Organic & Biomolecular Chemistry

Dynamic Article Links

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 7062

www.rsc.org/obc

PAPER

Resolving natural product epimer spectra by matrix-assisted DOSY†

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Received 6th July 2011, Accepted 19th July 2011
DOI: 10.1039/c1ob06097j

High resolution diffusion-ordered NMR spectroscopy allows the separation of signals from different species based on their diffusion coefficients. In general this requires that the NMR spectra of the components do not have overlapping signals, and that the diffusion coefficients are significantly different. Modifying the solvent matrix in which a sample is dissolved can change the diffusion coefficients observed, allowing resolution ("matrix-assisted DOSY"). We show here that dissolving the two naturally-occurring epimers of naringin in an aqueous solution of β-cyclodextrin causes both shift and diffusion changes, allowing the signals of the epimers to be distinguished. Chiral matrix-assisted DOSY has the potential to allow simple resolution and assignment of the spectra of epimers and enantiomers, without the need for derivatisation or for titration with a shift reagent.

Introduction

Derivatisation, shift reagents and chiral solvating agents can all allow NMR to distinguish diastereomers,[1] but without a pure sample of one stereochemistry, assigning signals to a given species usually requires titration with a shift reagent.[2,3] Here we show that assignment can be accomplished in a single NMR experiment.

DOSY[4] allows efficient separation of signals from different mixture components in solution, provided that they diffuse at different rates and do not overlap.[5] To address signal overlap, methods such as multi-exponential fitting[6] and multivariate analysis[7–9] can be used, but require a significant difference in diffusion coefficient.[10] Signal overlap can sometimes be avoided by using a parent experiment with better resolution, *e.g.* 3D,[11] pure shift[12] or ^{13}C DOSY[13] methods.

DOSY cannot separate signals of species that diffuse at the same rate. However, changing the matrix in which solutes diffuse, *e.g.* by introducing a co-solute which interacts differently with different substrates, can allow separation. This 'matrix-assisted' DOSY (MAD) approach allows similar species to be distinguished through the exploitation of selective binding,[14,15] and has been shown to allow resolution of constitutional isomers,[16] but diastereomers and enantiomers remain a challenge.

Epimers typically show some differences in chemical shift, but very similar diffusion coefficients; in achiral environments, enantiomers have identical spectra and diffusion coefficients. Chiral recognition is desirable for differentiating epimers, and a prerequisite for enantiomers, and is often achieved using a chiral selector, *e.g.* a chiral lanthanide shift reagent (LSR)[17] or solvating agent.[2,18]

MAD spectra reflect differences in diffusion caused by differences in binding. Here this is exploited to assign the signals of epimers, resolved in both the chemical shift and diffusion domains, in a single NMR experiment. We show that MAD using β-cyclodextrin allows resolution of the two native epimers of the chiral natural product naringin (Fig. 1). In the ^{1}H NMR spectrum of naturally-occurring naringin some doubling of signals occurs. However, the shift differences are small, and insufficient to allow use of the nuclear Overhauser effect to determine configuration. There is no detectable difference in the diffusion coefficients of the epimers in DOSY experiments on naringin alone.

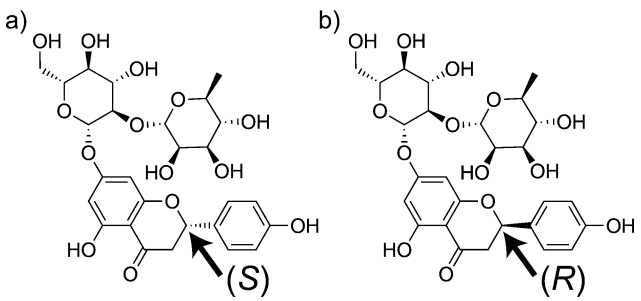

Fig. 1 Epimers of (a) (2*S*)-naringin and (b) (2*R*)-naringin, highlighting the position of the epimeric chiral centre.

Cyclodextrins are well-known to form inclusion complexes;[19] the hydrophobic cavity and peripheral hydroxyl groups bind species with hydrophobic moieties of appropriate size. The chirality of cyclodextrins was first exploited to resolve a racemic mixture as long ago as 1953,[20] and they have been widely used as chiral

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† This work was supported by the Engineering and Physical Sciences Research Council (Grant Numbers EP/H024336/1, EP/I007989/1, and EP/E05899X/1). JC thanks Consejo Nacional de Ciencia y Tecnología (CONACyT) for financial support.

