SPECIFIC ISOTOPIC LABELING OF SUGARS. SPECIFIC *C*-DEUTERATION OF 1,6-ANHYDRO-2,3-*O*-ISOPROPYLIDENE-β-D-TALOPYRANOSE THROUGH ENOLIZATION OF AN ALDOSULOSE DERIVATIVE: N.M.R. SPECTRAL STUDIES*†

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ABSTRACT

Treatment of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-4-ulose (**1**) with sodium deuteroxide in deuterium oxide leads to regiospecific and stereospecific incorporation of deuterium at C-3, to give the 3-*C*-deuterated analog (**2**) of the ketone **1**. Reduction of **1** with sodium borohydride gives 1,6-anhydro-2,3-*O*-isopropylidene-β-D-talopyranose (**3**) stereospecifically, and the 4-*C*-deuterated analog (**4**) was obtained when the reduction was effected with sodium borodeuteride. Similar reductions of the deuterated ketone **2** gave the 3-*C*-deuterated analog (**5**) of **3** and the 3,4-di-*C*-deuterated analog (**6**) of **3**, respectively. The 100-MHz n.m.r. spectrum of **3** was interpreted completely by comparison of its spectrum with those of the deuterated analogs **4**–**6**. Alterations in the appearance (but not the field position) of various signals in the n.m.r. spectrum of **3** as a function of isotopic substitution permitted complete, reliable determination of chemical shifts and coupling constants for all protons in the molecule.

INTRODUCTION

Various studies in this laboratory[1–8] have been concerned with the chemical and physical properties of 1,6-anhydro-β-D-hexopyranoses and their derivatives. In a preliminary report[1] of the present work, it was shown that 1,6-anhydro-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-4-ulose (**1**) undergoes alkali-catalyzed, enolic exchange by deuterium at C-3 exclusively. The proton at C-5 is prevented from undergoing exchange by the inability of the bicyclic molecule to support a trigonally hybridized atom at a bridgehead position (Bredt's rule). The orginal stereochemistry about C-3 is regenerated on reprotonation, because the alternative direction of attack by the incoming proton would generate *trans*-fusion of the 1,3-dioxolane system to the pyranoid ring, that thermodynamically is less favorable. It was suggested[1] that

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this exchange reaction, used in conjunction with isotopically and stereochemically specific reduction of the ketonic function, would provide a practical route, of potentially general applicability, for preparing specifically labeled sugar derivatives. In addition to the use of compounds labeled by this method in studies on enzyme-catalyzed reactions of sugars, such labeled molecules possess potential value for detailed n.m.r. spectral correlations and for evaluation of mass-spectral fragmentation pathways, because both of these analytical techniques exhibit critical sensitivity to the effects of replacement of one or more of the hydrogen atoms of a molecule by deuterium. Additional possibilities for use in the biochemical area include the obvious extension to the corresponding, specifically tritiated derivatives.

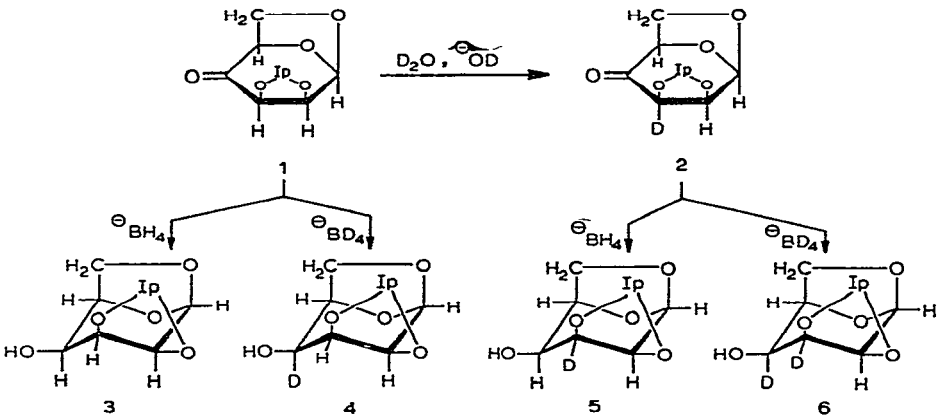
Several studies of 1,6-anhydro-β-D-hexopyranose systems by n.m.r. spectroscopy[1,2,5,9–12] and by mass spectrometry[13,14] have been reported, but, except for the present work[1], none have employed molecular systems in which deuterium atoms were attached directly to the carbon skeleton.

