e}	—	10.4	—	~10	—	—	—	—

*The results in brackets refer to calculated chemical shifts obtained by using the empirical rules presented in the text.
†1C-conformation.
‡C1-conformation.

TABLE II

Nuclear magnetic resonance parameters for hexoses in deuterium oxide

	Glucose		Mannose		Allose*		Galactose†		Talose	
	α	β	α	β	α	β	α	β	α	β
Chemical shifts in p.p.m. from tetramethylsilane (external)										
H_1	5.32	4.74	5.25	4.97	—	5.00	5.34	4.68	5.37	4.88
	[5.34]	—	[5.34]	[5.04]		[5.09]	[5.34]	[4.74]	[5.34]	[5.04]
H_2	3.60	3.32	4.01	4.03	—	—	3.87	3.55	—	—
	[3.62]	—	[3.92]	[3.92]			[3.97]	[3.67]		
Observed spacings (approximate coupling constants) in c.p.s.										
$J_{1,2}$	3.5	7.5	1.7	1.0	—	8.2	2.8	7.1	<1	<1

*The chemical shift for H_3^β was 4.28 p.p.m. with $J^\beta_{2,3} \simeq J^\beta_{3,4} \simeq 2.5$ c.p.s.

†Spin-decoupling experiments showed H_3^β and H_5^β to differ little in chemical shift at about 24 c.p.s. to higher field (3.73 p.p.m.) than H_4^β which gave a rough doublet at 3.97 p.p.m. with $J_{3,4}$ about 3 c.p.s.

β-forms of galactopyranose were not estimated with precision, the difference in the chemical shifts was the same as that found by experiment. It is quite evident, therefore, that these simple rules should find widespread application for determination of structure. For example, if the chemical shifts of the 2-hydrogens of a pyranose structure, as determined by spin decoupling of the signals for the anomeric hydrogens, are nearly the same, then it can be concluded that the 2-hydroxyl group is in an axial orientation. It should prove possible to extend this method to rules for differentiating between pyranose and furanose structures, and this matter will be examined primarily in an attempt to establish the ring forms of the rare hexoses in aqueous solution. In the case of D-ribose, the chemical shifts of the 2-protons for the α- and β-furanose forms were 4.19 and 4.07 p.p.m., respectively. These chemical shifts are to substantially lower field than those for the α- and β-pyranose forms, 3.85 and 3.60 p.p.m., respectively. It is to be expected that the ring protons of furanose structures will, in general, have chemical shifts intermediate to chemically similar protons in an axial and equatorial orientation. Also, the long-range configurational effects on chemical shift should be diminished in furanose structures.

The spectra of allose, talose, and altrose were determined but not subjected, as yet, to extensive investigation. The spectrum for allose at equilibrium clearly required the compound to exist virtually entirely in the β-D-allopyranose form. It was apparent that some mutarotation occurred, since the signal for the anomeric proton diminished in intensity as compared with the signal for the 3-proton at 4.28 p.p.m. when the solution was allowed to come to equilibrium. However, the signal of no other anomeric proton was readily observable. It is therefore apparent that the anchoring effect of the hydroxymethyl group for the chair conformation destabilizes the α-pyranose form by forcing a strong interaction between the axial 1- and 3-hydroxyl groups. Also, it is apparent that the introduction of a hydroxymethyl group on C-5 of the ribose structure leads to substantial destabilization of the furanose rings relative to that of the β-pyranose ring. The intensities of the signals attributable to anomeric protons found in the spectrum of D-talose require the compound to exist almost entirely in two tautomeric forms. If talose assumed either one of the furanose forms, then the 1-hydrogen would be in very much the same environment as in the corresponding ribofuranose. For this reason, the signal at 4.88 p.p.m. appears to be at too high a field to be assigned to H_1 of a furanose form. Instead, this signal likely arises from the β-pyranose structure in a distorted-chair or twist-boat conformation. The form of talose which gives a signal for H_1 at 5.37 p.p.m. could be either α-pyranose or β-furanose

(19). The spectrum for D-altrose in deuterium oxide at 35° showed four signals attributable to anomeric protons with a total intensity of one proton. The chemical shifts were 5.00, 5.13, 5.26, and 5.32 p.p.m. with spacings of 4.0, 1.5, 2.0, and 4.5 c.p.s., respectively. The relative intensities were about 29:37:11:23. Obviously, therefore, altrose exists in aqueous solution to a large extent in the furanose forms. This is not surprising in view of the axial hydroxyl groups at the 2- and 3-positions of the pyranose forms (especially the axial group at the 3-position) and the favorable all-*trans* configuration of the furanose form (17). Although it is possible to speculate on the origin of the signals, this matter seems best to be reserved for a future publication when more detailed information will be available. It may be noted, however, that, according to the above rules for the correlation of chemical shift with the configuration of sugars, the chemical shift of H_1 (equatorial) for α-D-altropyranose in the C1-conformation is expected to be at 5.34 p.p.m. and that for the β-anomer (H_1 axial) in the same conformation to be at 5.39 p.p.m.

