Improving the Use of Hydroxyl Proton Resonances in Structure Determination and NMR Spectral Assignment: Inhibition of Exchange by Dilution

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Hydroxyl resonances in NMR spectra potentially provide much structural information in the form of chemical shifts, *J* couplings, NOEs and isotope shifts but this information is largely destroyed by intermolecular exchange; these applications are briefly reviewed and the exchange mechanism is described. It is shown that exchange of the hydroxyl protons of alcohols in chloroform solution can be slowed simply by dilution of the alcohol because the rate-determining step for exchange requires a bimolecular encounter. When exchange is slow on the NMR chemical shift and coupling timescales, separate signals are observed for each different hydroxyl site, allowing spectral and structural assignment by correlation techniques such as decoupling, COSY, and related techniques. In addition, the limiting chemical shifts and the concentrations required for fast exchange are characteristically different for protons which are intramolecularly hydrogen-bonded. It is shown that propane-1,3-diol and ethane-1,2-diol are strongly hydrogen-bonded intramolecularly in chloroform solution.

It is widely believed by organic chemists that it is difficult or impossible to see *J* coupling or dipolar connections (NOEs) to the hydroxyl protons of alcohols. It is well known that intermolecular exchange of hydroxyl protons destroys coupling information, but it is less well known that such exchange can be easily controlled and manipulated, allowing one to extract far more useful information from an NMR spectrum. In this article we outline some of the factors which control the appearance of the spectra of alcohols, and show how they can be manipulated simply by dilution and careful solvent handling to give more useful spectra. A simple description of the theory of NMR timescales has been given elsewhere,[1] as have detailed strategies for spectrum assignment and structure determination.[2]

The complete suppression of intermolecular exchange allows the use of all the standard one- and two-dimensional techniques for determining NOEs and couplings. This is a major advantage in the determination of structure and conformation:

- Primary hydroxyls in RCH_2OH appear as triplets (or double doublets) due to H–O–C–H coupling, secondary hydroxyls in R_2CHOH appear as doublets, and tertiary hydroxyls in R_3COH appear as singlets; all may be separately resolved in polyols.
- In two-dimensional heteronuclear correlation spectra, two- or three-bond couplings between a hydroxyl proton and a carbon (H–O–^{13}C or H–O–C–^{13}C) can provide important connections across or to spectroscopically-silent quaternary carbons.
- Non-exchanging hydroxyls behave like any other proton in NOE difference or NOESY spectra, enabling alcohol groups to be located in space directly.
- Replacement of a hydroxyl proton by deuterium leads to small chemical shift effects on nearby carbons; these are most easily observed in a 1:1 slowly-exchanging OH/OD mixture, when carbons which experience isotope effects are split into more than one signal. This can be valuable for distinguishing carbons near to hydroxyl oxygen from those near to ether oxygen.[1,3]
- Hydroxyl chemical shifts themselves contain useful information on hydrogen bonding, as described below.

In practice, complete suppression of exchange is unnecessary: exchange simply needs to be slow relative to the appropriate timescale.[1] Why then do chemists not routinely look for hydroxyl couplings? This may be a prejudice that derives from the early days of continuous wave NMR spectroscopy: when concentrations of 0.1 mol dm^{-3} or higher were required it was indeed difficult to inhibit the bimolecular exchange process shown in eqn. (1).

$$R^1OH + R^2OH^* \longrightarrow R^1OH^* + R^2OH \quad (1)$$

However, modern Fourier transform techniques make routine the acquisition of spectra on millimolar solutions. In these dilute conditions the bimolecular collision rate between alcohol molecules falls sufficiently that τ, the average lifetime of a hydroxyl proton attached to a given molecule, increases dramatically. When τ exceeds J^{-1}, the coupling is no longer 'washed-out' by exchange, and when τ exceeds T_1 then one can observe separate NOEs to and from the various hydroxyl protons.[1,4,5]

The exchange process is acid catalysed and should more properly be written as in eqn. (2). The bimolecular rate constant

$$(R^1OHH^*)^+ + R^2OH \longrightarrow R^1OH + (R^2OHH^*)^+ \quad (2)$$

for this process is more than 10^{10} dm^3 mol^{-1} s^{-1} at 25 °C,[6] which means that even minute traces of acid lead to rapid hydroxyl exchange. The rigorous removal of acid from sample, solvent and NMR tube can lead to slow hydroxyl exchange in neat alcohols or even in aqueous solutions[7] but such observations require an experimental rigour which is well beyond the routine. Dilution provides a simpler and more accessible route to the same outcome. A useful corollary, described below, is that dilution is a powerful method for the detection of intramolecular hydrogen bonding through characteristic chemical shifts.