DISCUSSION

A solution of 1,6-anhydro-2,3-*O*-isopropylidene-α-D-*lyxo*-hexopyranos-4-ulose (**1**) in deuterium oxide containing a catalytic amount of sodium deuteroxide underwent, during 8 h at room temperature, a regiospecific and sterospecific conversion into the 3-*C*-deuterated analog (**2**), and no further incorporation of deuterium into the molecule took place at longer times of reaction. The specificity of this reaction is clearly established by n.m.r. spectral measurements, and detailed 100-MHz spectral data are recorded in the Experimental section for the parent ketone **1** and its 3-*C*-deuterated analog **2**. In line with the principle of Bredt's rule, ring strain in the [3.2.1] bicyclic system makes it impossible for C-5 to achieve sp^2 hybridization, so that the intermediate 4,5-enol required for exchange of H-5 cannot be formed. Planarity at C-3, a bridgehead having steric requirements that are much less critical, can be achieved readily, so that replacement of H-3 is facile; this rationalization is firmly established by the fact that a stable enediol acetate of the ketone **1** can be prepared[2].

As borohydride reduction of the ketone **1** affords[5] 1,6-anhydro-2,3-*O*-isopropylidene-β-D-talopyranose (**3**) stereospecifically, a route to the 4-deuterated analog (**4**) of **3** is provided by use of sodium borodeuteride. Similar reduction of the 3-deuterated ketone **2** gave the 3-deuterated alcohol (**5**) when borohydride was used, and the 3,4-dideuterated alcohol (**6**) when borodeuteride was used. Detailed analysis of the n.m.r. spectra of the four products **3**–**6** established that each was fully labeled at the positions indicated, and that no migration or reverse exchange of deuterium occurred during the reduction step.

The n.m.r. spectra of **3** and the labeled derivatives **4**–**6**, measured at 100 MHz in chloroform-*d* containing 5% of tetramethylsilane (see Fig. 1 and Table I) illustrate the phenomenon of spin-decoupling by isotopic substitution, and permit definitive assignment of the ring-proton signals of compound **3**; attempts to effect this interpretation by double resonance would have been tenuous at best, owing to the similarity of chemical shift between certain of the ring protons.



In the spectra of **3**–**6**, one-proton doublets are observed at τ 5.81 (splitting of 7.7 Hz) and at τ 4.71 (splitting of 3.1 Hz), with the latter slightly broadened in **3** and **4**. Additionally, a one-proton quartet, slightly broadened in the spectra of **3** and **5**, appears at τ 6.31 (splittings of 7.7 and 5.4 Hz) in all four spectra. The invariance of multiplicity of these three signals requires that they be assigned to H-1 and to the