Experiments with 2-deoxy-D-ribose conducted with L. Anderson confirmed the X-ray evidence (20) for the β-pyranose form in the crystals, m.p. 77–84°, $[\alpha]_D^{22}$ −97° → −48° after 20 min (*c*, 2 in water). The mutarotation was readily followed in the n.m.r. spectrometer at 0° and found to lead to a mixture of four tautomers. The α- and β-pyranose forms were present in about equal amounts at equilibrium and comprised about 70% of the mixture at 60°. The quartet signal for the anomeric proton of the α-anomer was at 4.90 p.p.m. with spacings of 2.5 and 8.2 c.p.s., and requires the 1C-conformation for this compound. The anomeric proton of the β-form gave a triplet signal at 5.36 p.p.m. with a spacing of 3.5 c.p.s. Certainly, therefore, the compound must exist, at least to a substantial extent, in the C1-conformation which is present in the crystalline solid (20). The furanose forms gave signals for the anomeric protons of about equal intensity at 5.69 p.p.m. (triplet with spacing of 4.5 c.p.s.) and 5.63 p.p.m. (quartet with spacings of 2.1 and 5.5 c.p.s.), which for obvious reasons likely arose from the α- and β-forms, respectively.

Since this manuscript was first submitted for publication, a paper appeared on the same subject by Rudrum and Shaw (18). In general, the conclusions are the same as those we communicated in 1962 from spectra measured at 60 Mc.p.s. The generally larger "coupling constants" reported herein are no doubt caused by a greater absence of second-order effects resulting from reduced virtual long-range coupling. A comparison of the spacings in these two publications further emphasizes (3) the hazard of using spacings as coupling constants. In the absence of knowledge related to this effect, Rudrum and Shaw (18) assigned the 1C-conformation to β-D-arabinopyranose. This conclusion was based on the belief that an equatorial orientation was required to account for the chemical shift of H_1. The present data indicate that the agreement between the observed and expected values would be even better with the compound in the C1-conformation. Thus, as was found for the acetylated sugars (3), the simple rule that equatorial protons give their signals to lower field than chemically similar but axial protons can have exceptions depending on the chemical nature and orientation of nearby substituent atoms or groups.

Finally, it is of interest to compare the amounts of the anomeric forms found by integration of the signals for the anomeric protons with the values obtained by other methods. The results given in Table III show excellent agreement for xylose, glucose, mannose, and galactose. The values for lyxose and arabinose agree well with Angyal's calculated values (21) but definitely differ from those determined by rotation and bromine oxidation (22) to an extent greater than the experimental error (about ±2%). Perhaps these pentoses exist to a small extent in the furanose form. The signal for the anomeric proton of β-talose was directly under the DOH signal; therefore, the n.m.r. value is only a rough estimate.

TABLE III

Percentage proportions of the α-aldopyranose form at equilibrium

	Nuclear magnetic resonance at 35°	Rotation*	Bromine oxidation*	Calculated†
Xylose	33	34.8	32.1	36
Glucose	36	36.2	37.4	36
Lyxose	71	76	79.7	73
Mannose	67	68.8	68.9	68
Ribose	38‡	—	—	11
Allose	<10?	—	—	—
Arabinose	63	73.5	67.6	61
Galactose	27	29.6	31.4	36
Talose	66?	—	65.9	77.5

*Reference 22.
†Reference 21.
‡Of the pyranose forms.

