Isotopic Perturbation of Intramolecular Hydrogen Bonds in Rigid 1,3-Diols: NMR Studies Reveal Unusually Large Equilibrium Isotope Effects

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Introduction

Intramolecular hydrogen bonds can be detected with equilibrium isotope effects manifest in the ^{1}H or ^{13}C NMR spectra of partially deuterated compounds,[1] a method referred to in the literature as SIMPLE (*S*econdary *I*sotope *M*ultiplets of *P*artially *L*abeled *E*ntities) NMR.[1b] The SIMPLE technique is easy to implement, as extra hydroxyl resonances resulting from partial deuteration of nearby hydroxyl groups are taken as evidence of intramolecular hydrogen bonds. Not surprisingly, the technique has found application in studies of carbohydrates and natural products. In general, the solvent of choice for SIMPLE experiments has been DMSO-d_6, a polar aprotic solvent which provides sharp hydroxyl resonances due to intermolecular exchange rate slowing.[2] To date, the largest ^{1}H shift observed by this method has been ca. −13 ppb, or 6.5 Hz on a 500 MHz instrument. The technique has several peculiarities which have yet to be explained. For example, the sign of the isotope effect has been found to vary. In a 1987 study of bafilomycin A_1, Everett observed the sign of a specific isotope shift could change with solvent (DMSO vs $CDCl_3$) and urged caution in using the sign convention for assigning of donor vs acceptor hydroxyls.[1j] In an earlier study of sucrose in DMSO, Christofides and Davies found that both positive (downfield shift) and negative (upfield shift) isotope effects could be observed for a single hydroxyl participating in multiple hydrogen bonds.[1e] The rationale was offered that the sign was indicative of whether a hydroxyl group was acting as a hydrogen bond donor (negative) or as a hydrogen bond acceptor (positive). Additionally, these researchers postulated the magnitude of the isotope shift could be correlated to the strength of the hydrogen bond. Along these lines, Reuben has reported extensive studies of deuterium-perturbed intramolecular hydrogen bonding in acyclic 1,3-diols using ^{13}C detection.[1g]

To investigate the origins of deuterium isotope effects manifest in ^{1}H spectra of hydroxyl-containing compounds, we have undertaken the synthesis and NMR analysis of several *myo*-inositol monoorthoformates (**1**, **4**, and **5**). The 1,3-diaxial hydroxyl groups in these compounds offer a favorable geometry to study intramolecular hydrogen bonding. Equilibrium isotope effects in these systems are much larger than any reported in the literature thus far and are useful for exploring the origin of the isotope effect as well as further defining the scope and limitations of the SIMPLE technique.

Results and Discussion

Compound **1** was prepared from *myo*-inositol monoorthoformate according to a known procedure.[3] The di-*tert*-butyldimethylsilyl ether **2** was prepared[4] and oxidized with DMSO/Ac_2O[5] to provide ketone **3** in excellent yield. Oxidation of this hindered secondary alcohol was also attempted using Cr(VI)/pyridine conditions as well as the standard Swern conditions; however, only 40−50% yields were observed in these cases, even when excess reagents were employed. The reaction of **3** with methylmagnesium chloride in THF was found to proceed with concomitant loss of the axial TBDMS ether to yield the axial alcohol **4** directly. To prepare ester **5**, **3** was added to a solution of lithio ethyl acetate[6] in THF at −78 °C, which provided the bis-TBDMS ethyl acetate derivative in excellent yield. Diol ester **5** was prepared by utilizing an axial-selective TBDMS removal promoted by brief exposure to 1 equiv of tetrabutylammonium fluoride.

