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Short Communication

Derivatized β-cyclodextrins combined with high field NMR for enantiomer analysis: application to ICI 185,282 (5(*Z*)-7-([2*RS*,4*RS*,5*SR*]-4-*o*-hydroxyphenyl-2-trifluoromethyl-1,3-dioxan-5-yl) heptenoic acid)

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Introduction

Despite the widespread use of cyclodextrins in chiral chromatography for the resolution and analysis of enantiomers (e.g. see ref. 1 for a review) their application to the determination of optically active compounds by NMR has received scant attention. However, such studies as have been performed [2–4] have clearly demonstrated that cyclodextrins are capable of producing diastereomeric complexes with sufficiently different NMR spectra to enable the measurement of optical purity. A particular advantage of cyclodextrins is that such measurements may be performed in aqueous (H_2O or ^2H_2O) solution rather than being restricted to the organic solvents required by other reagents (e.g. lanthanide shift reagents).

This not only allows the optical composition of salts to be determined but also opens up the possibility of performing such measurements on samples of biological fluids such as urine [an area where NMR is finding increasing application (see ref. 5 for a review)]. The main difficulty associated with the use of β-cyclodextrin in this type of application is its limited water solubility (*ca* 18 mg ml^{-1}). However, recently a variety of chemically modified β-cyclodextrins have become available which exhibit greatly enhanced water solubility.

Here we describe preliminary observations on the use of a variety of these derivatized β-cyclodextrins for the analysis of 5-(*Z*)-7-([2*RS*, 4*RS*, 5*SR*]-4-*o*-hydroxyphenyl-2-trifluoromethyl-1,3-dioxan-5-yl) heptenoic acid (ICI 185,282) (Fig. 1), using ^{19}F NMR, compared to those obtained using α, β and γ cyclodextrins.

Experimental

Materials

α, β and γ cyclodextrins were obtained from the Aldrich Chemical Co. Ltd (Gillingham, UK). Derivatized cyclodextrins (methyl-β-cyclodextrin, hydroxyethyl-β-cyclodextrin, hydroxypropyl-β-cyclodextrin), with varying degrees of molar substitution, were obtained

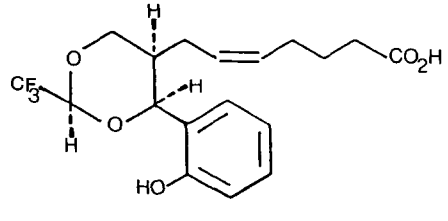

Figure 1
The structure of ICI 185,282.

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from Wacker Chemie AG (Consortium für Electrochemische Industrie, Munich, FRG) and were a gift to the authors from Dr B. Clarke (University of Bradford, UK). ICI 185,282 was synthesized in the Chemistry Department of ICI Pharmaceuticals. Deuterated solvents were purchased from Fluorochem Ltd (Glossop, UK) and fluorotrichloromethane was obtained from BDH Ltd (Poole, UK).

Methods

Spectroscopy was carried out using a Jeol GX 270 NMR spectrometer equipped with an H5 probe tuned to 254 MHz. Proton-coupled ^{19}F spectra were obtained using 90° pulses (6 μs), with a 50 kHz spectral width. Typically 64 free induction decays (fids) were collected into 64 k data points, with zero filling to 128 k prior to Fourier Transformation (FT). Aquisition time was 0.66 s with a delay of 2.35 s between pulses. Fluorotrichloromethane was used to provide a chemical shift reference (δ = 0 ppm).

Solutions of ICI 185,282 (approximately 2.5 $\times$ 10^{-3} M, ~1 mg ml^{-1}) were prepared in ^2H_2O and basified with a small amount of 20% sodium deuteroxide (1 drop 10 ml^{-1}) to aid dissolution. Cyclodextrins were then added to this solution in varying amounts and the effects on the ^{19}F NMR spectrum of ICI 185,282 examined (described more fully later in the text).