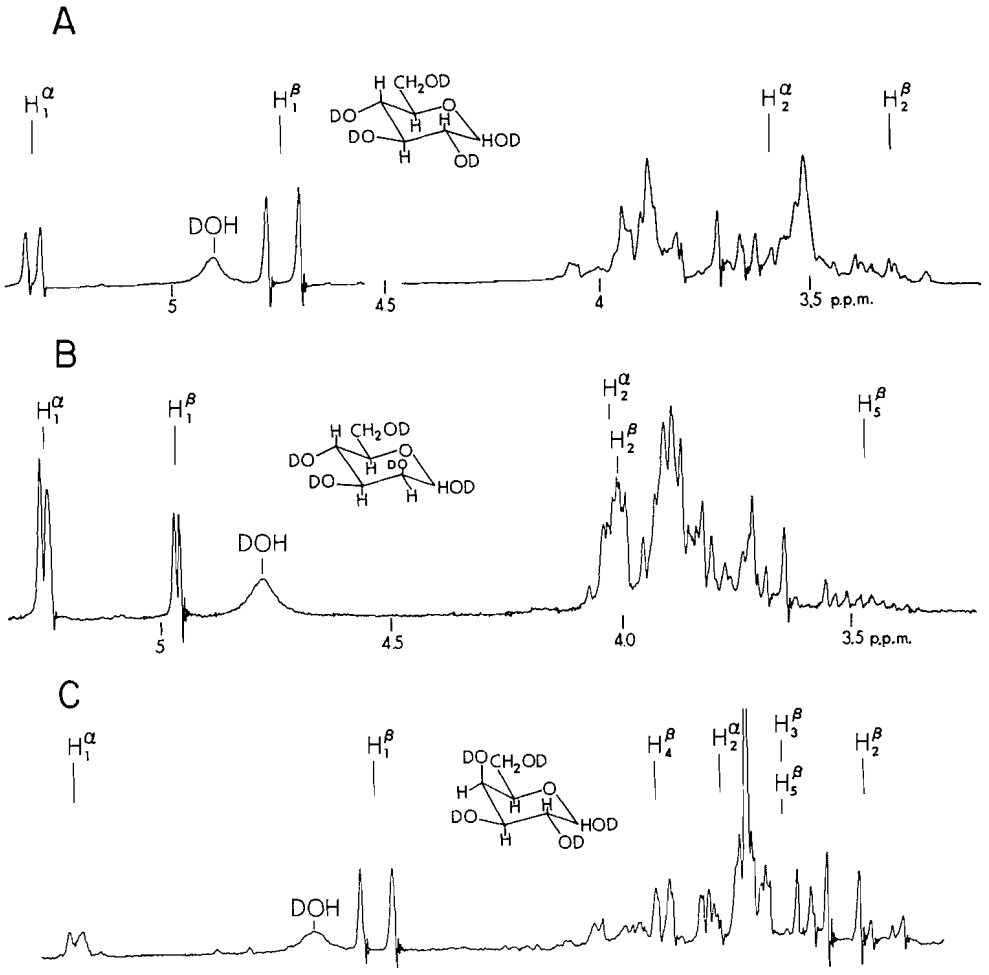

FIG. 3. Nuclear magnetic resonance spectra at 100 Mc.p.s. of aldohexoses at tautomeric equilibrium in deuterium oxide at 35°. LEGEND: A, D-glucose; B, D-mannose; C, D-galactose.

EXPERIMENTAL

The physical measurements were made as described previously (10).

6,6′-Dideuterio-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose

1,2-*O*-Isopropylidene-α-D-glucofuranurono-6,3-lactone, m.p. 120–122°, was prepared from D-glucurone according to the directions described by Owens *et al.* (23). The compound, 32.5 g, was dissolved in 200 ml of tetrahydrofuran freshly distilled from lithium aluminium hydride, and the solution was added dropwise to a stirred solution of 5.06 g of lithium aluminium deuteride (Metal Hydrides Inc., 96.3%) in 600 ml of the tetrahydrofuran. The addition took 3.5 h and the reaction mixture was refluxed for a further 1 h. Water, 15 ml, was then carefully added and this was followed by the addition of 10 ml of 15% aqueous sodium hydroxide and 55 ml of water (24). The precipitated aluminium hydroxide was removed by filtration with Celite as a filter aid. The filtrate was deionized with Amberlite IR 120, H^+, and evaporated to dryness *in vacuo*. The residue was triturated with acetone and ethanol to provide 30.5 g of a product which was dissolved in 900 ml of dry acetone. The solution was cooled to 0° and 36 ml of concentrated sulfuric acid was added dropwise to the stirred solution. After the mixture had been kept at about 15° for 4.5 h, it was cooled to below 10° and neutralized with a solution of 54 g of sodium hydroxide in 60 ml of water. The precipitated salts were removed by filtration and the filtrate was evaporated under reduced pressure. A chloroform solution of the syrupy residue was extracted with water, and the material in the aqueous extract was dried and retreated with acetone and sulfuric acid as described above. The combined chloroform solutions were evaporated and the residue recrystallized from benzene – petroleum ether. The total yield was 16.7 g (41.4% based on the lithium aluminium deuteride) of 6,6′-dideuterio-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, m.p. 108–110°, in agreement with the melting point of the undeuterated compound prepared in a similar manner (25).