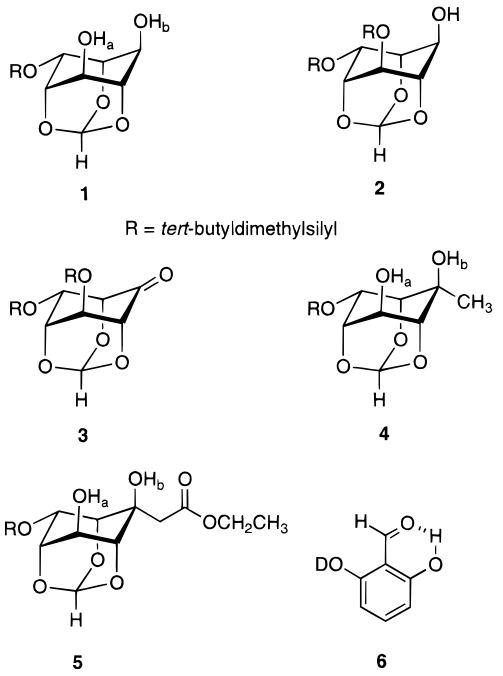

Table 1 summarizes deuterium isotope effects in the ^{1}H NMR spectra of diols **1**, **4**, and **5** in several solvents and temperatures. The results for the symmetric **1** are discussed within the context of a model shown in Figure 1. Under conditions of slow intermolecular exchange, the observed hydroxyl chemical shift in **1** is the average (δ_{av}) of the interior hydrogen bond donor site (δ_{in}) and the exterior hydrogen bond acceptor site (δ_{out}). For the OH−

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Table 1. Six-bond Isotope Effects (ppb) in Diols 1, 4, and 5

compd	solvent	T (°C)	$^6\Delta H_a$	$^6\Delta H_b$
1	DMSO-d_6	22	+29.9	+29.9
1	acetone-d_6	22	+14.0	+14.0
1	acetone-d_6	0	+17.4	+17.4
1	acetone-d_6	−60	+20.9	+20.9
1	$CDCl_3$	22	−12.7	−12.7
4	acetone-d_6	22	+14.7	+3.6
4	DMSO-d_6	22	+46	+20.0
4	C_6D_6[a]	22	−43.8	−59.5
4	C_6D_6[b]	22	−57.0	−70.0
5	DMSO-d_6	22	+43.8	0.0
5	C_6D_6	22	0.0	+9.0

[a] Sample prepared by adding 10−40 μL of CD_3OD to **4** in C_6D_6. [b] Sample prepared by dissolving predeuterated **4** in C_6D_6.

OH isotopomer, δ_{av} is expected to be the true average, as eq 1 (Figure 1) is an isoenergetic process. Addition of deuterium to the sample gives rise to the presence of an OH−OD isotopomer; the presence of any new hydroxyl resonances in the ^{1}H spectrum are due to a non-unitary equilibrium constant caused by the presence of deuterium (eq 2).[7] An upfield isotope shift of −12.7 ppb was observed for **1** in $CDCl_3$. The magnitude of this value, readily observed as a 3.8 Hz shift on a 300 MHz spectrometer, is comparable to previously reported ^{1}H SIMPLE effects.[1] In DMSO-d_6, a substantially larger and positive $^6\Delta$, +29.9 ppb, was recorded. As a point of comparison, this six-bond equilibrium isotope effect approaches the magnitude of the two-bond intrinsic isotope shift for HOD dissolved in DMSO (−23 ppb) or acetone (−30 ppb).[8]

The negative isotope shift observed for **1** in $CDCl_3$ is consistent with deuterium having a preference for the intramolecular hydrogen bond, assuming this site is deshielded relative to the acceptor site ($\delta_{in} > \delta_{out}$, Figure 1A). Thus for **1** in $CDCl_3$, it appears that the O−D···O bond is preferred to an O−H···O bond. By itself, this result is interesting in light of the recent work of Hansen, who found the opposite to be true in a study[9] of 2,6-dihydroxybenzoyl compounds, e.g., **6**.