Results and Discussion

The proton coupled ^{19}F NMR spectrum of ICI 185,282 in ^2H_2O, at a concentration of approximately 2.5 $\times$ 10^{-3} M, is shown in Fig. 2A. The signal for the three identical fluorine atoms of the trifluoromethyl group of ICI 185,282 appears as a doublet, (J ~ 3.05 Hz) centred on −81.6 ppm, due to spin–spin coupling with the proton on the adjacent carbon atom. The effect of adding a α, β or γ cyclodextrin, together with a variety of substituted β-cyclodextrins, is summarized in Table 1. No separation of the resonances for the enantiomers of ICI 185,282 was observed in the ^{19}F NMR spectrum in the presence of either α or γ cyclodextrins. However, as illustrated in Fig. 2B, the addition of β-cyclodextrin resulted in the partial resolution of the ^{19}F NMR signal into two peaks of equal intensity, presumably corresponding to the two enantiomers. This effect was only obtained when saturated solutions of β-cyclodextrin were employed. The absence of any separation with α-cyclodextrin was probably therefore due to the inability of ICI 185,282 to form an inclusion complex because the cavity of this cyclodextrin was too small. Conversely, in the case of the γ-cyclodextrin the cavity was probably too large to form an effective inclusion complex. On the basis of these results the effect on the resolution of the two enantiomer's ^{19}F NMR signals using a range of derivatized β-cyclodextrins was examined. These included methyl, hydroxyethyl and hydroxypropyl derivatized β-cyclodextrins with molar substitution (MS) ratios of between 0.6 and 1.8 (see Table 1 for details). As summarized in the table, hydroxypropyl derivatives of β-cyclodextrin were not observed to cause any resolution of the resonances for the

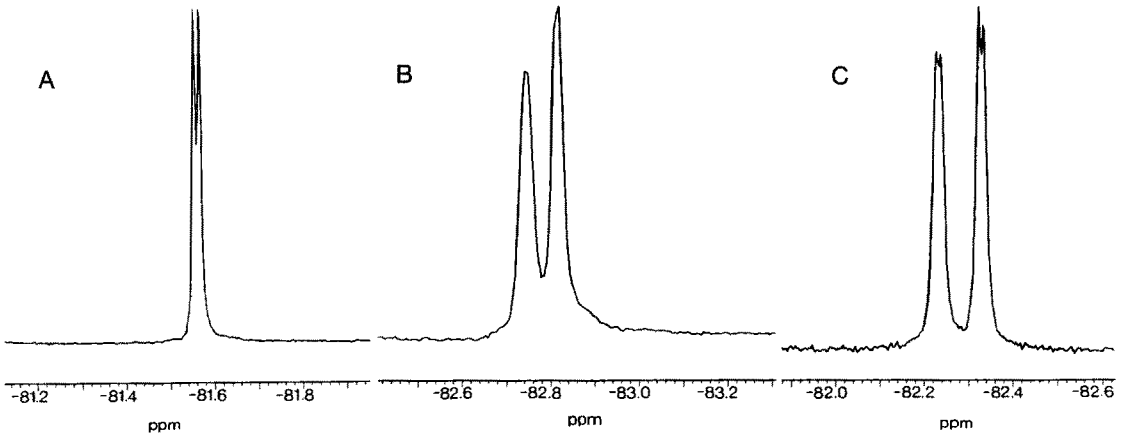

Figure 2
^{19}F NMR Spectra of ICI 185,282 in (A) ^2H_2O; (B) a saturated solution of β-cyclodextrin in ^2H_2O; (C) a solution of methyl-β-cyclodextrin (molar ratio of ICI 185,282 to methyl-β-cyclodextrin = 1:2.5) in ^2H_2O.

Table 1
Effect of cyclodextrins on the ^{19}F NMR resolution of the resonances of ICI 185,282 in $^{2}H_2O$*

Cyclodextrin (water solubility g 100 ml^{-1})	MS	Resolution	Peak separation (ppm)
α-Cyclodextrin (14)	—	—	—
β-Cyclodextrin (1.8)	—	Partial	0.08
γ-Cyclodextrin (23)	—	—	—
Methyl-β-cyclodextrin (80)	1.8	Yes	0.11
Hydroxyethyl-β-cyclodextrin (55)	0.6	Partial	0.06
Hydroxyethyl-β-cyclodextrin (65)	1.0	Partial	0.05
Hydroxyethyl-β-cyclodextrin (100)	1.6	Partial	0.06
Hydroxypropyl-β-cyclodextrin (45)	0.6	—	—
Hydroxypropl-β-cycodextrin (50)	0.9	—	—

*A molar ratio of ICI 185,282 to cyclodextrin (or derivative) of 1:2.5 was used except for β-cyclodextrin where a saturated solution was employed.