Scattered examples of the observation[8] and use[9] of slowly exchanging hydroxyls in chloroform solution have been published, but few chemists are aware of them and we know of no systematic studies at the low concentrations that have recently become more readily accessible. The results described here derive from our own accidental discovery of slow exchange during the structure determination of natural products where only very small amounts of material were available.[9b,10] Our study has been sufficiently extended to

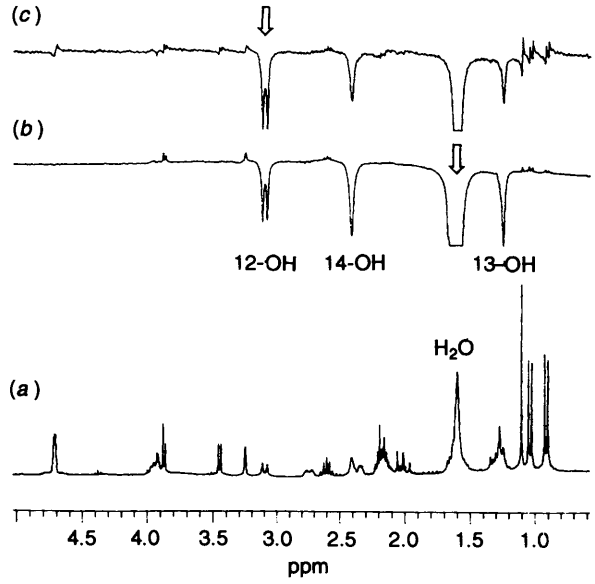


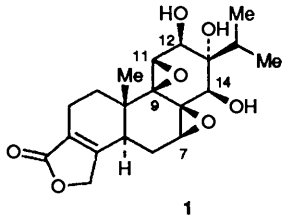
Fig. 1 300 MHz ^{1}H spectra of the diol **1** dissolved in $CDCl_3$ solution: (*a*) control spectrum, *ca.* 10 mmol dm^{-3}; (*b*) nOe difference spectrum resulting from irradiation of the water resonance; (*b*) nOe difference spectrum resulting from irradiation of the 12-hydroxyl resonance

uncover general principles, but is far from complete, rigorous or quantitative.

Not surprisingly, hydroxyl exchange is generally slower in hydrogen-bonding solvents such as DMSO or pyridine, and this has been much exploited in structural studies, particularly of carbohydrates and other polar natural products.[3c,11]

Results

Fig. 1 shows three spectra acquired during the structure elucidation of the triptolide derivative **1**: around 1 mg of



1

material was dissolved in $CDCl_3$ which had been passed through dry basic alumina to remove acid and some water, but no other precautions were taken. The 300 MHz spectrum (Fig. 1*a*) shows many well resolved resonances as well as the familiar signal at 1.6 ppm due to water dissolved in the organic solvent.* Irradiation of the water signal in an NOE difference experiment reveals, by saturation transfer,[1] the presence of three well-resolved hydroxyl resonances (Fig. 1*b*). This is, at first sight, a very surprising spectrum for a chloroform solution: not only are the three hydroxyls separately resolved, but one of them is a 12 Hz doublet due to coupling to 12-H; the chemical shift range of these signals is almost 2 ppm. Furthermore, the similar extent of saturation transfer to each signal implies similar rates of exchange for each hydroxyl with dissolved water even though the 12-hydroxyl is believed to be hydrogen-bonded to the 9,11-epoxide.[12] Similarly, irradiation of the 12-OH resonance at 3.1

* Water dissolved in non-polar solvents appears at 1–2 ppm while water in the aqueous environment of water droplets resonates around 4.5–5 ppm.