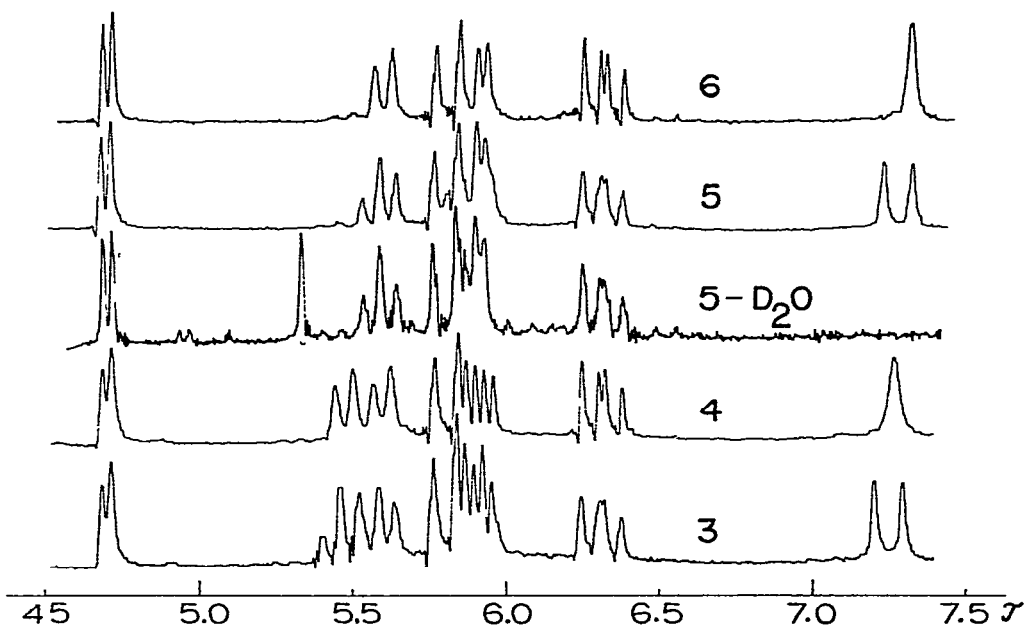


Fig. 1. Partial n.m.r. spectra of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-talopyranose (**3**) and its 4-deuterio (**4**), 3-deuterio (**5**), and 3,4-dideuterio (**6**) derivatives in chloroform-*d*; the spectrum of **5** after addition of deuterium oxide is also shown.

two C-6 protons, as all of the other signals should be significantly coupled to either H-3 or H-4; the 7.7-Hz coupling common to the two high-field signals must, therefore,

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TABLE I

N.M.R. SPECTRAL PARAMETERS OF 1,6-ANHYDRO-2,3-*O*-ISOPROPYLIDENE-β-D-TALOPYRANOSE (3) AND SOME DEUTERATED ANALOGS, MEASURED IN CHLOROFORM-*d*

Compound	*Position(s) deuterated*	*Chemical shifts (τ) and multiplicities[a] of signals*									
		H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*endo	*H-6*exo	*OH*	$C(Me)_2$	
3	—	4.71 bd	5.92 q	5.47 bt	5.88 bo	5.60 bt	5.81 d	6.31 bq	7.12 d	8.42, 8.65	
5	3	4.71 d	5.92 d	—	5.88 bq	5.60 t	5.81 d	6.31 bq	7.12 d	8.42, 8.65	
4	4	4.71 bd	5.92 q	5.47 bd	—	5.60 bd	5.81 d	6.31 q	7.12 s	8.42, 8.65	
6	3,4	4.71 d	5.92 d	—	—	5.60 d	5.81 d	6.31 q	7.12 s	8.42, 8.65	
		Spin–spin coupling constants (Hz)									
		$J_{1,2}$	$J_{1,3}$	$J_{2,3}$	$J_{3,4}$	$J_{3,5}$	$J_{4,OH}$	$J_{4,5}$	$J_{4,6exo}$	$J_{5,6exo}$	$J_{5,6endo}$	$J_{6exo,\ 6endo}$
3	—	3.1	< 1	6.0	6.0	< 1	9.4	5.4	< 1	5.4	0	7.7
5	3	3.1	—	—	—	—	9.4	5.4	< 1	5.4	0	7.7
4	4	3.1	< 1	6.0	—	< 1	—	—	—	5.4	0	7.7
6	3,4	3.1	—	—	—	—	—	—	—	5.4	0	7.7

[a]Multiplicities are designated b, broadened; d, doublet; o, octet, q, quartet; s, singlet; and t, triplet.

be $J_{6exo,6endo}$, thus confirming that these two signals are caused by the C-6 protons. Examination of a model of **3** discloses three salient features involving the ring protons. Firstly, the dihedral angle between H-5 and H-6*endo* is very nearly 90°, so that the coupling between these two protons would be expected[15,16] to be approximately zero. Secondly, H-4 and H-6*exo*, and H-1 and H-3, are held rigidly in a "1,3-diequatorial" ("W") disposition, an arrangement that has been shown[5,9] to produce a small, long-range coupling between pairs of protons so related. It is evident, therefore, that the signal of H-6*exo* is the H-6 signal at higher field, that H-6*endo* resonates at τ 5.81, that $J_{1,2}$ and $J_{5,6exo}$ equal 3.1 Hz and 5.4 Hz, respectively, that $J_{5,6endo}$ is approximately zero, and that $J_{4,6exo}$ and $J_{1,3}$ are "W couplings" of magnitude somewhat less than 1 Hz.