The NMR evidence for a preferred intramolecular O-D···O bond in **1** is in agreement with results obtained from a number of experimental and theoretical studies of intermolecular hydrogen bonds. In low-temperature matrix isolation IR studies of HOD dimers, the D-bonded form is exclusively observed.[10,11] The preference for intermolecular D-bonding has also been observed experimentally in matrix-isolation studies of HDO complexed with ammonia[12] and formaldehyde[13] as well as in molecular beam studies of HF-DF dimers.[14] Buckingham[15] has investigated several of these systems from a theoretical standpoint and has shown that differences in zero-point energies of stretching and bending vibrations are responsible for the heavy atom preferring one bond over another. Buckingham concluded that predictions of relative stability in hydrogen bonding systems are difficult to make due to uncertainty in the contribution of stretching and bending vibrational terms that can have opposite sign for a given bond. Scheiner and Cuma have reported recent *ab initio* calculations of hydrogen bonded water dimers showing a linear O-D···O bond is preferred to the O−H···O bond.[16] In their study, the zero-point vibrational energy difference was traced to two intermolecular bending modes that represent the wagging motion of the bridging hydrogen.

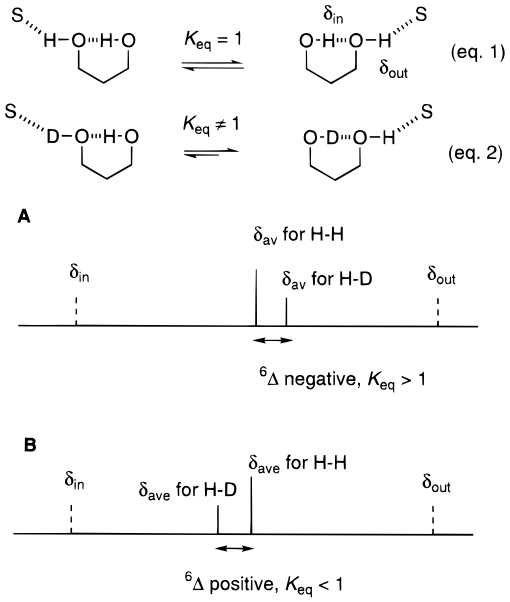

Figure 1. Hypothetical ^{1}H NMR spectra for hydroxyl groups in a symmetrical 1,3-diol (eq 1) and deuterated isotopomer (eq 2). (A) A model consistent with upfield (negative) isotope shifts observed in noninteracting solvents (e.g., benzene). (B) A model consistent with downfield (positive) isotope shifts observed in strong hydrogen bond-accepting solvents (e.g., acetone and DMSO).

In DMSO-d_6 and acetone-d_6 solution, $^6\Delta$ in **1** changes sign in a manner similar to the earlier observations of Everett.[1j] Unlike the case of solvents such as $CDCl_3$ and benzene-d_6, solvent interactions must be taken into account when attempting to explain the sign inversion in DMSO. In the absence of knowing the limiting (slow site exchange) chemical shifts for δ_{in} and δ_{out} in any solvent, one must resort to empirical chemical shift data. Two explanations for the downfield shift observed for **1** in DMSO-d_6 are offered here: (1) The downfield shift of the monodeuterated species in DMSO is consistent with deuterium favoring the hydrogen bond with solvent, resulting in a downfield shift for protium as it experiences a slight preference for the internal site. (2) As in $CDCl_3$, deuterium still prefers the internal hydrogen bond between the hydroxyl groups, but in DMSO the internal and external chemical shifts change such that δ_{in} is upfield relative to δ_{out}. The first explanation seems preferable on the basis of well-known hydrogen bond shifts, e.g., the average chemical shift of water and methanol in CD_3OD is 4.87 ppm while the HOD impurity in DMSO-d_6 is at higher field, ca. 3.4 ppm with nominal amounts present, shifting to low field with the addition of more H_2O.