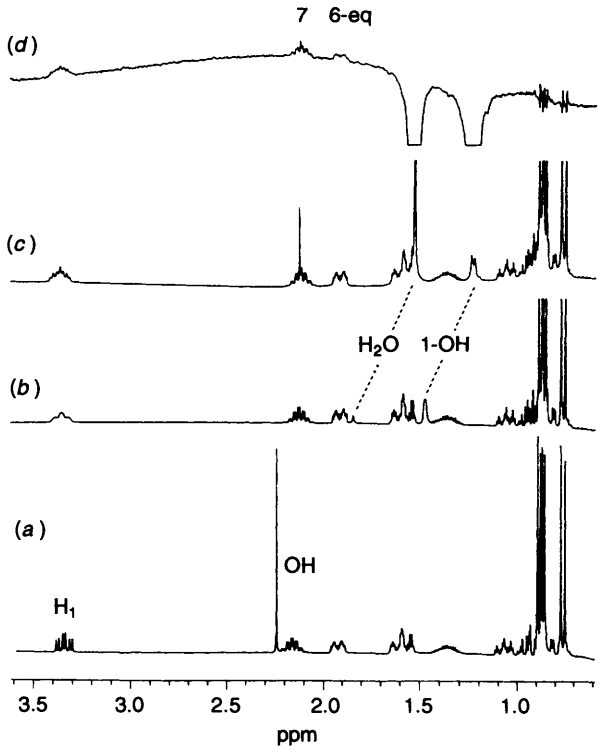
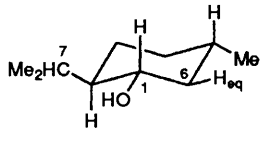


Fig. 2 300 MHz ^{1}H spectra of menthol, **2**, dissolved in $CDCl_3$ solution: (*a*) 2 mol dm^{-3}; (*b*) 250 mmol dm^{-3}; (*c*) 15 mmol dm^{-3}; (*d*) nOe difference spectrum resulting from irradiation of the hydroxyl resonance in the 15 mmol dm^{-3} solution. The apparent increase in size of the water resonance in (*c*) is simply a result of the smaller concentration of menthol

ppm gives essentially the same degree of saturation transfer (≈40%) to the other hydroxyl resonances and water (Fig. 1*c*). The explanation for these results is discussed below.

Prompted by these and many similar observations in several natural product investigations[9b,10] we had to ask why it was so easy to observe these slow-exchange effects in this work but so much more difficult in 'routine' spectra. Since shortage of natural material was the only common factor, we looked at the concentration dependence of the ^{1}H spectra of a range of simple alcohols. Fig. 2 shows the 300 MHz ^{1}H spectra of 2 mol dm^{-3}, 0.25 mol dm^{-3} and 15 mmol dm^{-3} solutions of menthol, **2**, in



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$CDCl_3$. At the highest concentration (Fig. 2*a*), the hydroxyl resonance appears as a sharp singlet at 2.24 ppm, and 1-H is a doublet of triplets at 3.34 ppm: clearly, the hydroxyl is in rapid intermolecular exchange. At 0.25 mol dm^{-3}, the hydroxyl has moved to 1.5 ppm, a small separate water signal is visible in slow exchange at 1.9 ppm and 1-H shows exchange broadening; a COSY spectrum of this sample (not shown) displays a correlation between 1-H and the hydroxyl proton so exchange is now slower than, or comparable with, J^{-1}. At 15 mmol dm^{-3} (Fig. 2*c*) the 1-H–OH coupling is well resolved in both proton resonances and a large separate water peak, integrating for around 20 mmol dm^{-3}, is also obtained. Irradiation of the hydroxyl (Fig. 2*d*) is accompanied by saturation transfer to

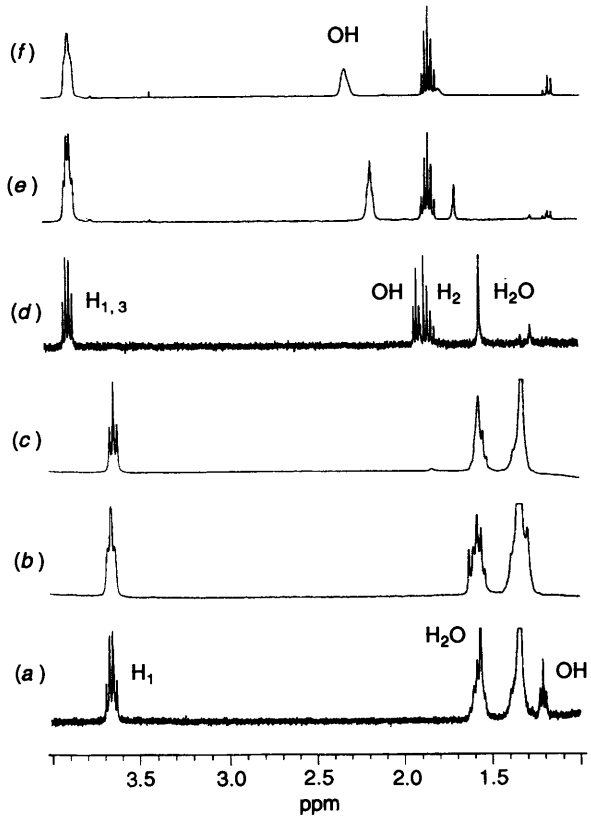
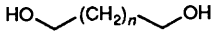