The compound, 1.0 g, was dissolved in 50 ml of 50% aqueous methanol, and 0.2 ml of concentrated sulfuric acid was added. The solution was heated at 100° for 3 h and then neutralized with barium hydroxide. Removal of solvent left a syrup which was acetylated with acetic anhydride and sodium acetate in the usual manner to provide 6,6′-dideuterio-β-D-glucopyranose pentaacetate, m.p. 132–133°, in 0.69 g yield. Treatment of a sample of this product with a 1:1 mixture of acetic acid – acetic anhydride containing sulfuric acid provided 6,6′-dideuterio-α-D-glucopyranose pentaacetate, m.p. 111.5–112.5°. The n.m.r. spectra of these two pentaacetates are reported in a separate communication (3).

2-O-Benzyl-D-arabinose

3-*O*-Benzyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (26), 201 g, was dissolved in a mixture of 400 ml of ethanol and 200 ml of *N* sulfuric acid. The solution was heated at 75° for 7.5 h, and then evaporated *in vacuo* to a light syrup which was dissolved in about 500 ml of water. This solution was heated at 90° for 1.5 h. The cooled solution was extracted twice with chloroform and then neutralized with barium hydroxide. Evaporation of the filtered solution and crystallization of the residue from acetone–benzene gave 128 g (95% yield) of 3-*O*-benzyl-D-glucopyranose (27), m.p. 134–136°.

The procedures (28) for the conversion of this compound into 2-*O*-benzyl-D-arabinose were modified (29) as follows. Sodium metaperiodate, 45 g, was dissolved by heating in 150 ml of water, and the rapidly cooled solution was added to 54 g of 3-*O*-benzyl-D-glucose in 150 ml of water. The resulting solution was cooled in ice water and then left at room temperature. The mass of crystals which formed in 15 min was collected and washed with cold water. The yield was 37 g of 2-*O*-benzyl-4-*O*-formyl-D-arabinose, m.p. 124–130°. Methanol, 500 ml, was added to the filtrate and the mixture was refrigerated for 2 h. The precipitated salts were removed by filtration and washed with methanol. The combined filtrates were evaporated to a syrup which was dissolved in 25 ml of water. On cooling, a further crop of the formyl ester, 11.3 g, was deposited. The total yield was 90% of material of sufficient purity for the next stage in the preparation.

The above compound was saponified in aqueous solution with sodium hydroxide. The solution was deionized with Amberlite IR 120, H^+, and then evaporated to a light syrup which deposited crystals after refrigeration for several days. These crystals, plus a second crop obtained from the mother liquors, were recrystallized from ethyl acetate. The yield was 83% of 2-*O*-benzyl-D-arabinose (28), m.p. 111–113°, $[\alpha]_D$ −73° after 0.5 h (*c*, 2.1 in water).

2-Deuterio-β-D-arabinose

2-*O*-Benzyl-D-arabinose, 12.0 g, was dissolved in 8 ml of deuterium oxide and the solvent was removed by freeze-drying. This procedure was repeated and the product was then dissolved in 30 ml of deuterium oxide which had been treated with 550 mg of sodium. After standing at room temperature for 7 days, the solution was neutralized with *O*-deuterated acetic acid and extracted three times with 300 ml volumes of ethyl acetate. The combined extracts were evaporated to dryness *in vacuo* and the residue was crystallized from ethyl acetate to give 3.4 g of 2-deuterio-2-*O*-benzyl-D-arabinose, m.p. 108–109.5°, $[\alpha]_D$ −71.5 (*c*, 1.7 in water).

The compound was dissolved in ethanol for hydrogenolysis at atmospheric pressure with 10% palladium-on-charcoal catalyst. The yield of 2-deuterio-β-D-arabinose isolated in the usual manner and crystallized from 80% aqueous methanol (30) was nearly quantitative.

2,5,5′-Trideuterio-β-D-arabinose

The compound was prepared from the above described 6,6′-dideuterio-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose by the same procedures as described for the preparation of 2-deuterio-β-D-arabinose from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose. The overall yield of material melting at 151–153° was 8%.

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