A low-temperature study of **1** dissolved in acetone-d_6 revealed a temperature-dependent isotope shift, indica-

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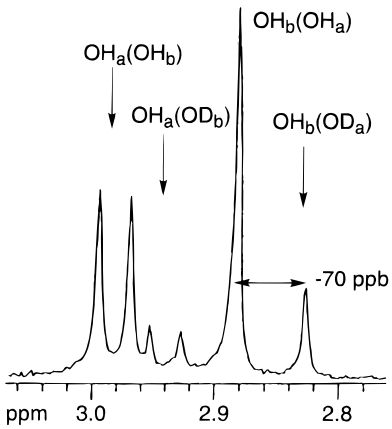

Figure 2. 300 MHz ^{1}H NMR spectrum of the hydroxyl resonances in partially deuterated (OH/OD) **4** dissolved in C_6D_6.

tive of an equilibrium isotope effect.[17] $^6\Delta$ in **1** at −60 °C is ca. 50% greater than the room temperature value, demonstrating that small isotope shifts can be magnified by working at temperatures easily reached on most modern NMR spectrometers. Further low temperature measurements could, in principle, reveal the limiting chemical shifts for the two sites and thus allow calculation of the energetic preference of deuterium for a particular site. Given the expected low barrier (3−5 kcal/mol[18]) for this process, substantially lower temperatures will have to be employed for this to be feasible.

When dissolved in benzene-d_6, unsymmetrical **4** yielded the largest negative $^6\Delta$ value measured to date, at room temperature, for the hydroxyl−hydroxyl interaction (Table 1, Figure 2). Under conditions of slow intermolecular exchange two sharp hydroxyl resonances (OH_a and OH_b) were observed, each signal averaged by rapid in-out site exchange. Initially, the isotope shift was measured by dissolving 10−15 mg of **4** in C_6D_6 and then partially deuterating the compound by adding microliter quantities of CD_3OD to the NMR tube. In this manner, $^6\Delta$ values of −59.5 and −43.8 ppb were measured for OH_b and OH_a, respectively. These values were observed to decrease in magnitude as additional CD_3OD was added to the NMR tube. To find the true isotope shifts, partially deuterated **4** was added to dry C_6D_6. When the tertiary and secondary hydroxyl groups were measured under this condition, $^6\Delta$ values of −70.0 and −57.0 ppb were obtained (Figure 2). In benzene, $^6\Delta$ was found to be quite sensitive to extraneous amounts of protic solvents. This is not the case for studies conducted in DMSO, probably due to strong solvation of HOD or CD_3OD. In benzene solvent, localized solvation of the 1,3-diol by methanol could cause $^6\Delta$ to diminish. This behavior might be expected, as the δ_{in}/δ_{out} chemical shift difference would be expected to decrease as the local environment begins to approximate that of CD_3OD.

We next wanted to explore the behavior of a 1,3-diol unsymmetrically perturbed by a hydrogen bond acceptor, e.g., **5**. The hydroxyl isotope shifts in **5** also exhibit a strong solvent dependence. In C_6D_6, for example, OH_b shows a small downfield shift (+ 9.0 ppb) while OH_a shows none. In this solvent, one might expect the hydrogen bonds to be strongly polarized to form an intramolecular hydroxyl−carbonyl hydrogen bond. In DMSO, this trend reverses, with a large effect (+43.8 ppb) measured for OH_a and no effect observed for OH_b. The large differences in these isotope effects serve to illustrate just how sensitive this method is to external perturbation. The interplay of stretching and bending effects could be responsible, and theoretical studies will be used to address these effects.

In summary, a study of proton SIMPLE effects in rigid inositol derivatives has revealed isotope effects much larger than those found in conformationally mobile systems. The work presented here shows that deuterium prefers the bridging site in 1,3-diaxial hydrogen bonds, a finding that can be compared with earlier theoretical and experimental studies of intermolecular hydrogen bonds. Extending the SIMPLE method to intramolecular hydrogen bonding studies in apolar solvents (e.g., $CDCl_3$, C_6D_6) is facilitated by prior deuteration and low-temperature NMR studies. Future work will examine other hydrogen bonding geometries and different arrangements of donors and acceptors, and these results will communicated in due course.