Fig. 3 300 MHz ^{1}H spectra of **3** and **4** in $CDCl_3$ solution; (*a*) 13 mmol dm^{-3} nonane-1,9-diol, **3**; (*b*) 25 mmol dm^{-3} nonane-1,9-diol, **3**; (*c*) 123 mmol dm^{-3} nonane-1,9-diol, **3**; (*d*) 6 mmol dm^{-3} propane-1,3-diol, **4**; (*e*) 110 mmol dm^{-3} propane-1,3-diol, **4**; (*f*) 390 mmol dm^{-3} propane-1,3-diol, **4**

the water but also by positive NOEs to the protons near the hydroxyl group.

The fact that exchange is concentration dependent in this way implies that under these conditions, the rate-determining step requires the encounter between one alcohol molecule and another (or water). The results in Fig. 1*c* further imply that in most cases there is no great preference for exchange between any particular sites, *i.e.* the exchange process is either simply dominated by intermolecular collision or is a process that occurs within an encounter complex but is insensitive to chemical environment.

We looked at the concentration dependence of the spectra of the three simple diols **3**–**5** in order to elucidate the influence of



3 $n = 7$
4 $n = 1$
5 $n = 0$

intramolecular hydrogen bonding. Figs. 3 and 4 summarise the results. Nonane-1,9-diol, **3**, behaves in essentially the same manner as menthol: in dilute solution the hydroxyl resonance appears around 1.3 ppm and the transition from slow to fast exchange on the *J* timescale occurs around 20 mmol dm^{-3} (Fig. 3*a*–*c*). Propane-1,3-diol differs in the chemical shift of the hydroxyl resonance at low concentration, 1.9 ppm (Fig. 3*d*) and in the fact that the transition to fast exchange on the *J* timescale does not occur at room temperature at any accessible concentration: at 110 mmol dm^{-3} there is some broadening but coupling is still visible (Fig. 3*e*) while a saturated solution (390 mmol dm^{-3}) is barely on the fast side of coalescence (Fig. 3*f*).* The latter solution when heated to 313 K gives the sharp triplet

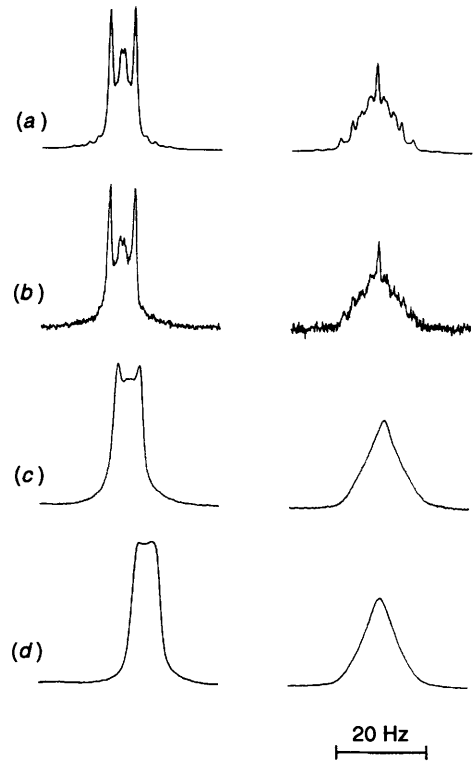
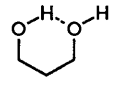