Table 2
Effect of concentration of methyl-β-cyclodextrin on the ^{19}F NMR resolution of the resonances of ICI 185,282* in $^{2}H_2O$

Methyl-β-cyclodextrin concentration (mg ml^{-1})	Resonance (ppm) +ve	Resonance (ppm) −ve	Separation (ppm)
1.31	NS	NS	—
2.9	NS	NS	—
4.71	−81.82	−81.86	0.04
6.89	−81.52	−81.68	0.12
10.21	−81.50	−81.64	0.14
15.93	−81.49	−81.64	0.15

*ICI 185,282 was present at 2.5×10^{-3} M, 1 mg ml^{-1}.
NS = no separation of the resonances for the +ve and −ve enantiomers.

two enantiomers of the drug. However, the three-hydroxyethylated β-cyclodextrins (MS 0.6–1.6) all produced a separation of the ^{19}F resonances of about the same magnitude.

The peak to peak separation of between 0.05 and 0.06 ppm produced by the hydroxyethyl derivatives was similar to the 0.08 ppm resolution resulting from saturated solution of β-cyclodextrin.

Interestingly, the degree of molar substitution of these hydroxyethylated β-cyclodextrins appeared to have little effect on the magnitude of the peak splitting observed in the ^{19}F NMR spectrum. By far the best results were obtained with the methyl-substituted β-cyclodextrin. Addition of this derivative to the ICI 185,282 solution (at a molar ratio of 1:2.5) resulted in a peak to peak separation of the ^{19}F NMR signals for the two enantiomers of some 0.11 ppm (see Table 1 and Fig. 1c).

The observed effects were strongly dependent upon the concentration of methyl-β-cyclodextrin employed as shown in Table 2. Thus, below approximately 5 mg ml^{-1}, no separation was observed in the ^{19}F NMR spectrum. However, with increasing concentration the peak to peak resolution produced on addition of the methyl-substituted cyclodextrin increased from 0.04 ppm (4.71 mg ml^{-1}) to 0.15 ppm (15.93 mg ml^{-1}).

In order to provide an unequivocal demonstration that the observed peak splitting did indeed result from the separation of the resonances for the two enantiomers (rather than some non-specific achiral inclusion phenomenon) studies were undertaken with individual

enantiomers. ^{19}F NMR of the pure enantiomers, in the presence of methyl-substituted β-cyclodextrin, did indeed result in the appearance of only one resonance for each enantiomer. Thus the positive enantiomer had a signal centered at −83.23 ppm whilst the negative enantiomer's signal appeared at −82.22 ppm. The resonances corresponded to those observed for the racemic compound when analysed under these conditions.

As one of our aims is to use β-cyclodextrins in biological fluids, in order to study enantioselective clearance and metabolism, we also briefly investigated the use of this technique for urine. ICI 185,282 was spiked into a fresh, control, urine at 1 mg ml^{-1} and the methylated β-cyclodextrin added. The resulting ^{19}F NMR spectrum showed a clear separation of the signals for the two enantiomers. This demonstrates that the presence of the endogenous constituents of urine did not interfere with the chiral inclusion complexation process.

Derivatized β-cyclodextrins appear to offer an attractive alternative to β-cyclodextrin itself in this type of analysis. A major advantage of these materials (apart from the superior ^{19}F NMR resolution obtained) is their greater water solubility, compared to β-cyclodextrin itself, which greatly facilitates their use.

Whilst we have concentrated here on describing our ^{19}F NMR results, it should be emphasized that the use of this approach is not limited to this nucleus and that, like Greatbanks and Pickford [3], we have also observed changes in the ^{1}H NMR spectrum of ICI 185,282 which would have enabled enantiomeric analysis. However, the ^{1}H NMR spectrum of a molecule as complex as ICI 185,282 in the presence of intererences from water protons and those of the β-cyclodextrins, is complex. In this instance the presence of a ^{19}F resonance on ICI 185,282 enabled us to exploit the specificity provided by this nucleus and greatly simplified interpretation.

Further NMR and computer-aided molecular modelling studies are in progress to extend these preliminary studies and attempt to provide some insight into the underlying mechanisms associated with the improved chiral recognition shown by the methyl-substituted β-cyclodextrin.

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