Experimental Section

(±)-Di-*tert*-butyldimethylsilyl Ether 2. *myo*-Inositol monoorthoformate (1.0 g, 0.526 mmol) was added portionwise to a stirred solution of *tert*-butyldimethylsilyl chloride (1.66 g, 2.1 equiv) and imidazole (0.75 g, 2.1 equiv) in DMF (5 mL). The reaction was allowed to stir for 3 h after which the DMF was removed *in vacuo* and the residue partitioned between ethyl acetate and dilute ammonium chloride. The organic phase was separated, shaken with brine, and dried over sodium sulfate. Solvent removal *in vacuo* gave material (1.966 g, 92%) of sufficient purity for the following transformation. An analytical sample (mp 75−78 °C) was obtained by flash chromatography. ^{1}H-NMR (300 MHz, C_6D_6) δ −0.27 (6H, s); 0.07 (6H, s); 0.66 (9H, s); 0.94 (9H, s); 3.71 (1H, d, J = 9.0 Hz); 3.98 (1H, m); 4.08 (1H, m); 4.25 (1H, m); 4.38 (1H, m); 4.46 (1H, m); 4.53 (1H, m); 5.57 (1H, s); ^{13}C-NMR (75 MHz, C_6D_6) δ −5.73, −5.49, −4.55, −4.55, 17.66, 18.45, 25.45, 26.00, 61.29, 69.17, 69.45, 70.07, 74.91, 75.69, 103.11; IR (KBr pellet) ν 3475, 2957, 2931, 1472, 1166, 1000, 853 cm^{-1}; DCI HRMS (ammonia) $M^+ + H$ calcd for $C_{19}H_{39}O_6Si_2$ 419.2285, found 419.2276.

(±)-Ketone 3. Acetic anhydride (4.8 mL, 51 mmol) was added to DMSO (7.1 mL, 100 mmol), and the mixture was allowed to stir at room temperature for 30 min. **2** (1 g, 2.39 mmol) was added in portions, and the reaction was stirred under an argon atmosphere at room temperature for 20 h. The reaction was then diluted with ethyl acetate (20 mL), saturated bicarbonate solution (5 mL), and distilled water (15 mL). The organic layer was separated, washed with brine, and dried over sodium sulfate. The ethyl acetate was removed *in vacuo*, and the residue was dissolved in 3−4 mL of CH_2Cl_2 and purified by passage through a plug of silica gel (2.5 × 5 cm), eluting with 2:1 hexanes/ethyl acetate. Removal of solvent in vacuo yielded ketone **3** as a white solid, mp 80−83 °C (0.978 g, 98%). ^{1}H-NMR (300 MHz, C_6D_6) δ −0.28 (3H, s), −0.23 (3H, s), −0.06 (3H, s), −0.03 (3H, s), 0.69 (9H, s), 0.87 (9H, s), 4.10−4.18 (3H, m), 4.35 (1H, m), 4.37 (1H, q, J = 1.9 Hz), 5.59 (1H, d, J = 1.1 Hz); ^{13}C-NMR (75 MHz, C_6D_6) δ −5.43 to 4.78 (4C), 17.78, 18.33, 25.45 (3C), 25.83 (3C), 65.87, 66.16, 74.74, 79.23, 82.67, 103.26, 199.21; IR (KBr pellet) ν 2958, 2930, 1759, 1100, 1000, 843 cm^{-1}; DCI HRMS (ammonia) $M^+ + H$ calcd for $C_{19}H_{37}O_6Si_2$ 417.2129, found 417.2136.

(±)-Methyl Derivative 4. Ketone **3** (97 mg, 0.251 mmol) was azeotropically dried once with toluene, and dissolved in THF (2 mL), and cooled to 0 °C in an ice bath. A 3.0 M THF solution of methylmagnesium chloride (420 μL, 1.26 mmol) was added dropwise via syringe, and the reaction mixture was brought to room temperature and allowed to stir for 2 h. The reaction was then diluted with diethyl ether (10 mL) and quenched with dropwise addition of saturated aqueous ammonium chloride solution (5 mL). The organic layer was separated, washed with