Fig. 4 Expanded sections of the 300 MHz ^{1}H spectra of **5** in $CDCl_3$ solution; (*a*) simulation of (*b*) using parameters in Table 1; (*b*) 7 mmol dm^{-3} ethane-1,2-diol; (*c*) 70 mmol dm^{-3} ethane-1,2-diol, **5**; (*d*) 226 mmol dm^{-3} ethane-1,2-diol, **5**

Table 1 Significant coupling constants in ethanediol **5**

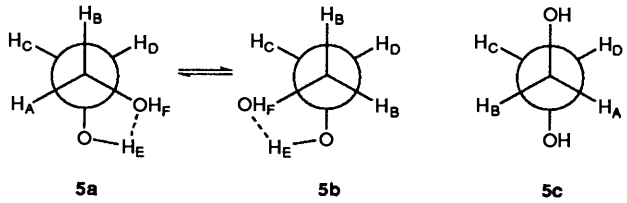
Proton pair	*J*/Hz
AB = CD	−10
AC = BD	3
AD = BC	7
AF = BF = CE = DE	5.5

and singlet expected of fast exchange. This contrast in behaviour must surely result from intramolecular hydrogen bonding as shown in **4a** and discussed below.



4a

Ethanediol, **5**, displays similar behaviour (Fig. 4*b*) to **4** in that it never achieves exchange decoupling at any accessible concentration at ambient temperature. In the slow exchange limit it displays unusual multiplet patterns which could be effectively simulated (Fig. 4*a*) using the coupling parameters summarised in Table 1; the derived couplings provide further support for a hydrogen-bonded structure, **5a** and **5b**, as



5a **5b** **5c**

* A saturated solution of propane-1,3-diol in the presence of immiscible droplets of excess of diol shows separate spectra for the latter, the hydroxyl protons resonating at 5.2 ppm and the protons on C-1 and C-3 at 3.85 ppm.

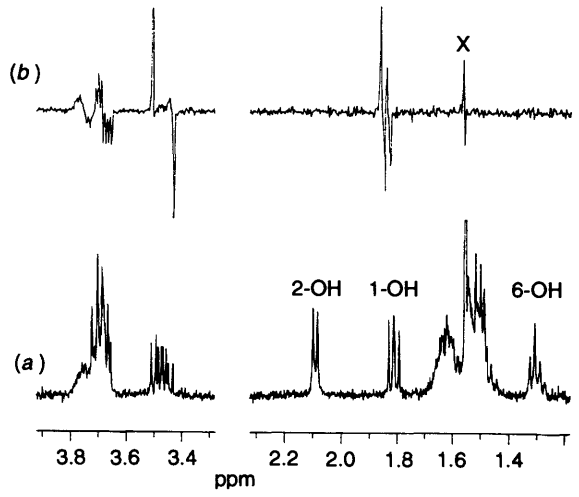
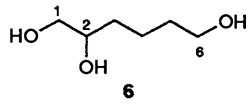


Fig. 5 300 MHz ^{1}H spectra of hexane-1,2,6-triol, **6**: (*a*) one-dimensional spectrum of a 5 mmol dm^{-3} solution in $CDCl_3$ solution; (*b*) cross-section through a double-quantum-filtered COSY spectrum showing responses between the 1-OH resonance and the inequivalent geminal pair on C-1. X marks t_1-noise from the sharp water signal

discussed below. At higher concentrations some exchange broadening is observed, and is accompanied by downfield shifts of the hydroxyl resonance (Fig. 4*c* and *d*).

It was clear from all these results that slow exchange was relatively easy to achieve in chloroform solution using some care in sample preparation, and that the presence of intramolecular hydrogen bonding gave characteristic limiting shifts to a hydroxyl resonance. The diagnostic power of this approach is illustrated by the 300 MHz spectrum of 5 mmol dm^{-3} hexane-1,2,6-triol **6** (Fig. 5*a*). As we could now predict,