Replacement of H-4 by deuterium (compound **4**) resolves a two-proton multiplet (centered at about τ 5.52), in the spectrum of **3**, into two discrete, one-proton doublets at τ 5.47 (spacing of 6.0 Hz) and τ 5.60 (spacing of 5.4 Hz). Further deuteration by replacement of H-3 (compound **6**) leads to loss of the lower-field doublet, which is thus identified as the H-3 signal. Because H–D couplings are only about 15% of the magnitude of the corresponding H–H couplings, the spectrum of **4** can be regarded as that of **3** with H-4 decoupled. The spacing of the doublet at τ 5.47 (H-3) must, therefore, represent $J_{2,3}$ (6.0 Hz). The spacing (5.4 Hz) of the higher-field doublet (τ 5.60) is identical with $J_{5,6exo}$, and this signal is thus identified as the H-5 resonance. When H-3 is deuterated (in compound **5**) the H-5 signal is observed as a triplet, because $J_{4,5}$ is equal to $J_{5,6exo}$ (5.4 Hz). The slight broadening of the H-5 peaks observed with compounds **3** and **4** arises from 1,3-diequatorial ("W") coupling of H-5 with H-3.

Deuteration of H-4 causes, also, a one-proton diminution in the integrated intensity of the spectrum near τ 5.9, as is seen when the spectrum of **4** is compared with that of **3**, and exposes a quartet at τ 5.92 (spacings of 6.0 and 3.1 Hz). This quartet can be assigned to H-2, because of the identity of the large and small spacings with $J_{2,3}$ and $J_{1,2}$, respectively. The assignment is verified by the collapse of this signal to a doublet (spacing 3.1 Hz, $= J_{1,2}$) in the spectrum of the 3,4-dideuterated derivative **6**. Vicinal, H–D coupling can be observed in the H-2 signal in the spectrum of the 3,4-d_2 derivative **6**; this proton is axially disposed, so that "W" coupling is negligible. The signal is seen at τ 5.92 as a broadened doublet ($J_{1,2}$ 3.1 Hz, $J_{2,3d} < 1$ Hz).

A third effect of deuteration at C-4 is the collapse of a doublet at τ 7.12 (spacing of 9.4 Hz) in the spectra of **3** and **5**, to a singlet in the spectra of **4** and **6**. As this signal could also be removed completely by treatment of the sample with deuterium oxide, the resonance at τ 7.12 is attributed to the proton of the 4-hydroxyl group, and the spacing of 9.4 Hz is assigned to $J_{4,OH}$.

Replacement of H-3 by deuterium (compound **5**) exposes a rather indistinct, four-line pattern (partially concealed by the H-2 doublet), centered at τ 5.88 and showing spacings of 9.4 and 5.4 Hz. These spacings correspond to $J_{4,OH}$ and $J_{4,5}$, respectively, and identify the signal as that of H-4. Additionally, removal of the

proton of the 4-hydroxyl group of **5** by treatment with deuterium oxide collapses the four-line pattern of the H-4 signal at τ 5.88 to a doublet of narrowly spaced doublets ($J_{4,5}$ 5.4 Hz, $J_{4,6exo}$ ~1 Hz) partially concealed by the H-2 signal. In the n.m.r. spectrum of **3**, the H-4 signal gives rise to an octet (partially overlapped by other signals) that is broadened by "W" coupling to H-6*exo* (see Fig. 1). Deuteration of H-3 also causes a narrowing of the lines of the H-5 pattern at τ 5.60, indicating the existence of a "W"-type of long-range coupling, also less than 1 Hz in magnitude, between H-3 and H-5 (two equatorially disposed protons).

The remaining signals of the spectrum, namely, three-proton singlets at τ 8.42 and τ 8.65, are assigned to the nonequivalent methyl groups of the isopropylidene acetal group.

The chemical shifts and coupling constants thus deduced (see Table I) from the spectra depicted in Fig. 1 afford a total analysis of the p.m.r. spectrum of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-talopyranose (**3**).

Although the idealized, chairlike, conformational depiction of compound **3** has H-1,2,3,4 and 5 mutually *gauche*-disposed, with dihedral angles of 60° between vicinal protons, the observed coupling data show that the actual structure is substantially distorted from this model; in particular O-5 appears to be displaced farther, and C-3 less, from the approximate plane of C-1, C-2, C-4 and C-5, than would have been the case for a strainless-ring model based on tetrahedral angles. Inspection of Dreiding models indicates the need for additional puckering at C-1–O-5–C-5 to accommodate the 1,6-anhydro bridge, and the dioxolane ring that includes C-2 and C-3 generates torsion along C-2–C-3 that presumably causes partial flattening of the pyranoid ring at C-2–C-3–C-4 and a decrease in the H-2–H-3 and H-3–H-4 dihedral angles to values substantially below 60°. These predictions accord well with evidence from the observed couplings; the values (6.0 Hz) found for $J_{2,3}$ and $J_{3,4}$ are much larger than those anticipated for a dihedral angle of 60°, and approach values expected[16] for eclipsed, vicinal protons.