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brine, and dried over sodium sulfate. Evaporation of ether gave a residue which was purified with flash chromatography (1 × 4 cm, eluting with 2:1 hexanes/ethyl acetate) to yield a white solid, mp 127−131 °C. (68 mg, 92%). ^{1}H-NMR (300 MHz, C_6D_6) δ 0.12 (6H, s), 1.01 (9H, s), 1.43 (3H, s), 2.67 (1H, s), 2.78 (1H, d, J = 9.0 Hz), 3.58 (1H, m), 3.79 (1H, m), 4.08 (1H, m), 4.31 (1H, m), 4.35 (1H, m), 5.52 (1H, d, J = 1.2 Hz); ^{13}C-NMR (75 MHz, C_6D_6) δ −4.57, −4.54, 18.48, 24.91, 26.01, 26.01, 26.01, 62.20, 69.05, 71.12, 73.31, 74.34, 79.00, 103.03; IR (KBr pellet) ν 3475, 3422, 2959, 2930, 1472, 1375, 832 cm^{-1}; DCI HRMS (ammonia) $M^+ + H$ calcd for $C_{14}H_{27}O_6Si$ 319.1577, found 319.1583.

(±)-Ethyl Acetate Derivative, *tert*-Butyldimethylsilyl Ether 5. Anhydrous ethyl acetate (0.21 mL, 2.1 mmol) was added dropwise to a stirred −78 °C solution of lithium bis-(trimethylsilyl)amide (2.18 mL of a 1.0 M THF solution, 2.1 mmol) in THF (3 mL). Stirring continued at this temperature for 15 min, after which azeotropically dried (toluene, 2×) inositol ketone **3** (303 mg, 0.720 mmol) in THF (3 mL) was added dropwise via cannula. The reaction mixture was stirred at −78 °C for 1.5 h, after which it was diluted with ether (20 mL), quenched with the dropwise addition of saturated aqueous ammonium chloride (10 mL), and brought to room temperature. The organic phase was separated, washed with brine, and dried over sodium sulfate. The aqueous phases were reextracted once with additional ether. The combined organics were evaporated *in vacuo*, and the residue was purified with flash chromatography (2 × 10 cm, eluting with 2:1 hexanes/ether) to yield a white solid, mp 96−99 °C (338 mg, 92%). ^{1}H-NMR (300 MHz, C_6D_6) δ −0.25 (3H, s), −0.20 (3H, s), 0.07 (6H, s), 0.67 (9H, s), 0.88 (3H, t, J = 6.0 Hz), 0.93 (9H, s), 2.92 (1H, d, J = 15.0 Hz), 3.19 (1H, d, J = 15.0 Hz), 3.89 (2H, q, J = 6.0 Hz), 4.05 (1H, m), 4.27 (1H, m), 4.47 (1H, m), 4.53 (2H, m), 4.58 (1H, m), 5.47 (1H, s). The di-TBDMS ether (178.3 mg, 0.353 mmol) was dissolved in THF (6 ml), cooled to −20 °C, and treated with TBAF (353 μL of a 1.0 M THF solution, 1 equiv). The reaction was allowed to stir at this temperature for 15 min, after which hexane (10 mL) was added and the flask allowed to warm to room temperature. The solvents were removed *in vacuo* and the residue was purified by flash chromatography (2 × 10 cm, eluting with 2:1 hexanes/ether) to yield a white solid, mp 110−113 °C (115.7 mg, 84.7%). ^{1}H-NMR (300 MHz, C_6D_6) δ 0.07 (6H, s), 0.76 (3H, t, J = 9.0 Hz), 0.94 (9H, s), 2.78 (2H, s), 3.69 (2H, q, J = 9.0 Hz), 3.90 (1H, br d), 4.02 (1H, m), 4.18 (1H, m), 4.45 (1H, m), 4.50 (1H, m), 5.17 (1H, s), 5.41 (1H, s); ^{13}C-NMR (75 MHz, C_6D_6) δ −5.17 (2C), 13.26, 17.92, 25.44 (3C), 38.43, 60.62, 61.31, 68.36, 71.21, 71.28, 74.36, 76.62, 102.47; IR (KBr pellet) ν 3402, 3391, 2927, 1720, 1158, 1012, 961 cm^{-1} DCI HRMS (ammonia) $M^+ + H$ calcd for $C_{17}H_{31}O_8Si$ 391.1788, found 391.1777.

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