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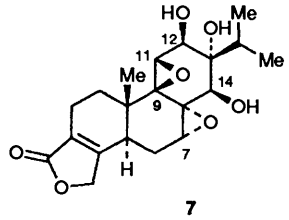
this shows all three hydroxyl resonances well resolved from each other and readily assigned by their characteristic shift and coupling patterns. The 6-hydroxyl is a 5.3 Hz triplet at the non-hydrogen bonded shift of 1.3 ppm, while the 1-hydroxyl is a double-doublet ($J \approx 5.8$ and 5.4 Hz) at 1.8 ppm and the 2-hydroxyl is a 4.3 Hz doublet at 2.1 ppm. Conventional COSY spectra then allow us to locate all the remaining resonances; for example, Fig. 5*b* shows a single COSY cross section which connects 1-hydroxyl to the two inequivalent protons on C-1. This compound is not miscible with chloroform at high enough concentrations to induce visible exchange in the absence of added acid. In the same vein, the ^{1}H spectrum of 1 mmol dm^{-3} β-1-octyl glucoside in $CDCl_3$ shows all four hydroxyl signals, well resolved in the 1.8–2.8 ppm region and readily assignable by decoupling or COSY (R. P. Bonar-Law, unpublished).

These slow exchange effects are lost in solutions which are stored and accumulate acid either by chloroform decomposition or leaching from the tube. All the spectra illustrated were obtained on freshly prepared solutions made up with chloroform which had just been treated with alumina. Even in the most dilute solutions, hydroxyl exchange gradually accelerates and moves into fast exchange if no precautions are taken to inhibit acid formation.

Discussion

The dependence of hydroxyl chemical shift on temperature, concentration and hydrogen bonding has, of course, been studied since the earliest days of NMR spectroscopy,[13] but most organic chemists have historically agreed with Jackman and Sternhell that these studies 'have little direct application in organic chemistry'.[14] This is a pity: in 1964 Bhacca and Williams[8] showed a spectrum of a steroidal diol in dilute solution in chloroform, and demonstrated the presence of two separate hydroxyl resonances (one of which even showed coupling) but the observation has been forgotten or ignored. However, the ease with which one can now observe slow exchange in a solvent like chloroform provides an unexpectedly powerful approach for assigning spectra and determining structures: for example, COSY and long-range heteronuclear correlation exploiting hydroxyls become routinely accessible. The fact that the hydroxyl chemical shift[13,14] and the concentration at which fast exchange sets in are both diagnostic for intramolecular hydrogen bonding is an additional bonus. Fig. 5 illustrates graphically the power of this smple approach to resolve and assign three superficially very similar hydroxyl groups. The use of slowly exchanging hydroxyls in structural studies in hydrogen-bonding solvents is better established,[1,2] but still surprisingly little exploited except in the carbohydrate field. By contrast, the use of slowly-exchanging amide protons for the study of structure and hydrogen bonding is very common.[15] Proton exchange in basic primary and secondary amines is faster than that of alcohols and is much more difficult to control.[1] Inhibition is best achieved by protonation with strong acid to ensure the complete absence of any basic nitrogen lone pairs in the solution; under these conditions, slowly exchanging amino proton signals can be observed and all the techniques described above can be employed.[16,17]

The subtlety and power of using hydroxyl resonances is illustrated by the triptolide derivative **1**: the hydroxyl chemical shifts in Fig. 1, together with the H–O–C–H couplings are strongly indicative of the hydrogen-bonding pattern shown in structure **1a**; clearly, the 13-hydroxyl is not intramolecularly hydrogen bonded, while both the 12- and 14-hydroxyls are. Furthermore, the lack of observable coupling between the 14-hydroxyl and 14-H supports the geometry shown, with a dihedral angle between the two protons of *ca.* 90°. This arrangement is supported by molecular mechanics calculations (unpublished). More remarkably, isomer **7**, which differs only



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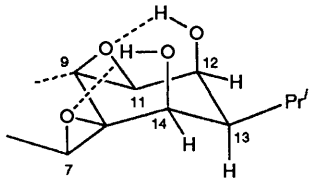
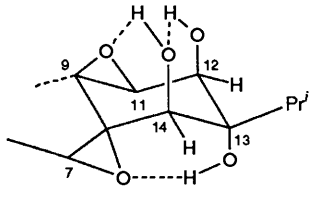
in the orientation of the 7,8-epoxide oxygen, has a spectrum (not shown)*[18] with a completely different pattern of hydroxyl chemical shifts and couplings; this indicates the hydrogen-bonding pattern shown in **7a**. Now all three hydroxyls show hydrogen bonding, and the 10.7 Hz coupling* between the 14-hydroxyl and 14-H indicates a new geometry, the 7,8-epoxide oxygen no longer being available; again this pattern is supported by our (unpublished) calculations. Where intramolecular hydrogen bonding is extremely strong, as in azadirachtin for example, then this is reflected in slower intermolecular exchange, a smaller temperature coefficient of the hydroxyl shift and even larger downfield shift.[9c] In alcohols

* 12-OH (2.23 ppm, d, 10.7 Hz); 13-OH (2.75 ppm, s); 14-OH (3.64 ppm, d, 10.7 Hz).