N.m.r. spectral data at 100 MHz for the ketone **1** and its 3-deuterated derivative **2** are given in the Experimental section; these values quantitatively upgrade the previously reported, 60-MHz data[1,3] and confirm the earlier assignment that the lowest- and highest-field, ring-proton resonances are those of H-1 and H-6*exo*, respectively. Even at 100 MHz, virtual coupling of H-3 with H-1, resulting from the close proximity of the H-2 and H-3 resonances, is observed; deuteration at C-3 (compound **2**) causes the H-1 signal to be resolved into a sharp doublet, and, simultaneously, a sharp doublet is observed for H-2, while the rest of the spectrum is essentially unchanged.

EXPERIMENTAL

General. — N.m.r. spectra of compounds **3–6** were recorded with a Varian HA-100 spectrometer in the frequency-sweep mode; concentrations of samples were ~10% in chloroform-*d* containing 5% (v/v) tetramethylsilane as the internal standard and provider of a lock signal.

*1,6-Anhydro-2,3-O-isopropylidene-β-*D-lyxo-*hexopyranos-4-ulose-3-*d (**2**). — A solution of 1,6-anhydro-2,3-*O*-isopropylidene-*β*-D-*lyxo*-hexopyranos-4-ulose[3] (**1**, 250 mg) in deuterium oxide (1.5 ml) showed the following n.m.r. spectral data (100 MHz, external tetramethylsilane): τ 4.13 (1-proton multiplet, width between outer peaks ~4 Hz, H-1), 5.18 (1-proton doublet of narrow doublets, $J_{5,6exo}$ 6.2 Hz, $J_{5,6endo}$ 1.2 Hz, H-5), 5.30 (2-proton broadened, apparent singlet, H-2,3), 5.31 (1-proton doublet of narrow doublets, $J_{6exo,6endo}$ 8.3 Hz, H-6*endo*), 5.79 (1-proton quartet, H-6*exo*), 8.03, 8.19 (3-proton singlets, CMe_2). A catalytic amount (~0.1 mg) of sodium deuteroxide was added, and the spectrum was recorded at intervals, the sample being kept at ~25°. After the elapse of 8 h, the sample of **1** had been completely converted into **2**, as observed by the collapse of the signal at 4.13 (H 1) into a sharp doublet ($J_{1,2}$ 3.0 Hz), the collapse of the signal at τ 5.30 into a sharp, 1-proton doublet (H-2) of spacing 3.0 Hz, and the appearance of an HOD signal of intensity 1 proton. No further change was observed in the spectrum during 12 h at 25°.

The 3-deuterated ketone **2** was extracted into chloroform, and the dried (sodium sulfate) extract was evaporated to give crystalline, chromatographically homogeneous **2** in almost quantitative yield. The product was indistinguishable from **1** by chromatography, mixed m.p., and X-ray powder diffraction pattern.

*1,6-Anhydro-2,3-O-isopropylidene-β-*D-*talopyranose* (**3**), *and its 4-deuterio* (**4**), *3-deuterio* (**5**), *and 3,4-dideuterio* (**6**) *derivatives.* — Treatment of samples of the ketone **1** (100 mg) in separate ethanolic solutions (5 ml) with (a) excess sodium borohydride or (b) excess sodium borodeuteride, followed by neutralization (aqueous ammonium chloride), extraction into chloroform, and drying (sodium sulfate), with subsequent evaporation of the extracts, gave crystalline **3** (100 mg) and **4** (90 mg), respectively. The products were recrystallized from petroleum ether (b.p. 30–60°). The same procedures were repeated, starting from the 3-deuterated ketone **2** (100 mg), to give **5** (100 mg) and **6** (95 mg), respectively. The products **3**–**6** were indistinguishable from one another by m.p. (104–105°, lit.[5] m.p. 104–105°), mixed m.p., t.l.c. mobility (R_F 0.28, silica gel G, 3:1 chloroform–ether), and X-ray powder diffraction patterns. For n.m.r. spectral data, see Table I and Fig. 1.

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