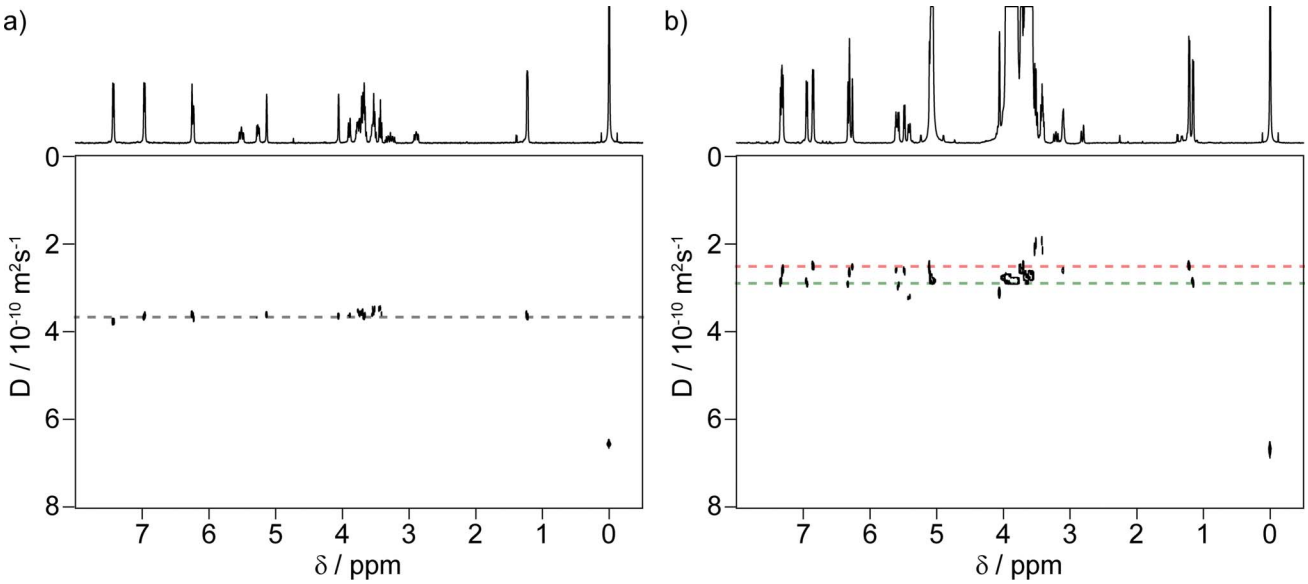

Fig. 2 Oneshot DOSY spectra of naringin (1.4 mM) in D_2O, with TSP (17 mM) in D_2O coaxial reference in a 5 mm NMR tube. Experiments were recorded (a) without and (b) with 4.7 mM β-cyclodextrin. Diffusion coefficients are indicated by the dashed lines and, in (b), show the separation of the epimer signals. 2*R* (green) and 2*S* (red) epimers of naringin are assigned based on previous studies.[24]

solvating agents.[1,21] Previous DOSY studies with cyclodextrins have concentrated on the separation of the signals of different species,[22,23] while the interaction of β-cyclodextrin with naringin has been shown to produce epimer-specific shift changes.[24]

Results and discussion

Here we show that the DOSY spectrum of a naringin/cyclodextrin solution allows signals to be assigned directly to one or other epimer, where previously titration with the shift reagent would have been required.[24]

Both epimers of naringin complex with β-cyclodextrin, but the binding strengths are different. Fast exchange between bound and free naringin gives a single set of signals for each epimer and a population-weighted diffusion coefficient, D_{av}, given by Lindman's Law

$$D_{av} = D_u\, p_u + D_b\, p_b,$$

where D_u and p_u and D_b and p_b are the diffusion coefficients and the fractions of unbound and bound molecules respectively.[25] In the DOSY spectrum of Fig. 2a, all naringin signals show the same diffusion coefficient, as if only one species were present in the sample. Adding β-cyclodextrin gives the spectrum of Fig. 2b. Here the different shift changes in the epimers and the differential binding distinguish the species, and the majority of the signals can be assigned directly with DOSY, giving assignments in agreement with titration.[24] A few naringin signals overlap with β-cyclodextrin, preventing detailed analysis of the region 3.2–4.0 ppm. Similar experiments with the aglycone naringenin did not show diffusion resolution.

Conclusions

Using chiral MAD we have demonstrated resolution of these two epimers of naringin, allowing signals to be assigned in a single experiment. We believe this represents the first example of the use of MAD to separate the signals of epimers. As naringin and β-cyclodextrin are of similar size, the maximum effect on diffusion is relatively small, implying a significant difference in binding between the two epimers. A more slowly-diffusing chiral recognition matrix, *e.g.* a polymer-bound cyclodextrin, would give a larger effect. This should be weighed against the line-broadening effect on the signals of a small molecule that interacts strongly with a large species. Clearly many other chiral matrices, *e.g.* chiral LSRs[26] or amidic aggregates,[27] could be used similarly. It is also interesting to speculate on the possibility of using molecular modelling of binding to aid chiral assignment here.[28] The combined changes in chemical shift and diffusion coefficient observed here suggest that the method has significant promise.

Experimental section

Spectra were measured for 0.07% w/w naringin (Sigma) in D_2O, with and without 0.49% w/w β-cyclodextrin (Sigma). 0.27% w/w sodium trimethylsilylpropionate-d_4 (TSP) reference in D_2O was used in a coaxial insert, to allow reference deconvolution but avoid complexation of TSP. Measurements were performed using a Varian VNMRS 500 MHz spectrometer, non-spinning, at 30 °C. The Oneshot DOSY pulse sequence[29,30] was used with 16 gradient amplitudes incremented in equal steps of nominal gradient squared from 10.5 to 56.4 G cm^{-1}. The diffusion time was 0.2 s and the total diffusion-encoding gradient pulse duration 1 ms; HDO was presaturated for 2.5 s. 32k complex points were collected with 128 transients for each gradient level, giving total experiment times of 195 min. Processing used correction for non-uniform field gradients.[31]

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