1a



7a

where there is no strong intramolecular hydrogen bonding, the observed three bond H–C–O–H coupling is generally around 5 Hz, consistent with the expected averaging of *gauche* and *trans* couplings.

The curious appearance of the resonances in ethanediol, **5**, was readily simulated using the coupling parameters in Table 1. This is simply a case of magnetic non-equivalence:[4] protons A and B have the same chemical shift as C and D, but they are differently coupled to each other and to the hydroxyl protons E and F. The appearance of the simulated multiplet was relatively insensitive (±1 Hz) to changes in most of the couplings but very sensitive (±0.2 Hz) to the three-bond H–C–O–H coupling. Note that the best fit was obtained with the four-bond J_{AE} and J_{CF} set to zero. The magnitudes of the derived couplings are consistent with the conformational equilibrium between **5a** and **5b** as shown; a substantial population of **5c** would be reflected in larger values for the AC and BD couplings. It is no surprise that ethanediol takes up this well-established[19] conformation which must result from intramolecular hydrogen bonding,[20] but we are not aware of any previous NMR observations which have demonstrated it so directly. It is remarkable that the two hydroxyl protons within an individual molecule are not in rapid exchange with each other on the *J* timescale, but that seems the only interpretation of the observed splittings.

The results presented here bring out very clearly the difference between the various NMR timescales:[1] the saturation transfer evident in Fig. 1 is the result of fast exchange on the T_1 timescale, but is only visible because the various signals are in slow exchange on the chemical shift timescale: *i.e.* τ, the lifetime of a proton on a single molecule, exceeds $(\Delta\delta)^{-1}$, where $\Delta\delta$ is the chemical shift difference in Hz between different hydroxyl sites. At the appropriate temperature or concentration the various hydroxyls can still be seen separately but are decoupled by exchange, because τ exceeds $(\Delta\delta)^{-1}$ but is smaller than J^{-1}.

We have made no attempt to measure or calculate bimolecular proton exchange rates in this work: that would require a rigorous suppression of both acid catalysis and adventitious water. Qualitatively however, and unsurprisingly, it is clear from the results for propanediol and ethanediol that intramolecular hydrogen bonding can reduce the rate of intermolecular exchange; the apparently slower exchange in propanediol would seem to correlate with the relative hydrogen bond strengths measured by IR spectroscopy.[20] The potential clearly exists for measuring both inter- and intra-molecular exchange rates as a function of temperature and so obtaining activation parameters and hydrogen bond strengths.

Experimental Methods

Triptolide derivatives were provided by Professor S. A. Matlin, University of Warwick, and all other alcohols were commercially available. All alcohols were used as supplied without any treatment to remove water or acid. Spectra were obtained on solutions of alcohols in 100% deuteriated chloroform (supplied by CEA, France, in 750 μl sealed ampoules). Immediately before use, chloroform was passed through a short column of chromatography grade basic alumina (10–250 mesh, pH 9.3–9.7, Fisons Scientific Equipment, Loughborough) to remove traces of acid.

All ^{1}H spectra were obtained at 300 MHz on Bruker WM 300 and AC 300 instruments. One-dimensional spectra were acquired into 16 K data points with spectral windows of 3–10 ppm, depending on the compound under study. NOE difference spectra of the triptolide derivative **1** and menthol **2** were obtained using 5 s and 15 s pre-irradiation times respectively; no relaxation delay was allowed between pulses. NOE difference spectra were processed with 1 Hz exponential line broadening.

The DQF-PS COSY spectrum of **6** was obtained using a standard microprogram. A 3.4 ppm spectral window was used in f_2, acquired into 2 K data points; 16 transients were collected for each of 512 increments, and the data set was zero-filled to 1 K in f_1 before Fourier transformation. Gaussian weighting was applied in each dimension, and symmetrisation was not applied.

The spectrum of ethanediol was simulated using Bruker's PANIC program.

Acknowledgements

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